

GUIDELINE LEVELS OF CHEMICAL CONTAMINATION USED TO ASSESS SEDIMENT QUALITY IN COASTAL AREAS : A CONTRIBUTION TO UNDERSTANDING FALSE NEGATIVE OR FALSE POSITIVE RESPONSES

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ABSTRACT

In the framework of the Oslo convention, the first step of the assessment of the ecological risk induced by dredged contaminated sediments relies upon the determination of global concentrations of the most common contaminants. They are compared to guideline levels of contamination : under level 1, sediment-bound contaminants are considered as having negligible impact whereas above level 2 a possible negative impact must be envisaged. Paired sites, contaminated vs control, were investigated in the Gulf of Biscay and the Channel. Despite of concentrations well below level 1 (French regulation), sediments from Fier d'Ars showed toxicity towards mussel embryogenesis. On the contrary, Boulogne harbor sediments showed metal concentrations higher than level 2 but did not induce any additional metal accumulation in worms *Hediste diversicolor* compared to specimens from the Bay of Somme. This lack of effect has been explained by the low mobility of sediment-bound metals in Boulogne harbor.

RÉSUMÉ

Dans le cadre de la convention d'Oslo, la première étape de l'évaluation du risque écologique induit par le dragage de sédiments contaminés repose sur la détermination des concentrations globales des polluants les plus communs. Elles sont comparées à des niveaux de référence: en-dessous du niveau 1, le risque est considéré comme négligeable tandis qu'au-dessus du niveau 2, un impact néfaste est à craindre. Nous avons étudié des paires de sites contaminés et comparativement propres dans le Golfe de Gascogne et la Manche. En dépit de concentrations très inférieures au niveau 1, les sédiments du Fier d'Ars perturbent le développement des larves de moules. Au contraire, les sédiments du port de Boulogne, avec des concentrations métalliques nettement supérieures au niveau 2 n'induisaient aucune accumulation supplémentaire de métaux dans les vers *Hediste diversicolor* par comparaison aux spécimens de la Baie de Somme. Cette absence d'effet a été expliquée par la faible mobilité des métaux liés au sédiment de Boulogne.

1. INTRODUCTION

Developed countries have proposed different strategies to address the problem of contaminated sediments (Chapman et al. 1998; Alzieu 1999; Clarke et al. 2000; MacCauley et al. 2000; McDonald et al. 2000; Babut and Alzieu 2003).

In the framework of the Oslo convention, recommendations have been published to assess the ecological risk induced by dredging. The first step of the assessment relies upon the determination of global concentrations of the most common contaminants (metals: As, Cd, Cr, Cu, Hg, Ni, Pb, Zn; total PCBs and congeners 28, 52, 101, 118, 138, 153, 180; total PAHs). The values thus determined are compared to two guideline levels of contamination adopted by different European countries, the values retained in the French regulations (Table 1) being among the most severe. Under level 1, sediment disposal is considered as having negligible impact on the receptor medium. Between levels 1 and 2, further investigations on the potential impact of dredged sediment disposal are needed, particularly when toxic contaminants such as Cd or Hg are involved. Above

level 2, a detailed risk assessment is needed, including at least sediment ecotoxicity tests.

Numerous bioassays have been developed to assess sediment toxicity, using crustaceans, molluscs, annelids, echinoderms and fish species (Chapman et al. 1998; Quiniou and Alzieu 1999). The embryotoxicity test with bivalves (particularly in *Crassostrea sp* and *Mytilus sp*) is recognised as one of the most rapid, easy and sensitive test, constituting a very good tool to assess the toxicity of bioavailable pollutants (Magnuson et al. 1996; His et al. 1997; Thompson et al. 1999a; Geffard et al. 2002a).

