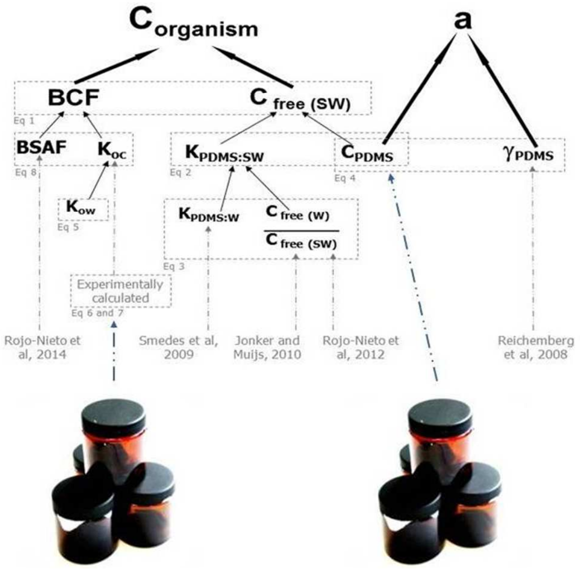
Environmental Science Processes & Impacts

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

rsc.li/process-impacts



Using C_{free} and specific BSAFs, the concentrations in target organs of benthic fish, hypothetically exposed to sediments can be estimated.

In sediments contaminated by hydrophobic organic compounds, the total concentrations are less indicative of potential exposure and distribution than the associated freely dissolved concentrations (C_{free}) or chemical activity, being recently proposed chemical activity as a key parameter for the baseline toxicity. Measurements of C_{free} can also be multiplied by an appropriate factor (BCF or BSAF) in order to predict bioconcentration/bioaccumulation. In this work, C_{free} and chemical activity associated to PAHs of marine sediments from a chronically polluted area have been studied to predict baseline toxicity and potential bioaccumulation from these sediments into target organs of flatfish. This new approach allows predicting the concentration in biological tissues under the study of C_{free} in sediments, as a useful tool in risk assessment.

0Title: Estimating baseline toxicity of PAHs from marine chronically polluted sediments and bioaccumulation in target organs of fish hypothetically exposed to them, a new tool in risk assessment.

Authors: E. Rojo-Nieto* and J. A. Perales

Andalusian Centre of Marine Science and Technology (CACYTMAR), Department of Environmental Technologies, University of Cadiz, 11510 Puerto Real, Spain.

*Corresponding author: elisa.rojo@uca.es; telephone 034 956016587; fax 0034 956 016746

Keywords:

Passive sampling, Sediment, PAH, Freely dissolved concentration, Chemical activity, Bioaccumulation.

Abstract

In soils and sediments contaminated by hydrophobic organic compounds (HOCs), the total concentrations are less indicative of potential exposure and distribution than the associated freely dissolved concentrations (C_{free}) or chemical activity. Therefore, these two parameters are increasingly used to assess sediment contamination with regards to their (1) partitioning into the water column, (2) bioaccumulation and (3) baseline toxic potential. In this work, sediments from a chronically polluted coastal area, with similar total PAH concentrations, were studied using PDMS coated glass jars (obtaining $C_{free\ (SW)}$ and chemical activity) to predict baseline toxicity and potential bioaccumulation from these sediments. Results indicate that, on one hand, the chemical activity of the sediments differed by up to one order of magnitude and were below the level at which lethal

baseline toxicity is expected, but are still a cause for concern due to the presence of other pollutants and different mechanisms of action. On the other hand, the combination of C_{free} measurements and Biota to Sediment Accumulation Factors (BSAFs) allowed concentrations in different target organs of benthic flatfish, hypothetically exposed to these chronically polluted sediments, to be estimated. This new approach allows to predict the concentration in biological tissues under the study of $C_{free(SW)}$ in sediments, as a useful tool in risk assessment.

1. INTRODUCTION

Due to their properties and the environmental implications, 8 polycyclic aromatic hydrocarbons (PAHs) are included in the list of Priority Pollutants of the European Water Framework Directive[1,2] and 16 PAHs in the United States Environmental Protection Agency (USEPA) Priority Pollutant List[3]. Levels of PAHs in the environment are characterized by low solubility in aqueous media, and once dissolved in water they tend to partition into organic phases including living organisms[4]. PAHs levels in marine sediments have a highly heterogenic spatial distribution depending on several characteristics of the study location, i.e., source, abundance, local atmospheric/marine circulation regime, sediment properties (grain size, content of organic carbon and black carbon), etc. Not only there is heterogeneity in concentrations within a region, but also the composition of the PAH mixture profile varies. This is rarely addressed in traditional risk assessment and management, which focus on the exposure and effects of single compounds and might lead to an underestimation of the actual risks.

Hydrophobic pollutants such as PAHs accumulate in biological membranes, disturbing their structure and function. This type of acute toxic action is called baseline toxicity or nonpolar narcosis[5]. Although baseline toxicity is the minimal toxicity that a compound may elicit, it is relevant for assessing the risk of complex mixtures in the environment[6]. According to several authors[7,8,9], total concentrations of hydrophobic organic compounds (HOCs) in soils and sediments are less indicative of potential exposure and distribution than the associated freely dissolved concentrations (C_{free}) or chemical activity. C_{free} can be understood as the concentration of freely dissolved molecules (not sorbed or bound to other phases). Chemical activity is not related to total concentrations, but to C_{free}. The chemical activity is a measure of the "effective concentration" of a species in an environmental compartment (e.g., water, sediment, biota, air). It has recently been proposed and experimentally confirmed that chemical activity is a key parameter for the baseline toxicity of hydrophobic organic chemicals, and that some highly hydrophobic substances can exert baseline toxicity at low aqueous concentrations but at relatively high chemical activities[9,10,11,12,13,14,15]. These, and other studies, suggest that exposure to hydrophobic organic substances is better described by freely dissolved concentrations and chemical activity than the total chemical concentration, since partitioning and diffusive uptake are spontaneous processes that are driven by differences in chemical activity. Because they can accumulate in sediments, PAHs have often been examined in this environmental compartment, in relation to their role in exposure and their effects on aquatic life. On the other hand, some PAHs are considered to be mutagenic and carcinogenic, and, consequently, their bioaccumulation is relevant and of special interest because it provides information about long-term toxicity[16,17]. Furthermore, even without detectable acute or chronic effects, bioaccumulation should be regarded as a hazard criterion in itself, since some effects may only be recognized in a later phase of life[18]. C_{free} is not only useful to determine the partitioning of pollutants among marine compartments, but also is interesting to determine other parameters. Measurements of C_{free} can be multiplied by an appropriate bioconcentration factor

(BCF) in order to predict bioconcentration in, for example, soil and sediment living organisms[19,20,21], according to:

$C_{organism} = C_{free} * BCF$ [1]

with $C_{organism}$ being the lipid-normalized concentration in the biota, and BCF being the lipid-normalized aqueous bioconcentration factor. In turn, K_{ow} allows us to relate BCF and BSAF (Biota to Sediment Accumulation Factors).

