

# Shell growth of large benthic foraminifera under heavy metals pollution: Implications for geochemical monitoring of coastal environments

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## Supplementary material 2: Results and statistics

All statistics were performed using R software version 3.5.3. Most of the data was found to have non-normal distribution according to the Shapiro-Wilk test, so the Mann-Whitney nonparametric test was used between the control and the HMs treatments. Significant differences were accepted at  $P < 0.05$ .

Supplementary Table 1: Added new chambers and area differences of all specimens in the experiments. Error is the standard deviation of three replicates. \*\*\* Represents data that doesn't fit the required statistical test assumptions.

| Specie              | Time [day] | Treatment | Number of Specimens | New Chamber | Difference from the control      |
|---------------------|------------|-----------|---------------------|-------------|----------------------------------|
| <i>A. lobifera</i>  | 31         | Control   | 84                  | 3.6±1.1     |                                  |
|                     |            | Cd        | 87                  | 3.0±1.4     | ***Mann-Whitney test, $P < 0.05$ |
|                     |            | Cu        | 90                  | 3.0±1.0     | Mann-Whitney test, $P < 0.05$    |
|                     |            | Pb        | 90                  | 3.6±1.2     |                                  |
| <i>A. lessonii</i>  | 24         | Control   | 86                  | 3.2±1.4     |                                  |
|                     |            | Cd        | 88                  | 3.1±1.6     |                                  |
|                     |            | Cu        | 89                  | 3.1±1.6     |                                  |
|                     |            | Pb        | 89                  | 3.4±1.7     |                                  |
| <i>S. orbiculus</i> | 32         | Control   | 49                  | 2.5±0.8     |                                  |
|                     |            | Cd        | 49                  | 2.0±0.9     | Mann-Whitney test, $P < 0.05$    |
|                     |            | Cu        | 51                  | 1.8±0.7     | Mann-Whitney test, $P < 0.05$    |
|                     |            | Pb        | 49                  | 1.7±0.8     | ***Mann-Whitney test, $P < 0.05$ |
|                     |            |           |                     |             | Area Addition [mm <sup>2</sup> ] |
|                     |            | Control   | 49                  | 318±169     |                                  |
|                     |            | Cd        | 49                  | 247±161     |                                  |
|                     |            | Cu        | 51                  | 273±158     |                                  |
|                     |            | Pb        | 49                  | 230±157     |                                  |

Supplementary Table 2: F test (compare two variances) and Shapiro-Wilk test (normality) to compare newly added chambers between two independent treatments of *A. lobifera*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | F test to compare two variances |    |                 | Shapiro-Wilk normality test |                  |
|------------|---------------------------------|----|-----------------|-----------------------------|------------------|
|            | F                               | df | p-value         | W                           | p-value          |
| Control-Cd | 1.803                           | 86 | <b>0.007454</b> | 0.94453                     | <b>3.189e-06</b> |
| Control-Cu | 0.84099                         | 89 | 0.422           | 0.91716                     | <b>2.273e-08</b> |
| Control-Pb | 1.3156                          | 89 | 0.2074          | 0.92755                     | <b>1.222e-07</b> |

Supplementary Table 3: Mann-Whitney test to compare newly added chambers between two independent treatments of *A. lobifera*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | Mann-Whitney test |                  |
|------------|-------------------|------------------|
|            | W                 | p-value          |
| Control-Cd | 2649.5            | <b>0.001388</b>  |
| Control-Cu | 2158              | <b>3.695e-07</b> |
| Control-Pb | 3801              | 0.9487           |

Supplementary Table 4: F test (compare two variances) and Shapiro-Wilk test (normality) to compare newly added chambers between two independent treatments of *A. lessonii*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | F test to compare two variances |    |                | Shapiro-Wilk normality test |                  |
|------------|---------------------------------|----|----------------|-----------------------------|------------------|
|            | F                               | df | p-value        | W                           | p-value          |
| Control-Cd | 1.3873                          | 87 | 0.1315         | 0.91347                     | <b>1.29e-08</b>  |
| Control-Cu | 0.80185                         | 85 | 0.3073         | 0.91818                     | <b>2.477e-08</b> |
| Control-Pb | 0.63825                         | 85 | <b>0.03852</b> | 0.94122                     | <b>1.32e-06</b>  |

