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GROWTH AND DEVELOPMENT OF *EISENIA FETIDA SAV.* WHEN CULTIVATED ON A MIXTURE OF POPLAR LEAVES AND PEAT

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Abstract
Urban leaf litter is a potential source of vermicompost, but its possible contamination with heavy metals raises safety concerns for its use. We assessed the growth and reproduction of *Eisenia fetida Sav.* when cultivated on mixtures containing poplar leaves collected from five locations in Tomsk, Russia, with varying anthropogenic loads (train station, Mikhailovsky park, river port, University park, and suburban control). After two months, the greatest biomass increase was observed in the mixture with leaves collected from the control point (8.87 g, 78.5%) and University park (8.74 g, 70.5%), while the smallest increase was in mixtures with leaves from the river port (5.70 g, 39.9%) and Mikhailovsky park (5.50 g, 43.0%). Cocoon production was highest in mixtures containing leaves from the control point (+138%) and University park (+94.8%), but decreased in mixtures containing leaves from the railway station (-40.9%). Juvenile growth was highest in mixtures containing leaves from Mikhailovsky park (+33.4 individuals). Overall, substrate quality in less polluted areas promotes better earthworm development. The data obtained will serve as a basis for rational leaf litter management in cities and for ensuring the safety of vermicompost production.

Keywords: *Eisenia fetida*; vermicomposting; poplar leaves; organic fertilizer; earthworm growth; earthworm reproduction.

ТЕРЕК ПЕН ШЫМТЕЗЕК ЖАПЫРАҚТАРЫНЫҢ ҚОСПАСЫНДА ӨСІРГЕНДЕ *EISENIA FETIDA SAV.* ӨСУІ ЖӘНЕ ДАМУЫ

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Аңдатпа
Қалалық жапырақ қалдықтары вермикомпосттың әлеуетті көзі болып табылады, алайда олардың ауыр металдармен ластануы пайдалану қауіпсіздігіне қатысты алаңдаушылық туғызады. Біз әртүрлі антропогендік жүктемесі бар Томск қаласының (Ресей) бес аймағынан (теміржол вокзалы, Михайловский саябағы, өзен порты, университет паркі және қала маңындағы бақылау аймағы) жиналған терек жапырақтары қосылған қоспаларда өсірілген *Eisenia fetida* Sav. өсуі мен көбеюін бағаладық. Екі айдан кейін биомассаның ең жоғары өсімі бақылау аймағынан жиналған жапырақтары бар қоспада байқалды (8,87 г, өсім 78,5%) және университет саябағында (8,74 г, 70,5%); ал ең төмен өсім өзен порты аймағынан алынған жапырақтары бар қоспада (5,70 г, 39,9%) және Михайловский саябағында (5,50 г, 43,0%) тіркелді. Салынған кокондар саны бақылау аймағының (+138%) және университет саябағының (+94,8%) жапырақтары бар қоспаларда ең жоғары болды, ал теміржол станциясы аймағынан алынған жапырақтары бар қоспада төмендеді (-40,9%). Жас дарақтардың өсуі Михайловский саябағы жапырақтары бар қоспада ең жоғары болды (+33,4 дарақ). Жалпы алғанда, ластануы аз аудандардағы субстрат сапасы жауын құрттарының жақсы дамуына ықпал етеді. Алынған мәліметтер қалалардағы түскен жапырақтарды тиімді басқару мен биогумус өндірісінің қауіпсіздігін қамтамасыз етуге негіз болады.

Кілт сөздер: *Eisenia fetida*; вермикомпосттау; терек жапырақтары; органикалық тыңайтқыш; жауын құрттарының өсуі; жауын құрттарының көбеюі.

