**Genotoxicity assessment ofsoils near heavy traffic roads using native populations of earthworm *Aporrectodea caliginosa***

Šrut Majaa, Štambuk Anamariaa, Husnjak Ivanab, Merkaš Martinac, Traven Lukad,e, Cvetković Želimiraf, Klobučar Göran I.V.a

1. Department of Zoology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, 10000 Zagreb, Croatia
2. Ministry of Environmental Protection, Physical Planning and Construction, Ulica Republike Austrije 14, Zagreb
3. Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Šalata 12, 10000 Zagreb, Croatia
4. Department of Environmental Medicine, Medical Faculty, University of Rijeka, Braće Branchetta 20a, 51000 Rijeka, Croatia
5. Teaching Institute of Public Health of the Primorsko-goranska county, Krešimirova 52a, 51000 Rijeka, Croatia
6. Department of Ecology, Institute of Public Health, Mirogojska c. 16, 10000 Zagreb, Croatia

Correspondence: msrut@biol.pmf.hr

**Abstract**

The application of biomarkers to field collected earthworms receives increasing interest due to their important role in the terrestrial ecosystems. Therefore, the aim of this study was to evaluate the usability of native populations of endogeic, widely distributed earthworm species, *Aporrectodea caliginosa* in theassessment of soil genotoxicity using the Comet assay. Validation of the Comet assay on earthworm coelomocytes has been established using *A. caliginosa* exposed to copper in a filter paper contact test. Furthermore the method has been applied on native populations of *A. caliginosa* sampled from the soils with different pollution intensity. Two of the sites were in the urban area (city of Zagreb) polluted by vehicular traffic, with high concentrations of heavy metals, PAHs, and mineral-oil hydrocarbons while the third site was considered as a reference site. To additionally assess the toxic effect neutral red retention time (NRRT) assay, a well established earthworm biomarker of toxicity, was conducted on copper exposed and field collected earthworms. Both methods revealed significant biological effect in copper exposed earthworms as well as in populations of *A. caliginosa* from polluted sites in comparison to the earthworms from the reference site. These results confirm the applicability of *A. caliginosa* as a suitable earthworm species for the *in situ* soil toxicity and genotoxicity surveys.

Keywords: Comet assay, DNA damage, NRRT assay, coelomocytes, *Aporrectodea caliginosa*