Heliyon 10 (2024) e27229

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Impact of heavy metals (Cu, Fe, Pb, Zn) on carbon and nitrogen uptake of the diatom-bearing benthic foraminifera *Heterostegina depressa*

Mario Bubl [a], Petra Heinz [a,*], Wolfgang Wanek [b], Michael Schagerl [c], Thilo Hofmann [d], Michael Lintner [a,e]

[a] Department of Palaeontology, University of Vienna, Vienna, Austria
[b] Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria
[c] Department of Functional and Evolutionary Ecology, University of Vienna, Vienna, Austria
[d] Department of Environmental Geosciences, University of Vienna, Vienna, Austria
[e] ING PAN - Institute of Geological Sciences, Polish Academy of Sciences, Research Centre in Kraków, Poland

A B S T R A C T

Foraminifera are protists primarily living in benthic marine and estuarine environments. We studied uptake of inorganic carbon (C) and nitrogen (N) of the photosymbiont-bearing benthic coral reef foraminifera *Heterostegina depressa* in the presence of heavy metals.

Incubation experiments were accomplished with artificial seawater enriched with copper, iron, lead and zinc at two different concentration levels (10 and 100 fold enriched in contrast to the usual culture medium). Additionally, isotopically labelled $^{13}$C-sodium bicarbonate and $^{15}$N-ammonium chloride were added to trace their assimilation over time (1 d, 3 d, 5 d, 7 d). Pulse-amplified modulated fluorescence measurements were performed to measure the potential impacts of heavy metals on chlorophyll fluorescence of the photosymbiont. Increased levels of copper (430.5 μg Cu/l) exhibited the greatest toxicity, while for low levels no effect on the overall metabolism of the foraminifera and the fluorescence activity of the photosymbiont could be detected. Iron (III) increased the symbiont activity, independent of concentration applied (44.5 and 513.3 μg Fe/l), which indicates Fe-limitation of the algal symbiont. Lead enrichment showed no detectable effect even at high concentration. Low concentrations of zinc (35.1 μg Zn/l) promoted the metabolism of the foraminifera, while high concentrations (598.4 μg Zn/l) were toxic. At low levels, two metals (Fe and Zn) promoted symbiont activity, at high levels, iron still boosted photosynthesis, but Zn and Cu had a negative impact on the obligatory photosynthetic symbionts.

## 1. Introduction

### 1.1. General introduction

Anthropogenic impacts are recognized in most habitats; elevated levels of pollutants with special respect to marine environments have been in focus for decades. Halpern [1] et al., analyzed a global pattern of anthropogenic impact on marine ecosystems and found out, that a large fraction of 41% of all investigated ecosystems are strongly affected by human activity. A relatively small impact can be found near the poles. But nowadays the amount of studies who report pollution in the Antarctic are increasing [2,3].

Heavy metal contamination of costal environment is a recently often discussed topic. This pollutant can be uncontrolled released to the environment via several industrial processes like coal mining, agricultural activities or due to steel industry [4]. The

* Corresponding author.
*E-mail address:* petra.heinz@univie.ac.at (P. Heinz).

https://doi.org/10.1016/j.heliyon.2024.e27229
Received 4 August 2023; Received in revised form 13 December 2023; Accepted 26 February 2024
Available online 7 March 2024

bioaccumulation of heavy metals is directly dependent on the uptake and storage mechanisms of the organisms as well as the bioavailability of the metals in the ecosystem [5]. A high concentration of some metals can lead to a rapid failure of the metabolism and the organisms will die [5]. However, in a small concentration heavy metal are essential trace elements and can push the organism's activity [6]. In this context, foraminifera have been utilized as bioindicators since the 1960s [7]. Foraminifera are single-celled organisms, which mainly occupy marine environments [8]. Benthic foraminifera can be found either above (epifaunal) or within (infaunal) the sediment-water interface [9,10]. Because of their high reproduction rates and their short life cycle, foraminifera also show great proxy abilities which enables them to derive information about different parameters of their surrounding environment [11,12]. Foraminifera respond quickly to an increase of heavy metal concentrations and are therefore used as early indicators of such events in the environment [13–15].

Larger benthic foraminifera (LBF) are characterised and identified by their large size in contrast to other foraminifera. In order to stabilize their shells, they developed an internally complex architecture [16]. This group accounts for 80 % of foraminiferal reef carbonate production on planet earth [17]. Several forms of symbiotic relationships occur between LBFs and other organisms, such as diatoms, chlorophytes, cyanobacteria, dinoflagellates, haptophytes or rhodophytes [18–20]. LBF benefit from photosynthetic products of the photobionts like glycerol and sugar [21]. In turn, photobionts are protected by the host and have a higher availability of nutrients compared to free-living algae [20,22].

