IN SITU KINETICS OF MERCURY BIOACCUMULATION IN *MYTILUS GALLOPROVINCIALIS* ESTIMATED BY TRANSPLANTATION EXPERIMENTS

Stellio Casas[1], Daniel Cossa[2], Jean-Louis Gonzalez[1], Cedric Bacher[3], Bruno Andral[1]

[1]Institut français de recherche pour l'exploitation durable de la mer (Ifremer), BP 330, F.83507 La Seyne-sur-mer, France. Stellio.Casas@ifremer.fr
[2]Institut français de recherche pour l'exploitation durable de la mer (Ifremer), BP 21105, F.44311 Nantes cedex 03, France
[3]Institut français de recherche pour l'exploitation durable de la mer (Ifremer), BP 70, F.29280 Plouzané, France

Abstract : Monitoring coastal contamination by trace metals pollution using mollusks bivalves as quantitative bioindicators is widely performed in many international biomonitoring programs. **For** this purpose, studying mercury dynamic in marine mussels is a reliable tool. *In situ* experiments on uptake and elimination kinetics were conducted in three Mediterranean sites, chosen on the basis of their contamination levels. Mussels were transplanted from a clean area to a highly contaminated one and then transferred back to a clean site. This approach makes it possible to define bioaccumulation factors and uptake and elimination rates consistently with the real environmental conditions.

Keywords : Bioindicator, *Mytilus galloprovincialis*, bioaccumulation, heavy metals, transplantation.

Introduction

Mussels (*Mytilus spp*.) are used as quantitative bioindicators of metal contamination in coastal waters throughout the world. The common term for this monitoring technique is "Mussel Watch" (e.g., Goldberg, 1975). The traditional approach (or passive approach) for a Mussel Watch program is to collect and analyze indigenous populations from designated sites. However, the natural variability of trace metals in tissue is a major concern for temporal and geographical trend interpretations of the data (Cossa, 1989). Active monitoring approaches have been proposed to partially overcome these problems. They consist in using mussels transplanted from a reference population to the monitored sites (e.g., Riget et al., 1997). The data collected from the active monitoring are however dependent on animal equilibration time with the ambient levels of the chemicals concerned. The resulting bioaccumulation is also influenced by environmental factors and biological processes. For example, as proxies for growth rate, the variation of the soft tissue weight has been shown to produce significant changes in metal concentrations in the tissues during the starvation or spawning period and this must be provided for in the monitoring system.

The present experiments address the question of the exposure period required for the equilibration for both uptake and depuration situations in the case of mercury in the mussel, *Mytilus galloprovincialis*. Many comparative studies of food and environmental contamination have been achieved in short term laboratory experiments, using tracer technique labeling algae and seawater to estimate the metal kinetics with mollusks (Borchardt, 1983; Fowler, 1982; Lee et al., 1998; Roditi and Fisher, 1999; Roesijadi et al., 1984). The significant differences in the results observed in these studies emphasize the need for caution when predicting the *in situ* bioaccumulation factors (BF) of metals. Our experiments have been designed to explore the mercury kinetics *in situ* within *M. galloprovincialis*, with the simultaneous measurements of the metal concentrations in water and suspended particles, and

measurements of the mussel biometrics. They make it possible to simultaneously observe both the metal accumulation and physiological change within the mollusk.

M. galloprovincialis used in the experiments were from a pristine mussel farm (Les Aresquiers, Gulf of Lions, Northwestern Mediterranean Sea) with a dissolved mercury concentration in water of around 0.35 ng L^{-1}. They have been transplanted for 6 months in Lazaret Bay where a permanent mercury contamination exists (the dissolved mercury concentration in water amounts to 2.2 ng L^{-1}). This contamination period was followed by a 3-month depuration period in Port-Cros Island. The same transplantation was performed in the Bages lagoon, where mercury contamination is low (dissolved mercury is 0.5 ng.L^{-1}).

Results and discussion

The uptake of mercury in mussels transplanted in Lazaret Bay showed an accumulation curve characterized by an initially rapid period followed by a slower one (Figure 1A). The kinetics looked like an asymptotic curve reaching a pseudo-equilibrium. During the 6-month exposure, the rate of uptake was steady except between 9 and 16 December 2002, which was the period in which there was a visibly abrupt loss of weight in the mussel soft tissue due to spawning (Figure 1B). A pseudo-equilibrium was attained after 110 days at a mercury concentration of 0.5 mg. kg^{-1}. During the 3-month experiment, the pseudo-equilibrium (0.42 mg.kg^{-1}) was reached somewhat rapidly after 60 days. These two mercury accumulation curves are similar and attain a saturation plateau at the same time. At the end of the experiments, observations were made pertaining to amounts of mercury of 1.57 and 1.64 µg per mussel for the 3 and 6-month experiments respectively.