РОСТ И РАЗВИТИЕ *EISENIA FETIDA SAV.* ПРИ ВЫРАЩИВАНИИ НА СМЕСИ ЛИСТЬЕВ ТОПОЛЯ И ТОРФА

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Аннотация
Городской листовой опад является потенциальным источником вермикомпоста, но его возможное загрязнение тяжелыми металлами вызывает опасения по поводу безопасности при использовании. Мы оценили рост и размножение *Eisenia fetida Sav.* при культивировании на смесях, содержащих листья тополя, собранных в пяти местах Томска (Россия), с различной антропогенной нагрузкой (железнодорожный вокзал, Михайловский парк, речной порт, Университетский парк и пригородный контроль). Через два месяца наибольший прирост биомассы наблюдался в смеси с листьями, собранными в контрольной точке (8,87 г, прирост - 78,5%) и Университетском парке (8,74 г, 70,5%), в то время как наименьший прирост наблюдался в смеси с листьями из речного порта (5,70 г, 39,9%) и Михайловского парк (5,50 г, 43,0%). Количество отложенных коконов было самым высоким в смесях, содержащих листья из контрольной точки (+138%) и Университетского парка (+94,8%), но снизилось в смесях, содержащих листья с железнодорожной станции (-40,9%). Рост молоди был самым высоким в смесях, содержащих листья из Михайловского парка (+33,4 особи). В целом, качество субстрата в менее загрязненных районах способствует лучшему развитию дождевых червей. Полученные данные послужат основой для рационального управления опавшими листьями в городах и обеспечения безопасности производства биогумуса.

Ключевые слова: *Eisenia fetida*; вермикомпостирование; листья тополя; органическое удобрение; рост дождевых червей; размножение дождевых червей.

Introduction

A large amount of organic waste is generated annually as a result of agricultural activity. Incineration is still a common method of disposal, although it pollutes the environment. Vermicomposting is an alternative method of agricultural waste disposal. During the processing of various wastes, the greatest amount of humic acid, nitrogen (N) and potassium (K) was found in vermicompost from cow manure. The maximum electrical conductivity (EC), the content of organic substances, magnesium (Mg) and manganese (Mn) were found in vermicompost from leaf litter. Vermicompost from oilcake has the highest levels of phosphorus, iron, zinc and copper. Vermicompost obtained from different types of raw materials differs in the total nutrient content and quality [1]. Some studies have examined the negative consequences of excessive use of chemical fertilizers, including low crop yields, increased pest and disease infestations, soil degradation, and adverse environmental impacts, by evaluating biohumusification as a sustainable organic farming practice [2].

The researchers studied the effect of vermicompost on vegetables and cereals and described its beneficial effects on most plant species. Vermicompost helps improve soil structure, texture, porosity, moisture retention, drainage and aeration, and reduces soil erosion. Vermicompost increases the microbiological activity of the soil, increases the number of beneficial microbes and reduces the level of damage to plants by pests and diseases. It also contains several micro- and macronutrients, vitamins, enzymes, and hormones such as auxins and gibberellins. [3].

Assessing the feasibility of processing leaf litter by earthworms with subsequent use of the resulting vermicompost as a fertilizer is an important task in the context of sustainable urban waste management and green infrastructure. Previous studies have demonstrated that vermicomposting of leaf litter produces a high-quality organic fertilizer rich in macro- and micronutrients as well as humic substances. The quality of the resulting compost depends on the composition of the initial substrate, earthworm species, and processing conditions. However, leaf litter in urban environments can accumulate heavy metals and other pollutants, which limits the potential use of such compost in agriculture and urban landscaping. Therefore, it is necessary to assess not only the processing efficiency but also the safety of the resulting product.

It has previously been noted that the presence of *E.fetida* alters the microbiological structure of fallen poplar leaves during vermicomposting. Notably, nitrogen-fixing Azotobacter species are found only in vermicomposted substrates – not in traditional composts – and their activity is higher in poplar-leaf-based systems than manure-based ones [4].