The present study has been undertaken to try to link the chemical characterisation of sedimentary media with different biological responses which can be used as tools to evaluate the health of benthic environments. Gross concentrations of chemicals have been determined in sediments from the Bay of La Rochelle, Gulf of Biscay, Fr. (exposed to anthropogenic activities including urbanisation, industrialisation and the presence of one of the biggest marina in Europe) and compared to a nearby coastal zone,

Fier d'Ars, devoted to oyster culture. Gross concentrations of metals have been determined in sediments collected in the vicinity of a shipbuilding yard (Boulogne harbor, The Channel, Fr.) and compared to the nearby Bay of Somme, harboring a nature reserve. The mobility of the most common metals has been determined using desorption tests as proposed by Amiard et al. (1995). However this approach provides only an insight of "potential bioavailability", thus bioaccumulation has been also determined in organisms living in close contact with the sediment such as microphytobenthos, meiofauna and intrasedimentary polychaetes (*Hediste diversicolor*). Sediment ecotoxicity tests have been also performed, based on the disturbance of embryogenesis in mussel larvae.

2. MATERIAL AND METHODS

Sediments were sampled monthly from March to November 1999 at La Rochelle and Fier d'Ars and in January 1999 in Boulogne harbor and the Bay of Somme. Superficial sediments (oxic fraction, 1st cm) were scraped using a plastic blade and placed immediately in a plastic box which was filled brimful to eliminate air bubbles. In the laboratory, the sediments were homogenised and organic matter and grain size were determined according to standard procedures. Aliquots of fresh sediment were submitted to desorption tests (effect of different pHs after Amiard et al. 1995). Another part of each sediment was immediately frozen, then freeze-dried and stored in sealed bottles in the dark at 4°C for chemical analysis and embryotoxicity tests.

In the paired sites from the Gulf of Biscay, microphytobenthos was recovered from the very superficial sediment (1-2 mm, 3 replicates, 1 L each) and nematofauna from the two top cm (3 replicates, a quarter of m² each) according to techniques based on their respectively positive and negative phototropism (Fichet et al. 1999, after Couch 1988). On the paired sites of the Channel, 20 worms *H. diversicolor* were collected then placed for 24 h in clean aerated seawater from the site of origin to allow them to eliminate their gut content. All the biological samples were then submitted to acid digestion with a view to metal analysis.

Reversed-phase high-performance liquid chromatography was used to determine oxytetracycline, oxolinic acid and flumequine concentrations in sediments (Pouliquen et al. 1992, 1994). PCBs and HAPs were quantified by using GC/MS and GC/ECD after focussed microwaved-assisted extraction (Baumard and Budzinski 1997; Budzinski et al. 1999; Thompson et al. 1999b). This procedure was validated in internal quality controls using standard reference materials (SRM1941a, SRM 1944, NIST, USA) and European intercalibration exercises (CRM 524, CRM 535). As, Cr, Hg and Ni in sediments were determined according to standardised methods (NF EN ISO 9965, 11885, 13506). Other trace metals were analysed in biological matrices and sediments by Flame or Flameless AAS after acid digestion. This procedure was submitted to both internal (mussels BCR/278R, CEC; sediments PACS-1 and MESS-2, NRC Canada) and external quality controls

(Campbell et al. 2000). Chemical concentrations were expressed per dry weight unit, except in the case of diatoms. In these samples chlorophyll concentrations were measured by fluorimetry in acetone extracts and metal concentrations expressed in µg g⁻¹ chlo_a then transformed to µg g⁻¹ of carbon according to de Jonge's data (1995).

Fertilized eggs of mussels *Mytilus edulis* were exposed to a water extract of sediment (elutriate) containing the contaminants remobilized from sediments (Geffard et al. 2001) originating from the Bay of La Rochelle and Fier d'Ars. The toxicity of bioavailable pollutants was determined considering the relationship between the percentages of abnormal larvae and the concentrations of elutriate (expressed in % of the total water extract) (His et al. 1997).

