



FED 4116

## Effects of heavy metals on sediment microbial diversity

# Sediment cores: a valuable tool to assess the anthropogenic contamination of a drainage bassin

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# Scientific context

Seine estuary

High industrialised and urbanised drainage basin of approximately 79,000 km<sup>2</sup>

Chemical and microbial contamination

Fecal bacteria (Touren *et al.*, 2007; Berthe *et al.*, 2008)

Trace metals (Meybeck *et al.*, 2007)

PAH and drugs (Tamtam *et al.*, 2008)

Interaction with hydrology and sedimentary processes  
(Ramond *et al.*, 2008; Dupont *et al.*, 1999; Deloffre *et al.*, 2007)

Estuarine fine sediments → a reservoir for contaminants



# Objectives

Does the microbial community of sediments reflects the impact of human activities on the drainage bassin ?

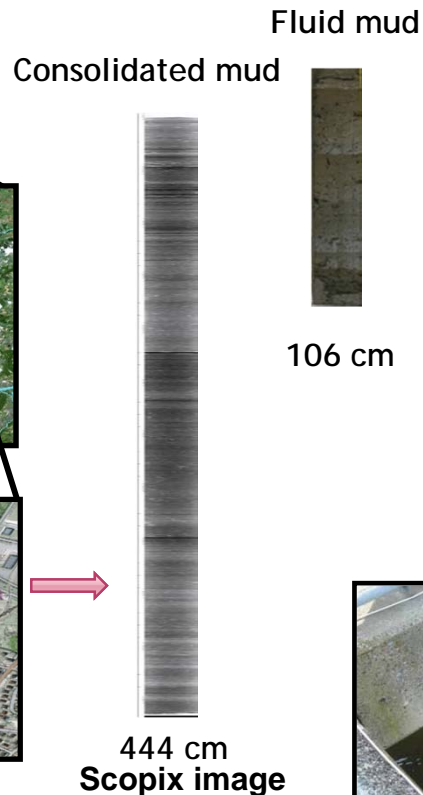
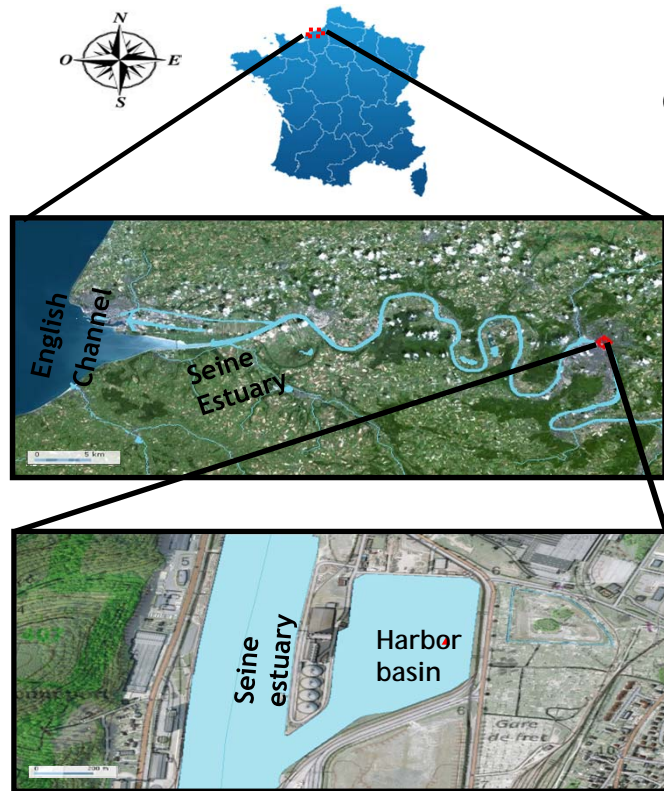


Sediment core : an historical record of sediment fluxes and associated contaminants over several years

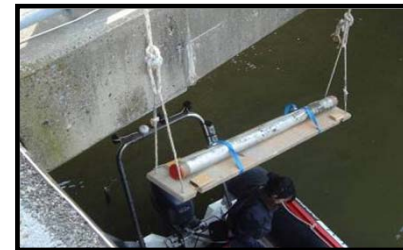
- 1 Determine the impact of the contamination of the Seine estuary sediment on the structure of microbial community
- 2 Occurrence and diversity of bacterial resistance genes to trace metals
- 3 Relationship between use of antibiotics and occurrence of antibiotic resistance genes among the microbial community

