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Research article

Effects of heavy elements (Pb, Cu, Zn) on algal food uptake by *Elphidium excavatum* (Foraminifera)

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ABSTRACT

Foraminifera are unicellular organisms and play a pivotal role in the marine material cycles. Past observations have shown that the species *Elphidium excavatum* is the most common foraminifera in the Baltic Sea. Feeding experiments showed that the food uptake and thus the turnover of organic matter are influenced by changes of physical parameters (e.g., temperature, salinity). Since many areas of the Baltic Sea are strongly affected by anthropogenic activity and are strongly contaminated by heavy elements from shipping in the past, this study examined the effect of heavy elements pollution on the food uptake of the most common foraminiferal species of the Baltic Sea, *E. excavatum* which was a subject of several previous studies. Therefore, Baltic Sea seawater was enriched with metals at various levels above normal seawater levels and the uptake of ^{13}C- and ^{15}N-labelled phytodetritus was measured by isotope ratio mass spectrometry. For each combination of metal type, concentration and time point 20 individuals of *E. excavatum* (three replicates) were fed with the green algae *Dunaliella tertiolecta*. The effect of dose parameters was measured in a two-way analysis of variance. Significant differences of food uptake were observable at different types and levels of heavy elements in sea water. Even a 557-fold increase in the Pb concentration did not affect food uptake, whereas strong negative effects were found for higher levels of Zn (144 and 1044-fold) and especially for Cu (5.6 and 24.3-fold). In summary it can be stated, that an increase in the heavy elements pollution in the Kiel Fjord will lead to a significant reduction in the turnover of organic matter by foraminifera such as *E. excavatum*.

1. Introduction

Foraminifera are marine unicellular organisms, playing a key role in marine organic matter cycling. Foraminifera species of the genus *Elphidium* have a calcitic test, occur worldwide (Freyling-Hanssen, 1972) and in large numbers in shallow marine habitats such as the German Wadden Sea or the Baltic Sea (e.g. Moodley, 1990). Abnormalities in test formation were discovered in some foraminifera (e.g. Polovodova and Schönfeld, 2008). The formation of such abnormalities was not only observed in the natural environment, but also in laboratory experiments (Wennrich et al., 2007). Frequent causes of this phenomenon are rapid changes in salinity (Stouff et al., 1999), seawater acidification (Le Cadre et al., 2003) or heavy elements pollution (Polovodova and Schönfeld, 2008; Le Cadre and Debenay, 2006). Deformed tests often exhibit an increased content of Pb, Zn, Cu, Cr or Cd (Sharifi et al., 1991; Samir and El Din, 2001). In other words, the chemical composition of the foraminiferal test reflects the metal concentration of the sediment, where the foraminifera live (Boehnert et al., 2020).

High population densities of foraminifera with deformed tests were found in areas characterized by eutrophication (Lidz, 1965; Sharifi et al., 1991; Samir and El Din, 2001). There are several types of test deformation with different origins (Boltovskoy et al., 1991). Since fossilized foraminifera tests are preserved, knowledge of deformations and the associated environmental causes helps to draw conclusions about past environmental changes (Rhumbler, 1911; Boltovskoy and Wright, 1976). Types of test deformations and their possible causes are reviewed by Boltovskoy et al. (1991), Boltovskoy and Wright (1976) and Haynes (1981).

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In two fjords of the Bay of Kiel, 18 different types of abnormalities were found during past investigations (Polovodova and Schönfeld 2008). Abnormalities were more common in *Ammonia beccarii* than in *Elphidium excavatum*. However, *E. excavatum* is a very abundant foraminifera in the Kiel Fjord and can even make up over 90% of all living foraminifera there (Schönfeld and Numberger, 2007). The Kiel Fjord is an area heavily affected by shipyards, military and heavy civil infrastructure (Nikulina et al., 2007). In the neighborhood of Flensburg Fjord human activities have significantly increased the nutrient input as well as the heavy element content in sediments in the past 30 years (Nikulina and Dullo, 2009). Compared to other areas in the Baltic Sea, the concentrations of Zn, Cu or Pb of the Flensburg Fjord, which is closely related to the Kiel Fjord, are considered moderate with about 45 µg Cu, 137 µg Zn and 40 µg Pb per gram sediment (Nikulina et al., 2008). Nevertheless, the abundance of deformed tests increased significantly in recent decades, which was attributed to environmental stress (Nikulina et al., 2008). Despite the important role of foraminifera in marine organic matter cycling we are not aware of any study of the effect of heavy element pollution on food uptake and metabolism of foraminifera thus far. Feeding experiments with foraminifera have shown that the food uptake of shallow marine species such as *Ammonia tepida* or *Haynesina germanica* is significantly dependent on salinity and water temperature (Lintner et al., 2020a; Wukovits et al., 2017), and on light conditions in *E. excavatum* (Lintner et al., 2020b).

