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Article *in* Journal of Ecological Engineering · March 2025

DOI: 10.12911/22998993/201236

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JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(5), 220–229
https://doi.org/10.12911/22998993/201236
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Received: 2025.02.05
Accepted: 2025.02.25
Published: 2025.03.16

Species-dependent population-level responses of composting earthworms *Eisenia fetida* and *Dendrobaena veneta* to a regular stress factor

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ABSTRACT

Earthworms of the species *Eisenia fetida* and *Dendrobaena veneta*, referred to as composting earthworms, are commonly used in the processing of organic waste of plant origin. The product of this process is vermicompost, i.e. fertilizer used in agriculture. Both species, despite morphological and physiological similarities, are characterized by different life strategies, but also show different reactions to stress factors. The aim of the study was to analyze the population-level response of two species of composting earthworms, *E. fetida* and *D. veneta*, to a repeated stress factor – low-voltage electric current (4.5 V). The studies confirmed that both analyzed earthworm species show different sensitivity to the stress factor. In *D. veneta* earthworms, a negative effect of stress was observed in the form of a decrease in numbers, body weight and parameters related to cocoons, compared to *E. fetida* earthworms, which turned out to be resistant to this type of stress factor.

Keywords: life cycle, population parameters, electric current, species diversity.

INTRODUCTION

Eisenia fetida (Savigny) and *Dendrobaena veneta* (Rosa) are earthworms species often called ‘composting earthworms’ (Reinecke and Viljoen, 1990; Viljoen *et al.,* 1991). These invertebrates are commonly used in the decomposition of organic plant-derived matter, specifically in the production of vermicompost (Kostecka *et al.,* 2018). This form of organic fertilizer is gaining increasing popularity in sustainable agriculture, where it has been demonstrated to enhance crop productivity by promoting the breakdown of organic residues, enriching the soil with essential nutrients and stimulating microbial activity (e.g. Enebe & Erasmus, 2023). Apart from the role of earthworms in processing organic waste into vermicompost, these invertebrates have also been used in environmental and laboratory research as bioindicators for assessing soil quality, given their sensitivity to environmental stress as pollutants, including heavy metals, organic contaminants and microplastics (Wang *et al.,* 2024).

A stressor is any external or internal stimulus that disrupts an organism’s physiological balance, requiring an adaptive response (Asres & Amha, 2014). Stress factors acting on the earthworm organism can vary and elicit different responses. In their natural environment, earthworms, upon exposure to a stressor, such as a predator attack, a sudden temperature change, exposure to intense light, or contact with a chemical substance, begin to rapidly contract and relax their muscles, which is observed as wriggling (Singh *et al*., 2019). As a result of this response, coelomic fluid containing coelomocytes is expelled from the pores located along the body (Podolak-Machowska *et al.,* 2014). This method is commonly used by researchers to collect coelomic fluid, which has a wide range of applications (Roch 1979). The species’ adaptability to different environmental conditions has been an area of active research, especially in relation to its reproductive strategies and survival mechanisms under stress (Hawkins *et al.*, 2020). In this type of study, the selection of the right species for testing plays an important role. International ISO (1993) and OECD standards (1984) describe the *E. fetida* species as the test organism. However, due to the different sensitivities to environmental stress, it seems justified to use several species of earthworms that show varying levels of sensitivity to the stress factor (Dittbrenner *et al.,* 2011). Consequently, different species exhibit varied responses to the same stressor, a variation that stems from their distinct life strategies, genetic traits, and previous ecological experiences.

Both species of earthworms, living in a similar environment, which is soil rich in organic substances, show a number of differences in morphological structure, behavior and represent a different life strategy (Kasprzak 1986; Dominguez & Edwards, 2011a). Previous studies comparing the life cycle this species have shown that *D. veneta* individuals achieve a significantly higher individual mass and lay larger and heavier cocoons compared to *E. fetida* (Podolak *et al.,* 2020). Other studies have shown the differences in the sensitivity of both analyzed earthworm species to the chemical stress factor in the form of selected anesthetics. It has been proven that *D. veneta* earthworms are more sensitive to stress, in contrast to *E. fetida*, which appears to have a wider range of tolerance to different stress factors (Podolak *et. al.,* 2019).

Considering the differences in the life strategies of both „composting" earthworm species, the objective of the present study was to analyze the population-level response of two species of composting earthworms, *Eisenia fetida* and *Dendrobaena veneta*, to a repeated stress factor - low-voltage electric current (4.5 V).

MATERIAL AND METHODS

Animals

The experiment was conducted using *Eisenia fetida* (Savigny, 1826) and *Dendrobaena veneta* (Rosa, 1893) (Annelida, Oligochaeta), which were sourced from laboratory colonies at the University of Rzeszów (Poland). The earthworm cultures were kept under controlled conditions in the laboratory (18 ± 2 °C, 24L), housed in plastic containers filled with horticultural substrate/soil (Kronen Universalerde). The substrate's properties, as provided by the manufacturer, were as follows: pH ($CaCl_2$) 5.5–6.5; nitrogen (N) 200–450 mg/l; phosphorus pentoxide (P2O5) 200–400 mg/l; potassium oxide (K_2O) 300–500 mg/l; and ISO 9001 (2000) certified. The earthworms were regularly supplied *ad libitum* with organic household waste.

