KICK-OFF MEETING, 22.5.2025.

Exposure, biological effects and fate of microplastics in aquatic organisms under different anthropogenic impacts

PlastOrgAnoTox

The application of microscopy in microplastic effect research

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Where you can find us?

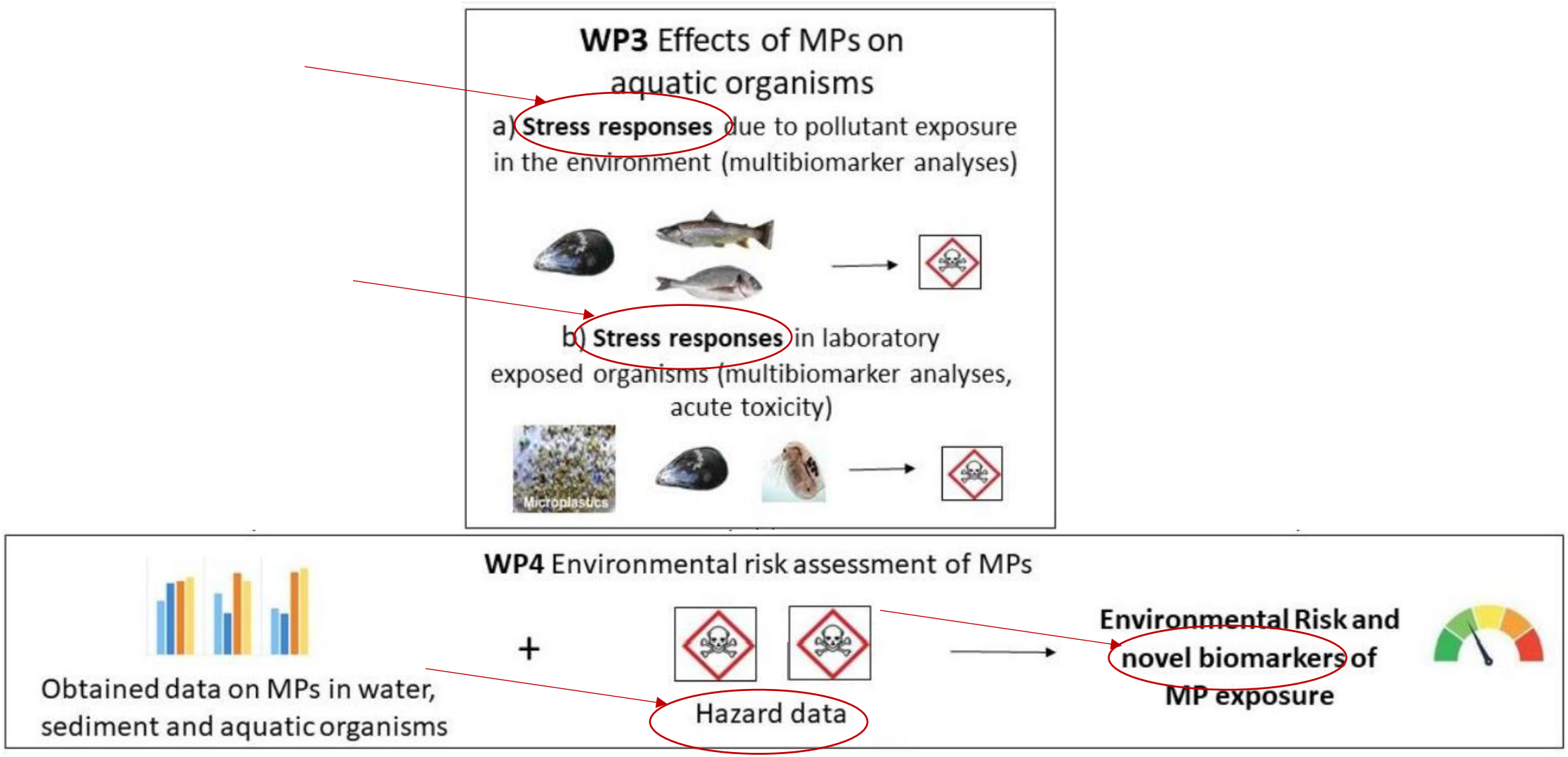
University of Ljubljana

Biotechnical faculty

Bionanoteam
Nanobiology & Nanotoxicology Research

Department of biolgy

Contribution of Bionanoteam Nanobiology & Nanotoxicology Research to PlastOrgAnoTox



