

# FORAMINIFERA AS BIOINDICATORS OF HEAVY METALS POLLUTION IN THE HINSHOLMEN HARBOUR

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**Abstract**

This study presents for the first time recent foraminiferal assemblage data from the Hinsholmen harbour, Gothenburg, Sweden. Previous studies have shown that the concentration of heavy metals in Hinsholmen harbour is very high but based on results of this study likely have none-significant impact on foraminifera richness and abundance. The foraminiferal assemblages are mainly dominated by opportunistic species *Ammonia* spp. subordinated by *E. williamsoni*, *E. clavatum* and *E. oceanense*, *Quinqueloculina* spp. and *Trochammina* sp. Only *Trochammina* sp. appears to have a positive correlation with heavy metals. Based on the shell morphology, *Trochammina* sp. looks identical to an invasive species *T. hadai*, originating from Japan. Hence, due to its potential invasive nature, it is possible that the *Trochammina* sp. distribution is driven by the most frequented areas rather than the pollution itself. Future studies should consider additional environmental parameters and include a large number of stations to draw significant conclusions.

**Keywords** – Benthic foraminifera, leisure boat, invasive species, bio-monitoring

**Résumé**

Cette étude présente des données récentes sur les assemblages de foraminifères du port de Hinsholmen, Gothenburg, Suède. Des études précédentes ont montré que la concentration en métaux lourds dans ce port est très élevée, mais d'après cette étude, cela n'a probablement pas d'impact significatif sur la richesse et l'abondance des foraminifères. Les assemblages de foraminifères sont principalement dominés par des espèces opportunistes *Ammonia* spp. subordonnées par *E. williamsoni*, *E. clavatum*, *E. Oceanense*, *Quinqueloculina* spp. et *Trochammina* sp.. *Trochammina* sp. semble présenter une corrélation positive avec les métaux lourds et la morphologie de sa coquille semble identique à l'espèce envahissante *T. hadai*, originaire du Japon. En raison de sa nature potentiellement invasive, il est possible que la fréquentation maritime influe davantage la distribution de *Trochammina* sp. que la pollution elle-même. A l'avenir prendre en compte des paramètres environnementaux supplémentaires et multiplier le nombre de stations serait judicieux afin de parvenir à des conclusions significatives.

**Mots-clés** – Foraminifères benthiques, bateau de plaisance, espèces invasives, bio-monitoring

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## PREAMBLE

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My internship took place at the University of Gothenburg in Sweden, in the Department of Marine Sciences, focuses within oceanography, geology, chemistry, biology and conservation. One of their research subjects is Marine Geology where sediment and microfossil analysis are conducted to trace and interpret changes in marine environments during geological and historical times.

During this internship, I was under the supervision of Irina Polovodova Asteman, a specialist in benthic foraminifera and geochemical proxies in coastal waters, fjords, and estuaries. She works on topics such as marine pollution and the introduction of invasive species, using sediments as a natural archive of changes and a benchmark for environmental baseline assessment.

In 2019, a mapping of heavy metals concentration due to human activities was carried out in Hinsholmen harbour, located near the University of Gothenburg, by Agata Olejnik as part of her master's internship also conducted in the Department of Marine Sciences, under the supervision of Lennart Bornmalm. My internship is a continuation of this project with the objective to understand the influence of the concentration of these heavy metals on the biodiversity of Hinsholmen harbour. It was during this time that the samples containing the foraminifera studied in this report were collected.

Finally, during the identification of foraminiferal species present in the various samples, we realized that based on the shell morphology, *Trochammina* sp. looks identical to an invasive species, *T. hadai*, originating from Japan. Living specimens were collected, and cellular DNA analysis will soon be conducted to confirm the presence or absence of *Trochammina hadai* in Hinsholmen harbour and if it is *T. hadai*, it would be the first time this species has been found in Northern Europe.

# 1 INTRODUCTION

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Coastal ecosystems provide essential goods and services to society such as the provision of food, trade and recreational opportunities (Costanza et al., 2014) therefore, increasingly threatened by anthropogenic impacts, especially in harbours and marinas. Leisure boats can contribute to water pollution through the release of chemicals from cleaning products, antifouling coatings, fuels, and oils. These substances can have detrimental effects on marine ecosystems and the organisms that inhabit them by increasing environmental pressure. For example, heavy metals, are a particularly toxic class of inorganic pollutants which have been released to the environment after intense anthropogenic activities (Manzetti, 2020). Metals can leak for the most part from boats but also from wooden constructions as docks and piers that come in contact with water. Today, copper is the most frequently used active substance in anti-fouling paints (Bolam, 2007) since the application of tributyltin (TBT) on vessels was banned in 2003 as a response to the severe negative effects that TBT has on marine organisms (Gibbs & Bryan, 1986).

Foraminiferal response to heavy metal pollution has been often studied (Ellison, Broome, & Ogilvie, 1986) (Frontalini, et al., 2009) (Dijkstra, et al., 2017) because they are excellent bioindicators. Foraminifera have a short life cycle and therefore can respond rapidly to changes in environment. Foraminifera moved really slowly, which prevents them from avoiding disturbances (Fouet, 2022).

