

Measurement of engineered nanomaterial exposure in microorganisms using direct and indirect methods

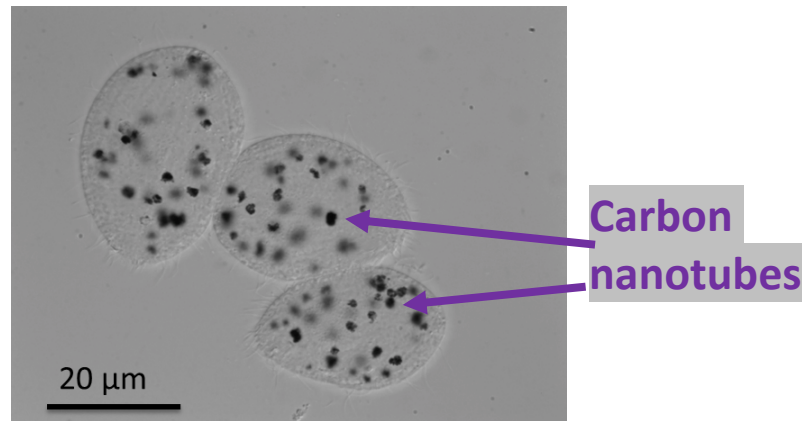
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Naresh Devarajan, Patricia A. Holden*

University of California, Santa Barbara

QEEN II: 2nd Quantifying Exposure to Engineered Nanomaterials from
Manufactured Products Workshop

October 9, 2018

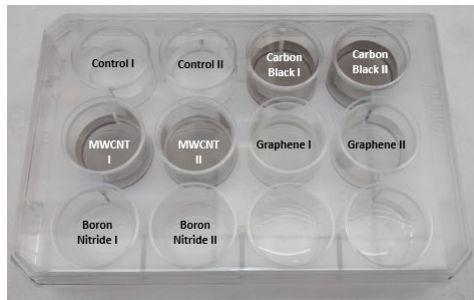
Bioaccumulation – an important exposure-related parameter



Protozoa *Tetrahymena thermophila* exposed to multiwall carbon nanotubes

Microscopy and image analysis for measuring ENM uptake and depuration kinetics

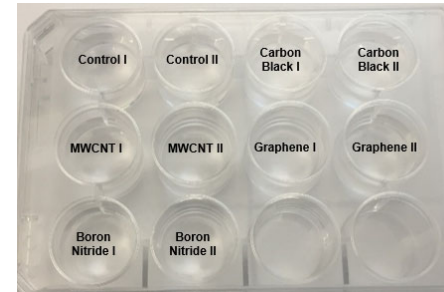
Exposure to 10 mg/L of nanomaterials (uptake)



Protozoa transferred to fresh medium



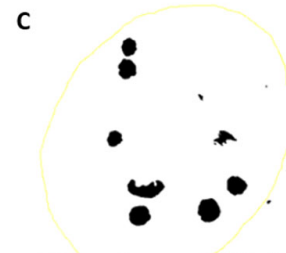
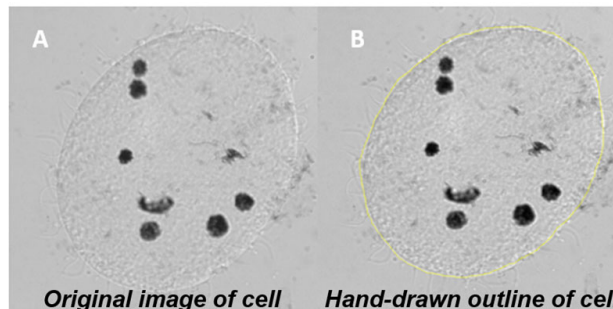
Depuration