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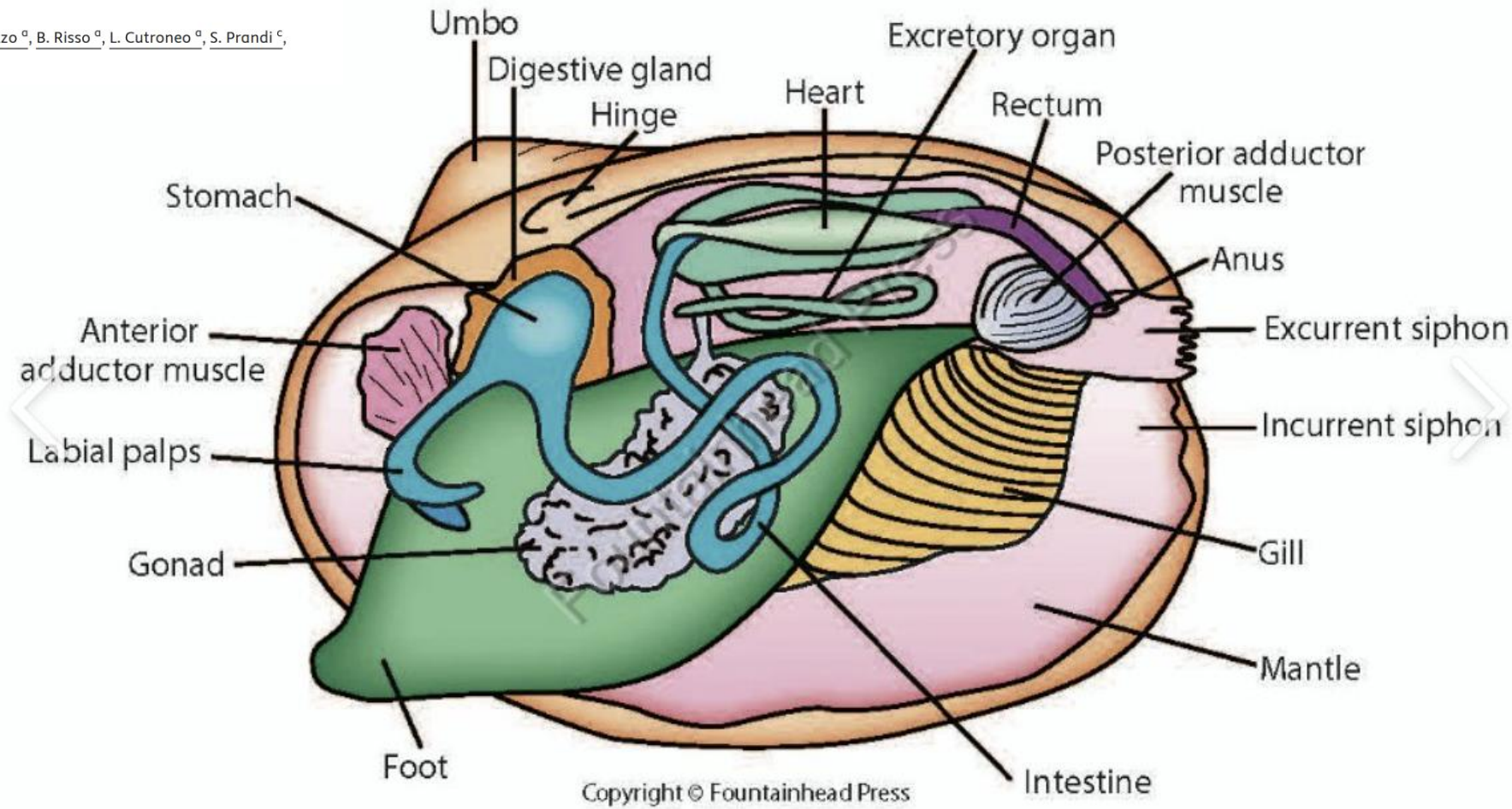
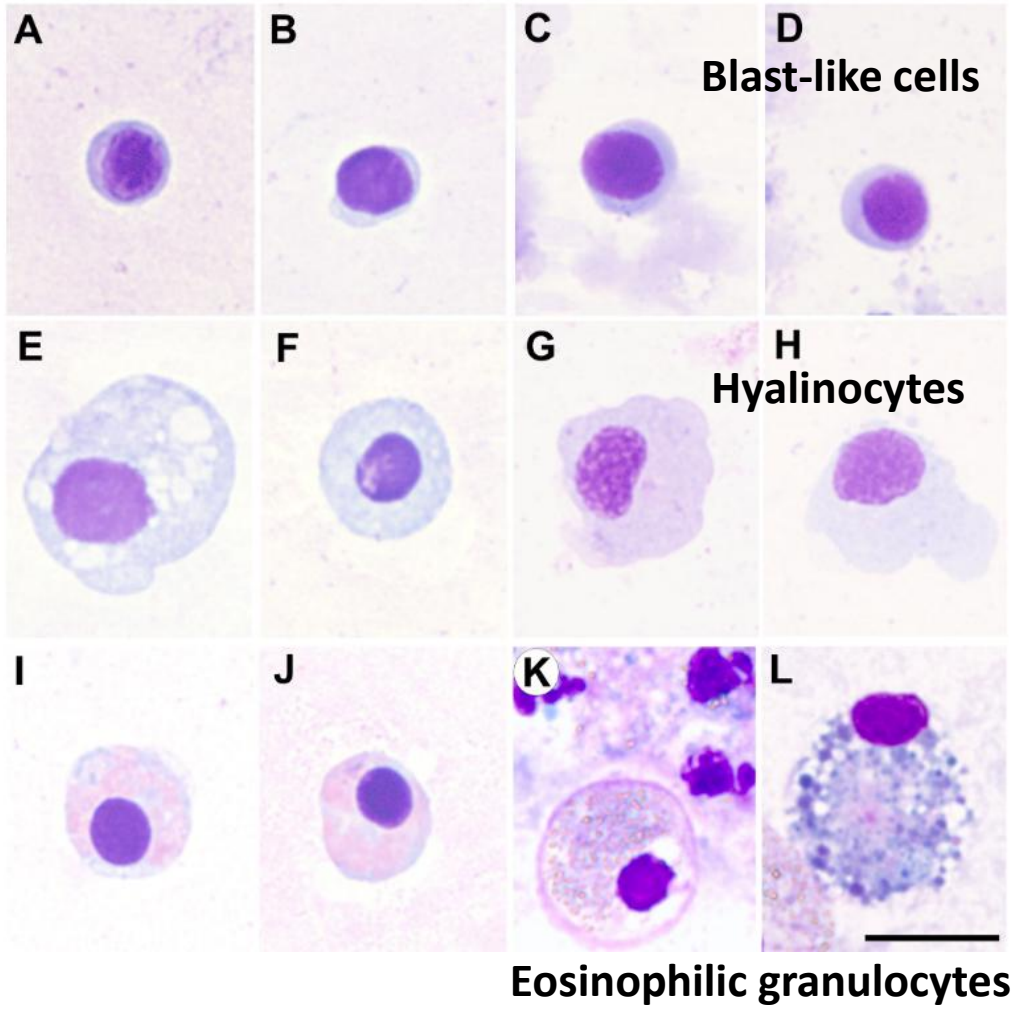
Multiple responses of *Mytilus galloprovincialis* to plastic microfibers

M. Auguste [a 1], M. Leonessi [a b 1], M. Bozzo [a], B. Risso [a], L. Cutroneo [a], S. Prandi [c], A. Jemec Kokalj [d], D. Drobne [d], L. Canesi [a b]



Haemocyte types of various bivalve species



Micrographs of haemocyte types of various bivalve species, all them corresponding to haemolymph samples collected from the adductor muscle, cytocentrifuged onto slides and fixed and stained with the kit Hemacolor® (Merck).
(A–D) **Blast-like cells** of Ruditapes decussatus, Ruditapes philippinarum,
Aequipecten opercularis and Mimachlamys varia, respectively.
(E–H) **Hyalinocytes** of R. decussatus, R. philippinarum, A. opercularis and M. varia, respectively.
(I–K) **Eosinophilic granulocytes** of R. decussatus, R. philippinarum and Ostrea edulis, respectively. (L) Basophilic granulocyte of O. edulis. Scale bar: 10 µm

de la Ballina NR, Maresca F, Cao A and Villalba A (2022) Bivalve Haemocyte Subpopulations: A Review. Front. Immunol. 13:826255. doi: 10.3389/fimmu.2022.826255

