POLSKA AKADEMIA NAUK
INSTYTUT ZOOLOGII

FRAGMENTA FAUNISTICA

Tom 35 Warszawa, 1991.12.15 Nr 14

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The influence of urbanization on the earthworm infection by monocystid gregarines

[With 2 Tables and 3 Figures in the text]

Abstract. Earthworm infection by monocystid gregarines was studied in the soil of Warsaw green areas differently exposed to urbanization, particularly to heavy metal pollution. The incidence of infection was significantly higher within earthworm populations of road adjacent plots compared to those of park centers. Significant correlation was found between earthworm infection and heavy metal concentration in soil. Values of incidence of earthworm infection may provide an adequate indication of trends in the soil pollution.

Key words: earthworms, monocystid gregarines, Apicomplexa, infection, parasites, heavy metals, urbanization, Warsaw.

INTRODUCTION

A modern city possessing vast green areas is generally stated to be an ecosystem in which the basic elements of its structure and principles of functioning are similar to those of natural ecosystems (PISARSKI and TROJAN, 1976; GEPP, 1977).

The urbanization of an area involves many serious impacts on both abiotic and biotic components of the environment. Besides changes in trophic and climatic relations, those proceeding in soil belong to the most important. Hence, in order to assess the degree of urban stress, soil animals are often used as bioindicators. Naturally, bioindicators are mainly searched among the most susceptible soil animals, however, some representatives of the non susceptible ones may also be occasionally utilized for the purpose of bioindication. Earthworms are of that type due to their capability to accumulate poisonous substances in their bodies. Analysis of earthworm tissue may then serve as a useful and rapid method for monitoring the degree of pollution at different sites (CZARNOWSKA and JOPKIEWICZ, 1978; EBING et al., 1984). Another way of earthworm utilization in bioindication was suggested by PURRINI (1983, 1987) and PIŽL (1985, 1989). They found strong correlations between the soil pollution with SO_2 deposits and/or herbicides, and the susceptibility of earthworms for diseases, and presumed that the results of parasitological examination of earthworms may indicate the degree of soil pollution. The objective of the present study was to examine the earthworm populations in different Warsaw green areas for monocystid gregarines and to find out, at the instance of the effect of urbanization, whether the above hypothesis is valid on a larger scale.

SITE, MATERIAL AND METHODS

Study areas

The investigation was carried out in five urban green areas of the Warsaw City, where study plots differently influenced by anthropogenous pressure were selected (Fig. 1). Soil characteristics are given in KUBICKA et al. (1986), STERZYŃSKA (1990).

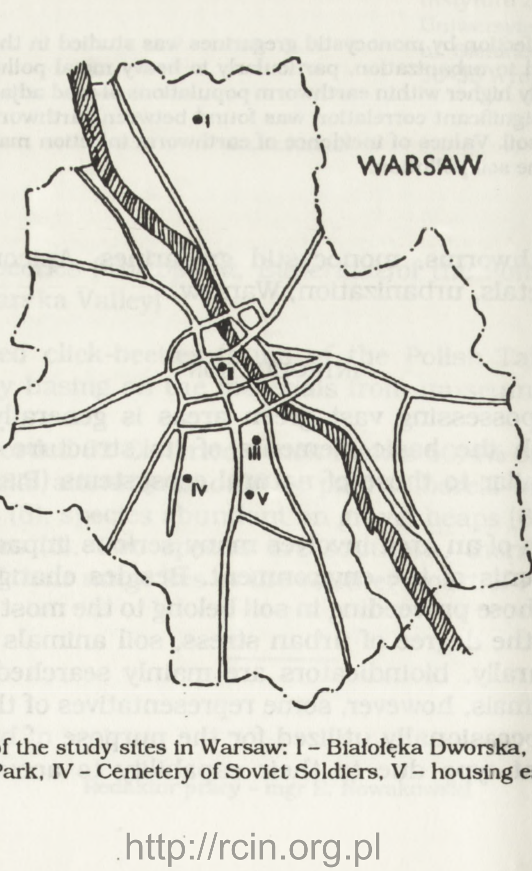

Fig. 1. Location of the study sites in Warsaw: I – Białołęka Dworska, II – Saxon Garden, III – Łazienki Park, IV – Cemetery of Soviet Soldiers, V – housing estate Wierzbno.