We studied the effect of heavy elements on the food uptake of *E. excavatum*. We assessed the effect of elevated levels of Cu, Zn and Pb as these metals are among the most common pollutants in eutrophic shelf regions and are strongly suspected of causing changes in the foraminiferal community (Ferraro et al., 2006).

2. Materials and methods

2.1. Sampling

Sediment and water samples were collected from the Kiel Fjord with the F. S. ALKOR on October 22nd and 23rd, 2019. Surface sediment and porewater samples were obtained using a grap corer and a rumohr lot in Laboe (N54° 25.261′ and E10° 12.331′), where the Kiel Fjord opens into the Baltic Sea. The water depth was 16.5 m, the water temperature 13.2 °C and the salinity 20.3 PSU (practical salinity unit).

On board, the top 5 cm sediment of 6 grap corer (20 × 20 cm) were wet-sieved through a 125 µm sieve with sea water and kept in a cool box with natural seawater collected at the sampling site. A permanent culture of foraminifera was setup in the laboratory in Vienna, at a temperature of 20 °C and a salinity of 20 PSU.

2.2. ^{13}C and ^{15}N enriched algal food

The green algae *Dunaliella tertiolecta* served as the food source for these experiments because it is a standard food source for foraminifera culturing experiments. This alga was cultivated in the f/2 nutrient medium (Guillard and Ryther, 1962), which was enriched with the stable isotopes ^{13}C and ^{15}N. For this purpose, $NaH^{13}CO_3$ and $Na^{15}NO_3$ were added to obtain a final concentration of 1.5 mmol/L ^{13}C and 0.44 mmol/L ^{15}N in the medium. The algal culture was incubated at 20 °C and a light/dark rhythm of 16:8 h. The culture was harvested when the culture medium became intensely green. The algae were centrifuged at 800 *g* for 10 min and then washed three times with artificial seawater (Enge et al., 2011). After each washing step, the algae were centrifuged again and the supernatant was removed. Finally, the algal pellet was shock frozen in liquid nitrogen and lyophilized for 3 days at 0.180 mbar. In order to maintain the quality of the food, the dried algae were wrapped in aluminum foil and kept in the dark. The resulting algal powder had a ^{13}C content of 4.14 at% and ^{15}N content of 39.0 at%, and its C: N ratio was 5.78.

3. Experimental setup

3.1. Analysis and preparation of seawater enriched with heavy elements

The concentration of Cu, Zn and Pb in natural seawater and porewater (3 replicates) at the sampling site was analyzed using inductively coupled plasma mass spectrometry (ICP-MS, – Agilent IPC-MS 7900 quadrupole mass spectrometry system, quality control reference material: US Canadian certified reference material: Lake Sediment LKSD-1). For the ICP-MS measurements of the water samples, immediately after sampling 1 µl HCl (36%) were added per ml natural seawater or porewater. Water-soluble salts ($CuSO_4$*5 H_20, $ZnSO_4$*7 H_2O, $PbCl_2$) were used to enrich these metals in natural seawater used for culturing experiments. Sea water was amended with heavy elements at different levels and heavy elements measured using ICP-MS before starting the experiment (a list of the measured concentrations of Cu, Zn and Pb in amended seawater is given in Table 1). A wide range of Zn concentration was investigated by increasing the Zn concentrations up to 1000-fold from natural seawater Cu should be lower enriched (5 and 25-fold) because this element is probably known as toxic metal for many organisms. A higher Cu enrichment (100-fold) was planned, but not validated in the following manuscript due to Cu precipitation. Following the low Pb concentration in the sea water a wide range from 2.5 up to 500-fold enrichment was used to test the influence of the foraminiferal behavior. The exact enrichments are given in Table 1, which differ slightly from the calculated ones, because the used salts were hydrophile, which made them more difficult to handle.