The seas surrounding Sweden are under pressure in the form of toxic emissions, shipwrecks leaking oil, increasing boat traffic and substances that cause eutrophication (Swedish Agency Marine and Water Management, 2021). It is why in this context this investigation focuses on the distribution of benthic foraminifera in surface sediments from the heavy metal contaminated Hinsholmen harbour located in Gothenburg city, on the Swedish west coast. The purpose of this study is to detect an influence of heavy metal concentration on species richness and abundance of benthic foraminiferal assemblages in Hinsholmen harbour.

# 2. METHODS

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## 2.1 Study area

The Hinsholmen Harbour located in Västra Frölunda, on the Swedish west coast, was built in the 1970's and today is one of the larger marinas in Gothenburg area (Göteborg Stad, 2023). It is located in a shallow bay, surrounded mostly by private villas and a popular nature trail. The water circulation in the bay is therefore limited and is mostly driven by the sea currents outside and by the prevailing winds (Olejnik, 2020). The marina consists of 28 piers, mostly wooden but some are made of concrete. There are two cleaning plants, equipped with a drainage-and filtering system. According to the Gothenburg City Hall website, the harbour has the capacity for 1500 boats during the high season and 850 during winter season. On one hand this popular marina provides mooring and docking services for recreational boats and yachts, highly appreciated by users, but on the other hand it is an area with high anthropogenic pollution.

## 2.2 Material and method

### ***Sediment sampling and foraminiferal analysis***

The samples used in the present study were collected in 2019 with a purpose to map heavy metal distribution in the harbour (Olejnik, 2020). In total 36 surface sediment sample (0-2 cm) were collected. At each station samples were taken manually from the piers by using Kayak corer from 36 stations along the 28 piers of Hinsholmen harbour (**Fig. 1**). Since the time dedicated to this study was limited, only 23 out of the 36 samples were processed. The samples were selected based on their locations in order to obtain an even distribution in the harbour. However, the original station numbers (1 to 36) have been retained to facilitate the comparison process with data from Olejnik (2020).

Since the original study (Olejnik, 2020) focused on metals, no staining with Rose Bengal was done to distinguish living foraminifera. Hence, this study presents total foraminiferal fauna combining living and dead individuals. All samples were weighed and washed with tap water through a 1000 µm sieve to remove large



debris such as shells, pebbles and macro algae and a 63  $\mu\text{m}$  sieve to remove clay, silt. The number of species will decrease with increasing sieve size and may give misleading information (Schroeder & Scott David, 1987). The sediment residue  $>63\ \mu\text{m}$  was then dried at  $50^\circ\text{C}$ . The samples were split based on the desired final quantity for hand picking, as observing a too large fraction of sediment under a microscope can reduce the visibility of foraminifera. At least three hundred specimens were picked from each sample and identified until species level if possible. The result of individual identification provides a table available in the appendix (**Appendix. A**), which includes the standardized relative abundance in individuals/ $\text{g}^{-1}$  sediment for each species at each sampling station.

The final faunal data set from this study contains 13 benthic foraminiferal species/genera. Analysis and interpretation were only performed on a group of 11 benthic species and/or genera due to the exclusion of species occurring with frequencies below 2%. The selected species and/or genera are *Ammonia* spp., *Quinqueloculina* spp., *Elphidium oceanense*, *Elphidium selseyensis*, *Elphidium williamsoni*, *Elphidium clavatum*, *Elphidium albiumbilicatum*, *Trochammina* sp., *Haynesina germanica* and *Miliammina fusca*. After observations under the optical microscope, a distinction was made between two *Quinqueloculina* sp. species into specimens with a smooth test and specimens with stripes or costae (Bouchet, 2007).

### **Hierarchical cluster analysis & diversity indices**

A dendrogram grouping the stations based on the distribution of foraminifera assemblages was performed using PAST software (version 4.03: (Hammer, et al., 2001)) calculated by Q-mode hierarchical cluster analysis using the Bray-Curtis dissimilarity (1957) (**Fig. 2**). Diversity indices (**Appendix. D**) like Shannon-weaver (1963), Simpson (1949) and ES(100) (how many different species are expected in a random sample of 100 individuals) (Hurlbert, 1971), were calculated using PAST software (version 4.03: (Hammer et al., 2001)) based on the standardized abundance data (**Appendix. A**) and are utilized to quantify the Ecological Quality Status (EcoQS) (Aasgaard, 2020) a concept that

has been implemented in the European Water Framework Directive (WFD 2000/60/EC).

