

EFFECTS OF HERBICIDES ON TARGET AND NONTARGET ORGANISMS

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Abstract. Pesticides have significant environmental and public health impact. This study examined the target and nontarget effects of herbicide Sekator OD 375 (amidosulfuron and iodosulfuron) to target weed species *Chenopodium album* L., to two nontarget plant species – barley *Hordeum vulgare* L.; duckweed *Lemna minor* L. and one aquatic microinvertebrates *Daphnia magna*. The terrestrial plants were sprayed directly with various dilutions of herbicide sprays (solutions equivalent to 0.1-0.001 of recommended field application rate), the aquatic plants and microinvertebrates were affected by the same concentrations of herbicide in their growth medium. The measured endpoints in plants bioassays were: survival, height of the shoots, the dry biomass, concentrations of malondialdehydes (MDA) the growth rate of *L. minor*. The mortality of *D.magna* was observed. All plant species had a strong phytotoxic response to herbicide application, though the response of target species was more pronounced. The growth of shoots of *Chenopodium album* and *Hordeum vulgare* was inhibited by 37.1% and by 18.4% respectively. The dry biomass of barley was by 33 %, of *Chenopodium album* – by 71,5% lower than in control. *Lemna minor* showed extremely strong response: in the highest concentrations of herbicide the breakdown of all colonies was observed. The biomass of *Lemna minor* in lowest concentrations of herbicide was approximately by 31 % lower than in control. Application of herbicide provoked an oxidative stress, the concentrations of MDA in *Ch. album* and *H. vulgare* were, respectively, by 8.4, and 1.2-fold higher than in control. The microinvertebrates were very sensitive and application of herbicide caused 50-100 % mortality of microinvertebrates. The study revealed that application of herbicides causes the adverse effects not only in target vegetation, but in nontarget organisms as well.

Keywords: aquatic invertebrates, herbicides, nontarget organism; oxidative stress, phytotoxicity, target organism.

1. Introduction

Pesticides are ubiquitous in the environment and have significant environmental and public health impact. Recently the usage of the herbicides has grown up. Unfortunately herbicides affect not only the species of plants they are used to destroy (targeted ones), but also other ones (not-targeted ones). Intensive herbicide use causes soil, surface and groundwater water pollution. Field studies have shown that nontarget vegetation is at risk of accidental herbicide exposure, by direct overspray or indirectly by spray drift from aerial or ground applications (Mars *et al.* 1989; de Snoo and van der Poll 1999). Wild species are more or equally sensitive to herbicide application as crop species (Boutin and Rogers 2000; Clark *et al.* 2004).

Moreover, indirect herbicide impact to whole ecosystem may be relatively high as their affects to target and nontarget plant species may disturb communities structure and ecosystems functions. Interest in the potential effects of herbicides on non-target organisms has been increasing rapidly in recent years. Although herbicides are expected to affect aquatic biota in Lithuania, though were no systematic studies in our country on this issue. Most of the works focus on in inventories of old

pesticides and their storage (Bagdonas *et al.* 2005; Kadūnas *et al.* 2006) and few studies have been conducted on herbicide effects on biota (Deveikytė 2005; Četkauskaitė *et al.* 2006).

The sulfonylurea herbicides are strong acetolactate synthase (ALS) inhibitors. They are widely used for control weeds in cereal and broad-leaved crops. ALS is the first common enzyme of brached-chain amino acid (valine, leucine and isoleucine) biosynthesis. Their mode of action and the way they exert their toxicity in higher plants has been well studied (Duke 1990; Binder *et al.* 2007).

However, little is known about the toxicity of these herbicides to nontarget terrestrial and aquatic vegetation. Studies showed that sulfonylureas may act as gametocides (Yu *et al.* 2009), can evoke oxidative stress in plants (Wang and Zhou 2006; Duman *et al.* 2010), decrease chlorophyll content (Frankart *et al.* 2003). Previously it was recorded that even 1 % of the recommended dose of herbicide application might adversely affect the growth, morphology and reproduction of nontarget plants (Boutin *et al.* 2000). Sometimes low doses of herbicides stimulate the growth of non-target plants, though higher doses lead to decrease in growth, content of photosyn-

thetic pigments and increase in lipid peroxidation (Duman *et al.* 2010).

