

YELLOW LUPIN IS A GOOD BIOINDICATOR OF SOIL CONTAMINATION WITH SULFAMETHAZINE

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ABSTRACT

Sulfamethazine is an antimicrobial sulphur drug used to treat big animals suffering from lung and digestive track diseases. The aim of the current study was to evaluate the suitability of selected leguminous plants as bioindicators of sulfamethazine contamination in the soil. Pea, lentil, soybean, adzuki bean, alfalfa and yellow lupin seeds were germinated for six days using PHYTOTOKKIT™ (MicroBio Test Inc., Belgium). The soil was watered with distilled water supplemented with different concentrations of sulfamethazine: 10, 100 µM and 1, 5, 15, 20 mM. No-observed-effect-concentration (NOEC) for all germinated seeds was equal 20mM. Effective concentration causing a 50% (EC₅₀) reduction of the root growth after 3, 6 or 9 days was the same for lupin, pea and lentil and equalled 10 µM. The EC₅₀ determined for soybean after 3 and 6 days was higher and amounted 0.25mM and 1.0 mM after 9 days. It was shown that root elongation was a better indicator of sulfamethazine phytotoxicity than the seed germination. Increasing concentrations of sulfamethazine led to decrease of the fresh mass and slight increase of the dry mass and electroconductivity. Yellow lupin proved to be the best bioindicator of soil contamination with sulfamethazine among all tested plants.

KEYWORDS

Lupinus luteus, *Pisum sativum*, *Lens esculenta*, *Glycine max*, *Vigna angularis*, *Medicago sativa*, sulfamethazine, Phytotoxkit™

INTRODUCTION

Pharmaceuticals are used in veterinary medicine as preventive and therapeutic measures (Dolliver *et al.* 2007). The pollution of the environment with veterinary therapeutic agents and their metabolites increases due to the rising doses of applied pharmaceuticals caused by emergence of resistant microorganisms. It results in implementation of new drugs in veterinary medicine. Moreover, the industrial, multi-stand animal farms are responsible for occurrence of places with locally high concentration of drugs (Jjemba 2002). Veterinary

medicines are often applied massively (whole herds and not individual animals are treated) and excreted in unmodified form (anti-worm medicines and some antibiotics) or as metabolites. They are transported from the farms to the environment through open sewage system or are distributed in the fields together with manure (Hirsch *et al.* 1999). Sulfamethazine (4-amino-N-(4,6-dimethyl-2pyrimidinyl)benzenesulfonamide) is an antimicrobial sulphur drug. Sulfonamides are commonly incorporated into animal feedstuffs for prophylactic and therapeutic purposes as well as for production enhancement (Dolliver *et al.* 2007). They are used in veterinary prophylaxis of infections, in the treatment of diseases and as growth promoters (Babić *et al.* 2006). The aim of the present study was to determine morphological and physiological response of seeds, roots and seedlings of selected plants to a veterinary medicine, sulfamethazine, present in varying concentration in the soil.

MATERIAL AND METHODS

SEED GERMINATION AND ROOT GROWTH TEST

Seeds of yellow lupin (*Lupinus luteus*), pea (*Pisum sativum*), lentil (*Lens esculenta*), soybean (*Glycine max*), azuki bean (*Vigna angularis*) and alfalfa (*Medicago sativa*) were germinated for three, six and nine days using PHYTOTOKKIT™ (MicroBio Test Inc., Belgium). Germination was carried out in controlled climatic conditions with temperature set at 25°C and 90 % RH humidity, in darkness. Ninety ml of soil (sand, vermiculite, peat 1:0.3:1, v/v/v) were placed in plastic microbiotest plates. The soil was covered with Whatman No. 1 filter-paper and watered with 27 ml distilled water supplemented with different sulfamethazine (Sigma-Aldrich) final concentrations: 10, 100µM and 0.25, 1, 5, 15 and 20 mM. The control plants were watered with pure distilled water. The root length was estimated using Image Tool for Windows. The effective concentration causing a 50% response (EC₅₀) was calculated for inhibition of root growth and no observed effect concentration (NOEC) that did not affect germination was noted. Dry and fresh mass and electroconductivity were determined according to ISTA 1997.