Experimental design

Adult earthworms (*clitellate*) were placed in plastic boxes containing 8 dm^3 of soil, in groups of 10 individuals per box for each species. Every four weeks, the containers with earthworms were carefully checked using the hand-sorting method of the substrate and the cocoons and earthworms found were counted and weighed separately after thorough cleaning. After that pre-mature and mature earthworms in groups of 10 individuals were placed in Petri dishes containing 20 ml of 0.9% NaCl (B. Braun Melsungen, Na – 154 mmol/l; Cl – 154 mmol/l) and stimulated with a low-voltage electric current supplied from the battery (4.5 V VARTA Superlife) for 1 minute according to the safe method of obtaining coelomocytes modified for the purpose of the experiment (Roch, 1979 as cited in Płytycz *et al.,* 2006). After that all cocoons and animals were returned to the appropriate containers. Each container was fed *ad libitum* with organic household waste. The experimental conditions (e.g. temperature, humidity, type of substrate) reflected experimental conditions in vermiculture. The experiment was carried out in triplicate and lasted 52 weeks.

Statistical analysis

The results were shown as means ± SD. *Two-way ANOVA* and multiple comparisons of means (*NIR*) (independent variables – time, stress or no stress) were used to determine differences in the mean number, weight, fertility of the observed earthworm populations. Before performing the appropriate tests, it was checked whether the data distributions followed a normal distribution (*Shapiro-Wilk test*) and whether the variances across groups were homogeneous (*Brown-Forsythe test*). STATISTICA v. 13 (*StatSoft*) was used for statistical analyses.

RESULTS

Changes in earthworm population size

The results of the study revealed that after 52 weeks of the experiment, the population of *E. fetida* consisted of 3265 ± 420 individuals, whereas the population of *D. veneta* comprised only 1092±57 individuals. Analysis of the developing population of *E. fetida* throughout the experimental period showed no significant effect of the applied stress factor on the total population size ($p > 0.05$). However, it was demonstrated that the population of *D. veneta*, regularly exposed to stress, exhibited a significantly lower population size compared to the control group ($p < 0.001$) (Table 1).

No significant differences were observed in the number of earthworms classified as immature, pre-mature, and adult individuals between the stress-exposed group and the control group for *E. fetida* ($p > 0.05$). However, *D. veneta* earthworms were found to be sensitive to the applied stress, as evidenced by a significantly lower number of individuals in each age class ($p < 0.01$) (Fig. 1a-c). The first cocoons were observed as early as the 4th week of the experiment in both studied species. No significant differences in the number of cocoons were found compared to the control group for *E. fetida* ($p > 0.05$), whereas for *D. veneta*, significantly less cocoons were recorded in the populations exposed to the stress factor compared to the control group (Fig. 1d).

Table 1. Change in the number of the population of *E. fetida* and *D. veneta* earthworms (individual·container^{-1} ± SD) exposed to a regular stress factor (4.5 V) compared to the control group in the annual cycle

Week	0	4	8	12	16	20	24	28	32	38	44	52	Coefficient of variation
Eisenia fetida													
Control	10±0	10±0	32±9	393±63	575±140	813±148	999±135	1548±178	2449±367	2417±265	3047±662	3265±420	85%
Stress	10±0	10±0	45±4	458±137	590±195	903±225	1188±290	1376±327	1921±342	2458±78	2836±329	3817±719	86%
Dendrobaena veneta													
Control	10±0	10±0	25±4	87±9	162±3	258±7	350±19	443±22	542±41	500±16	713±27	1092±57	94.5%
Stress	10±0	10±0	23±4	80±13	155±33	241±58	321±96	385±71	461±119	398±107	497±211	595±139	78.3%

