



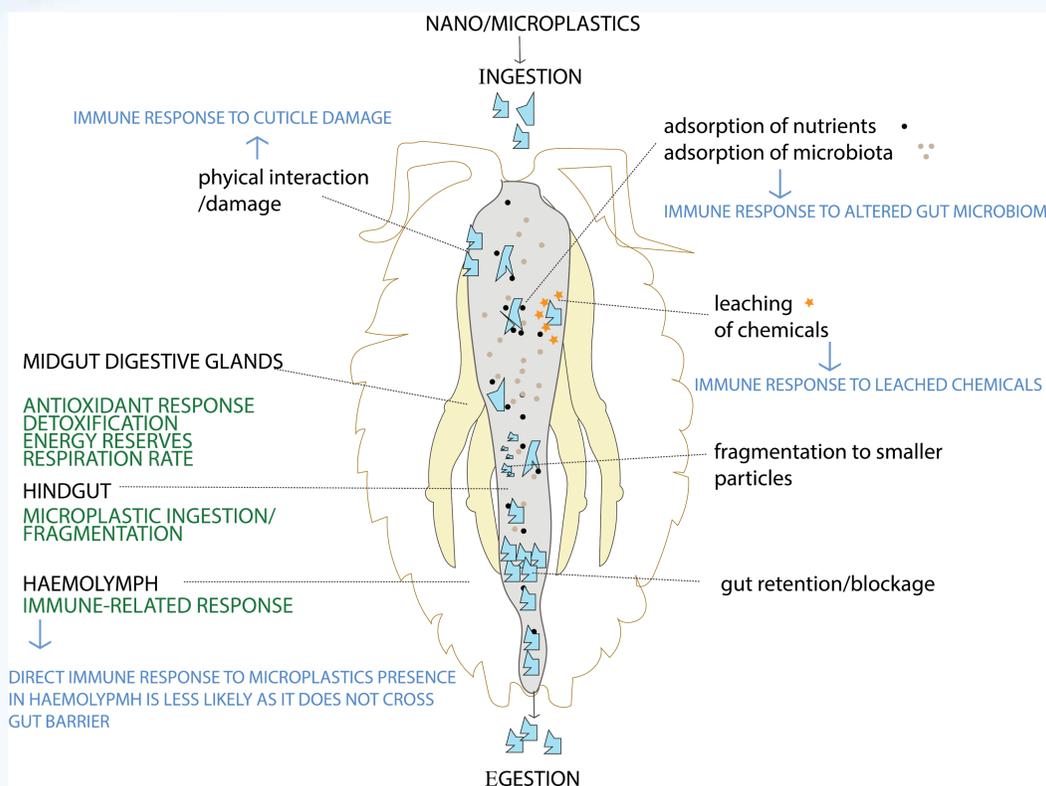
# IMMUNE RESPONSE OF TERRESTRIAL CRUSTACEAN *PORCELLIO SCABER* TO MICROPLASTICS EXPOSURE

Anita Jemec Kokalj, Damjana Drobne, Andraž Dolar

University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia; E-mail contact: [anita.jemec@bf.uni-lj.si](mailto:anita.jemec@bf.uni-lj.si)

**INTRODUCTION AND AIM:** Microplastics can potentially enter the terrestrial environment by fragmentation of plastic wastes, via microplastics contaminated sewage sludge deposition on agricultural land, or as a result of extreme agricultural practices. Despite the potential presence of microplastics in terrestrial environments, data regarding the effects of microplastics on terrestrial organisms are scarce, in particular it is unclear how long-term exposure affects immune processes. In this study, we investigated the immune response of terrestrial isopod *Porcellio scaber* to crumb rubber and polyester fibers.

## ANTICIPATED IMMUNOMODULATORY EFFECTS OF MICROPLASTICS



**METHODS:** Animals were exposed to polyester textile microfibers or crumb rubber from end-of-life tyres (0.05 to 1.5% w/w) in Lufa 2.2 soil for 3 weeks (Selonen et al., 2020). Immune response was followed during and after the exposure. The following immune parameters in the haemolymph were assessed: total haemocyte count (THC); differential haemocyte count (DHC); haemocyte viability.