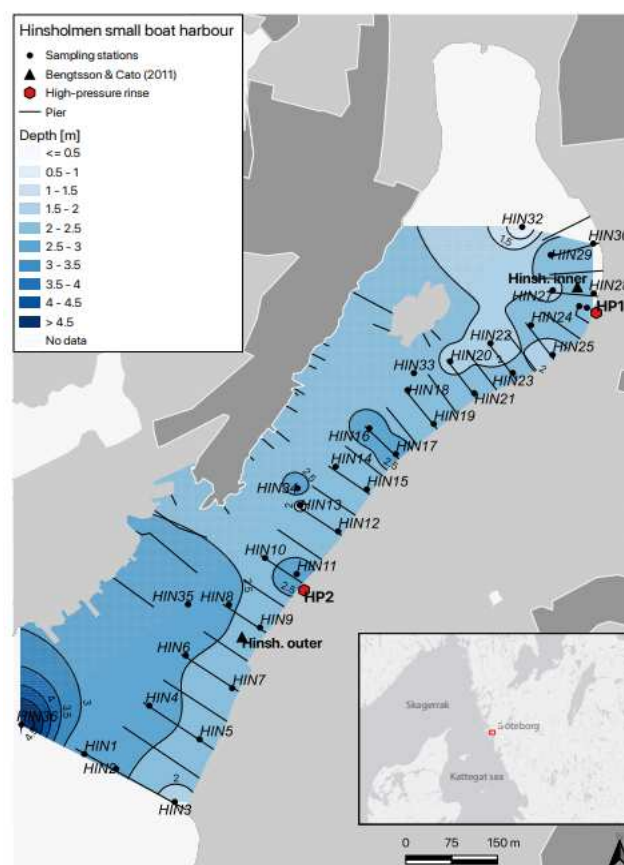
## **2.3 Heavy metals analyses**

### **Origin of the data**

Heavy metal analysis has been conducted in the area in 2019 by A. Olejnik, as a part of master thesis study. The same stations had been used for both foraminifera and heavy metals analysis. The results of heavy metal analysis and water depth (**Appendix. B**) includes the concentration of Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pb), Vanadium (V), Zinc (Zn). These values in addition with water depth will be used as references for the environmental parameters in the results section.

### **Hierarchical cluster analysis**

A dendrogram was obtained calculate by Q-mode hierarchical cluster analysis using Bray-Curtis (1957) distance on PAST (version 4.03). The stations were ordered based on their concentration of heavy metals (**Appendix. B**). The objective was to form groups of stations that



**Figure 1** Bathymetrical map of the study area with black dots representing the sampling stations adapted from Olejnik (2020). The red points indicate the two cleaning plants in the harbour.

have similar values to compare with foraminiferal assemblage and EcoQS values obtained before.

### Canonical correspondence analysis

The relationship between benthic foraminifera and environmental variables was evaluated by canonical correspondence analysis (CCA) using PAST software (version 4.03).

To statistically support the results multiple linear regression was performed using PAST (version 4.03), with the relative abundance of each species as the dependent variable and the concentration of heavy metals at each station as the independent variable.

## 3. RESULTS

### 3.1. Benthic foraminiferal assemblages

#### Foraminifera density

No benthic foraminifera were observed at station 27, 32, 33, 34 and 35. At the other stations, the absolute abundances of the foraminifera vary between 150 (station 5) and 596 (station 28) individuals per gram sediment (**Appendix. A**). Excluding stations with no specimens, the mean abundance per station is 309 ind/g<sup>-1</sup> sediment per gram.

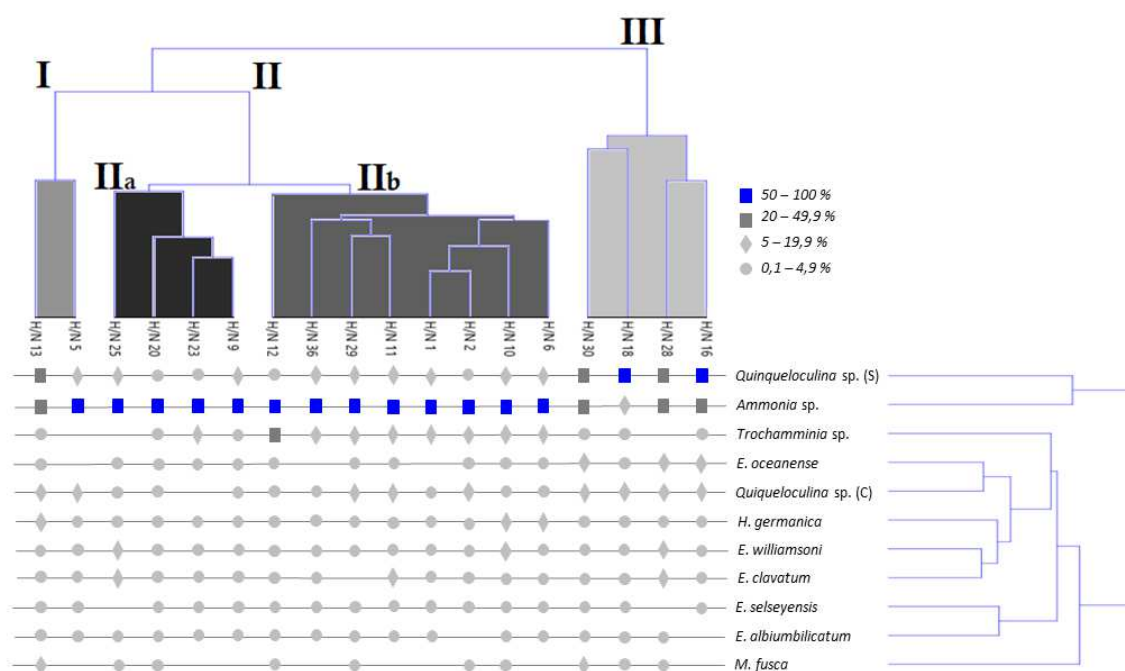
#### Foraminifera diversity

The most dominant genus is *Ammonia* spp. for almost all of the stations sampled, except in the stations 13, 28 and 30 where an assemblage *Quinqueloculina* spp. and a great proportion of *Ammonia* spp. was found (**Appendix. C**). Station 18 obtained a Shannon index of 1.4 and a Simpson index of 0.6, and appears to be dominated by a “smooth” morphotype of *Quinqueloculina* sp.