Due to all of this, in this work, C_{free} and the chemical activity of PAHs were determined in sediments from a chronically polluted environment, in order (1) to study the baseline toxic potential of these sediments and (2) to determine if it is possible to assess and predict bioaccumulation in the target organs of fish hypothetically exposed to them.

2. MATERIAL AND METHODS

2.1. Sampling area

The Bay of Algeciras (36º7’51.91’’N, 5º23’45.63’’W) is located in the south of Spain, on the Strait of Gibraltar (Figure S1). This coastal area has suffered chronic anthropogenic impact for several decades from urban and industrial sources. It is an important industrial area of the Mediterranean Sea, with a large refinery (capable of processing twelve million tons of crude annually) and its associated petrochemical industry, in addition to steel, and power production (4 thermal power plants, natural gas and one with coal). Another significant source of pollution is the intense maritime traffic associated with the transportation of oil to and from the refinery, bunkering activities in the Bay and the commercial shipping activities of the Port of Algeciras, ranked among the most important in the world[22], which handled 43 million tons of goods in 2007. Five large population clusters are also located around the Bay, comprising more than 250,000 inhabitants (a population 'agglomeration' according to EU Directive 96/62/CE[23]).

2.2. Sample collection

Three sediment samples were obtained with a Van-Veen grab at depths ranging from 10 to 16 metres, from different locations in different sampling campaigns, during three consecutive years (P1A in 2007, P2A in 2008 and P2B in 2009), being P2A and P2B in a very close location (Figure S1). These samples were selected after the analysis of total PAHs in a previous study (Rojo-Nieto et al[24] and supplementary data). The three sediments analysed were chosen from a wide set of samples for being representative of a chronically polluted coastal area (close to the main sources of pollution) and because they present similar concentrations of total PAHs (on a dry weight basis). From each sampling point, approximately 400 g of sediment was placed in amber borosilicate containers with PTFE top and stored at -20ºC for subsequent analyses. Additionally, an extra 400 g was collected in plastic bags for purposes of sediment characterisation.

2.3. Analytical procedure for total PAHs

In the sediments, PAH analysis was based on the analytical procedures proposed by the USEPA[25,26] and described in Rojo-Nieto et al.[24,27]. These data of total PAHs, on a dry weight basis, are from a previous study[24] and can be found in the supplementary material. Briefly, approximately 4 g of sediment was weighed to an accuracy of 0.001 g, Soxhlet-extracted with dichloromethane-acetone, purified on Florisil columns, and concentrated using a rotary evaporator after changing the solvent from dichloromethane-hexane to acetonitrile. The concentration of 16 US EPA priority list of PAHs was determined by gas chromatography-mass selective detection (GC-MSD) using a Voyager (ThermoElectron) gas chromatograph, according to Rojo-Nieto et al. [24,27]. A reference material (soil

LGC6182) was analyzed with 81-102% of certified value of PAHs obtained, excepting Benzo (g,h,i) perylene.

2.4. Artificial seawater

Artificial seawater was prepared by dissolving NaCl (21.03 g L^{-1}), Na_2SO_4 (3.52 g L^{-1}), KCl (0.61 g L^{-1}), KBr (0.088 g L^{-1}), $Na_2B_4O_7 \times 10H_2O$ (0.034 g L^{-1}), $MgCl_2 \times 6H_2O$ (9.5 g L^{-1}), $CaCl_2 \times 2H_2O$ (1.32 g L^{-1}), $SrCl_2 \times 6H_2O$ (0.02 g L^{-1}), and $NaHCO_3$ (0.17 g L^{-1}) in Millipore water as recommended by the USEPA for acute toxicity tests with marine organisms[28]. The final total salt concentration was 0.45 M.

2.5. Sediment characterisation

All the samples were dry-sieved in laboratory using a Ro-Tap machine and seven sieves at 1 phi intervals; this procedure provided the statistical parameters described by Folk and Ward[29] for granulometry characterization. Following García-Robledo et al.[30], organic matter was determined by loss on ignition (LOI). Carbon content was analysed using a LECO CHNS 932 Analyser. Inorganic carbon content was quantified by analysing carbon content in samples burned at 550°C for 5 h. The organic fraction (TOC, total organic carbon) was determined as the difference in the carbon content between the initial dried and the ashed samples. Five replicates were used.

2.6. Coated glass jars

120 mL amber glass jars were coated with nominal 2, 4, and 8 µm layer of silicone polydimethylsilosane (PDMS) Silastic ® (Dow Coning Corporation, Midland, MI). The coating procedure has been described in detail by Reichenberg et al[31]. Jars were filled with each sediment (79±9 g fresh weight), and with 40 mL aqueous solution of sodium azide (0.5 g L^{-1}) in artificial

seawater to inhibit microbial activity and to create a suspension. For each sediment, 6 jars were studied, including duplicates for each coating thickness. The jars were sealed with aluminium foil and capped airtight. The analytes were allowed to equilibrate for two weeks at 20ºC, rotating horizontally at 8 rpm. After this period, the sediment was removed, and the jars rinsed with a little distilled water, then cleaned and dried with lint-free tissue. The analytes were extracted from PDMS with hexane, by rolling the solvent in the capped jars for 30 min, repeating the extraction 3 times and combining the aliquots. The extracts were concentrated using a rotatory evaporator after changing the solvent to acetonitrile. The composition and concentration of 16 different PAHs were determined by gas chromatography coupled with mass spectrometry detector (GC-MS/MS). This passive sampling method allows disclosing artefacts as sample depletion and polymer surface adsorption. The equilibrium between the sediments and the PDMS was demonstrated using different coating thicknesses. The approach have been successfully applied elsewhere[8,31,32,33] to sediments and soils, determining different HOCs, such as PCBs and PAHs.

2.7. Data analysis

A schematic representation of this section can be found in figure 1, allowing a better comprehension of the data calculation procedure.

2.7.1. Calculation procedure to obtain $C_{free(SW)}$

With coated glass jars, C_{PDMS}, the concentration of analyte in silicone, was obtained. Using equation 2, it can be translated to $C_{free(SW)}$:

$$C_{free(SW)} = C_{PDMS} / K_{PDMS:SW} \quad [2]$$

where $K_{PDMS:SW}$ ($L\ L^{-1}$) is the PAH equilibrium partitioning ratio between PDMS silicone and the seawater used in this study, and $C_{free(SW)}$ is free concentration of analyte in the seawater. However, these specific $K_{PDMS:SW}$ were not available, so there were calculated using the following equation:

$$K_{PDMS:W} = K_{PDMS:SW} * C_{free(SW)} / C_{free(W)} = K_{PDMS:SW} * 10^{(-Ksalt\ x\ Csalt)}\ [3]$$

where $K_{PDMS:W}$ is the PAHs equilibrium partitioning between this specific PDMS (Silastic) and MilliQ water, obtained from Smedes et al [34], C_{salt} is the molar concentration of electrolyte, K_{salt} is the Setschenow constant and $C_{free(SW)} / C_{free(W)}$ is the ratio between concentrations in equilibrium in Seawater and in MilliQ water (from Rojo-Nieto et al[35] and Jonker and Muijs[36]). Jonker and Muijs[36] and Rojo-Nieto et al[35] used the exactly same artificial seawater as this study.

