JEE Journal of Ecological Engineering
Volume 21, Issue 5, July 2020, pages 114–119
https://doi.org/10.12911/22998993/122237

Received: 2020.03.23
Revised: 2020.04.17
Accepted: 2020.05.08
Available online: 2020.05.25

Effectiveness of Lumbricidae Extracting with an Environmentally Friendly Method

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ABSTRACT

Lumbricidae, as representatives of edaphone, are used in ecological research. Over the years, various attempts to improve the ways of earthworm extraction have been made. One of the pro-environmental methods for extracting these animals from the soil is the method involving the application of electric current (*octet*). The aim of the study was to compare the efficiency of the earthworm extraction using the mixed method (manual segregation along with the use of 0.5% formalin solution) with the method applying the electroshocking. The species composition of earthworms extracted using both methods was the same (seven species of earthworms belonging to three ecomorphological groups were found). A lower number and biomass of earthworms were found in the permanent grassland and arable land with the help of *octet* than using the mixed method. Further research is needed to improve the existing methods for Lumbricidae extraction and to implement new ones that would be safe for the edaphon representatives.

Keywords: earthworms sampling, non-destructive extraction method, electroshocking, mixed method, permanent grassland, arable land

INTRODUCTION

Due to the specific lifestyle, Lumbricidae play a key role in soil environments as "ecosystem engineers" [Jouquet *et al.* 2006]. These organisms are subject to ecological worldwide research. In order to determine the qualitative and quantitative composition of earthworms in a given environment, both correct soil sampling and subsequent animal extraction are necessary. There are two groups of methods for obtaining Lumbricidae from the environment, i.e. physical and behavioral [Bartlett *et al.* 2010, Valckx *et al.* 2012]. The first group consists of manual sorting of the soil or washing the soil sample with a stream of water and then sieving it on sieves of varying mesh size. In turn, the behavioral methods are based on the extraction of Lumbricidae using, e.g. chemical, electrical or thermal means. A combination of the methods from both groups is also used, i.e. manual segregation and extraction using a chemical agent [EN ISO 23611–1: 2006]. The advantage of manual soil sorting is the ability to find Lumbricidae in all developmental stages: mature, young individuals and cocoons as well as active forms and those being at rest – diapause [Gutierrez-Lopez *et al.* 2016]. However, the young and small specimens are often omitted [Jiménez *et al.* 2006]. In turn, the disadvantages of the screening method include low efficiency and damage to individuals arising under the influence of e.g. a water jet. This makes it difficult to identify the collected material [Valckx *et al.* 2012].

The behavioral methods usually use a solution of formaldehyde (formalin), mustard suspension and allyl isothiocyanate (AITC) [Butt *et al.* 2010, Singh *et al.* 2015]. High efficiency and shorter extraction time, e.g. compared to manual

screening, have made the chemotactic methods widely used in the studies of Lumbricidae clusters [Valckx *et al.* 2012]. Despite the high efficiency in earthworm extraction, formalin is a toxic compound for the plant, animal and human organisms [Conolly *et al.* 2002, Čoja *et al.* 2008]. The irritants contained in mustard (allyl isothiocyanate) are less harmful compounds to the environment. For this purpose, a ready-made commercial mustard emulsion is used [Pelosi *et al.* 2009] or mustard powder extract is prepared [Eisenhauer *et al.* 2008]. The disadvantage, however, is that the concentration of AITC in mustard and mustard powder is not exactly known and may vary in the starting material used to make solutions, which hinders the standardization of the method [Valckx *et al.* 2012]. The work on the application of onion (*Allium cepa* L.) extracts is also underway; however, implementation of this method requires further research in diverse habitats [Steffen *et al.* 2013, Singh *et al.* 2017].

In addition to the use of mustard and onion, the method using low intensity electric current (*octet*) is characterized by low harmfulness to the soil organisms [Thielemann 1986, Schmidt 2001]. In Poland, the use of this pro-environmental method is not widespread.

The aim of the study was to determine the efficiency of Lumbricidae extraction from the soil using low intensity electric current, compared to the mixed method recommended by ISO [EN ISO 23611–1: 2006].

MATERIAL AND METHODS

The research was carried out at two sites – permanent grassland (50 a) and arable land sown with cereals (5 ha) (Table 1) located at the Didactic and Research Station of the University of Rzeszów in Krasne near Rzeszów (SE Poland).

