



Current research in bio-nano interactions

In vivo experiments

Professor and Associate Dean Zheng Hai-Lei, PhD and Professor Damjana Drobne, PhD

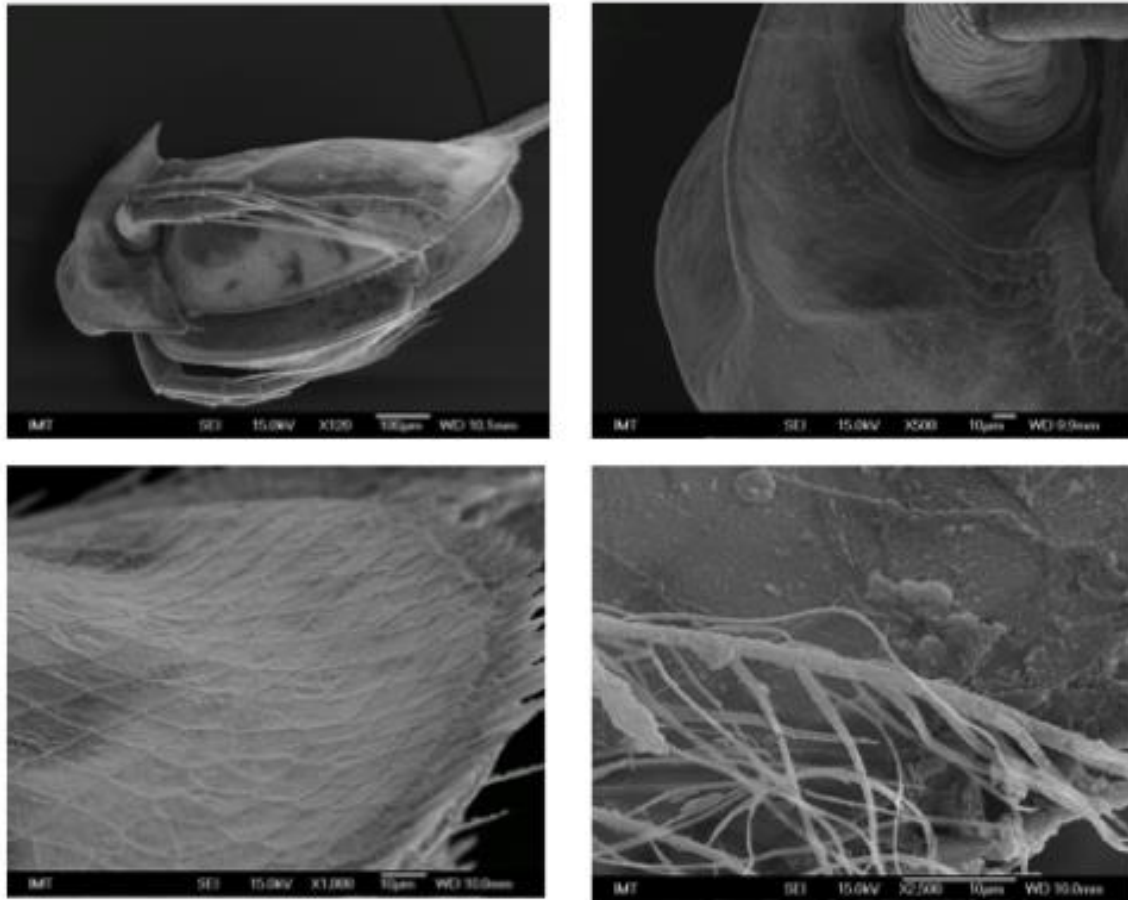
Dr. Sara Novak

Nanobiology and Nanotoxicology Research Group

Xiamen University, 18.9.2015

Water flea *DAPHNIA MAGNA*

Adsorbtion of NPs



After exposing *D.magna* to TiO₂ NPs (ISO 6341:2013) no acute toxicity was observed.
NPs were adsorbed to body surface of daphnids.

Brine shrimp ARTEMIA SALINA

Ingestion of NPs

Aquatic Toxicology 163 (2015) 121–129



Contents lists available at ScienceDirect

Aquatic Toxicology

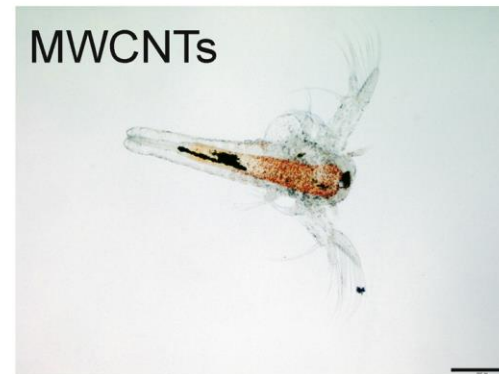
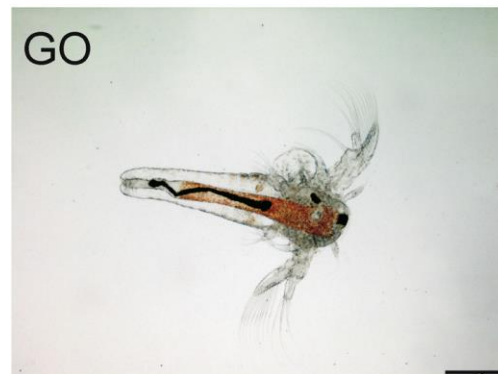
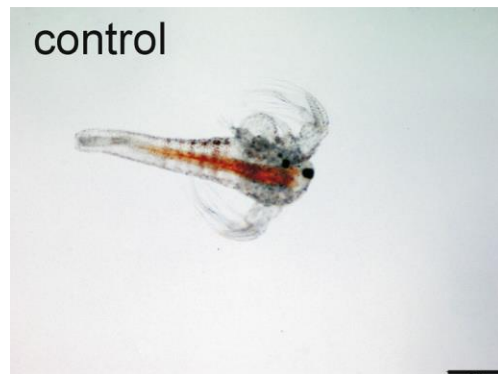
journal homepage: www.elsevier.com/locate/aquatox



High surface adsorption properties of carbon-based nanomaterials are responsible for mortality, swimming inhibition, and biochemical responses in *Artemia salina* larvae



Tina Mesarič^a, Chiara Gambardella^b, Tamara Milivojević^a, Marco Faimali^b,
Damjana Drobne^{a,c,d}, Carla Falugi^e, Darko Makovec^f, Anita Jemec^a, Kristina Sepčić^{a,*}