2.7.2. *Calculation procedure to obtain chemical activity (a)*

To translate the concentrations of the individual PAHs in the silicone into chemical activities, activity coefficients (γ_{PDMS}) of this specific PDMS from Reichemberg et al[31] were used, through the following equation:

$$a = \gamma_{PDMS}\ x\ C_{PDMS}\quad [4]$$

For two PAHs (benzo(b)fluoranthene and benzo(ghi)perylene), activity coefficients were not available, so the activity coefficients of an isomer with same molecular weight and similar K_{ow} (benzo(k)fluoranthene and indene(123-cd)pyrene respectively) were used in this case, as an approximation.

2.7.3. Calculation procedure to obtain Generic and Site-specific Sediment organic carbon partition coefficient (K_{oc})

According to DiToro et al[37], for non-ionic chemicals, the partitioning between sediment and pore water ($C_{free(SW)}$) is primarily determined by the organic carbon concentration of the sediment (as long as equilibrium between pore water and sediment is achieved). Generic sediment organic carbon partition coefficient (K_{oc}) can be estimated, and several authors reported log K_{oc} to be close to log K_{ow}. In fact, according to USEPA[38] and DiToro et al[7], Generic-K_{oc} can be estimated as a function of K_{ow} as follows:

$$\text{Log}(K_{oc,estimated}) = 0.00028 + 0.983 \log(K_{ow}) \quad [5]$$

On the other hand, according to Maruya et al[39], Pardue et al[40], Van der Hoop et al[41], and Katagi[42], Site–specific K_{oc} values, more accurate and rigorous, can be calculated from measurements of pollutant concentrations in sediment and pore water concentrations according to:

$$K_{oc,calculated} = K_p / f_{oc} \quad [6]$$

$$K_p = C_{sediment}/C_{free(SW)} \quad [7]$$

Where K_p is the sediment-pore water partition coefficient, f_{oc} is the mass fraction of organic carbon (sediment) and $C_{sediment}$ is the concentration of studied chemical in the sediment.

2.7.4. Calculation procedure to obtain concentration in organisms ($C_{organism}$)

$C_{organism}$ of organisms hypothetically exposed to these sediments can be calculated from $C_{free(SW)}$ and BCF using equation 1. On the other hand, according to Kraaij et al[20], the equilibrium partitioning model assumes that the concentration of HOCs in the pore water (C_{free}) is related to the HOC concentration in the organic carbon-normalized sediment ($C_{sediment,oc}$) so knowing that K_{oc} can be expressed as $C_{sediment,oc}$ / $C_{free(SW)}$, BCF can be calculated through the following equation:

$$BCF = C_{organism} / C_{free(SW)} = (C_{organism} / C_{sediment,oc}) \times (C_{sediment,oc} / C_{free(SW)}) = BSAF \times K_{oc} \quad [8]$$

BSAF is the specific Biota to Sediment Accumulation Factors (BSAFs) for a fish species, being:

$$BSAF = C_{organism} / C_{sediment,oc} \quad [9]$$

where $C_{organism}$ is the lipid-normalized tissue concentration and $C_{sediment,oc}$ is the organic carbon-normalized sediment concentration. The site/species-specific BSAFs used were determined in previous studies[43], where these factors were calculated for two target organs (muscle and liver) of feral Senegalese sole (*solea senegalensis*) living in the study area.

3. RESULTS AND DISCUSSION

3.1. Total concentration of PAHs and C_{free}

The three samples chosen for this study presented a similar total concentration of the 16 PAHs (µg/Kg dry weight) studied, being 1732.00 µg Kg^{-1}, 1728.34 µg Kg^{-1} and 1432.77 µg Kg^{-1} in samples P1A, P2A and P2B respectively. Concentrations of individual PAHs can be found in Table S1, in the supplementary material. The three sediments studied can be classified as sandy sediments, having a fine fraction (<0.063 mm) of 0.88%, 1.65% and 2.55%. According to Mucci and Ederborn[44], these sediments can be classified as having organic-poor carbon content, the TOC obtained being, respectively, 1.1%, 0.2% and 0.7%. However, the TOC value for P1A could have some interference in its determination, due to the presence of some small algae fractions in this sediment sample. In fact, previous samples taken in this point reported TOC values of 0.2-0.4% [27].

Although at first glance these sediments might seem as nearly pristine according to NOAA SQuiRTs[45] regarding their dry weight concentration, taking into consideration the low content of organic carbon and correcting the total chemical concentration based on this, it can be observed that the sediments are not "pristine", being, on the contrary, Moderately Polluted according to Kamer and Swartz guidelines[24].

For the sediments, $C_{free(SW)}$ of equilibrium with artificial seawater was determined, using coated glass jars (using six jars for each sediment, duplicates of nominal thickness 2, 4, and 8 μm). Results obtained for individual PAHs are shown in Figure 2, with total C_{free} (sum of 16 PAHs) of 73.67 ng L^{-1} in P1A, 6.62 ng L^{-1} in P1B and 9.22 ng L^{-1} in P2B. In this figure it can be observed that, despite the sediments having a similar total concentration of PAHs, $C_{free(SW)}$ differ by one order of magnitude between first sediment (P1A) and the two others (P2A and P2B). It is extensive to some individual PAHs that, despite having similar total concentration in the samples, differ by one order of magnitude according to their C_{free}, or even are below the detection limit. In this figure it can also be noticed that in sediment P1A besides having higher concentrations of $C_{free(SW)}$ also present a higher variety of PAHs above the detection limit. In addition, in this figure it can be observed that P2A and P2B, which are sediments from very close places in different sampling campaigns, have very similar profiles, both in composition and in concentrations. It is interesting to note that in all samples, individual PAHs with higher free concentrations were Phenanthrene and Pyrene, together with Fluorene and Fluoranthene. Other studies[39,46] have obtained similar profiles of $C_{free(SW)}$ from superficial marine sediment, with a predominance of Pyrene and Phenanthrene, together with Fluoranthene.

