



Effects of organic pollutants on the locomotor activity of *Rossiulus kessleri* (Diplopoda, Julida)

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Rossiulus kessleri (Lochmander, 1927) is one of the commonest Diplopoda species in the moderate climate zone of Eurasia. This millipede is often subject to numerous industrial pollutants, but their effects on this species have not been studied so far. For laboratory studies, we made a 150 cm-long and 10 cm-wide experimental chamber, with marks every 10 cm. In the middle of the chamber, we placed 60 individuals that had an opportunity to move toward the source of odor or away from it. The behavior of the millipedes was recorded on video, and the direction in which each individual moved was observed for 5 minutes. In the experiments, we used 30 organic compounds: butyl acetate, diethyl oxalpropionate, butyl acrylate, diethyl malonate, ortho-xylene, thymol, α -methylbenzylamine, carvacrol, hexane, pentane, biphenyl, cyclohexane, 2-methylfuran, 5-methylfurfural, furfuryl alcohol, 2-methylbutanoic acid, 3,7-dimethyl-6-octenoic acid, isovaleric acid, tert-butylacetic acid, propionic acid, 4-methyl-2-pentanol, 3-methyl-2-butanone, 2-pentanone, methyl acetoacetate, ethyl pyruvate, isobutyraldehyde, 2-ethoxyethanol, potassium sulfite, calcium sorbate, and sodium diacetate. These compounds are used in various industrial spheres, construction, agriculture, and food industry. With industrial waste, they are released into the environment and can potentially affect populations of *R. kessleri*. Of the 30 compounds, 23 had no significant effect on the locomotor activity of *R. kessleri*. The strongest repellent activity was exerted by isobutyraldehyde (the attractant coefficient measured 0.43 ± 0.40) and biphenyl (0.49 ± 0.11). A weak repellent effect on *R. kessleri* was displayed by thymol (the attractant coefficient equaled 0.66 ± 0.26), pentane (0.73 ± 0.43), tert-butylacetic acid (0.82 ± 0.72), and cyclohexane (0.88 ± 0.34). A weak attractant effect on *R. kessleri* was produced by 2-methylbutanoic acid (the attractant coefficient measuring 1.32 ± 0.48). Therefore, seven of the 30 compounds that are broadly used in industries and agriculture exerted either repellent (six compounds) or attractant (one compound) effect on the millipede.

Keywords: attractants; repellents; isobutyraldehyde; biphenyl; thymol; pentane; tert-butylacetic; cyclohexane; 2-methylbutanoic acid.

Introduction

The main sources of environmental pollution in the regions of Eurasia are metallurgy and construction, agriculture, and electric power industry. Among chemical pollutants, pesticides are considered to be some of the most significant factors of pollution due to their ability to accumulate in different components of ecosystems (Siegfried, 1993; Kozak & Brygadyrenko, 2018; Kozak et al., 2020). Those chemicals concentrate in plants and via food chains travel to animal organisms (Martynov & Brygadyrenko, 2017; Faly et al., 2023; Faly & Brygadyrenko, 2024).

Despite the diplopods' significance for ecosystems, little research has been performed on the mechanisms through which pollutants from industrial and municipal waste affect the species compositions of their groups, for example, trophic, respiratory, or contact through the cuticle (Makarov, 2015). Representatives of the Diplopoda class are mostly prime decomposers of the litter, which process foliage and wood (Striganova, 1972; Brygadyrenko, 2014, 2015). Those animals occur in forests, and in lower numbers in meadow and steppe areas (Svyrydchenko & Brygadyrenko, 2014). In settlements, populations of Diplopoda are subject to an array of factors, in particular, pesticides, heavy metals, persistent organic pollutants, microplastic, fragmentation of biotopes, soil compaction, recreation, changes in the species compositions of autotrophs, burning of leaves and litter, etc. (Brygadyrenko & Ivanyshyn, 2015; Brygadyrenko, 2016; Green & Boots, 2018; Tóth & Hornung, 2019). As a result of a complex impact on organisms living in urbanized settlements, the number of Diplopoda remains low, while their species composition is quite impoverished (Pakhomov et al., 2008; Tóth & Hornung, 2019).

