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SHORT COMMUNICATIONS

Glass or Plastic? Influence of Funnel Material on the Efficiency of Nematode Extraction using the Baermann Method

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Nematodes are one of the most widespread and numerous groups of invertebrates: they are found almost everywhere [1], and the number can reach 20 million individuals/m^2 [2]. Nematode abundance is an important indicator of soil health [3–5]. To correctly estimate abundance and be able to compare values obtained in different studies, standardization of the extraction technique is necessary. Isolation of nematodes by the Baermann method is the most commonly used technique, developed at the beginning of the 20th century, and repeatedly modified to maximize efficiency [6]. When standardizing it, attention was paid to choosing the optimal extraction time, sample weight, and amount of water [6], and also considered equipment variations: sieves with different mesh sizes, different types of filters [7, 8]. However, the question of the possibility of nematodes settling on the walls of extraction funnels, as well as the influence of the material from which they are made, on the extraction efficiency has not been previously considered. Meanwhile, in various studies authors use funnels made of glass [9–11] and from plastic [12–14]. Most often, authors do not indicate the funnel material at all, for example [7, 15]. The purpose of this work is to answer the question of whether the material of the funnels affects the efficiency of nematode extraction.

Soil sampling (organic and organomineral horizons) was carried out in two areas of pine forest in the park area of Yekaterinburg (Southwestern Forest Park and the territory of the Botanical Garden of the Ural Branch of the Russian Academy of Sciences). In both areas, from each of the five sample plots, five samples were taken from each horizon using a spatula and a frame (10 × 10 cm) to a depth of 5 cm. The distance between the collection points was at least 2 m, between the sample plots, 150 m. Then, samples of each horizon taken from one site were combined and thoroughly mixed to achieve sample homogeneity. The combined sample was divided into six equal parts (organic horizon—10 g, organomineral—25 g, with an accuracy of 0.02 g)—subsamples. To extract nematodes from each sample (six subsamples), three glass and three plastic funnels (low-density polyethylene, Gigant GT-67830 brand) were simultaneously used. The diameter of all types of funnels was 16 cm, the angle of inclination of the glass funnel was 60°, the plastic funnel was 50°, and the rest of the equipment was identical (Fig. 1). A steel sieve with a diameter of 14 cm was also used, not touching the walls of the funnel; the mesh size was 100 μm.

According to the standard protocol of the Baermann method, nematode extraction was carried out using a setup consisting of a funnel, a silicone tube, and a 2 mL tube. A sieve with a filter (thin paper napkin) and a sample placed on it was placed in a funnel and the system was filled with water so that the water wetted the sample but did not cover it completely. The extraction duration was 48 h at room temperature in the dark. After removing the sieve with the sample and draining the remaining water in the installation, the nematodes were washed into a separate test tube from the walls of the funnels using a laboratory rinse in one circular motion. In this way, 120 tubes with a suspension of nematodes were obtained (2 horizons × 10 areas × 6 subsamples), as well as 100 tubes with washings from the walls of the funnels (20 washings were not made for technical reasons).

To count the number of nematodes, three aliquots (0.1 mL) were taken from each 1 mL tube after shaking. Nematode abundance (ind./100 g) was calculated as the average number of nematodes in an aliquot multiplied by 10 and then normalized by the dry weight of the subsample. For data analysis and visualization, the nlme, ggpubr, and multcomp packages were used in the Rv.4.2.2 programming environment [16]. Statistical analysis included two-factor analysis of variance: fixed factors – funnel material, soil horizon and their interaction (hereinafter referred to as “material × horizon”), random factor—site. Multiple comparisons were performed using Tukey’s test. Abundance