In the uncontaminated Bages lagoon, the kinetics of mercury concentration in soft-tissue were flat and devoid of any significant accumulation (Figure 1). The mercury concentration in soft tissue at the end of the experiments was about 5 times lower compared to the contaminated site at Lazaret Bay. A perturbation of kinetics was observed in each location, around the 17/12/02, during a substantial loss of soft weight corresponding to the spawning period.

The decontamination kinetics of mercury in soft-tissue of mussels transplanted from Lazaret Bay to Port-Cros was significant and comprised a decrease of 23% after 103 days (Figure 1A). Consistently with the absence of mercury contamination in the Bages lagoon, no mercury change in the mussel soft-tissue could be observed after the transplantation of mussels from Bages to Port-Cros.

Bioaccumulation is the result of interaction between physiological factors (growth, reproduction, storage, etc.), chemical factors (uptake, excretion, accumulation, etc.) and environmental factors (temperature, salinity, etc.). These factors influence the bioaccumulation process in time and space, and thus are forcing variables. In kinetic term, these factors interact on each others in relation to time, to the characteristics of the sites and to the magnitude of the contamination. During the accumulation process in the contaminated site, the uptake attained a maximum rate at the beginning of the experiment and the major forcing variable was the contamination level of the environment.

Growth and reproduction are the two major biological factors influencing the bioconcentration process. On the one hand, the spawning of mussel, which corresponds to a significant loss of weight (up to 40% of the soft tissue mass here), coupled to the accumulation process, causes an abrupt increase of mercury concentration in tissue. On the other hand, the growth counteracts the mercury accumulation and its increase seemingly dilutes the mercury content of the animals.

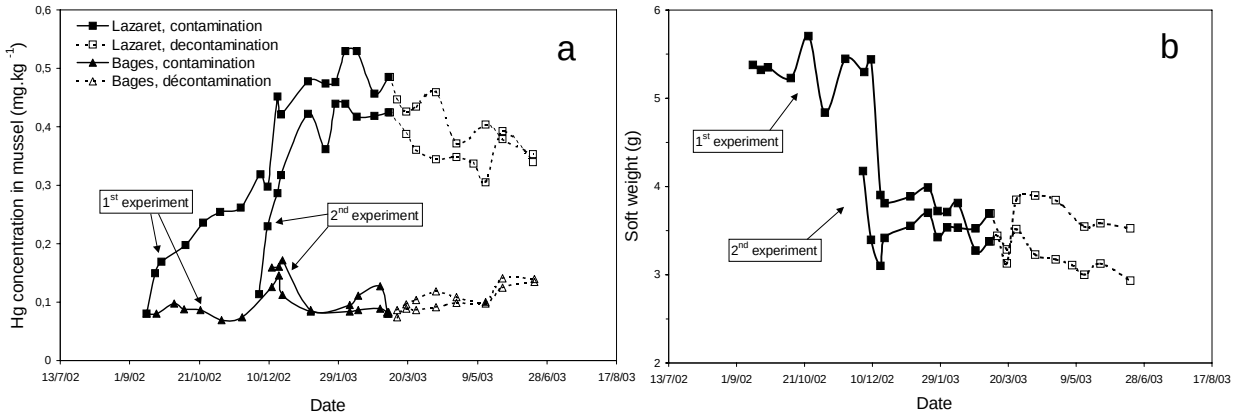

Figure 1. Mussel transplantation experiments. (a) Mercury concentrations in the mussel dry soft tissue, (b) Dry mass of the soft tissue per mussel.

Our results show that mercury uptake did not linearly proceed over time in mussels, contrary to many studies in other bivalves. Mussels may require a long period of exposure to reach equilibrium with ambient metals. Therefore, the bioconcentration factor (BF: mercury concentration in the mussel soft tissue on a wet weight basis divided by the dissolved mercury concentration in the water), calculated during the experiments increase with time (Table 1). The highest values of the BF have to be taken into account with a steady state assumption. The BF calculated for the decontamination experiments are indicative of non equilibrium situations.

