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Reaction of Mollusk Population to Emissions from the Middle Ural Copper Smelter

A. V. Nesterkov

Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, ul. Vos'mogo Marta 202, Yekaterinburg, 620144 Russia

e-mail: nesterkov@ipae.uran.ru

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Abstract—A quantitative study of the mollusk population of the herbaceous layer has been carried out in the secondary upland meadows in a gradient of pollution with emissions from the Middle Ural Copper Smelter (MUCS) (city of Revda, Sverdlovsk oblast). The total abundance of mollusks is 1.3 times lower in a moderately polluted area than in reference areas. The mollusks disappear entirely from the grass cover in close proximity to the smelter. The reasons for the change in the mollusk population structure may be as follows: a heavy-metal toxic effect under conditions of environmental acidification, a lack of available Ca due to its depletion from the upper soil horizons, a reduction in species diversity, and a simplification of meadow grass-cover architecture (which modifies the hydrothermal regime in the herbaceous layer). Apparently, a combination of these effects eliminates mollusks from the herbaceous layer, a trend which is not observed in the case of environmental pollution with only heavy metals.

Keywords: terrestrial mollusks, grass cover, industrial pollution, copper smelter, heavy metals, calcium, Middle Urals

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The largest proportion of terrestrial mollusks found in the herbaceous layer fall into the category of stratochortophiles, i.e., mobile stratified invertebrates closely connected to grass cover, but performing periodic migrations in litter horizons (Lagunov, 1994). The association of mollusks with both ecosystem layers increases their sensitivity to external effects (including those of an anthropogenic nature) in comparison with regular chortobionts. Thus, under conditions of pollution, it is the litter which normally displays the natural acid-base reaction that accumulates the highest amounts of pollutants (Vorobeichik, 2003a). Terrestrial mollusks are generally considered macroconcentrators of a wide range of heavy metals; they possess effective mechanisms of detoxication and, consequently, are potentially capable of inhabiting substrates with high concentrations of heavy metals. Mollusks were frequently found in close proximity to smelters (Hopkin, 1989; Rabitch, 1996; Fritsch et al., 2008) and pollution sources of other natures (Ireland, 1979; Zaldibar et al., 2008). At the same time, mollusks are entirely missing in the meadow grass cover close to the Middle Ural Copper Smelter (MUCS). This fact was first mentioned in 1989 (Vorobeichik et al., 1994) and confirmed in the later paper (Nesterkov, Vorobeichik, 2009). The absence of similar examples in known publications allows us to assume that such a reaction to pollution is not altogether typical of this group. The need to interpret the disappearance of the mollusks from the areas next to the studied copper smelter explains the relevance of the present study.

The purpose of the present paper is to reveal possible reasons for the disappearance of terrestrial mollusks from the grass cover of areas most polluted by emissions from the MUCS.

This study has been carried out in the environs of the MUCS, which is located on the outskirts of the city of Revda in Sverdlovsk oblast. The MUCS has been in operation since 1940 and is one of the largest sources of industrial pollution in Russia. The main components of its emissions are gaseous compounds (SO_2, HF, and nitrogen oxides) and heavy metals (Cu, Zn, Cd, Pb, etc.) associated with dust particles.

The ecotoxicological profile was laid to the west of the MUCS, against the direction of prevailing winds. The nature of anthropogenic transformation of ecosystems has been thoroughly described earlier (Vorobeichik et al., 1994). The studied plots were located in the impact (1 km from the smelter), buffer (4 km), and background (30 km) pollution zones in the depressed topographic areas represented by the secondary upland meadows formed in glades about 5000 m^2 in size as a result of forest clear-cutting around 50 years ago.