I. Białołęka Dworska. A suburban district of Warsaw. Seminatural site, studies for a comparative purpose.The study plot was a fallow supporting plant community of the class Artemisietea. The soil was typically brown soil developed from alluvial loam on loose sands.

II. Saxon Garden. One of the oldest and most polluted parks in Warsaw, situated in the city center. Banked anthropogenic soil was prevailing (DOBRZANSKI et al., 1977).

Study plot a. Marszałkowska street adjoining lawn on the brink of the-park.Mechanicalcompositionofthesoil – strong medium sand on very fine light medium sand.

Study plot b. An open lawn located in the center of the park. Sward vegetation resembled plant associations of the alliance Cynosurion and Arrhenatherion elatioris. Upper soil layer composed of very fine sandy loam. Soil relatively strongly contaminated with heavy metals (Table I).

Table I. Some soil characteristics of Warsaw green areas.

No.	Locality		organic matter C (%)	pH KCl 1 n	Ca-CO3 (%)	C/N	heavy metals (ppm)			
							Zn	Cu	Pb	CD
I	Białołęka Dworska		5.98	5.6	ND	ND	ND	ND	ND	ND
II	Saxon Garden	a	6.98	7.1	1.53	15.7	250	46	155	1.18
		b	ND	ND	ND	ND	66	23	64	ND
III	Łazienki Park	a	3.80	6.1	0.00	15.4	248	19	32	0.32
		b	4.13	6.6	0.19	13.5	92	13	12	1.18
IV	Cemetery of Soviet Soldiers	a	1.93	6.4	0.54	11.5	129	18	27	ND
		b	3.00	6.3	0.09	13.3	78	14	27	0.19
V	Wierzbno		3.39	6.6	0.13	15.1	134	22	50	0.23

a – streetside plot, b – inside plot. ND – not determined

III. Łazienki Park. An old park established in the 18th century in the place of a forest. The soil ranks among the mechanically transformed soils classified by DOBRZANSKI et al. (1975) as anthropogenic black earth.

Study plot a. A narrow lawn located on the brink of the park, along the Ujazdowskie Avenue. Upper soil layer – sandy loam.

Study plot b. Open lawn located in the park center. The plants growing there ranked among species characteristic of the class Molinio-Arrhenatheretea and the alliance Cynosurion. The upper soil layers composed of very fine sandy loam, sandy loam and strongly medium sand.

IV. Cemetery-Mausoleum of Soviet Soldiers. A park set up in the place of crop fields. Its soils were classified as mechanically transformed anthropogenic soils (CZARNOWSKA, 1975).

Study plot a. A large lawn fenced on one side with Ulmus laevis POLL. separating it from the street. The composition of the herb layer vegetation resembled a pasture association of the alliance Cynosurion. The upper soil layers composed of a very fine sandy soil.

Study plot b. A large lawn, 150 m off the street. Species composition of the sward resembled plant associations of the alliance Cynosurion. Uppersoillay-er196 a very fine sandy soil changing to silty loam.

V. Wierzbno housing estate. A modern housing estate with a characteristic dispersed settlement. Strongly mixed with building materials used for the estate construction, the soil resembled debris soils found in the city center.

Study plot was a small (0.8 ha) orderly lawn, not trampled, surrounded with apartment houses. Vegetation of the herbaceous layer was resembling the composition of the alliance Cynosurion. The soil – very fine sandy loam with 5–10 cm layer of very fine sandy soil.

Earthworm sampling

In the above described plots, earthworms were sampled during May–June 1988 to assess earthworm density and to obtain animals for the subsequent parasitological investigations. Five quadrats, each $0.1m^2$ uniformly spaced throughout each plot, were sampled for earthworms using the formalin method of RAW (1959). The numbers and species composition of earthworms were determined after preservation in 4% formalin. The nomenclature of earthworms follows EASTON (1983).

Parasitological investigations

Before preservation, adult earthworms were separated and examined for parasites. Each specimen was opened along the mid-dorsal line from the mouth to the clitellum, and various stages of monocystid gregarines (*Apicomplexa, Monocystidae*) were searched in the body cavity, nephridia, blood vessels, spermathecae, testes and seminal vesicles. The incidence of infection was recorded for each earthworm species and plot studied. Subsequently, 5 wet mount smears were prepared from the lobes of seminal vesicles of each specimen of *Lumbricus terrestris L.*, and cysts of gregarines were counted to assess the rate of infection. According to the number of the cysts found, earthworms were classified as heavily (> 50 sporocysts per ind.), medium (25–50) and slightly (< 25) infected. To determine species, permanent smears of trophozoites were prepared, fixed in sublimat – alkohol, stained in Erlich's hematoxylin, counter-stained with eosin and mounted in Canada balsam.