Supplementary Table 5: Mann-Whitney test to compare newly added chambers between two independent treatments of *A. lessonii*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | Mann-Whitney test |         |
|------------|-------------------|---------|
|            | W                 | p-value |
| Control-Cd | 3721.5            | 0.8475  |
| Control-Cu | 4040              | 0.5142  |
| Control-Pb | 3549              | 0.3962  |

Supplementary Table 6: F test (compare two variances) and Shapiro-Wilk test (normality) to compare newly added chambers between two independent treatments of *S. orbiculus*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | F test to compare two variances |    |         | Shapiro-Wilk normality test |                  |
|------------|---------------------------------|----|---------|-----------------------------|------------------|
|            | F                               | df | p-value | W                           | p-value          |
| Control-Cd | 1.238                           | 48 | 0.4624  | 0.87364                     | <b>1.242e-07</b> |
| Control-Cu | 1.1979                          | 48 | 0.5283  | 0.8597                      | <b>2.753e-08</b> |

|            |        |    |        |         |                  |
|------------|--------|----|--------|---------|------------------|
| Control-Pb | 1.1351 | 48 | 0.6625 | 0.87775 | <b>1.833e-07</b> |
|------------|--------|----|--------|---------|------------------|

Supplementary Table 7: Mann-Whitney test to compare newly added chambers between two independent treatments of *S. orbiculus*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | Mann-Whitney test |                  |
|------------|-------------------|------------------|
|            | W                 | p-value          |
| Control-Cd | 804               | <b>0.002808</b>  |
| Control-Cu | 1783.5            | <b>8.244e-05</b> |
| Control-Pb | 1798              | <b>4.214e-06</b> |

Supplementary Table 8: F test (compare two variances) and Shapiro-Wilk test (normality) to compare area differences between two independent treatments of *S. orbiculus*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | F test to compare two variances |    |         | Shapiro-Wilk normality test |                  |
|------------|---------------------------------|----|---------|-----------------------------|------------------|
|            | F                               | df | p-value | W                           | p-value          |
| Control-Cd | 0.90011                         | 48 | 0.7169  | 0.91525                     | <b>9.607e-06</b> |
| Control-Cu | 1.1496                          | 48 | 0.626   | 0.91276                     | <b>5.933e-06</b> |
| Control-Pb | 1.1587                          | 48 | 0.6119  | 0.92094                     | <b>1.897e-05</b> |

Supplementary Table 9: Mann-Whitney test to compare area differences between two independent treatments of *S. orbiculus*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | Mann-Whitney test |                |
|------------|-------------------|----------------|
|            | W                 | p-value        |
| Control-Cd | 863.5             | <b>0.0168</b>  |
| Control-Cu | 1484              | 0.1066         |
| Control-Pb | 1641,             | <b>0.00177</b> |

Supplementary Table 10: Average Chl *a* concentrations normalized to the area of the specimens. Error is the standard deviation of the three replicates.

| Specie              | Treatment | Number of Specimen | Chl a [ng mm <sup>-2</sup> ] | Difference from the control |
|---------------------|-----------|--------------------|------------------------------|-----------------------------|
| <i>A. lobifera</i>  | Control   | 12                 | 0.11±0.03                    |                             |
|                     | Cd        | 12                 | 0.14±0.07                    |                             |
|                     | Cu        | 12                 | 0.14±0.03                    |                             |
|                     | Pb        | 12                 | 0.11±0.03                    |                             |
| <i>A. lessonii</i>  | Control   | 18                 | 0.05±0.02                    |                             |
|                     | Cd        | 18                 | 0.05±0.02                    |                             |
|                     | Cu        | 18                 | 0.06±0.02                    | Mann-Whitney test, P<0.05   |
|                     | Pb        | 18                 | 0.03±0.02                    | Mann-Whitney test, P<0.05   |
| <i>S. orbiculus</i> | Control   | 11                 | 0.09±0.02                    |                             |
|                     | Cd        | 11                 | 0.09±0.02                    |                             |
|                     | Cu        | 12                 | 0.09±0.02                    |                             |
|                     | Pb        | 11                 | 0.11±0.02                    | Mann-Whitney test, P<0.05   |

Supplementary Table 11: F test (compare two variances) and Shapiro-Wilk test (normality) to compare Chl a concentrations between two independent treatments of *A. lobifera*. Statistical differences accepted at P<0.05 are mark in bold.