3. RESULTS

3.1 Physico-chemical characterisation of sediments

In sites studied in the Gulf of Biscay, the organic matter content was significantly higher (paired t-tests, p = 0.0324) in sediments originating from La Rochelle (annual mean: 9.3%, SD: 3.0%) than in those from Fier d'Ars (mean: 5.7%, SD: 2.5%). The grain size was lower (p = 0.0339) at La Rochelle (annual mean of the percentage of particles < 63 µm: 95%, SD: 3%) than at Fier d'Ars (mean: 89%, SD: 7%).

In sites studied in The Channel, the differences were much marked with 77% of particles < 63 µm and 4.8% of organic matter in Boulogne harbor versus 1.3% and 0.9% respectively in sediment from the Bay of Somme.

Table 1. Guideline levels of sediment contamination retained in the French regulation and levels of chemicals determined in sediments from two sites of the French coast of the Gulf of Biscay. Metals: annual means and SD in mg kg⁻¹ dry weight (n=9). Organic chemicals in µg kg⁻¹ dry weight (n=2)

	Level 1	Level 2	Fier d'Ars	La Rochelle
As	25	50	15 (3)	
Cd	1.2	2.4	0.30 (0.09)	0.26 (0.07)
Cr	90	180	38 (6)	
Cu	45	90	10 (4)	20 (7)
Hg	0.4	0.8	0.037 (0.003)	
Ni	37	74	13 (2)	
Pb	100	200	21 (5)	30 (12)
Zn	276	552	90 (32)	158 (47)
Σ PCB	500	1000	1.40 – 1.63	20.66 – 35.25
CB 28	25	50	0.04	0.20 – 0.31
CB 52	25	50	<0.2 – 0.09	1.28 – 1.47
CB 101	50	100	<0.13 – 0.01	1.61 – 2.92
CB 118	25	50	0.10 – 0.17	1.80 – 4.02
CB 138	50	100	0.18 – 0.22	2.25 – 4.75
CB 153	50	100	0.20 – 0.27	2.39 – 4.43
CB 180	25	50	0.04 – 0.05	1.04 – 1.16
Σ PAH	1000 ^a	3000 ^a	381-504	657-989

^a German regulation (in Alzieu 1999)

3.2 Global concentrations in sediments

In sites studied in the Gulf of Biscay, metals (Cu, Pb, Zn), PCBs and PAH concentrations were significantly higher in the sediments from La Rochelle compared to those of Fier d'Ars (paired t-tests, $p = 0.0001$) whereas no significant differences were noticed for Cd ($p = 0.10$). On the other hand, metals and PCBs concentrations in both sediments were consistently lower than the recommended level 1 retained in the French regulation (tab. 1). No guideline values are proposed for Σ PAH in the French regulation but the levels determined in these sites were lower than the level 1 indicated by other European countries (in Alzieu, 1999). Moreover, three antibacterial agents commonly used in fish-farming (oxytetracycline, oxolinic acid and flumequine) were searched in sediments from the Fier d'Ars. None of these antibacterial agents was present at detectable levels in sediments from Fier d'Ars.

Table 2. Metal concentrations in sediments and worms from the Bay of Somme and Boulogne harbor (mg kg⁻¹ dry weight). Sediments : n=3. Worms : n=20, analysed individually (Somme) or pooled (Boulogne).

Metal	Sediment		<i>Hediste diversicolor</i>	
	Somme	Boulogne	Somme	Boulogne
Cd	0.05 (0.00)	0.38 (0.01)	0.05 (0.03)	0.06
Cu	0.6 (0.1)	153 (10)	11 (4)	15
Zn	7.3 (0.2)	1092 (87)	105 (23)	88

In the Channel, the control site had Cd, Cu and Zn concentrations in the sediment (tab. 2) far below the level 1. In Boulogne harbor, Cd concentrations were higher than in the Bay of Somme but still below the level 1 whereas Cu and Zn concentrations were consistently higher than level 2.

3.3 Mobility of sediment-bound metals

The results of desorption tests with sediments from the Gulf of Biscay are depicted in fig. 1 for sediments exposed at pH 4 for 2 hours at ambient temperature. In both sites Cd showed the highest percentages of desorption (several tens of units) whereas these percentages were generally lower than 10 for Cu and Zn. However, for all three of these elements, the highest remobilization was always observed in sediments collected in the Bay of La Rochelle, in agreement with an anthropogenic origin of metals at this site.

