DESIGN AND APPLICATION OF A BATTERY OF BIOMARKERS AS A NEW ACHIEVEMENT IN DREDGED MATERIAL CHARACTERIZATION AND MANAGEMENT

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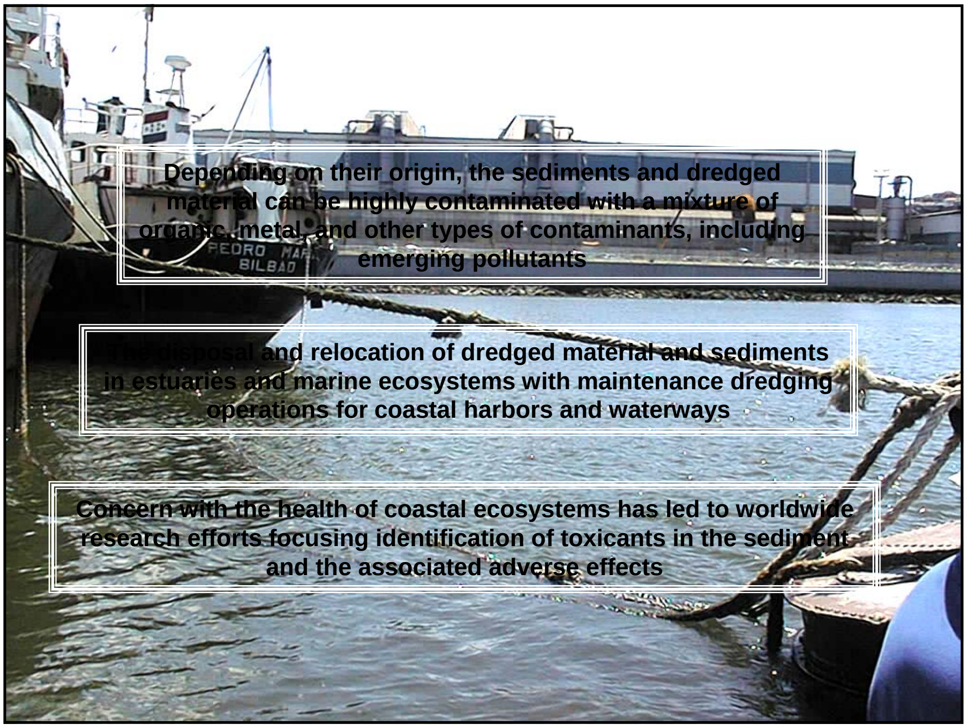


Depending on their origin, the sediments and dredged material can be highly contaminated with a mixture of organic, metal, and other types of contaminants, including emerging pollutants

The disposal and relocation of dredged material and sediments in estuaries and marine ecosystems with maintenance dredging operations for coastal harbors and waterways

Concern with the health of coastal ecosystems has led to worldwide research efforts focusing identification of toxicants in the sediment and the associated adverse effects

Dredged material is managed in the countries that belong to the OSPAR and Helsinki Conventions where Spain is a member

Recommendations for this activity not included in a regulatory framework

Therefore, each country has adopted these recommendations for dredged material characterization and management and developed their regulatory guidelines, which are mainly based in physic-chemical characterization of the sediment.

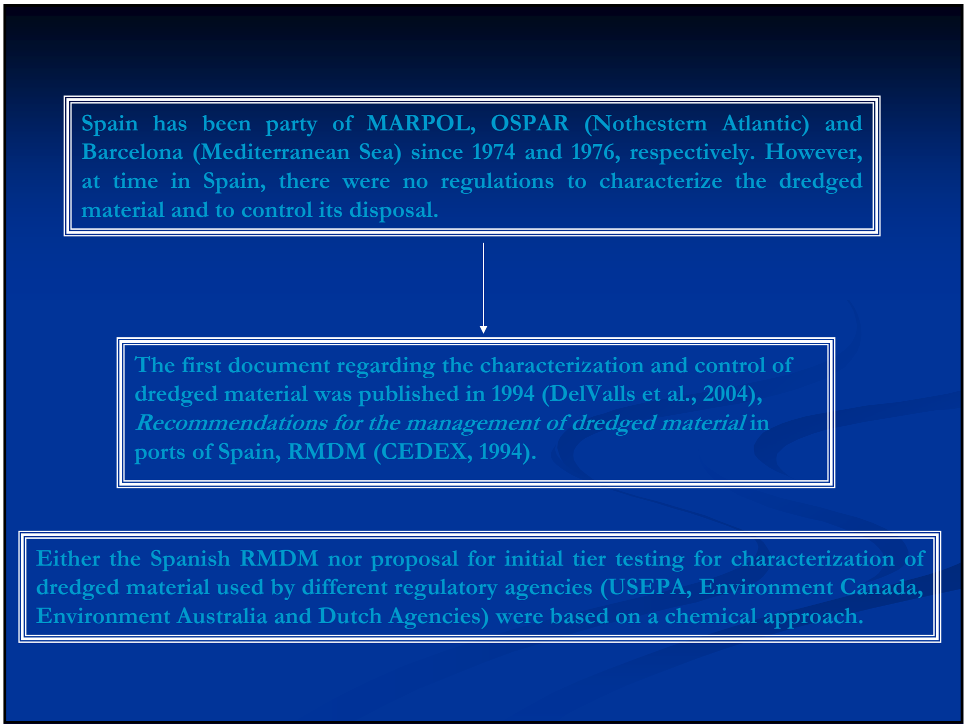
This kind of characterization has allowed the derivation of numerical Sediment Quality Guidelines (SQGs) which are widely utilized.