At research sites, earthworms were sought using the electroshocking method. The *octet* device is built of eight soil probes in an octagonal arrangement (6 mm in diameter and 65 cm active length) with insulated handles around the circle (52 cm in diameter covering an area of 0.22 m^2). Outside, the wires are terminated with clamps that give the possibility of electrical connection with the soil probe. The adjacent bars are spaced 20 cm apart and the opposite bars 52 cm apart. The opposite bars were paired according to the code (1–5, 2–6, 3–7, 4–8), which was marked on the front panel of the device with letters A, B, C, D. In the *octet* device, the electric field circulates successively in the sampling area, where the soil probe pairs are electrified in eight sequences: AB, ABC, BC, BCD, CD, ACD, AD, ABD. The 12 V

Table 1. Characterization of research site in Krasne

Site	Vegetation	Soil type	Soil properties
Permanent grassland N 50º 02′ 53.9″ E 22º 03′ 16.8″	goat rue, orchard grass, alse oat-grass, meadow fescue, red clover	brown earth of loess-origin, granulometric composition of ordinary dust, average agronomic category (soil of wheat, good complex, soil class IIIa)	mean soil temperature • (0–20 cm) 14.1 ± 3.4 °C; • (< 20 cm) 13.9 ± 3.3 °C mean soil moisture • (0–20 cm) 19.9 ± 6.1%; • (< 20 cm) 19.8 ± 6.4% pH in KCl 5.95; humus 1.91%; • 11.1 mg P_2O_5/100 g DM; • 18 mg K_2O/100 g DM; • 2.6 mg Mg/100 g DM
Arable land N 50º 03′ 13.9″ E 22º 05′ 26.7″	in 2012, the field was sown with winter rye (90 kg · ha^{-1}) in 2013, spring barley (140 kg · ha^{-1}) was sown in the field	brown earth of loess-origin, granulometric composition of ordinary dust, average agronomic category (soil of wheat, good complex, soil class IIIa)	mean soil temperature • (0–20 cm) 13.9 ± 3.9 °C; • (< 20 cm) 13.8 ± 3.9 °C mean soil moisture • 0–20 cm) 19.7 ± 6.2 %; • (< 20 cm) 19.1 ± 6.7 % pH in KCl 5.78; humus 1.52%; • 9.3 mg P_2O_5/100 g DM; • 16 mg K_2O/100 g DM; • 2.1 mg Mg/100 g DM

After Mazur-Pączka *et al.* [2019], amended.

direct voltage is supplied from the battery and directed to the inverter, where it is converted into 230 V alternating voltage. From there, i.e. the regulated autotransformer, it is fed to the transformer, where depending on the needs, it is possible to increase the voltage to 450 V. The current with the appropriate voltage is directed from the transformer to the soil probes. The voltage and current parameters at the output to the soil probes are measured by a voltmeter and ammeter (Figure 1). The time for each sequence is 2.5 min.

The earthworms for analysis were collected from the central part defined by the ring and probe (Figure 1).

In the test sites, apart from the collection of earthworms with the *octet* method, a mixed method of Lumbricidae extraction proposed by ISO [EN ISO 23611–1: 2006] was used in parallel. For this purpose, the 25×25×25 cm soil blocks were sorted manually. From the deeper layers of the soil profile, the earthworms were extracted by pouring the surface of the well formed after selecting the sample with 10 liters of 0.4% formalin solution (HCHO from CHEM-PUR). Applying Zisci's recommendations [1962], eight test samples were taken each time at the sites (using the current method and the mixed method), determining them randomly by casting a metal frame. The samples were collected annually from May to November 2012 and from April to November 2013. Identification of the found species was carried out using the keys for marking the Polish terrestrial Oligochaeta [Kasprzak 1986].

At the test sites, the soil temperature and humidity were measured each time at a depth of 0–20 cm and beneath. The soil moisture was determined with a drying method at 105°C [PN ISO 11465: 1999]. The soil was also taken from each site for pH analysis in KCl, organic carbon (using the Tiurin method in the Nikitin modification), the content of available phosphorus, potassium and magnesium forms according to Mehlich III [MAFF1981].

In order to characterize the Lumbricidae population, Shannon-Wiener (H) indices were determined:

$$H = -\Sigma\, pi \ln pi \qquad (1)$$

where: *pi* is the number of specimens of a particular species vs. population size of all organisms.

The data obtained are presented as mean ± standard deviation (SD). The results were statistically analyzed in the STATISTICA ver. 10 software using the Student's t-test or Mann-Whitney U test (if the assumptions for parametric tests were not met).

