## Supporting Information

# Engineered Nanoselenium Supplemented Fish Diet: Toxicity Comparison with Ionic Selenium and Stability against Particle Dissolution, Aggregation and Release

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**S1. ICP-MS and ScICP-MS Performance Conditions**

All spICP-MS measurements were performed on a PerkinElmer NexION 300D ICP-MS operating in a single-particle mode, using the conditions in Table S1.

**Table S1.** ICP-MS and spICP-MS operating conditions

| Parameter/Component | Value/Description |
|---|---|
| Sample Uptake Rate | 0.27 mL/min |
| Nebulizer | MEINHARD HEN |
| Nebulizer gas flow | 1.1 L/min |
| Spray Chamber | Glass cyclonic |
| RF Power | 1600 W |
| Analytes | $^{80}$ Se |
| Dwell Time | 50 s |

**S2. Electron Microscope**

Both samples of Se-ENNs in MQ water and internalized in tissues were pictured at room temperature using a SEM Magellan 400L equipped with an energy dispersive spectroscopy (EDS) detector Octane Elect Super (EDAX) [1]. Secondary electrons micrographs were detected by an in-chamber Everhart-Thornley detector. Transmitted electrons (bright and dark field STEM signals) were captured by a retractable-segmented semiconductor detector (STEM III). For all SEM investigations, an acceleration voltage of 30 kV was used, and the used probe current was in the range from 50 pA to 3.2 nA depending on the acquired signal type. In the case of picturing Se-ENNs in MQ water using the SEM, the samples were investigated on a copper grid with a density of 300 mesh covered by a thin carbon layer [2].

**S3. The Percentage of Different Mineral Components in the Commercial Fish Diets**

The percentage of the different components in the diets based on information provided by the manufacturer are reported in Table S2.

**Table S2.** The mineral ingredient of the commercial diet used in this study.

| Composition | Amount |
|---|---|
| Calcium | < 15000 mg/kg |
| Phosphorus | < 20000 mg/kg |
| Sodium | > 5000 mg/kg |
| Manganese | 40 mg/kg |
| Potassium | 3321 mg/kg |
| Copper | 10 mg/kg |
| Iron | 220 mg/kg |
| Zinc | 170 mg/kg |
| Selenium | 1.7 mg/kg |
| Iodine | 5 mg/kg |

**S4. Condition of the Used Tap Water**

The aged tap water was used with basic parameters: temperature 21 ± 1°C; total ammonia 0.02 mg/L; $NO_3$ 1.55 mg/L; $NO_2$ 0.006 mg/L; chemical oxygen demand 0.6 mg/L, $PO_4$ 3-1 ± 0.01 mg/L, $Ca^{2+}$ 32.9 ± 0.8 mg/L, and $Mg^{2+}$ 2.9 ± 0.0 mg/L.

**S5. Analysis of the Swimming Activity**

Mostly, fish behavior investigations are based on analyses by expert observations and/or monitoring tools, such as video recording of the behavior and subsequent offline analysis of video records [3]. Video analyses of the behavioral data are less time consuming and minimize the subjective interpretation of the data.

The swimming behavior analysis operates by detection of individual fishes from the image obtained by the 3D camera and applies image-processing methods to detect the fish, determine the 3D position in the region of interest, create a fish short-term trajectory, and calculate appropriate features (speed,

orientation, length of trajectory, etc.). The monitoring system was located on the top of each aquarium and it provided the 3D trajectories of the fish group (Figure S1). The monitoring was conducted for 40 minutes of swimming activity (Figure S2). The monitoring was performed simultaneously for all aquaria. The video output from the camera for all the controls and treatments was automatically quantified for swimming activity behavior by software implemented in-house (Figure S2) for fish 3D position processing [4]. The software captured the position of the fish in each axes as x, y and z coordinates, together with a timestamp (Figure S3). The average swimming speed was provided by the system for each experimental group separately. The movement of each fish was recorded without any disturbances. The average velocity of the fish per second was used to determine the swimming activity. The swimming activity was calculated on the frame-by-frame basis as the ratio between the length of the fish trajectory and the time of swimming was determined. The obtained data were then transformed into centimeters per second for individual fish and averaged for the fish group.

**Figure S1**. Positioning of 3D camera on top of the aquarium for the monitoring. Five 3D cameras were moved in between 21 aquariums to perform the monitoring.

**Figure S2**. The monitoring system consist of the 3D camera and computer with the software for real-time determination of fish 3D positions. One system monitors one aquarium at a time.

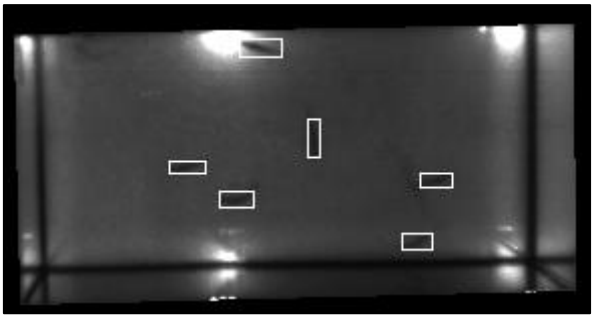


**Figure S3**. The infrared image of the 3D camera with detected fish. The area outside aquarium is masked by black colour. The detected fish are marked by white rectangles.

