

Liège, December the 12th



Coastal pollution of the Mediterranean and extension of its biomonitoring to trace elements of emerging concern







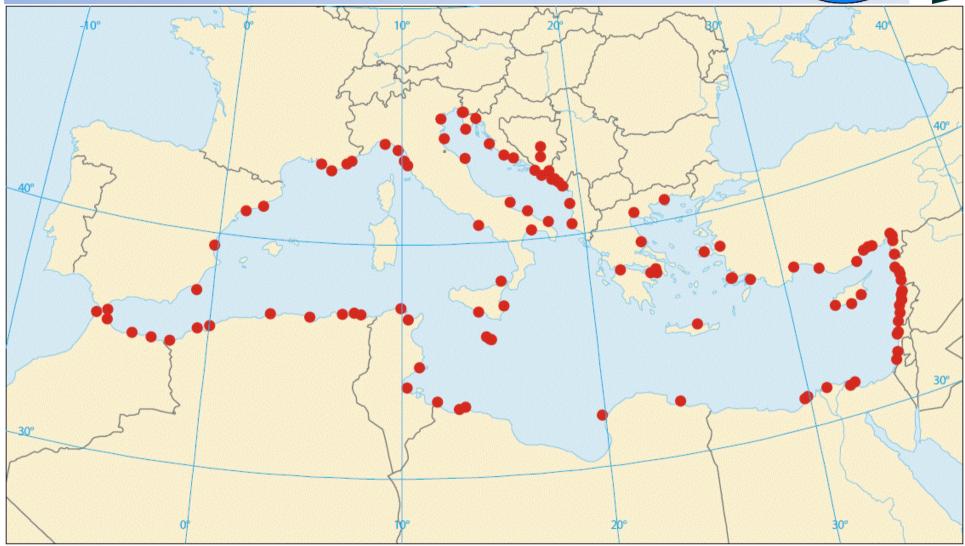
Dissertation presented by **Jonathan Richir** for the fulfilment of the degree of PhD in Sciences

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Coastal pollution of the Mediterranean







Pollution hot spots (full red circles) along the Mediterranean coasts (EEA 2006)

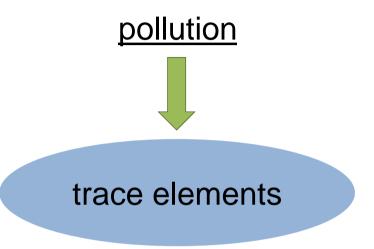
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Trace elements



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Trace elements

C, N, O, Fe, Zn, As, Sn, Sb, Bi, Cu, Pb



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Trace elements

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Abundant elements

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Trace elements

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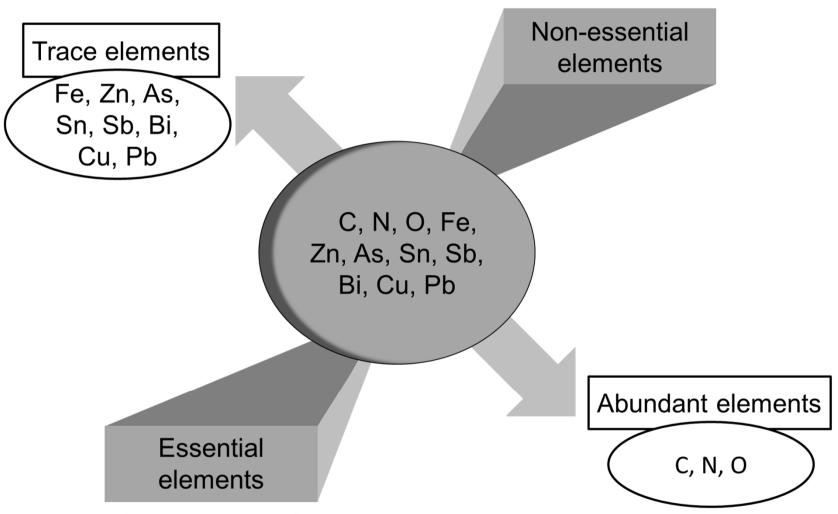
C, N, O

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Trace elements



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Trace elements

Trace elements

Fe, Zn, As, Sn, Sb, Bi, Cu, Pb Non-essential elements

As, V, Sn, Sb, Pb, Bi

C, N, O, Fe, Zn, As, Sn, Sb, Bi, Cu, Pb

Essential elements

Fe, Zn, Cu

Abundant elements

C, N, O

(after Amiard 2011)







Monitoring of the pollution by trace elements





Group 1	Group 2										
0.98	4 1,57										
941	Be 9.012										
1 0.93	12 1.31										
la 2.990	Mg 24,305	Group: 3	4	5	6	7 I transition e	B laments —	9	10	11	12
0.82	20 1.00	21	22	23	24	25	26	27	28	29	30
9.102	Ca 40.08	Sc 44.956	Ti 47.90	V 50.941	Cr 51.996	Mn 54.938	Fe. 55.847	Ce 58.933	NI 58.71	Cu 63,546	Zn 65.37
7 0.82 b 5.47	38 0.95 Sr 87,62	39 Y 88.906	40 2 r 91,22	41 Nb 92.906	42 Mo 95.94	43 Tc (99)	44 Ru 101.07	45 Rh 102.91	46 Pd 106.4	47 Ag 107.87	48 Cd 112.40
5 0.79 s 32,91	56 0.89 Ba 137.34	57 La 138.91	72 Hf 178.49	73 Ta 180.95	74 W 183.85	75 Re 186.2	76 Os 190.2	77 lr 192.22	78 Pt 195.09	79 Au 196.97	80 Hg 200.59
7 r 223)	88 Ra 226.025	89 Ac 227.0	104 Rf (261)	105 Db (262)	106 Sg (263)	107 Bh	108 Hs	109 Mt	110 Uun	111 Vuu	112 Unb

Group 13	Group 14	Group 15	Group 16	
5 2.04	6 2.55	7 3.04	8 3.44	
B	C	N	O	
10,811	12.011	14.007	15.999	
13 1.61	14 1.90	15 2.19	16 2.58	
Al	Si	p	S	
26.98	28.086	30,974	32.064	
31 1,81	32 2.01	33 2,18	34 2.55	
Ga	Ge	As	Se	
69.72	72.59	74,922	78.96	
49 1.78	50 1.96	51 2.05	52 2.10	
In	Sn	Sb	Te	
114.82	118.69	121.75	127.60	
81 2.04	82 2.32	83 2,02	84	
TI	Pb	Bi	Po	
204.37	207.19	208,98	(210)	

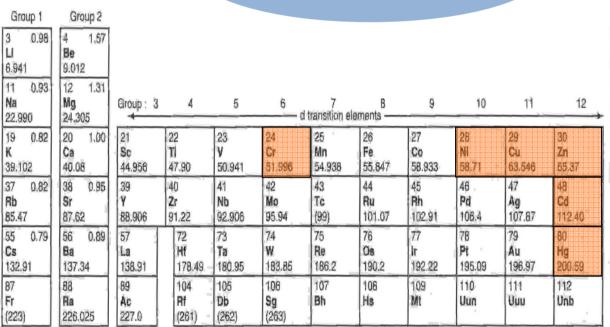






Monitoring of the pollution by trace elements





trace elements

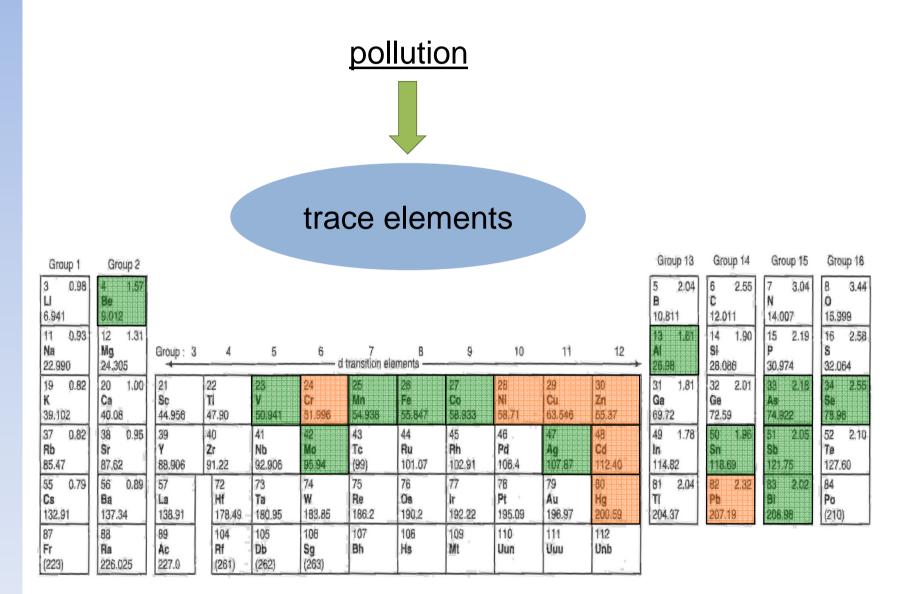
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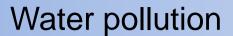




Monitoring of the pollution by trace elements

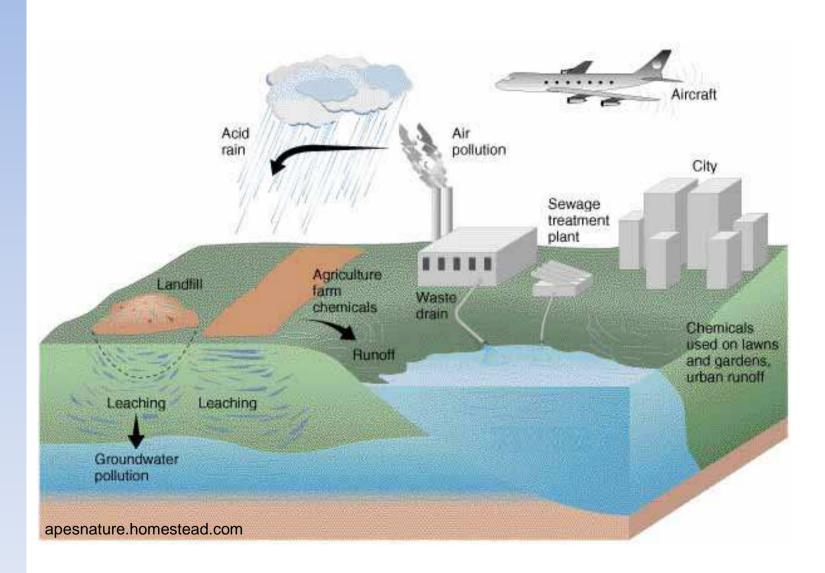




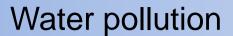






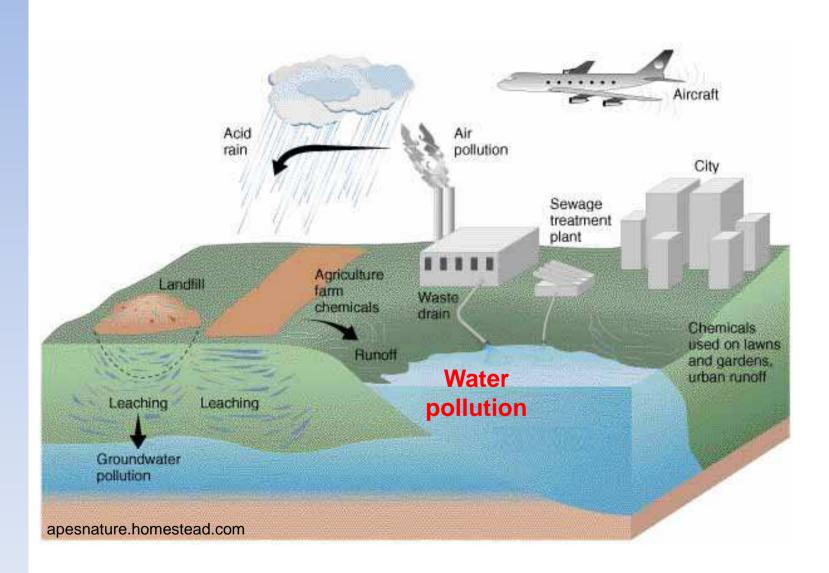






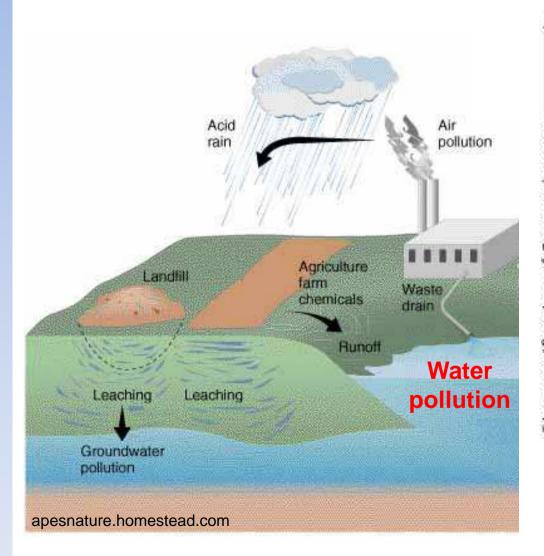


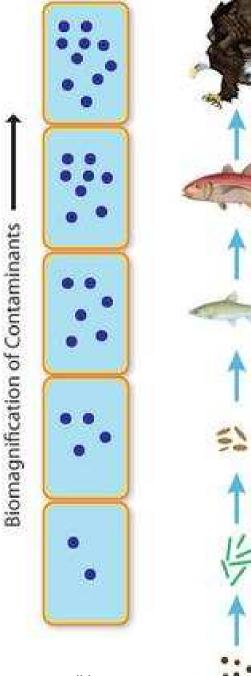






Water pollution







Public health





COUNTERTHINK













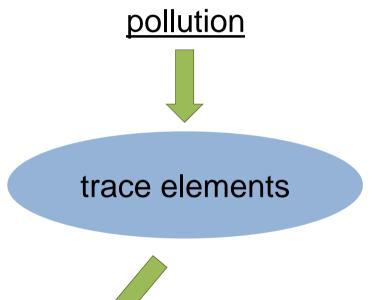
trace elements











direct measurements in the environment



Biomonitoring









trace elements





direct measurements in the environment

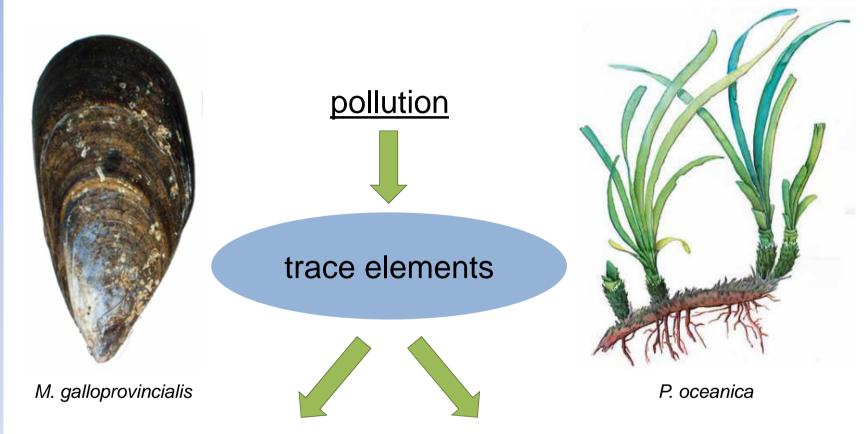
bioindicators = organisms accumulating pollutants to levels representative of their habitat pollution status.



Biomonitoring







direct measurements in the environment

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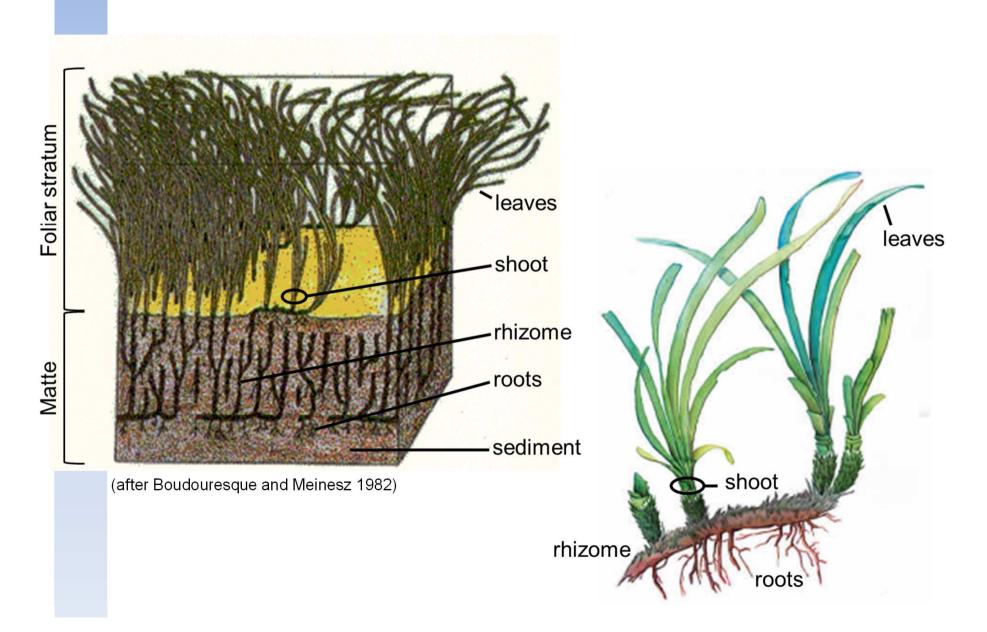
Posidonia oceanica









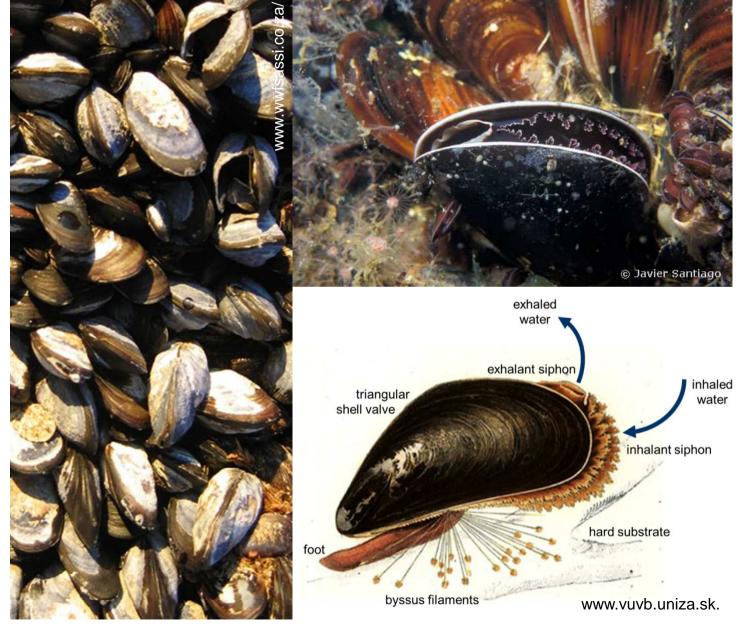




Mytilus galloprovincialis



















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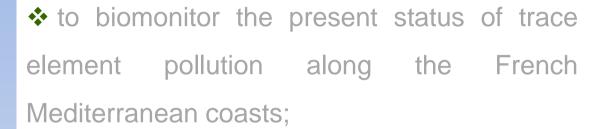
❖ to investigate the potential use of P. oceanica and M. galloprovincialis to biomonitor trace elements of environmental "emerging concern";









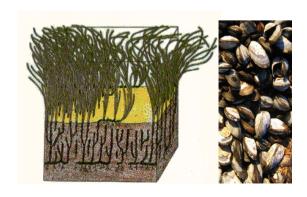




❖ to investigate the potential use of P. oceanica and M. galloprovincialis to biomonitor trace elements of environmental "emerging concern";



❖ to study the mechanisms determining trace element accumulation in both species, under reference conditions or when exposed to environmental changes of pollutant loads.









spatial variation of trace element levels in *P. oceanica* shoots along the French Mediterranean coasts ↔ polluting human activities;





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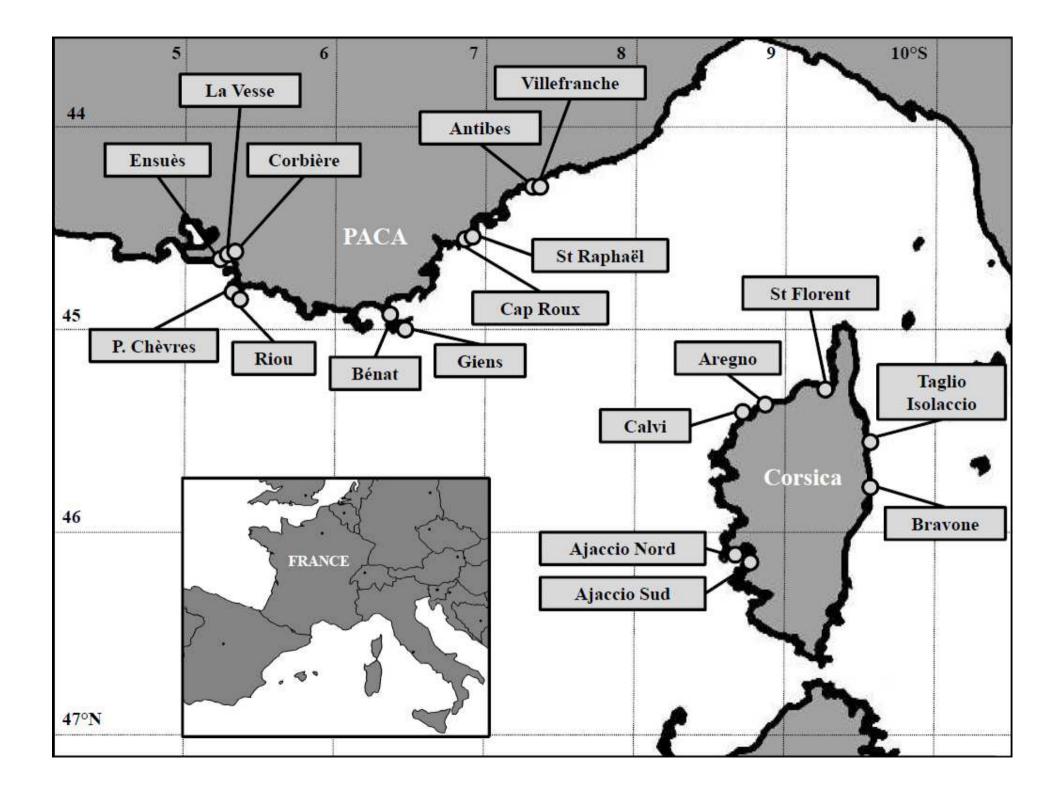
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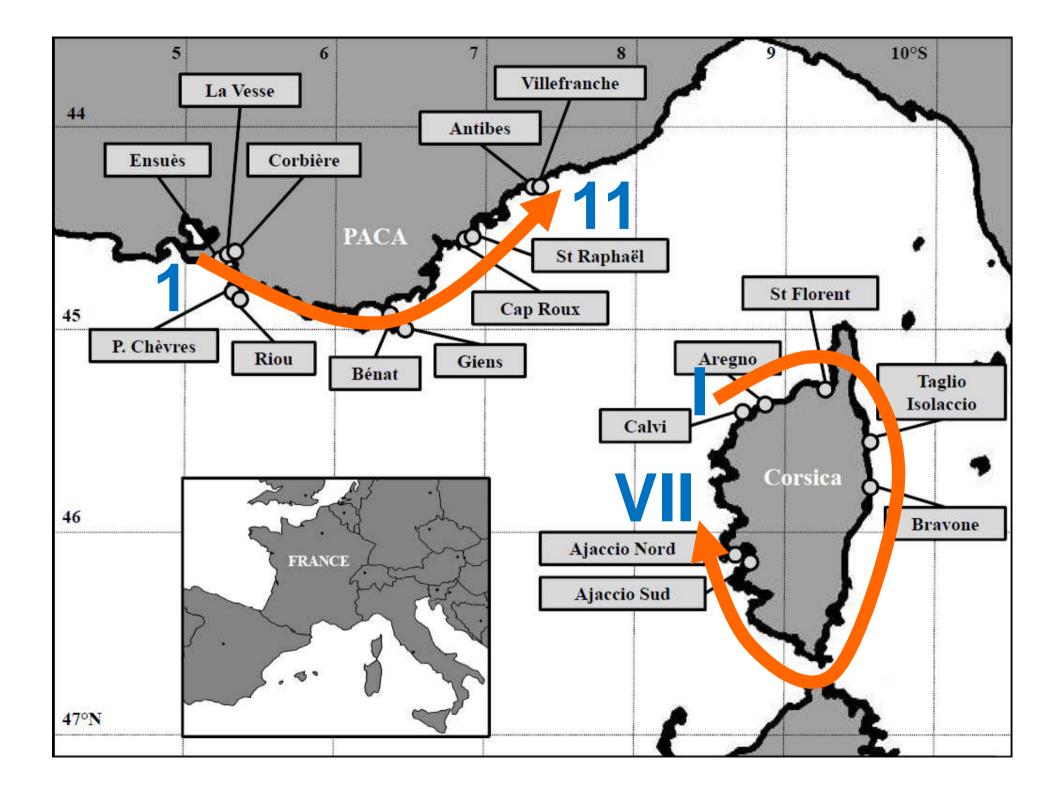


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1 - petroleum products (V)