Since *Rossiulus kessleri* (Lochmander, 1927) is one of the commonest diplopods in the steppe zone of Eurasia (Kobahidze, 1963; Brygadyrenko & Svyrydchenko, 2015; Bachvarova, 2016; Kokhia & Golovatch, 2018), peculiarities of its development have been explored

more or less in depth, compared with other diplopods (Striganova, 1972). The *Rossiulus* genus belongs to the Julidae family and is represented by two species: *R. vilnensis* (Jawłowski, 1925) and *R. kessleri*. The latter, common in the plains of Ukraine, is most numerous in the steppe zone (Cherny & Golovach, 1993; Pokhylenko & Korolev, 2013; Pakhomov et al., 2022). In Ukraine, *R. kessleri* is abundant in Zhytomyr, Poltava, Cherkasy, Kyiv, Luhansk, Mykolaiv, Dnipropetrovsk, Kharkiv, and Sumy oblasts (Cherny & Golovach, 1993). This species plays an important role in biogeochemical cycles, in particular, in the process of decomposition of organic matter, although it is often overlooked in ecological studies (Lokšina, 1964; Helb, 1975; Lokšina & Golovatch, 1979; Zakharov & Gorbach, 2019).

Svyrydchenko & Brigadyrenko (2014) found that the representatives of *R. kessleri* in the forests of the Ukrainian steppe zone have a trophic specialization. Of the offered feeds made of foliage from 16 species of trees, the representatives of *R. kessleri* better consumed foliage of *Quercus robur* L., *Acer negundo* L., *Cerasus vulgaris* L., and *Malus domestica* Borkh. Using *R. kessleri* as a model organism can shed light on the processes of decomposition of organic compounds and interactions in ecosystems (Kicaj, 2023). *Rossiulus kessleri* is a significant link in the food chain in forest ecosystems. This millipede is food for various predators, including amphibians, reptiles, birds, mammals, and many invertebrates. Furthermore, this diplopod can be used as an indicator of the ecological state of ecosystems due to its sensitivity to changes in the environment (Wytwer, 1993). The behavioral peculiarities of *R. kessleri* make it a perfect candidate for a pollution bioindicator (David, 2015; Kovalchuk & Loseva, 2017). This calciphilous subendemic species of the Russian Plain extends from Central Belarus in the west to the regions of Bashkiria and Orenburg in the east, and from Archangelsk Oblast in the north – where the northernmost record is from the Severnaya Dvina River delta north of Archangelsk – southward to Dagestan, beyond Europe's eastern boundary. The life cycle and reproductive strategy of *R. kessleri* was studied by Striganova (1972) studied (Kime &