### 1.2. Heavy metal pollution

Elements featuring density >5 g/cm$^3$ are termed heavy metals (HM) and naturally occurring in the Earth's crust [23]. A variety of HM are essential trace elements for organisms, however, in elevated concentrations they can be toxic and potentially bioaccumulate through the food chain [23,24].

Through certain events such as ocean oxidation periods or elevated anthropogenic impacts, HM are released in elevated concentrations into the environment, whereupon they are considered bioavailable [23,25]. Potential sources are for instance biosolids, fertilizers, landfills, and household waste [24,25]. However, besides man-made contamination, also natural sources need to be considered, such as weathering of volcanic minerals [26].

With respect to HM contamination, benthic foraminifera are a great monitoring tool [27]. In coastal habitats, the elements Cu, Pb, and Zn are considered as the most frequent pollution [28]. HM impact on foraminiferal communities along continental coastlines is stronger compared to areas located further away from the mainland [15]. Foraminiferal test abnormalities significantly dependent on the type of pollutant and the concentration [29]. The foraminiferal species *Elphidium excavatum* can adapt to these events and outcompete other species in polluted near-shore environments [29]. In contrast to that, Miliolids display a strong sensitivity to such pollution events [29].

Copper is an essential trace element for organisms, but can cause negative effects at higher concentrations [30], e.g. oxidative stress, which damages DNA, lipids, and proteins [31]. The availability of iron is associated with the regulation of algal communities in coastal upwelling regimes and assists in the development of diatom blooms [32]. Zinc (oxide) nanoparticles deriving from sunscreens released by human into the environment at global beaches are presumably considered harmful to a variety of marine microorganisms [33,34]. Highly increased (1044 x Zn enrichment) concentrations of Zn negatively affected the metabolism of foraminifera [35]. Human extraction of lead and its utilization reaches far back in history, however, the impact on marine organisms is rather diverse [35–37]. Recent studies based on metabolic observations showed that some foraminiferal species might adapt to increasing lead concentrations [35,38].

The present study aims to obtain further knowledge on anthropogenically induced HM pollution (Cu, Fe, Pb, Zn) and the metabolic responses of the symbiont-bearing coral reef LBF *Heterostegina depressa* d'Orbigny 1826, since HM enrichment in coral reefs increased in the last decades. For that purpose we will test two different concentrations of HM. The first (10-fold enriched) reflects common metal concentrations in pore waters. These metals can be released by stronger mixing events triggered by storm surges. The second concentration (enriched 100 times) is an experimentally selected concentration and is intended to represent heavily polluted water. During these experiments, we will investigate whether the foraminifera would survive under such extreme conditions. Our hypothesis is that copper, even in low concentrations, has a negative effect on the metabolism of the foraminifera, since copper is also used to combat algae. On the other hand, iron supplementation should lead to activation of photosymbionts, since iron is normally the limiting factor for marine phytoplankton.

## 2. Material and methods

### 2.1. Experimental setup

A main culture of *H. depressa*, hosted at the Department of Palaeontology at the University of Vienna provides a sufficient number of individuals for the experiments. All selected foraminifera had a diameter of approximately 1.2 mm. We prepared artificial seawater enriched with HM (interference factor) and stable isotopes (measured parameters). Artificial seawater was used to reduce the impact of dissolved organic matter or other pollutants that could arise when filtered seawater will be used from the sampling site. For the basic medium, 33 g of reef salt were dissolved in 1 L of purified, sterile-filtered water.

Inductively coupled plasma mass spectrometry, as well as optical emission spectroscopy (ICP-MS & OES) measurements (3 replicates) were done at the Department of Environmental Geoscience/University of Vienna to estimate the natural content of Cu, Fe, Pb, Zn in the applied artificial seawater. For HM stocking, water-soluble salts were used: Cupric sulphate Pentahydrate (CuSO4*5H2O),

Ferric Chloride Hexahydrate (FeCl3*6H2O), Lead (II) chloride (PbCl2), and Zinc Sulphate Heptahydrate (ZnSO4*7H2O) were dissolved in distilled water. In the present study, two concentrations c1 and c2 are applied, whereby c1 is considered an elevated (10-fold) HM concentration and c2 a highly elevated (100-fold) concentration, compared to c0 (concentration of the main culture). Then stable isotopes 13C (NaH13CO3 = 0.235 mmol/l) and 15 N (15NH4Cl = 0.220 mmol/l) were added to each setup (c0, c1, c2).

Foraminifera were separated from the main culture and cleaned with a small brush from adhering particles. Afterwards, the specimens were placed in crystallization dishes for the isotopic uptake with HM treatments.

The exact HM concentrations of the synthetic seawater are listed in Table 1.