Sampling for microscopy



Image analysis

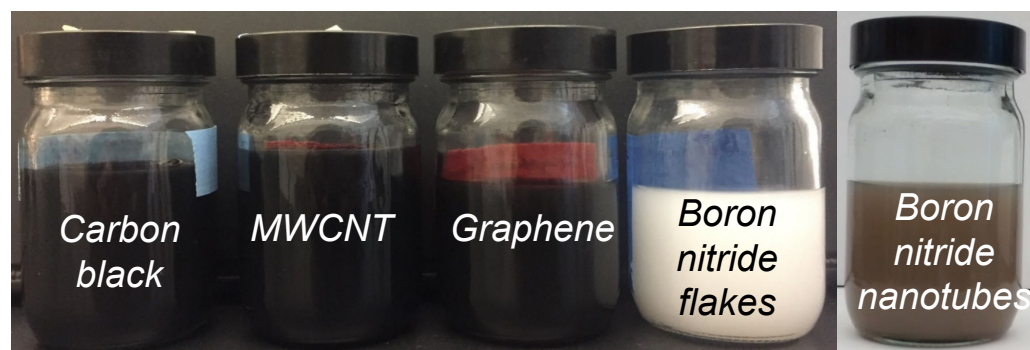


Binary image of filled vacuoles

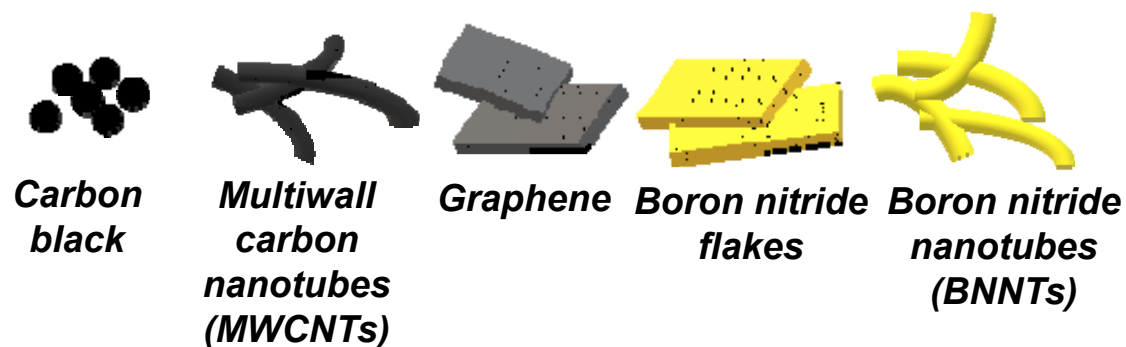


Particle analysis of filled vacuoles

How do shape and chemistry affect ENM uptake and depuration rates?



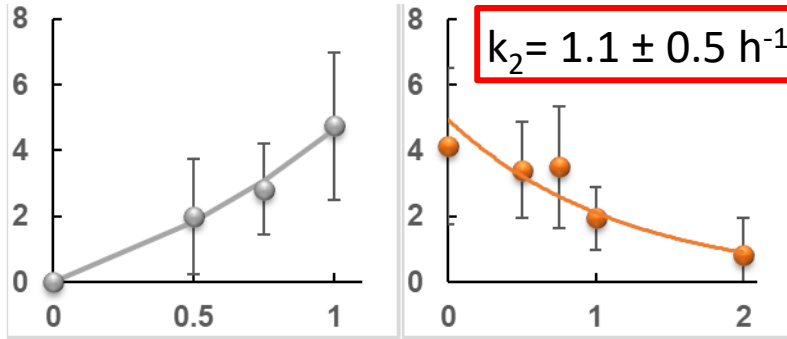
Nanomaterials (200 mg/L) were dispersed in Nanopure water with 400 mg/L alginic acid by probe sonication



