Vermiremediation of micropollutants from sewage sludge

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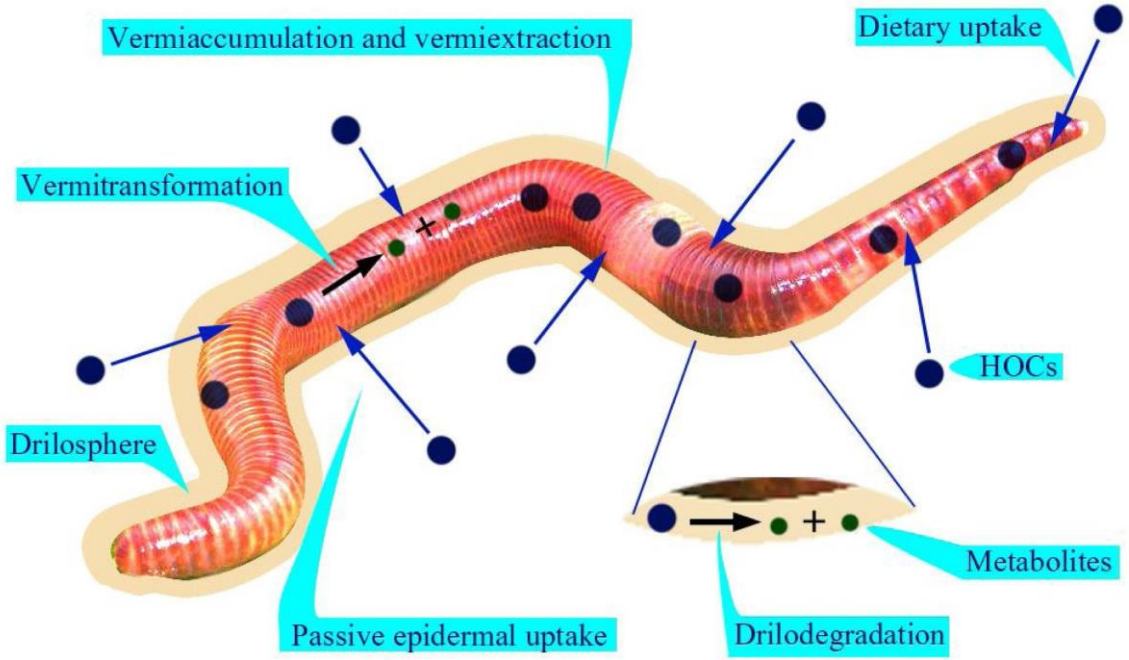
Vermicomposting

- using earthworms to decompose organic matter
- earthworm's muscular action and enzymes, microorganisms
- product vermicompost is rich in nutrients
- biological decontamination of sewage sludge

http://g2nursery.com/product/vermicompost/

Vermiremediation

- remediation of pollutants by earthworm activity
- heavy metals, PCBs, PAHs, crude oil
- micropollutants?



Shi et al., 2020. Vermiremediation of organically contaminated soils: Concepts, current status, and future perspectives

Micropollutant analysis

1. freeze – drying of samples
2. extraction to methanol
3. purification if needed
4. analysed on LC/MS triple quadrupole for targeted analysis

