



Caged *Gammarus fossarum* (crustacea) as a robust tool for the characterization of bioavailable contamination levels in continental waters. Toward the determination of threshold values

J.P. Besse, Marina Coquery, C. Lopes, Arnaud Chaumot, H. Budzinski, P. Labadie, Olivier Geffard

► To cite this version:

J.P. Besse, Marina Coquery, C. Lopes, Arnaud Chaumot, H. Budzinski, et al.. Caged *Gammarus fossarum* (crustacea) as a robust tool for the characterization of bioavailable contamination levels in continental waters. Toward the determination of threshold values. *Water Research*, 2013, 47 (2), p. 650 - p. 660. 10.1016/j.watres.2012.10.024 . hal-00773522

HAL Id: hal-00773522

<https://hal.science/hal-00773522>

Submitted on 14 Jan 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Caged *Gammarus fossarum* (crustacea) as a robust tool for the
characterization of bioavailable contamination levels in continental waters.
Toward the determination of threshold values**

Authors

BESSE Jean-Philippe (First author) ^a

COQUERY Marina ^a

LOPES Christelle ^a

CHAUMOT Arnaud ^a

BUDZINSKI Hélène ^b

LABADIE Pierre ^b

GEFFARD Olivier ^{a*}

mail: olivier.geffard@irstea.fr

phone number : (+33) 4 72 20 89 05

a: Irstea, UR MALY (Freshwater Systems, Ecology and Pollution), 5 rue de la Doua,

CS70077, 69626 VILLEURBANNE Cedex, France

Phone : (+33) 4 72 20 87 85

Fax : (+33) 4 78 47 78 75

b: Université Bordeaux 1, Environnements et Paléoenvironnements Océaniques et
Continentaux (EPOC) - UMR 5805 CNRS, Laboratoire de Physico- et Toxico-Chimie de
l'environnement (LPTC), 351 cours de la Libération, 33405 Talence, France.

* corresponding author

Abstract

We investigated the suitability of an active biomonitoring approach, using the ecologically relevant species *Gammarus fossarum*, to assess trends of bioavailable contamination in continental waters. Gammarids were translocated into cages at 27 sites, in the Rhône-Alpes region (France) during early autumn 2009. Study sites were chosen to represent different physico-chemical characteristics and various anthropic pressures. Biotic factors such as sex, weight and food availability were controlled in order to provide robust and comparable results. After one week of exposure, concentrations of 11 metals/metalloids (Cd, Pb, Hg, Ni, Zn, Cr, Co, Cu, As, Se and Ag) and 38 hydrophobic organic substances including polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyles (PCBs), pentabromodiphenylethers (PBDEs) and organochlorine pesticides, were measured in gammarids. All metals except Ag, and 33 organic substances among 38 were quantified in *G. fossarum*, showing that this species is relevant for chemical biomonitoring. The control of biotic factors allowed a robust and direct inter-site comparison of the bioavailable contamination levels. Overall, our results show the interest and robustness of the proposed methodological approach for assessing trends of bioavailable contamination, notably for metals and hydrophobic organic contaminants, in continental waters.

Furthermore, we built threshold values of bioavailable contamination in gammarids, above which measured concentrations are expected to reveal a bioavailable contamination at the sampling site. Two ways to define such values were investigated, a statistical approach and a model fit. Threshold values were determined for almost all the substances investigated in this study and similar values were generally derived from the two approaches. Then, levels of contaminants measured in *G. fossarum* at the 27 study were compared to the threshold values obtained using the model fit. These threshold values could serve as a basis for further

implementation of quality grids to rank sites according to the extent of the bioavailable contamination, with regard to the applied methodology.

Keywords

Active biomonitoring; threshold values; bioaccumulation; gammarids; freshwaters, priority substances

1. Introduction

The use of biota to monitor levels and trends of chemical contamination in water (i.e., chemical biomonitoring) was suggested in the mid-1970s for costal waters (Goldberg, 1975), and has been thereafter used in several monitoring programmes in costal and continental waters (Besse et al., 2012). As an integrative matrix, biota enables reliable measures for trace metals and hydrophobic contaminants, as the higher levels retained by the organisms can be more easily measured. Moreover, biota reflects the bioaccumulative and bioavailable fraction of contaminants in receiving waters, which are of direct ecotoxicological relevance. Finally, biota enables time-integrated measures over the exposure period, so it can be used to establish spatial and temporal trends of a bioavailable contamination (EC, 2010; Andral et al., 2004; Rainbow 1995).

Currently, two different strategies of chemical biomonitoring can be adopted: passive and active biomonitoring. Passive approaches rely on indigenous organisms (Sudaryanto et al. 2002; Goldberg 1975), while active ones rely on transplanted (or caged) individuals from a reference site (Benedicto et al., 2011; Andral et al., 2004). Even though passive approaches have proved useful for monitoring contamination trends for metals and several organic contaminants, they are recognized as suffering from two major drawbacks: i) they depend on the effective presence of the native organism at the sampled sites; and, ii) several factors (e.g., variability in the exposure time, age and size of sampled organisms) may hinder accurate interpretation of the results (Besse et al., 2012). Active approaches, based on transplanted organisms, have been developed more recently with the aim of resolving these limitations. Indeed, active approaches can be applied even if study sites are devoid of native organisms, they allow limiting biological variability as organisms are collected from the same population, and the exposure time can be controlled (Bourgeaut et al., 2010; Bervoets et al. 2005; Andral

et al. 2004; Mersch et al. 1996). If some active biomonitoring programs have been implemented in the marine environment (Benedicto et al., 2011; Andral et al., 2004), no such approaches have been undertaken at a large scale to monitor contamination in continental waters.

Therefore, the first objective of this study was to investigate the relevance and robustness of an active biomonitoring strategy, using the amphipod *Gammarus fossarum* to monitor trends of bioavailable contamination for trace metals and hydrophobic contaminants in continental waters.

The amphipod species *Gammarus fossarum* was selected as the test organism as gammarids are widespread and common in rivers and streams of Western Europe, where they are often present at high density. They are ecologically relevant as they represent an important reserve of food for macroinvertebrate, fish, bird and amphibian species and also play a major part in leaf litter breakdown processes (Macneil et al. 1997; Welton 1979). Moreover, *G. fossarum* is easy to identify down to the species level and its physiology is quite well known (Coulaud et al., 2011; Lacaze et al., 2011; Dedourge-Geffard et al., 2009). Finally, a well defined caging protocol is available for this species, allowing to control biological characteristics such as size, sex, supply of food; and it has been widely used for several studies in ecotoxicology (Coulaud et al., 2011; Lacaze et al., 2011; Dedourge-Geffard et al., 2009). As organisms are caged and as their only source of food is the one provided at the beginning of the experiment, concentrations measured in gammarids are to be regarded as mainly proceeding from the dissolved phase and not from the trophic route.

The second objective of this study was to benefit from such a caging approach to determine threshold values of bioavailable contamination, assuming that measured concentrations above the threshold values could be considered as representative of a bioavailable contamination at

the sampling site, whereas values under the threshold values would only reflect the background level of contamination.

Such threshold are expected to i) allow the identification of problematic contaminants on the basis of the bioavailable fraction, and ii) serve as a basis for any further implementation of quality grids that would allow to rank sites according to the extent of the bioavailable contamination. For instance, in continental waters, threshold values have been determined in aquatic bryophytes (by passive monitoring) to improve the interpretation of results and to build quality grids of metal contamination (AE, 1998). Furthermore, with regard to the marine environment, the OSPAR Commission has built some statistical tools that enable testing whether the mean measured concentrations (in sediment or biota) can be considered to be near background concentrations or is representative of an effective contamination of the sampling site (ICES, 2004).

To fulfil the two objectives of this study – investigating the relevance and robustness of the caging strategy, and determining threshold values of bioavailable contamination – individuals of *Gammarus fossarum* were caged for 7 days at 27 sites of rivers of the Rhône-Alpes region (France) and 49 contaminants (11 metals and 38 hydrophobic organic substances) were monitored. Sites were chosen for their differences in watershed size, physico-chemical characteristics and anthropic pressures.

2. Materials and methods

2.1. Sampling and handling of tests organisms

Gammarids were collected at “La Tour du Pin”, a known unpolluted upstream part of the Bourbre River (France). This station displayed good water quality according to RNB data

records (French Watershed Biomonitoring Network), and a high density of gammarids was found. Sexually mature *G. fossarum* were collected using a hand-held net. Gammarids were sieved (2-2.5 mm) to separate juveniles and adults, and were stored in plastic bottles containing ambient freshwater, then quickly brought to the laboratory. The organisms were kept during a 15 days acclimatisation period in 30 L tanks under constant aeration. A 10/14 h light/dark photoperiod was maintained and the temperature was kept at 12 (± 1) °C. They were continuously supplied with groundwater mixed with osmosis water at two different constant water hardness: 112 or 223 mg L⁻¹ of CaCO₃, depending on the hardness level of the subsequent studied site. Organisms were fed *ad libitum* with alder leaves (*Alnus glutinosa*) collected in a pristine site and previously conditioned for at least 6 (± 1) days in groundwater. Twice a week freeze-dried *Tubifex* sp. worms were added as a dietary supplement.

2.2. Caging procedure

The caging procedure applied here is derived from the one formerly used for *in situ* toxicity assessment and for the development of biomarkers (Coulaud et al., 2011; [Lacaze et al., 2011](#); [Dedourge-Geffard et al., 2009](#)).

Gammarids were caged in polypropylene cylinders (length, 10 cm; diameter, 5.5 cm) capped at their ends with pieces of net (mesh: 1 mm) to guarantee free water flow. Twenty-four hours before initiating the experiment, pools of 25 individual *G. fossarum* were placed in the cylinders. Eight experimental cylinders were placed into a rigid plastic container to protect them. The containers were subsequently positioned parallel to the direction of water flow and secured to the streambed using natural rocks. To limit the influence of body length, growth and sex on contaminants accumulation, only mature and same age-ranked gammarids were exposed: male *G. fossarum* with an average body length of 9 \pm 1 mm and an average weight

ranging from 4 to 6 mg (dry weight) were selected. To avoid the influence of starvation on survival rate and contaminants uptake, 20 alder leaf (*A. glutinosa*, same as in laboratory) discs (20 mm in diameter) were supplied into each cage.

After 7 days of exposure, gammarids were collected, counted (for survival rate assessment), dried, weighted, frozen in liquid nitrogen and stored at -80°C until chemical analyses.

Temperature probes were fixed to one cage at each site, so that measurements could be carried out twice a day throughout the experiment. The measure of hardness was also carried out at the laboratory from grab samples collected at the beginning and at the end of the exposure.

2.3. Study sites

The experiment was conducted in September 2009. Twenty-seven sites were selected in the Rhône-Alpes region aiming at covering a large range of geographical locations (approximately 20 000 km²), various types of hydrological systems and a large range of physico-chemical characteristics (Figure 1, Table S1 and S2).

For our study, the sites were also selected seeking to cover diverse anthropogenic pressures (industrial, urban and agricultural activities). Within these 27 sites, 12 sites (1-12) were chosen as non- impacted sites among the national reference network (Water Framework Directive- WFD - implementation) in collaboration with the regional public water agency (<http://sierm.eaurmc.fr/eaux-superficielles>). According to expert judgement based on data on land use, chemical monitoring (macropollutants and some micropollutants), and ecological diagnosis, these sites were considered to be devoid of (or to showing limited) anthropic pressure. The 15 other sites (13-27) were chosen as impacted sites among the national control network (WFD implementation) by the regional water agency (<http://sierm.eaurmc.fr/eaux->

[superficielles](#)). According to the expert classification based on data on land use, degraded water chemical quality (macropollutants and some micropollutants) and/or poor faunistic indices, these sites were considered impacted by anthropic activities. Detailed physico-chemical characteristics for all studied sites (i.e., surface water temperature, dissolved oxygen, pH and hardness), and pressure types for the 15 anthropically impacted sites are presented in supplementary data, Tables S1 and S2. For data treatment, and notably for the determination of threshold values, study sites were used together, not taking into account their *a priori* contamination level.

2.4. Choice of contaminants and chemical analysis

A total of 49 contaminants were investigated in this study, including 22 chosen with reference to the list of WFD priority substances (EC, 2008). The list included 11 metals or metalloids: Cd, Pb, Hg, Ni (WFD priority substances; EC, 2008) and Ag, As, Co, Cr, Cu, Se and Zn. Also, 38 hydrophobic organic substances ($\log K_{ow} > 3$) were investigated including chlorinated pesticides (among which 8 WFD priority substances): lindane, hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT) isomers and metabolites (2,4' DDE; 4,4' DDE + dieldrin; 2, 4'-DDD; 4, 4'-DDD; 2, 4'-DDT; 4,4'-DDT); heptachlor and heptachlor epoxide; 7 indicator PCBs: CB n° 28, 52, 101, 118, 138, 153 and 180; 4 congeners of PBDEs (among which 3 WFD priority substances): BDE n° 47, 99, 119 and 153; and 16 PAHs (among which 7 WFD priority substances): naphthalene, anthracene, fluoranthene, benzo(a)pyrene (BaP), benzo(b,k,j)fluoranthenes, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene (BP); and acenaphthylene, acenaphthene, fluorene, phenanthrene, pyrene, benzo(a)anthracene, benzo(e)pyrene, triphenylene + chrysene, perylene and dibenzo(a)anthracene + dibenzo(c)anthracene (DaA + DaC).

2.4.1. Metal analysis

Individuals of *G. fossarum* were pooled (5 organisms per sample) to obtain an average mass of 30 mg dry weight (about 150 mg wet weight). Three replicates of each pooled sample were subjected to analysis. All chemical analyses were conducted at the Irstea of Lyon. Metals (Cd, Pb, Hg, Ni, Ag, As, Co, Cr, Cu, Se and Zn) were analysed by inductively coupled plasma mass spectrometry (ICP-MS, Thermo X7 series II), after mineralization with nitric acid in a microwave oven. For Hg, samples were analyzed by automated atomic absorption spectrometry (MILESTONE, Direct Mercury Analyser 80).

Blank tests were carried out systematically to detect any possible contamination along the analytical chain. The following certified reference materials (CRM) were used for quality control for metals: National Research Council Canada (NRCC) TORT-2, lobster hepatopancreas, and International Atomic Energy Agency IAEA-407, fish. For Hg, National Institute of Standards and Technology Standard Reference Material (NIST) SRM-2976 and Institute for Reference Materials and Measurements (IRMM) CRM 278 R, mussel tissue, were used. The limits of quantification (LQ), determined according to NF XPT 90-210 (AFNOR, 1999), are detailed in Table 1. The CRM results were generally well within certified values. Relative standard deviations of triplicate pooled sample analyses (including sampling and analytical variability) were generally below 20%.

2.4.2. Organic substances analysis

All chemical analysis were carried out at the Centre de Développement et de Transfert Analytique (CDTA, Bordeaux). Individuals of *G. fossarum* were pooled (75 organisms per

sample) to obtain an average mass of 2300 mg wet weight (i.e., approximately 400 mg dry weight). Extraction and quantification methods for PAHs, PCBs and organochlorine pesticides are described elsewhere in detail (Cailleaud et al., 2007; Thompson and Budzinski, 2000). Briefly, contaminants accumulated in *G. fossarum* were extracted using dichloromethane by microwave-assisted extraction (Maxidigest 350 VWR, Fontenay sous Bois, France). The organic extracts were then purified. Concentrations of PCBs and organochlorine pesticides on the one hand and of PAHs on the other hand were measured, respectively, using a gas chromatography (GC)/electron capture detector (Hewlett-Packard 5890A series IIGC, Avondale, MA, USA) equipped with a ^{63}Ni electron-capture detector) and GC/mass spectrometry (Hewlett-Packard model series 6890A GC and an Agilent Networks 5973 mass selective detector, Agilent Technologies, Santa Clara, CA, USA). The LQ are detailed in Table 2.

Procedural blanks (glass material and solvents) were regularly performed. The effectiveness of the different analytical procedures was evaluated by analyzing National Institute of Standards and Technology Standard Reference Material (NIST) SRM-2978, mussel tissue, for PCBs and PAHs.

2.5. Determination of threshold values of contamination

To determine threshold values of bioavailable contamination, *G. fossarum* concentrations for each contaminant were first sorted by increasing value. From such a representation, we subsequently determined the threshold value from which the concentration measured in *G. fossarum* could be considered as significant. For that, two different approaches were investigated: the first one based on a statistical approach (normality assumption), and the second one based on a model fit (bacterial growth model).

2.5.1. Statistical approach

The first method was based on the assumption that contamination levels in organisms would be normally distributed only at sites devoid of any bioavailable anthropogenic and/or geochemical background contamination. For each substance and values higher than the LQ, we tested if the overall data set followed a Gaussian distribution (using Shapiro test). If not, the most contaminated site was removed from the data set and the normality tested again. Such an iterative process was conducted until a data set normally distributed was obtained. The threshold value for each substance was determined from the respective Gaussian distribution obtained by the 95th percentile, with a risk of false negative set at 5% (Figure 2). The iterative procedure was implemented using the R statistical computing program (R Development Core Team, 2007).

2.5.2. Model fit

The second method was based on the observation that contamination levels in gammarids followed the same overall pattern as bacterial growth kinetic: with a latent period (corresponding here to the background level of contamination in organisms), and an exponential phase (corresponding to the significative phase of accumulation). Therefore, the distribution of contamination levels, sorted by increasing values, was fitted using a bacterial growth model, the Barranyi's model, following Equation 1 (Baranyi et al., 1993).

$$\log_{10}(C(t)) = \log_{10}(C_{\max}) + \log_{10} \left(\frac{-1 + \exp(\mu * t) + \exp(\mu * lag)}{-1 + \exp(\mu * t) + \exp(\mu * lag) * 10^{(C_{\max} - C_0)}} \right) \quad \text{Equation 1}$$

where $C(t)$ is the concentration at site t ; C_{max} is the maximal concentration; μ is the accumulation rate; lag is the point from which the exponential phase of accumulation begins; and C_0 is the minimal concentration.

The “ lag ” allowed separating the background level of contamination from the significant phase of accumulation, and was used as the break point from which the threshold value of contamination was estimated (Figure 3). The calculation was performed using the R statistical computing program. The parameters of the Baranyi’s model fitted to the data were estimated by nonlinear regression using the “ $nlstools$ ” package (<http://cran.r-project.org/web/packages/nlstools/nlstools.pdf>).

3. Results

3.1. Quantification of contaminants in *Gammarus fossarum*

After the 7 days of exposure, gammarids survival rate remained high, with a mean survival rate higher than 75% at all but one site (site 27, Supplementary data, Figure S1).

Contamination levels in *G. fossarum* are presented in Table 1 for metals (or metalloids) and in Table 2 for organic substances. Considering metals/metalloids, nearly all measured values were higher than the LQ. Cd, Hg, As, Cu, Co, Se and Zn were always quantified, while Pb, Ni, and Cr were quantified at all but 2, 4, and 8 sites, respectively. Only Ag was not quantified at all among the 27 sites. For organic substances, most of them were also quantified in caged *G. fossarum*, with only 5 substances never detected: heptachlor, heptachlor epoxide, 2,4'-DDD and BDE congeners n° 119 and 153 (Table 2). Some DDT isomers (namely 4,4'-DDE + dieldrin; 4,4'-DDT and 2,4'-DDT), BDE congeners (47 and

153), some PAHs (naphthalene, acenaphthene, pyrene, benzo(a)anthracene, and triphenylene + chrysene), and all PCB congeners were always quantified.

To estimate the capacity of accumulation of *G. fossarum* with regard to the investigated contaminants, empirical factors (ratio maximal concentration/minimal) were assessed. Except for Zn and Cu, all ratios were higher than 2, with values up to 100 for organic contaminants (phenanthrene, 2,4'-DDT).

3.2. Threshold values of contamination

Threshold values determined for contaminants investigated here are displayed in Table 3. The statistical approach provided a threshold value for all studied substances with concentrations higher than the LQ (i.e., 43 substances), whereas the model fit could not provide threshold values for 8 contaminants: 5 metals (As, Cr, Cu, Se and Zn), and 3 organic substances (naphthalene, DaA-DaC, and BDE 47).

For a single substance, threshold values calculated using the two approaches were very close to each other (Table 3): the maximum interval observed between two values was of $0.1 \mu\text{g.g}^{-1}$ for metals (observed for Ni) and 3.1 ng.g^{-1} for organics (observed for phenanthrene).

4. Discussion

4.1. Suitability *G. fossarum* as a biomonitor of chemical contamination in continental waters.

Contrary to other freshwater invertebrates, such as the invasive bivalve *Dreissena polymorpha*, gammarids have not been much used to monitor chemical contamination in continental waters. For metals, very few data from passive (Schaller et al., 2011; Amyot et al.,

1996) and active biomonitoring (Lacaze et al., 2011; Dedourge-Geffard et al., 2009; Khan et al., 2011) are available. Mean and median values reported in these latter studies (for Cd, Ni, Pb, As, Co, Cr, and Zn) for rivers are in the same range as values observed here. For organic substances investigated here, and for continental waters, no data from active biomonitoring were found in the scientific literature and only very few data from passive biomonitoring are available (Blais et al., 2003). Heptachlor was measured in *G. lacustris*, at around 0.1 ng.g^{-1} wet weight (about 0.5 ng.g^{-1} dry weight, assuming 80% moisture). These results suggest that, even if uptake rates may differ between the two species, the absence of quantification of this substance in our study was not linked to a low accumulation in *G. fossarum*, but rather to an absence of contamination of the study sites by this contaminant. Our results show that almost all investigated substances accumulated well and could be quantified in *G. fossarum*, on a relatively short exposure period (Tables 1 and 2). Moreover, the high Max/Min ratios measured for most of the organic contaminants studied suggest that *G. fossarum* is a good accumulator and a suitable species for monitoring chemical contamination in continental waters. Indeed, as stated by Rainbow (2002), the fact that the sampled organism is a strong accumulator increases the power of resolution between sites. Nonetheless, the suitability of *G. fossarum* for chemical monitoring is limited in the specific case of Cu: indeed, gammarids are known to be poor indicators for this metal, as Cu is involved in haemocyanin synthesis, and is therefore highly regulated in all gammarid species (Dedourge-Geffard et al., 2009; Taylor and Anstiss, 1999).

Finally, considering the analytical methodologies used in this study, only a limited amount of tissue matrix was necessary to quantify the contaminants investigated here: about 5 organisms (approximately 150 mg wet weight) for all metals but Hg, 5 organisms for Hg, and 75 organisms (approximately 2300 mg wet weight) for all organic substances.

4.2. Robustness of the active methodology for chemical monitoring

During this study, we focused on showing the robustness of the methodology and the comparability of the results. The methodology proposed here (organisms of same sex, same weight and supplemented with food) allowed obviating any influence of biotic factors on tissue levels of contaminants. At the end of the exposure, only minor variations of weight were observed (mean of 5.8 mg dry weight per organism, with a standard deviation of 0.7 mg). Selecting mature organisms with the same weight allowed avoiding any confounding effect of this parameter, which is considered by several authors as one of the main factor that can influence tissue levels of contaminants (Geffard et al., 2007; Mubiana et al., 2006; Andral et al., 2004; Boyden, 1974).

