DOI: 10.2428/ecea.2012.19(09)109 ECOL CHEM ENG A. 2012;19(9):1133-1137

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HERBICIDE TOXICITY TO THE CALIFORNIA EARTHWORMS *Eisenia fetida* Sav. and *Dendrobaena veneta* Rosa

WPŁYW TOKSYCZNOŚCI HERBICYDÓW NA DŻDŻOWNICE KALIFORNIJSKIE *Eisenia fetida* Sav. i *Dendrobaena veneta* Rosa

Abstract: This paper aims at estimating influence of selected herbicides on earthworms *Eisenia fetida* and *Dendrobaena veneta* survival abilities in toxic environment and on their reproduction cycle. Parameters taken into account:

– changes in fatality rate after applying toxins compared with the control,
– number of laid cocoons and hatchings,
– lethal concentration (LC_{50}).

Herbicides containing the following active substances: urea, aminophosphonic and phenoxyacid were chosen, as chemical material.

Earthworm responded differently to every herbicide. Significant decrease in body weight under the influence of linuron and decrease of the hatching rate under the influence of gliphosate and phenoxyacids.

Keywords: herbicides, *Lumbricidae,* life activity, reproduction

Earthworms (*Lubricidae*) being an important part of soil mesofauna decide upon biological activity of the soil and indirectly – upon its fertility. They are specifically adapted to variable habitat and life conditions [1–3].

Earthworms' activity creates favourable life conditions for many soil animals and for plants. *Lubricidae* are also an excellent source of information on environmental pollution due to their easy culture and broad range of occurrence. Because of these reasons, earthworms are considered indicator animals and are used in field and laboratory tests. Application of plant protection chemicals is not harmless to natural environment affecting eg the life cycle of oligochaetes. The effect might be direct or

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indirect. Pesticides, including herbicides, may affect earthworms directly causing their death or indirectly by changing their reproduction and/or behaviour. Moreover, herbicides *via* earthworms may enter higher links of the trophic chain [4–6].

Almost all herbicides are phytotoxic also for plants they have to protect. They show lower acute toxicity as compared with other plant protection means. Circa 13 % of herbicides is included in the I or II class of toxicity [7].

Material and methods

Two species of the California earthworms *E. fetida* and *D. veneta* kept in universal soil were used in experiments. They were taken from the own culture of the Department of Zoology, Warsaw University of Life Sciences. Adult individuals were used in tests which lasted circa 50 days for each experimental variant. The following herbicides were applied: Bofix 260EC, Afalon 450SC and Glifocyd 360 SL (Table 1).

Table 1

Characteristics of plant protection chemicals

Herbicide	Common name	Chemical name	Chemical group	Chemical formula
Afalon 450 SC	linuron	3-(3,4-dichlorophenyl)-1-methoxy-1--methylurea	derivative of urea	$C_9H_{10}Cl_2N_2O_2$
Bofix 260 EC	fluoroxypyr	((4-amino-3,5-dichloro-6-fluoro-2--pyridinyl)oxy)acetic acid,	derivative of pyridinocarboxylic acid (phenoxy acids)	$C_7H_5Cl_2N_2O_3$
	chlopyralid	3,6-dichloropyridine-2-carboxylic acid,	derivative of carboxylic acid	$C_6H_3Cl_2NO_2$
	MCPA	4-chloro-*o*-tolyloxyacetic acid	derivative of phenoxy acids	$C_9H_9ClO_3$
Glifocyd 360 SL	gliphosate	*N*-(phosphonomethyl)glycine	aminophosphoniates	$C_3H_8NO_5P$

Concentrations applied in experiments were 2–3 times lower than those recommended by the producers. Afalon and Bofix were used in concentration of 2.5 cm^3 per 600 cm^3 of water and Glifocyd – in concentration of 5 cm^3 per 600 cm^3 of water. The effect of the active substances of the herbicides was observed for 24 and 48 hours. Earthworms were not fed during that time. The control group consisted of earthworms kept in boxes to which distilled water was added. Experiments were carried out at a temperature of 25–28 ^oC.

After 48 hours the earthworms were left alone until the appearance of cocoons which were collected and transferred to other containers. Each experiment was triplicated with the use of 20 mature individuals per sample. To estimate mean *lethal concentration* (LC_{50}) for earthworms the containers were filled with 200 cm^3 of scalded and dried soil and poured with 50 cm^3 of herbicide solutions. Observations were carried out for 2 days.

Results and discussion

Limited mobility and migration at short distances made earthworms the appropriate organisms for monitoring chemical pollutants [8, 9]. *E. fetida* due to high genetic homogeneity and reproduction became a model species in tests for the harmfulness of chemical substances to saprophagous invertebrates [10]. When monitoring environmental pollution with earthworms one should consider their ability to accumulate heavy metals and other components of plant protection chemicals.