Table 1. *Variations of the bioconcentration factor (BF) for different sites and kinetics.*

Site	**Contamination**		**Decontamination**	
	Mean	Range	Mean	Range
Lazaret Bay	2.32×10^5	$1.64 - 3.83 \times 10^5$	1.12×10^6	$0.72 - 1.81 \times 10^6$
Bages Lagoon	1.73×10^5	$0.96 - 2.17 \times 10^5$	2.44×10^6	$2.86 - 7.13 \times 10^5$

Bioaccumulation can also be predicted on the basis of the measurements of the rate constants of uptake and elimination. A simple bioaccumulation model made up of one input *via* water, impact of weight changes and of one output *via* faeces can be calculated:

$$\frac{dC_{mussel}}{dt} = r_{da}.C_{water} - r_{ad}.C_{mussel} - \frac{dP}{P.dt}.C_{mussel}$$

with C_{mussel} : Hg concentration in soft-tissue of mussels ($\mu g.g^{-1}$) ; C_{water} : Hg concentration in water ($\mu g.l^{-1}$) ; P : Soft-tissue weight (g) ; r_{da} : uptake rate ($l.g^{-1}.d^{-1}$) ; r_{ad} : elimination rate (d^{-1}).

Table 2. *Uptake and decontamination rates for mercury in* Mytilus spp. *BF: bioconcentration factor at pseudo-equilibrium; r_{da}: uptake rate; r_{ad}: elimination rate*

Species	r_{da}	r_{ad}	BF	Ref.
M. galloprovincialis	1.8 $l.g^{-1}.d^{-1}$	0.0065 d^{-1}	$4x10^5$	This study *(in situ)*
M. edulis	2.3 ± 0.2 $l.g^{-1}.d^{-1}$	0.08 d^{-1}	$1x10^5$	Roditi et al., 2000 *(lab.)*
M. edulis	1.84 - 4.75 $l.g^{-1}.d^{-1}$	0.05 d^{-1}	$1x10^5$	Roditi & Fisher, 1999*(lab.)*

Values of uptake and elimination rates calculated here are similar to lower values found in lab-studies (Table 2). Uptake rates may be overestimated in lab-studies, due to the short time of the experiments (15-30 days) and without reaching a pseudo-equilibrium. Thus, only the first rapid period of uptake is observed.

Conclusions

This "kinetic" approach provides an understanding of metal bioaccumulation and a validation of the use of mussel transplantation for mercury monitoring purposes. The parameters determined here will be used in a biokinetic model coupling mussel growth and mercury uptake and excretion which is the next step in our research. By combining environmental and biological data, the model could constitute an optimized biomonitoring tool which can be applied to various coastal environments.

References

Borchardt, T., 1983, Influence of food quantity on the kinetics of cadmium uptake and loss via food and seawater in *Mytilus edulis*, *Mar. Biol.* **76:** 67-76.

Cossa, D. 1989. A Review of the Use of *Mytilus* Spp. as Quantitative Indicators of Cadmium and Mercury Contamination in Coastal Waters, *Oceanol. Acta*. **12**: 417-432.

Fowler, S. W., 1982, Biological transfer and transport processes, in: *Pollutant transfer and transport in the sea* (G. Kullenberg, ed.), CRC press. Boca Raton, pp. 2-65.

Goldberg, E. D., 1975, The Mussel Watch, *Mar. Pollut. Bull.*, **6:** 111-113.

Lee, B. G., Wallace, W. G., and Luoma, S. N., 1998, Uptake and loss kinetics of Cd, Cr and Zn in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: effects of size and salinity, *Mar. Ecol. Prog. Ser.* **175:** 177-189.

Riget, F., Johansen, P., and Asmund, G., 1997, Uptake and release of lead and zinc by blue mussels (*Mytilus edulis*). Experience from transplantation experiments in greeland, *Mar. Pollut. Bull.* **34:** 805-815.

Roditi, H. A., and Fisher, N. S., 1999, Rates and routes of trace element uptake in zebra mussels, *Limnol. Oceanogr*. **44:** 1730-1749.

Roesijadi, G., Young, J. S., Drum, A. S., and Gurtisen, J. M., 1984, Behaviour of trace metals in *Mytilus edulis* during a reciprocal transplant field experiment, *Mar. Ecol. Prog. Ser.* **18:** 155-170.