RESEARCH ARTICLE

Heavy metal incorporation in foraminiferal calcite under variable environmental and acute level seawater pollution: multi-element culture experiments for *Amphisorus hemprichii*

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Abstract

The accelerated release of heavy metals into the coastal environments due to increasing anthropogenic activities poses a severe threat to local marine ecosystems and food chains. Although some heavy metals are essential nutrients for plants and animals, higher concentrations can be toxic and hazardous. To mitigate this threat, developing quantifiable proxies for monitoring heavy metal concentrations in near-shore marine environments is essential. Here, we describe culture experiments to quantify uptake of some heavy metals using live juvenile specimens of the large benthic foraminifera (LBF) *Amphisorus hemprichii* collected from the subtropical waters of Rottnest Island located ~20 km offshore Perth, South West Australia. The uptake of Mn, Ni, Cd, and Pb in the newly precipitated chambers of *Amphisorus hemprichii* in the laboratory was characterized using the micro-analytical technique, laser ablation inductively coupled plasma mass spectrometry. We found no significant increase in Mn, Ni, Cd, and Pb incorporation in the tests of *Amphisorus hemprichii* with increasing temperature and light intensities. Importantly, we found that changes in the concentrations of Mn, Ni, and Cd in the *A. hemprichii* tests are directly proportional to those in the culture solution over a wide range of concentrations. The calculated partition coefficients for Mn, Ni, and Cd from our culture experiments are 1.3 ±0.2, 0.3±0.04, 2.6±0.3, respectively. These multi-element calibration studies now enable *A. hemprichii* to be utilized as a naturally occurring bio-archive to quantitatively monitor the anthropogenic pollution of Mn, Ni, and Cd in coastal waters.

Keywords Coastal monitoring · Heavy metal pollution · Large benthic foraminifera · *Amphisorus hemprichii* · Laboratory culture · LA-ICP-MS

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Introduction

Monitoring of heavy metals in the coastal marine environment

Intensification of anthropogenic activities (e.g., industries and agriculture) along coastal environments has resulted in widespread heavy metal contamination (Hart and Lake 1987; Hill 2010). This poses a direct threat to marine ecosystems and increased risks to trophic food chains, including those upon which humans are dependent. While some heavy metals are essential nutrients for various biochemical and physiological functions (WHO/FAO/IAEA 1996), at elevated levels, they can be toxic to plants and animals. Some heavy metals (e.g., Pb, Cd) also have bio-accumulative characteristics (DeForest et al. 2007; Zhou et al. 2008; Hosono et al. 2011; Zuykov et al. 2013) leading to much higher concentrations in the trophic food chain than those in seawater. Higher initial seawater

concentrations of these metals can thus be toxic at higher trophic levels and are known to have affected cell components and some enzymes involved in metabolism, detoxification, and cell repair (Wang and Shi 2001). Furthermore, with the expansion of coastal facilities and increased dredging, many sediments-bound heavy metals are released into the seawater and become bio-available. Hence, effective heavy metal monitoring of pollution in the marine environments is essential in preventing and mitigating both event-based pulsed changes and longer-term increases in "baseline" concentrations. Traditionally, monitoring of heavy metals in the marine environment has been done by direct measurement of water concentrations and/or extracts from sediment samples. Additionally, in some cases, abnormally high concentrations have been inferred by analysis of organic tissues in the local biota (Phillips 1977) and human blood samples (Buchman 2008; Gulson et al. 2009). Generally, daily measurements of water samples are logistically impractical and expensive. Furthermore, sediment extracts represent time-averaged concentrations that are influenced by environmental factors. At the same time, organisms can have different biological responses (i.e., vital effects) and hence variable uptake rates even though they are exposed to the same concentrations of heavy metals in the environment. Thus, despite improvements in analytical sensitivity, continuous and long-term monitoring of coastal water quality using traditional sampling methodology remains a challenge. To overcome these shortcomings, continuous records from geochemical analysis of shells of marine calcifiers such as corals (Shen and Boyle 1987), bivalves (Gillikin et al. 2005), and foraminifera (Titelboim et al. 2018) are increasingly being utilized. These approaches require sampling of shell geochemistry along time series of growth trajectories (i.e., sclerochronology) and known elemental partitioning to extract continuous records of heavy metal concentrations in seawater.

Foraminifera as geochemical proxies

Compared to corals and bivalves, foraminifera are highly abundant and diverse eukaryotic microorganisms (Protozoa) found in all marine environments (Sen Gupta 2003). Most species of foraminifera secrete tests composed of calcium carbonate that are often well preserved in coastal environments. During the process of test-building, minor and trace elements present in the seawater are incorporated by substituting for calcium or carbonate ion in the foraminiferal crystal lattice (Katz et al. 2010). This geochemical property of foraminiferal shells provides the foundation upon which many geochemical proxies for oceanography and paleoclimate studies are based (e.g., Boyle 1981; Hester and Boyle 1982; Delaney and Boyle 1986; Boyle 1988; Delaney 1989; Lea and Boyle 1989; Boyle 1992; Boyle et al. 1995; Nürnberg et al. 1996; Mashiotta et al. 1997; Rickaby and Elderfield 1999; Marchitto 2004). A

prerequisite for the quantitative application of these proxies, however, requires knowledge of foraminiferal elemental uptake and foram test/seawater partitioning under seawater conditions, which can most readily be obtained through laboratory culture studies. The first foraminiferal laboratory culture experiments were carried out by Delaney et al. (1985), who studied the incorporation of Li, Sr, Mg, and Na in the calcareous tests of planktonic foraminifera *Globigerinoides sacculifer*. Subsequently, the partitioning of other trace metals (e.g., Cd, Ba, U, Cu, Mn, Ni, Zn, and Pb) into the foraminifera tests was obtained through laboratory culture experiments by various researchers (Table 1).

Based on the quantitatively defined elemental partitioning, the shell geochemistry of foraminiferal tests is increasingly being utilized as environmental proxies for monitoring heavy metal pollution in marine environments (Maréchal-Abram et al. 2004; Herut et al. 2007; De Nooijer et al. 2007; Frontalini et al. 2009; Madkour and Ali 2009; Munsel et al. 2010; Nardelli et al. 2013, 2016; Youssef 2015; van Dijk et al. 2017; Titelboim et al. 2018, 2021; Ben-Eliahu et al. 2020; Boehnert et al. 2020; Smith et al. 2020; Sagar et al. 2021). These unicellular protists have a number of advantages over other calcareous organisms in biomonitoring of heavy metal pollution: (1) they are highly abundant and diverse (Sen Gupta 2003) and (2) relatively easy to culture in the laboratory (Sagar et al. 2021 and references therein) under a range of realistic environmental conditions, and, (3) since they produce their carbonate shells incrementally, each successive layer represents the seawater chemistry at that specific time of formation. Nevertheless, it is crucial to determine how reliable these environmental proxies are in the natural environment, which is affected by multiple factors, e.g., multi-element concentrated seawater, seasonal temperature, and varying light intensities.

