

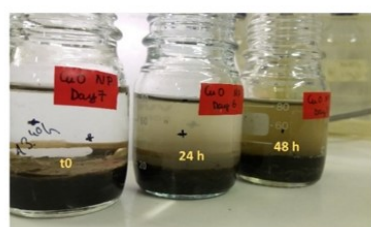
## Supplementary Information (SI)

**Table S1: Total amount and individual body wet weight (ww) of *T. tubifex* worms in sediment exposures.**

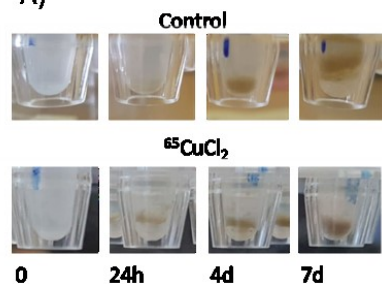
| Total amount of worms (g ww) added to 30 g wet sediment (i.e., per beaker)      |         |         |         |         |         |         |         |
|---------------------------------------------------------------------------------|---------|---------|---------|---------|---------|---------|---------|
| Day Treatment                                                                   | 1       | 2       | 3       | 4       | 5       | 6       | 7       |
| Control                                                                         | 0.414   | 0.405   | 0.400   | 0.419   | 0.417   | 0.426   | 0.427   |
| <sup>65</sup> CuCl <sub>2</sub>                                                 | 0.411   | 0.404   | 0.403   | 0.413   | 0.413   | 0.405   | 0.406   |
| <sup>65</sup> CuO NP                                                            | 0.403   | 0.0408  | 0.409   | 0.401   | 0.419   | 0.429   | 0.414   |
| Body ww of worms [g] in sediment exposure (t=0, i.e., when added to the beaker) |         |         |         |         |         |         |         |
| Day Treatment (sub-sample)                                                      | 1       | 2       | 3       | 4       | 5       | 6       | 7       |
| Control                                                                         |         |         |         |         |         |         |         |
| Individ.1                                                                       | 0.0026  | 0.0026  | 0.0050  | 0.0027  | 0.0049  | 0.0041  | 0.0036  |
| Individ.2                                                                       | 0.0038  | 0.0026  | 0.0031  | 0.0031  | 0.0032  | 0.0020  | 0.0047  |
| Individ.3                                                                       | 0.0030  | 0.0032  | 0.0030  | 0.0059  | 0.0026  | 0.0028  | 0.0033  |
| Individ.4                                                                       | 0.0024  | 0.0024  | 0.0031  | 0.0045  | 0.0021  | 0.0038  | 0.0060  |
| Individ.5                                                                       | NA      | 0.0050  | 0.0031  | 0.0023  | 0.0025  | 0.0049  | 0.0038  |
| Individ.6                                                                       | NA      | 0.0032  | 0.0032  | 0.0021  | 0.0028  | 0.0031  | 0.0032  |
| Individ.7                                                                       | NA      | 0.0052  | 0.0051  | 0.0034  | 0.0029  | 0.0041  | 0.0028  |
| Individ.8                                                                       | NA      | 0.0027  | 0.0040  | 0.0030  | 0.0033  | 0.0035  | 0.0031  |
| Individ.9                                                                       | NA      | 0.0050  | 0.0023  | 0.0026  | 0.0018  | 0.0057  | 0.0037  |
| Individ.10                                                                      | NA      | 0.0042  | 0.0024  | 0.0028  | 0.0031  | 0.0022  | 0.0031  |
| mean                                                                            | 0.0030  | 0.0036  | 0.0034  | 0.0032  | 0.0029  | 0.0036  | 0.0037  |
| sd                                                                              | ±0.0005 | ±0.0011 | ±0.0009 | ±0.0011 | ±0.0008 | ±0.0011 | ±0.0009 |
| <sup>65</sup> CuCl <sub>2</sub>                                                 |         |         |         |         |         |         |         |
| Individ.1                                                                       | 0.0022  | 0.0037  | 0.0023  | 0.0013  | 0.0033  | 0.0051  | 0.0028  |
| Individ.2                                                                       | 0.0029  | 0.0024  | 0.0033  | 0.0036  | 0.0022  | 0.0037  | 0.0025  |
