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ORIGINAL ARTICLE

Effect of heavy metals accumulation on locomotor activity of Ixodid ticks

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Our research aimed to study the ability of ixodid ticks to accumulate heavy metals in their bodies within a large industrial city. We used 2128 units of adult hungry ixodid ticks and 10 units of vegetation samples, collected from March 2018 to September 2020 in Zaporizhzhia (large industrial city, southeastern Ukraine). The samples were examined for the content of Cd, Pb, and Ca by the spectrometer with inductively coupled plasma series ICP-OES (Shimadzu). We also used the method of determining the locomotor activity for ticks' behavior study. Our results demonstrated that the concentration of toxic elements in the ticks enlarges along with the content of these elements in the ground litter (r = 0.98). Analyzed the calcium content in the body of ixodid ticks, we clustered them in two groups according to the element susceptibility. Two–way analysis of variances regards cadmium and calcium accumulation in the body of Ixodid ticks proved the dependence of calcium content in ticks on the habitat degree of cadmium accumulation (P = 44.90, 0.0001, ANOVA). We suggested that the high industrialization level of industrial cities directly affect the ecology of Ixodid ticks, which, in turn, could cause the susceptibility of ticks to the pathogens.

Key words: ixodid ticks, bioaccumulation, heavy metals.

Introduction

Nowadays, infection diseases, namely, Lyme disease, tick-borne encephalitis, and West Nile fever are rapidly spreading in many regions and are of great international concern (Kowalec et al., 2019). Scientists identify several reasons for these phenomena: global warming, anthropogenic factors, including outdated technologies in most industrial enterprises, noncompliance with current legislation on emissions treatment, migration processes, etc. (Allen et al., 2017).

Ukraine is the second-largest country in Europe, with a population of more than 43 million people; most of them are urban residents (Migration, 2016). While industrial production and locomotor transport are the main sources of environmental pollution, various harmful substances are released into the environment, including heavy metals (Pattenden et al., 2000). It is known, that heavy metal ions lead to the degradation of ecosystems and affect all types of habitats. As a result, their structure may be simplified, which affects the animal populations and human health (Tchounwou et al., 2012). Most pollutants are deposited in the soil from atmospheric air (Pattenden et al., 2000), they are accumulated in plants and then through the food chains get to the animal and human organism. It is obvious, that organisms, whose vital activity is associated with the soil and vegetation, suffer from human-induced factors, like ixodid ticks - vectors of various tick-borne infections, which spend most of their lives in the ground litter (Balashov, 2010). Thus, we are dealing with a change in communicable diseases under the influence of technological progress, which entails the potential for disease outbreaks in urban and semi-urban areas (Kubiak et al., 2019).

According to Babushkin et al. (2010), in Zaporizhzhia, there are many industrial enterprises, whose emissions of harmful substances account for 60% of total gross emissions, and the harmful emissions from vehicles compile approximately 40%. In recent decades, scientists have noted an increase in the number of ixodid ticks in many European countries (Kjaer et al., 2019; Zajac et al., 2020) and Ukraine (Rogovskyy et al., 2017). In our previous publications, this trend was also noted (Voronova et al., 2012). We also reported six species of ixodid ticks, two of which can be the vectors of human pathogens in the Zaporizhzhia region (Voronova et al., 2011, 2009).

Application of a relatively new direction – the study of the impact of anthropogenic pollution on vectors of natural and focal diseases is connected with the possibility of using arthropods, including the ixodid ticks, as the bioindicators (Alekseev, 2002). According to Zharkov et al. (2000), soil contamination with toxic metals affects the biology and morphology of ticks, leading to a decrease in their immunity and, consequently, increase the susceptibility of ticks to pathogens.