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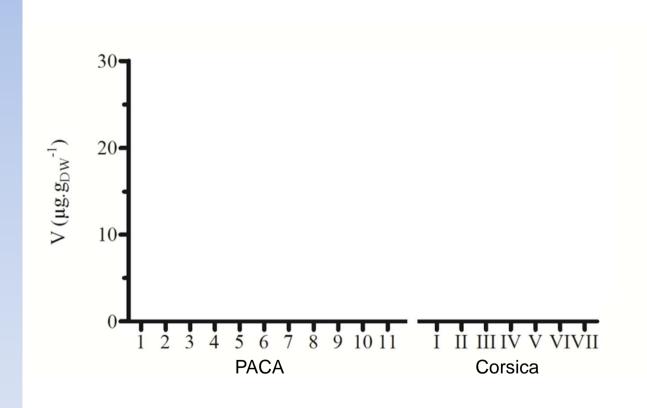
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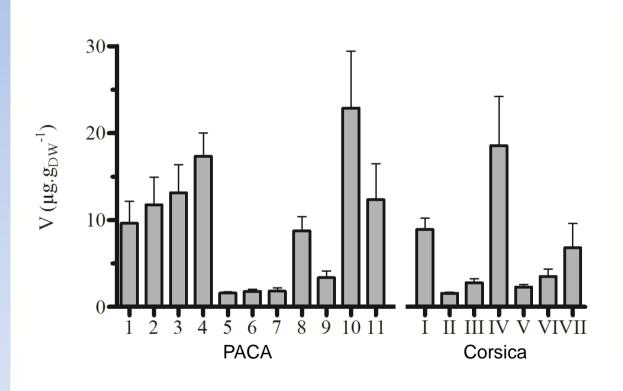












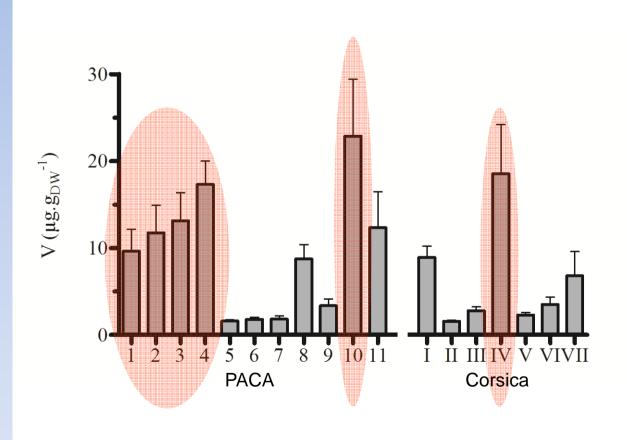












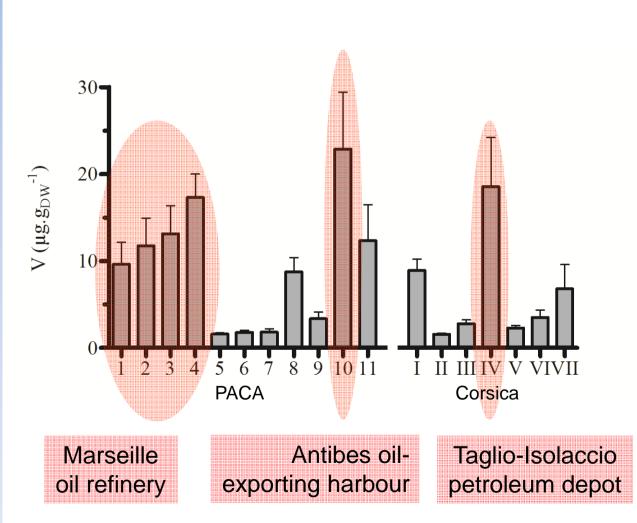


















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2 - mining (Sb, Zn) activities

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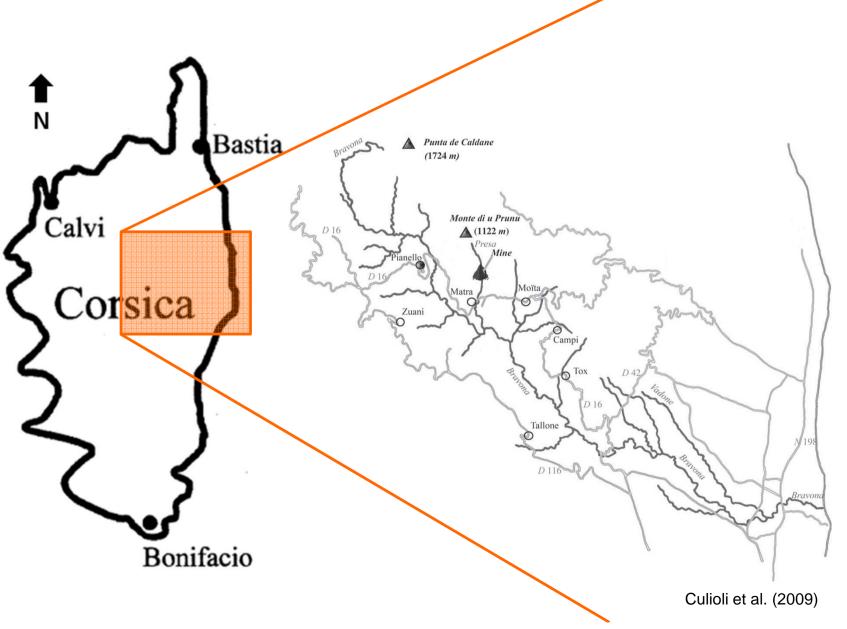










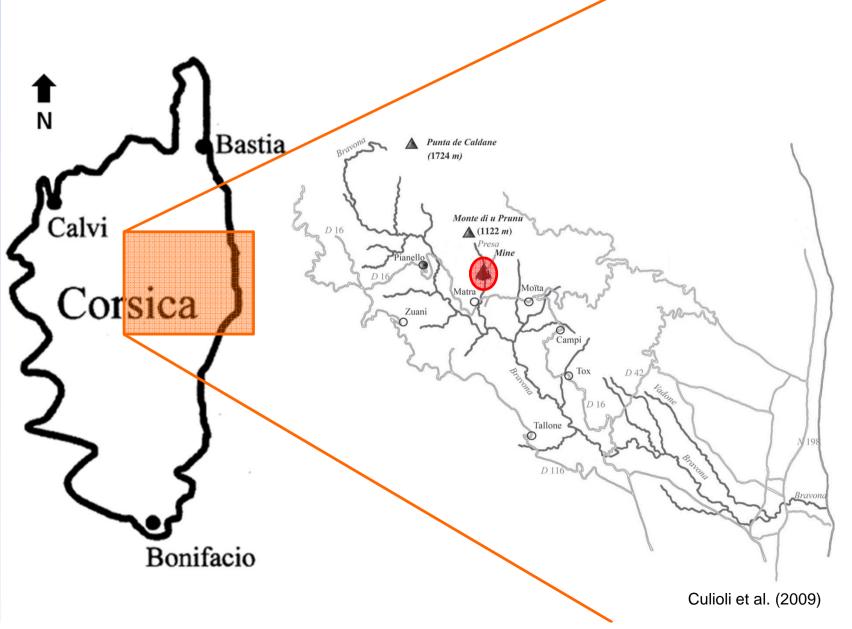








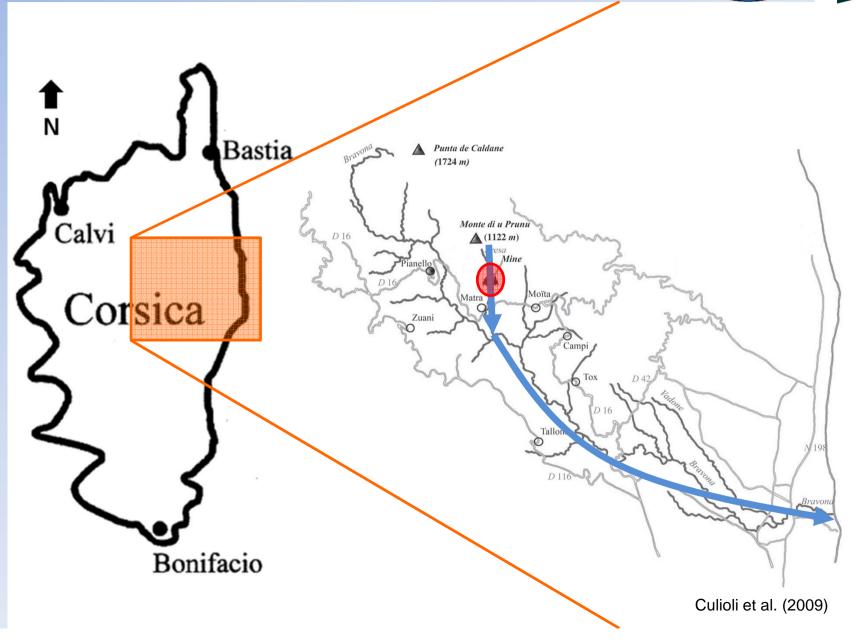








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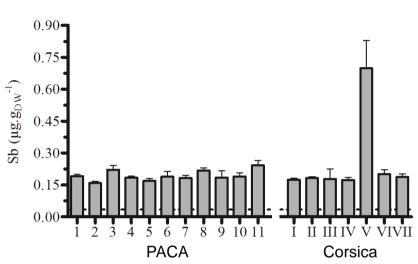










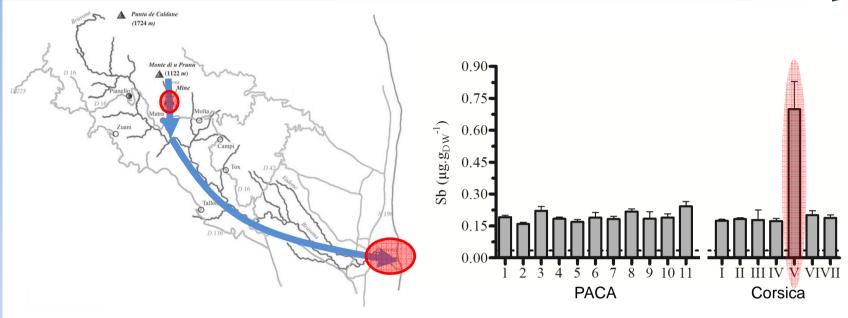








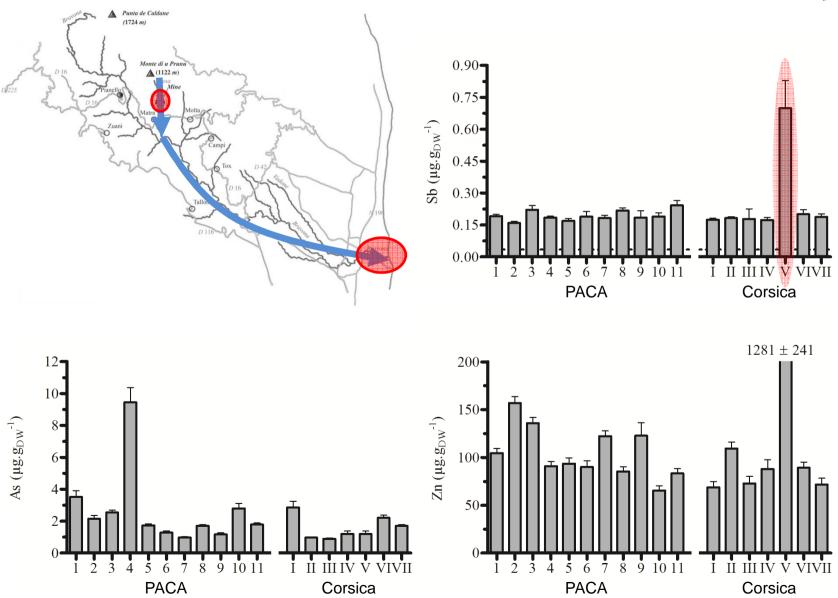








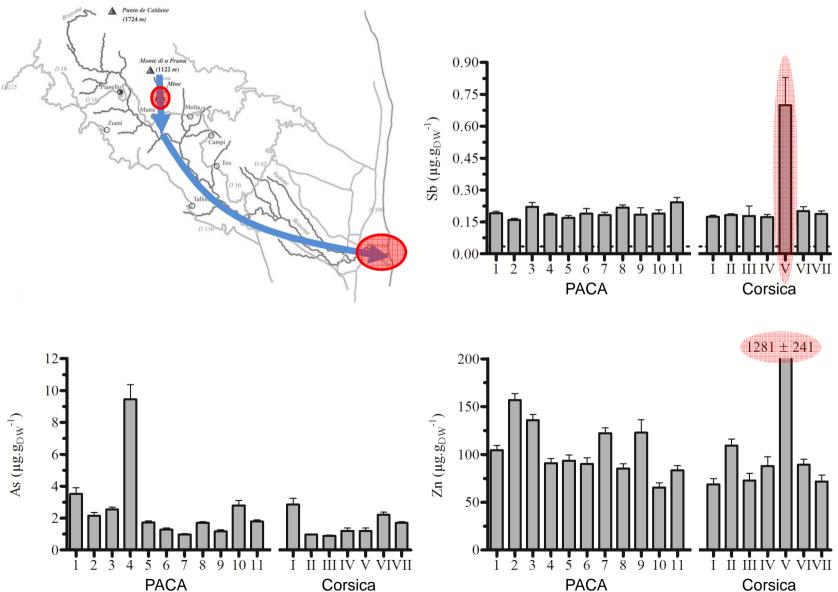








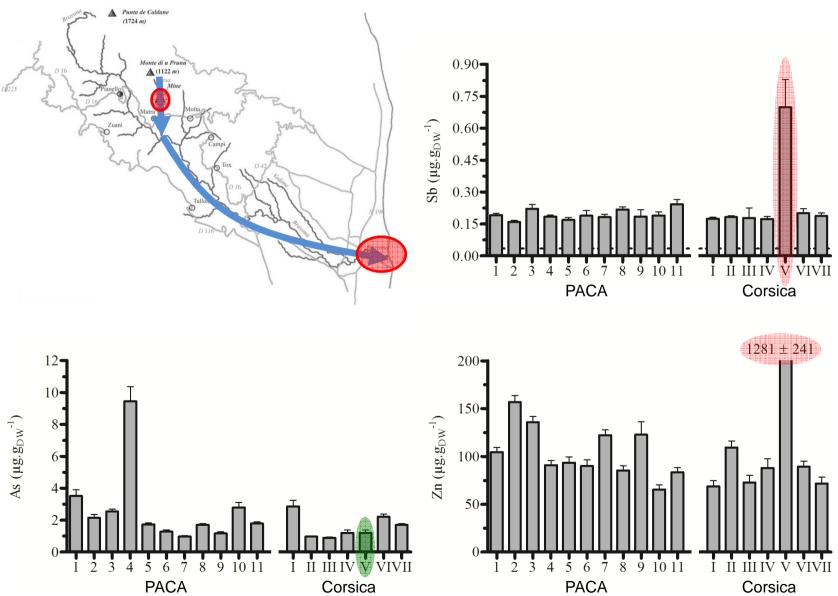




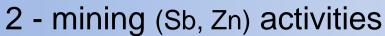






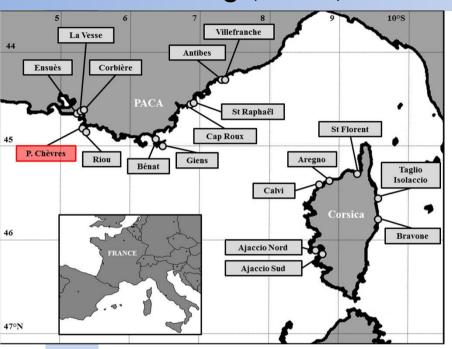


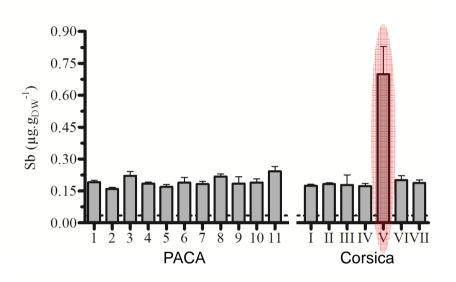


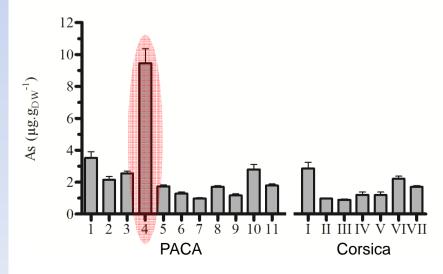


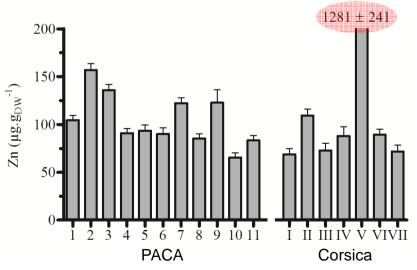






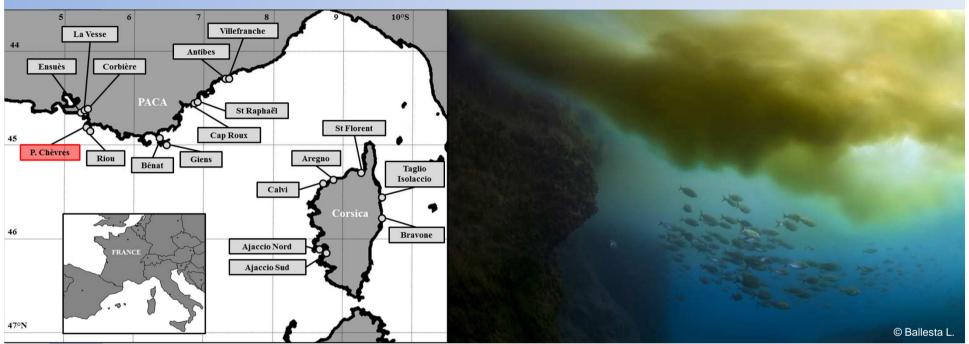


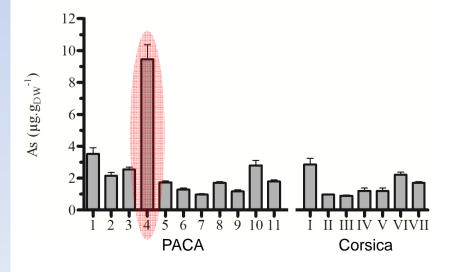






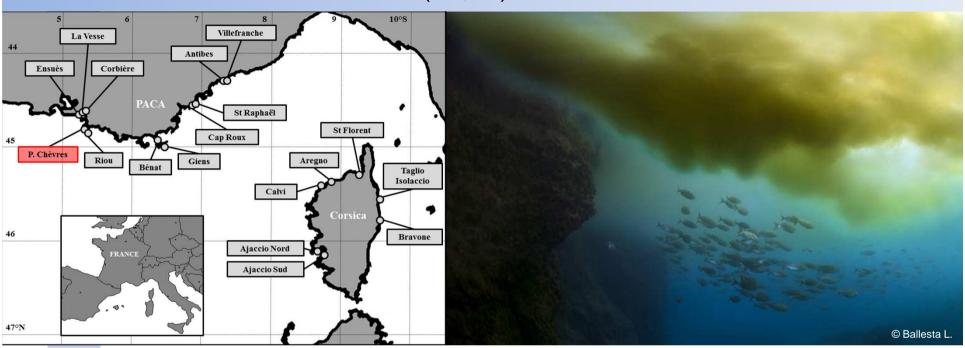
3 - cities and indutries (As, Bi)

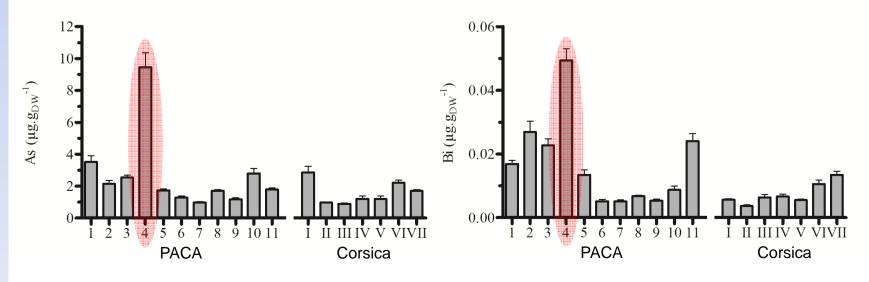


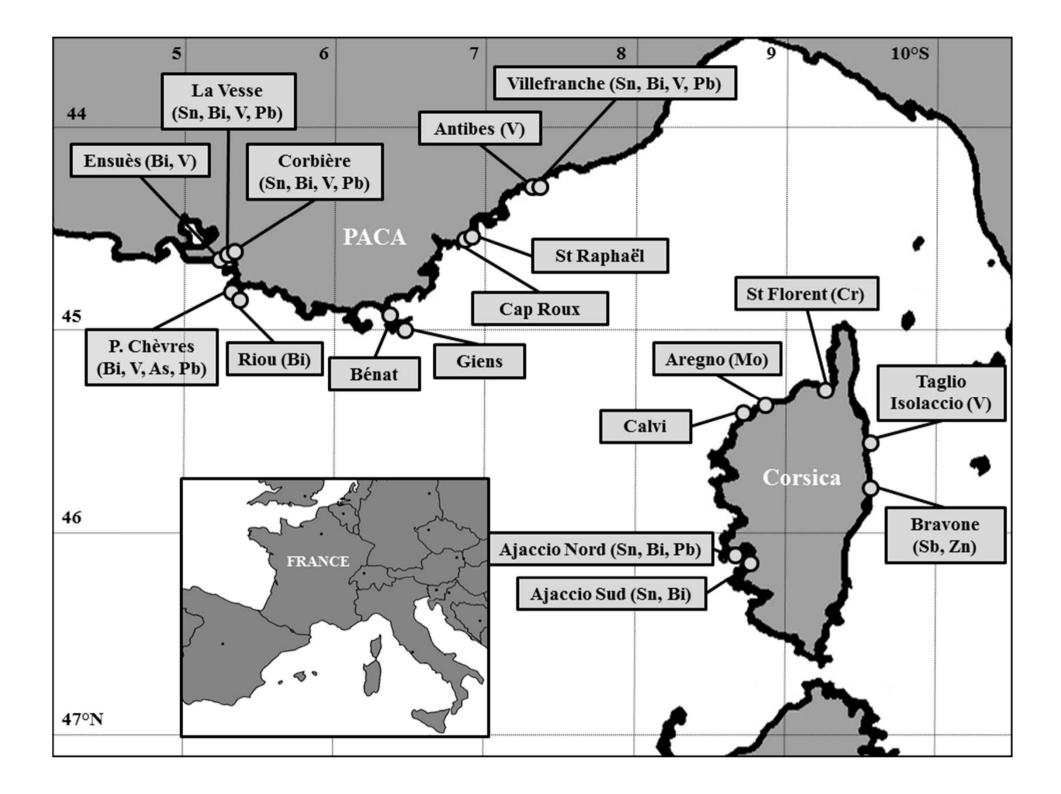


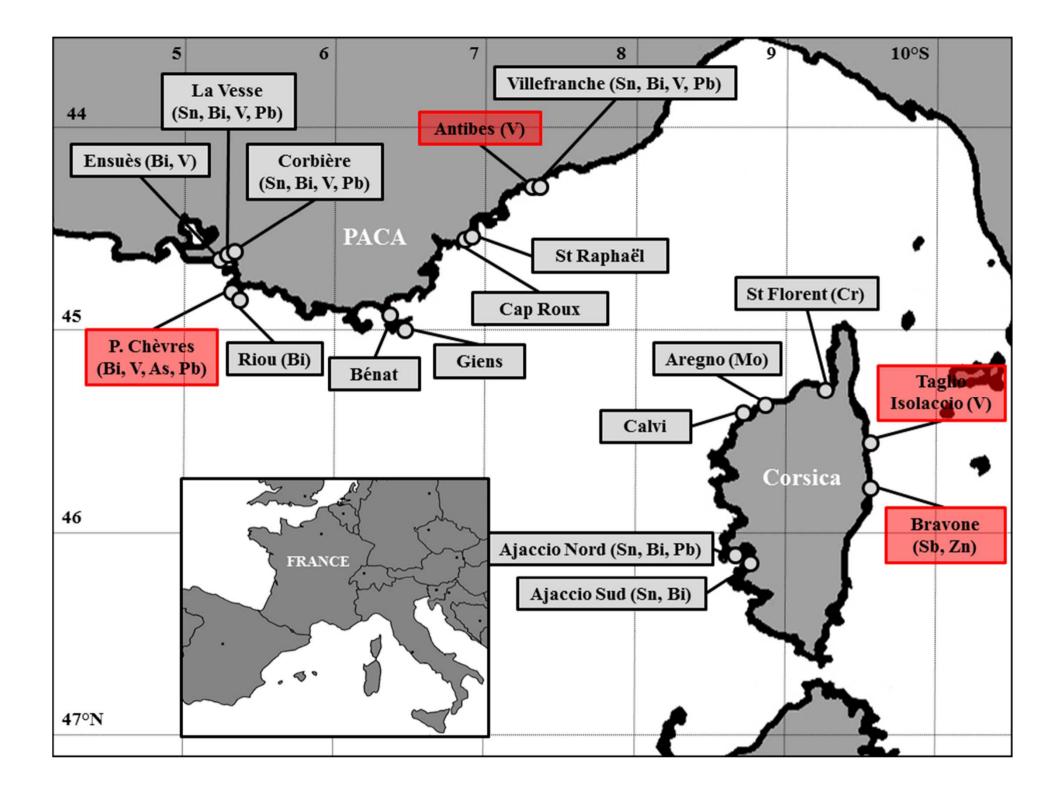


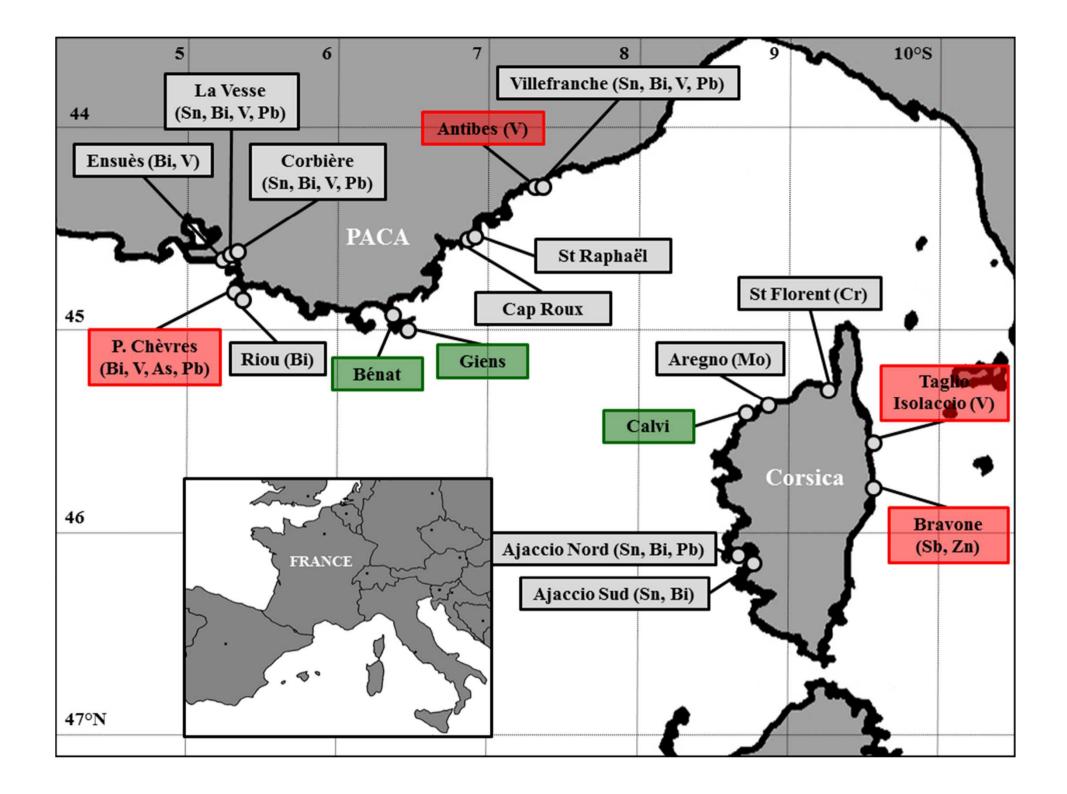
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Conclusions

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Conclusions

The French Mediterranean coasts are submitted to local (Sb, V), diffuse (Ag) and/or chronic (As) contaminations by trace elements.









