ISSN 1067-4136, Russian Journal of Ecology, 2020, Vol. 51, No. 5, pp. 430–439. © Pleiades Publishing, Ltd., 2020.
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Bait-Lamina Test in the Assessment of Polluted Soils: Choice of Exposure Duration

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Received March 16, 2020; revised March 30, 2020; accepted April 16, 2020

Abstract—Measurement of feeding activity of soil saprophages by the consumption of bait made of plant material—the bait-lamina test—is used for solving many problems of functional ecology. It is important to choose the correct exposure duration of bait to obtain unbiased data. This requires the analysis of time dependence of bait consumption, however, it has not been performed for technogenically polluted soils. We have compared two study sites of spruce–fir forest in the area affected by long-term emissions of the Middle Ural Copper Smelter: background (30 km away from the plant) and strongly polluted by heavy metals (1 km away). The feeding activity was evaluated on days 3, 6, 9, 12, and 15. The dependence of the consumed bait portion on the exposure duration sharply differs at the background and polluted sites: it is clear linear in the period from the third till the twelfth day on the former and is absent on the latter. This is related to the difference not only in the abundance, but also in the species composition of saprophages. The pollution effect is the most pronounced on the ninth day of exposure, and deviations from this period for more than two days both to the higher and smaller sides are undesirable. The recalculations of the original values should be carefully applied: the linear interpolation may be used to recalculate the activity in the background area to adjust it to the exposure duration in the polluted site, but not vice versa.

Keywords: feeding activity, soil health, organic matter decomposition, soil fauna, soil invertebrates, saprophages, detritivores, earthworms, enchytraeids, forest litter, heavy metals, industrial pollution, copper smelter, southern taiga

DOI: 10.1134/S1067413620050136

The assessment of feeding activity of soil saprophages with the use of laminas with bait made of plant material—the bait-lamina test (BLT) [1]—is a popular method, which is repeatedly recommended to be included in the system of soil health indicators as one of the few available and simple approaches for the assessment of functional ecology of soil animals [2–4]. Since the 1990s, the BLT has been widely used in various fields, including the assessment of technogenically polluted soils [5–8]. Its application in ecotoxicological studies is regulated by an international standard [9]. The attention to the BLT has become greater in recent years, because it is important to take into account the contribution of soil saprophages to the destruction of organic matter when predicting the effect of global climate change on the carbon cycle [10].

Despite the fact that the method is simple, there are numerous difficulties in its application. Therefore, the attention of researchers to various methodological aspects is reasonable, for example, to the selection of the optimal bait composition [11] or dyes for its contrasting color [12]. The choice of exposure duration is one of the most important methodological aspects of the BLT, because it may greatly affect the results of comparing different experiment variants. For example, conclusions about the impact of uranium mining operations on the feeding activity of saprophages did not coincide for 7- and 14-day exposure periods [13].

The author of the method Von Törne [1] proposed exposing laminas from 13 hours to five days (or even four hours). In the following experiments, the exposure period was usually longer: from 7–10 to 14–20 days in temperate climate and 4–8 days in the tropic region [14]. Sometimes, the exposure was much longer: 32–33 [15, 16], 40–42 [16, 17], 66 [18], and even 90 days [19]. The international standard recommends choosing the exposure duration based on reconnaissance experiments, so that bait consumption in the control variant in the soil layer with the maximal feeding activity is not less than 30% [9]. This recommendation is not always followed, and the exposure duration is chosen from previous works. Only a few published issues are devoted to the analysis of the dependence of feeding activity on the duration of bait exposure in laboratory [20, 21] and field [22, 23] experiments.

The duration cannot be universal: it should be chosen on the basis of local conditions, because the outcomes of the BLT depend on both the abundance of saprophages and on many environmental factors, soil humidity and temperature in particular [10, 20, 22]. The comparison of habitats, contrasting with respect to the abundance of saprophages or to the external conditions, is the most difficult task. The problem of this kind arises during the assessment of technogenically polluted soils, when it is necessary to compare sites with high and very low abundance of saprophages and with different composition of bait consumers. As far as we know, the choise of exposure duration has not been studied for these particular conditions.

