Supplementary Information

Establishing baseline assessment levels for monitoring coastal heavy metals using foraminiferal shells: A case study from the South-eastern Mediterranean

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S1: Cleaning protocol description (modified after [37])

Specimens were placed in precleaned 2mL vials, were performed 1. Rinsing in Milli-Q water and sonication for 30 seconds (twice). The water was replaced with methanol and the vial was sonicated for another 30 seconds (twice). 2. An oxidative step to remove remaining organic matter. A 250 µL aliquot composed of equal volumes of 30% H_2O_2 and Suprapur 0.1M NaOH solution was added to each vial. Next, samples were placed on a prewarmed hot plate (65°C) for 5 minutes and were sonicated for another 5 minutes (twice). The entire procedure of step 2 was repeated twice. 3. Finally, the samples were rinsed in Milli-Q water (three times). The cleaning was performed at the ultraclean lab in the Interuniversity Institute for Marine Sciences (IUI, Eilat).

S2: Quality control – blanks and standards

Table S2.1: Average and standard deviation values of in-house standards from three analytical sessions:

Sampling date	Standard	Cu/Ca [µmol/mol]	Zn/Ca [µmol/mol]	Pb/Ca [µmol/mol]	Sr/Ca [µmol/mol]	Mg/Ca [mmol/mol]
March 2021	*Amphistegina* tests	1.51±0.07	20.9±0.7	74.3±1.5	1716±34	10.5±0.2
	local Red Sea sediments	5.2±0.1	14.4±0.4	4.02±0.09	103±2	2.7±0.1
August 2021	*Amphistegina* tests	1.48±0.05	20.1±0.5	75.9±0.8	1879±36	5.3±0.1
	local Red Sea sediments	5.1±0.5	13.4±0.4	4.86±0.15	111±3	1.3±0.1
2013	*Amphistegina* tests	1.61±0.02	22.8±1.3	73.4±0.4	1971±19	12.6±0.2
	local Red Sea sediments	4.8±0.1	13.5±0.5	4.80±0.04	111±2	3.0±0.1

Table S2.2: Foraminiferal dissolution blanks (defined as 3 SD of the procedural blank) and detection limits.

Sampling date		Cu [µg/L]	Zn [µg/L]	Pb [µg/L]	Sr [µg/L]	Ca [µg/L]	Mg [µg/L]
March 2021		0.10±0.07	0.95±0.81	0.018±0.009	0.025±0.012	24±16	2.2±0.4
	Detection limit	0.2	2.5	0.03	0.04	50	1.5
August 2021		0.04±0.04	0.16±0.10	0.017±0.018	0.028±0.068	20±23	0.4±0.7
	Detection limit	0.1	0.3	0.06	0.2	70	2.3
2013		0.05±0.01	0.91±0.77	0.006±0.002	0.012±0.005	16±10	1.3±0.4
	Detection limit	0.03	2.3	0.01	0.01	30	1.3

Table S2.3: Seawater analyses: recovery, procedural blanks and detection limits (defined as 3 SD of the procedural blank) for the studied metals. Results of measured values CASS-6 and NASS-7 Certified Reference Materials are presented against certified values.

	Recovery (%)	Procedural blank	Detection limit	CASS-6 Certified value	CASS-6 Measured value (n=2)	NASS-7 Certified value	NASS-7 Measured value (n=5)
Zn [µg/L]	100	0.09	0.02	1.24±0.18	1.25±0.03	0.41±0.08	0.375±0.004
Cu [µg/L]	93	0.004	0.001	0.52±0.03	0.73±0.01	0.20±0.01	0.203±0.03
Pb [ng/L]	101	0.39	0.10	10±4	11.66±0.01	2.5±0.8	2.8±0.5

S3: Seawater data

Table S3.1: Seawater dissolved concentrations collected in March 2021 in Nachsholim Site:

Replicate	Cu [µg/L]	Zn [µg/L]	Pb [ng/L]
1	0.35±0.01	0.28±0.05	9.97±0.03
2	0.34±0.02	0.31±0.10	10.1±0.6

S4: Statistical analyses

Statistical analyses were performed using R software [45]. For each dataset, assumptions of normality of the residuals and homogeneity of variances were tested, if both assumptions were valid, ANOVA was performed, and in cases of normality validation and homogeneity violation, Welch's ANOVA test was applied. Compressions for unbalanced design or in cases where normality was violated, non-parametric Kruskal–Wallis test was applied. Asterisk and the red text indicate significant differences.