| Individ.3                                                                       | 0.0034  | 0.0026  | 0.0051  | 0.0017  | 0.0037  | 0.0047  | 0.0030  |
| Individ.4                                                                       | NA      | 0.0032  | 0.0036  | 0.0037  | 0.0041  | 0.0030  | 0.0027  |
| Individ.5                                                                       | NA      | 0.0034  | 0.0025  | 0.0021  | 0.0028  | 0.0038  | 0.0020  |
| Individ.6                                                                       | NA      | 0.0038  | 0.0030  | 0.0037  | 0.0029  | 0.0052  | 0.0032  |
| Individ.7                                                                       | NA      | 0.0035  | 0.0049  | 0.0048  | 0.0027  | 0.0023  | 0.0031  |
| Individ.8                                                                       | NA      | 0.0029  | 0.0029  | 0.0029  | 0.0046  | 0.0051  | 0.0041  |
| Individ.9                                                                       | NA      | 0.0052  | 0.0029  | 0.0027  | 0.0053  | 0.0034  | 0.0032  |
| Individ.10                                                                      | NA      | 0.0044  | 0.0082  | 0.0017  | 0.0041  | 0.0053  | 0.0034  |
| mean                                                                            | 0.0028  | 0.0035  | 0.0039  | 0.0028  | 0.0036  | 0.0042  | 0.0030  |
| sd                                                                              | ±0.0005 | ±0.0008 | ±0.0017 | ±0.0011 | ±0.0009 | ±0.0010 | ±0.0005 |
| <sup>65</sup> CuO NP                                                            |         |         |         |         |         |         |         |
| Individ.1                                                                       | 0.0049  | 0.0034  | 0.0040  | 0.0033  | 0.0026  | 0.0026  | 0.0037  |
| Individ.2                                                                       | NA      | 0.0024  | 0.0063  | 0.0020  | 0.0070  | 0.0061  | 0.0023  |
| Individ.3                                                                       | NA      | 0.0017  | 0.0031  | 0.0031  | 0.0037  | 0.0053  | 0.0034  |
| Individ.4                                                                       | NA      | 0.0017  | 0.0025  | 0.0033  | 0.0033  | 0.0033  | 0.0030  |
| Individ.5                                                                       | NA      | 0.0029  | 0.0039  | 0.0025  | 0.0037  | 0.0030  | 0.0024  |
| Individ.6                                                                       | NA      | 0.0045  | 0.0052  | 0.0029  | 0.0030  | 0.0023  | 0.0037  |
| Individ.7                                                                       | NA      | 0.0031  | 0.0049  | 0.0019  | 0.0044  | 0.0032  | 0.0044  |
| Individ.8                                                                       | NA      | 0.0049  | 0.0057  | 0.0029  | 0.0034  | 0.0032  | 0.0026  |
| Individ.9                                                                       | NA      | 0.0033  | 0.0032  | 0.0023  | 0.0049  | 0.0048  | 0.0045  |
| Individ.10                                                                      | NA      | 0.0034  | 0.0030  | 0.0032  | 0.0024  | 0.0051  | 0.0041  |
| mean                                                                            | 0.0049  | 0.0031  | 0.0042  | 0.0027  | 0.0038  | 0.0039  | 0.0034  |
| sd                                                                              | 0.0000  | 0.0010  | 0.0012  | 0.0005  | 0.0013  | 0.0012  | 0.0008  |
| Average (all treatment groups)                                                  |         |         |         |         |         |         |         |
| sd                                                                              | 0.0032  | 0.0034  | 0.0038  | 0.0029  | 0.0034  | 0.0039  | 0.0034  |
|                                                                                 | ±0.0008 | ±0.0010 | ±0.0014 | ±0.0010 | ±0.0011 | ±0.0011 | ±0.0008 |