Seawater without additional salts (Zn 0, Cu 0 and Pb 0) was used as a control to obtain the food uptake of foraminifera under natural environmental conditions. For the other experiments only one heavy element was enriched, the other two have still the same concentration like the natural seawater (Table 1). So, a clear response of the foraminifera attributed to one added metal dose can be observed. To avoid a salinity impact of the treatment, the salinity of the culture medium was measured after adding heavy element salts and was corrected, if necessary, with distilled water.

3.2. Culturing of foraminifera

All experiments were set up in triplicates. First, the filtered (pore size 0.45 µm) natural seawater was enriched with the metals to the desired concentrations and added to crystallization dishes. Afterwards, 20 individuals of *E. excavatum* (Figure 1, categorized S5 after Darling et al., 2016) were placed in each dish and the dishes sealed with parafilm. For the experiments only individuals were selected, which have no test abnormalities and were fully filled with brownish cytoplasm, which highlighted a healthy state of the foraminifera. The cultures were incubated for 24 h in the incubator at 20 °C and a light/dark rhythm of 16:8 h. After these 24 h, the food source (lyophilized *D. tertiolecta* powder) was added and the foraminifera were fed for several days without removing the algae (1, 5, 10 and 15).

At the end of the experimental period, the foraminifera were picked from the cultures and any food residues attached to their tests were removed with a brush. Finally, the foraminifera were washed three times with distilled water. This process was carried out with particular care, as the arising osmotic shock can cause the tests to burst with loss of parts of the cytoplasm.

3.3. Isotope ratio measurements

For element and isotope measurements, foraminifera were transferred into tin capsules (Sn 99.9, IVA Analysentechnik GmbH & Co. KG) and air dried for 3 days. The natural abundance of the isotopes (blanks) was measured from foraminifera (triplicates which each 20 individuals) which were taken from the main culture and are not further cultivated in any other solutions. Foraminiferal tests were then decalcified by adding 5

Table 1. Heavy element concentrations of natural seawater (= control = Zn/Cu/Pb 0, n = 3) and the sea water (Zn 1–3, Cu 1–3, Pb 1–3, standard deviations in parenthesis, n = 3), measured using ICP-MS.

Sample ID	Zn enrichment [x-fold vs. SW]	Zn measured [μg/l]	Cu enrichment [x-fold vs. SW]	Cu measured [μg/l]	Pb enrichment [x-fold vs. BW]	Pb measured [μg/l]
natural seawater (control = Zn 0, Cu 0, Pb 0)	-	69.5 (5.43)	-	40.8 (8.22)	-	<0.02 (-)
Zn 1	9.2	640 (102)	-	-	-	-
Zn 2	144	10000 (554)	-	-	-	-
Zn 3	1044	72530 (7963)	-	-	-	-
Cu 1	-	-	5.6	230 (7.54)	-	-
Cu 2	-	-	24.3	991 (257)	-	-
Pb 1	-	-	-	-	2.4	4.0 (1.5)
Pb 2	-	-	-	-	48.5	81 (10)
Pb 3	-	-	-	-	557	931 (139)

μl 4% hydrochloric acid twice. The dissolution took place in a drying cabinet at 60 °C. As a final step, the samples were left in the drying cabinet at 60 °C for another 24 h to remove any residual moisture. The samples were weighed to the nearest μg and kept in the desiccator until isotope ratio measurement. These measurements took place in the Stable Isotope Laboratory for Environmental Research (SILVER) at the University of Vienna. The ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were determined using an isotope ratio mass spectrometer (IRMS, Delta^{PLUS}, coupled by a ConFlo III interface to an elemental analyzer, EA 1110, Thermo Finnigan). Vienna PeeDee Belemnite ($R_{VPDB} = 0.0112372$) and atmospheric dinitrogen ($R_{atmN} = 0.0036765$) were used as the isotope standards for C and N. A detailed description of the methodology for calculating the amount of C (pC) and N (pN) ingested from the *D. tertiolecta* food source is available in Lintner et al. (2020a). For statistical evaluation of the effects of heavy element level and time multifactorial ANOVA and multiple range test was carried out using StatGraphics Centurion 18 (significant cut of: α = 0.05).