All these SQGs guidelines can be used to asses individual chemicals by comparing the chemical concentration with the limit concentrations or to estimate the probability of acute sediment toxicity and to determine the possible biological effect of combined toxicant groups by calculating mean quotients for a large range of contaminants (Long et al., 1998).

Mechanistic and empirical approaches

SQG: numerical chemical concentrations intended to be either protective of biological resources, or predictive of adverse effects to those resources or both

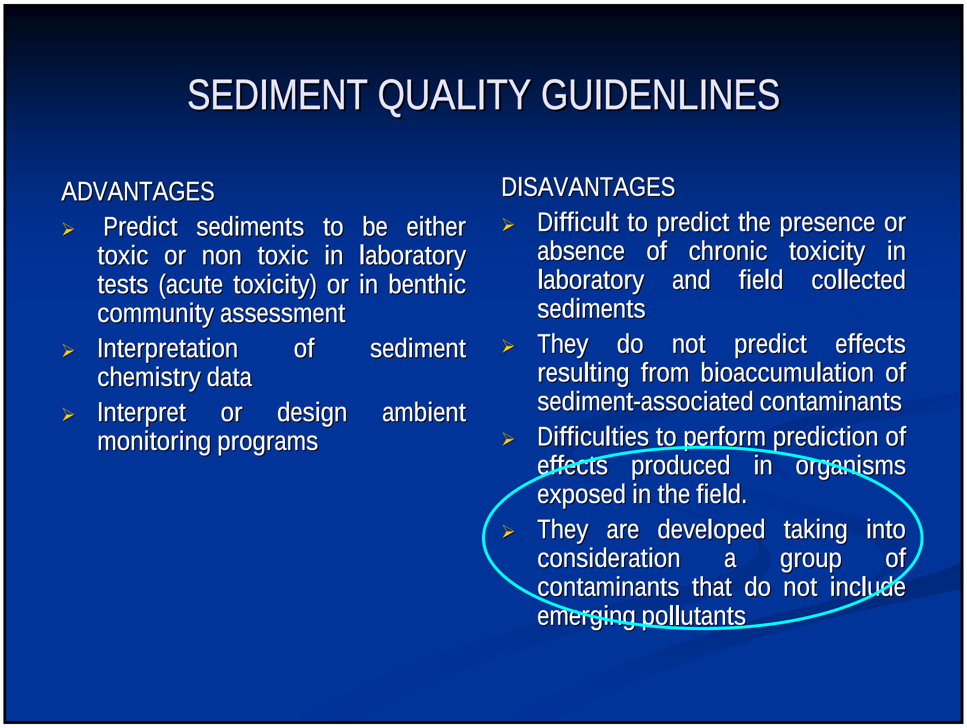
- EqP approach (Di Toro and McGrath, 2000)
- Screening level concentration approach (Persaud et al., 2003)
- Effects range-low (ERL) and Effects range-median (ERM) approaches (Long et al., 1995; USEPA)
- Treshold-effects level (TEL) and Probable-effects level (PEL) approaches (USEPA 1996)



Spain has been party of MARPOL, OSPAR (Nothestern Atlantic) and Barcelona (Mediterranean Sea) since 1974 and 1976, respectively. However, at time in Spain, there were no regulations to characterize the dredged material and to control its disposal.

The first document regarding the characterization and control of dredged material was published in 1994 (DelValls et al., 2004), *Recommendations for the management of dredged material* in ports of Spain, RMDM (CEDEX, 1994).

Either the Spanish RMDM nor proposal for initial tier testing for characterization of dredged material used by different regulatory agencies (USEPA, Environment Canada, Environment Australia and Dutch Agencies) were based on a chemical approach.



