Tissue accumulation and toxic potential of ingested TiO_2 nanoparticles by a terrestrial isopod (*Porcellio scaber*, Isopooda, Crustacea)

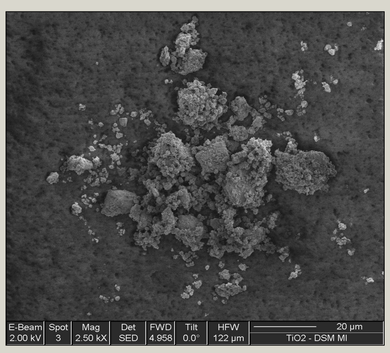
Novak S[1], Drobne D[1], Valant J[1], Pipan Tkalec Ž[1], Pelicon P[2], Vavpetič P[2], Grlj N[2], Falnoga I[3], Mazej D[3], Remškar M[4],

[1] Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia.
[2] Institute "Jožef Stefan", Department of Low and Medium Energy Physics, Jamova 39, SI-1001, Ljubljana, Slovenia
[3] Institute "Jožef Stefan", Solid State Physics Department, Jamova 39, SI-1001, Ljubljana, Slovenia
[4] Institute "Jožef Stefan", Department of Environmental Sciences, Jamova 39, SI-1001, Ljubljana, Slovenia

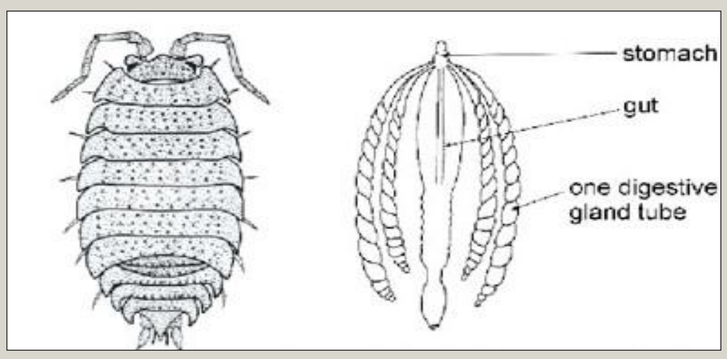
ABSTRACT

The aim of our work presented here was to study internalisation of ingested nano-TiO_2 by digestive gland epithelial cells of a terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea). Data on particle internalisation will be coupled to data on the toxicity (feeding rate, weight change and mortality) as well as cytotoxicity to digestive gland cells (cell membrane destabilisation). A model invertebrate (*Porcellio scaber*, Isopoda, Crustacea) was fed with nano-TiO_2 dosed food where the concentrations of TiO_2 were 100, 1000, 3000, 5000 µg Ti/g dry weight of food. After two weeks of feeding exposure, amount of Ti in the entire body was analysed by IC-PMS and elemental analyses of tissue cross sections were performed using particle induced x-ray emission (PIXE). The results show that ingested TiO_2 particles are neither internalized by digestive gland cells nor cause toxic effect if cell membrane is intact. When cell membrane was destabilized, Ti was detected inside cells. Our results confirm literature report on low toxic potential of nano-TiO_2. However our results are not entirely in agreement with literature data which repot that TiO_2 particles are not internalized.