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trace elements of environmental emerging concern **ARE** of concern.





Conclusions

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trace elements of environmental emerging concern **ARE** of concern.

Extension of the list of trace elements classically biomonitored to the 19 trace elements studied in this work (multielement analyser such as ICP-MS).





Specific objectives

- spatial variation of trace element levels in P. oceanica shoots along the French Mediterranean coasts ↔ polluting human activities;
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STARESO (Calvi, Corsica)







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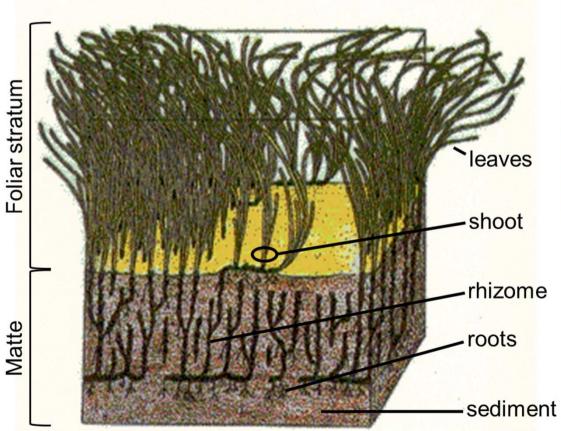




Site of reference for the biomonitoring of the pollution by trace elements in the Northwestern Mediterranean?



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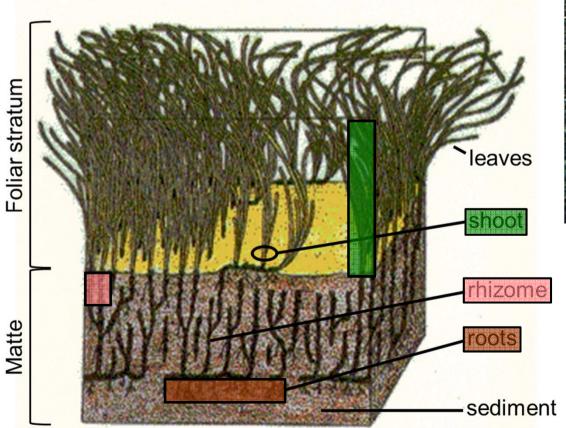






Site of reference for the biomonitoring of the pollution by trace elements in the Northwestern Mediterranean?

P. oceanica shoots, rhizome and roots;

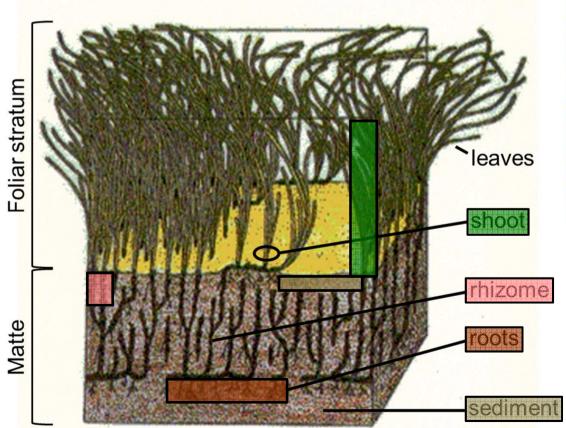






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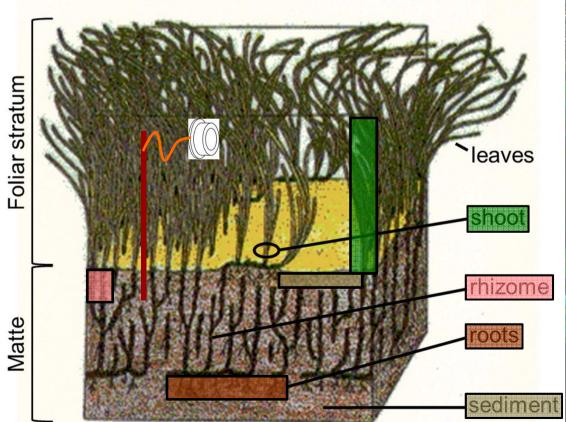






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- P. oceanica shoots, rhizome and roots;
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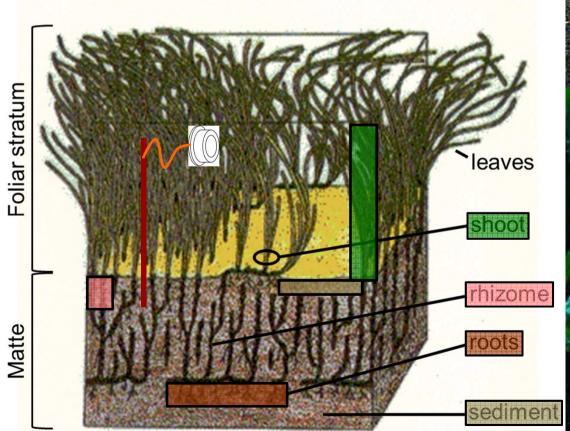






Site of reference for the biomonitoring of the pollution by trace elements in the Northwestern Mediterranean?

► [trace elements] low to very low → YES: site of reference.











Trace element concentrations in *P. oceanica* shoots (in µg.g⁻¹_{DW})

Site	Pb	Zn	V	Bi	Ref.
Sounion (Greece)					Sanz-Lázaro et al. (2012)
Spanish coasts					Sanchiz et al. (2000)
Favignana Island (Sicily)					Campanella et al. (2001)
Stareso (Corsica)					This study
General mean: French Med. coasts					This study
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Conclusions

REFERENCE







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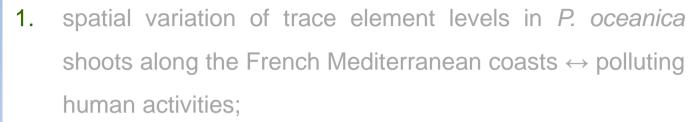
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STARESO meadow shelter an abundant, complex and diverse community of amphipod crustaceans (Michel 2011, Sturaro 2012).



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P. oceanica vs. M. galloprovincialis



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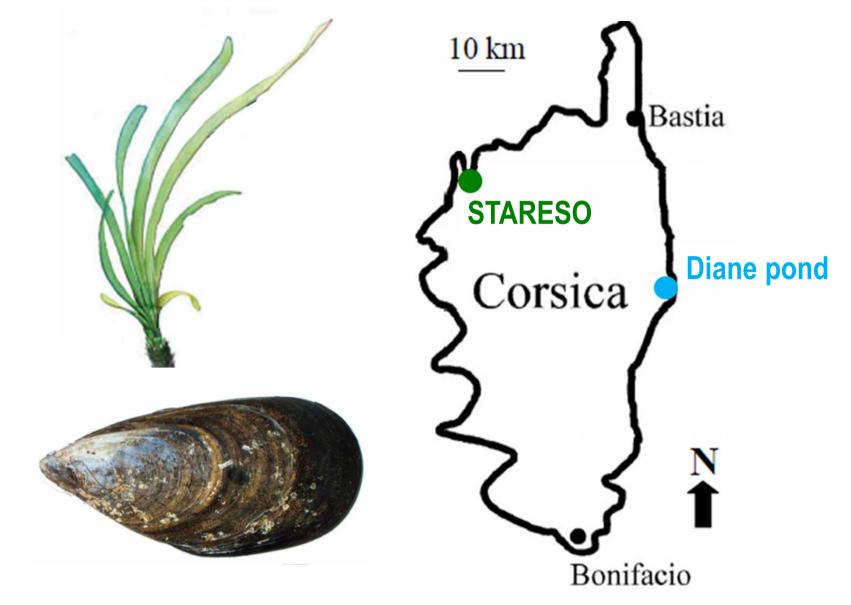
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P. oceanica vs. M. galloprovincialis



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Diane pond















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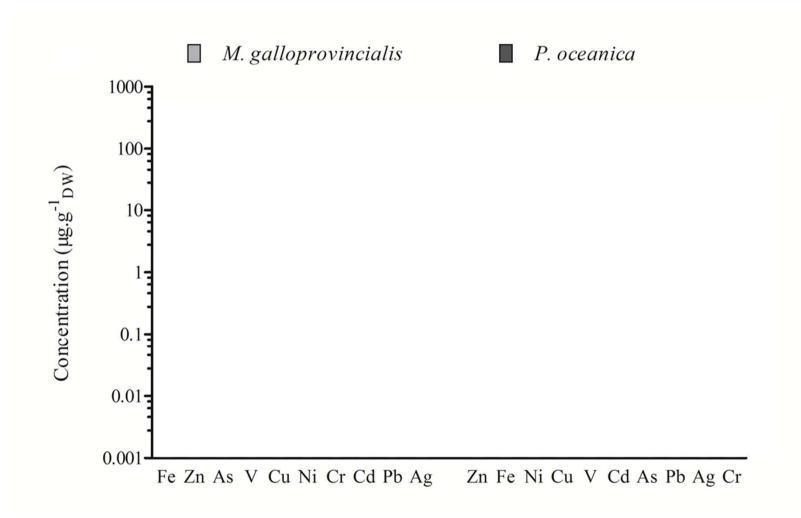
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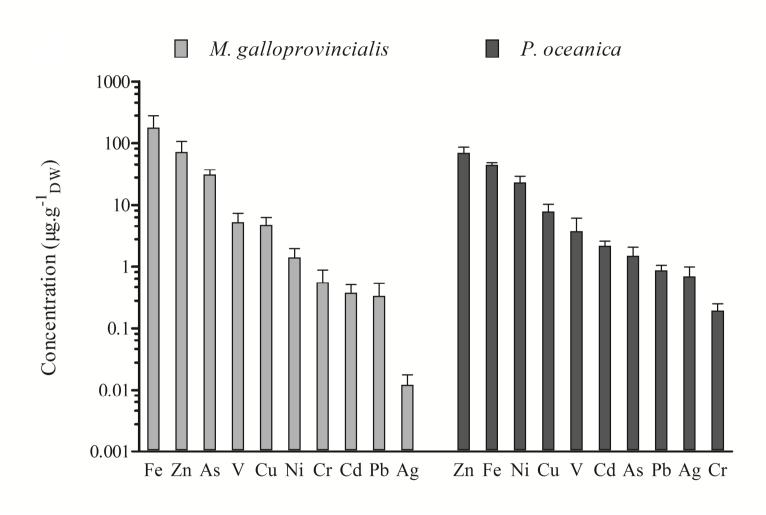
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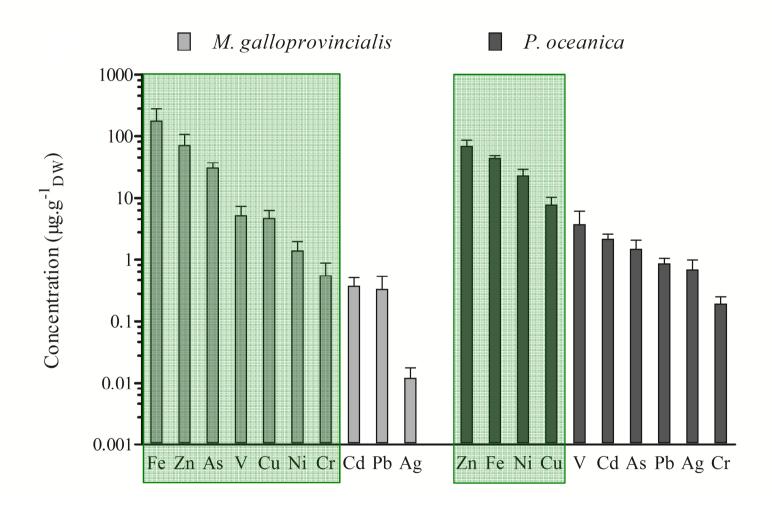
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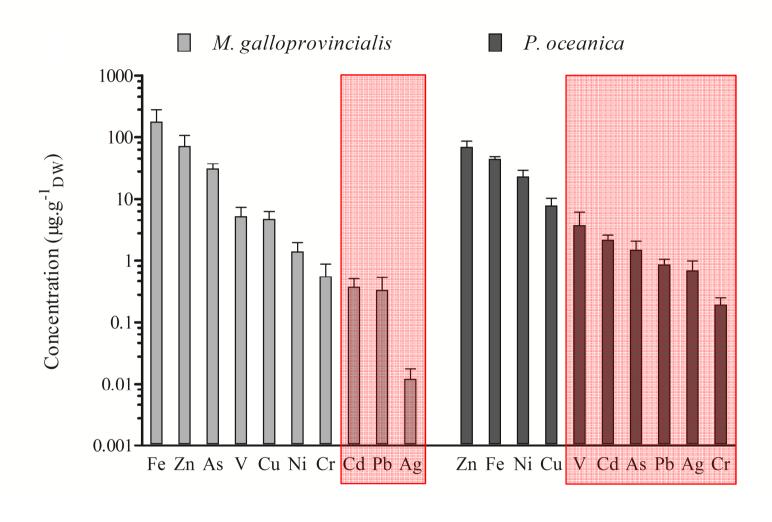
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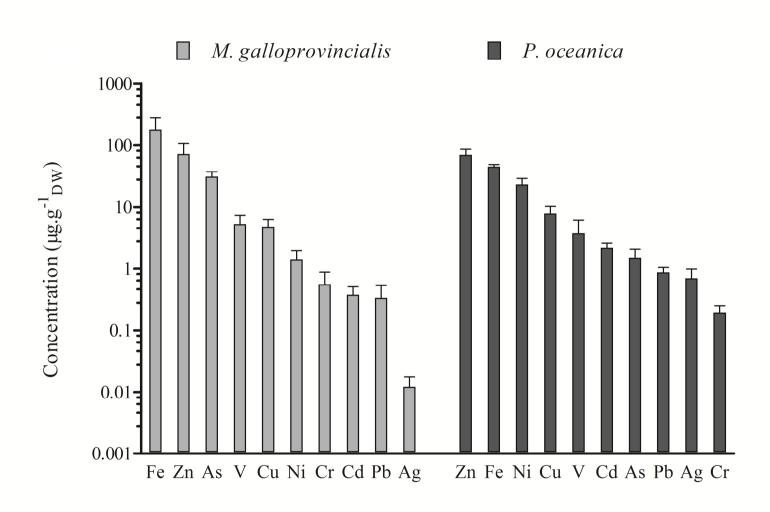








Trace elements broadly studied



Bioaccumulation factors from seawater to:

- $M. galloprovincialis = 2.7 10^4$
- *P.* oceanica = $2.5 \cdot 10^4$

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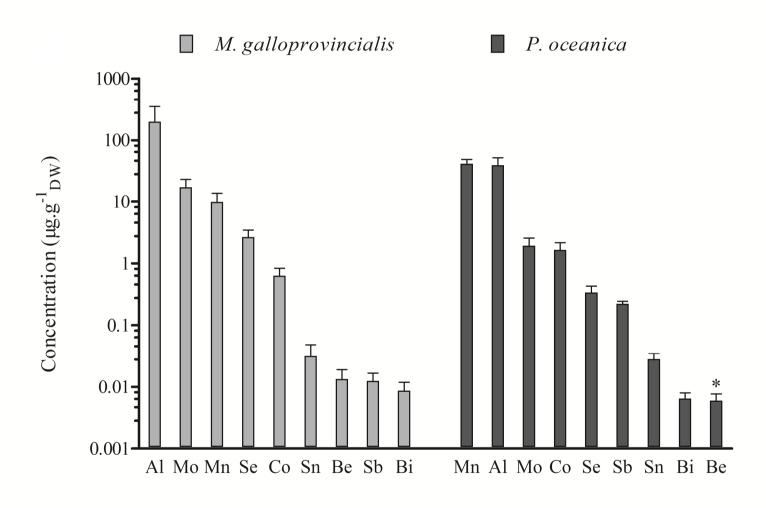
M

N





Trace elements little monitored



U

M

0

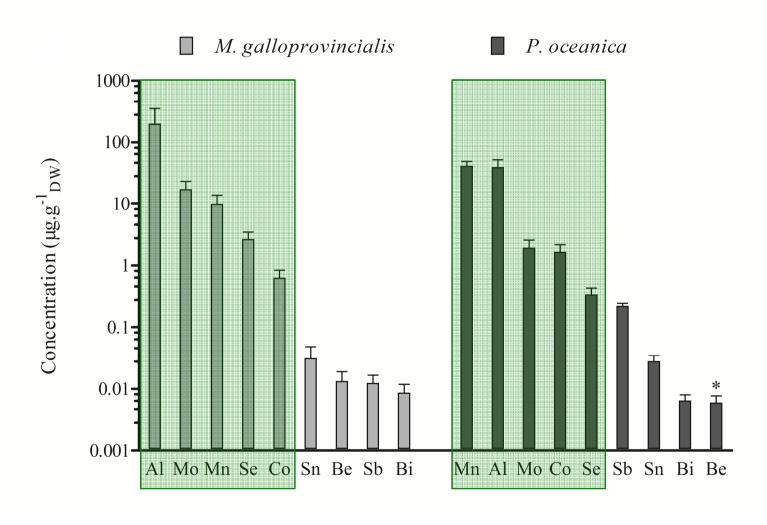
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Cean old



Trace elements little monitored





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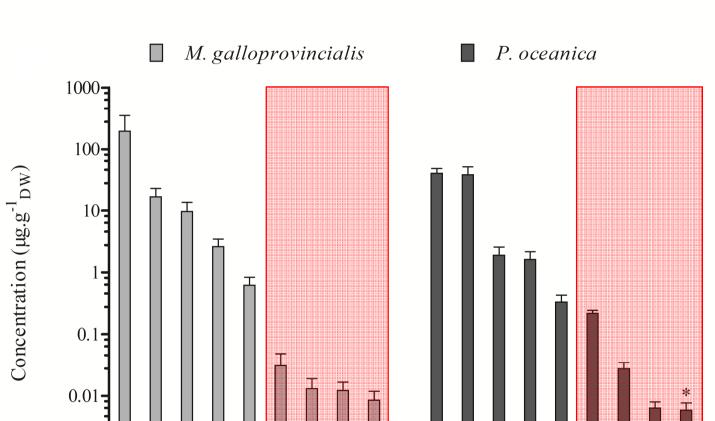


0.001



Mn Al Mo Co Se Sb Sn Bi Be

Trace elements little monitored



Al Mo Mn Se Co Sn Be Sb Bi

U

M

0

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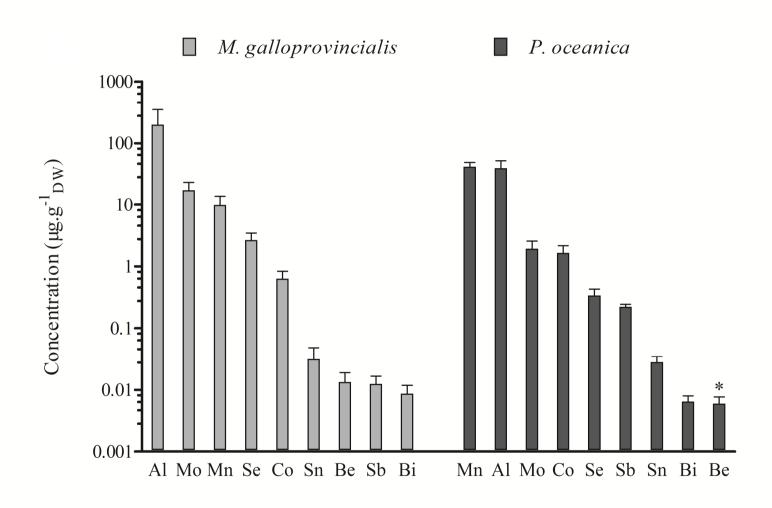