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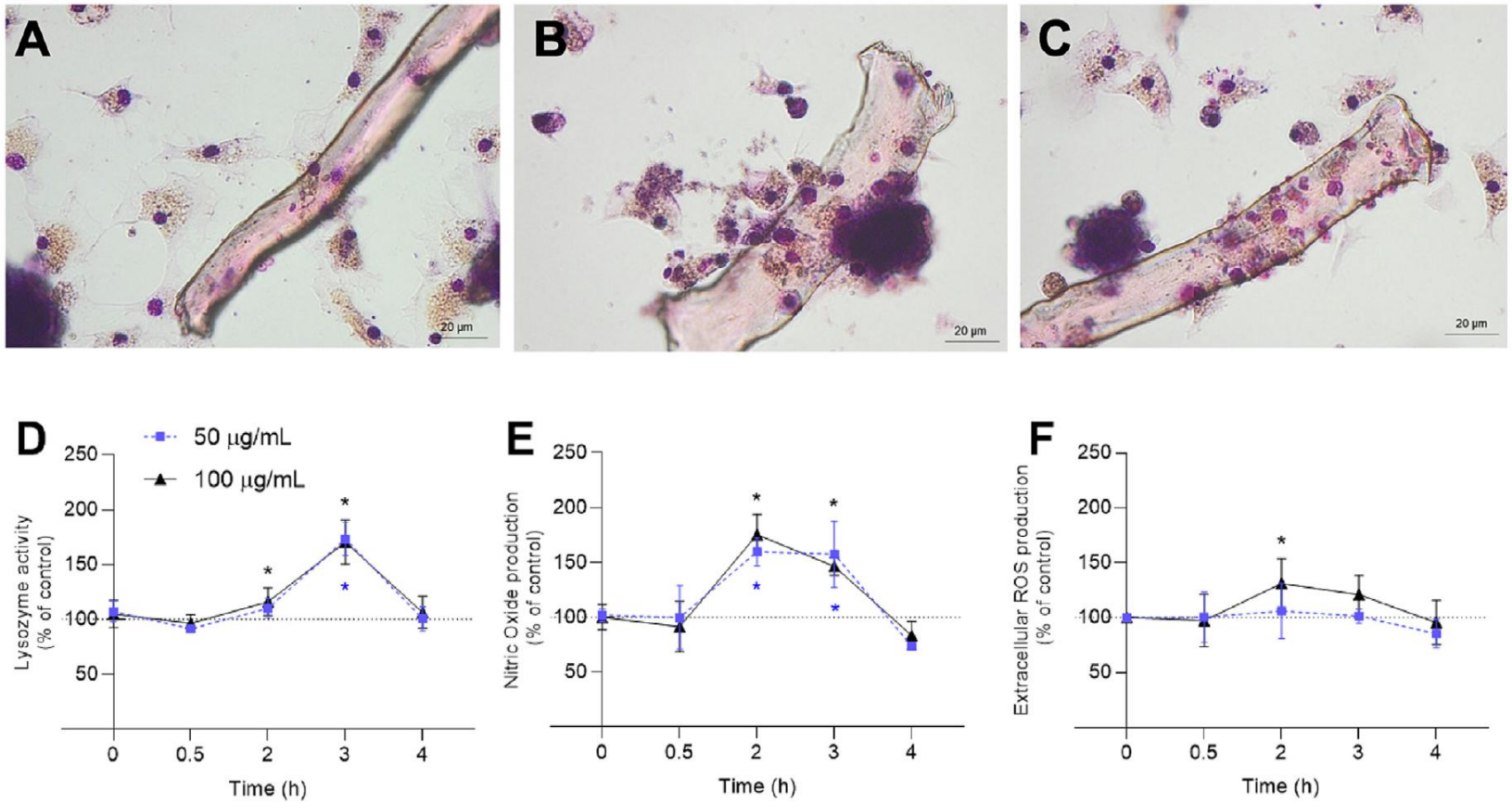

Time dependent **in vitro** interactions of M. galloprovincialis hemocytes with MF and effects on functional immune parameters. **A–C) Hemocyte adhesion:** representative images of hemocytes stained with Giemsa after exposure to MF (50 µg/mL) for different times (0.5 h, 3 h and 4 h, respectively) showing progressive cell adhesion to MF over time.

Scale bar: 20 µm. D–E) Immune responses: D) Lysozyme activity; E)Nitric oxide production and F) Extracellular ROS production. Data, representing the mean±SDof 4 independent experiments performed in triplicate, are reported as % of control values. Statistical analyses were performed by non-parametric Kruskal–Wallis followed by Dunn's multiple comparisons test (*$p \leq 0.05$).

Hematoxylin and eosin (H&E) staining of **gills from control and MF exposed mussels** (10 and 100 µg/L, 96 h). Representative images of apical sections of the gill lamellae. A) Control group, with intact cilia and regular organization of filaments; B) 10 µg/L exposure group: disorganization of cilia, enlargement of hemolymph vessels and C) 100 µg/L exposure group: thickened epithelia, and heavy hemocytic infiltration, pigmented brown granules (details in inset).

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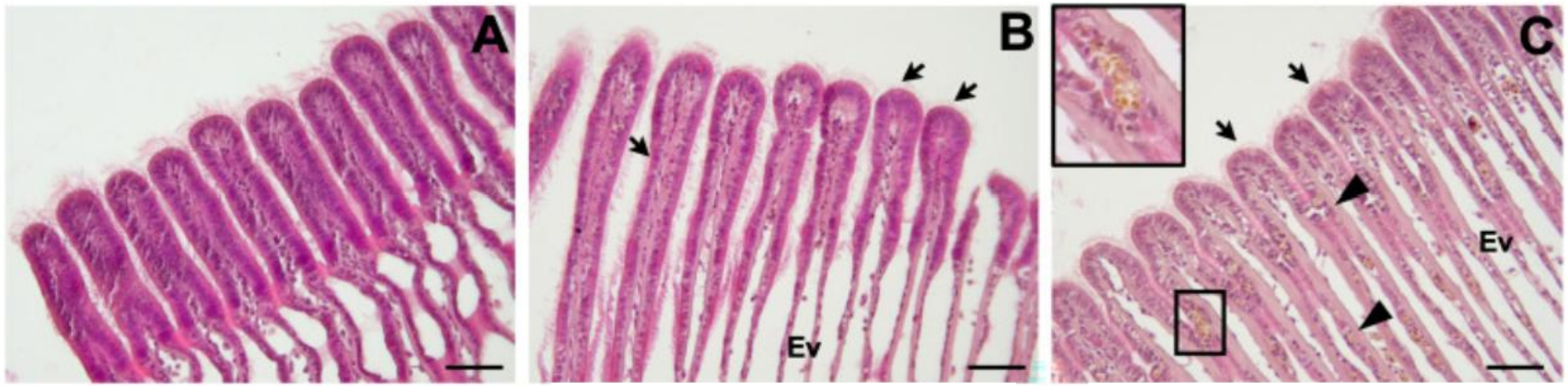

Control | 10 µg/L exposure group | 100 µg/L exposure group

Arrows: **disorganization of cilia**. Ev: **enlargement of hemolymphatic vessel**. Arrowheads: thickened epithelia with connective tissue and hemocytic infiltration. Scale bar: 50 µm.