Station 23 has the lowest diversity with a Shannon index value of 0.8 and a Simpson index value of 0.3 with a predominance of *Ammonia* spp. Stations 28, 13 and 30 have the highest diversity with a Shannon index value ranges from 1.7 to 1.8 and a Simpson index value of 0.8 (**Appendix. D**) with an assemblage of *Quinqueloculina* spp. and *Ammonia* spp. For other stations, Shannon index ranges from 1.1 to 1.6 and Simpson index range from 0.5 to 0.7.

#### Foraminiferal assemblage

The Q-mode cluster analysis, results in the grouping of samples into three main clusters (I, II, III) (**Fig. 2**) related to the distribution of each species. Stations 27, 32, 33, 34, and 35 have no bullets because no foraminifera were counted, and therefore they will not be taken into account.



**Figure 2** Dendrogram of each sample from the relative abundance values of each species. Blue squares represent an abundance of over 50% in the sample. Grey squares represent a presence between 20 – 49.9%. Grey triangles and circles correspond to less than 19.9% presence.

The first cluster is composed of the stations 5 et 13, as they show both the lowest abundance of individuals (**Appendix. A**) with a Shannon index of 1,8 and 1,4 respectively (**Appendix. D**). The second cluster is characterized by dominant *Ammonia* spp. Here, two sub-clusters IIa and IIb can be recognised. Cluster IIa is subordinated with *Quinqueloculina* sp. "smooth" morphotype and some *Elphidium* species. Cluster IIb is subordinated by *Trochammina* sp. The third cluster consists of an assemblage of *Quinqueloculina* sp. "smooth" morphotype with *Ammonia* spp., subordinated with *E. williamsoni* and *E. clavatum*. *Quinqueloculina* sp. "smooth" morphotype is the dominant species at stations 18 and 16

### 3.2 Influence of heavy metals on distribution of foraminifera species

#### **Hierarchical cluster**

The Q-mode cluster analysis, results in the grouping of samples into three main clusters (I, II, III) (**Appendix. E**) related to the concentration of each heavy metal. Station 18 does not fit into any group since it is the only one with an extremely high concentration of As (50.3 [mg/kg]). Station 32 has not much similarity with the other stations as it is the least polluted site (for example, it is 6.5 times less polluted by copper as compared to the most polluted station 28).

The first cluster is composed of the most polluted stations (Cr, V, Zn and Hg and extreme values of Cu) (**Appendix. E**). The second cluster is characterized by lower pollution levels, except for Cu. Finally, the third cluster consists of stations with lower pollution levels than the previous two clusters, despite the fact that the concentration of Cu is consistently high, but much lower than the other stations.

#### **Canonical correspondence analysis**

The canonical correspondence analysis identified two axes, which explain 45.8 % and 18.7% of the variance in the assemblage (**Appendix. Fa, b**).

On the species/environment plots (**Appendix. Fa**) species were divided into many groups. *Ammonia* spp., *E. albiumbilicatum*, *E. selseyensis* and *H. germanica* appears influenced by Hg and Cd. *E. williamsoni*, *E.*

*clavatum* and *E. oceanense* show a positive relationship with As, Cr, Zn, Cu and to a lesser degree with V, Pb, Ni and Co. *Quinqueloculina* sp. "smooth" and "costae" morphotypes plot separately from all heavy metals. Also, remarkably, there are two species (*Trochammina* sp. and *M. fusca*) that diverge from the other species in the graph.

For the station/environment plot (**Fig. 3b**) two groups emerged. A group included stations 13, 16, 18, 28, 30 and the other groupe constituted of all the other stations. Positive relationship was observed for this second group for heavy metals and water depth except for Zn, Cu, Cr and As. But the results suggest that the influence of heavy metals is low.

#### **Linear regression**

The linear regressions analysis showed that heavy metals (individually or together) cannot significantly explain the distribution of the majority of the foraminiferal assemblages. Only the distributions of *Trochammina* sp, *E. selseyensis*, and *E. williamsoni* tend to be explained at 67, 68, and 66 % respectively by the interaction of heavy metals (**Appendix. G**). These results should be taken with caution because their significance levels (p-values) are 0.08, 0.09, and 0.06 respectively. However, it is important to mention them in order to provide potential reflection for further research.

## 4. DISCUSSION

Stations 13 and 5 in the first cluster are the least abundant and belong to the moderately polluted group of stations in the harbour but with poor EcoQS values for ES(100) and Shannon index .

The foraminiferal assemblage dominated by *Ammonia* spp., with lesser quantities of *Elphidium* spp. and *Quinqueloculina* spp., corresponds to the most polluted stations (25, 20, 23, and 9) with poor and bad EcoQS values for ES(100) and Shannon index.