**Table S2: Total and individual body ww of *T. tubifex* worms collected from the sediment after the exposure.**

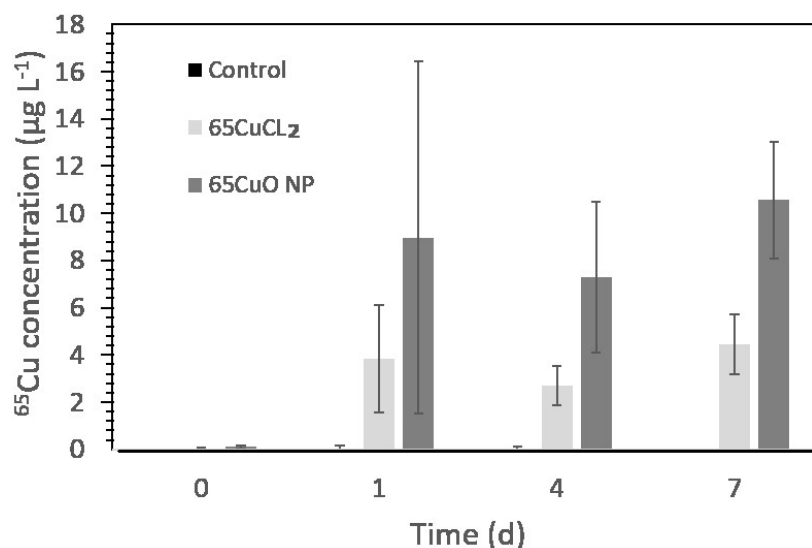
| Day                                                                                                            | 1      | 2      | 3      | 4      | 5      | 6      | 7      |
|----------------------------------------------------------------------------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| <b>Total ww of worms [g] recovered after 7 days of exposure in spiked sediment (before gut-emptying phase)</b> |        |        |        |        |        |        |        |
| Control                                                                                                        | 0.487  | 0.468  | 0.546  | 0.574  | 0.531  | 0.531  | 0.505  |
| <sup>65</sup> CuCl <sub>2</sub>                                                                                | 0.502  | 0.578  | 0.516  | 0.567  | 0.488  | 0.534  | 0.507  |
| <sup>65</sup> CuO NP                                                                                           | 0.501  | 0.540  | 0.569  | 0.515  | 0.516  | 0.523  | 0.412  |
| <b>Number of worms recovered after 7 days of exposure in spiked sediment (before gut-emptying phase)</b>       |        |        |        |        |        |        |        |
| Control                                                                                                        | 122    | 105    | 119    | 118    | 108    | 106    | 108    |
| <sup>65</sup> CuCl <sub>2</sub>                                                                                | 116    | 124    | 116    | 122    | 104    | 109    | 104    |
| <sup>65</sup> CuO NP                                                                                           | 115    | 121    | 122    | 112    | 107    | 94     | 108    |
| <b>Individual worm body ww [g] (calculated from above data)</b>                                                |        |        |        |        |        |        |        |
| Control                                                                                                        | 0.0040 | 0.0045 | 0.0046 | 0.0049 | 0.0049 | 0.0050 | 0.0047 |
| <sup>65</sup> CuCl <sub>2</sub>                                                                                | 0.0043 | 0.0047 | 0.0044 | 0.0046 | 0.0047 | 0.0049 | 0.0049 |
| <sup>65</sup> CuO NP                                                                                           | 0.0044 | 0.0045 | 0.0047 | 0.0046 | 0.0048 | 0.0056 | 0.0038 |



A)



B)



C)

**Figure S1:** Turbidity and Cu concentrations in overlying water during *T. tubifex* exposures. A) Images obtained during exposures illustrating the increase in water turbidity due to resuspended material. B) Images of water samples collected at the beginning of exposure, after 24 h, 4 days and 7 days, and after left to sediment. C) <sup>65</sup>Cu concentrations in samples of overlying water collected at several time points during *T. tubifex* exposures presented as mean ± sd (n = 3).



**Figure S2:** *T. tubifex* (with cleared guts) proportioned for fish feeding. *T. tubifex* originating from control (A), <sup>65</sup>CuCl<sub>2</sub>-spiked (B), and <sup>65</sup>CuO NP-spiked sediment (C).

**Table S3.** Average body ww of *T. tubifex* worms fed to the fish in the different treatment groups and at the different days.