The floristic composition of the meadow vegetation differs considerably along different load zones, which can be attributed to the disappearance of sensitive species of herbs and their replacement by grasses. The meadows in the background zone are of a herb type, with the dominance of melancholy thistle (*Cirsium heterophyllum* Hill.), lady's mantle (*Alchemilla sp.*), meadowsweet (*Filipendula sp.*), oxeye daisy (*Leucanthemum vulgare* Lam.), Swiss centaury (*Centaurea Phrygia* L.), and globeflower (*Trollius europaeus* L.). The plant cover is dense, multilayered, and with a developed architecture formed by branching and intertwining herbaceous plants. The meadows in the buffer zone are herb–grass meadows dominated by colonial bentgrass (*Agrostis tenuis* Sibtb.), tufted hairgrass (*Deschampsia caespitosa* Beauv.), timothy-grass (*Phleum pratense* L.), melancholy thistle, and meadow vetchling (*Lathyrus pratensis* L.). The plant-cover characteristics are similar to those in the background zone (considerable density; developed stratification and architecture). The meadows in the impact zone are of a grass type, with absolute dominance of colonial bentgrass and a minor proportion of tufted hairgrass and marsh gilled (*Coronaria flos-cuculi* A.Br.). The plant cover is sparse, without distinct stratification. During the period of study, neither harvesting nor livestock grazing took place in the study areas.

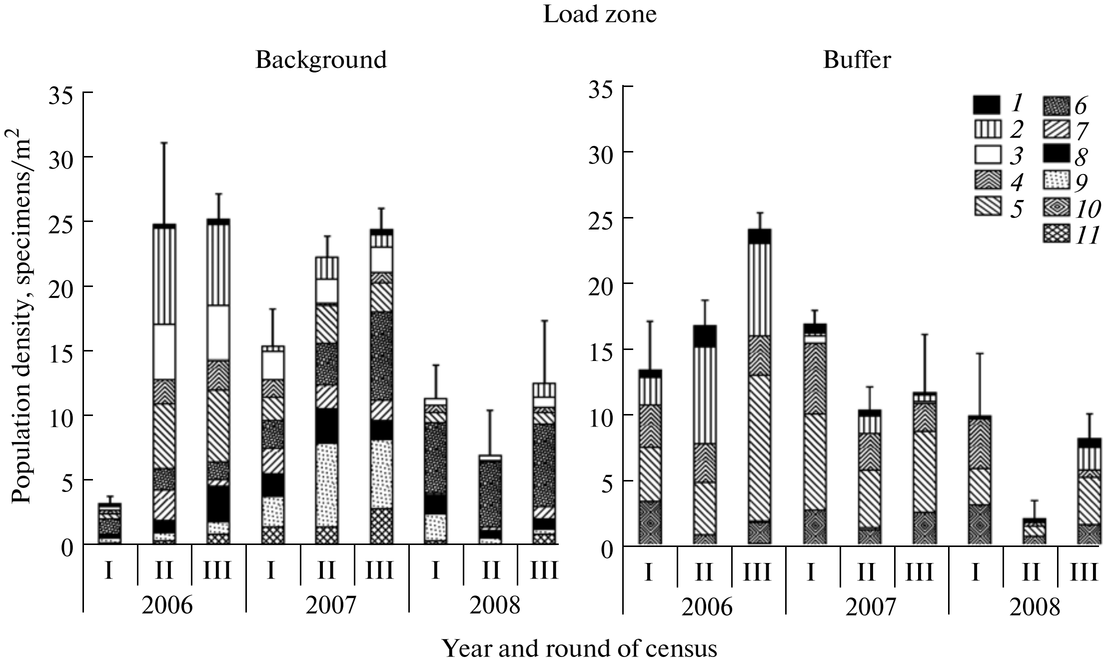
MATERIALS AND METHODS

The material was collected over three years, each year in three rounds coinciding to the second half of summer months. The work was performed using the modified Konakov–Onisimova biocenometer with a square footing 0.25 m^2 in area. Censuses were taken on the sampling plots positioned at the central area of meadow and, thus, distanced from the forest frontier. Three sampling plots 50 × 50 m in size were marked in each load zone and positioned at distances of 100–300 m from each other. Each sample was collected during the one-time placement of a biocenometer on a sampling plot, with all the invertebrates inside being subsequently collected. Herbaceous plants trapped in the biocenometer in a sampling plot were cut, and the plant material sample was formed and analyzed separately. The points for installing the biocenometer were chosen randomly, but at intervals of no less than 5 m. The detailed construction of the biocenometer and methodological aspects of material collection have been described earlier (Nesterkov, Vorobeichik, 2009). Ten samples per sampling plot were taken during each round. Thus, a total of 810 samples (270 each year) were taken and 1917 specimens of terrestrial mollusks were collected over the entire study period. For the samples of plants (total of 810), the total air-dry weight and the total weight of graminoids (grasses and sedges) were measured with 0.1-g accuracy. Mathematical treatment included a nonparametric multivariate analysis of variance (Scheirer–Ray–Hare test (H) based on (Sokal, Rohlf, 1995) and a nonparametric correlation analysis (Spearman's rank correlation coefficient). The community structure was analyzed with *Chekanovskii–Sorensen's* similarity indices (Ics) for quantitative data (form b based on (Pesenko, 1982). The analysis of mollusks community structure was carried out using the most abundant species (91.18–99.35% of the total abundance). Numerically insignificant species (*Discus ruderatus* (Feruss.), *Euconulus fulva* (Mull.), *Euomphalia strigella* (Drap.), *Punctum pygmaeum* (Drap.), *Vallonia costata* (Mull.), *Vitrina pellucida* (Mull.), and *Carychium* sp.) were not examined in this paper. Along with the specimens of mollusks whose taxonomic identity needs to be verified, they were classified as "others."