The initial amount of bait for the BLT is fixed. Hence, the relative rate of its consumption is estimated, and incorrect exposure duration may result in overestimation or underestimation of data. If such biases are different for various sites or variants of the experiment, there is a high risk that mistaken conclusions will be made by their comparison. If the saprophage activity is high, too long exposure will result in rate underestimation. For example, if the bait is completely consumed in the middle of the exposure period, empty laminas will be exposed during the second half of the period. Hence, the rate will be reduced twice, because the amount of consumed bait is determined relatively to the duration of the entire period. If the exposure period is too short, the difference between the compared sites may not be detected due to insufficient time for its manifestation, because bait consumption is a stochastic process. Thus, an optimal exposure period should be chosen.

The kind of time dependence of bait consumption is also an important problem. If this relationship is linear, the values of feeding activity determined on different days may be standardized by reducing them to the same unit. Otherwise, such conversions are incorrect and may also result in mistaken conclusions.

The aim of this work was to analyze the dependence of the bait consumption rate by soil saprophages on the duration of its exposure in the background and strongly polluted areas. We tested the hypothesis of the linear relationship, at least within a particular time period, both at background and polluted sites. We assume that the pollution affects only the line slope. In addition, we attempted to reveal the optimal time period for the BLT, at which the differences between the background and polluted areas are the most pronounced.

MATERIALS AND METHODS

The Study Area. This work was performed in the southern taiga, in the area affected by long-term atmospheric emissions of the Middle Ural Copper Smelter, at two study sites of spruce–fir forest: background and polluted (30 and 1 km, respectively, to the west of the emission source).

Long-term (since 1940) atmospheric emissions of heavy metals (Cu, Pb, Cd, Zn, Fe, etc.) and metalloids (As) near the plant resulted in a manifold (by one or two orders of magnitude) excess of their background content in the topsoil (Table 1). The combination of this factor with acidification of naturally slightly acidic soils due to emissions of sulfur and nitrogen oxides exerted dramatic effects on terrestrial ecosystems, which were described many times [24–30]. The background site is characterized by relatively undisturbed forest ecosystems, while the polluted area represents extreme variants of their technogenic digression. The parameters of soil, vegetation, and soil fauna important for the interpretation of the outcomes are given in Table 1.

Although the emissions have almost ceased since 2010 due to the plant reconstruction, neither a reduction in the content of metals in soil, nor revegetation occurred near the plant [24, 26]. The normalization of soil acidity [26] in recent years favored the recovery of soil invertebrate communities, and earthworms [30, 31], and, related to them, the common mole [32], advanced closer to the plant. However, they are not yet present in the polluted area studied in this work.

Data Collection. We used standard bait laminas: strips of hard plastic 12 cm long with 16 perforated holes 1.5 mm in diameter located every 5 mm, which were preliminary filled with a bait composed of wet paste made of a mixture of nettle leaf powder and microcrystalline cellulose (at the ratio of 3 : 7). The laminas filled with bait were dried at room temperature for two days.

The feeding activity was measured in the first half of the growing season of 2019 with the start on May 29 and the end on June 13. According to the data of the nearest weather station (the city of Revda), the mean day air temperature during this period (May–June) was 13.4°C, and the precipitation amount was 91 mm, which is close to the climate norm (of the last 60-year period): 12.9°C and 119 mm, respectively.

Four microplots 25 × 25 cm were laid at each site. The location of microplots was chosen randomly, and the distance between them within the site was 15–25 m. We set 25 laminas on each microplot in the nodes of a regular grid with a step of 5 cm (200 plates in total).

At the installation point, the forest litter and humus horizon were incised by a sharp knife. The laminas were set strictly vertically, and the upper hole corresponded to a depth of 0.5 cm from the forest litter surface. The laminas were removed from the soil (and were not placed there again) on the third, sixth, ninth, 12th, and 15th days. Five laminas were randomly taken from each microplot on a particular day (a random number generator implemented in Microsoft Excel was used). The laminas were then examined at a laboratory, where the perforation rate of each hole (i.e., the consumption of bait by saprophages) was visually assessed by the five-point scale: 0—not eaten; 0.25—about 25%; 0.5—50%; 0.75—75%; and 1—100% of the area eaten. Contrary to the traditional two-point scale,