37 pharmaceuticals and personal care products

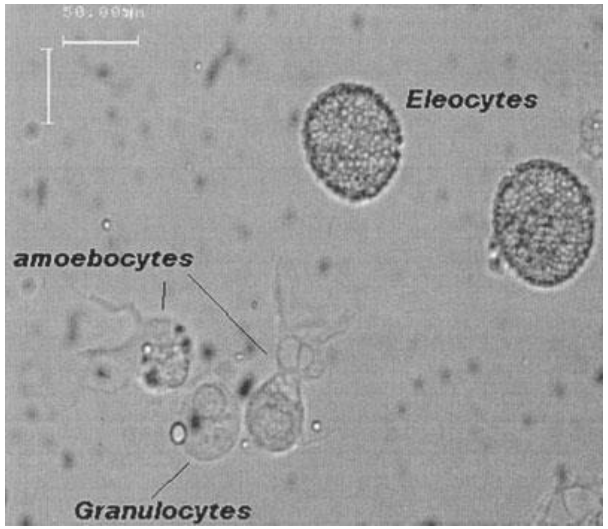
15 endocrinne disruptors

37 per/polyfluorinated compounds

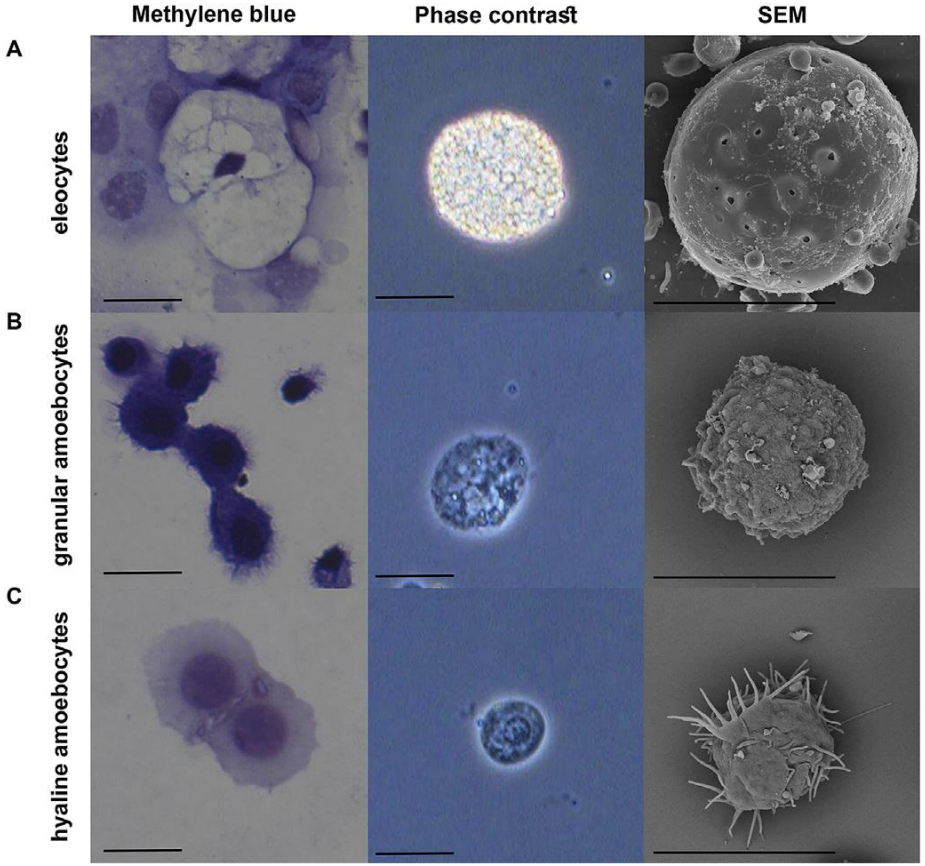
Cytotoxicity effects

- earthworm immune cells coelomocytes

- coelomocytes
  - eleocytes
  - amoebocytes
    - hyaline
    - granular



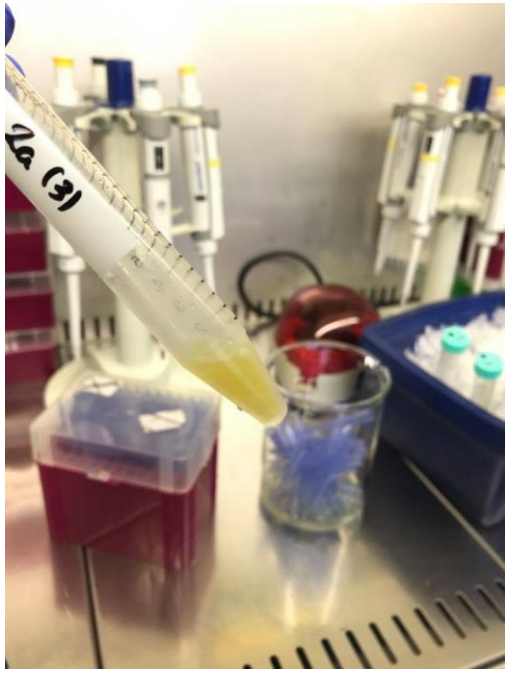
Di Marzio et al., 2005. Improved Single-Cell Gel Electrophoresis Assay for Detecting DNA Damage in *Eisenia foetida*