RESULTS AND DISCUSSION

Mollusks are entirely missing in the meadow grass cover of the impact zone. The abundance of mollusks decreases in a statistically significant way while moving from the background zone to the buffer zone (from 16.22 ± 1.87 to 12.65 ± 1.46 specimens/m^2 on average over three years; $H(1;405) = 5.10$; $p = 0.024$). The results of the 3-year observations showed pronounced year to year variations in the abundance of mollusks ($H(2;405) = 32.43$; $p < 0.001$) highly modified by pollution ($H(2;405) = 7.55$; $p = 0.023$). The lowest abundance values were identified in 2008, whereas they were similar in 2006 and 2007. In 2006, the increase in mollusk abundance was identified in the buffer zone, reaching values similar to those in the background zone (figure). The seasonal variation in each observation year was translated in an increase in the total abundance during summer ($H(2;405) = 17.14$; $p < 0.001$). Seasonal dynamics depended on the level of pollution ($H(2;405) = 9.13$; $p = 0.010$) and the census year ($H(4;405) = 22.34$; $p < 0.001$). The difference between the background zone and the buffer zone is marked even under superficial consideration of the most abundant species of mollusks communities (Table 1). The species composition changed as the emission source was approached: *Fruticola fruticum* (Mull.) and *Succinea putris* (L.) disappeared, while *Zonitoides nitidus* (Mull.), which was not found in the grass cover of the nonpolluted areas, emerged. However, this species is a hydrophilic one and is drawn towards the humid zones that are found in the depressed topographic areas on one of the sampling plots of the buffer zone; thus, its presence is hardly conditioned by pollution. The proportions of most species found on both studied areas are considerably different. *Columella columella* (G.Mart), *Cochlicopa* sp., and *Vertigo* sp. are abundant in the background pollution zones; slugs *Arion subfuscus* (Drap.), *Euconolus fulva* (Mull.), and *Perpolita hammonis* (Strom.) inhabit the polluted areas. The proportion of slugs *Deroceras agreste* (L.) is rather high and similar in both zones of anthropogenic load.

The example of a smelter in Finland proved that the considerable decrease in the abundance of mollusks close to the emission source may be caused by both



Population dynamics of the most abundant species of mollusks in different load zones ((*1*) *Arion subfuscus*, (*2*) *Deroceras agreste*, (*3*) *Cochlicopa* sp., (*4*) *Euconulus fulva*, (*5*) *Perpolita hammonis*, (*6*) *Fruticicola fruticum*, (*7*) *Columella columella*, (*8*) *Succinea putris*, (*9*) *Vertigo* sp., (*10*) *Zonitoides nitidus*, (*11*) others. (I–III) census rounds; accounting unit, sampling plot, $n = 3$; vertical line shows the error of mean for the total abundance).

direct and indirect effects (Eeva et al., 2010). The first group includes the direct toxic effect of industrial pollutants, mainly heavy metals and sulfur oxides. On the other hand, the indirect effect of pollutants is translated by the modifications of the living environment, i.e., the meadow grass cover. Its deterioration may cause both the modification of the hydrothermal regime of a station and the depletion of mollusk forage reserves. Let us analyze the mentioned causes in this paper.

