A study of the influence of brevetoxin exposure on trace element bioaccumulation in the blue mussel *Mytilus edulis*

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ABSTRACT

Marine organisms are exposed to and affected by a multitude of chemicals present in seawater and can accumulate in their tissues a wide range of contaminants as well as natural biotoxins associated with harmful algal blooms (HABs). Trace elements and biotoxins may modify physiological functions in exposed organisms, and studies have been conducted to better understand their respective kinetics and effects in marine species. Despite the increasing concern of concurrent toxic HABs and pollution events due to anthropogenic pressures and global change, very little information is available on their combined effects. Chemical interactions between biotoxins and trace elements have been reported, and exposure to certain biotoxins is known to modify ion transport pathways, suggesting that biotoxins have the potential to alter trace element uptake. Using specific and sensitive radiotracer techniques (radioligand receptor binding assay and γ-spectrometry), this laboratory study examined the influence of pre-exposure to the brevetoxins (PbTxs)-producing microalgae *Karenia brevis* on the bioaccumulation of selected non-essential (Cd) and essential (Co, Mn and Zn) trace elements in the blue mussel *Mytilus edulis*. PbTxs are a group of neurotoxins known to accumulate in bivalves but also to have lethal effects on a number of marine organisms including fish and mammals. We found that, over 23 days exposure to the radiotracers, the bioaccumulation of the dissolved essential trace elements Co, Mn and Zn in *M. edulis* was not significantly affected by pre-exposure to toxic *K. brevis*. In contrast, the uptake rate constant k_u of Cd was significantly higher in the pre-exposed group (p < 0.05), likely caused by a decrease in mussel clearance rates after *K. brevis* exposure. These results suggest that the effects of algal toxin exposure on bioaccumulation of trace elements in mussels may be trace element-dependent.

1. Introduction

In coastal marine environments, dissolved organic and inorganic elements coming from both natural or anthropogenic sources are prevalent and can play an important role in the physiology of inhabiting organisms. Their concentrations and distributions in the marine environment have been increasing over the last decades, mostly due to human activities such as industrialization, urbanization and agriculture (e.g. Förstner and Wittmann, 2012). Some trace elements such as Co, Mn, and Zn are considered essential to marine organisms because they are required for basic metabolic processes; they are components of various enzyme functional groups, play a structural role in respiratory pigments and metalloenzymes, and can activate co-factors for various proteins (Simkiss, 1979; Williams, 1981). Other trace elements such as Cd, having no known biological function for organisms, are considered

as non-essential. Nevertheless, all trace elements, both essential and non-essential, may cause adverse effects on organismal physiology if their concentrations exceed thresholds beyond which homeostatic regulation processes are saturated (Förstner and Wittmann, 2012). In addition to those chemicals resulting from geological weathering processes (Morel and Price, 2003), naturally occurring compounds such as biotoxins associated with harmful algal blooms (HABs) can also accumulate in marine organisms and may affect physiological functions.

Growth in the frequency and intensity of toxin-producing HAB events over the last decades (Hallegraeff, 1993; Smayda, 1990; Sournia, 1995; Van Dolah, 2000) increases the likelihood that filtering, planktivorous and even upper trophic level organisms are exposed to biotoxins. Bivalve molluscs in particular have been found to be capable of accumulating biotoxins including azaspiracid, okadaic acid, saxitoxins, domoic acid and brevetoxins (PbTxs) (FAO, 2011). Toxic to humans,

those biotoxins may also cause severe physiological and behavioral changes in bivalve species (Manfrin et al., 2012). Modification of valve closure and of feeding and filtration activities have been reported in various bivalve species after exposure to different toxin-producing microalgae (Contreras et al., 2012; Echevarria et al., 2012; Shumway et al., 2006; Shumway and Cucci, 1987). For example, reduction in filtration activity, as estimated by the measurement of clearance rates, has been demonstrated in four bivalve species exposed to PbTxs (e.g. Leverone et al., 2007). Nevertheless, such effects may be variable or even conflicting, where reduced filtration rates have been observed in the soft shell clam *Mya arenaria* and the ribbed mussel *Geukensia demissa* while the oyster *Ostrea edulis* increased rates of filtration under identical experimental conditions (Shumway and Cucci, 1987).