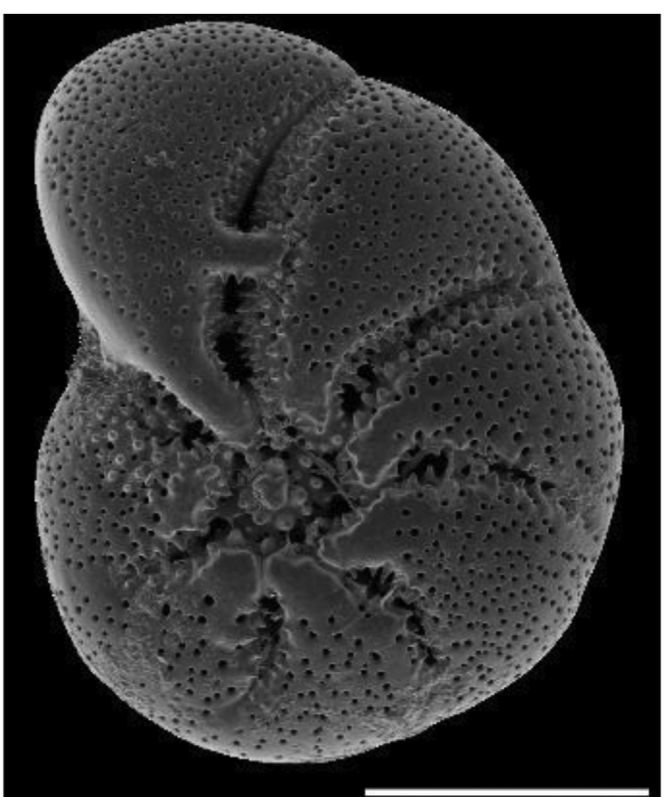

Figure 1. SEM – picture of the incubated foraminifera *E. excavatum* S5 (Darling et al., 2016). Bar scale = 100 μm.

4. Results

4.1. Analysis of water samples

The enrichment values described in Table 1 refer to the measured concentrations in the untreated natural seawater, relative to surface water for Zn and Cu, and to porewater for Pb at 0.5 cm sediment depth. The concentrations of Zn were very similar in surface water (69.5 μg/l) and porewater (65.8 μg/l in 0.5 cm depth). The Cu concentration in surface seawater (40.8 μg/l) was more than twice as high as in the porewater (<20 μg/l in 0.5 cm depth). However, the Pb concentration in surface water (<0.02 μg/l) was below detection limit and only measurable in porewater (1.67 μg/l in 0.5 cm depth). Detailed measurements of the porewater are given in the appendix.

4.2. C and N uptake at different heavy element concentrations

The effect of different levels of heavy elements on the absorbed amount of C (pC) and N (pN) is shown in Figure 2 compared to controls that did not receive any heavy element enrichment. For all data a single set of three replicates was used for each time period, which were also used to perform ANOVA. A detailed explanation of the statistical test results can be found in the appendix. Generally (over all doses, metal types and sampling intervals), food C uptake (pC) decreased ($P = 0.0002$ $df = 3$) while food N uptake (pN) increased over time ($P < 0.0001$ $df = 3$). Pb triggered a slight stimulation of pC (P metal type <0.0001 $df = 3$, P time $= 0.0002$ $df = 3$, P interaction $= 0.0503$ $df = 9$). pN was also affected by metal type ($P = 0.0456$ $df = 3$, Pb/Zn ≥ control ≥ Cu) and time ($P < 0.0001$ $df = 3$), but the interaction was non-significant ($P = 0.5515$ $df = 9$).