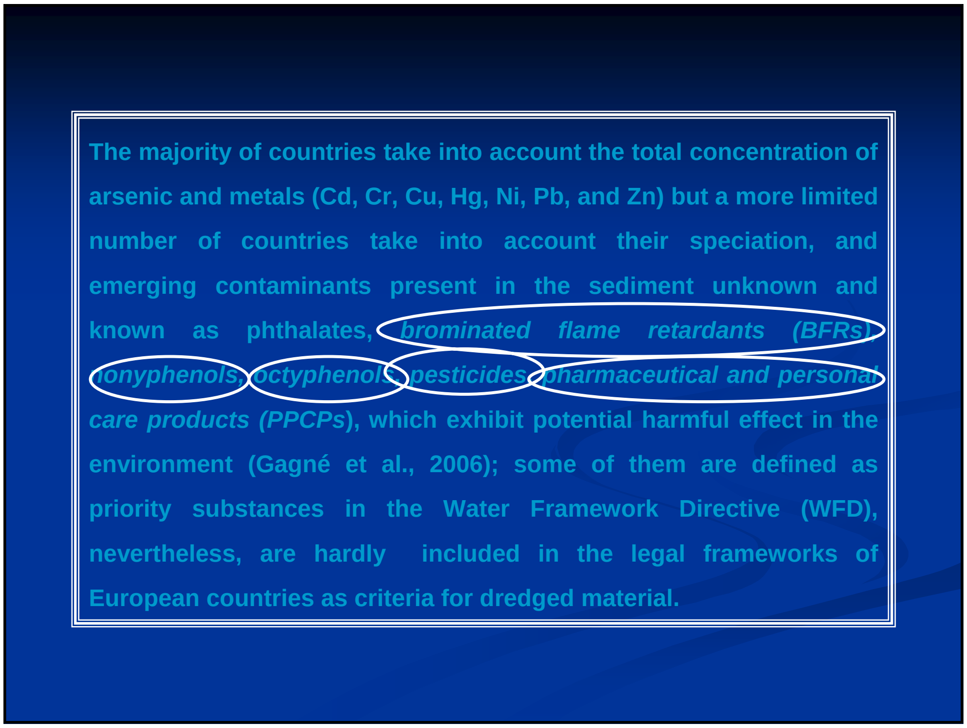
SEDIMENT QUALITY GUIDENLINES

ADVANTAGES

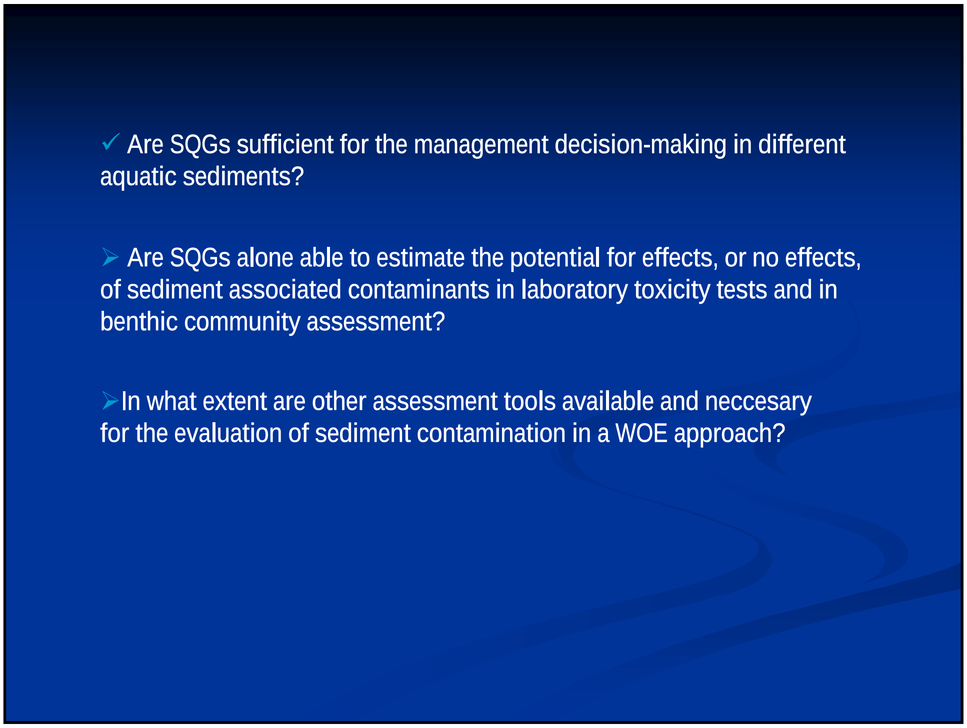
- Predict sediments to be either toxic or non toxic in laboratory tests (acute toxicity) or in benthic community assessment
- Interpretation of sediment chemistry data
- Interpret or design ambient monitoring programs

DISAVANTAGES

- Difficult to predict the presence or absence of chronic toxicity in laboratory and field collected sediments
- They do not predict effects resulting from bioaccumulation of sediment-associated contaminants
- Difficulties to perform prediction of effects produced in organisms exposed in the field.
- They are developed taking into consideration a group of contaminants that do not include emerging pollutants



The majority of countries take into account the total concentration of arsenic and metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn) but a more limited number of countries take into account their speciation, and emerging contaminants present in the sediment unknown and known as phthalates, *brominated flame retardants (BFRs), nonyphenols, octyphenols, pesticides, pharmaceutical and personal care products (PPCPs)*, which exhibit potential harmful effect in the environment (Gagné et al., 2006); some of them are defined as priority substances in the Water Framework Directive (WFD), nevertheless, are hardly included in the legal frameworks of European countries as criteria for dredged material.