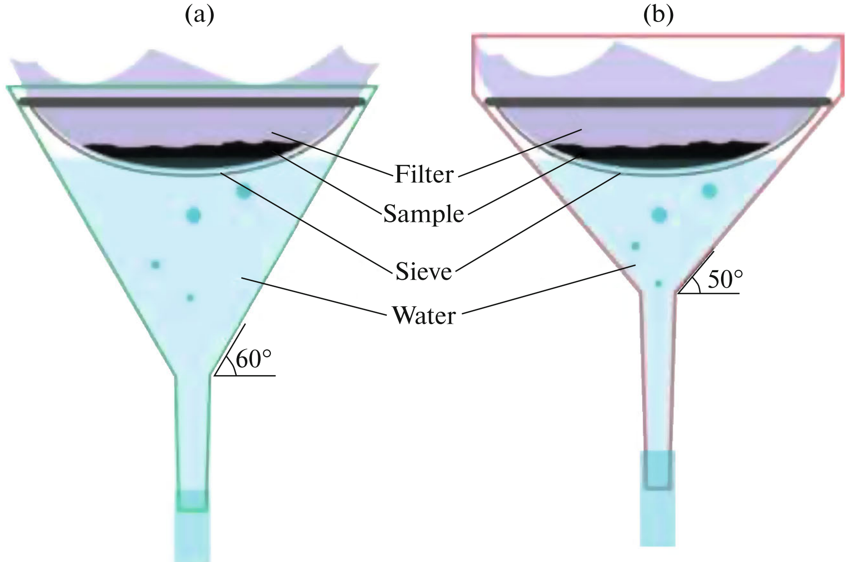

Fig. 1. Prefabricated design of glass (a) and plastic (b) funnel for extraction of nematodes using the Baermann method.

values were pre-logarithmized and data expressed as fractions of unity were converted to arcsin (√p).

The number of nematodes in the organic and organomineral horizons was (median, confidence interval in parentheses) 13 114 (12 229–15 257) and 3271 (3055–3985) specimens/100 g, respectively (Fig. 2), which is consistent with the data for the average and southern taiga [17–19]. In the mineral horizon, which contains less organic residues compared to the forest litter, the abundence of nematodes is significantly lower (F (1;105) = 392.7; $p < 0.001$, Fig. 2a), which also agrees well with literature data [19, 20].

The number of nematodes extracted using a plastic funnel is significantly higher than using a glass funnel (F (1;105) = 8.7; $p = 0.039$), in the absence of interaction between the factors “material × horizon” (F (1;105) = 2.16; $p = 0.144$). When using Tukey’s criterion, differences in the abundance of extracted nematodes were found only for the organomineral horizon (see Fig. 2a). Thus, the material of the funnel influences the efficiency of nematode extraction. The effect on the extraction results from samples with low nematode abundance is more pronounced.

The number of nematodes remaining on the walls of the funnels also depends on the material of the funnel (F (1;87) = 61.1; $p < 0.001$) – more of them remain on glass funnels (see Fig. 2b). The interaction of factors is also statistically insignificant (F (1;87) = 2.19; $p = 0.14$). The losses of nematodes averaged over horizons from glass funnels amounted to 21.7 (17.4–25.2)%, while from plastic funnels it was almost 3 times less, 7.6 (6.4–8.8)% (see Fig. 2d). The authors of most works and textbooks do not mention the need to wash off nematodes from the walls of funnels at the end of extraction [6, 7, 21]. Meanwhile, our data indicate a significant shift in estimates of the abundance of nematodes due to their settling on the walls, and when using any funnel material.

The differences between different types of funnels can be explained in terms of material properties: glass has hydrophilic properties [22], which means that during extraction more nematodes adhere to it compared to plastic. We also note that in our experiment the angle of glass funnels is smaller compared to plastic ones, and the losses, on the contrary, are greater. This means that at the same inclination angle, an even greater difference in extraction efficiency between funnels made of these materials is possible.

The total number of nematodes, i.e., the amount extracted from the sample and washed off from the walls does not differ between different types of funnels: F (1;87) = 1.52; $p = 0.22$ (see Fig. 2c), interactions between factors are insignificant (F (1;87) = 1.23; $p = 0.27$). This indicates that washouts from funnels level out the extraction error caused by the adhesion of nematodes to the walls of the funnels.

Thus, the choice of material for funnels is important because it influences estimates of nematode abundance due to differences in the proportion of individuals settling on their walls. Therefore, caution should be exercised when combining data from different studies if the publications do not specify funnel material. Washing off the nematodes remaining on the walls of the funnels makes it possible to reduce the underestimation of their numbers, but increases the operating time several times, which is undesirable due to the short shelf life of the samples. An alternative to washing off nematodes from the walls to minimize losses may be the use of funnels and other installation com-

