Supporting Information

for Global Challenges, DOI: 10.1002/gch2.202100091

ZnO Nanomaterials and Ionic Zn Partition within Wastewater Sludge Investigated by Isotopic Labeling

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Table S1. Summary of past literature on the behavior of ZnO nanomaterials in wastewater media, compared to this work.

| Wastewater media and their characteristics | ZnO ENM characteristics a) | Spiking concentration [µg g⁻¹] | Incubation period | References |
|------------------------------------------|---------------------------|-------------------------------|-------------------|------------|
| Primary sludge, Anglian Water, UK (pH = 5.2) | NPs: 7.6 nm in diameter | 10.2 | 4 h | This work |
| Activated sludge from California, USA | Three types of NPs | 2, 10 | 7 days | Smeraldi et al.¹¹ |
| Simulated influent (pH = 7) | Highly heterogeneous in size (10−130 nm) and shape | 5, 10, 20 | 21 days | Chaüque et al.²² |
| Real sewage from South Australia, Australia | NPs: 22.9 nm in diameter | 700 | 3 days | Brunetti et al.³¹ |
| Simulated sludge (humic acid; pH = 4.5) | Nanorods: 139 nm in diameter and ~5.3 in aspect ratio | 1000 | 3 h | Gomez-Gonzalez et al.⁴⁴ |
| A mixture of primary and activated sludge from South Australia, Australia (pH = 7.2) | Three types of NPs: a. 30−40 nm in diameter | 1000 | 10 days | Lombi et al.⁵⁵ |
|                                           | c. Co-doped ZnO with hydrodynamic size d = 35 nm | | | |

a) Unless otherwise specified, NP size/diameter refers to bare size as measured by transmission or scanning electron microscopy.
Standard operating procedure for the synthesis of $^{68}$ZnO nanoparticles

1) 35 mL acetic acid are heated to 89°C under stirring and with use of a reflux condenser.

2) 390 mg $^{68}$Zn metal are added to the acetic acid and the reaction is left to run for 72 hours.

3) After cooling, the $^{68}$Zn acetate present in the bottom of the vessel is removed, transferred to a petri dish and dried in an oven at 60°C overnight.

4) 100 mg $^{68}$Zn acetate is dispersed in 50 mL diethylene glycol (DEG) by stirring for 15 min. The glass beaker with this mixture is then placed in oven at 60°C for 72 hours.

5) Silicone oil is heated in a metal or glass bath to 177°C. Once a stable temperature has been reached, the glass beaker with the DEG-$^{68}$Zn acetate mixture (covered with a foil lid with pierced holes) is placed in the silicone oil bath.

6) When the DEG-$^{68}$Zn acetate mixture reaches 170°C, 100 μL ultrapure water is added to hydrolyze the $^{68}$Zn acetate and the mixture is then stirred at 350 rpm.

7) The DEG starts to turn yellow after about 30 minutes; at this point the glass beaker is removed from the silicone oil bath and left to cool.
Figure S1. Procedure followed for sieving the sludge samples and general view.
### Table S2. Summary of the primary sludge samples studied with and without enriched Zn addition.

| Samples  | Added species | Incubation time | Characteristics                                                                 |
|----------|---------------|-----------------|---------------------------------------------------------------------------------|
| 1        | Solid         | -               | Solid sludge (>500 µm) with no added ZnO NPs                                   |
| 2        | Solid         | $^{68}$ZnO $^{64}$ZnCl$_2$ | 30 min | Solid sludge (>500 µm) with labeled Zn added, and incubated during 30 min under orbital shaking |
| 3        | Solid         | $^{68}$ZnO $^{64}$ZnCl$_2$ | 4 h     | Solid sludge (>500 µm) with labeled Zn added, and incubated during 4 hours under orbital shaking |
| 4        | Liquid        | -               | Liquid sludge with no added ZnO NPs                                           |
| 5        | Liquid        | $^{68}$ZnO $^{64}$ZnCl$_2$ | 30 min | Liquid sludge with labeled Zn added, and incubated during 30 min under orbital shaking |
| 6        | Liquid        | $^{68}$ZnO $^{64}$ZnCl$_2$ | 4 h     | Liquid sludge with labeled Zn added, and incubated during 4 hours under orbital shaking |
| 7        | UF fraction   | -               | Ultrafiltered fraction (< 2-3 nm) with no added ZnO NPs                      |
| 8        | UF fraction   | $^{68}$ZnO $^{64}$ZnCl$_2$ | 30 min | Ultrafiltered fraction (< 2-3 nm) with labeled Zn added, incubated during 30 min under orbital shaking |
| 9        | UF fraction   | $^{68}$ZnO $^{64}$ZnCl$_2$ | 4 h     | Ultrafiltered fraction (< 2-3 nm) with labeled Zn added, incubated during 4 hours under orbital shaking |
Table S3. Summary of the concentrations and masses of natural and the two enriched Zn species measured by MC-ICP-MS for the experimental systems.