Unexpectedly, no desorption was observed for Cd and Cu present in sediments from Boulogne harbor whereas desorption was observed for Zn but it was not significant (Zn concentration in the treated sediment vs that in original sediment compared using Kruskal-Wallis tests).

3.4 Metal uptake in sediment-dwelling species

In microphytobenthos (mainly diatoms) extracted from sediments, Cd and Zn levels were significantly higher in specimens collected from La Rochelle than in those from

Fier d'Ars (fig. 2). No intersite differences were noted for Cu and Pb. In nematofauna, the levels of the four studied metals did not differ significantly between these sites.

In worms collected from the clean and contaminated sites studied in The Channel, the concentrations of all of the three studied metals were very similar (Tab. 2).

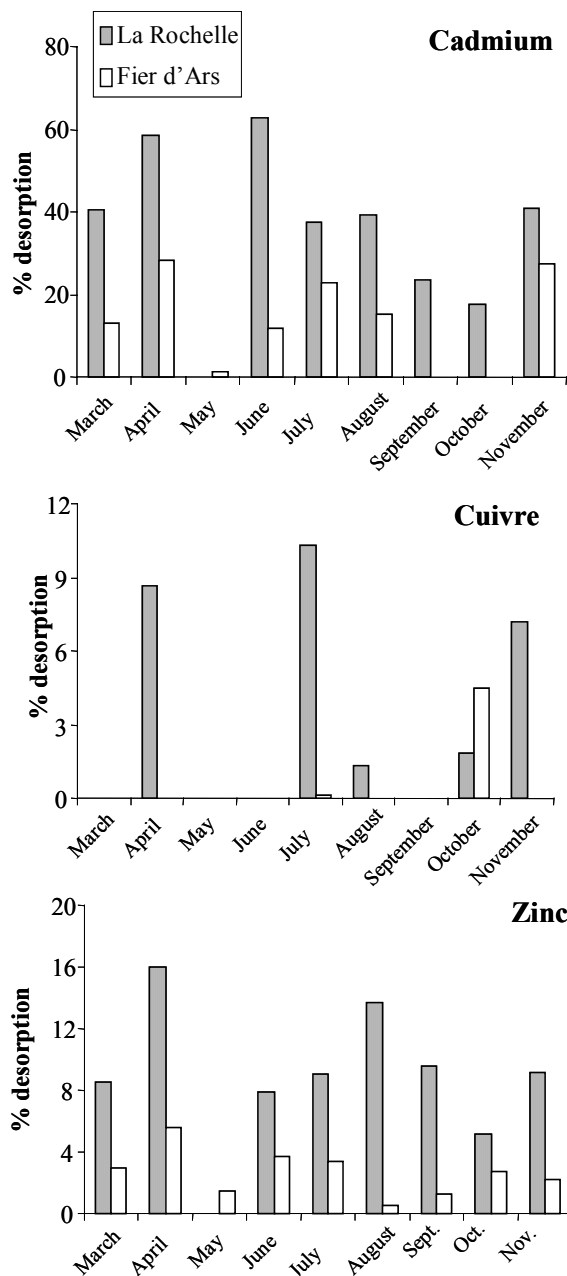


Figure 1. Comparative mobility of metals present in sediments from a control (Fier d'Ars) and a contaminated (Bay of La Rochelle) sites. Results are expressed as percentages of desorption at pH 4.