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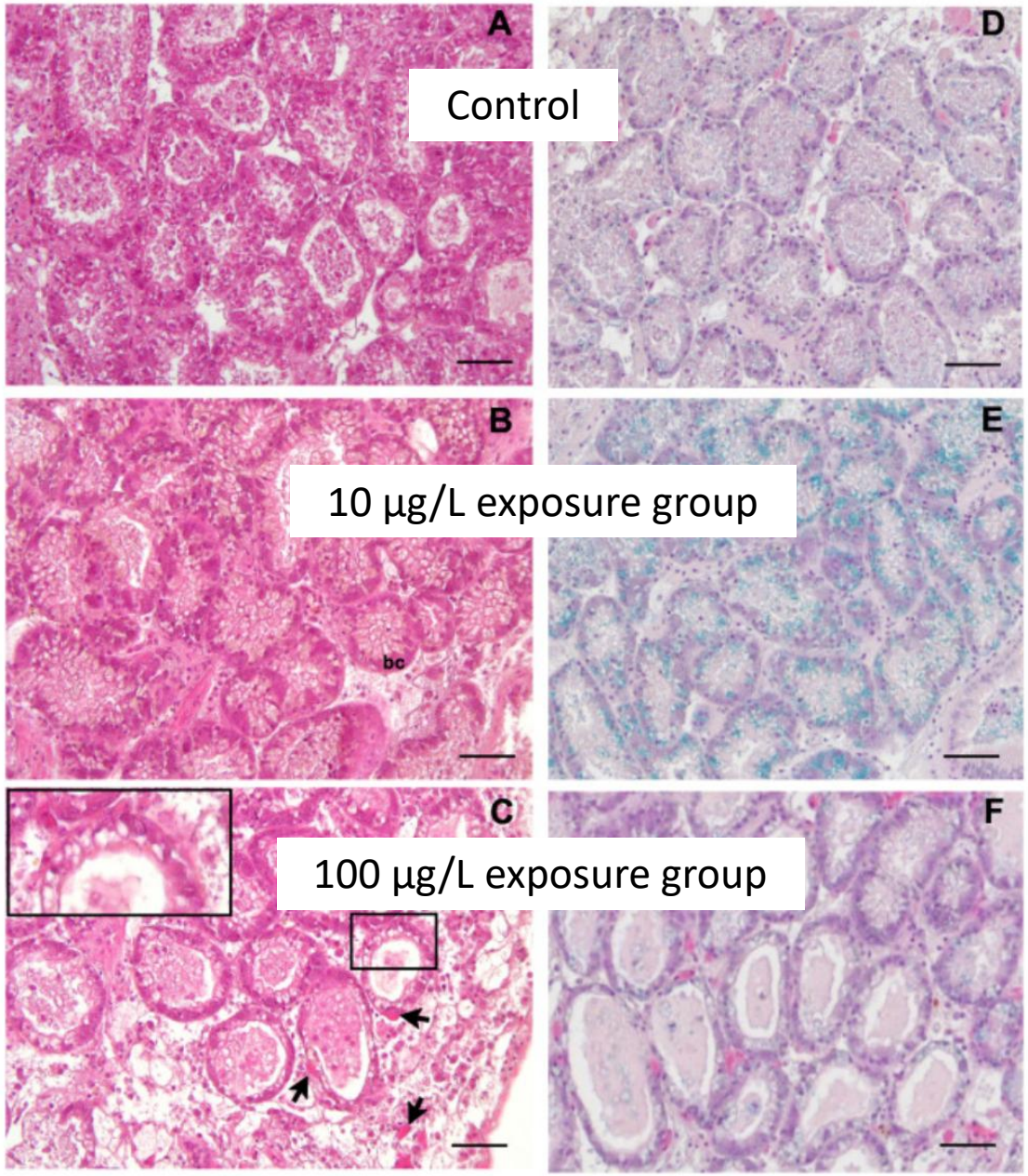


Hematoxylin and eosin (H&E) and Alcian Blue staining of **digestive gland** from control and MF exposed mussels (10 and 100 µg/L, 96 h).

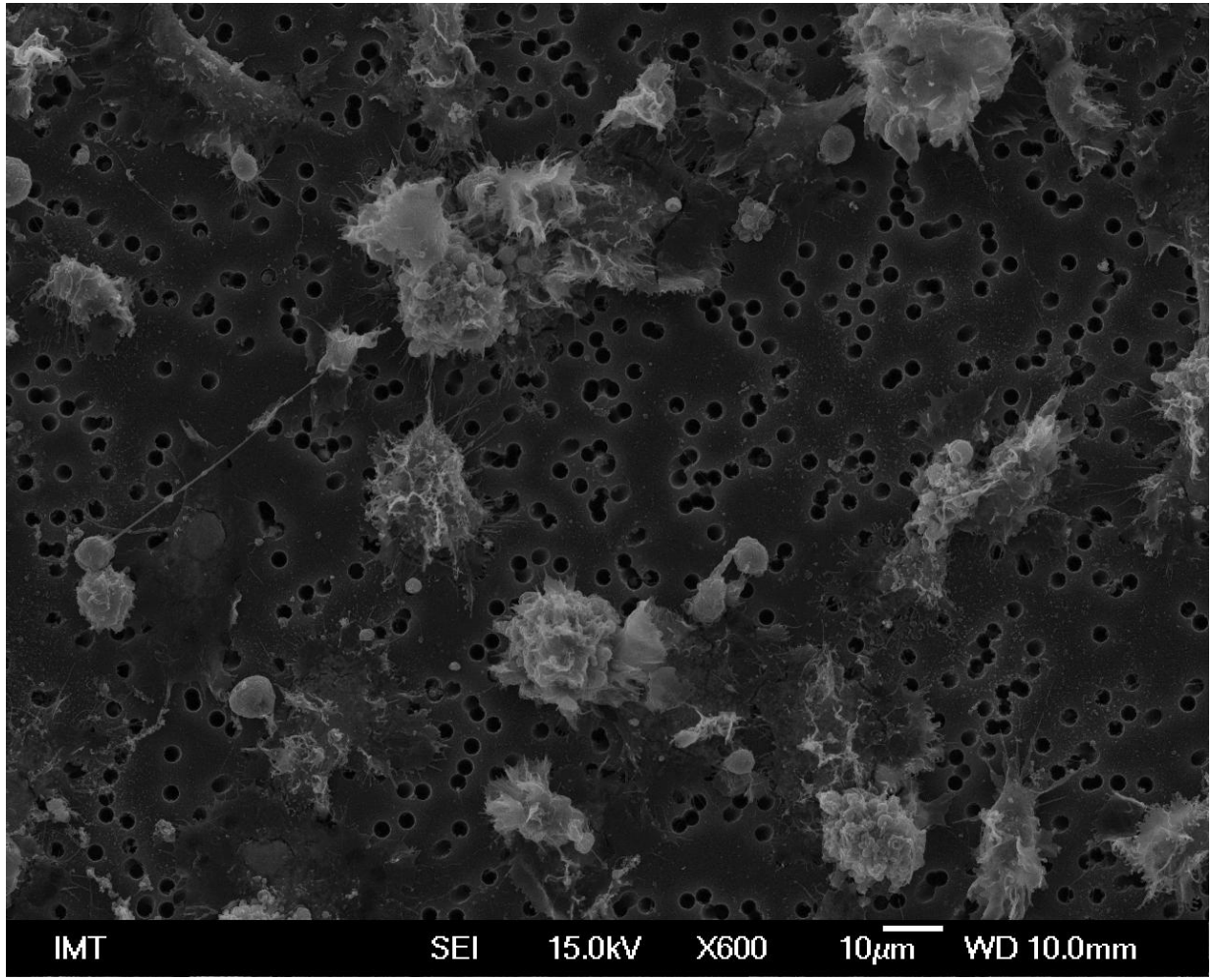
Left panel (A–C) stained only with H&E and right panel (D–F) were first stained with Alcian Blue (for mucus) followed by H&E.