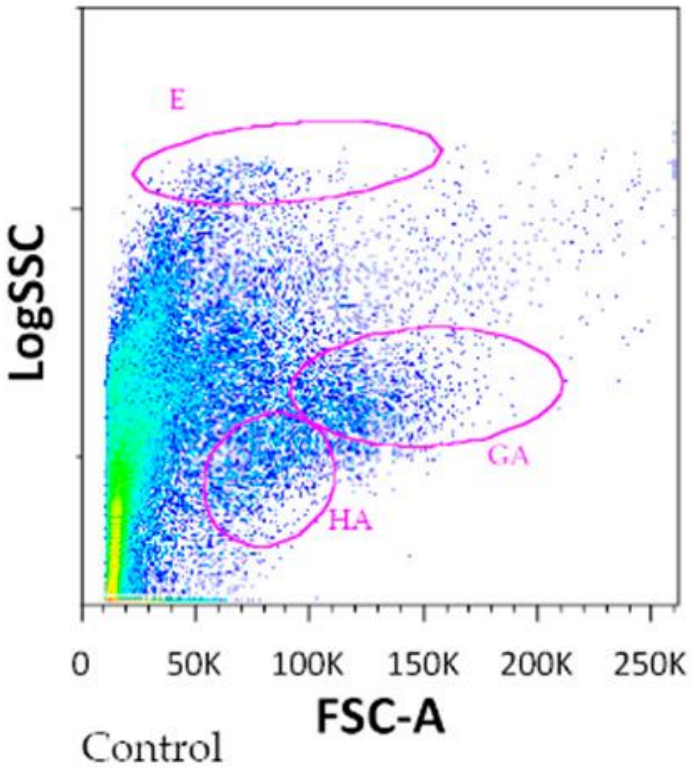


Prochazkova et al., 2020. Pattern recognition receptors in annelids

Battery of cytotoxicity tests

- exposition *in vivo* or *in vitro*
- cell harvesting: extrusion buffer – non-invasive method (chemical)
- staining
- flow cytometer measurement

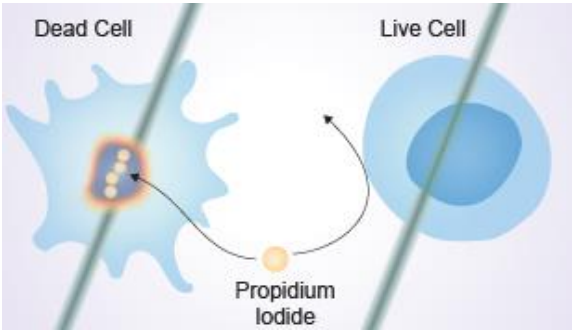




Semerad et al., 2020. In Vitro Study of the Toxicity Mechanisms of Nanoscale Zero-Valent Iron (nZVI) and Released Iron Ions Using Earthworm Cells

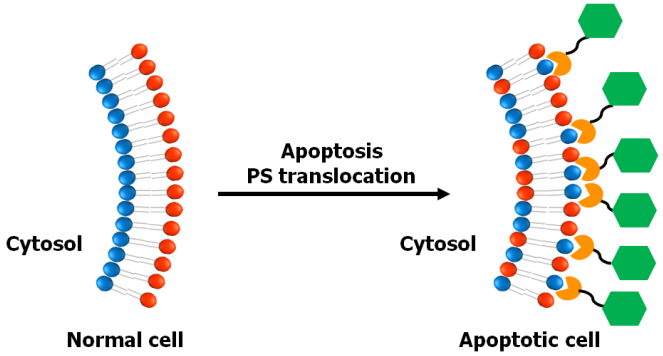
Fluorescent staining

- cell viability – propidium iodide



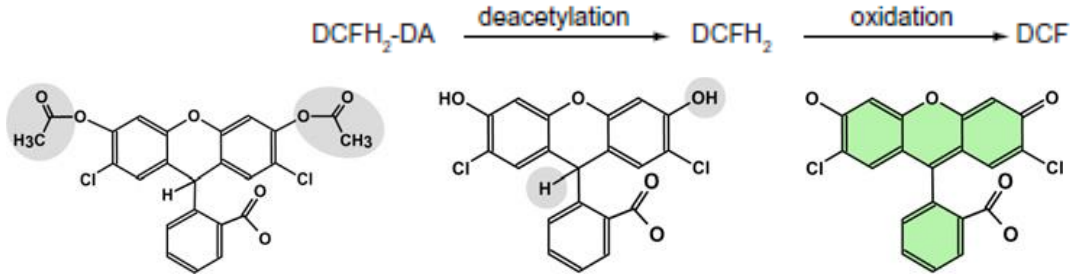
https://www.rndsystems.com/resources/protocols/flow-cytometry-protocol-analysis-cell-viability-using-propidium-iodide