Additionally, an incubation of foraminifera in untreated artificial seawater (no metals) was done as a control. After the incubation period of 1, 3, 5 and 7 days, the foraminifera were prepared for isotope ratio measurements. This time period was also used in other treatments and was found to be optimal for incubation experiments with foraminifera.

Specimens were isolated from the dishes, cleaned via flush-washing with distilled water, and placed in previously weighed tin capsules. Afterwards they were air-dried for three days and decalcified with 12.5 μl HCl (2 M) to dissolve the calcite test. The remaining cytoplasm in the capsules was finally dried in a dry chamber at 50 °C for three days and reweighed. Analyses of C:N ratios and the C and N contents were done with an isotope ratio mass spectrometer (IRMS, DeltaPLUS, coupled by a ConFlo III interface to an elemental analyser EA 1110, Thermo Finnigan) at the Stable Isotope Laboratory for Environmental Research (SILVER) at the University of Vienna. Calculation of incorporated isotopes was done according to Lintner et al. [39].

In another set of experiments, pulse-amplified modulated (PAM) fluorescence measurements of the photosynthetic active area were performed to analyse the impact of HM treatments on the photosymbiont. Foraminifera were incubated in 6 well-plates (one individual per well) and were covered with 10 ml artificial seawater enriched with HM. The plates were sealed with parafilm to avoid evaporation and ensure stable salinity conditions and measured after 1, 3, 5, and 7 days. The foraminifera were incubated at a temperature of 25 °C and a salinity of 33. The light intensity was measured at 40 μmol photons m−2s−1 and a light dark cycle of 12:12 h was provided.

We used Imaging-PAM (microscopy version, Walz company, Eiffeltrich, Germany) to get insight into the photoactive area of the photosymbionts. The instruments consist of a modified epi-fluorescence microscope, equipped with a modulating LED light source and a photomultiplier for detection of modulated chlorophyll fluorescence (Zeiss microscope Scope. A1 – AXIO, camera system model: IMAG-KG - AVT Manta G145B A56; IMAGING-PAM model IMAG- CG). Fluorescence measurements of the chloroplasts were determined via the software program developed for PAM-analysis, ImagingWin v 2.56p FW MULTI GigE3b (Walz GmbH). Settings throughout the entire period of the software were constant. The PAM fluorometer stimulates the symbionts and their photosystem II in the chloroplasts, which was transferred graphically into the photoactive area (%) for each time point (0, 1, 3, 5 and 7 days).

### 2.2. Data analysis

Two-way ANOVA was performed for isotopic uptake experiments to test significant differences of type of HM, concentration and incubation time. For PAM-series two-way repeated measurement ANOVA was applies to test the influence of HM and time on the photosynthetic active area of the symbionts. All statistical tests were performed with the software Past 4.03 and a level of significance = 95%. Detailed information about the raw data and the ANOVA tables are given in the supplementary file.

## 3. Results

### 3.1. Isotopic uptake of the foraminifera

The type of HM, significantly influenced the carbon and nitrogen uptake (ANOVA, df = 3; $p < 0.001$). The isotopic uptake of all experiments is shown in Fig. 1. At the lower metal concentration (c1), the uptake of carbon and nitrogen only differed significantly from the control in the presence of copper ($p < 0.001$, df = 1). At higher metal levels (c2), the carbon and nitrogen uptake were significantly lower in comparison to the control ($p < 0.001$, df = 1), with the exception of lead ($p = 0.867$, df = 1).

Copper: The isotope uptake (IC & IN) throughout the copper experiment is highly significant depending on the concentration ($p < 0.001$, df = 1) and the time ($p < 0.001$, df = 3), as well as their interaction ($p < 0.001$). For concentration c2, the metabolism of the foraminifera is decreased, and less carbon is incorporated, whereas, at c1, the foraminifera can compensate for the slightly elevated

**Table 1**
Heavy metal concentration of all synthetic seawater media, as well as the natural content of the medium.

| Sample ID | Cu [μg/l] | Fe [μg/l] | Pb [μg/l] | Zn [μg/l] |
|---|---|---|---|---|
| Synthetic seawater (Cu0, Fe0, Pb0, Zn0) | <10 | <10 | <0.5 | <10 |
| Cu_c1 | 19.5 | – | – | – |
| Cu_c2 | 430.5 | – | – | – |
| Fe_c1 | – | 44.5 | – | – |
| Fe_c2 | – | 513.3 | – | – |
| Pb_c1 | – | – | 2.4 | – |
| Pb_c2 | – | – | 41.3 | – |
| Zn_c1 | – | – | – | 35.1 |
| Zn_c2 | – | – | – | 598.4 |