Trace elements little monitored



Bioaccumulation factors from seawater to: •

- $M. galloprovincialis = 5.2 10^4$
- *P.* oceanica = $3.1 \ 10^4$



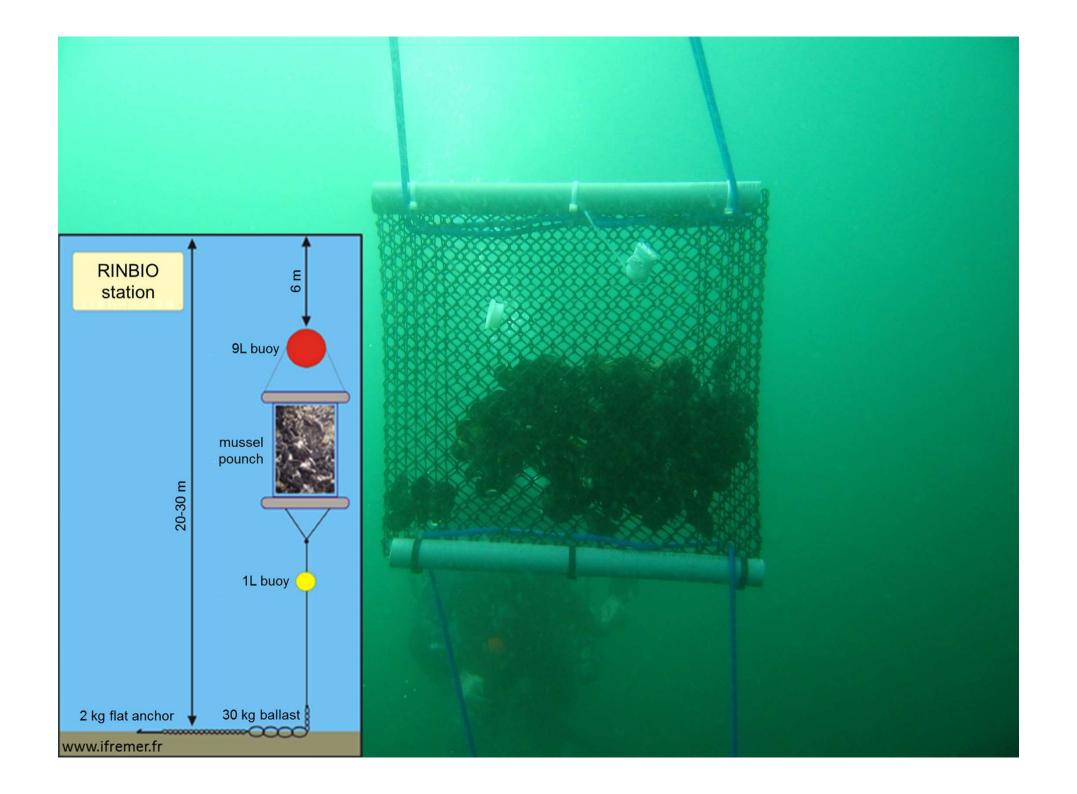


Cear object



Mussel caging

В U M 0 N



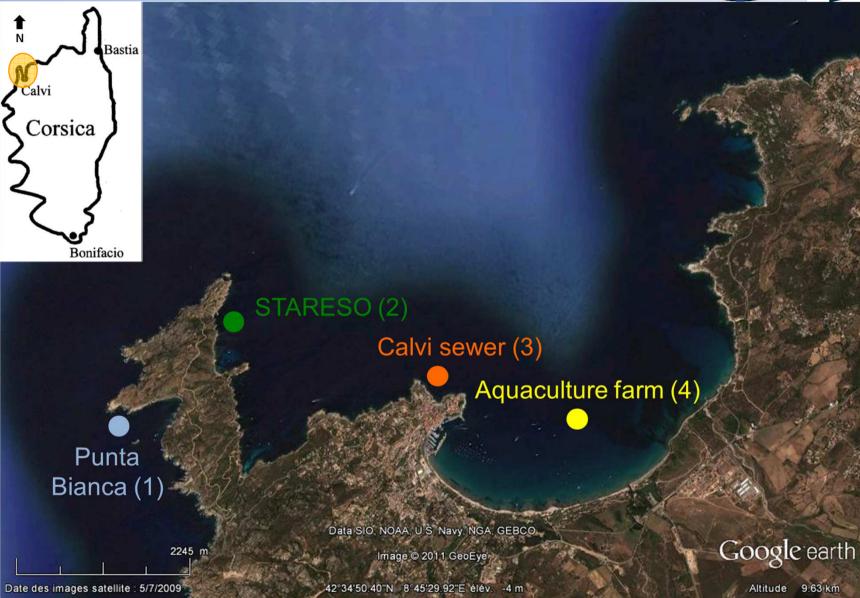














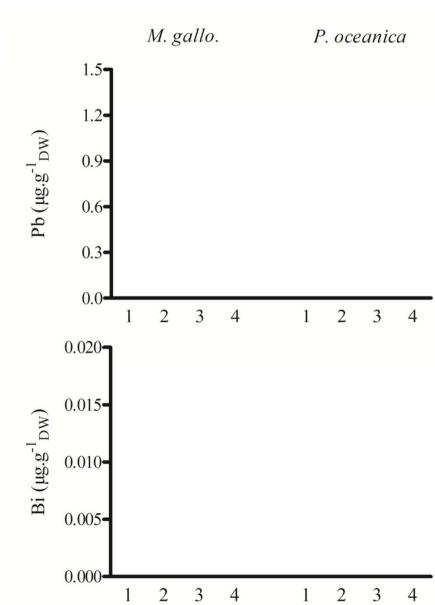
Mussel caging





Lifestyle of organisms







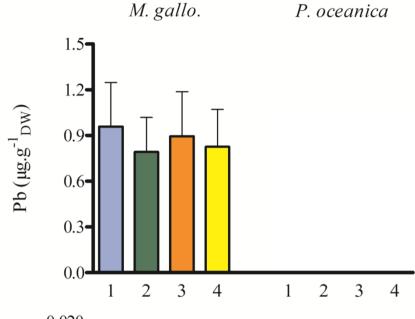




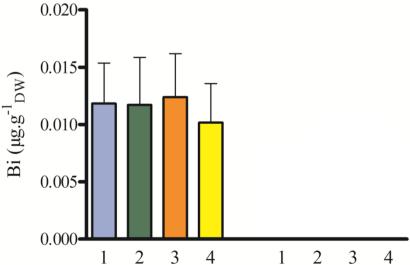


Lifestyle of organisms

M. galloprovincialis = filter feeder
 → homogenous and clean status of the water column;









Mussel caging

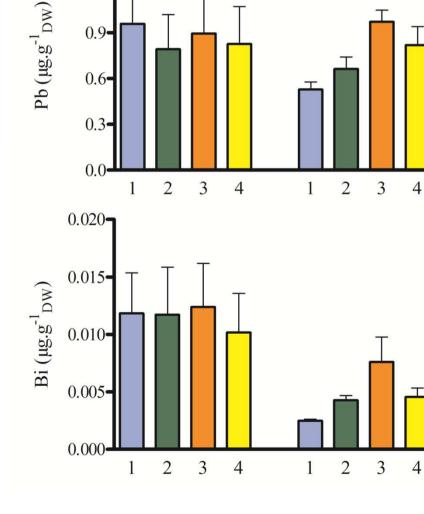


P. oceanica



Lifestyle of organisms

- *M. galloprovincialis* = filter feeder \rightarrow homogenous and clean status of the water column;
- P. oceanica = rooted primary producer
- → weak point sources of long-term accumulations of pollutants in sediments.



M. gallo.

1.5

1.2-

0.9-

0.6







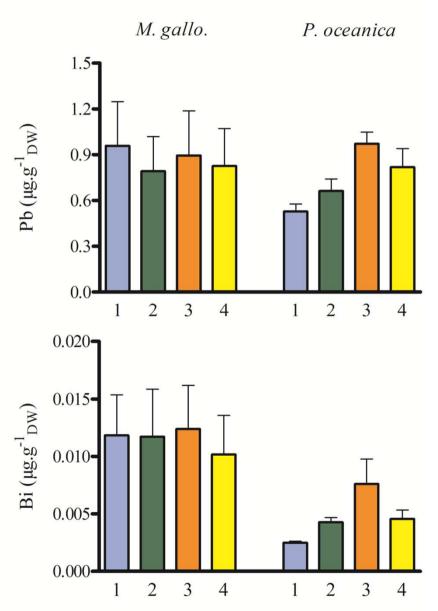




Lifestyle of organisms

- M. galloprovincialis = filter feeder
 → homogenous and clean status of the water column;
- P. oceanica = rooted primary producer
 → weak point sources of long-term
 accumulations of pollutants in sediments.
- ▶ 2 complementary species concomitantly used.







U

M

U

0

N







Conclusions

Ν









Conclusions

Both bioindicators effectively bioaccumulate trace elements and give complementary information on the health status of their environment — lifestyle.

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Conclusions

Both bioindicators effectively bioaccumulate trace elements and give complementary information on the health status of their environment → lifestyle.

M. galloprovincialis: overall health status of the water body; P. oceanica: time-integrated response to weak human impact.







Both bioindicators effectively bioaccumulate trace elements and give complementary information on the health status of their environment \rightarrow lifestyle.

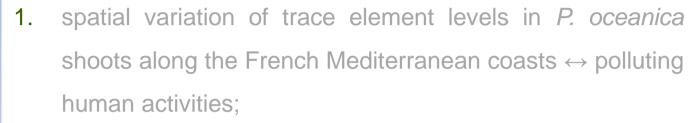
── *M. galloprovincialis*: overall health status of the water body; P. oceanica: time-integrated response to weak human impact.

→ Important to sample appropriate stations to properly determine the health status of a specific site, mostly in monitoring surveys using *P. oceanica*.



•000000000000

Trace element kinetics



- 2. definition of conditions/levels of reference for the Northwestern Mediterranean;
- 3. influence of *P. oceanica* and *M. galloprovincialis* lifestyle on trace element bioaccumulation behaviour;
- 4. trace elements uptake and loss kinetics by both species.









In situ contamination of P. oceanica

KINETICS

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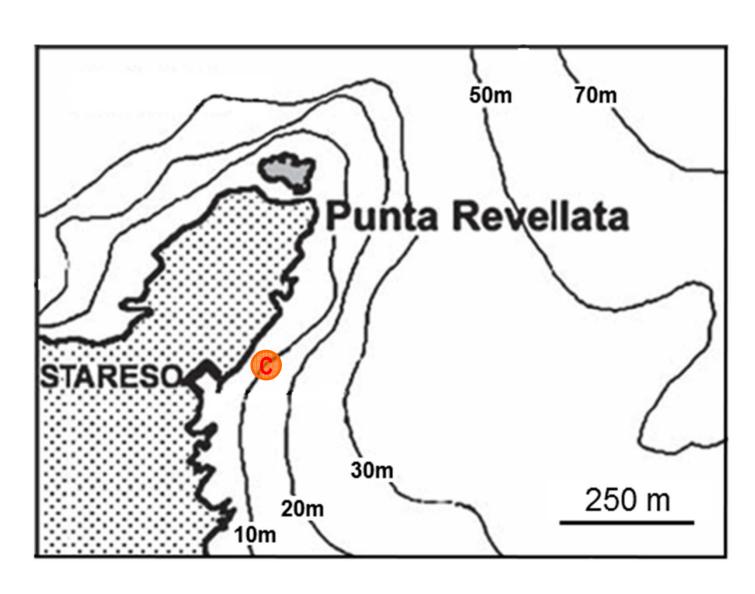


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In situ contamination of P. oceanica





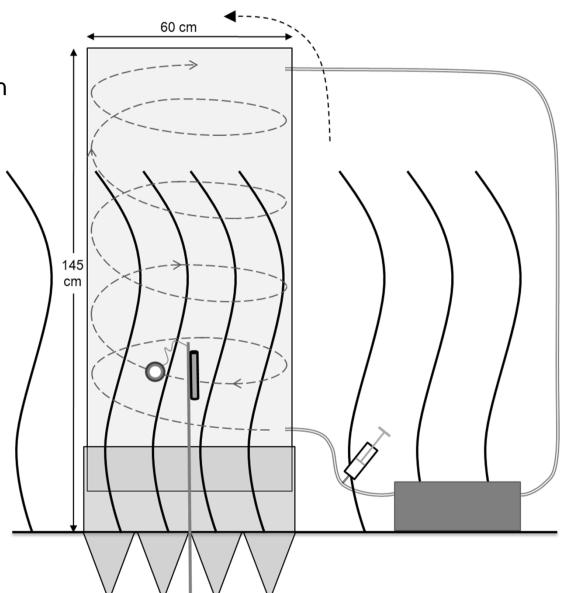
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Mesocosm

- 5 days of contamination in June 2009;
- 410L bell-shaped mesocosm;
- Contamination every 12 hours (9am-9pm);
- 15 days of decontamination.



C S









Mesocosm



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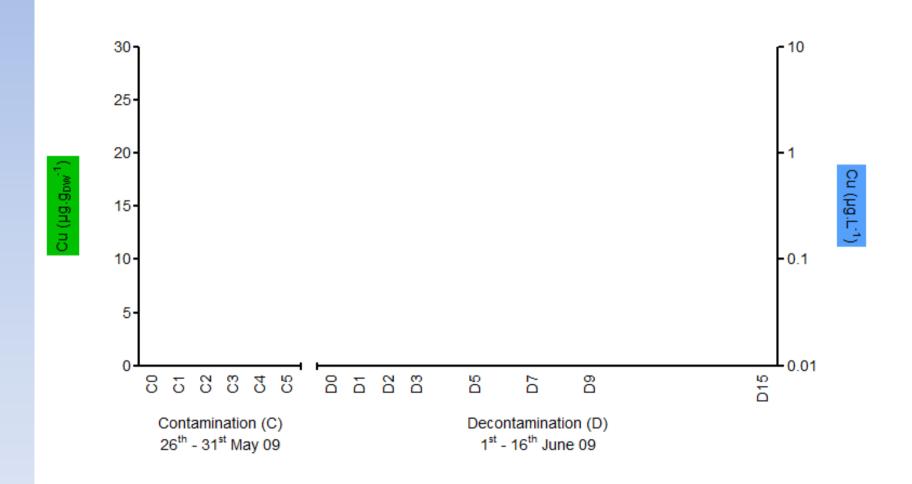
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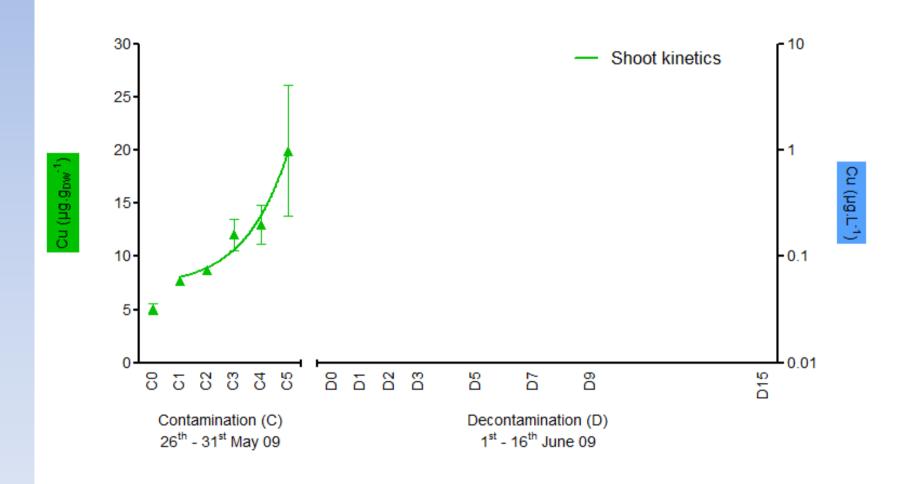
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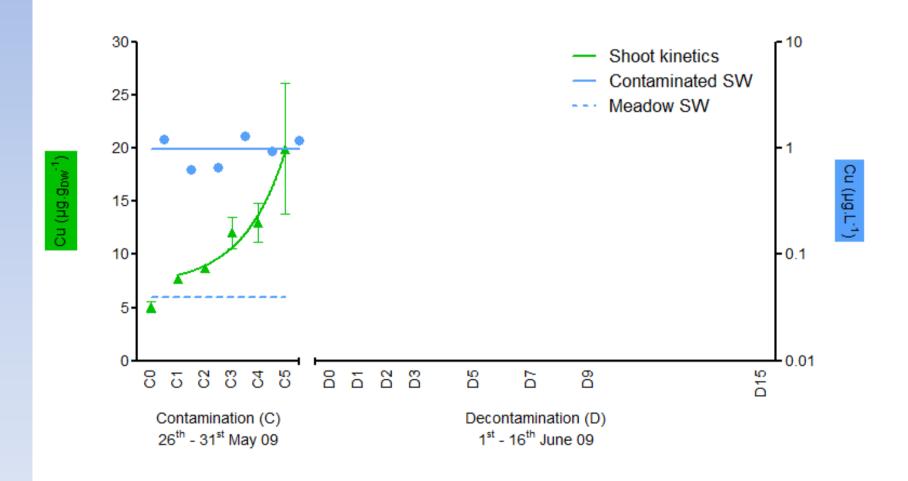
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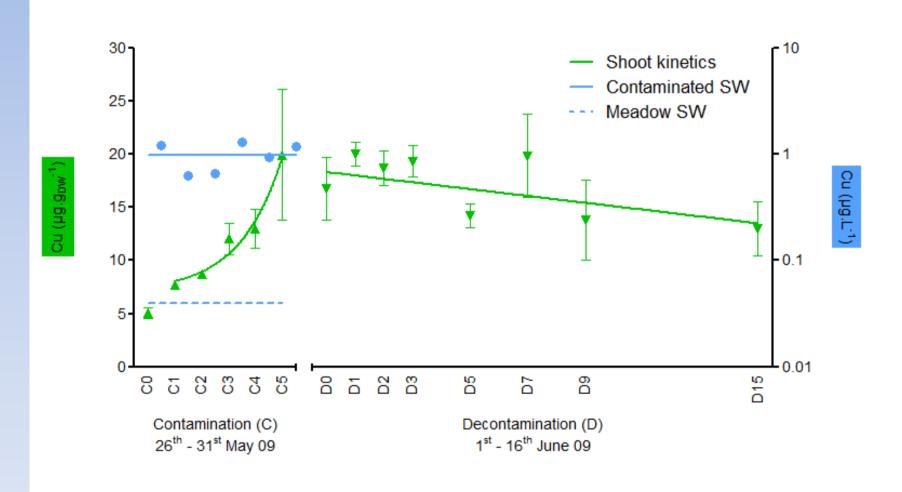
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Con object



Cu in rhizomes

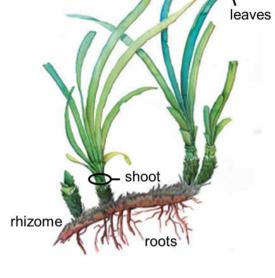
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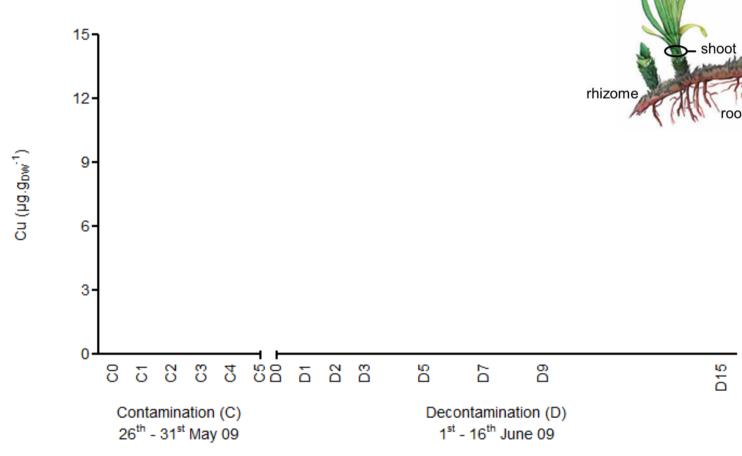
E

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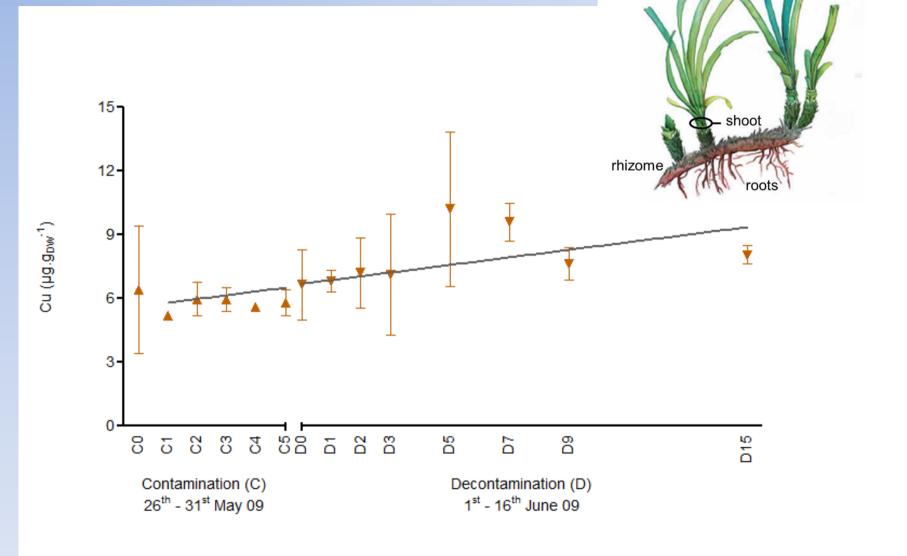
Cu in rhizomes







Cu in rhizomes



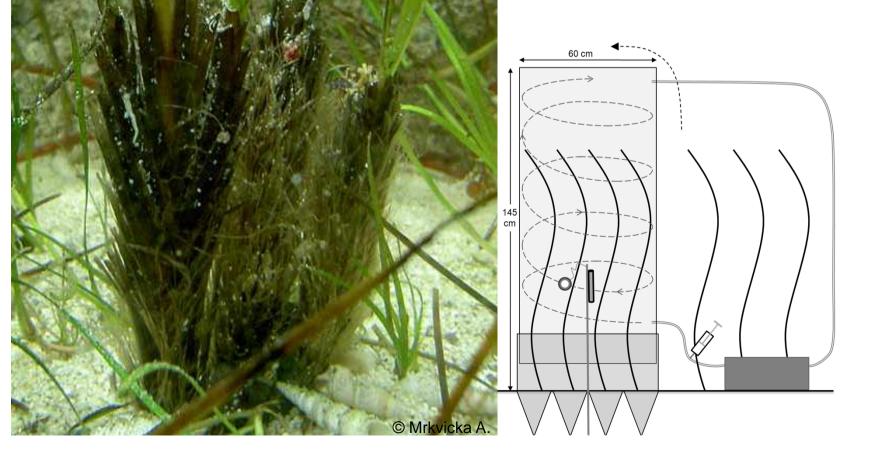
leaves

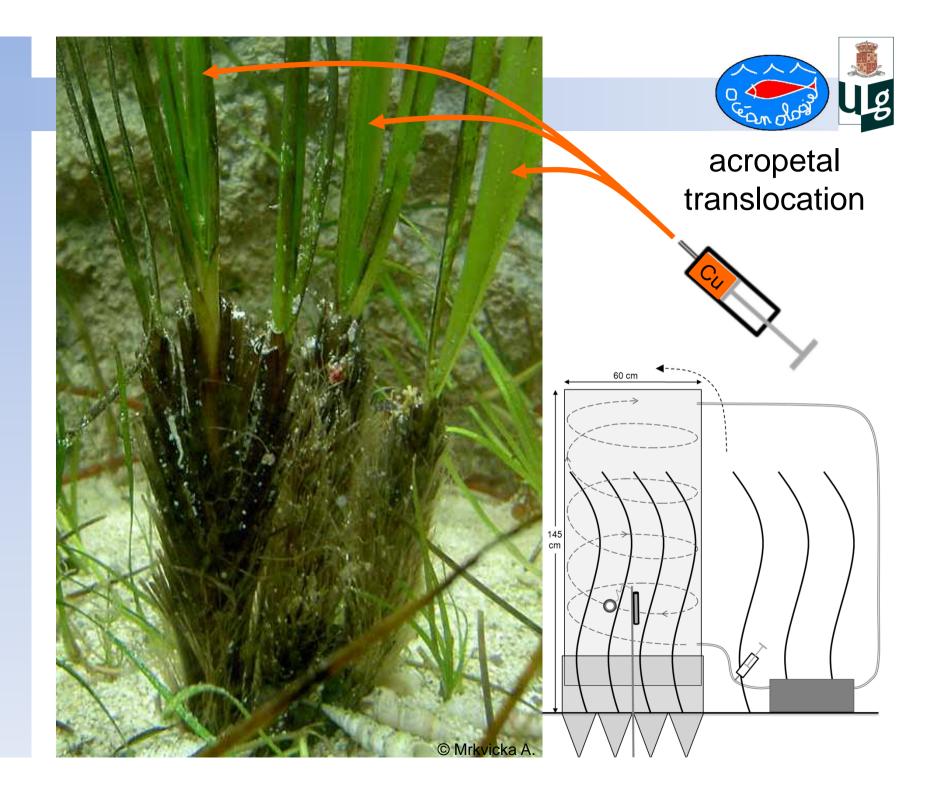
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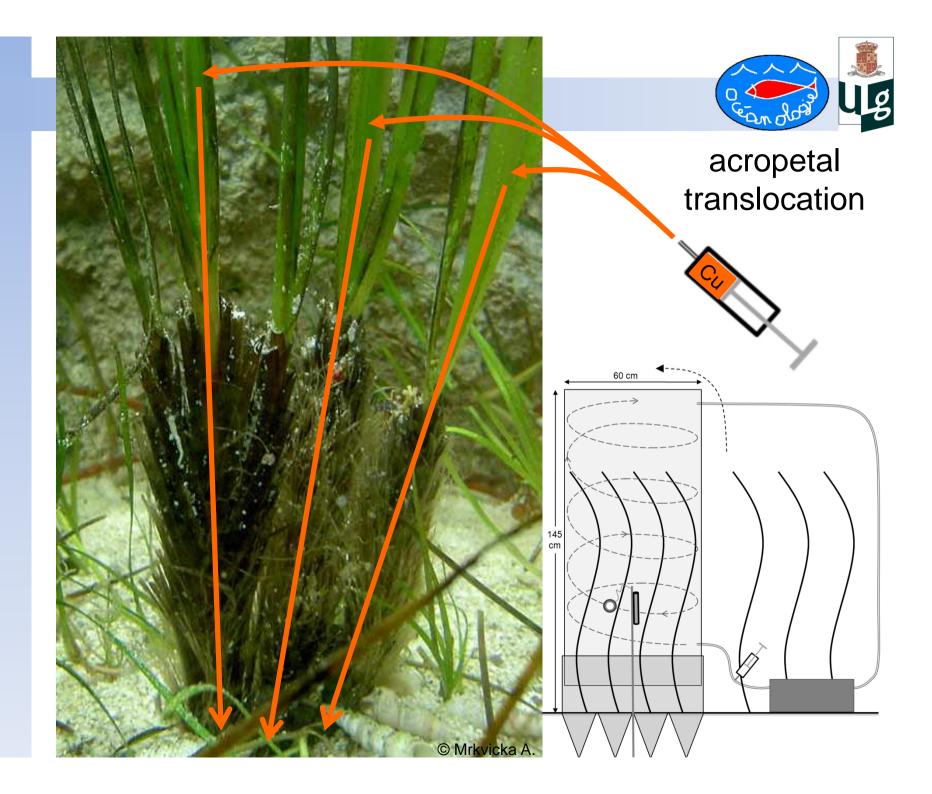




acropetal translocation











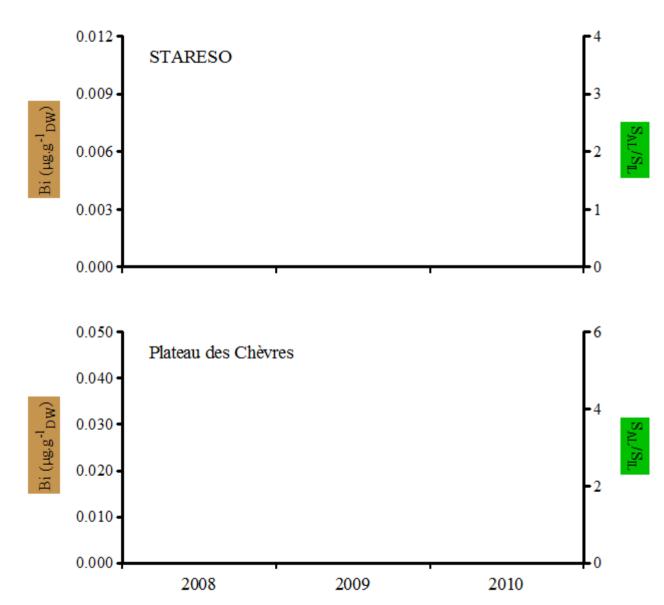
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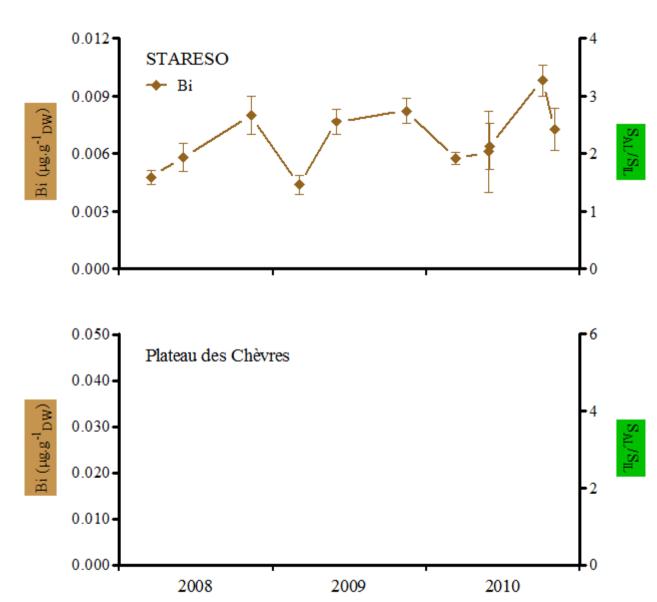