- apoptosis/necrosis – annexin V



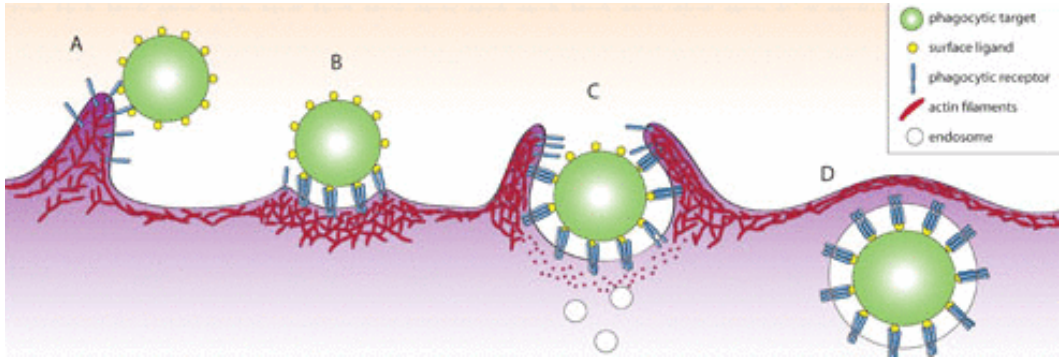
https://www.abpbio.com/product/annexin-v-binding-assays/

- reactive oxygen species – 2′,7′-dichlorofluorescein diacetate



Reiners et al., 2012. 2′,7′ -Dichlorofluorescein is not a probe for the detection of reactive oxygen and nitrogen species