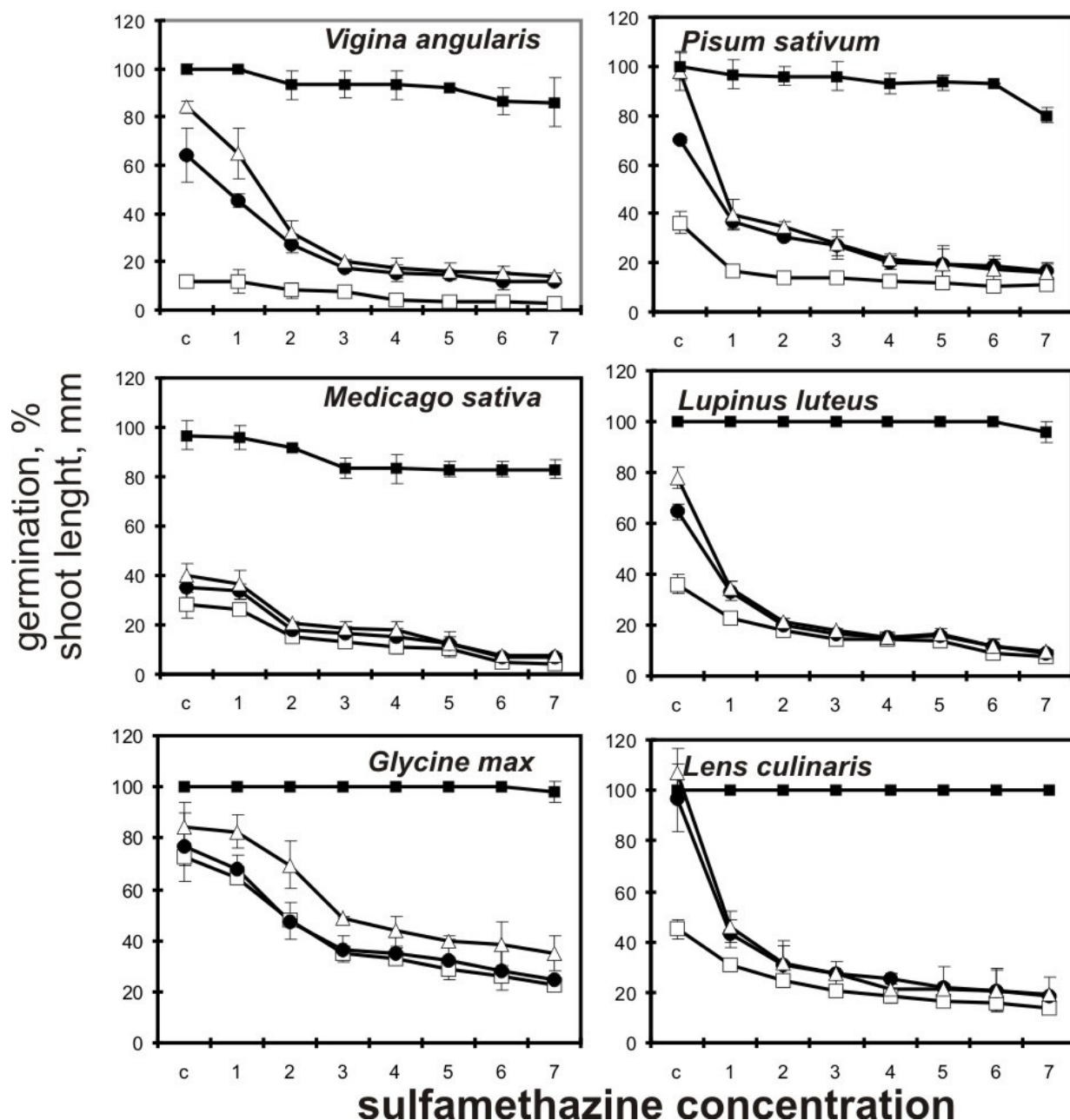


Fig. 1. Seed (■) germination [%] and root length [mm] of *Vigna angularis*, *Pisum sativum*, *Medicago sativa*, *Lupinus luteus*, *Glycine max* and *Lens esculenta* using three (□), six (●) nine (Δ) days on soil supplemented with different sulfmethazine concentrations (c-control, 1-10 μM, 2-100 μM, 3-0.25 mM, 4-1 mM, 5-5 mM, 6-15 mM and 7-20 mM). Data points represent the means ± SD for nine replicate samples.