3.2. Chemical activity and baseline toxicity

Diffusion across membranes occurs spontaneously across chemical activity rather than down concentration gradients, with equilibrium partitioning being defined by equal chemical activities[14]. In this study, chemical activities of PAHs were calculated, and are shown in Figure 2. The sum of chemical activities is an indicator of the baseline toxic potential of the mixture[10]. In this figure it is represented a grey area, which shows the chemical activity range where baseline toxicity is lethal[10,11,14,15,35]. In all cases, chemical activity was below the baseline lethal area. However, it is interesting to remark that the chemical activity of sediment P1A is above the 1% contribution to baseline toxicity line. According to Mayer and Reichenberg[14], the contribution of a chemical (or a family of chemicals) to the baseline toxicity of a mixture can be kept low when keeping its chemical activity less than a certain threshold level, which could be set to less than 1%. This would protect the aquatic environment against baseline toxicity. In a chronic polluted site such as the studied area this result above 1% is of special concern, due to the existence of other pollutants different from the 16 measured PAHs[47,48,49], some of them exerting nonpolar toxicity (as PCBs, PBDEs, alkylated PAHs, etc). It is important to highlight that this grey zone indicates the chemical activity range where baseline toxicity is lethal, and this is a very severe and drastic end point, so sublethal effects might occur below these values. Further studies should be conducted to establish chemical activity ranges for sublethal effects. These chemical activities and concentrations also are of concern because although they may not exert nonpolar narcosis, some of these compounds are carcinogenic.

3.3. Partition coefficients for sediment organic carbon

In Table 1 are shown log $K_{ow,}$ log $K_{oc,\ estimated}$ (Generic-Sediment organic carbon partition coefficient), average (across the samples) of log $K_{oc,\ calculated}$ (Site–specific-Sediment organic carbon partition coefficient) and its variation coefficient for those individual PAHs which appeared in the three samples. Results showed a very good concordance, in most of the PAHs studied, between theoretically estimated and experimentally calculated values of K_{oc}, in these organic-poor carbon content sediments and using artificial seawater. As has been proposed by several authors[7,38], the use of K_{ow} (or an equation based on it) is a good and quick approximation to $K_{oc.}$ However, as is shown in table 1 and was highlighted by Baker et al[50] and Witt et al[46], the estimated or Generic-K_{oc} values are only approximations, which could underestimate the actual sediment to water distribution of HOCs by one to two orders of magnitude. So, despite estimated or Generic K_{oc} being a good approximation, Site-specific K_{oc} and $C_{free(SW)}$ are required for accurate determinations (for example, to determine toxicity or bioaccumulation), being necessary to measure and experimentally calculate these parameters whenever possible.

3.4. Application of C_{free} to estimate concentration in different target organs of feral fish hypothetically chronically exposed to sediments

According to Ribeiro et al[51], Pyrene is frequently used as a marker of the total PAH contamination in fish, with Naphthalene and Phenanthrene also being abundant in them. In previous studies, Site/species-specific Biota to Sediment Accumulation Factors (BSAFs) of Phenanthrene and Pyrene from sediment were calculated for two target organs (muscle and liver) of a species of feral fish, *Solea senegalensis*, living in the Bay of Algeciras[43] (supplementary data). These BSAFs were calculated through the measurement of concentrations of Phenanthrene and Pyrene in the sediment at the exact location where the fish were collected and measuring concentrations of PAHs in different organs of the fish. Samples used to obtain these BSAFs were taken in two sampling campaigns in different points of the bay, in 2008 and 2009[43] (supplementary data), during the spawning period of this species (May-June), as the lipid content of different organs changes through the reproductive cycle and therefore the lipid-normalized concentration changes, and using adult specimens of very similar length and weight. In those fish, the main PAHs found were Phenanthrene and Pyrene[43], which also is in agreement with the C_{free} profile of the three sediments object of this study (Figure 2).

Although traditionally the fish are not used as biomonitors of contamination by PAHs[18,52], under chronic exposure situations (like adult Senegalese sole in the case of the area under study) it leads to accumulation of PAHs from sediment or food[53,54,55] , with fish, especially benthic species such as flatfish (as *Solea* sp.), being used as indicators of PAHs sediment pollution[56]. These BSAFs used were calculated from a data set of sediments and fish different from the data set of sediments used in the present study, but from the same study area and under the same environmental conditions. The sediments used in the present study were collected during the same sampling campaigns of those used to calculate BSAFs, but in the case of the sediments of the current study, there were no target-fish captured associated to the sediment, so there is a lack of data on bioaccumulation in organs of *S. Senegalensis* at these sampling points. It is therefore very interesting, in order to obtain a complete picture of the study area, to apply novel techniques to estimate the concentration in target organs of fish hypothetically exposed to sediments in those points where samples were not available.

Table 2 shows the average values of these BSAFs for muscle and liver, from 25 *S. senegalensis* individuals, from the study of Rojo-Nieto et al[43]. Most of these BSAFs are lower than theoretically predicted (1-2)[57]. *Solea senegalensis* is a benthic fish, which is in closer contact with sediment than pelagic or demersal fish. A relative difference among the individual BSAFs can be observed (Standard Deviation of the Mean in table 2), due to this being experimental data from field long term exposure under non-standardized conditions. According to Wong et al[58] the BSAF model may be applicable to benthic fish in environmental situations, with the caveats that this model assumes that HOCs are in equilibrium between sediments, pore water and organisms. In the environment, biota concentrations will often deviate from equilibrium, and equilibrium partitioning predictions can then serve as a reference value for the evaluation and comparison of monitoring data. Monitoring data below the equilibrium partitioning level might indicate biotransformation or that the fish have migrated from a less polluted area. In that regard the BSAF model assumes that the chemical analyte is not transformed in the sediment or the biota. It is known that fish metabolize PAHs, with high molecular weight PAHs being the most metabolized[59,60,61,62,63]. Wong et al[58] obtained results below the predicted values of the model for some organochlorines that are metabolized by fish. Despite these limitations, BSAFs have been proposed as a simple screening tool to estimate bioaccumulation in fish, as the first tier of a multi-tier evaluation[18,58,64], and experimental site/species-specific BSAFs are highly recommended for this purpose.

These experimentally calculated BSAFs have allowed estimating concentrations in two target organs for individuals of *S. senegalensis* hypothetically exposed to the sediments of this study, through the equation 1, knowing the $C_{free(SW)}$ associated with the sediments obtained using the coated glass jars and the Site-specific K_{oc}. Table 2 shows the predicted concentrations of these two PAHs in liver and muscle (during the sparring period) if some individuals of adult Senegalese sole of 25-30 cm were long-term exposed to these sediments. These lipid-normalized concentrations are of the same order of magnitude of those found previously in Senegalese sole from the study zone[43], by direct measuring in biological tissues. This concordance between estimated concentrations and measured concentrations reported by Rojo-Nieto et al[43] from same study area confirms the potential of using $C_{free(SW)}$ and BSAFs as a tool to estimate concentrations in organs of benthic feral fish exposed to polluted sediments. However, these BSAFs should be calculated with very high accuracy for each species, and for each stage of life, reproductive period, etc, in order to be able to use them, in combination with $C_{free(SW)}$, to estimate the concentration in biological tissues. The determination of HOCs in organs of feral organisms is laborious and expensive (because it requires fishing campaigns), and it is sometimes difficult to obtain a representative number of specimens of the same length and weight, etc. The estimation of biota concentrations on the basis of C_{free} measurements in combination with BCF or BSAFs values might thus be an alternative and a useful screening tool in site specific risk assessments.