Table 1. Proportion ratio (%) of the most abundant species and the mean density of mollusk population in different load zones over different census years

Taxon	Load zone and census year					
	background			buffer		
	2006	2007	2008	2006	2007	2008
Arion subfuscus (Drap.)	1.50	0.65	–	5.88	3.07	4.58
Deroceras agreste (L.)	26.07	4.95	3.46	30.39	5.46	9.15
Cochlicopa sp.	16.54	9.68	5.63	–	1.71	0.65
Euconulus fulva (Mull.)	8.27	3.66	3.03	16.67	26.28	22.22
Perpolita hammonis (Strom)	20.80	11.40	6.06	35.29	45.73	35.29
Fruticicola fruticum (Mull.)	7.77	19.57	54.98	–	–	–
Columella columella (G.Mart.)	5.76	8.82	4.33	0.25	–	–
Succinea putris (L.)	7.27	9.46	9.09	–	–	–
Vertigo sp.	3.76	23.01	9.96	–	0.34	–
Zonitoides nitidus (Mull.)	–	–	–	10.78	17.41	27.45
Others	2.26	8.82	3.46	0.74	–	0.65
Mean density, specimens/m^2	17.73 ± 4.09	20.67 ± 1.79	10.27 ± 2.30	18.13 ± 2.08	13.02 ± 1.88	6.80 ± 2.08

Blank stresses the absence of species; mean value and the error of mean are indicated for the density; accounting unit: census round × sampling plot, $n = 9$.

Table 2. Concentration of mobile forms of heavy metals and pH in the upper (0–5 cm) soil layer of the meadow in different load zones

Index	Load zone		
	background (n = 15)	buffer (n = 20)	impact (n = 25)
Concentration, μg/g:			
Cu	22.98 ± 0.85	376.10 ± 36.53	827.55 ± 35.72
Pb	21.59 ± 1.03	127.23 ± 16.87	288.04 ± 15.97
Cd	0.79 ± 0.03	2.55 ± 0.17	2.78 ± 0.19
Zn	27.74 ± 1.50	62.49 ± 3.46	87.44 ± 3.47
pH_{water}	5.42 ± 0.03	5.60 ± 0.17	5.16 ± 0.02

Metals were extracted with the ammonium acetate buffer with pH = 4.8; the samples were collected in 1999–2000. Mean value and the error of mean are indicated; accounting unit, sample; n, number of samples. The data were furnished by E.L. Vorobeichik, Doctor of Biology (Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences).

Heavy metals and their compounds are the most thoroughly studied anthropogenic pollutants in terms of their effects on mollusks. The capacity of mollusks to accumulate considerable amounts of heavy metals without damage to their organism has been mentioned plenty of times (Hopkin, 1989; Rabitsch, 1996). Terrestrial mollusks assimilate about 60% of Zn, 68% of Cd, 43% of Pb, and 90% of Cu via consumption of food, lead being the leader in terms of metabolic regulation: its concentration in tissues is always considerably lower than that in food (Laskowski, Hopkin, 1996). Along with the ability to accumulate heavy metals without damaging themselves, mollusks are capable of exuding them with feces (Brooks et al., 1992) and mucus (Ireland, 1979); bacteria inhabiting the alimentary canal can also diminish the toxic effect of metals (Brooks et al., 1992). On the other hand, products used to eliminate mollusks for agricultural purposes were based on Cu, Sn, and Zn. However, the effect in this case was achieved due to the mollusks' refusal to consume the treated substrate and their death by starvation afterwards (Hopkin, 1989). A high concentration of heavy metals in a ration may still cause developmental disorders and even the death of mollusks following intoxication. However, such data were achieved in laboratory experiments, where there was no choice of forage substrate (Boyd, Jhee, 2005).