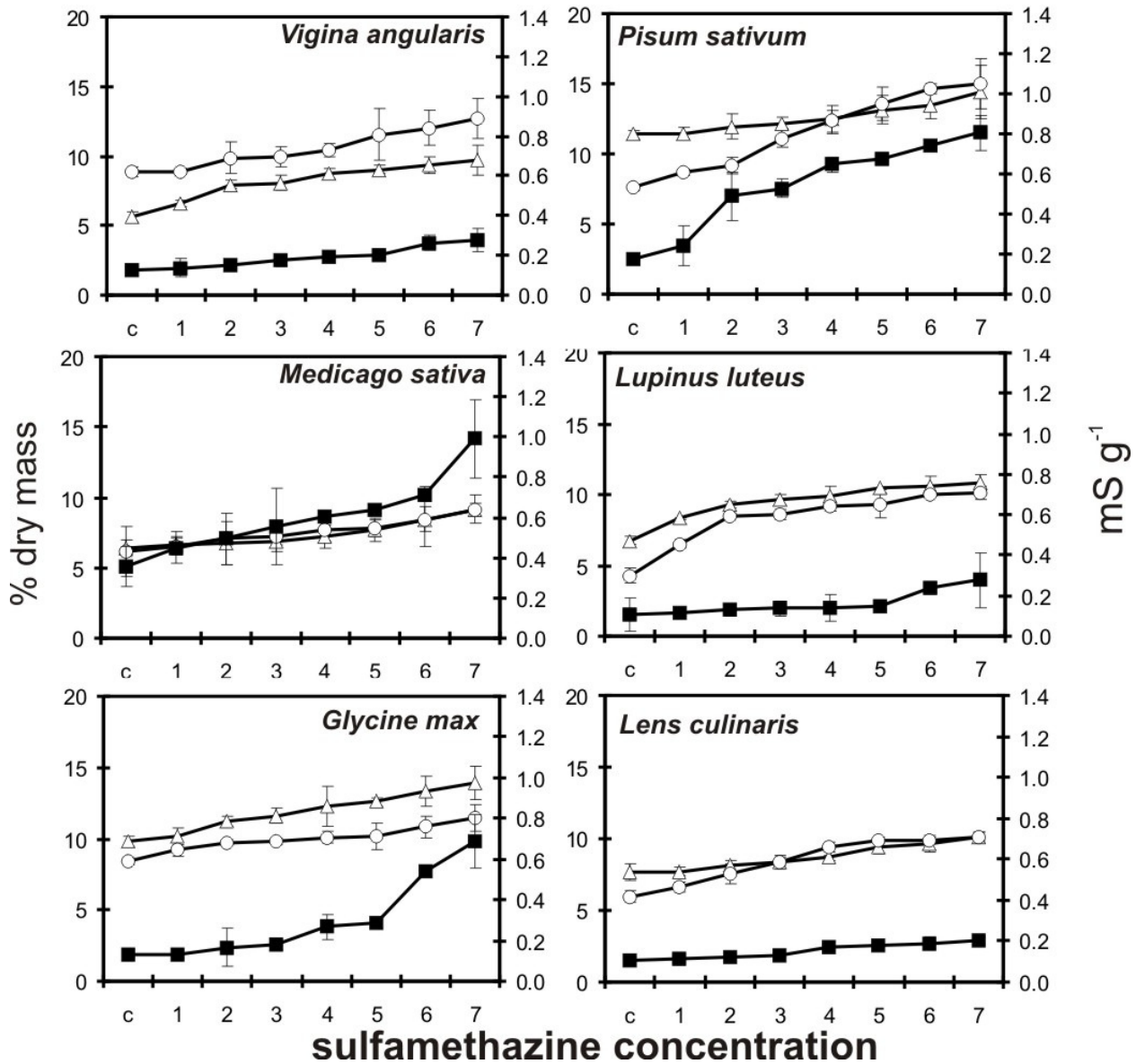


Fig. 2. Shoot length [mm] of *Vigna angularis*, *Pisum sativum*, *Medicago sativa*, *Lupinus luteus*, *Glycine max* and *Lens esculenta* using three (□), six (●) nine (Δ) days on soil supplemented with different sulfmethazine concentrations (c- control, 1-10 μM, 2-100μM, 3- 0.25 mM, 4-1 mM, 5- 5 mM, 6-15mM and 7-20 mM). Data points represent the means ± SD for nine replicate samples.