Table 1. Characteristics of study sites

Parameter	Study site		Reference
	background	polluted	
Location and landscape description	N 56°47′51″, E 59°25′03″, 455 m above sea level, 30 km from the plant. Spruce-fir forest on the watershed of the Bol'shaya Talitsa and Belyi Atig rivers	N 56°50′37″, E 59°52′44″, 370 m above sea level, 1 km from the plant. Spruce-fir forest on the lower part of the eastern slope of the Shaitanskii Ridge	[27]
Stand description	Composition: *Abies sibirica* (50%), *Picea obovata* (20%), *Populus tremula* (20%), and *Betula* spp. (10%). The age is 100 years. The stock is 413 m^3/ha	Composition: *Picea obovata* (50%), *Abies sibirica* (30%), *Betula* spp. (10%), and *Salix* spp. (10%). The age is 77 years. The stock is 113 m^3/ha.	[29]
Herb–dwarf-shrub layer description	60.8 species per SS, dominants: *Oxalis acetosella*, *Dryopteris* spp., *Calamagrostis arundinacea*, *Aegopodium podagraria,* and *Ajuga reptans*	6.8 species per SS, *Agrostis capillaries* is the absolute dominant.	[24, 27]
Soil type	Albic Retisol (Cutanic)	Stagnic Retisol (Cutanic, Toxic)	[26]
Prevailing humus form	Dysmull	Eumor	[25]
Forest litter thickness, cm	1.5	5.0	[25]
Concentration in forest litter*, μg/g:			
Cu	37.3	3484.3	[25]
Pb	67.3	2462.5	[25]
Cd	2.4	16.6	[25]
pH (water) in forest litter	5.9	4.9	[25]
Abundance, ind./m^2:			
earthworms	377.5	0**	[30]
enchytraeids	1005.3	4.1	[30]
Diplopoda	11.3	3.4	[30]
Gastropoda	337.4	0.4	[30]
wireworms	58.3	71.7	[30]
Nematocera larvae	103.8	34.2	[30]
collembolans	35333	12376	[34]

SS is study site, * acid-soluble forms, ** abundance at a distance of 1 km from the plant, where the BLT was performed (in the cited work, abundance at a distance of 1–2 km is given).

this scale enabled us to interpret the mean values not as a conditional index of the feeding activity of saprophages, but as the rate of bait consumption by them.

Data Analysis. The feeding activity was analyzed both for the entire lamina (i.e., 16 holes 8.0 cm deep) and individually for four successive layers (i.e., four holes in each 2-cm-thick layer). We evaluated the differences between the sites, layers, and exposure periods by the three-way ANOVA, between exposure periods and microplots within each study site by the two two-way ANOVA, and between particular exposure periods by several one-way ANOVA. When it was necessary, we used the White correction for heteroskedasticity, and also applied the angular transformation of the amount of consumed bait. The index of relationship between the feeding activity and exposure period was approximated by the linear regression. The index of effect size relative to the background was calculated as log Response Ratio; the effects, for which the 95% confidence interval did not include zero, were considered significant. The microplot, i.e., the mean value for five laminas, was the sampling unit in all cases. Since the distance between the laminas within the microplot is considerably (300–500 times) smaller than the distance between the microplots within site, the microplots were correctly considered independent replications. Statistical analysis was performed in the R 3.6.3 computing environment.

Table 2. Outcomes of the two-way ANOVA for differences between the exposure periods and microplots at the background and polluted sites

Layer	Study site and source of variation					
	background			polluted		
	Time (T), $df_{factor} = 1$	Microplot (P), $df_{factor} = 3$	T × P $df_{factor} = 3$	Time (T), $df_{factor} = 1$	Microplot (P), $df_{factor} = 3$	T × P $df_{factor} = 3$
I–IV	37.9 (<0.001)	2.6 (0.100)	0.4 (0.764)	3.6 (0.084)	22.5 (<0.001)	1.1 (0.391)
I	28.5 (<0.001)	1.1 (0.379)	0.2 (0.901)	2.9 (0.116)	7.4 (0.004)	0.9 (0.448)
II	31.8 (<0.001)	1.7 (0.226)	1.1 (0.370)	3.0 (0.110)	9.9 (<0.001)	0.5 (0.690)
III	20.1 (<0.001)	3.6 (0.047)	1.0 (0.439)	0.1 (0.716)	20.6 (<0.001)	0.1 (0.983)
IV	18.0 (<0.001)	5.9 (0.011)	2.4 (0.117)	4.0 (0.070)	11.5 (<0.001)	1.1 (0.406)

The Fischer F-test is used, the *p*-values are given in parentheses; df_{factor} is the number of degrees of freedom of the factor; $df_{error} = 12$. The angular transformation is used for the percentage of consumed bait. Layers: I—1–4 holes (0.5–2.0 cm from the surface of the forest litter), II—5–8 (2.5–4.0 cm), III—9–12 (4.5–6.0 cm), IV—13–16 (6.5–8.0 cm), and I–IV—1–16 (0.5–8.0 cm, i.e. the entire lamina).