**S6. Physicochemical Characterization of the Se-ENNs in MQ water**

The hydrodynamic size (nm) and the SEM measured size of the 60 nm and 120 nm Se-ENNs measured in MQ water (Table S3).

**Table S3.** size and zeta potential of the Se-ENNs in MQ water

| **Sample** | **Particle hydrodynamic size [nm]** | **SEM measured size [nm] (average of 100 counts)** | **Zeta potential mV** |
|---|---|---|---|
| 60 nm Se ENNs | 168-215 | 54-82 | -24 ± 0.2 |
| 120 nm Se ENNs | 253-321 | 106-153 | -21 ± 0.4 |

**S7. Characterization of the Se-ENNs Using Scanning Electron Microscope**

In the case of the SEM analysis of Se ENNs in MQ water, the samples were investigated on a carbon layer covered copper grids with a density of 300 mesh. The carbon layer was prepared by carbon evaporation on a freshly pinched mica sheet in the sputter coater ACE600 (Leica Microsystems). Thickness of the layer was estimated by oscillating crystal to approximately 8 nm. During the sample preparation, a solution of particles was dropped on the carbon coated grid in an amount of 7 µl, left to sediment for 10 seconds, and washed by one drop of bi-distilled water. To ensure hydrophilicity, the pure carbon layer placed on the grids was before application of the sample placed under glow discharge for 10 s.

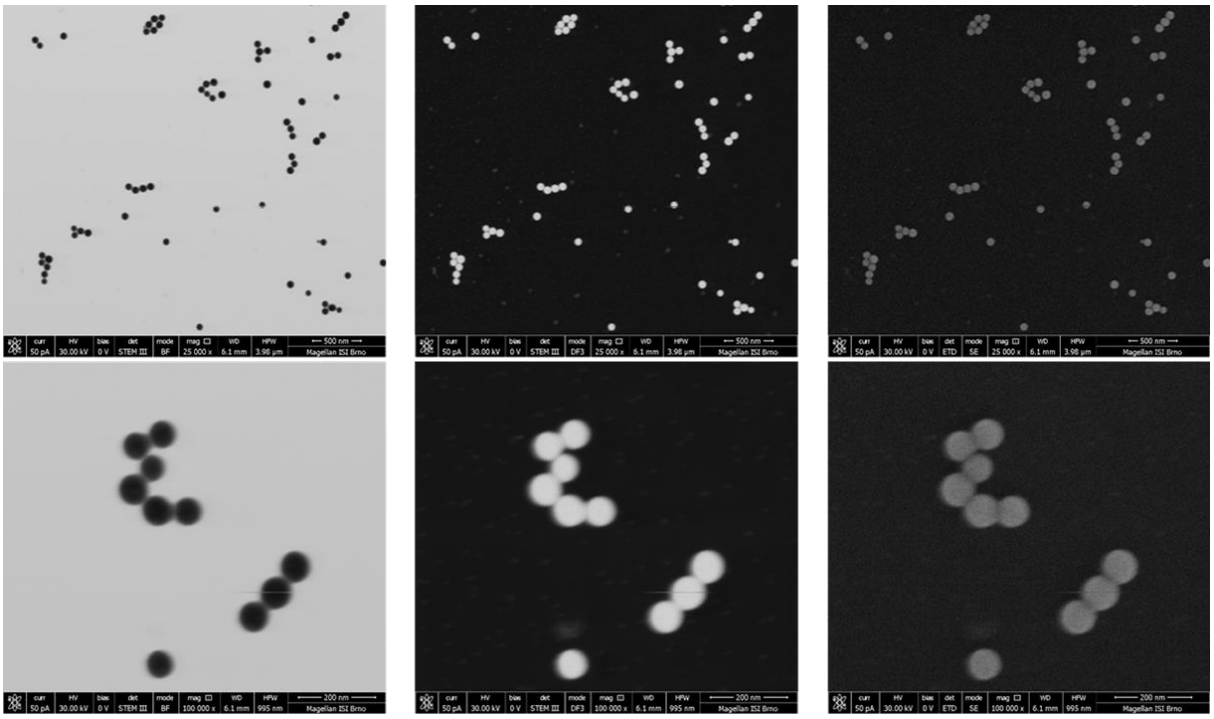
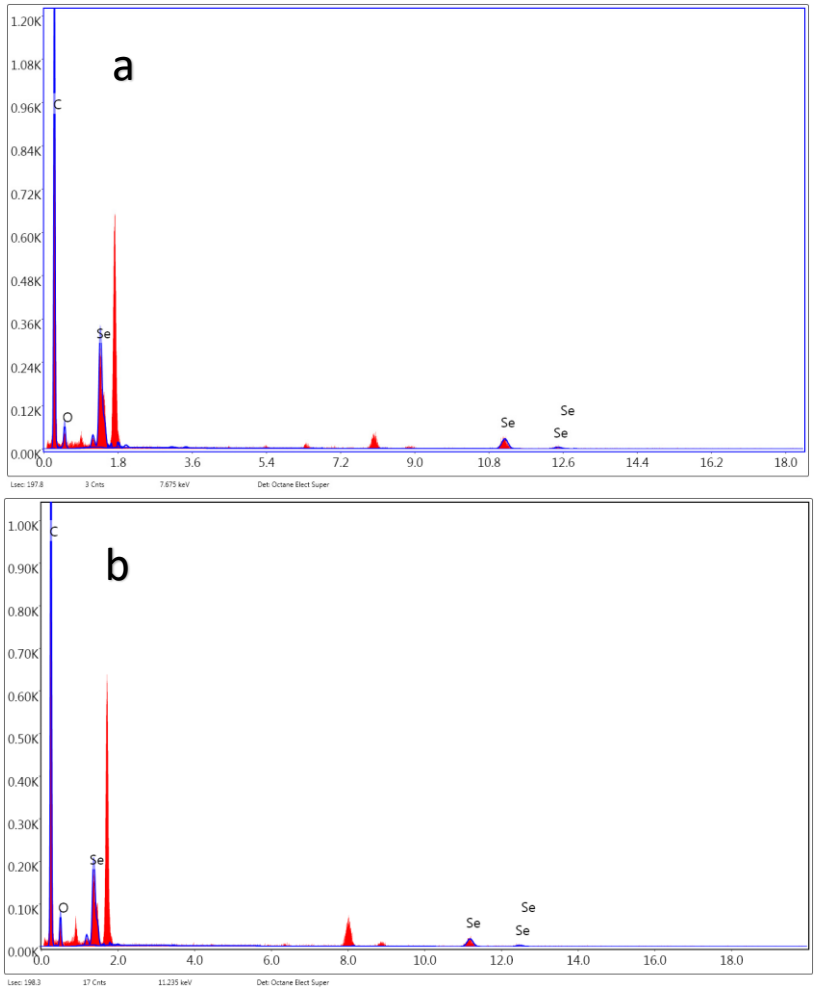