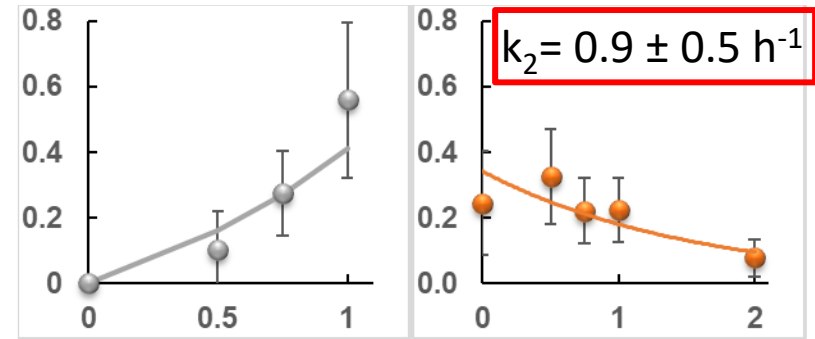
Uptake and depuration kinetics results

y-axis: ENM concentration in protozoa, $\text{mg kg}^{-1} \text{ dw} \times 10^3$

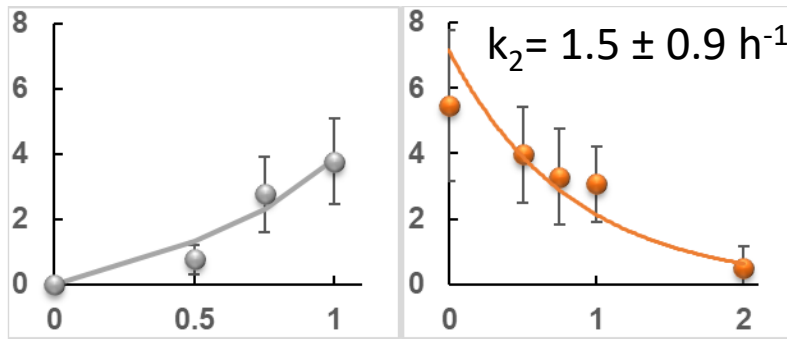
Multiwall carbon nanotubes (MWCNTs)



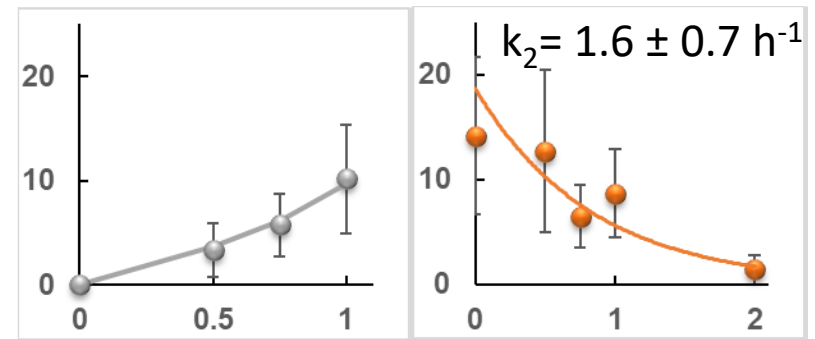
Boron nitride nanotubes (BNNTs)



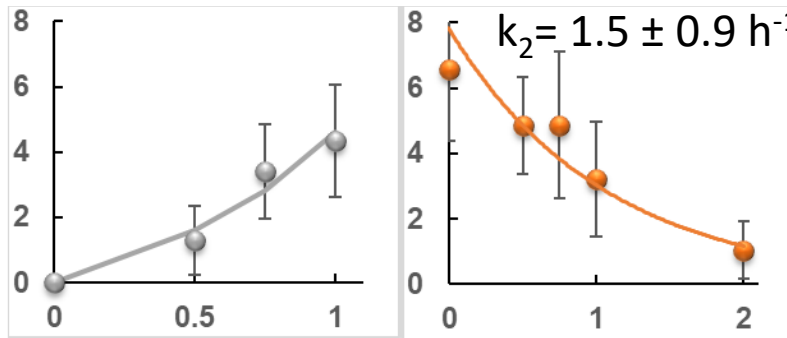
Graphene



Boron nitride flakes



Carbon black



x-axis: Time (h)