Changes in filtration activity in the presence of biotoxins, as have been previously observed, could affect the ability of bivalves to bioaccumulate trace elements from the dissolved pathway (Wang, 2001). Thus, it can be postulated that trace element bioaccumulation in bivalves may be altered by exposure to toxin-producing harmful algae. Moreover, trace element bioavailability and potential transfer in aquatic food chains may be altered by the formation of complexes with algal toxin molecules which often contain functional groups such as amino, carboxyl, phenol, sulfhydryl and hydroxyl groups (Humble et al., 1997; Rue and Bruland, 2001). For example, Cu bioavailability has been shown to be affected by the formation of a domoic acid-Cu complex (Rue and Bruland, 2001). In addition, changes in membrane potential that occur with exposure to algal neurotoxins such as PbTxs may impact cellular ion transport (Berman and Murray, 2000; Liberona et al., 2008; Tian et al., 2011) and consequently may affect the distribution of trace elements to target tissues and cells, and hence their accumulation in bivalves.

This study assessed the effects of pre-exposure to *Karenia brevis* culture (containing *K. brevis* cells and solubilized PbTxs) on bioaccumulation of non-essential (i.e. Cd) and essential (i.e. Co, Mn and Zn) trace elements in the blue mussel *Mytilus edulis*. Specific nuclear techniques were used (1) to quantify PbTx concentrations accumulated in mussels using a radioligand receptor binding assay (RBA) and (2) to determine the uptake kinetics of studied metals using γ-radiotracers (^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn). Two levels of biological organization, individual tissues and the entire blue mussel, were considered in this study in order to evaluate the whole-body uptake kinetics and the among compartment transfer dynamics of trace elements after exposure to PbTxs.

2. Materials and methods

2.1. Origin and maintenance of organisms

One hundred blue mussels *M. edulis*, a species widely used as a bioindicator for monitoring of trace elements, were purchased from a seafood seller (Les Halles du Midi, Monaco) and were transported to the International Atomic Energy Agency (IAEA-EL) premises in Monaco. Mussels were acclimated to laboratory conditions for 1 month (continuously aerated, open-circuit 300-L plastic tank; flux: 150 L h^{-1}; salinity: 38; temperature: 20 ± 1 °C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) prior to experimentation. During this period, mussels were fed a daily algal diet of *Isochrysis galbana* and were confirmed to be PbTx-free by RBA analysis.

K. brevis culture (NOAA-1 strain isolated from Charlotte Harbor, FL, USA) was grown in a 20-L plastic container containing 0.45 μm filtered aged seawater (dark-incubated for 1 month minimum; salinity: 38; temperature: 20 ± 1 °C; pH: 8.0 ± 0.1; bilateral luminosity 66–80 μE m^{-2} s^{-1}; light/dark: 12h/12h) spiked with an atypical f10k medium (Holmes et al., 1991). *K. brevis* were harvested at the stationary phase (i.e. approx. 10000 cells mL^{-1}) for use in the experiment.

2.2. Experimental procedures

2.2.1. Exposure to K. brevis culture

Mussels *M. edulis* (33.1 ± 5.1 g wet wt) were evenly distributed across the bottom of three 20-L aquaria (n = 40; same environmental conditions as described in section 2.1.). Prior to distribution, byssal threads were cut with a scissors to limit mussel movement and thus maintain a homogeneous distribution of the mussels throughout the tank over the experimental duration. An experimental treatment was randomly assigned to each aquarium. Mussels from aquaria 1 and 2 were exposed to *K. brevis* culture (concentration of 980 ± 20 cells mL^{-1} in the exposure tank) while aquarium 3 served as a control without algal exposure. *K. brevis* culture was added to aquaria 1 and 2 for 1 h daily (i.e. the time required for the complete filtration of *K. brevis cells* by the mussels, data not shown) in a closed circuit over a period of 4 days. Culture was added to the aquaria with extreme care to prevent cell lysis. Cell abundance was checked manually by optic microscopy in 3 replicate counts. After the 1-h exposure period, seawater was changed and mussels fed briefly (30 min with *I. galbana*) in *K brevis*-free conditions. After the last exposure to *K. brevis* (day 4), eight mussels from aquaria 1 and 2 were collected, sacrificed and dissected to quantify the PbTx concentrations in combined soft tissues for some mussels and in the following individual body compartments for others: (1) gills, (2) digestive gland and (3) remaining soft-parts. To follow PbTx depuration, mussels from the first *K. brevis*-exposed aquarium (aquarium 1) were kept in *K. brevis*-free conditions (open-circuit; conditions as described in section 2.1) for 23 days, after which eight mussels were sacrificed and dissected as described above. The remaining mussels of the second *K. brevis*-exposed aquarium (aquarium 2) were exposed to dissolved radiotracers for 23 days as described in section 2.2.2.