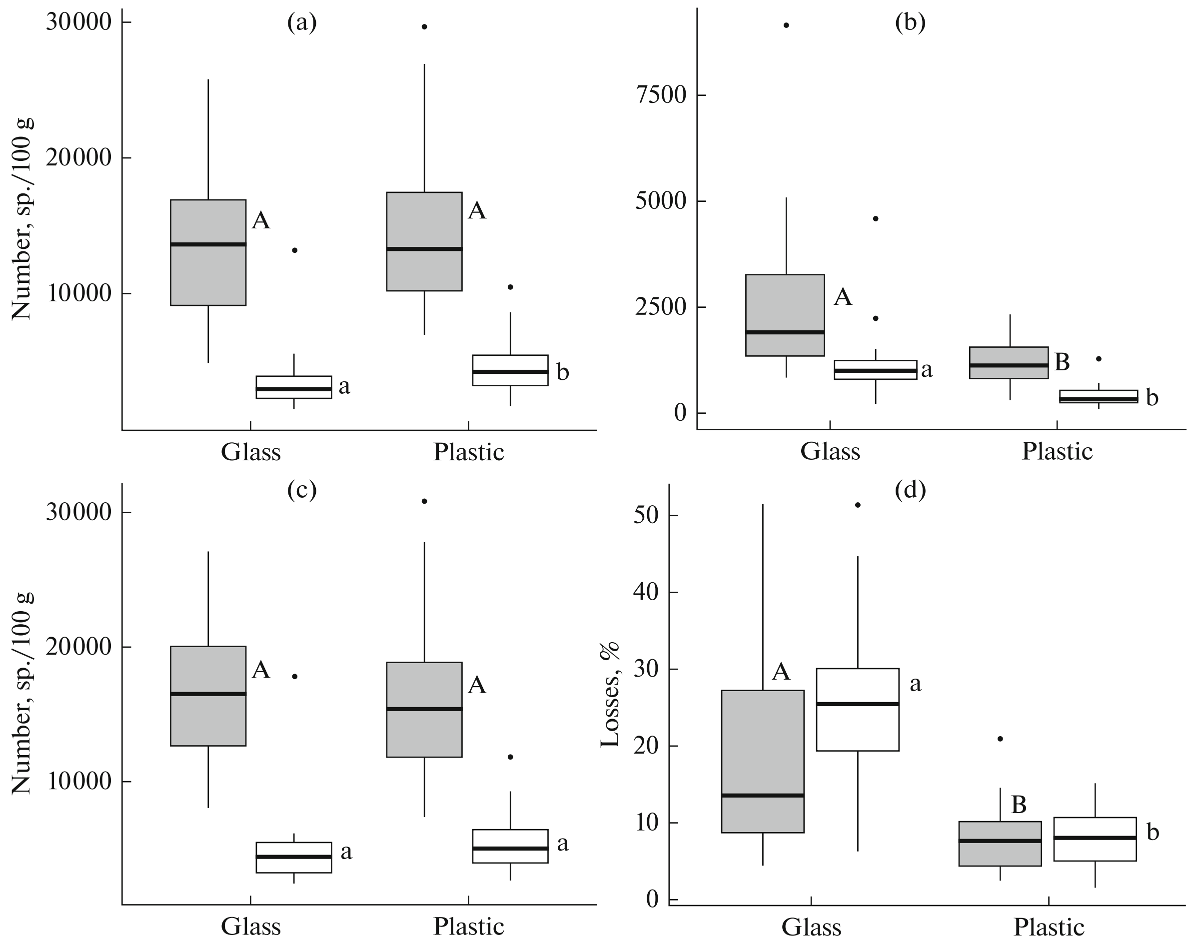

Fig. 2. The influence of funnel material and soil horizon on the number of extracted nematodes (a); the number of nematodes washed away from the walls of the funnels (b); total number of nematodes (extracted and washed off) (c); percentage of nematode losses (g). (The numbers are calculated on the dry mass of the soil, $n = 120$). Gray fill is the organic horizon, white fill is the organomineral horizon. Identical letters mean the absence of statistically significant differences according to Tukey's test ($p < 0.05$): capital letters are used when comparing options for the organic horizon, and lowercase letters for the organomineral horizon. The horizontal bar is the median, the boundaries of the box are the interquartile range, the whiskers are the range, and the dot is the outlier.

ponents made of materials with hydrophobic properties.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures performed on the animals complied with the ethical standards of the Institute of Plants and Animals of Ecology, the Ural Branch of the Russian Academy of Sciences (Protocol no. 14 dated May 12 2023).

CONFLICT OF INTEREST

The author of this work declares that she has no conflicts of interest.

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