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Is *Arenicola marina* a suitable test organism to evaluate the bioaccumulation potential of Hg, PAHs and PCBs from dredged sediments?

M.C. Casado-Martínez [a,*], V. Branco [b], C. Vale [b], A.M. Ferreira [b], T.A. DelValls [a]

[a] *Departamento de Química-Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro, s/n, 11510 Puerto Real, Cádiz, Spain*

[b] *Departamento do Ambiente Aquatico, Instituto de Investigaçao das Pescas e do Mar (INIAP-IPIMAR), Av. Brasilia, 1400 Lisboa, Portugal*

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Abstract

This paper presents the results of investigations on the suitability of lugworms (*Arenicola marina*) to study the bioaccumulation potential of Hg, PCB and PAH compounds from dredged sediments upon laboratory exposure. The results of tissue concentrations for several sediments from Spanish ports showed that it is possible to identify increased levels of contaminants in lugworms just after 10 days of exposure although different bioaccumulation trends were shown amongst compounds and sediments. Total and organic Hg compounds were accumulated following a non-linear trend, with a sharp increase of tissue concentrations in lugworms exposed to levels of contamination associated to a significant increase in mortality. Interestingly organic Hg compounds accounted for an average of 40% of the total Hg in lugworms exposed to sediments presenting sublethal concentrations while, when exposed to sediments presenting lethal concentrations, organic Hg compounds only accounted for 4% of the total Hg accumulated in lugworms. While lugworms seem to readily accumulate Hg and PCB compounds, with some variability explained by the organic matter content in sediments or other factor for which it accounts for, the results for PAHs suggest a more complex process of bioaccumulation as no relationship was observed between the measured concentrations in sediments and in lugworms, not even after correcting the results for this factor. Besides, the differences in the calculated BSAFs for each compound and for each sediment supported the use of bioassays for evaluating the bioaccumulation potential of sediment-bound contaminants as part of the assessment framework required in pre-dredging investigations, as they still offer unique information about the bioavailability of sediment-bound contaminants.

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1. Introduction

In the last decades much effort has been devoted to develop effective assessment frameworks for dredged material characterization and management (den Besten et al., 2003). While there is a general agreement in the use of bioassays together with chemical sediment quality guidelines to establish the risks associated with the aquatic disposal of these materials, there is still considerable discrepancy on the assessment framework when sediments are contaminated with compounds that are liable to bioaccumulate/biomagnify (e.g. Hg, PCB or PAH compounds). Addressing questions concerning the potential of contaminants in dredged materials to move into the food chain and produce effects in organisms begins with assessing bioaccumulation potential (PIANC, 2006). Various models have been described to predict the bioaccumulation potential based on sediment physico-chemical characteristics (e.g. chemical concentrations and organic carbon) and they seem recommended to some extent for organic compounds (Stronkhorst, 2003). Nevertheless, it seems that these models

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[*] Corresponding author. Tel.: +34 956 016794; fax: +34 956 016040.
E-mail address: mcarmen.casado@uca.es (M.C. Casado-Martínez).

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should only be used for screening purposes and supported by following secondary biological tests (USEPA/USACE, 1991; PIANC, 2006) to avoid lengthy and costly investigations caused by overprotective assumptions (Bradham et al., 2006).

This paper presents results of Hg, PAH and PCB concentrations in tissues of the marine benthic organism *Arenicola marina* (Polychaete) exposed to dredged materials under laboratory conditions. This species is an infaunal benthic invertebrate living buried and feeding on material obtained from the sediment surface, thus it can be considered a representative species for environmental quality assessments. Its use as test organism for acute toxicity testing is supported by several recommendations from international agencies (OSPAR, 1995; Thain and Bifield, 2001) but this species has been also used to study bioaccumulation processes (Bernds et al., 1998; Kaag et al., 1998a,b; Christensen et al., 2002; Geiszinger et al., 2002). This species is commonly found in contaminated sites such as ports, harbours, estuaries and bays, where it accounts for a high proportion of the total biomass. Furthermore, it has a dominant role in habitat and community structure and provides an important food source for species of interest such as flatfish, wading birds and ragworms. Then it is an important prey item, which can contribute to the transport of contaminants to higher levels in the food chain. The objective of this work is to test the suitability of this approach for studying the bioaccumulation potential of sediment-bound contaminants in pre-dredging investigations. The results are further discussed in relation to the sediment physico-chemical properties to investigate on the predictability of bioaccumulation processes from the information retrieved through the classical dredged material assessment frameworks.

2. Material and methods

2.1. Test sediments and chemical characterization

The sediments used were sampled at several Spanish harbors: Barcelona and Cádiz (named B# and CA#, respectively), which are important fishing and shipping ports near major centers of population, and Cartagena and Huelva (named C# and H#, respectively), located in areas mainly affected by historic mining activities but also hosting several industrial activities. The sediments represented a broad range of physico-chemical properties, namely grain size distribution and organic matter content, and levels of contamination. The physico-chemical characterization of sediments followed the recommendations for dredged material characterization used in Spain (CEDEX, 1994). Detailed protocols and results of the sediment sampling and physico-chemical characterization have been previously described elsewhere (Casado-Martínez et al., 2006). Briefly, grain size distribution followed UNE 103 101 and organic matter content was measured by loss-on-ignition at 550 °C and gravimetric determination as recommended for small dredged volumes. For Hg the cold vapor technique was used and was quantified using atomic absorption spectrometry. Polychlorinated biphenyl congeners (CB28, 52, 101, 118, 138, 153 and 180) and polycyclic aromatic hydrocarbons (acenaphtylene, acenaphtene, anthracene, benz(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benz(*a*)pyrene, benzo(*g*,*h*,*i*)perylene, chrysene, dibenz(*a*,*h*)anthracene, fluoranthene, fluorene, indene, phenanthrene and pyrene) were quantified after extraction with cyclohexane and dichloromethane by means of ultrasound treatment and concentration and clean up with column chromatography. Determination of PCBs was made with gas chromatography with electron capture detection (GC-ECD) (EPA 8080) and is expressed as the sum of the measured congeners, while PAHs were determined with HPLC with fluorescence detection (EPA 8310). All the analytical procedures were checked with reference materials (MESS-1 for trace metals and NRC-CNRC HS-1 for organic compounds) and obtained concentrations that did not differ significantly ($p < 0.05$) from certified values.