RESULTS

During investigations, following five species of earthworms were found: *Allolobophora chlorotica* (SAVIGNY, 1826), *Aporrectodea caliginosa* (SAVIGNY, 1826), *A. rosea* (SAVIGNY, 1826), *Lumbricus castaneus* (SAVIGNY, 1826), *L. terrestris Linnaeus*, 1758. The dominating species was *L. terrestris*.

The earthworm densities in park centers were found to be similar to each other as well as to those in the lawns of Wierzbno and Białołęka Dworska, ranging between 126.0–156.0 ind.m^{-2} (Table II). On the contrary, significantly lower earthworm densities (ANOVA, $p < 0.01$) were noted in the streetside plots of the parks, forming 61.8, 36.5 and 34.5% of the values found in the inside plots of the Saxon Garden, the Cemetery of Soviet Soldiers and the Łazienki Park, respectively (Table II). No significant correlation was found, however, between earthworm density and the pollution of surface soil layer with heavy metals.

Table II. Earthworm populations in the soil of Warsaw green areas.

No.	Locality		Earthworm density (ind. m^{-2})	Number of adult worms*
I	Białołęka Dworska		148.0	36
II	Saxon Garden	a	94.0	16
		b	152.0	30
III	Łazienki Park	a	50.0	14
		b	145.0	32
IV	Cemetery of Soviet Soldiers	a	46.0	16
		b	126.0	30
V	Wierzbno		156.0	34

* total in 5 samples, each of 0.1 m^{-2}
a – streetside plot, b – inside plot,

As results of parasitological analyses ascertained there were little differences in the proportion of adult earthworms infected by monocystid gregarines among inside park plots of the Warsaw green areas studied (Fig. 2). The lowest incidence of infection was established in the suburban control plot of Białołęka Dworska, 33.3%, and the highest ones in those of the Saxon Garden and Wierzbno, 53.3% and 58.8% respectively. However much higher proportions of infected worms (ANOVA, $p < 0.01$) were found in streetside plots, ranging between 85.7 and 93.7% of the total adult population. The incidence of infection was found to be significantly correlated with the total metal pollution of the soil ($r = 0.79$, $p < 0.05$), and especially with that with zinc ($r = 0.82$, $p < 0.05$). The correlations between earthworm infection and concentrations of Cu, Pb and Cd were not significant.

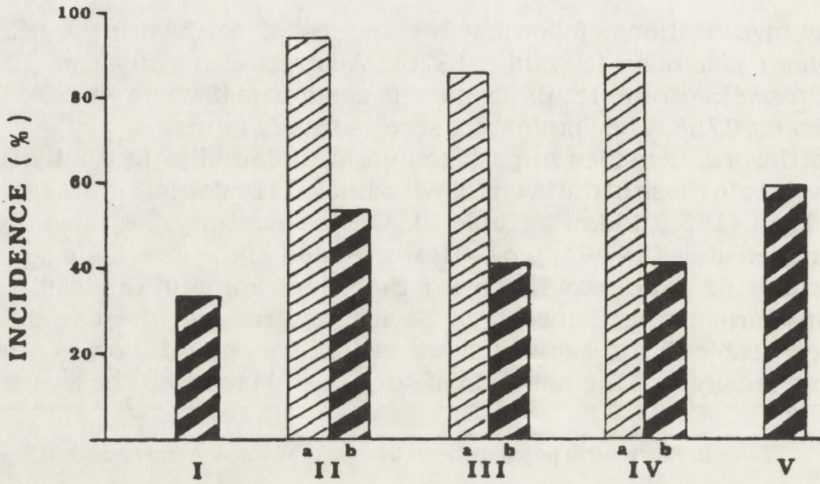

Fig. 2. Incidence of earthworm infection by monocystid gregarines (%) in the study sites of Warsaw (for explanation of I i, II a, b; III a,b; IV a, b; V – see Tab. I).