Mussels from the third 20-L aquarium (aquarium 3: control) were maintained in the same conditions as the mussels in aquarium 1 but were not exposed to cultures of *K. brevis*. All handlings (water changes, feeding) were repeated on this aquarium to ensure potential levels of stress and numbers of individuals were identical between all the aquaria throughout the experiment.

2.2.2. Exposure to dissolved radionuclides

Remaining mussels (n = 32), in both aquarium 2 (pre-exposed to *K. brevis*) and aquarium 3 (control), were exposed in the same 20-L aquaria to a mixture of dissolved γ-radiotracers (aquaria conditions as previously described in section 2.2.1) over 23 days (nominal activities of 0.5 Bq mL^{-1} for ^{57}Co and ^{54}Mn and 1 Bq mL^{-1} for ^{109}Cd and ^{65}Zn). The duration of exposure was chosen to allow accurate determination of kinetic parameters. To keep the environmental radioactivity constant, spiked seawater was renewed regularly (i.e. at the time of each γ-counting as detailed below), with a maximum of 3 days between two renewals of seawater (following Metian et al., 2009). Seawater radioactivity in the two radiotracer-exposed aquaria (2 and 3) was checked before and after each spike renewal by γ-counting 150-ml water samples (time-integrated activities available in Table 1). After each water

Table 1

Time-integrated activities measured during the 23-day exposure to the dissolved radiotracers for mussels pre-exposed to *K. brevis* culture (aquarium 2) and for non-exposed mussels (aquarium 3, i.e. control group). Data are Means ± SD.

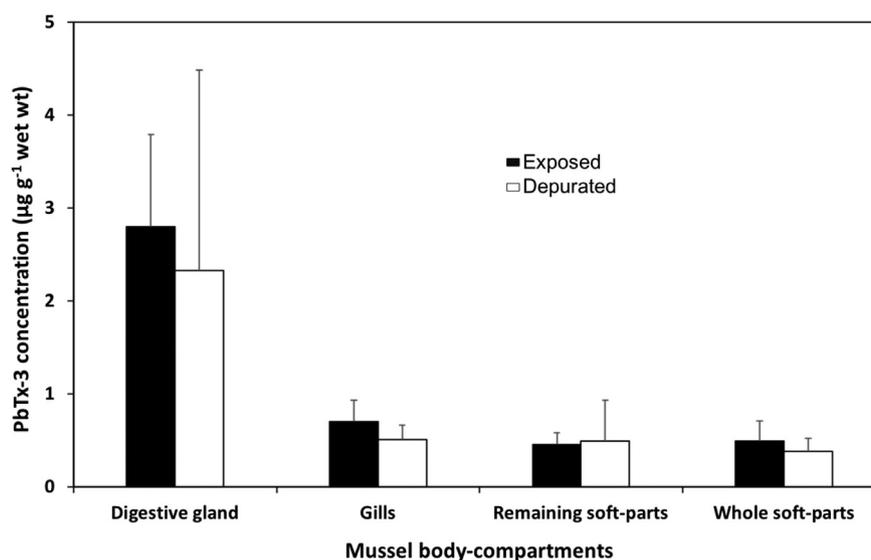| Radiotracer	Aquarium 2 (Pre-exposed to *K. brevis,* Bq mL^{-1})	Aquarium 3 (Control group, Bq mL^{-1})
^{109}Cd	0.93 ± 0.17	1.00 ± 0.12
^{57}Co	0.43 ± 0.11	0.43 ± 0.12
^{54}Mn	0.39 ± 0.17	0.41 ± 0.16
^{65}Zn	0.80 ± 0.39	0.78 ± 0.40

Fig. 1. Concentration of PbTx-3 in four mussel compartments (digestive gland, gills and remaining soft-parts and whole-soft parts, n = 4) after four periods of exposure to *K. brevis* cells (black) and subsequently after three weeks of depuration (white). Data are Means + SD.

renewal and before radiotracer spiking, mussels were fed 30 min with *I. galbana*. Eight tag-identified mussels from each aquarium were collected using forceps at different time intervals (after 1, 3, 6, 8, 10, 13, 15, 17, 20 and 23 days of exposure), rinsed three times in clean seawater, weighed and then radioanalyzed (γ-counted) alive to quantify whole-body radiotracer concentrations. After counting, mussels were returned to their respective aquarium. After 3, 9 and 23 d of exposure, mussels were collected (n = 4), sacrificed, and dissected into 4 compartments: (1) gills, (2) digestive gland, (3) remaining soft-parts and (4) shell. Samples were γ-counted in order to assess the radionuclide body distribution.