ENM shape-dependent depuration

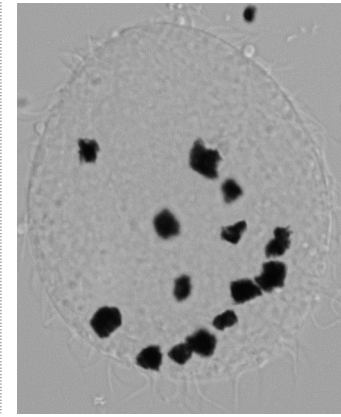
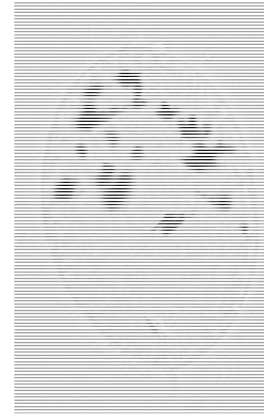
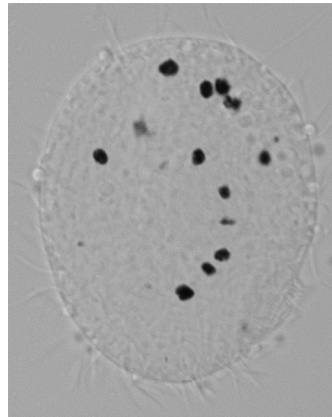
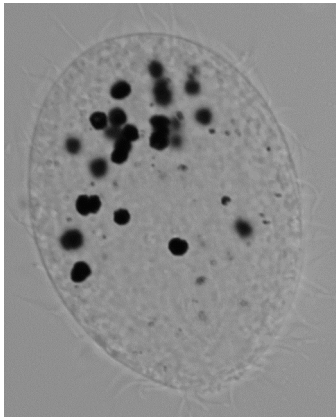
Carbon black



Graphene



Multiwall carbon nanotubes (MWCNTs)



$k_2 = 1.5 \pm 0.9 \text{ h}^{-1}$

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$k_2 = 1.1 \pm 0.5 \text{ h}^{-1}$

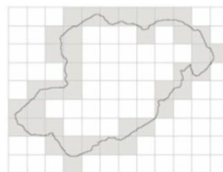
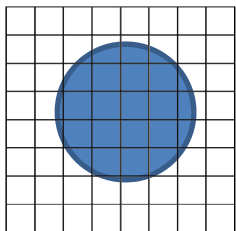
Spherical food vacuoles

Polygonal food vacuoles

Fractal dimension:
 1.020 ± 0.05

Fractal dimension:
 1.064 ± 0.07

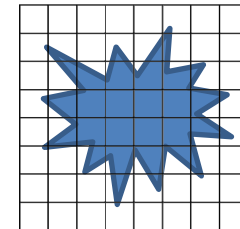
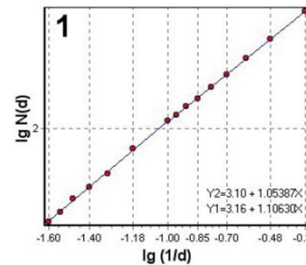
Fractal dimension:
 1.096 ± 0.05