✓ Are SQGs sufficient for the management decision-making in different aquatic sediments?

➢ Are SQGs alone able to estimate the potential for effects, or no effects, of sediment associated contaminants in laboratory toxicity tests and in benthic community assessment?

➢In what extent are other assessment tools available and neccesary for the evaluation of sediment contamination in a WOE approach?

The use of these Sediment Quality Guidelines, alone, has been widely discussed and different and important limitations as a tool for the assessment and management of sediment and dredged material have been stated.

The complex matrix of dredged material places limitations on the use of chemical analytical methods alone for estimating the bioavailability and the toxicity of contaminants present (DelValls et al., 2004). These values only permit the characterization of the sediment in a predictable way, they are not site specific and they do not take into consideration the bioavailability and effects of the contaminants present in the sediment.

GROWING CONCERN: THE USE OF SQGs TOGETHER WITH OTHER TOOLS: SEDIMENT TOXICITY TESTS, BIOACCUMULATION AND BENTHIC COMMUNITY ALTERATION

MULTIPLE CHEMICAL AND BIOLOGICAL LINES OF EVIDENCE (LOE)

APPROPIATE FRAMEWORK

IN A SCIENTIFICALLY DEFENSIBLE WEIGHT OF EVIDENCE APPROACH (WOE)

Biological testing is becoming widely accepted for characterizing the chemical hazards in dredged material, and for providing information to support the process of evaluating the impact of the dredged material. By exposing relevant organisms under controlled conditions to samples of the material to be dredged and then measuring toxicological effects (e.g. mortality or reduced growth) and/or the bioaccumulation of contaminants in tissues, estimates can be made in the chemical hazards present (DelValls et al., 2004).

In this sense, different countries have developed toxicity methods applicable to whole sediment, sediment elutriate, sediment suspension, porewater and /or sediment extract. The scientific community has been developing science –based tools to identify sediments that are impaired and, ultimately, to support effective management decisions and priorities for dealing with contaminated sediments.

Toxicity testing of contaminated sediments has focused primarily on acute toxicity (lethality) effects of organisms, with highly contaminated material showing correlations between sediment contaminant concentrations and survival in some cases but not in others (Burton & Scott 1992).

More recent work has been developing sublethal end-points for sediment tests. ‘Whole sediment’ testing with in faunal species has the greatest relevance for predicting ecologically-relevant end-points. However, natural variability in sediment particle size, natural contaminants (e.g. ammonia, hydrogen sulfide) and interspecies competition may result in a number of factors which may confound interpretation of sediment assay results.

Monitoring Programmes

- Recently the biomarker approach has been incorporated into several pollution monitoring programmes and practical workshops:
  - Europe and the USA e.g. the North Sea Task Force Monitoring Master Plan and the NOAA’s National Status and Trends Program .
  - The International Council for the Exploration of the Sea ICES and the Intergovernmental Oceanographic Commission IOC , such as those in the North Sea Stebbing and Dethlefsen, 1992 .
  - The United Nations Environment Programme in the Mediterranean Sea including a variety of biomarkers UNEP, 1997.
  - They have also been included in the Joint Monitoring Programme of the OSPAR convention where Portugal and Spain are members.

- **Sweden**
  - **long-term programme**
    - **EROD, blood parameters, histopathology, MT**
- **Norway**
  - **imposex**
  - **JAMP (Norwegian OSPAR programme)**
    - **cod, flatfish**
    - **MT, EROD, PAH-metabolites**
- **Germany**
  - **fish larval embryonic aberrations**
  - **fish diseases**

- **France, UK. Netherlands, Belgium, Spain, Italy, Croatia**
  - **various species (fish, bivalves, crustaceans)**
  - **a range of biomarkers**

METAL	Sediment Quality Guidelines			
	CEDEX, 1994		Long et al., 1995	
	AL1	AL2	ERL	ERM
As	80	200	8.2	70
Cd	1.0	5.0	1.2	9.6
Cu	100	400	34	270
Cr	200	1000	81	370
Hg	0.6	3.0	0.15	0.71
Ni	100	400	20.9	51.6
Pb	120	600	46.7	218
Zn	500	3000	150	410
Σ_7PCBs	30	100	22.7	180
Σ_{13}PAHs	-	-	0.35	2.36