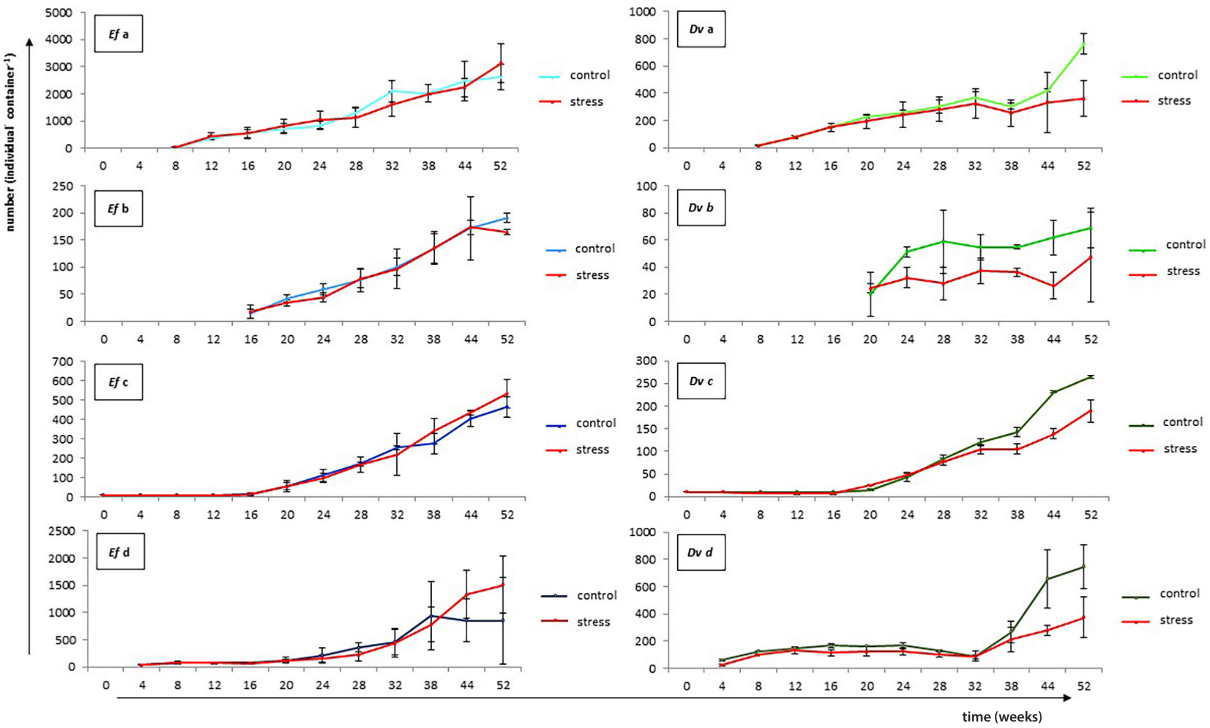

Figure 1. Change in the number of earthworms (individuals·container^{-1} ± SD): a) immature, b) pre-mature, c) mature d) cocoon of *E. fetida* (left side) and *D. veneta* (right side) exposed to a regular stress factor (4.5 V) compared to the control group over the annual cycle

Changes in the biomass of the earthworm population

To investigate the response of both „composting species" to the regularly applied stress factor in the form of low-voltage electrical current, data on the body mass of individuals from the experimental groups were also analyzed and compared to the control groups. Analysis of the total biomass of the populations revealed no detrimental effect of stress on *E. fetida* ($p > 0.05$) over the 52-week study period. However, a significant response was observed in *D. veneta*, with a marked difference in the total biomass of the population exposed to electrical current ($p < 0.001$) (Fig. 2).

In populations with increasing animal density, the average body weight of individual individuals decreased significantly, which was observed at the second population status check, for both species of earthworms studied ($p < 0.001$). However, the regular stress factor did not affect the average individual weights of individuals exposed to electric current in this case, compared to the control for the species *E. fetida* ($p > 0.05$). A similar pattern was observed for all separately analyzed age groups of earthworms of this species ($p > 0.05$), which confirms that the species *E. fetida* was not sensitive to this type of stress factor (Fig. 2 a-c left side).

Classifying the *D. veneta* earthworms into distinct age groups revealed no effect of stress on the total biomass of immature individuals ($p > 0.05$), although a significant effect was observed for the average body mass ($p < 0.05$) (Fig. 2a, right side). The average total biomass of pre-mature individuals in the stressed groups was 16.23 ± 2.96 g, significantly lower than that of the control group individuals, which was 24.50 ± 6.66 g ($p < 0.001$). Analysis of the average body mass of individual pre-mature earthworms showed no effect of stress on this parameter ($p > 0.05$) (Fig. 2b, right side). In the case of adult *D. veneta* individuals, electrical current significantly reduced the total biomass of the population ($p < 0.001$), although no significant effect was observed on individual body mass (p 0.05) (Fig. 2c, right side).

When analyzing the mass of cocoons laid by earthworms of both species, no response to stress in the form of a decrease in their total biomass and individual mass was observed ($p > 0.05$) (Fig. 2d left and right side).

DISCUSSION

Earthworms, like other organisms living in the soil, are constantly exposed to various abiotic and biotic factors, such as radiation, temperature,