- phagocytosis – Fluoresbrite microspheres 1,00 µm

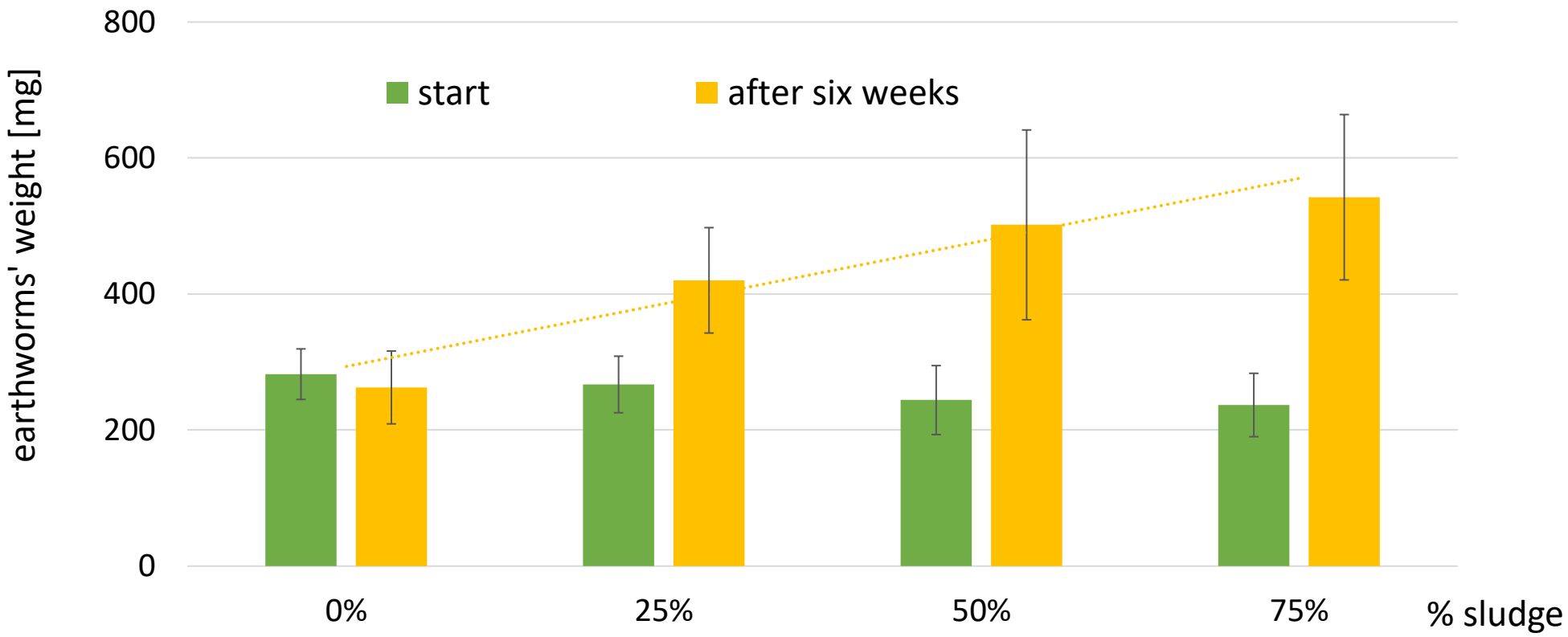


Jaumouillé, Grinstein, 2016. Molecular Mechanisms of Phagosome Formation

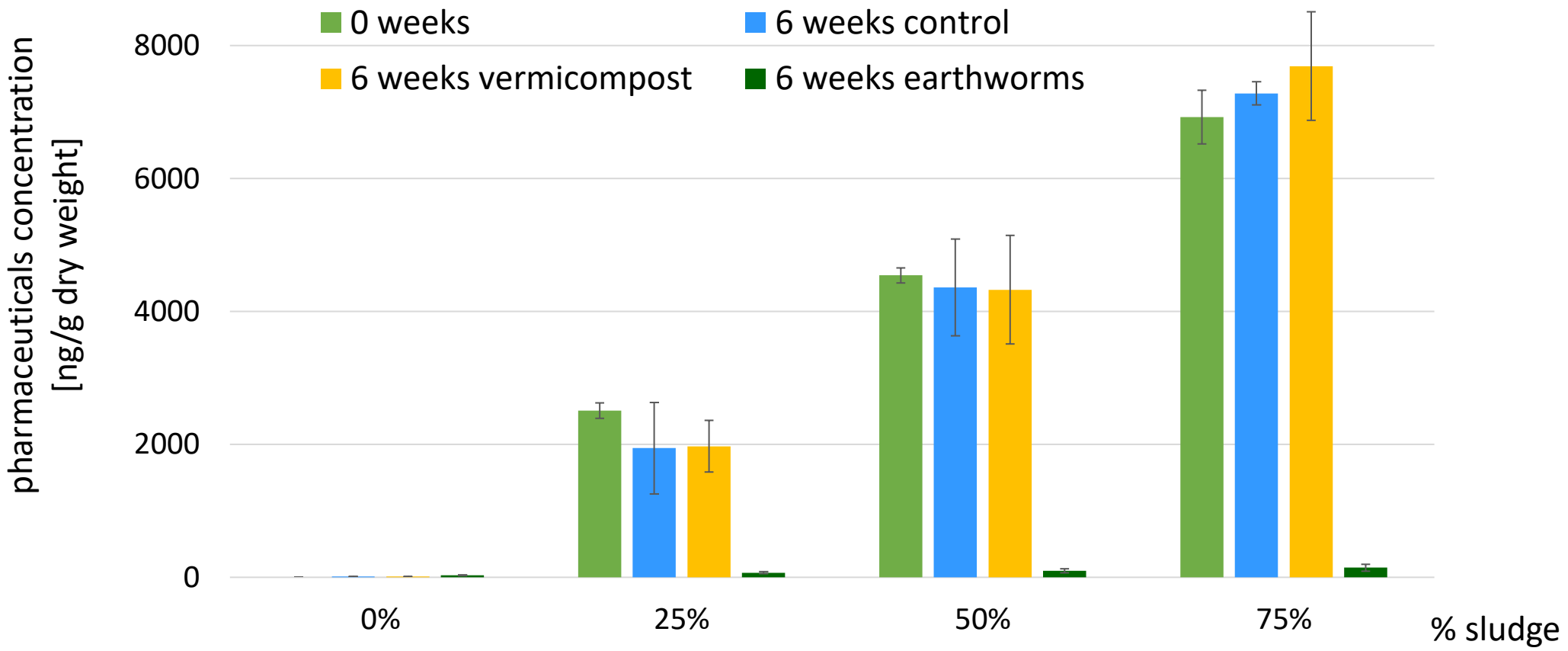
Experimental design

- 3 batches of sewage sludge from different WWTPs
- sewage sludge mixed with straw – 0, 25, 50 and 75% sludge
- triplicates
- earthworm *Eisenia andrei*
- 20 earthworms/200 g substrate
- control samples – without earthworms
- 6 weeks
- textile sacks
- controlled temperature and humidity
- darkness