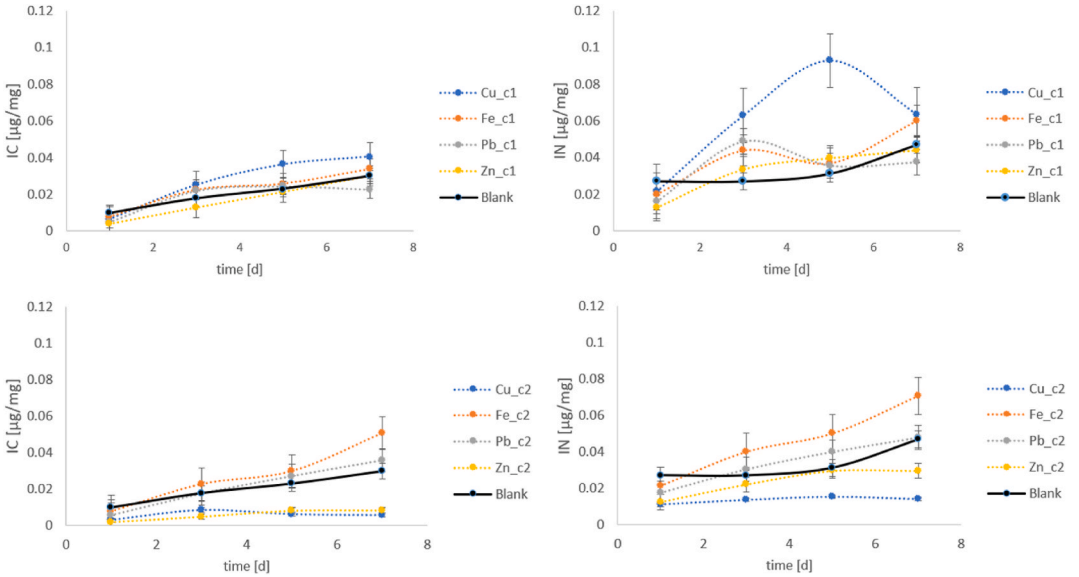


**Fig. 1.** Mean values of the isotopic uptake of *H. depressa* at different heavy metals and concentrations (n = 8, error bars indicate the standard error). The x-axis shows the four different time points and the y-axis displays the incorporation of carbon (IC) on the left and nitrogen (IN) on the right column in µg/mg.

interference factor. The carbon uptake, show a constant increas during the experiment at c1. In contrast to that, uptake at c2 is decreasing after three days close to zero (Fig. 1). A similar pattern can be observed for the nitrogen uptake at c2.

Iron: Compared to the control, we generally found an increased carbon and nitrogen uptake over time ($p < 0.001$, df = 3). Also, the concentration treatments different significantly ($p < 0.001$, df = 1), as well as their interaction (conc x time) ($p < 0.01$).

Lead: No significant differences between c1 and c2 were observed on the carbon and nitrogen uptake ($p = 0.044$ for IC & $p = 0.345$ for IN, both df = 1). The incorporated carbon, as well as the incorporated nitrogen, is highly significant on the time ($p < 0.001$, df = 3) and high significant in the interaction of time and concentration ($p < 0.01$). The carbon uptake at c1 is decreasing after day 5, while the uptake at c2 follows a linearly upward trend. For the IN plot, a similar linearly increasing trend occurs for the measurements at concentration c2. The uptake at c1 shows that the isotopic uptake of nitrogen was at day 3 higher compared to the uptake level within the c2 specimens. After day 3 the uptake decreased until day 5 and raised again slightly until the end of the observation period (day 7).

Zinc: The amount of incorporated carbon and nitrogen isotopes is highly significant with time ($p < 0.001$, df = 3) and concentration of zinc ($p < 0.001$, df = 1). However, for IN, no statistical significance with a focus on the interaction of time and concentration is calculated. The isotopic carbon uptake at c1 followed a linear increase and already started to separate from the uptake values of c2 (decreasing trend) after the third day. In contrast to that, the higher concentration of c2 is depressing the metabolic behavior of the foraminifera and the amount of carbon isotope uptake is constantly low. A similar trend can be observed for nitrogen uptake. Hereby, the values of nitrogen uptake at c1 and c2 start to separate on day 3 and higher nitrogen uptake was observed at concentration c1.

### 3.2. *Effect on photobionts*

The impact of heavy metals on the activity of the algae hosted by *H. depressa* is visualised via Fig. 2(a) and (b). The photoactive area

