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# INFLUENCE OF LEAD AND CADMIUM IONS ON THE ENTOMOPATHOGENIC NEMATODES *Steinernema feltiae* Filipjev

## WPŁYW JONÓW OŁOWIU I KADMU NA NICIENIE ENTOMOPATOGENNE *Steinernema feltiae* Filipjev

**Abstract:** The aim of this study was to determine the influence of lead and cadmium compounds on the entomopathogenic nematodes *Steinernema feltiae* Filipjev. The conducted research allowed to determine the sexual strucure of the nematodes developing inside the body cavity of the host as well as the survival rate of IJs before leaving the insect and after the migration to the external environment. Chemical and biological material as used in the conducted experiments. Chemical material included lead nitrate at the concentration of 40,100,500 ppm and cadmium nitrate at the concentration of 1, 2, 3 ppm. Biological material included the entomopathogenic nematodes *Steinernema feltiae* and test insects – *Galleria mellonella* L. – larvae from own culture.

**Keywords:** *Steinernema feltiae*, *Galleria mellonella*, lead, cadmium

Ability of bioaccumulation in invertebrates is influenced not only by position in the trophic chain but first of all by their physiology and ability of detoxification and removal of metals from the organism [1]. Invertebrates, which appear in soil environment, constitute approximately 99 % of animal species. There are small animals and they are not able to quickly leave the place of their appearance. These animals constitute a significant source of information about various types of pollutions. Evaluating content of heavy metals in direct way, we gain information about physical amount of a given element. Many studies focused on selection of plant or animal

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organisms, which are accumulating toxic substances in their tissues in order to determine toxicity of compounds in the soil [2].

Contamination of the environment with fluoride and metals is the one of the most important ecological problems in modern civilization due to possibility of their toxic action on plants, animals and humans.

Some heavy metals, such as Pb, Zn, and Cu are classified to the group of toxic elements, with very high degree of risk for the environment [3]. Not only invertebrates but also mammals are sensitive indicator of lead in the environment, because especially their bone tissue, reflect environmental pollution [4]. Many investigators have paid attention to ecological features of the nematodes, such as: species and trophic diversity, short time of the development, ability of fast colonization and easy isolation and identification of the respective species, which facilitate their use as bioindicators of the environmental pollution [5]. Species diversity is influenced by soil salinity, fertilization and agricultural intensification, as well as environmental pollution, including pollution with heavy metals. Variety of nematode groups comparing to natural habitats causes increase in number of bacteriophages [6]. In soil, where the limestone or the waste from the lead works was used, higher concentration of bacteriophagic and mycophagic nematodes was established. However, higher concentration of the predatory and omnivorous nematodes was not established. Lead directly and indirectly influences general number of nematode species, their diversity and biomass [7]. Among soil nematodes, we distinguish entomopathogenic nematodes, and there are some of them, which are feeding in the soil, but actively looking for the host and having contact with heavy metal ions. Some ions of heavy metal ions stimulate pathogenicity of the nematodes, but the other heavy metal ions limit it [1, 8]. Many heavy metals are included in coenzymes, active parts of vitamins, and respiratory dyes. They become toxic, if they enter biochemical reactions, which they normally do not take part in, and replace suitable substances [9]. Heavy metals penetrate into mitochondria and cause disturbances in gas exchange; they also influence ability of reproduction. Together with food, an animal organism may be penetrated by the xenobiotic elements (Cd, Pb), which do not take part in metabolism, they are very harmful, but they are consumed and assimilated by the organism, and become toxic when their concentrations exceed the allowable level [3, 10].

## Material and methods

Experimental material constituted of entomopathogenic nematodes *Steinernema feltiae* (bio-preparation "Owinema") and test insects – larvae *Galleria mellonella* L. (own culture). Also chemical material was used for the experiments: lead nitrate $Pb(NO_3)_2$ in concentrations of 40, 100, 500 ppm, cadmium nitrate $Cd(NO_3)_2 \cdot 4H_2O$ in concentrations of 1, 2, 3 ppm and the control containing distilled water. Cycle of experiments took place in accordance with items a–c.

a) 10 insects from each container were placed in Petrie dishes and 50 IJs (*S. feltiae*) per one host were introduced. After two days following the death of *G. mellonella*, the dissection was performed in order to specify number of females and males of

the giant generation of the nematodes developing in the host fed with contaminated food,

b) 10 insects of *G. mellonella* from each container were contacted in the Petrie dishes with invasive larvae of *S. feltiae* (in initial dose of 50 IJs/host). After 8 days following death, insect dissection was conducted in order to determine living and dead larvae L3 placed in the body of the host before migration to the external environment,

c) 10 insects of *G. mellonella* from each box were contacted with 50 IJs (*S. feltiae*) insect. Dead insects were placed in the White's traps in order to obtain living larvae of nematodes migrating from the insect into the external environment. Every week for one month, living and dead nematodes were counted in order to determine their survival rate in the external environment. Each experiment comprised three repetitions.

## Results and discussion

Together with the increase in the lead ions, the sex structure of the parasitic generation of *S. feltiae* developing in the body cavity of the insect was changing. Global number of the parasitic generation of developing nematodes was significantly lowered. Lead ions more intensively influenced the number of developing males of *S. feltiae* than number of females. However, high concentration of lead also adversely influenced number of females of *S. feltiae*. Together with increase in the lead ions, number of females as well as males of *S. feltiae* was decreasing. In case of the cadmium nitrate concentration at the level of 3 ppm, number of the "giant" males and females of the parasitic generation of *S. feltiae* in the insect, with reference to other concentrations, was the highest, even higher than the value in the control experiment. Based on data, it may be concluded that the cadmium nitrate revealed more significant influence on males than on females. The least development was established at concentration of 2 ppm (Fig. 1).