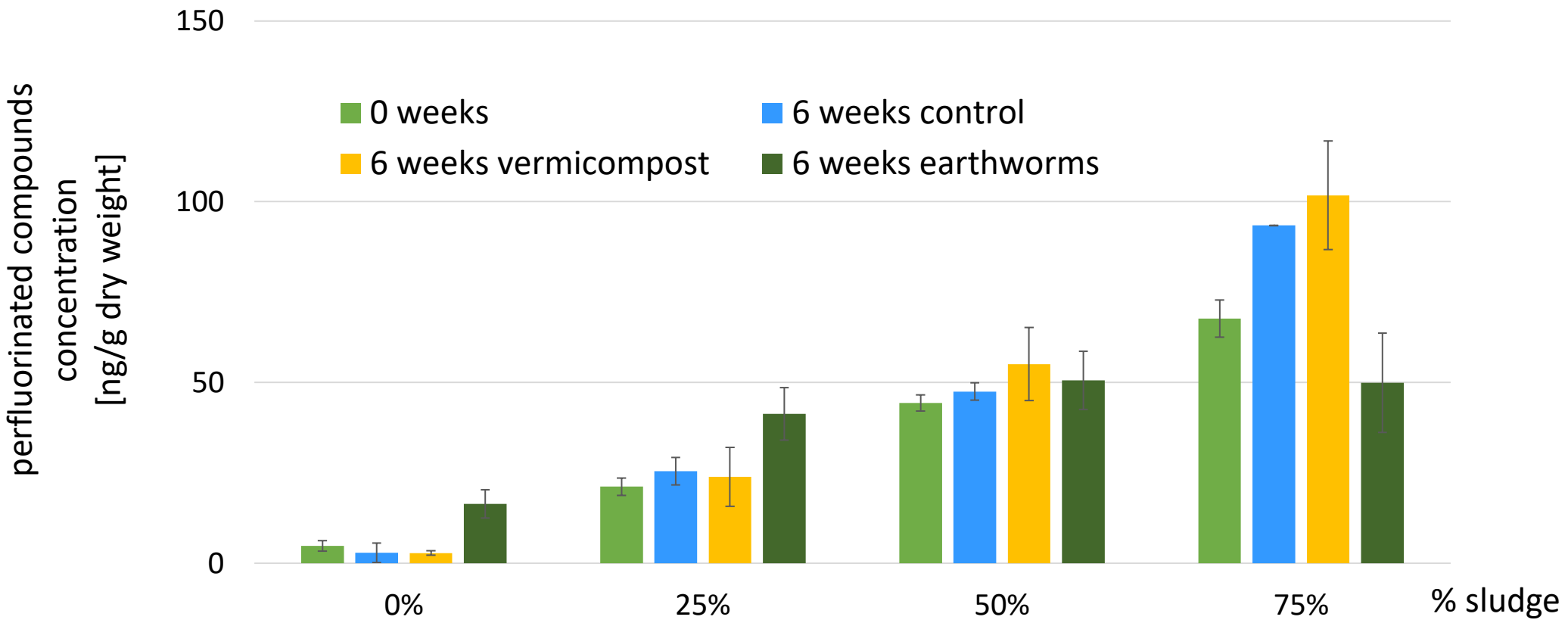
Earthworms‘ weight



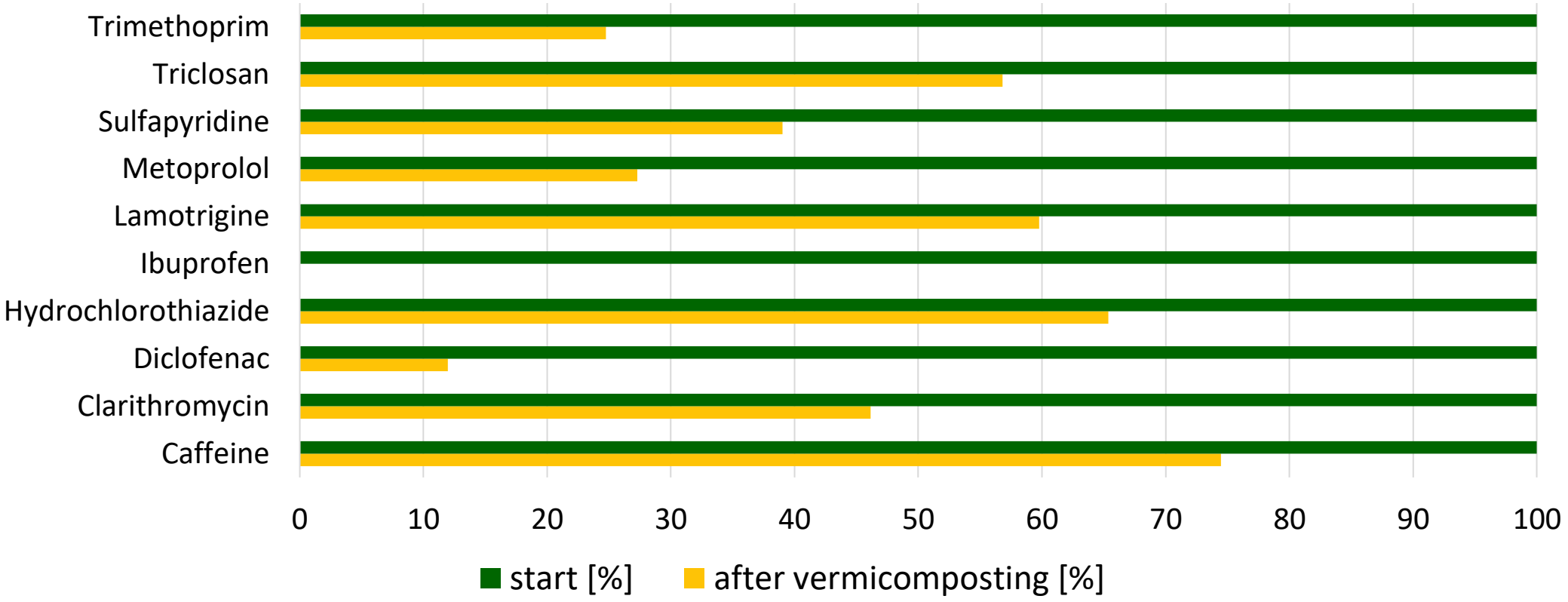
Pharmaceuticals & endocrinne disruptors



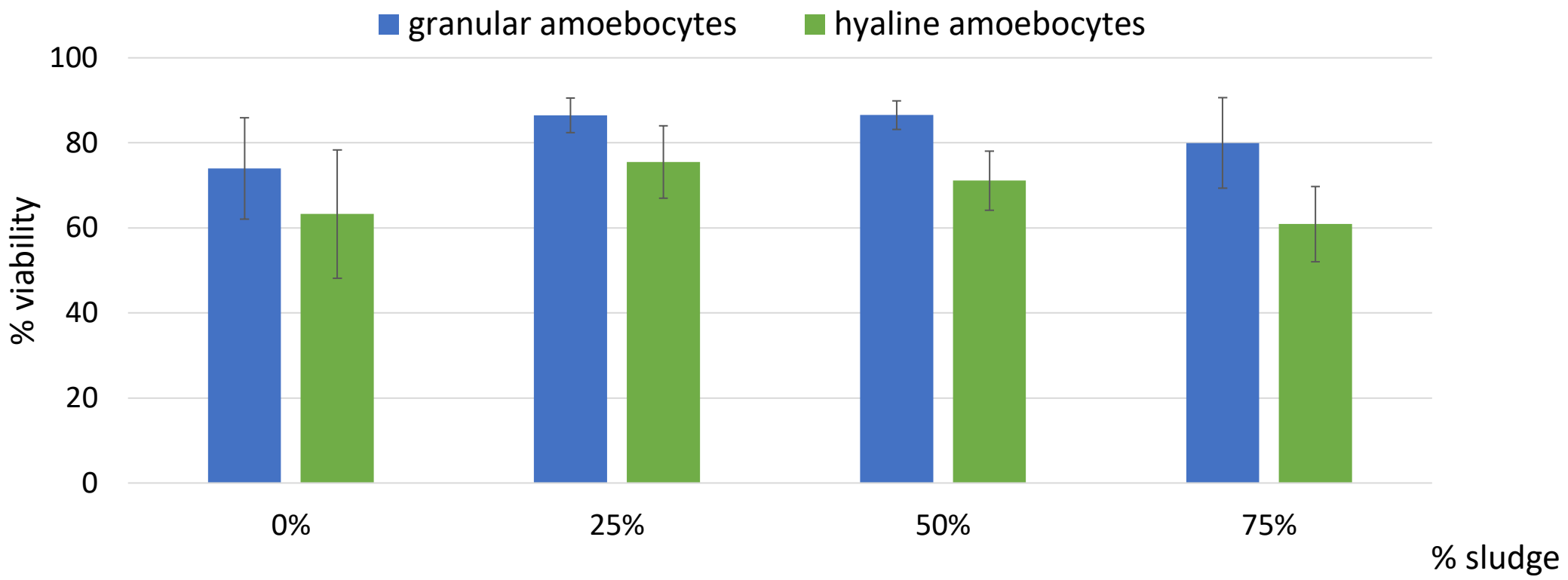
Poly/perfluorinated compounds