2.2. Test organisms and experimental set-up

Individuals of *A. marina* (lugworms) were collected in the Cantabric coast (north of Spain) by hand digging and were shipped refrigerated to the laboratory in the University of Cádiz in clean seawater. Once in the laboratory the organisms were immediately transferred into 20 l holding tanks with a 5 cm clean sediment layer and clean seawater for acclimatization to test conditions (temperature and salinity) for at least one week prior to tests. Before being used, organisms were transferred to 20 l holding tanks with clean seawater for at least 24 h for depuration. The bioassay followed protocol by Thain and Bifield (2001). Sediments (2 l) were loaded into 12 l aquaria providing a layer of sediment 5 cm deep. After 12 h approximately clean sea water (6 l) was added to each container and then exposure chambers were left to settle for 12 h before aeration was started. After 12 h of aeration five lugworms were placed in three replicate containers. No extra food was added to the test organisms and overlying water was renewed every three days or if any mortality was registered. After 10 d, surviving worms were placed in clean seawater to depurate for 24 h, then blotted dry, pooled and frozen at −80 °C.

2.3. Tissue measurements

2.3.1. Hg

Results of Hg accumulation are presented for a total of 10 sediment samples: four in the port of Cadiz and four in the port of Cartagena (CA# and C#, respectively) and one sediment sample from Huelva (H2) and Barcelona (B4). Total mercury (Hg_T) was measured on the lyophilized organisms using an Automated Mercury Analyzer AMA-254 (Altec Ltd., Prague, Czech Republic) after drying (200 °C) and burning (700 °C) under constant oxygen flow. The analytical results were quality checked by analysis reference materials (DORM-2 and TORT-2) before and after each batch of samples. Obtained concentrations did not differ significantly ($p < 0.05$) from certified ones. Organic mercury (Hg_{ORG}) was extracted from samples with concentrated HBr followed by extraction of the organic phase into toluene and back extraction with an aqueous cysteine solution, according to the method described by Scerbo and Barghigiani (1998). Mercury in cysteine solutions was measured as described for Hg_T.

2.3.2. PAHs

Five samples were considered to study the accumulation of PAHs in lugworms according to the levels of concentrations reported in the sediments: three sampling sites located in the port of Barcelona, B4 in the inner harbor, B1 at the external part near open sea and B3 located along the harbor, and two sediments from Cartagena, C1 in the inner bay where the harbor is allocated, and C4 in the entrance of the bay. PAH were extracted from whole tissues using soxhlet extraction (24 h, hexane:dichloromethane, 1:1), after lyophilization. The extracts were further cleaned up and fractionated by silica:alumina column chromatography, concentrated and quantified by capillary gas chromatography/mass spectrometry/electron capture detector. Lugworms' tissues were quantified for the compounds acenaphtylene, acenaphtene, anthracene, benz(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benz(*a*)pyrene, benzo(*g*,*h*,*i*)perylene, chrysene, dibenz(*a*,*h*)anthracene, fluoranthene, fluorene, indene, phenanthrene and pyrene. Detection limits were 0.2–4.5 ng g^{-1} dry weight for PAHs. The average recoveries ($n = 7$) of the analytical procedure were performed with certified reference mussel tissues SRM 2977 (NIST, USA) and the recoveries data ranged between 75% and 120%. All the results were expressed on a dry weight basis.

2.3.3. PCBs

For the study of bioaccumulation of PCBs congeners, we present results for three sediment samples collected at different ports with levels of concentrations of concern: those from the inner fishing harbor in Cádiz (CA2), B2 in the port of Barcelona and C2 in the port of Cartagena. Briefly, lyophilized organisms were soxhlet extracted in *n*-hexane for 6 h. The extracts were purified in Fluorisil column and eluted with hexane and dichloromethane/hexane. Seventeen congeners were quantified in the lugworms tissues (#18, 31, 52, 49, 44, 101, 151, 149, 118, 153, 105, 138, 187, 128, 180, 170 and 194) using a Hewlett–Packard 6890 gas chromatograph with an electron capture detector and a capillary column (DB5, JW, 60 m) following conditions described by Ferreira and Vale (1995). The analytical detection limit was 0.1 ng g^{-1} and the mean recovery was 93 ± 16.2%. Lipid content was determined by the weight of hexane extracted components.

2.4. Data treatment and analysis

For Hg_T, bioaccumulation was evaluated through the development of bioaccumulation ratios (BARs) and sediment concentration ratio (SCRs) using the results from control sediment (namely CA1)

$$\mathrm{BAR} = C_{\mathrm{OS}}/C_{\mathrm{OC}}$$

where $[C_{\mathrm{OS}}]$ is the corresponding mean concentration of Hg_T in lugworms exposed to the sediment (S) and $[C_{\mathrm{OC}}]$ is the concentration of Hg_T in lugworms exposed to the control sediment. For comparative purposes sediment concentration ratios (SCRs) were calculated as follows:

$$\mathrm{SCR} = C_{\mathrm{S}}/C_{\mathrm{C}}$$

where $[C_{\mathrm{S}}]$ is the concentration of Hg_T in the sediment (S) and $[C_{\mathrm{C}}]$ is the concentration of Hg_T in the control sediment (C).

