Supplementary Information

Co-exposure to foodborne and waterborne ZnO nanoparticles in aquatic sediment environments enhances DNA damage and stress genes expression in freshwater Asian clam *Corbicula fluminea*

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Supplementary Figure Captions

Figure S1. Analysis of hydrodynamic diameter of ZnO-NPs in EPA water

The distribution of the hydrodynamic diameter of ZnO-NPs in EPA water (5 mg Zn/L) was analyzed using dynamic light scattering (DLS, Delsa Nano C; Beckman Coulter, CA, USA) after the ZnO-NPs suspension was sonicated.

Figure S2. Kinetic release of ionic Zn from ZnO-NPs in EPA water.

The release of ionic Zn from ZnO-NPs in EPA water (5 mg Zn/L) was analyzed at <0.5, 4, 7, 24, 48, and 72 h after the ZnO-NPs suspension was sonicated. The suspension was filtered through Amicon Ultra-15 Ultracel 3 centrifuge tubes (3 kDa cutoff ≈0.9 nm, Millipore, Billerica, MA) to eliminate undissolved ZnO-NPs. The concentration of Zn ions in the filtrate was measured using inductively coupled plasma - atomic emission spectroscopy (ICP-AES, Spectro Ciros 120, Kleve, Germany). The results are presented as the mean ± SD.

Figure S3. Effect of ZnO-NPs on lipid peroxidation in Asian clam *C. fluminea* in aquatic sediment environments.

Clams were cultured with two exposure routes: “untreated algae” and “ZnO-NPs-pretreated algae + ZnO-NPs” and were sampled and dissected on day 14 during exposure. To assess lipid peroxidation, malondialdehyde (MDA) concentration was measured using the Lipid Peroxidation (MDA) Assay Kit (Sigma-Aldrich Chemicals Co., USA) according to the manufacturer’s

instructions. The MDA concentration was further normalized to the total protein measured with the Coomassie Plus (Bradford) Assay Kit (Thermo Fisher Scientific, Rockford, IL, USA). The results are presented as the mean of two biological trials

Figure S1

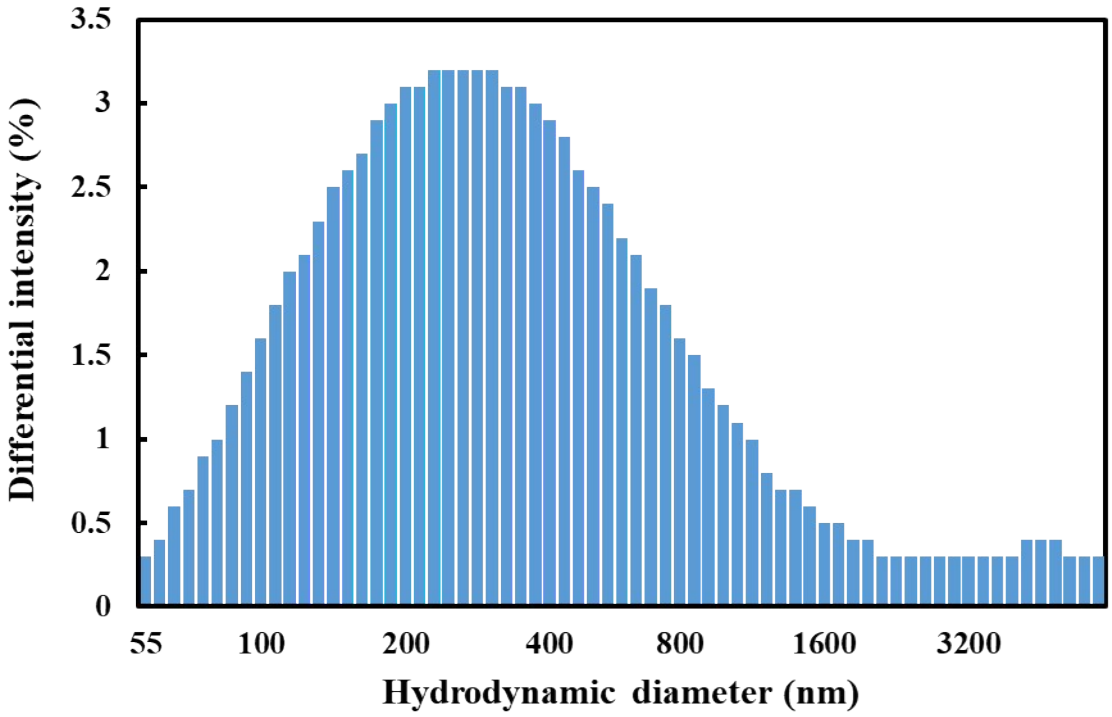

Differential intensity (%)
3.5
3
2.5
2
1.5
1
0.5
0
55
100
200
400
800
1600
3200
Hydrodynamic diameter (nm)