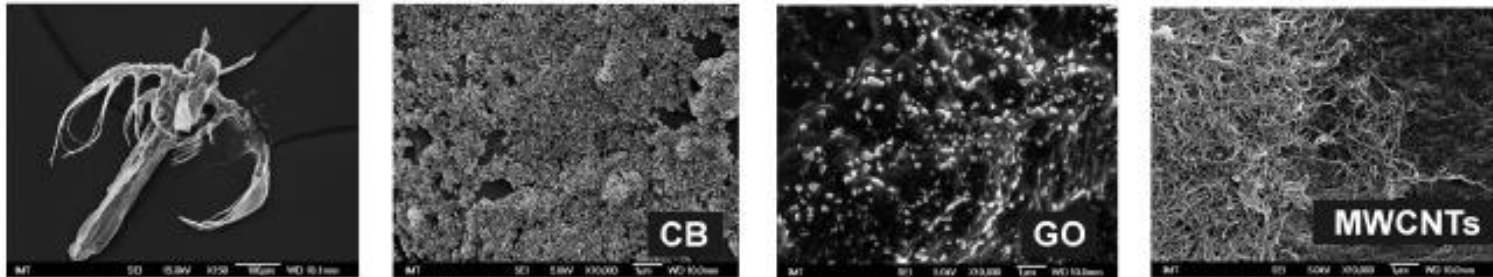
Brine shrimp *ARTEMIA SALINA*

Adsorption of NPs

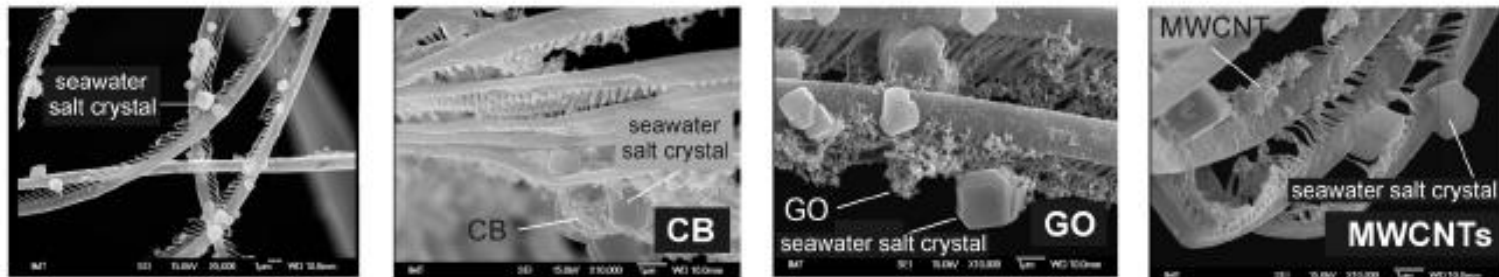
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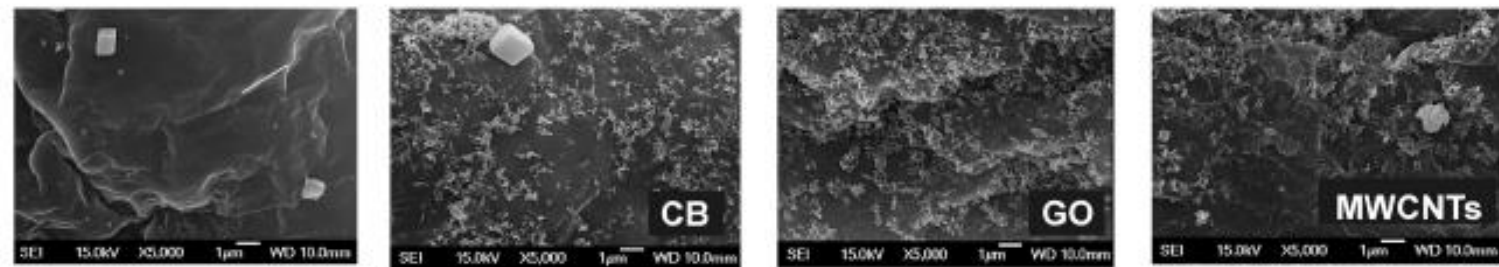
Nanomaterials on membrane surface



Nanomaterials on *A. salina* gills

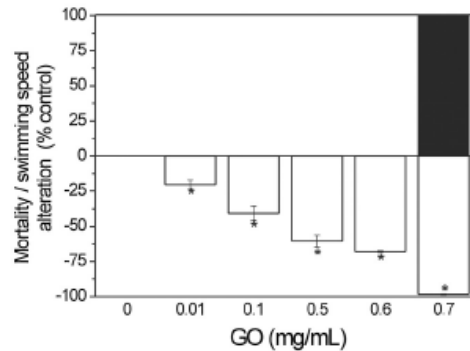
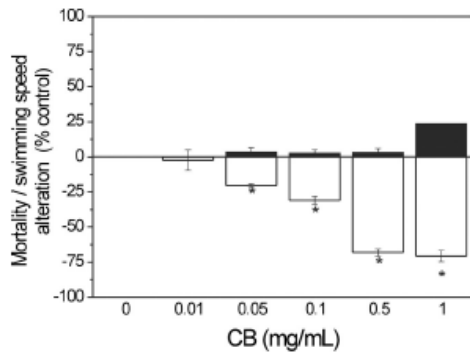


Nanomaterials on *A. salina* abdomen

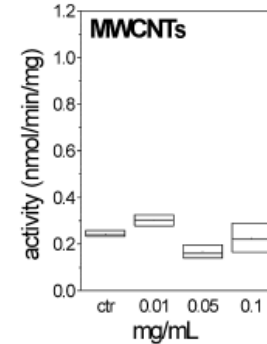
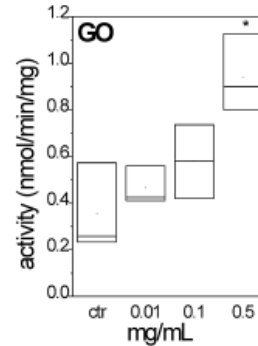
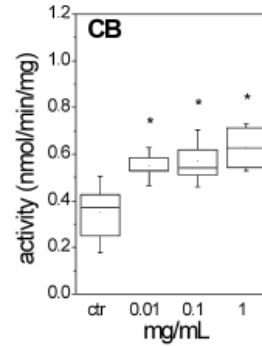


Brine shrimp ARTEMIA SALINA

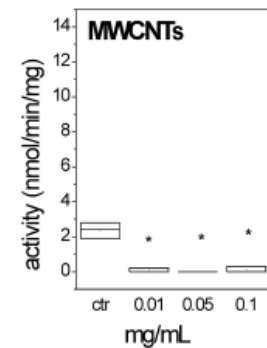
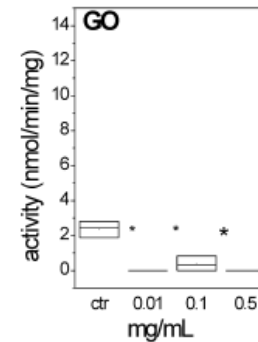
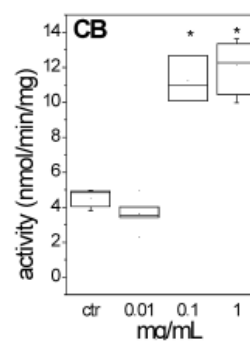
Mortality and biochemical biomarkers



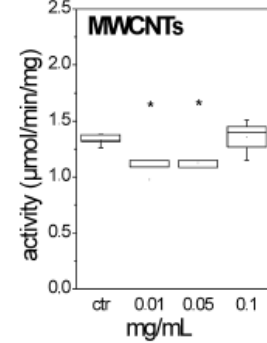
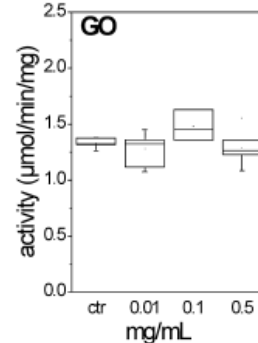
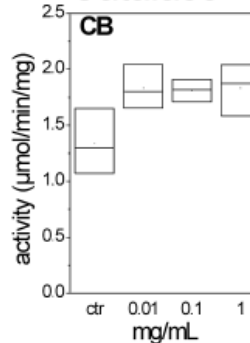
Cholinesterase



Glutathione-S-transferase



Catalase



SEA URCHIN

% fertilisation



Aquatic Toxicology 163 (2015) 158–166



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Aquatic Toxicology

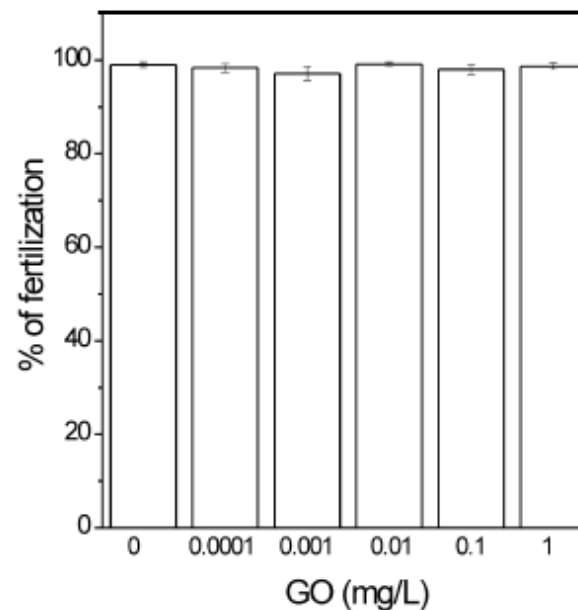
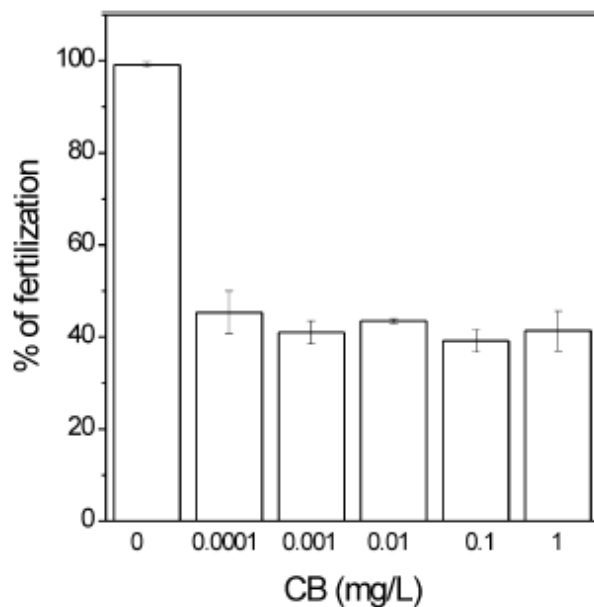
journal homepage: www.elsevier.com/locate/aquatox