4. CONCLUSIONS

In the study of marine sediments from a chronically polluted area (the Bay of Algeciras) and with similar total concentration of PAHs, the $C_{free(SW)}$ differ by one order of magnitude, highlighting the importance of study this parameter in addition to the traditional ones, such as total chemical concentration. In all cases the chemical activity of these sediments (related to the 16 PAHs analyzed) was below the level at which lethal baseline toxicity is expected. However, in one case the sum of chemical activities was above the 1% contribution to lethal baseline toxicity, being this of special concern due to the presence of other pollutants (the contribution to the chemical activity of a chemical (or a family of chemicals) of a mixture should be less than 1%, to protect the aquatic environment against baseline toxicity). It has been confirmed that K_{oc} can be approximated as a function of K_{ow} in these organic-carbon poor content sediments, but for accurate determinations (needed to calculate toxicity or bioaccumulation) $C_{free(SW)}$ and Site-specific K_{oc} should be measured and experimentally calculated whenever possible. Using $C_{free(SW)}$ obtained by sediment passive sampling, and previously established well-defined Site/species-specific BSAFs, the lipid-normalized concentrations in different target organs of benthic flatfish hypothetically exposed to these sediments under specific environmental real conditions can be estimated, allowing us to predict the concentration in biological tissues under the study of $C_{free(SW)}$ in sediments, as a useful tool in risk assessment.

ACKNOWLEDGEMENTS

Elisa Rojo-Nieto would like to thank the Ministry of Education (Spain) for funding her research fellowship (FPU). The authors deeply acknowledge all the valuable advice and support provided during this study by Prof. Dr. Philipp Mayer (Department of Environmental Engineering, Technical University of Denmark). Skilled technical help from Edward Carpio (Department of Environmental Technologies, University of Cadiz) and Margit Fernqvist Møller (Department of Environmental Science, Aarhus University) is also gratefully acknowledged. We are also grateful to Ms. Claire Owen for English correction. This is the International Campus of Excellence of the Sea (CEIMAR) journal publication nº100.

REFERENCES

1. Council Directive 2008/105/EC. 2008. On environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Official Journal of the European Union L348, 21/11/1996, 0084-0097.

2. Council Directive 2013/39/EU. Amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Official Journal of the European Union, L226: 1-17

3. Code Of Federal Regulation. 1982. Title 40, Appendix A to part 423-126 Priority pollutants. 47 FR 52304.

4. de Maagd, P.G., ten Hulscher, D.T.E.M., van den Heuvel, H., Opperhuizen, A. & Sijm, D.T.H.M., 1998. Physicochemical properties of polycyclic aromatic hydrocarbons: aqueous solubilities, n-octanol/water partition coefficients, and Henry's law constants. Environ. Toxicol. Chem. 17 (2), 251–257.

5. Escher, B. I. & Schwarzenbach, R. P. 2002. Mechanistic studies on baseline toxicity and uncoupling of organic compounds as a basis for modeling effective membrane concentrations in aquatic organisms. Aquatic Sciences - Research Across Boundaries, 64**,** 20-35.

6. Schwarzenbach R.P., Escher B.I., Fenner K., Hofstetter T.B ., Johnson C.A ., von Gunten U., Wehrli B., 2006. The challenge of micropollutants in aquatic systems Science,313, (5790),1072-1077

7. Di Toro, D. M., Zarba, C. S., Hansen, D. J., Berry, W. J., Swartz, R. C., Cowan, C. E., Pavlou, S. P., Allen, H. E., Thomas, N. A. & Paquin, P. R. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environmental Toxicology and Chemistry, 10: 1541–1583.

8. Mäenpää, K., Leppänen, M. T., Reichenberg, F., Figueiredo, K. & Mayer P., 2011. Equilibrium Sampling of Persistent and Bioaccumulative Compounds in Soil and Sediment: Comparison of Two Approaches to Determine Equilibrium Partitioning Concentrations in Lipids. Environmental Science & Technology 45 (3), 1041-1047

9. Reichenberg, F. & Mayer, P. 2006. Two Complementary Sides Of Bioavailability: Accessibility And Chemical Activity Of Organic Contaminants In Sediments And Soils. Environmental Toxicology and Chemistry, 25, 1239-1245.

10. Engraff, M., Solere, C., Smith, K. E. C., Mayer, P. & Dahllöf, I. 2011. Aquatic Toxicity of PAHs and PAH Mixtures At Saturation To Benthic Amphipods: Linking Toxic Effects To Chemical Activity. Aquatic Toxicology, 102, 142-149.

11. Mackay, D., Arnot, J. A., Petkova, E. P., Wallace, K. B., Call, D. J., Brooke, L. T. & Veith, G. D. 2009. The physicochemical basis of QSARs for baseline toxicity. SAR and QSAR in Environmental Research, 20, 393-414.

12. Mackay, D., Arnot, J. A., Wania, F. & Bailey, R. E. 2011. Chemical activity as an integrating concept in environmental assessment and management of contaminants. Integrated Environmental Assessment and Management, 7, 248-255

13. Mayer, P. & Holmstrup, M. 2008. Passive Dosing Of Soil Invertebrates With Polycyclic Aromatic Hydrocarbons: Limited Chemical Activity Explains Toxicity Cutoff. Environmental Science & Technology, 42, 7516-7521.

14. Mayer, P. & Reichenberg, F. 2006. Can Highly Hydrophobic Organic Substances Cause Aquatic Baseline Toxicity And Can They Contribute To Mixture Toxicity? Environmental Toxicology and Chemistry, 25, 2639-2644.

15. Smith, K. E. C., Dom, N., Blust, R. & Mayer, P. 2010. Controlling And Maintaining Exposure Of Hydrophobic Organic Compounds In Aquatic Toxicity Tests By Passive Dosing. Aquatic Toxicology, 98, 15-24.

16. Milinkovitch, T., R. Kanan, Thomas-Guyon, H., Le Floch, S. (2011). "Effects of dispersed oil exposure on the bioaccumulation of polycyclic aromatic hydrocarbons and the mortality of juvenile Liza ramada." Science of The Total Environment 409(9): 1643-1650.