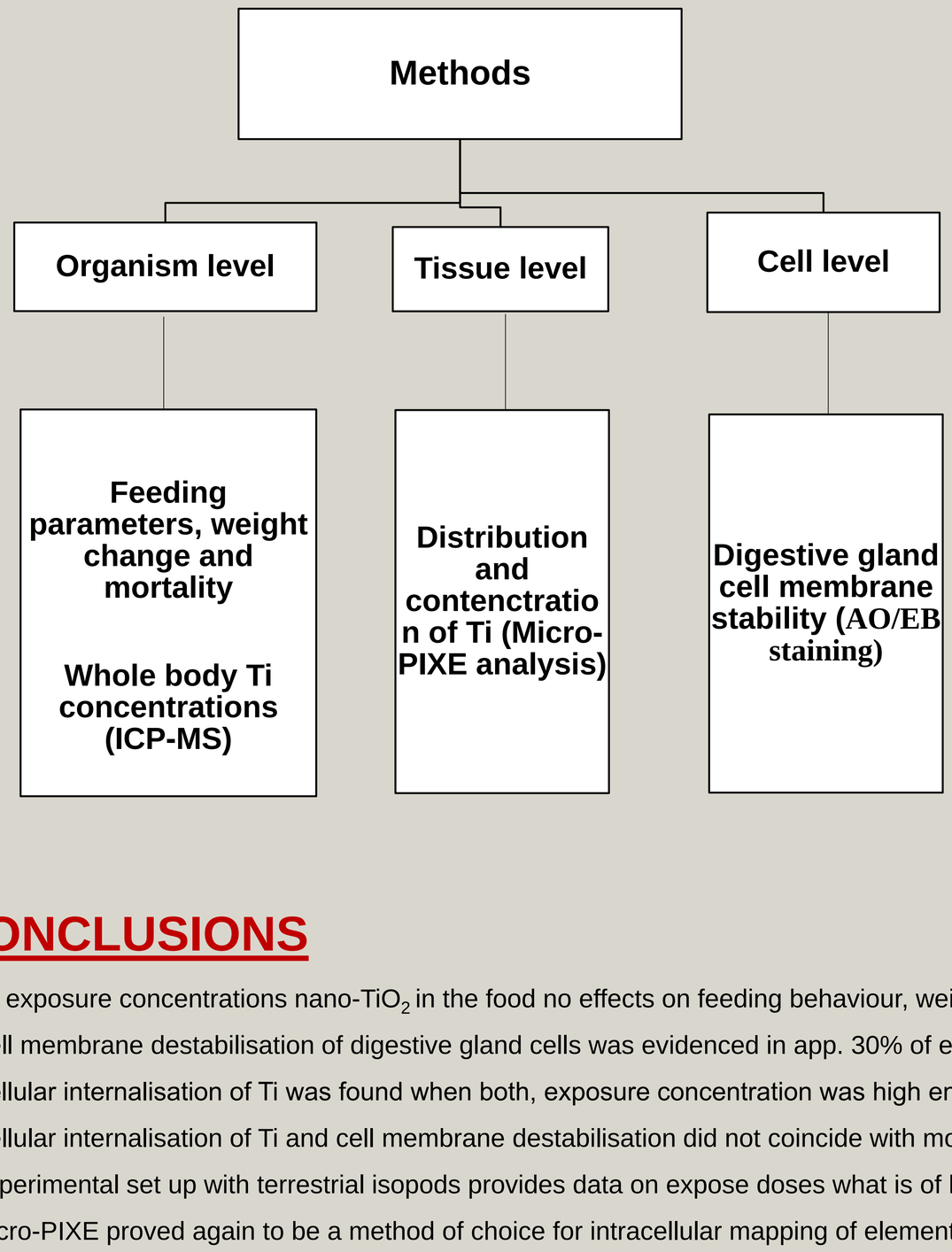
METHODS AND MATERIALS



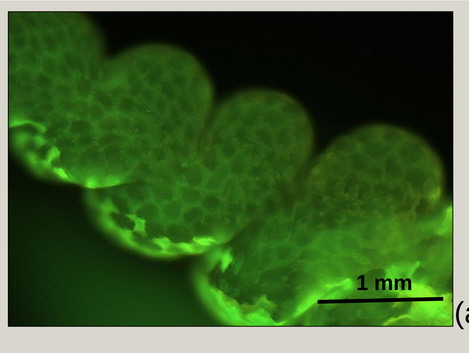
Isopods were collected and placed in a Petri dish. One hazelnut leaf treated with particles was placed in each Petri dish. The TiO_2 nanoparticles were suspended in distilled water to get different final concentrations (100, 1000, 3000 and 5000 µg ml^{-1}) on leafs.



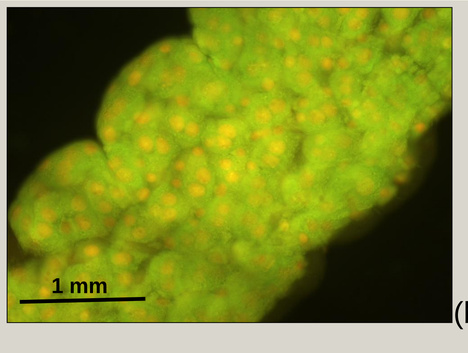
After the exposure, the digestive glands of animals were isolated.



RESULTS



(a)



(b)

Micrographs of hepatopancreatic tissue of *P. scaber* taken by fluorescent microscope. (a) Negative control, no nuclei are stained with EB and (b) positive control, cells with destabilized membranes have nuclei stained with EB (orange).

