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Using the polychaete *Arenicola marina* to determine toxicity and bioaccumulation of PAHS bound to sediments

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Abstract The present study was conducted to evaluate a sediment toxicity and bioavailability test with the polychaete *Arenicola marina* as a potential tool to assess sediments contaminated by oil spills. A bioassay using the lugworm *Arenicola marina* was carried out in order to determine toxicity and bioaccumulation associated with the contaminants present in the fuel oil extracted from a sank tanker. After 10 and 21 days of exposure to sediments with different proportions of fuel oil (0.5, 1, 2, 4 and 8%) polychaetes were sampled to determine the mortality and the levels of individual PAHs in the organisms. During the experiment, mortality was recorded and the concentration (percentage of fuel oil) that provokes the mortality of the 50% of the *Arenicola marina* population exposed was calculated for both sampling dates (LC50(10)=6.4%; LC50(21)=2.4%). Bioaccumulation was mainly produced for fluoranthene, pyrene, benzo(b)fluoranthene and benzo(k)fluoranthene, whereas phenantrene and anthracene where initially accumulated and then metabolized. The results obtained in the present study suggest *Arenicola marina* can be a suitable species for assessing PAHs toxicity and bioaccumulation as part of oil spill management.

Keywords LC50 · Quality values · Sediment toxicity · Oil spill

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Introduction

The biological effects associated with the chemicals from the oil spill depend on the nature of the affected ecosystem (DelValls 2003). Petroleum can adversely affect organisms by physical action (smothering, reduced light), habitat modification (altered pH, decreased dissolved oxygen, decreased food availability) and toxic action (Albers 2003). The remaining fuel from an important oil spill in the North of Spain (*Prestige*, 2002) that was still in the tanker was eventually extracted in 2004; the composition of this heavy fuel-oil (type M-100) was a mixture of saturated hydrocarbons, aromatic hydrocarbons, resins and asphaltenes, being most of the polycyclic aromatic hydrocarbons – PAHs – of an intermedium-

high molecular weight (Alzaga et al. 2004; Blanco et al. 2006). PAHs are a ubiquitous group of contaminants and can accumulate and persist in marine sediments (Neff 1979), and affect organisms through toxic action (Albers 2003).

Sediment bioassays (toxicity and bioaccumulation) are instruments used to test the toxicity and bioavailability of chemical compounds in sediments to benthic organisms. In the present study we selected *Arenicola marina*, in order to test the toxicity and bioaccumulation of the fuel oil extracted from the tanker. *A. marina* is a bulk sediment feeding polychaete worm that lives in a U-shaped burrow. This species was chosen for the bioassay because it: (a) is continuously exposed to contaminants in the sediment, which it ingest while feeding; (b) is available all the year round, often in reasonably high densities; (c) tolerates a wide range of particle sizes and salinities, (d) has a broad geographic range; and (e) supposes an important species in coastal food chain (Bat and Raffaeli 1997). In addition this species has been recommended by Oslo-Paris Commission (1995) as monitoring organism.

Accumulation of hydrophobic organic contaminants by benthic organisms can occur either from aqueous phase or dietary exposure (Lamoureux and Brownawell 1998). Uptake of hydrophobic compounds from ingested material has been reported as a major contributor to an animal's total body burden of toxicants (Kaag et al. 1996; Kaag et al. 1998; Penry and Weston 1998; Selck et al. 2003). Also bioaccumulation studies have shown that lugworms accumulate organic contaminants to higher concentrations than filter feeding animals (Kaag et al. 1997).

The ability of organisms to metabolize and excrete PAHs also has been shown to be related to bioaccumulation (Rust et al. 2004a, 2004b), where species with limited metabolic ability tend to accumulate higher PAHs concentrations in their tissue (Varanari et al. 1985; Driscoll and McElroy 1996; Rust et al. 2004b). Even though the presence of a PAH metabolising system in *A. marina* (Christensen et al. 2002) has been strongly suggested, invertebrates tend to excrete metabolites more slowly than vertebrate species (Rust et al. 2004b). Metabolites have been found to be eliminated at rates either greater or lower than those of the parent compound (Spacie and Hamelink 1995). Therefore, toxicity will depend on a combination of relative retention time and relative toxicity of parent versus metabolites (Selck et al. 2003). Previous studies agree that *A. marina* appears to be an appropriate choice as indicator species for PAH bioaccumulation (Rust et al. 2004b), and a suitable organism to monitor PAH pollution.