Although the nitrogen conversion process during vermicomposting is slower than in manure systems, leaf litter can still be a valuable raw material for producing vermicompost due to its ability to produce high-quality fertilizer. The initial nitrate level of the mixture of soil and leaf litter in the greenhouse ensures a high nitrate content in the final vermicompost. This makes leaf litter an attractive alternative to manure, particularly in the Siberian region where a large amount of fallen leaves is currently being sent to landfills [5].

Similar trends were observed by Aalok [6], who investigated the nutrient dynamics during composting and vermicomposting of leaf litters from different plant species (*Eucalyptus hybrid, Pinus roxburghii, Populus deltoides, Shorea robusta*) and *Parthenium hysterophorus* mixed with municipal solid waste. Vermicomposting resulted in a significant reduction in pH (2.29–19.99%) and C/N ratio (47.55–69.21%), alongside an increase in total nitrogen (47.0–145.5%), phosphorus (26.45–121.84%), potassium (20.96–106.18%), calcium (11.07–24.66%), and magnesium (14.47–66.05%) compared to initial values. However, the final nutrient quality of the vermicompost varied depending on the initial properties of the leaf litter.

Vermicomposting of some tree species produced vermicomposts with high nutrient content, including phosphorus, nitrogen, potassium, magnesium, and calcium. Although nutrient levels varied during the vermicomposting period, the final products demonstrated potential for soil fertility improvement and reducing reliance on synthetic fertilizers in crop production. Mokgophi et al. [7] investigated the impact of vermicomposting on the chemical and biological characteristics of three agroforestry species: *Chamaecytisus tagasaste* (tree lucerne), *Vachellia karroo*, and *Moringa oleifera*. Leaves and small twigs were vermicomposted with *Eisenia fetida* for six weeks under laboratory conditions. The results showed that vermicomposting significantly enhanced macronutrient content in all three feedstocks, with *M. oleifera* producing the highest quality vermicompost — macronutrients (Ca, K, Mg, P) were 50–170 % higher compared to the other species. Vermicomposting increased molybdenum (Mo) content, while other micronutrients (Zn, Mn, Fe, B) decreased significantly over time. Importantly, *E. fetida* reproduction increased dramatically, with more than a 450% increase in earthworm numbers across all three feedstocks. The authors concluded that vermicompost from agroforestry species has the potential to improve soil fertility and reduce the use of synthetic fertilizers in crop production [7].

Some researchers have noted that regardless of the processed substrate, the nutrient content in vermicompost has always been higher than in the control organic waste. It was shown that neem leaves and wheat straw were the most suitable raw materials for producing vermicompost. The C/N ratio in organic waste decreased significantly during composting, which indicates an increase in the concentration of nitrogen, which is easily accessible to plants.

Consequently, plants grown on these vermicompost showed much better growth compared to the control [8-10].

When processing poplar leaves, it should be borne in mind that they accumulate a significant amount of dangerous chemicals. Thus, black poplar accumulates Zn, Cd, Pb, Cu, Ni and other substances in its leaves in proportions that, under intact conditions, indicate a corresponding contamination of Zn (173.3 ppm), Cd (0.70 ppm) and Co (1.10 ppm), in and As (0.20 ppm). This study shows that the studied species can provide an effective biomonitoring service in urbanized environments, providing valuable information on the occurrence and biological consequences of heavy metal pollution [11].

In another study, elements were found in black poplar leaves in the order Zn>>Fe>Mn>Al>Pb>Ni>Cu>Co>Cd and increased with increasing PM10 concentrations, indicating that poplar leaves are sensitive to air pollution. All the analyzed poplar species demonstrated a high potential for phytoremediation of PAHs, PCBs and TM, as evidenced by the detected levels in their tissues. Based on these results, urban decision makers are encouraged to select poplar species that correspond to the specific pollutants prevailing in the area [12].