17. Ramachandran S.D., P.V. Hodson, C.W. Khan, K. Lee, 2004. Oil dispersant increases PAH uptake by fish exposed to crude oil. Ecotoxicol Environ Saf, 59, 300–308

18. Van der Oost R., Beyer J., Vermeulen N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, 13, 57–149

19. Trimble, T. A., You, J. & Lydy, M. J. 2008. Bioavailability of PCBs from field-collected sediments: Application of Tenax extraction and matrix-SPME techniques. Chemosphere, 71**,** 337-344.

20. Kraaij, R., Mayer, P., Busser, F. J. M., Van Het Bolscher, M., Seinen, W., Tolls, J. & Belfroid, A. C. 2003. Measured Pore-Water Concentrations Make Equilibrium Partitioning Work A Data Analysis. Environmental Science & Technology, 37**,** 268-274.

21. Van Der Wal, L., Jager, T., Fleuren, R. H. L. J., Barendregt, A., Sinnige, T. L., Van Gestel, C. A. M. & Hermens, J. L. M. 2004. Solid-Phase Microextraction To Predict Bioavailability and Accumulation of Organic Micropollutants in Terrestrial Organisms after Exposure to a Field-Contaminated Soil. Environmental Science & Technology, 38**,** 4842-4848.

22. AAPA, American Association of Port Authorities. 2007. World Port Ranking. www.aapa-ports.org

23. Council Directive 96/62/EC, 1996. On ambient air quality assessment and management. Official Journal of the European Union L296, 21/11/1996, p. 0055-0063

24. Rojo-Nieto E., Sales D., Perales J. A., 2013. Sources, transport and fate of PAHs in sediments and superficial water of a chronically polluted semi-enclosed body of seawater: Linking of compartments. Environmental Science: Processes & Impacts 15-5, 986-995.

25. USEPA. 1996. Method 3540C: Soxhlet extraction. www.epa.gov

26. USEPA. 2000. Method 3620C: Florisil cleanup. www.epa.gov

27. Rojo-Nieto E., Garrido-Pérez C., Anfuso-Melfi G., Lopez-Aguayo F., Sales-Marquez D. and Perales-Vargas-Machuca J.A. (2011). The Zoning of Semi-Enclosed Bodies of Water According to the Sediment Pollution: The Bay of Algeciras as a Case Example. Estuaries and Coasts 34(6): 1129-1139.

28. USEPA. 2002. Methods For Measuring The Acute Toxicity Of Effluents And Receiving Waters To Freshwater And Marine Organisms. Fifth Edition. EPA-821-R-02-012.

29. Folk, R.L. and Ward, W.C., 1957. Brazos River Bar. A study in the significance of grain size parameters. Journal of Sedimentary Petrology, 27: 3 - 26.

30. García-Robledo E., Corzo A., García de Lomas J., and van Bergeijk S.A., 2008. Biogeochemical effects of macroalgal decomposition on intertidal microbenthos: a microcosm experiment. Marine Ecology Progress Series, Vol. 356: 139–151

31. Reichenberg, F., Smedes, F., Jönsson, J.-Å. & Mayer, P. 2008. Determining the chemical activity of hydrophobic organic compounds in soil using polymer coated vials. Chemistry Central Journal, 2**,** 1-10.

32. Jahnke, A.; Mayer, P.; McLachlan, M. S. 2012. Sensitive equilibrium sampling to study polychlorinated biphenyl disposition in Baltic Sea sediment. Environ. Sci. Technol. 46, 10114−10122.

33. Jahnke, A.; Mayer, P.; McLachlan, M. S.; Wickström, H.; Gilbert, D.; MacLeod, M. 2014. Silicone passive equilibrium samplers as “chemometers” in biota and sediment of a Swedish lake. Environ. Sci.: Processes Impacts 16, 464−472.

34. Smedes, F., Geertsma, R. W., Zande, T. V. D. & Booij, K. 2009. Polymer−Water Partition Coefficients of Hydrophobic Compounds for Passive Sampling: Application of Cosolvent Models for Validation. Environmental Science & Technology, 43, 7047-7054.

35. Rojo-Nieto E., Smith K. E. C., Perales J.A. and Mayer P. 2012. Recreating the seawater mixture composition of HOCs in toxicity tests with Artemia franciscana by passive dosing. Aquatic Toxicology. 120–121(0): 27-34.

36. Jonker, M. T. O. & Muijs, B. 2010. Using solid phase micro extraction to determine salting-out (Setschenow) constants for hydrophobic organic chemicals. Chemosphere, 80, 223-227.

37. Di Toro, D. M. & Mcgrath, J. A. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. Environmental Toxicology and Chemistry, 19, 1971-1982.

38. USEPA. 1996. Soil Screening Guidance: Technical Background Document. EPA/540-R-95-128. Washington, DC: U.S. EPA, Office of Solid Waste and Emergency Response, May 1996.

39. Maruya, K. A., Risebrough, R. W. & Horne, A. J. 1996. Partitioning of Polynuclear Aromatic Hydrocarbons between Sediments from San Francisco Bay and Their Porewaters. Environmental Science & Technology, 30, 2942-2947.

40. Pardue, J. H., Masscheleyn, P. H., Delaune, R. D., Patrick, W. H. & Adrian, D. D. 1993. Assimilation of hydrophobic chlorinated organics in freshwater wetlands: sorption and sediment-water exchange. Environmental Science & Technology, 27, 875-882.

41. Van Den Hoop, M. A. G. T., Kreule, P. & Gustav Loch, J. P. 1999. Sorption Kinetics and Transformation of DDT in Sediment. Water, Air, & Soil Pollution, 110, 57-66.

42. Katagi, T. 2006. Behavior of Pesticides in Water-Sediment Systems. Reviews of Environmental Contamination and Toxicology. In: Ware, G. W., Albert, L. A., Voogt, P., Gerba, C. P., Hutzinger, O., Knaak, J. B., Mayer, F. L., Morgan, D. P., Park, D. L., Tjeerdema, R. S., Whitacre, D. M., Yang, R. S. H. & Gunther, F. A. (eds.). Springer New York.

43. Rojo-Nieto E., Oliva M., Sales-Márquez D., Perales J.A., 2014. Feral Finfish, and their Relationships with Sediments and Seawater, as a tool for PAHs Risk Assessment in Chronically Polluted Environments. Science of the Total Environment, 470-411, 1030-1039.

44. Mucci, A., and H. M. Edenborn. 1992. Influence of an organic-poor landslide deposit on the early diagenesis of iron and manganese in a coastal marine sediment. Geochim. Cosmochim. Acta 56: 3909-392 1.

45. NOAA, Sediment Quality Guidelines Developed for the National Status and Trends Program, National Oceanic and Atmospheric Administration, United States, 1999.

46. Witt, G., G. A. Liehr, Dörthe Borck and Philipp Mayer.2009. "Matrix solid-phase microextraction for measuring freely dissolved concentrations and chemical activities of PAHs in sediment cores from the western Baltic Sea. Chemosphere 74(4): 522-529.