The aim of this study is to asses the sensitivity of the polychaete *A. marina* to the contamination associated with PAHs from oil spills by using the remaining fuel oil extracted from a tanker and to determine the bioavailability of PAHs present in the fuel oil by measuring the bioaccumulation in the exposed organisms. The results obtained will permit the calculation of the Sediment Quality Guidelines (SQGs) for this group of PAHs and will help to predict toxicity and bioaccumulation of the fuel oil in invertebrates. In order to reach these objectives, a bioassay was conducted exposing a population of the polychaete *Arenicola marina* to different dilutions of fuel oil with clean sediment.

Material and methods

Toxicity test

Intertidal clean sediment from the Bay of Cádiz (South of Spain) was mixed with fuel oil extracted from the tanker (0.5, 1, 2, 4 and 8% dry weight). The sediment was filtered (0.6 mm) prior to the toxicity to remove inorganic and organic debris and benthic organisms capable of preying *A. marina* (Riba et al. 2003). These sediments were dried and homogenized at room temperature prior to chemical analysis.

The *A. marina* lugworms were sampled in field by hand-digging and immediately transported to laboratory in containers with sea water. Once there, lugworms were placed in aquariums with sieved sediment from the Bay of Cádiz (5 cm thick) and acclimated for 10 days; air was provided and water was replaced three times per day. Water temperature was kept at 18°C and natural photoperiod was selected. The dilutions of fuel oil (0.5, 1, 2, 4 and 8% dry weight) in sediment and the clean sediment without fuel oil (2 kg) were placed in replicates (three) in 11 L tanks and clean sea water was added. Lugworms were put into the tanks (six per tank) which were covered to avoid evaporation. The experiment lasted 21 days. Mortality was daily recorded and after 10 and 21 days of exposure sampling was performed by transferring individuals

to aerated clean sea water without sediment, where they were held for approximately 4 h to empty the sediment of the body. Organisms were then frozen at −20°C.

Chemical analysis

Sediment was digested as described by Loring and Rantala (1992) for trace metal analysis (Ni, V, Cd, Pb, Cr, Co). Measurement was performed by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer 4100 ZL) (USEPA 1984), et al. Results are expressed as milligrams per kilogram of dry sediment. The analytical procedures were checked using reference material (MESS-1 NRC and CRM 277 BCR) and showed a recovery greater than 90% of the certified concentration.

Polycyclic aromatic hydrocarbons (fluorene, acenaphthene, naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzofluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, dibenzo[ah]anthracene, indene[123-cd]pyrene, benzo[ghi]perilene) were analyzed by using a gas chromatograph equipped with an electron capture detector (GC/MS) (US Environmental Protection Agency 1984). Briefly, dried samples were soxhlet extracted with *n*-hexane for 18 h, and the extracts were isolated by column chromatography on Florisil–alumino–silica. PAHs were eluted and their fractions were dried in a rotatory evaporator and re-dissolved in isooctane. Aromatic fractions were analyzed on a Hewlett-Packard (HP) 5890 Series II gas chromatograph coupled with HP 5970 mass spectrometer. Chromatographic resolution was achieved with a 30 m×0.250 mm DB-5 capillary column, which has a 0.25 μm film thickness, with helium as carrier gas. Quality control was carried out using NRC-CNRC HS-6 sediment reference material. The analytical procedure showed a recovery greater than 90% of the certified concentration.

The individuals of each treatment were put together and liophilized for the PAHs analysis. Briefly, the samples were soxhlet extracted with hexane/acetone 1:1 during 24 h; then, the extracts were transferred into tubes and dissolved with hexane until a final volume of 20 ml. Samples of each tube were evaporated to 2 ml. These extracts were isolated by column chromatography on alumino–silica using 20 ml hexane, then 30 ml hexane/methane 9:1 (Fraction I: aliphatic hydrocarbons) and finally 40 ml of hexane/methane 4:1 (Fraction II: PAHs). Flasks with Fraction II were dried in a rotatory evaporator and re-dissolved in hexane (final volume 10 ml). These extracts were evaporated until a final volume of 0.5 ml. Aromatic fractions were analyzed with a gas chromatograph coupled with a mass spectrometer (Finnigan Mat, GCQ tm). Chromatographic resolution was achieved with a 30 m×0.250 mm DB-5 capillary column, which has a 0.25 μm film thickness, with helium as carrier gas.