To evaluate the magnitude of bioaccumulation of organic compounds no comparisons between BARs and SCRs could be performed due to the low levels of these contaminants in the control sediment. To have a measure of the bioaccumulation, the biota-sediment accumulation factors (BSAFs) were calculated for each compound and for the sum of compounds as follows:

$$\mathrm{BSAF} = C_{\mathrm{OS}}/(C_{\mathrm{S}}/f_{\mathrm{oc}})$$

where $[C_{\mathrm{OS}}]$ is the mean concentration of the compound (C) in lugworms exposed to the dredged material (S) expressed on the basis of the lipid content, $[C_{\mathrm{S}}]$ is the concentration of the compound C in the dredged material (S) and $[f_{\mathrm{oc}}]$ is the sediment organic content.

3. Results

3.1. Grain size distribution and organic matter content

The sediment samples tested presented very different physico-chemical properties. Only one sample was a typical sand with low organic matter content while the rest of sediments differed in properties within and between ports, with organic matter ranging from 7.19% to 24.33% (corresponding to C3 and CA4, respectively) and a proportion of fines between 31.87% and 99.59% (corresponding to these same two sediments). These two sediment properties also varied a great deal inside each port area, possibly due to the different sedimentation and hydrodynamic conditions in the inside. Organic matter seems to be preferentially accumulated in sediments deposited in inner parts (stations CA4, B4 and C1), being significantly correlated with the % of fines ($r = 0.84$, $p < 0.05$).

3.2. Hg compounds

The concentrations of Hg_T in sediments ranged between 0.05 and more than 136 mg kg^{-1} dry weight (CA1 and C1, respectively; can be calculated from data shown in Table 1).

Table 1
Results of the concentrations of Hg_T in lugworms (C_O) expressed as $mg\,kg^{-1}$ on dry weight basis and calculated ratios for each of the sediments assessed (BAFs, BARs and SCRs; see text for calculation expressions)

Sample	C_O	BAF	BAR	SCR	Hg_{ORG}
CA1	0.05	1	1	1	–
CA2	0.08	0.04	1.6	39.6	–
CA3	0.01	0.04	0.2	5.6	–
CA4	0.02	0.40	0.4	1	–
H2	0.11	0.06	2.2	39.8	39.1
B4	0.08	0.02	1.6	82.4	39.5
C1	10.52	0.08	210.4	2728	3.8
C2	0.12	<0.01	2.4	654.2	39.4
C3	1.98	0.02	39.6	2304	4.1
C4	0.14	0.01	2.8	431.8	39.4

Concentrations of organic mercury in lugworms (Hg_{ORG}) are expressed as percentage of the concentration of total Hg in organisms (C_O).

The lowest Hg_T concentrations were reported in sediments from the port of Cádiz although the sediments at the inner fishing harbor presented an intermediate level of contamination according to the limit values used in Spain for dredged material characterization ($1.98\,mg\,kg^{-1}$). Similarly, the sediments from Huelva (H2) and Barcelona (B4) reported intermediate and high levels of contamination, with concentrations exceeding the lowest and highest limit concentrations of Hg_T for dredged material management, respectively (1.99 and $4.12\,mg\,kg^{-1}$). The most significant concentrations were reported for the sediments at the Cartagena harbor, which were one order of magnitude higher than the highest national limit value in the case of sites C2 and C4 (32.71 and $21.59\,mg\,kg^{-1}$, respectively), both located in the western part of the Cartagena bay, and two orders of magnitude for sediments C1 and C3 (136.4 and $115.2\,mg\,kg^{-1}$, respectively). Even if the sediments from inner harbors are generally enriched in fine sediments, organic matter and Hg_T, there was no statistical significant correlation between these three variables due to the determining results found in Cartagena, which presented the most conspicuous concentrations of Hg_T but not associated with high proportion of fines and organic matter.

Lugworms exposed to the sandy sediment (CA1) presented tissue concentrations in the lowest range for all samples ($0.05\,mg\,kg^{-1}$ dry wt). The exceptions were lugworms exposed to CA3 and CA4 with 0.02 and $0.01\,mg\,kg^{-1}$ dry wt, respectively. When exposed to sediments from the inner fishing harbor (CA2), which presented the highest contamination, lugworms accumulated higher concentrations of Hg_T ($0.08\,mg\,kg^{-1}$ dry wt), which are within the range of those presented in lugworms exposed to the sediments from Barcelona and Huelva (0.08 and $0.11\,mg\,kg^{-1}$ dry wt, respectively). Upon exposure to the lowest contaminated sediments from Cartagena, lugworms accumulated Hg_T at slightly higher concentrations (0.12 and $0.14\,mg\,kg^{-1}$ dry wt for C2 and C4, respectively), while upon exposure to sediments C3 and C1, with concentrations of Hg_T above $100\,mg\,kg^{-1}$ dry wt, tissue concentrations rose to 1.98 and $10.52\,mg\,kg^{-1}$ dry wt, respectively. These results and the calculated BARs and SCR (Table 1) evidenced that concentrations in lugworms after 10 days of exposure were less variable than concentrations in sediments. While the rank of samples according to sediment concentrations ($C1 > C3 > C2 > C4 > B4 > H2 > CA2 > CA3 > CA4 > CA1$) was consistent with the rank of samples according to concentrations in lugworms ($C1 > C3 > C4 > C2 > H2 > B4 = CA2 > CA1 > CA3 > CA4$), this leads to a high variability in the calculated BAFs, which is a measure of the proportion of Hg present in sediments that has been accumulated by lugworms.