The given examples lead us to assume that terrestrial mollusks can, in theory, inhabit areas highly polluted by heavy metals. Shell-bearing mollusks were indeed identified 1 km from a smelter in England (Hopkin, 1989), as well as 250 m from a Pb–Zn smelting plant in Austria (Rabitsch, 1996). In the latter case, the author specified the mean concentrations of heavy metals in soil (μg/g): Cu, 100.2; Pb, 2440.0; Cd, 23.6; and Zn, 2743.0. Populations of shell-bearing mollusks were also found in northern France next to a liquidated smelter (concentrations in soil (μg/g): Pb, 1201.6; Cd, 21.3; Zn, and 1497.3) (Fritsch et al., 2011). It should be specified that the concentration of Cu is higher in soils of the impact zone meadows, while the content of Pb, Cd, and Zn is considerably lower than that registered in the mentioned papers (Table 2). Similar evidences exist with regard to pollution sources of a different nature. Thus, slugs *Arion ater* L. were found in close proximity to the Pb–Zn mine in Wales (Ireland, 1979) and next to the zinc mine in Basque Country (concentrations in soil (μg/g): Cu, 26.18; Pb, 321.75; Cd, 14.26; and Zn, 6981.99 (Zaldibar et al., 2008).

The abovementioned examples may be interpreted in such a way that the toxic effect of heavy metals per se does not constitute a reason for the disappearance of mollusks in the MUCS impact zone. All cited papers examine industrial facilities that do not practice the initial metal melting, as opposed to the MUCS, and, therefore, do not entail the acidification of soil with SO_2 and SO_3 hydration products. A decrease in pH of soil (Table 2) leads to an increase in the solubility of salts of heavy metals and stimulates their mobility in abiotic environments. This, in turn, results in an interaction between emission components and increases their toxic effect on all biotic components (Vorobeichik, 2003b). Moreover, terrestrial mollusks may be considerably affected by the increase in Ca mobility in acidified environments and Ca depletion from the upper soil horizons. It was revealed that Ca concentration in the background zone litter is 45 mg-eq/100g of soil; in the buffer zone it was 31 and in the MUCS impact zone it was only 14 (Kaigorodova, Vorobeichik, 1996). Calcium constitutes the main ingredient of the shell: its absence in soil leads to a considerable decrease in the abundance of mollusks (Wareborn, 1969). Ca is also part of the digestive gland basal-cell pellets responsible for the storage of a wide range of heavy metals, including Zn, Cu, and Pb (*The biology of terrestrial molluscs*, 2001).

Curiously enough, despite the fact that the mollusks living by the MUCS disappear both from grass cover and the forest litter (Vorobeichik et al., 1994; Vorobeichik et al., 2007), they occasionally appear in the ration of nestlings in the impact zone (Bel'sky et al.,

Table 3. Correlation between the total abundance of mollusks and the phytomass of meadow plants in different space and time scales

Year	Grass-cover fraction	In different load zones		In entire area
		background	buffer	
2006	Graminoids	–0.03	–0.17	–0.08
	Nongraminoids	0.73*	0.35	0.56*
	All plants	0.55	0.09	0.40
2007	Graminoids	0.34	–0.25	–0.38
	Nongraminoids	0.59	–0.37	0.44
	All plants	0.68*	–0.4	–0.08
2008	Graminoids	–0.38	–0.82**	–0.55**
	Nongraminoids	0.42	0.55	0.45
	All plants	0.02	–0.42	–0.23
Over the entire period	Graminoids	–0.22	0.07	–0.19
	Nongraminoids	0.63***	0.48*	0.58***
	All plants	0.32	0.44*	0.34**

Significance level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; accounting unit: sampling plot (in different load zones: $n = 9$ in different years and $n = 27$ over the entire period; in the entire area, $n = 18$ in different years and $n = 54$ over the entire period).

1998). By all appearances, small populations of particular species (*Discus ruderatus* (Feruss.) and *Perpolita hammonis* (Strom)) can still live next to the smelter. This indicates that at least some species do not fall into the category of those having entirely disappeared; one should rather speak about the decrease in their abundance to extremely low values which can not be revealed using standard methods.

The strengthening of the heavy-metal toxic effect coupled with the acidification of the environment negatively affects all biotic components (Vorobeichik et al., 1994). Along with terrestrial mollusks, the entire range of invertebrate taxa disappears completely next to the MUCS: Lumbricidae, Enchytraeidae, Diplopoda, and Geophilidae disappear from the soil mesofauna composition (Vorobeichik et al., 1994; Vorobeichik et al., 2007); Coreidae and Phalangiidae vanish from the grass-cover layer (Zolotarev, 2009; Nesterkov, 2009).