RESULTS

The feeding activity at the polluted site was on the average significantly lower for all layers as compared to the background (three-way ANOVA: $F_{1;120} = 223.7$, $p \ll 0.001$). However, the differences between the sites were not the same for different layers, similarly to the differences between sites with different exposure periods: the interactions of the factors site × layer and the site × exposure duration were significant ($F_{3;120} = 33.6$, $p \ll 0.001$ and $F_{4;120} = 8.8$, $p < 0.001$, respectively). These results are well visualized by confidence intervals of the effect sizes: the polluted site significantly differed from the background for all the exposure period in layer I, for nine-, 12-, and 15-day periods in layer II, and only for nine- and 12-day periods in layer III. For layer IV, the differences between the study sites were insignificant at all the exposure periods (Fig. 1).

For all the layers, the change of the effect size with time was nonlinear: it increased first and then decreased. The maximal differences between the sites were recorded for the nine-day exposure period independently of the layer: at this duration, the activity at the background site was higher than at the polluted site: 21 times for the entire lamina, 81 times for layer I, 14 times for layer II, 13 times for layer III, and five times for layer IV.

The portion of consumed bait clearly increased with time at the background site, while in the polluted area, the dependence was absent (Fig. 1). The effect of the exposure period was statistically significant for all the layers in the first case and insignificant in the second case (Table 2).

The dependence of feeding activity on the exposure duration at the background site may be satisfactorily approximated by the linear regression: for the entire lamina, the adjusted R^2 is equal to 0.63, and the slope is 3%/day, $p < 0.01$. Although five points with particular exposure period are insufficient for precise analysis of the dependence, it is obvious that it reaches the plateau at the last exposure stages: the feeding activity for 12- and 15-day periods did not significantly differ (one-way ANOVA) for the entire lamina and for particular layers ($p = 0.318$–0.992).

Contrary to the differences between the exposure periods, the effect of spatial heterogeneity was statistically significant at the polluted site and only in the two lower layers of the background area (Table 2). The interactions of the factors exposure period × microplot were insignificant in all cases, i.e., the portion of consumed bait equally depended (at the background site) or did not depend (on the polluted one) on the exposure duration on all microplots.

The variation coefficient, which reflects the spatial inhomogeneity at a distance of dozens of meters, was significantly (2–8 times) higher at the polluted site as compared to the background (Fig. 2). It decreased with time at the background site and was constant in the polluted area.

The feeding activity decreased sharply with the depth at the background site for all exposure periods. For example, for the 9-day period, the activity in deeper layers was lower as compared to layer I: 1.7 times in layer II, 6.1 times in layer III, and 8.4 times in layer IV. The slope of the linear regression approximating the time dependence also regularly decreased with the depth: it was 4.4%/day in layer I ($p < 0.01$), 3.8%/day in layer II ($p < 0.01$), 2.2%/day in layer III ($p < 0.01$), and 1.4%/day in layer IV ($p = 0.027$). The goodness of fit of the linear approximation decreased in the same direction: the adjusted R^2 was 0.65 in layer I, 0.58 in layer II, 0.37 in layer III, and 0.20 in layer IV. At the polluted site, the vertical stratification of the feeding activity was not pronounced; the values were similar in all the layers.