Sperm exposure to carbon-based nanomaterials causes abnormalities in early development of purple sea urchin (*Paracentrotus lividus*)



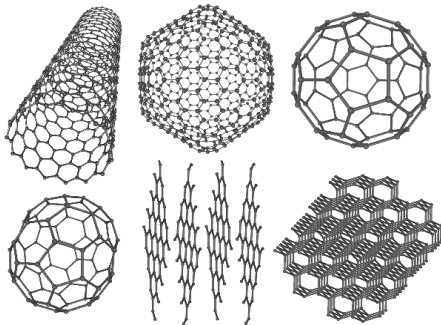
Tina Mesarič^a, Kristina Sepčić^a, Damjana Drobne^{a,b}, Darko Makovec^{b,c}, Marco Faimali^d, Silvia Morgana^d, Carla Falugi^e, Chiara Gambardella^{d,*}



Cholinergic signalling system



CHOLINERGIC system has a central role in the fertilisation of sea urchin.



SEA URCHIN

Abnormalities in skeleton

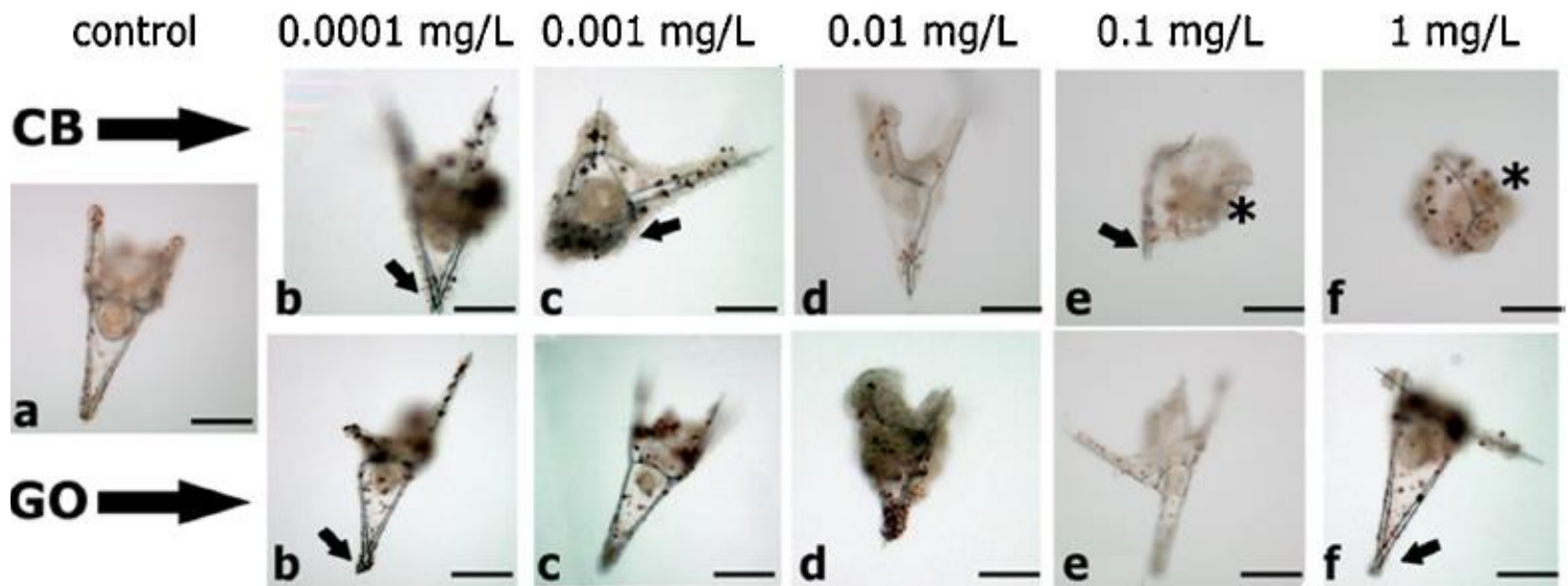


Fig. 4. Representative morphological features of embryos 48 h (A) and 72 h (B) after egg fertilisation (pluteus stages) by the *P. lividus* sperm exposed to CB or GO (as indicated). Asterisks, incomplete or erroneous migration of skeleton elements; arrows, anomalous arrangement of skeletal rods (crossed tips). Scale bar: 50 μ m.

SEA URCHIN Abnormalities

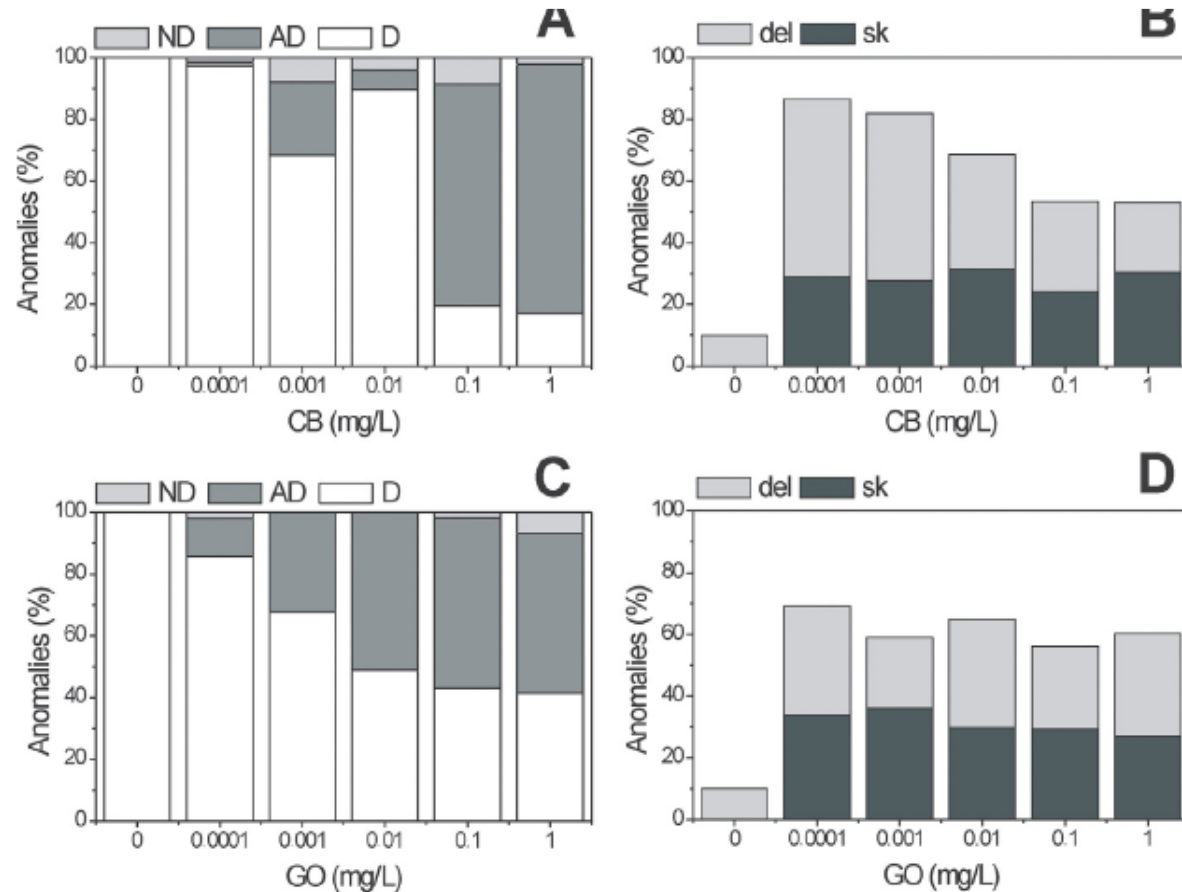
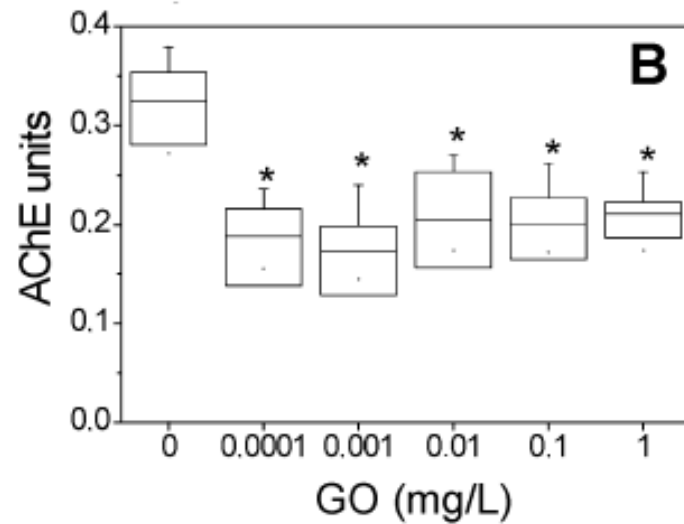
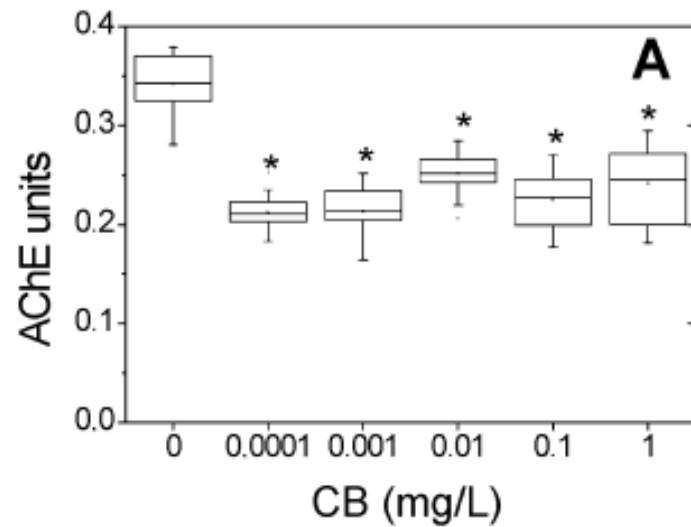