Data calculations

The toxic parameter associated with the fuel oil (LC50) was obtained from the mortality data of *Arenicola marina* measured after 10 and 21 days of exposure to the fuel dilutions (0.5, 1, 2, 4 and 8%). LC50 was defined as the concentration (percentage of fuel oil) that provokes the mortality of 50% of the *Arenicola marina* population exposed. The LC50 was calculated by linear regressions of log toxicant dilution of fuel oil on declining probit values (Probit-Analysis-Program, version 1.5). Sediment Quality Values were calculated basing in the LC50 results.

Results and discussion

Acute toxicity

The mortality of the lugworm *Arenicola marina* in each treatment was recorded after 10 and 21 days of exposure. Figure 1 shows how mortality in control (clean sediment from the Bay of Cádiz) was 0 after 10 days of exposure, while after 21 days 5.6% of the organisms exposed died. However, mean survival in all the replicates of clean sediment from the control (Ca1) was higher than 94% after 10 and 21 of exposure. Death of test organisms was positively related to dose at time of exposure. No survival was detected in the highest dilutions of fuel oil (8%) after 21 days of exposure. For all treatments, the day 21 of exposure shows higher mortality of *Arenicola* than the day 10. Survival results for *Arenicola* show substantial variability among replicates which has been already observed in previous studies (Matthiessen et al. 1998). Other authors obtained differences in tolerance to toxicants, including PAHs, depending on

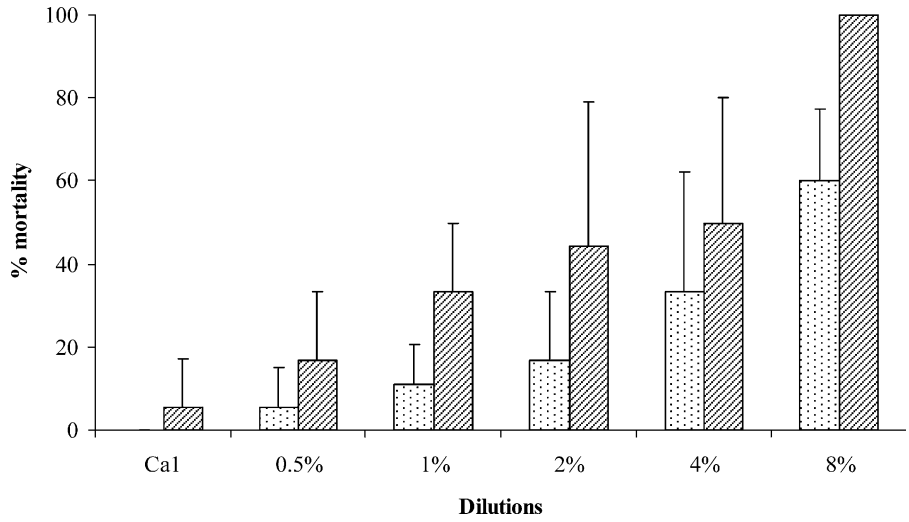

Fig. 1 Average and standard deviations of the percentage of mortality of the polychaete *Arenicola marina* after 10 (*dotted bars*) and 21 (*striped bars*) days of exposure to each dilution

the polychaete specie (Bach et al. 2005) whereas low percentages of oil contaminated sediment inhibited *Arenicola* feeding almost completely (Grant and Briggs 2002). The mortality of individuals of *A. marina* exposed to dredged material demonstrated a slight correlation with the organic contaminants (PAHs and PCBs) even though these correlations were not significant (Casado-Martínez 2007). Results obtained in this study show a toxicity related to time of exposure, what was previously confirmed by previous studies (Rossi and Neff 1978).