Supplementing gammarids with food ensures an optimal survival rates and prevents from any growth variation linked with food availability, which is a clear advantage over bivalves. In fact, the accumulation of contaminants depends on the organism growth (Andral et al., 2004). Hence, as shown for bivalves, caged organisms exposed in sites of different trophic potential may exhibit different growth rates, which prevent a direct comparison of tissue concentrations. In such cases, there is a need to correct raw data to account for various growth rates (Bourgeault et al., 2010; Andral et al. 2004; Mersch et al., 1996).

Contrary to biotic factors, the influence of abiotic factors cannot be controlled in our methodology. Such factors can be suspected to perturb the physiology of organisms and therefore to influence levels of contamination in *G. fossarum*. As an example, the role of temperature on bivalves' physiology and on bioaccumulation is commonly emphasised (Minier et al., 2006; Gossiaux et al., 1996). Considering the conditions of this study, no influence of temperature was observed. This could be linked to the fact that the observed temperatures during the exposure (minimum of 8.6°C, maximum of 19.7°C; Supplementary

data, Table S3) were all within the tolerance range for *G. fossarum*, which is estimated to be from 0°C to 25°C, with an optimum temperature of 12°C (Wijnhoven et al., 2003).

Furthermore, for gammarids, results of a laboratory experiment indicated that temperature has only a weak effect on metals accumulation (Pellet et al., 2009). Moreover, results of a field study on the accumulation of organic pesticides and PCBs in gammarids suggested that temperature has a negligible influence on tissue levels of contaminants when compared to the growth rate of organisms (Blais et al., 2003).

Although the impact of hardness on metal speciation and bioavailability is well known (Lebrun et al., 2011; Peters et al., 2011; Heijerick et al., 2003; Wright & Frain, 1981), its influence on the physiology of organisms, in particular on the density of sites of action of metal transporters, and subsequently on the accumulation of metals in organisms, has been poorly studied to date. Ma et al. (1999) showed that Cu uptake in *Ceriodaphnia dubia* did not change when organisms were grown in water with high or low Ca^{2+} concentration levels. In contrast, Pellet et al. (2009) showed that Cd influx in *G. pulex* decreased as Ca^{2+} concentrations increased due to the decrease of Cd bioavailability resulting from the competition between Ca^{2+} and Cd. In the study sites, hardness ranged from 15 to 290 mg.L^{-1} of CaCO_3 ((Supplementary data, Table S1, S2); no influence of hardness on contamination levels of organisms was observed. To suppress the effect of hardness, *G. fossarum* was acclimated to appropriate level of hardness (see section 2.1.) prior to the biomonitoring. Thus, the effects of hardness on the physiology of gammarids are expected to be negligible.

4.3. Threshold values of bioavailable contamination

4.3.1. Validity of calculated threshold values

This is the first study dealing with the determination of threshold values of bioavailable contamination in biota for metals and hydrophobic substances. Consequently, it is only possible to propose preliminary statements on the validity of the calculated threshold values. As a first approach, threshold values were considered to be valid for a given substance when i) a threshold value could be calculated via the two different approaches and ii) the values given by the two approaches were close to each other. To verify the second statement, we compared the difference between the two values to the maximum concentration measured in gammarids, using Equation 2 ("Reliability ratio"). We consider that the lower the ratio, the more reliable the threshold value.

$$\text{Reliability ratio} = \left| \frac{\text{Threshold}_{stat} - \text{Threshold}_{fit}}{\text{Maximum measured concentration}} \right| \times 100 \quad \text{Equation 2}$$

Results are displayed in Table 3. For the 35 contaminants for which the two methodologies provided a threshold value, reliability ratios were very low (<0.1) for all but 5 contaminants, and the highest ratio (0.3) was observed for Hg and BDE 99. Therefore, we consider these threshold values as valid.

4.3.2. *Invalid threshold values*

Threshold value for Cu, obtained using the statistical approach only, was expected to be invalid. In fact, as discussed in section 4.1., gammarids are poor indicators for this metal as they are able to regulate it. Thus, contrary to other contaminants, distribution of the measured concentrations of Cu showed a specific profile (i.e., not gaussian, or without an exponential phase; Supplementary data, Figure S2).

For 7 other contaminants (As, Cr, Se, Zn, naphthalene, DaA-DaC and BDE 47), only the statistical approach provided a threshold value (Table 3, Figure S2 of supplementary data) so

their validity is questionable. Unvalidity may stem from the overall absence of contamination, or conversely, from the presence of a significant bioavailable contamination at all study sites. In fact, for these 7 contaminants, the whole datasets followed a normal distribution. Hence, if we assume that contamination levels in organisms are normally distributed only at sites devoid of any anthropogenic pressure, such results suggest that measured concentrations at the 27 sites were only representative of the “background” impregnation. This is also in agreement with the fact that no threshold value could be determined using the model fit (i.e., measured concentrations only representative of the latent phase of bioaccumulation).

Such an hypothesis could be verified in the case of Zn. We included additional bioaccumulation data obtained from a previous study on metal impacted sites, based on the same gammarus species and a similar exposure protocol, and showing concentrations in gammarids up to $237 \mu\text{g.g}^{-1} \text{ dw}$ (Lacaze et al., 2011). We obtained threshold values using both calculation methods, slightly above maximum concentrations measured in the present study. Such results underline the need to obtain bioaccumulation data representative of contaminated areas to determine valid thresholds.

Overall, our results support the relevance of the two methodologies (i.e., statistical approach or model fit) to determine threshold values of bioavailable contamination in caged *G.*

fossarum.

4.3.3. Application of the threshold values

As a preliminary application of our defined threshold values, we classified the study sites according to valid thresholds obtained with the model fit (Table 4).

For organic contaminants, this classification showed that 15 sites (#1 to 11, 13, 16, 18 and 26) displayed less than 5 substances exceeding thresholds. For these 15 sites - except sites 16, 18 and 26 - these results support the expertise of water agency (Supplementary data, Table S1 and S2), as these sites did not show a bioavailable contamination for most of the investigated substances. For sites 16, 18 and 26, observed discrepancies with the expertise of water agency may stem from one of the following reasons : i) conclusions obtained from experts did not take into account the physico-chemical parameters of study sites, whereas they play a key role in the bioavailability of pollutants, ii) contamination levels were determined in caged organisms for a duration exposure of 1 week, and therefore, do not integrate contamination variability that may exist in aquatic systems, and iii) sites may be contaminated by other organic substances than the ones investigated here.

Conversely, 7 other sites (# 12, 14, 19, 22, 23, 24 and 27) had concentrations in gammarids higher than the threshold values for several organic substances and displayed distinguishable profiles of contamination. For instance, sites 12, 23, 24 and 27 showed a clear contamination of PAHs and PCBs, and site 19 displayed a specific contamination of pesticides and PCBs. Hence, these results show that threshold values are valuable tools to identify sites showing a bioavailable contamination, to identify problematic contaminants and to draw typologies of contamination. This is of value for river basin authorities for establishing strategic framework for the management of waterbodies.

For metals, the threshold classification showed that only 10 sites among the 27 did not display any concentration higher than the threshold values, while most of the sites showed a contamination by Cd and Pb, and site 18 had a specific contamination profile with Hg, Ni and Co. With regard to the expert classification of the Water Agency, numerous discrepancies were observed. Indeed, sites 1 to 12 were expected to be devoid of any anthropic pressure (Section 2.3.). Such discrepancies may stem from the presence of i) an anthropogenic source

of contamination not pre-identified, or ii) a local geochemical background. Currently, available information is too scarce to draw any definitive conclusion, but we note that these sites are localized in the Western part of the Rhône-Alpes region, near the “Massif Central” mountain range, known to present elevated geochemical backgrounds for As, Cd and Pb (Cf. FOREGS Geochemical Baseline Mapping Programme; <http://www.gsf.fi/publ/foregsatlas>).

5. Conclusion

Results of this study showed that caged *Gammarus fossarum* is a robust and useful tool to monitor bioavailable contamination trends of metals and hydrophobic organic substances in continental waters. The two most important advantages of this methodology are i) that it can be applied even if the study site is devoid of native organisms and ii) that it provides results that enable a direct comparison of bioavailable contamination trends among different sites.

Moreover, using two simple calculation approaches, we were able to determine valid threshold values of bioavailable contamination in *G. fossarum*. These threshold values allowed discriminating background levels of contamination from any significant bioaccumulation in gammarids, thus indicating a bioavailable contamination at the sampling site. Such threshold values could further serve as a basis for the implementation of a quality grid that would allow ranking sites according to the extent of the bioavailable contamination, and with regard to the applied methodology. To our knowledge, this was the first study to investigate the implementation of such threshold values in the context of active biomonitoring.

Next step in our work will focus on the following objectives: investigating the bioaccumulation of other organic substances - notably WFD priority substances - and further

validating the threshold values by conducting additional studies at national scale in France, taking into account various river systems and different anthropic pressures. Such an effort is necessary to clearly establish if the defined threshold values are dependent or not of hydrogeochemical characteristics and if they need some adjustment. Finally, investigating longer duration of exposure will allow assessing whether threshold values also depend on the exposure time.

Acknowledgments

Authors thank ONEMA (the French National Agency for Water and Aquatic Ecosystems) for its financial support. Authors also thank technical staff of Irstea for their assistance in the field experiments and for analyses of trace elements.

References

AE (1998) Les bryophytes aquatiques comme outil de surveillance de la contamination des eaux courantes par les micropolluants métalliques. Concept, méthodologie et interprétation des données. Etude inter-agences n° 55.

AFNOR, Norme NF XPT 90-210, Protocole d'évaluation d'une méthode alternative d'analyse physico-chimique par rapport à une méthode de référence, 1999, 58 pp.

(www.boutique.afnor.org/).

Amyot, M., Pinel-Alloul, B., Campbell, P.G.C., Désy, J.C. (1996) Total metal burdens in the freshwater amphipod *Gammarus fasciatus*: Contribution of various body parts and influence of gut contents. *Freshwater Biology* 35(2), 363-373.

Andral, B., Stanisiere, J.Y., Sauzade, D., Damier, E., Thebault, H., Galgani, F. and Boissery, P. (2004) Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. *Marine Pollution Bulletin* 49(9-10), 704-712.

Baranyi, J., Roberts, T.A., McClure, P. (1993) A non-autonomous differential equation to model bacterial growth. *Food Microbiology* 10 (1) 43-59.

Benedicto, J., Andral, B., Martínez-Gómez, C., Guitart, C., Deudero, S., Cento, A., Scarpato, A., Caixach, J., Benbrahim, S., Chouba, L., Boulahdid, M. and Galgani, F. (2011) A large scale survey of trace metal levels in coastal waters of the Western Mediterranean basin using

caged mussels (*Mytilus galloprovincialis*). *Journal of Environmental Monitoring* 13(5), 1495-1505.

Bervoets, L., Voets, J., Covaci, A., Chu, S., Qadah, D., Smolders, R., Schepens, P. and Blust, R. (2005) Use of transplanted zebra mussels (*Dreissena polymorpha*) to assess the bioavailability of microcontaminants in flemish surface waters. *Environmental Science and Technology* 39(6), 1492-1505.

Besse, J.P., Geffard, O., Coquery, M. (2012) Relevance and applicability of active biomonitoring in continental waters under the Water Framework Directive. *Trends in Analytical Chemistry*. 6, 113-127.

Blais, J.M., Wilhelm, F., Kidd, K.A., Muir, D.C.G., Donald, D.B. and Schindler, D.W. (2003) Concentrations of organochlorine pesticides and polychlorinated biphenyls in amphipods (*Gammarus lacustris*) along an elevation gradient in mountain lakes of western Canada. *Environmental Toxicology and Chemistry* 22(11), 2605-2613.

Bourgeault, A., Gourlay-Francé, C., Vincent-Hubert, F., Palais, F., Geffard, A., Biagianti-Risbourg, S., Pain-Devin, S. and Tusseau-Vuillemin, M.H. (2010) Lessons from a transplantation of zebra mussels into a small urban river: An integrated ecotoxicological assessment. *Environmental Toxicology* 25(5), 468-478.

Boyden, C.R. (1974) Trace element content and body size in molluscs. *Nature* 251(5473), 311-314.

- Cailleaud, K., Forget-Leray, J., Souissi, S., Hilde, D., LeMenach, K., Budzinski, H. (2007) Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to a planktonic species *Eurytemora affinis* (Calanöida, copepoda). Part 1: PCBs and PAHs. Chemosphere 70, 270–280.
- Coulaud, R., Geffard, O., Xuereb, B., Lacaze, E., Quéau, H., Garric, J., Charles, S., Chaumot, A. (2011) In situ feeding assay with *Gammarus fossarum* (Crustacea): Modelling the influence of confounding factors to improve water quality biomonitoring. Water Research 45(19), 6417-6429.
- Dedourge-Geffard, O., Palais, F., Biagianti-Risbourg, S., Geffard, O., Geffard, A. (2009) Effects of metals on feeding rate and digestive enzymes in *Gammarus fossarum*: an in situ experiment. Chemosphere 11, 1569-1576.
- European Commission (EC) (2008) Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/799 EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and 800 amending Directive 2000/60/EC of the European Parliament and of the Council, Official Journal of European Commission L 348 (2008) 84.
- European Commission (EC) (2010) CMA. Guidance on surface water chemical monitoring under the Water Framework Directive. Guidance document no 25. Technical report 210.3991. Common Implementation Strategy for the Water Framework Directive, EC, Brussels, Belgium.

Geffard, A., Quéau, H., Dedourge, O., Biagianti-Risboug, S. and Geffard, O. (2007) Influence of biotic and abiotic factors on metallothionein level in *Gammarus pulex*. Comparative Biochemistry and Physiology - C Toxicology and Pharmacology 145(4), 632-640.

Goldberg, E.D. (1975) The mussel watch. A first step in global marine monitoring. Marine Pollution Bulletin 6(7), 111.

Gossiaux, D.C., Landrum, P.F. and Fisher, S.W. (1996) Effect of temperature on the accumulation kinetics of PAHs and PCBs in the zebra mussel, *Dreissena polymorpha*. Journal of Great Lakes Research 22(2), 379-388.

Heijerick, D.G., Janssen, C.R., De Coen, W.M. (2003) The combined effects of hardness, pH, and dissolved organic carbon on the chronic toxicity of Zn to *D. magna*: Development of a surface responses model. Archives of Environmental Contamination and Toxicology 44, 210-217.

ICES (2004). OSPAR/ICES Workshop on the evaluation and update of background reference concentrations (B/RCs) and ecotoxicological assessment criteria (EACs) and how these assessment tools should be used in assessing contaminants in water, sediment and biota. 9 – 13 February, La Hague. Final report. Available at www.ospar.org.

Khan, F.R., Irving, J.R., Bury, N.R., Hogstrand, C. (2011) Differential tolerance of two *Gammarus pulex* populations transplanted from different metallogenic regions to a polymetal gradient. Aquatic Toxicology 102(1-2), 95-103.

Lacaze, E., Devaux, A., Mons, R., Bony S., Garric, J., Geffard, A., Geffard, O. (2011) DNA damage in caged *Gammarus fossarum* amphipods : A tool for freshwater genotoxicity assessment. *Environmental Pollution* 159 (6), 1682-1691.

Lebrun, J.D., Perret, M., Uher, E., Tusseau-Vuillemin, MH., Gourlay-Francé, C. (2011) Waterborne nickel bioaccumulation in *Gammarus pulex* : Comparison of mechanistic models and influence of water cationic composition. *Aquatic Toxicology* 104, 161-167.

Macneil, C., Dick, J.T.A. and Elwood, R.W. (1997) The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): Problems and perspectives concerning the functional feeding group concept. *Biological Reviews of the Cambridge Philosophical Society* 72(3), 349-364.

Ma, H., Kim, S.D., Cha, D.K., Allen, H.E. (1999) Effect of kinetics of complexation by humic acid on toxicity of copper to *Ceriodaphnia dubia*. *Environmental Toxicology and Chemistry* 18 (5), 828-837.

Mersch, J., Wagner, P. and Pihan, J.C. (1996) Copper in indigenous and transplanted zebra mussels in relation to changing water concentrations and body weight. *Environmental Toxicology and Chemistry* 15(6), 886-893.

Minier, C., Abarnou, A., Jaouen-Madoulet, A., Le Guellec, A.M., Tutundjian, R., Bocquené, G. and Leboulenger, F. (2006) A pollution-monitoring pilot study involving contaminant and

biomarker measurements in the Seine Estuary, France, using zebra mussels (*Dreissena polymorpha*). *Environmental Toxicology and Chemistry* 25(1), 112-119.

Mubiana, V.K., Vercauteren, K. and Blust, R. (2006) The influence of body size, condition index and tidal exposure on the variability in metal bioaccumulation in *Mytilus edulis*. *Environmental Pollution* 144(1), 272-279.

Pellet, B., Geffard, O., Lacour, C., Kermoal, T., Gourlay-FrancÉ, C. and Tusseau-Vuillemin, M.H. (2009) A model predicting waterborne cadmium bioaccumulation in *gammarus pulex*: the effects of dissolved organic ligands, calcium, and temperature. *Environmental Toxicology and Chemistry* 28(11), 2434-2442.

Peters, A., Lofts, S., Merrington, G., Brown, B., Stubblefield, W., Harlow, K. (2011) Development of biotic ligand models for chronic manganese toxicity to fish, invertebrates, and algae. *Environmental Toxicology and Chemistry* 30(11), 2407-2415.

R Development Core Team. 2007. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://cran.r-project.org/>.

Rainbow, P.S. (2002) Trace metal concentrations in aquatic invertebrates: Why and so what? *Environmental Pollution* 120 (3) , pp. 497-507.

Rainbow, P.S. (1995) Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin* 31(4-12), 183-192.

Schaller, J., Dharamshi, J., Dudel, E.G. (2011) Enhanced metal and metalloid concentrations in the gut system comparing to remaining tissues of *Gammarus pulex* L. *Chemosphere* 83 (4), 627-631.

Sudaryanto, A., Takahashi, S., Monirith, I., Ismail, A., Muchtar, M., Zheng, J., Richardson, B.J., Subramanian, A., Prudente, M., Hue, N.D. and Tanabe, S. (2002) Asia-Pacific mussel watch: Monitoring of butyltin contamination in coastal waters of Asian developing countries. *Environmental Toxicology and Chemistry* 21(10), 2119-2130.

Taylor, H.H., Anstiss, J.M. (1999) Copper and haemocyanin dynamics in aquatic invertebrates. *Marine and Freshwater Research* 50 (8), 907-931.

Thompson, S., Budzinski, H. (2000) Determination of polychlorinated biphenyls and chlorinated pesticides in environmental biological samples using focused microwave-assisted extraction. *International Journal of Environmental Analytical Chemistry* 76 (1), pp. 49-60.

Welton, J.S. (1979) Life-history and production of the amphipod *Gammarus pulex* in a Dorset chalk stream. *Freshwater Biology* 9, 12.

Wijnhoven, S., Van Riel, M.C. and Van Der Velde, G. (2003) Exotic and indigenous freshwater gammarid species: Physiological tolerance to water temperature in relation to ionic content of the water. *Aquatic Ecology* 37(2), 151-158.

Wright, D.A. and Frain, J.W. (1981) The effects of calcium on cadmium toxicity in the freshwater amphipod, *Gammarus pulex* (L.). Archives of Environmental Contamination and Toxicology 10, 321-328.

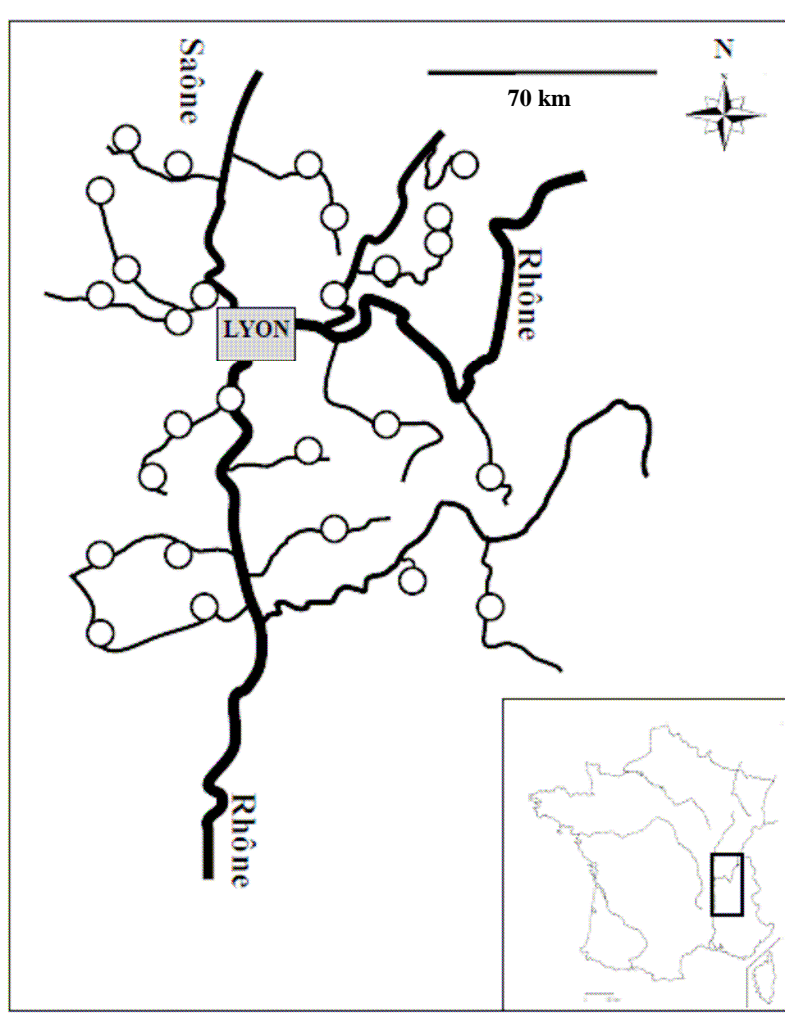


Figure 1. Location of study sites (n=27) along the Rhône-Alpes region (France).