Stations dominated by *Quinqueloculina* sp. "smooth" morphotype (18, 16) are slightly less polluted than stations that include both *Quinqueloculina* sp. "smooth" and *Ammonia* spp., and some *Elphidium* species. Several authors have concluded that *Quinqueloculina* species.

appeared to be more sensitive to pollution, whereas *Elphidium* spp. and *Ammonia* spp. were more tolerant and can be regarded as opportunistic (Valenti, et al., 2008), (Ferraro, et al., 2006).

In this study *Ammonia* spp. is the most abundant and dominant genus and tend to be correlated with Hg pollution. Some authors found the same result with a reduction in benthic foraminiferal abundance, and the dominance of opportunistic species in sediments affected by mercury (Di Leonardo, et al., 2007).

A positive correlation was found between the genera *Trochammina* sp. and heavy metal concentration. In this study, microscopic observations as well as the outliers of the canonical correspondence analysis suggest that specimens belong to the species *Trochammina hadai*. This invasive species is native to Japan (Uchio, 1962), and cellular DNA analysis of observed *Trochammina* sp. is needed to confirm presence of *Trochammina hadai* in Hinsholmen Harbour. Recently, *Trochammina hadai* has arrived to European waters and has been reported as frequent in French harbours (Bouchet, et al., 2023). In this study, the majority of *Trochammina* specimens were sampled at stations located at the entrance of the harbour (36, 1, 2, and 6) and close to the cleaning plants (12, 11, 10). These stations are where maritime traffic is highest, in other words, where the ship density in the port is the most important. It is possible that the presence of this putative *Trochammina hadai* is firstly explained by the physical transport of the species by boats, rather than by a competitive advantage in a polluted environment. The positive correlation between the genera *Trochammina* sp. and heavy metal concentration may potentially hide a bias in the selection of environmental parameters. In the future study of Hinsholmen harbour it might be appropriate to add other parameters such as temperature, salinity, total organic carbon (TOC) as well as the average boat density per station.

## 5. CONCLUSION

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Based on diversity of foraminifera EcoQS is bad or poor, similar to what is assessed by heavy metals pollution in Hinsholmen harbour.

However, qualitatively heavy metals pollution slightly impacts the distribution of foraminifera in the Hinsholmen harbour, but no significant conclusions could be drawn.

In the future, studying the samples from untreated stations may potentially overcome the lack of significance in these results. A study conducted over several years and at different time periods could support or refute the findings. Furthermore, although the heavy metals studied here have limited impact, the addition of other environmental parameters (temperature, salinity, TOC...) in the future may provide a clearer explanation for the distribution of foraminiferal assemblages.

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## APPENDIXES

**Appendix A** Raw data of foraminifera abundance by station. Stations 27, 32, 33, 35 are in grey because no dead benthic foraminifera were observed at these stations. In red, station with the highest abundance and in blue station with the lowest abundance all the others stations appears in green. Total of abundance at each station appear in yellow.