Field study in *Beta vulgaris* L. treated with herbicides carbamate and cyclohexamediones showed that the treatment decreased the yield of *B. vulgaris* (Deveikyte, 2005).

The recommended field application rate of sulfonyleureas is relatively low (approximately 2-75 g ha⁻¹) and residues in soil in the range 0.01-0.07 ng g⁻¹ can reduce the growth of plants (Moyer *et al.* 1990).

These herbicides may end up in surface waters and present potential risks for aquatic vascular plants, algae and invertebrates. In general, the toxicity usually is evaluated with plants and it is supposed to be of relatively low acute and chronic animal toxicity.

The aim of the study was to determine the herbicide effect to target weed species (*Chenopodium album*) and to two nontarget plant species – barley *Hordeum vulgare* L.; duckweed *Lemna minor* L. and one aquatic microinvertebrate *Daphnia magna*.

2. Materials and methods

An investigative study was carried out to explore the effects of amidosulfuron and iodosulfuron methyl, sodium salt on three plants species and one aquatic invertebrate species.

Chosen organisms were exposed to five test concentrations (0.01, 0.025, 0.05, 0.075, and 0.1 ml l⁻¹) of commercially available Sekator OD 375 (Bayer Crop-Science). Sekator OD 375 is composed of amidosulfuron (9 %), iodosulfuron methyl, sodium salt (2.2 %) and mephenpyr diethyl (22 %). Herbicide was dissolved in water or growth medium of tested organisms to imitate the condition of field spray in agriculture.

Phytotoxicity test was performed on three higher plants: target species common lambsquater (*Chenopodium album*) and two nontarget species – spring barley (*Hordeum vulgare* L.) and aquatic plant common duckweed (*Lemna minor* L.). Terrestrial plants were grown in 10 cm diameter pots in a potting mixture of peat substrate, containing all necessary macro- and micro-nutrients. After emergence the number of plants was reduced to 10 in each pot. Plants were kept in regime of 21 °C ± 2°C temperature under 16:8 h light:dark.

The following endpoints were measured to evaluate phytotoxicity: shoot height (mm), and shoot mass (g), content of malondialdehyde. The plants were sprayed at the four- to five-leaf stage with a laboratory pot sprayer. Experiment has lasted 10 days and has been conducted in 3 replicates with five concentrations of herbicide.

The standardised growth inhibition test with duckweed was performed according to OECD Guideline 221 (OECD 2004). The stock culture of *L. minor* was grown in modified Steinberg medium in growth chambers at 24 °C ± 2°C with a light/dark cycle of 16/8 h. 10 double-fronded healthy common duckweed *L. minor* colonies were transferred to Petri dishes containing different concentrations of herbicide. The herbicide was dissolved in

growth medium of *L. minor*. Experiment has lasted 7 days and has been conducted in 3 replicates.

Toxicity was recorded as percent inhibition of growth (fronds number and biomass) (relative to control) of *L. minor* as a result of 7-day exposure to the toxicant in its growth medium.

Specific growth rate was calculated from the following equation with measured fronds number (N) at the end (t₁) and the start of the test (t₀):

$$r = \frac{\ln(N_{t_1}) - \ln(N_{t_0})}{t_1 - t_0} \quad (1)$$

Acute 24-48 h immobilisation test was performed with *Daphnia magna* according to OECD Guideline 202. The control was synthetic freshwater (growth medium of *D. magna*).

5 organisms (less than 24 h old) were exposed to each dilution sample in 3 replicates. After 24 h and 48 h incubation in light/dark cycle 16:8 h at 20±1 °C, the number of dead individuals was recorded. The test was considered valid if the mortality in control with synthetic freshwater did not exceeded 10 %.

Results for the toxicity tests were expressed as the concentration of the sample that produced a 50% effect (e.g., growth inhibition, mortality) (EC₅₀ or LC₅₀). EC₅₀ (LC₅₀) values were expressed as a percentage of effluents tested. Toxicity values (LC₅₀) were converted in Toxic Units (TU) (Eq. 1), i.e. inverse of LC₅₀ expressed in %:

$$TU = [1/EC_{50}] \times 100 \quad (2)$$

3. Determination of lipid peroxidation

Concentration of malondialdehyde (MDA), the by-product of lipid peroxidation, was used as biomarker of membrane oxidative damage. MDA content was determined by reaction with thiobarbituric acid (TBA). The sample of fresh leaves tissue was homogenized with Tris-HCl buffer solution containing 1.5% of PVPP (pH 7.4) and centrifuged at 10 000 g for 30 min at 4° C. Equal amounts of tissue extract and 0.5 % TBA in 20 % trichloroacetic acid (TCA) (w/v) was mixed and heated at 95° C for 30 min. After centrifugation of reaction mixture at 10 000 g for 15 min. absorbance of the colored supernatant was measured at 532 nm and corrected for unspecific turbidity by subtracting the value of absorbance at 600 nm. The concentration of MDA was expressed in nmol g⁻¹ fresh weight using extinction coefficient of 155 mM⁻¹cm⁻¹ (Buege and Aust, 1978).

Each species endpoint per herbicide treatment sample was compared to the corresponding reference sample mean using a Students' *t* test. The difference was significant than *p* < 0.05.

4. Results and discussion

Both terrestrial target and nontarget plants species had a strong phytotoxic response to herbicide application,

though the response of target species was more pronounced. The growth of shoots of *Chenopodium album* and *Hordeum vulgare* treated with highest herbicide concentration was inhibited by 37.1 % and 18.4 %, respectively (Fig 1).

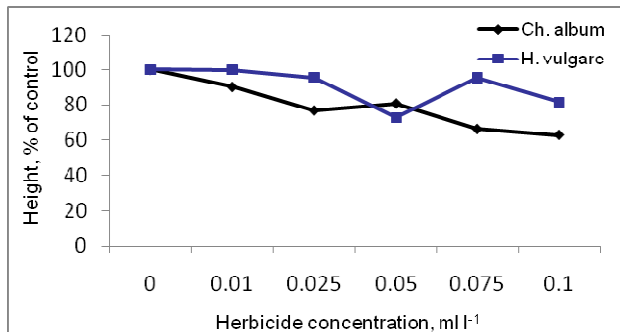


Fig 1. Shoot height of *Ch. album* and *H. vulgare* exposed to different concentrations of herbicide

The regression analysis revealed strong negative relationship between herbicide concentration and the growth of shoots (*Ch. album*: $r = -0.85$, $p < 0.05$; *H. vulgare*: $r = -0.59$, $p < 0.05$). Relationship between the sulfonylureas content in soil and plant growth was determined in several studies (Parrish et al. 1995; Boutin et al. 2000; Alonso-Prados et al. 2002).

The biomass was more sensitive endpoint than shoot height (Fig 2): even the lowest Sekator OD375 concentrations resulted in *Ch. album* and *H. vulgare* biomass reduction by 48.5 % and 9 %, respectively. The highest herbicide concentration statistically decreased the dry weight of barley by 33 %, of *Chenopodium album* by 71.5 % ($p < 0.05$).

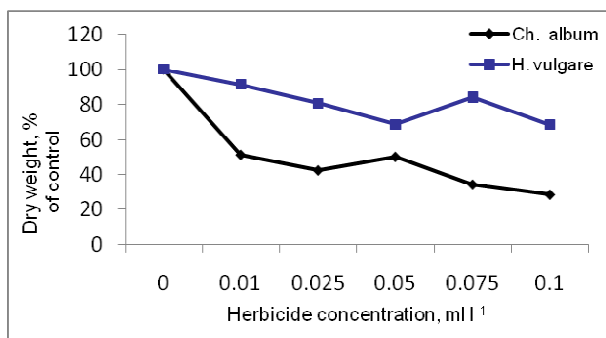


Fig 2. Dry weight of *Ch. album* and *H. vulgare* exposed to different concentrations of herbicide

The regression analysis revealed strong negative relationship between herbicide concentration and the dry weight (*Ch. album*: $r = -0.69$, $p < 0.05$; *H. vulgare*: $r = -0.59$, $p < 0.05$). The decrease of terrestrial plant biomass due to herbicide application was observed in numerous studies and it corresponds well to our data (Boutin et al. 2000; Riemens et al. 2008).

Lipid peroxidation is a sensitive measure of oxidative damage and is useful as a biomarker for oxidative stress. Herbicide provoked an oxidative stress; the concentration of MDA in *Ch. album* and *H. vulgare* were,

respectively, by 8.4 and 1.2-fold higher than in control (Fig 3).