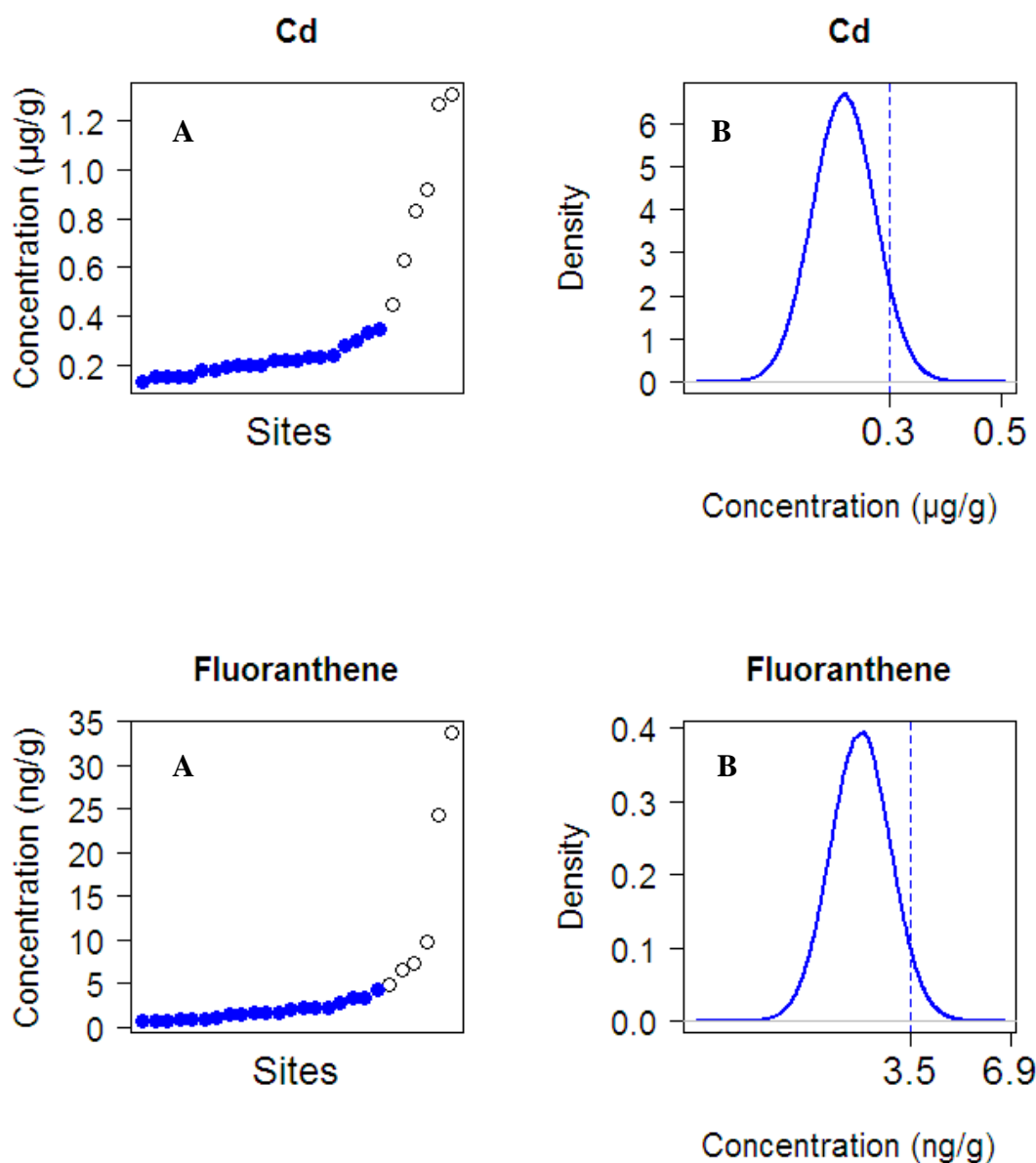


Figure 2. Example of the threshold value calculation for cadmium and fluoranthene, using the proposed statistical approach (see section 2.5.1. for details). A: cadmium / fluoranthene concentrations measured in *G. fossarum* at each site, sorted by increasing values. B: Distribution of the full circles of graph A constituting the larger data set following a Gaussian distribution; the threshold value derived with this method for cadmium and fluoranthene is indicated with the dotted line (percentile 95%).

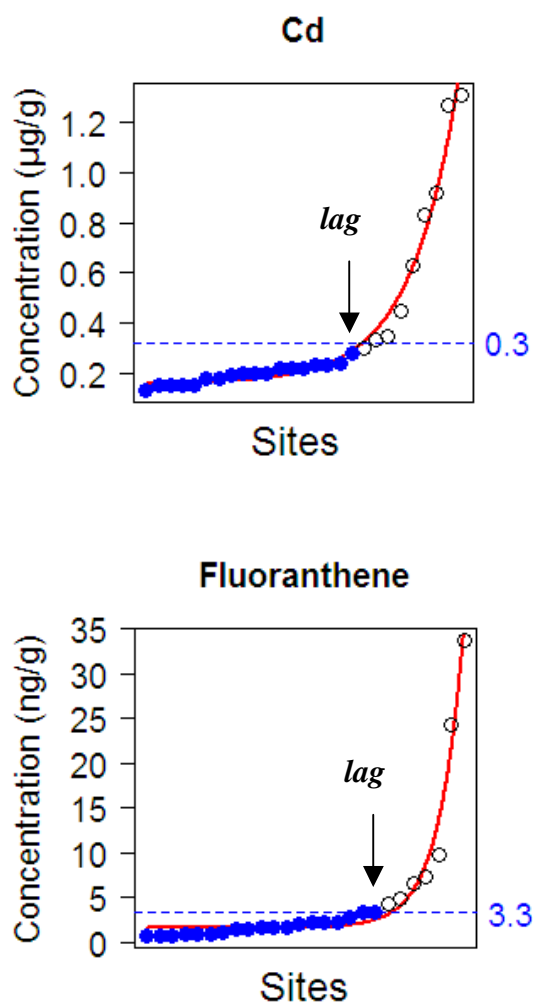


Figure 3. Example of the threshold value calculation for cadmium and fluoranthene, determined using the model fit (Baranyi's model). Concentrations measured at each site are sorted by increasing values. Fit of the Baranyi's model is showed in plain line. The *lag* is the break point where the accumulation enters an exponential phase; the concentration corresponding to the *lag* is defined as the threshold value. The full circles are the sites below the *lag*, representing the background contamination in organisms.

Table 1. Metal concentrations, limits of quantification (LQ) and frequency of quantification (% data \geq LQ) in caged *Gammarus fossarum* after 7 days of exposure for the 27 studied sites. Concentrations are expressed in $\mu\text{g.g}^{-1}$ dw (dry weight). Med, Min and Max are the median, minimal and maximal of the measured concentrations, respectively.

| Metals | Concentration ($\mu\text{g.g}^{-1}$ dw) | | | | LQ | Frequency (%) |
|-----------|------------------------------------------|-------|-------|---------|-------|---------------|
| | Med | Min | Max | Max/Min | | |
| Cd | 0.22 | 0.13 | 1.31 | 10.4 | 0.04 | 100 |
| Pb | 0.28 | 0.14 | 1.39 | 9.9 | 0.07 | 93 |
| Hg | 0.049 | 0.040 | 0.107 | 2.7 | 0.010 | 100 |
| Ni | 0.48 | 0.27 | 1.29 | 4.8 | 0.19 | 85 |
| Ag | < LQ | < LQ | < LQ | - | 0.80 | 0 |
| As | 1.62 | 0.95 | 2.72 | 2.9 | 0.20 | 100 |
| Cr | 0.48 | 0.20 | 1.06 | 5.3 | 0.20 | 70 |
| Cu | 72.1 | 50.7 | 85.3 | 1.7 | 0.20 | 100 |
| Co | 0.32 | 0.13 | 0.96 | 7.4 | 0.08 | 100 |
| Se | 2.05 | 1.28 | 2.58 | 2.0 | 0.40 | 100 |
| Zn | 69.7 | 49.5 | 81.9 | 1.6 | 0.74 | 100 |

Table 2. Concentrations of organic substances, limits of quantification (LQ) and frequency of quantification (% data \geq LQ) in caged *Gammarus fossarum* after 7 days of exposure for the 27 study sites. Concentrations are expressed in ng.g^{-1} dw. Med, Min and Max are respectively the median, minimal and maximal of the measured concentrations.

| Organic substances | Concentration (ng.g^{-1} dw) | | | | | Frequency (%) |
|--------------------------|----------------------------------------|------|------|---------|-----|---------------|
| | Med | Min | Max | Max/Min | LQ | |
| Hexachlorobenzene | 0.5 | 0.4 | 10.0 | 27.5 | 0.3 | 41 |
| Lindane | 0.3 | 0.1 | 7.8 | 78.0 | 0.3 | 37 |
| Heptachlor | < LQ | < LQ | < LQ | - | 0.3 | 0 |
| Heptachlor epoxide | < LQ | < LQ | < LQ | - | 0.3 | 0 |
| 2, 4' DDE | 1.0 | 0.3 | 10.6 | 35.3 | 0.3 | 44 |
| 4, 4' DDE + Dieldrin | 3.4 | 1.9 | 62.9 | 33.1 | 0.3 | 100 |
| 2, 4' DDD | < LQ | < LQ | < LQ | - | 0.3 | 0 |
| 4, 4' DDD | 2.9 | 1.4 | 44.6 | 31.8 | 0.3 | 100 |
| 2, 4' DDT | 1.3 | 0.6 | 59.2 | 98.7 | 0.3 | 100 |
| 4, 4' DDT | 1.7 | 1.0 | 48.4 | 48.4 | 0.3 | 52 |
| Naphthalene | 19.9 | 8.5 | 36.8 | 4.3 | 0.3 | 100 |
| Anthracene | 0.6 | 0.3 | 9.1 | 91.0 | 0.3 | 41 |
| Fluoranthene | 2.1 | 0.7 | 33.7 | 48.1 | 0.3 | 96 |
| Benzo(b,k,j)fluoranthene | 1.8 | 0.4 | 19.4 | 48.5 | 0.3 | 96 |
| Benzo(a)pyrene | 1.0 | 0.3 | 3.3 | 11.0 | 0.3 | 63 |
| Indeno(1,2,3-cd)pyrene | 0.9 | 0.4 | 4.8 | 12.0 | 0.3 | 59 |
| Benzo(g,h,i)perylene | 0.9 | 0.3 | 3.8 | 12.7 | 0.3 | 63 |
| Acenaphthylene | 0.7 | 0.3 | 2.7 | 9.37 | 0.3 | 85 |
| Acenaphthene | 1.5 | 0.8 | 5.6 | 6.84 | 0.3 | 100 |
| Fluorene | 2.0 | 0.4 | 8.3 | 20.9 | 0.3 | 96 |
| Phenanthrene | 4.1 | 0.4 | 39.9 | 100.5 | 0.3 | 93 |
| Pyrene | 2.0 | 0.4 | 47.7 | 124 | 0.3 | 100 |
| Benzo(a)anthracene | 1.2 | 0.3 | 14.7 | 48.9 | 0.3 | 100 |
| Benzo(e)pyrene | 0.7 | 0.3 | 5.7 | 20.6 | 0.3 | 70 |
| Triphenyl + Chrysene | 1.7 | 0.6 | 23.8 | 41.1 | 0.3 | 100 |
| Perylene | 0.4 | 0.3 | 1.9 | 7.4 | 0.3 | 59 |
| DaA + DaC | 0.9 | 0.4 | 2.6 | 6.84 | 0.3 | 52 |
| CB 50+28 | 1.8 | 0.3 | 13.2 | 44.0 | 0.3 | 100 |
| CB 52 | 3.9 | 1.9 | 33.8 | 17.8 | 0.3 | 100 |
| CB 101 | 3.9 | 1.9 | 24.5 | 12.9 | 0.3 | 100 |
| CB 118 | 3.6 | 1.0 | 20.7 | 20.7 | 0.3 | 100 |
| CB 138 | 6.5 | 3.3 | 45.5 | 13.8 | 0.3 | 100 |
| CB 153 | 8.0 | 4.1 | 50.8 | 12.4 | 0.3 | 100 |
| CB 180 | 2.3 | 1.3 | 16.6 | 12.8 | 0.3 | 100 |
| BDE 47 | 2.4 | 0.9 | 5.0 | 5.5 | 0.3 | 100 |
| BDE 119 | < LQ | < LQ | < LQ | - | 0.3 | 0 |
| BDE 99 | 1.2 | 0.5 | 3.2 | 6.4 | 0.3 | 100 |
| BDE 153 | < LQ | < LQ | < LQ | - | 0.3 | 0 |

Table 3. Calculated threshold values of bioavailable contamination determined by two different approaches, a statistical approach and a model fit (see section 2.5); and threshold validity (see section 4.3.). Threshold values are expressed in $\mu\text{g.g}^{-1}$ dw for metals and in ng.g^{-1} dw for organic substances.

5

| | Investigated substances | Threshold values | | Threshold validity | | 6 |
|----------------------------------------------------------|----------------------------|----------------------|-----------|--------------------|-----------|----|
| | | Statistical approach | Model fit | Reliability ratio | Validity | 7 |
| Metals ($\mu\text{g}\cdot\text{g}^{-1}$ dw) | Cd | 0.3 | 0.3 | 0.00 | valid | 8 |
| | Pb | 0.4 | 0.3 | 0.07 | valid | 9 |
| | Hg | 0.06 | 0.09 | 0.30 | valid | 10 |
| | Ni | 0.7 | 0.7 | 0.00 | valid | 11 |
| | As | 2.5 | nd | nd | not valid | 12 |
| | Co | 0.5 | 0.5 | 0.00 | valid | 13 |
| | Cr | 0.9 | nd | nd | not valid | 14 |
| | Cu | 73.9 | nd | nd | not valid | 15 |
| | Se | 2.5 | nd | nd | not valid | 16 |
| | Zn | 84.7 | nd | nd | not valid | 17 |
| Pesticides ($\text{ng}\cdot\text{g}^{-1}$ dw) | Hexachlorobenzene | 0.6 | 1.0 | 0.04 | valid | 18 |
| | Lindane | 0.4 | 0.7 | 0.04 | valid | 19 |
| DDTs ($\text{ng}\cdot\text{g}^{-1}$ dw) | 2,4' DDE | 1.9 | 1.6 | 0.03 | valid | 20 |
| | 4, 4' DDE + Dieldrine | 4.8 | 6.0 | 0.02 | valid | 21 |
| | 4, 4' DDD | 3.9 | 5.0 | 0.02 | valid | 22 |
| | 2, 4' DDT | 1.6 | 3.1 | 0.03 | valid | 23 |
| | 4, 4' DDT | 2.8 | 3.2 | 0.01 | valid | 24 |
| HAPs ($\text{ng}\cdot\text{g}^{-1}$ dw) | Naphthalene | 32.7 | nd | nd | not valid | 25 |
| | Anthracene | 1.5 | 1.3 | 0.02 | valid | 26 |
| | Fluoranthene | 3.5 | 3.3 | 0.01 | valid | 27 |
| | Benzo (b,k,j) fluoranthène | 3.8 | 3.1 | 0.04 | valid | 28 |
| | Benzo(a)pyrène | 1.3 | 0.9 | 0.12 | valid | 29 |
| | Indeno (1,2,3-cd) pyrene | 1.7 | 1.6 | 0.02 | valid | 30 |
| | Benzo (g,h,i) perylene | 1.3 | 1.1 | 0.05 | valid | 31 |
| | Acenaphtene | 2.6 | 2.7 | 0.02 | valid | 32 |
| | Acenaphtylene | 1.3 | 0.9 | 0.15 | valid | 33 |
| | Fluorene | 2.9 | 1.6 | 0.16 | valid | 34 |
| | Phenanthrene | 6.8 | 3.7 | 0.08 | valid | 35 |
| | Pyrene | 3.3 | 3.1 | 0.00 | valid | 36 |
| | Benzo(a)anthracene | 2.2 | 1.8 | 0.03 | valid | 37 |
| | Benzo(e)pyrene | 1.3 | 1.1 | 0.04 | valid | 38 |
| | Triphenyl-chrysene | 2.6 | 2.9 | 0.01 | valid | 39 |
| | DaA-DaC | 1.6 | nd | nd | not valid | 40 |
| | Perylene | 0.3 | 0.6 | 0.16 | valid | 41 |
| PCBs ($\text{ng}\cdot\text{g}^{-1}$ dw) | 50 + 28 | 3.5 | 2.9 | 0.05 | valid | 42 |
| | 52 | 4.9 | 7.9 | 0.01 | valid | 43 |
| | 101 | 5.8 | 6.6 | 0.03 | valid | 44 |
| | 118 | 5.5 | 4.3 | 0.06 | valid | 45 |
| | 138 | 8.3 | 10.9 | 0.06 | valid | 46 |
| | 153 | 11.5 | 13.3 | 0.04 | valid | 47 |
| | 180 | 3.3 | 3.8 | 0.03 | valid | 48 |
| PBDEs ($\text{ng}\cdot\text{g}^{-1}$ dw) | 47 | 4.3 | nd | nd | not valid | 49 |
| | 99 | 2.1 | 1.1 | 0.30 | valid | 50 |

37 nd: not determined.

Table 4. Classification of study sites using the threshold values defined with the model fit approach. Grey squares indicate concentrations in gammarids higher than the threshold value (values are given in $\mu\text{g.g}^{-1}$ dw for metals and in ng.g^{-1} dw for organics). Sites numbered from 1 to 12 are those considered as non subjected to anthropic activities with reference to the expert classification made by the Water Agency. Sites numbered from 13 to 27 are those considered as subjected to anthropic activities with reference to the expert classification made by Water Agency (see section 2.3).