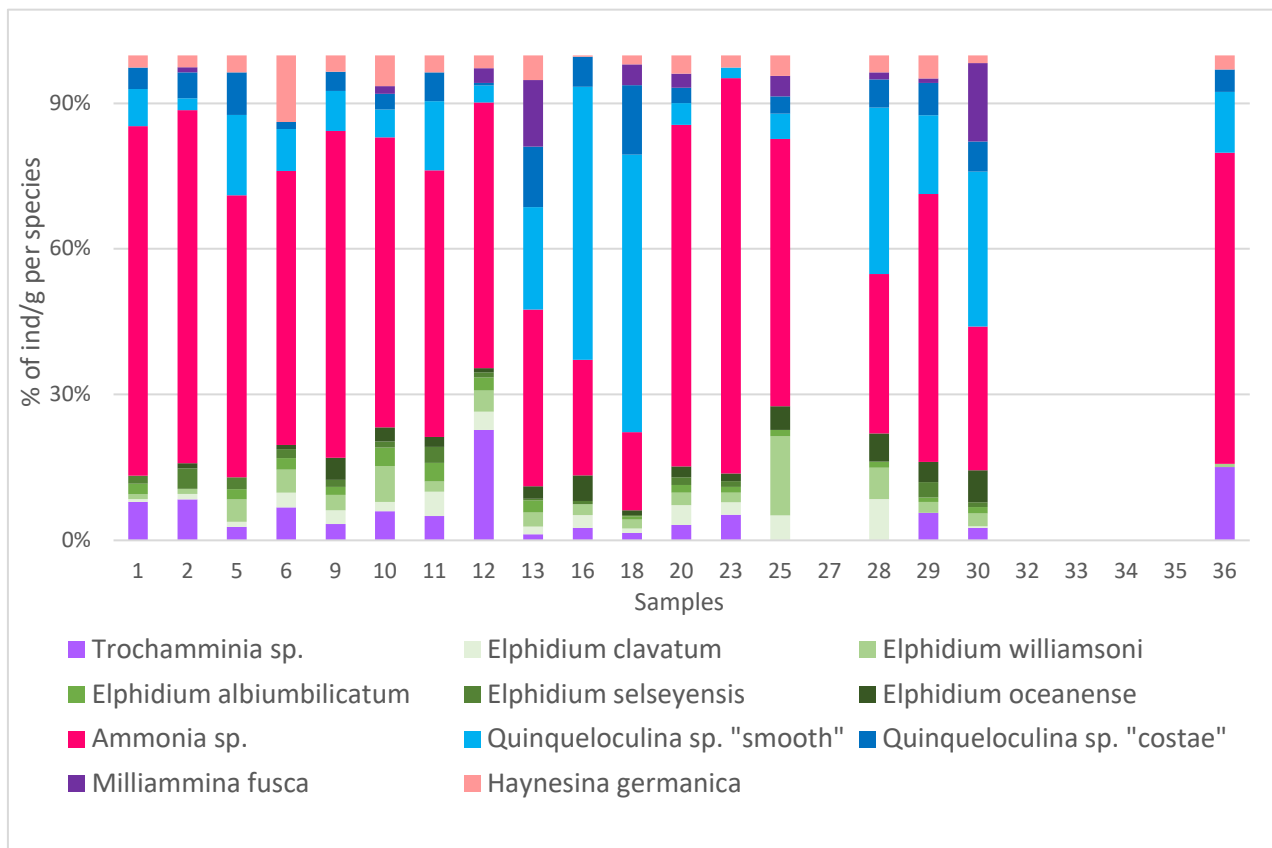
Samples/species	Trochammina sp.	Elphidium clavatum	Elphidium williamsoni	Haynesina germanica	Elphidium albiumbilicatum	Ammonia sp.	Quinqueloculina sp. "smooth"	Quinqueloculina sp. "costae"	Elphidium fusca	Elphidium selseyensis	Elphidium oceanense	Total
H/N 1	20	2	2	6	6	184	19	11	0	4	0	255
H/N 2	22	3	3	7	0	192	7	14	3	11	3	264
H/N 5	4	2	7	5	3	87	25	13	0	4	0	150
H/N 6	19	8	14	39	7	160	25	4	0	5	3	284
H/N 9	12	10	11	12	6	230	28	14	0	5	15	341
H/N 10	20	6	24	21	12	193	19	10	5	4	9	324
H/N 11	13	13	5	9	10	144	37	16	0	9	5	262
H/N 12	64	11	12	8	8	154	10	2	8	3	2	282
H/N 13	2	3	5	9	5	64	37	22	24	1	5	176
H/N 16	11	11	10	1	0	100	235	26	0	3	22	418
H/N 18	4	2	5	5	2	42	149	37	11	1	2	260
H/N 20	13	17	10	16	6	287	18	13	12	6	9	408
H/N 23	16	7	6	7	3	237	6	0	0	3	5	292
H/N 25	0	18	57	15	5	192	18	13	15	0	17	349
H/N 27	0	0	0	0	0	0	0	0	0	0	0	0
H/N 28	0	51	39	21	7	196	204	34	9	0	34	596
H/N 29	18	0	7	15	3	172	50	21	3	10	13	311
H/N 30	9	1	9	6	5	103	111	22	56	3	23	346
H/N 32	0	0	0	0	0	0	0	0	0	0	0	0
H/N 33	0	0	0	0	0	0	0	0	0	0	0	0
H/N 34	0	0	0	0	0	0	0	0	0	0	0	0
H/N 35	0	0	0	0	0	0	0	0	0	0	0	0
H/N 36	36	0	2	7	0	154	30	11	0	0	0	239



**Appendix B** Raw data of heavy metals concentration and water depth (WD) by station (Olejnik, 2020). Stations 27, 32, 33, 35 are in grey because no dead benthic foraminifera were observed at these stations. In red, heavy metal with very large deviation, in orange = large deviation, in yellow = distinct deviation, in green = little deviation and in blue = no deviation, according to Ecological Status classification based on Swedish Environmental Protection Agency regulation. (Presented as the ratio between measured concentration and background level for each element).

Stations	As	Cd	Co	Cr	Cu	Hg	Ni	Pb	V	Zn	WD
H/N 1	27,5	0,340	6,18	63,1	70,6	0,173	16,9	19,5	38,5	139,0	2,75
H/N 2	23,8	0,284	6,39	55,0	67,5	0,143	17,7	18,3	41,6	144,0	2,5
H/N 5	13,0	0,350	<sup>2</sup>	42,2	89,9	0,288	21,4	33,3	42,3	169,0	2,5
H/N 6	13,5	0,542	8,95	45,7	85,6	0,297	22,7	31,2	53,5	165,0	2,5
H/N 9	28,0	0,301	7,81	73,3	292,0	0,257	22,5	35,1	50,2	227,0	2,4
H/N 10	15,9	0,307	9,64	50,1	109,0	0,281	23,9	33,2	58,1	199,0	2,5
H/N 11	13,3	0,269	8,03	45,5	283,0	0,257	22,0	35,9	48,6	229,0	2,6
H/N 12	8,2	0,202	5,80	29,0	88,4	0,389	14,5	25,5	33,3	138,0	2,3
H/N 13	15,4	0,284	7,54	46,5	99,2	0,240	19,5	28,2	46,1	183,0	1,9
H/N 16	13,4	0,302	9,89	51,2	141,0	0,356	26,5	36,5	64,2	223,0	2,75
H/N 18	50,3	0,135	7,10	113,0	123,0	0,207	16,4	25,7	41,9	172,0	2
H/N 20	14,3	0,256	10,90	48,6	154,0	0,287	27,9	39,5	68,3	244,0	1,9
H/N 23	23,7	0,283	11,70	72,4	251,0	0,470	30,8	56,4	72,8	294,0	2
H/N 25	43,1	0,323	9,70	123,0	320,0	0,379	26,3	46,9	60,1	315,0	1,9
H/N 27	10,9	0,152	7,09	38,0	122,0	0,190	20,1	28,8	50,6	163,0	1,9
H/N 28	28,1	0,307	8,41	81,5	312,0	0,268	23,0	41,3	54,3	290,0	2
H/N 29	14,0	0,246	9,83	51,3	161,0	0,277	25,8	35,9	35,5	227,0	2,3
H/N 30	12,6	0,330	9,50	45,0	226,0	0,256	25,5	36,3	63,2	258,0	2,1
H/N 32	5,2	0,201	5,97	23,6	49,7	0,079	13,9	13,9	38,5	104,0	0,6
H/N 33	9,0	0,483	10,20	47,4	161,0	0,283	26,4	36,4	66,8	246,0	2,5
H/N 34	9,5	0,631	8,77	35,8	82,6	0,300	22,4	28,7	53,4	179,0	2,75
H/N 35	11,1	0,453	9,14	42,1	49,1	0,444	23,5	34,0	59,6	144,0	3
H/N 36	11,2	0,420	9,93	43,4	113,0	0,395	25,1	34,7	66,1	199,0	5,3



