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Comparative study of the bioaccumulation and elimination of trace metals (Cd, Pb, Zn, Mn and Fe) in the digestive gland, gills and muscle of bivalve *Pinna nobilis* during a field transplant experiment

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ABSTRACT

The purpose of this study was to investigate the long-term bioaccumulation and elimination of Cd, Pb, Mn, Zn and Fe by *Pinna nobilis* tissues after their 90 day-transplantation period at Téboulba fishing harbor. During the transplantation period, the Cd, Pb, Mn, Zn and Fe concentrations in the different tissues of the mussels were measured before and after exposure period. Metal (Cd, Pb, Mn, Zn and Fe) accumulation in *P. nobilis* mussels varied depending on the analyzed tissue and the caging times. Notable differences in Cd, Pb, Mn, Zn and Fe accumulation patterns within the digestive gland, gills and muscle were found and may be due to the ability of each tissue to accumulate metals. During the depuration phase, the elimination of Cd, Pb, Mn, Zn and Fe depended on the target tissue and the metal speciation. Cd, Pb, Mn and Fe were eliminated rapidly from one organ and increased in other when compared to those of 90 day transplanted mussels. The increase of metal loads during the elimination phase is not clear and particularly what kind of processes is responsible for such response. However, it is reasonable to assume that metals increase is related to the existence of an accumulation/detoxification mechanism, which involves the transport of metals from an organ to another. The data obtained indicate that because of the significantly high quantities of Cd, Pb, Mn, Zn and Fe accumulated during the exposure phase, the transplanted mussels are suitable bioindicators for monitoring trace metals in marine ecosystem.

Introduction

Due to the intensive anthropogenic activities, a significant amount of metals has been found in coastal areas, causing several biological effects from molecular dysfunctions to ecological degradation depending on the time of exposure and concentration levels [1,2]. Among the significant sources with potential threat to marine ecosystem are harbors, which are known from as a chronic source of pollutants [3,4].

Bivalves are commonly used to detect metal pollution in the marine environment due to their wide distribution, sedentary state, ease of collection, transplantation, and maintenance in the laboratory; and ability to accumulate high concentrations of a wide range of contaminants including heavy metals [5]. In this sense, the fan mussel *Pinna nobilis* L. is the largest endemic bivalve in the Mediterranean Sea [6] and could be an interesting organism test for ecotoxicological studies due to the large size (60–120 cm) and high contaminant accumulation [7]. However, population of *P. nobilis* has greatly reduced during the last few decades because of several factors including the reduction and loss of their natural habitat by an increase in anthropogenic inputs into coastal waters [7].

Most studies of bivalve metal bioaccumulation have been carried out on mussels or clams under laboratory controlled conditions or collected from a polluted area with high metal inputs [8,9]. Large size mussel has a big contact surface with external environments allowing a greater metal uptake than other mussels or clams do. Abiotic and biotic factors such as temperature, salinity, food supply, age, and reproductive status also influence, heavy metal accumulation and thus have to be considered in pollution assessment studies. Bivalves assimilate metals by the ingestion of particulate material suspended in water, ingestion of food, ion uptake of dissolved metals across gills, and the absorption on tissues and membrane surfaces [10]. Metal tissues depends on the type of exposure, whether through diet or water, and can be used as a pollution indicator. In view of future national monitoring of large mussels *P. nobilis* environment, we investigated the long-term bioaccumulation and elimination of Cd, Pb, Mn, Zn and Fe by *P. nobilis* tissues after their transplantation at Téboulba fishing harbor (Tunisia). The use of caged bivalves is a useful strategy for monitoring marine pollution through the analysis of metals accumulated [11]. The use of

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caged organisms provides a time-integrated assessment of environmental quality, and reveals the early biological effects induced by accumulated pollutants [12]. In this study, we investigated the accumulation and elimination of trace metals in different tissues of *P. nobils* following their long-term caging into the Téboulba fishing harbor for better assessing the risk of multimetal exposure under real conditions.

Materials and methods

P. nobilis sampling and experimental caging technique

Wild *P. nobilis* specimens (30) were manually collected at an uncontaminated area of Monastir bay in September 2008. After collection, bivalves were immediately transported in a cold container by boat to the Téboulba fishing harbor (35°39′N, 10°57′E), where they were exposed to ambient conditions for 3 months, and then transferred immediately out of harbor; to the uncontaminated area for a 3-month depuration period. Twenty-four specimens of 30 ± 10 cm shell length were placed in a rectangular cage (70 cm × 70 cm × 100 cm) and maintained at contact with the bottom. The remaining 6 bivalves were immediately transferred to the uncontaminated area for a 3-month depuration period.

After 0, 1, 2, and 3 months of exposure period and a 3-month depuration, 6 bivalves were collected and immediately dissected. The tissues, i.e. gills, digestive gland and the anterior and posterior adductor muscles, were carefully removed, placed in tubes and were stored at −40 °C until analysis.