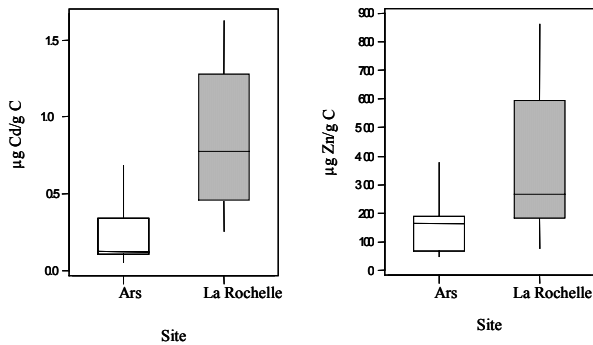


Figure 2. Concentrations of Cd and Zn in microphytobenthos extracted from sediments collected in control (Fier d'Ars) and contaminated (Bay of La Rochelle) sites from the Gulf of Biscay.

3.5 Sediment toxicity

Disturbances of embryogenesis in mussel *M. edulis* larvae were generally observed following exposures to sediment elutriates (fig. 3). The percentage of elutriate needed to induce 50% of larval abnormalities (EC 50) varied from less than 10 % in August to 100 % in May for sediments collected in the Bay of La Rochelle; from less than 10 % in May to 50 % in April for sediments from Fier d'Ars. However, except in August, the quantities of elutriates needed to provoke toxicity was always significantly higher in the contaminated site compared to the so-called control site.

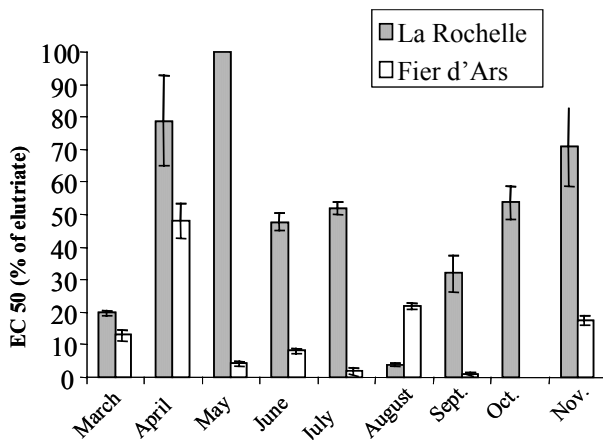


Figure 3. Intersite comparison of effective concentrations of sediment elutriates (expressed as percentages of total elutriate) on the embryogenesis of mussel *Mytilus edulis* larvae.

Since it has been shown that the storage of freeze-dried sediments is responsible for an enhancement of NH_4^+ concentrations at levels eventually higher than the NOEC ($200\mu\text{mol.l}^{-1}$, quoted in Geffard et al. 2002b), this parameter has been determined in monthly samples from each site (unpublished). Values higher than the NOEC were determined in sediments collected from Fier d'Ars in July

and August only. If toxicity was important in July (but no more than in September or October), in August the EC50 was one of the highest determined all over the study. Annual means were respectively 144 and $129\mu\text{mol.l}^{-1}$ in sediments from Fier d'Ars and the Bay of La Rochelle respectively (SD 39 and 53) and they did not differ significantly (paired t-tests, $p = 0.23$).

4. DISCUSSION AND CONCLUSION

Taking into account the French regulations about sediment quality controls, the sediments collected from Fier d'Ars and the Bay of La Rochelle may be considered as "clean" sediments since the total concentrations of metals and organic contaminants are well below the recommended level 1. However, sediments from La Rochelle were more contaminated, considering metals, total PCBs as well as different congeners, and PAHs. Since significant intersite differences were noted considering the organic matter content and the grain size of sediments, correlation coefficients between these factors and metal concentrations were determined. No significant correlation was established between metal concentrations and physico-chemical characteristics of these sediments.

Surprisingly, the sediments of Fier d'Ars were more toxic to mussel larvae than sediments from La Rochelle. The hypothesis that the bacterial degradation of organic matter would be responsible for increased NH_4^+ levels in sediment elutriates has been examined. The toxicity of ammonia to many marine organisms is well-documented and has been identified as an important factor in several studies about sediment biological quality (Ankley et al. 1990; Kohn et al. 1994; Huber et al. 1997; Van Sprang and Janssen, 1997). In the present study, ammonia concentrations cannot be responsible for the observed intersite differences and the high toxicity of sediments from Fier d'Ars.