| Groups     | F test to compare two variances |    |                | Shapiro-Wilk normality test |                  |
|------------|---------------------------------|----|----------------|-----------------------------|------------------|
|            | F                               | df | p-value        | W                           | p-value          |
| Control-Cd | 3.5339                          | 11 | <b>0.04709</b> | 0.82022                     | <b>0.0006426</b> |
| Control-Cu | 1.0785                          | 11 | 0.9025         | 0.95787                     | 0.2168           |
| Control-Pb | 1.0421                          | 11 | 0.9467         | 0.94557                     | 0.2168           |

Supplementary Table 12: Mann-Whitney test to compare Chl a concentrations between two independent treatments of *A. lobifera*. Statistical differences accepted at P<0.05 are mark in bold.

| Groups     | Mann-Whitney test |         |
|------------|-------------------|---------|
|            | W                 | p-value |
| Control-Cd | 84,               | 0.5065  |
| Control-Cu | 42.5              | 0.09335 |
| Control-Pb | 85                | 0.4703  |

Supplementary Table 13: F test (compare two variances) and Shapiro-Wilk test (normality) to compare Chl a concentrations between two independent treatments of *A. lessonii*. Statistical differences accepted at P<0.05 are mark in bold.

| Groups     | F test to compare two variances |    |         | Shapiro-Wilk normality test |         |
|------------|---------------------------------|----|---------|-----------------------------|---------|
|            | F                               | df | p-value | W                           | p-value |
| Control-Cd | 0.4498                          | 17 | 0.109   | 0.96986                     | 0.4218  |
| Control-Cu | 2.3042                          | 17 | 0.09444 | 0.98986                     | 0.9813  |
| Control-Pb | 2.0343                          | 17 | 0.1532  | 0.94823                     | 0.09198 |

Supplementary Table 14: Mann-Whitney test to compare Chl *a* concentrations between two independent treatments of *A. lessonii*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | Mann-Whitney test |                  |
|------------|-------------------|------------------|
|            | W                 | p-value          |
| Control-Cd | 166.5             | 0.8992           |
| Control-Cu | 97.5              | <b>0.04279</b>   |
| Control-Pb | 268               | <b>0.0008384</b> |

Supplementary Table 15: F test (compare two variances) and Shapiro-Wilk test (normality) to compare Chl *a* concentrations between two independent treatments of *S. orbiculus*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | F test to compare two variances |    |         | Shapiro-Wilk normality test |         |
|------------|---------------------------------|----|---------|-----------------------------|---------|
|            | F                               | df | p-value | W                           | p-value |
| Control-Cd | 0.91866                         | 10 | 0.8959  | 0.916                       | 0.06285 |
| Control-Cu | 1.5477                          | 10 | 0.484   | 0.94428                     | 0.2219  |
| Control-Pb | 1.1184                          | 10 | 0.8631  | 0.94185                     | 0.2161  |

Supplementary Table 16: Mann-Whitney test to compare Chl *a* concentrations between two independent treatments of *S. orbiculus*. Statistical differences accepted at  $P < 0.05$  are mark in bold.

| Groups     | Mann-Whitney test |                |
|------------|-------------------|----------------|
|            | W                 | p-value        |
| Control-Cd | 51.5              | 0.5761         |
| Control-Cu | 64                | 0.9264         |
| Control-Pb | 28.5              | <b>0.03843</b> |

Supplementary Table 17: Growth rate results calculated by the area differences in *S. orbiculus* and the number of new chambers added, in *A. lobifera*, *A. lessonii* and *S. orbiculus*. Error is the standard deviation of the three replicates.

| Specie              | Time [day] | Treatment                               | Growth [Chamber Week <sup>-1</sup> ] |
|---------------------|------------|---|--------------------------------------|
| <i>A. lobifera</i>  | 31         | Control                                 | 0.8±0.3                              |
|                     |            | Cd                                      | 0.7±0.3                              |
|                     |            | Cu                                      | 0.7±0.2                              |
|                     |            | Pb                                      | 0.8±0.3                              |
| <i>A. lessonii</i>  | 24         | Control                                 | 0.9±0.4                              |
|                     |            | Cd                                      | 0.9±0.5                              |
|                     |            | Cu                                      | 0.9±0.5                              |
|                     |            | Pb                                      | 1.0±0.5                              |
| <i>S. orbiculus</i> | 32         | Control                                 | 0.5±0.2                              |
|                     |            | Cd                                      | 0.4±0.2                              |
|                     |            | Cu                                      | 0.4±0.2                              |
|                     |            | Pb                                      | 0.3±0.2                              |
|                     |            | Growth Rate [Area % Day <sup>-1</sup> ] |                                      |
|                     |            | Control                                 | 2.1±0.7                              |
|                     |            | Cd                                      | 1.6±0.6                              |
|                     |            | Cu                                      | 1.8±0.5                              |
|                     |            | Pb                                      | 1.5±0.7                              |