*Values expressed as mg/kg expect Σ_7PCBs expressed as ug/kg

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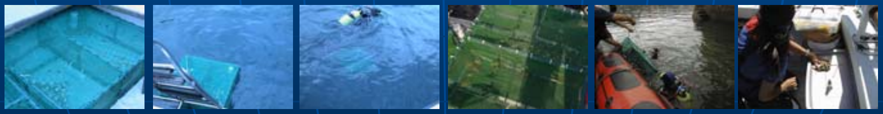
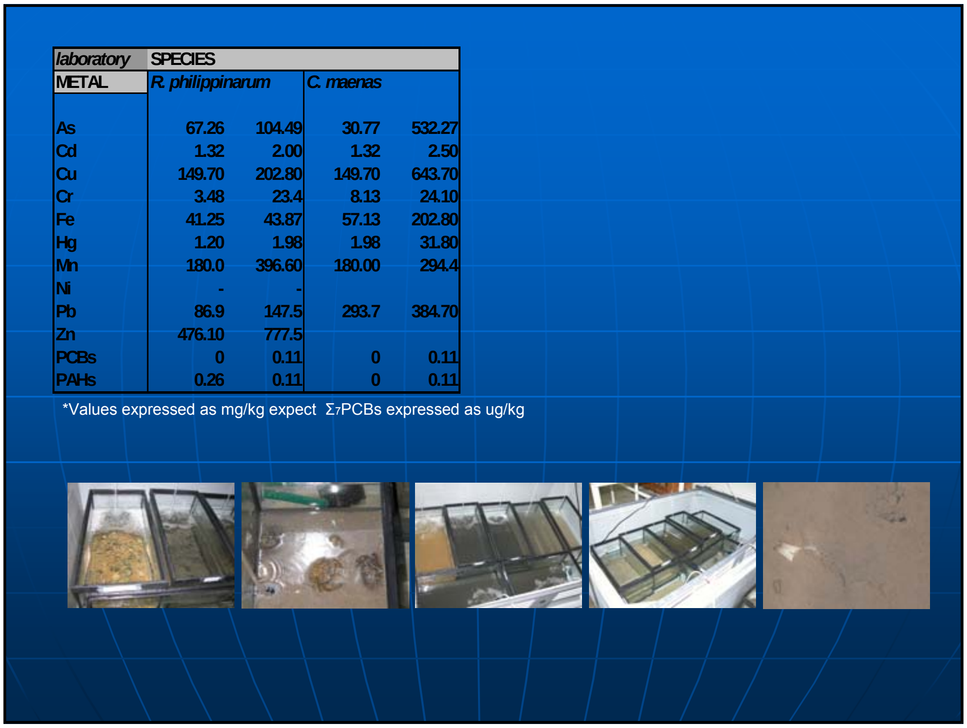
in situ	SPECIES			
METAL	*R. philippinarum*		*C. maenas*	
As	16.61	104.49	16.61	104.49
Cd	0.04	2.50	0.04	2.00
Cu	46.76	204.1	23.03	204.1
Cr	3.48	24.10	3.48	23.42
Fe	41.25	42.00	16.98	42.00
Hg	1.20	1.98	0.18	11.43
Mn	191.35	354.45	191.35	354.45
Ni	16.90	32.00	15.72	32.00
Pb	147.5	293.7	17.61	293.70
Zn	135.5	1857.00	135.5	1857.00
PCBs	0.01	0.11	0.01	0.11
PAHs	0.01	62.77	0.11	0.26