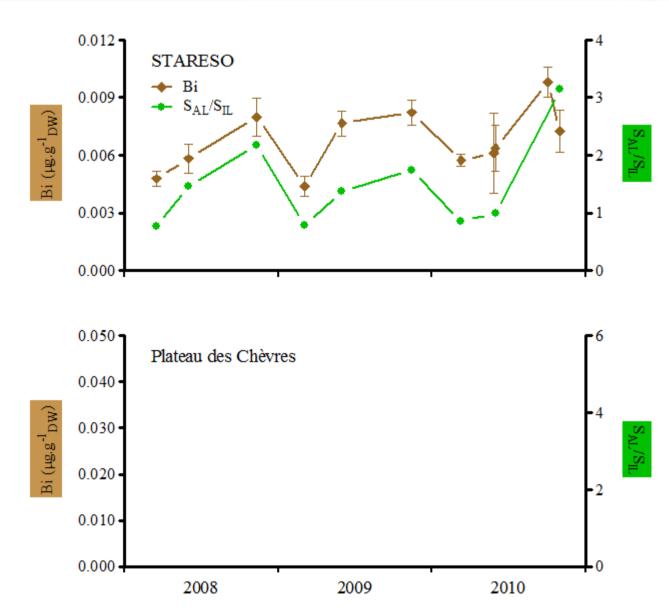










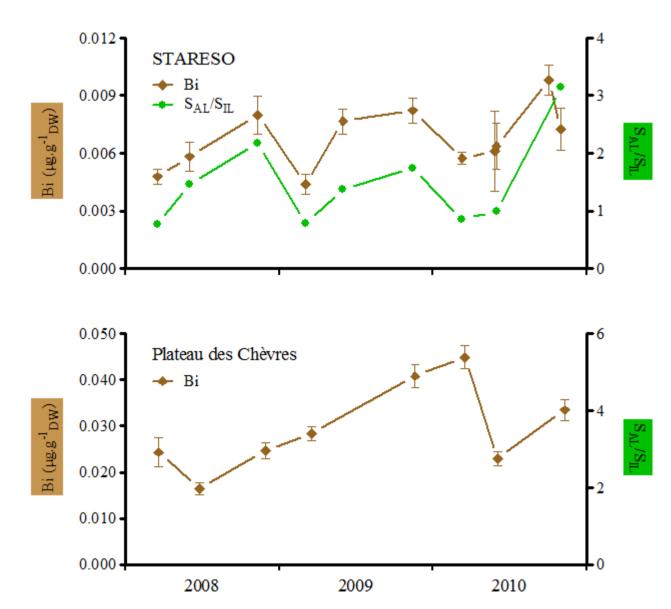










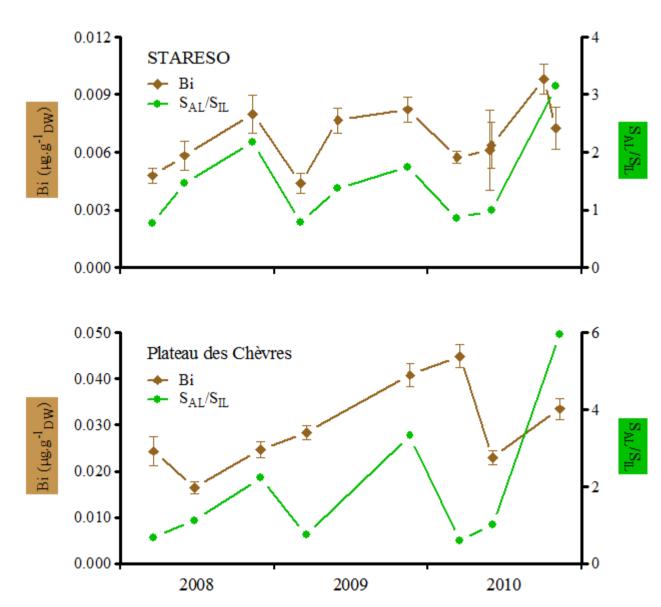














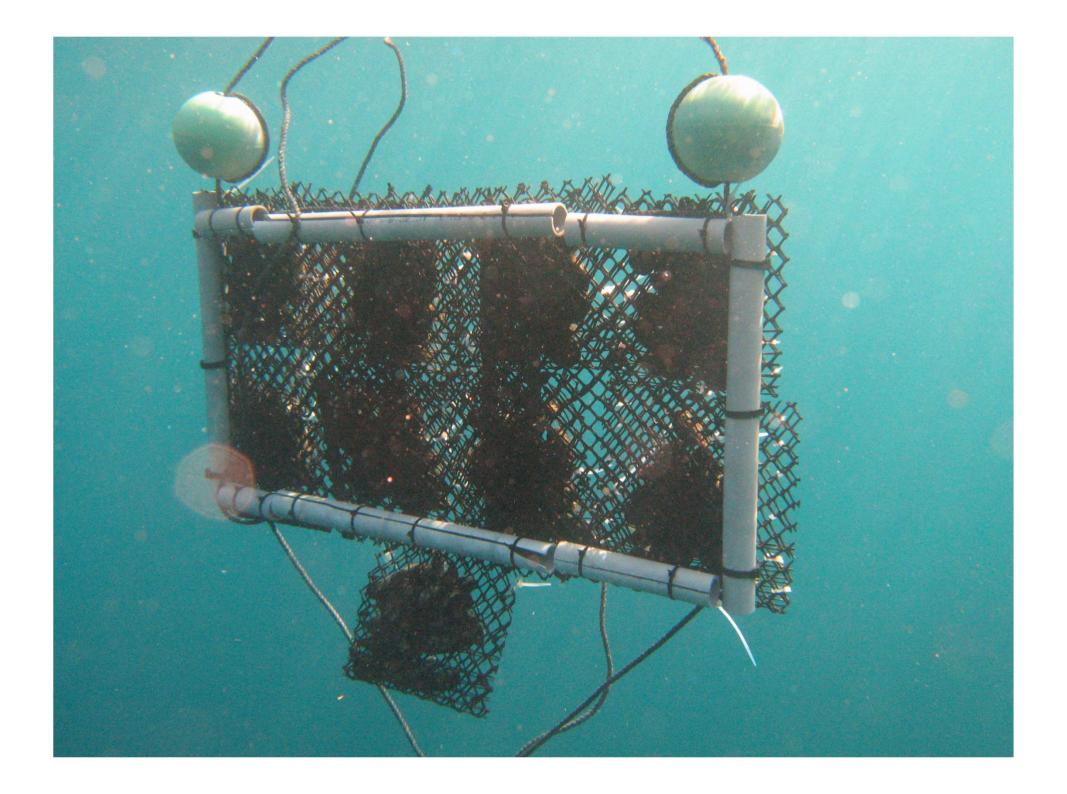






Mussel caging

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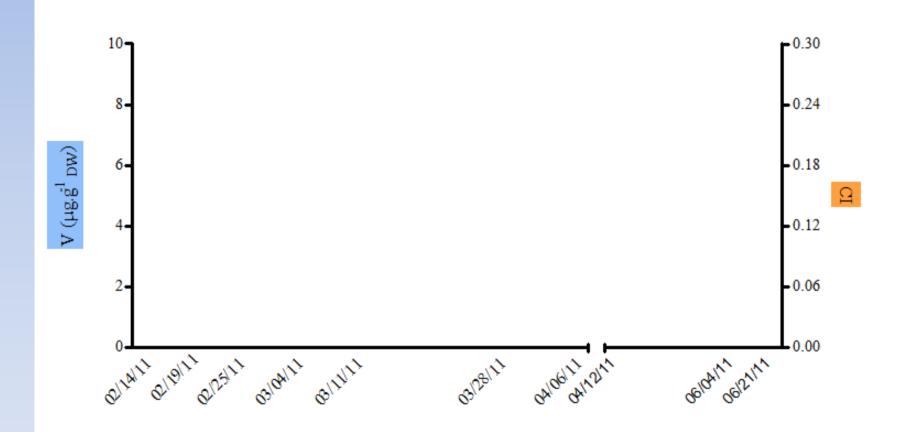
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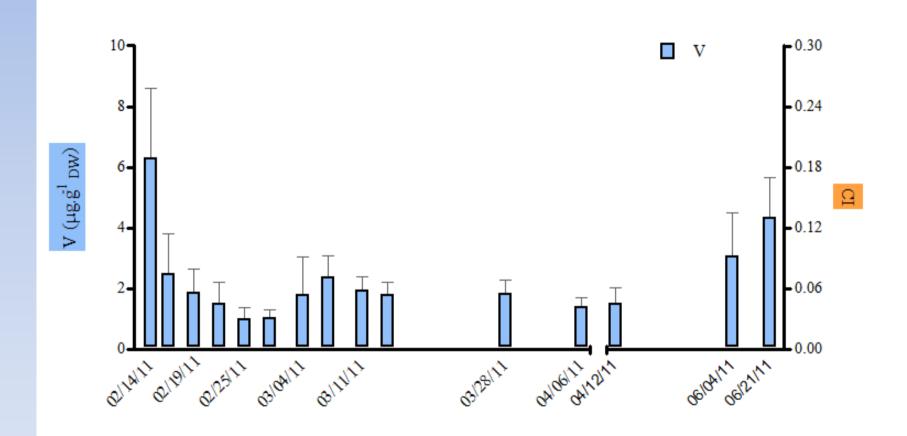
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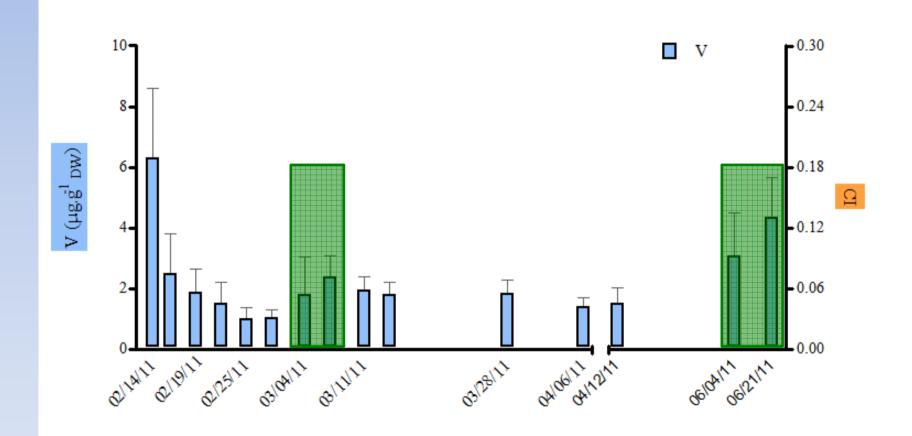
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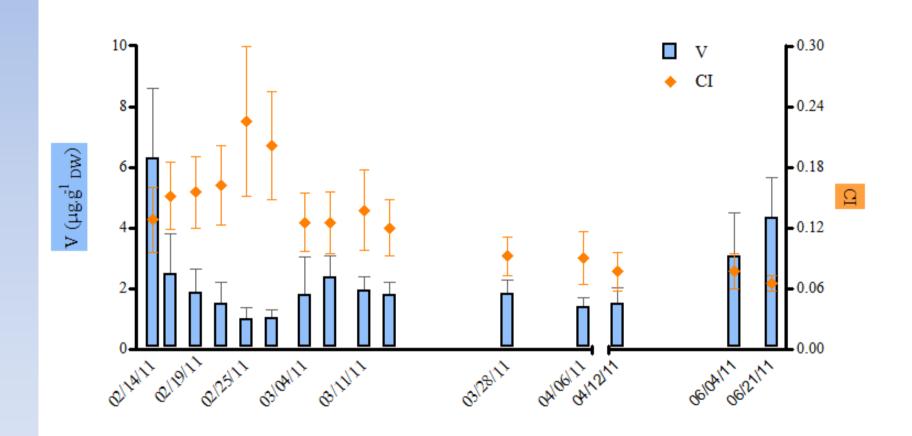
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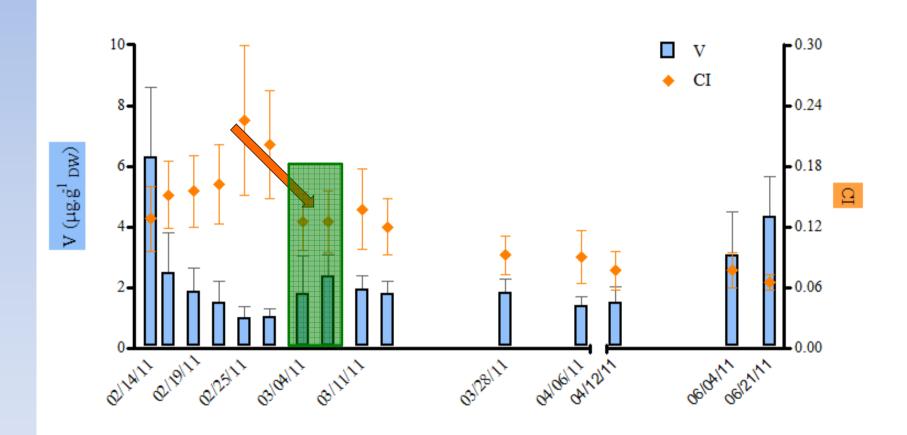
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Cean olds





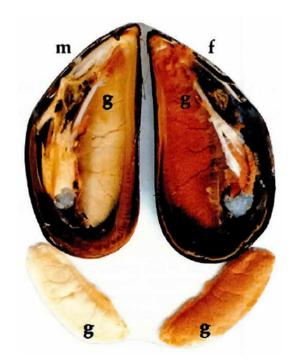






- Influence of size on TE accumulation by mussels grown on ropes:
 - → YES?
 - \rightarrow NO?
- Influence of sex on TE accumulation prior to spawn:
 - → males vs females.



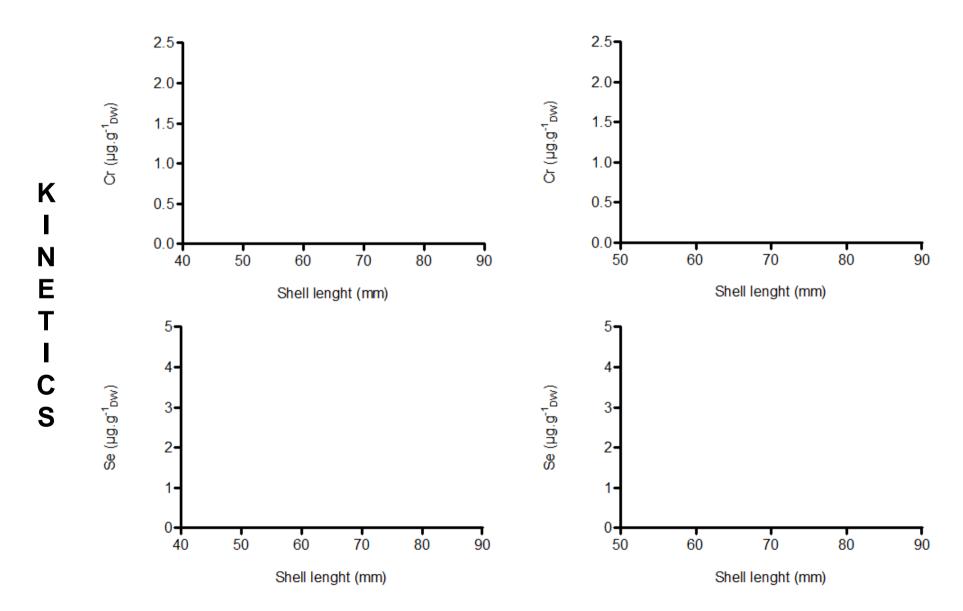










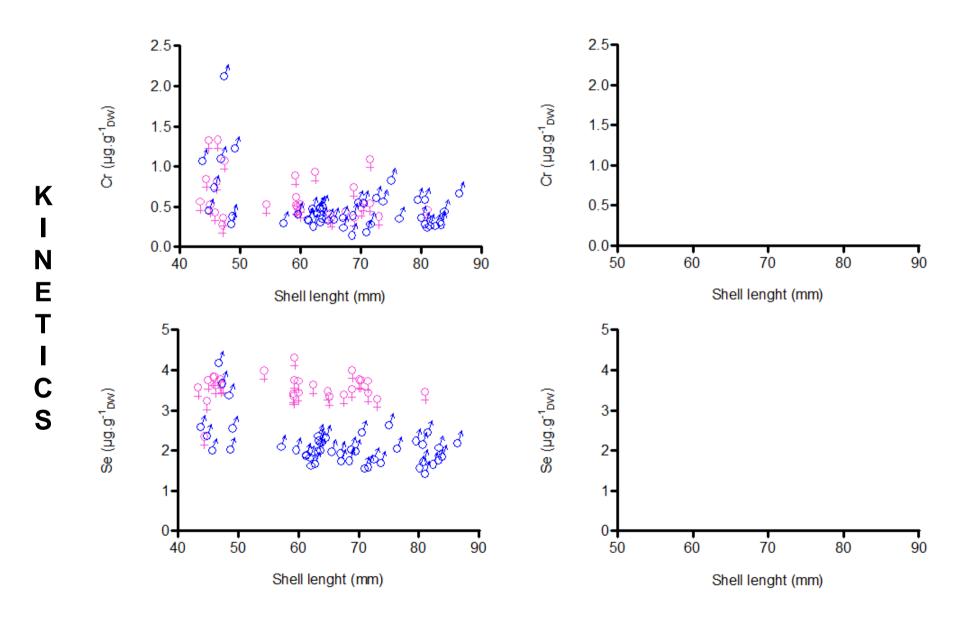










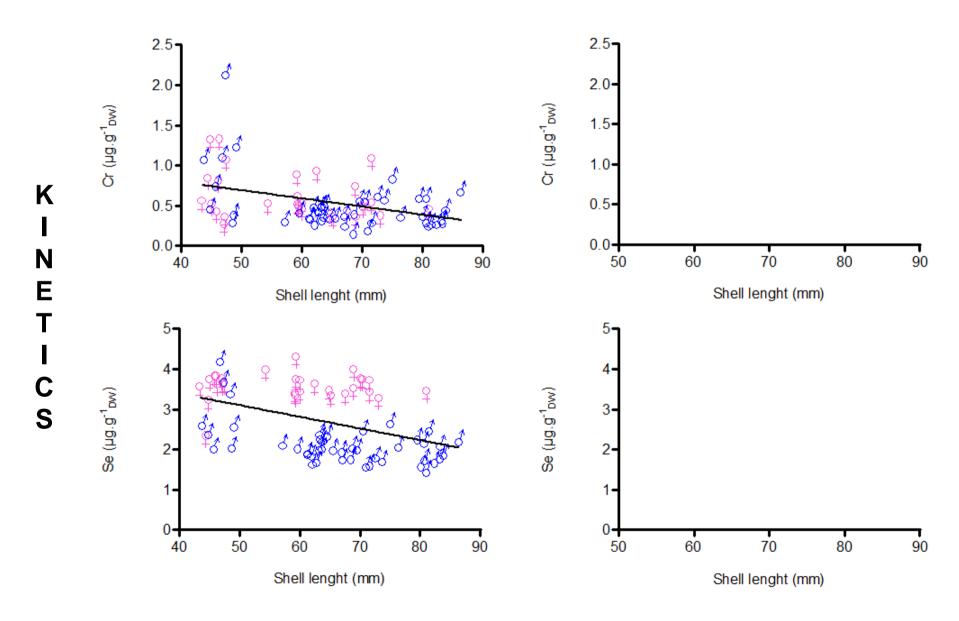






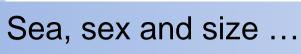






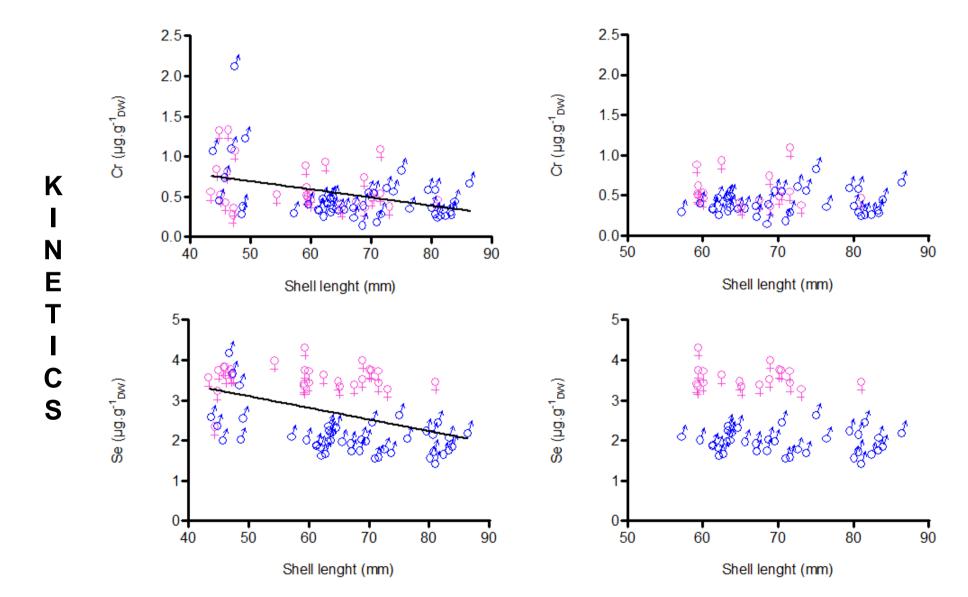










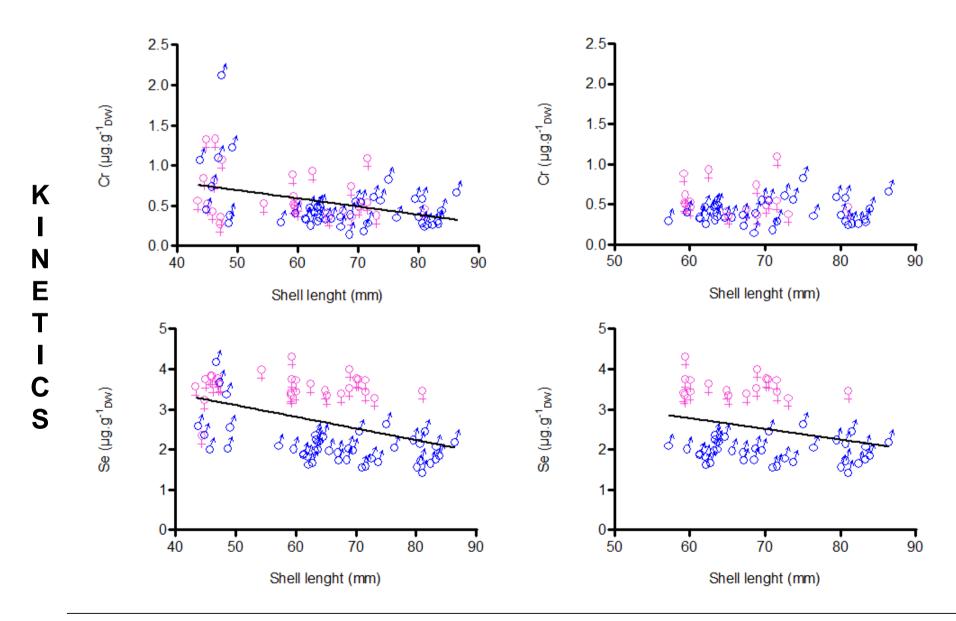




















Conclusions

KINETICS









Conclusions

M. galloprovincialis and P. oceanica = complementary bioindicator species (lifestyle) that can be used concomitantly.



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Conclusions

M. galloprovincialis and P. oceanica = complementary bioindicator species (lifestyle) that can be used concomitantly.

TE kinetics under the influence of various parameters that interact: environmental conditions, physiology of the species etc.



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Conclusions

- M. galloprovincialis and P. oceanica = complementary bioindicator species (lifestyle) that can be used concomitantly.
- TE kinetics under the influence of various parameters that interact: environmental conditions, physiology of the species etc.
- M. galloprovincialis: consensual monitoring protocols (e.g. sexual dormancy) and kinetic models (Casas et al. 2005, 2006, 2008); P. oceanica: no general rule → common rules of use in monitoring surveys.