Study sites

| Investigated substances | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
|--------------------------|-------------|-------------|-------------|------|------------|------|------|-------------|------|-------------|-------------|-------------|-------------|-------------|-------------|------------|------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Cd | 0.83 | 1.31 | 1.27 | 0.18 | 0.15 | 0.15 | 0.17 | 0.23 | 0.23 | 0.22 | 0.63 | 0.2 | 0.45 | 0.33 | 0.21 | 0.19 | 0.12 | 0.15 | 0.28 | 0.24 | 0.3 | 0.91 | 0.21 | 0.14 | 0.2 | 0.2 | 0.35 |
| Pb | <LQ | 0.17 | 0.31 | 0.22 | 0.16 | 0.14 | 0.14 | 0.43 | 0.19 | 0.27 | 0.46 | 0.85 | <LQ | 0.4 | 0.27 | 0.2 | 0.24 | 0.17 | 0.28 | 1.39 | 0.82 | 0.89 | 0.95 | 0.29 | 0.46 | 0.18 | 0.92 |
| Hg | 0.05 | 0.05 | 0.05 | 0.04 | 0.04 | 0.04 | 0.04 | 0.05 | 0.06 | 0.05 | 0.05 | 0.04 | 0.06 | 0.05 | 0.05 | 0.05 | 0.04 | 0.05 | 0.11 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Ni | 0.53 | 1.28 | 0.43 | 0.6 | 0.68 | 0.33 | 0.26 | <LQ | <LQ | 0.5 | 0.35 | 0.45 | 0.48 | 1.27 | 0.37 | 0.37 | 0.34 | 0.59 | 0.88 | 0.45 | <LQ | <LQ | 1.01 | 0.69 | 0.58 | 0.4 | 0.36 |
| Co | 0.33 | 0.23 | 0.27 | 0.33 | 0.13 | 0.14 | 0.14 | 0.47 | 0.27 | 0.31 | 0.33 | 0.39 | 0.46 | 0.32 | 0.28 | 0.27 | 0.29 | 0.26 | 0.96 | 0.24 | 0.43 | 0.44 | 0.5 | 0.39 | 0.36 | 0.23 | 0.34 |
| Hexachlorobenzene | 0.4 | <LQ | <LQ | <LQ | 0.4 | 0.5 | <LQ | <LQ | <LQ | 0.4 | <LQ | 0.9 | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | 10.0 | <LQ | <LQ | <LQ | <LQ | 0.6 | <LQ | 0.6 | 0.5 |
| Lindane | 0.3 | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | 0.4 | <LQ | <LQ | <LQ | 0.3 | 0.4 | 0.4 | <LQ | 0.4 | <LQ | 7.8 | 0.4 | <LQ | 0.4 | 1.4 | <LQ | 0.6 | <LQ | <LQ |
| 2, 4' DDE | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | 1.7 | 0.3 | 1.1 | 1.5 | <LQ | 0.6 | 0.3 | 10.6 | <LQ | <LQ | 0.4 | 2.1 | 0.9 | 1.0 | <LQ | 0.7 |
| 4, 4' DDE+Dieldrin | 2.4 | 2.9 | 2.6 | 2.5 | 3.2 | 2.3 | 2.4 | 1.9 | 2.3 | 2.3 | 3.9 | 3.4 | 4.2 | 4.2 | 3.3 | 3.1 | 3.6 | 3.5 | 37.0 | 3.9 | 3.9 | 6.9 | 5.5 | 5.1 | 3.4 | 10.4 | 62.9 |
| 4, 4' DDD | 2.0 | 1.9 | 1.9 | 1.9 | 1.9 | 2.0 | 2.3 | 1.6 | 2.4 | 2.1 | 3.6 | 3.7 | 1.4 | 3.5 | 2.3 | 3.0 | 1.8 | 3.3 | 44.6 | 3.4 | 2.9 | 4.3 | 6.3 | 5.2 | 3.3 | 5.9 | 24.3 |
| 2, 4' DDT | 1.0 | 1.0 | 1.0 | 1.0 | 1.1 | 0.8 | 0.9 | 0.6 | 0.8 | 0.7 | 0.9 | 1.3 | 2.2 | 1.3 | 3.1 | 2.9 | 2.3 | 2.6 | 59.2 | 2.3 | 2.3 | 2.4 | 2.3 | 1.9 | 1.5 | 1.2 | 2.8 |
| 4, 4' DDT | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | 1.2 | 1.3 | 1.7 | 1.9 | 1.7 | 1.7 | 48.4 | 2.8 | 2.3 | 2.1 | 2.9 | 1.4 | 1.0 | <LQ | 12.5 |
| Anthracene | <LQ | <LQ | 0.7 | <LQ | 0.4 | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | 1.7 | <LQ | <LQ | 0.5 | 1.0 | <LQ | 0.3 | <LQ | 0.9 | <LQ | 0.7 | 3.7 | 9.1 | 0.4 | 0.3 | 1.2 |
| Fluoranthene | 0.9 | 0.7 | 1.4 | 1.7 | 1.6 | 1.0 | 0.8 | 1.2 | <LQ | 1.0 | 1.7 | 7.3 | 0.7 | 2.0 | 3.4 | 2.3 | 3.4 | 2.9 | 1.5 | 6.5 | 2.3 | 4.8 | 33.7 | 24.2 | 4.3 | 2.3 | 9.8 |
| Benzo(b,k,j)fluoranthene | 0.4 | 0.6 | 1.0 | 1.2 | 1.7 | <LQ | 0.6 | 1.4 | 0.6 | 1.6 | 1.5 | 5.6 | 0.8 | 1.7 | 3.2 | 2.1 | 2.8 | 2.0 | 1.0 | 4.7 | 3.2 | 3.7 | 19.4 | 7.8 | 3.1 | 1.9 | 8.7 |
| Benzo(a)pyrene | <LQ | <LQ | <LQ | 0.5 | <LQ | <LQ | <LQ | 0.5 | <LQ | <LQ | 0.3 | 0.6 | <LQ | 1.1 | 1.0 | 0.9 | 1.1 | 0.7 | <LQ | 1.2 | 1.1 | 1.2 | 3.0 | 3.3 | 0.3 | 0.6 | 2.8 |
| Indeno(1,2,3-cd)pyrene | <LQ | <LQ | <LQ | 0.5 | 0.4 | <LQ | <LQ | <LQ | <LQ | <LQ | <LQ | 1.4 | <LQ | 0.5 | 0.9 | 0.9 | 1.0 | 0.7 | <LQ | 0.9 | 1.3 | 1.1 | 4.8 | 1.7 | 0.7 | 0.6 | 1.8 |
| Benzo(g,h,i)perylene | <LQ | <LQ | <LQ | 0.3 | 0.4 | <LQ | <LQ | 0.6 | <LQ | <LQ | <LQ | 1.6 | <LQ | 0.5 | 0.9 | 0.6 | 0.7 | 0.6 | 0.1 | 0.9 | 1.0 | 1.1 | 3.8 | 2.2 | 0.9 | 0.9 | 2.1 |
| Acenaphthylene | 0.4 | 0.5 | 0.3 | <LQ | 0.7 | 0.4 | 0.3 | <LQ | <LQ | 0.3 | 0.3 | 0.9 | 0.6 | 0.6 | 0.9 | <LQ | 1.0 | 0.7 | 1.1 | 1.4 | 0.9 | 1.1 | 1.2 | 2.7 | 1.0 | 0.5 | 0.8 |
| Acenaphthene | 1.5 | 1.5 | 1.9 | 0.8 | 1.7 | 1.4 | 1.3 | 1.0 | 0.9 | 1.3 | 0.9 | 5.6 | 1.7 | 1.3 | 1.9 | 1.2 | 1.5 | 1.5 | 2.2 | 2.5 | 1.4 | 1.6 | 3.1 | 2.9 | 1.9 | 1.5 | 2.2 |
| Fluorene | 1.2 | 1.2 | 2.7 | 0.7 | 1.9 | 1.4 | 0.7 | 0.7 | <LQ | 0.5 | 0.6 | 6.1 | 2.6 | 1.8 | 2.2 | 1.5 | 2.0 | 2.1 | 2.6 | 2.7 | 1.4 | 2.4 | 8.3 | 6.5 | 4.8 | 0.4 | 5.1 |
| Phenanthrene | 2.2 | 2.0 | 8.7 | 2.1 | 4.4 | 3.5 | 0.6 | 2.1 | <LQ | 1.6 | 0.7 | 17.2 | 4.2 | 3.0 | 6.3 | 2.6 | 4.1 | 5.5 | 4.8 | 3.3 | 0.4 | 5.3 | 39.9 | 37.8 | 12.5 | <LQ | 26.6 |
| Pyrene | 0.7 | 0.5 | 2.1 | 1.1 | 1.3 | 0.5 | 0.8 | 1.1 | 0.4 | 1.2 | 1.5 | 6.0 | 0.8 | 2.1 | 2.4 | 1.9 | 2.6 | 3.0 | 2.0 | 8.0 | 2.0 | 4.1 | 40.7 | 47.7 | 5.4 | 2.9 | 10.8 |
| Benzo(a)anthracene | 0.6 | 0.3 | 0.6 | 0.9 | 0.8 | 0.5 | 0.6 | 1.0 | 0.3 | 0.6 | 0.8 | 2.2 | 0.6 | 1.3 | 1.8 | 1.3 | 1.5 | 1.2 | 1.1 | 4.8 | 1.9 | 2.2 | 14.7 | 11.4 | 2.4 | 1.3 | 3.9 |
| Benzo(e)pyrene | <LQ | <LQ | <LQ | 0.3 | 0.4 | <LQ | <LQ | 0.3 | <LQ | 0.4 | 0.4 | 1.5 | <LQ | 0.4 | 0.7 | 0.6 | 0.8 | 0.5 | <LQ | 1.2 | 0.9 | 1.1 | 5.7 | 2.6 | 0.9 | 0.7 | 2.3 |
| Triphenene + Chrysene | 0.6 | 0.6 | 1.1 | 1.5 | 1.5 | 0.7 | 0.6 | 1.2 | 0.6 | 1.5 | 1.6 | 4.1 | 0.7 | 1.7 | 2.4 | 1.8 | 2.1 | 2.0 | 1.4 | 6.0 | 2.4 | 4.0 | 23.8 | 13.4 | 4.6 | 2.8 | 9.1 |
| Perylene | <LQ | <LQ | <LQ | 0.3 | 0.4 | <LQ | <LQ | 0.3 | <LQ | 0.4 | 0.4 | 1.5 | <LQ | 0.3 | 0.3 | 0.7 | 0.5 | 0.3 | <LQ | <LQ | 0.3 | 0.5 | 1.2 | 0.8 | 0.4 | 0.3 | 1.9 |
| PCB 50+28 | 2.0 | 1.6 | 1.6 | 1.5 | 3.8 | 1.8 | 0.6 | 0.3 | 1.0 | 1.6 | 0.6 | 5.9 | 1.2 | 2.2 | 2.1 | 1.1 | 2.9 | 1.8 | 13.2 | 1.2 | 0.7 | 1.5 | 4.7 | 3.2 | 5.3 | 2.6 | 2.3 |
| PCB 52 | 3.9 | 2.6 | 3.5 | 3.5 | 4.2 | 2.7 | 2.5 | 2.7 | 2.7 | 3.9 | 2.4 | 8.6 | 4.1 | 7.7 | 9.8 | 2.2 | 4.3 | 3.8 | 33.8 | 3.2 | 1.9 | 2.7 | 8.3 | 4.6 | 9.4 | 3.9 | 5.6 |
| PCB 101 | 3.9 | 4.7 | 3.6 | 3.8 | 3.9 | 2.3 | 2.9 | 1.9 | 2.6 | 3.6 | 3.3 | 9.3 | 3.7 | 9.8 | 13.5 | 2.7 | 4.0 | 3.7 | 24.5 | 5.0 | 3.6 | 7.3 | 14.9 | 7.6 | 8.7 | 4.8 | 4.8 |
| PCB 118 | 2.9 | 3.4 | 2.7 | 3.5 | 2.7 | 1.4 | 1.2 | <LQ | 1.0 | 2.7 | 2.8 | 13.5 | 3.7 | 11.0 | 16.3 | 3.9 | 4.0 | 3.8 | 20.7 | 1.8 | 2.0 | 5.7 | 8.7 | 3.6 | 7.1 | 2.9 | 4.0 |
| PCB 138 | 5.3 | 6.1 | 6.6 | 7.4 | 6.6 | 3.4 | 4.1 | 3.3 | 3.4 | 4.9 | 5.8 | 10.6 | 6.0 | 14.0 | 16.6 | 5.3 | 6.1 | 5.8 | 45.5 | 9.9 | 5.1 | 23.5 | 31.8 | 12.9 | 12.0 | 10.8 | 6.5 |
| PCB 153 | 6.8 | 8.4 | 8.2 | 8.9 | 8.0 | 4.6 | 5.4 | 4.1 | 4.9 | 6.9 | 7.5 | 10.6 | 7.6 | 16.7 | 16.5 | 6.5 | 7.4 | 6.8 | 50.8 | 12.1 | 5.7 | 30.8 | 36.6 | 16.7 | 13.7 | 16.7 | 7.6 |
| PCB 180 | 1.6 | 1.6 | 2.3 | 2.6 | 2.5 | 2.0 | 1.9 | 1.7 | 2.0 | 2.2 | 2.8 | 2.4 | 1.6 | 3.8 | 4.1 | 1.8 | 1.4 | 1.3 | 16.6 | 3.0 | 1.4 | 9.1 | 14.2 | 4.9 | 3.8 | 3.5 | 2.1 |
| BDE 99 | 1.3 | 1.9 | 1.0 | 1.1 | 1.8 | 0.9 | 1.0 | 0.5 | 1.0 | 0.7 | 1.7 | 1.4 | 0.8 | 1.4 | 1.4 | 0.6 | 0.6 | 0.9 | 1.5 | 1.3 | 0.5 | 0.6 | 3.2 | 1.7 | 2.7 | 1.2 | 2.2 |

LQ: Limit of quantification

Table S1. Location and water physico-chemical characteristics for the 12 study sites selected among the national reference network (WFD implementation). According to the expert classification of the Water Agency (i.e., no known anthropic pressure and overall good quality indices) these sites were considered not subjected by anthropic pressures.

| Site information | | | | Physicochemical characteristics | | | |
|---------------------------------------|-----------------|--------------|-----------|----------------------------------------------|----------------------------|------------|--------------------------------------------------------|
| Site (river / location) | GPS coordinates | | Site code | Water temperature (°C) min – max [med] | Dissolved oxygen (%) | pH | Hardness (mg.L ⁻¹ of CaCO ₃) |
| | E | N | | | | | |
| Doux Labatie d'Andaure | 04°29' 41.5" | 45°01' 23.6" | 1 | 11.2 - 16.1 [14.0] | 98 100 | 7.1 7.2 | 14.2 15.3 |
| Cance Saint Julien Vocance | 04°30' 11.9" | 45°10' 39.5" | 2 | 9.8 - 15.2 [12.5] | 100 100 | 7.0 6.6 | 16.1 17.2 |
| Gier La Valla en Gier | 04°30' 36.4" | 45°26' 36.3" | 3 | 9.9 - 14.2 [12.2] | 100 100 | 7.2 6.5 | 16.5 16.9 |
| Ain Saint Maurice de Gourdans | 05°11' 20.0" | 45°48' 27.5" | 4 | 12.2 - 16.1 [14.0] | 99 100 | 8.0 8.3 | 173.4 164.9 |
| Albarine <i>Chaley</i> | 05°32' 31.8" | 45°57' 22.8" | 5 | 9.7 - 12.5 [11.0] | 100 100 | 8.2 8.3 | 208.3 206.2 |
| Mandorne Oncieux | 05°28' 23.7" | 45°58' 36.1" | 6 | 8.7 - 13.0 [10.9] | 100 99 | 8.2 8.3 | 157.6 157.5 |
| Vareze Cours et Buis | 04°58' 52.0" | 45°26' 15.3" | 7 | 10.4 - 15.3 [12.7] | 100 100 | 7.9 7.9 | 180.9 168.1 |
| Galaveyson Saint Clair sur Galaure | 05°07' 50.3" | 45°15' 26.5" | 8 | 10.2 – 14.7 [12.4] | 100 100 | 7.8 7.5 | 163.7 173.1 |
| Drevenne Rovon | 05°27' 55.5" | 45°12' 11.6" | 9 | 10.6 - 14.7 [12.8] | 100 100 | 8.2 8.3 | 175.1 176.6 |
| Guiers Mort Saint Laurent du Pont | 05°45' 17.4" | 45°21' 42.2" | 10 | 8.6 - 10.7 [9.6] | 100 100 | 8.4 8.5 | 172.3 175.7 |
| Ardières Les Ardillats | 04°31' 15.9" | 46°11' 11.8" | 11 | 8.9 - 14.5 [12.0] | 100 100 | 7.9 8.2 | 43.7 39.7 |
| Ergues Poule les Echarmeaux | 04°26' 45.5" | 46°08' 21.2" | 12 | 8.0 - 14.7 [11.6] | 100 100 | 7.7 7.8 | 55.0 48.6 |

For physicochemical characteristics, 2 values are given for each site. Except for temperature, these values correspond to measurements performed at the beginning and at the end of the exposure (i.e., at 7 days). Contrary to other parameters, temperature was measured every hour during the 7 days of exposure.

Table S2. Location and water physico-chemical characteristics for the 15 study sites chosen among the national control network (WFD implementation). According to the expert classification of the Water Agency (i.e., degraded water chemical quality and/or degraded faunistic indices) these sites were considered subjected to anthropic pressures.

| Site information | | | Impact type | | | | Physicochemical characteristics | | | |
|-----------------------------------|----------------------------------|--------------|-----------------------------------------|--------|------------|--------------------------------------|-------------------------------------------------|----------------------------|------------|-----------------------------------------------------------|
| Site (river / location) | GPS coordinates | Site code | Pressure type and intensity | Metals | Pesticides | Other organic contami nants | Water temperature (°C) min – max [med] | Dissolved oxygen (%) | pH | Hardness (mg.L ⁻¹ of CaCO ₃) |
| Doux Saint Jean de Muzols | 04°49' 39.5" E 45°04' 40.2" N | 13 | Industrial Agricultural Urban | + | + | + | 15.3 – 19.5 [17.0] | 100 100 | 7.2 7.1 | 34.9 38.9 |
| Cance Sarras | 04°47' 47.6" E 45°11' 30.9" N | 14 | Industrial Urban | + | + | + | 12.0 – 17.6 [14.9] | 100 100 | 7.7 7.6 | 118.7 126.5 |
| Albarine Saint Rambert | 05°26' 01.8" E 45°56' 32.1" N | 15 | Industrial 1 Urban 2 | | | + | 10.4 – 15.1 [12.7] | 100 100 | 8.0 8.5 | 161.4 161 |
| Veyle Lent | 05°11' 48.4" E 46°06' 58.7" N | 16 | Agricultural 3 | | ++ | | 11.1 – 14.1 [12.7] | 100 98 | 8.0 7.8 | 231.1 244.4 |
| Veyle Servas | 05°10' 31.3" E 46°07' 37.9" N | 17 | Agricultural 3 | | +++ | | 11.0 – 16.4 [13.9] | 100 86 | 8.3 7.9 | 228.7 227.9 |
| Ange Brion | 05°33' 05.3" E 46°10' 12.3" N | 18 | Industrial 3 Urban 2 | ++ | | +++ | 9.8 - 15.8 [12.8] | 100 89 | 8.3 7.9 | 200.4 289.6 |
| Drac Fontaine | 05°42' 04.3" E 45°11' 36.6" N | 19 | Industrial 3 | +++ | +++ | +++ | 13.1 - 16.9 [15.4] | 100 100 | 7.9 7.9 | 130.5 164.6 |
| Turdine Arbresle | 04°36' 09.1" E 45°50' 15.5" N | 20 | Industrial 3 Urban | + | | + | 10.0 - 17.0 [13.6] | 100 100 | 8.4 8.2 | 174.1 165.6 |
| Azergues Legny | 04°34' 21.4" E 45°54' 24.6" N | 21 | Agricultural 2 Industrial 2 Urban | +++ | + | | 11.5 - 15.4 [12.9] | 100 100 | 8.1 8.1 | 133.2 133.2 |
| Azergues Lucenay | 04°43' 33.1" E 45°54' 41.5" N | 22 | Agricultural 3 Industrial 2 | +++ | +++ | + | 13.3 - 18.2 [15.8] | 100 100 | 8.1 8.1 | 256.2 268.5 |
| Gier Givors | 04°45' 42.3" E 45°35' 15.4" N | 23 | Urban | ++ | + | ++ | 11.8 - 19.2 [16.7] | 100 100 | 7.2 7.6 | 204.0 143.5 |
| Rhône Givors | 04°47' 03.4" E 45°35' 36.4" N | 24 | Urban Industrial | ++ | + | ++ | 18.0 - 19.8 [19.2] | 100 100 | 7.7 7.6 | 188.6 190.0 |
| Bourbre Pont de Cheruy | 05°10' 29.9" E 45°04' 00.3" N | 25 | Urban 2 Industrial 2 | + | + | + | n.a. | 100 100 | 7.7 7.4 | 230.4 260.6 |
| Saône Ile Barbe | 04°49' 57.3" E 45°47' 49.4" N | 26 | Urban Industrial | ++ | +++ | + | 18.5 - 19.8 [18.8] | 100 100 | 7.9 7.7 | 110.2 235.4 |
| Ardières Saint Jean d'Ardières | 04°44' 00.9" E 46°07' 18.4" N | 27 | Agricultural 3 Industrial 2 Urban | +++ | +++ | ++ | 12.7 - 15.6 [14.3] | 100 100 | 8.2 8.9 | n.a. 106.1 |

n.a.: not available

Impact type: + indicate the pressure's intensity (+: low; ++: moderate; +++: strong).

Values displayed in the column “pressure type and intensity” indicate, when available, the impact level of the selected contaminants on the receiving environment (1: moderate; 2: moderate to strong; 3: strong). These values were defined regarding data on land use, chemical monitoring and ecological diagnosis (<http://www.rhone-mediterranee.eaufrance.fr/gestion/dce/documents-locaux.php>)

For physicochemical characteristics, 2 values are given for each site. Except for temperature, these values correspond to measurements performed at the beginning and at the end of the exposure (i.e., at 7 days). Contrary to other parameters, temperature was measured every hour during the 7 days of exposure.

Figure S1. Survival rate (mean \pm standard deviation of 4 replicates) of caged *G. fossarum* after 7 days of exposure for all studied sites (n=27)

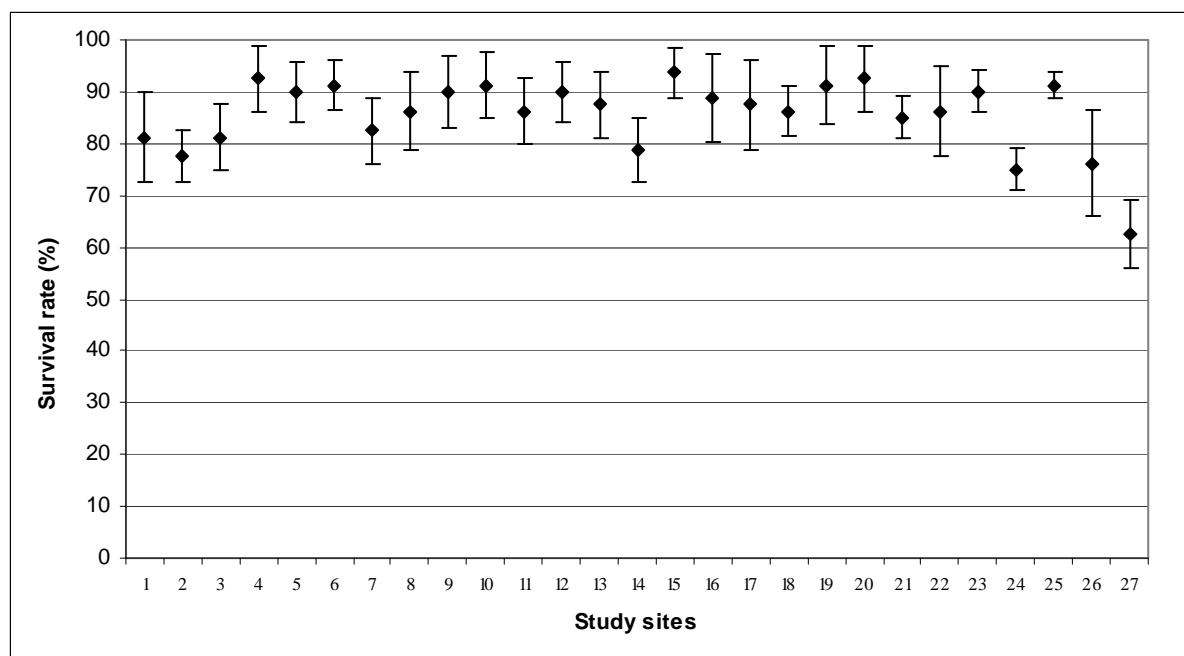
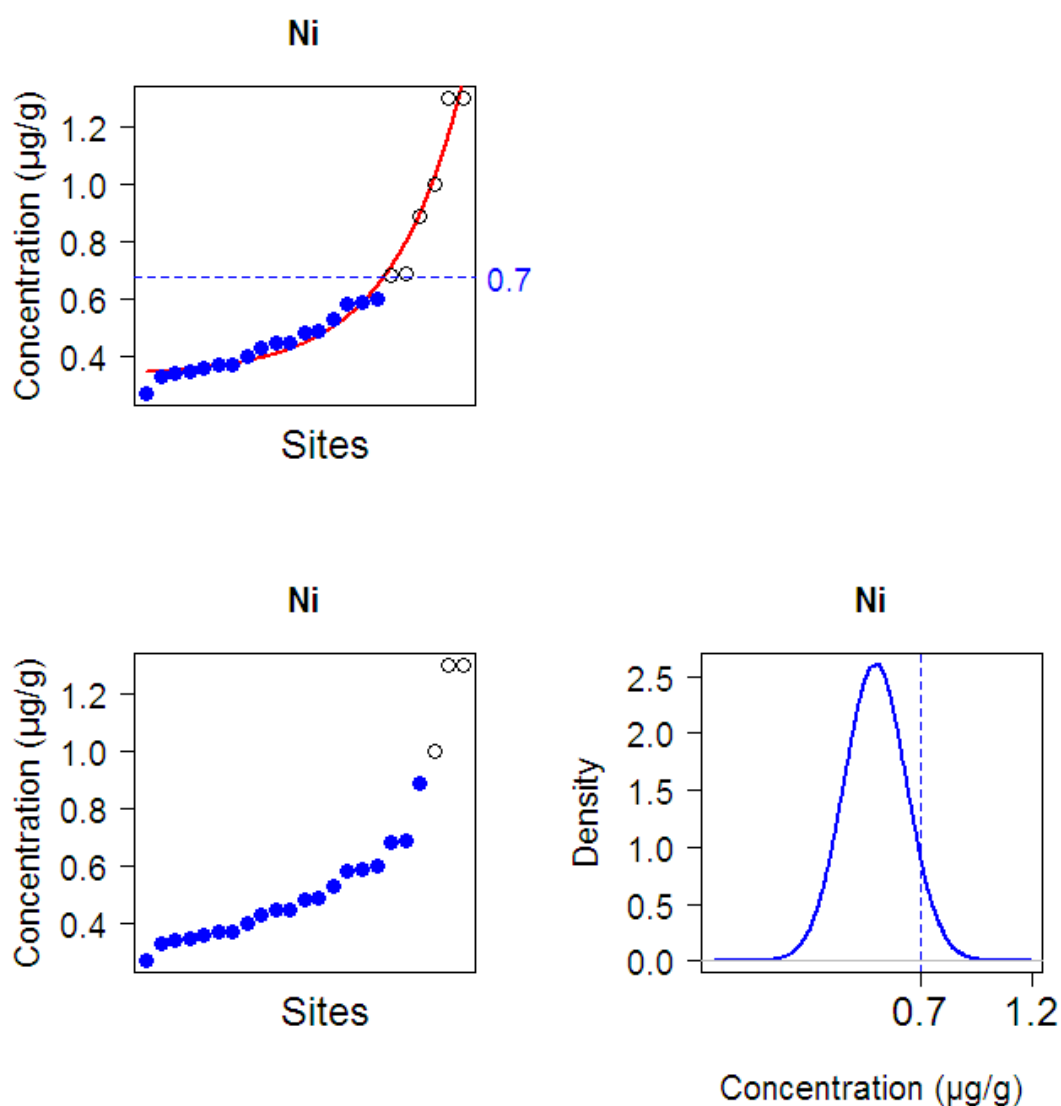
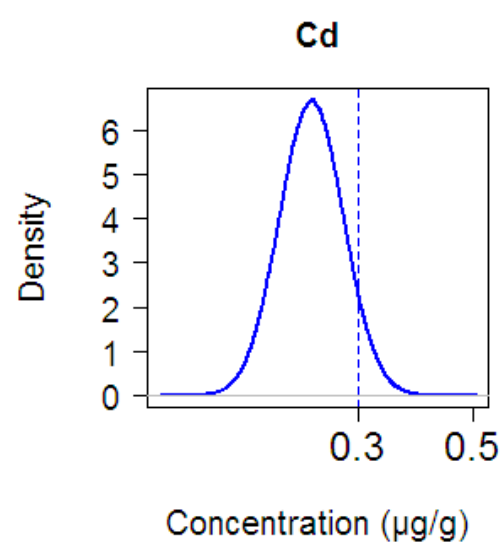
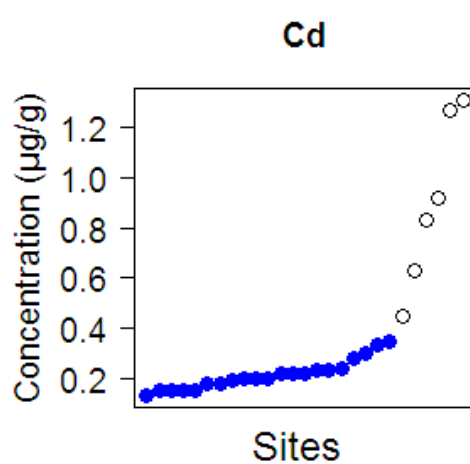
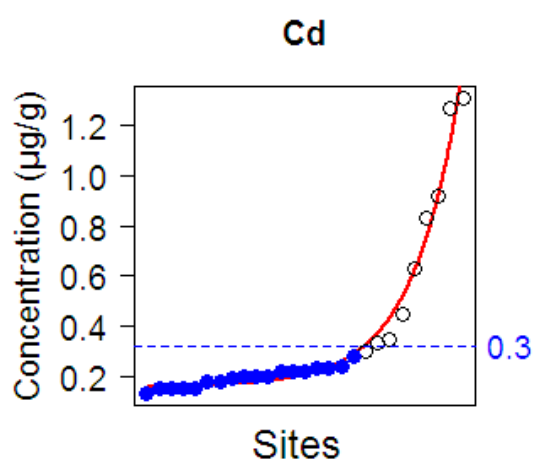
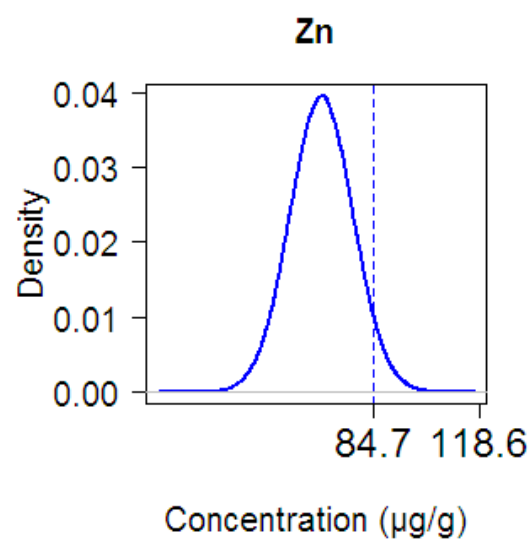
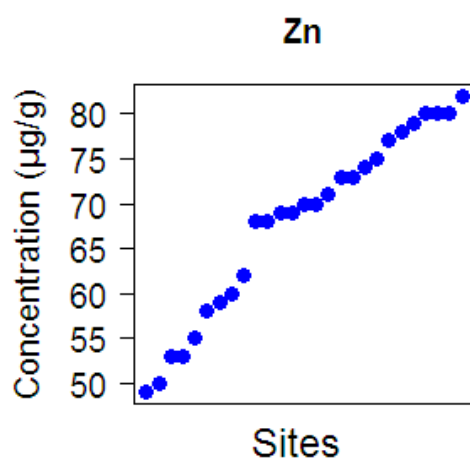