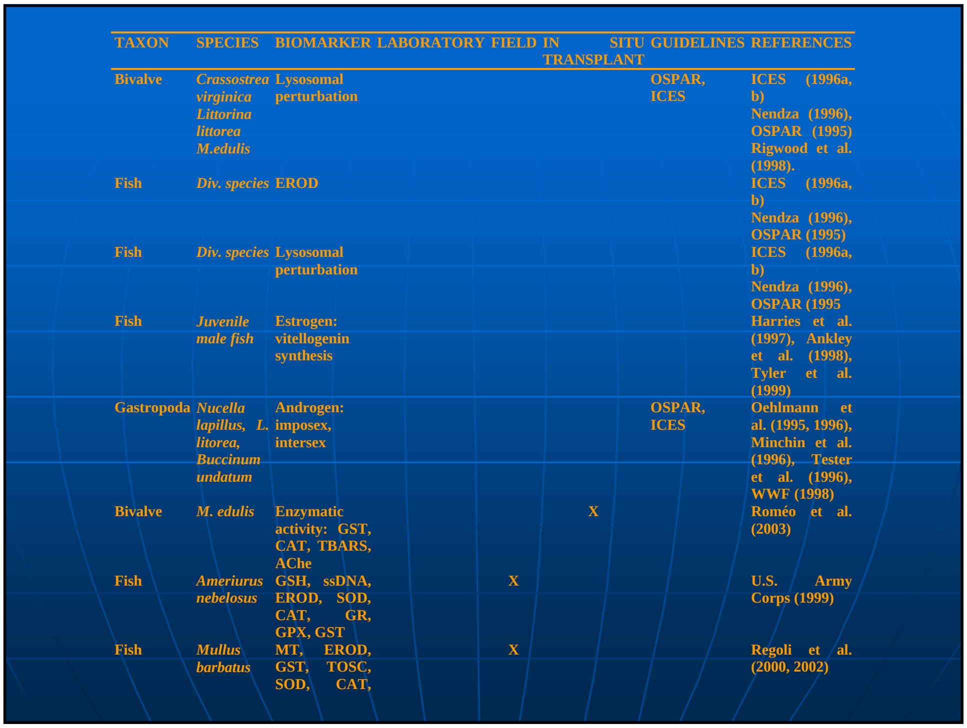
*Values expressed as mg/kg expect Σ_7PCBs expressed as ug/kg





laboratory	SPECIES			
METAL	*R. philippinarum*		*C. maenas*	
As	67.26	104.49	30.77	532.27
Cd	1.32	2.00	1.32	2.50
Cu	149.70	202.80	149.70	643.70
Cr	3.48	23.4	8.13	24.10
Fe	41.25	43.87	57.13	202.80
Hg	1.20	1.98	1.98	31.80
Mn	180.0	396.60	180.00	294.4
Ni	-	-		
Pb	86.9	147.5	293.7	384.70
Zn	476.10	777.5		
PCBs	0	0.11	0	0.11
PAHs	0.26	0.11	0	0.11

*Values expressed as mg/kg expect Σ_7PCBs expressed as ug/kg



TAXON	SPECIES	BIOMARKER	LABORATORY	FIELD	IN SITU TRANSPLANT	GUIDELINES	REFERENCES
Bivalve	*Crassostrea virginica* *Littorina littorea* *M.edulis*	Lysosomal perturbation				OSPAR, ICES	ICES (1996a, b) Nendza (1996), OSPAR (1995) Rigwood et al. (1998).
Fish	*Div. species*	EROD					ICES (1996a, b) Nendza (1996), OSPAR (1995)
Fish	*Div. species*	Lysosomal perturbation					ICES (1996a, b) Nendza (1996), OSPAR (1995
Fish	*Juvenile male fish*	Estrogen: vitellogenin synthesis					Harries et al. (1997), Ankley et al. (1998), Tyler et al. (1999)
Gastropoda	*Nucella lapillus, L. litorea, Buccinum undatum*	Androgen: imposex, intersex				OSPAR, ICES	Oehlmann et al. (1995, 1996), Minchin et al. (1996), Tester et al. (1996), WWF (1998)
Bivalve	*M. edulis*	Enzymatic activity: GST, CAT, TBARS, AChe			X		Roméo et al. (2003)
Fish	*Ameriurus nebelosus*	GSH, ssDNA, EROD, SOD, CAT, GR, GPX, GST		X			U.S. Army Corps (1999)
Fish	*Mullus barbatus*	MT, EROD, GST, TOSC, SOD, CAT,		X			Regoli et al. (2000, 2002)

- Biomarker response may indicate the presence of *biologically available* contaminant, rather than a biologically inert form of contamination

- Using a suite a *biomarkes* may reveal the presence of contaminants that were not *suspected initially*

- Biomarker responses *often persist long after* a transient exposure to a *contaminant* that has then *degraded* and is no longer *detectable.* Thus biomarkers may detect intermitent pollution events that routine chemical monitoring may miss

- Biomarker analyses, are in many cases, *much easier to perform* and are *considerably less expensive* than a wide range of chemical analysis

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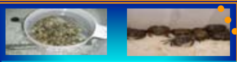

TAXON	Test specie	Water	Sediment	Characteristics
Crustacean	Crab: e.g. *Carcinus maenas*	X	X	Salinity Tolerance; Deposit feeding
Bivalve	Clam: e.g. *Ruditapes philippinarum*	X	X	Salinity Tolerance; Deposit feeding

Three sentinel specie, three ecological life style: filter feeding, deposit feeding and burrowing

+

Natural environmental variables tolerance (pH, salinity, temperature…)

+

Test of biomarkers responses in these tests organisms under physic chemical environment fluctuations

RECOMMENDATIONS:

✓ *Indicator species selection.* It should be taken into account the sensitivity of the specie, life stage tested, its degree of phylogenetic and ecological relatedness to receptors at the disposal site, its preferences and tolerance to the particle size makeup of the test sediment, and so on. The use of native species improves the ecological relevance of the tests results. It is important also to take into account the condition index of the test species (Amiard et al., 1998).