**Figure S4**. Images of 60 nm Se-ENNs in BF, HAADF and ETD



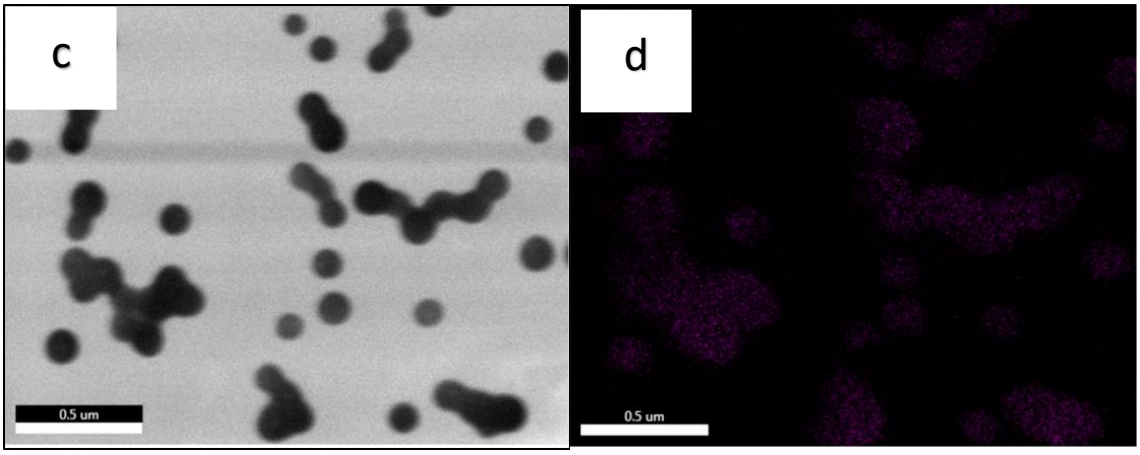



**Figure S5.** The EDX spectrum of 60 nm (a) and 120 nm (b) particles in the MQ water. c) The EDX map for 120 nm particles in MQ water. d) The Elemental overlay map of the 120 nm particles.

**References**

1. S. Marvanová, P. Kulich, R. Skoupý, F. Hubatka, M. Ciganek, J. Bendl, J. Hovorka and M. Machala, Size-segregated urban aerosol characterization by electron microscopy and dynamic light scattering and influence of sample preparation, *Atmospheric Environment*, 2018, **178**, 181-190.
2. V. Krzyzanek, N. Sporenberg, U. Keller, J. Guddorf, R. Reichelt and M. Schönhoff, Polyelectrolyte multilayer capsules: nanostructure and visualisation of nanopores in the wall, *Soft Matter*, 2011, **7**, 7034-7041.
3. E. S. Mikhailova and A. O. Kasumyan, Taste preferences and feeding behavior in threespine stickleback Gasterosteus aculeatus in marine and fresh waters, *Journal of Ichthyology*, 2010, **50**, 795-807.
4. M. M. Saberioon and P. Cisar, Automated multiple fish tracking in three-Dimension using a Structured Light Sensor, *Computers and Electronics in Agriculture*, 2016, **121**, 215-221.