2.2.3. PbTx quantification using β-radioligand receptor binding assay (RBA)

PbTxs from the whole *K. brevis* culture were extracted using 500 mg C-18 SPE cartridges (recovery: 99–125%, Twiner et al., 2007). Solvent extraction for PbTxs from mussel tissues was performed as previously described (Poli et al., 2000; Pouil et al., 2018) and tested (recovery: 90–108%, Dickey et al., 1999). Briefly, samples of each tissue type (n = 4) were homogenized (T25 Ultra-Turrax Basic, IKA) in acetone, sonicated, and centrifuged at 3000 g for 10 min. Each supernatant was collected, and the process was repeated two more times. Combined supernatants were evaporated under a stream of nitrogen in a water bath at 40 °C. Dried samples were resuspended in 80% methanol, mixed by inversion after the addition of equal volumes of n-hexane, and centrifuged at 3000 g for 1 min to facilitate phase separation. The methanol phase was collected in glass tubes and evaporated under a stream of nitrogen. All dry extracts (from *K. brevis* culture and mussel tissues) were resuspended in 100% methanol and stored at −18 °C until analysis. The activity-based RBA was used to detect and quantify composite PbTx concentrations in the different tissue extracts (according to Bottein et al., 2010; Dechraoui Bottein and Clausing, 2017, with some modification) using PbTx-3 as the standard. Samples were analysed at three dilutions in triplicate in a 96-well filterplate format (MSFB N6B 50 MultiscreenHTS), and radioactivity was counted in a liquid scintillation β-counter (MicroBeta2 Microplate Counter, PerkinElmer) after a 1-h period of dark incubation. Toxin quantification of samples was calculated from PbTx-3 standard curves (4-parameter logistic regressions) determined using GraphPad Prism software version 6.0 (San Diego, USA). As γ-emitters (here ^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn) can cause inference in β-detection with liquid scintillation counting (here tritiated PbTx: [^{3}H]PbTx-3), quantification of PbTx

concentrations cannot be performed on radiotracer-exposed samples.

2.2.4. Radiotracers and γ-spectrometry measurements

Uptake kinetics of radionuclides in mussels were determined using high specific activity γ-radiotracers (^{109}CdCl$_2$ in 0.5M HCl, T$_{1/2}$: 464 days, ^{57}CoCl$_2$ in 0.1M HCl, T$_{1/2}$: 271 days, ^{54}MnCl$_2$ in 0.5M HCl, T$_{1/2}$: 312 days, and ^{65}ZnCl$_2$ in 0.1M HCl, T$_{1/2}$: 244 days) purchased from Polatom, Poland. Radioanalyses were carried out using a γ-spectrometer system composed of 4 Germanium - N or P type - detectors (EGNC 33–195-R, Canberra® and Eurysys®) connected to a multi-channel analyser, and information was treated using a computer equipped with a spectra analysis software (Interwinner 6, Intertechnique®). The radioactivity in living mussels and samples was determined by comparison with standards of appropriate geometry spiked with known activity (Cresswell et al., 2017). Briefly, calibration was conducted with mussel "phantoms" created by filling empty shells with paper towels soaked with known quantities of radiotracers in 2M HCl. For samples of seawater and soft tissues, the calibration was done by using similar containers filled with the same volume of a 2M HCl solution spiked with known concentrations of radiotracers. Corrections were made for background and physical radioactive decay (Rodriguez y Baena et al., 2006).

2.3. Data treatment and statistical analyses

Uptake kinetics of radiotracers were expressed in terms of change in concentration factor (CF) over time, where the CF is the ratio between whole-body activity (Bq g^{-1} wet wt) and time-integrated activity of radiotracers in seawater (Bq g^{-1}). Radiotracer uptake kinetics were described using a simple linear regression model (Eq. (1)) or by a saturation exponential kinetic model (Eq. (2)) if the observed kinetics tended to reach a steady-state equilibrium:

$$CF_t = k_u t \tag{1}$$

$$CF_t = CF_{ss}\left(1 - e^{-k_e t}\right) \tag{2}$$

with $CF_{ss} = \frac{k_u}{k_e}$

Here CF_t and CF_{ss} are the concentration factors at time t in days (CF_t) and at steady-state (CF_{ss}), respectively, k_u is the uptake rate constant (d^{-1}) and k_e is the depuration rate constant (d^{-1}; e.g. Warnau et al., 1996; Whicker and Schultz, 1982).