In particular, *Populus euramericana* shows high potential in the field of purification from PAHs and PCBs, which makes it an ideal choice for areas with significant industrial or vehicle emissions. *P. nigra* demonstrates excellent ability to accumulate heavy metals, especially Zn, and can be a priority plant in areas with high concentrations of pollutants, containing heavy metals. Although *P. alba* shows a slightly lower overall xenobiotic accumulation potential, it noticeably accumulates the most carcinogenic PAHs (CPAH), which underscores its importance in strategies for restoring human health in urban areas with high health risk factors [13].

It was previously stated that the pretreatment of leaf litter with white rot fungi under solid-phase fermentation conditions before adding *Eisenia fetida* to vermicompost significantly improved the decomposition of lignocellulose and nutrient enrichment. Fallen leaves mixed with cow manure in a ratio of 1:1 and 2:1 produced the best results by increasing the levels of total Kjeldahl nitrogen (TKN), available phosphorus (Paval) and calcium (Ca). The activity of microbiological enzymes, as well as the growth of earthworms and their fertility were significantly increased in the pretreated leaf biomass. The authors suggested that the use of fungal consortia for pretreatment and subsequent vermicomposting is a promising area for future research [14].

Another study investigated the vermicomposting of waste biomass from three littoral plants – *Typha latifolia*, *Iris pseudacorus*, and *Ceratophyllum demersum* – using *Eisenia fetida*. The results showed that *E. fetida* could be effectively used for the rapid utilization of pure *I. pseudacorus* and *C. demersum* waste, but *T. latifolia* waste could not be fully recycled within the experimental timeframe. Vermicomposts obtained were characterized by higher N, P, K, Ca, and Mg content compared to the initial plant biomass. Heavy metal content (Cu, Mn, Zn, Cd, Pb) in the vermicomposts did not exclude their use as fertilizers [15]

The processing of nutrients from silver oak (*Grevillea robusta*) and waste litter from bamboo leaves mixed with cow dung using *E.fetida* was studied. Composting led to a significant decrease in pH, total organic carbon, exchangeable potassium and the C: N ratio. Conversely, there was an increase in electrical conductivity, ash content, total nitrogen content, total P, total Ca and nitrate nitrogen. Beds with silver oak showed a higher degree of mineralization than beds with bamboo. Mixtures of vermicompost waste contained a large number of microorganisms: bacteria (423-684 CFU × 10 g/l), fungi (22-37 CFU × 10 g/l) and actinomycetes (108-142 CFU × 10 g/l). *E. fetida* demonstrated a good increase in biomass and cocoon formation on all vermicomposts, with the largest number of earthworms An increase in the population was observed in the control with 100% cow manure (50.0 ± 3.0 specimens). The authors concluded that the fallen leaves of urban forests can be converted into value-added manure for sustainable soil fertility management [16].

The purpose of our study is to evaluate the effect of poplar leaf litter from various urban areas on the growth and reproduction of earthworms *Eisenia fetida*.

Materials and methods of study

As a component for vermicomposting, we used the leaf litter of poplars growing on the territory of Tomsk. In total, five samples of poplar leaves were selected in different parts of the city (a mixture of *Populus balsamifera, Populus suaveolens* and *Populus nigra*).

Different levels of anthropogenic impact were taken into account when selecting poplar leaf sampling sites. The prevailing wind in Tomsk is southwesterly, so the general transport of airborne pollutants occurs toward the northeast. However, whether a specific sampling site receives wind-transported emissions depends on its location relative to nearby highways. For example, the river port area (point 3) is located downwind from Lenin Street when the wind blows from the southwest. In contrast, Mikhailovskaya Park may receive emissions from the highway primarily when wind directions have an easterly component. Thus, the correlation between vehicle emissions and wind direction is not uniform across all sampling points and must be interpreted with consideration of the local orientation of roads relative to the prevailing wind (figure 1).