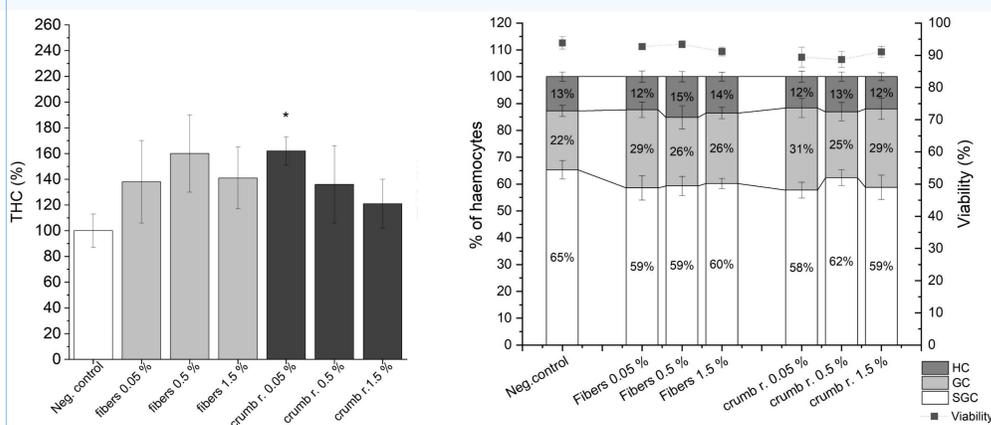
**Crumb rubber:** produced from mixed end-of-life passenger car tires by cryo-milling. The powder contained several different synthetic rubbers, 10-35% natural rubbers and 25-35% carbon black. Traces of metals and PAH were detected. The dominant fraction of particles lies between 80 and 110  $\mu\text{m}$  (mean 102.9  $\mu\text{m}$ ).

**Polyester fibers** were prepared by cutting a fleece blanket followed by cryo-milling using a homogenizer (MillMix 20, Domel, Slovenia). The fibers had a shape of narrow strips with an approximate length  $220 \pm 200 \mu\text{m}$ , ranging from 12 to 2 870  $\mu\text{m}$  and height of 6  $\mu\text{m}$  (Selonen et al., 2020).

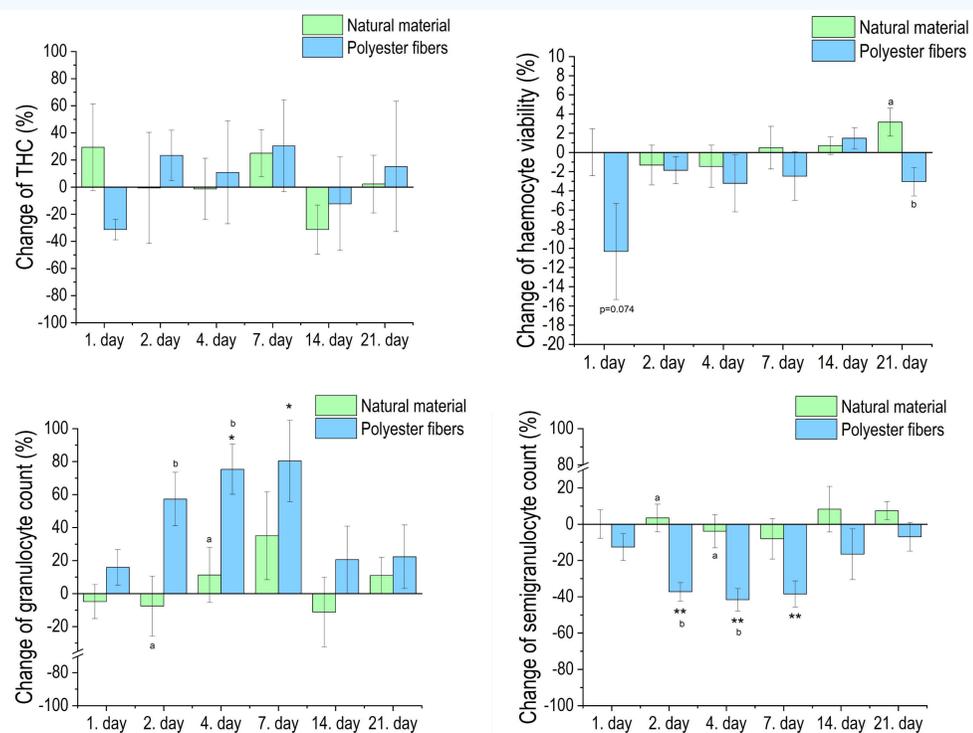
**Natural control:** wooden particles from beech tree, mean size 212  $\mu\text{m}$

**RESULTS:** Immune parameters are modulated upon exposure to microplastics. Total haemocyte count (THC) was slightly, but insignificantly, increased and also their viability was not affected. The number of granulocytes was increased and semigranulocyte count was decreased implying a shift in metabolic action of haemocytes. The response was time-dependent with the peak of changes in period 2-7 days. A gradual return to control levels was found after 3 weeks of exposure, however some parameters were not entirely the same as the controls. No clear microplastics dose-related trend was observed. Natural control (wooden fibers) did not induce any effects.

### Different concentrations of microplastics after 21 days:



### Time-dependent immunomodulation (all conc. 1.5%, w/w) example: polyester fibers



**CONCLUSIONS:** Our results reveal a clear time-dependent immunoactivation in *Porcellio scaber* exposed to microplastics. The response was most pronounced after 4-7 days and a gradual decline to nearly control levels were evident after 21 days. What this alteration in immune processes means for the immunocompetence of the organism as well as its general fitness remains to be investigated.

\* in comparison to control (control=0; source culture)  
a,b = comparison between treatments/day