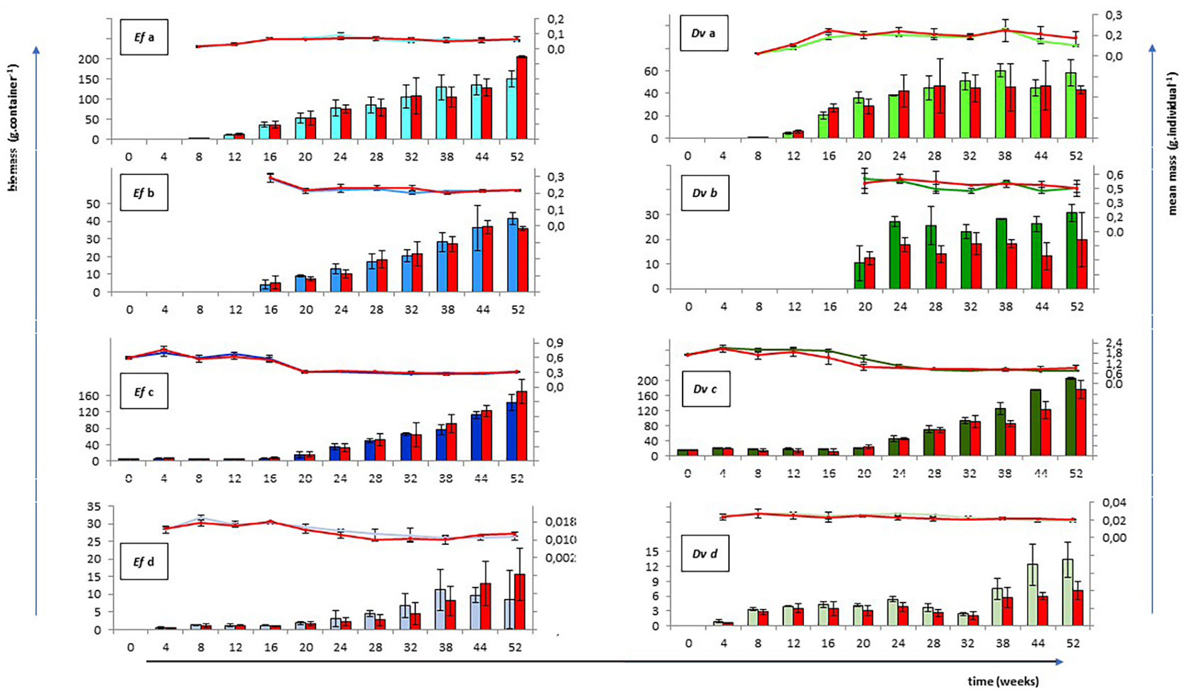

Figure 2. Change in the total biomass (bar chart), mean body mass (line graph) (g·container^{-1} ± SD): a) immature, b) pre-mature, c) mature d) cocoon of *E. fetida* (left side) and *D. veneta* (right side) exposed to a regular stress factor compared to the control group over the annual cycle

water, chemical substances, mechanical stressors and other organisms. Operating in the variable conditions of the external environment, organisms use homeostatic mechanisms to maintain a state of relative internal stability. When exposed to various stressors that exceed their tolerance range, these mechanisms are disrupted, leading to a state that, from a biological perspective, can be understood as the result of the organism's interaction with the external environment (Rasmussen and Holmstrup, 2002; Frindt *et al.,* 2006; Mackenzie *et al.,* 2009; Suthar, 2014).

There are various concepts describing the mechanism of stress, the biological foundations of which are based on Cannon's 1939 theory of homeostasis. The main assumption of this theory is that each organism has biochemical and physiological regulatory mechanisms that allow it to maintain a state of relative balance. According to Selye's theory (1977), physiological stress is a non-specific reaction of any living organism caused by the action of strong stimuli. As a result of the stressor, both a single cell and the entire organism apply the "fight or flight" principle. If the proverbial escape is not possible for various reasons, the organism tries to adapt to the new conditions in which it finds itself. Among the changes occurring in the organism in stressful conditions, two basic adaptive mechanisms can be distinguished. The first is the local adaptation syndrome, which includes changes occurring only at the site of the stressor, and the second is the general adaptation syndrome, which concerns the reaction of the entire organism. The reaction of the entire organism consists of three phases: an alarm reaction that initiates the mobilization of the body's defense forces, immunity, or adaptation to new conditions, which lasts for some time, and the exhaustion phase. The latter is a consequence of a long-term or too intense stress factor that affects the loss of the body's defense capabilities (Selye, 1956, 1977; Grygorczuk, 2008; Rice, 2012; Szabo *et al.,* 2012).

Selye (1977) emphasizes that all animals are sensitive to stressors, including those lower organized, which do not have a well-developed nervous system. Earthworms belonging to the Annelida have a simplified nervous system (ladder type), consisting of, among others, a nerve trunk connected to nerve ganglia. The subesophageal ganglion connects with nerve fibers surrounding the esophagus, creating a paraesophageal ring. On the dorsal side, there is a supraesophageal (cephalic) ganglion, which is the equivalent of the brain. It controls body movements, but also complex forms of behavior such as searching for food and avoiding noxious stimuli (Kasprzak, 1986; Jura, 2007; Sadowski, 2012). Having a nervous system, earthworms, like other animals, have developed the ability to avoid or minimize the effects of stimuli that are dangerous to them because they cause, for example, damage to their body tissues (Rożen, 1994; Christensen & Mather, 2004, cited in Rożen, 2004; Elwood, 2011).

The effects of stress can be seen at various levels of the organization of life of organisms and can affect their cells, single individuals or entire populations. A stress factor can disrupt enzyme activity, increase mortality, reduce fertility, growth and development and influence changes in individual behavior (Pelosi *et al.,* 2014).

Earthworms, as a result of their stress response, expel coelomic fluid along with the coelomocytes contained in it. They can lose it, for example, under the influence of a sudden change in temperature, intense light, high concentration of chemical substances or as a result of mechanical stimuli (Wikiera *et al.,* 1996; Hendawi *et al.,* 2004; Engelmann *et al.,* 2005; Jura, 2007). Scientists involved in research related to earthworm coelomocytes have long been using this phenomenon, using, among others, modifications of the method of obtaining coelomic fluid described by Roch in 1979 (Roch, 1979; Hamed *et al.,* 2005; Plytycz *et al.,* 2006, 2007, 2009, 2010, 2011; Molnar *et al.,* 2015; Santocki *et al.,* 2016). When using this method, coelomic fluid is obtained in a non-invasive way, because the entire procedure, despite requiring a stress response such as violent writhing of animals, does not directly cause their death. However, it is not entirely clear what the consequences of its long-term use are and, therefore, exposing earthworms to chronic stress and how this affects the development of the earthworm population.

Due to its short life cycle, high reproductive rate and ease of breeding, *E. fetida* is the recommended species and therefore commonly used in standardized toxicity tests (ISO, 1993; OECD, 1984; Lowe & Butt, 2007). However, studies based on only one earthworm species may lead to the omission of certain environmental hazards, which indicates the need to use several earthworm species in such studies (Velki & Hackenberger, 2013; Jovana *et al.,* 2014).