Concentration effects of specific heavy elements were analyzed separately for each heavy element (Cu: level 1–2, Pb/Zn: level 1–3), and compared to controls (level 0). Increasing concentrations of Cu significantly suppressed pC, where Cu at low concentration stimulated pN and high levels decreased pN relative to controls. The effect of Cu on C and N uptake is highly significant with time ($P < 0.0001$ $df = 3$) and concentration ($P < 0.0001$ $df = 2$) Concentration effects of Pb were non-significant for pC ($P = 0.6012$ $df = 3$) or pN ($P = 0.3579$ $df = 3$). Concentration effects of Zn were significant on pC, with low concentrations stimulating pC and higher concentrations inhibiting pC ($P < 0.0001$ $df = 3$). pN was stimulated by low and intermediate concentrations and suppressed by the highest concentration of Zn ($P = 0.0002$ $df = 3$).

5. Discussion

5.1. Food uptake at different heavy element enrichments

The results (Figure 2) indicate that not all metals influenced food uptake in *Elphidium excavatum* equally. Also, the concentration of the

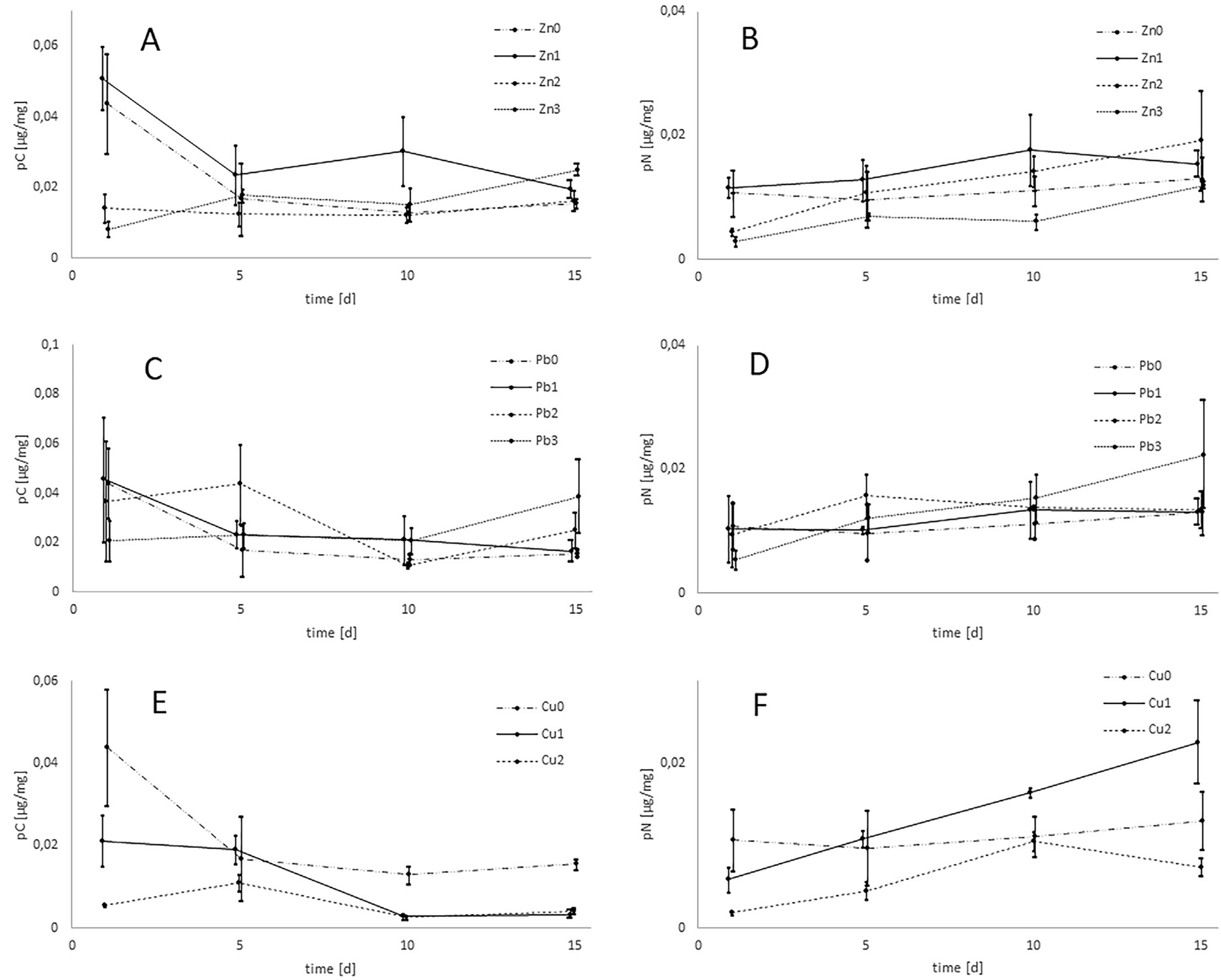

Figure 2. Foraminiferal carbon (pC) and nitrogen (pN) uptake over time. Graphs A and B show food uptake in the Zn experiment (pC and pN), C and D represent Pb and E and F the Cu experiment (for concentrations of the metals see Table 1). The exact concentrations of the metals are listed in Table 1. The error bars show the standard deviations with n = 3 replicates, the data points are the mean of three replicates to each 20 individuals.

heavy elements plays a major role. In the experiments with Zn, noticeable decreases in C and N uptake only occurred at the 144- and 1044-fold higher concentration than is found in natural seawater, while the lowest Zn amendment (9.2-fold) actually stimulated food uptake. In contrast, not even the 557-fold higher Pb concentration than in natural seawater had a significant negative impact on food uptake of *E. excavatum*. This highlights that Pb has no impact on the nutrition of *E. excavatum*. The results of the Cu experiment were markedly different. A significant decrease in food uptake was recorded at the 5.6- and 24.3-fold increase in Cu concentration relative to surface seawater. Other studies have shown an effect of heavy elements on pseudopodial activity, an important factor of foraminiferal foraging. For instance, the response of pseudopodial activity was sensitive to heavy elements such as Cd at 1000 μg/L in *Ammonia parkinsoniana* and at 2500 μg/L in *Ammonia tepida* (Denoyelle et al., 2012; Ros et al., 2020). Growth in terms of chamber formation or maximum growth was inhibited by Cd (Denoyelle et al., 2012; Linshy et al., 2013), with very different sensitivities between species. Chamber