

## **Earthworms (Lumbricidae) reaction to stress at the population and cell level**

### Abstract

Earthworms (Lumbricidae) play an important role in numerous processes occurring within ecosystems. As "*ecosystem engineers*" they influence the physical and chemical properties of soil and participate in the functioning of generally understood environment.

Moreover, they are of a considerable practical importance, for instance, in waste management. Vermicomposting with the presence of earthworms may be one of the efficient methods of waste disposal in the place of its production. It is an effective method, commonly utilised in the world and constituting an object of a multi-discipline research, however still requiring updating of solutions to numerous problems.

Furthermore, due to their high sensitivity to environmental stress earthworms can be used for soil ecotoxicology. The animals can be used as bio-indicators in examining soil contamination, e.g. with various xenobiotics. Thanks to such defence mechanisms as cellular response system, it is possible to use them in monitoring the level of soil contamination also at the level of their coelomocytes. Earthworms exposed to a stress factor release the coelomic fluid with coelomocytes contained in it. The method of obtaining coelomocytes is utilised by scientists investigating the immunological system in earthworms. Thanks to their sensitivity to various types of stress, earthworms and coelomocytes are used as a model in environmental contamination research.

Lumbricidae may also be used as an experimental model at the first stage of testing of various chemical substances, including local anaesthetics used to anaesthetise vertebrates. The promising results of observations of procaine and lidocaine effects on earthworms allow to believe that using these animals as models could replace the costly experiments on guinea pigs, rabbits, rats and mice that require approval of the ethical committee.

The objective of this work was to determine the reactions of earthworms to stress, including: () presentation of selected properties of earthworm populations of *Eisenia fetida* (Sav.) and *Dendrobaena veneta* (Rosa) species in an annual cycle, which has a practical meaning, for instance, in species selection for vermiculture and vermicompost production; () determination of the impact of a regular stress factor on selected properties of researched earthworm populations and their coelomocytes (stress is understood as an effect of an electrical current stimulation with the voltage of 4.5V); () determination of the effect of procaine and lidocaine on the examined earthworm species for the purpose of detection of toxic concentrations – LC<sub>05</sub>, LC<sub>50</sub>, LC<sub>95</sub> (stress is understood as an effect of selected local

anaesthetics); () determination of procaine and lidocaine concentrations that are safe and efficient in reversible anaesthesia of earthworms.

The results of the last part of the work are important both in the selection of a proper mean to immobilise earthworms in undertaking such a demanding research, as well as building of a new platform of knowledge connected with the possibility to utilise earthworms in preliminary testing of various pharmaceuticals.

The research object were two earthworm species of *Eisenia fetida* (Sav.) and *Dendrobaena veneta* Rosa. The first part of the work analyses changes in the populations of researched species occurring over the year. The second part investigates the reaction of earthworms to stress induced with an electrical stimulation (changes within the population, coelomocyte count). The last part of the paper defines the reaction of earthworms to anaesthetics – procaine and lidocaine.

The analysis of a yearly cycle of development of medium-sized populations of *E. fetida* and *D. veneta* showed that the equally sized start populations (of 10 specimens) of both species kept developing until research conclusion, which proves that proper conditions had been ensured. The population of *E. fetida* earthworms was characterised by a quick pace of development – after 52 weeks its size was three times the size of the *D. veneta* population. With the three times larger population, *E. fetida* earthworms reached a significantly lower average individual mass as compared to *D. veneta*. This concerned all the immature, premature and mature specimens. The lower individual mass of *E. fetida* was however compensated with a larger population, and, consequently, with similar total biomass as compared with *D. veneta*. Cocoons laid by *E. fetida* were lighter, narrower and shorter as compared with those laid by *D. veneta*, however the time of appearance of hatched young specimens was similar. *E. fetida* earthworms were first to achieve sexual maturity, whereas in the case of *D. veneta* species this process was longer – premature, mature specimens and cocoons appeared later. Both species demonstrated similar "vitality" – the mortality rate of both populations in the experiments reached 17%.

On the basis of an analysis and comparison of selected properties of vermicomposts produced from household waste it was confirmed that in the case of *D. veneta* presence, vermicomposts were richer in potassium, sodium and magnesium as well as contained more cadmium and chromium. Despite the use of various earthworm species the obtained vermicomposts were characterised by a similar content of nitrogen, phosphorus, calcium, lead, zinc, copper, nickel, manganese and iron. The vermicompost produced by *D. veneta* was characterised by a higher pH value and lower salinity.

The research showed diversified reactions of *E. fetida* and *D. veneta* to stress induced with an electrical stimulation on a population level. In the case of *E. fetida* regular stimulations with electrical current (4.5 V) did not have an effect on the average population size, total biomass and average body weight of specimens constituting complete populations and cocoons. The sensitivity was revealed in the form of a decrease in the average individual body mass, the average biomass sum of specimens constituting incomplete populations and the average mass of a single cocoon.

In the case of earthworms of *D. veneta* species the applied stress factor induced vaster changes visible on a population level. The electrical stimulation caused a decrease in the count of all analysed age groups and cocoons. Moreover, specimens reacted with a reduction in the average individual mass and average biomass within the population. This concerned all age classes of *D. veneta* specimens in all populations.

The electrical stimulation did not affect the life expectancy among the *E. fetida* population, however the *D. veneta* population showed a significant reduction in the likelihood of survival of the stressed population.

The effects of regular electrical stimulations, and thus the deprivation of *E. fetida* and *D. veneta* earthworms of coelomic fluid were manifested at the cellular level in the form of reduction of the total number of coelomocytes and a lower percentage of eleocytes. The low content of cells in coelomic fluid may not only confirm the effect of an electrical stimulation, but also the fact that earthworms need a period longer than 4 weeks to restore the full count of coelomocytes.

The opinions expressed among earthworm breeders were that electrical current may stimulate a more rapid development in earthworms. Although the research failed to confirm this hypothesis it is still required to search for other possible development stimulators for the populations of earthworms used in verminocomposting of organic wastes.

The reactions of earthworms to anaesthetics were diversified. The possibility to use a higher concentration range of procaine and lidocaine for *E. fetida* as compared to *D. veneta* confirms a higher immunity of *E. fetida* to the applied stress in the form of chemical substances. The reaction to this kind of stress was diversified and depended on the applied concentration. Higher anaesthetic concentrations caused rapid body movements, excretion of coelomic fluid and morphological changes observed upon the lapse of 48 hours. Mortality among earthworms grew along with the increase of concentrations of the applied substances.

The LC<sub>05</sub>, LC<sub>50</sub>, LC<sub>95</sub> values showed significant differences within the analysed anaesthetics and species of tested earthworms. Procaine was inefficient in immobilising

earthworms, whereas it was confirmed that lidocaine may be used in reversible immobilisation of both species.