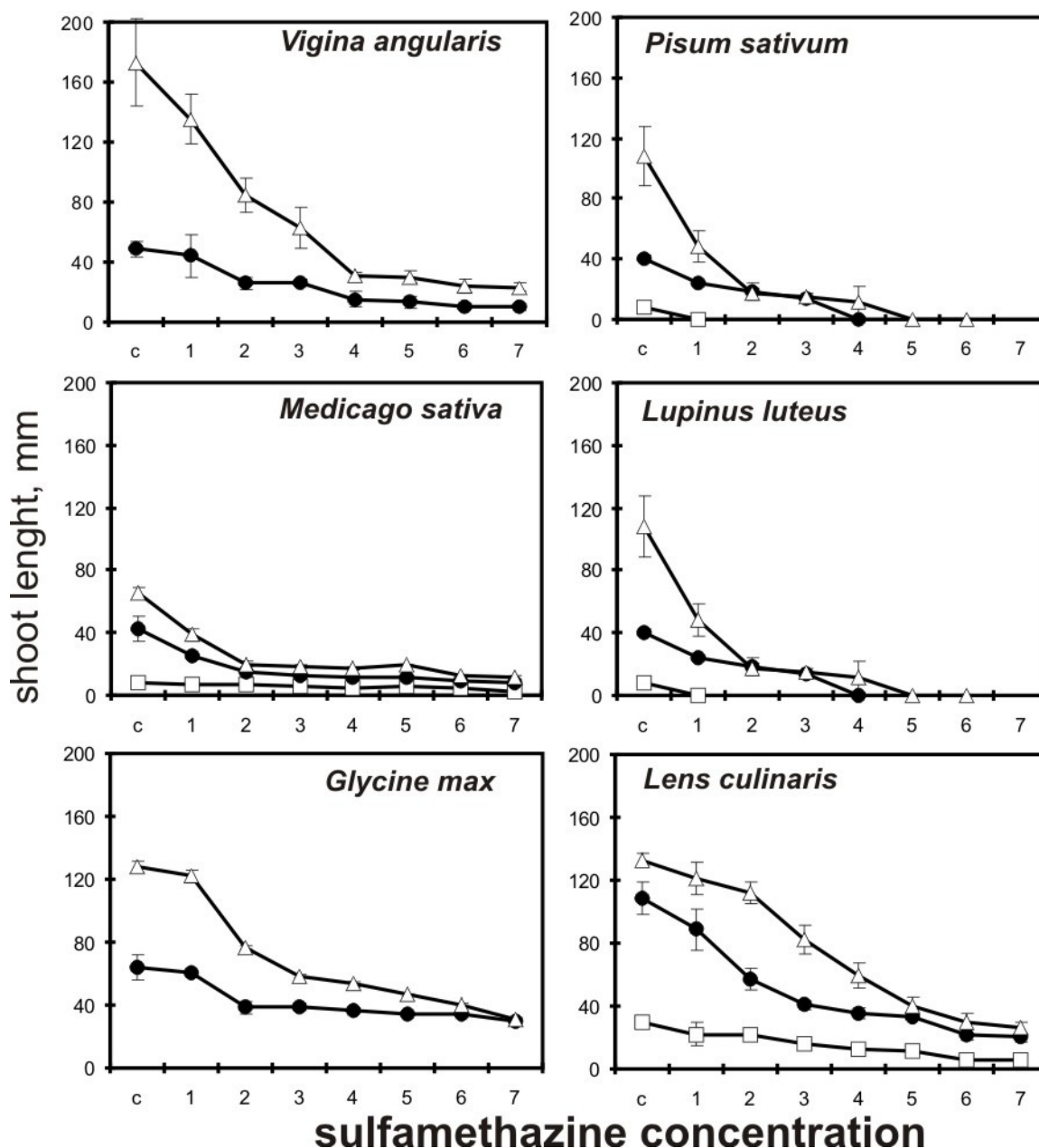


Fig. 3. Roots (o) and shoot (Δ) dry mass [%] and electroconductivity (\blacksquare , [mS fresh mass⁻¹]) of *Vigna angularis*, *Pisum sativum*, *Medicago sativa*, *Lupinus luteus*, *Glycine max* and *Lens esculenta* seedlings growing for nine days on soil supplemented with different sulfmethazine concentrations (c-control, 1-10 μ M, 2-100 μ M, 3-0.25 mM, 4-1 mM, 5- 5 mM, 6-15 mM and 7-20 mM). Data points represent the means \pm SD for nine replicate samples.

STATISTICAL ANALYSIS

The experiment was conducted in nine replicates. The results were statistically evaluated using analysis of variance (F test) for two factor experiments (split-plot). The mean values of the plots were compared using q SNK test (Student-Newman-Keuls).

RESULTS AND DISCUSSION

SEED GERMINATION, ROOT AND STEMS ELONGATION TEST

Plant sensitivity to environment contamination is often used to estimate environment degradation. Plants respond in different manners to many kinds of toxic substances. The phytotoxic effect is a result of interaction between the compound and the plant in given environmental conditions. The symptoms include morphological deformations and changes in plant biochemistry (Mohammad *et al.* 2006, Marchiol *et al.* 2007). Plant sensitivity to human and veterinary medical compounds, which are introduced into the environment, can be used to evaluate the environment degradation (Dolliver *et al.* 2007). The effect of sulfamethazine concentration (10, 100 μ M and 0.25, 1, 5, 15, 20 mM) on germination and roots and stems elongation of six plant species (yellow lupin (*Lupinus luteus*), pea (*Pisum sativum*), lentil (*Lens esculenta*), soybean (*Glycine max* L.), adzuki beans (*Vigna angularis*) and alfalfa (*Medicago sativa*), their fresh and dry mass and seedlings electroconductivity was analysed. The seed germination and root elongation (Fig. 1) measurements were taken three, six and nine days after sulfamethazine application.