Name of the compound	CAS registry number	Empirical formula	Structural formula	Use
2-Methylfuran	534-22-5	C ₅ H ₆ O		A flavoring or a supplementary agent in chemical synthesis.
5-Methylfurfural	620-02-0	C ₆ H ₆ O ₂		It has applications as a synthetic intermediate relevant to the fields of medicine, agriculture and cosmetics; it is a food additive, and has FEMA number 270s and JECFA number 745
Furfuryl alcohol	98-00-0	C ₅ H ₆ O ₂		A solvent for tannins, dyes, and resins; resins prepared based on this compound are chemically stable and non-flammable.
Hexane	110-54-3	C ₆ H ₁₄		It is used as a solvent, especially in shoemaking and glue production, and also as a solvent for polymers, plant oils, and paints.
Pentane	109-66-0	C ₅ H ₁₂		It is used as a component in liquids for lighters, motor and aviation fuel, in laboratory solvents, as an additive to petroleum and aerosol propellants.
Biphenyl	92-52-4	C ₁₂ H ₁₀		A dye carrier and a preservative preventing mold on food products, an intermediate product for synthesis of organic chemicals, fluid for heat transfer, and fungicide.
2-methylbutanoic acid	600-07-7	C ₅ H ₁₀ O ₂		It is used in food supplements and flavorings.
3,7-Dimethyl-6-octenoic acid	502-47-6	C ₁₀ H ₁₈ O ₂		The compound is used in flavorings and food supplements.
Isovaleric acid	503-74-2	C ₅ H ₁₀ O ₂		It is used in the production of fungicides, rodenticides, drugs, sedatives and other pharmaceutical products, for solvents, perfumes, and for making of complex ethers.
tert-Butylacetic acid	1070-83-3	C ₆ H ₁₂ O ₂		An intermediate product in the synthesis of drugs and agricultural chemicals.
Propionic acid	79-09-4	C ₃ H ₆ O ₂		An intermediate product for fungicides and herbicides, plastifiers, pharmaceutical products; food preservative that is naturally present in cheese; grain preservative.
4-Methyl-2-pentanol	108-11-2	C ₆ H ₁₄ O		It is a solvent for dyes, oils, resins, natural gum, wax, nitrocellulose, and ethylcellulose.
3-Methyl-2-butanone	563-80-4	C ₅ H ₁₀ O		It is used as an intermediate product for the production of herbicides, pharmaceutical preparations, and dye precursors; solvent for nitrocellulose lacquers.
2-Pentanone	107-87-9	C ₅ H ₁₀ O		The compound is used as a solvent for lacquers and surface coatings, flavoring, and also in organic synthesis.
Methyl acetoacetate	105-45-3	C ₅ H ₈ O ₃		It is used for the synthesis of nifedipine, as a solvent for cellulose ethers, and a chemical intermediate product for organic compounds.
Ethyl pyruvate	617-35-6	C ₅ H ₈ O ₃		It is used as a flavoring.
Isobutyraldehyde	78-84-2	C ₄ H ₈ O		It is used in the organic synthesis (cellulose esters, pantothenic acid, flavorings); intermediate compound for precursors of neopentyl glycol and natural gum antioxidants.
Potassium sulfite	10117-38-1	K ₂ SO ₃		The compound is used in anti-inflammatory and diuretic drugs; component of fixing agents in photography, food supplement, ingredient of cosmetics.
Calcium sorbate	7492-55-9	C ₁₂ H ₁₄ CaO ₄		It is used as a chemical preservative in food products and for the preparation of fungistatic agents.
Sodium diacetate	126-96-5	C ₄ H ₇ NaO ₄		Agricultural fungicide and bactericide, food preservative, sequestrant in animal feeds, mordants, varnish hardeners, and anti-tarnishing agents.

Note: the data are provided according to Hawley (1977), Ullmann's Encyclopedia of Industrial Chemistry (2000), Sullivan & Krieger (2001), Hawley-Lewis (2007), Burdock (2010), Dionisio et al. (2018).

The effects of the aromatic compounds on the locomotor activity of the millipedes was assessed using a one-factor disperse analysis (ANOVA). The results were considered statistically significant at $P < 0.05$.

Results

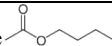
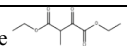
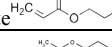
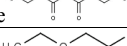
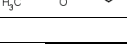





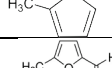
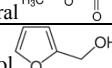
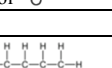
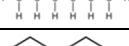
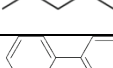
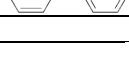

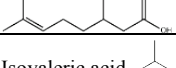




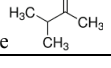
Of the 30 compounds, 23 had no effect on the locomotor activity of *R. kessleri* (Table 2). The strongest repellent effect was exerted by isobutyraldehyde and biphenyl. Thus, the attractant coefficient – the ratio


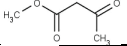
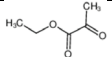

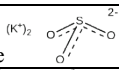
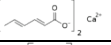

of the number of millipedes located within 40 cm closest to the odor source to the number of millipedes within 40 cm farthest from the odor source – equaled 0.43 ± 0.40 for isobutyraldehyde and 0.49 ± 0.11 for biphenyl. That is, the number of millipedes that ran away from the odor source was over twice the number of those that migrated toward it.