✓ *Sediment handling.* Sediment storage: duration, temperature, container material; animals acclimatization, transport; Food utilized, photoperiod should be standardized.

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✓ An integration through the TRIAD methodology should be performed, taking into account the sediment characterization, biomarkers responses in laboratory assays and in situ assays.

TIER TESTING RECOMMENDED FOR DREDGED MATERIAL ASSESSMENT

TIER 0: RECOPILATION OF INFORMATION

TIER 1: SCREENING AND DETECTION OF TOXIC IMPACTS

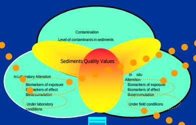
Physical and Chemical analysis of the sediment

TIER 2: CHARACTERIZATION OF TOXIC IMPACTS

Wide range of effects parameters short term, long term toxicity tests and bioaccumulation

TIER 3: VERIFICATION OF *IN SITU* ALTERATIONS

Assess biomarkers of exposure and effect and/or verify the laboratory measurements in the field



INCORPORATION OF BIOMARKERS TO DREDGED MATERIAL MONITORING

✓Screening of biomarkers

✓Bioavailability of chemicals in sediment: TRIAD methodology containing chemical characteristics of sediment, exposure (bioaccumulation and biomarkers of exposure) and effect (biomarkers of effect)

✓Biomarkers testing : time, site and physic chemical conditions

✓Extrapolation of biomarkers from laboratory to field

✓Biomarkers analysis in different species

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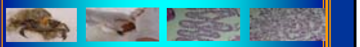


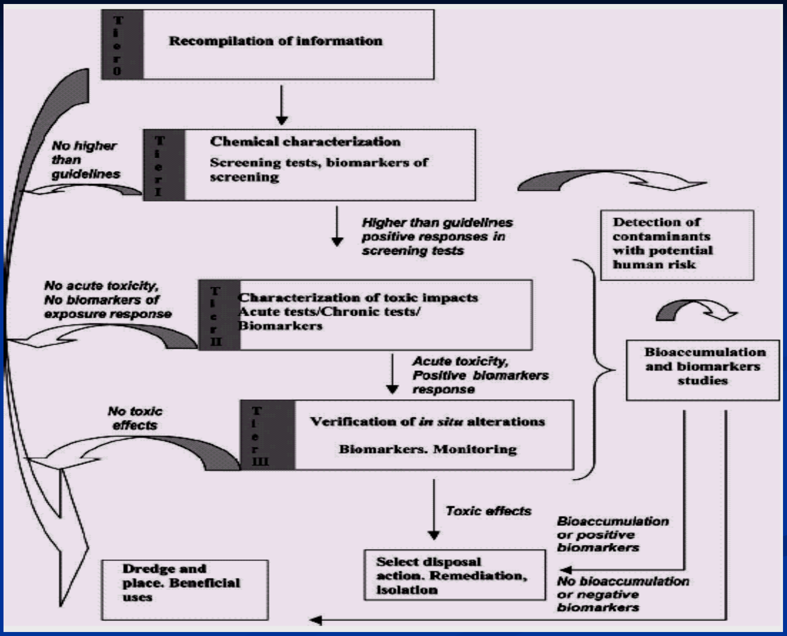
BIOMARKERS	DESCRIPTION
Metallothionein	Induction of this protein indicates the exposure to metals
GSH	Assay that determines the total glutathione content, a natural antioxidant
DNA damage	Assay that detects single strand breaks in DNA, a measure of damage of DNA
EROD	Assay for Ethoxyresorufin-O-deethylase, Phase I detoxification enzyme
CAT	Assay for catalase, antioxidant enzyme
SOD	Assay for superoxide dismutase, an antioxidant enzyme
GR	Assay for glutathione reductase, an antioxidant enzyme
GPX	Assay for glutathione peroxidase, an antioxidant enzyme
GST	Assay for glutathione-S-transferase, a Phase II detoxification enzyme
LPO	Assay to determine the level of thiobarbituric reactive substances from lipid peroxide breakdown
Vitellogenin/vitellin	Induction of this protein indicates the exposure to substances that could perturbe the endocrine function