Therefore, the study of environmental factors influencing the distribution of Ixodid ticks vectors is crucial in the ecology and epidemiology of transmissible natural-fire diseases. Previous studies of heavy metals impact on tick ecology (Alekseev, 2006), showed that the high content of cadmium in ticks leads to changes in their exoskeleton (abnormalities) and behavioral reactions (Dubinina et al., 2004). Some authors also reported the ticks with anomalies, associated with the environmental pollution impact (Alekseev et al., 2007; Chitimia-Dobler et al., 2017; Molaei & Little, 2020). Alekseev (2002) shows that anomalies of the exoskeleton are associated with the loss of calcium in the body of parasites, but his hypothesis was not broadly supported so far.

Our study aimed to test the ability of ixodid ticks to accumulate heavy metals and to trace the changes in ticks' locomotor activity.

Materials and methods

The objects of our research were *Ixodes ricinus* L. females, collected in natural and urbanized habitats of the Zaporizhzhia region (Ukraine) from March 2018 to September 2020. The collection of ticks was performed on the flag in position sample (Kuznetsov, 1968), taking into account the recommendation of Rulison et al. (2013). Humidity and temperature in the ground litter were measured using portable humidity and temperature data logger LOG 32.

Before identification in the laboratory, the hungry ticks were placed in 10 ml tubes, in which a piece of wet sterile bandage was placed and capped. The collection place, date, and time were recorded on a test tube. In the laboratory, ticks were identified by species based on morphological features using the determinant (Yemchuk, 1960). Determination of Cd (cadmium) and Pb (lead) in a pool of hungry ixodid tick females were done by the method of emitting optical spectrometry with inductively coupled plasma (ICP-OES) after treatment with a mixture of concentrated nitric hydrochloric acid in a specially designed microwave system. Measurements were performed by a spectrometer with an inductively coupled plasma series ICP-OES (Shimadzu). Two-factor analysis of variance was used to assess the habitat influence on ixodid ticks in different years.

We also study the locomotor activity of certain groups of ticks, which were distributed at the place of their collection. While the locomotor activity is the only measured characteristic, which contains information about the changes in the body under the influence of certain factors (Alekseev, 2002), we used the method of determining the locomotor activity, based on monitoring the tick movement on a horizontal platform (Alekseev et al., 2000), to assess the anthropogenic changes in tick habitats.

The relationship between the content of heavy metals in the ground litter and ixodid ticks was estimated by the Pearson correlation coefficient. Statistical processing of the results was performed using PAST v. 3.25 software. The obtained results are presented like mean ± standard error (x ± SE) from not less than 10 independent samples.

Results

We determined the dependence between the concentrations of toxic elements in the ground litter and *Ixodes ricinus.*

Table 1. The content of toxic elements in natural and urban habitats ground litter and *Ixodes ricinus* (mg/kg, 2018–2020).

Source	Place	2018		2019		2020	
		Cd	Pb	Cd	Pb	Cd	Pb
Ground litter	Natural habitat (background zone)	0.34±0.06	0.64±0.19	0.45±0.12	0.92±0.26	0.46±0.15	1.02±0.18
	Urbanized habitat	0.90±0.04	1.91±0.15	1.01±0.10	1.99±0.12	0.93±0.30	2.29±0.40
Female ticks	Natural habitat (background zone)	2.21±0.12	0.51±0.14	2.61±0.25	0.68±0.22	2.81±0.11	0.79±0.17
	Urbanized habitat	3.94±0.34	1.14±0.07	4.05±0.15	1.17±0.07	4.18±0.08	1.21±0.10

We revealed a high susceptibility of ticks toward the toxic elements and high ability to accumulate them in their bodies. The concentration of toxic elements in the body of ticks increased along with elements concentration in the ground litter. In particular, the highest correlation was registered for cadmium ($r = 0.98$ $p < 0.05$). The trend line shows a general tendency to the growth of the concentration of heavy metals in both natural and urban habitats (linear correlation coefficient 0.90).