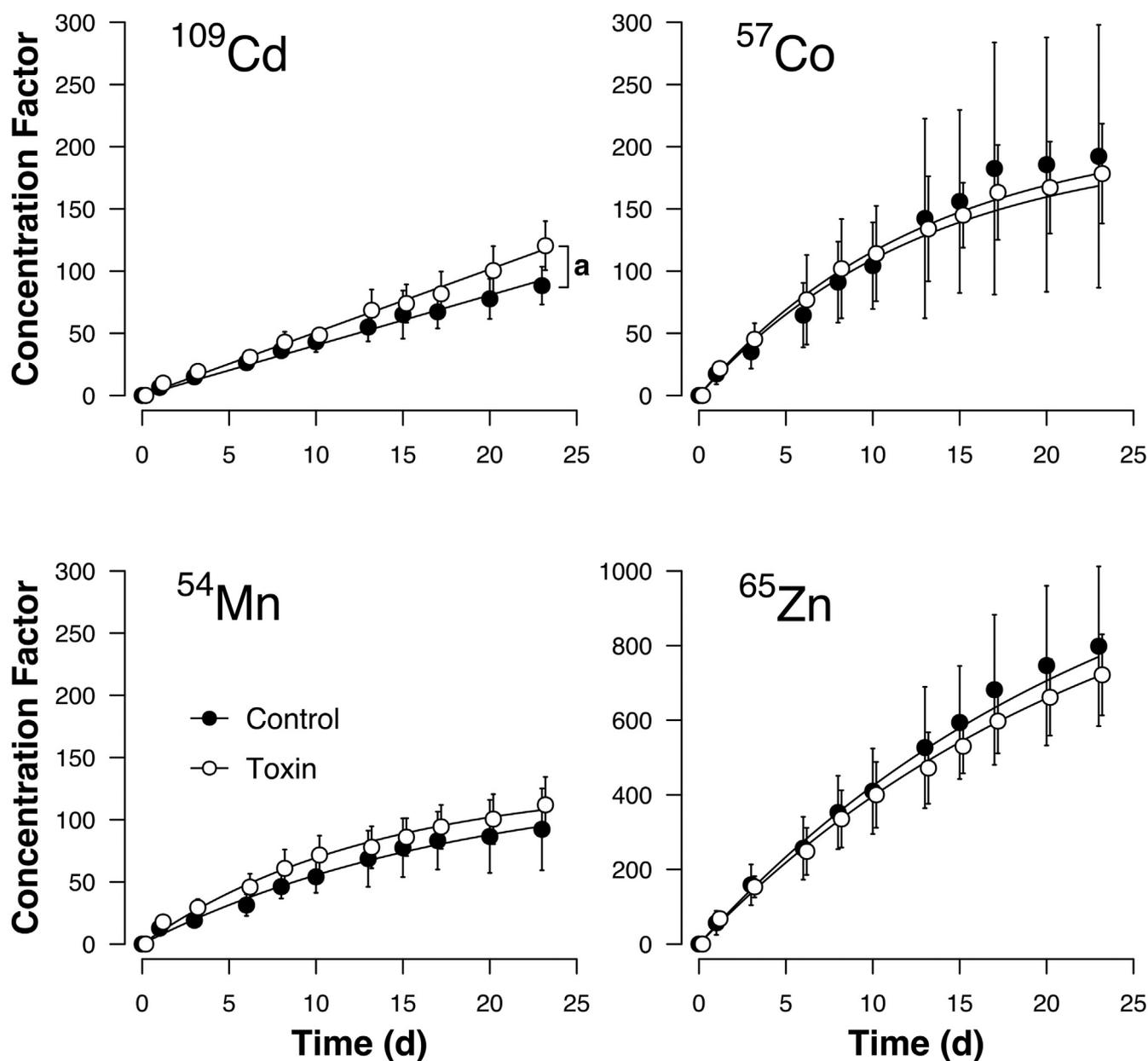In order to capture the influence of *K. brevis* pre-exposure on

Fig. 2. Whole-body uptake of [109]Cd, [57]Co, [54]Mn and [65]Zn expressed as Concentration Factors (means ± SD) in *K. brevis* pre-exposed (white) or control (black) blue mussels (*M. edulis*, n = 8). Parameters of uptake kinetics and their statistics are given in Table 1. Letter denotes significant difference (p < 0.05) between k_u of the mussels from the two experimental conditions.