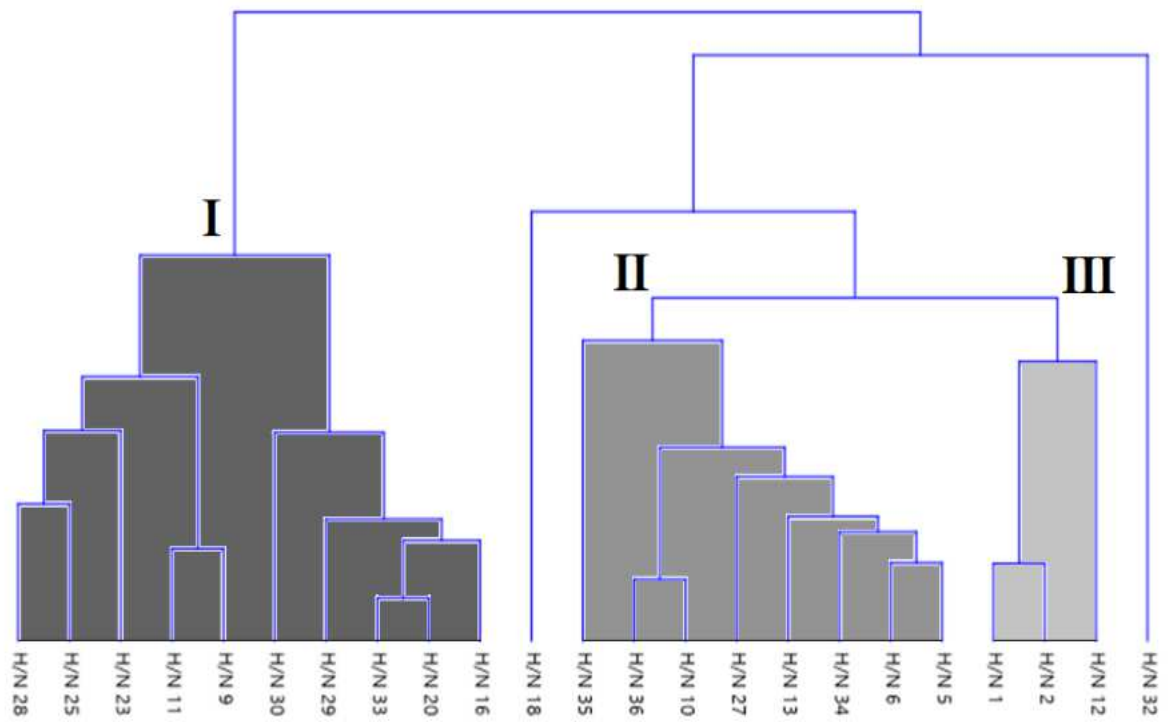


**Appendix C** Species richness per station expressed as a percentage. *Ammonia* spp. is represented in hot pink and *Quinqueloculina* sp. are represented in light blue for "smooth" and dark blue for "costae"