production was inhibited by Cd already at 2–14 μg/L in *Pararotalia nipponica,* but only at >10000 μg/L in *Ammonia tepida* (Denoyelle et al., 2012; Linshy et al., 2013). Three foraminifera species (*Amphistegia lobifera, A. lessonii,* and *Sorites orbicularis*) also showed very different sensitivities (pseudopodial activity) towards Cd, Zn, Cu and Pb (Ben-Eliahu et al., 2020). The pseodupodial activity is directly linked to the food uptake by foraminifera. Based on our experiments we can assume, that a reduced food uptake correlates directly with a reduction of pseudopodial activity. Therefore, Cu and Zn led to a reduction of the pseudopodial activity of the foraminifera *E. excavatum*, whereas Pb does not negatively influence the activity of this foraminifera.

Past studies have shown that deformed tests of foraminifera had increased concentrations of metals such as Pb, Zn, Cu, Cr or Cd, compared to non-deformed tests (Samir and El-Din, 2001; deNooijer et al., 2007). Certain areas of the Kiel Fjord are particularly enriched with the heavy elements Pb and Zn (Polovodova and Schönfeld, 2008), probably due to the strong eutrophication and industrialization in these regions. According to our results, an increase in heavy element concentration for a short period (tested time period in our study 15 days) do not leads to test deformations as previously described (Polovodova and Schönfeld, 2008), but generally to a significant reduction in metabolism and food uptake, particularly in response to Cu and Zn (Figure 2). At this point it should be mentioned, that we tested the metabolic adaptions of the foraminifera for each metal separately, so no metal-metal interactions are measured by now.

Interesting insights can be gained from the results of the Cu experiment. The negative effect of Cu amendment on the amount of absorbed C (pC) was highly significant at the start of the experiment (Figure 2E). From day 10 onwards, pC decreased at all concentrations to approximately 0.005 μg/mg but remained high in controls (0.02 μg/mg). This extremely low value actually indicates no further food uptake. Considering the amount of N (pN) uptake in the Cu experiment, it can be seen that the difference in pN increased with duration of experiment and at the intermediate Cu level even increased and exceeded pN in controls. Other series with Zn or Pb amendments showed less difference in the behavior of foraminiferal C uptake and N uptake. These two elements (C and N) are used in different metabolic pathways of a cell. Some aspects are discussed in Lintner et al. (2020b), but for detailed discussion further studies are necessary. By now we can assume, that the presence of Cu influences the metabolic C processes more negatively than N processes.