Supplementary Table 18: Two-way ANOVA test comparing relative growth rates variances between different treatments of Cd and Cu at different CMC additions. Statistical differences accepted at  $P < 0.05$  are mark in bold.

|               | df | Sum Sq | Mean Sq | F value | p-value          |
|---------------|----|--------|---------|---------|------------------|
| CMC           | 1  | 8855.3 | 8855.3  | 90.787  | <b>1.869e-08</b> |
| Treatment     | 1  | 1732.5 | 1732.5  | 17.763  | <b>0.0005211</b> |
| CMC:Treatment | 1  | 3840.8 | 3840.8  | 39.377  | <b>6.447e-06</b> |
| Residuals     | 18 | 1755.7 | 97.5    |         |                  |

Supplementary Table 19: Number of specimens used in the Image analyses. Dead specimens were excluded from the analyses.

| Treatment | Replicate | Number of specimens (Replicate) | Number of specimens (Treatment) |
|-----------|-----------|---------------------------------|---------------------------------|
| Control   | Rep-1     | 27/29                           | 81/86                           |
|           | Rep-2     | 25/28                           |                                 |
|           | Rep-3     | 29/29                           |                                 |
| Cd        | Rep-1     | 23/30                           | 82/88                           |
|           | Rep-2     | 30/30                           |                                 |
|           | Rep-3     | 29/28                           |                                 |
| Cu        | Rep-1     | 28/29                           | 82/87                           |
|           | Rep-2     | 27/29                           |                                 |
|           | Rep-3     | 27/29                           |                                 |
| Pb        | Rep-1     | 27/29                           | 84/89                           |
|           | Rep-2     | 29/30                           |                                 |
|           | Rep-3     | 28/30                           |                                 |

Supplementary Table 20: Average  $I_{red}$  values. For imaging settings refer to S1.  $I_{red}$  error of the replicates is the standard deviation of specimens  $I_{red}$  values. Treatment error is the standard deviation of three replicates.

| Treatment | Replicate | Average $I_{red}$ [DN s <sup>-1</sup> ]<br>Replicate | Average $I_{red}$ [DN s <sup>-1</sup> ]<br>Treatment | Difference from<br>the Control |
|-----------|-----------|--|--|--------------------------------|
| Control   | Rep-1     | 386±75   | 359±53   |                                |
|           | Rep-2     | 332±26   |  |                                |
|           | Rep-3     | 358±30   |  |                                |
| Cd        | Rep-1     | 340±32   | 331±32   |                                |
|           | Rep-2     | 322±31   |  |                                |
|           | Rep-3     | 333±32   |  |                                |
| Cu        | Rep-1     | 303±34   | 323±31   | Mann-Whitney<br>P<0.05         |
|           | Rep-2     | 323±14   |  |                                |
|           | Rep-3     | 344±26   |  |                                |
| Pb        | Rep-1     | 301±29   | 293±26   | Mann-Whitney<br>P<0.05         |
|           | Rep-2     | 294±20   |  |                                |
|           | Rep-3     | 284±25   |  |                                |

Supplementary Table 21: F test (compare two variances) and Shapiro-Wilk test (normality) to compare  $I_{red}$  values between two independent treatments of *A. lessonii*. Statistical differences accepted at P<0.05 are mark in bold.

| Groups     | F test to compare two variances |    |         | Shapiro-Wilk normality test |                  |
|------------|---------------------------------|----|---------|-----------------------------|------------------|
|            | F                               | df | p-value | W                           | p-value          |
| Control-Cd | 1.0429                          | 81 | 0.8506  | 0.92116                     | <b>8.859e-05</b> |
| Control-Cu | 1.4751                          | 81 | 0.07991 | 0.95392                     | <b>0.005068</b>  |
| Control-Pb | 1.4751                          | 81 | 0.07991 | 0.89376                     | <b>4.225e-06</b> |
| Cd-Pb      | 1.5383                          | 81 | 0.0525  | 0.93519                     | <b>7.81e-07</b>  |
| Cu-Pb      | 1.4751                          | 81 | 0.07991 | 0.97158                     | <b>0.001727</b>  |
| Cd-Cu      | 1.0429                          | 81 | 0.8506  | 0.97294                     | <b>0.002662</b>  |