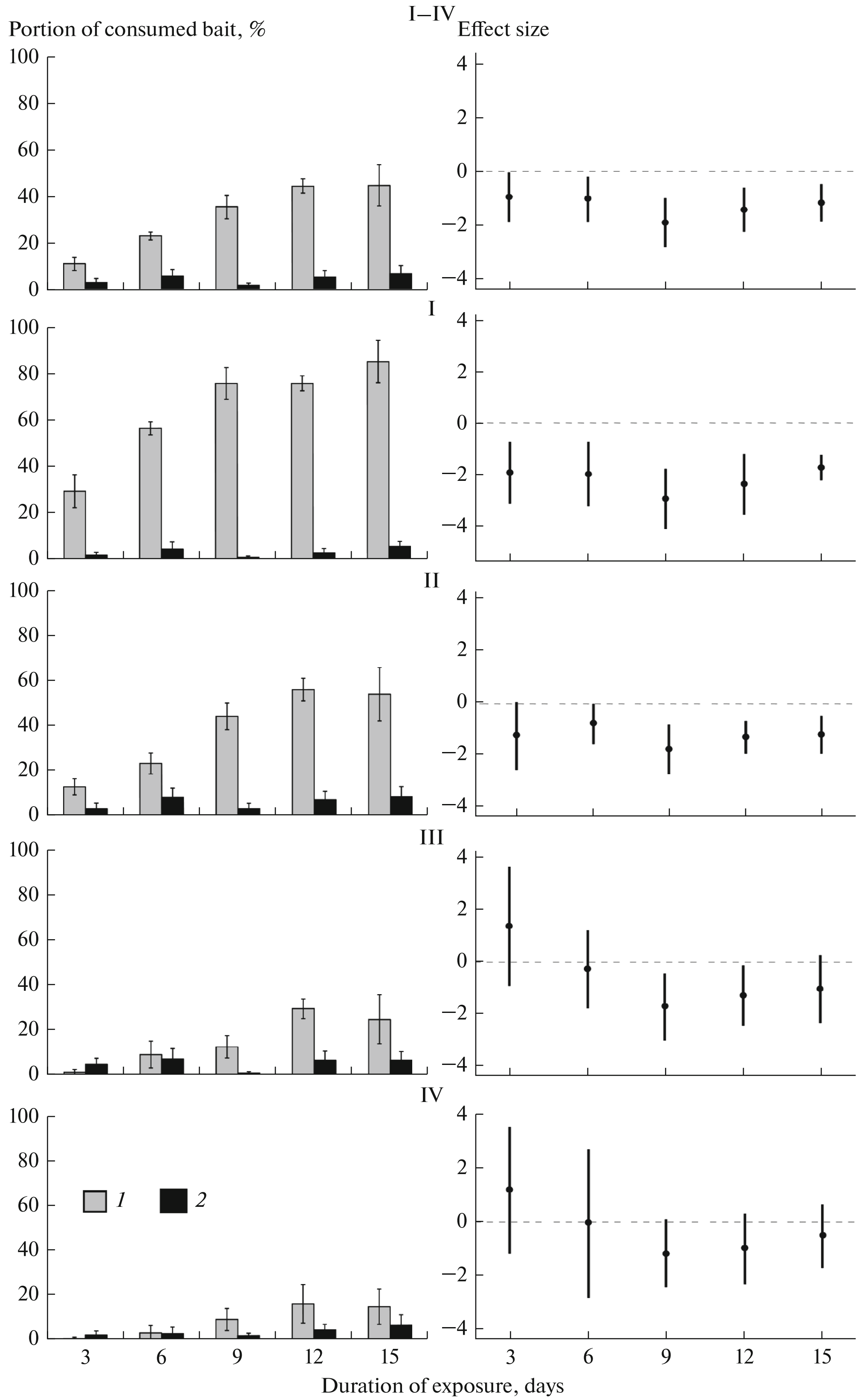

Fig. 1. Feeding activity of soil saprophages (the portion of consumed bait, % of the initial values, is on the left) and the effect size index (the log responce ratio is on the right), depending on the exposure duration of the bait. Study sites: *1*—background and *2*—polluted; layers: I—upper four holes (0.5–2.0 cm from the surface of forest litter), II—5–8 holes (2.5–4.0 cm), III—9–12 holes (4.5–6.0 cm), IV—13–16 (6.5–8.0 cm), and I–IV—1–16 holes (0.5–8.0 cm, i.e. the entire lamina). Error bars: the standard error for the feeding activity and 95% confidence interval for the effect size. Microplot is the sampling unit ($n = 4$).