| Day                                            | 1       | 2       | 3       | 4       | 5       | 6       | 7       | Mean (d1-7) ±sd |
|------------------------------------------------|---------|---------|---------|---------|---------|---------|---------|-----------------|
| Treatment                                      | ww [g]  | ww [g]  | ww [g]  | ww [g]  | ww [g]  | ww [g]  | ww [g]  |                 |
| Control                                        | 0.0047  | 0.0047  | 0.0042  | 0.0044  | 0.0041  | 0.0045  | 0.0030  |                 |
| Control                                        | 0.0022  | 0.0051  | 0.0031  | 0.0032  | 0.0032  | 0.0046  | 0.0029  |                 |
| Control                                        | 0.0034  | 0.0021  | 0.0027  | 0.0032  | 0.0042  | 0.0040  | 0.0041  |                 |
| Control                                        | 0.0031  | 0.0050  | 0.0061  | 0.0051  | 0.0049  | 0.0045  | 0.0029  |                 |
| Control                                        | 0.0081  | 0.0066  | 0.0041  | 0.0048  | 0.0045  | 0.0047  | 0.0019  |                 |
| Control                                        | 0.0041  | 0.0054  | 0.0030  | 0.0029  | 0.0024  | 0.0029  | 0.0031  |                 |
| Control                                        | 0.0042  | 0.0047  | 0.0034  | 0.0035  | 0.0047  | 0.0034  | 0.0032  |                 |
| Control <sup>a</sup>                           | 0.0029  | 0.0045  | 0.0034  | 0.0036  | 0.0041  | 0.0068  | 0.0027  |                 |
| Control <sup>a</sup>                           | 0.0053  | 0.0044  | 0.0040  | 0.0023  | 0.0050  | 0.0037  | 0.0041  |                 |
| Control <sup>a</sup>                           | 0.0027  | 0.0068  | 0.0031  | 0.0039  | 0.0050  | 0.0053  | 0.0041  |                 |
| <b>Mean (Control)</b>                          | 0.0041  | 0.0049  | 0.0037  | 0.0037  | 0.0042  | 0.0044  | 0.0032  | 0.0040          |
| <b>sd</b>                                      | ±0.0016 | ±0.0012 | ±0.0009 | ±0.0008 | ±0.0008 | ±0.0010 | ±0.0007 | ±0.0005         |
| <sup>65</sup> CuCl <sub>2</sub>                | 0.0052  | 0.0037  | 0.0034  | 0.0035  | 0.0032  | 0.0060  | 0.0036  |                 |
| <sup>65</sup> CuCl <sub>2</sub>                | 0.0039  | 0.0060  | 0.0041  | 0.0047  | 0.0040  | 0.0052  | 0.0021  |                 |
| <sup>65</sup> CuCl <sub>2</sub>                | 0.0025  | 0.0046  | 0.0025  | 0.0023  | 0.0032  | 0.0040  | 0.0049  |                 |
| <sup>65</sup> CuCl <sub>2</sub>                | 0.0045  | 0.0023  | 0.0037  | 0.0034  | 0.0035  | 0.0048  | 0.0031  |                 |
| <sup>65</sup> CuCl <sub>2</sub>                | 0.0051  | 0.003   | 0.0037  | 0.0047  | 0.0031  | 0.0038  | 0.0023  |                 |
| <sup>65</sup> CuCl <sub>2</sub>                | 0.0045  | 0.0027  | 0.0039  | 0.0030  | 0.0028  | 0.0042  | 0.0039  |                 |
| <sup>65</sup> CuCl <sub>2</sub>                | 0.0054  | 0.0048  | 0.0023  | 0.0038  | 0.0038  | 0.0044  | 0.0055  |                 |
| <sup>65</sup> CuCl <sub>2</sub> <sup>a</sup>   | 0.0033  | 0.0030  | 0.0028  | 0.0056  | 0.0033  | 0.0035  | 0.0014  |                 |
| <sup>65</sup> CuCl <sub>2</sub> <sup>a</sup>   | 0.0032  | 0.0035  | 0.0053  | 0.0037  | 0.0044  | 0.0043  | 0.0032  |                 |
| <sup>65</sup> CuCl <sub>2</sub> <sup>a</sup> ) | 0.0038  | 0.0037  | 0.0040  | 0.0036  | 0.0073  | 0.0050  | 0.0041  |                 |
| <b>Mean (CuCl<sub>2</sub>)</b>                 | 0.0041  | 0.0037  | 0.0036  | 0.0038  | 0.0039  | 0.0045  | 0.0034  | 0.0039          |
| <b>sd</b>                                      | ±0.0009 | ±0.0011 | ±0.0008 | ±0.0009 | ±0.0012 | ±0.0007 | ±0.0012 | ±0.0003         |
| <sup>65</sup> CuO NP                           | 0.0028  | 0.0060  | 0.0030  | 0.0047  | 0.0050  | 0.0060  | 0.0033  |                 |
| <sup>65</sup> CuO NP                           | 0.0058  | 0.0042  | 0.0046  | 0.0053  | 0.0049  | 0.0037  | 0.0032  |                 |
| <sup>65</sup> CuO NP                           | 0.0025  | 0.0044  | 0.0003  | 0.0024  | 0.0028  | 0.0034  | 0.0028  |                 |
| <sup>65</sup> CuO NP                           | 0.0038  | 0.0038  | 0.0028  | 0.0035  | 0.0011  | 0.0052  | 0.0029  |                 |
| <sup>65</sup> CuO NP                           | 0.0052  | 0.0038  | 0.0030  | 0.0019  | 0.0019  | 0.0038  | 0.0031  |                 |
| <sup>65</sup> CuO NP                           | 0.0023  | 0.0054  | 0.0033  | 0.0023  | 0.0032  | 0.0060  | 0.0043  |                 |
| <sup>65</sup> CuO NP                           | 0.0043  | 0.0028  | 0.0040  | 0.0032  | 0.0037  | 0.0042  | 0.0041  |                 |
| <sup>65</sup> CuO NP <sup>a</sup>              | 0.0060  | 0.0056  | 0.0038  | 0.0026  | 0.0040  | 0.0054  | 0.0040  |                 |
| <sup>65</sup> CuO NP <sup>a</sup>              | 0.0040  | 0.0038  | 0.0036  | 0.0037  | 0.0032  | 0.0037  | 0.0027  |                 |
| <sup>65</sup> CuO NP <sup>a</sup>              | 0.0040  | 0.0040  | 0.0066  | 0.0038  | 0.0034  | 0.0066  | 0.0057  |                 |
| <b>Mean (<sup>65</sup>CuO NP)</b>              | 0.0041  | 0.0044  | 0.0038  | 0.0033  | 0.0033  | 0.0048  | 0.0036  | 0.0039          |
| <b>sd</b>                                      | ±0.0012 | ±0.0009 | ±0.0011 | ±0.0010 | ±0.0011 | ±0.0011 | ±0.0009 | ±0.0005         |

<sup>a</sup> Worms used for determination of <sup>65</sup>Cu WSBB.