The accumulation of organic Hg compounds (Hg_{ORG}) was also assessed in lugworms after 10 days of exposure to some of the sediments (Table 1). The results showed a clear increase in the accumulation of Hg_{ORG} in lugworms as the Hg_T concentration increases, being the rank of samples according to the Hg_{ORG} content in lugworms in agreement with that generated through the concentrations of Hg_T. The concentrations ranged between 0.404 and 0.082 ppm registered upon exposure to sediments C1 and C3, respectively, 0.039 and 0.042 when exposed to H2 and CA2, and 0.049 and 0.055 ppm to C2 and C4. These concentrations represented a 3.8% and 4.1% of the Hg_T in lugworms exposed to sediments C1 and C3, respectively, while for the rest of sediments Hg_{ORG} accounted for the $39.35 \pm 0.17\%$ of Hg_T.

3.3. PAHs

The total PAH concentrations in sediments ranged between 280 and $1800\,mg\,kg^{-1}$ (data can be calculated from Table 2). In the port of Barcelona (samples B#) the concentrations increased in the inner sediments (B4) as the proportion of fines and organic matter also increased, while the significant increase in PAHs in the sediments at the entrance of the port of Cartagena was not related to a higher proportion of fines and organic matter although in both cases the fluoranthene/pyrene ratio indicated a higher contribution of petroleum hydrocarbons. Generally, the highest sediment concentrations were present for high molecular weight compounds, namely pyrene, indene, fluoranthene, benzo(*g*,*h*,*i*)perylene, benzo(*b*)fluoranthene and benzo(*a*)anthracene.

While the total concentration in sediments ranked the samples from $B4 > C4 > C1 > B3 > B1$, the concentrations in lugworms exposed to these sediments were 477, 389, 443, 453 and $640\,mg\,kg^{-1}$ dry wt, respectively (Table 2). These results determined the highest BSAF for the less contaminated sample B1 (BSAF = 2.67), while for the rest of sediments BSAF values ranged between 0.26 and 0.74. When considering the individual PAH compounds, the highest tissue concentrations were reported for the PAH benzo(*b*)fluoranthene, phenanthrene, pyrene and fluoranthene, between 33 and $74\,mg\,kg^{-1}$, while the rest of compounds reported tissue concentrations between 20

Table 2
Concentrations of PAHs in sediments (C_S) and organisms (C_O) expressed as mg kg^{-1} dry wt

PAH[a]	B1	BSAF[b]	B3	BSAF	C1	BSAF	C4	BSAF	B4	BSAF
ANY	46.65	–	30.88	–	30.16	–	27.08	–	31.52	–
ANA	40.80	–	27.70	–	27.05	–	24.34	4.87	28.64	5.73
F	37.14	–	25.84	–	24.80	–	22.46	–	26.07	1.74
P	74.68	14.94	51.56	1.29	50.19	1.00	44.77	0.56	52.56	0.44
A	19.44	–	13.22	0.13	13.08	–	11.75	0.12	13.58	0.45
FL	54.51	1.82	40.03	0.57	38.73	0.43	33.41	0.20	41.77	0.23
PY	56.97	1.90	41.87	0.52	41.77	0.46	35.56	0.25	48.75	0.20
BA	34.08	1.70	23.27	0.58	21.63	0.36	18.92	0.21	22.14	0.74
C	34.35	1.72	25.04	0.50	24.39	0.35	20.27	0.20	25.66	0.18
BBF	67.75	3.39	50.56	1.26	50.42	0.42	42.42	0.33	55.10	0.26
BKF	27.59	1.38	19.57	0.49	18.56	0.37	16.39	0.23	20.52	0.21
BAP	34.34	1.14	23.14	0.33	22.89	0.25	20.69	0.16	24.21	0.13
IN	37.04	0.93	26.06	0.33	25.07	0.19	23.41	0.16	27.45	0.12
DBA	36.07	0.36	24.08	0.24	23.81	0.60	21.34	0.53	25.35	0.51
BPE	41.00	0.82	28.44	0.41	27.70	0.23	25.72	0.18	29.94	0.16
Total C_S	240		610		910		1240		1800	
C_O	640.5	2.67	453.0	0.74	443.7	0.49	389.8	0.31	476.7	0.26

[a] ANY, acenaphtylene; ANA, acenaphtene; F, fluorene; P, phenanthrene; A, anthracene; FL, fluoranthene; PY, pyrene; BA, benz(*a*)anthracene; C, chrysene; BBF, benzo(*b*)fluoranthene; BKF, benzo(*k*)fluoranthene; BAP, benzo(*a*)pyrene; IN, indene; DBA, dibenz(*a*)anthracene; BPE, benzo(*g*,*h*,*i*)perylene.

[b] BSAFs not calculated if PAH were not detected in sediments. For concentrations lower than a determined value BSAF are calculated assuming a sediment concentration half the determined value.