Past feeding experiments with *Elphidium excavatum* showed that significant differences in food uptake due to changes in physical parameters (salinity) could only be noticed in longer lasting experiments (>7 days) (Lintner et al., 2020b). In comparison to the experiments in this study, greatest heavy element effects were evident in the short-term, i.e., up to 5 days (see pC in Zn or Cu treatments in Figure 2). In the Kiel Fjord, seasonal fluctuations in natural salinity are common (Christiansen et al., 1996). Therefore, *E. excavatum* may be inherently better adapted to fluctuations in salinity whereas apparently an increase in toxic elements quickly leads to a decrease in metabolic and feeding activity. In summary, *E. excavatum* copes better with short-term fluctuations in salinity than with increasing heavy element concentrations.

5.2. Toxicity of metals for foraminifera

Not all heavy elements were toxic for *E. excavatum*, and even not at all concentrations. We could show that there was sometimes a higher uptake of food by slightly increased heavy element concentrations compared to the untreated samples. Considering the metals in detail, it is known that lead has been used in industrial productions for at least 5000 years, which goes hand in hand with an increase of Pb in populated areas (Gidlow, 2004). For *E. excavatum*, the increase in Pb concentration did not lead to metabolic restrictions. Differential sensitivity of foraminifera species to Pb at ~1000 μg/l, close to the highest level in our study, was also reported earlier, with growth of two *Amphistegina* species being non-responsive while growth of *Sorites orbicularis* decreased by 32% (Ben-Eliahu et al., 2020). Frontalini et al. (2015) showed that foraminifera, particularly *Ammonia parkinsiana*, produce more lipid droplets in seawater enriched with Pb than those living in enriched seawater. These modifications indicate pollutant-induced stress in *A. perkinsiana* (Frontalini et al., 2015). The authors suspected that some types of foraminifera thus have a potential cytological mechanism to protect themselves against increasing heavy element concentrations. These results would correspond to the data from our study, according to which *Elphidium* may also adapt to increased Pb concentrations by cytological modifications. For a precise assessment of this hypothesis, transmission-electronic examinations of the cytoplasm of *E. excavatum* would need to be carried out. Finally, it should be mentioned that our experiments only took place over a relatively short period of 15 days. In a study by Frontalini et al. (2018) an increase in Pb in seawater led to a decrease in the richness and diversity of foraminiferal communities, but only after 8 weeks. Such long-term studies are difficult to carry out with our experimental setup, because we did not incubate with natural sediment and therefore cannot offer the naturally occurring diversity of food sources.

Considering Zn, strong accumulations of Zn can be detected not only in the Kiel Fjord but also on the Polish Baltic Sea coast near Gdansk (Szefer et al., 1995). It is reasonable to assume that many of these pollutants are discharged from the terrestrial realm via neighboring rivers (Pawlik, 1980). However, it must be taken into account that Zn is an essential metal for all living organisms and a toxic effect only arises at excessive concentrations (Eisler, 1993). This relation could also be established in our experiments. The 144- and 1044-fold increase in Zn concentration clearly lowered food uptake (Figure 2A, B), while a 9.2-fold increase stimulated food uptake. This relationship could also explain the deformations in the foraminifer tests with increased heavy element concentrations. Deformations were not detected in our experiments (microscopic analysis of each foraminifer after the incubation time was performed), but the experimental times may have been too short for *Elphidia* to form new (deformed) tests.