The decrease of bioconcentration and bioaccumulation of pesticide is a result of reduced absorption caused by metabolic processes of soil organisms and by defence mechanisms of earthworms such as the release of larger amounts of slime [11, 12]. In performed experiments Glifocyd 360 SL caused such a reaction in both earthworm species. The defence mechanism in *D. veneta* lasted during the whole experiment, which consequently affected the number of laid cocoons (Table 2). The response to herbicides involved also the irritation or burn of dermatitis and muscular sack and increased sensitivity of the nervous system. These results confirm observations done by Kamionek et al (2005) [13]. Irritation of the nervous system was observed in both species 48 hours after exposition. Later, non-coordinated movements and hypersensitivity to light were observed in the case of Bofix 260 EC. In the case of Glifocyd application the symptoms subsided after 10 days. Earthworms are also characterised by remarkable ability to adapt to changing environmental conditions. However, herbicides caused increased mortality short after their application [5, 13].

Table 2

The effect of herbicides on the California earthworms

Herbicide	Reaction to active substance of herbicide		Number of cocoons		Number of young individuals	
	E. fetida	*D. veneta*	*E. fetida*	*D. veneta*	*E. fetida*	*D. veneta*
Control	n.c.	n.c.	634	664	668*	572*
Afalon 450 SC	losses of coelomatic fluid, death, convulsions and non-coordinated movements, micro--wounds	losses of coelomatic fluid, death, convulsions and non-coordinated movements	1	2	0	0
Bofix 260 EC	sensitivity to the touch, death and escapes	sensitivity to the touch, death and escapes	201	383*	292	468
Glifocyd 360 SL	loss of body firmness, slowed movements, infrequent death	loss of firmness or "bloat" of the body, overproduction of slime, slowed movements	458*	206	627	234*

* Correlation significant at $p < 0.01$ (two-sided).

High earthworm mortality was observed after application of Afalon 450 which was finally reflected in their reproductive abilities. The least toxic appeared glyphosate containing Glifocyd 360 SL which reduced the population by circa 18 %. Mosleh et al

[11] demonstrated that isopoturon accumulated in the earthworms' bodies did not necessarily cause their death. Active substances and their metabolites may, however, exert sublethal effects like eg growth inhibition [11, 14]. In performed experiment such response was observed for Bofix 250 EC and Glifocyd 360 SL. The strongest reaction was observed in Glifocyd treated young individuals of *E. fetida* and *D. veneta* which reached the size of 1 cm and matured (Photo 1) lying deformed cocoons (Photo 2).

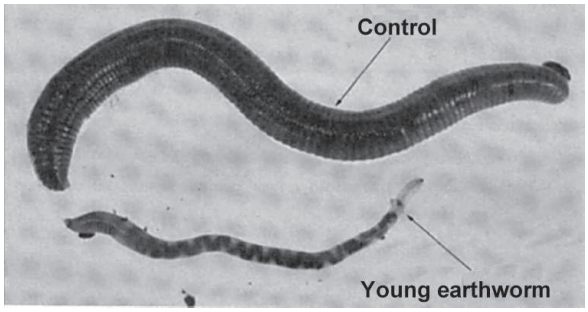

Photo 1. Young individual of *D. veneta* after the contact with herbicide compared with the control one

Photo 2. Deformed cocoons of *E. fetida* after the contact with Glifocyd

Experimentally measured mean lethal concentration (LC_{50}) of Bofix 260 EC was 4.17 mg/dm^3 for both species being lower than the dose recommended by the producer. The lethal concentrations of Glifocyd 360 SL were many times higher and amounted to 160 and 320 mg/dm^3 for *D. veneta* and *E. fetida*, respectively.

Conclusions

1. Applied herbicides caused irritation of the nervous system and of dermatitis and muscular sack dermatitis in *E. fetida* and *D. veneta*.
2. Herbicides containing linuron and glyphosate as active substances inhibited the growth of earthworms and development of young individuals after hatching.

3. All applied herbicides negatively affected reproduction of analysed oligochaete species.

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Abstrakt: Celem przeprowadzonych badań było określenie toksycznego wpływu wybranych środków ochrony roślin na dżdżownice *E. fetida i D. veneta* na ich zdolność do przeżywania w środowisku oraz reprodukcję. Pod uwagę wzięto takie parametry, jak:
– zmiany w liczebności po zastosowaniu środka chemicznego w porównaniu z kontrolą,
– liczba składanych kokonów i wylęg,
– średnie stężenie śmiertelne (LC_{50}).

Jako materiał chemiczny wybrano herbicydy zawierające substancje czynne z grupy: mocznikowej, aminofosfonianowej i fenoksykwasów. Reakcja dżdżownic na zastosowane herbicydu była różna. Zaobserwowano znaczne zmniejszenie masy ciała pod wpływem linuronu oraz spadek liczby wylęgających się osobników pod wpływem glifosatu i związków z grupy fenoksykwasów.

Słowa kluczowe: herbicydy, *Lumbricidae*, reprodukcja, aktywność życiowa