A weak repellent effect on *R. kessleri* was displayed by thymol (the attractant coefficient equaled 0.66 ± 0.26), pentane (0.73 ± 0.43), tert-butylacetic acid (0.82 ± 0.72), and cyclohexane (0.88 ± 0.34). A weak attractant effect on *R. kessleri* was exhibited by 2-methylbutanoic acid (the attractant coefficient equaled 1.32 ± 0.48).

Table 2

Effects of organic compounds on the locomotor activity of *Rossius kessleri* (mean \pm standard deviation, $n = 20$)

Compound	Within 40 cm closest to the odor source	Within 40 cm farthest from the odor source	Attractant coefficient ($I \pm SD$), individuals	F, $F_{0.05} = 4.01$	Significance of the number of specimens per 40 cm closest to and farthest away from the odor source (P)	Effect on the locomotor activity of <i>R. kessleri</i>
Simple and mixed ethers						
Butyl acetate 	8.29 ± 1.11	8.90 ± 2.53	0.99 ± 0.46	0.614	0.436	no activity
Diethyl oxalpropionate 	6.68 ± 1.34	7.61 ± 1.41	0.96 ± 0.47	1.711	0.196	no activity
Butyl acrylate 	7.03 ± 1.77	6.65 ± 1.49	1.07 ± 0.51	0.142	0.708	no activity
Diethyl malonate 	5.36 ± 1.08	5.31 ± 1.13	0.97 ± 0.34	0.007	0.935	no activity
2-Ethoxyethanol 	4.68 ± 1.31	5.74 ± 1.19	0.86 ± 0.45	1.172	0.284	no activity
Aromatic cyclic compounds						
ortho-xylene 	9.10 ± 1.53	9.42 ± 2.51	1.15 ± 0.99	0.180	0.673	no activity
Thymol 	7.38 ± 1.52	11.79 ± 2.84	0.66 ± 0.26	25.666	4.4×10^{-6}	weak repellent activity
α -Methylbenzylamine 	8.90 ± 1.72	8.81 ± 1.75	1.12 ± 0.47	0.015	0.903	no activity
Carvacrol 	8.44 ± 2.02	9.72 ± 2.55	0.89 ± 0.26	2.566	0.115	no activity
Cyclohexane 	9.33 ± 2.32	11.13 ± 2.85	0.88 ± 0.34	4.529	0.038	weak repellent activity
2-Methylfuran 	7.15 ± 1.70	5.81 ± 1.12	1.28 ± 0.28	2.380	0.128	no activity
5-Methylfurfural 	4.97 ± 0.88	5.88 ± 0.99	1.02 ± 0.66	1.686	0.199	no activity
Furfuryl alcohol 	6.77 ± 1.42	7.19 ± 2.05	1.02 ± 0.65	0.156	0.694	no activity
Carbohydrates						
Hexane 	6.49 ± 2.09	8.27 ± 2.06	1.10 ± 0.81	2.072	0.155	no activity
Pentane 	6.90 ± 2.03	9.83 ± 2.97	0.73 ± 0.43	8.418	0.005	weak repellent activity
Biphenyl 	4.76 ± 1.10	9.92 ± 2.68	0.49 ± 0.11	33.993	3×10^{-7}	repellent activity
Acids						
2-Methylbutanoic acid 	8.18 ± 1.39	6.47 ± 1.31	1.32 ± 0.48	4.445	0.039	weak attractant activity
3,7-Dimethyl-6-octenoic acid 	4.25 ± 1.15	3.62 ± 1.16	1.76 ± 2.32	0.519	0.474	no activity
Isovaleric acid 	6.94 ± 1.19	8.32 ± 2.04	0.81 ± 0.24	1.945	0.168	no activity
tert-Butylacetic acid 	5.81 ± 1.70	9.58 ± 2.41	0.82 ± 0.72	12.596	0.001	weak repellent activity
Propionic acid 	3.79 ± 1.86	5.31 ± 0.61	1.59 ± 1.50	1.674	0.201	no activity
Alcohols						
4-Methyl-2-pentanol 	7.88 ± 1.25	8.36 ± 1.82	0.99 ± 0.41	0.531	0.469	no activity
Ketones						
3-Methyl-2-butanone 	3.81 ± 0.75	5.38 ± 1.31	1.69 ± 2.08	3.253	0.076	no activity