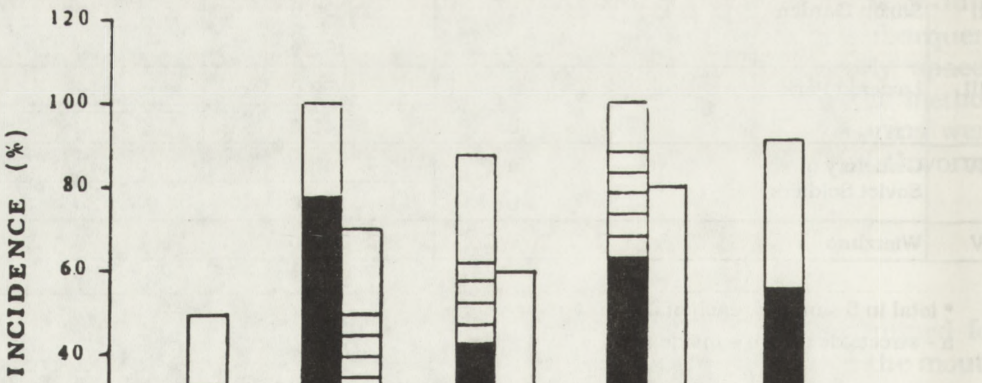

Fig. 3. Incidence and rate of infection by monocystids (%) of in Warsaw predominating earthworm, *Lumbricus terrestris L.* For explanation of I; II a, b; III a, b; IV a, b; V – see Tab. I.

Although the differences among individual plots were similar to those observed for the total earthworm community, the incidence of infection of *Lumbricus terrestris* was generally higher compared to other species and formed 50% of population in Białołęka Dworska, 60–80% in the inside plots of the city parks and 91% in Wierzbno, and it was highest in the streetside plots of the Saxon Garden and the Cemetery of Soviet Soldiers (Fig. 2, 3). On these two plots there were no specimens without infection. It was probably due to the broader variety of gregarine parasites found infecting this species; the data concerning gregarine species and their host specificity has been published elsewhere (PIŻL, 1990). Nevertheless, a higher sensitivity of *L. terrestris* to infection cannot be excluded as well. As regards the rate of infection, the highest proportions of heavily infected worms were found in streetside plots of the city parks and in Wierzbno (Fig. 3), significantly different from those in inside park plots and the control plot of Białołęka Dworska (ANOVA, $p < 0.01$). A significant correlation ($r = 0.83$, $p < 0.05$) was also found between the proportion of heavily infected specimens and the soil pollution with heavy metals.

DISCUSSION

The impact of pollutants on soil living organisms received much attention during the last decades, and an interest developed in the use of earthworms as indicators of environmental contamination. However, most studies have presented LD_{50} figures and/or the data on pollutant concentrations in earthworm tissues, but ignored estimating the changes of populations to survive in a habitat subjected to pollution at sublethal levels. Obviously, the effects of soil pollution may be manifested in a lower survival and a reduced growth and reproduction of earthworms (BENGTSSON et al., 1986).

In Warsaw, atmospheric pollution is 2 to 30 times higher than in non-urbanized areas (TROJAN, 1981), and considerable contents of heavy metals were observed in the soils of all plots under study (Table I). However, the impact of urban stress on earthworms was not so great as expected, and in the inside plots of the city parks, earthworm densities reached relatively high values, similar to that found in the control suburban plot. Hence, anthropogenically transformed soils of the urban green areas seem to be able to support large populations of earthworms, and the differences in soil physical characteristics, pH and organic matter content appear not to be of great importance. Significantly lower densities were found in park plots adjacent to roads, indicating worse environmental conditions. Nevertheless, though soil pollution was higher in these plots compared with those situated inside parks, no correlations were found between heavy metal concentrations and earthworm density. That is why it is necessary to consider other factors such as soil contamination with salts and/or oil, soil compaction, changes in microclimatic conditions, etc.