# Sampling strategy

The Rhapsodis project (Seine Aval program) : an interdisciplinary approach



- Two types of corer:
- Gravity corer  
"Amaury" → Fluid mud sampling
  - Percussion corer  
→ deeper core sampling



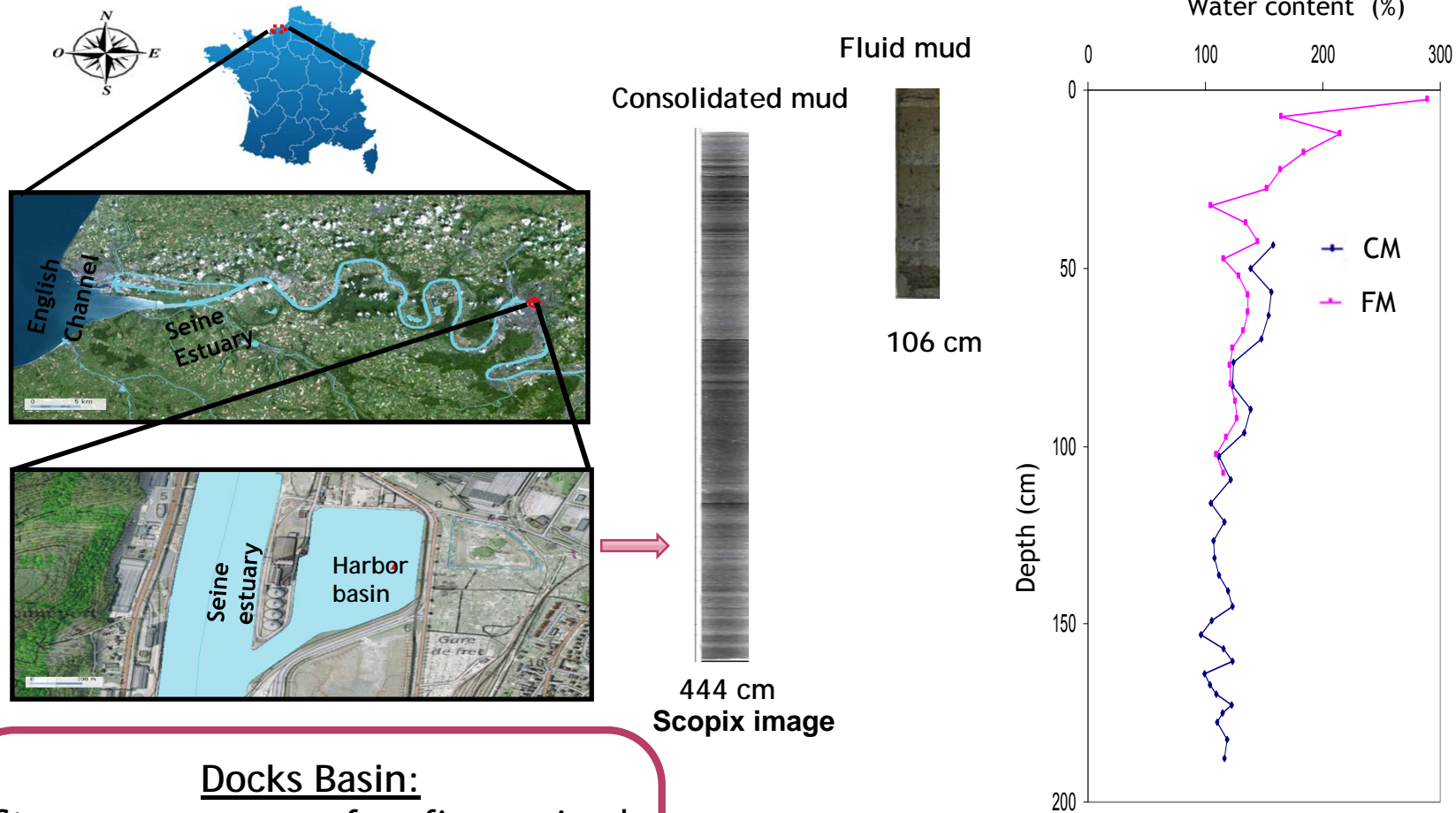
Docks Basin:  
Storage area of fine-grained sediment and contaminants, situated in the river part of the Seine estuary and away from dredging → **undisturbed sedimentary record**