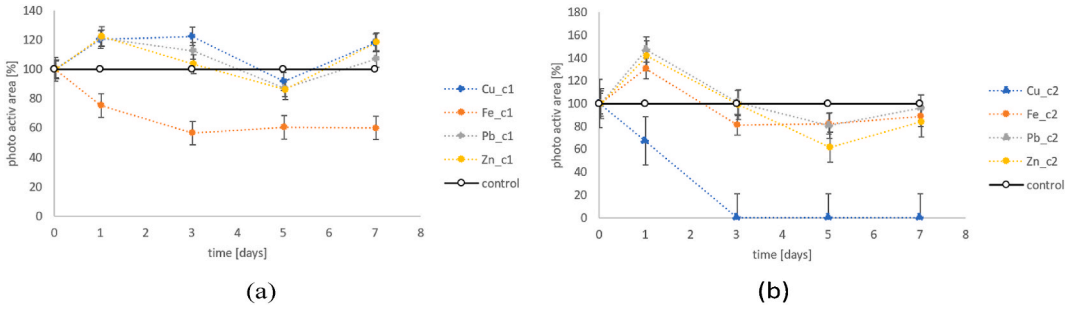


**Fig. 2.** The plot displays the photoactive area [%] (x-axis) against the time (y-axis). The black line is the control line and the metals copper, iron, lead, and zinc are individually color-coded and concentration level c1 under (a) and c2 under (b)

is given in percent of the total foraminiferal area, including the standard error for six replicates. All plotted data were normalised against the control values (black control line), which are the percental shifts of the specimens, that were incubated without the heavy metals as an interference factor.

The increasing and decreasing effects on the activity of the symbionts at higher concentration level c2 are highly significant depending on the type of metal ($df = 3$; $p < 0.001$), time ($df = 4$; $p < 0.001$) and their interaction ($p < 0.001$).

The symbiotic activity of the symbionts at **copper** level c1 is throughout the total incubation time close to the control line. However, on day 7, the longest observation period, the symbiotic activity is above the control line. In contrast to that, the activity at c2 visualises a strong decrease trend from the start of the experiment and after day 3 no activity can be observed. The symbiotic activity during the copper-experiment is highly significant depending on the time ($p < 0.001$) and the concentration ($p < 0.001$), as well as the interaction ($p < 0.001$).

The measurements of the photoactive area, corresponding to c1 of **iron**, need to be considered as a calculation artifact, which is explained in more detail in the chapter 4.2. The measured area of iron at concentration level c2 is on every measured timepoint close to the control line.

**Lead** at c1 and c2 resulted in increased symbiotic activity on day 1, however throughout the rest of the experiment the values were close to the control line. Statistical evaluations resulted in no significant activity differences between foraminiferal specimens incubated in c1 and c2.

Both concentration levels of **zinc** resulted in increased activity on day 1, overlayed on day 3 with the control line, and on day 5, the values of photoactive areas of c1 and c2 started to separate.

## 4. Discussion

### 4.1. Impact of heavy metals on the metabolism of H. depressa

Copper influences the behavior of *H. depressa* highly depending on the provided concentration. At low levels, the foraminifera are still able to cope with this HM. We assume that copper is considered as a trace element for foraminifera, as it was already proved for other organisms [40]. At high concentrations, the metabolism of H. depressa and/or their symbionts is highly impaired. Lintner et al. [35] analyzed the effects of elevated copper concentrations on the algal food uptake by the foraminiferal species *Elphidium excavatum* and found a comparable pattern. Because *Elphidium excavatum* does not host symbionts, we assume an adverse effect on the foraminifera itself, and not only on the photosymbionts. Carbon and nitrogen uptake of concentration levels c1 and c2 are differing from each other, probably because carbon is mainly involved in respiration, whereas nitrogen is mainly incorporated in amino acids, proteins, and DNA [41]. Throughout the metabolic process, the foraminifera are releasing the digested carbon and nitrogen in parts, such as respiratory CO2 or excreta, to their surrounding environment [41,42]. The reduced metabolic activity of benthic foraminifera caused by environmental stress is already discussed in various studies [35,43–45].

Iron led independently of concentration levels to increased metabolic activity. The increased carbon isotope uptake is depending on the concentration level. In contrast to that, nitrogen uptake is comparable between c1 and c2. Iron is probably a limiting resource in the main culture so its addition during the experiments promotes growth. In the world's oceans, iron is also considered as limiting factor, and seawater is featuring only a very small content of this HM [46]. Throughout the iron experiment, one possible adaptation reaction could be that the carbon is diluted by a stronger nitrogen intake, and by the fact that the algae have an increased photosynthetic activity. Following that, the algae assimilate more carbon at higher Fe concentrations, which are then available for the host.

In accordance to Lintner et al. [35], even increased levels of lead did not affect the food uptake. Applied lead concentrations do not cause chances in metabolic activities at least for short periods of time.