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General conclusions



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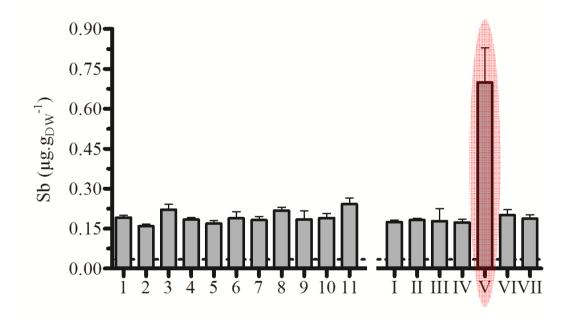
General conclusions

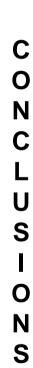
Contamination of the French Mediterranean coasts:
 TEs of emerging concern ARE of concern.





Contamination of the French Mediterranean coasts:
 TEs of emerging concern ARE of concern.









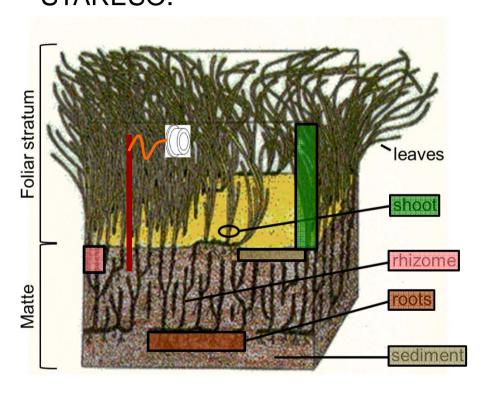
Conditions of reference for the NW Mediterranean: STARESO.







2. Conditions of reference for the NW Mediterranean: STARESO.









3. P. oceanica and M. galloprovincialis lifestyle:

Complementary bioindicators.

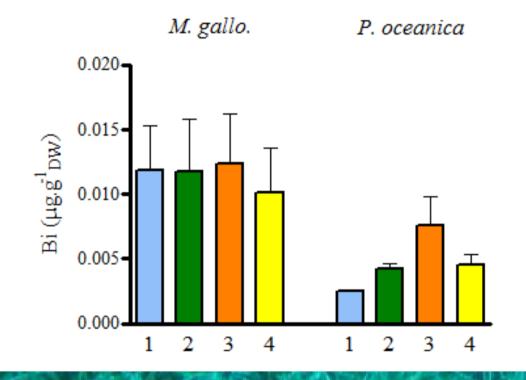






3. P. oceanica and M. galloprovincialis lifestyle:

Complementary bioindicators.









4. Uptake and loss kinetics:

Rapid balance modulated by biological cycles.

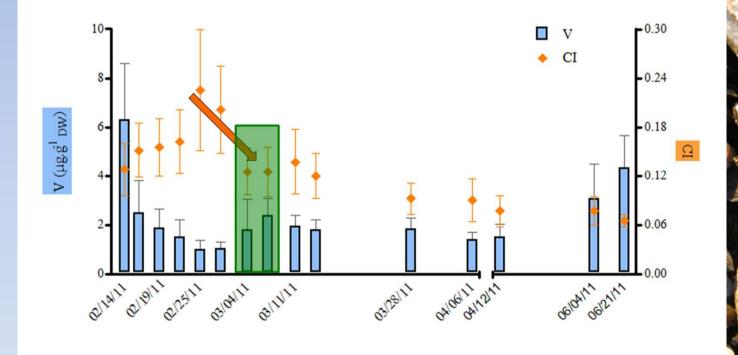






4. Uptake and loss kinetics:

Rapid balance modulated by biological cycles.









- Contamination of the French Mediterranean coasts:
 TEs of emerging concern ARE of concern.
- Conditions of reference for the NW Mediterranean: STARESO.
- 3. P. oceanica and M. galloprovincialis lifestyle: Complementary bioindicators.
- 4. Uptake and loss kinetics:

Rapid balance modulated by biological cycles.



Questions

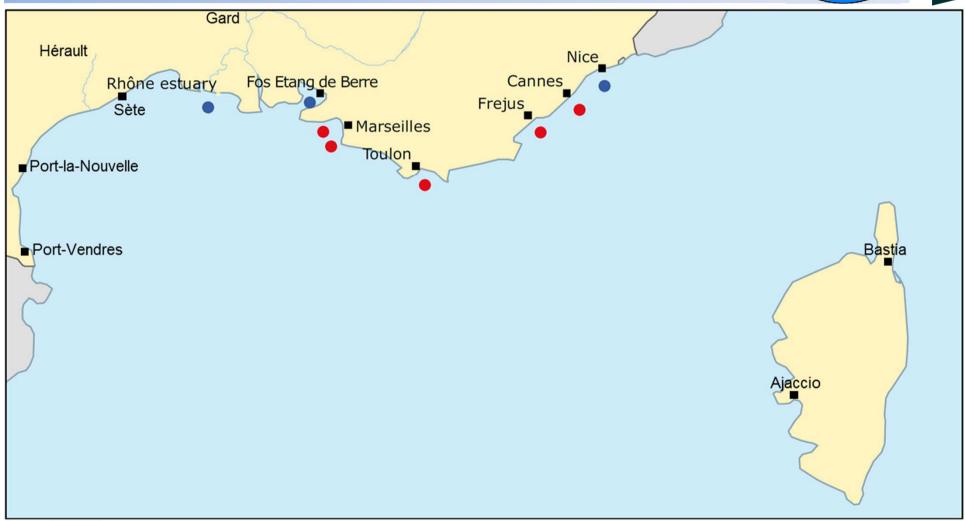




Pollution of the Mediterranean French coasts





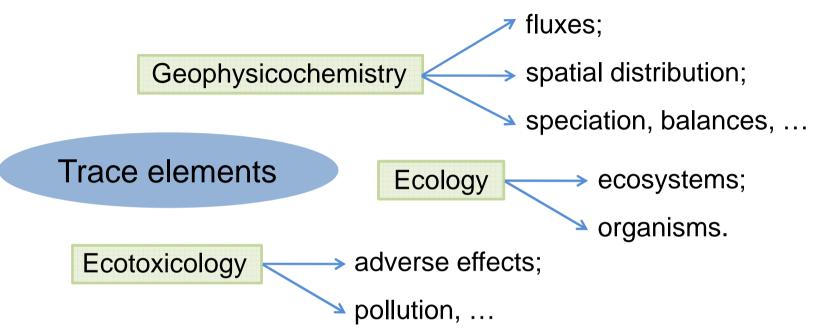


Zoom on the Mediterranean coastlines of France. Full red circles: pollution hot spots; blue full circles: areas of major concern; full black squares: coastal cities (modified from EEA 2006).

Ce an olds

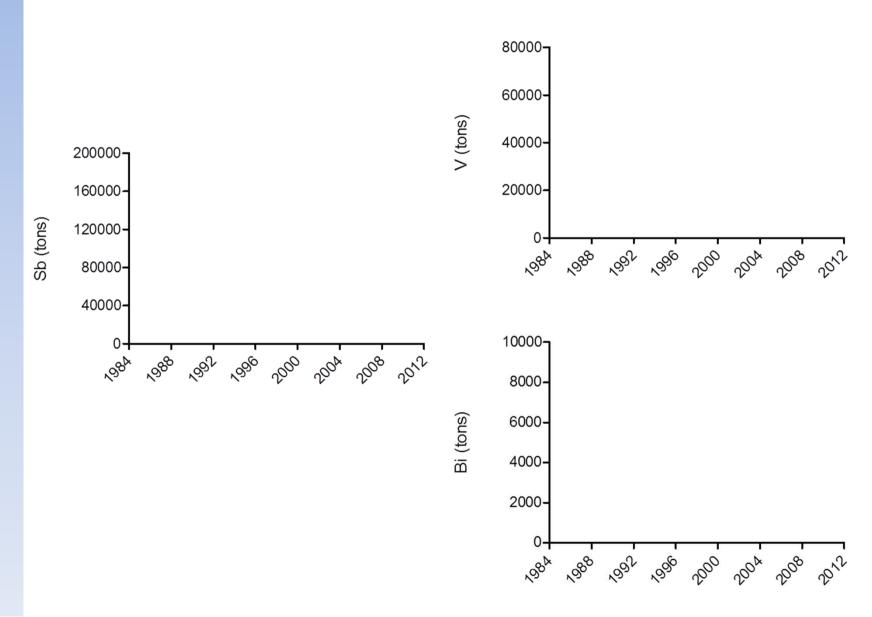


Trace elements

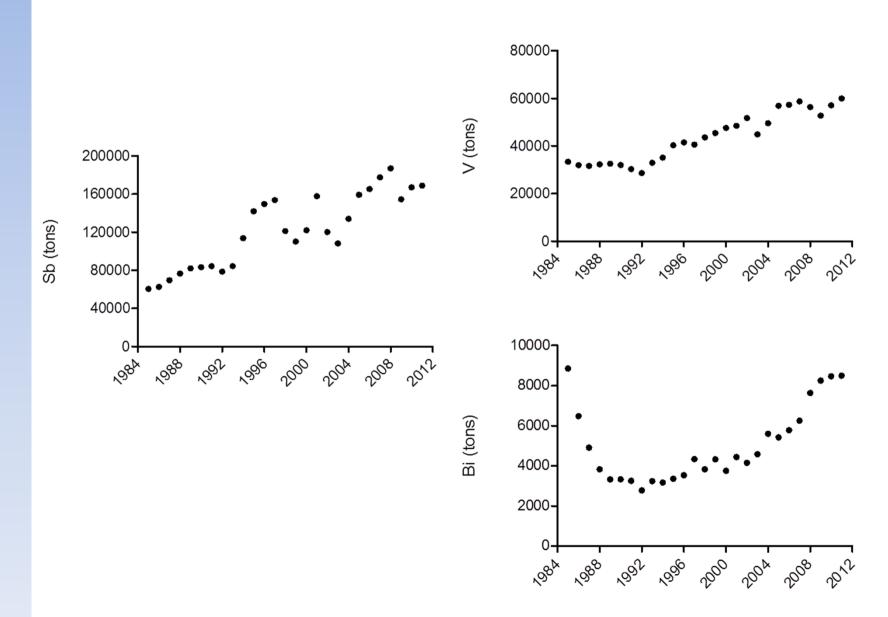




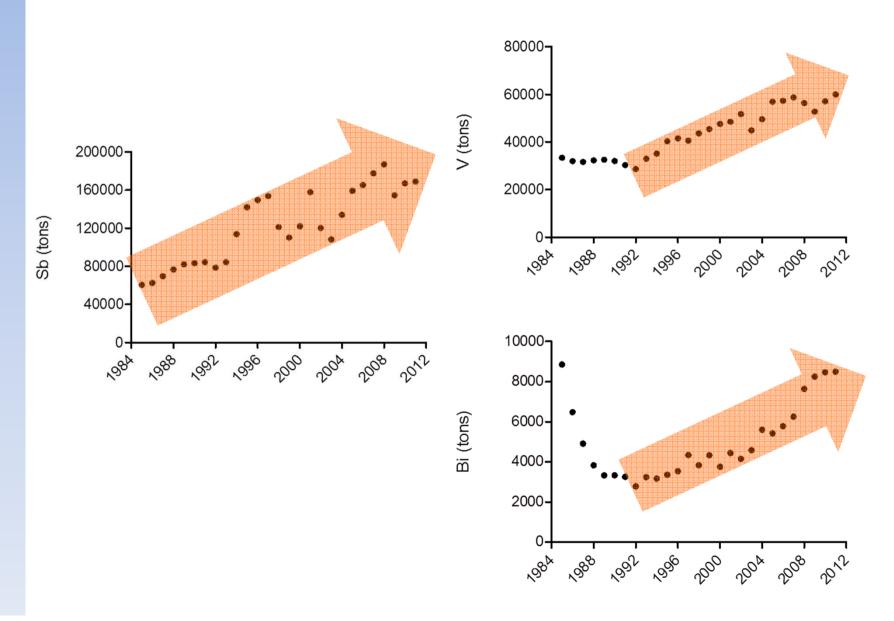




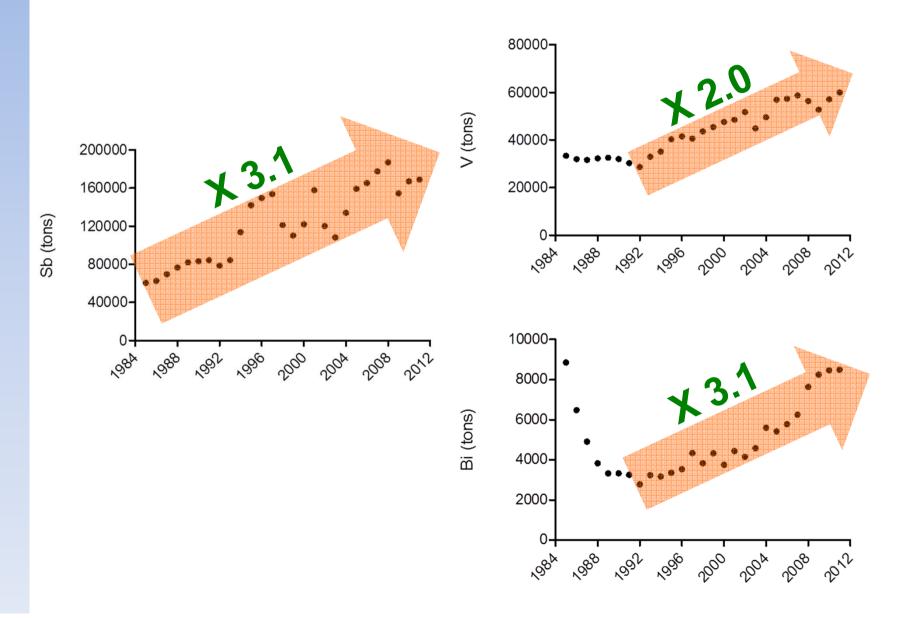








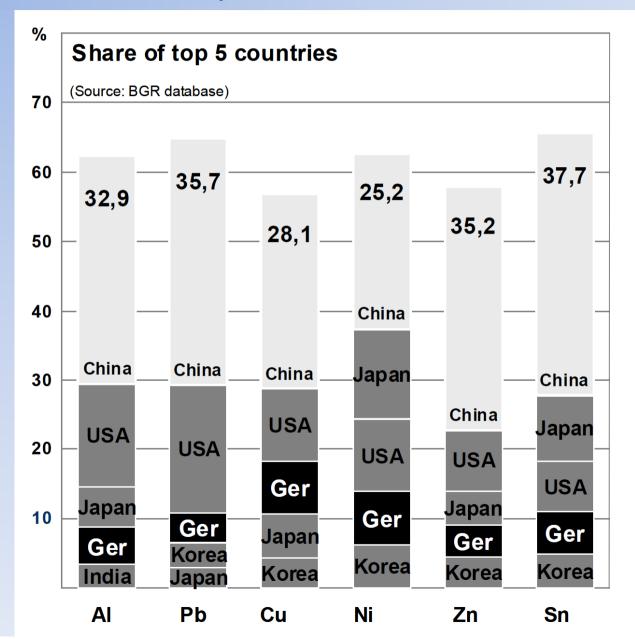








World consumption of trace elements



(modified after Sievers et al. 2010)

Biomonitoring



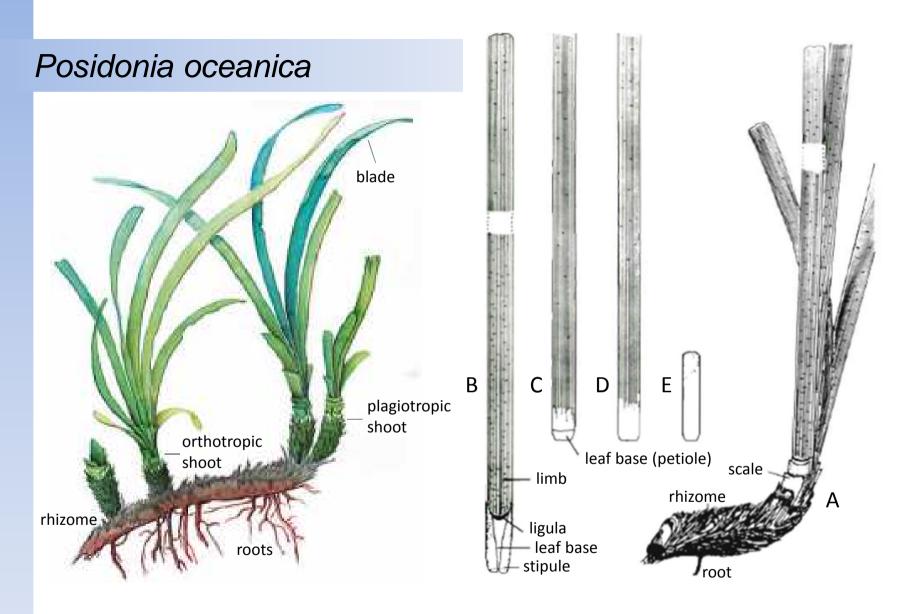


Number of references (1975-2010) on trace elements studied in *Posidonia oceanica*

TE	Cd	Pb	Cu	Zn	Cr	Fe	Ni
# ref.	34	29	27	25	22	14	13

TE	As	Se	Ag	Co	Mn	Al
# ref.	3	3	2	2	2	1
TE	Ве	Bi	V	Мо	Sn	Sb
# ref.	0	0	0	0	0	0



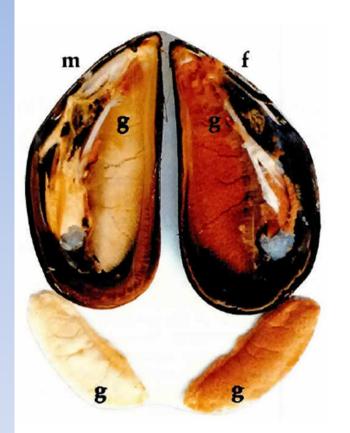


Left: *P. oceanica* shoots fixed on a plagiotropic rhizome. Right: (A) shoot of leaves on a plagiotropic rhizome; (B, C) adult leaves; (D) intermediate leaf; (E) juvenile leaf (modified after Libes and Boudouresque 1987).

Mytilus galloprovincialis









Tissue morphology in *M. galloprovincialis*. **Left** - opened sexually matured male (m) and female (f) mussels and isolated gonads (g) (Mikhailov et al. 1995). **Right** - 1 = mantle (gonad), 2 = muscular mantle edge, 3 = foot, 4 = hepatopancreas, 5 = labial palps, 6 = gills, 7 = visceral mass, 8 = foot retractor muscle, 9 = posterior adductor muscle, *circles* = gonad follicle regions spread over the mantle, *small white lines* = indicate gonad duct). Scale bar 1 cm (Torrado and Mikhailov 2000).

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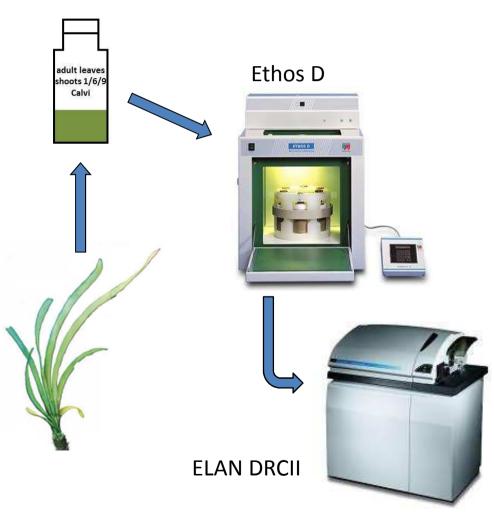
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Laboratory analyses







Analytical steps:

homogeneous sample



• acidic digestion in a microwave oven



measures :

> inductively coupled plasma mass spectrometer

N











List of metrics used in the PREI, unit and methodology used to obtain the results for each metric,

PREI metrics and units	Methodology and references
Shoot density (shoot m ⁻²)	Quadrat 20 × 20 cm Soullard et al. (1994) 20 measurements at 15 m depth
Lower depth limit (m)	Noted in situ by scuba diver
Type of this limit (regressive, progressive, stable)	Noted in situ by scuba diver according to the classification of Meinesz and Laurent (1978)
E/L	On 20 shoots sampled at 15 m depth; measurement of total dry weight of epiphyte and leaf (adult + intermediate) Dauby and Poulicek (1995)
Leaf surface area (cm ² shoot ⁻¹)	On 20 shoots sampled at 15 m depth: measurement of total leaf surface area according to the methodology of Giraud (1979)

Sampling strategy:

Cean old



PREI: 42 sites sampled in april 2007 at 15m

PREI values calculated in Corsica and PACA.

CORSICA		PACA		
Station	PREI	Station	PREI	
Cargèse	0.668	Ponteau	0.360	
Tiucca	0.630	Couronne	0.525	
Ajaccio-nord	0.564	Carry	0.680	
Ajaccio-sud	0.495	Ensuès	0.686	
Porto Pollo	0.386	La Vesse	0.465	
Figari-Bruzzi	0.619	Corbière	0.305	
Piantarella	0.597	Nord Pomègues	0.628	
Sant'Amanza fdb	0.542	Prado	0.636	
Sant'Amanza eb	0.671	P. Chèvres	0.477	
Large Diana	0.689	Riou	0.677	
Bravone	0.779	Calanque	0.584	
Taglio Isolaccio	0.690	Cassis	0.563	
Large Biguglia	0.721	Figuerolle	0.660	
Erbalunga	0.741	Bandol	0.682	
Maccinagio	0.650	Brusc	0.634	
St Florent	0.478	Carqueiranne	0.708	
Aregno	0.789	Garonne	0.583	
Calvi	0.724	Levant	0.802	
		Giens	0.819	
		Bénat	0.764	
		St Raphael	0.690	
		Cap Roux	0.847	
		Antibes	0.560	
		Villefranche	0.280	

Boundaries and colour codes for the different levels of ecological status.

EQR	Ecological status	Color code
1-0.775	High	Blue
0.774-0.550	Good	Green
0.549-0.325	Moderate	Yellow
0.324-0.100	Poor	Orange
<0.100-0	Bad	Red

List of metrics used in the PREI, unit and methodology used to obtain the results for each metric.