**Table S4. Number of *T. tubifex* fed to each of the fish during the experiment.**

| Treatment         | Fish ID | Day of feeding |   |   |   |   |   |   |
|-------------------|---------|----------------|---|---|---|---|---|---|
|                   |         | 1              | 2 | 3 | 4 | 5 | 6 | 7 |
| Control           | 04A     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 04C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 10A     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 10B     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 10C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 13A     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 13C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 16A     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
| CuCl <sub>2</sub> | 02C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 05C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 08B     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 08C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 11A     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 11B     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 11C     | 7              | 7 | 7 | 7 | 5 | 6 | 6 |
|                   | 17B     | 7              | 7 | 7 | 7 | 5 | 6 | 6 |
| CuO NP            | 03C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 06A     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 06B     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 09A     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 09B     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 09C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 12A     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |
|                   | 12C     | 7              | 7 | 7 | 7 | 6 | 6 | 6 |

Table S5. Fish-specific food rations and dietary exposure doses.

|                   |         |     | Fish ww [g]     |                | Food ration |       |       |       |       |       |       |               |                          |                                                                    | Dietary exposure dose |  |
|-------------------|---------|-----|-----------------|----------------|-------------|-------|-------|-------|-------|-------|-------|---------------|--------------------------|--------------------------------------------------------------------|-----------------------|--|
|                   |         |     | Before exposure | After exposure | [g ww worm] |       |       |       |       |       |       |               | [g dw worm] <sup>1</sup> | [ $\mu\text{g } ^{65}\text{Cu g}^{-1} \text{ fish}$ ] <sup>2</sup> |                       |  |
| Treatment         | Fish ID | Sex |                 |                | Day 1       | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Daily average | Total                    | Total                                                              | Total                 |  |
| Control           | 04A     | m   | 0.506           | 0.502          | 0.028       | 0.035 | 0.026 | 0.026 | 0.025 | 0.027 | 0.019 | 0.027         | 0.186                    |                                                                    |                       |  |
|                   | 04C     | m   | 0.586           | 0.562          | 0.028       | 0.035 | 0.026 | 0.026 | 0.025 | 0.027 | 0.019 | 0.027         | 0.186                    |                                                                    |                       |  |
|                   | 10A     | f   | 0.686           | 0.689          | 0.028       | 0.035 | 0.026 | 0.026 | 0.025 | 0.027 | 0.019 | 0.027         | 0.186                    |                                                                    |                       |  |
|                   | 10B     | f   | 0.611           | 0.609          | 0.028       | 0.035 | 0.026 | 0.026 | 0.025 | 0.027 | 0.019 | 0.027         | 0.186                    |                                                                    |                       |  |
|                   | 10C     | m   | 0.733           | 0.649          | 0.028       | 0.035 | 0.026 | 0.026 | 0.025 | 0.027 | 0.019 | 0.027         | 0.186                    |                                                                    |                       |  |
|                   | 13A     | m   | 0.682           | 0.593          | 0.028       | 0.035 | 0.026 | 0.026 | 0.025 | 0.027 | 0.019 | 0.027         | 0.186                    |                                                                    |                       |  |
|                   | 13C     | m   | 0.794           | 0.725          | 0.028       | 0.035 | 0.026 | 0.026 | 0.025 | 0.027 | 0.019 | 0.027         | 0.186                    |                                                                    |                       |  |
|                   | 16A     | f   | 0.563           | 0.575          | 0.028       | 0.035 | 0.026 | 0.026 | 0.025 | 0.027 | 0.019 | 0.027         | 0.186                    |                                                                    |                       |  |
|                   |         |     |                 |                |             |       |       |       |       |       |       |               | 0.026                    | 0                                                                  |                       |  |