RESULTS

Seven earthworm species were identified in the study area using the *octet* method: *Dendrodrilus rubidus tenuis* (Savigny 1826), *Dendrobaena octaedra* (Savigny 1826), *Lumbricus rubellus* (Hoffmeister 1843), *Aporrectodea caliginosa* (Savigny 1826), *Aporrectodea rosea* (Savigny 1826), *Octolasion lacteum* (Oerley 1881), *Lumbricus terrestris* (L. 1758). The individuals found belonged to three ecomorphological groups (epigeic, endogeic and anecic). The quality composition of the earthworms found by means of this method coincided with the one determined using the mixed method [EEN ISO 23611–1: 2006] on

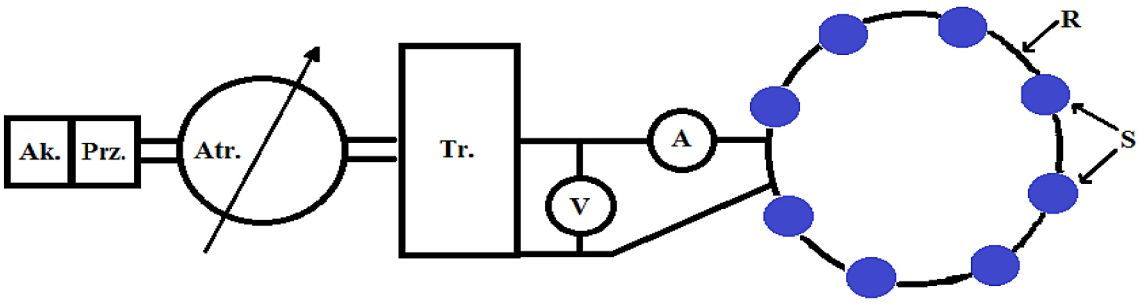

Figure 1. Block diagram of the *octet* device:
Ak. – 12 V battery; Prz. – inverter; Atr. – autotransformer (regulating output voltage); Tr. – transformer (increases voltage from 230 V to a maximum of 450 V); A – ammeter; V – voltmeter; S – probes (rods around the ring; R – limiting the operating surface

permanent grassland as well as on the arable land (Table 2).

With the use of electricity, both the permanent grassland and the arable land have lower density of earthworms (22.03 ± 4.12 ind.·m^{-2} and 18.21 ± 4.15 ind.·m^{-2}, respectively) compared to mixed extraction method (101.01 ± 18.03 ind.·m^{-2} and 82.12 ± 18.26 ind.·m^{-2}) ($p < 0.001$) (Table 3). A similar trend was observed for Lumbricidae biomass ($p < 0.001$). Shannon-Wiener diversity index on the meadow and arable land was similar for both methods ($p > 0.05$) (Table 3).

The density and biomass of earthworms from individual ecomorphological groups extracted applying the *octet* method were lower compared to the mixed method ($p < 0.05$) (Table 4).

DISCUSSION

The use of electricity to extract the earthworms has been tested, among others, by Tiho and Josens [2000], Schmidt [2001], Čoja *et al.* [2008] and Butt *et al.* [2010]. In this method, the voltage is transmitted from the soil probes of the *octet* device to the soil column, which irritates Lumbricidae and causes them to escape to the soil surface. In this study, the species structure of Lumbricidae extracted by means of the *octet* method coincided with that obtained applying the mixed method recommended by ISO [EN ISO 23611–1: 2006]. Seven earthworm species belonging to all three ecomorphological groups were found using both methods (Table 2). It can be assumed that the use of electric current reflects the qualitative structure of earthworms in a given area. This is confirmed by the similar values of the Shannon-Wiener index at the tested sites for the earthworms extracted with electric current and the mixed method (Table 3). The analysis of the qualitative structure of earthworms extracted with the help of the *octet* device is recommended in the places where the chemical extraction is prohibited or ineffective. This method can be more effective on slopes, in urban areas or in the areas where minimal environmental disturbances are required, i.e. in protected areas or natural communities [Schmidt 2001]. Staddon *et al.* [2003] have shown that this method is safe for the plant and animal organisms. The electroshocking did not have any negative effect on the groups of Enchytraeidae, nematodes, Collembola and mites [Blair *et al.* 1995]. Lower density and biomass of earthworms in the study area obtained with the help

Table 2. Species of earthworms found with the *octet* method (OM) and the mixed method (MM) at research sites in Krasne

Species of Lumbricidae	Research sites			
	permanent grassland		arable land	
	OM	MM	OM	MM
D. rubidus tenuisa a	+	+	+	+
D. octaedra a	+	+	-	-
L. rubellus a	+	+	+	+
A. caliginosa b	+	+	+	+
A. rosea b	+	+	+	+
O. lacteum b	+	+	+	+
L. terrestris c	+	+	+	+

+ – presence, – – absence.
a – epigeic earthworms, b – endogeic earthworms, c – anecic earthworms.