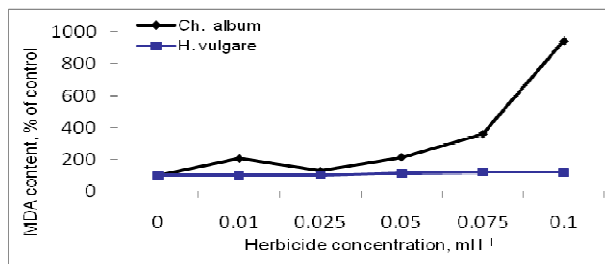


Fig 3. MDA content in *Ch. album* and *H. vulgare* exposed to different concentrations of herbicide

Content of MDA in both species increased along with increasing herbicide concentration ($r > 0.85$, $p < 0.05$). Our results support the data of other studies reporting that even doses under 1 % of the recommended application rate can have significant effects on nontarget terrestrial plants growth (Fletcher et al. 1996; Boutin et al. 2000). Though Cedergreen et al. (2004) observed no effect of metsulfuron-methyl to the growth rate of barley (*H. vulgare*), but oil-seed rape (*Brassica napus*) growth rate was dramatically reduced due to exposure to herbicide.

Common duckweed (*Lemna minor* L.) has showed extremely strong response: after exposure of *L. minor* to the highest concentration of Sekator OD 375 (0.075-0.1 ml l⁻¹), the breakdown of all colonies was observed.

Dry weight of *L. minor* significantly decreased along with herbicide concentration in solution (Fig 4).

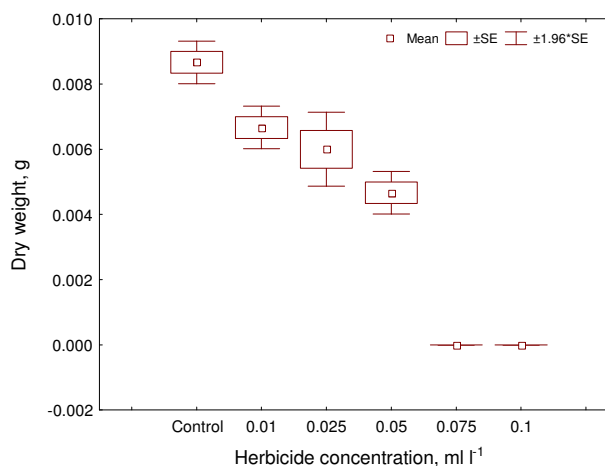


Fig 4. Dry weight of *L. minor* treated with herbicide

The biomass of *L. minor* in solution containing 0.05 ml l⁻¹ of Sekator OD 375 was by 31 % lower than in control. Between the dry weight of frond and herbicide concentration in solution was detected strong negative relationship ($r = -0.88$, $p < 0.05$).

Along with increasing herbicide concentration in solution, inhibition of growth rate of *L. minor* was observed (Fig 5). In case of the highest concentrations (0.075-0.1 ml l⁻¹) no one new frond was protruded before the break-

down of the colonies, so it was considered as 100 % inhibition.

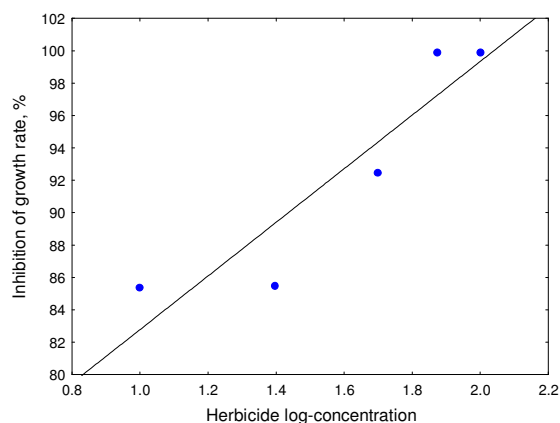


Fig 5. The growth rate inhibition by herbicide

Exposure to 0.05 ml l⁻¹ led to decrease of growth rate by 93 %. The regression analysis showed that inhibition of *L. minor* growth rate significantly increases with herbicide concentration in solution (R = 0.92, p < 0.05). As even the lowest tested herbicide concentration inhibited the growth of *L. minor* more than 50 %, we could only make prognostic calculations that EC₅₀ may be approximately 0.1 µl l⁻¹. Only EC₉₀ was calculated and it was 0.027 ml l⁻¹.