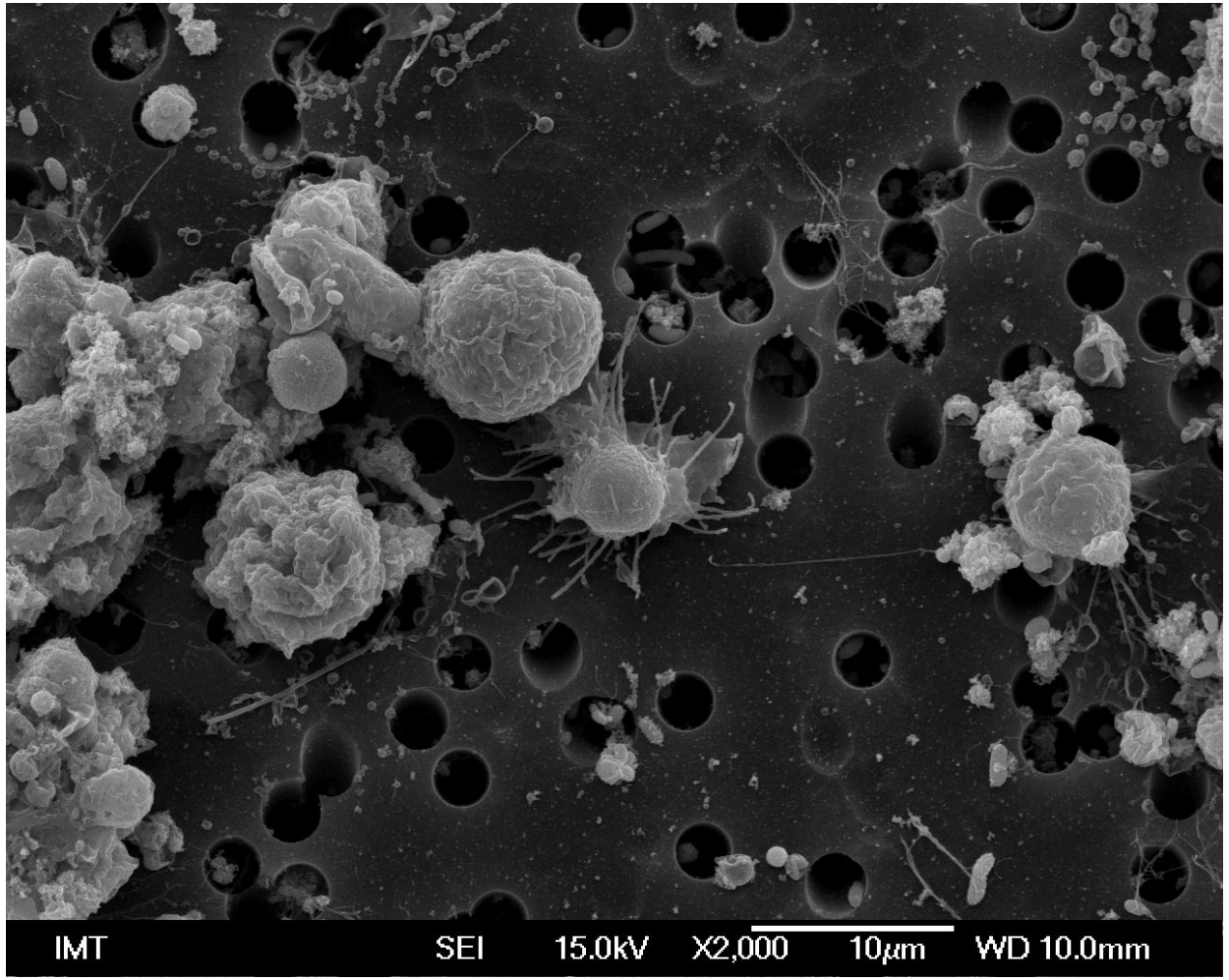
A to D) **Control**;

B–E) 10 µg/L exposure group: **tubules with thicker epithelium and smaller lumen.** Large presence of mucus in tubule epithelium

C–F) 100 µg/L exposure group: **heterogeneous and degenerated tubules, and hemocytic infiltration** (arrows). dc: digestive cell. bc: basophilic cell. Inset: detail of vacuolated epithelium. Scale bar: 50 µm.

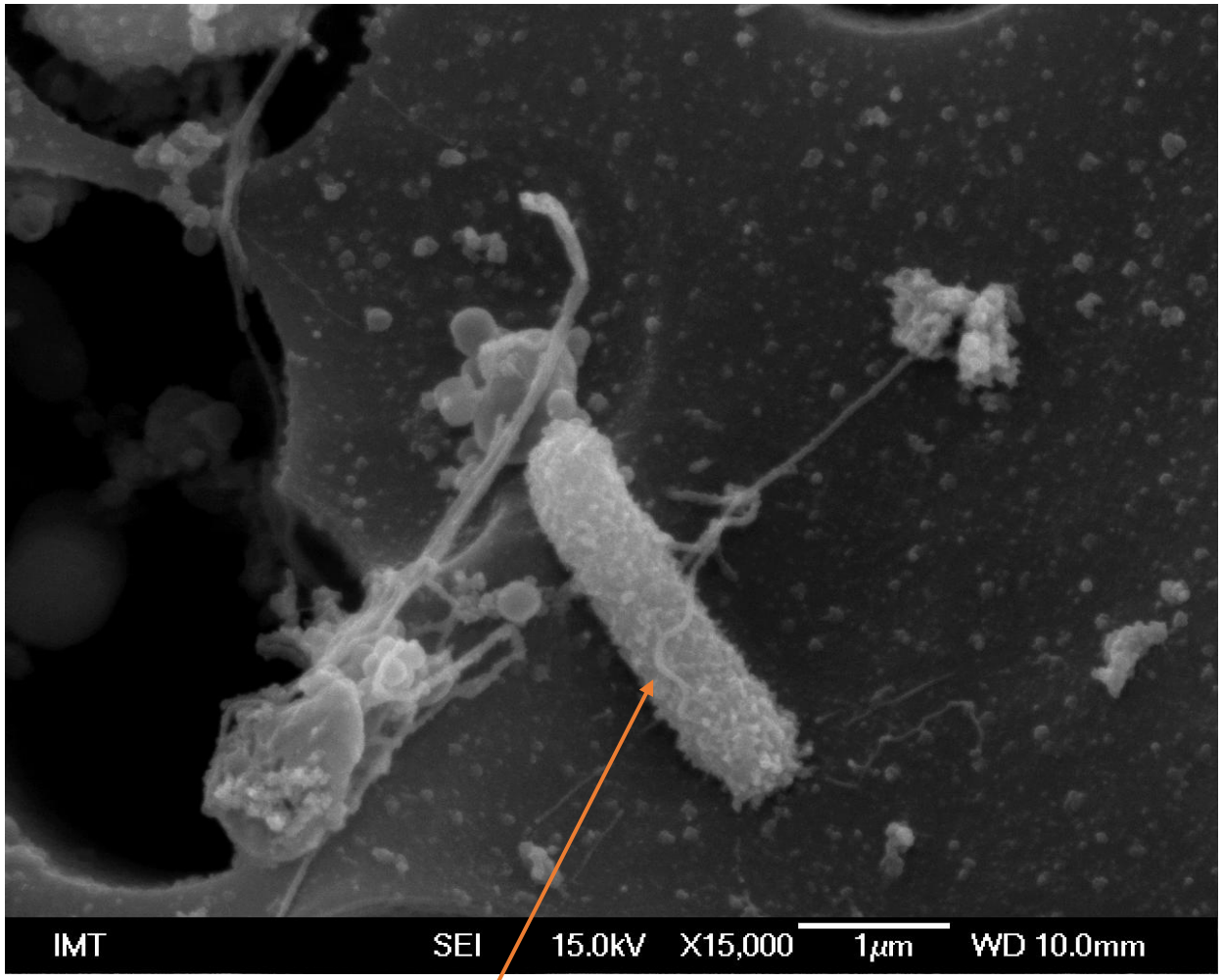
Scanning electron microscopy (SEM)
haemocytes of *Mytilus galloprovincialis*