Table 3
Concentrations of PCB congeners in organisms in μg kg^{-1} dry wt

	CA2	B2	C2
PCB-18	0.22	0.27	0.33
PCB-31	0.31	0.45	0.83
PCB-52	0.47	0.79	0.69
PCB-49	n.d.	0.49	0.33
PCB-44	0.28	2.64	0.44
PCB-101	1.82	2.64	2.55
PCB-151	1.47	2.26	2.49
PCB-149	3.82	4.83	6.76
PCB-118	1.56	1.55	1.82
PCB-153	7.60	10.36	11.45
PCB-105	0.79	1.24	1.35
PCB-138	5.06	7.27	7.70
PCB-187	5.57	7.97	8.80
PCB-128	0.57	0.94	0.89
PCB-180	2.26	3.73	4.13
PCB-170	1.20	2.01	2.11
PCB-194	0.26	0.56	0.57
Total PCBs	33.27	47.80	53.23
BSAF	0.23	0.56	0.11

n.d. means not detected.

and 40 mg kg^{-1}, except anthracene, which reported the lowest tissue concentrations between 10 and 20 mg kg^{-1}.

3.4. PCBs

Only the total concentrations for the sum of PCB congeners were available in this study for the sediments studied, which is actually the information required for dredged sediments assessments. In this case, the levels of concentrations varied from 140 μg kg^{-1} dry wt for CA2 and B2 to 468 μg kg^{-1} dry wt for C2 (Table 3). While the concentrations of total PCBs in sediments were similar for CA2 and B2, the concentrations in lugworms were 33.27 and 47.80 μg kg^{-1} dry wt, respectively. According to the higher sediment concentrations, the concentration of PCBs in lugworms exposed to sediments C2 was also higher than those reported in lugworms exposed to the other two samples (53.23 μg kg^{-1} dry wt) although this determine a calculated BSAF of 0.17, 0.23 and 0.03 for CA2, B2 and C2, respectively when considering only PCBs congeners measured in sediments.

In the case of tissue concentrations, information was also available for the levels of concentrations of single compounds. The highest levels of concentrations in lugworms' tissues were reported for congener number 153, followed by 187, 138 and 149 while congeners 18, 31, 49, 194 and 128 were present at considerably lower levels. The rest of congeners were present at intermediate levels.

3.5. Mortality of lugworms exposed to sediments

Sediments from Cádiz and Barcelona did not cause a significant increase in the lugworm mortality, with the exception of sediment CA4 and CA1, which resulted in a mean mortality of approximately 20% and 15%, and the sediments from Huelva, with 25% of mortality. The sediments from Cartagena presented very variable mortality results. Sediments C3 and C1, from the eastern bay, were highly toxic inducing a mortality rate of 67% and 80%, respectively, while sediments from the western bay (C2 and C4) did not decrease survival in lugworms even though they presented high levels of both metallic and organic contaminants. A significant correlation ($r = 0.76$, $p < 0.05$; Fig. 1) between lugworm mortality and Hg concentration

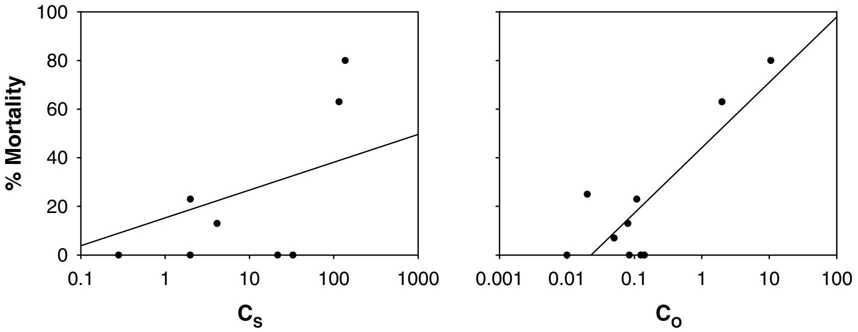

Fig. 1. Relationship between concentrations of Hg in sediments (C_S) and in lugworms (C_O) and mortality registered during the exposure to the sediments.

in sediments was observed, nonetheless as the results of the control sediment show, 15–20% of mortality may be caused by other sediment condition but not related to chemical stress. When the mortality results are represented according to the concentrations of Hg in lugworms the plot of all data fitted a logarithmic curve ($R^2 = 0.71$; Fig. 1). Organic pollutant (PAH and PCB) concentrations were not correlated with mortality.

4. Discussion

The results for Hg_T depicted a clear non-linear bioaccumulation trend in the lugworm *A. marina*, with slight increases in the concentrations in organisms over dramatic increases in sediment concentrations (Fig. 2a). This bioaccumulation trend has been also found for other metals in this species (unpublished results) although it is difficult to interpret these results when they are derived from environmental samples. Some physiological regulation of metals by *A. marina* may exist as it has been previously reported for some essential metals such as Zn in different polychaetes (EEDP, 1987 cited in McGeer et al., 2003). This hypothesis would also explain that the threshold for significant toxic effects corresponds to a threshold for a dramatic incorporation of Hg_T by lugworms, indicating the disruption of this hypothetic biological system that regulates the internal concentrations of metals in this organism. Conversely, at intermediate exposure concentrations it is more difficult to associate the toxic effects with the levels of Hg_T accumulated in the organisms, possibly due to the cocktail of contaminants in sediments or the different bioavailability of sediment-bound contaminants.