The exposure to another representative of sulfonureas – metsulfuron-methyl, dramatically reduced the relative growth rate of *L. minor* and *L. minor* showed the highest susceptibility to this herbicide among 12 tested aquatic plant species. It was observed that fast growing species with a small exposed leaf area were more sensitive than slow growing species with large exposed leaf area (Cedergreen *et al.* 2004).

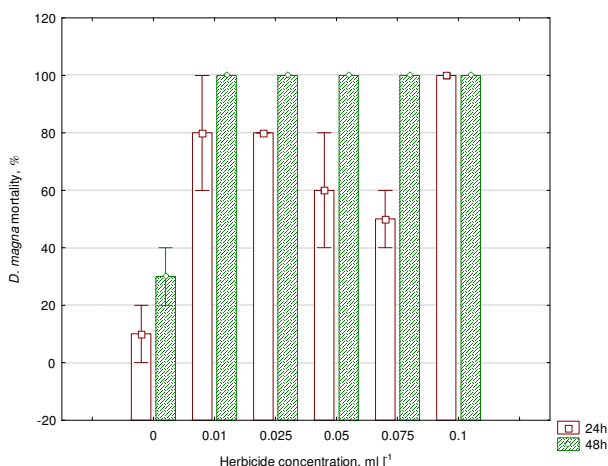


Fig 6. Mortality of *Daphnia magna* exposed to different concentration of herbicide

Herbicide treatment showed very high acute toxicity to invertebrates *Daphnia magna*. Even the lowest concen-

tration of Sekator OD 375 (0.01 ml l⁻¹) led to death of *D. magna* (Fig 6) and the mortality reached 100 % in the case of the highest tested concentration. All the concentrations were lethal after 48 h of exposure.

The results of logistic regression show that the risk of death of tests organisms increases with the concentration of herbicide ($\chi^2=35,16$, p<0,05) (Fig 7).

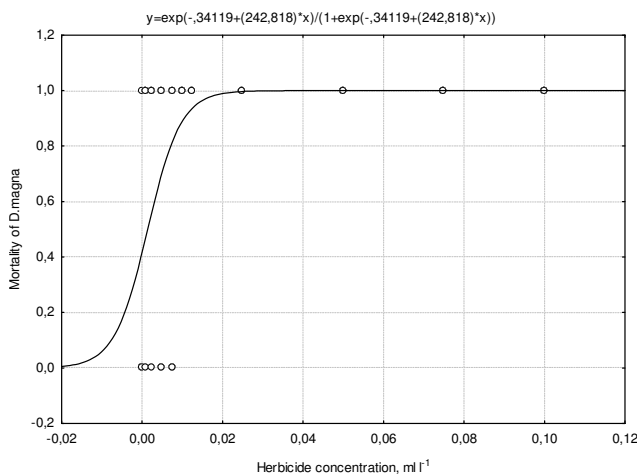


Fig 7. Relationship between concentration of herbicide and the mortality of *Daphnia magna*

The strong statistically significant relationship between concentrations of herbicides and the acute and chronic toxicity to invertebrates has previously been found by several authors (Carejeira *et al.* 1998; Pereira *et al.* 2000), though there is a lack of such studies investigating herbicide impact on aquatic fauna.

5. Conclusions

1. The study revealed that application of herbicides causes the adverse effects not only in target vegetation, but in nontarget species as well, though target species had the stronger response. The herbicide application evoked oxidative stress and altered the growth of terrestrial plants. The growth of shoots of target species *Ch. album* and nontarget species *H. vulgare* was inhibited by 37.1% and by 18.4% respectively. The dry biomass of barley was by 33 %, of *Chenopodium album* – by 71.5% lower than in control. The content of MDA in *Ch. album* and *H. vulgare* were, respectively, by 8.4, and 1.2-fold higher than in control.
2. The aquatic plants are more susceptible to herbicide than terrestrial ones. *Lemna minor* showed extremely strong response: in the highest tested concentrations of herbicide the breakdown of all colonies was observed.
3. The microinvertebrates *Daphnia magna* were very sensitive and application of herbicide caused 50-100 % mortality of organisms.
4. Our results suggest that herbicides runoff in drainages and rivers could have an adverse effect on the growth of aquatic plants and invertebrates.

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