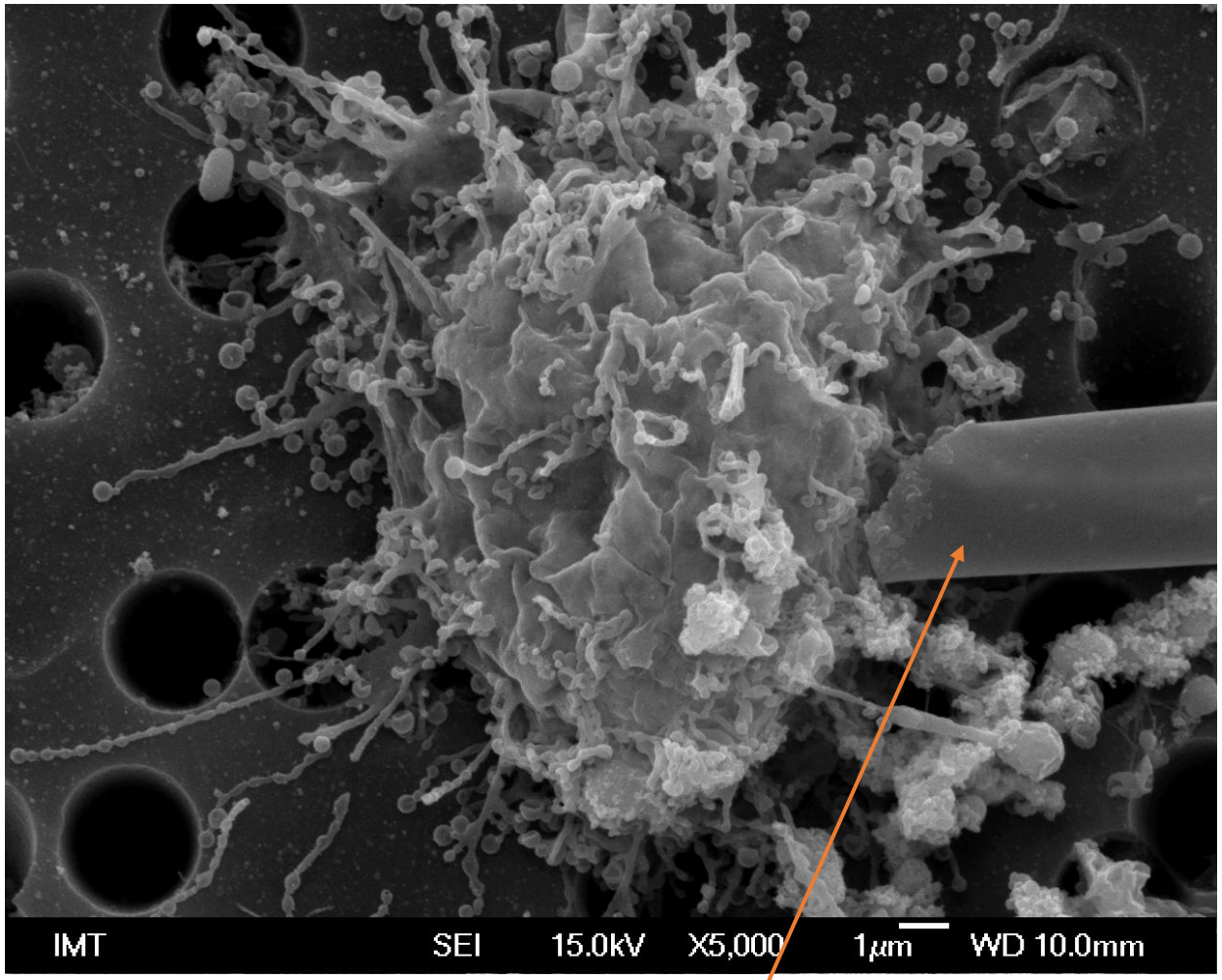


Different types of hemocytes could be seen

Scanning electron microscopy (SEM)
Haemocyte of *Mytilus galloprovincialis*

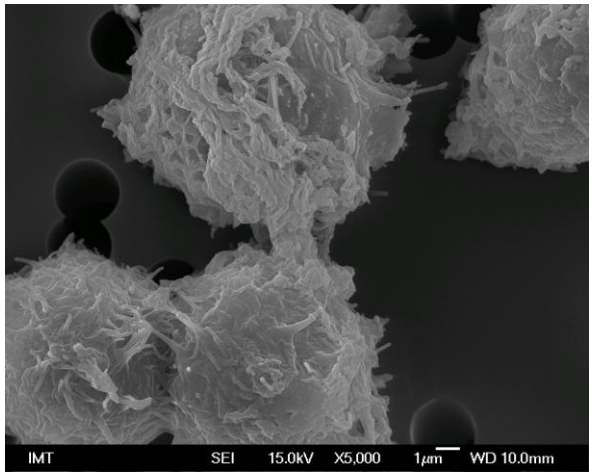


Bacterial cell

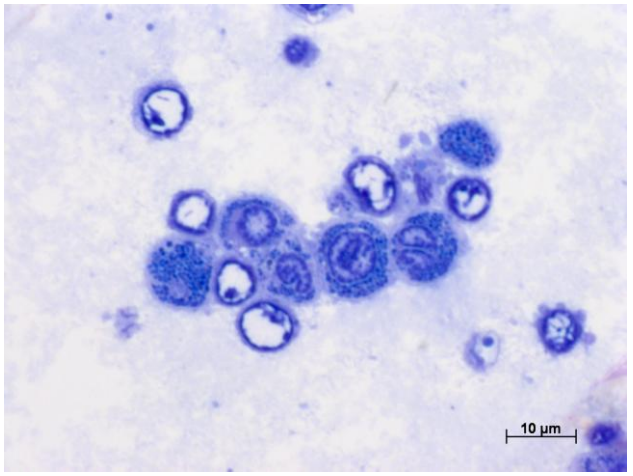


Plastic fibre

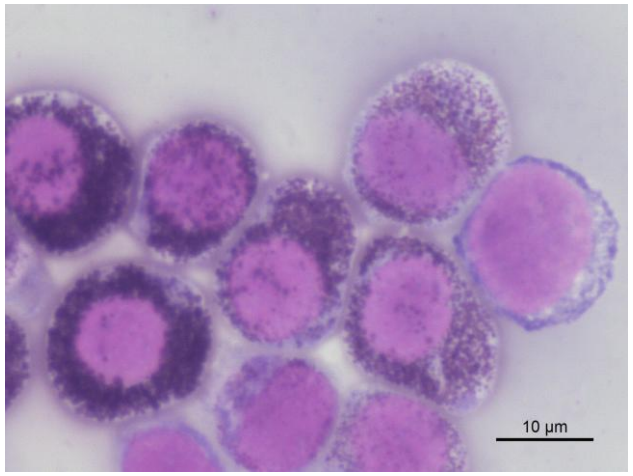
Microscopy of hemocytes of terrestrial crustacean *Porcellio scaber*