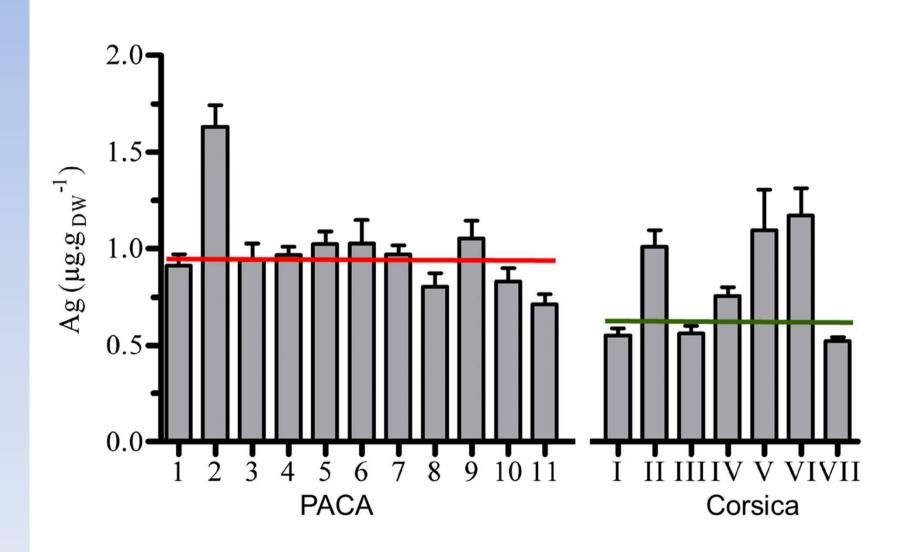
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Ν



Ag - diffuse pollution



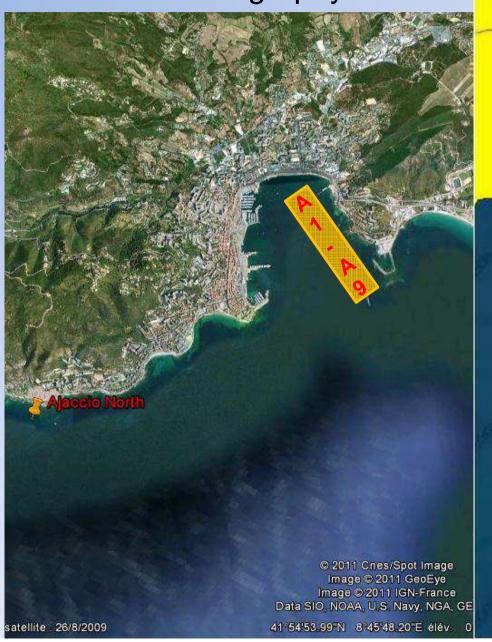


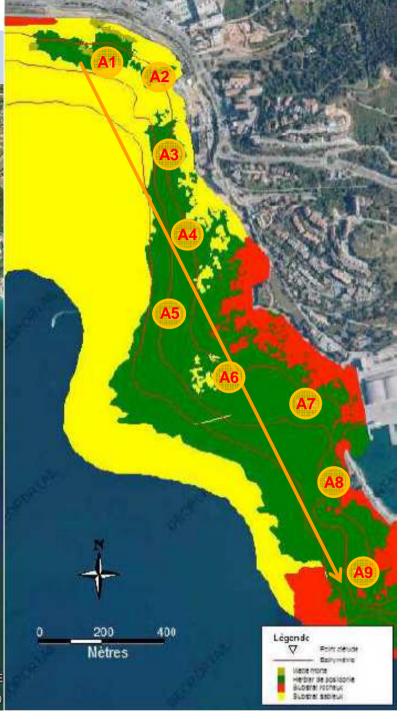






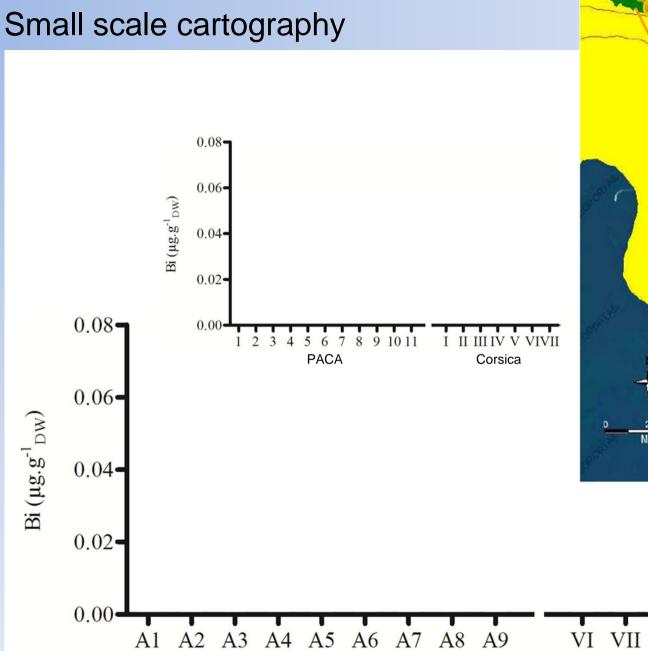
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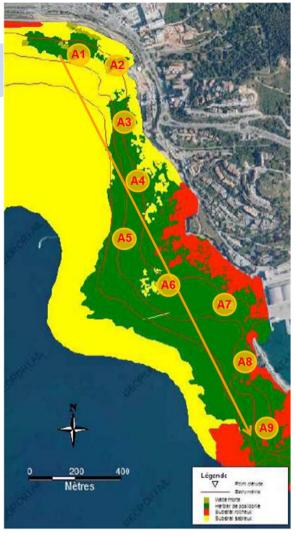




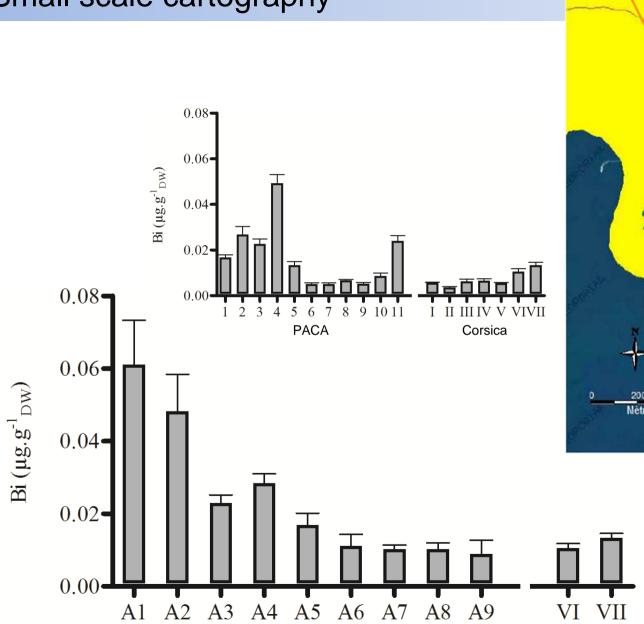
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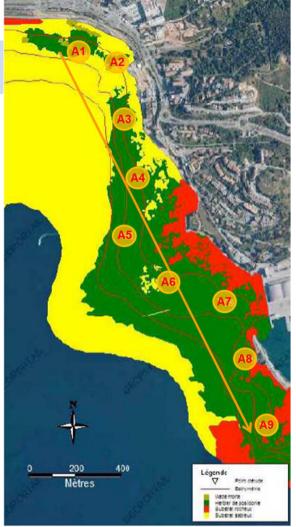




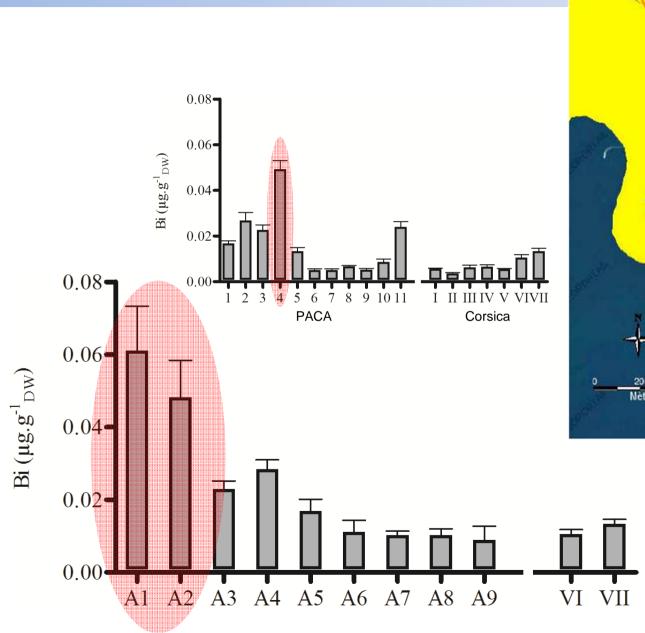


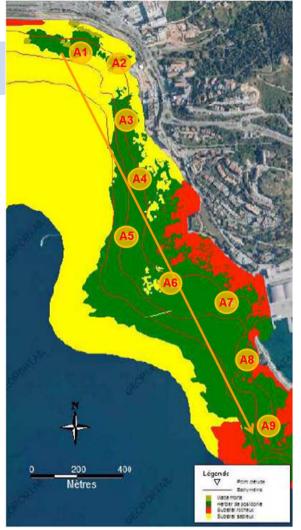




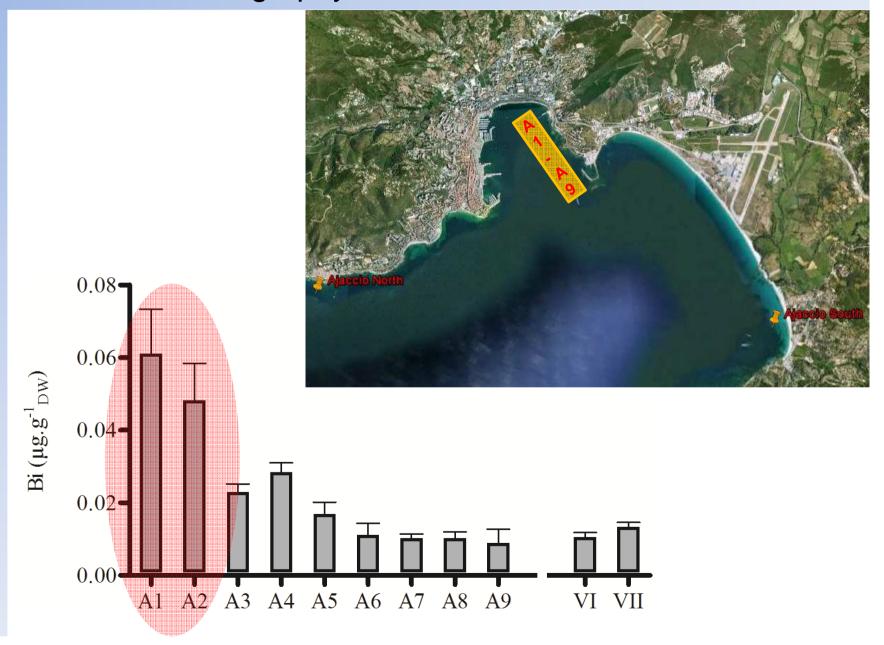










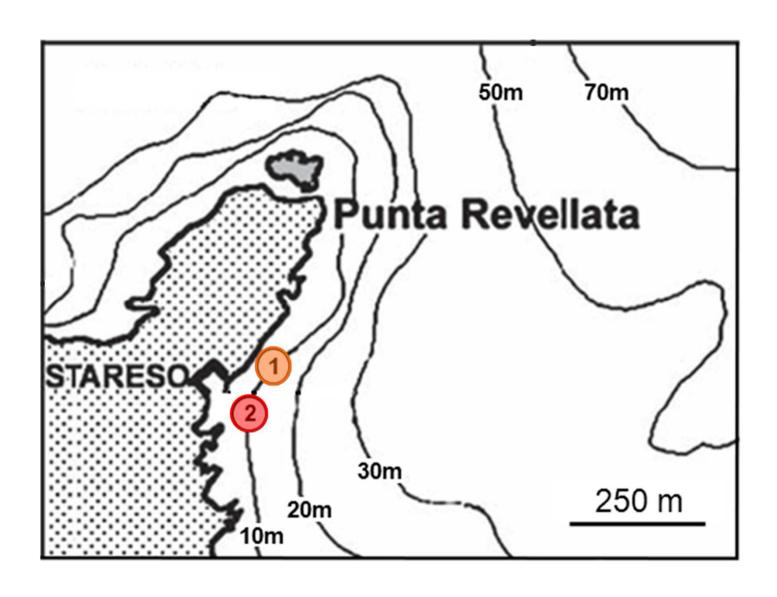








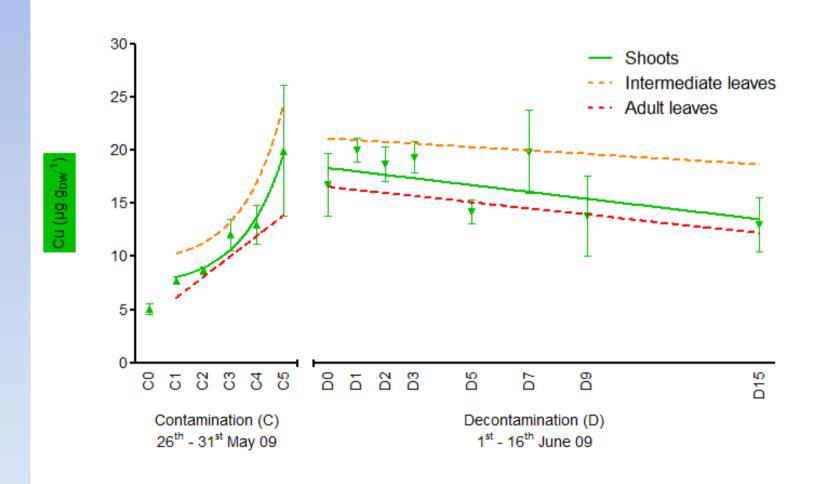
In situ contaminations of P. oceanica







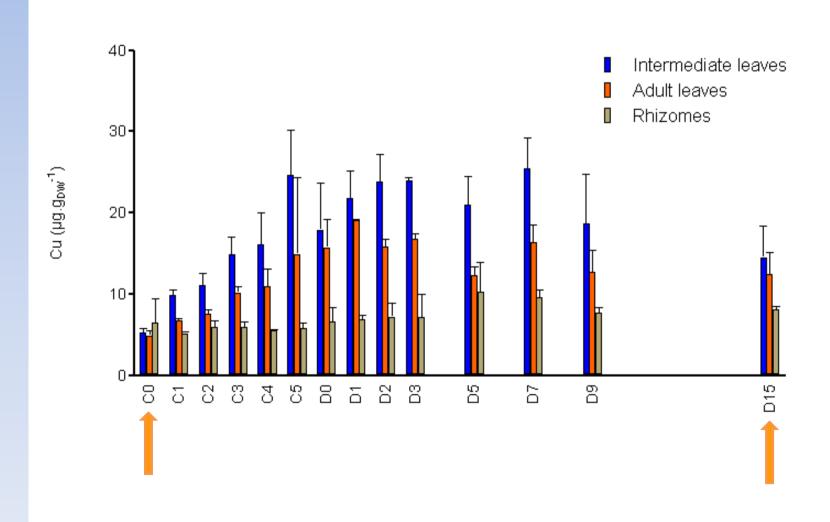
Cu: moderate conta. - shoots and leaves







Cu: moderate conta. - compartmentalization



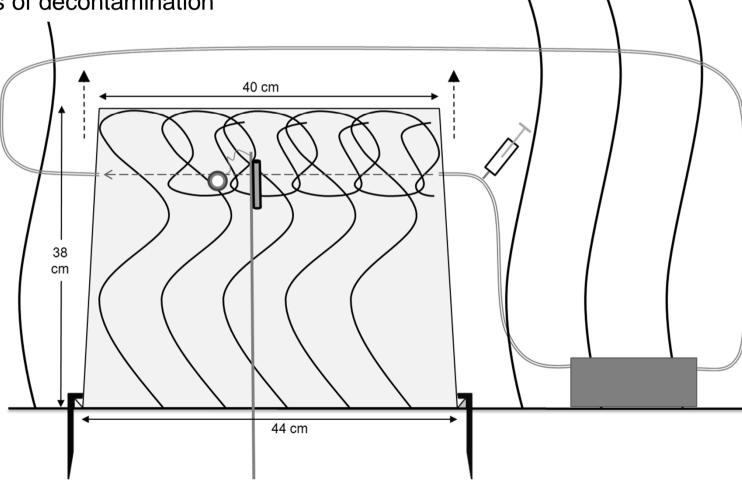






Acute mesocosm

- 24h00 of contamination in June 2009;
- 54L rectangular-box mesocosm;
- 5 contaminations, 1 every 3 hours (9am-9pm);
- 15 days of decontamination

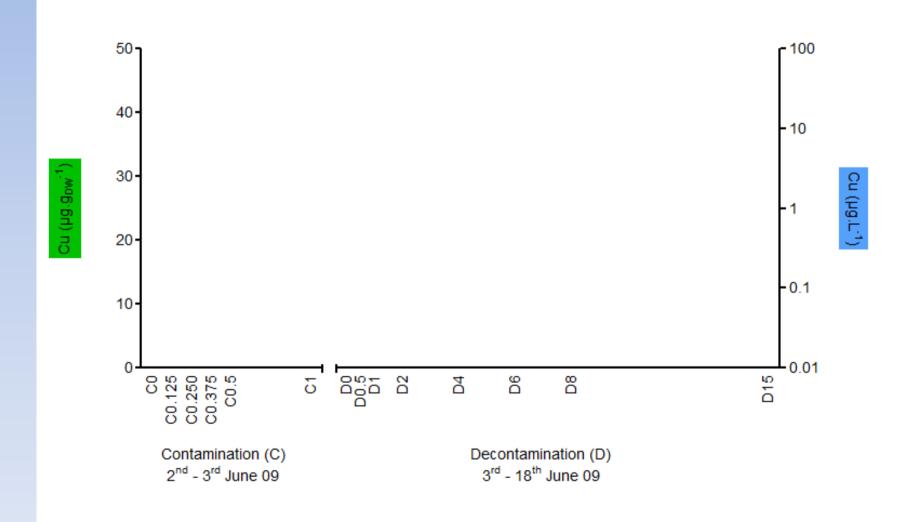








Cu: acute contamination - shoots

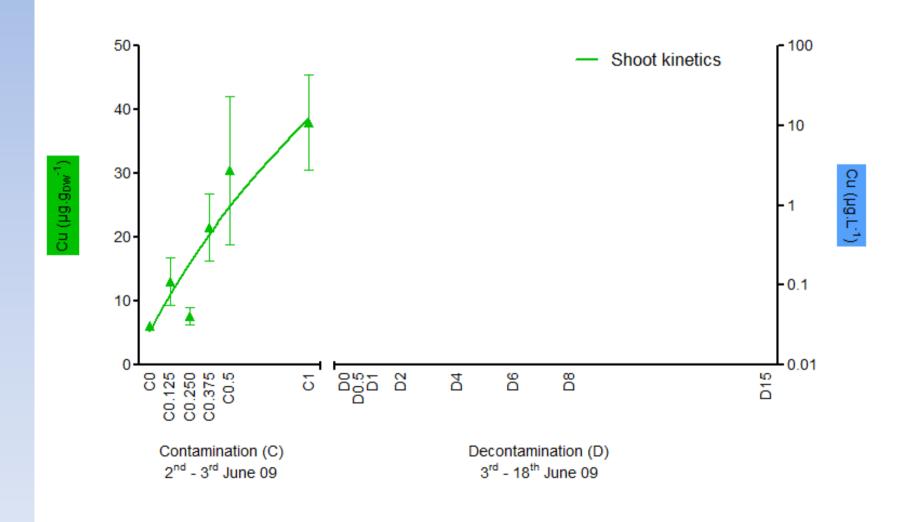








Cu: acute contamination - shoots

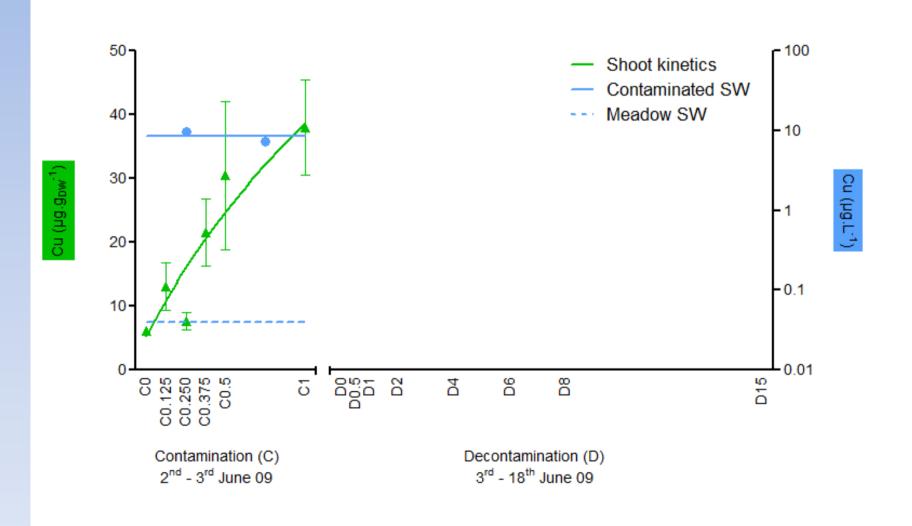








Cu: acute contamination - shoots

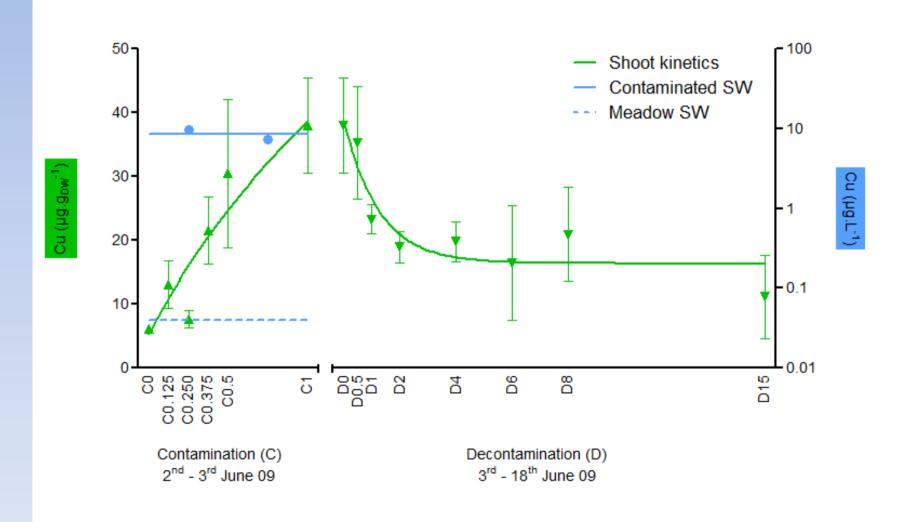








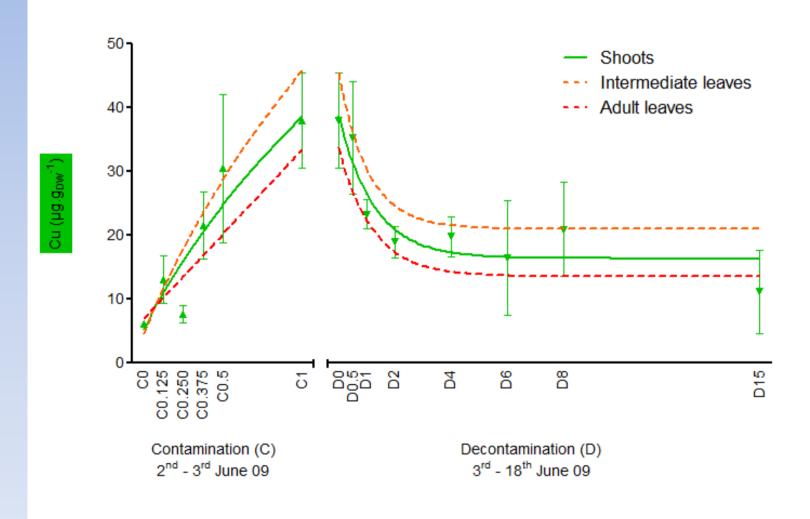
Cu: acute contamination - shoots







Cu: acute contamination – shoots and leaves

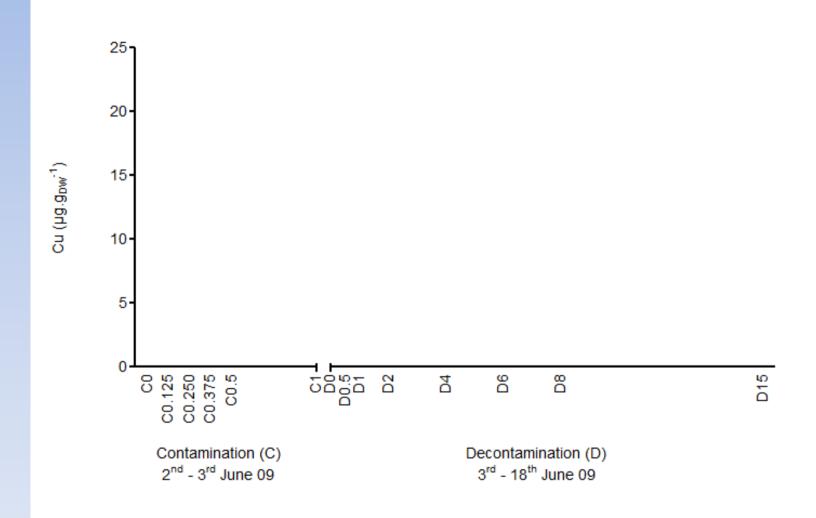








Cu: acute contamination - rhizomes

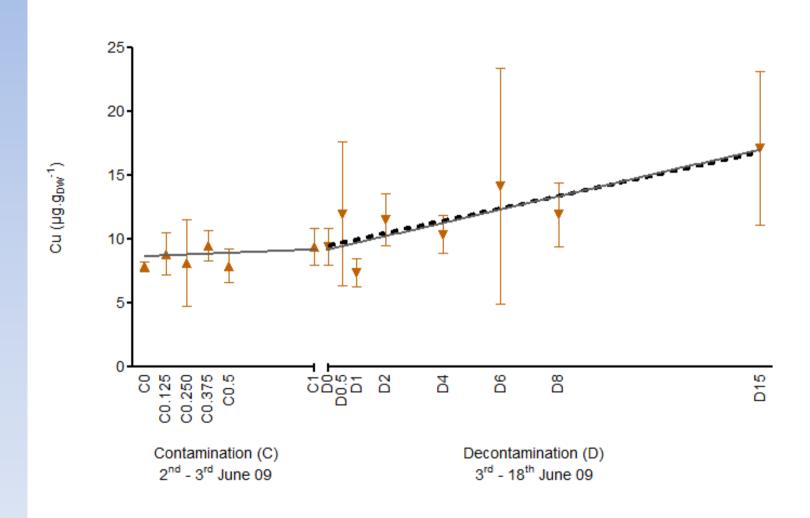








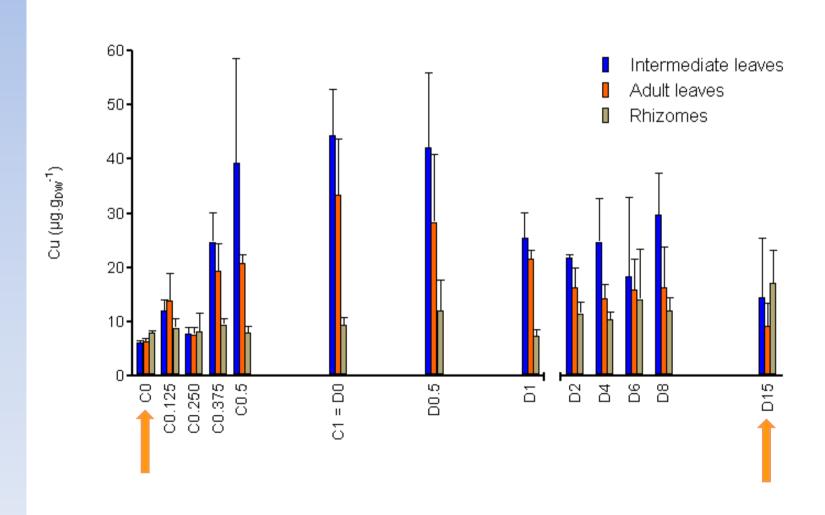
Cu: acute contamination - rhizomes







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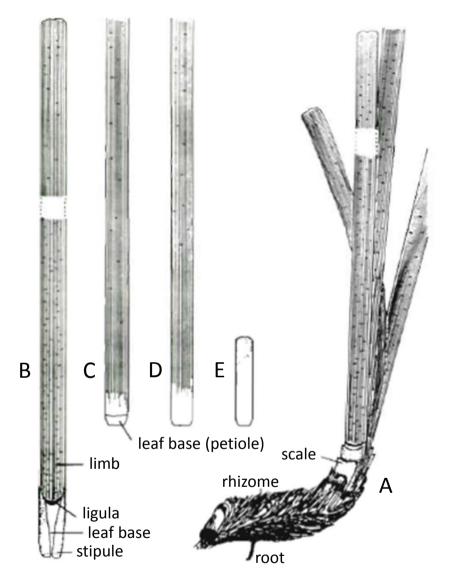




Tissue compartmentalization



1 = mantle (gonad), 2 = muscular mantle edge, 3 = foot, 4 = hepatopancreas, 5 = labial palps, 6 = gills, 7 = visceral mass, 8 = foot retractor muscle, 9 = posterior adductor muscle, *circles* = gonad follicle regions spread over the mantle, *small white lines* = indicate gonad duct) (Torrado and Mikhailov 2000).