The enrichment of zinc stimulates the foraminifera *E. excavatum* for slightly higher food uptake. In contrast to that, they showed a statistical tendency, that a further increase of Zn concentration decreases the element uptake. At low HM concentrations, the specimens showed a constant increase in isotopic carbon and nitrogen uptake over time. The amount of incorporated carbon and nitrogen followed a logarithmic trendline ($y = 0.017\ln(x) + 0.106$; $R2 = 0.85$). This trend can be explained as uptake/acclimation of the foraminifera to the lower concentration of zinc after day 3, followed by a saturation after day 5. Zn is known to act as essential trace element at low concentrations also in other organisms. At high concentrations, the metabolism of H. depressa was strongly reduced. Other studies have shown that Zn is released into the environment as ZnO nanoparticles, which are harmful to a variety of organisms [33,47]. Lintner et al. [34] tested the influence of sunscreens containing such nanoparticles on *H. depressa* and they noticed ZnO as a potential ingredient reduced the photosynthetic area of the symbionts.

### 4.2. The effect of heavy metals on the symbiont activity of H. depressa

According to the very similar results of the effect of metals copper, lead, and zinc at concentration level c1, it can be stated that the lower enrichment is immaterial for the symbionts of *H. depressa*. No greater negative impact on the photoactive area could be observed. However, on day 7, which is the longest observation period, the symbiont activity of the symbionts in the Cu, Pb, and Zn experiments is above the control line. This can be explained due to the nutritious effect on foraminiferal health of slightly elevated concentrations. Iron at concentration c1 needs to be considered as a calculation artifact, because the incubated individuals (Fe c1) were by mistake on average larger than the other foraminifera at the start of the measurements. Thus, they also had a larger area of active chloroplasts, which resulted in different values.

The effect of copper at concentration c2 is separated from the three other metals. The symbiotic activity starts to decrease from the

beginning, which is due to the toxicity of this metal in such high concentrations. The symbiotic activity of *H. depressa*, which was in contact with iron, lead, and zinc is at day 7 below the control line, which can be explained due to the higher stressful environment compared to c1. Longer observation periods are needed to gain a better understanding of symbiotic behavior under the influence of these heavy metals. We assume that the longer the observed period, the better the trend can be observed, because reproduction of symbionts will intensity the effects.

## 5. Conclusions

We found various metabolic responses of HM additions to specimens of *H. depressa*. The selection of the type of metal was of particular importance in the context of observe different adaptation behaviors of this LBF. Additionally, the choice of concentrations and enrichment factors of the heavy metal contents compared to natural levels was crucial as well. At low concentrations of Cu and Zn, C and N uptake was promoted indicating the nutritious properties of these trace metals. As a reminder, these concentrations reflect natural levels of metals in pore water. By mixing events, these levels of metals can be released from the sediment, become available to the foraminifera and can potentially increase their metabolism. However, at higher concentrations (very polluted environment), both Cu and Zn induced toxic effects, as shown in a strong decrease in C and N uptake and reduction of the photosynthetic active area. In contrast to that, Fe proved to be generally advantageous for foraminiferal health independent of the concentration level.

## Data availability statement

Data included in article/supp. material/referenced in article.

## CRediT authorship contribution statement

**Mario Bubl:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Petra Heinz:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Wolfgang Wanek:** Writing – review & editing, Resources, Methodology. **Michael Schagerl:** Writing – review & editing, Software, Resources, Methodology. **Thilo Hofmann:** Writing – review & editing, Resources, Methodology. **Michael Lintner:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27229.

## References

[1] Benjamin S. Halpern, Shaun Walbridge, Kimberly A. Selkoe, Carrie V. Kappel, Fiorenza Micheli, Caterina d'Agrosa, John F. Bruno, et al., A global map of human impact on marine ecosystems, Science 319 (5865) (2008) 948–952.
[2] R. Bargagli, E. Rota, Microplastic interactions and possible combined biological effects in antarctic marine ecosystems, Animals 13 (1) (2022) 162.
[3] T.A. Palmer, A.G. Klein, S.T. Sweet, P.A. Montagna, L.J. Hyde, T.L. Wade, J.B. Pollack, Anthropogenic effects on the marine environment adjacent to Palmer Station, Antarctica, Antarct. Sci. 34 (1) (2022) 79–96.
[4] V. Singh, N. Singh, S.N. Rai, A. Kumar, A.K. Singh, M.P. Singh, V. Mishra, Heavy metal contamination in the aquatic ecosystem: toxicity and its remediation using eco-friendly approaches, Toxics 11 (2) (2023) 147.
[5] R. Mishra, E. Singh, A. Kumar, A.K. Singh, S. Madhav, S.K. Shukla, S. Kumar, Metal pollution in marine environment: sources and impact assessment, in: Metals in Water, Elsevier, 2023, pp. 175–193.
[6] G. Cheloni, V.I. Slaveykova, Combined effects of trace metals and light on photosynthetic microorganisms in aquatic environment, Environments 5 (7) (2018) 81.
[7] M. Denoyelle, E. Geslin, F.J. Jorissen, L. Cazes, F. Galgani, Innovative use of foraminifera in ecotoxicology: a marine chronic bioassay for testing potential toxicity of drilling muds, Ecol. Indicat. 12 (1) (2012) 17–25.
[8] J. Pawlowski, M. Holzmann, J. Tyszka, New supraordinal classification of Foraminifera; molecules meet morphology, Mar. Micropaleontol. 100 (2013) 1–10.
[9] J. Pawlowski, M. Holzmann, Diversity and geographic distribution of benthic foraminifera: a molecular perspective, Biodivers. Conserv. 17 (2) (2007) 317–328.
[10] A. Holbourn, A.S. Henderson, N. Macleod, Atlas of Benthic Foraminifera, edition, Wiley-Blackwell, New York, 2013, p. 1, aufl.
[11] C. Hillaire-Marcel, A. De Vernal, Proxies in Late Cenozoic Paleoceanography, in: Of Developments in Marine Geology, vol. 1, Elsevier Science & Technology, Oxford, 2007.
[12] P.P.B. Eichler, C.P. Barker, Benthic Foraminiferal Ecology: Indicators of Environmental Impacts, Springer Nature, 2020.
[13] J. Murray, E. Alve, Benthic foraminifera as indicators of environmental change: estuaries, shelf and upper slope, in: S. Haslett (Ed.), Environmental Quaternary Micropalaeontology, Hodder Arnold, 2002, pp. 59–90.
[14] F. Frontalini, R. Coccioni, Benthic foraminifera for heavy metal pollution monitoring: a case study from the central Adriatic Sea coast of Italy, Estuar. Coast Shelf Sci. 76 (2) (2008) 404–417.