Analysis of metals

The concentration of Cd, Pb, Zn and Fe in seawater of Téboulba harbor was determinated according to the methods previously described by Ben-Khedher et al. [41]. Briefly, seawater samples were filtered at the time of collection through a 0.45-μm membrane and acidified for the determination of dissolved elements. Sample was analyzed by inductively coupled plasma/atomic emission spectrometry (ICP/AES) using OPTIMA 3300 RL (Perkin Elmer®) according to the standard NF EN ISO 11885.

Metals were analyzed in the freeze-dried organs by atomic absorption spectrophotometry (AAS) according to the methods published previously by Ennouri et al. [13] and Ghedira et al. [14]. After freezing, two pooled out of three bivalve tissues per caging time were lyophilized and homogenized using a ball mill made of agate. The total element analyses were performed with 0.2 g of freeze-dried organs sample digested at 180 °C for 20 min, using high pressure Teflon reactors and 6 ml concentrated nitric acid (HNO_3) in microwave (Millestone, type Ethos) oven system. After digestion, samples were transferred to 50 ml flasks and the volume was adjusted to 50 ml using Milli-Q water. The concentrations of Cd, and Pb were determined by graphite furnace atomic absorption spectrophotometry with Zeeman background correction (AAS-ZGF instrument, Varian 220Z). Analysis of Fe, Zn and Mn were performed by flame atomic absorption spectrophotometry (Varian AA 10). The analytical and quality assurance was performed by analyzing certified reference materials of shellfish tissue (International Atomic Energy Agency (IAEA 407)) duplicate samples and individual blanks.

The analytical results indicated good agreement between the certified and found values and the recovery of metals near quantitative (95%) for most of analytes (Table 1).

All metal concentrations are reported in $\mu g\,g^{-1}$ of dry weight of sample. Values represent the mean of two independent extractions from the organ samples.

Table 1
Mean and standard deviation of data for IAEA-407.

Element	Certified value	Measured value
Cd	0.189 ± 0.019	0.176 ± 0.022
Pb	0.12 ± 0.06	0.17 ± 0.05
Mn	3.52 ± 0.32	2.28 ± 0.43
Zn	67.1 ± 3.8	67.33 ± 2.75
Fe	146 ± 14	139 ± 15

Statistical analysis

Results were expressed as the mean of two independent extractions of two pools out of three tissues each. The data of Cd, Pb, Mn, Zn and Fe accumulation obtained in control and transplanted mussels into the harbor for 30, 60 and 90 days were used for regression analysis. The relations between metals concentrations for each tissue were also investigated using Pearson correlations. All the statistical results were obtained using a statistical package (SPSS 9.0 for Windows), with a probability of $p < 0.05$ considered significant.

The bioaccumulation of trace metals into mussels during transplantation was expressed as bioconcentration factor (CF), which is the ratio between the metal in mussels ($\mu g\,g^{-1}$ of dry weight) and the metal concentration in seawater ($\mu g\,ml^{-1}$). Bioconcentration factor was best described using a simple equation: $CF = [(C_t - C_c)/C_w]$ [15], where C_c and C_t were the metal concentrations in the tissue of mussels before and after transplant experiment, C_w represented the metal concentration in the harbor seawater and determined at the end of the transplant experiment. When $C_t < C_c$, the CF was considered as 0.

The elimination of metals was expressed in percentage of lost metal concentration. Elimination factor (EF) was described by the equation $EF = 100 - [(C_e/C_t) \times 100]$, where EF is the percentage of lost metal concentration, C_e is the metal concentration in mussel tissue after depuration period, C_t is the metal concentration in tissue of 3-months transplanted mussels. When $C_e > C_t$, the EF was considered as 0.

Results and discussion

Marine bivalves including *P. nobilis* mussel are very sensitive to metals and are known for their capacity to concentrate them from their environment, including potentially toxic elements such as cadmium or lead. Transplanting bivalves from a reference site to a polluted area avoids bias related to age and reproductive status of the organisms that influence contaminant bioaccumulation and allows a more accurate assessment of the real biological effects of contaminants over a predetermined exposure period [16]. In this study, wild mussels (*P. nobilis*) were manually collected at uncontaminated area of Monastir bay (center of Tunisia) then transferred to Téboulba fishing harbor, where they were exposed to ambient conditions for three months.

During the transplantation period, the metal of the mussels was measured before and after caging exposures (Table 2). No mortality of mussels was observed during the transplantation experiment. The temporal variation of Cd, Pb, Mn, Zn and Fe concentrations in digestive gland (DG), gills (G) and muscle (M) of the transplanted *P. nobilis* into the Téboulba fishing harbor during three-month exposure and three-month depuration periods was shown in Fig. 1. Metal (Cd, Pb, Mn, Zn and Fe) accumulation in mussels *P. nobilis* varied, depending on the analyzed tissue and the caging times. The data are summarized in Fig. 1. Metal concentrations in mussels are comparable with already published values for other species of bivalves [40,17].

Most studies concerning metal bioaccumulation by bivalve mollusks have focused on whole-body [18–20], while very little data