| CuCl <sub>2</sub> | 02C     | f   | 0.512           | 0.513          | 0.029       | 0.026 | 0.025 | 0.027 | 0.023 | 0.027 | 0.020 | 0.025         | 0.178                    |                                                                    | 0.032                 |  |
|                   | 05C     | f   | 0.503           | 0.514          | 0.029       | 0.026 | 0.025 | 0.027 | 0.023 | 0.027 | 0.020 | 0.025         | 0.178                    |                                                                    | 0.032                 |  |
|                   | 08B     | f   | 0.643           | 0.610          | 0.029       | 0.026 | 0.025 | 0.027 | 0.023 | 0.027 | 0.020 | 0.025         | 0.178                    |                                                                    | 0.027                 |  |
|                   | 08C     | m   | 0.580           | 0.682          | 0.029       | 0.026 | 0.025 | 0.027 | 0.023 | 0.027 | 0.020 | 0.025         | 0.178                    |                                                                    | 0.024                 |  |
|                   | 11A     | f   | 0.785           | 0.740          | 0.029       | 0.026 | 0.025 | 0.027 | 0.023 | 0.027 | 0.020 | 0.025         | 0.178                    |                                                                    | 0.022                 |  |
|                   | 11B     | m   | 0.643           | 0.595          | 0.029       | 0.026 | 0.025 | 0.027 | 0.023 | 0.027 | 0.020 | 0.025         | 0.178                    |                                                                    | 0.028                 |  |
|                   | 11C     | m   | 0.638           | 0.608          | 0.029       | 0.026 | 0.025 | 0.027 | 0.019 | 0.027 | 0.020 | 0.025         | 0.174                    |                                                                    | 0.026                 |  |
|                   | 17B     | f   | 0.645           | 0.574          | 0.029       | 0.026 | 0.025 | 0.027 | 0.019 | 0.027 | 0.020 | 0.025         | 0.174                    |                                                                    | 0.028                 |  |
|                   |         |     |                 |                |             |       |       |       |       |       |       |               | 0.025                    | 0.028 ± 0.003                                                      |                       |  |
| CuO NP            | 03C     | m   | 0.528           | 0.487          | 0.028       | 0.031 | 0.026 | 0.023 | 0.020 | 0.029 | 0.022 | 0.026         | 0.179                    |                                                                    | 0.059                 |  |
|                   | 06A     | m   | 0.637           | 0.605          | 0.028       | 0.031 | 0.026 | 0.023 | 0.020 | 0.029 | 0.022 | 0.026         | 0.179                    |                                                                    | 0.047                 |  |
|                   | 06B     | f   | 0.733           | 0.675          | 0.028       | 0.031 | 0.026 | 0.023 | 0.020 | 0.029 | 0.022 | 0.026         | 0.179                    |                                                                    | 0.042                 |  |
|                   | 09A     | m   | 0.650           | 0.661          | 0.028       | 0.031 | 0.026 | 0.023 | 0.020 | 0.029 | 0.022 | 0.026         | 0.179                    |                                                                    | 0.043                 |  |
|                   | 09B     | f   | 0.684           | 0.639          | 0.028       | 0.031 | 0.026 | 0.023 | 0.020 | 0.029 | 0.022 | 0.026         | 0.179                    |                                                                    | 0.045                 |  |
|                   | 09C     | f   | 0.619           | 0.61           | 0.028       | 0.031 | 0.026 | 0.023 | 0.020 | 0.029 | 0.022 | 0.026         | 0.179                    |                                                                    | 0.047                 |  |
|                   | 12A     | f   | 0.656           | 0.625          | 0.028       | 0.031 | 0.026 | 0.023 | 0.020 | 0.029 | 0.022 | 0.026         | 0.179                    |                                                                    | 0.046                 |  |
|                   | 12C     | f   | 0.585           | 0.564          | 0.028       | 0.031 | 0.026 | 0.023 | 0.020 | 0.029 | 0.022 | 0.026         | 0.179                    |                                                                    | 0.051                 |  |
|                   |         |     |                 |                |             |       |       |       |       |       |       |               | 0.026                    | 0.048 ± 0.005                                                      |                       |  |

<sup>1</sup>calculated using average dw/ww-ratio of T. tubifex worms (0.14 ± 0.04, n = 42).

<sup>2</sup>calculated based on measured <sup>65</sup>Cu tissue concentration in T. Tubifex worms after <sup>65</sup>CuCl<sub>2</sub> and <sup>65</sup>CuO NP exposure (0.65 ± 0.38 and 1.12 ± 1.03  $\mu\text{g g}^{-1}$  dw tissue, respectively; n = 14).