In this study, we use the reef-dwelling cosmopolitan large benthic foraminifera (LBF), for our culture experiments aimed to quantify the intake of some heavy metals (Mn, Ni, Cd, and Pb) in their tests under three experimental settings. *Amphisorus hemprichii* was chosen for our culture experiments because these miliolids are plentiful along the coasts of Western Australia and are commonly found along other Indian Ocean coastlines. Moreover, its close relative *Marginopora* sp. is abundant in the tropical and subtropical near-shore marine waters of the Western Pacific Ocean. We describe here the laboratory settings and analytical results obtained from our culture experiments using *Amphisorus hemprichii*; (1) multi-element partitioning from seawater at varying temperatures but uniform light intensity, (2) multi-element partitioning from a multi-element spiked solution at uniform temperature but varying light intensities, and (3) multi-element partitioning from multi-element spiked culture solutions at a uniform temperature and light intensity. We report the partitioning coefficients for Mn, Ni, and Cd

Table 1 Partitioning coefficient of trace metal (K_D^{Me}) obtained from laboratory culture experiments using foraminifera

Trace metals	Lead author and year of publication	Metal partition coefficient (K_D^{Me}); remarks	Foraminifera used in laboratory cultures and their habitat	
			Planktonic	Benthic
Li, Sr, Mg, Na	Delaney et al. 1985	$K_D^{Li} = (5.3\pm0.6)\times10^{-3}$ $K_D^{Sr} = 0.16\pm0.02$ Mg/Ca: No clear pattern observed with changes in seawater Mg/Ca ratios Na/Ca: No correlation of shell Na/Ca with solution chemistry	*Globigerinoides sacculifer* [subsurface (20–75m) waters; tropical to subtropical]	
Ba	Lea and Spero 1992	Between 0.14 and 0.17	*Orbulina universa* [surface waters; tropical, subtropical and transition zones]	
	Lea and Spero 1994	0.147±0.004	*Globigerina sacculifer* and *Orbulina universa*	
	Havach et al. 2001	0.24±0.07 0.24±0.06 0.20±0.04		*Bulimina marginata* [deep sea] *Uvigerina peregrine* [deep sea] *Ammonia beccarii* [shallow water; littoral and neritic environments]
U	Russel et al. 1994	$(10.6\pm0.3)\times10^{-3}$ for *A. lobifera* $(7.9\pm0.1)\times10^{-3}$ for *G. calida*	*Globigerinella calida* [surface waters; tropical, subtropical and temperate regions]	*Amphistegina lobifera* [shallow water; tropical, and subtropical]
Cd	Mashiotta et al. 1997	1.9±0.2 (for *G. bulloides*) 0.095±0.021 (for *O. universa*)	*Globigerina bulloides* and *Orbulina universa* [*G. bulloides*: subsurface (75–100m) waters; between 10°N and 40°S]	
	Havach et al. 2001	3.0±1.0 2.0±1.0 1.0±0.5 4.0±2.0		*Bulimina marginata* *Uvigerina peregrine* *Ammonia beccarii* *Cibicidoides pachyderma* [deep sea]
	Maréchal-Abram et al. 2004	~1.1 average of their culture experiments 2, 3, and 5 ~2.3 average of cultures 1 and 4		*Ammonia beccarii*
Cu	De Nooijer et al. 2007	Between 0.14 and 0.17		*Ammonia tepida*, [shallow water; temperate] *Heterostegina depressa* [shallow water; tropical]
	Munsel et al. 2010	0.14±0.02		*Ammonia tepida*
Mn	Munsel et al. 2010	At least 2.4; high scatter in data		*Ammonia tepida*
Ni	Munsel et al. 2010	1.0±0.5		*Ammonia tepida*
Zn	Nardelli et al. 2016	From 4.03±0.06 to 0.2±0.01; decreases with increasing Zn concentrations		*Pseudotriloculina rotunda* [shallow water; temperate]
	Titelboim et al. 2021	Average = 6.3		*Amphistegina lobifera*, *Amphistegina lessonii*
Pb	Sagar et al. 2021	8.37±0.3		*Amphisorus hemprichii* [shallow water; tropical, warm temperate]
	Titelboim et al. 2021	Average = 12.9		*Amphistegina lobifera*, *Amphistegina lessonii* [*A. lessonii*: shallow water; tropical, and subtropical]
Mn, Ni, Cd	This study	$K_D^{Mn} = 1.3\pm0.2$ $K_D^{Ni} = 0.3\pm0.04$ $K_D^{Cd} = 2.6\pm0.3$		*Amphisorus hemprichii*

obtained from calibration equations based on the incorporation of these metals into the tests of cultured *Amphisorus hemprichii* specimens from the artificially enriched seawater. This study further demonstrates the utility of benthic foraminiferal recorder as a quantitative method to monitor heavy metal pollution in coastal environments.

## Materials and methods

Sampling and culturing foraminifera

Specimens of the shallow water-dwelling *Amphisorus hemprichii* were collected from the subtropical waters of Parker Point in Rottnest Island (Fig. 1a), located ~20 km offshore Perth, Western Australia. Live juvenile *A. hemprichii* were picked from the blades of seagrass *Posidonia australis* and placed in culture flasks and ziplock plastic bags precleaned with acid. The culture flasks were pre-filled with recently filtered seawater, and the plastic bags contained fresh seawater. The seawater was filtered at the site using 0.2-μm polytetrafluoroethylene syringe filters from Merck Millipore attached with 60-ml syringes from Henke-Sass Wolf GmbH. All collected specimens were acclimatized for 30 days by housing them in 100-l water tanks in the University of Western Australia (UWA) seawater culturing facility at Watermans. These tanks were supplied with fresh seawater pumped continuously from offshore. Culture experiments were then performed in the temperature-controlled laboratory at UWA's Indian Ocean Marine Research Centre (IOMRC) at the Crawley campus using seawater batches transferred from Watermans. Three different culture studies were carried out on specimens of the LBF *Amphisorus hemprichii*: (1) experiments with uniform light intensity but varying temperatures in undoped seawater; (2) multi-element enrichment culture experiments with one spiked solution, same temperature, but four varying light intensities; and (3) multi-element enrichment culture experiments with varying heavy metal concentrations under constant temperature and uniform light intensity. In these experiments, randomly picked juvenile *A. hemprichii* specimens showing active pseudopodial activity under a binocular were used.

Culture studies 1: temperature experiments

These studies were carried out under three different temperature settings, 23 °C, 25 °C, and 27 °C±0.3 °C, in three separate 25-l glass aquariums: AQ1, AQ2, and AQ3, respectively. A filtration system and a water pump were fitted in each aquarium for better circulation of the culture water. The temperature