(A) shoot of leaves on a plagiotropic rhizome; (B, C) adult leaves; (D) intermediate leaf; (E) juvenile leaf (modified after Libes and Boudouresque 1987).

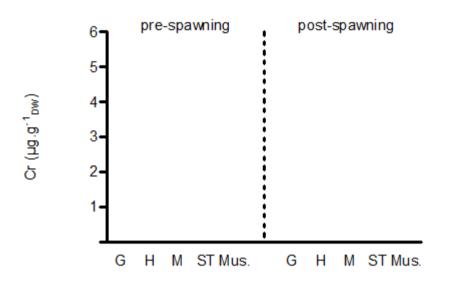


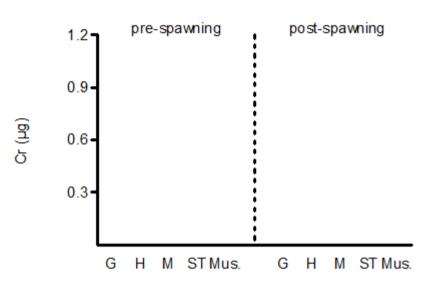








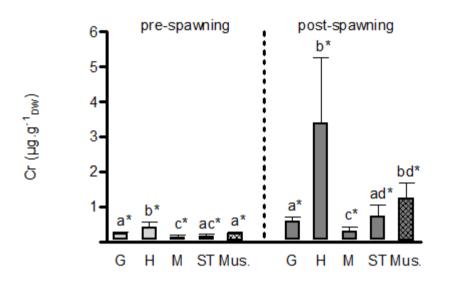


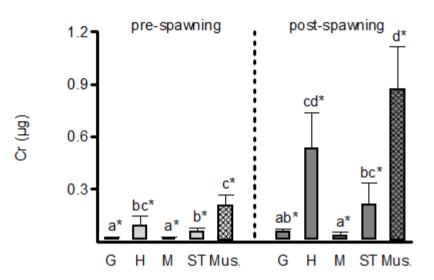








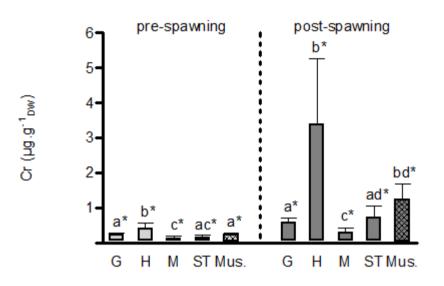


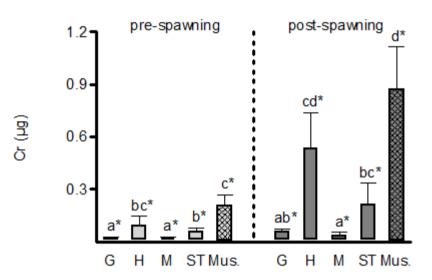


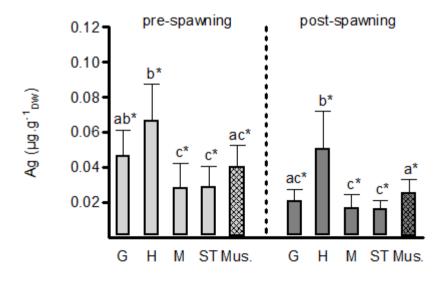


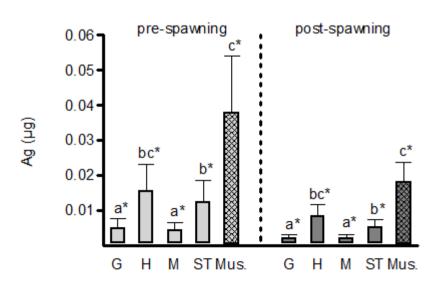








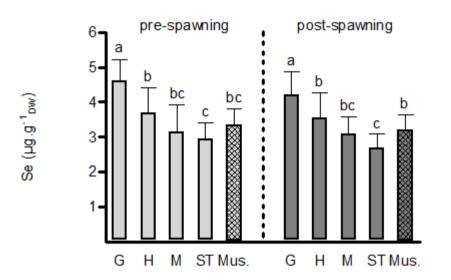


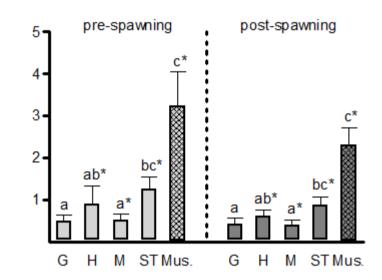














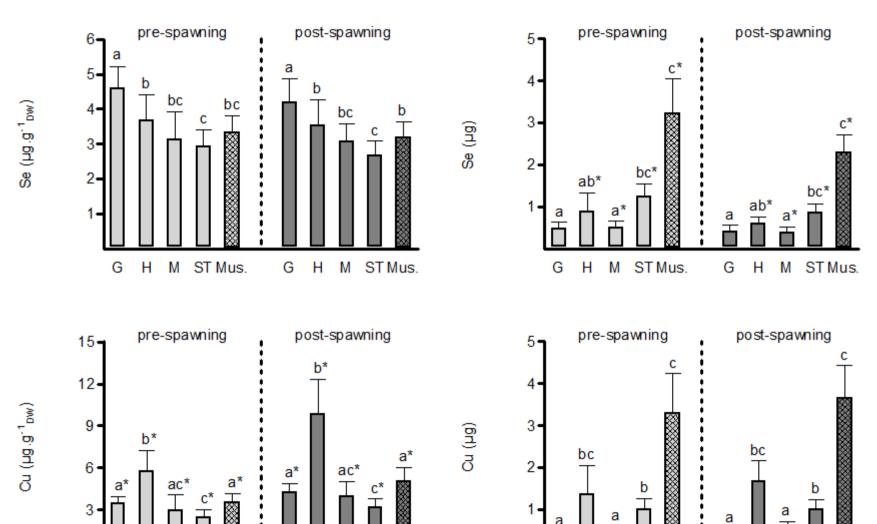


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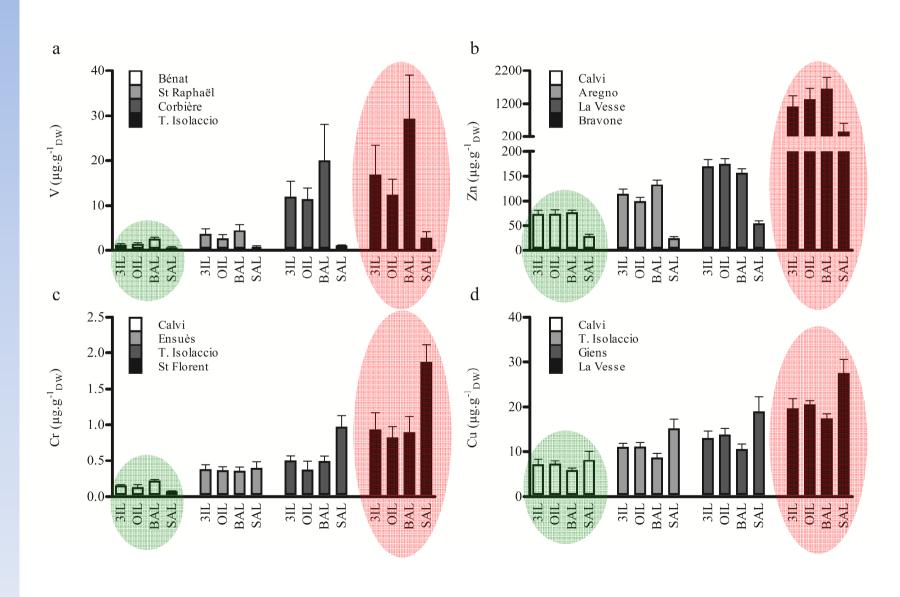












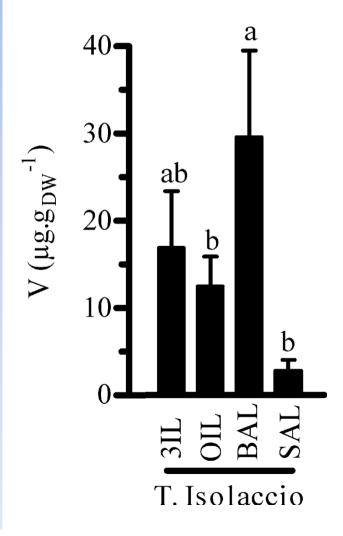


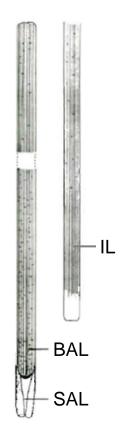




P. oceanica

1. V (Mn, Co, Ni, As, Se, Mo, Sb, Pb, Bi).





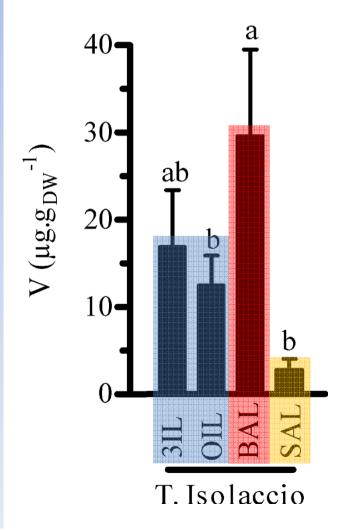


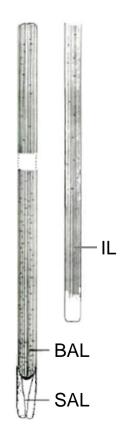




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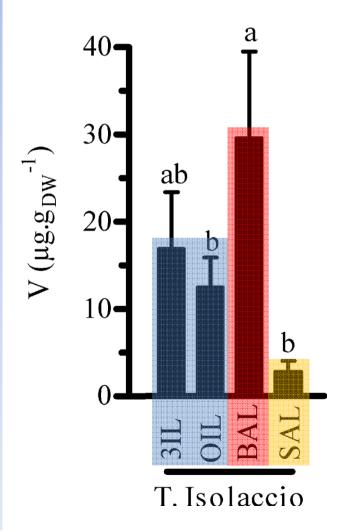






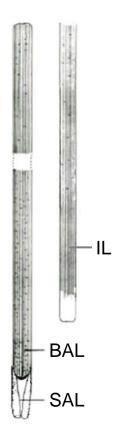
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Hypotheses:

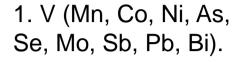
- > water column accumulation
- > residence time
- dilution effect

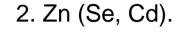






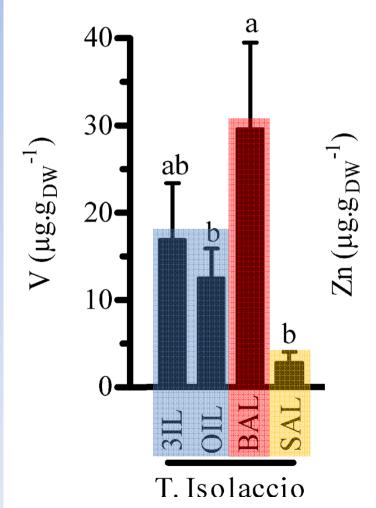
P. oceanica

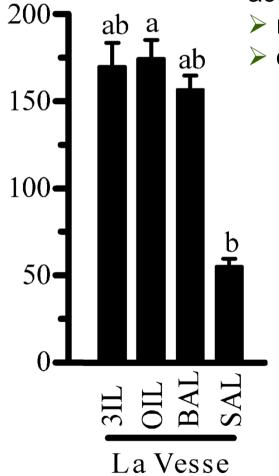


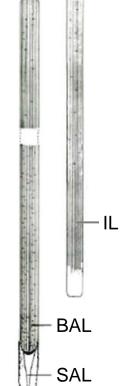


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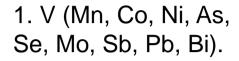


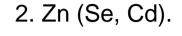






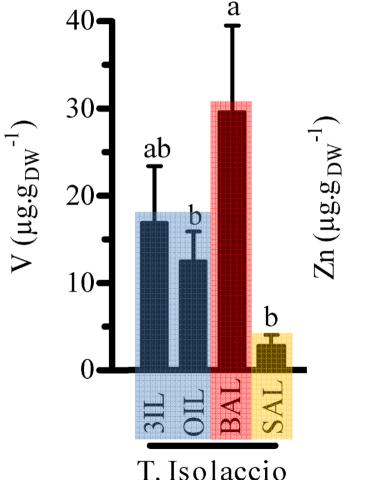


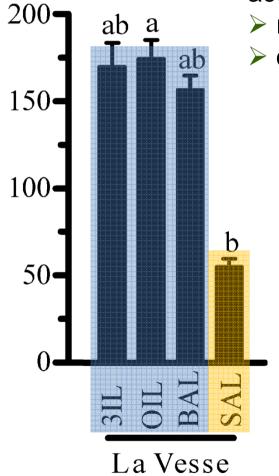


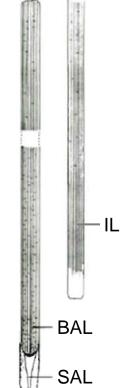




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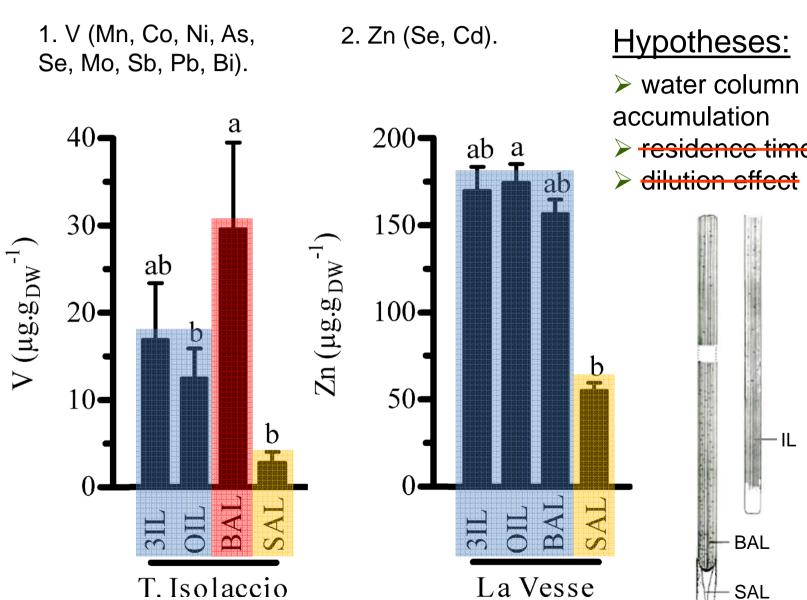






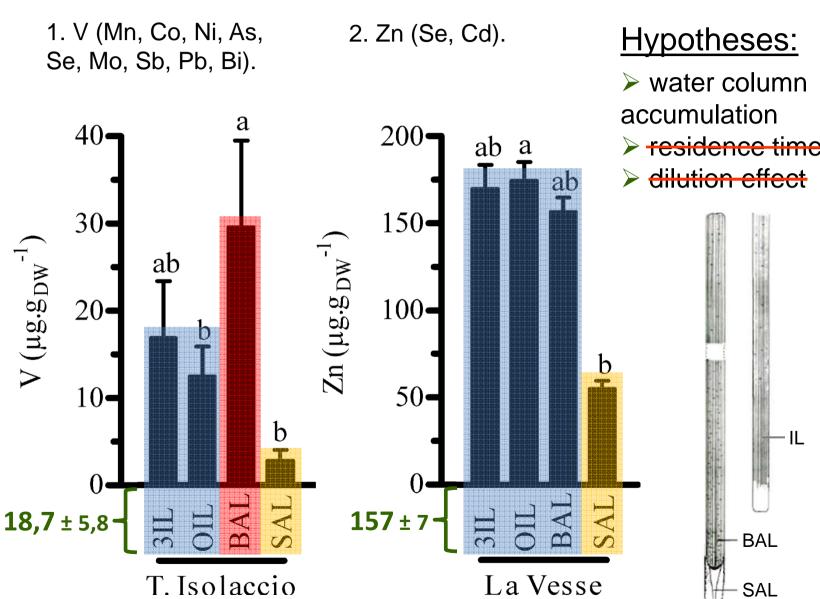


SAL









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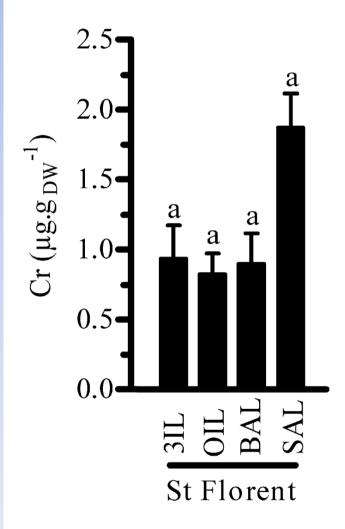


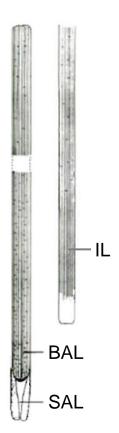




P. oceanica

3. Cr (Al, Fe, Ag).





Ν

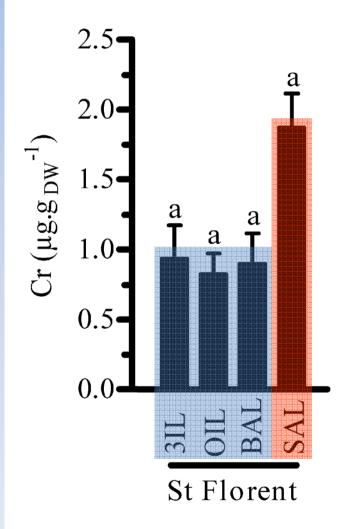


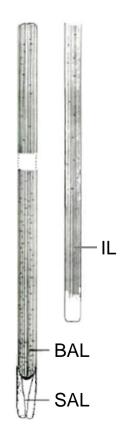




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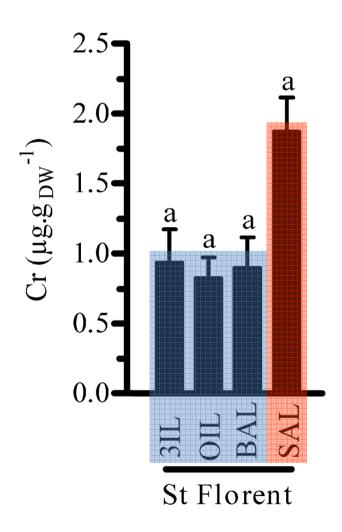




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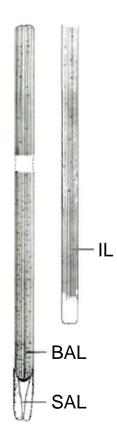






Hypotheses:

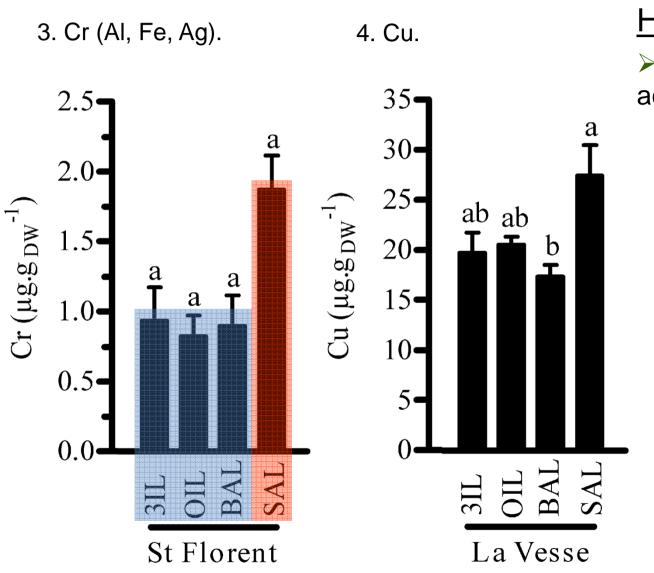
> sediment accumulation





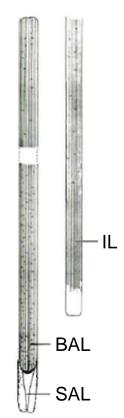






Hypotheses:

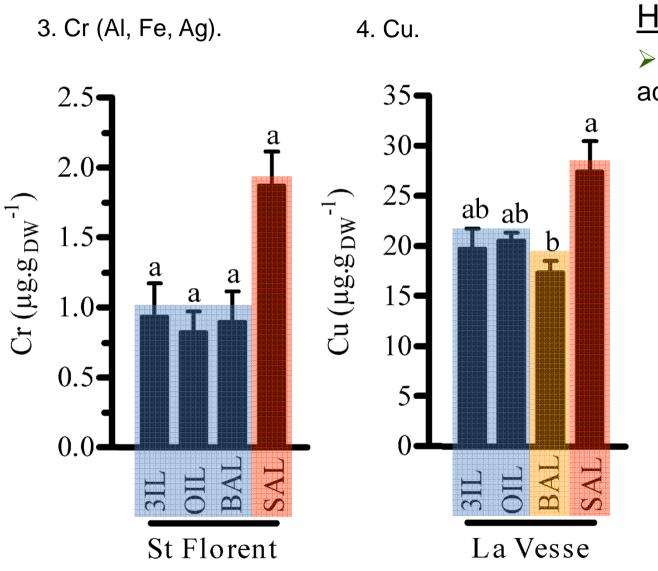
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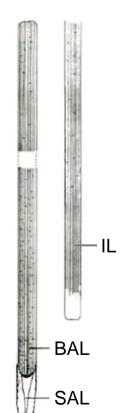






Hypotheses:

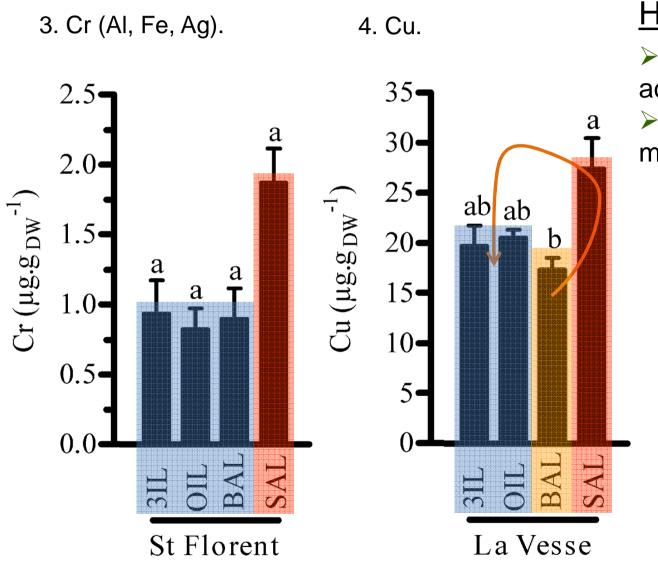
> sediment accumulation





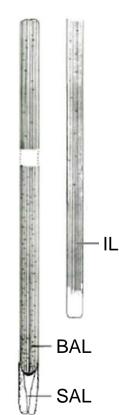


P. oceanica



Hypotheses:

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- ➤ Cu = essential micronutrient

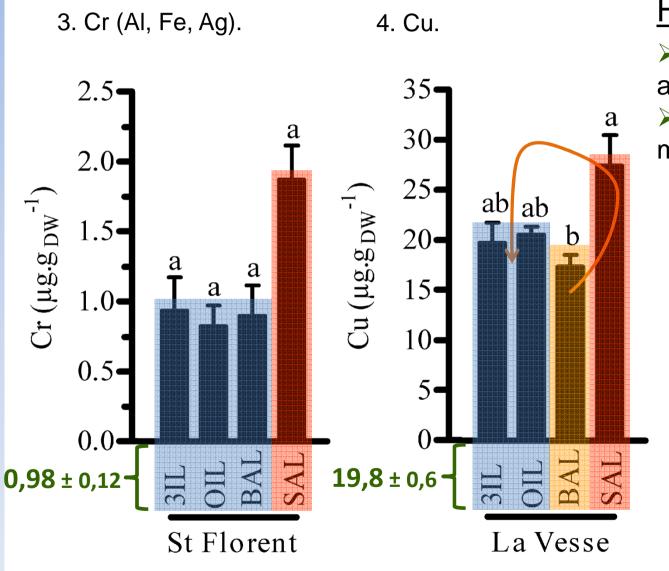






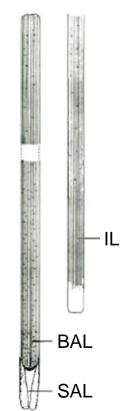


P. oceanica



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C 0 M Α R M Ε N Α Z Α 0 N







Conclusion

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TE compartmentalization can differ between clean and contaminated sites.







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Use entire individuals in monitoring surveys