**Table S6. Newly added  $^{65}\text{Cu}$  and background Cu concentrations in diet, feces, intestine, liver, and carcass of three-spined stickleback following 7-day dietary exposure to *T. tubifex* worms collected from clean,  $^{65}\text{CuCl}_2$ - and  $^{65}\text{CuO}$  NP- spiked sediment.** Values represent the mean  $\pm$  sd. No significant differences between the means of the  $^{65}\text{CuCl}_2$ - and  $^{65}\text{CuO}$  NP-exposure were detected (t-test).

| Sample               | Measure       |                                                 | <i>n</i> | Control               | $^{65}\text{CuCl}_2$ | $^{65}\text{CuO}$ NP |
|----------------------|---------------|-------------------------------------------------|----------|-----------------------|----------------------|----------------------|
| New $^{65}\text{Cu}$ |               |                                                 |          |                       |                      |                      |
| Diet (worms)         | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 14       | $0.155 \pm 0.333$     | $0.647 \pm 0.379$    | $1.117 \pm 1.030$    |
|                      | Daily dose    | $[\mu\text{g g}^{-1} \text{ ww fish day}^{-1}]$ | 14       |                       | $0.004 \pm 0.001$    | $0.007 \pm 0.001$    |
|                      | Total dose    | $[\mu\text{g g}^{-1} \text{ ww fish}]$          | 14       |                       | $0.028 \pm 0.003$    | $0.048 \pm 0.005$    |
|                      | Total amount  | $[\mu\text{g}]$                                 | 14       |                       | $0.016 \pm 0.001$    | $0.029 \pm 0.000$    |
| Feces                | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 8        | $0.153 \pm 0.259$     | $6.291 \pm 2.390$    | $13.553 \pm 9.925$   |
| Intestine            | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 8        | $0.033 \pm 0.055$     | $0.081 \pm 0.038$    | $0.065 \pm 0.074$    |
| Liver                | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 8        | $0.000 \pm 0.000$     | $0.054 \pm 0.094$    | $0.011 \pm 0.030$    |
| Remainder            | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 8        | $0.006 \pm 0.007$     | $0.007 \pm 0.008$    | $0.010 \pm 0.010$    |
| Background Cu        |               |                                                 |          |                       |                      |                      |
| Diet (worms)         | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 14       | $22.316 \pm 9.634$    |                      |                      |
| Feces                | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 8        | $117.453 \pm 100.616$ |                      |                      |
| Intestine            | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 8        | $9.528 \pm 1.499$     |                      |                      |
| Liver                | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 8        | $389.771 \pm 100.903$ |                      |                      |
| Remainder            | Concentration | $[\mu\text{g g}^{-1} \text{ dw}]$               | 8        | $4.100 \pm 0.792$     |                      |                      |

**Table S7: Correlation analysis of gene expression levels ( $\Delta C_q$ ) and  $^{65}\text{Cu}$  tissue concentration ( $\mu\text{g g}^{-1}$  dw) in intestinal tissue of fish fed with worms collected from  $^{65}\text{CuCl}_2$ -spiked sediment.** The cells in the first, second and third row show the correlation coefficient (Pearson's  $r$ ),  $p$ -value and number of independent samples ( $n$ ) for each variable pair. The pair(s) of variables with positive correlation coefficients and  $p$  values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and  $p$  values below 0.050, one variable tends to decrease while the other increases. For pairs with  $p$  values greater than 0.050, there is no significant relationship between the two variables (Pearson Product Moment Correlation, SigmaPlot for Windows Version 14.0). Statistically significant correlations are highlighted by means of asterisks (\*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ ).

|              | <i>gcl</i> | <i>gr</i> | <i>gpx</i> | <i>sod-1</i> | <i>Cat</i> | <i>zo-1</i> | <i>ctr1</i> | [Cu]      |
|--------------|------------|-----------|------------|--------------|------------|-------------|-------------|-----------|
| <i>mta</i>   | 0.309      | 0.438     | -0.113     | 0.158        | 0.323      | 0.68        | 0.207       | -0.0142   |
|              | 0.457      | 0.277     | 0.791      | 0.709        | 0.436      | 0.0635      | 0.623       | 0.973     |
|              | 8          | 8         | 8          | 8            | 8          | 8           | 8           | 8         |
| <i>gcl</i>   |            | -0.023    | -0.666     | 0.896        | 0.72       | 0.118       | 0.812       | 0.632     |
|              |            | 0.957     | 0.0716     | **0.00263    | *0.0439    | 0.781       | *0.0144     | 0.0925    |
|              |            | 8         | 8          | 8            | 8          | 8           | 8           | 8         |
| <i>gr</i>    |            |           | 0.549      | -0.375       | -0.431     | 0.572       | -0.286      | -0.552    |
|              |            |           | 0.159      | 0.36         | 0.287      | 0.138       | 0.492       | 0.156     |
|              |            |           | 8          | 8            | 8          | 8           | 8           | 8         |
| <i>gpx</i>   |            |           |            | -0.759       | -0.701     | 0.0541      | -0.565      | -0.908    |
|              |            |           |            | *0.029       | 0.0528     | 0.899       | 0.145       | **0.00182 |
|              |            |           |            | 8            | 8          | 8           | 8           | 8         |
| <i>sod-1</i> |            |           |            |              | 0.846      | -0.0757     | 0.864       | 0.75      |
|              |            |           |            |              | **0.0081   | 0.859       | **0.00562   | *0.0321   |
|              |            |           |            |              | 8          | 8           | 8           | 8         |
| <i>cat</i>   |            |           |            |              |            | -0.0654     | 0.941       | 0.606     |
|              |            |           |            |              |            | 0.878       | ***0.000491 | 0.112     |
|              |            |           |            |              |            | 8           | 8           | 8         |
| <i>zo-1</i>  |            |           |            |              |            |             | -0.121      | 0.106     |
|              |            |           |            |              |            |             | 0.775       | 0.803     |
|              |            |           |            |              |            |             | 8           | 8         |
| <i>ctr1</i>  |            |           |            |              |            |             |             | 0.509     |
|              |            |           |            |              |            |             |             | 0.198     |
|              |            |           |            |              |            |             |             | 8         |