Lupin was the most sensitive to sulfamethazine among the tested leguminous plants. Plants sensitivity to toxic compounds present in soil was confirmed by research (Dolliver *et al.* 2007) which show that extension of the exposure time to the toxic factor increases plants sensitivity. Biotests, opposite to instrumental (chemical) methods, allow estimation whether very low levels of active substance residues in the soil can be phytotoxic to crop plants (Wolska *et al.* 2007). In the reported study all the seeds, irrespectively of the concentration of applied sulfamethazine, germinated in the range between 100% (lupin, lentil and soybean) and 73%. No observed effect concentration (NOEC) for germination of all tested plants was 20 mM. Similar lack of germination inhibition (germination above 85%) after application of other toxic compounds was observed in transgenic and non-transgenic soybean (Torres *et al.* 2003).

Root length is in ecotoxicology an important morphological parameter of soil contamination. Under effect of increasing sulfamethazine concentration an inhibition of the root elongation was observed in tested plants (Fig. 1). All the plants responded in a similar manner. The exposure time to the toxic compound (3, 6 or 9 days) affected root elongation. The control plants

had the longest roots. The fastest root elongation in the absence of the toxic compound after 6 and 9 days was observed in lentil, adzuki bean and lupin and the slowest in alfalfa. The increase of root exposure time to sulfamethazine contributed to inhibition of root elongation. Lupin was the most sensitive to sulfamethazine. The length of lupin root, starting from the sulfamethazine concentration of 100 μ M after 3, 6 and 9 days, was similar and decreased steadily to reach 8.5mm at the concentration of 20mM. Soybean was the most tolerant plant to the 9-day exposure to the studied pharmaceutical. After 9 days of exposure to all tested sulfamethazine concentrations soybean plants formed the longest roots.