In this sense, the concentrations of Hg_T in lugworms upon exposure to low concentrations of Hg_T in sediments were in the lowest range for all samples, but the lowest concentration in lugworms did not correspond to the lowest sediment concentrations but to the uncontaminated fine grained sediments (CA3 and CA4). These results may be explained by the differential mercury speciation and bioavailability in the various sediment types (water > sand > sand/mud > mud; Pesch, 1979 cited by Muhaya et al., 1997), which is also related to different uptake rates on polychaetes (Jarvinen and Ankley, 1999). In this line Muhaya et al. (1997) found that Hg concentrations in polychaete worms were not proportional to those in marine surface sediments, being the spatial distribution of Hg in sediments and in worms determined by the sediment composition and the organic matter content. Previous results on the sediments used in this study indicated that metals, Hg included, tend to link with fine grained sediments, with organic matter as one of the key binding phases (Casado-Martínez, 2006). Considering that organic matter (e.g. humic/fulvic acids) present in sediments complexes Hg, and thus may result in less bioavailability, some negative correlation between the proportion of fine sediments and organic matter content, and the concentrations of Hg in lugworms would be reasonable.

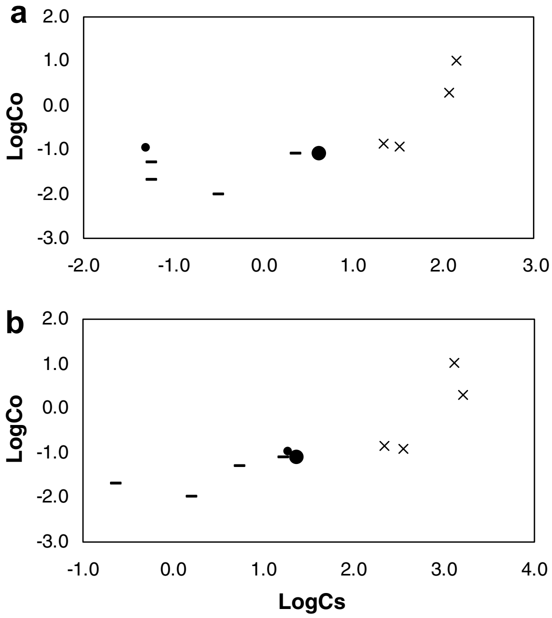

Fig. 2. Concentrations of Hg in lugworms (C_O) and in sediments (C_S) before (a) and after the correction for the organic matter content of the sediments (b). (–) sediments from Cádiz; (×) sediments from Cartagena; (•) sediments from Huelva; (●) sediments from Barcelona. All concentrations expressed as mg kg^{-1} dry wt.

To assess the effect of this factor sediment concentrations were corrected for the organic matter content and the new individual pairs of data were plotted (Fig. 2b). This new plot maintained the general bioaccumulation trend according to the determining concentrations of Hg in Cartagena and not determining organic matter content in these sediments, while the variability found for other sediments may be explained by organic matter content or some other factor for which it accounts for. It seems that this factor is the sole cause of the reduced bioavailability of Hg_T at site B4, the inner sediments from Barcelona, causing similar tissue concentrations to sediments H2 and CA2, thus leading to a reduction in the BAF value for B4 to half of that calculated for CA2 and H2. The empirically derived BAF, which is one of the most simplified models for bioaccumulation, considers a single-compartment model to predict partitioning between exposure medium (sediment) and biota. In our study BAFs varied among two orders of magnitude (three considering the uncontaminated sandy sediment) and, moreover, some inverse relationship with the concentrations of Hg in sediments was observed thus being dependent on the exposure concentrations considered for its calculation (McGeer et al., 2003).

In the last years especial attention has been paid to the transformation of inorganic Hg to its organic forms. Specifically inorganic Hg is known to be transformed by anaerobic sediment microorganisms into methyl-Hg, which is known to bioaccumulate at successive trophic levels in the food chain. If high trophic level feeders, such as birds and mammals, ingest sufficient methyl-Hg in prey and drinking water, Hg toxicoses resulting in damage to nervous, excretory and reproductive systems (Wolfe et al., 1998). Few studies on Hg speciation in relation to bioaccumulation in lugworms have been performed but it seems that there is a large variability in the proportion of Hg that is accumulated in its organic forms by different polychaete species: while in *Nereis diversicolor* methyl-Hg accounts for 18% of the total Hg concentration (Muhaya et al., 1997), a 100% have been reported for the polychaete *Maldani sarsi* (Ali et al., 1997). No information is available for the lugworm *A. marina,* but our first results on whole organisms after 10 days of exposure showed that Hg_{ORG} may account for 4–40% of the Hg_T accumulated in tissues. The highest concentrations of Hg_{ORG} were associated to the highest concentrations of Hg_T in surviving organisms exposed to the most contaminated sediments, although in this case these compounds accounted for only 4% of the Hg_T and the sediments caused a significant decrease in lugworm survival (samples C1 and C3). In the case of lugworms exposed to the other two samples from Cartagena, namely C2 and C4, the results were very similar to those reported for other sediments and Hg_{ORG} accounted for 40% of the Hg_T accumulated in lugworms. Since no data of Hg_{ORG} in sediments are available, further research should be directed to elucidate if such difference is caused by sediment geochemical characteristics or rather there is some biological process implied in the transformation of internal concentrations in lugworms from its inorganic to organic forms.