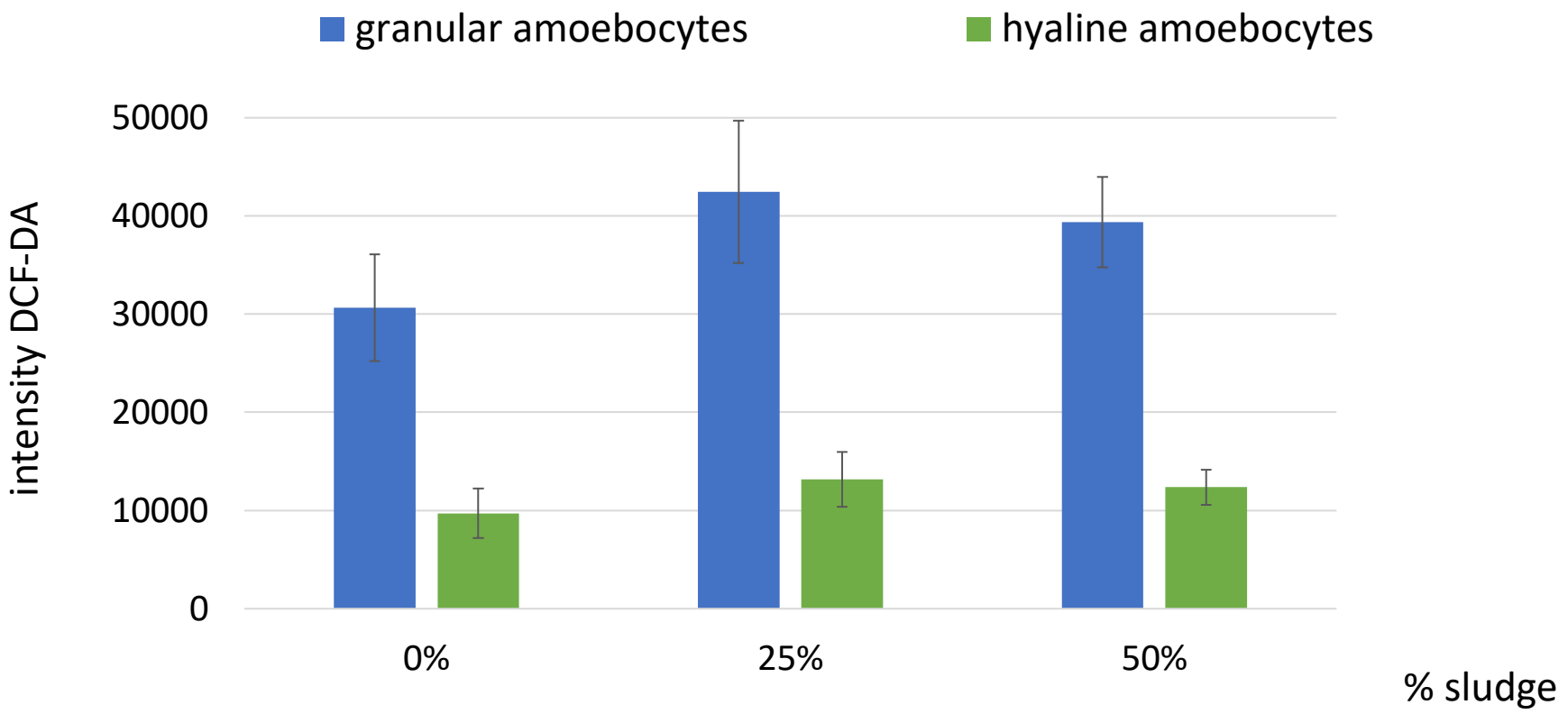
Selected micropollutants degradation (75 % sludge)



Cytotoxicity - viability



Cytotoxicity – ROS



Conclusion

- increase in earthworms' weight
- decrease of some micropollutants in the substrate
- bioaccumulation of micropollutants in earthworms
- no changes in cell viability
- increase in reactive oxygen species production

Thank you for your attention

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