Despite the variability of mortality results in the Arenicola bioassays the estimation of LC50 values can be performed. The mortality data were used to calculate two LC50s: LC50(10) to describe toxicity after 10 days of exposure and LC50(21) to describe toxicity after 21 days of exposure. The LC50 value and the concentration of PAHs or metals in the sediment permits calculation of the Sediment Quality Values (SQVs) for each contaminant (Table 1). The LC50(10) value of fuel oil associated with the toxic responses for *Arenicola marina* is 6.4% of fuel oil, which corresponds with a concentration of 92.42 mg kg^{-1} of total PAHs ([PAH]oil*6.4/100) (SQV_1). On the other hand the LC50(21) of fuel oil associated with toxicity for this polychaete is 2.4%, which accounts for a concentration of 34.52 mg kg^{-1} of total PAHs ([PAH] oil*2.4/100) (SQV_2). Previously, a 10-day exposure study with the amphipod *Corophium volutator* and fuel oil from the tanker produced an LC50 of 1.37% (Morales-Caselles, ICMAN-CSIC, personal observations) which suggests that *Arenicola* presents lower sensitivity to the fuel than *Corophium*. Previous studies have demonstrated that *Arenicola marina* shows markedly lower acute toxicity to hydrocarbon and other contaminants bound to sediments than *Corophium* (Matthiessen et al. 1998; Grant and Briggs 2002).

The values of total PAHs concentration obtained from the LC50s calculations (SQV_1 and SQV_2) may be compared with international Sediment Quality Guidelines (SQGs). National Oceanic and Atmospheric Administration (1999) explains the 10th percentile values named the ERL (Effects Range-Low) as the concentrations below which adverse effects rarely occur, whereas the 50th percentiles named ERM (Effects Range-Median) values are representative of concentrations above which effects frequently occur. SQV_1 for total PAHs (92.42 mg kg^{-1}) is higher than the guidelines ERL and ERM calculated (4,022 μg kg^{-1} and 44,792 μg kg^{-1} respectively) while SQV_2 (34.52 $mgkg^{-1}$) keeps higher than ERL and lower than ERM. The justification about why the SQVs obtained for *Arenicola marina* are higher than the ERM could be because of the fact that this polychaete species presents lower sensitivity to the PAHs toxicity than other marine organisms, which allows *Arenicola* to survive in an environment highly contaminated by these compounds. On the other hand results of SQVs for metals are lower than the National Oceanic and Atmospheric Administration guidelines, hence metals are probably not a toxicity factor.

Table 1 Total PAHs and metal concentration measured in the negative control (Ca1) and in the fuel oil

		Fuel	Ca1	ERL	ERM	SQV_1	SQV_2
PAHs (μg kg^{-1})	Total PAHs	1443	n.d	4022	44792	92424	34517
	Fluorene	99.3	n.d	19	540	–	–
	Acenaphthene	75.3	n.d	16	500	–	–
	Naphthalene	395	n.d	160	2100	–	–
	Phenanthrene	385	n.d	240	1500	–	–
	Anthracene	51.4	n.d	85	1100	–	–
	Fluoranthene	28.5	n.d	600	5100	–	–
	Pyrene	111	n.d	665	2600	–	–
	Benzo[a]anthracene	55.9	n.d	261	1600	–	–
	Chrysene	102	n.d	384	2800	–	–
	Benzofluoranthene	16.0	n.d	n.a.	n.a.	–	–
	Benzo[e]pyrene	45.7	n.d	n.a.	n.a.	–	–
	Benzo[a]pyrene	29.7	n.d	430	1600	–	–
	Perilene	11.4	n.d	n.a.	n.a.	–	–
	Dibenzo[ah]anthracene	5.70	n.d	63	260	–	–
	Indene[123-cd]pyrene	5.23	n.d	n.a.	n.a.	–	–
	Benzo[ghi]perilene	17.1	n.d	n.a.	n.a.	–	–
Metals (mg kg^{-1})	Ni	55	14.1	20.9	51.6	8.3	7.5
	V	170	80.0	n.a.	n.a.	42.7	41.1
	Cd	n.d	n.d	1.2	9.6	n.d	n.d
	Pb	n.d	23.0	46.7	218	10.8	11.2
	Cr	0.31	31.0	81	370	14.6	15.1
	Co	n.d	3.40	n.a.	n.a.	1.6	1.7

Sediment quality values for PAHs are obtained from the LC50 and calculated for the *Arenicola marina* used in the sediment toxicity test. Sediment Quality Guidelines were derived using previous studies data (ERL= Effects Range-Low and ERM= Effects Range-Median, National Oceanic and Atmospheric Administration (1999) (n.d, not detected; n.a., not analyzed).