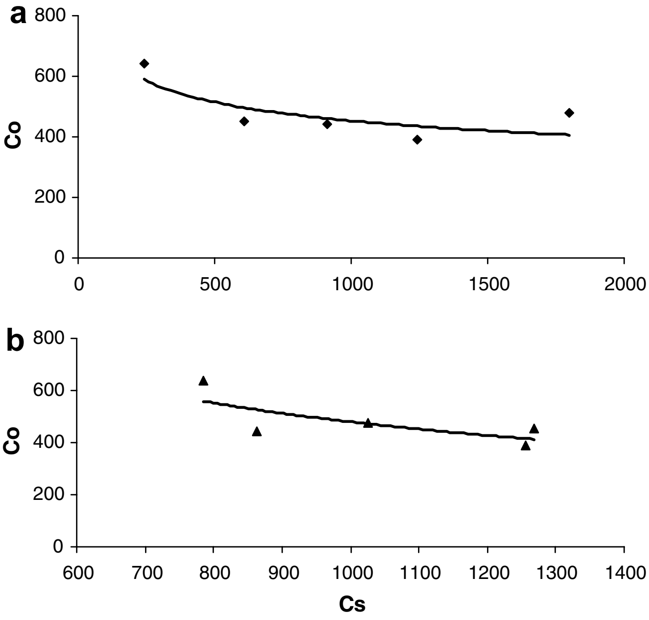

Fig. 3. Concentrations of PAHs in lugworms (C_O) and in sediments (C_S) before (a) and after the correction for the organic matter content of the sediments (b). All concentrations expressed as mg kg^{-1} dry wt.

Something similar occurs when considering results for PAHs compounds. The general bioaccumulation trend for the sum of PAHs showed a slight negative relationship between the concentrations in sediments and in lugworms (Fig. 3a). Moreover no trend was identified when comparing sediments from a same port. Non-polar organic chemicals such as PAHs have a low affinity for the water phase and high for poorly soluble organic phases such as tissues of aquatic organisms, organic particles or colloids. However marine sediments are an extreme heterogeneous mixture of different chemical composition and grain size, which may result in a very different fate of sediment-bound chemicals (Neff, 2002). Among the different attempts to normalize the bioavailability of PAHs the most common approach is the normalization to the concentration of total organic carbon in the sediment (Timmerman and Andersen, 2003; Ruus et al., 2005). However, the application of this correction to the sum of the total PAHs did not explain the variability in the relationship between concentrations in sediments and in lugworms (Fig. 3b) and this inverse correlation prevailed possibly due to the mixture of compounds that is considered, with different environmental behavior.

BSAFs are used on the evaluation of the suitability of dredged sediments for disposal at open water sites in theoretical bioaccumulation potential estimations according to procedures given in the implementation manuals for regulating dredging (USEPA/USACE, 1991). Generally a mean value of four was accepted for screening purposes although different validating projects have been undertaken to identify more predictive rather than protective models (McFarland, 1995). For the sediments considered in this study the calculated BSAFs for the sum of compounds ranged between 0.81 and 0.22, which are one order of magnitude lower than this theoretical value indicating that it is possible that steady-state was not reached after the exposure period considered. Nevertheless our results are still in agreement with those reported by McFarland (1995), which stressed the overestimation of the bioaccumulation to benthic invertebrates when using the standard value of four compared to those calculated empirically in laboratory experiments or field-collected organisms. This same report suggested that bioaccumulation may not be a linear function of organic carbon-normalized PAHs concentrations because a large fraction of these compounds may not be readily capable of desorption. In particular, even source matrix can affect the bioavailability of polycyclic aromatic hydrocarbons to deposit-feeding benthic invertebrates (Rust et al., 2004). The dominance of pyrogenic PAHs in the sediments may hamper the comparison between stations because a great proportion of PAHs may be associated with the soot fraction in the sediments rendering them to be unavailable (Kaag et al., 1998a,b). Soot/water partitioning coefficients for phenanthrene and fluoranthene in harbor sediments have been reported about two orders of magnitude higher than the predicted sediment organic carbon/water partitioning coefficients (K_{oc}), indicating very strong binding of the PAHs to the soot particles (Neff, 2002). For the sediments reported in this study, fluoranthene/pyrene phenanthrene/anthracene ratio values identified a clear contribution of pyrogenic PAHs at C4 (Behlahcen et al., 1997), which would explain the lowest bioavailability of sediment-bound PAHs according to the lowest BSAFs at this station. But in the case of other sediments the contribution of point sources of petrogenic and pyrogenic PAHs, such as accidental oil spills or discharges from tankers and ships, hampers the use of such approaches.

When individual PAH compounds are considered, results are in agreement with previous studies on *A. marina* exposed to harbor and reference sediments, which reported higher tissue concentrations after exposure to reference sediments for fluoranthene, pyrene, chrysene, benzo(*e*)pyrene and benzo(*b*)fluoranthene while exposure to harbor sediments enhanced the bioaccumulation of anthracene, benzo(*e*)pyrene, benzo(*a*)anthracene and chrysene (Kaag et al., 1998a). In our study, the sediments accumulated anthracene and benzo(*a*)anthracene to a higher extent from sediment B4 that is actually in the inner harbor (notice the higher BSAFs in Table 2) although in general the highest accumulation was registered for phenanthrene, fluoranthene, pyrene, benzo(*b*)fluoranthene and dibenzo(*a*)anthracene. These results are also in agreement with the values available in the BSAF database for this species (http://el.erdc.usace.army.mil/bsaf/what.html), which presents the highest values for these compounds, namely 0.95 for phenanthrene, 0.85 for fluoranthene and 1.61 for pyrene. Although our values are similar to these, those from pyrene are more similar to the BSAFs reported by Meador et al. (1997) and Ruus et al. (2005) of 0.40 and 0.20, respectively. Similarly, we found lower BSAFs than four for the compounds benzo(*a*)pyrene and benzo(*k*)fluoranthene, even though they were in agreement with those from previous studies (0.12 value present in the literature and 0.21 mean value in this study for benzo(*a*)pyrene and 0.24 value present in the literature and 0.32 mean value in this study for benzo(*k*)fluoranthene). The exception was BSAF calculated for B1, which were considerably higher. On the contrary, these results are only partially in agreement with experiments on the solubilization for PAHs in *A. marina* gut fluids, which identified some enhanced solubilization of phenanthrene and benzo(*a*)pyrene in relation to predictions based on equilibrium partitioning theory (Voparil and Mayer, 2000; Weston et al., 2000; Voparil et al., 2004).