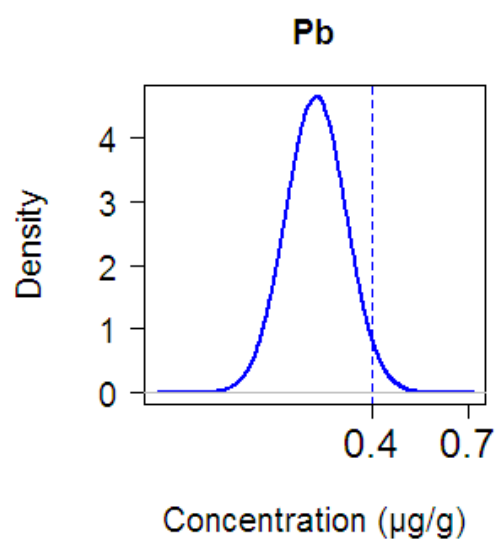
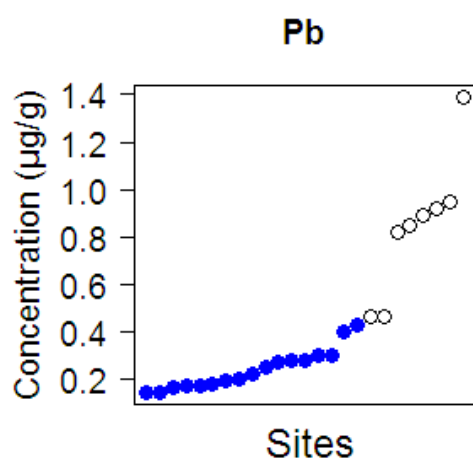
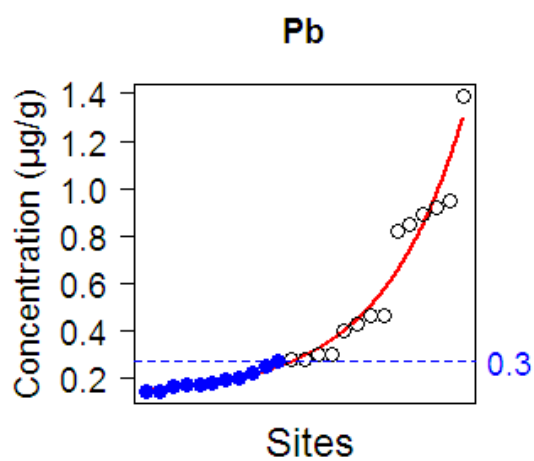
Figure S2. Threshold values determined for investigated metals and organic substances. For each substance, the top figure gives the threshold value determined with the model fit (Baranyi's bacterial growth model), and the bottom figure, the threshold value determined with the statistical approach (normality assumption).

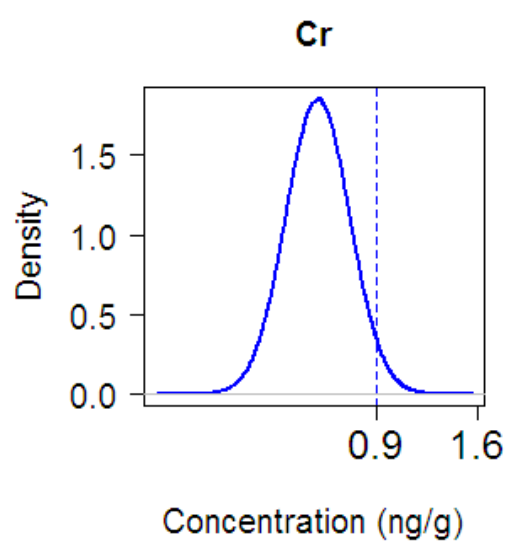
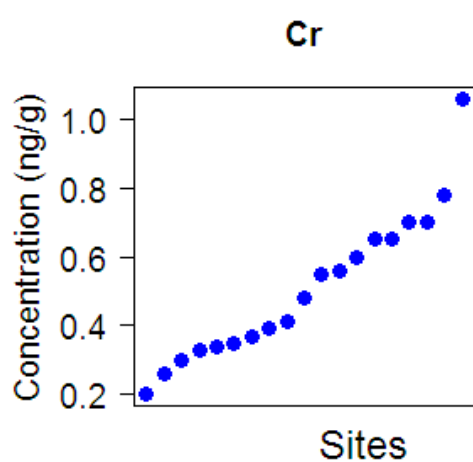
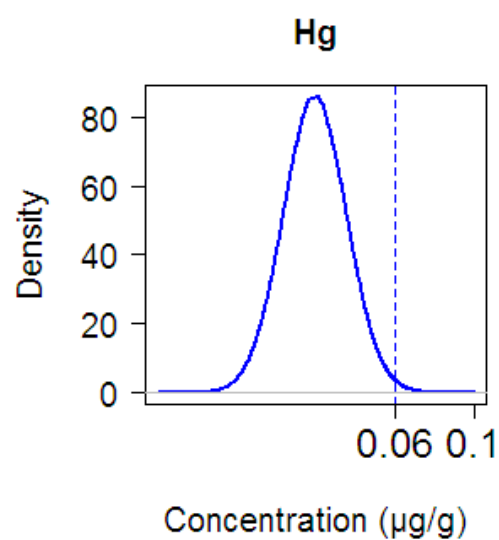
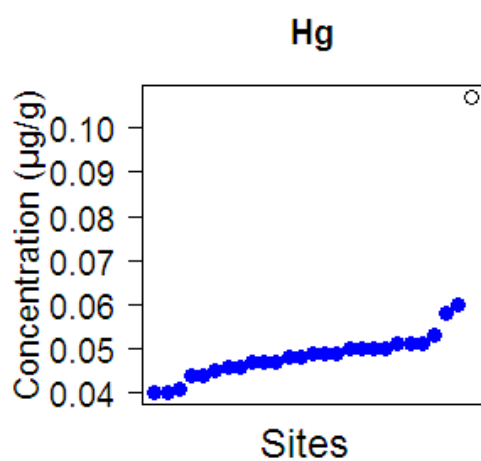
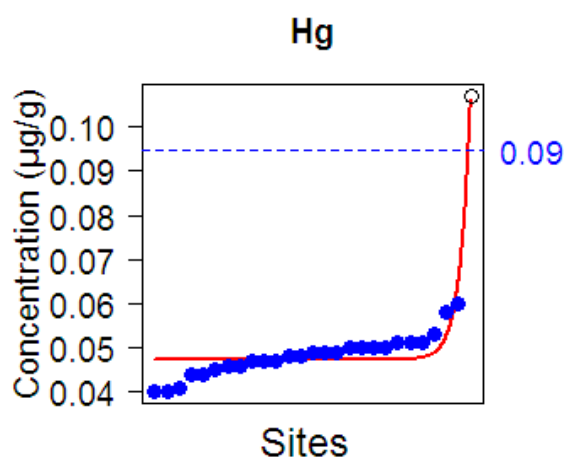
Threshold values are given in $\mu\text{g.g}^{-1}$ (dry weight) for metals and in ng.g^{-1} (dry weight) for organic substances.

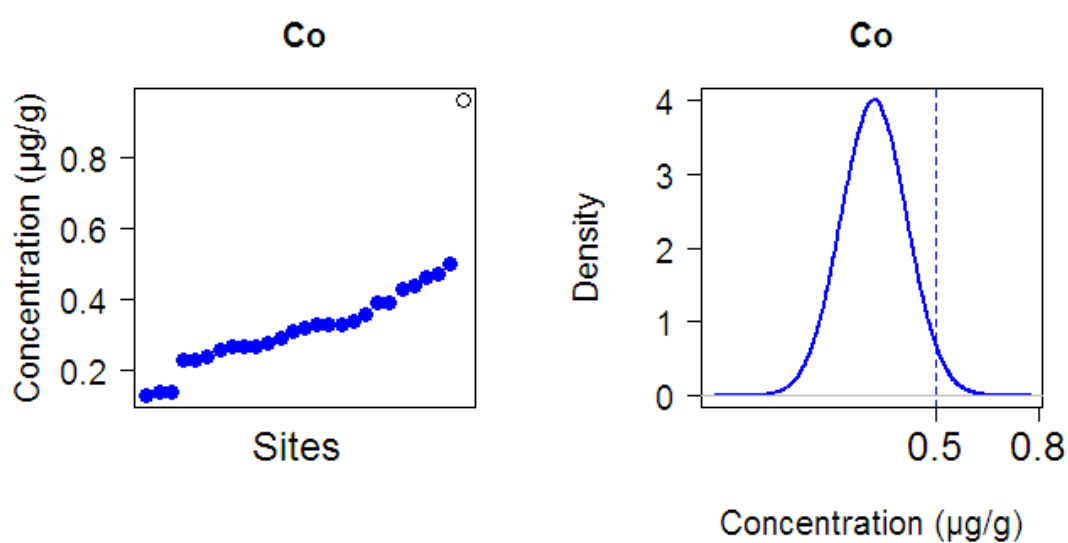
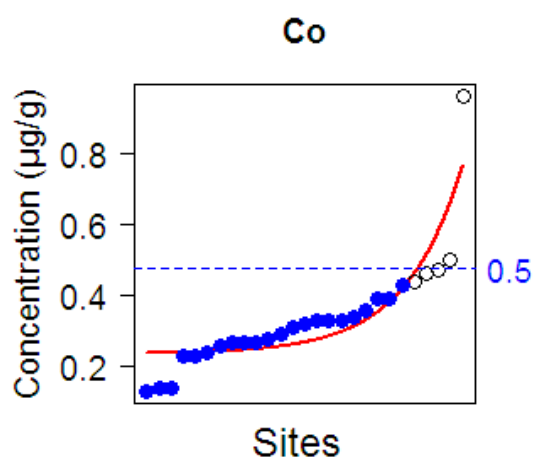
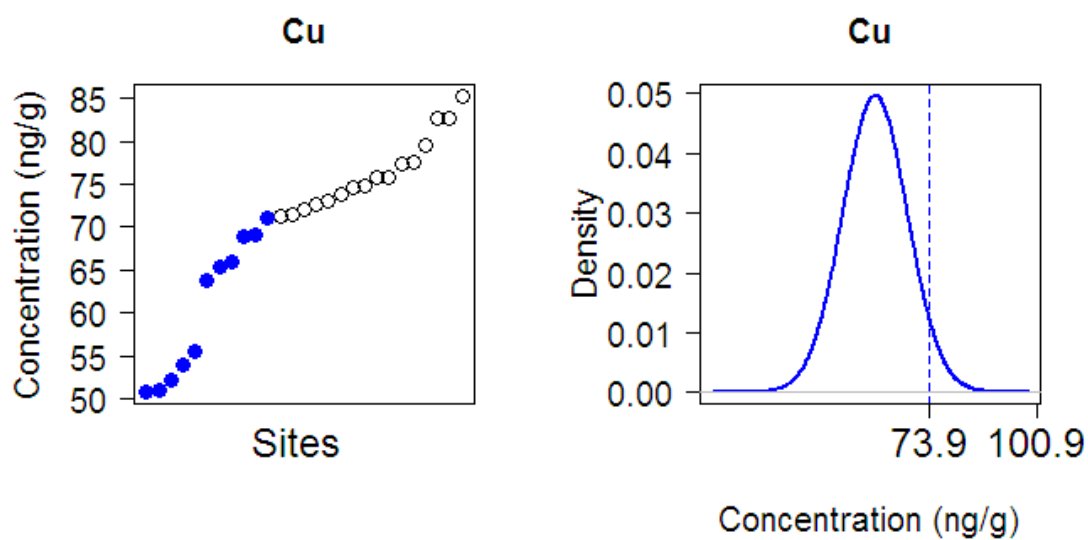
I. METALS

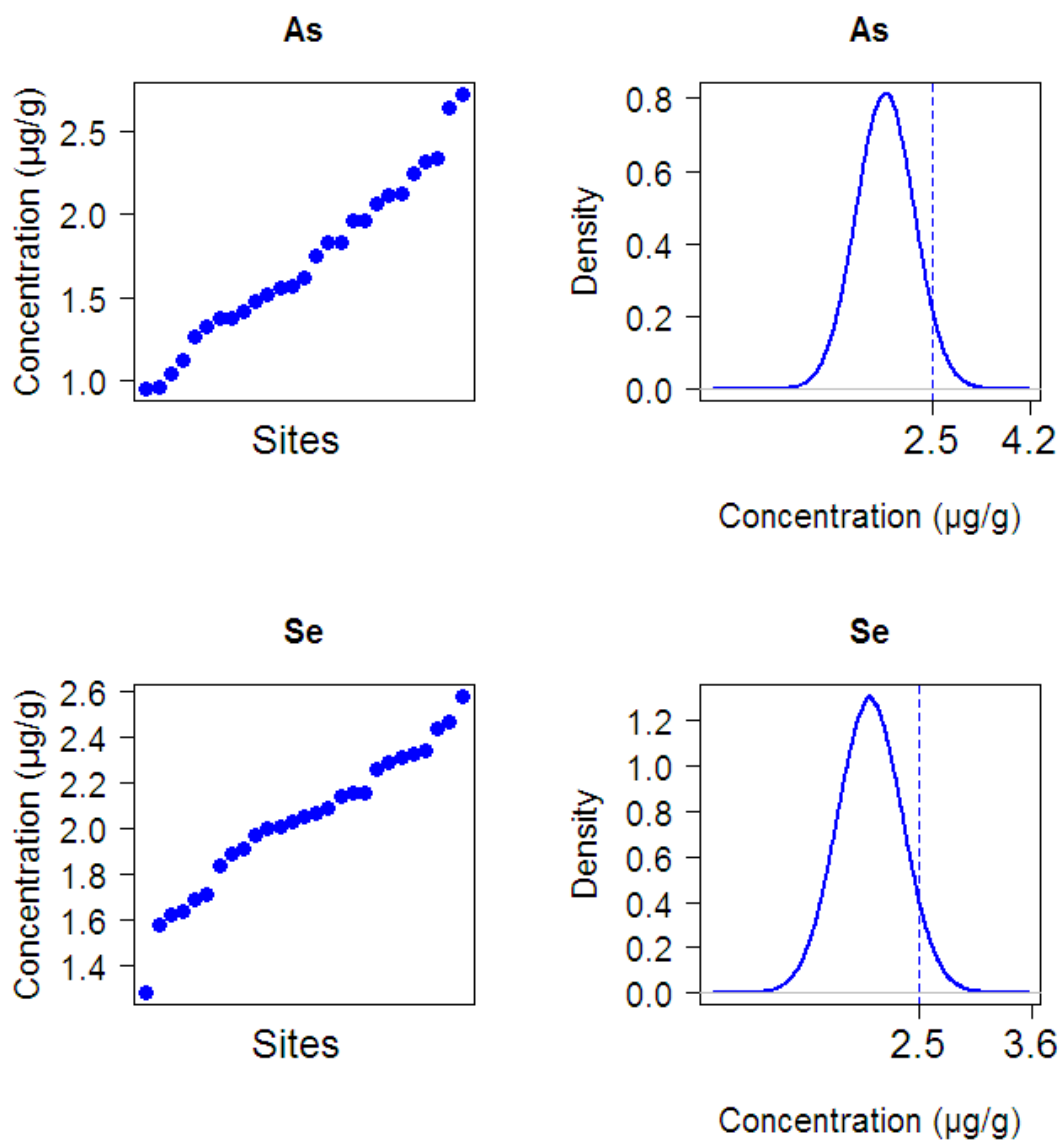




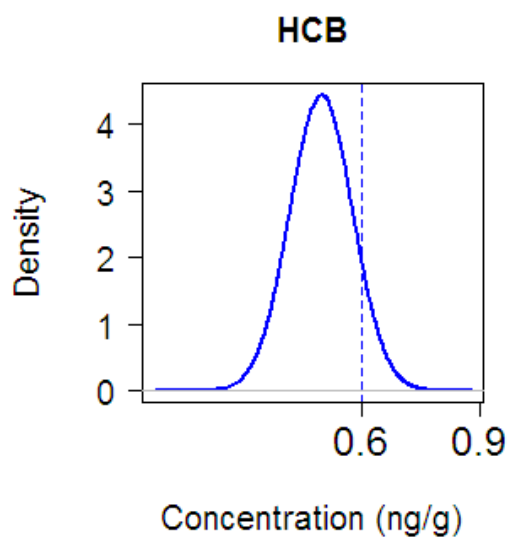
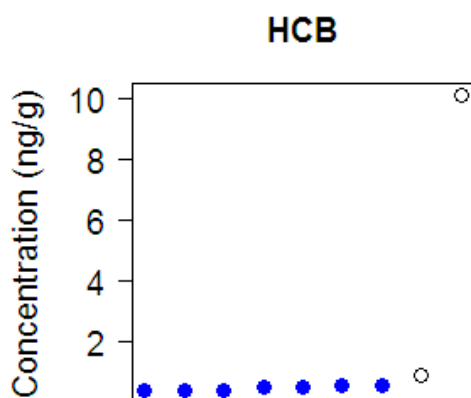
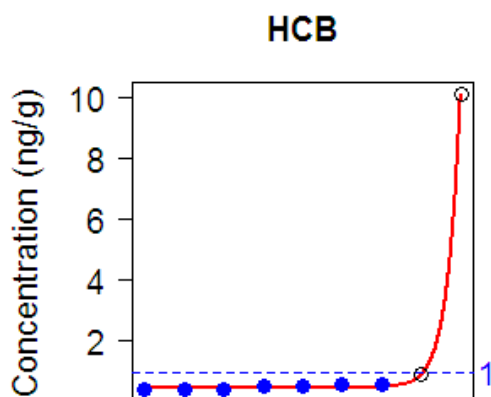




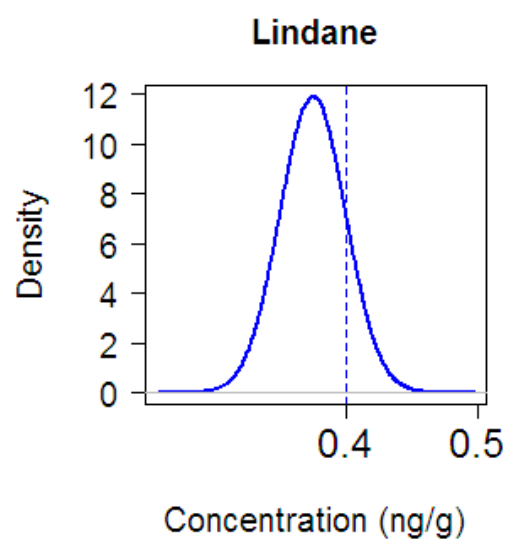
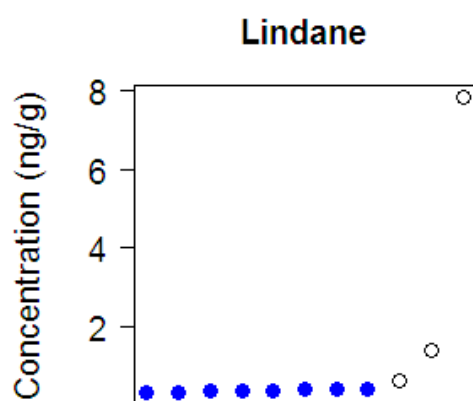
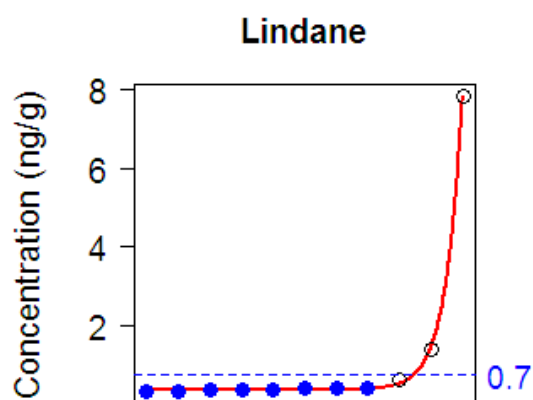