Fig. 1 **a** Map of Rottnest Island showing Parker Point, the sampling location. The inset shows the map of Western Australia, and the white spot represents Rottnest Island. **b** Scanning electron microscope (SEM) image of a portion of *Amphisorus hemprichii* showing pre-ablated laser track for cleaning surface contaminants. **c** High-resolution SEM image showing a laser burned spot (100×30 μm). SEM images were obtained using instrument TESCAN VEGA3
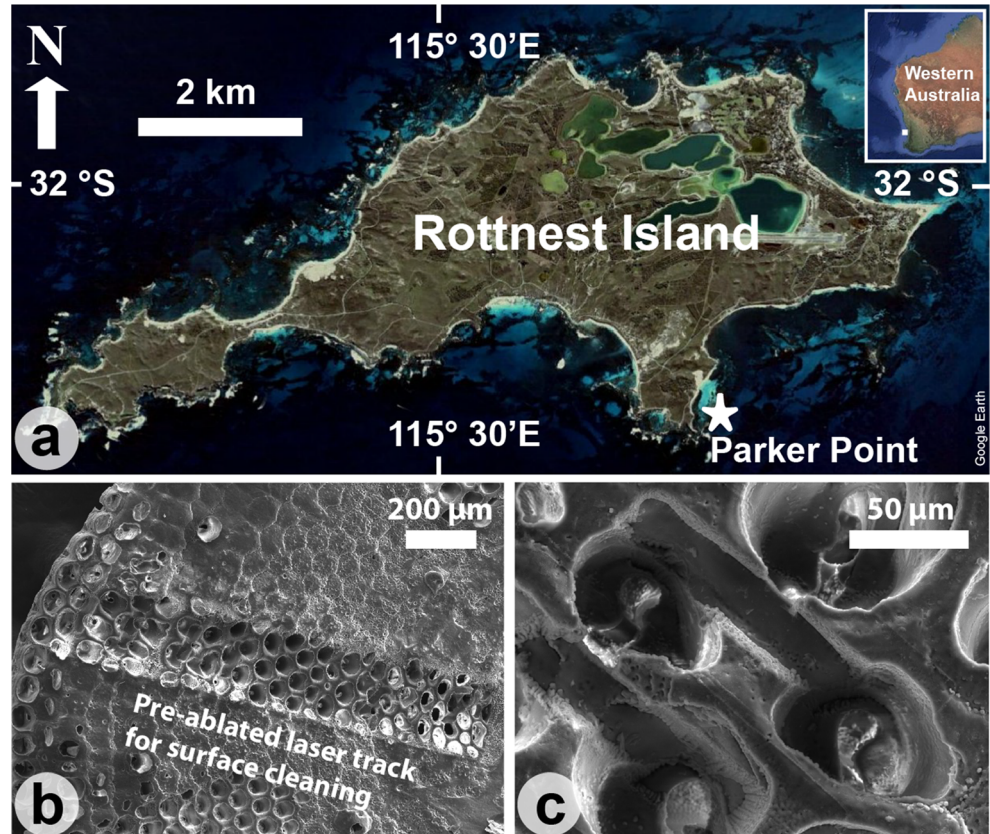
in the aquariums was monitored and controlled by using temperature sensors and water heaters connected to an on-line APEX water control system. The pH of the culture water in each aquarium was monitored by using a pH sensor connected to the APEX system. In these experiments, 20 live juvenile *Amphisorus hemprichii* specimens were placed in each aquarium, which was filled with fresh, un-filtered, and non-spiked seawater. The temperature of the aquariums was gradually increased at an increment of 0.2 °C per 3 days to help acclimatize the *Amphisorus hemprichii* specimens. The initial temperature of the aquariums was 20 °C±0.5 °C. Once the final desired temperature condition was obtained, the culture studies were carried out over an 8-week period. The culture water was exchanged weekly with freshly collected seawater to limit algal and bacterial buildup. Before changing the seawater, the inner walls of the glass aquariums were wiped clean to remove algal growth. This experiment was conducted with uniform light intensity in all aquariums with the peak value reaching 177 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) at noon and with 12 h on and 12 h off cycle mimicking the light intensity of the sample collection site (Ross et al. 2015). The *A. hemprichii* foraminifera in these experiments had to find their own food from the culture medium as they normally do in the natural environment (Goldstein 2003).

Culture studies 2: light intensity experiments

These experiments were conducted on *Amphisorus hemprichii* specimens with four different light intensity set-tings: 22, 55, 299, and 483 μmol photons m^{-2} s^{-1} PAR at noon with 12 h on and 12 h off cycle. Each light intensity experiment was tested with 26 juvenile *A. hemprichii* speci-mens showing active pseudopodial activity; 18 specimens were kept in Petri dishes and the rest in breathable Corning culture bottles. These light experiments were conducted in an otherwise dark temperature-controlled room, which was set at 23 ± 0.5°C. The Petri dishes and the culture bottles contain-ing the specimens were placed in a tub filled with circulat-ing water to prevent the increase of temperature from the light intensities. All four sets of light intensity experiments were kept in individual barriers so that the light intensity from one set of experiment did not interfere with the others. Only one multi-element spiked solution having the same concentration as spiked experiment "S3" (Table 2) was used as the culture medium during the 8-week duration of these experiments. The spiked culture solution was changed every week to limit the algal and bacterial buildup in the specimen holders. The spiked solution was prepared with filtered seawater, which was obtained using a filtration unit from Sartorius, model SM 16510, using 0.2-μm hydro-philic nylon membrane filter paper from Merck Millipore.

The foraminifera were supplied with food that consisted of a mixture of two microalgae (*Tetraselmis* sp., and *Nannochloropsis* sp.,) and a diatom (*Phaeodactylum*), once a week 2 h before replacing the culture solution. These algae were cultivated at the Algae R&D Centre of Murdoch University (Ishika et al. 2018, 2019) under opti-mized growth conditions of temperature and salinity.

Culture studies 3: multi-element experiments

The multi-element and multi-spiking culture studies were con-ducted for 16 weeks using the collected *Amphisorus hemprichii* specimens in two batches (batches 1 and 2). Batch 1 comprised five experiments named S1, S2, S3, S4, and S5. Batch 2 included three experiments named A1, A2, and A3. Batch 1 was spiked with relatively high metal con-centrations, whereas batch 2 was spiked within environmental ranges (Table 2). For example, based on the guidelines devel-oped for toxicants (ANZECC, ARMCANZ 2000) in marine waters around Australia and New Zealand, the concentration of Cd should be below 5.5, 14, and 36 μg/L to protect 95, 90, and 80% of species, respectively. In our batch 2 experiments, we cultured *Amphisorus hemprichii* specimens with Cd con-centrations ranging from 4.2 to 16.8 μg/L, which were within the abovementioned trigger range, whereas experiments in batch 1 were cultured with Cd concentrations ranging from 24.4 to 195.0 μg/L (Table 2). All the experiments were cul-tured using filtered seawater spiked with multi-element metal concentrations except S1, the control experiment, which was carried out using non-spiked filtered seawater. In batch 1, the concentration of heavy metals for experiment S5 was used as the stock solution, and serial dilutions were made to prepare the spiked concentrations for experiments S2, S3, and S4. Every experiment in batch 1 (S1, S2, S3, S4, and S5) contained six 100-ml Corning culture flasks, and each flask housed 10 live juvenile specimens of *Amphisorus hemprichii*. The culture medium for experiments in batch 2 was prepared by serial dilutions from a stock solution, A0 (Table 2). All the three experiments in batch 2 (A1, A2, and A3) had two Corning culture flasks, and each housed 10 live and juvenile *Amphisorus hemprichii* specimens. The spiked stock solutions (S5 and A0) were prepared by mixing metal chlorides; $MnCl_2$, $NiCl_2$, $CdCl_2$, and $PbCl_2$ (Sigma Aldrich) in filtered seawater to minimize suspended particles and bacterial content. The culture solution for all experiments was replaced once a week to prevent bacterial buildup in the culture flasks. These multi-element spiking culture experiments were con-ducted under controlled light intensity with a peak value of 177 μmol photons m^{-2} s^{-1} PAR at noon and with 12 h on and 12 h off cycle. The temperature of the culture labora-tory was kept similar to the seawater temperature of the sample collection site, which was 20 °C ± 0.5 °C.