| Sample                  | Concentration of Zn species [µg g⁻¹]  | Mass of Zn species µg for 10 g sludge sample b) |
|-------------------------|----------------------------------------|-------------------------------------------------|
|                         | Natural Zn  | ⁶⁸Zn-en | ⁶⁴Zn-en | Natural Zn  | ⁶⁸Zn-en | ⁶⁴Zn-en |
| Solid (no Zn added)     | 45.5        | 0.00    | 0.01    | 18.7        | 0.00    | 0.00    |
| Solid 30 min            | 50.9        | 30.5    | 14.9    | 21.0        | 12.6    | 6.14    |
| Solid 4 h               | 44.6        | 24.1    | 11.4    | 18.4        | 9.91    | 4.69    |
| Liquid (no Zn added)    | 6.26        | 0.0     | 0.0     | 60.1        | 0.00    | 0.00    |
| Liquid 30 min           | 2.02        | 2.31    | 1.15    | 19.4        | 22.2    | 11.0    |
| Liquid 4 h              | 2.96        | 2.65    | 1.25    | 28.4        | 25.4    | 12.0    |
| UF (no Zn added)        | 0.89        | 0.00    | 0.00    | 8.52        | 0.00    | 0.00    |
| UF 30 min               | 1.35        | 2.67    | 1.33    | 12.9        | 25.6    | 12.7    |
| UF 4 h                  | 1.90        | 3.01    | 1.43    | 18.2        | 28.9    | 13.7    |

a) The unit µg g⁻¹ denotes µg of detected Zn species per gram of a given phase (solid, liquid, or UF = ultrafiltrate) of the primary sludge.

b) Mass of Zn present in the three phases of the experimental system with 10 g of sludge; 10 g of sludge encompass 0.412 g solid and 9.588 g liquid. The Zn mass amounts and concentrations have a bias of less than 8%.
Figure S2. Zn mass budget, showing re-equilibration of Zn species within sludge samples over time. Slight variations observed from 30 min to 4 h reflect slow re-equilibration of Zn within the experimental system, including the surfaces of the polyethylene bottles (see text for details). The Zn mass amounts have a repeatability and bias of less than 1 and 8%, respectively.
Table S4. Calculated solid-liquid partition coefficients for the added $^{68}$Zn-en and $^{64}$Zn-en.

|                  | $^{68}$ZnO NPs |                  | $^{64}$ZnCl$_2$ salt |                  |
|------------------|----------------|------------------|----------------------|------------------|
|                  | Concentration of $^{68}$Zn in the solid [µg g$^{-1}$] | Concentration of $^{68}$Zn in the liquid [µg g$^{-1}$] | Solid-liquid partition coefficient $D_{\text{SL}}$ | Concentration of $^{64}$Zn in the solid [µg g$^{-1}$] | Concentration of $^{64}$Zn in the liquid [µg g$^{-1}$] | Solid-liquid partition coefficient $D_{\text{SL}}$ |
| **30 min**       | 30.5           | 2.31             | 13.2                 | 14.9             | 1.33          | 12.9          |
| **4 h**          | 24.1           | 2.65             | 9.1                  | 11.4             | 1.43          | 9.1           |
References

[1] J. Smeraldi, R. Ganesh, T. Hosseini, L. Khatib, B. H. Olson, D. Rosso, *Water Environ. Res.* **2017**, 89, 880.

[2] E. F. C. Chaüque, J. N. Zvimba, J. C. Ngila, N. Musee, *Water SA* **2016**, 42, 72.

[3] G. Brunetti, E. Donner, G. Laera, R. Sekine, K. G. Scheckel, M. Khaksar, K. Vasilev, G. De Mastro, E Lombi, *Water Res.* **2015**, 77, 72.

[4] M. A. Gomez-Gonzalez, M. A. Koronfel, A. E. Goode, M. Al-Ejji, N. Voulvoulis, J. E. Parker, P. D. Quinn, T. B. Scott, F. Xie, M. L. Yallop, A. E. Porter, M. P. Ryan, *ACS Nano* **2019**, 13, 11049.

[5] E. Lombi, E. Donner, E. Tavakkoli, T. W. Turney, R. Naidu, B. W. Miller, K. G. Scheckel, *Environ. Sci. Technol.* **2012**, 46, 9089.

[6] M. A. Gomez-Gonzalez, M. A. Koronfel, H. Pullin, J. E. Parker, P. D. Quinn, M. D. Inverno, T. B. Scott, F. Xie, N. Voulvoulis, M. L. Yallop, M. P. Ryan, A. E. Porter, *Adv. Sustain. Syst.* **2021**, 5, 2100023.

[7] A. L. Fabricius, L. Duester, B. Meermann, T. A. Ternes, *Anal. Bioanal. Chem.* **2014**, 406, 467.