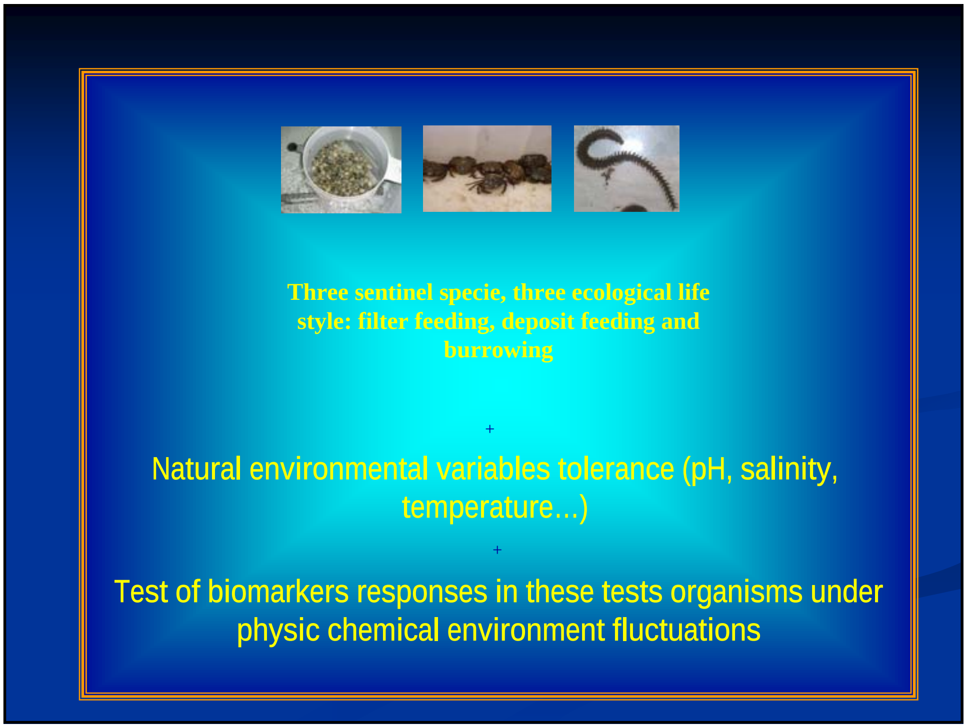
Biomarkers applied in a tier-testing approach for sediment management could allow the performance of more sensitive SQGs for dredged material assessment and management.

Their inclusion in a tier- testing approach, starting with screening biomarkers together with chemical characterization on TIER I.

Then, it is advised the determination, on TIER II, of oxidative stress responses (cytochrome P450 enzymes, lipid peroxidation…) and metallothionein like-proteins (MTLP) as biomarkers of exposure to organic and metallic contaminants, together with biomarkers of effect (genotoxicity, endocrine disruption, inmunotoxicity…).

Finally, it is proposed the verification of these responses *in situ* assays on a TIER III.





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Aknowledgements

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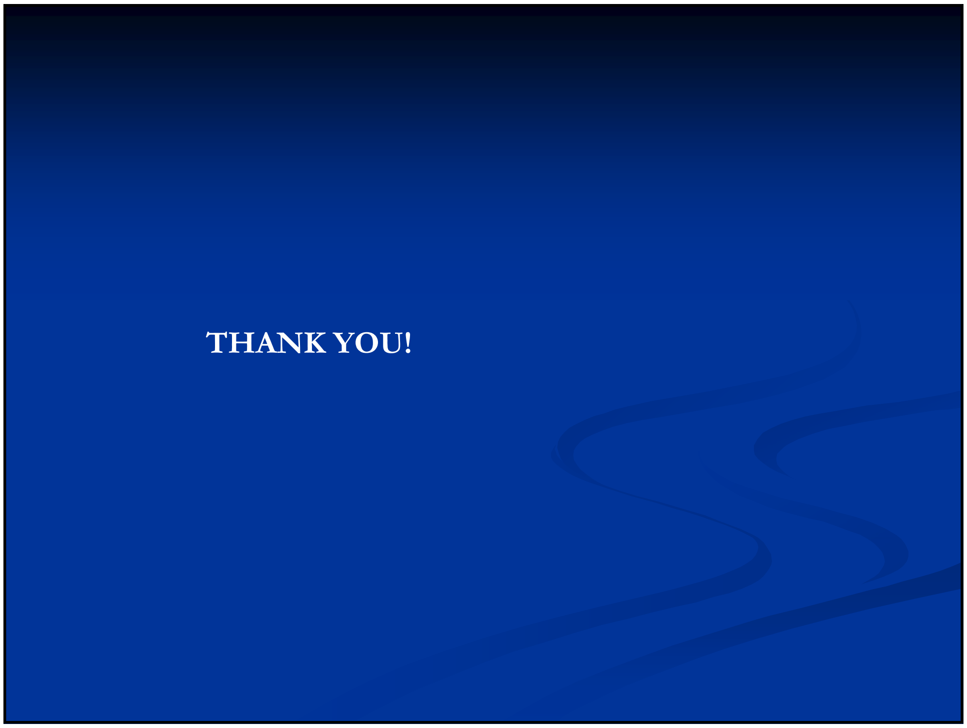

THANK YOU!