The second possible cause of mollusk disappearance is related to the living environment modification following anthropogenic pollution. It is known that species composition, diversity, and structural organization of herbaceous plants are the main determinants of structure and variety of the invertebrates population (Woodcock., Pywell, 2010). We identified 54 species of herbaceous plants in nonpolluted areas and only 46 in the buffer load zone. The proportion of graminoids (grasses and sedges) increased: from 27.8 ± 2.5% (over about three census years) in the background zone to 43.8 ± 2.6% in the buffer zone; at the same time, the values of the aboveground phytomass in the studied areas were similar (265.65 ± 13.62 and 280.63 ± 14.72 g/m^2, respectively). Revealingly, only four plant species were found in the grass cover of the impact zone (1 km from the source emission), where the mollusks were not identified; the proportion of graminoids accounted for 98.8 ± 0.4%, while the total phytomass decreased by 1.6 times in comparison with the background zone values (166.33 ± 9.12 g/m^2). Thus, the modification of the herbaceous-plant community structure following the pollution is quite pronounced in the meadows.

A direct correlation was revealed between the total abundance of mollusks and the total phytomass, as well as the total phytomass of plants that do not belong to grasses and sedges; however, the correlation is inverse between mollusks and graminoids phytomass (Table 3). A direct correlation between mollusks and the nongraminoid grass-cover fraction was well pronounced across the study area throughout the study. In case of graminoids, the correlation is inverse and manifests itself during particular study years when analyzing particular load zones.

The changes in the meadow grass cover in the MUCS environs have been thoroughly described (Vorobeichik et al., 1994; Khantemirova, 2004). A similar situation was identified in meadow ecosystems in England polluted with heavy metals (Cu and Cd), where the grass cover of the area most highly exposed to pollutants was dominated by species of the genus *Agrostis* and *Festuca* (Hunter et al., 1987). The increase in the proportion of graminoids characterized by vertical linear organization, poorly marked branching of stems, and narrow lamina considerably affects the spatial organization of the herbaceous-plant layer. This will result in the disappearance of multilayered structures of intertwining shoots and leaves that create a developed three-dimensional space. The complex vegetation architecture hinders the free circulation of the air, which creates the microclimatic gradient. It is then reasonable to assume that the fluctuations in temperature and humidity would be more pronounced in the grass cover with simplified architecture consisting mainly of grasses and sedges. Meanwhile, the grass-cover moisture regime (along with the taxonomic variety of plants) of steppe ecosystems providing similar conditions to those having been described constitutes an essential factor that determines the structure of the invertebrate community (Wenninger, Inoue, 2008). It should be noted that it is the nature of the moisture regime that determines a great many aspects of the vital activity of terrestrial mollusks, in particular, their diurnal migrations in the herbaceous layer (Mel'nichenko, 1936). Arguably, this is the reason for the inverse correlation between the abundance of mollusks and the phytomass of graminoids: an increase in the proportion of grasses and sedges leads to the simplification of the grass-cover architecture, which, in turn, makes the moisture conditions less favorable for mollusks. This assumption confirms the generally direct nature of correlation between the abundance of mollusks and the total phytomass, as well as the nongraminoid phytomass fraction. One can also assume that an unfavorable moisture regime in grass and sedge communities hinders slugs, which are less sensitive to the lack of calcium than shell-bearing mollusks from polluted areas.

CONCLUSIONS

The present paper is part of a comprehensive study into the invertebrate communities inhabiting the herbaceous layer of the MUCS operating area. Terrestrial mollusks disappear completely from the grass cover close to the smelter following the pollution. The abundance of mollusks decreases in the moderately polluted area compared to the background zone; the difference between their community species structures in the background and the buffer zones is marked even under superficial consideration. The correlation between the abundance of mollusks and the phytomass of grass cover is revealed in both load zones: it is positive with the total phytomass and the nongraminoids phytomass and negative with the grass and sedge phytomass.

The toxic effects of MUCS emissions on the mollusks communities may be divided into direct and indirect ones. The first is conditioned by the combined effect of environment acidification (with SO_2 and SO_3) and heavy metals, as well as depletion of calcium from soil; the second effect is translated by the reduction in species composition of the meadow grass cover and the increase in proportion of graminoids as the emission source is approached. Apparently, the combination of two types of effects results in the elimination of mollusks from the herbaceous layer, a trend which is not observed in case of pollution of the environment with only heavy metals.

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