N = 38
d = 30 pixels



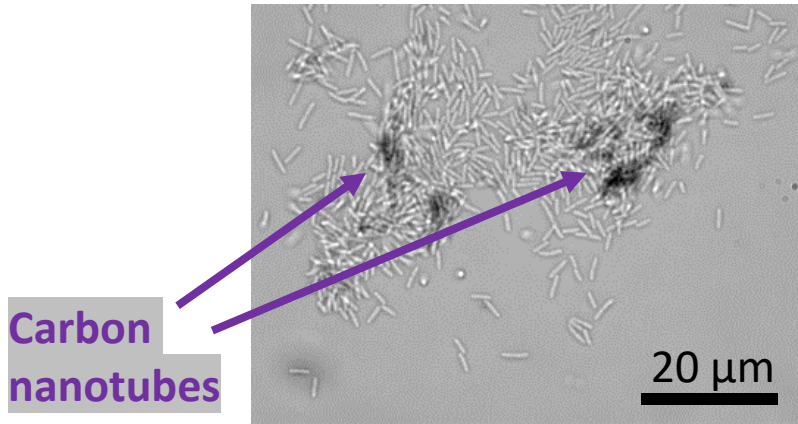
N = 59
d = 20 pixels



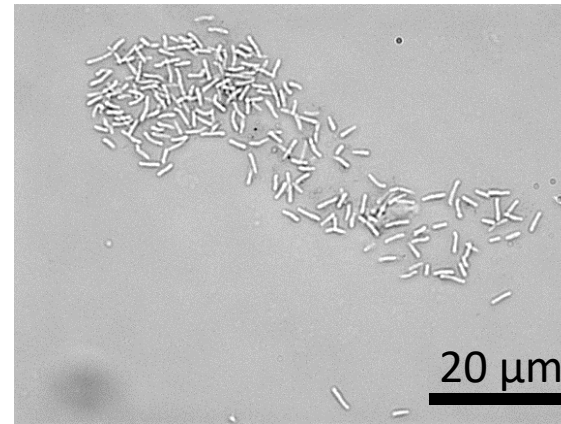
Advantages of microscopy-based approach

- Easy quantification of phagocytosed carbon-based ENMs
- Robust comparison of uptake and depuration of different ENMs
- Acquisition of both single-cell and population level information
- Do not require ENM extraction from cells prior to quantification