radionuclide uptake capacities in mussels, statistical comparisons were conducted using individual uptake kinetics: individual parameters (k_u and CF_{ss}) were obtained from the best-fit model at the global scale (Eq. (1)) for each individual. Differences between these parameters were then tested using two-tailed Wilcoxon-Mann-Whitney non-parametric tests. For each body compartment, the same statistical test was used to compare trace element distribution during the uptake period between control mussels and those pre-exposed to *K. brevis* culture. The same procedure was used to compare PbTx concentrations in the body compartments of the mussels from the exposed and the depurated mussels. The level of significance for statistical analyses was always set at α = 0.05. All the statistical analyses were performed using R software 3.0.1 (R Development Core Team, 2014).

3. Results and discussion

We found that blue mussels accumulated PbTxs in non-negligible

quantities (i.e. 0.49 ± 0.22 µg g^{-1} wet wt in whole soft tissues after the last exposure to the *K. brevis*, Fig. 1). Moreover, concentrations of PbTxs were found to be more than 4-fold higher in the digestive gland (up to 2.80 ± 1.00 µg g^{-1} wet wt, Fig. 1) compared to other tissues, suggesting that mussels accumulated PbTxs mainly by ingestion of *K. brevis* cells. As PbTxs were also found in the gills, this suggests that PbTxs may have been accumulated in part from dissolved PbTxs in the water during exposure to *K. brevis* culture (i.e. *K. brevis* intact cells and solubilized PbTxs). After the 23-d depuration period, PbTxs were strongly retained in mussel tissues (78% of initial values: 0.38 ± 0.14 µg g^{-1} wet wt in the whole-soft tissues after three weeks of depuration, n = 4). McFarland et al. (2015) showed that another mytilid species, the green-lipped mussel *Perna viridis*, retained high levels of PbTxs after a *K. brevis* bloom, where tissue concentrations remained above the regulatory limit for human consumption for 4–5 months. This timeline is significantly longer than the depuration time of 2–8 weeks that has been found for oyster and clam species (McFarland

Table 2

Parameters of whole-body uptake of ^{54}Mn, ^{57}Co, ^{65}Zn and ^{109}Cd in *M. edulis* exposed for 23 d to waterborne radionuclide (n = 8) 1) after a pre-exposure to toxic *K. brevis* and 2) in the control group (non-exposed).

Radiotracer	Model	CF$_{ss}$ ± ASE	k$_u$ ± ASE	R^2
1) Pre-exposed to *K. brevis*				
^{109}Cd	Linear	–	5.1 ± 0.1 [a]	0.90
^{57}Co	Exponential	214.8 ± 26.5 [a]	16.6 ± 1.9 [a]	0.78
^{54}Mn	Exponential	130.7 ± 13.4 [a]	9.9 ± 0.9 [a]	0.85
^{65}Zn	Exponential	1172.3 ± 332.3 [a]	49.4 ± 3.3 [a]	0.91
2) Control group (non-exposed)				
^{109}Cd	Linear	–	4.0 ± 0.1 [a]	0.87
^{57}Co	Exponential	218.5 ± 46.0 [a]	14.6 ± 2.3 [a]	0.75
^{54}Mn	Exponential	134.1 ± 33.6 [a]	8.5 ± 1.3 [a]	0.75
^{65}Zn	Exponential	1136.4 ± 287.3 [a]	52.0 ± 4.5 [a]	0.87

Kinetic parameters: CF$_{ss}$: Concentration Factors at steady state; k$_u$: uptake rate constant (d^{-1}). ASE: asymptotic standard error; R^2: determination coefficient of the uptake kinetics.

Probability of the model adjustment: [a] p < 0.001.

et al., 2015). These findings demonstrate that PbTxs were present in the tissues of the mussel throughout the trace element exposure period and suggest that depuration and detoxification processes operate on a longer timescale than that of this experiment (three weeks) in mussels exposed to *K. brevis*.