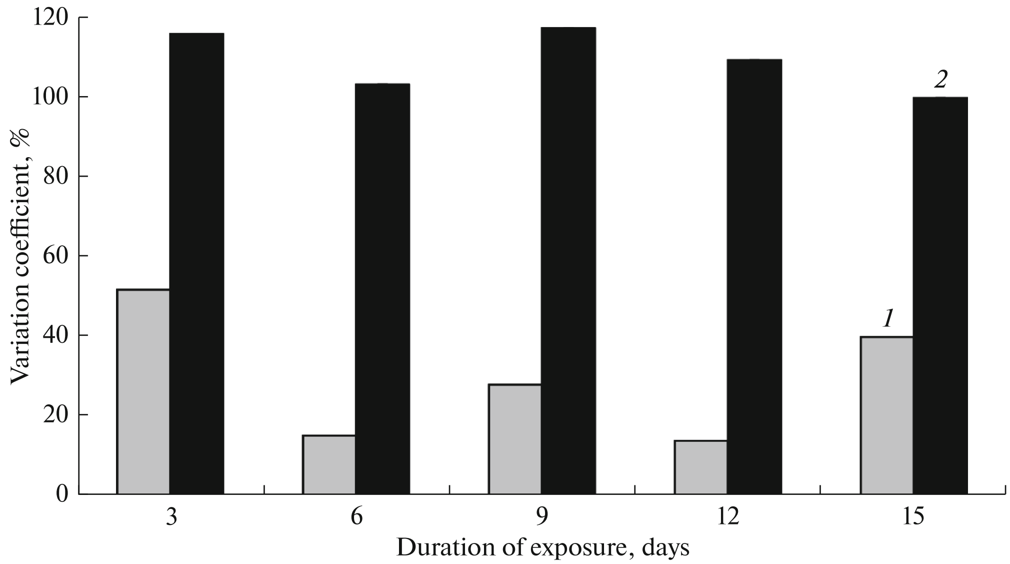

Fig. 2. Variation coefficient (%) of the feeding activity for the entire bait lamina at different exposure periods. Study sites: *1*—background and *2*—polluted. Microplot is the sampling unit ($n = 4$).

DISCUSSION

The main result of our work consists in the conclusion that the dynamics of bait consumption is radically different at the background and polluted sites. In the former case, the feeding activity is linearly related to the duration of the exposure in the range from 3 to 12 days, and in the latter case, the correlation is absent. This is obviously related to changes not only in the abundance, but also in the species composition of saprophages under the effect of pollution.

The Composition of Bait Consumers. Earthworms and enchytraeids are considered the main bait consumers in ecosystems of the temperate climate, while microarthropods (collembolans and oribatids) play a smaller role [11, 20, 33]. In addition, there are experimental data that baits are also consumed by woodlice [1]. Although other saprophages have not been specially tested, the potential consumers may be represented by diplopods, gastropods, larvae of some families of Coleoptera (Elateridae and others), and Nematoceran flies (Diptera, Nematocera: Tipulidae, Limoniidae, Bibionidae, Sciaridae, Chironomidae, Cecidomyiidae, etc.).

There are no woodlice in dark coniferous forests of the studied area. The opinions, concerning microarthropods, are contradictory: the correlation between the abundance of collembolans and the amount of bait consumed during the 15—25-day exposure period [11] was direct at a high number of animals (108000—375000 ind./m^2) in mesocosms, but was absent even on the 60th day at their low number (1400—12700 ind./m^2) [21]. In another laboratory experiment with the exposure period of 14 days, the bait remained completely uneaten even at a higher number of collembolans (34—49 thousand ind./m^2) [20]. The correlation between the outcomes of the BLT and the abundance of collembolans or oribatids was not revealed at field experiments [33]. According to data by N.A. Kuznetsova [34], the abundance of collembolans in polluted areas in the region of our research is 12000 ind./m^2. A significant participation of this low-number group in bait consumption in the polluted area during 3—15-day exposure is hardly probable.

Soil pollution by metals in the area affected by emissions from the copper smelter exerts an adverse impact on all groups of saprophages, especially earthworms (Table 1). They dominate among soil macroinvertebrates at the background site, but disappear near the plant [30, 35]. The abundance of other potential bait consumers (Enchytraeidae, Diplopoda, and Gastropoda) at the polluted site is strongly reduced, but they have not completely disappeared. Enchytraeids occupy an intermediate position between macro- and mesofauna, and our conclusion concerns only large representatives (of size more than 2 mm), which are taken into account during hand sorting. We do not have data on the wet funnel method of enchytraeids from mesofauna in the research area. Their number may be tens of thousands of organisms per 1 m^2 [36]. However on the basis of data in areas near other copper smelters, where the number of enchytraeids was estimated by the wet funnel method [36, 37], we can expect that the abundance of representatives of this size in the polluted area is significantly reduced. On the other hand, the spatial distribution of enchytraeids may be very uneven, especially in a polluted environment [37], so there may be local rise in their density near the laminas.