Fig. 2. Larval development anomalies and their classification following egg fertilisation by the *P. lividus* sperm exposed for 1 h to CB (A and B) and GO (C and D), according to the 24-h-old gastrulae (A and C) and 72-h-old plutei (B and D) and the exposure concentrations, as compared to the controls (0). ND, non-developed gastrulae or arrested development; AD, anomalous development; D, normal development; del, delayed forms; sk, skeletal anomalies. Data are means $\pm$ standard error of three independent experiments.

SEA URCHIN

AChE activities



Honeybee *APIS MELLIFERA*

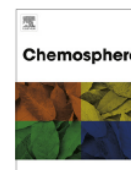
Chemosphere 120 (2015) 547–554



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Chemosphere

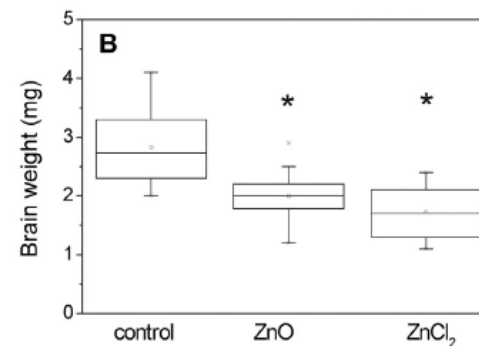
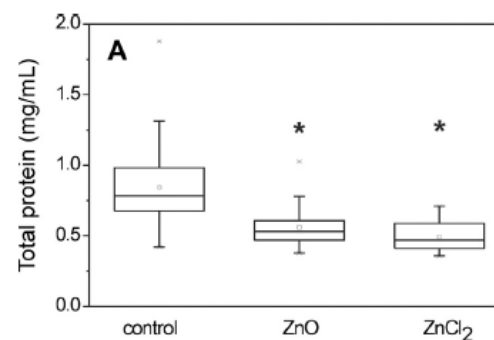
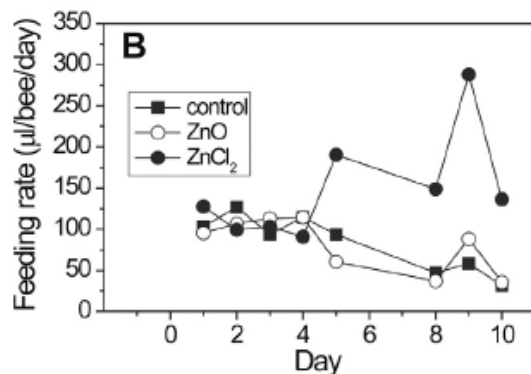
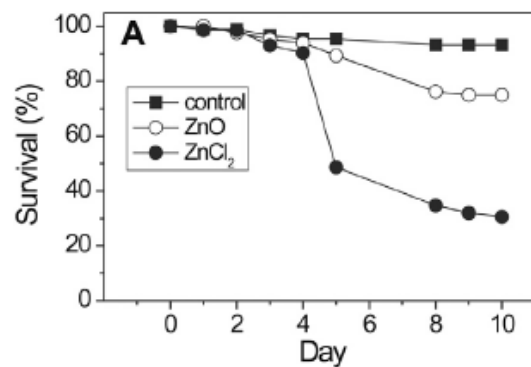
journal homepage: www.elsevier.com/locate/chemosphere