47. González-Fernández, D., Garrido-Pérez, M., Nebot-Sanz, E. & Sales-Márquez, D. 2011. Source and Fate of Heavy Metals in Marine Sediments from a Semi-Enclosed Deep Embayment Subjected to Severe Anthropogenic Activities. Water, Air, & Soil Pollution, 221**,** 191-202.

48. Morales-Caselles, C., Kalman, J., Riba, I. & Delvalls, T. A. 2007. Comparing sediment quality in Spanish littoral areas affected by acute (Prestige, 2002) and chronic (Bay of Algeciras) oil spills. Environmental Pollution, 146, 233-240.

49. Jiménez B., L. Blanco, M. J. González, J. B. Ortiz-Delgado, O. Campana, C. Garrido, J. A. Perales, C. Sarasquete, 2010. Evaluation of persistent organic pollutants in Algeciras Bay (Spain). Proceeding in the Abstract Book of the SETAC European meeting.

50. Baker, J. R., Mihelcic, J. R. & Shea, E. 2000. Estimating Koc for persistent organic pollutants: limitations of correlations with Kow. Chemosphere, 41**,** 813-817.

51. Ribeiro, C. A. O., Vollaire, Y., Sanchez-Chardi, A. & Roche, H. 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the Eel (Anguilla anguilla) at the Camargue Nature Reserve, France. Aquatic Toxicology, 74**,** 53-69.

52. Patrolecco, L., N. Ademollo, S. Capri, R.Pagnotta, S.Polesello(2010). Occurrence of priority hazardous PAHs in water, suspended particulate matter, sediment and common eels (Anguilla anguilla) in the urban stretch of the River Tiber (Italy). Chemosphere 81(11): 1386-1392.

53. Arnot J.A., Gobas FAPC (2006) A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Rev 14:257–297

54. Farrington JW (1991) Biogeochemical processes governing exposure and uptake of organic pollutant compounds in aquatic organisms. Environ Health Perspect 90:75–84

55. Simcik MF, Offenberg JH (2006) Polycyclic aromatic hydrocarbons in the Great Lakes. Handbook of environment chemistry, vol. 5. Springer-Verlag, Berlin, 307–353

56. Levengood J, Schaeffer D. 2011. Polycyclic aromatic hydrocarbons infish and crayfish from theCalumet region of southwestern Lake Michigan. Ecotoxicology 20 (6):1411–21.

57. Oen, A. M. P., Schaanning, M., Ruus, A., Cornelissen, G., Källqvist, T. & Breedveld, G. D. 2006. Predicting low biota to sediment accumulation factors of PAHs by using infinite-sink and equilibrium extraction methods as well as BC-inclusive modeling. Chemosphere, 64**,** 1412-1420.

58. Wong, C. S., Capel, P. D. & Nowell, L. H. 2001. National-Scale, Field-Based Evaluation of the Biota−Sediment Accumulation factor Model. Environmental Science &Technology, 35(9), pp 1709–1715

59. Varanasi, U.and Stein, J.E 1991 Disposition of xenobiotic chemicals and metabolites in marine organisms. Environ. Hlth. Persp., 90, 93–100

60. Hellou J., D. Mackay, B. Fowler, 1995 Bioconcentration of polycyclic aromatic compounds from sediments to muscle of finfish Environ. Sci. Technol., 29, 2555–2560

61. Hellou J., J. Leonard , T.K. Collier , F. Ariese, 2006. Assessing PAH exposure in feral finfish from the Northwest Atlantic. Marine Pollution Bulletin 52, 433–441

62. Baumard P., H. Budzinski, P. Garrigues, J.C. Sorbe, T. Burgeot, J. Bellocq 1998 Concentrations of PAHs (polycyclic aromatic hydrocarbons) in various marine organisms in relation to those in sediments and to trophic level Mar. Pollut. Bull., 36, 951–960

63. Neff J.M. 2002. Polycyclic aromatic hydrocarbons in the ocean. In: Neff JM, editor. Bioaccumulation in marine organisms. Oxford: Elsevier. p. 241–318. [Chapter 15]

64. Liang Y, Tse MF, Young L, Wong MH. 2007. Distribution patterns of polycyclic aromatichydrocarbons (PAHs) in the sediments andfish at Mai Po Marshes Nature Reserve,Hong Kong. Water Res 41(6):1303–11.

Table 1: Log K_{ow}, Log $K_{oc,\ estimated}$ (generic sediment organic partition coefficient), and average Log $K_{oc,\ calculated}$ (site specific sediment organic partition coefficient) and its coefficient of variation (CV). *FLUO* Fluorene, *PHE* Phenanthrene, *ANT* Anthracene, *FLUOR* Fluoranthene, *PYR* Pyrene, *B(a)A* Benzo(a)Anthracene, *CHRY* Chrysene, *B(b)F* Benzo(b)Fluoranthene, *B(k)F* Benzo(k)Fluoranthene, *B(a)P* Benzo(a)Pyrene, *B(ghi)P* Benzo(g,h,i)Perylene, *I(123)P* Indeno(1,2,3-cd)Pyrene.

	Log K_{ow}	**Log $K_{oc,\ estimated}$ "Generic"**	**Average Log $K_{oc,\ calculated}$ "Site specific"**	**CV (Log $K_{oc,\ calcualted}$)**
FLUOR	5.22	5.13	5.31	0.21
PHE	4.57	4.49	4.80	0.18
PYR	5.18	5.09	5.17	0.16
B(b)F	5.80	5.70	6.18	0.09
B(ghi)P	6.4	6.29	7.02	0.10
I(123cd)P	6.65	6.54	6.64	0.05
FLUO	4.18	4.11	3.85	0.03
B(k)F	6.00	5.90	5.94	
CHRY	5.60	5.51	4.91	
ANT	4.54	4.46	4.46	
B(a)A	5.91	5.81	4.83	
B(a)P	6.04	5.94	5.79	

Table 2. Average BSAFs calculated from Rojo-Nieto et al (2014) data, BCFs obtained and lipid-normalized concentrations ($C_{organism}$) in target organs. In parentheses are shown the Standard Deviation of the Mean (SEM).