Supplementary Table 22: Mann-Whitney test to compare  $I_{red}$  values between two independent treatments of *A. lessonii*. Statistical differences accepted at P<0.05 are mark in bold.

| Groups     | Mann-Whitney test |                  |
|------------|-------------------|------------------|
|            | W                 | p-value          |
| Control-Cd | 3725              | 0.2295           |
| Control-Cu | 5522.5            | <b>1.629e-11</b> |
| Control-Pb | 5522.5            | <b>1.629e-11</b> |
| Cd-Pb      | 5707              | <b>1.006e-13</b> |
| Cu-Pb      | 5522.5            | <b>1.629e-11</b> |
| Cd-Cu      | 3725              | 0.2295           |

Supplementary Table 23: Survival rates. Living, dead, lost and specimens which overwent reproduction event. Specimens over going reproduction were considered as living specimens. Survival rate error is the standard deviation of three replicates.

| Specie              | Treatment | Replicate | Living Specimens | Dead Specimens | Lost Specimens | Reproduction | Survival rate [%] |
|---------------------|-----------|-----------|------------------|----------------|----------------|--------------|-------------------|
| <i>A. lobifera</i>  | Control   | Rep-1     | 30               | 0              | 0              | 0            | 98±2              |
|                     |           | Rep-2     | 24               | 1              | 5              | 0            |                   |
|                     |           | Rep-3     | 29               | 1              | 0              | 0            |                   |
|                     | Cd        | Rep-1     | 24               | 3              | 3              | 0            | 94±6              |
|                     |           | Rep-2     | 30               | 0              | 0              | 0            |                   |
|                     |           | Rep-3     | 28               | 2              | 0              | 0            |                   |
|                     | Cu        | Rep-1     | 28               | 2              | 0              | 0            | 93±4              |
|                     |           | Rep-2     | 27               | 3              | 0              | 0            |                   |
|                     |           | Rep-3     | 29               | 1              | 0              | 0            |                   |
|                     | Pb        | Rep-1     | 30               | 0              | 0              | 0            | 97±6              |
|                     |           | Rep-2     | 27               | 3              | 0              | 0            |                   |
|                     |           | Rep-3     | 30               | 0              | 0              | 0            |                   |
| <i>A. lessonii</i>  | Control   | Rep-1     | 27               | 1              | 1              | 1            | 96±2              |
|                     |           | Rep-2     | 24               | 2              | 2              | 2            |                   |
|                     |           | Rep-3     | 28               | 1              | 1              | 0            |                   |
|                     | Cd        | Rep-1     | 24               | 2              | 2              | 2            | 97±4              |
|                     |           | Rep-2     | 30               | 0              | 0              | 0            |                   |
|                     |           | Rep-3     | 28               | 1              | 0              | 1            |                   |
|                     | Cu        | Rep-1     | 28               | 2              | 0              | 0            | 90±3              |
|                     |           | Rep-2     | 26               | 4              | 0              | 0            |                   |
|                     |           | Rep-3     | 27               | 3              | 0              | 0            |                   |
|                     | Pb        | Rep-1     | 25               | 4              | 0              | 1            | 96±8              |
|                     |           | Rep-2     | 29               | 0              | 0              | 1            |                   |
|                     |           | Rep-3     | 28               | 0              | 1              | 1            |                   |
| <i>S. orbiculus</i> | Control   | Rep-1     | 17               | 0              | 0              | 0            | 100±0             |
|                     |           | Rep-2     | 15               | 0              | 0              | 0            |                   |
|                     |           | Rep-3     | 9                | 0              | 8              | 0            |                   |
|                     | Cd        | Rep-1     | 14               | 2              | 1              | 0            | 92±3              |
|                     |           | Rep-2     | 16               | 1              | 0              | 0            |                   |
|                     |           | Rep-3     | 16               | 1              | 0              | 0            |                   |
|                     | Cu        | Rep-1     | 16               | 1              | 0              | 0            | 94±6              |
|                     |           | Rep-2     | 15               | 2              | 0              | 0            |                   |
|                     |           | Rep-3     | 17               | 0              | 0              | 0            |                   |
|                     | Pb        | Rep-1     | 16               | 1              | 0              | 0            | 92±3              |
|                     |           | Rep-2     | 14               | 2              | 1              | 0            |                   |
|                     |           | Rep-3     | 16               | 1              | 0              | 0            |                   |