The response of earthworms to stress related to, for example, pesticides depends on their toxicity and duration of exposure, but is largely biologically determined. Therefore, different species of earthworms may have a different threshold of sensitivity to a specific substance (Frund *et al.,* 2011; Pelosi *et al.,* 2014), which results from, for example, morphological and physiological differences between them (Edwards & Bohlen, 1996). Homa *et al.* (2007) showed, for example, that *Allolobophora chlorotica* is much more sensitive to copper contamination than *E. fetida*, which was confirmed by analyzing animal mortality. In addition, Lukkari *et al.* (2005) proved, for example, that earthworms of the species *E. fetida* are more resistant to heavy metals than other species such as *Aporrectodea tuberculata*. In turn, the studies of Robidoux *et al.* (2004) showed that the species *E. andrei* tolerated chemicals at higher concentrations than *A. rosea* earthworms. When comparing mortality, growth and reproduction in earthworms exposed to different pesticides, *E. fetida* was more resistant to the exposed chemicals than the tropical earthworm *Perionyx excavatus* (De Silva *et al.* 2010). Additionally, Velki & Hackenberger (2013) compared the response of earthworms to insecticide application and showed that *E. fetida* was more resistant to chemical stress than *L. rubellus*.

The results of our own research on the effect of the stressor in the form of electrostimulation may indicate that *E. fetida* earthworms are resistant to a moderate stress factor, because it did not affect significant changes in the population size. As reported by Anderson *et al.* (2013), immature individuals show greater sensitivity to stress, which in the case of his research was a high concentration of heavy metals, than mature individuals. Despite such indications, a comparison of the number of *E. fetida* individuals in individual age classes did not confirm the differences in this parameter in relation to the control group.

In contrast to *E. fetida*, the populations of *D. veneta* studied were sensitive to the applied stress, which was expressed by a decrease in the average population size in the groups exposed to electric current compared to the control groups. A clearly negative effect of the stress factor for this species in the form of a decrease in the population size was observed in all age classes. Body mass is also an important indicator allowing for examining the response to stress (Jovana *et al.,* 2014). When undertaking this study, individuals were weighed at regular time intervals. This resulted in obtaining average body masses and average total biomasses of entire populations. In the case of *E. fetida*, the stress factor induced by the current did not affect the characteristics of individual mass and biomass of the entire population. Compared to the control groups, no differences were found in the average mass of individuals (both immature, premature and mature individuals) and the total mass of all individuals combined.

D. veneta earthworms responded differently to the applied stress factor. In the groups exposed to electric current, the total biomass of the population was lower than in the control groups, but the average masses of individual individuals did not decrease. When analyzing immature, premature and mature individuals separately, the negative effect of stress in most cases was visible in the form of a decrease in the average biomass sums due to the smaller size of this group, which was not synonymous with the effect on the individual mass of individuals.

Immature individuals react to the stress factor to a greater extent than mature individuals (Anderson *et al.,* 2013), but our own studies did not confirm this regularity.

The measurements of the body mass of both analyzed species showed that *D. veneta* earthworms responded to regular electric stress by reducing their body mass (in contrast to *E. fetida* earthworms). This may mean that despite their larger size and higher individual mass (Kasprzak 1986), *D. veneta* earthworms are more sensitive than *E. fetida* earthworms.

The electric current used in the study did not limit the cocoon-laying of *E. fetida* earthworms, which was confirmed by analyzing the average numbers of cocoons in their populations and the average numbers of cocoons per adult individual. The situation was different in *D. veneta* earthworms, in which electrical stress caused a decrease in the number of cocoons. A lower number of cocoons per reproductive individual was also observed in comparison to the control. Analyzing the sensitivity of both earthworm species to the applied stress factor, it turned out that also in this case *D. veneta* earthworms were more sensitive. In the studies by Simonsen & Scott-Fordsmand (2004), it was shown that earthworms of the species *L. rubellus* living in an environment contaminated with heavy metals invest more energy in cocoon production. These earthworms laid more cocoons than the animals in the control group.

However, reproductive success in this case does not reflect the number of cocoons laid, because significantly more young individuals hatched from the cocoons of earthworms living in an uncontaminated environment. The intensity of the stress factor or, as in the case of heavy metals, the concentration of the xenobiotic may affect, for example, the reproduction of earthworms. Anderson *et al.* (2013) report that under the influence of high concentrations of heavy metals, *L. rubellus* individuals laid fewer cocoons as a response to chemical stress.

The conducted studies show that the applied electric stress had no effect on the mass of laid cocoons and therefore on their total biomass in *E. fetida* populations. The opposite observations apply to *D. veneta* cocoons, the total biomass of which was lower in the stressed groups, but no differences were found in the data on the average masses of cocoons. Therefore, this part of the life history cycle of *D. veneta* also seems to be more sensitive to stress than in the earthworms of the species *E. fetida*.