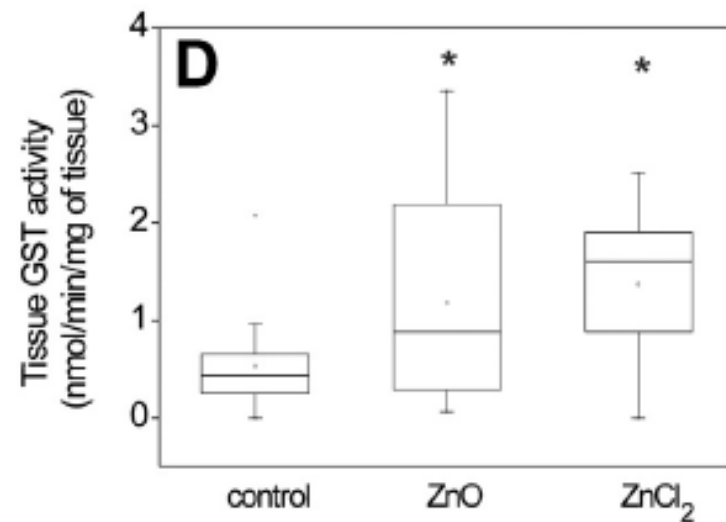
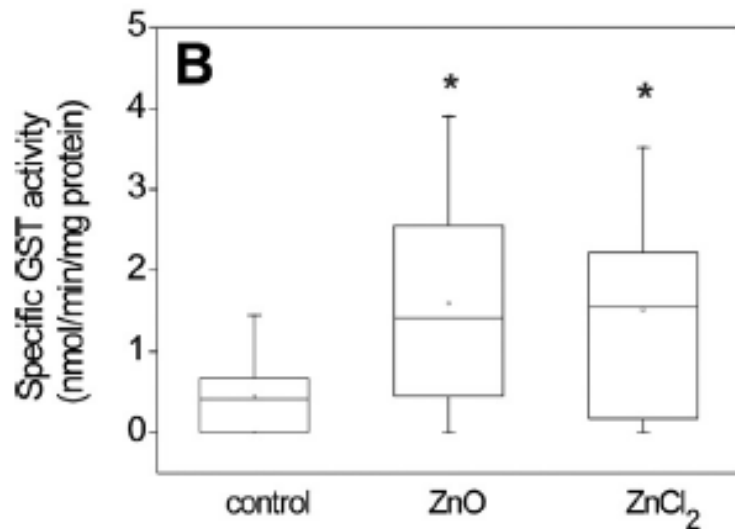
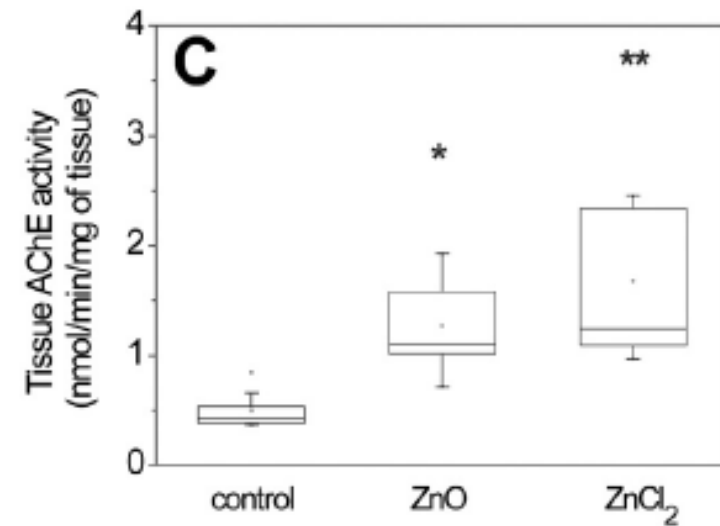
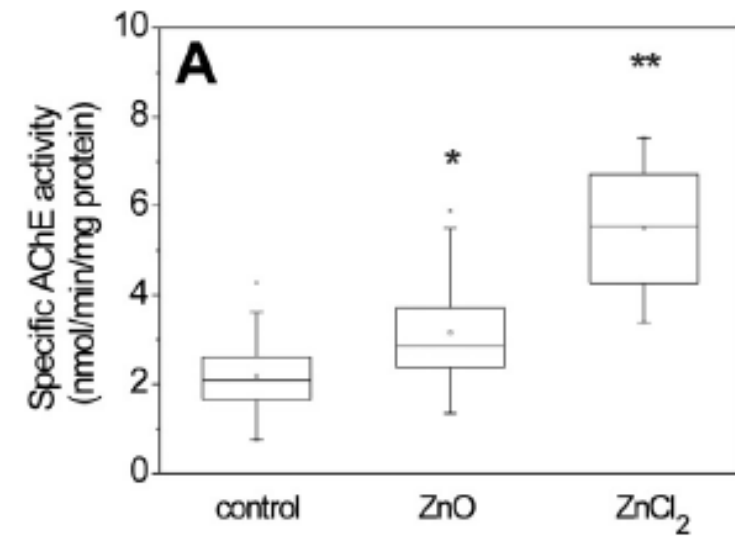
Neurotoxic potential of ingested ZnO nanomaterials on bees



Tamara Milivojević^a, Gordana Glavan^a, Janko Božić^a, Kristina Sepčić^a, Tina Mesarič^a,
Damjana Drobne^{a,b,c,*}



Honeybee *APIS MELLIFERA*

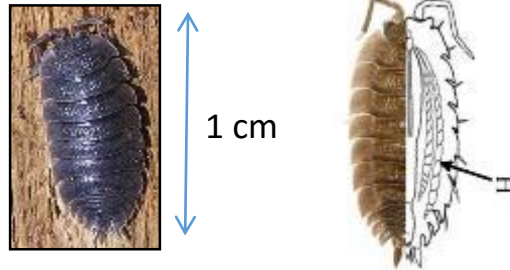


Model organism

PORCELLIO SCABER

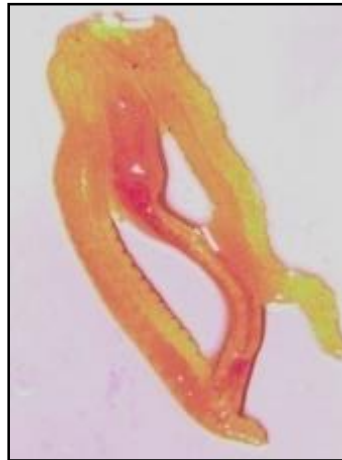
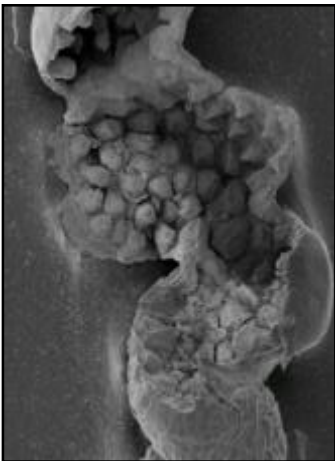
Woodlouse

- In terrestrial environment.
- Detritivores.
- Producing compost.

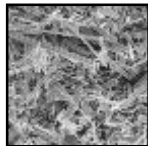


Model tissue:

Digestive glands-hepatopancreas



Feeding experiment – exposure of Isopods on food treated with nanomaterials

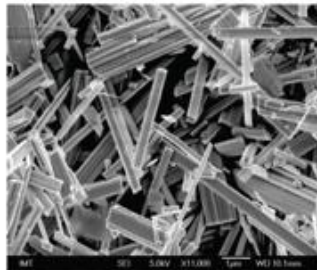


NPs suspension



Case study I: Exposure of Isopods to WO_x nanofibers

Feeding experiment



Tungsten oxide
nanofibers



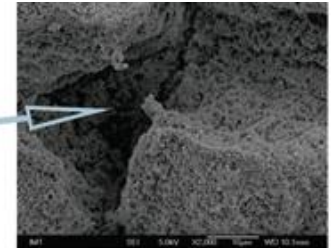
Model
organism



Scanning electron microscopy (SEM)

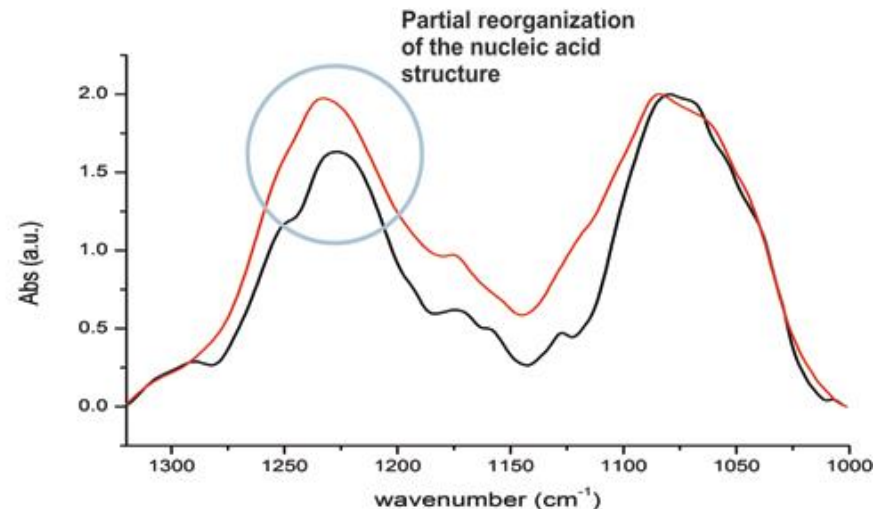
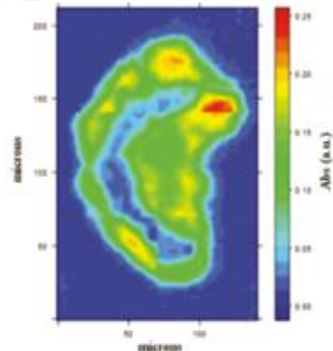
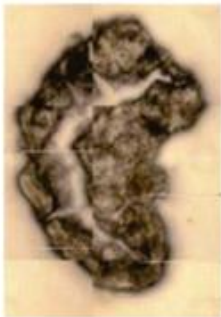


Model digestive tissue
(hepatopancreas)