Table 2 Heavy metal (Mn, Ni, Cd) concentrations of the culture medium in experimental batches 1 and 2 and the stock solution (A0) for batch 2. Experiment S1 represents the control set-up where the *Amphisorus hemprichii* specimens were cultured using non-spiked filtered (0.2 μm) seawater. Metal concentrations in the stock solutions of both batches (S5 and A0) and the control experiment (S1) were measured in HR-ICP-MS (Thermo Fisher Scientific). *BDL* below detection limit. See the section "Culture studies 3: multi-element experiments" for details

Multi-element culture studies using *Amphisorus hemprichii*	Experiments	Metal concentrations (μg/L) in the cultured seawater			Remarks
		Manganese (Mn)	Nickel (Ni)	Cadmium (Cd)	
Batch 1	S1 (control)	0.4	BDL	BDL	No spiking
	S2	220.0	212.9	24.4	Multi-element spiking
	S3	440.0	425.8	48.8	
	S4	880.0	851.5	97.5	
	S5	1760.0	1703.0	195.0	Multi-element stock solution
Batch 2	A1	9.3	22.5	4.2	Multi-element spiking
	A2	18.6	45.1	8.4	
	A3	37.3	90.2	16.8	
	A0	74.5	180.4	33.6	Multi-element stock solution

Chemical cleaning and geochemical analyses of cultured *Amphisorus hemprichii* specimens

The *Amphisorus hemprichii* specimens selected for geochemical analysis from the three culture studies mentioned above were first thoroughly rinsed and later soaked in MilliQ water for 48 h. The individual *A. hemprichii* specimens were then subjected to high-energy ultra-sonication pulses using an ultrasonic probe from Sonics Vibracell to break open the thin-transparent cover from their chambers. Specimens were then chemically cleaned using an oxidative cleaning reagent prepared in the laboratory from a mixture of 10% H_2O_2 and 0.1-M NaOH. Finally, the specimens were rinsed and ultra-sonicated with 18.2Ω MilliQ deionized water (Sagar et al. 2021). The chemically cleaned specimens were oven-dried at 40 °C for 15 h and then mounted on glass slides by double-sided carbon tape for metal-to-calcium ratios (Me/Ca) using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

In situ analysis of ^{55}Mn, ^{60}Ni, ^{111}Cd, and ^{208}Pb in the tests of cultured *Amphisorus hemprichii* specimens was conducted using a sector field ICP-MS (Thermo, Element XR) in combination with a G2 Analyte (Teledyne) laser ablation system housed in the Advanced Geochemical Facility for Indian Ocean Research (AGFIOR) at the UWA. Prior to actual measurements, each laser track was pre-ablated (Fig. 1b) to remove surface contaminants (Sadekov et al. 2008). Metal (Me/Ca) analysis was carried out by spot measurements (100×30 μm; Fig. 1c) on the calcite septa (terminology from Hottinger 2006) of the *A. hemprichii* specimens at a repetition rate of 6 Hz and 3.44 J cm^{-2} fluence (Sagar et al. 2021). For calibration of Me/Ca contents, the National Institute of Standards and Technology (NIST) glass 614 was used as the primary

reference material. Repeat standards of NIST glass 612 were used as the secondary reference material and run every half hour throughout the analytical sessions. The reproducibility of Mn/Ca, Ni/Ca, Cd/Ca, and Pb/Ca in NIST 612 for the analytical runs of various experiments is provided in Table 3. From the temperature, light intensity, and multi-element culture experiments, a total of 12, 16, and 64 *A. hemprichii* specimens were analyzed, respectively. Twelve spots marked carefully on each foraminifera specimen (one on each septum) were laser-fired that generated 1104 spots (92 foraminifera × 12 spots). Each spot was depth-profiled, and 14 measurements were taken from each spot. Finally, the laser spot analysis and depth profiling generated 15,456 (1104×14) laser data points. All Me/Ca data generated by LA-ICP-MS was processed using Igor Pro software.

Table 3 Reproducibility of National Institute of Standards and Technology (NIST) glass 612 in the analytical runs of various experiments

Me/Ca ratios in the tests of cultured *Amphisorus hemprichii* specimens	Reproducibility of NIST glass 612 (% 2RSE)		
	Temperature and light intensity experiments	Multi-element spike batch 1	Multi-element spike batch 2
$^{55}Mn/^{43}Ca$	1.5	1.0	0.97
$^{60}Ni/^{43}Ca$	2.2	2.7	1.6
$^{111}Cd/^{43}Ca$	2.4	2.5	1.5
$^{208}Pb/^{43}Ca$	1.9	1.4	1.8

Principal component analysis and calculation of partition coefficients

Principal component analysis (PCA) was applied to the Me/Ca data (from batches 1 and 2) generated using LA-ICP-MS from the outer septum of cultured *A. hemprichii* that formed under spiked conditions in the laboratory. Each Me/Ca was treated as an individual variable, and hence the data of the four variables (Mn/Ca, Ni/Ca, Cd/Ca, and Pb/Ca) were analyzed using PCA to reduce the dimensionality into a set of comprehensive principal components. Analysis and plots for PCA were generated using the "factoextra" and "FactoMineR" package in open-access software, R (RStudio 4.0.2), coded for statistical computing and graphics. Partition coefficients (K_D) of the metals, Mn, Ni, and Cd, used in our multi-element spiking culture experiments were calculated according to the following expression:

$$K_D^{Me} = (Me/Ca)_{foram-calcite} / (Me/Ca)_{seawater}$$

The K_D^{Me} was calculated for each individual experiment of batch 2 (experiments A1, A2, and A3) and then averaged to obtain the final K_D^{Me}. The results of multi-enrichment spiking culture experiments in batch 1 were not included in calculating the K_D^{Me}. For more details, see the section "Incorporation of metals in the tests of cultured *Amphisorus hemprichii* and their principal component analysis."

Results

Effects of temperature on metal incorporation into the tests of *Amphisorus hemprichii*