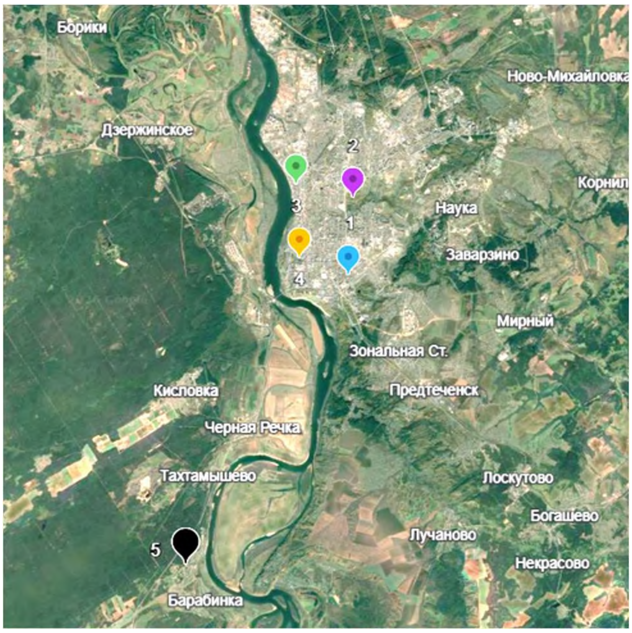

Figure 1. Map of poplar leaf sampling sites in Tomsk and surrounding areas. 1 - Tomsk railway station (blue), 2 - Mikhailovskaya Park (purple), 3 - Tomsk river port (green), 4 - The University Park (orange), 5 - Kaftanchikovo village (black)

Point 1 (high anthropogenic load). Samples were collected on September 8 and 26 near the Tomsk railway station. This area has heavy traffic and significant exhaust emissions, making it classified as an area with a high anthropogenic load.

Point 2 (park area near the highway). Samples were taken on September 8 and 27 in Mikhailovskaya Park, the closest edge of which is approximately 50 meters from the avenue, while the sampling itself was conducted at distances of 70 to 500 meters from the highway. The park is located in a lowland. This location was chosen because it is a large park in Tomsk located next to a busy highway.

Point 3 (spatially isolated area). Samples were collected on September 8 and 27 near the Tomsk river port. Sampling points are located 150-220 meters from the street with heavy traffic. Despite its proximity to a busy street, the street near the river port is spatially separated from it by a distance, allowing us to assess the role of wind transport of heavy metals and other elements.

Point 4 (roughly reference area in the city). Samples were taken on September 10 and 28-29 in the University Park. The park is located 200 meters from the avenue and 350-400 meters from the highway. Wind transport of heavy metals from these roads does not play a key role in vegetation contamination in this area.

Point 5 (control, suburban area). Samples were taken on September 10 and 28-29 in Kaftanchikovo village (a suburb of Tomsk). Leaves were collected in a park near a school at least 500 meters from the nearest highway. This site serves as the control site in this study.

The vermicomposting experiment was conducted using 1-liter plastic containers in 3 replicates (n=3) for four different treatments and a control (a total of 15 containers). Before the experiment, the leaves were shredded into a 4-5 mm fraction. 500 ml of shredded leaves and 100 g of water were added to each container to create a homogeneous mixture, followed by the addition of 300 ml of peat. 30 adult earthworms were weighed and placed in each container. One month after the experiment began, the number of adult earthworms, juvenile earthworms, and cocoons was counted. Two months later, the number of earthworms was counted again, and the worms were weighed in each container. The earthworms were dried in a GP-40 MO air sterilizer for subsequent analysis. The resulting vermicompost was also collected for further analysis. Data analysis included calculation of mean (M), standard deviation of mean (m).

Results and Discussion

The two-month experiment showed that the weight gain of Eisenia fetida differed significantly depending on the location of the poplar leaves used as a substrate. The highest absolute weight gain was observed in earthworms that were kept on a substrate with leaves from the village of Kaftanchikovo (control group) and the University Park (urban group), with an increase of 8.98 ± 0.83 g and 8.85 ± 0.83 g, respectively, corresponding to a relative growth rate of 73.8% and 78.2%. These locations have minimal direct anthropogenic impact and are located further away from major highways, suggesting that the fallen leaves from less polluted areas contribute to the more efficient development of earthworms.