[15] F. Frontalini, C. Buosi, S. Da Pelo, R. Coccioni, A. Cherchi, C. Bucci, Benthic foraminifera as bio-indicators of trace element pollution in the heavily contaminated Santa Gilla lagoon (Cagliari, Italy), Mar. Pollut. Bull. 58 (6) (2009) 858–877.
[16] D.M.K. BouDagher-Fadel, Evolution and Geological Significance of Larger Benthic Foraminifera, 2 edition, UCL Press, London, 2018.
[17] M.R. Langer, M.T. Silk, J.H. Lipps, Global ocean carbonate and carbon dioxide production; the role of reef Foraminifera, J. Foraminifer. Res. 27 (4) (1997) 271–277.
[18] J.J. Lee, Algal symbiosis in larger foraminifera, Symbiosis 42 (2006) 63–75.
[19] S. Uthicke, C. Altenrath, Water column nutrients control growth and C:N ratios of symbiont- bearing benthic foraminifera on the Great Barrier Reef, Australia, Limnol. Oceanogr. 55 (4) (2010) 1681–1696.
[20] C. Schmidt, P. Heinz, M. Kucera, S. Uthicke, Temperature-induced stress leads to bleaching in larger benthic foraminifera hosting endosymbiotic diatoms, Limnol. Oceanogr. 56 (5) (2011) 1587–1602.
[21] P. Hallock, Symbiont-bearing Foraminifera, Springer Nether- lands, Dordrecht, 2003, pp. 123–139.
[22] J.J. Lee, P. Hallock, Algal symbiosis as the driving force in the evolution of larger foraminiferaa, Ann. N. Y. Acad. Sci. 503 (1) (1987) 330–347.
[23] E. Merian, M. Anke, M. Ihnat, M. Stoeppler, et al., Elements and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance, Number second ed., Wiley-VCH Verlag GmbH & Co. KGaA, 2004.
[24] D. Roberts, M. Nachtegaal, D.L. Sparks, Speciation of Metals in Soils, John Wiley & Sons, Ltd, 2005, pp. 619–654 (chapter 13).
[25] D.L. Sparks, Toxic metals in the environment; the role of surfaces, Elements 1 (4) (2005) 193–196.
[26] L.E. Grecco, A.O. Marcos, E.A. Gomez, S. Botte, J. Marcovecchio, Natural and anthropogenic input of heavy metals in sediments from bahía blanca estuary (Argentina), J. Coast Res. (2006) 1021–1025.
[27] S. Boehnert, A. Birkelund, G. Schmiedl, H. Kuhnert, G. Kuhn, H. Hass, D. Hebbeln, Test deformation and chemistry of foraminifera as response to anthropogenic heavy metal input, Mar. Pollut. Bull. 155 (2020) 111112.
[28] L. Ferraro, M. Sprovieri, I. Alberico, F. Lirer, L. Prevedello, E. Marsella, Benthic foraminifera and heavy metals distribution: a case study from the Naples Harbour (Tyrrhenian Sea, Southern Italy), Environ. Pollut. 142 (2) (2006) 274–287, 1987.
[29] A. Samir, A. El-Din, Benthic foraminiferal assemblages and morphological abnormalities as pollution proxies in two Egyptian bays, Mar. Micropaleontol. 41 (3) (2001) 193–227.
[30] J. Trevors, C. Cotter, Copper toxicity and uptake in microorganisms, J. Ind. Microbiol. Biotechnol. 6 (2) (1990) 77–84.
[31] L.M. Gaetke, C.K. Chow, Copper toxicity, oxidative stress, and antioxidant nutrients, Toxicology 189 (1) (2003) 147–163.
[32] K.W. Bruland, E.L. Rue, G.J. Smith, Iron and macronutrients in California coastal upwelling regimes: implications for diatom blooms, Limnol. Oceanogr. 46 (7) (2001) 1661–1674.