Incorporation of Cd, Mn, Ni, and Pb in the tests of juvenile *Amphisorus hemprichii* cultured in aquariums was evaluated over a range of temperatures (23, 25, and 27 °C±0.3 °C). The temperature experiments were conducted using non-filtered and non-doped seawater (section "Culture studies 1: temperature experiments") as the culture medium. Four tests from each aquarium were laser-fired (12 spots on each test) to observe the partitioning of abovementioned heavy metals in the tests of *Amphisorus hemprichii*. The Cd to Ca ratios (Cd/Ca) in the tests of *A. hemprichii* specimens increased from 0.0003 ±0.0001 mmol/mol at 23 °C to 0.001±0.0002 mmol/mol at 25 °C. However, with further increase in temperature to 27 °C, the Cd/Ca ratios remained the same at 0.001±0.0002 mmol/mol (Fig. 2a). Although the Pearson correlation coefficient (*r*) value reveals a positive relationship (*r* = 0.8) between the Cd concentration in the *A. hemprichii* tests and temperature, it is statistically insignificant (*p* > 0.05). In contrast, the mean concentration of Mn/Ca in the tests of *A. hemprichii* increased linearly from around 0.008±0.0006 mmol/mol at 23 °C to
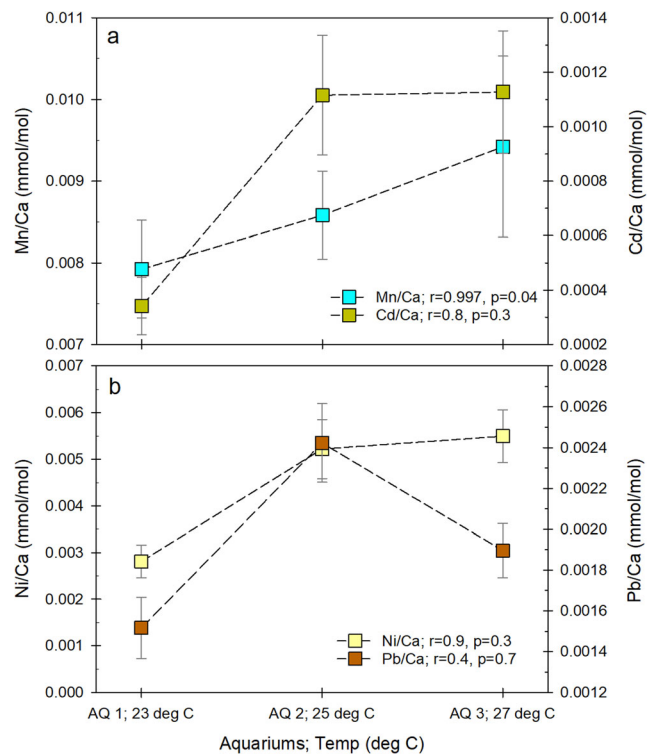
Fig. 2 Effect of temperature on the partitioning of heavy metals from the culture medium (zero spiked seawater) into the tests of *Amphisorus hemprichii* specimens with increasing temperature conditions from 23 to 27 °C; **a** Mn/Ca and Cd/Ca, **b** Ni/Ca and Pb/Ca. Error bars represent one standard error of the mean

0.009±0.001 mmol/mol at 27 °C (Fig. 2a), but the populations are not distinguishable within one standard error of analytical uncertainties. The concentration of Ni/Ca in the tests increased from 0.003±0.0003 mmol/mol at 23 °C to 0.005±0.0006 mmol/mol at 25 °C. However, an additional 2 °C increase in the aquarium temperature resulted in a negligible increase in Ni concentration (Fig. 2b). Thus, while the incorporation of Ni in the tests suggests a strong positive correlation (*r* = 0.9) with the increase in temperature, it is statistically insignificant (*p* > 0.05) relative to experimental uncertainties. Pb/Ca ratios also increased marginally from 0.0015±0.0001 mmol/mol at 23 °C to 0.0024±0.0002 mmol/mol at 25 °C. With a further increase in the aquarium temperature to 27 °C, the incorporation of Pb/Ca in the tests of *A. hemprichii* foraminifera decreased to 0.0019±0.0001 mmol/mol (Fig. 2b). The overall correlation between the incorporation of Pb in the tests and their corresponding aquarium temperatures is thus also weak and insignificant (*r* = 0.4, *p* > 0.05).

### Effects of light intensity on metal incorporation into the tests of *Amphisorus hemprichii*

The light intensity experiments focused on the amount of incorporation of Cd, Mn, Ni, and Pb in the tests of live *Amphisorus hemprichii* foraminifera from the culture solution

in four different light intensity settings (22, 55, 299, and 483 μmol photons m^{-2} s^{-1} PAR at noon; hereafter read as LI1, LI2, LI3, and LI4, respectively). In these experiments, the multi-element spiked concentration, S3 (Table 2), was used as the culture medium. The metals, Cd and Mn, follow nearly the same trend of partitioning with varying light intensities (Fig. 3a). A weak positive correlation, $r = 0.3$ and 0.2, is observed between the light intensities and the incorporation of Cd and Mn, respectively. Ni partitioning in the tests shows a strong positive association ($r = 0.9$) though statistically insignificant ($p > 0.05$) with increasing light intensities (Fig. 3b). The maximum amount of Ni incorporation, 0.8 ± 0.04 mmol/mol, is observed at LI3, and it is similar (0.8 ± 0.3 mmol/mol) at LI4. The concentration of Pb in the tests of *Amphisorus hemprichii* increased steeply from 0.08 mmol/mol at LI1 to 0.2 ± 0.01 mmol/mol at LI2. It was observed that the incorporation of Pb was nearly the same (around 0.2 mmol/mol) with further increase in light intensities; LI3 and LI4 (Fig. 3b). Our Pb data in the tests of *Amphisorus hemprichii* foraminifera showed a positive association ($r = 0.6$) with the increase in light intensities (Fig. 3b) but is statistically not significant ($p > 0.05$).

Incorporation of metals in the tests of cultured *Amphisorus hemprichii* and their principal component analysis

The amount of incorporation of various metals used in this study (Mn, Ni, and Cd) in the tests of cultured LBF *Amphisorus hemprichii* from the multi-element spiked solutions is shown in Fig. 4. The plots show results obtained from six spiked culture experiments A1, A2, and A3 (batch 2) and S2, S3, and S4 (batch 1). The results from spiked culture experiments S5 are not included in this study because the foraminifera *A. hemprichii* specimens from this experiment were stressed due to Pb toxicity, which resulted in minimal calcification with only one or two new septa formed over the 16-week culture duration (Sagar et al. 2021). The concentration of Mn in the abovementioned six multi-element spiked seawater solutions ranged from 9.3 μg/L in experiment A1 to 880.0 μg/L in experiment S4 (Table 2). A linear incorporation ($r = 0.96$) of Mn/Ca ratios in the tests was observed with the increase in seawater Mn-spiked concentrations from 9.3 μg/L (experiment A1) to 440 μg/L (experiment S3); five experiments (A1, A2, A3, S2, S3) with increasing Mn concentration in the cultured seawater. The highest Mn/Ca ratios of 3.1 mmol/mol in the *A. hemprichii* tests were observed in experiment S3 (Fig. 4a), which had 440 μg/L of Mn (Table 2). However, we noticed that with a further increase in Mn-spiked concentration to 880 μg/L (experiment S4), the amount of Mn/Ca ratios in the tests decreased to 2.4 mmol/mol (Fig. 4a). Another heavy metal, Ni, was spiked in the culture solutions ranging from 22.5 μg/L (experiment A1) to 851.5 μg/L (experiment S4). We observed a linear increase of Ni/Ca ratios in the tests of *Amphisorus hemprichii* specimens with an increase in seawater Ni concentrations ($r = 0.996$) from 22.5 μg/L (experiment A1) to 425.8 μg/L (in experiment S3) (Fig. 4b). However, it was noted that with further increase in Ni concentrations in the seawater (851.5 μg/L in experiment S4), there was no rise in the Mn/Ca ratios in the tests (Fig. 4b). The Cd concentrations in our spiked seawater culture experiments varied from 4.2 μg/L in experiment A1 to 97.5 μg/L in experiment S4 (Table 2). The LBF *Amphisorus hemprichii* specimens linearly incorporated Cd from the Cd-spiked seawater solutions ($r = 0.9$) ranging from 4.2 μg/L in experiment A1 to 48.8 μg/L in experiment S3 (Fig. 4c). However, the *Amphisorus hemprichii* specimens in experiment S4 (seawater containing 97.5 μg/L of Cd) incorporate a similar amount of Cd to experiment S3 having 48.8 μg/L of Cd but has a higher standard error (Fig. 4c).