Bioaccumulation

The polychaetes ingest sediment and thus are exposed to PAHs in solution in the interstitial water and those adsorbed to sediment particles (Neff 2002).

Organisms were sampled the day 10 and 21 of the fuel oil exposure experiment in order to analyze the content of PAHs in their bodies. A biota/sediment bioaccumulation factor (BCF) was defined to interpret the results obtained. This factor accounts for the concentration of PAHs in the organisms (Co) related to the concentration of that contaminant in the sediment (Cs) (BCF = Co/Cs). BCF results are shown in Fig. 2. In general, bioaccumulation decreases when the percentage of fuel in the sediment sample increases. This behaviour could be due to the fact that toxicity increases with the content of fuel. Casts were not found in those tanks with higher concentrations of hydrocarbons, so probably feeding was inhibited by the presence of contaminants in the sediment; this fact could lead to lower levels of bioaccumulation in those treatments with the highest amount of fuel oil. This trend has been shown in previous studies on other invertebrates (Landrum et al. 2003) where, in general, the uptake coefficient declined with increasing PAHs concentration, especially with pyrene. Also, lower accumulation factors were found to correspond to treatments for which significant mortality was observed (Rust et al. 2004a).

The BCF calculated for the day 10 of exposure is higher for phenanthrene, anthracene, fluoranthene, pyrene and benzofluoranthenes (Fig. 2); after 21 days of exposure the highest levels of bioaccumulation were for fluoranthene, pyrene and benzofluoranthenes but not for the lower molecular weight compounds phenantrene and anthracene. The decreased of the BCF for phenantrene and anthracene could be due to the fact that after 21 days the organisms have been able to metabolize the PAHs with lower molecular weight. On the other hand, during long-term contact between PAHs and sediment particles, PAHs become tightly bound to organic phases in the sediment, reducing their bioavailability (Neff 2002).