The presence of an important fish-breeding farm (Lefèvre, 2000) should be a possible source of disturbance through inputs of organic matter and pharmaceutical drugs in the environment (Gowen and Bradbury 1987). However, the effluents are efficiently recycled (Lefèvre, 2000) and occasional measures of nutrients in the water column have not revealed such a pollution in Fier d'Ars ($\text{NH}_4 < 0.05\text{ mg l}^{-1}$ compared to 0.40 in the Bay of La Rochelle; $\text{NO}_2 < 0.05\text{ mg l}^{-1}$ vs 0.27; $\text{NO}_3 < 0.5\text{ mg l}^{-1}$ vs 5.5; $\text{PO}_4 < 0.05\text{ mg l}^{-1}$ vs 0.18). Antibacterial drugs, especially oxytetracycline, oxolinic acid and flumequine, are the most commonly pharmaceutical drugs used in fish farming (Yndestad 1993). However, none of these three antibacterial drugs was detected in sediments from Fier d'Ars.

The presence of fungi, including toxic species, have been shown in marine areas devoted to bivalve culture (Sallénave-Namont et al. 2000). Thus, in the sediment samples from Fier d'Ars the toxicity of which has been demonstrated in the present study, the presence of toxic fungus metabolites has been investigated according to the chemical method described by Landreau et al. (2002). Such metabolites were detected (Pouchus, pers. comm.) and

precise quantification is now needed to correlate toxicological parameters (EC 50) and compound concentrations.

Despite the raised sediment metal concentrations, worms collected in winter from Boulogne harbor showed no enhancement of metal concentrations in their tissues compared to the Bay of Somme chosen as reference site. The ratio between metal concentrations in contaminated vs control sites were as high as 239, 150 and 7.6 respectively for Cu, Zn and Cd in sediments and only 1.3, 0.8 and 1.1 in worms.

In the case of Zn, these observations may be attributed to the ability of many marine organisms including *Hediste diversicolor* (Bryan and Hummerstone 1973; Bryan 1976a, b; Amiard et al. 1987) to regulate their Zn body burden to a relatively constant level when exposed in the field or in the laboratory to this element. On the contrary, it is known that Cu and Cd are accumulated by *H. diversicolor* and other annelids in proportion to their concentrations in the surrounding medium (Bryan 1976a, b; Bryan et al. 1980; Amiard et al. 1987).

However, the bioavailability of sediment-bound metal to *H. diversicolor* is not necessarily a simple direct relationship to sediment total metal concentration for metal bioavailabilities may be affected by other characteristics of the sediment that affect metal speciation. Luoma and Bryan (1982) have shown that the most important factors controlling Cd and Cu concentrations in the polychaete *H. diversicolor* are the concentrations of these metals in surface sediments but also the partitioning of sediment-bound metals between different sediment constituents.

The results of the desorption tests carried out in the present study are in agreement with these findings. In Boulogne harbor where remobilization was practically undetectable, bioaccumulation by worms was negligible. In the sites of the Gulf of Biscay, the highest concentrations of Cd and Zn were registered in the microphytobenthos from the contaminated site of La Rochelle, in agreement with both the highest gross concentrations and the highest mobilities. Cu, the mobility of which was comparatively low, was no more accumulated in biota from La Rochelle than in specimens from Fier d'Ars. The absence of intersite differences in metal accumulation in nematofauna remains unexplained in a zoological group which has not given rise to numerous studies.

In general, discrepancies between chemical characterisation of sediments and biological effects may be due to two different bias as shown in the present study: i) the toxicity of a given sediment may be ignored because it is not induced by one among the chemicals usually analysed; ii) the toxicity of a sediment may be overestimated when sediment-bound contaminants are engaged in physico-chemical forms so stable that chemicals are not easily remobilised, a situation which has been described on several occasions in harbor sediments (see Armynot du Châtelet et al., present symposium).

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