The Me/Ca data, Mn/Ca, Ni/Ca, Cd/Ca (this study), and Pb/Ca (Sagar et al. 2021), obtained from the outer septum of the *Amphisorus hemprichii* specimens, which formed in the laboratory with the spiked culture solutions (from batches 1 and 2), were analyzed using PCA. The Me/Ca (Mn/Ca, Ni/Ca, Cd/Ca, and Pb/Ca) data were treated as individual variables.
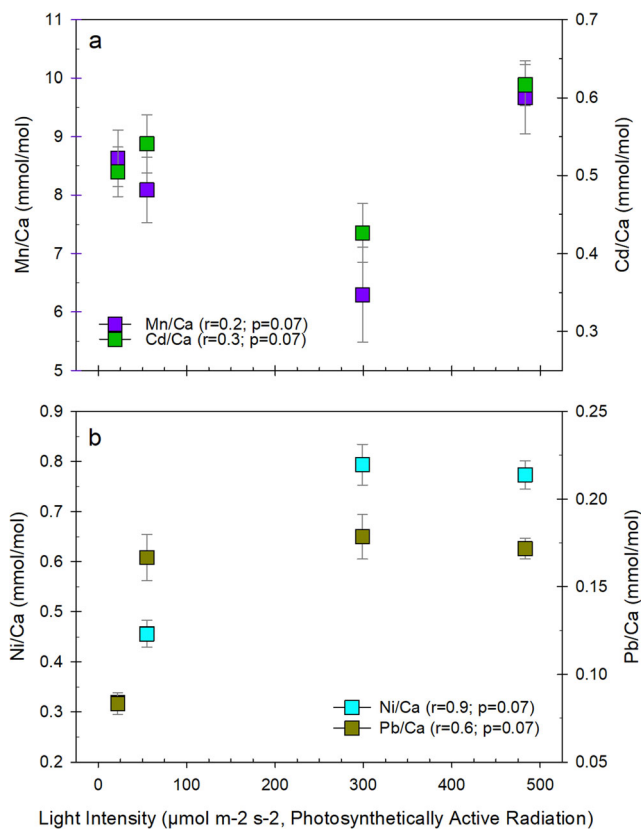
Fig. 3 Effect of varying light intensities on the partitioning of heavy metals from multi-element spiked culture solution (S3) into *A. hemprichii* tests; **a** Mn/Ca and Cd/Ca, **b** Ni/Ca and Pb/Ca. Error bars represent one standard error of the mean
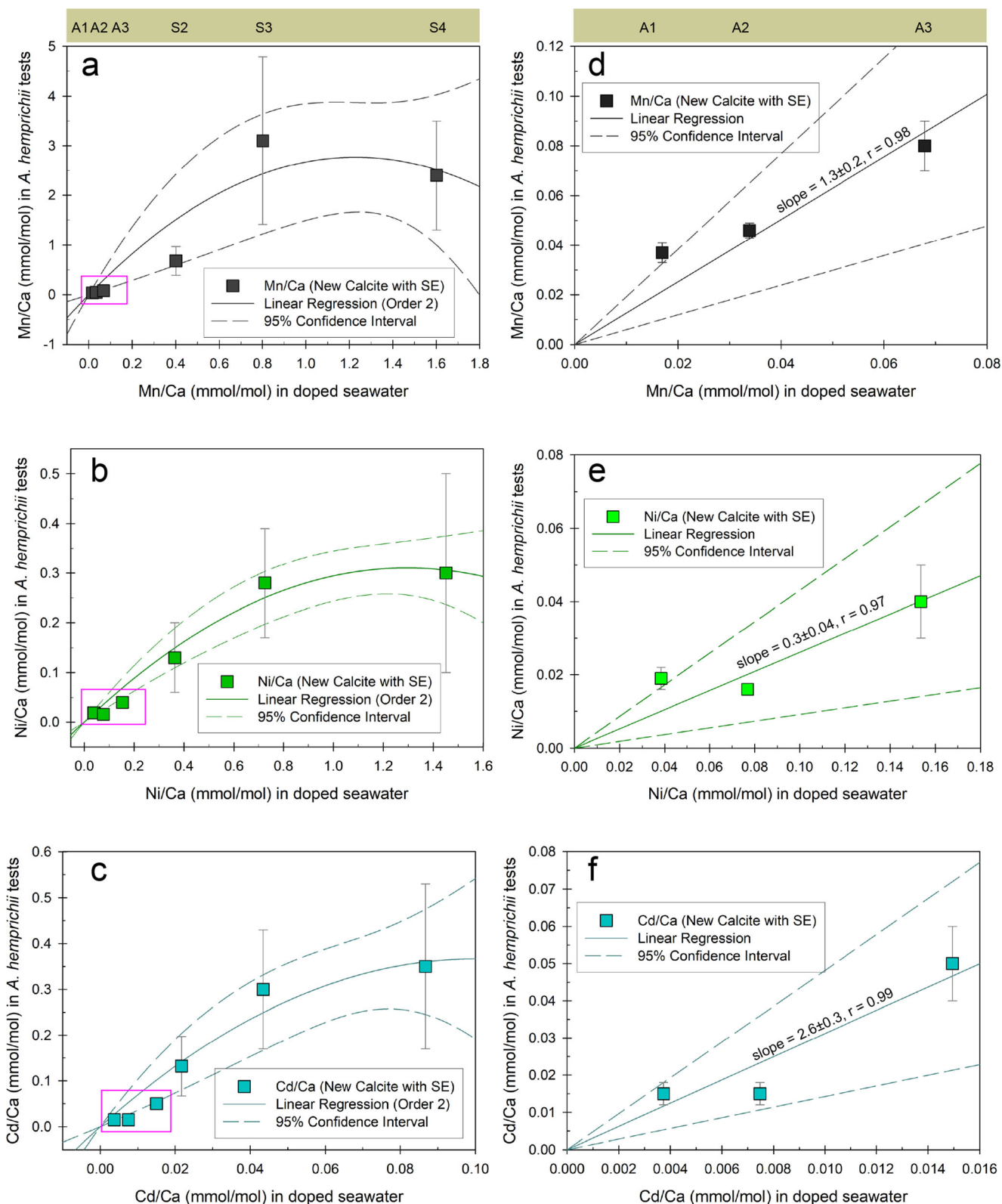
Fig. 4 Metal/Ca ratios in new calcite obtained from culture experiments A1, A2, and A3 (batch 2) and S2, S3, and S4 (batch 1) for the heavy metals, Mn, Ni, and Cd (**a**, **b**, **c**, respectively). Error bars represent one standard error of the mean. **d**, **e**, **f** The zoomed-in region for experiments A1, A2, and A3; marked as pink rectangles in **a**, **b**, and **c**, respectively. The slope of the linear regressions represents the partition coefficient of the heavy metals Mn (**d**), Ni (**e**), and Cd (**f**)

The variable correlation plots for batches 1 and 2 are shown in supplementary Fig. 1. For batch 1 (experiments S2, S3, S4), the first two principal components (PCs) accounted for 95.6% of the variance in the data sets and PC1 alone with 89.8%. In batch 2 (experiments A1, A2, and A3), the first two PCs accounted for 94.3% of the total variance in the data and PC1 alone with 86.8%. In batch 1, all the variables are grouped together; variables Ni/Ca, Mn/Ca, and Cd/Ca are grouped much closer (supplementary Fig. 1a). Similarly, all the variables are also grouped together in batch 2 (supplementary Fig. 1b). Ni/Ca and Cd/Ca appear to fall on the same line indicating a strong positive correlation. It was also observed that in both batches 1 and 2, all the variables are well away from the origin and positioned close to the circumference of the correlation circle (supplementary Fig. 1), indicating they all are equally important.