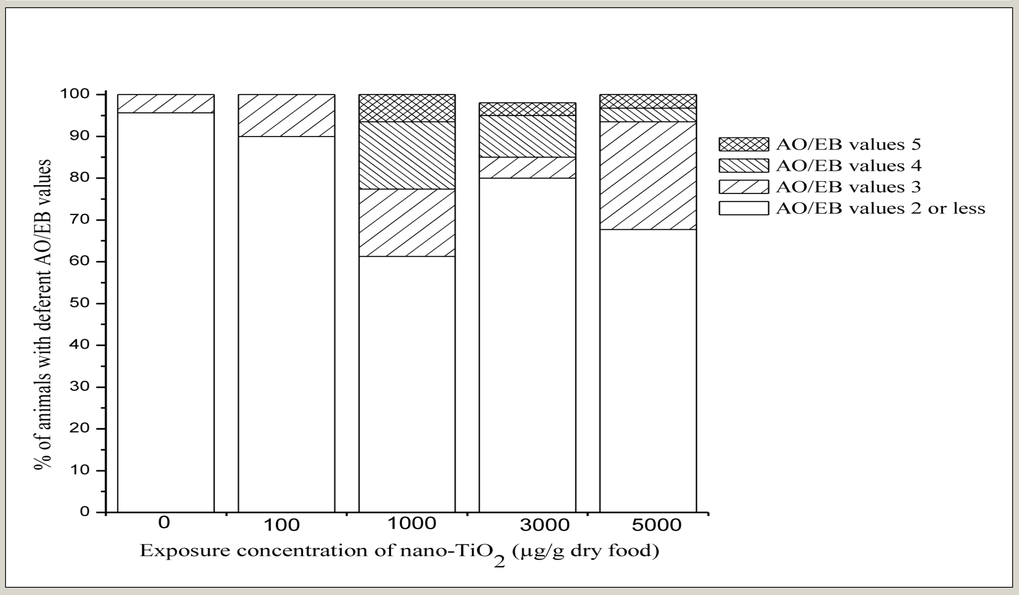
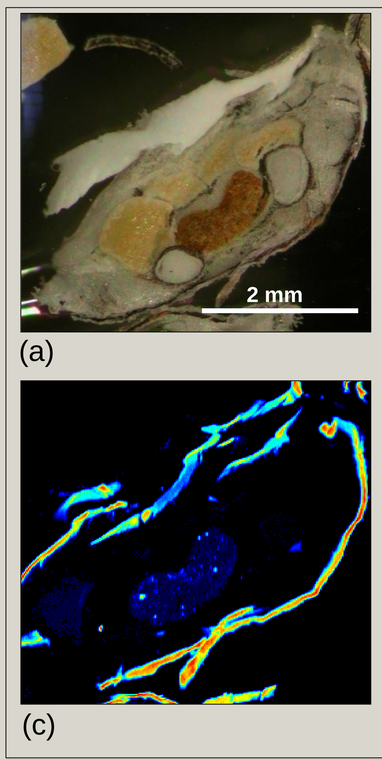
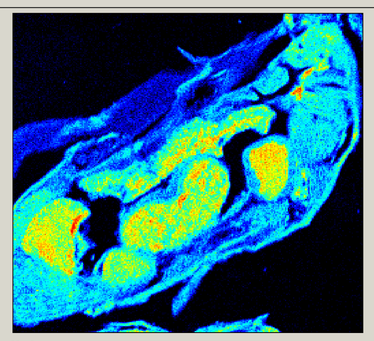


Table shows % of animals per group with different degrees of destabilised cell membrane, which was assessed visually and classified from 0 to 9 according to the predefined scale (0-control group,100,1000,3000,5000-nominal concentrations of nano-TiO_2 per 100 mg of individual leaf).

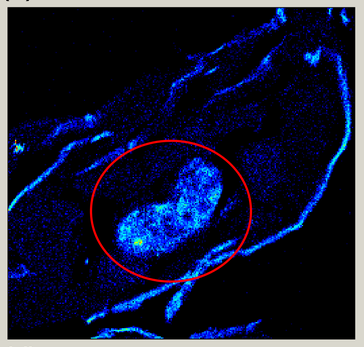


(a)



(b)

(c)



(d)

Transverse section of animal (a), distribution map of K (b), Ca (c) and distribution map of Ti.
Ti was present only in animal gut (red circled).

CONCLUSIONS

- At exposure concentrations nano-TiO_2 in the food no effects on feeding behaviour, weight change or mortality were evidenced in a *P. scaber* after 14 days of feeding
- Cell membrane destabilisation of digestive gland cells was evidenced in app. 30% of exposed animals to 1.000 – 5.000 µg/g nano-TiO_2 in the food.
- Cellular internalisation of Ti was found when both, exposure concentration was high enough (5.000 µg/g nano-TiO_2 in the food) and cell membrane was destabilised.
- Cellular internalisation of Ti and cell membrane destabilisation did not coincide with mortality, weight change or feeding behaviour.
- Experimental set up with terrestrial isopods provides data on expose doses what is of high value for environmental risk assessment.
- Micro-PIXE proved again to be a method of choice for intracellular mapping of elemental distribution taking advantage of high elemental sensitivity and low lateral resolution.