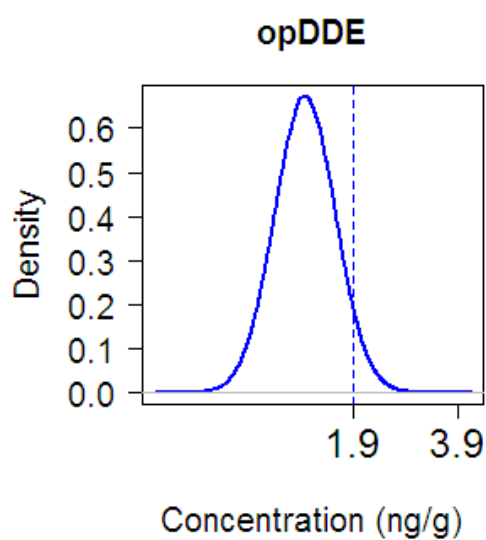
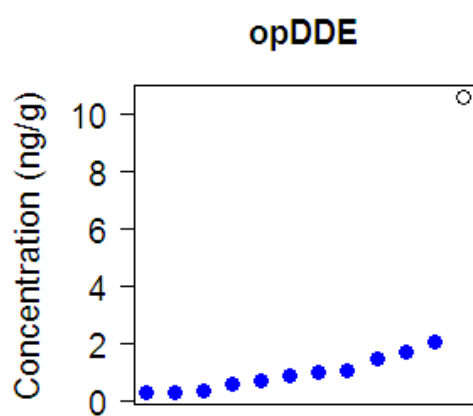
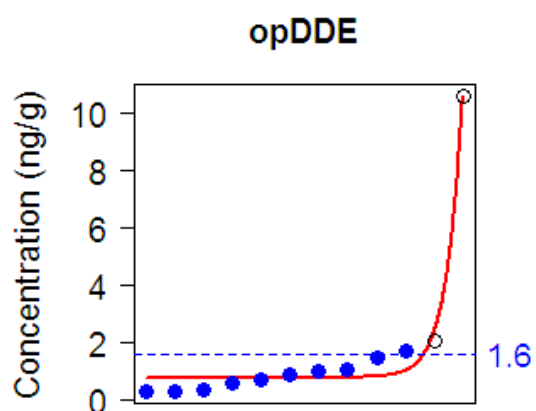


II. ORGANOCHLORINE PESTICIDES

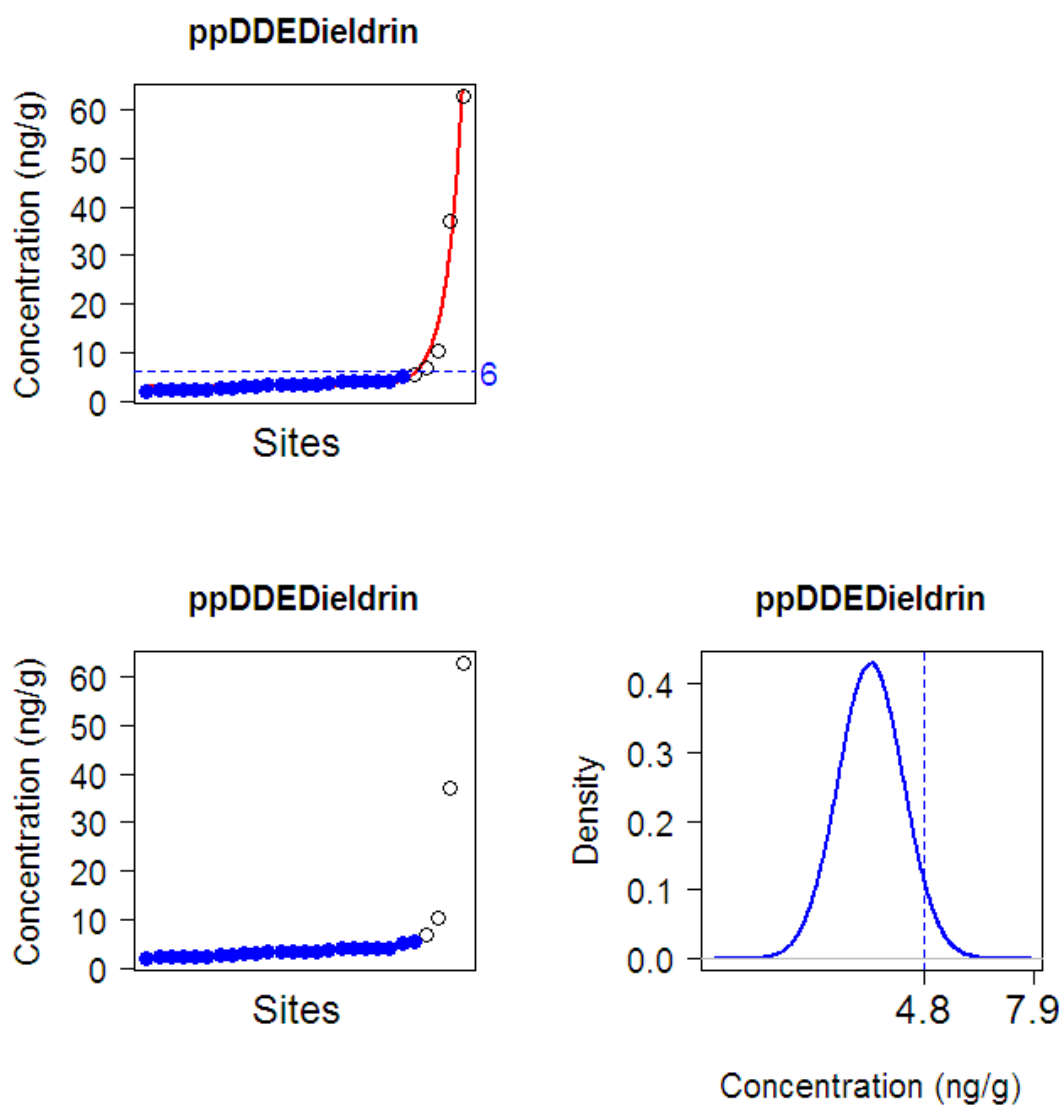


HCB : hexachlorobenzene

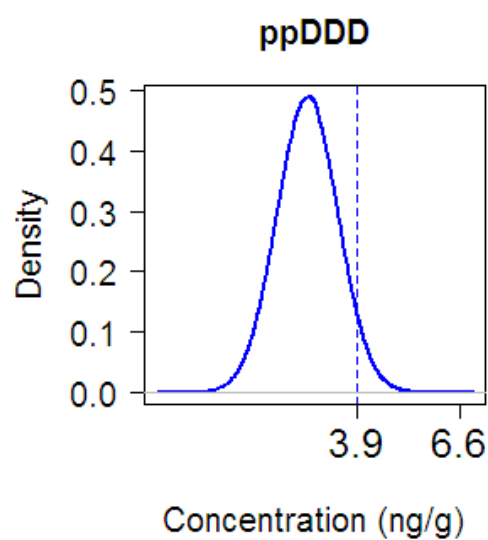
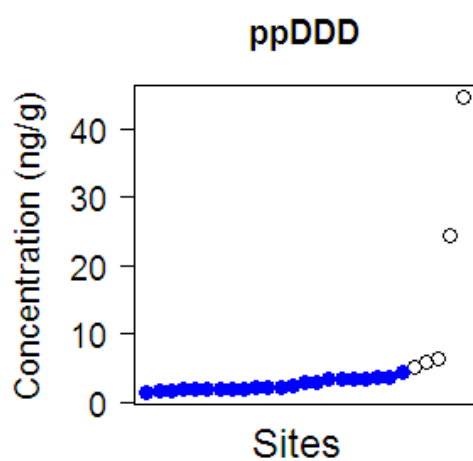
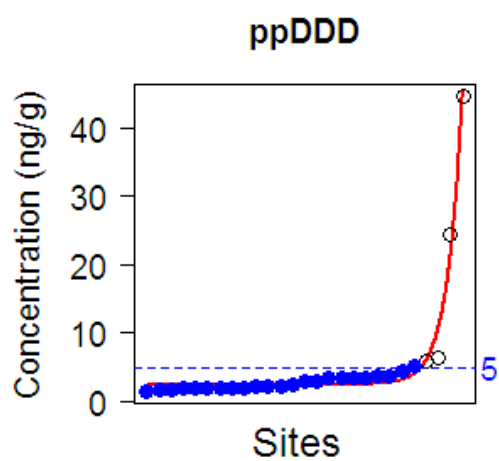




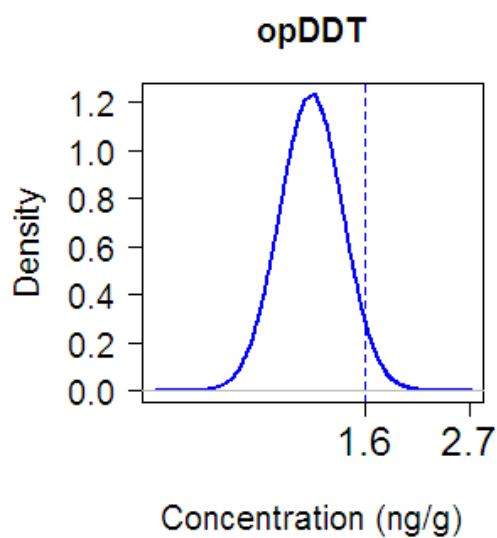
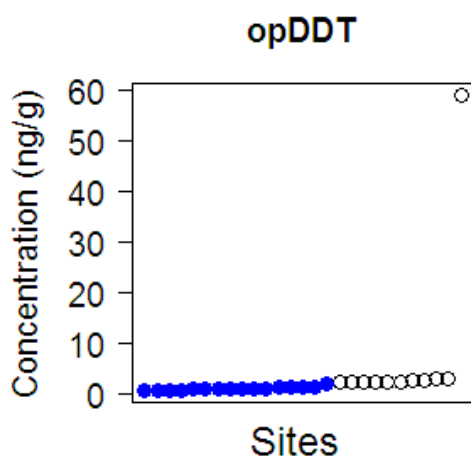
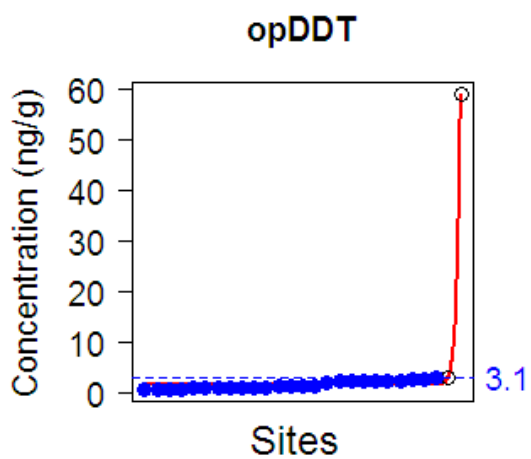
opDDE: 2,4'-DDE



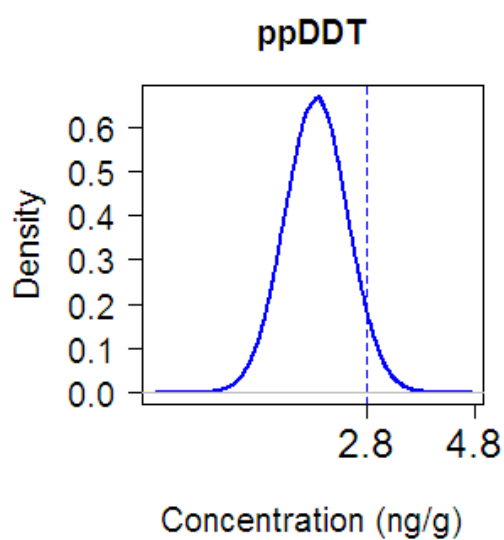
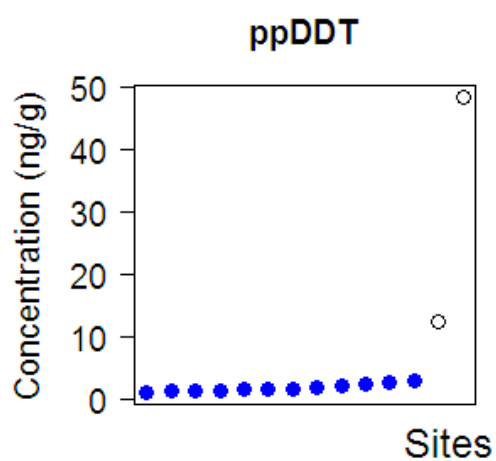
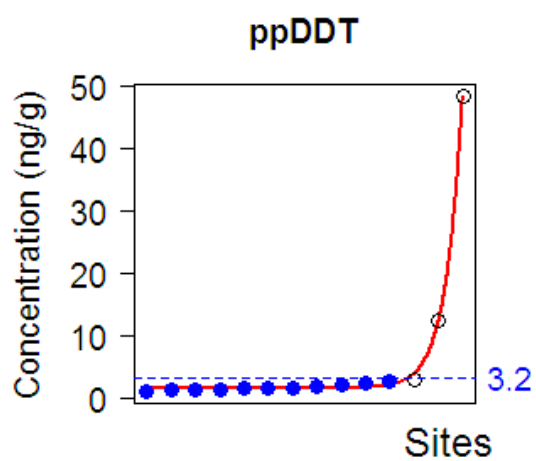
ppDDEdieldrin: 4,4'-DDE + dieldrin



ppDDD: 4,4'-DDD

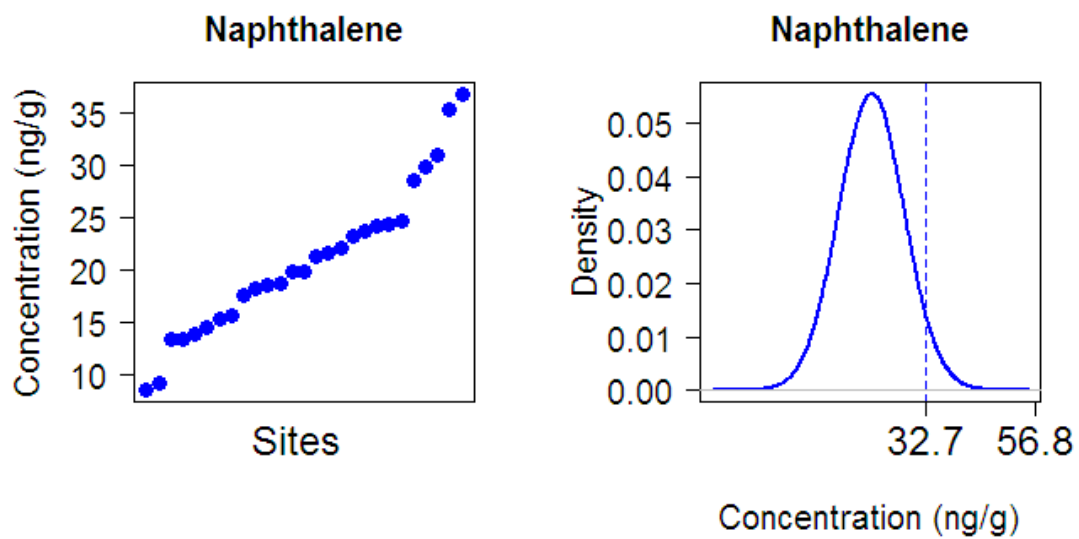


opDDT: 2,4'-DDT

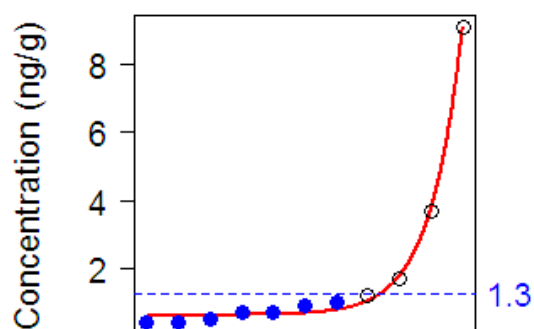


ppDDT: 4,4'-DDT

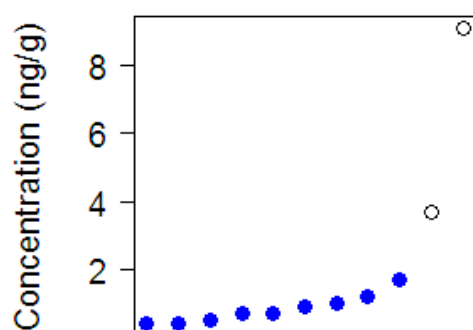
III. PAHs



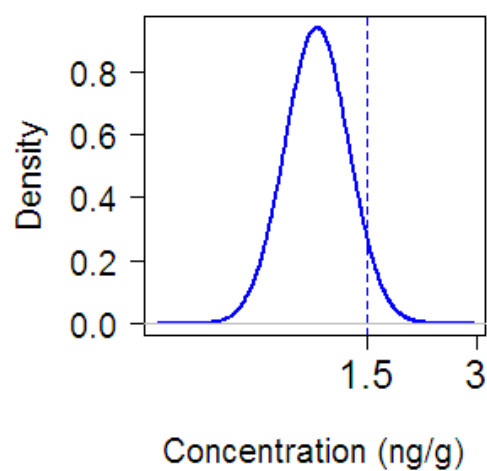
Anthracene

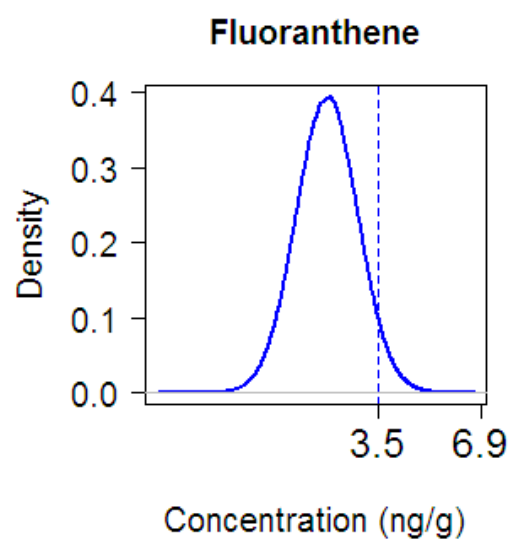
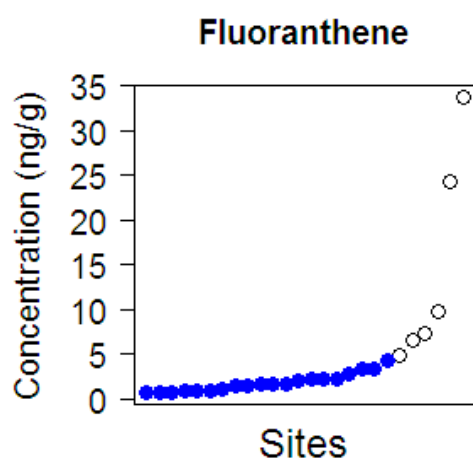
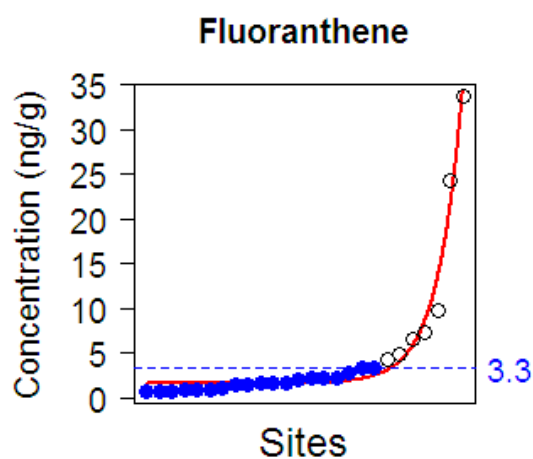


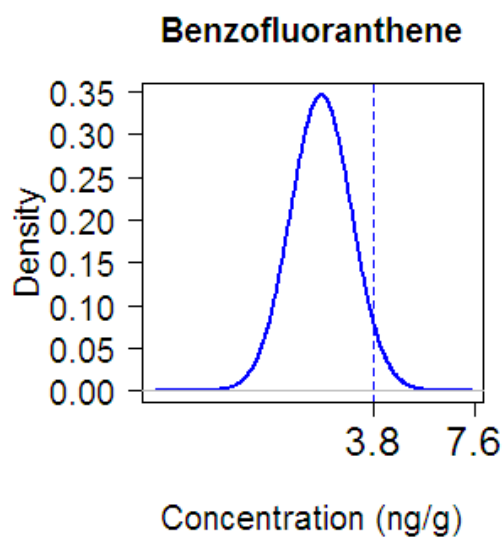
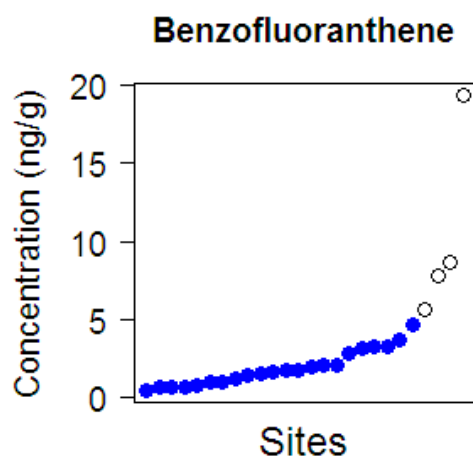
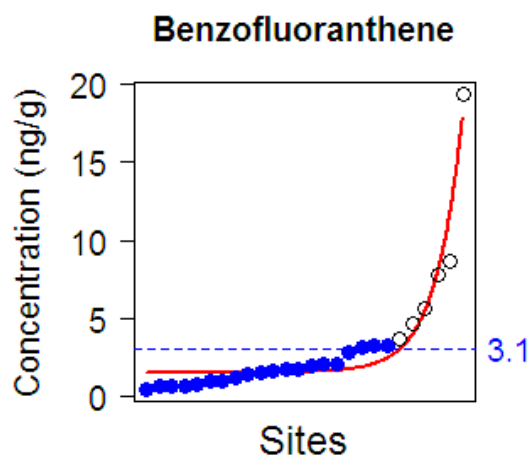
Anthracene



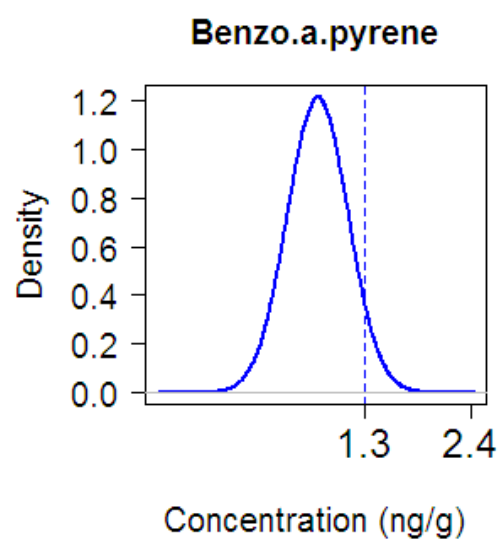
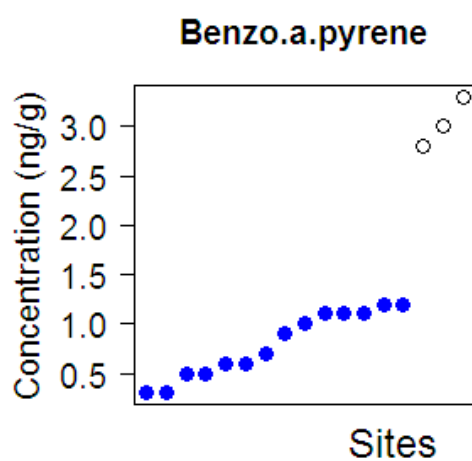
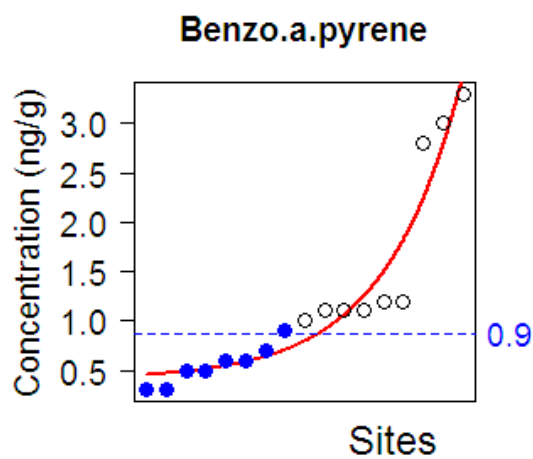
Anthracene