CONCLUSIONS

Regular electric stimulation did not affect the features of *E. fetida* at the population level. So the applied stress factor did not affect the average number, total biomass, average body weight of individuals constituting complete populations and cocoons. Furthermore, low voltage electric current did not affect the life expectancy of the stressed population of *E. fetida*. In the case of earthworms of the species *D. veneta*, stress with electric current caused significant changes in the population. As a result of coelomocyte ejection, a decrease in the number of all analyzed age groups and cocoons was observed. Individuals also responded by reducing the average individual mass and average total biomass. This applied to all age classes of *D. veneta* in all populations. Low voltage electric current did not affect the life expectancy of the stressed population of *E. fetida*, in contrast to the population of earthworms of the species *D. veneta*, where it significantly reduced the probability of survival of the stressed population. The commonly used method of obtaining coelomocytes for various analyses, which consists in exposing earthworms to the action of low voltage electric current, is an effective method because it allows obtaining samples of coelomic fluid and thus safe for earthworms due to the lack of influence on the population parameters of the studied species of earthworms. Moreover, popular opinion circulating among the growers of earthworms was that electric current could stimulate earthworms to grow faster. This was not confirmed – we should still look for possible stimulators of the development of the vermicomposting organic waste earthworm population. It is not known whether the results of the experiment can be generalized, for example, to other earthworm species. Different earthworm species are characterized by different sensitivity to environmental stress, therefore their sensitivity may be different, as seen in the case of *E. fetida* and *D. veneta*. The results may influence earthworm breeding practices in vermicompost production, because it is confirmed that regularly supplied stress can affect the decrease in earthworm vital parameters and thus affect the efficiency of vermicompost production.

Acknowledgments

I would like to thank Professor Joanna Kostecka from the Department of the Basis of Agriculture and Waste Management for her help in planning the experiment and interpreting the results.

REFERENCES

1. Anderson C. J., Kille P., Lawlor A. J., Spurgeon D. J. (2013). Life-history effects of arsenic toxicity in clades of the earthworm *Lumbricus rubellus*. *Environmental Pollution*. 172: 200–207. https://doi.org/10.1016/j.envpol.2012.09.005
2. Asres A., Amha N. (2014). Effect of stress on animal health: A review. *Journal of Biology, Agriculture and Healthcare.4*(27): 116–122.
3. Christensen O. M., Mather J. G. (2004). Pesticide-induces surface migration by lumbricid earthworms in grassland: life-stage and species differences. *Ecotoxicology and Environmental Safety*. *57*: 89–99. https://doi.org/10.1016/j.ecoenv.2003.08.007
4. De Silva P. M. C. S., Pathiratne A., Van Gestel C. A. M. (2010). Toxicity of chlorpyrifos, carbofuran, mancozeb and their formulations to the tropical earthworm *Perionyx excavatus*. *Applied Soil Ecology*. *44*: 56–60. https://doi.org/10.1016/j.apsoil.2009.09.005
5. Dittbrenner N., Schmitt H., Capowiez Y., Triebskorn R. (2011). Sensitivity of Eisenia fetida in comparison to *Aporrectodea caliginosa* and *Lumbricus*

terrestris after imidacloprid exposure. Body mass change and histopathology. *Journal of Soils and Sediments. 11*(6): 1000–1010. https://doi.org/10.1007/s11368-011-0397-5