# Sampling strategy

The Rhapsodis project (Seine Aval program) : an interdisciplinary approach



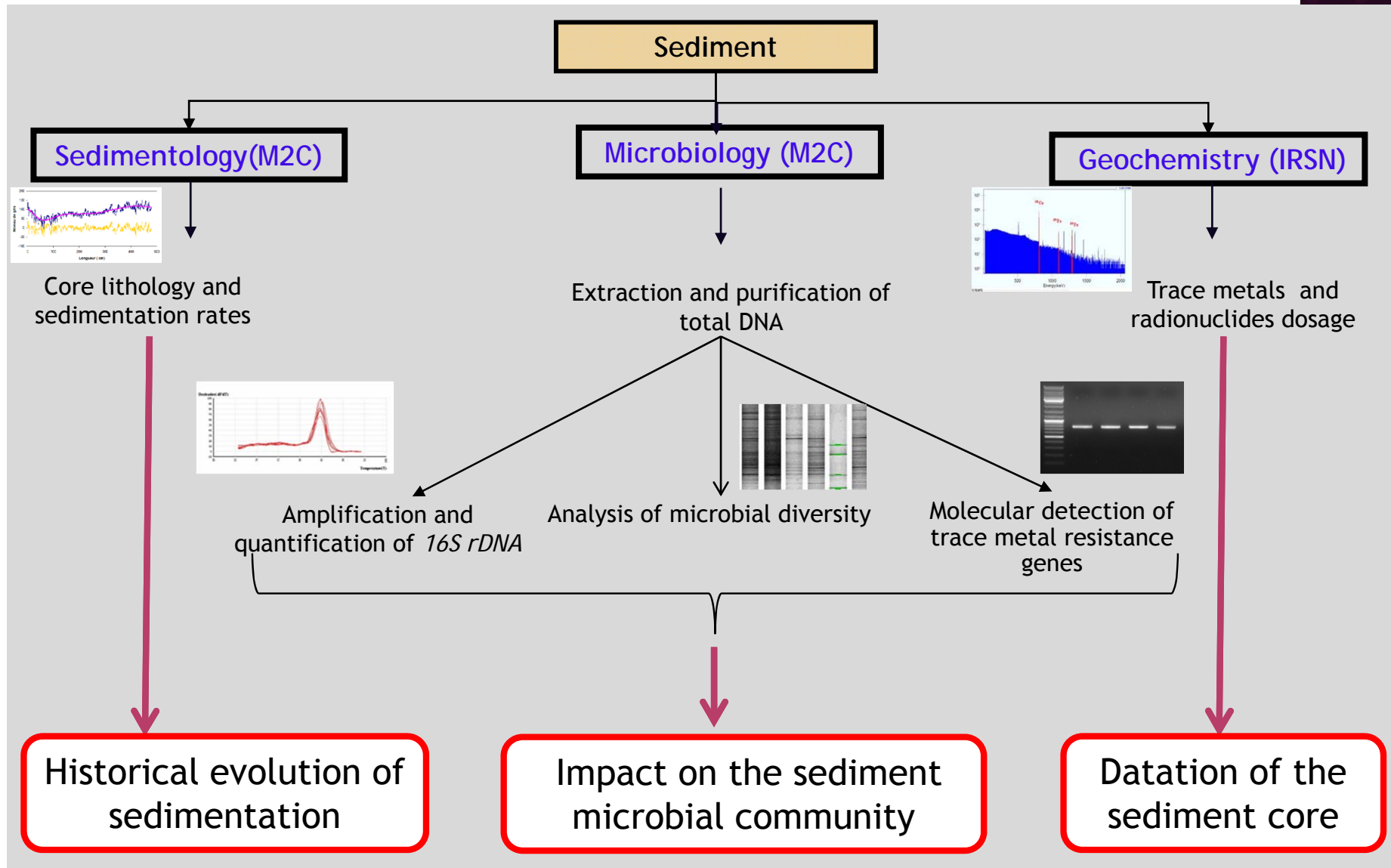
## Docks Basin:

Storage area of fine-grained sediment and contaminants, situated in the river part of the Seine estuary and away from dredging → **undisturbed sedimentary record**

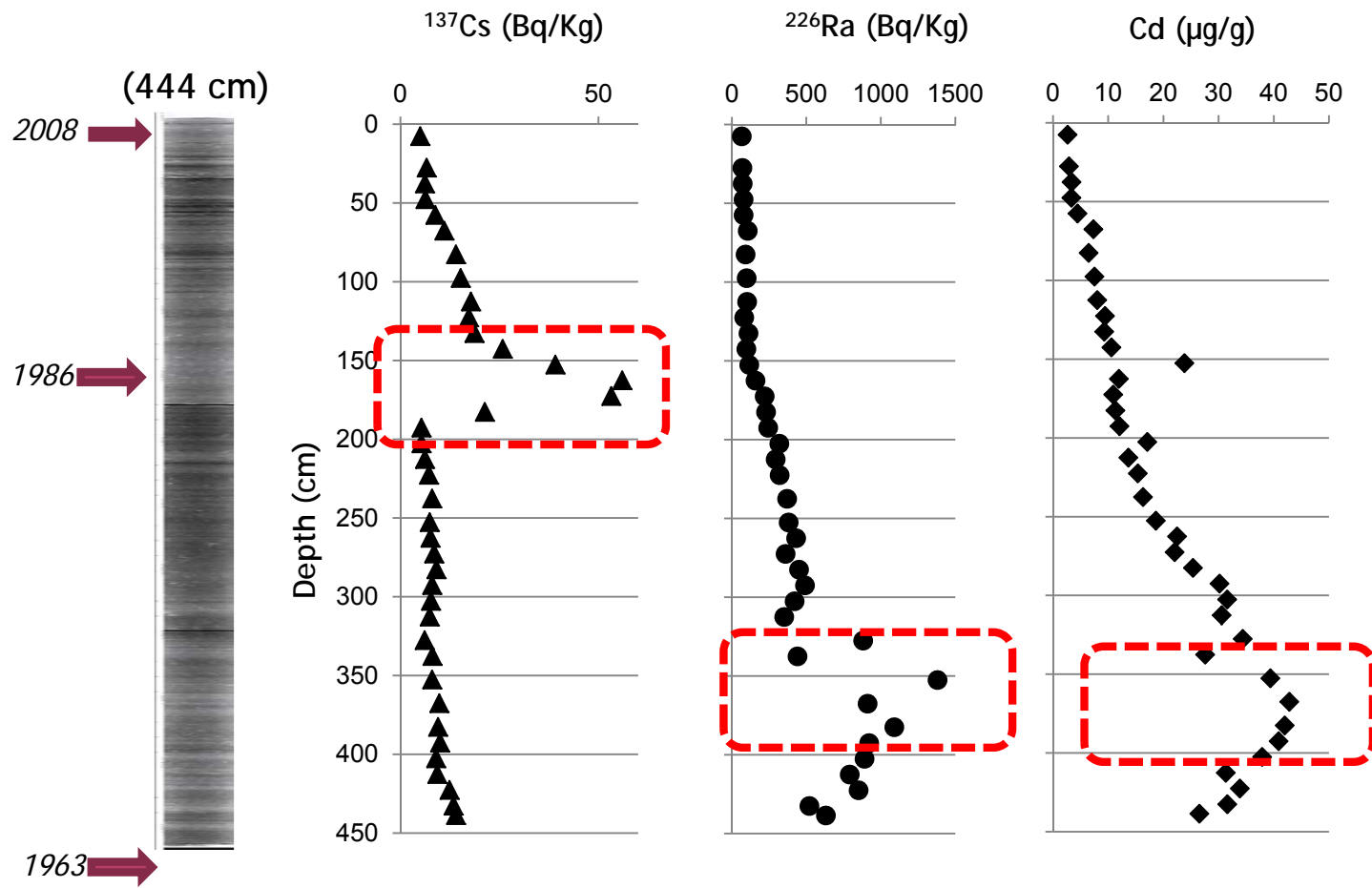
CM and FM cores phasing has been achieved Using Water content. The loss of surface sediment generated by the percussion corer and compaction of the sediment (signal analysis) were also computed.