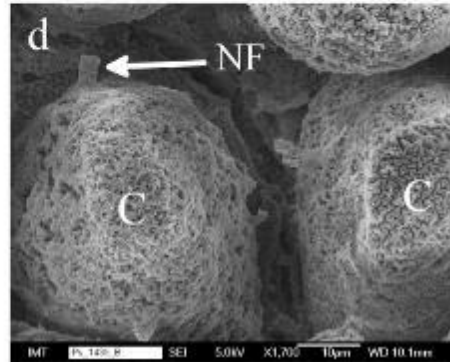
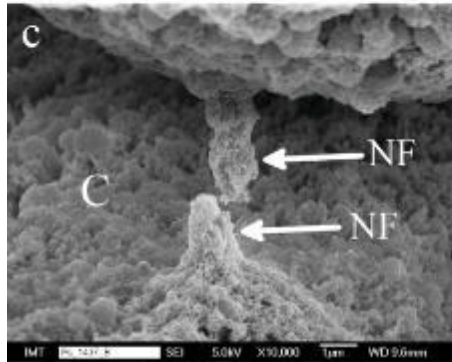
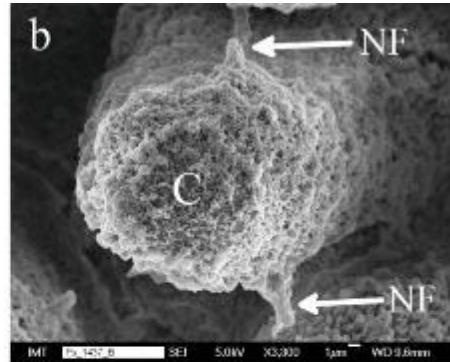
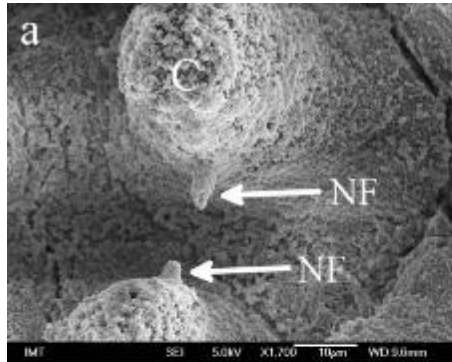
Nanofiber trusted in cell

Optical and Fourier transform
infrared (FTIR) image of digestive tissue

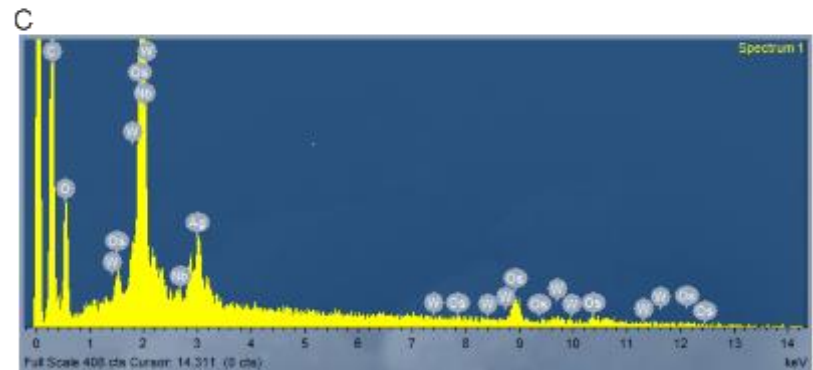
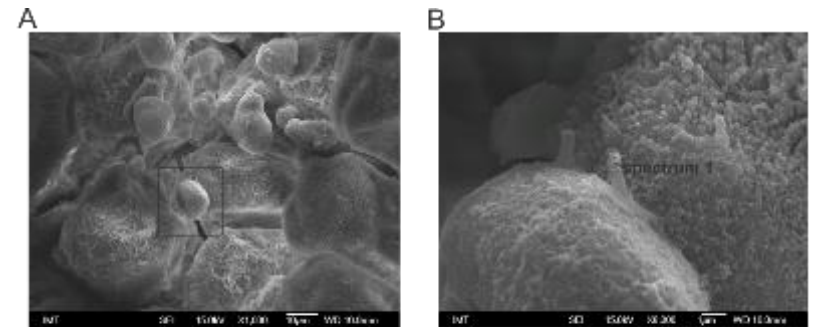


Average spectra of control (black line) and nanofiber
exposed (red line) of digestive tissue in nucleics acids region

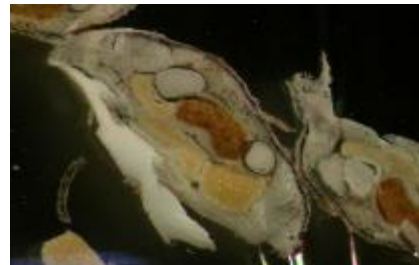
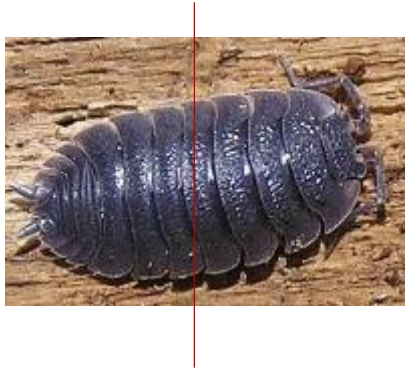
Hepatopancreatic cells of animals fed on tungsten oxide nanofibers



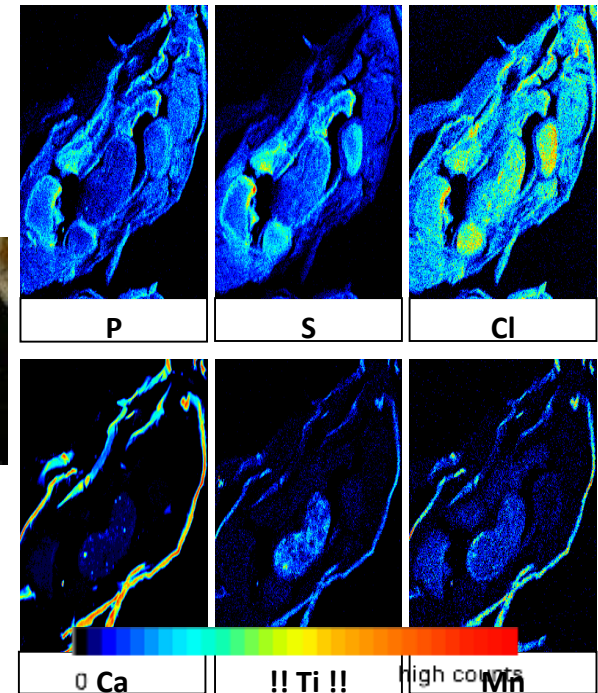
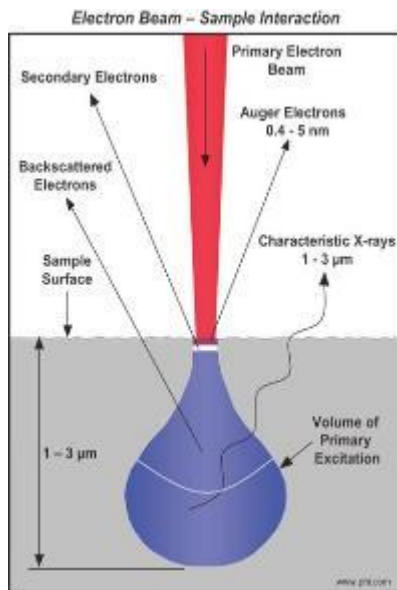
Scanning electron microscopy with
Energy-dispersive X-ray spectroscopy



Case study II: Elemental analyses of cross sections of Isopods exposed to TiO_2 NPs



Animal cross section (50 μm)



NO accumulation of Ti in heptopancreas.

Case study III:

Histopathology changes of Isopods digestive tissue after prolonged exposure to TiO_2 NPs



Preparation of histology sections



Changes in tissue morphology



Digestive gland
isolation after 28 days
of feeding experiment.

Nano-medicine

FIB/SEM EDX study of mouse urinary bladder tumorigenesis and internalisation of metal based nanoparticles by MB49 cells

Engineered nanoparticles are large enough to avoid rapid elimination through the kidney and small enough to penetrate the vasculature of the tumor tissues.