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Table S4.1: Comparing ICPMS and LA-ICPMS from July 2013:

Metal/Ca	Statistical test	Results
Pb	Kruskal–Wallis	χ_1^2= 8.35, p-value = 0.003*
Sr	Kruskal–Wallis	χ_1^2= 1.88, p-value = 0.170
Mg	Kruskal–Wallis	χ_1^2= 12.27, p-value <0.001*

Table S4.2: Statistical comparison of metal/Ca between March and August 2021:

Metal/Ca	Statistical test	Results
Pb	One way ANOVA	$F_{1,8}$= 0.29, p-value= 0.986
Cu	One way ANOVA	$F_{1,8}$= 0.36, p-value= 0.566
Zn	Welch	$F_{1,5.2}$ =2.43, p-value= 0.178
Sr	One way ANOVA	$F_{1,8}$= 12.84 p-value= 0.072
Mg	One way ANOVA	$F_{1,8}$= 31.46 p-value<0.001*

Table S4.3: Statistical comparison of metal/Ca between single chamber 2013-2014 LA-ICPMS analyses to whole shell 2021 ICPMS records:

Metal/Ca	Statistical test	Results
Pb	Welch	$F_{5,2}$ =23.99, p-value=0.002*
Cu	Welch	$F_{5,2}$ =7.21, p-value= 0.033*
Zn	Welch	$F_{2,6.1}$ = 40.87, p-value<0.001*
Sr	Kruskal–Wallis	χ_2^2=5.18 p-value= 0.075
Mg	Welch	$F_{2,5.8}$ = 50.00, p-value<0.001*

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Table S4.4: Comparing ICPMS and LA-ICPMS:

Sampling date	Metal/Ca	Statistical test	Results
July 2013	Pb	Welch	$F_{1,21}$ =1.38, p-value= 0.253
	Sr	Kruskal–Wallis	χ_1^2=5.92, p-value = 0.014*
	Mg	Kruskal–Wallis	χ_1^2=3.19, p-value = 0.074
March 2013	Pb	Kruskal–Wallis	χ_1^2=5.36, p-value = 0.020*
	Sr	Kruskal–Wallis	χ_1^2=4.15, p-value = 0.041*
	Mg	Kruskal–Wallis	$F_{1,18}$ =9.80 p-value= 0.006

Table S4.5: Statistical comparison of metal concentration between March and August 2021:

Metal/Ca	Statistical test	Statistical test
Pb	Welch	$F_{1,1}$ = 0.07, p-value= 0.828
Cu	One way ANOVA	$F_{1,3}$= 1.12, p-value= 0.367
Zn	One way ANOVA	$F_{1,3}$= 176.2, p-value<0.001*
Sr	One way ANOVA	$F_{1,3}$= 2.18 p-value= 0.236
Mg	One way ANOVA	$F_{1,3}$= 8.95 p-value=0.06

Table S4.6: Statistical comparison of metal/Ca between single chamber 2013-2014 LA-ICPMS analyses to whole shell 2021 ICPMS records:

Metal/Ca	Statistical test	Statistical test
Pb	Welch	$F_{2,1.8}$ =300.06, p-value=0.005*
Cu	Kruskal–Wallis	χ_2^2=6.46, p-value= 0.122
Zn	Kruskal–Wallis	χ_2^2= 136.63, p-value<0.001*
Sr	Kruskal–Wallis	χ_2^2=11.75 p-value= 0.002*
Mg	Kruskal–Wallis	χ_2^2=3.09, p-value=0.199