On the contrary of that found for PAHs, the plot of total PCBs concentrations in tissues versus total concentrations in sediments evidenced that lugworms accumulate PCBs upon laboratory exposure as the highest concentration in lugworms was associated to the highest sediment concentrations (Fig. 4a) while organic matter appeared as a determining factor determining the bioavailability of PCBs to this deposit feeder organism (Fig. 4b). This accumulation trend was generally seen for all congeners (Table 3), although we found the highest concentrations in lugworms for PCB-153, 187, 149 and 138. Studies on other polychaetes have reported the preferential accumulation of PCB-118, 153, 138 and 180 by *N. diversicolor* when exposed to harbor sediments for 28 days (Ruus et al., 2005) while *Neanther virens* accumulates PCBs-52, 49, 101 or 28 after exposure to contaminated sediments for longer exposure periods (http://el.erdc.usace.army.mil/bsaf/what.html). However this could be due to the different exposure duration needed to reach steady-state for each individual compound rather than species-specific preferences. This would also explain that the calculated BSAFs in this study are generally lower than

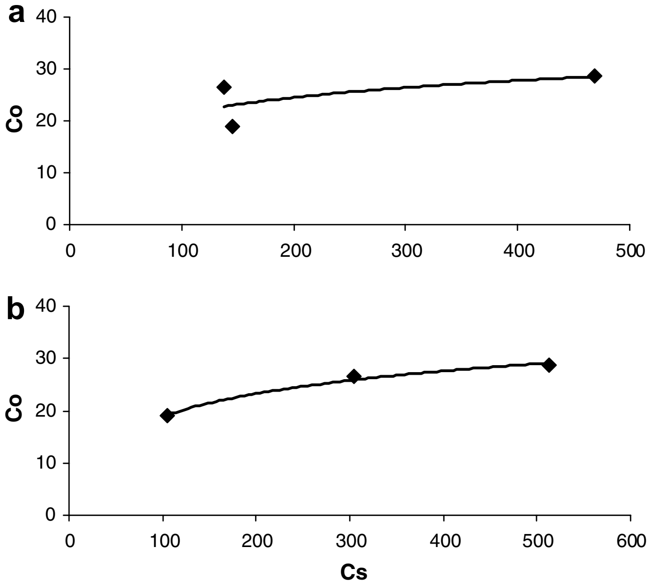

Fig. 4. Concentrations of PCBs in lugworms (C_O) and in sediments (C_S) before (a) and after the correction for the organic matter content of the sediments (b). All concentrations expressed as μg kg^{-1} dry wt.

those found in the literature for different polychaetes (Meador et al., 1997; Jonken et al., 2004; Ruus et al., 2005) and are more similar to those for other benthic organisms with different feeding regimes such as *Macoma nasuta* and *Mya arenaria* (Boese et al., 1997) although BSAFs for total PCBs as low as 0.22 have been also reported in the literature (http://el.erdc.usace.army.mil/bsaf/what.html), which stresses the large variation of PCB bioaccumulation factors among different studies.

In conclusion, the large variability in the results summarised in this paper are in agreement with other studies that already stressed the difficulties for predicting tissue concentrations based on chemical measurements alone. Bioaccumulation ratios may be very variable even for sediments coming from a single harbor and after correcting for the sediment organic matter content. Port and harbors are very complex environments where countless conditions converge to form multiple unique systems in a very short distance, and this hampers the use of simple models to predict bioaccumulation from dredged sediments to benthic invertebrates. Nevertheless, it is still the most reliable protective model as screening methodology in early steps of the characterization frameworks. To have a more complete picture on possible bioaccumulation processes affecting benthic invertebrates, sediment bioassays still offer unique and direct information. The keynote deposit-feeding polychaete *A. marina* has shown promising results as test organism especially for Hg compounds and PCBs. It is possible to identify increased levels of PCBs in lugworms just after 10 days of exposure, and the compounds that occur at the highest concentrations in lugworms are in the group of congeners considered most environmentally threatening (Meador et al., 1997). Similarly, our first results on the bioaccumulation of organic Hg compounds from dredged sediments indicate the importance of its consideration on assessment frameworks as far as the physico-chemical characterization for dredged materials does not foresee its quantification in sediments. For bioaccumulation potential assessments, *A. marina* may be a suitable test organism for routine bioaccumulation tests that accomplishes most of the requirements needed for routine testing and monitoring of these type of compounds. Further research is needed to attribute an ecological significance of the measured concentrations and to interpret the results in absolute terms. But its use and interpretation by comparing tissue concentrations with those measured in organisms exposed to reference materials and/or in organisms present at the disposal sites under consideration as it is already recommended under established regulatory frameworks for pre-dredging investigations seems a good compromise. This is of especial concern in relation to their propensity to biomagnify within aquatic food web and according to their toxicological importance, as they may have deleterious effects at low concentrations (PIANC, 2006). Regarding PAH compounds, our results should undergo a complete study with longer exposure periods to ensure that the variability observe is really caused by differences in the bioavailability of sediment-bound contaminants, as *A. marina* is among the benthic invertebrates that accumulate highest levels of both PAHs and PCBs upon exposure to contaminated sediments (Kaag et al., 1998a).

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