After four exposures to *K. brevis* culture (both cells and solubilized PbTxs) at ecologically relevant cell concentrations (~1000 cells mL^{-1}; Gannon et al., 2009; Stumpf et al., 2003), mussels were exposed to dissolved trace elements for 23 days. Our results showed that whole-body uptake kinetics of all essential trace elements (Co, Mn and Zn) followed saturation kinetics regardless of pre-exposure to PbTxs (R^2: 0.75–0.91, n = 8; Fig. 2 and Table 2). Uptake kinetics of the non-essential trace element Cd were best described by a linear model (Fig. 2 and Table 2). Comparisons among the rates at which elements were taken up in the whole organisms (k$_u$) and the estimated CF$_{ss}$ indicated that the essential elements (Co, Mn and Zn) were bioaccumulated similarly (p > 0.05, Fig. 2 and Table 2) between unexposed *M. edulis* and those exposed to *K. brevis* cells. In contrast, a statistically significant higher uptake rate constant k$_u$ was found for Cd in *K. brevis*-exposed mussels compared to controls (p = 0.049, Fig. 2) suggesting PbTx pre-exposure increased Cd uptake.

An important driver of variation in rates of bioaccumulation among bivalve species is the clearance rate. A negative relationship between trace element absorption efficiency (from the dissolved pathway) and clearance rates have been demonstrated for three marine bivalves (*P. viridis*, *Septifer virgatus*, and *Ruditapes philippinarum*; Wang, 2001), indicating that individuals filtering a larger quantity of water display
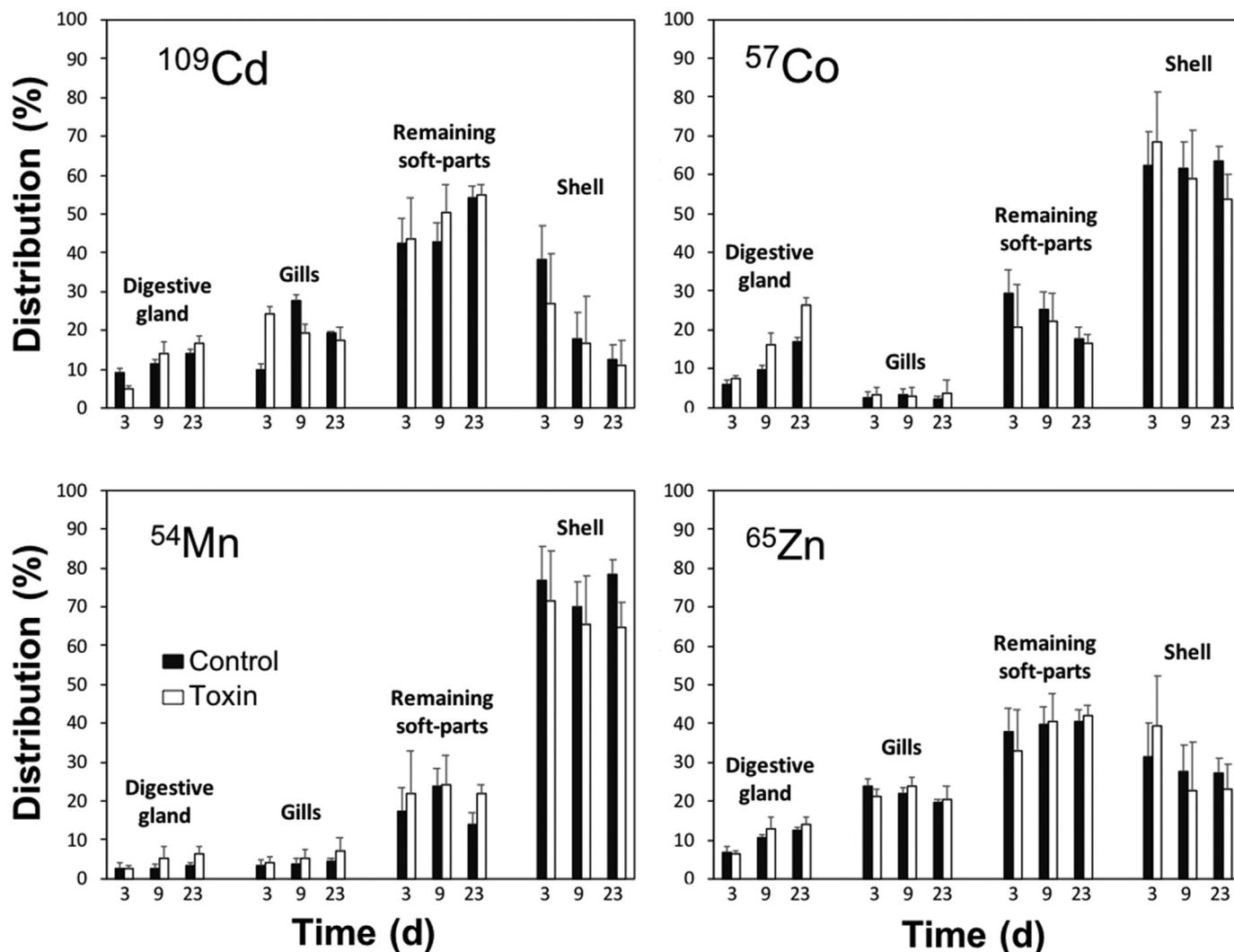