Figure S2

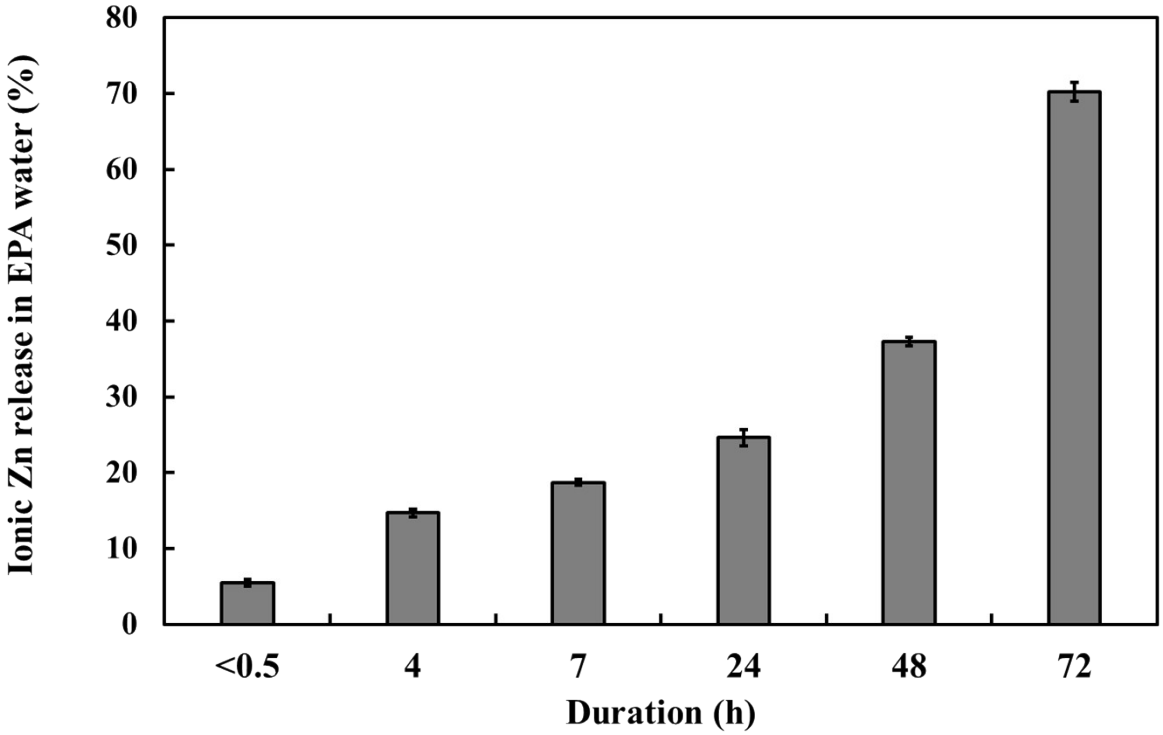
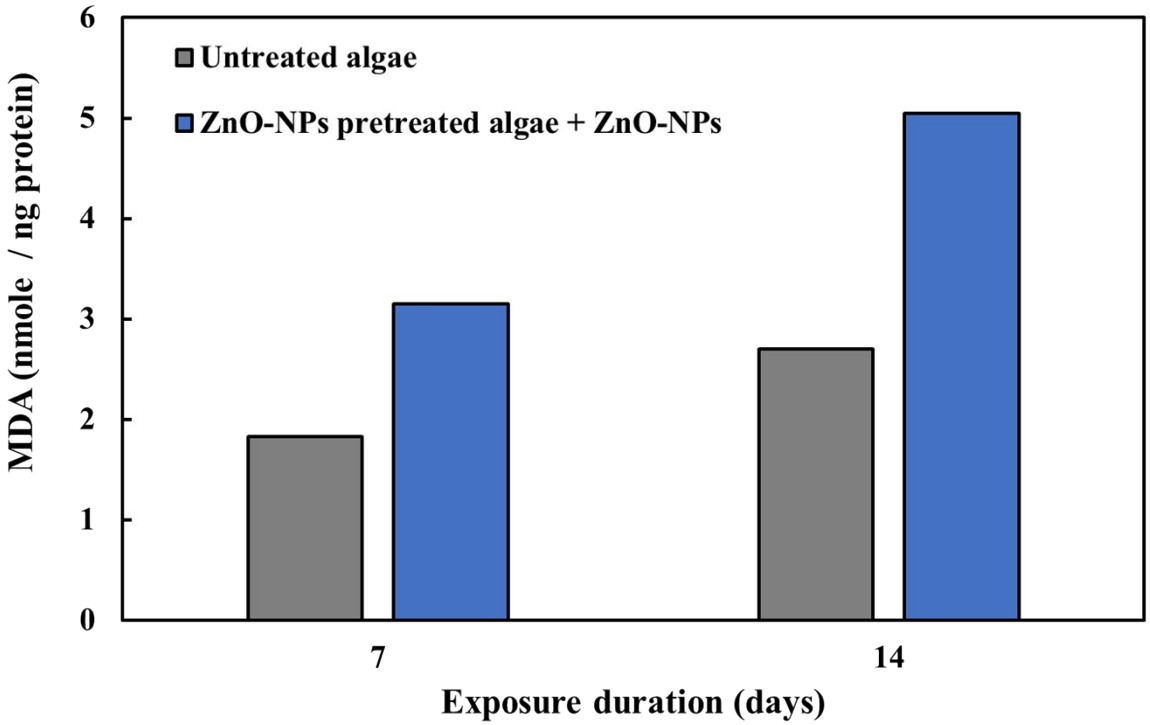

Ionic Zn release in EPA water (%)
80
70
60
50
40
30
20
10
0
<0.5
4
7
24
48
72
Duration (h)

Figure S3


Untreated algae
ZnO-NPs pretreated algae + ZnO-NPs
MDA (nmole / ng protein)
0
1
2
3
4
5
6
7
14
Exposure duration (days)