The lowest growth rates were observed in the earthworms that were fed with leaves from the Tomsk River Port (initial weight 14.27 ± 2.57 g, final weight 20.00 ± 1.54 g; gain 5.73 ± 3.00 g, 40.2%) and the Mikhailovsky Park (initial weight 12.82 ± 1.27 g, final weight 18.30 ± 3.36 g; gain 5.48 ± 3.59 g, 42.7%). Although the worms from the group that was fed with leaves from the river port had the highest initial weight (14.27 g), their relative growth rate was the lowest, indicating a possible slowdown in growth due to spatial isolation, but still contaminated leaf substrate. The group that was fed with leaves from the Tomsk Railway Station (high anthropogenic load) showed intermediate results (7.47 ± 0.77 g, 72.7%), which, despite the high level of pollution in this location, did not result in the lowest growth rates (table 1).

Overall, the data obtained suggest that the quality of the poplar leaf substrate collected from different urban areas directly affects the growth efficiency of Eisenia fetida. Leaves from suburban and park areas with less anthropogenic impact promote better development of worms, while leaves from areas with industrial or traffic pollution – even if they are collected in spatially isolated areas (river port) or in a park near a highway (Mikhailovsky Park) – promote slower growth rates.

Table 1. Changes in the biomass of earthworms during the experiment

Sampling site	Earthworm weight at the beginning of the experiment, g	Earthworm weight after 2 months, g	Growth, g	Growth, %
Kaftanchikovo village	12,16 ± 0,48	21,14± 0,68	8,98 ± 0,83	73,8
The University Park	11,32± 0,60	20,17± 0,57	8,85± 0,83	78,2
Tomsk railway station	10,27± 0,24	17,74± 0,73	7,47± 0,77	72,7
Tomsk river port	14,27± 2,57	20,00± 1,54	5,73± 3,00	40,2
Mikhailovskaya Park	12,8 ± 1,27	18,30 ± 3,36	5,48 ± 3,59	42,7

All values are the mean and standard deviation of three replicates

Significant differences in the dynamics of Eisenia fetida population over a two-month experimental period, depending on the location of the poplar leaves used as a substrate were found. In all locations, there was an increase in the number of adult earthworms, with the highest absolute increase observed in Mikhailovsky Park (+8.63 individuals, +29.1%) and the lowest in the Tomsk River Port (+4.03 individuals, +13.8%). This indicates that adult individuals survived and developed on all substrates, but the rate of growth of the adult population depended on the quality of the leaf litter in each location (table 2).

Table 2. Changes in the number of Eisenia fetida during cultivation on a substrate of poplar leaves and peat

Sampling site	Accounting period (days)	Adults, ind.	Juveniles, ind.	Cocoons, ind.
Tomsk railway station	30	29.7	0.7	29.3
	60	34.67 ± 0.94	8.33 ± 1.20	17.33 ± 1.48
The University Park	30	30.0	0.3	24.3
	60	36.33 ± 0.94	14.67 ± 1.95	47.33 ± 8.42
Mikhailovskaya Park	30	29.7	0.3	31.3
	60	38.33 ± 2.98	33.67 ± 9.31	42.67 ± 6.47
Tomsk river port	30	29.3	1.0	21.0
	60	33.33 ± 0.94	20.00 ± 5.81	24.00 ± 1.82
Kaftanchikovo village	30	30.0	0.7	27.3
	60	37.00 ± 0.91	13.33 ± 1.54	65.00 ± 17.57

All values are the mean and standard deviation of three replicates.

The greatest differences were observed in the number of newly emerged individuals. The highest number of newly emerged individuals was recorded in Mikhailovsky Park (33.67 ± 9.31 individuals, +11123%) and in the Tomsk River Port (20.00 ± 5.81 individuals, +1900%), despite the fact that these locations initially had relatively few newly emerged individuals (0.3–1.0 individuals). The extremely high percentage increase indicates active reproduction and successful hatching of worms from cocoons. However, the lowest number of newly emerged individuals was recorded in the Tomsk Railway station (8.33 ± 1.20 individuals, +1090%), which may indicate a possible negative impact of high anthropogenic load on reproductive function.