Compound	Within 40 cm closest to the odor source	Within 40 cm farthest from the odor source	Attractant coefficient (I ± SD), individuals	F, F _{0.05} = 4.01	Significance of the number of specimens per 40 cm closest to and farthest away from the odor source (P)	Effect on the locomotor activity of <i>R. kessleri</i>
2-Pentanone 	7.43 ± 1.26	8.53 ± 2.06	0.90 ± 0.39	1.723	0.194	no activity
Methyl acetoacetate 	8.03 ± 1.26	9.61 ± 2.42	0.92 ± 0.30	3.775	0.057	no activity
Ethyl pyruvate 	7.56 ± 1.32	8.97 ± 2.25	0.92 ± 0.34	2.959	0.091	no activity
Aldehydes						
Isobutyraldehyde 	3.46 ± 1.07	9.38 ± 2.15	0.43 ± 0.40	39.566	<0.001	repellent activity
Salts						
Potassium sulfite 	8.17 ± 1.98	6.76 ± 1.65	1.14 ± 0.38	1.679	0.200	no activity
Calcium sorbate 	6.83 ± 1.41	6.35 ± 1.54	1.20 ± 0.49	0.219	0.642	no activity
Sodium diacetate 	6.82 ± 1.39	6.44 ± 1.40	1.37 ± 1.34	0.119	0.731	no activity

Discussion

The most unexpected result of our study was that not all the compounds (not even a half), characterized by sharp odor for people, affected the migratory activity of *R. kessleri*. Perhaps, the olfactory receptors on the mucous membrane of the human nose and the nose of other mammals are absent in arthropods, *R. kessleri* in particular (Schönrock, 1981; Müller & Sombke, 2015). A repellent activity toward *R. kessleri* was exerted by isobutyraldehyde and biphenyl. A weak repellent effect on *R. kessleri* was produced by thymol, pentane, tert-butylacetic acid, and cyclohexane. A weak attractant effect on *R. kessleri* was demonstrated by 2-methylbutanoic acid.

Isobutyraldehyde is used in organic synthesis (cellulose esters, pantothenic acid, flavorings), and also in Amazonian rubber antioxidants (Dionisio et al., 2018). Its odour is described as that of wet cereal or straw – similar to straw that is decomposed by microorganisms in the conditions of high humidity, which is one of the food substrates for *R. kessleri*. Each year, the global industry produces several million tonnes of isobutyraldehyde (Ullmann's Encyclopedia of Industrial Chemistry, 2000), and therefore its influence on the ecosystems where diplopods live can be very significant.

Biphenyl is used as a dye carrier and a preservative, protecting food products from mold, and also as an intermediate product for synthesis of organic chemicals, a fluid for heat transfer, and a fungicide (Dionisio et al., 2018). This compound has a distinct unpleasant smell. It is known as a primary compound for the production of polychlorinated biphenyls (PCBs), which were earlier broadly used in manufacturing of electric transformers and condensers, but are now prohibited by the International Convention of Persistent Organic Pollutants. Biphenyl is extensively applied to the surfaces of apples, bananas, oranges, mandarin oranges, lemons, pineapples, and other fruits. Together with fruit skins, the E₂₃₀ preservative (fungicide) in large amounts ends up in landfills of solid municipal waste in most countries. Perhaps, this compound can repel millipedes and substantially impact natural ecosystems where *R. kessleri* lives.

Thymol is used as an antibacterial agent, preservative, stabilizer, flavoring, initial material for the synthesis of menthol, and antioxidant. Thymol is broadly used as a disinfectant in oral hygiene products, as an antiseptic, and also as a veterinary anthelmintic agent (Sullivan & Krieger, 2001; Burdock, 2010). It is one of the main components (around 30%) of the aroma of various species of *Thymus* (around 350 species of the genus, the Lamiaceae family) and oregano (around 45 species of the *Origanum* genus, the Lamiaceae family, including *Origanum vulgare* L.). Interestingly, plant associations with domination of species of the *Thymus* and *Origanum* genera often are living environments for most abundant populations of *R. kessleri* (pine forests, ravine banks, steppe areas). Nonetheless, in our experiment, thymol showed a weak repellent effect on *R. kessleri*.