6. Dominguez J., Edwards C. A. (2011a). Biology and Ecology of Earthworm Species used for Vermicomposting. [w:] Vermiculture technology. Earthworms, organic wastes and environmental management. [red.] Edwards C. A., Arancon N. Q., Shreman R. *CRC. Taylor and Francis Group. Press. Boca Raton. London, New York. 3*: 27–40.
7. Edwards, C. A., Bohlen, P. J. (1996). Biology and ecology of earthworms. *Chapman and Hall. London, New York, Melbourne, Madras.* ss. 426.
8. Elwood R. W. (2011). Pain and suffering in invertebrates? *ILAR Journal*. *52*: 175–184. https://doi.org/10.1093/ilar.52.2.175
9. Enebe M. Ch., Erasmus M. (2023). Vermicomposting technology – A perspective on vermicompost production technologies, limitations and prospects. *Journal of Environmental Management. 345*(1): 118585. https://doi.org/10.1016/j.jenvman.2023.118585
10. Engelmann P., Pal J., Berki T., Cooper E. L., Nemeth P. (2002). Earthworm leukocytes react with different mammalian antigen-specific monoclonal antibodies. *Zoology. 105*: 257–265. https://doi.org/10.1078/0944-2006-00068
11. Frindt A., Zoń A., Bielański P. (2006). Stres jako forma zachowania się zwierzęcia. *Wiadomości Zootechniczne*. *1*: 15–18.
12. Frund H-Ch., Graefe U., Tischer S. (2011). Earthworms as bioindicators of soil quality. Earthworm Innate Immune System. [w:] Biology of earthworms [red.] Karaca A. *Springer Heidelberg Dordrecht, London, New York. 16*: 261–278. https://doi.org/10.1007/978-3-642-14636-7_16
13. Grygorczuk A. (2008). The notion of stress in medicine and psychology. *Psychiatry*. *5*: 111–115.
14. Hamed S. S., Kauschke E., Cooper E. L. (2005). Cytochemical properties of earthworm coelomocytes enriched by percoll. *International Journal of Zoological Research. 1*.1: 74–83. https://doi.org/10.3923/ijzr.2005.74.83
15. Hawkins L. J., Storey K. B. (2020). Advances and applications of environmental stress adaptation research. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 240*. 110623. https://doi.org/10.1016/j.cbpa.2019.110623
16. Hendawi M., Sauve S., Ashour M., Brousseau P., Fournier M. (2004). A new ultrasound protocol for extrusion of coelomocyte cells from the earthworm *Eisenia fetida*. *Ecotoxicology and Environmental Safety*. *59*: 17–22. https://doi.org/10.1016/j.ecoenv.2003.07.015
17. Homa J., Stürzenbaum S. R., Morgan A. J., Płytycz B. 2007. Disrupted homeostasis in coelomocytes of *Eisenia fetida* and *Allolobophora chlorotica* exposed dermally to heavy metals. *European Journal of Soil Biology*. *43*: 273–280. https://doi.org/10.1016/j.ejsobi.2007.08.027
18. ISO 11268−1: (1993). Soil quality effects of pollutants on earthworms (*Eisenia fetida*) – Part 1: Determination of acute toxicity using artificial soil substrate.
19. Jovana M., Tanja M., Mirjana S. (2014). Effects of three pesticides on the earthworm *Eisenia fetida* (Savigny 1826) under laboratory conditions: Assessment of mortality, biomass and growth inhibition. *European Journal of Soil Biology*. *62*. 127–131. https://doi.org/10.1016/j.ejsobi.2014.03.003
20. Jura Cz. (2007). Bezkręgowce – Podstawy morfologii funkcjonalnej, systematyki i filogenezy. *Wydawnictwo Naukowe PWN. Warszawa*. *18*: 331–382.
21. Kasprzak K. (1986). Skąposzczety glebowe III, dżdżownice (Lumbricidae). PAN Instytut Zoologii. Klucz do oznaczania bezkręgowców Polski. *Wydawnictwo Naukowe PWN. Warszawa*. ss. 187.
22. Kostecka J., Garczyńska M., Podolak A., Pączka G., Kaniuczak J. (2018). Kitchen organic waste as material for vermiculture and source of nutrients for plants. *Journal of Ecological Engineering. 19*(6): 267–274. https://doi.org/10.12911/22998993/99691
23. Lowe Ch. N. Butt K. R. (2007). Earthworm culture, maintenance and species selection in chronic ecotoxicological studies: A critical review. *European Journal of Soil Biology*. *43*: 281–288. https://doi.org/10.1016/j.ejsobi.2007.08.028
24. Lukkari T., Marjo Aatsinki M., Vaisanen A., Haimi J. (2005). Toxicity of copper and zinc assessed with three different earthworm tests. *Applied Soil Ecology*. *30*: 133–146. https://doi.org/10.1016/j.apsoil.2005.02.001
25. Mackenzie A., Ball A. S., Virdee S. R. (2009). Ekologia – Krótkie wykłady. *Wydawnictwo Naukowe PWN. Warszawa*. ss. 427.
26. Molnar L., Pollak E., Skopek Z., Gutt E., Kruk J., Morgan A. J. (2015). Immune system participates in brain regeneration and restoration of reproduction in earthworm *Dendrobaena veneta*. *Developmental and Comparative Immunology*. *52*: 289–279. https://doi.org/10.1016/j.dci.2015.04.001
27. OECD Guideline for testing of chemicals. 1984. Earthworm, Acute Toxicity Tests. *OECD. Paris, France*. *207*: 1–9.