The number of cocoons also varied significantly depending on the location. The highest number of cocoons was found in the village of Kaftanchikovo (65.00 ± 17.57 individuals, +138%) and in the University Park (47.33 ± 8.42 individuals, +94.8%), indicating that the substrate from less polluted areas (suburban control and urban reference) promotes the formation of cocoons. However, there was a decrease in the number of cocoons in the Tomsk River Port (– 40.9%), which may indicate that the leaves from this area with heavy traffic negatively affect the formation of cocoons or reduce the reproductive capacity of the worms.

It is noteworthy that in Mikhailovsky Park (despite the fact that it is located next to the highway), the highest productivity of juveniles and a significant number of cocoons were noted, possibly due to the park's location in a lowland and the remoteness of sampling points (70-500 m) from the road, which can reduce the direct impact of vehicle exhaust fumes.

In general, these results demonstrate that the quality of the substrate obtained from poplar leaves collected in various urban areas significantly affects the reproductive function of Eisenia fetida. Leaves from suburban (Kaftanchikovo) and park areas with a lower direct anthropogenic load (University Park) contributed to an increase in cocoon production, while leaves from places with heavy traffic (railway station) were associated with a decrease in reproductive productivity. However, the high productivity of juveniles in Mikhailovsky Park and Tomsk River Port suggests that even moderately polluted areas can contribute to the active reproduction of earthworms, which underlines the ability of this species to adapt to various substrate conditions.

The relationship between growth and reproduction was not clear. In areas with high biomass growth (Kaftanchikova, University Park), high cocoon production was also observed, but only a moderate number of juveniles. On the contrary, in Mikhailovsky Park, despite the low increase in biomass, the highest productivity of juveniles was observed, which probably reflects a compromise between somatic growth and reproduction. On the other hand, a large number of juveniles in this area may indicate successful hatching, but the low weight gain of an adult individual indicates that the quality of the substrate was insufficient for simultaneous maintenance, growth and reproduction.

It is assumed that anthropogenic pollution is the main factor influencing *E. fetida*, although we cannot confirm this directly without chemical analyses of the vermiculture elements. Our planned chemical analyses of the samples will allow us to understand the migration paths of individual elements in the leaves-earthworms-vermicompost system.

Conclusion

The quality of the substrate obtained from poplar leaves harvested in various urban areas directly affects the growth efficiency and reproductive capacity of Eisenia fetida. Leaves from suburban and park areas with a lower anthropogenic load contribute to better worm development and increased cocoon production. Moving away from areas polluted by traffic, whether it's a busy divided street (river port) or a park next to a highway (Mikhailovsky Park), is associated with a decrease in growth efficiency. However, these same areas can still support active reproduction, which highlights the need to evaluate both growth and reproductive parameters when assessing the quality of the substrate for vermicompost. Tomsk Railway Station, despite the highest anthropogenic load, showed an intermediate growth, but reduced cocoon production, which indicates that various pollutants can affect the physiological processes of earthworms in different ways.

Vermicompost obtained from fallen poplar leaves collected in suburban or park areas with moderate traffic (for example, Kaftanchikova, University Park) may be recommended for use in urban landscaping without additional treatment, however, follow-up studies need to be conducted:

1. Quantitative chemical analysis of heavy metals and other hazardous elements in the initial leaf litter, obtained vermicompost and earthworm tissues;
2. Comparison of measured levels of pollutants with established safety standards;
3. Eco-toxicological assessment of vermicompost using biological tests (for example, seed germination tests). These steps will help determine whether vermicompost from fallen leaves can be safely used in urban environments for municipal and agricultural needs.

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