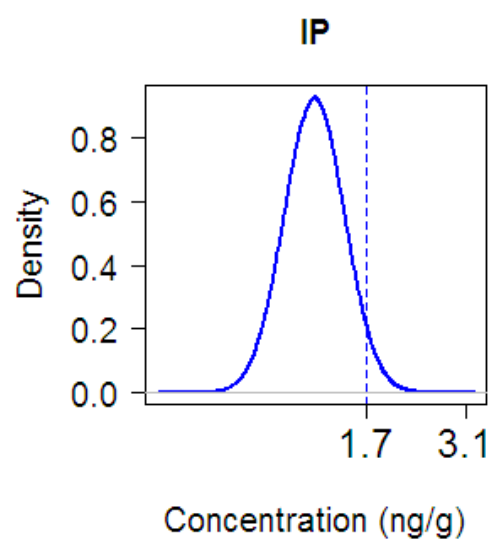
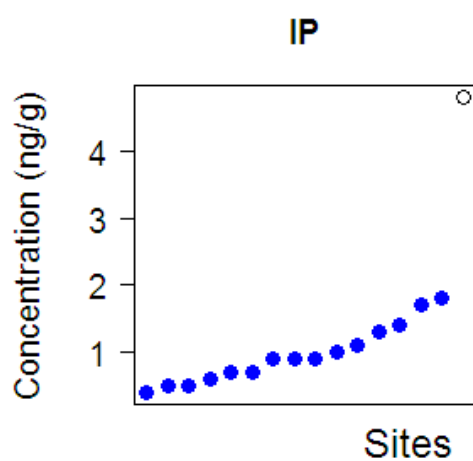
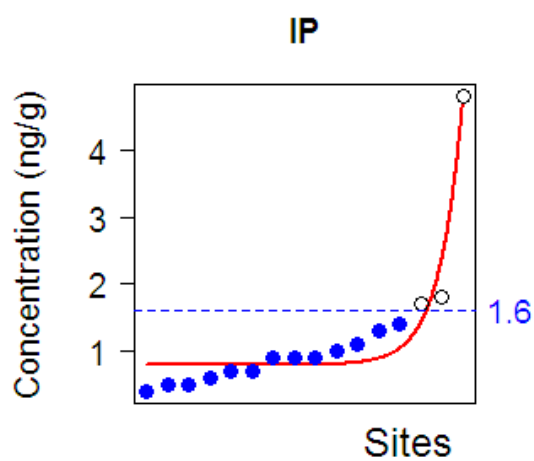




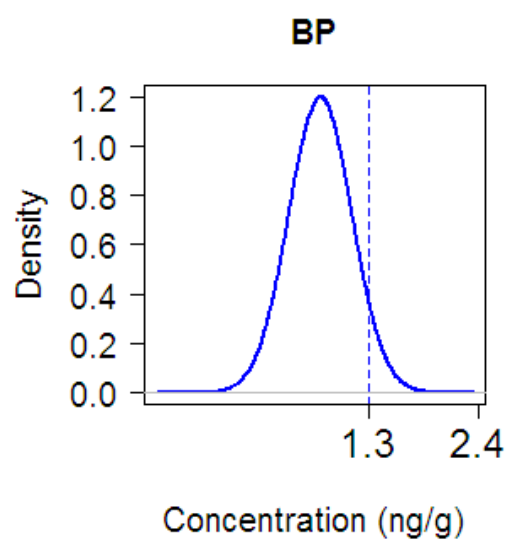
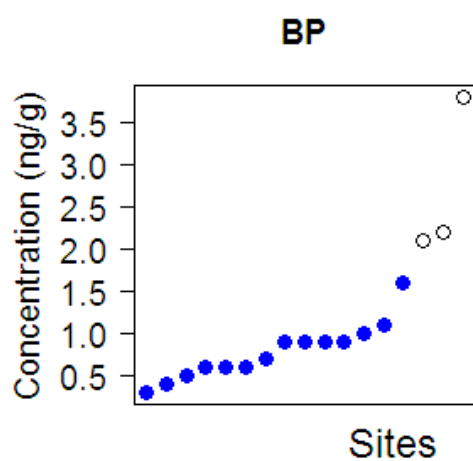
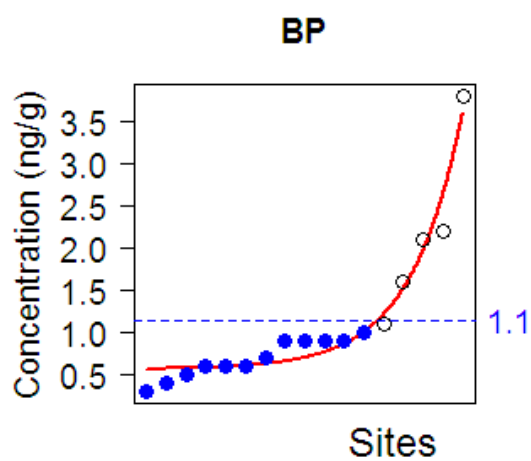


Benzofluoranthene: benzo(b,k,j)fluoranthene

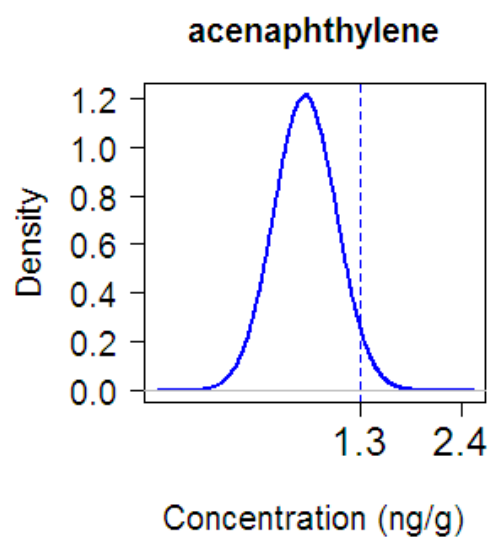
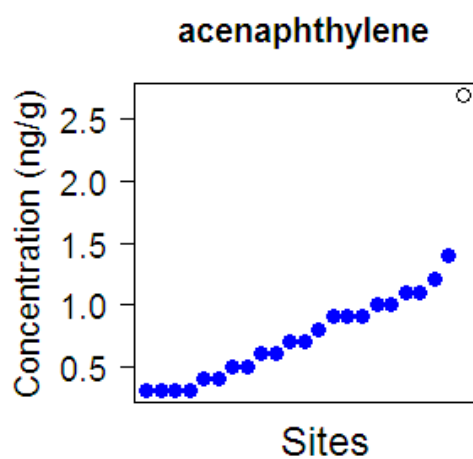
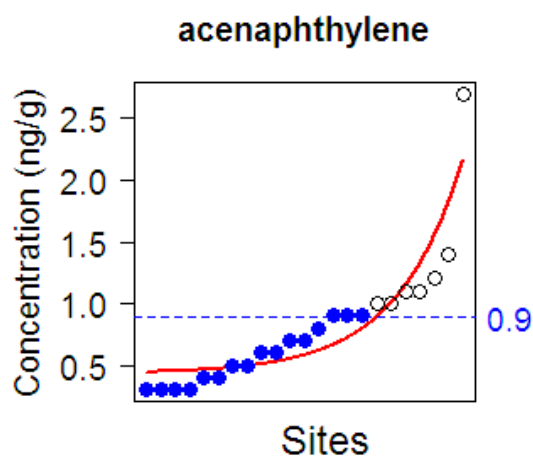


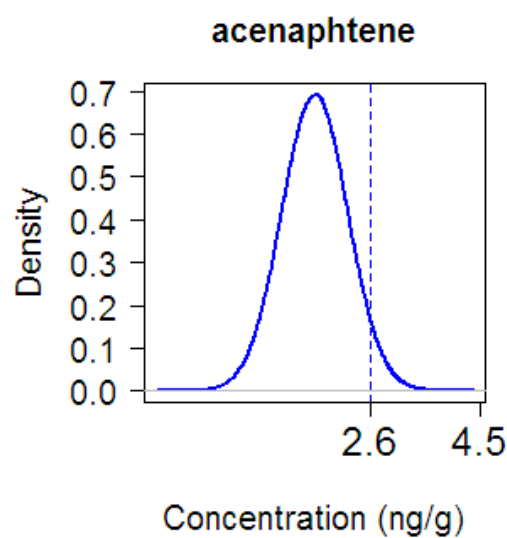
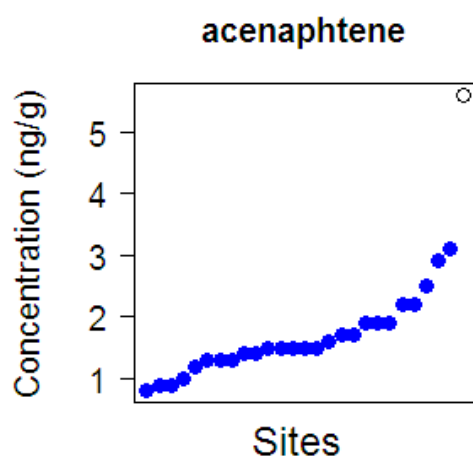
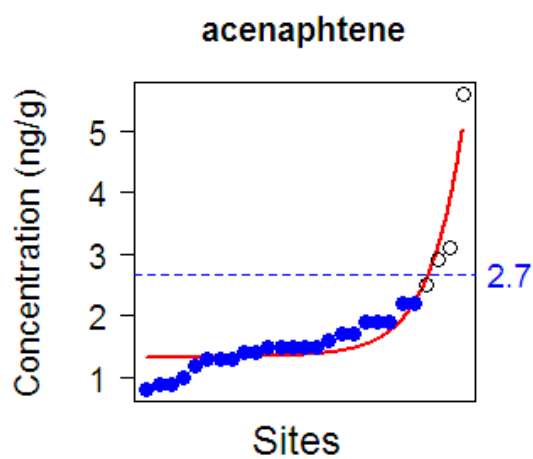


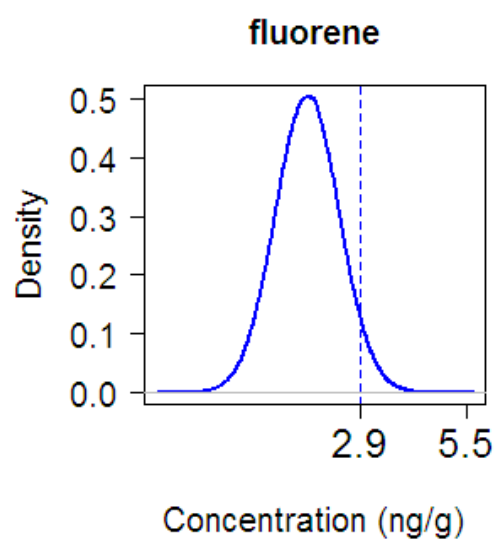
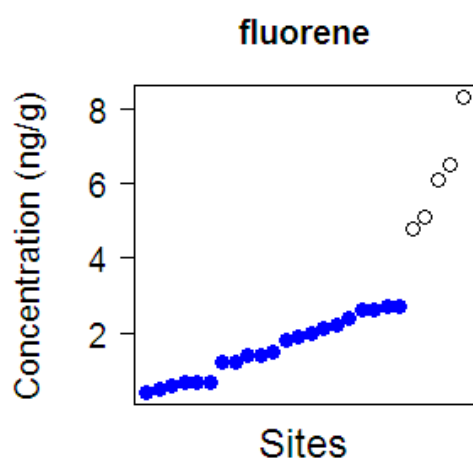
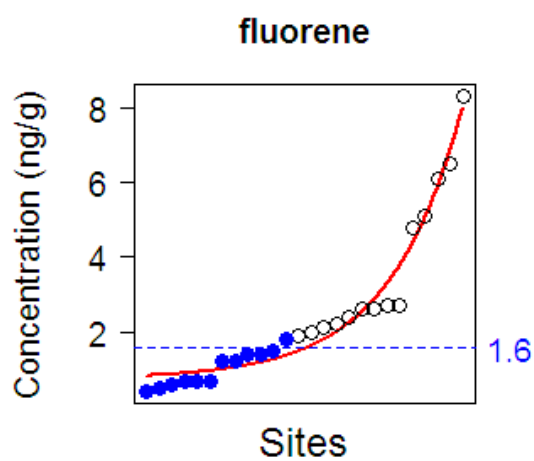
IP: indeno(1,2,3-cd)pyrene

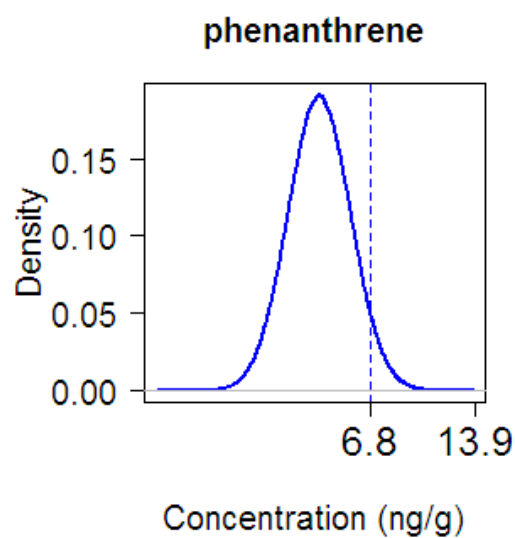
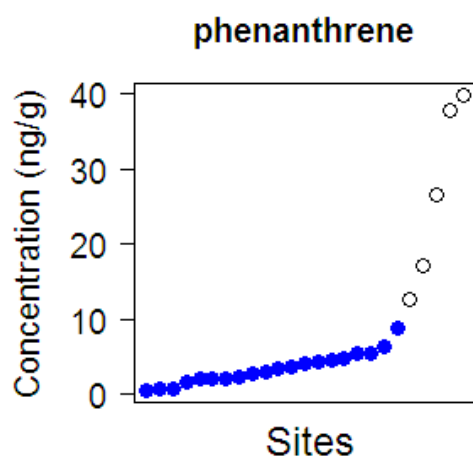
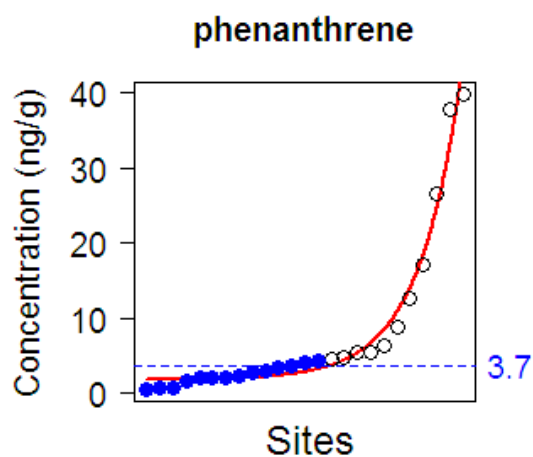


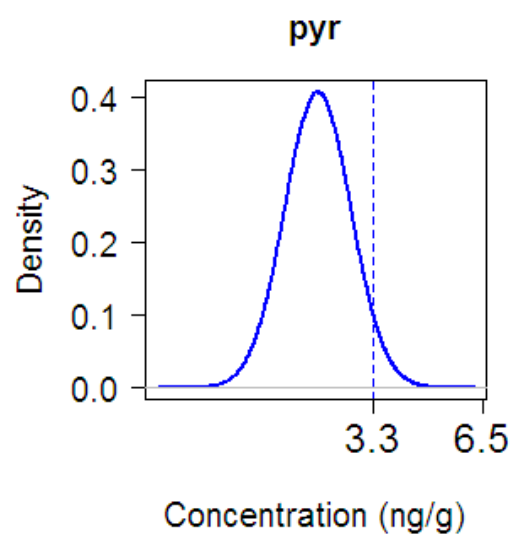
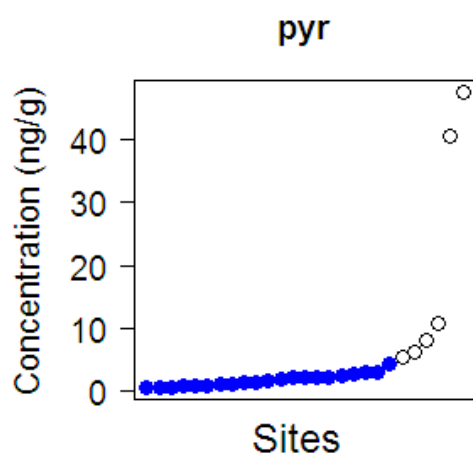
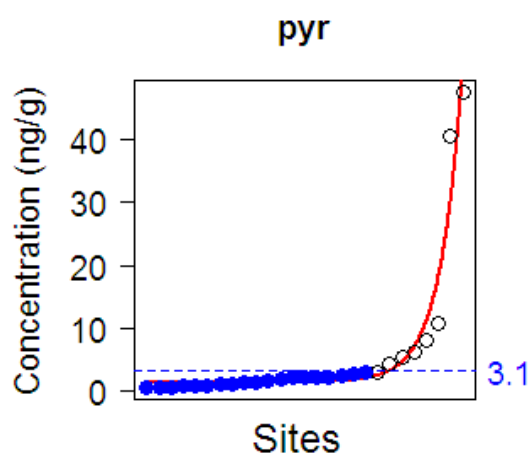
BP: benzo(g,h,i)perylene



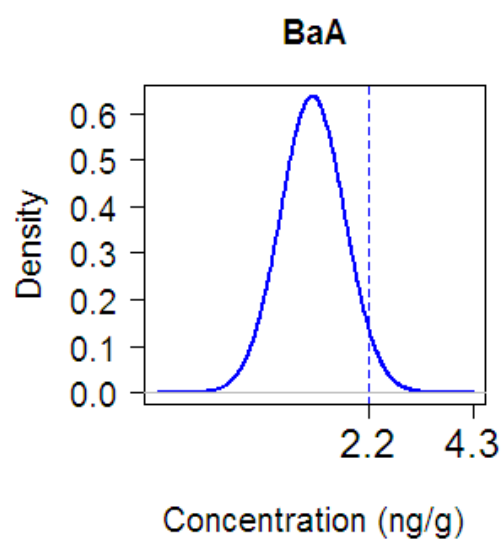
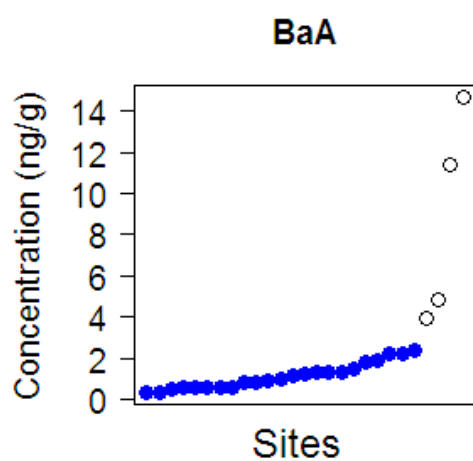
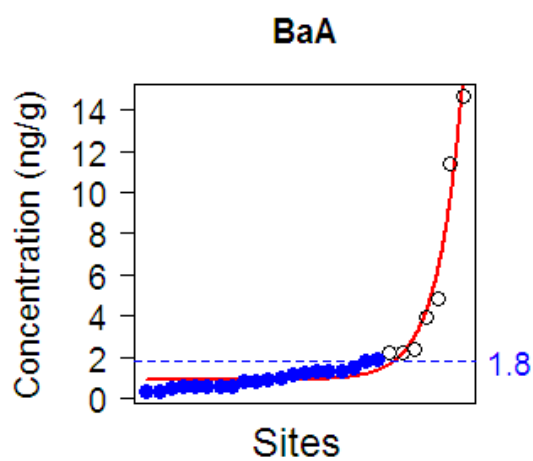




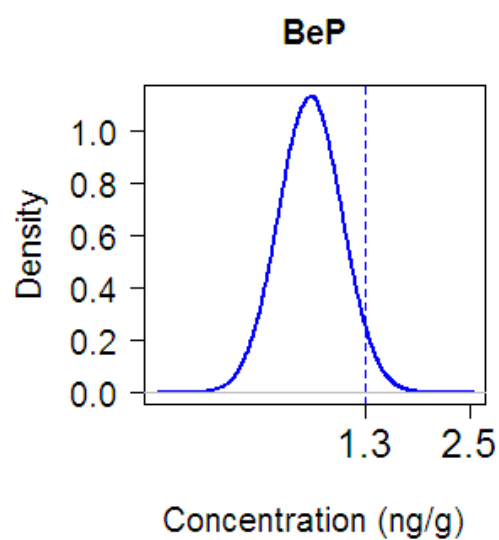
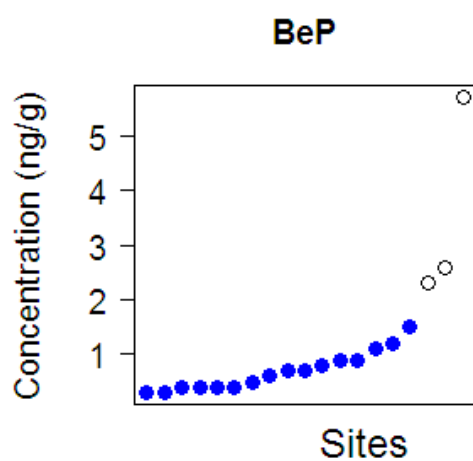
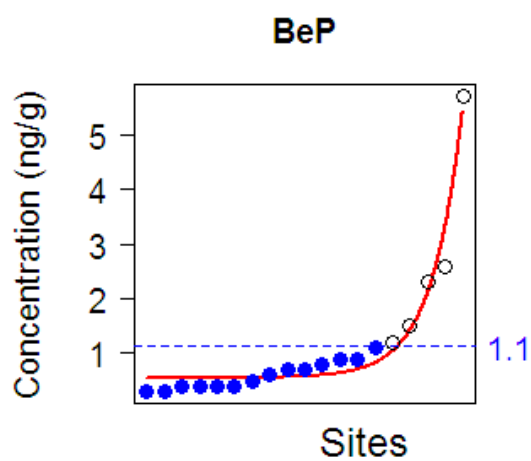