Additionally, interactions between earthworms and their parasites may also be affected and indirectly contribute to the above effects (GUNNARSSON and RUNDGREN, 1986). Earthworms are know to be exposed to numerous groups of parasites, ranging from protozoans to nematodes and dipteran larvae (EDWARDS and LOFTY, 1977; LEE, 1985). Monocystid gregarines are the commonest and best known. They have been studied by many authors (LEVINE, 1977) but little attention was paid to the influence of environmental factors on earthworm parasitology PURRINI (1983) recorded a mean incidence of infection of 25% in earthworms from two polluted localities in Germany, but found no gregarines, nor any other parasites in earthworms from four localities in Spain and one in Austria. More recently, PURRINI (1987) compared results of parasitological investigation of soil invertebrate populations including those of eartworms in several localities of Germany, Thailand and Tanzania, and concludedthat SO_2–deposits, industrial pollution and pesticides are probably involved in susceptibility of soil animals to diseases and parasites. It was supported by PIŽL (1985, 1989) who found strong correlations between earthworm infection by monocystid gregarines and herbicide treatment, and assumed that adverse effects of herbicides on physiological conditions of earthworm hosts resulted in the decrease of their pathogen resistance. Reduced defence capacity of the physiologically weakened worms is presumed to be mainly responsible also for the increased incidence of earthworm infection recorded in the mostly stressed plots of the Warsaw parks. The correlation found between incidence of infection and total concentration of heavy metals may then indicate adverse effects of sublethal levels of soil pollution on earthworms. Earthworms sampled in metal polluted soils may show high metal content in the seminal vesicles, the most common place of gregarine infection (BENGTSSON et al., 1983). Subsequently, it could be presumed that the occurence of metals there could lead to a lowered incidence of infection due to their adverse impact on gregarines. Opposite results, however, were found in our analyses, and further investigations of the effects of heavy metals on monocystid gregarines are needed.

Despite many conflicting data in the literature on the toxicity of individual metals (LEE, 1985) and in spite of the fact that the chemical form in which toxic heavy metals are presented is apparently an impotant factor in the level at which toxicity appears (MALECKI et al., 1982), there is evidence that, at least in some situations, toxic effects of Zn on earthworms may overlap those of other metals (RHEE, 1975). Additionally, high Zn concentrations in soil are known to reduce substantially the content of other metals, particularly Cd, in earthworms (BEYER et al., 1982). This may partly explain why correlation was found between incidence of infection and concentration of Zn only but not of other metals.

Little information is available on the effects of gregarine parasites on the hosts, but they are presumed to be not very great (CHENG, 1973). An asexual reproductive phase does not occur, so the host is not overwhelmed by tissue-invading parasites. However, some effects may be expected on the reproductive efficiency of worms, because considerable hypertrophy of the male funnel cells supporting parasites was found as well as the absorbtion of developing male cells by monocystids parasitizing the sperm morulae (STEPHENSON, 1930). Nevertheless, to estimate the part of gregarine infection in decreased earthworm density in road adjacent plots of the Warsaw parks, further studies clarifying the influence of monocystid parasites on earthworm mortality and reproduction were desirable.

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STRESZCZENIE

[Tytuł: Wpływ urbanizacji na stopień zainfekowania dżdżownic przez monocysty gregarin]

Badaniami objęto gleby zieleńców miejskich Warszawy, biorąc pod uwagę stopień ich zanieczyszczenia metalami ciężkimi oraz gleby łąkowe, położone na terenie podmiejskiej dzielnicy Warszawy w Białołęce Dworskiej. Dżdżownice były zbierane na przełomie maja i czerwca 1988 r. metodą formalinową Rawa; przy czym dojrzałe egzemplarze były oddzielane i oceniane pod kątem stopnia ich zainfekowania przez parazyty. Jednocześnie liczbę cyst gregarina liczono w pięciu kolejnych dojrzałych egzemplarzach *Lumbricus terrestris* L.

Stwierdzono, że stopień spasożytowania dżdżownic może być dobrym wskaźnikiem zanieczyszczenia gleby. Jest on istotnie wyższy w populacjach zasiedlających gleby trawników położonych w pobliżu jezdni w porównaniu do populacji zasiedlających gleby trawników położonych w głębi parków. Występuje pozytywna korelacja pomiędzy stopniem zainfekowania dżdżownic przez monocysty gregarina a stopniem zanieczyszczenia gleby metalami ciężkimi (Zn, Cu, Pb, Cd) łącznie i zanieczyszczeniem Zn. Największą wrażliwością na zainfekowanie gregarinami w warunkach stresu urbanizacyjnego charakteryzuje się gatunek *Lumbricus terrestris* L.

Redaktor pracy – dr I. Pilipiuk