Discussions

Influence of temperature on the incorporation of metals in foraminifera

Culture experiments with foraminifera *Amphisorus hemprichii* were conducted under different temperature settings (23 °C, 25 °C, and 27 °C) to determine whether seasonal variability in seawater temperature might affect the incorporation of Mn, Ni, Cd, and Pb. Of the heavy metals that are the focus of this experiment, only Cd/Ca ratios in the tests of benthic foraminifera (in different temperature settings) have been most widely studied and are a well-established proxy for seawater paleo-nutrient concentrations (Marchitto 2004 and references therein); however, laboratory culture experiments are limited. In laboratory culture experiments at 28–30 °C using planktonic foraminifera *Globigerinoides sacculifer*, *Orbulina universa*, and *Globigerinoides ruber*, Delaney (1989) found a wider Cd distribution coefficient, ranging between 2 and 4, indicative of species-dependent partitioning. Another culture experiment with planktonic foraminifera *Globigerina bulloides* at 22 °C by Mashiotta et al. (1997) obtained K_D^{Cd} of 1.9±0.2. Estimates from in situ collections by Boyle (1992) showed that the partitioning of Cd into the tests of benthic foraminifera (*Uvigerina* sp., *Cibicidoides kullenbergi*, *Cibicidiodes wuellerstorfi*, *Cibicidoides pachyderma*, and *Nutallides umbonifera*) also varied with depth. Here K_D^{Cd} ranged between 1.3 for benthic foraminifera collected from <1150 m (water temperatures of 6.8 and 10.8 °C) and 2.9 for the ones beyond >3000 m (water temperature around 3.6 °C). Boyle (1992) concluded that the depth dependency of Cd partitioning was probably related to pressure or other depth-correlated properties than to temperature. Additionally, Hester and Boyle (1982) analyzed Cd/Ca ratios in benthic foraminifera *Uvigerina* sp. and *C. kullenbergi* in

sediment core tops from cold deep waters (water temperature around 3 °C) of the Atlantic, Pacific, and the Mediterranean Sea and, assuming present-day Cd seawater concentrations, inferred a K_D^{Cd} of 2.0±0.4. A study by Rickaby and Elderfield (1999) using foraminifera *Globigerina bulloides* from core tops in the North Atlantic found an exponential increase of K_D^{Cd} (1.5 to 6.8) with a rise in temperature (from 8 °C to 15 °C). A similar study was undertaken by Marchitto (2004) using seven taxa of benthic foraminifera (*Cibicidoides kullenbergi*, *C. pachyderma*, *C. wuellerstorfi*, *Hoeglundina elegans*, *Planulina ariminensis*, *P. foveolata*, and *Uvigerina* sp.) picked from 14 core tops and two grab samples. The water temperature of these sites ranged from 4.2 to 17.7 °C with K_D^{Cd} values close to those predicted by Boyle (1992) and Boyle et al. (1995). They did not find any indication of the influence of temperature on the partitioning of Cd from the seawater to the tests of benthic foraminifera species. From our temperature experiments, the increase of Cd/Ca ratios by 0.0007 mmol/mol from 23 to 25 °C falls within the uncertainty (one SE) of Cd/Ca measurements for the new calcite formed in the laboratory in spiked solution (culture studies 1), implying a non-significant increase in Cd/Ca ratios with the increase in temperature. For the other studied metals, Mn/Ca, Ni/Ca, and Pb/Ca, the increase of 0.001, 0.002, and 0.0009 mmol/mol, respectively, falls within the uncertainty of our laser measurements for the respective metals. Hence, our studies suggested that there was no significant increase in metal incorporation into the tests of LBF *Amphisorus hemprichii* specimens with the increase in temperature.

Influence of light intensity on the incorporation of metals in foraminifera

Live juvenile specimens of *Amphisorus hemprichii* were grown in the laboratory under different light intensity settings to measure the effect of symbiont photosynthetic activity on the incorporation of metals (Mn, Ni, Cd, and Pb) from the multi-element spiked solution "S3" (Table 2), which was used as the culture medium. The different light intensity settings were 22, 55, 299, and 483 µmol photons m^{-2} s^{-1} PAR at noon (section "Culture studies 2: light intensity experiments"). These foraminifera host dinoflagellates as symbiont partners (Lee et al. 1991) who provide them with substantial energy and take their metabolites (Hallock 2003). It was shown experimentally that photosynthetically active symbionts are necessary for the existence of the host foraminifera (Lee et al. 1991). The symbiotic algae hosted by *Amphisorus hemprichii* use natural sunlight for their photosynthetic activity. Hence, it is important that these foraminifera get adequate sunlight for their own survival, growth, and reproduction (Lee 1995). Light also helps for enhanced growth, as was observed in increased calcification in three soritid species from the Caribbean: *Archaias angulatus*, *Cyclorbiculina compressa*,

and *Sorites marginalis* (Duguay 1983). It was also found that the intensity of light influenced the behavior of the symbiont-bearing foraminifera; they moved away from both strong light sources and dim light sources to optimal light conditions (Lee et al. 1980; Sinutok et al. 2013). Interestingly, it was observed that *Amphisorus hemprichii* foraminifera even sacrificed their feeding territories and moved towards optimal light conditions for their algal symbionts (Lee et al. 1980). This photoprotection is mediated by the hosts and is not symbiont driven, according to Petrou et al. (2017). Based on culture experiments with foraminifera *Marginopora vertebralis*, Petrou et al. (2017) found that the host foraminifera relocates the symbionts deeper within its calcium carbonate tests upon receiving a photo stress signal from its symbionts. Though there are a few studies on the importance of the symbiotic relationship of LBF and its symbionts in varying light conditions, there are no studies on the amount of incorporation (or variation) of toxic metals in the tests of foraminifera with increasing light intensities. From our laboratory culture experiments, Cd/Ca and Mn/Ca ratios in the tests of cultured *Amphisorus hemprichii* specimens do not show any strong positive correlation with varying light intensities. Both Ni/Ca and Pb/Ca ratios increased in the tests of cultured specimens when the light intensities increased from 27 to 55 μmol photons m^{-2} s^{-1}. The Pb/Ca ratios remained nearly constant from 55 to 483 μmol photons m^{-2} s^{-1}, which suggested that the best light intensity of Pb partitioning was above 55 and below 299 μmol photons m^{-2} s^{-1} as beyond that the Pb/Ca ratios decreased, though very small. For Ni partitioning, we observed the light intensity around 299 μmol photons m^{-2} s^{-1} was the ideal one. Overall, based on our results, we find that the partitioning of metals with varying light intensities is insignificant.