# Methodology

The Rhapsodis project (Seine Aval program) : an interdisciplinary approach

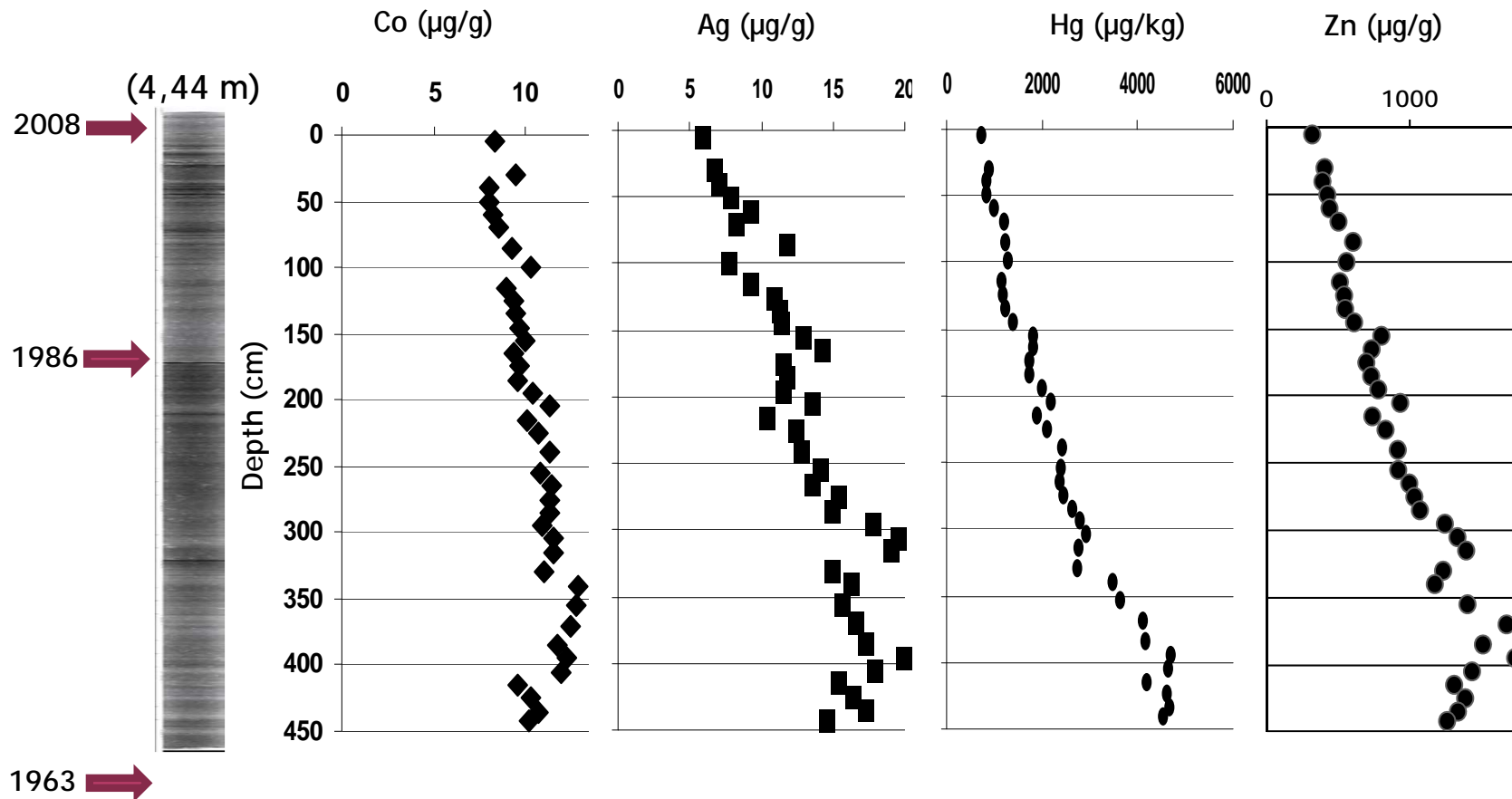


# Cores datation



- The signature of Chernobyl event (1986) was detected in the sediment core by  $^{137}\text{Cs}$   $\gamma$  ray spectrometry. Mean sedimentation rate  $\sim 10\text{cm}\cdot\text{year}^{-1}$
- The signature of phosphogypse release in the Seine estuary in the 1970s