28. Pelosi C., Barot S., Capowiez Y., Hedde M., Vandenbulcke F. (2014). Pesticides and earthworms. A review. *Agronomy for Sustainable Development*. *34*: 199–228.
29. Płytycz B., Homa J., Kozioł B., Różanowska M., Morgan A. J. (2006). Riboflavin content in autofluorescent earthworm coelomocytes in species-specific. *Folia Histochemica et Cytobiologica*. *44*: 275–280.
30. Płytycz B., Kiełbasa E., Grębosz A., Duchnowski M., Kruk J., Morgan A. J. (2010). Riboflavin mobilization from eleocyte stores in the earthworm *Dendrodrilus rubidus* aerially-contaminated Ni-Smelter soil. *Chemosphere*. *82*: 199–205. https://doi.org/10.1016/j.chemosphere.2010.06.056
31. Płytycz B., Klimek M., Homa J., Mazur A. I., Kruk J., Morgan A. J. (2011). Species-specific sensitivity of earthworm coelomocytes to dermal metal (Cd, Cu, Ni, Pb, Zn) exposures: Methodological approach. *Pedobiologia*. *54*: 203–210. https://doi.org/10.1016/j.pedobi.2011.06.002
32. Płytycz B., Klimek M., Homa J., Tylko G., Kołaczkowska E. (2007). Flow cytometric measurement of neutral red accumulation in earthworm coelomocytes: Novel assay for studies on heavy metal exposure. *European Journal of Soil Biology*. *43*: 116–120.
33. Płytycz B., Lis-Molenda U., Cygal M., Kiełbasa E., Grębosz A., Duchnowski M., Morgan A. J. (2009). Riboflavin content of coelomocytes in earthworm (*Dendrodrilus rubidus*) field populations as a molecular biomarker of soil metal pollution. *Environmental Pollution*. *157*: 3042–3050. https://doi.org/10.1016/j.envpol.2009.05.046
34. Podolak A., Kostecka J., Mazur-Pączka A., Garczyńska M., Pączka G., Szura R. (2020). Life Cycle of the Eisenia fetida and Dendrobaena veneta Earthworms (Oligohaeta, Lumbricidae). *Journal of Ecological Engineering. 21*(1): 40–45. https://doi.org/10.12911/22998993/113410
35. Podolak A., Kostecka J., Rożen A., Garczyńska M., Pączka G., Mazur-Pączka A., Szura R. (2019). New perspectives for the use of earthworms – testing of anesthetics. *Journal of Ecological Engineering. 20*(3): 253–261. https://doi.org/10.12911/22998993/100560
36. Podolak-Machowska A., Kostecka J., Librowski T., Santocki M., Bigaj J., Płytycz B. (2014). Effects of anesthetic compounds on responses of earthworms to electrostimulation. *Folia Biologica (Kraków)*. *62*: 155–162. https://doi.org/10.3409/fb62_2.155
37. Rasmussen L. M., Holmstrup M. (2002). Geographic variation of freeze-tolerance in the earthworm *Dendrobaena octaedra. Journal of Comparative Physiology B. 172:* 691–698. https://doi.org/10.1007/s00360-002-0298-4
38. Reinecke A.J., Viljoen S.A. (1990). The influence of worm density on growth and cocoon production of the compost worm *Eisenia fetida* (Oligochaeta). *Rev. Ecol. Biol. Sol, 27*, 221–230.
39. Rice V. H. (2012). Theories of stress and its relationship to health. [In:] Handbook of Stress, Coping, and Health Implications for Nursing Research, Theory, and Practice. (Ed.) *Rice V. H. SAGE Publications. Los Angeles.*
40. Robidoux P. Y., Svendsen C., Sarrazin M., Thiboutot S., Ampleman G., Hawari J. Weeks J. M., Sunahara Gl. (2004). Assessment of a 2,4,6-trinitrotoluene-contaminated site using Aporrectodea rosea and Eisenia andrei in mesocosms. Archives of Environmental Contamination *and Toxicology***.** *48*: 56–67. https://doi.org/10.1007/s00244-004-0217-7
41. Roch P. (1979). Protein analysis of earthworm coelomic fluid: I Polymorphic system of natural hemolysin of *Eisenia fetida andrei*. *Developmental and Comparative Immunology*. *3*: 599–608. https://doi.org/10.1016/S0145-305X(79)80055-5
42. Rożen A. (1994). Metale w dżdżownicach. *Zeszyty Naukowe AR w Krakowie*. *292*: 61–66.
43. Rożen A. (2004). Wpływ kadmu na rozmnażanie i przeżywalność dżdżownicy *Dendrobaena octaedra* (Savigny 1826). *Zeszyty Problemowe Postępów Nauk Rolniczych*. *498*: 181–191.
44. Sadowski B. (2012). Biologiczne mechanizmy zachowania się ludzi i zwierząt. *Wydawnictwo Naukowe PWN. Warszawa*. ss. 584.
45. Santocki M., Falniowski A., Płytycz B. (2016). Restoration of experimentally depleted coelomocytes in juvenile and adult composting earthworms *Eisenia andrei, E. fetida* and *Dendrobaena veneta*. *Applied Soil Ecology*. *104*: 163–173. https://doi.org/10.1016/j.apsoil.2015.08.022
46. Selye H. (1956). The stress of life. *McGraw-Hill. New York*. ss. 324.
47. Selye H. (1977). Stres okiełznany. *Państwowy Instytut Wydawniczy. Warszawa* (tytuł oryginału – Stress without distress).
48. Simonsen V., Scott-Foedsmand J. J. (2004). Genetic variation in the enzyme esterase, bioaccumulation and life history traits in the earthworm *Lumbricus rubellus* from a metal contaminated area, Avonmouth, England. *Ecotoxicology*. *13*: 773–786. https://doi.org/10.1007/s10646-003-4475-3
49. Singh J., Schadler M., Demetrio W., Brown G. G., Eisenhauer N. (2019). Climate change effects on earthworms – a review. *Soil Organisms 91*(3): 114–138. https://doi.org/10.25674/so91iss3pp114
50. Suthar S. (2014). Toxicity of methyl parathion on growth and reproduction of three ecologically different tropical earthworms. International *Journal of Environmental Science and Technology. 11*: 191–198.

https://doi.org/10.1007/s13762-012-0154-3

51. Szabo S., Tache Y., Somogyi A. (2012). The legacy of Hans Selye and the origins of stress research: A retrospective 75 years after his landmark brief „Letter" to the Editor of Nature. *Stress. 15*: 472–478. https://doi.org/10.3109/10253890.2012.710919
52. Velki M., Hackenberger B. K. (2013). Different sensitives of biomarker responses in two epigenic earthworm species after exposure to pyrethroid and organophosphate insecticides. *Archives of Environmental Contamination and Toxicology*. *65*: 498–509. https://doi.org/10.1007/s00244-013-9930-4
53. Viljoen S.A., Reinecke A.J., Hartman L. (1991). Life-cycle of the European compost worm Dendrobaena veneta (Oligochaeta). *South African Journal of Zoology, 26*, 43–48.
54. Wang J., Yang Y., Wu J., Zhao K., Zhang X. (2024). The interaction between biochar and earthworms: Revealing the potential ecological risks of biochar application and the feasibility of their co-application. *Science of The Total Environment. 950*(10): 175240. https://doi.org/10.1016/j.scitotenv.2024.175240
55. Wikiera A., Stankiewicz A., Chadzińska M., Plytycz B. (1996). Wpływ temperatury i osmolarności na celomocyty dżdżownic. *Zesz. Nauk. AK im. H Kołłątaja w Krakowie*. *310*: 155–162.