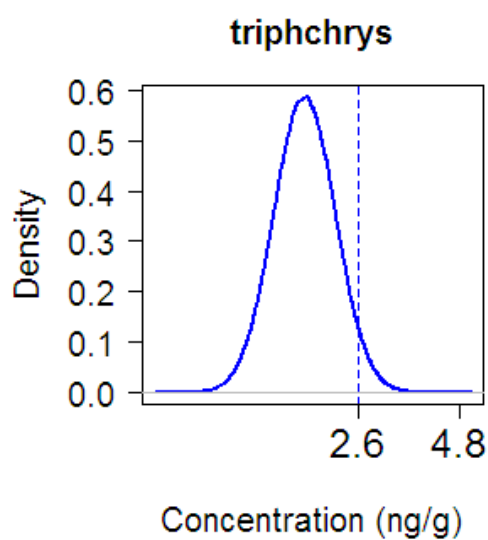
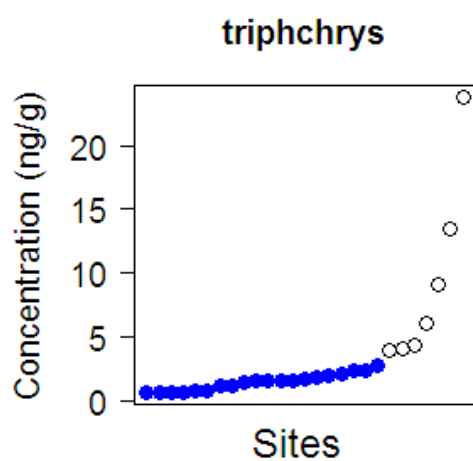
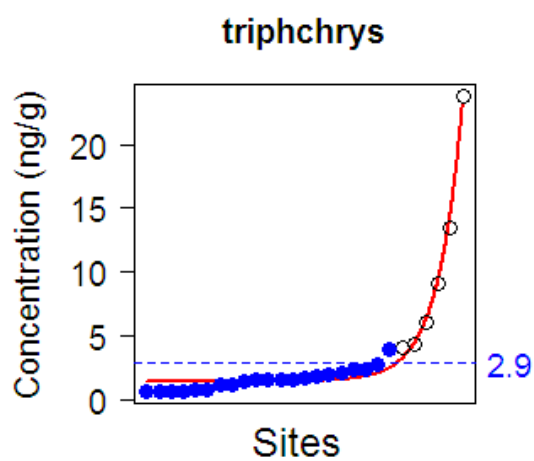
pyr : pyrene



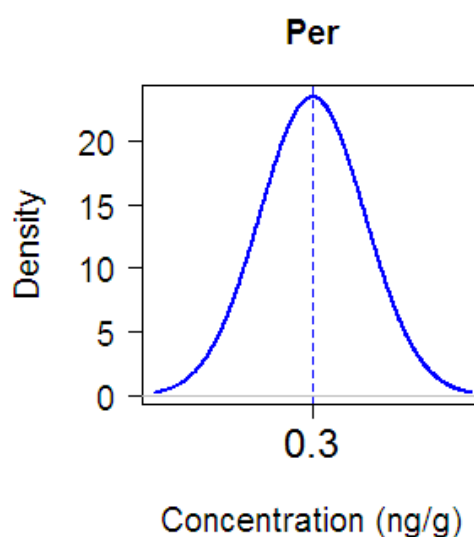
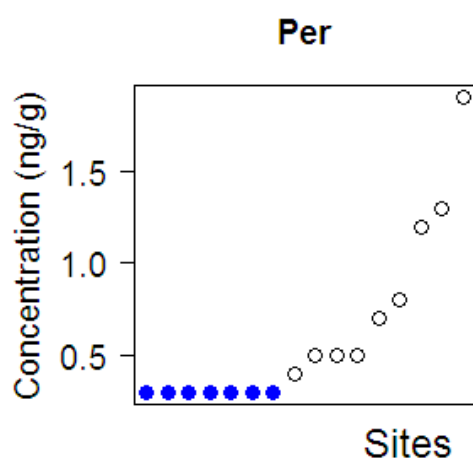
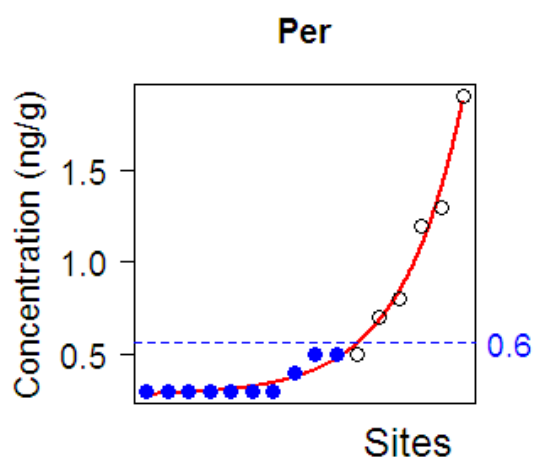
BaA: benzo(a)anthracene



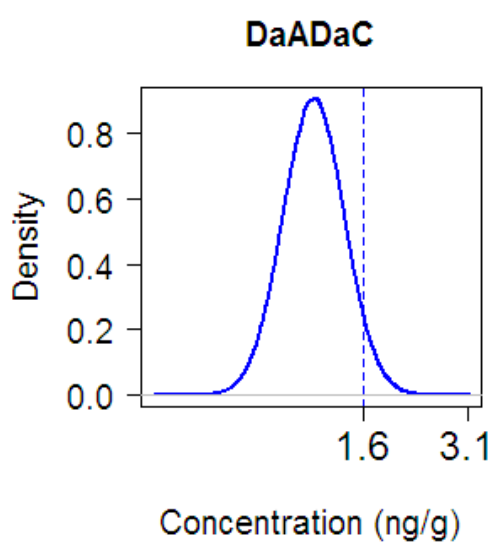
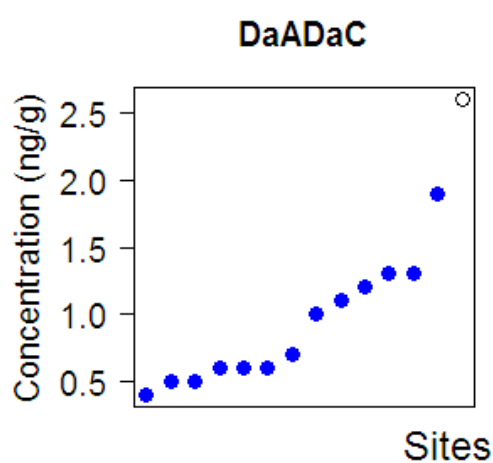
BeP: benzo(e)pyrene



triphchrys: triphenene + chrysene

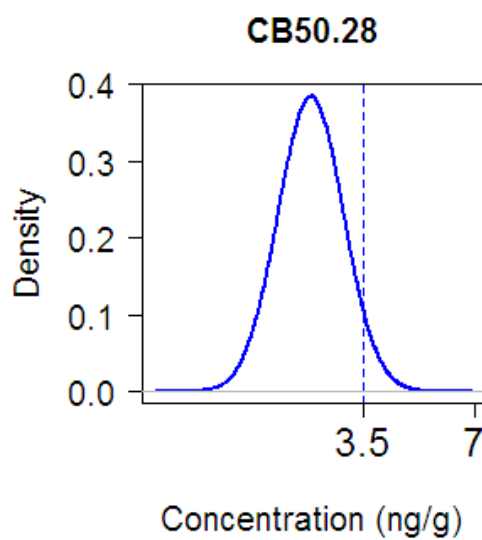
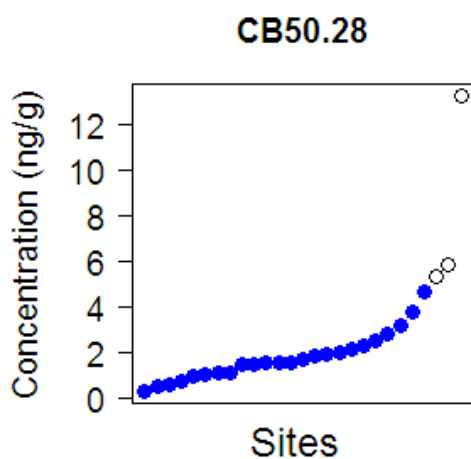
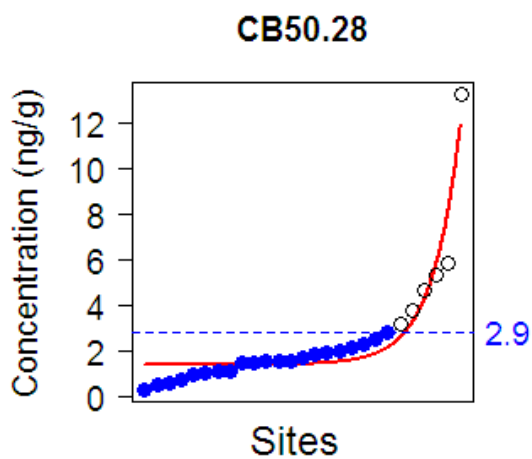


Per: Perylene

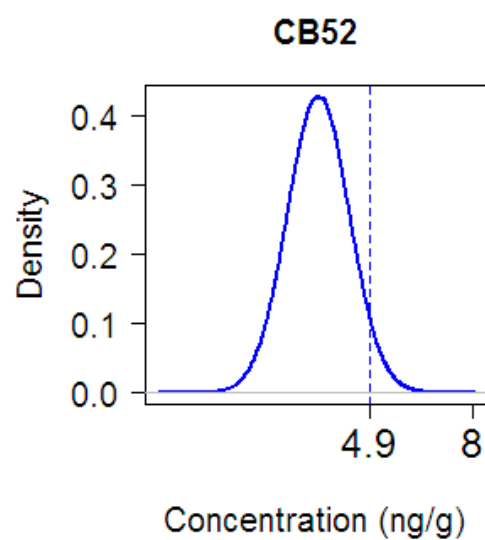
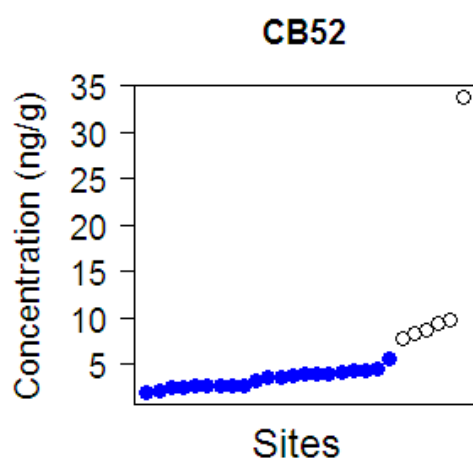
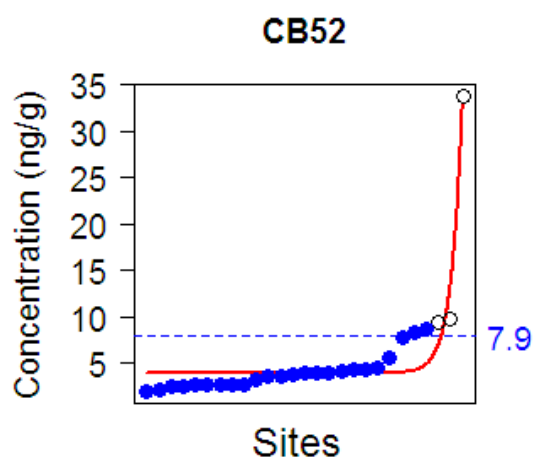


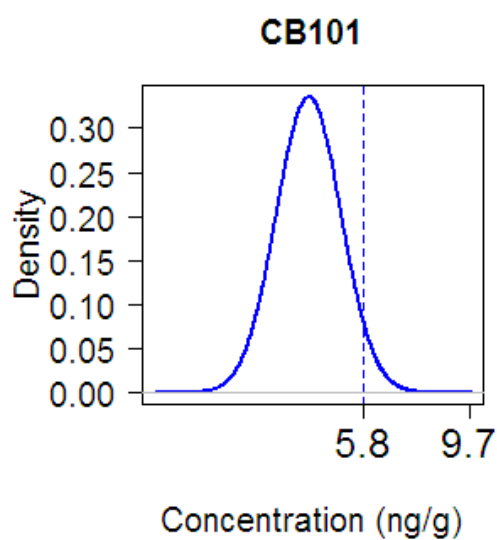
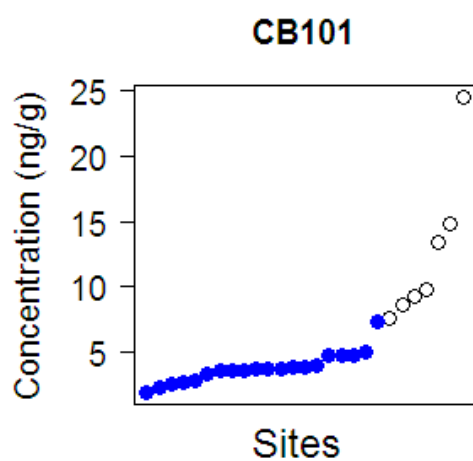
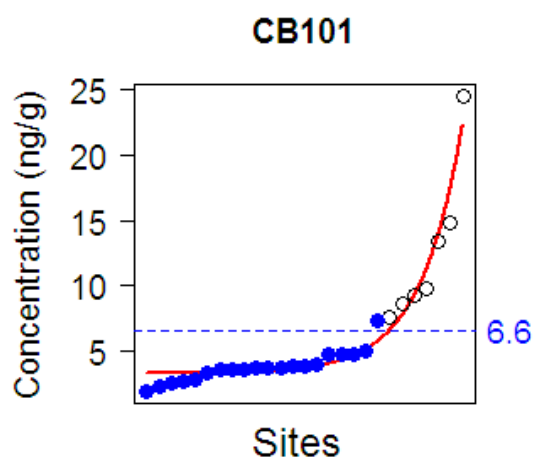
DaADaC : Dibenzo(a,c)anthracene

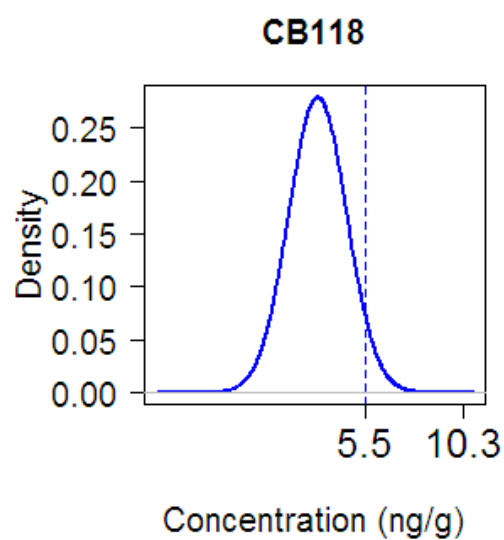
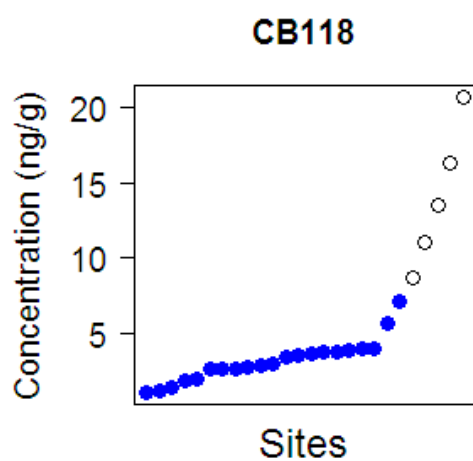
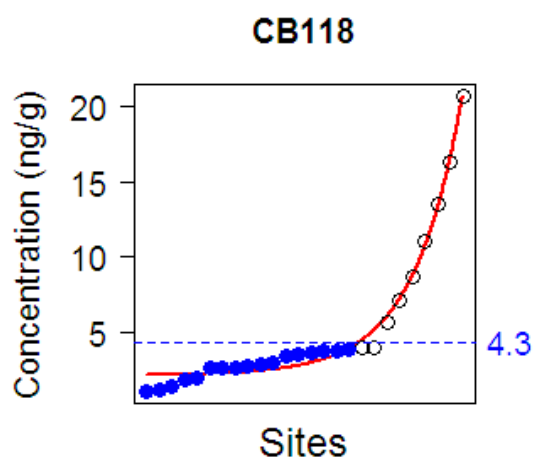
IV. PCB CONGENERS (INDICATOR PCBs)

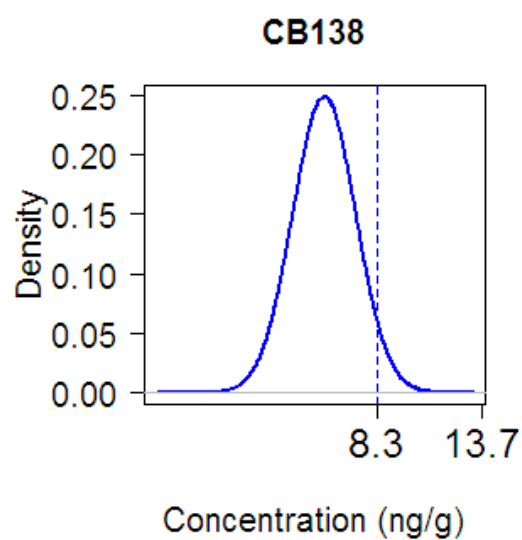
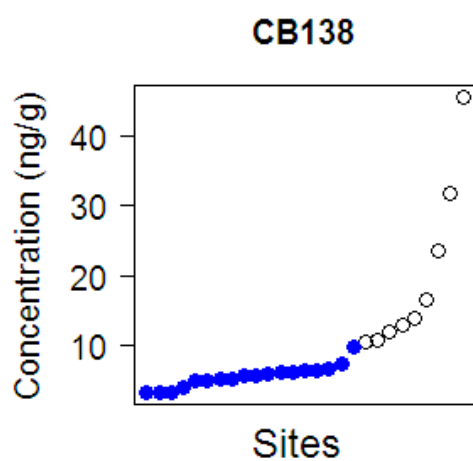
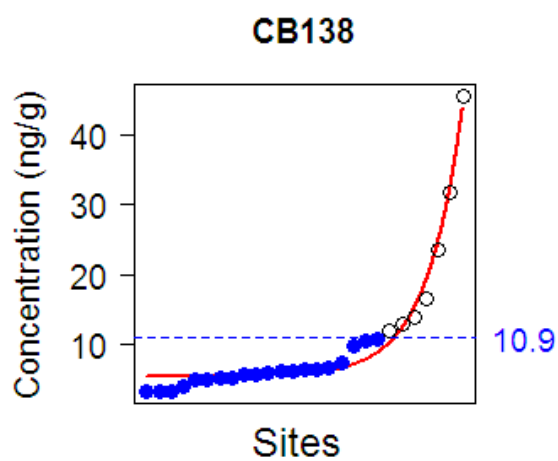


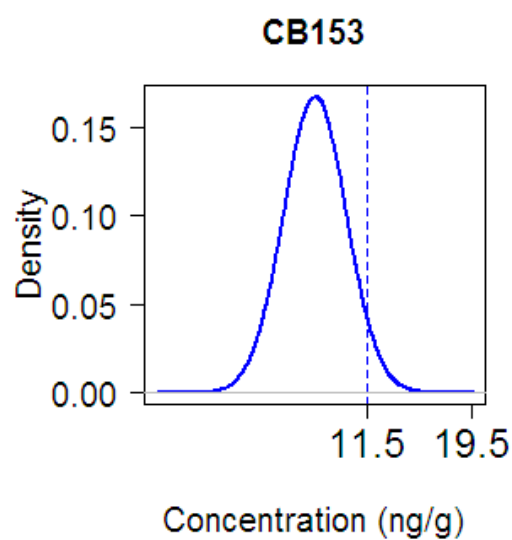
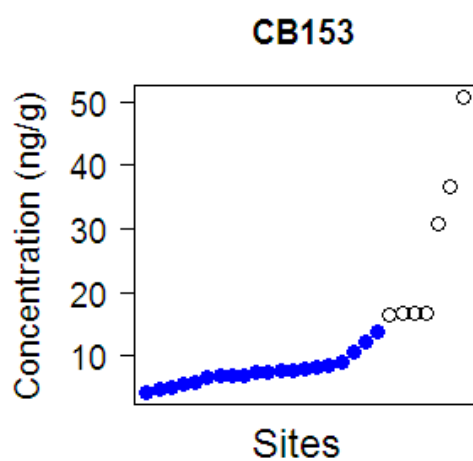
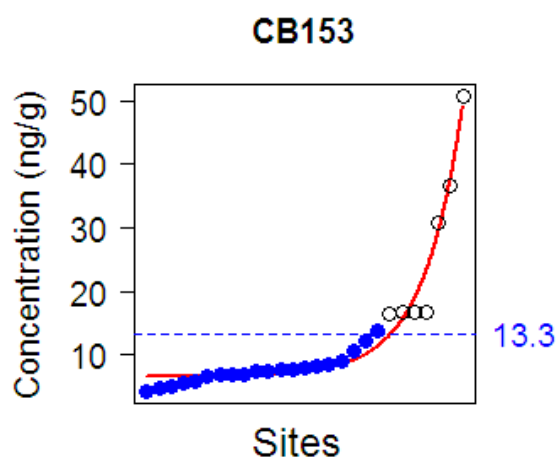
CB 50.28: CB 50 +CB 28

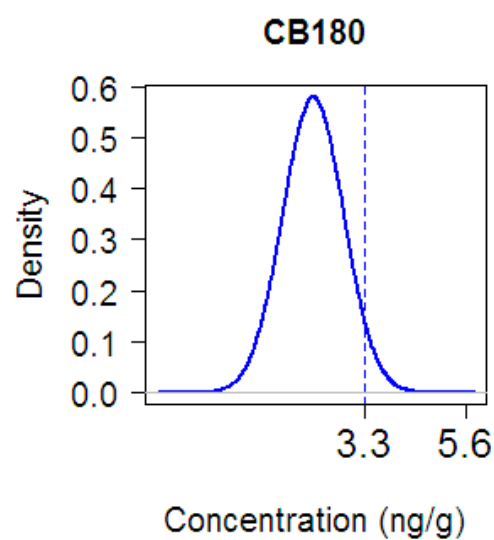
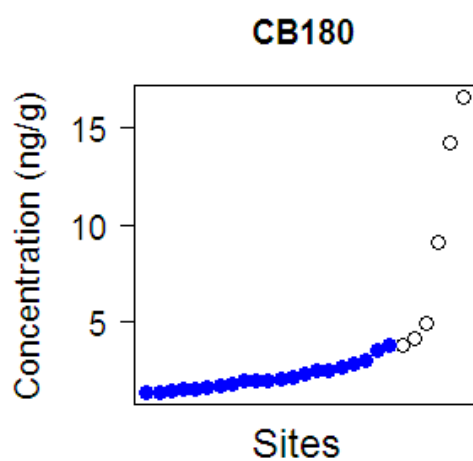
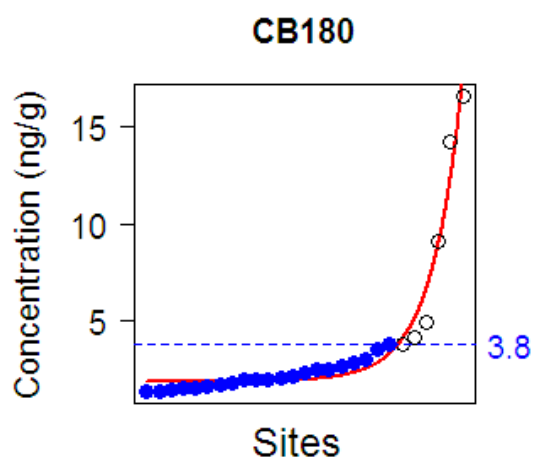












V. PBDE CONGENERS