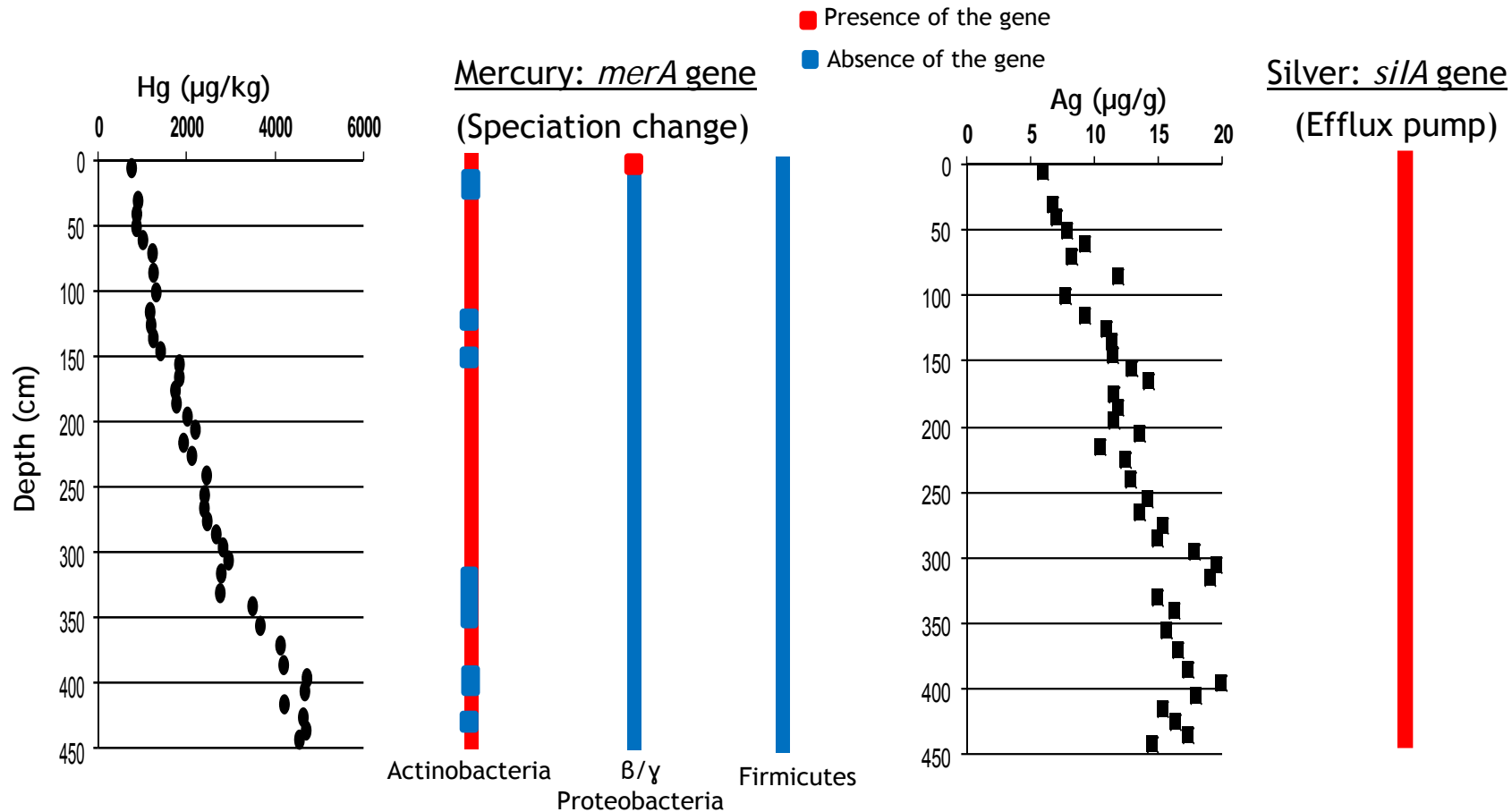
# Evolution of trace metals concentrations in the last 40 years