Pentane is used as a component in liquids for lighters, motor and aircraft fuel, in laboratory solutions, as an additive to petroleum and aerosol propellants (Ullmann's Encyclopedia of Industrial Chemistry, 2000; Hawley-Lewis, 2007). Its boiling temperature is 9 to 36 °C. It is one of the primary compounds responsible for odors released into the air from gas stations and cars with internal combustion engines (diesel and petroleum). Its annual emissions into the environment are extremely high – those are various products of oil refinement and gas condensate (Dionisio et al., 2018). It was characterized by a weak repellent effect on *R. kessleri*. Perhaps, it is one of the reasons for the low number of this diploped in urbanized territories.

Tert-butylacetic acid is used as an intermediate product in the synthesis of drugs and agricultural chemicals (Ullmann's Encyclopedia of Industrial Chemistry, 2000). The annual amounts of its production and emissions into the environment are thousands of times less than those analyzed earlier in this section. It is unlikely that this compound significantly limits the populations of *R. kessleri* in natural conditions.

Cyclohexane is used in the production of nylon, as an agent for removal of paints and varnishes, and as a solvent and an intermediate product for organic synthesis. It is broadly used as a solvent and extraction solvent in pharmaceutical industry (Ullmann's Encyclopedia of Industrial Chemistry, 2000; Hawley-Lewis, 2007). For people, cyclohexane has a specific odor of oil products. It exhibited a weak repellent effect on *R. kessleri*, and together with other components of fuels and lubricants is able to affect natural populations of this millipede.

The compound 2-methylbutanoic acid is used in food additives and flavorings (Burdock, 2010). It is a secondary component of essential oils of *Angelica archangelica* L. (Apiaceae), *Valeriana officinalis* L. (Caprifoliaceae), and *Arctium lappa* L. (Asteraceae). These and other plants of the families Apiaceae, Asteraceae, and Caprifoliaceae often occur in places with high number of *R. kessleri*. Therefore, it would be practical to study the trophic spectrum of *R. kessleri*: whether it includes *Angelica archangelica*, *Arctium lappa*, and *Valeriana officinalis*, or on the contrary, the millipede avoids consuming them. The compound 2-methylbutanoic acid also occurs in the fruits such as cacao, apricots, and apples. Interestingly, the odor of this acid is pleasant for humans (although it is subjective), and is also characterized as an attractant of *R. kessleri*.

Thus, *R. kessleri*, one of the commonest diploped (Enghoff, 1984), is a good model for studying effects of various compounds on the migratory activity due to their sensitivity to changes in the environment. Depending on the concentration and compound, the reaction of *R. kessleri* can vary from reduced activity to active avoidance of the odor source. Understanding the effects of organic compounds on *R. kessleri* is significant not only for scientific research in biology and ecology, but also for development of effective methods of pest control, and also for assessments of biological dangers of various compounds (Martynov & Brygadyrenko, 2017). Protection of populations of millipedes is critical-

ly important not only for the preservation of species, but also for the support of stability and health of the ecosystems where they live (Eng-hoff, 2015). Awareness and management of the influence of organic compounds on those organisms allows providing a more sustainable future for our natural resources.

Conclusion

The organic compounds (7 of the 30 studied compounds) had a significant effect on the locomotor activity of *R. kessleri*. The repellent effect was seen more often than attractive effect. The repellent activity toward *R. kessleri* was exerted by isobutyraldehyde and biphenyl. The weak repellent effect on *R. kessleri* was demonstrated by thymol, pentane, tert-butylacetic acid, and cyclohexane, whereas the weak attractant effect on *R. kessleri* was displayed by 2-methylbutanoic acid.

Thus, for the first time it was determined that Diplopoda can react to odors of organic compounds that are com-mon in industrial raw materials and municipal wastes. Until now, this influence has been unstudied. Perhaps, the olfac-tory irritants can have a significant effect on the distribution of millipedes near human settlements, in particular, be a cause of disappearance of some species of millipedes in urban agglomerations.

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