We clustered the ixodid ticks in two groups according to the calcium content. The calcium contamination in the first group was 3117 ± 19.1 mg/kg, and in the second group - 1842±22.7 mg/kg. Two-factor analysis of variance for cadmium accumulation and calcium content in the body of Ixodes ticks between groups collected in different habitats showed a dependence of calcium content in the body of Ixodes ticks on habitats and degree of cadmium accumulation ($F = 44.90$, $P < 0.0001$, ANOVA). We also found a high negative correlation between the degree of cadmium accumulation and calcium content in the body of Ixodes mites ($r = -0.93$ $p < 0.05$).

The study of the relationship between the accumulations of toxic elements with the locomotor activity of Ixodes ticks showed the following pattern: the more toxic elements are accumulated in the body of the parasite, the more behavioral reactions it demonstrated. It means that parasites become more active (Figs. 1-2). There was also a high correlation between the degree of cadmium accumulation and ticks locomotor activity ($r = -0.86$ $p < 0.05$).

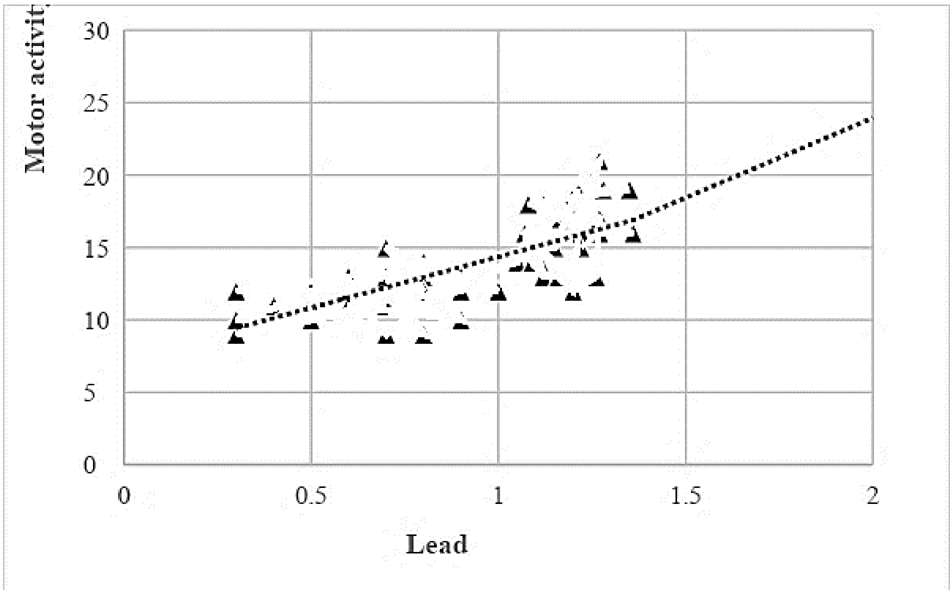

Fig. 1. The relationship between lead accumulation and locomotor activity of ticks in natural and urban habitats (Zaporizhzhia region, 2018–2020)