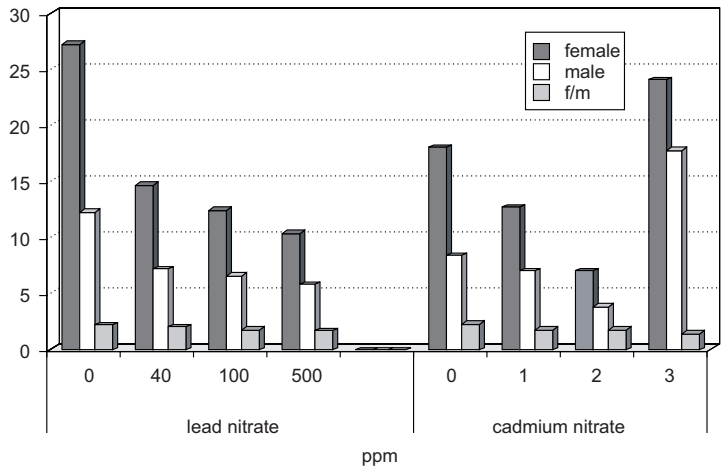


Fig. 1. Influence of lead nitrate and cadmium nitrate included in the food of the insects on the sex structure of the parasitic generation of *S. feltiae*

Influence of the lead nitrate and the cadmium nitrate included in the food of the insects on vitality of saprophagic, mono-phase generation of the (L3) *S. feltiae* larvae before migration from the host.

Increase in concentration of the lead influenced vitality of saprophagic population (L3) of the nematodes. Increase of the lead concentration contributed to reduction of number of living nematodes *S. feltiae* in the insect before migration. Similarly, the cadmium nitrate adversely influenced the nematode numbers. The highest proportion of living larvae of the nematodes to the dead ones was revealed at the cadmium nitrate concentration of 2 ppm (Fig. 2).

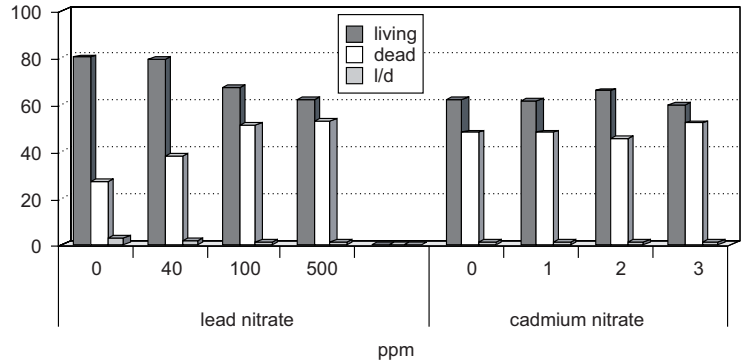


Fig. 2. Influence of the lead and cadmium ions included in the food of insects on vitality of saprophagic, mono-phase generation of the *S. feltiae* larvae before migration from the host

Conducted studies also include evaluation of influence of lead and cadmium ions on the survival rate of (L3) *S. feltiae* larvae in the external environment after migration from the host. Crucial factor influencing number of nematodes in this part of the experiments was not only concentration of the heavy metals, but also time of contact with heavy metal ions. Increase in the mortality of the nematodes inside the insect influenced their ability of migration to the external environment and their vitality. The longer time the larvae stayed in the external environment the higher was their mortality.

In all cases, there was the high correlation between concentration of the lead and cadmium ions, and the number of living and dead invasive larvae, sex structure of *S. feltiae* and the number of individual organisms of the saprophagic generation in the body cavity of the host.

Regression revealed that not only time but also concentration influenced number of males as well as females and there was the obvious influence on mortality rate of the nematodes. Statistical evaluation revealed more significant influence of the heavy metals on the whole development cycle of the nematodes, than on the host insect. Based on conducted evaluation, the conclusion may be drawn that the lead and cadmium ions cause changes in sex structure of the entomopathogenic nematodes *S. feltiae* and in mortality rate of invasive form of these nematodes, as well as in the speed of invasion of the entomopathogenic nematodes to the host. Heavy metal ions influence development of nematodes *S. feltiae* in insects *G. mellonella* and their vitality. Based on conducted

experiments, as well as data published in literature, it may be stated that entomopathogenic nematodes may be used as bioindicators of soil contamination and as biological preparations in battle with the plant pests.

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**Abstrakt:** Celem pracy było określenie wpływu związków ołowiu i kadmu na nicienie entomopatogenne *Steinernema feltiae* Filipjev. Prowadzone badania umożliwiły określenie struktury płciowej nicieni rozwijających się w jamie ciała żywiciela, jak również przeżywalność IJs przed opuszczeniem owada oraz po migracji do środowiska zewnętrznego. W prowadzonych badaniach użyto materiału biologicznego i chemicznego. Materiał chemiczny stanowił azotan ołowiu w stężeniach 40 100 500 ppm oraz azotan kadmu w stężeniach 1, 2, 3 ppm. Materiał biologiczny stanowiły nicienie entomopatogenne *Steinernema feltiae* i owady testowe – larwy *Galleria mellonella* L. – hodowla własna.

**Słowa kluczowe:** *Steinernema feltiae*, *Galleria mellonella*, ołów, kadm