- The maximum factors of enrichment are very high in particular for  $\text{Cd}^{2+}$  and  $\text{Ag}^+$  (respectively factor 300 and 400)
- A regular decrease of  $\text{Hg}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Zn}^+$  and  $\text{Cd}^{2+}$  concentrations since the 1970s



# Relationship between trace metals contamination and bacterial resistance genes

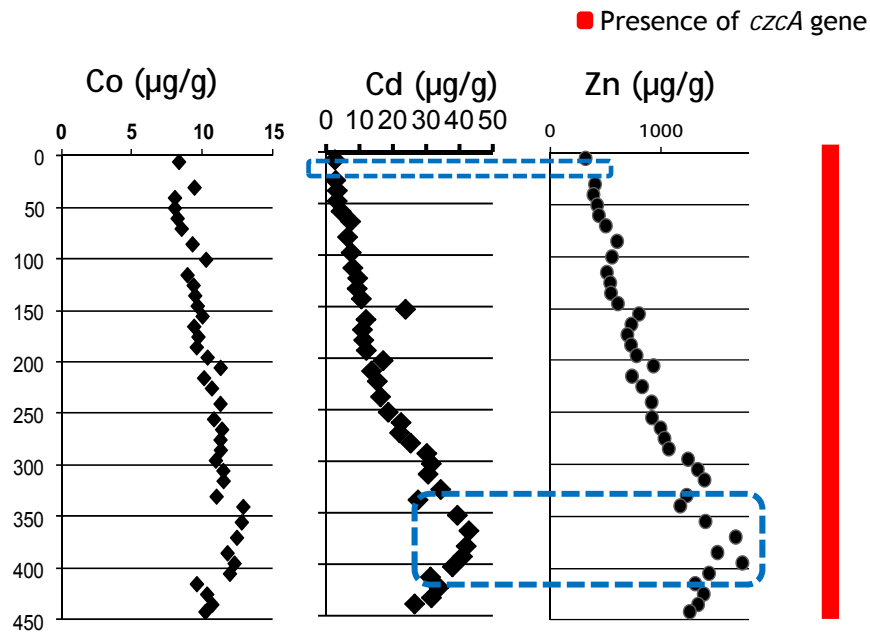


- *merA* gene response to mercury contamination is dependent on the bacterial phylum
- Response of microbial communities to permanent sediment contamination by Ag<sup>+</sup>

# Relationship between trace metals contamination and occurrence, diversity of *czcA* gene

## Occurrence of *czcA* gene

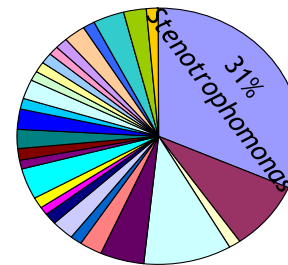
*czcA* is a proton/cation efflux pump induced by  $\text{Cd}^{2+}$ ,  $\text{Zn}^{+}$  and  $\text{Co}^{+}$ .



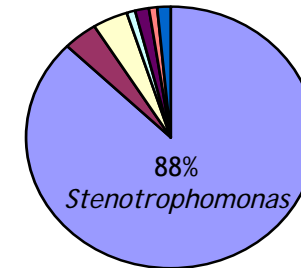
## Diversity of *czcA* gene

*czcA* gene diversity was investigated in two levels of the core (5 and 355 cm) with contrasting levels of cadmium (2.7 and 39.4 ppm) and zinc (311 and 1395 ppm)

[ $\text{Cd}^{2+}$ ]=2.7 ppm



[ $\text{Cd}^{2+}$ ]= 39.4 ppm



- Occurrence of *czcA* gene all along the sediment core.
- Diversity of the *czcA* gene is high in less contaminated sediments and drastically reduced at high concentrations of cadmium and zinc.