Specific NPs properties

- Versatile modification possibilities - retain efficient solubility and colloidal properties for use in complex environments
- High surface to volume ratio - important for optimizing drug payloads and other NP based interactions.
- Shape - direct effect on how NPs function *in vivo*.
- Unique optical properties - opportunity for exploiting spectral regime.

Promising applications

- Simultaneous drug delivery and molecular imaging.
- Diagnostic and therapeutic purposes.

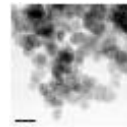
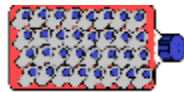
Case study IV:

FIB/SEM EDX study of mouse urinary bladder tumorigenesis and internalisation of metal based nanoparticles by MB49 cells

Aim of our work: use of FIB/SEM EDX method for confirming the presence of cancer cells with internalised nanoparticles in the tissue.

- The mouse urothelial carcinoma cell line used as an *in vitro* and *in vivo* model of urothelial carcinoma.

Cell line MB49 + CoFe_2O_4 NPs (24h).

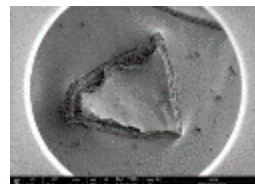


For magnetic drug delivery (attached or encapsulated therapeutic agents), radio-frequency hyperthermia, magnetic resonance imaging (MRI), diagnostics...

Cells injected in the mouse urinary bladder (1h).

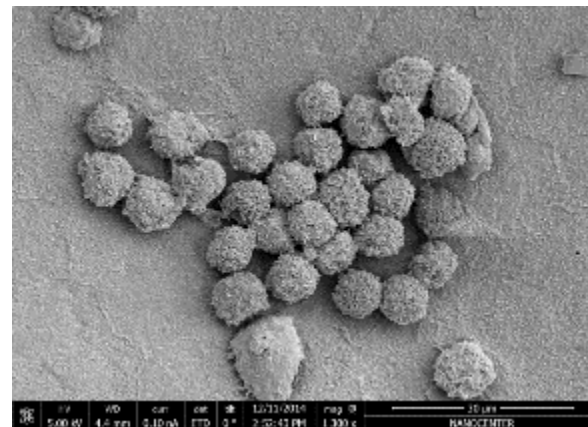
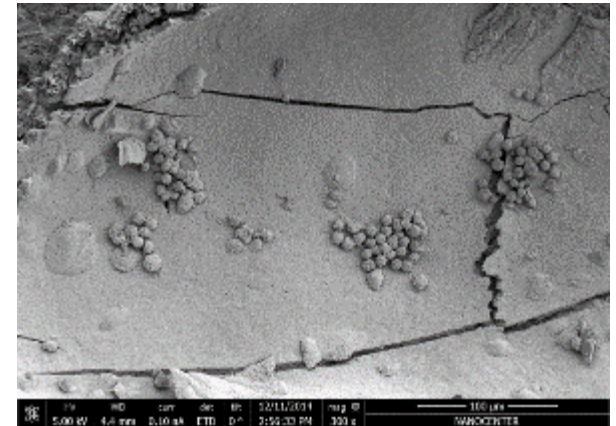
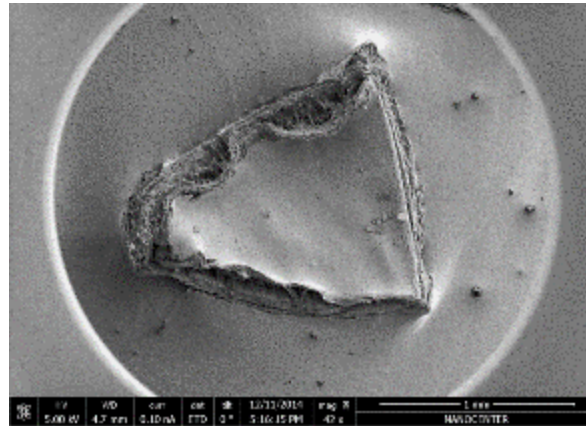


Fixation of isolated urinary bladders.



Scanning electron microscopy (SEM)

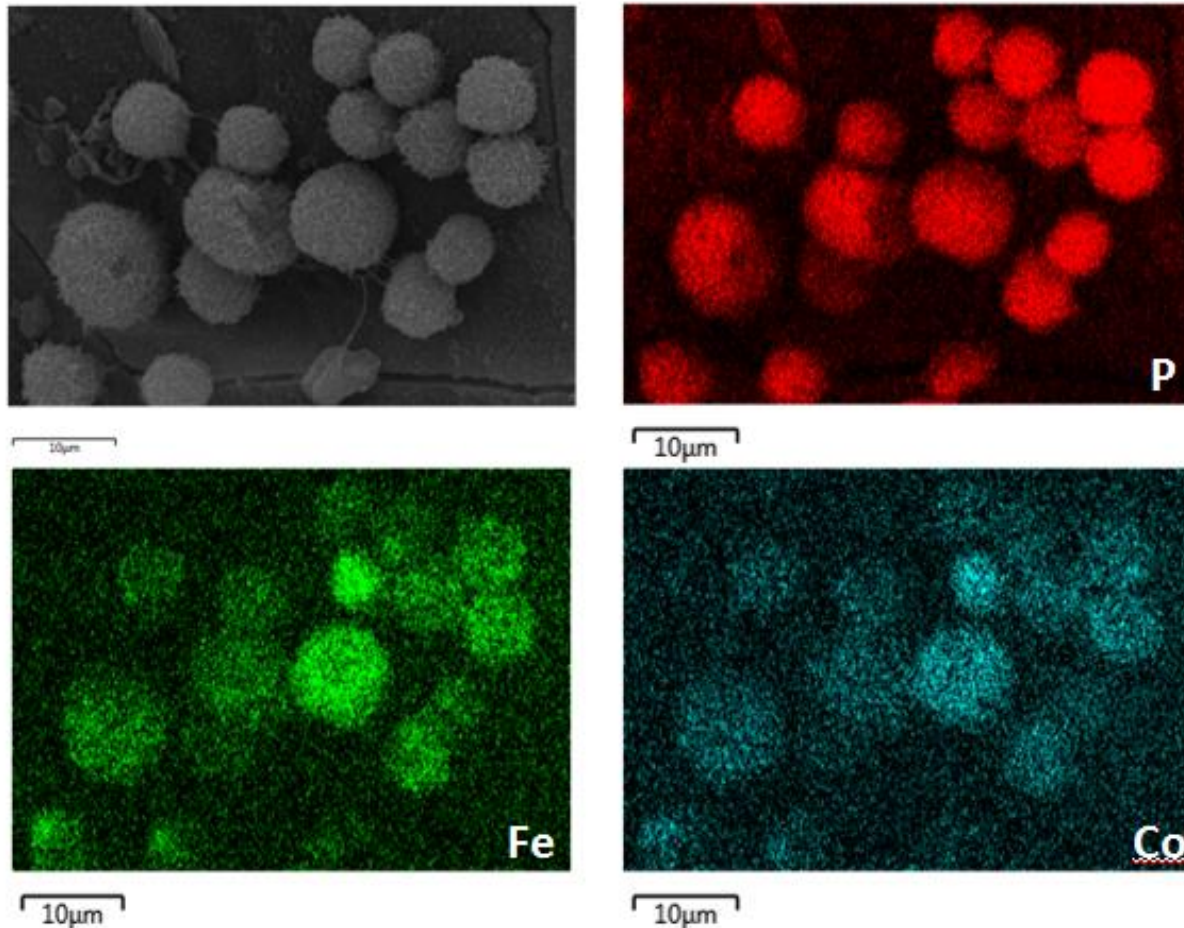
Selection of area with cancer cells on the base of their morphological characteristics.



Dual-beam system of Scanning Electron Microscope and Focused Ion Beam, Helios NanoLab™ 650 (Center of excellence in Nanoscience and Nanotechnology, Institute Jozef Stefan, Ljubljana, Slovenia).