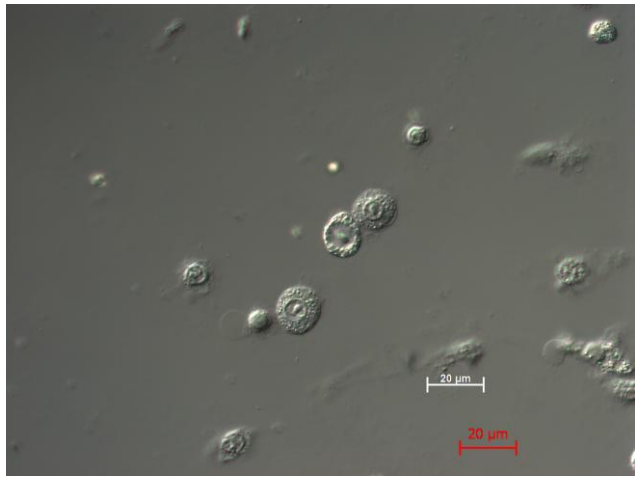
Scanning electron microscopy



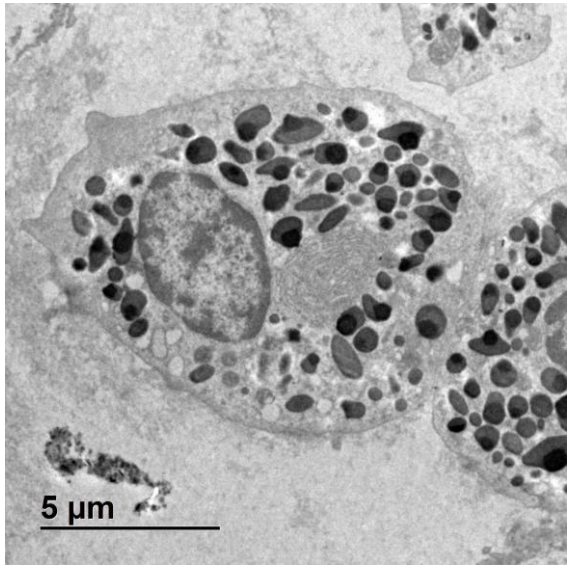
Richardson stain

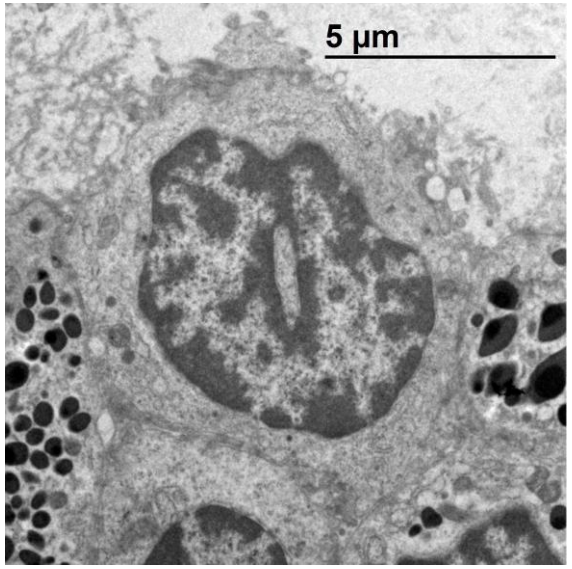


5% Giemsa stain

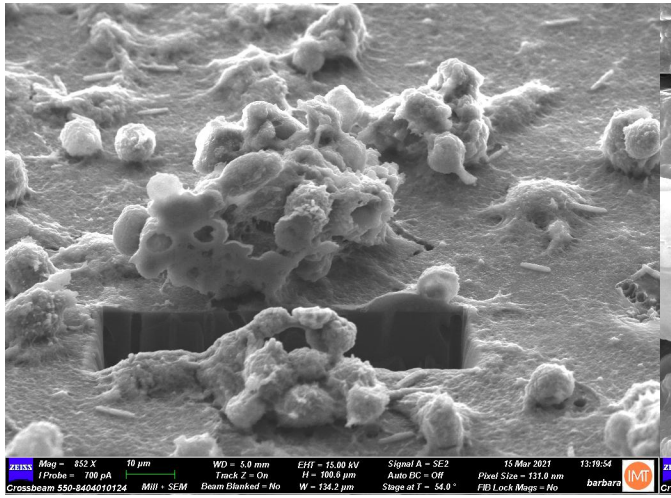


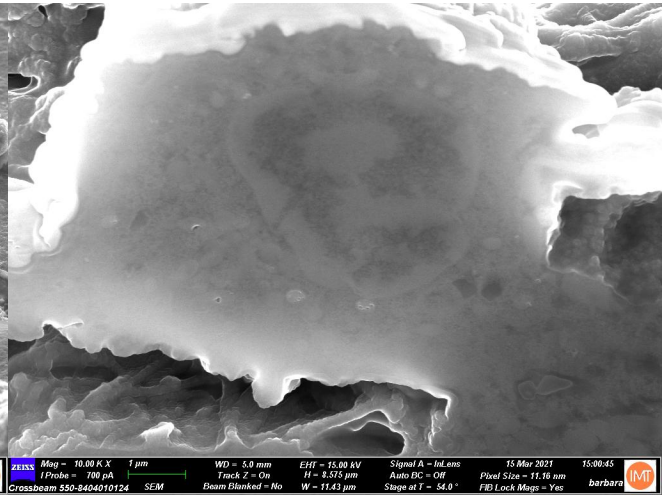
Differential interference contrast (DIC) microscopy