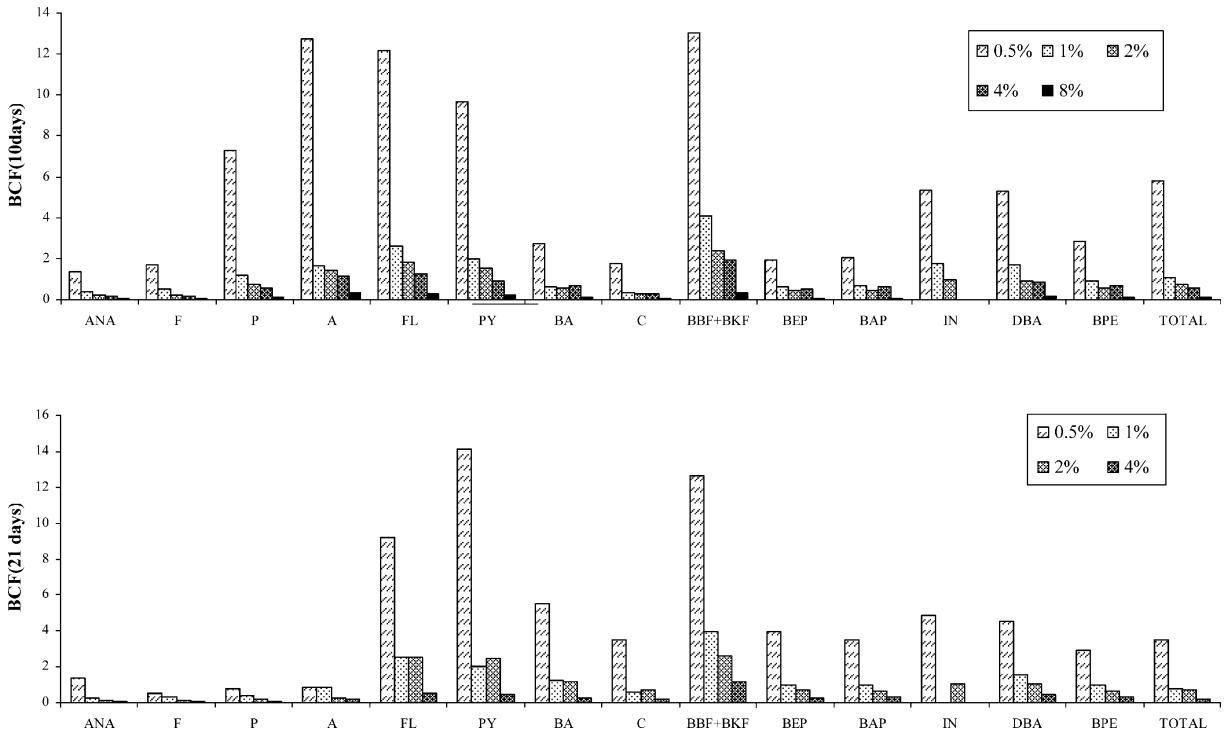

Fig. 2 Bioaccumulation factors (BCF) calculated for *Arenicola marina* after 10 and 21 days of exposure to the dilutions of fuel oil (0.5, 1, 2, 4 and 8%). *ANA* Acenaphtene, *F* fluorene, *P* phenantrene, *A* anthracene, *FL* fluoranthene, *PY* pyrene, *BA* benzoanthrazene, *C* chrysene, *BBF + BKF* benzo(b)fluoranthene and Benzo(k)fluoranthene, *BEP* benzo[e]pyrene, *BAP* benzo[a]pyrene, *IN* indene[123-cd]pyrene, *DBA* dibenzo[ah]anthracene, *BPE* benzo[ghi]perilene, *TOTAL* sum of individual PAHs

Levels of BCF confirm that those PAHs that present logKow values of 5–6 show the highest accumulation potential as reported in previous studies (Kaag et al. 1997; kaag et al. 1998; Rust et al. 2004a).

Fluoranthene presents high bioaccumulation potential relative to smaller or larger PAHs and it is known to be highly toxic to benthic invertebrates (Selck et al. 2003; Landrum 1989; Swartz et al. 1990). This compound may also possess genotoxic (mutagenic and carcinogenic) properties, though these effects are not associated directly with the parent compound, but arise largely as a result of biotransformation processes that lead to the formation of reactive intermediates (Rastetter et al. 1982; Babson et al. 1986; Bach et al. 2005). Pyrene presents a high bioaccumulation factor and associates strongly to sediment particles (Landrum 1989). Results of BCF for pyrene increase from day 10 to day 21, in contrast with other authors that found that the fraction of unmetabolized pyrene in tissues of *A. marina* was unaffected by the duration of exposure (Christensen et al. 2002). Benzo(b)fluoranthene and benzo(k)fluoranthene present similar values of BCF for the day 10 and 21 of exposure, which suggests that these PAHs with high molecular weight were initially bound to the organism tissues and were not metabolized probably due to their low solubility. On the other hand this could be as a result of the fact that threshold effect has been achieved and the availability of the high molecular weight compounds decreases as a consequence of tight organic bonding in the sediment.

Conclusions

In the present study the sensitivity of the polychaete *Arenicola marina* to the fuel oil from a tanker (*Prestige* 2002) has been tested, and in spite of the variability in mortality results, it showed a clear dose-related mortality but more endurance than other organisms. Bioaccumulation was mainly produced for fluoranthene, pyrene, benzo(b)fluoranthene and benzo(k)fluoranthene whereas phenantrene and anthracene where initially accumulated and then probably metabolized.

Although PAHs do not biomagnify through trophic levels (Neff 2002), *A. marina*, which is often used as a bite, is able to live in PAH-contaminated environments and accumulate PAHs. Although *A. marina* is less sensitive than other species, it is likely to be available even in at polluted sites, for studies of PAH bioaccumulation. Attending to this, we propose that *Arenicola marina* should be used in the assessment of oil impacts associated with spills included in a set of bioassays, in order to determine acute and sublethal toxicity responses; in addition further research towards including biomarkers in this species it is recommended.

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