Fig. 3. Distribution over the radiotracer uptake period of ^{109}Cd, ^{57}Co, ^{54}Mn and ^{65}Zn among four body compartments (digestive gland, gills, remaining soft parts and shell) of *K. brevis*-exposed (white) or control (black) blue mussels (*M. edulis*). At each time four mussels were dissected. All the values are expressed as percentage of the whole-body activity (Means + SD). For all the radiotracers, no significant difference was found between pre-exposed and control mussels at any time.

lower trace element absorption efficiency. Harmful algae have been shown to alter clearance rates in bivalves, although both the magnitude and direction of effects depend on the species. Hegaret et al. (2007) demonstrated in the blue mussel that the exposure to three harmful algal species (*Prorocentrum minimum, Alexandrium fundyense*, and *Heterosigma akashiwo*) increases clearance rates, while Leverone et al. (2007) found a species-dependent reduction in clearance rate (79% for the bay scallop *Argopecten irradians*, or 38% for the green mussel *P. viridis*) in the presence of 1000 cells mL^{-1} of *K. brevis* culture. Although the clearance rate was not measured in our study, a preliminary experiment showed that ingestion rate (i.e. phytoplankton cells ingested by individual h^{-1}) decreased in mussels pre-exposed to *K. brevis* (data not shown). Thus, we suggest that pre-exposure to *K. brevis* may also have induced a decrease in the clearance rate in mussels, leading to a higher Cd uptake rate constant k$_u$. Although it is a non-essential element, Cd was mainly distributed in the mussel soft tissues (82% after 9 days of waterborne exposure, Fig. 3). In contrast, the other elements were present predominantly in the shell, likely through adsorptive process (28–70% after 9 days, Fig. 3). This finding further suggests that an active mechanism such as a decrease of the clearance rate may be responsible for the effects on Cd bioaccumulation in the mussel tissues.

As previously stated, the distributions of trace elements among the mussel body compartments (i.e. digestive gland, gills and remaining tissues and shell) were determined throughout the exposure period. The distribution of each trace element among tissues was not significantly affected by pre-exposure to *K. brevis* (p > 0.05, Fig. 3), indicating that storage mechanisms of such elements remain constant despite the accumulation of PbTxs in mussels.

The current study presents the first findings on the effects of pre-exposure to toxin-producing microalgae on the bioaccumulation of dissolved trace elements in a bivalve species. Care needs to be taken in how to expand on these results. Some of the functional groups generally present in algal toxin molecules, such as amino, carboxyl, phenol, sulfhydryl and hydroxyl groups, have the potential to complex with metal ions. Such complexes could affect the bioavailability of metals throughout aquatic food chains, as has been demonstrated in the case of cyanobacterial microcystins and domoic acid produced by the diatom *Pseudo-nitzschia* spp. (Humble et al., 1997; Rue and Bruland, 2001). Thus, further investigations should be conducted to investigate the influence of concurrent exposure of metals and algal toxins in bivalves.

4. Conclusion

This study revealed statistically significant differences in the dissolved Cd whole-body uptake kinetics in common mussels pre-exposed to environmentally relevant concentrations of PbTx-producing *K. brevis* in comparison to control mussels. This difference is likely related to decrease of the clearance rate of the *K. brevis*-exposed mussels. Nevertheless, no difference was observed in the uptake of the studied essential trace elements (Co, Mn, Zn). These findings indicate that the occurrence of PbTxs in the environment at concentrations of ~1000 cells mL^{-1} appear to have a limited effect on the subsequent bioaccumulation of trace elements by this species of bivalves. More research is needed to confirm observed patterns at higher doses of PbTxs or more chronic exposures. In addition, as harmful algae can produce a variety of toxins that can potentially interact with trace elements and have diverse modes of action which can vary between bivalve species, further investigations are needed to study the influence of (1) simultaneous exposure to PbTxs and trace elements, (2) other biotoxins on trace element bioaccumulation in mussels, and (3) concurrent exposure using other species of bivalves.

Acknowledgments

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