Larvae of Click beetles and Nematoceran flies (with the exception of Tipulidae and Limoniidae) are the only groups among large saprophages, whose abundance is not considerably reduced or even increases near the plant [30, 35]. Special attention should be paid to the possible role of larvae of Click beetles (wireworms) in bait consumption. On the one hand, the specific structure of their oral apparatus does not enable us to consider this group to be consumers of bait *sensu stricto:* due to oral filters, they feed only on liquid, which larvae squeeze out of roots and other substrates with powerful cutting mandibles [38]. On the other hand, according to our observations, when the soil is sufficiently moistened, the bait in the holes of the laminas is very soft (mushy), so it can be mechanically damaged or even pressed out of the holes, when wireworms try to squeeze liquid from it. In addition, wireworms are characterized by active foraging and intensive migration in soil, when the main role is played by their head capsule [38]. According to modern concepts, most Click beetles are polyphages (detritivores in particular) at the larval stage [39], though some species were previously assigned exclusively to phytophages and some of them to zoophages. The dominant species in the research area (*Athous subfuscus* and *Dalopius marginatus*) [35] are also assigned to polyphages [39].

Thus, based on documented changes in the structure of communities of soil macroinvertebrates under the effect of pollution, it may be assumed that different groups of saprophages consume bait at different sites: earthworms and enchytraeids at the background site and larvae of Nematoceran flies, wireworms, and possibly small enchytraeids at the polluted site. The participation of large enchytraeids, diplopods, and gastropods in the polluted area cannot be completely excluded, but it is of low probability, because their abundance is very small.

Vertical Stratification. Differences in the vertical distribution of feeding activity at the background and polluted sites correspond to vertical stratification of invertebrates. The background area is absolutely dominated (90% in number) by epigeic worm species (mainly *Perelia diplotetratheca*), while endogeic species (*Aporrectodea rosea* and *Perelia tuberosa*) are less abundant [35]. This determines a significantly higher feeding activity in the forest litter and top layers of the humus horizon as compared to deeper ones. In the polluted area, most invertebrates inhabit exclusively the forest litter [40], which is not differentiated into subhorizons and represents a relatively uniform layer composed of plant debris with preserved initial structure and without visible evidences of zoogenic transformation [25]. Its thickness averages 5 cm (to 8–11 cm) [25]; i.e., almost all holes of bait laminas are allocated to this horizon. That is why there is no sharp decrease in feeding activity with the increase in the depth of the holes at the polluted site.

Shape of Time Dependence Curve. Our initial hypothesis about the linear correlation between the amount of consumed bait and the duration of the exposure was only confirmed for the background site (Fig. 1, Table 2). The same linear correlation (in the range from 3 to 14 days) was revealed during laboratory experiments with enchytraeids and native soil fauna under optimal soil temperature and humidity. Nevertheless, under less favorable conditions, the dependence was not linear [20]. The similar conclusion may be made by the outcomes of the BLT in three natural zones: in the period from 3 to 14 days, the dependence was linear in nemoral spruce forests, where moisture conditions were optimal for large soil saprophages [22, 23]. In less favorable habitats—in arid biotopes on the Black Sea coast or in cold waterlogged forests on the White Sea coast—the dependence was not linear [22]. Thus, there is a similarity between polluted areas and natural habitats unfavorable for soil invertebrates.

A clearly pronounced linear part of the dependence of feeding activity on the duration of the exposure at the background site may indicate that the processes, which form the basis of the BLT, are time homogeneous. The amount of consumed bait is the result of two factors: the presence of consumers in the immediate vicinity of the lamina at the time of its setting and the time-average distance of their movement. In turn, the former factor is determined by the abundance and uneven spatial distribution of consumers. The mean movement distance depends on the size, specific locomotion, and foraging of animals; on the compactness, porosity, and humidity of soil; on the availability of other substrates for consumption; and on many other factors. In other words, the initial period of exposure is a snapshot of the spatial distribution of bait consumers. Then, animals move, and the snapshots superimpose on one another and form a generalized pattern of the feeding activity.

Based on this model, we can conclude that the abundance of saprophages at the background site is high and they are relatively evenly distributed in space because the bait consumption on the third day is substantial. In addition, the great abundance is combined with high mobility of invertebrates, which is evidenced by a well pronounced linear increase in the bait consumption from the third to ninth day. Both the abundance of saprophages (zero or extremely low bait consumption in the initial period) and the mean distance of their movement (the absence of time dependence of bait consumption) are sharply reduced in the polluted area. In turn, a small mean movement distance may be a result of a decrease in the size of saprophages (related to changes in their composition) and in their mobility. The absence of time dependence of bait consumption may also be a consequence of low density and/or great spatial aggregation of invertebrates, which is proved by high variation coefficients (Fig. 2). Our experimental scheme does not enable strict differentiation of all these components, which requires special research.