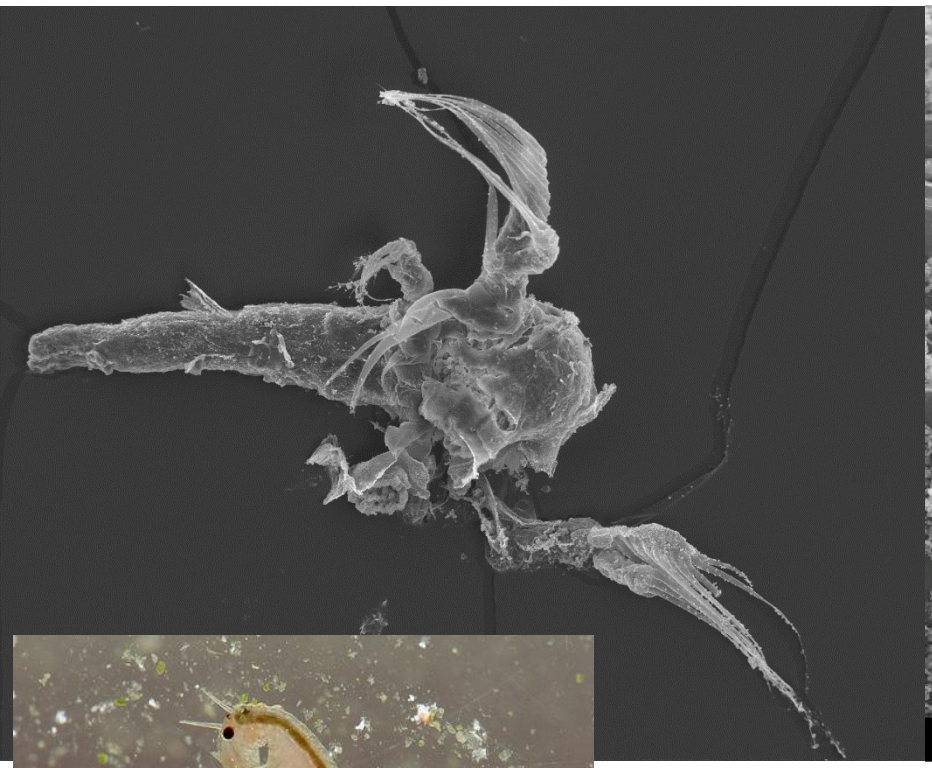
Transsmision electron microscopy (TEM)

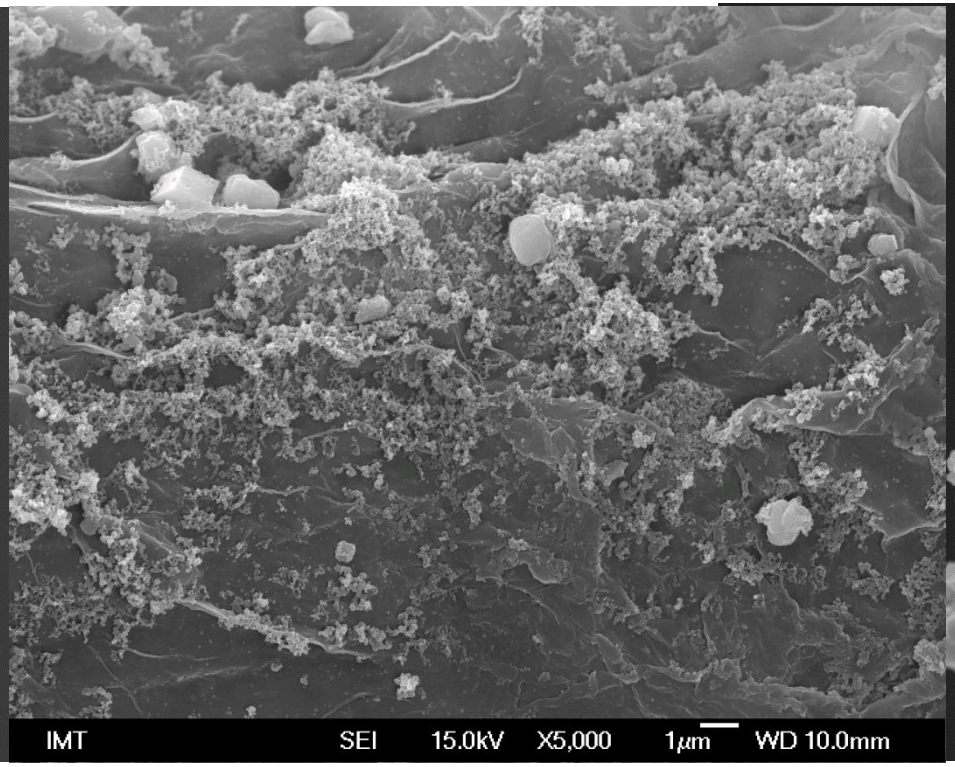


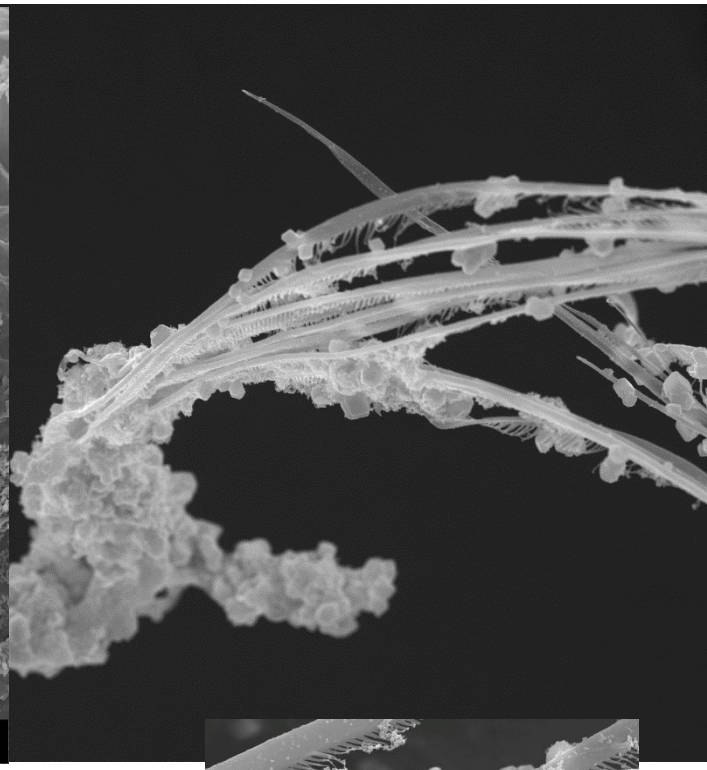


Focused ion beam/scanning electron microscopy (FIB/SEM)

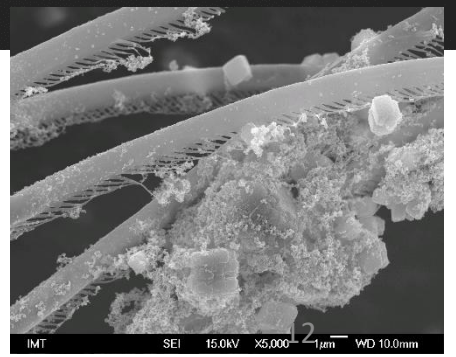
Microscopy of nanoparticles to surface of *Artemia salina*







Carbon black exposed *Artemia salina*



Thank you for attention!