Scanning electron microscopy with energy dispersive x-ray spectromicroscopy (SEM/EDX)

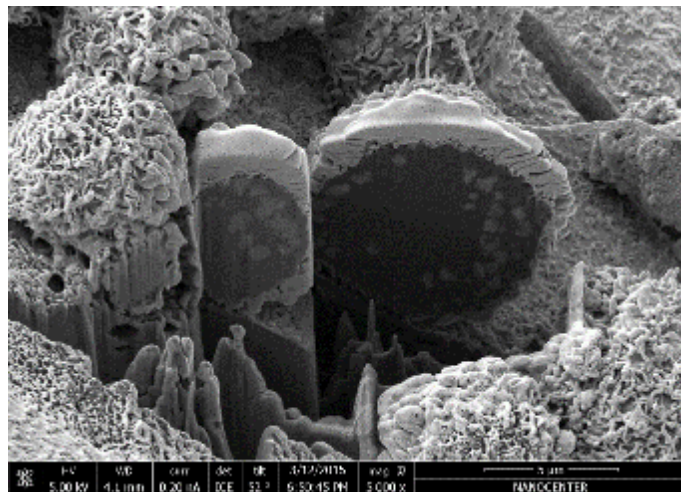
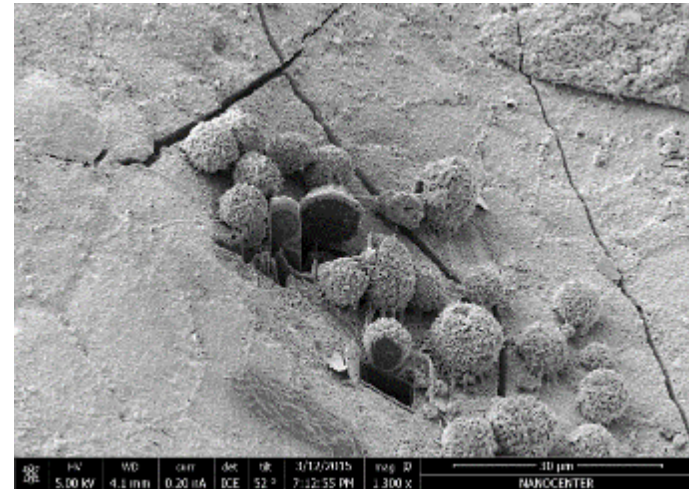
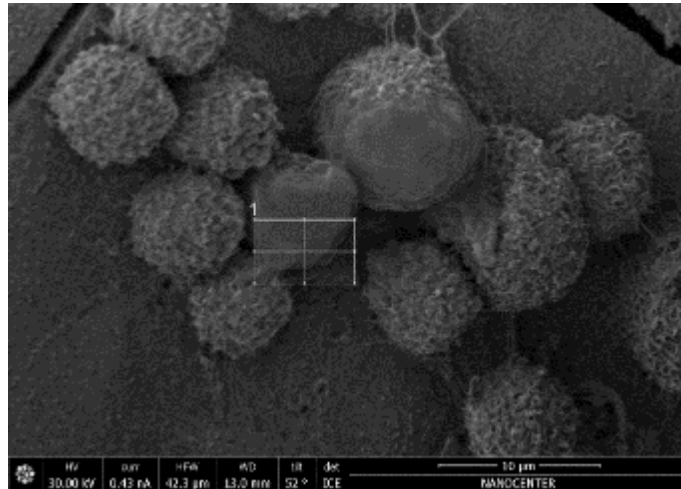
Confirming CoFe_2O_4 NPs internalization in selected cells.



Electron micrography of **cancer cells** with corresponding elemental maps of P, Co and Fe. P is shown for cells location; presence of Co and Fe confirmed NPs internalization.

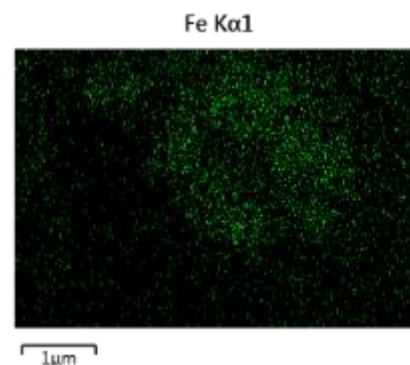
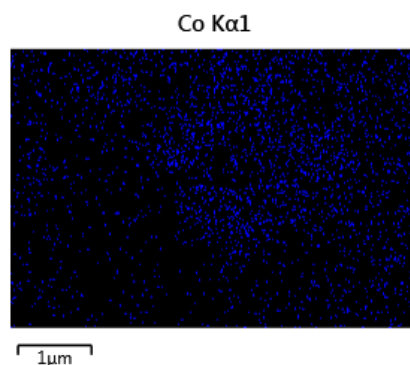
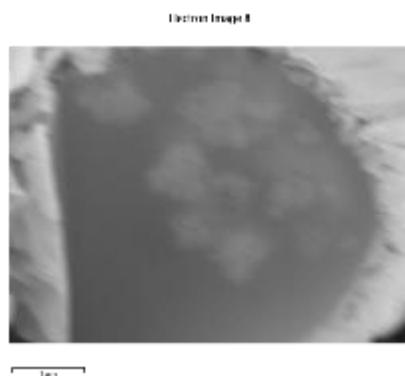
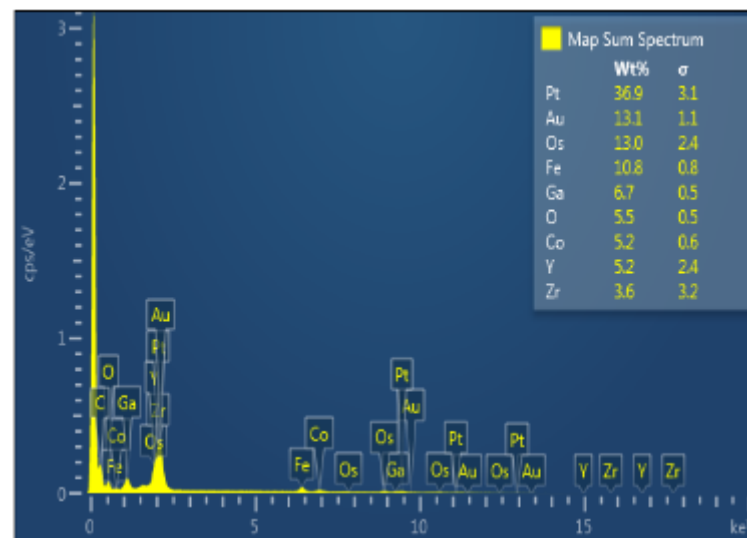
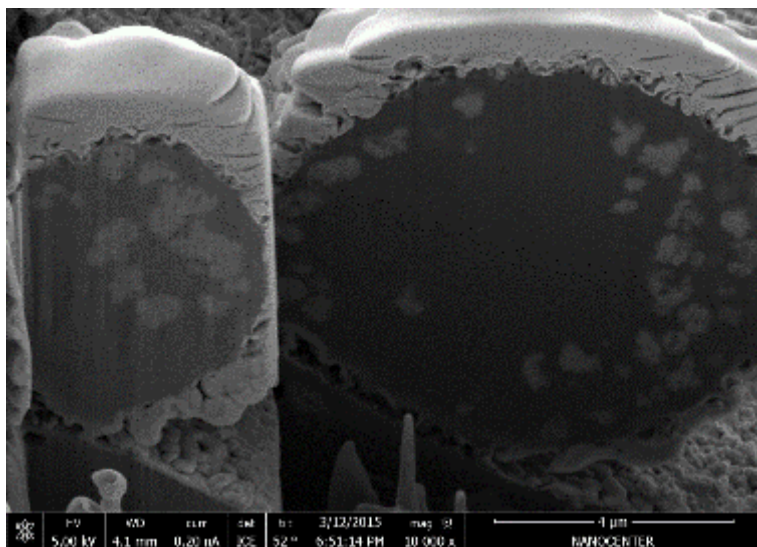
Focus ion beam scanning electron microscopy (FIB/SEM)

ROUGH MILLING: Ion currents of 0.43 nA at 30 kV, beam size was approximately 50 nm of diameter.
POLISHING: beam currents of 100 to 300 pA, beam size was approximately 10 nm of diameter.



- a) Selected cancer cells sputtered with Pt.
- b) Two regions of selected cancer cells milled with FIB.
- c) SEM imaging of two milled cancer cells.

SEM/EDX of of FIB milled cell interior



EDX spectrum of investigated region shows the presence of some most abundant elements. EDX maps show distribution of two elements belonging to CoFe_2O_4 NPs, Co and Fe.