The time dependence of bait consumption reaches the plateau after 12 days of exposure (Fig. 1), which may be a consequence not only of the depletion of the consumed substrate. The bait may also lose its attraction for invertebrates. The first cause is reasonable for the top 2-cm layer, because the bait consumption in it exceeds 80%. However, in the underlying layers, the consumption is significantly smaller. Since it is equal for these layers on the 15th and the 12th days, this indirectly testifies to the reasonability of the latter reason, but may be also the result of a large spatial heterogeneity of the bait consumption (Table 2). Special research is also needed to reveal the reasons, by which the time dependence curve reaches the plateau.

Methodological Results. The main methodological result of our research consists in the possibility to choose the optimal time period for the BLT. The maximal difference between polluted and background sites, i.e., the most pronounced effect of pollution on the feeding activity of saprophages, was found at a nine-day exposure period. Therefore, its duration in the experiments should be within the range of 7–11 days. Deviation from it in both sides rises the risk of a type II error in statistical hypotheses testing; i.e., it is possible to make an erroneous conclusion about the absence of the pollution effect on the feeding activity.

Another methodological aspect is related to the problem of whether the BLT outcomes could be transformed to the same unit, if the tests are performed with different exposure periods. This transformation is quite often performed, using linear interpolation. For example, the rate is given in %/7 days for a real duration from 7 to 10 days [41], in %/10 days for the duration from 15 to 43 days [11], and in %/28 days for the duration from 33 to 42 days [16]. The correctness of this transformation is completely determined by the factor of whether the real and interpolated time periods fall on the linear part of the time dependence. In our case, the interpolation is correct, first, only for the background area, and second, only in the range from 3 to 12 days. This means that if necessary, the values may be recalculated only for the background area (not for the polluted one), and only if the exposure period belongs to the linear part of the curve (not to the plateau).

CONCLUSIONS

There is a great difference in the dependence of the portion of consumed bait on the duration of the exposure period between the background area characterized by a pronounced linear correlation and the polluted site, where the correlation is absent. This is related to difference not only in the abundance, but also in the species composition of soil saprophages.

Two important methodological conclusions may be made on the basis of our research. First, the duration of the exposure affects the statistical inference on differences between the background and polluted areas. The effect size is the most pronounced at the exposure period of nine days, so it is reasonable to choose the BLT duration within the range of 7–11 days. Deviations from this interval in both directions are undesirable. At longer duration, the bait consumption rate on the background area is underestimated. At a smaller period, the data are strongly affected by stochastic processes at polluted sites due to the low abundance of saprophages and their uneven spatial distribution. Second, the recalculation of the original values of feeding activity to transform them to the same unit should be performed with care: it is permissible to recalculate the background values to adjust them to the exposure period in a polluted area, but not vice versa.

It should be mentioned that the feeding activity of soil saprophages in our experiment was not limited by climatic factors, because the weather conditions were close to the climatic norm. In addition, the maximal abundance of soil macroinvertebrates usually occurs at the beginning of the growing season, just when the measurements were performed. Therefore, the conclusions were made for these conditions and should be verified for less optimal ones, such as dry or cold periods. It is also important to assess the reproducibility of the results in other types of plant communities and natural zones, in addition to the studied southern taiga spruce forests.

Our results concern a special case: polluted areas near a particular copper smelter. However, the effect on the functional activity of soil fauna in areas strongly polluted by heavy metals is similar to other negative types of anthropogenic impact. In any case, our work has shown the importance and prospects of the introduction of time parameter into the BLT: this enable us not only to avoid bias, but also to obtain additional information (for example, the length of the lag period, the slope of the regression line, and the time of reaching the plateau), which may be used for assessing soil health.

ACKNOWLEDGMENTS

We are grateful to V.S. Mikryukov, K.B. Gongalsky, and A.I. Ermakov for the discussion and comments of the manuscript. We remember with gratitude Andrei Dmitrievich Pokarzhevskii (1946–2006), who informed us about the perspectives and details of the bait-lamina test in the mid-2000s.

This work was performed according to the State Assignment of the Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences. The analysis of data and manuscript preparation were supported by the Russian Foundation for Basic Research, project no. 19-29-05175.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

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Translated by I. Bel'chenko