[33] A.M. Schrand, M.F. Rahman, S.M. Hussain, J.J. Schlager, D.A. Smith, A.F. Syed, Metal- based nanoparticles and their toxicity assessment. Wileyo interdisciplinary reviews, Nanomedicine Nanobiotechnol. 2 (5) (2010) 544–568.
[34] M. Lintner, M. Schagerl, B. Lintner, M. Nagy, P. Heinz, Photosynthetic performance of symbiont-bearing foraminifera Heterostegina depressa affected by sunscreens, Sci. Rep. 12 (1) (2022), 2750- 2750.
[35] M. Lintner, B. Lintner, W. Wanek, N. Keul, F. von der Kammer, T. Hofmann, P. Heinz, Effects of heavy elements (Pb, Cu, Zn) on algal food uptake by Elphidium excavatum (foraminifera), Heliyon 7 (11) (2021) e08427.
[36] D. Gidlow, Lead toxicity, Occup. Med. 54 (2) (2004) 76–81.
[37] F. Frontalini, F. Semprucci, L. Di Bella, A. Caruso, C. Cosentino, A. Maccotta, G. Scopelliti, C. Sbrocca, C. Bucci, M. Balsamo, M.V. Martins, E. Armynot du Châtelet, R. Coccioni, The response of cultured meiofaunal and benthic foraminiferal communities to lead exposure: results from mesocosm experiments, Environ. Toxicol. Chem. 37 (9) (2018) 2439–2447.
[38] F. Frontalini, D. Curzi, F. Giordano, J. Bernhard, E. Falcieri, R. Coccioni, Effects of lead pollution on Ammonia parkinsoniana (foraminifera): ul- trastructural and microanalytical approaches, Eur. J. Histochem. 59 (1) (2015) 1–8.
[39] M. Lintner, B. Lintner, W. Wanek, N. Keul, P. Heinz, The e ffect of the salinity, light regime and food source on carbon and nitrogen uptake in a benthic foraminifer, Biogeosciences 18 (4) (2021) 1395–1406.
[40] Y. Wen, R. Li, X. Piao, G. Lin, P. He, Different copper sources and levels affect growth performance, copper content, carcass characteristics, intestinal microorganism and metabolism of finishing pigs, Animal Nutrition 8 (2022) 321–330.
[41] H. Nomaki, Y. Chikaraishi, M. Tsuchiya, T. Toyofuku, N. Ohkouchi, K. Uematsu, A. Tame, H. Kitazato, Nitrate uptake by foraminifera and use in con- junction with endobionts under anoxic conditions, Limnol. Oceanogr. 59 (6) (2014) 1879–1888.
[42] F. Hannah, R. Rogerson, J. Laybourn-Parry, Respiration rates and bio- volumes of common benthic foraminifera (Protozoa), J. Mar. Biol. Assoc. U. K. 74 (2) (1994) 301–312.
[43] J.M. Bernhard, E. Alve, Survival, ATP pool, and ultrastructural charac- terization of benthic foraminifera from Drammensfjord (Norway): response to anoxia, Mar. Micropaleontol. 28 (1) (1996) 5–17.
[44] B.J. Ross, P. Hallock, Dormancy in the foraminifera: a review, J. Foraminifer. Res. 46 (4) (2016) 358–368.
[45] C. LeKieffre, J.E. Spangenberg, G. Mabilleau, S. Escrig, A. Meibom, E. Geslin, Surviving anoxia in marine sediments: the metabolic response of ubiquitous benthic foraminifera (Ammonia tepida), PLoS One 12 (5) (2017) e0177604-e0177604.
[46] E. Bazzani, C. Lauritano, M. Saggiomo, Southern Ocean iron limitation of primary production between Past knowledge and future projections, J. Mar. Sci. Eng. 11 (2) (2023) 272.
[47] A.-J. Miao, X.-Y. Zhang, Z. Luo, C.-S. Chen, W.-C. Chin, P.H. Santschi, A. Quigg, Zinc oxide- engineered nanoparticles: dissolution and toxicity to marine phytoplankton, Environ. Toxicol. Chem. 29 (12) (2010) 2814–2822.