Basing on the performed tests comparing soybean and lupin, it can be stated that soybean was less sensitive to the investigated veterinary drug. Therefore, lupin was a better bioindicator of soil contamination with sulfamethazine. The conclusion was corroborated by the obtained EC₅₀ values. Effective concentration causing a 50% reduction (EC₅₀) of root growth after 3, 6 and 9 days was identical for lupin, pea and lentil and equalled 10 μ M. The EC₅₀ determined for soybean after 3 and 6 days was higher and equalled 0.25mM and 1.0 mM after 9 days.

The study definitely showed that root growth is a better criterion for evaluation of soil toxicity than germination. Results of Dolliver *et al.* (2007) from a 45-day greenhouse experiment showed that sulfamethazine was taken up by corn (*Zea mays* L.), lettuce (*Lactuca sativa* L.), and potato (*Solanum tuberosum* L.) with concentrations in plant tissue ranging from 0.1 to 1.2 mg kg⁻¹ dry weight. Sulfamethazine concentrations in plant tissue increased with corresponding increase of sulfamethazine in manure.

Plant stems were the next evaluated morphological characteristic. The stem length of all tested plants was measured after 3, 6 and 9 days of exposure to sulfamethazine (Fig. 2). In the absence of the toxic compound alfalfa had the shortest stems (55.7mm on average), while adzuki bean had the longest (173 mm on average). Pea growing for 3 days in the soil with any of the tested sulfamethazine concentrations did not form stems, except for the control plants. The concentrations of 1 mM and 5 mM sulfamethazine after 6 and 9 days, respectively, inhibited stem formation in pea. Noteworthy, inhibition of stem growth in lupin was documented in 82% already after 3 days at a concentration of 10 μ M.

A similar effect was observed after 6-day exposure at a concentration of 5 mM and after 9 days at the highest tested sulfamethazine concentration. Soybean and adzuki bean formed stems only after 6 says of the experiment duration. The inhibition of soybean stem growth in 50% and adzuki bean in 80% was documented after 3 and 9 days of exposure to the

highest sulfamethazine concentration. Similarly as root elongation, stem elongation indicated that lupin was a good bioindicator. Alfalfa and lentil growing in the soil contaminated with sulfamethazine for 3, 6 or 9 days

formed stems but even the lowest concentration of the drug inhibited the elongation. It was shown that the highest concentration of sulfamethazine that did not inhibit stem growth (NOEC – no observed effect concentration) after 6 or 9 days for adzuki bean, soybean and lentil was 20 mM. The root dry mass and seedling electroconductivity were measured 9 days after sulfamethazine application (Fig. 3). The dry mass increased slightly but steadily together with the increase of sulfamethazine concentration in the soil. At the highest sulfamethazine concentration, the dry mass of both roots and stems did not exceed 15% mg/g dry mass.

The electrical conductivity method was used to evaluate the degree of seedlings damage after glyphosate application. The method was successfully used to ascertain the degree of freezing injury (Prášil, Zámečník 1998). Electroconductivity, similarly like dry mass increased steadily. However, the electroconductivity increase was slower than the dry mass increase. In the control plants electroconductivity of lupin and soybean was 0.11 mS/gdm on average, adzuki bean and lentil 0.13 mS/gdm and pea 0.17 mS/gdm, while in the case of alfalfa it was higher and equal 0.35 mS/gdm. A sudden increase of electroconductivity in pea was observed starting at the concentration of 100 μ M, while for soybean and lupin the threshold value was 1.0 mM and 15 mM sulfamethazine, respectively. At the highest sulfamethazine concentration soybean electroconductivity did not exceed 1.57 mS/gdm (Fig. 3).

Summing up, basing on the evaluated morphological (germination, root and stem length, dry mass) and physiological characteristics (electroconductivity of seedlings) and EC₅₀ it can be stated that among all the tested leguminous plants lupin was the best bioindicator of soil contamination with sulfamethazine.

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