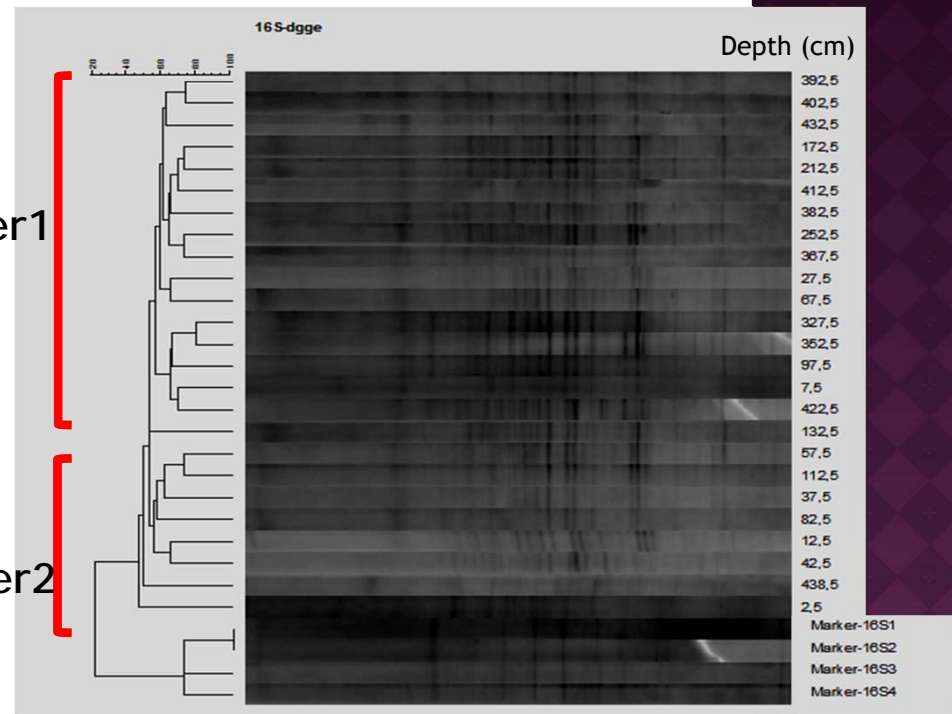
# Relation between bacterial structure community and chemical contamination

Structure of bacterial community was investigated using DGGE fingerprints.



2 clusters distinguished using a non-parametric statistical tests ( $\alpha = 0.05$ ) showed a structuration of microbial communities according to PAH and trace metals concentrations. Cluster2

Cluster1



➤ Structure of bacterial community reflects the chemical contamination

# Impact of the use of antibiotics and resistant bacteria in the environment

Date	Depth (cm)	<i>bla-TEM</i>	
2008	5-10		
	25-30		
	35-40		
2000 (CTX-M)	55-60	■	
	65-70	■	
	80-85		
	95-100	■	
	110-115	■	
	130-135		
	150-155		
	1986	170-175	■
		190-195	■
		210-215	■
230-235		■	
250-255			
325-330		■	
335-340		■	
350-355		■	
365-370		■	
380-385		■	
385-390	■		
1990 (TEM)	390-395		
	400-405	■	
	410-415	■	
	420-425	■	
	430-435	■	
	435-442	■	
	1970		

■ PCR detection of *bla*-CTX-M gene

## Epidemiology

Increasing prevalence of  $\beta$  lactam-resistant strains among pathogens in hospital since 1989.

Sediments: a reservoir of antibiotic-resistant genes ?

2 antibiotic resistance genes (encoding ESBL) were investigated by PCR: *bla*-TEM and *bla*-CTX-M

- Detection of *bla*-TEM gene in the deep sediments (since the 1970's)
  - related to antibiotic resistant fecal bacteria ?
  - autochthonous/environmental bacteria ?

- Absence of detection of the *bla*-CTX-M

- Evolution of the environmental resistome ?
- Possible retrotransfer to human pathogens ?

# Conclusions and perspectives

## Conclusions:

- The multidisciplinary approach has allowed the reconstruction of the sediment core contamination history.
- The metagenome of sediment bacterial communities reflects the contamination of the drainage basin

## Perspectives:

- Intercomparison of sites with different anthropic pressures.
- Sampling the antibiotic resistome since 1970 by a metagenome approach.



**THANKS FOR YOUR ATTENTION!**