Indirect measurement of ENM exposure

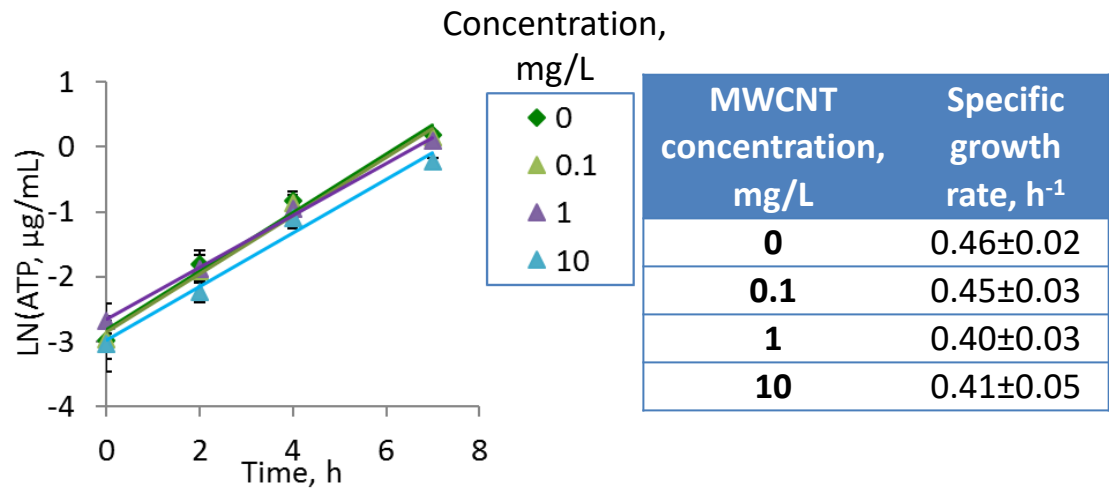


Pseudomonas aeruginosa
exposed to 10 mg/L MWCNTs

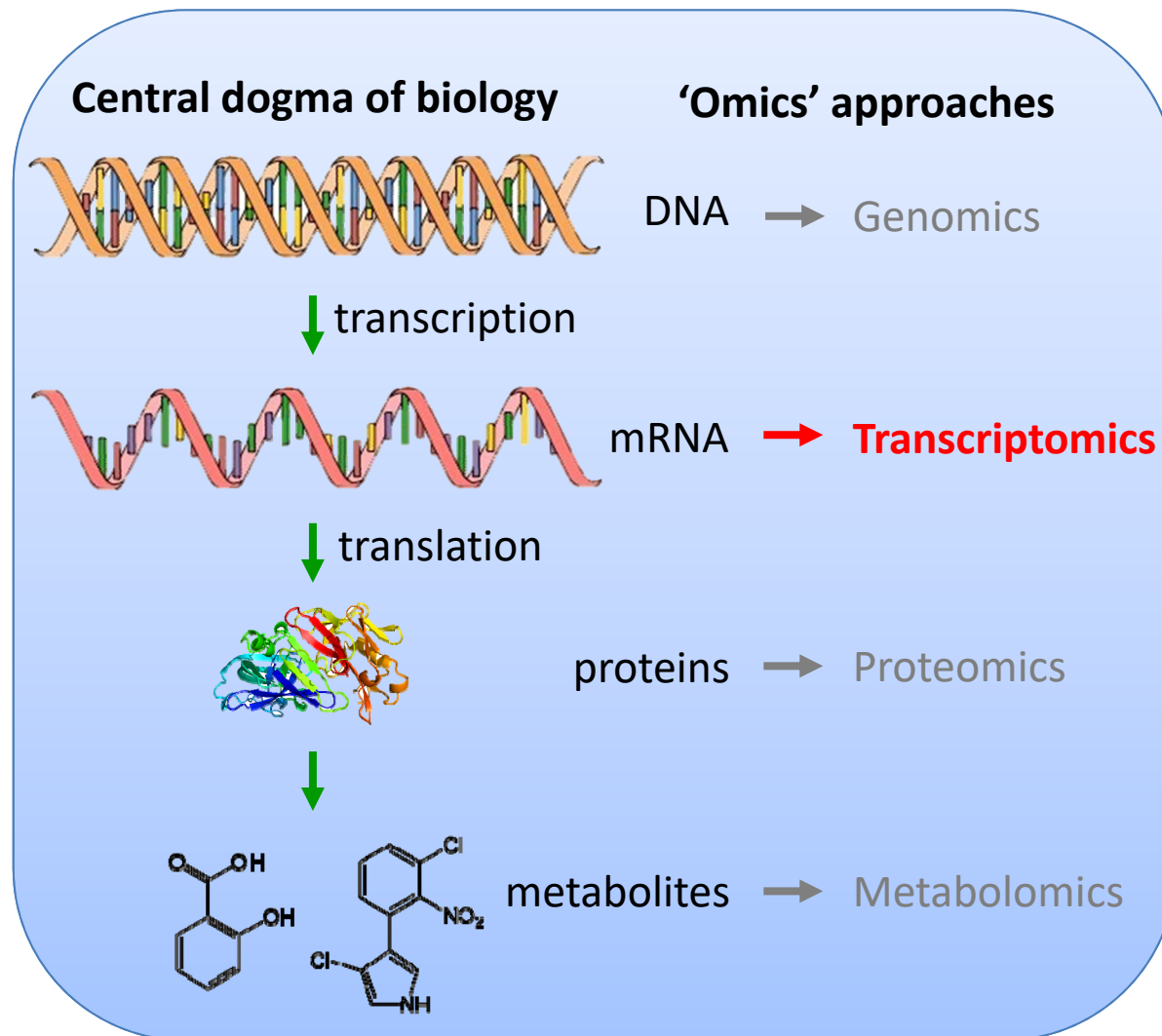


Bradyrhizobium diazoefficiens
exposed to 10 mg/L MWCNTs

MWCNTs did not inhibit bacterial growth or induce significant membrane damage

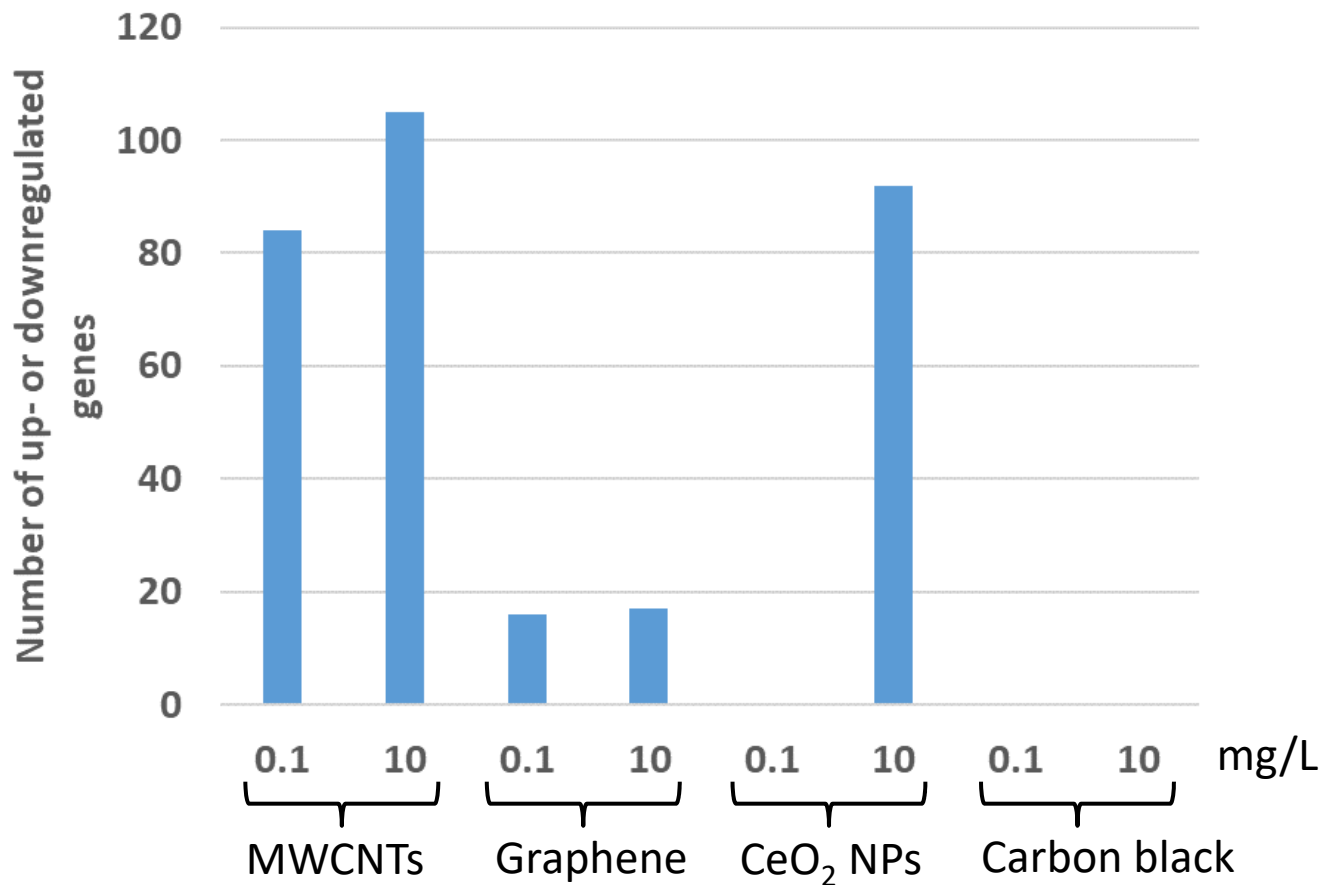


Omics-Based Approaches in ENM Exposure Assessment



Global transcriptomic response to ENMs at non-growth-inhibitory concentrations

Bacteria *B. diazoefficiens* were grown in ENM-amended medium and gene expression was quantified using RNA sequencing



Summary

- Microscopy-based approach enabled easy and robust measurement of ENM uptake and depuration kinetics.
- Tubular ENMs had longer residence times in protozoa, thus may have a higher potential for harmful effects and trophic transfer.
- Non-targeted 'omics approaches are promising in developing biomarker-based methods for indirect ENM exposure assessment.

Acknowledgements

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