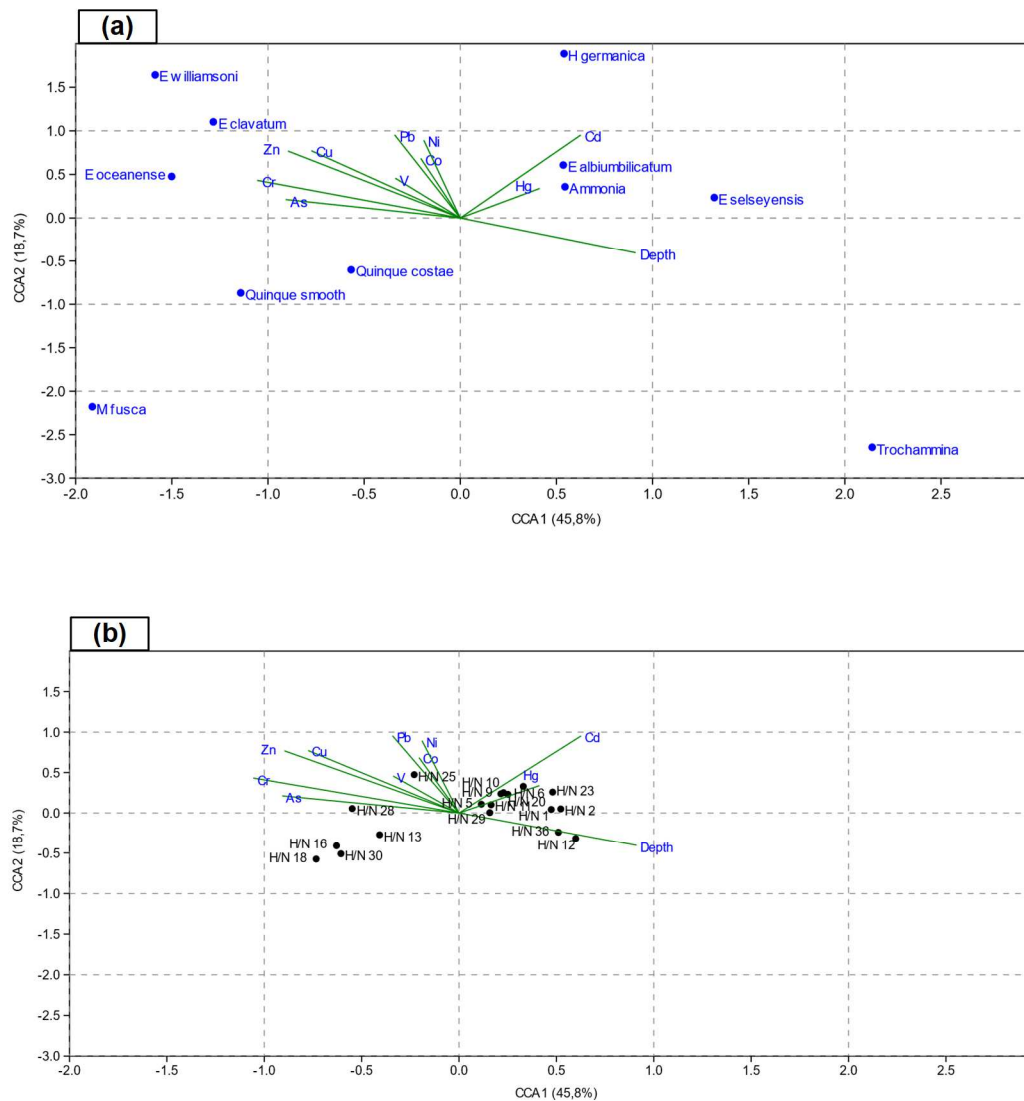
**Appendix D** Diversity indices (Shannon, Simpson and ES100) obtained using PAST. Colours corresponding to the Ecological Quality Status (EcoQS) index from the European Water Framework Directive (WFD 2000/60/EC). Blue is the best quality status and red is the worst quality status.

	Shannon index	Simpson index	ES(100)
H/N 1	1,08	0,46	8
H/N 2	1,12	0,49	8
H/N 5	1,41	0,62	9
H/N 6	1,51	0,64	9
H/N 9	1,32	0,55	9
H/N 10	1,54	0,66	10
H/N 11	1,58	0,66	10
H/N 12	1,47	0,62	10
H/N 13	1,820	0,79	10
H/N 16	1,32	0,62	7
H/N 18	1,38	0,62	8
H/N 20	1,27	0,54	10
H/N 23	0,83	0,33	8
H/N 25	1,53	0,65	9
H/N 27	0,00	0,00	0
H/N 28	1,68	0,75	8
H/N 29	1,53	0,66	9
H/N 30	1,75	0,77	9
H/N 32	0,00	0,00	0
H/N 33	0,00	0,00	0
H/N 34	0,00	0,00	0
H/N 35	0,00	0,00	0
H/N 36	1,11	0,46	5

Status class intervals		
	H'log2_f	ES100
High	5,0-3,4	35-18
Good	3,4-2,4	18-13
Moderate	2,4-1,8	13-11
Poor	1,8-1,2	11-9
Bad	1,2-0	9-0



**Appendix E** Dendrogram of each sample from the heavy metal concentration. Three clusters have been creating. On the left stations with highest concentration. on the right stations with the lowest concentration of heavy metals. Station 18 is the only one with high concentration of As.



**Appendix F** Species-environment (a) and sample-environment (b) biplots (the first two axes) of the CCA results using dead foraminiferal assemblages. Green lines refer to the gradient direction of environmental variables.

**Appendix G** Most significant results of linear regression on the three dependant variables *Trochammina* sp. *E. williamsoni* and *E. selseyensis* by the interaction of heavy metals. P-value corresponded to the significance of the results and should tend to zero. the commonly accepted threshold being 0.05.  $R^2$  is the degree of explanation of the independent variables. It can be converted into percentage by multiplying it by 100.

	<i>Trochammina</i> sp	<i>E.</i> <i>williamsoni</i>	<i>E.</i> <i>selseyensis</i>
(p-value)	0.08	0.09	0.06
$R^2$	0.67	0.66	0.68