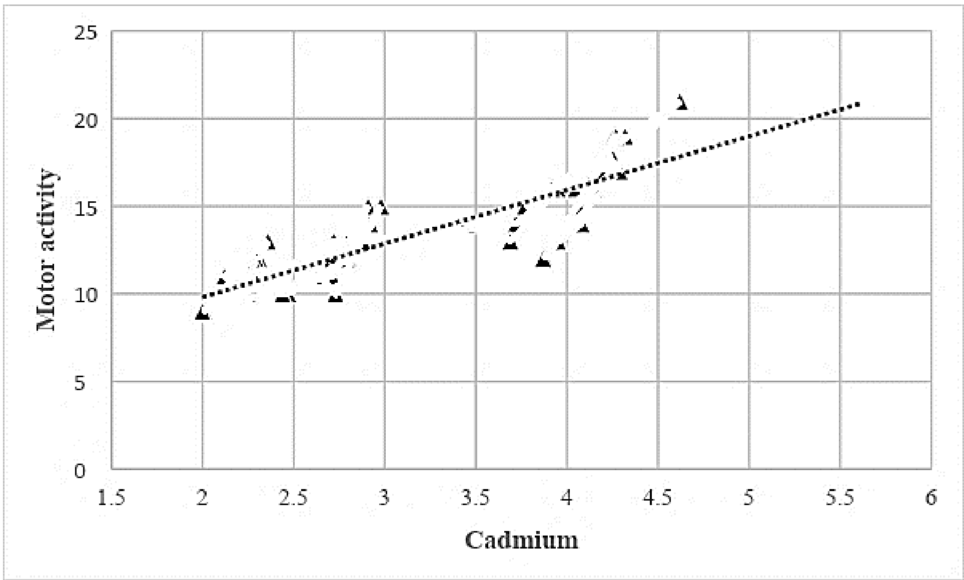

Fig. 2. The relationship between cadmium accumulation and ticks locomotor activity in natural and urban habitats (Zaporizhzhia region, 2018–2020).

Discussion

Our research proved that ixodid ticks *(Ixodes ricinus)* accumulate toxic elements regardless of natural or urban habitats. *Ixodes ricinus* collected from industrial areas had higher rates of toxic elements accumulation than in relatively natural habitats. These results confirm our suggestion that ixodes are largely influenced by technological factors. Soil pollution was observed throughout Ukraine, the average cadmium concentration in the experimental area is similar to the level of soil contamination in the Sub Dnieper region, where it was three times higher (Tsvetkova & Gunko, 2015). We registered accumulation of cadmium by *Ixodes ricinus* was 2.21 ± 0.12 mg/kg in natural and 3.94 ± 0.34 mg/kg in urban habitats, which confirms the study of other scientists, reported the high ability of invertebrates from industrial areas to accumulate a considerable number of heavy metals (Azam et al., 2015; Jelaska et al., 2007). Heikens et al. (2001) showed that the concentration of lead and cadmium in the body of invertebrate growth with increasing of the concentration of these elements in the soil, which is also confirmed by our study.

It was reported that with the accumulation of toxic elements the ticks become more active (Alekseev et al., 2000). Thus, the changed behavior of *Ixodes ricinus* can affect their ability to transmit the pathogens. Zharkov et al. (2000) noted anomalies in ixodid ticks connected with heavy metals exposure. This caused the deformation in the external skeleton, anomalies in body structure or dents on the tick shields (Alekseev et al., 2007). These morphological changes are relevant indicators of the weakening of tick the immune system (Guglielmone et al., 1999; Alekseev & Dubinina, 2008; Keskin et al., 2016; Chitimia-Dobler et al., 2017). This anomaly is directly related to ticks' disability to resist infection with pathogens (Alekseev et al., 2007). Dubinina et al. (2004) have shown that Ixodid ticks have similar abnormalities when cadmium and lead accumulate in their body. Such ticks highly likely contaminated the viruses and bacteria, and the level of dangerous human diseases is several times higher, compared to the natural areas.

We did not detect the *Ixodes ricinus* with signs of mutation, but we selected two groups of ticks with a difference in calcium accumulation in their body in Zaporizhzhia region within 2018–2020. This confirms hypothesis (Alekseev 2002), that the high amount of cadmium affects the calcium content in the shield, which leads to various structural abnormalities. Thus, our experimental data confirmed that the toxicity of cadmium could be increased due to calcium deficiency (Dubinina et al., 2004). Our data indicate that the technogenic load of large industrial city directly affects the life processes of ixodid ticks. This increases the susceptibility of ticks to pathogens, so they become more epidemiologically dangerous. In this regard, a promising research area should be monitoring of toxic elements in tick populations from industrial urban areas.

Conclusions

The accumulation of cadmium in Ixodes ticks affects the calcium content, which leads to abnormalities in their body structure. Toxic elements negatively affect the biology and behavior of Ixodes, which are pathogenic of many human diseases. We suggested that under anthropogenic load, the behavior of ixodid ticks could be changed toward increasing their locomotor activity and could lead to deterioration of the epidemiological situation.

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