	Phenanthrene muscle	Pyrene muscle	Phenanthrene liver	Pyrene liver
Average BSAF	0.319 (0.25)	2.094 (1.35)	0.080 (0.063)	0.422 (0.20)
BCF	20137	324273	5039	65430
$C_{organism}$ sediment P1A (µg/Kg lipid)	659.84	3588.93	165.14	724.15
$C_{organism}$ sediment P2A (µg/Kg lipid)	80.16	551.29	20.06	111.24
$C_{organism}$ sediment P2B (µg/Kg lipid)	59.54	383.13	14.90	77.31

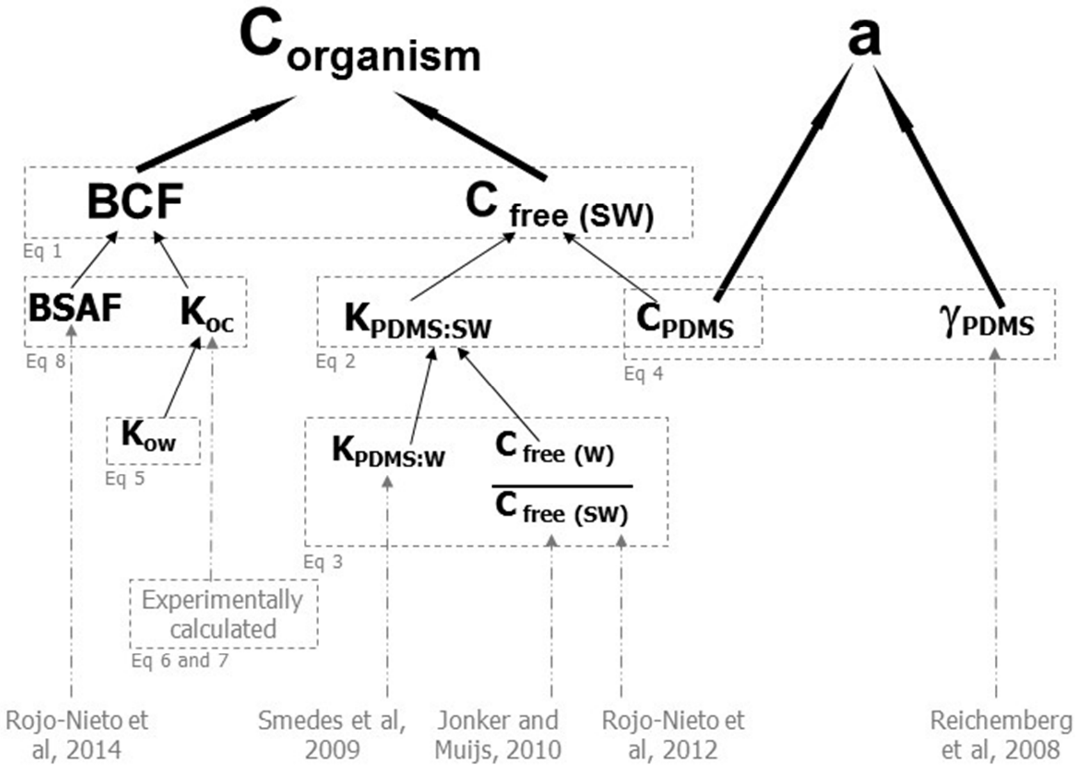


Figure 1. $C_{organism}$ **(bioaccumulation) and "a" chemical activity (toxicity) calculation scheme.**

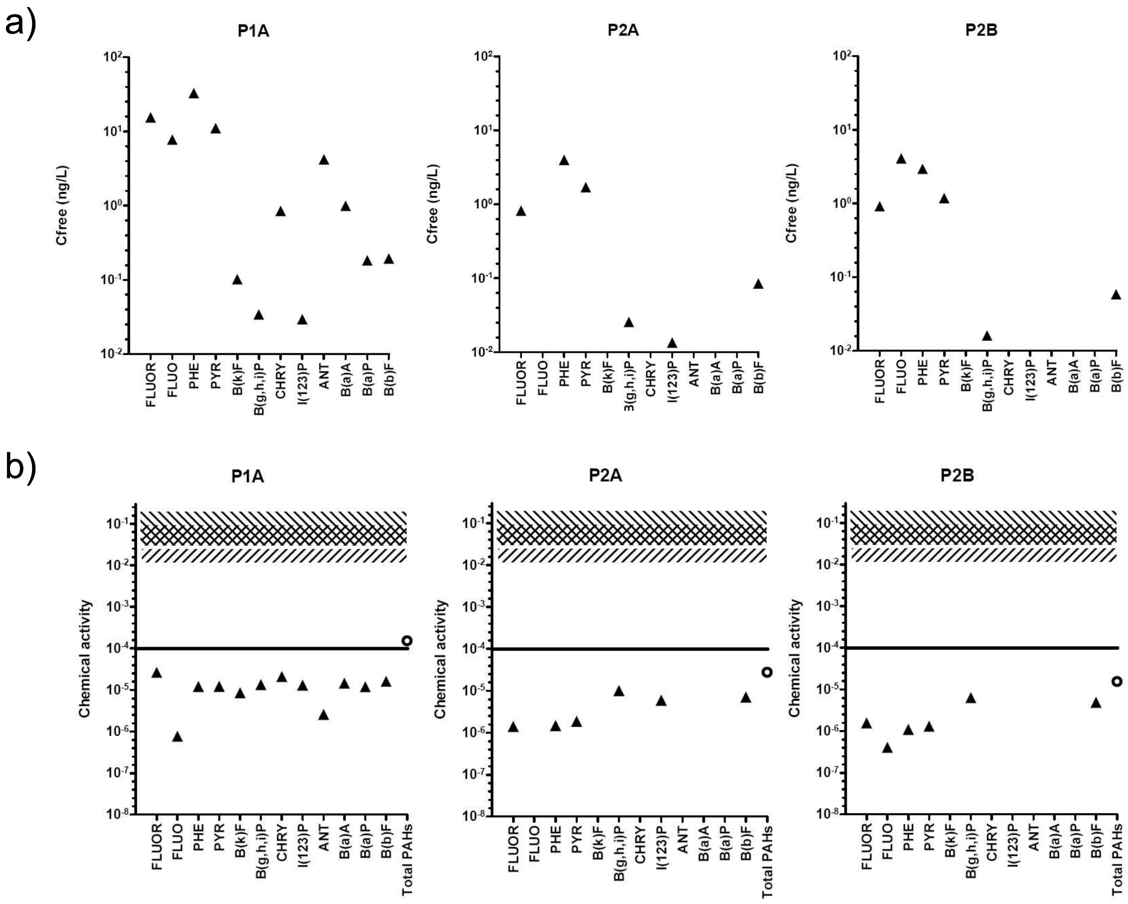


Figure 2. a) Measured freely dissolved pore seawater concentrations of PAHs ($C_{free(SW)}$) from sediments. **b)** Chemical activities of different PAHs and the sum of the 16 studied. Grey zone indicates risk of lethal baseline toxicity and line the 1% contribution to baseline toxicity. *FLUO* Fluorene, *PHE* Phenanthrene, *ANT* Anthracene, *FLUOR* Fluoranthene, *PYR* Pyrene, *B(a)A* Benzo(a)Anthracene, *CHRY* Chrysene, *B(b)F* Benzo(b)Fluoranthene, *B(k)F* Benzo(k)Fluoranthene, *B(a)P* Benzo(a)Pyrene, *B(ghi)P* Benzo(g,h,i)Perylene, *I(123)P* Indeno(1,2,3-cd)Pyrene.