The metal that induced the most significant negative effects on food uptake by *E. excavatum* was Cu. Copper is also an essential metal for organisms and plays an important role in a variety of biological processes (Gaetke and Chow, 2003). Past studies have shown that an increase of Cu concentrations by a factor of 100 led to a change in the living foraminiferal community structure (Alve and Olsgard, 1999). Regarding our results, even an increase by a factor of 5- to 24-fold (230–990 μg/l) decreased the feeding activity of the foraminifera. Cu as low as 400 μg/l completely suppressed growth and reproduction of two *Ammonia* species (Le Cadre and Debenay 2006). In contrast, one long-term study (32 weeks) showed no significant influence on the formation of deformed tests with increasing Cu concentration (Alve and Olsgard, 1999). However, the proportion of Cu in newly formed tests increased the higher the Cu concentration in the sea water was (Munsel et al., 2010). This means, so far, that the metabolic mechanism of Cu toxicity in foraminifera is not well understood. However, it has been shown that increasing Cu concentrations inhibit the respiration of microorganisms (e.g., Trevors and Cotter, 1990). The toxic form of Cu is usually Cu^{2+} (Summers and Silver, 1978), which was also used in this study. As mentioned above, Cu also seems to have a negative influence on the respiration of *E. excavatum*, which might explain the difference between the trend in C uptake compared to N uptake (Figure 2 E, F). At high concentrations, Cu^{2+} ions also have a negative direct effect on proteins, enzymes and nucleic acids (Flemming, 1987). Since N uptake increased linearly with time in *E. excavatum*, it can be assumed that Cu had less impact on the N metabolism than on the C metabolism.

5.3. Ecological aspects

A high level of eutrophication in the region favors the input of heavy elements into the Baltic Sea. Many studies have investigated the eutrophication of the Baltic Sea and how these heavy elements can be removed (e.g., Rönnberg and Bonsdorff, 2004). Moreover, a large number of chemical compounds has been synthesized to sequester heavy elements such as mercury and remove them from the water column (Crockett et al., 2015). The harmfulness of metals to organisms is also related to the bioavailability of the metals (Bryan and Langston, 1992). Many metals are bound in the sediment and are only released to the environment under certain redox conditions, pH values or desorption processes (Oakley et al., 1980). *Elphidia* make up the majority of living foraminifera in the Baltic Sea (Schönfeld and Numberger, 2007). Our experiments have shown that an increase of dissolved heavy elements in sea water reduces the metabolic activity of *Elphidia*. It should be mentioned that the average population size of foraminifera increased 6-fold between 1970 and 2007 in the Baltic Sea (Schönfeld and Numberger, 2007). The populations were influenced by the increase in nutrient inputs due to increased agricultural activity (Nehring, 1991), the changing oxygen content in the bottom water (Nausch et al., 2003), and the decline in the use of some heavy elements such as Pb in the shipping industry. Foraminifera contribute significantly to the turnover of organic matter in the oceans and are abundant in areas with increased organic C inputs (e.g., Moodley, 1990). According to our data, a further increase of the heavy element concentrations would result in foraminifera metabolizing less organic matter. However, foraminifera make a significant contribution to the transfer of energy in the form of organic matter to higher trophic levels (e.g., Beringer et al., 1991). Heavy element pollution could disturb this energy flow and probably have a major impact on the ocean ecosystem. The past 70 years have shown that the Kiel Fjord is increasingly characterized by anthropogenic influences such as shipyards, military or infrastructure (Nikulina et al., 2007). Not only foraminifera are affected by heavy elements, an increased concentration of Cu or Zn has also been detected in fish and mussels (e.g., Senocak, 1995). Since most of the pollutants come from urban areas (Gerlach, 1984), further damage to the ecosystem can be prevented by the reduction of industrial influence. Finally, we will highlight two aspects based on our observation on heavy element pollution and foraminiferal activity. First, the generally toxicity of heavy elements: The environmental heavy element change will definitively affect the food uptake and therefore also the activity of foraminifera. The results of this study showed, that rapid increased heavy elements concentrations decreased the metabolism of foraminifera within 5 days (see Figure 2). The second aspect belongs to the specific toxicity of heavy elements by foraminifera: This study showed, that some elements like Cu are even more toxic for foraminifera than others (e.g., Pb). So, following the results here, an environmental contamination with heavy elements like Pb (up to 557-fold) does not affect the activity of the tested foraminifera, hence an enrichment of 5.6-fold Cu dramatically decreased the metabolisms of these here investigated foraminifera.

Declarations

Author contribution statement

Michael Lintner: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bianca Lintner: Performed the experiments; Analyzed and interpreted the data.

Wolfgang Wanek, Nina Keul & Petra Heinz: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Frank von der Kammer & Thilo Hofmann: Contributed reagents, materials, analysis tools or data.

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Data availability statement

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

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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