Principal component analysis and partition coefficients

The PCA of Me/Ca ratios, Mn/Ca, Ni/Ca, Cd/Ca (this study), and Pb/Ca (Sagar et al. 2021), obtained from the outer septum of the cultured *Amphisorus hemprichii* foraminifera specimens in multi-element spiked solutions showed 89.8 % and 86.8 % variance for batches 1 and 2, respectively, which was explained by the first PC alone (Supplementary Fig. 1). This was expected as the Me/Ca ratios in the outer septum of the cultured foraminifera specimens were a direct result of our laboratory spiking with multi-element spiked solutions of batches 1 and 2 (Table 2). All of the four original variables (Me/Ca) are well represented and equally important, which is evident from the variable correlation plots (as the vector of the variables nearly touches the circumference of the correlation circle). In both batches, the variables are clustered together, indicating a high correlation between them. The maximum angle between the variables in both batches is around 45°

indicating that all of the four variables (Me/Ca) are positively correlated and follow nearly the same systematic process of incorporation from the spiked solutions into the calcite lattice of *Amphisorus hemprichii* foraminifera.

Partition coefficients (K$_D$) for metals, Mn, Ni, and Cd, were deduced from molar foraminiferal test ratios and their corresponding seawater (multi-element culture solution) values. Based on the geochemical results from our culture experiments, we observed that the concentration of spiked metals in the tests of *Amphisorus hemprichii* specimens in experiments S4 were similar to S3 or even lower (Fig. 4a, b, c). As discussed, the concentration of the spiked solution in experiment S4 was 2× higher than experiment S3 (Table 2). The similar or lower concentrations of metals (Me/Ca) in the *A. hemprichii* tests of experiments S4 compared to S3 possibly indicate metal saturation in the calcite tests. Similar observations of decrease in Zn incorporation with increasing concentration of Zn-spiked solution (at 10 mg/L) were found by Nardelli et al. (2016) in their culture studies with benthic foraminifera *Pseudotriloculina rotunda*. These authors suggested that this could be partially due to the precipitation of Zn in the form of zinc oxides/hydroxides. Munsel et al. (2010) also reported similar observations of decreased Ni concentrations in the tests of benthic foraminifera *Ammonia tepida* at higher Ni-spiked seawater (20-fold higher than natural seawater). They suggested that higher Ni concentrations could have had toxic effects on foraminiferal cells, which inhibited calcification. Reduced calcification with increased Zn toxicity was also observed in culture studies using *Pseudotriloculina rotunda* by Nardelli et al. (2013). Despite the abovementioned biological and chemical controls in foraminifera, we observed linear incorporation of Mn, Ni, and Cd in the calcite tests of cultured LBF *Amphisorus hemprichii* specimens for a wide range of seawater concentrations (see the section "Incorporation of metals in the tests of cultured *Amphisorus hemprichii* and their principal component analysis").

The K$_D^{Me}$ for the studied heavy metals (Mn, Ni, and Cd) was calculated by using the following formula (Fig. 4d, e, f):

$$K_D^{Me} = [Me/Ca]_{test}/[Me/Ca]_{seawater}$$

We report the K$_D^{Me}$ obtained from the multi-element spiked culture batch 2 (experiments A1, A2, and A3) because (1) the concentration of metals in the spiked solution in these experiments was near to environmental range and (2) the SE of the new calcite (Me/Ca) incorporated in this batch was lower compared to the experiments in batch 1 (experiments S2, S3, S4). The K$_D^{Mn}$, K$_D^{Ni}$, and K$_D^{Cd}$ obtained from our culture studies with *Amphisorus hemprichii* specimens are 1.3 ±0.2, 0.3±0.04, and 2.6±0.3, respectively (Fig. 4d, e, f). Munsel et al. (2010), from their laboratory culture experiments with benthic foraminifera *Ammonia tepida*, estimated K$_D^{Mn}$ to be at least 2.4, based on the scatter of Mn

concentrations in their culture solutions. They suggested that the wide scatter of Mn concentrations was possibly due to the formation of Mn oxides and hydroxides under oxic conditions, a feature that was not observed in our experiments. The K_D^{Ni} obtained from our studies (0.3 ± 0.04) is markedly lower than the K_D^{Ni} of 1.0 ± 0.5 determined by Munsel et al. (2010). In contrast, the partition coefficient of cadmium (K_D^{Cd}) for *A. hemprichii* tests in our study, 2.6 ± 0.3, falls within the range of K_D^{Cd} values (ranging from 1.0 ± 0.1 to 2.8 ± 0.6) obtained by Maréchal-Abram et al. (2004), based on their culture experiments with *Ammonia beccarii*. Another laboratory culture study aimed at determining the K_D^{Cd} using a planktonic and non-symbiont bearing foraminifera *G. bulloides* (Mashiotta et al. 1997) reported K_D^{Cd} value of 1.9 ± 0.2, which is slightly lower compared to the K_D^{Cd} obtained from our culture experiments with *Amphisorus hemprichii*.

Conclusions

This study presents the results of cultured LBF *Amphisorus hemprichii* to determine (1) the effects of temperature and light intensities on the partitioning of Mn, Ni, Cd, and Pb and (2) the partitioning coefficients (K_D) of the heavy metals, Mn, Ni, and Cd, which are typically associated with environmental coastal pollution. We find that:

1. Over the temperature range from 23 to 27 ± 0.3 °C, uptake of Cd, Ni, and Pb by *Amphisorus hemprichii* is insensitive to temperature ($p > 0.05$). For incorporation of Mn, there is a marginally positive relationship ($r = 0.997, p < 0.05$), but the increased Mn/Ca values were within one standard error of the mean.
2. The results from the light intensity experiments (22, 55, 299, and 483 μmol photons m^{-2} s^{-1} PAR at noon) also showed a non-significant increase of Mn, Ni, Cd, and Pb concentrations in the tests of *A. hemprichii* foraminifera with the increase in light intensities ($p > 0.05$ for all studied metals).
3. Based on our high-resolution geochemical analysis, we observed that under acute pollution, the concentration of Mn, Ni, and Cd in the tests of *Amphisorus hemprichii* specimens reached saturation levels, possibly due to vital effects. However, we observe a strong linear association ($r > 0.9$) between incorporation of these metals and the doped seawater concentrations in our culture experiments for a non-lethal range of seawater pollution, Mn, 9.3 to 440 μg/L; Ni, 22.5 to 425.8 μg/L; and Cd, 4.2 to 48.8 μg/L.
4. The results from the principal component analysis show that the spiked metals, Mn, Ni, Cd, and Pb, are positively correlated and also well represented in the variable correlation plots, which indicated that all the four variables (Mn/Ca, Ni/Ca, Cd/Ca, and Pb/Ca in this study) were

equally important. The first principal component alone was found to explain 89.8 % and 86.8 % variance for experimental batches 1 and 2, respectively.
5. The K_D of Mn, Ni, and Cd determined from our multi-element and multi-concentration culture studies of batch 2 with *Amphisorus hemprichii* foraminifera specimens are 1.3 ± 0.2, 0.3 ± 0.04, and 2.6 ± 0.3, respectively.

The quantitative results on the partitioning of heavy metals, Mn, Ni, and Cd (this study), and Pb (Sagar et al. 2021), obtained from a wide range of multi-element enrichment culture experiments into the calcite shells of *Amphisorus hemprichii* demonstrate the robustness of the LBF *Amphisorus hemprichii*. These specimens can be used for monitoring heavy metal contamination in the coastal environments, especially in the reefs where these LBF are abundant.

Declarations

Ethics approval and consent to participate Not applicable

Consent for publication   Not applicable

Competing interests   The authors declare no competing interests.

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