Table 3. Characteristics of earthworm population extracted by *octet* method (OM) and mixed method (MM) on permanent grassland and in arable land

Ecological parameters	Research sites			
	permanent grassland		arable land	
	OM	MM	OM	MM
Earthworm density [ind. · m^{-2}]	22.03^{a} ± 4.12	101.01^{b} ± 18.03	18.21^{a} ± 4.15	82.12^{b} ± 18.26
Earthworm biomass [g · m^{-2}]	12.64^{a} ± 2.62	77.72^{b} ± 15.30	10.71^{a} ± 2.67	54.34^{b} ± 11.72
Shannon-Wiener diversity index (H)	0.64^{a} ± 0.23	0.60^{a} ± 0.18	0.66^{a} ± 0.25	0.57^{a} ± 0.16

ab – statistically significant differences ($p < 0.05$).
aa – statistically insignificant differences ($p > 0.05$).

Table 4. Earthworm ecomorphological group characteristics (D – density [ind.·m^{-2}], B – biomass [g·m^{-2}]) extracted by *octet* method (OM) and mixed method (MM) on sites in Krasne

Ecomorphological group		Research sites			
		permanent grassland		arable land	
		OM	MM	OM	MM
Epigeic	D	2.83^{a} ± 1.75	5.33^{b} ± 2.42	2.32^{a} ± 1.20	4.50^{b} ± 3.19
	B	0.6^{a} ± 0.42	1.46^{b} ± 0.97	0.45^{a} ± 0.25	1.25^{b} ± 0.74
Endogeic	D	6.87^{a} ± 4.09	29.25^{b} ± 12.07	8.92^{a} ± 5.22	23.91^{b} ± 9.20
	B	2.37^{a} ± 1.36	10.82^{b} ± 6.07	3.35^{a} ± 1.54	10.85^{b} ± 4.15
Anecic	D	2.37^{a} ± 1.07	15.50^{b} ± 8.97	2.49^{a} ± 1.05	6.25^{b} ± 4.76
	B	6.22^{a} ± 3.25	40.04^{b} ± 25.80	5.04^{a} ± 2.27	16.99^{b} ± 12.99

ab – statistically significant differences ($p < 0.05$).
aa – statistically insignificant differences ($p > 0.05$).

of *octet* (Table 3) could be due to low soil moisture in the summer months. Low water content in the soil is one of the factors limiting this method [Schmidt 2001, Szlavecz *et al.* 2013]. Eisenhauer *et al.* [2008] verified the improvement of *octet* efficiency by adding water to the dry soil, from which the samples were previously taken. The addition of water to dry soil did not increase the efficiency of the method. This was probably due to the earlier transition of some species into the state of anabiosis caused by adverse environmental factors. It was also found that the extraction of the earthworms from the endogeic group was more effective with the *octet* method than using formalin. In this study, with the help of the *octet* method, in turn, a lower number and Lumbricidae biomass from all three ecomorphological groups were recorded (Table 4). The effectiveness of the *octet* method largely depends on the type of soil, vegetation cover and pH of the substrate [Weyers *et al.* 2008]. More earthworms are extracted from the low pH soil [Čoja *et al.* 2008].

CONCLUSIONS

1. In the studied ecosystems, the species composition of Lumbricidae found with the help of the *octet* method coincided with the structure of earthworms extracted by means of the mixed method. The *octet* method, due to its pro-environmental nature, can be used to identify the qualitative structure of Lumbricidae in the protected areas.
2. Lower number and biomass of earthworms were found at the tested sites applying *octet* compared to the mixed method. Extraction of earthworms using electric current did not reflect the quantitative structure of earthworms. The effectiveness of the electroshocking method depends largely on the moisture content of the substrate, soil type and type of plant cover.
3. The research on the effectiveness of the Lumbricidae extraction with a help of an *octet* device requires continuation in diverse ecosystems.

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