**Table S8: Correlation analysis of gene expression levels ( $\Delta C_q$ ) and  $^{65}\text{Cu}$  tissue concentration ( $\mu\text{g g}^{-1}$  dw) in intestinal tissue of fish fed with worms collected from  $^{65}\text{CuO}$  NP-spiked sediment.** The cells in the first, second and third row show the correlation coefficient (Pearson's  $r$ ), p-value and number of independent samples ( $n$ ) for each variable pair. The pair(s) of variables with positive correlation coefficients and p values below 0.050 tend to increase together. For the pairs with negative correlation coefficients and p values below 0.050, one variable tends to decrease while the other increases. For pairs with p values greater than 0.050, there is no significant relationship between the two variables (Pearson Product Moment Correlation, SigmaPlot for Windows Version 14.0). Statistically significant correlations are highlighted by means of asterisks (\*  $p < 0.05$ ).

|                     | <i>mta</i> | <i>gcl</i> | <i>gr</i> | <i>gpx</i> | <i>sod-1</i> | <i>cat</i> | <i>zo-1</i> | <i>ctr1</i> |
|---------------------|------------|------------|-----------|------------|--------------|------------|-------------|-------------|
| <b>[Cu]</b>         | 0.404      | -0.786     | -0.615    | -0.123     | -0.0611      | 0.469      | -0.698      | 0.384       |
|                     | 0.322      | 0.0207     | 0.104     | 0.772      | 0.886        | 0.241      | 0.0544      | 0.347       |
|                     | 8          | 8          | 8         | 8          | 8            | 8          | 8           | 8           |
| <b><i>mta</i></b>   |            | -0.642     | -0.36     | 0.463      | -0.0345      | 0.585      | 0.0394      | 0.421       |
|                     |            | 0.0861     | 0.381     | 0.248      | 0.935        | 0.128      | 0.926       | 0.299       |
|                     |            | 8          | 8         | 8          | 8            | 8          | 8           | 8           |
| <b><i>gcl</i></b>   |            |            | 0.794     | -0.255     | -0.319       | -0.74      | 0.457       | -0.376      |
|                     |            |            | *0.0186   | 0.543      | 0.441        | *0.0357    | 0.254       | 0.358       |
|                     |            |            | 8         | 8          | 8            | 8          | 8           | 8           |
| <b><i>gr</i></b>    |            |            |           | -0.423     | -0.0694      | -0.684     | 0.531       | -0.0754     |
|                     |            |            |           | 0.296      | 0.87         | 0.0614     | 0.176       | 0.859       |
|                     |            |            |           | 8          | 8            | 8          | 8           | 8           |
| <b><i>gpx</i></b>   |            |            |           |            | 0.0627       | 0.519      | -0.0223     | -0.0684     |
|                     |            |            |           |            | 0.883        | 0.187      | 0.958       | 0.872       |
|                     |            |            |           |            | 8            | 8          | 8           | 8           |
| <b><i>sod-1</i></b> |            |            |           |            |              | 0.438      | 0.128       | 0.365       |
|                     |            |            |           |            |              | 0.277      | 0.763       | 0.373       |
|                     |            |            |           |            |              | 8          | 8           | 8           |
| <b><i>cat</i></b>   |            |            |           |            |              |            | -0.0817     | 0.684       |
|                     |            |            |           |            |              |            | 0.848       | 0.0616      |
|                     |            |            |           |            |              |            | 8           | 8           |
| <b><i>zo-1</i></b>  |            |            |           |            |              |            |             | 0.258       |
|                     |            |            |           |            |              |            |             | 0.537       |
|                     |            |            |           |            |              |            |             | 8           |