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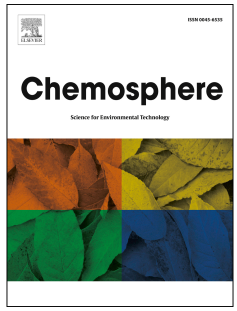

The elemental composition of small mammals in a commercial orchard–meadow system

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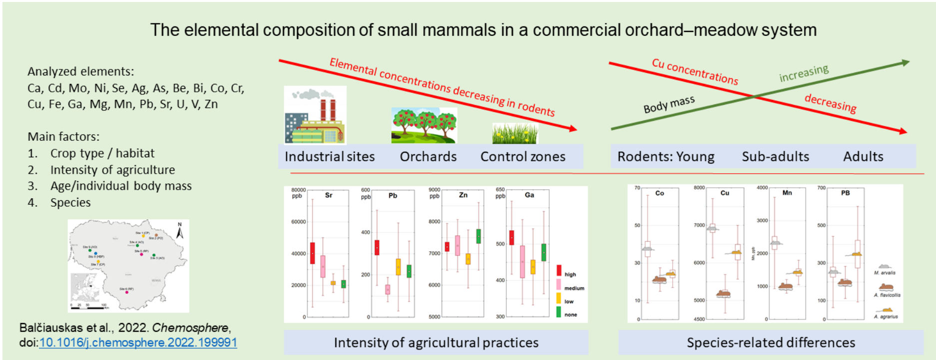
The elemental composition of small mammals in a commercial orchard–meadow system
Analyzed elements:
Ca, Cd, Mo, Ni, Se, Ag, As, Be, Bi, Co, Cr, Cu, Fe, Ga, Mg, Mn, Pb, Sr, U, V, Zn
Main factors:
1. Crop type / habitat
2. Intensity of agriculture
3. Age/individual body mass
4. Species
Balčiauskas et al., 2022. Chemosphere, doi:10.1016/j.chemosphere.2022.199991
Elemental concentrations decreasing in rodents
Industrial sites
Orchards
Control zones
Cu concentrations
decreasing
Body mass
increasing
Rodents: Young
Sub-adults
Adults
Intensity of agricultural practices
Species-related differences

The elemental composition of small mammals in a commercial orchard–meadow system

Linas Balčiauskas[a], Žilvinas Ežerinskis[b], Vitalijus Stirkė[a], Laima Balčiauskienė[a], Andrius Garbaras[b], Vidmantas Remeikis[b]

[a]Nature Research Centre, Akademijos 2, 08412 Vilnius, Lithuania; linas.balciauskas@gamtc.lt; vitalijus.stirke@gamtc.lt; laima.balciauskiene@gamtc.lt

[b]Center for Physical Sciences and Technology, Saulėtekio av. 3, 02300 Vilnius, Lithuania; zilvinas.ezerinskis@ftmc.lt; andrius.garbaras@ftmc.lt; vidmantas.remeikis@ftmc.lt

* Correspondence: Linas Balčiauskas; Nature Research Centre, Akademijos 2, 08412 Vilnius, Lithuania; linas.balciauskas@gamtc.lt;Tel.: +370-685-34141

Abstract

We analyze concentrations of various elements in small mammals from commercial orchards and berry plantations and test differences between them depending on species, individual body mass, age, gender, type and age of crop and intensity of applied agricultural measures. Skinned front legs (muscle and bones) were used to register the presence and concentration of Ca, Cd, Mo, Ni, Se, Ag, As, Be, Bi, Co, Cr, Cu, Fe, Ga, Mg, Mn, Pb, Sr, U, V and Zn. The most abundant species were common vole (*Microtus arvalis*), yellow-necked (*Apodemus flavicollis*) and striped field (*A. agrarius*) mice. The maximum recorded concentrations exceeded the minimums by 1.7–7.7 times in Mg, Zn, Cu, Ga, Ni and Ca, and 11.0–23.2 times in Mn, Be, Mo, Co, Sr, V, Pb and As. The hypothesis that the use of fertilization and pesticides in commercial orchards should induce differences in the elemental concentrations between crop areas and control habitats was confirmed by the higher concentrations of Cu, Mn, Bi, Co, Cr, Fe, Ni, Sr and Pb in rodents from the crop areas. Spatially controlled generalized linear mixed model confirmed the cumulative influence of species and crop, explaining 30–80% of the distribution of Ca, Ni, Co, Cu, Ga, Mn, Pb and U. The effect of species and the age of the individual was significant for Ni, Co, Cu, Fe, Ga, Mn and Pb, while effect of gender was not expressed. Depletion of Cu in older individuals was found in all three species. With species as a grouping factor, the effect of crop type and the intensity of agricultural practices were significant factors in the accumulation of Ca, Ni, Co, Cu, Ga, Mn and Pb, while effect of crop age was not expressed. The obtained elemental concentrations in rodents indicated orchards to be cleaner than heavy polluted areas.

Keywords: rodents; agricultural areas; elemental composition; pollution

1. Introduction

Farming activities and other anthropogenic activities in the agro-ecosystem and adjacent habitats have a negative impact on the environment and ecological status (Moss, 2008). Although metals are naturally present in the environment (Pereira et al., 2006), their levels in various parts of the environment are significantly increased by human activities, including by industrial and agrochemicals such as pesticides, fertilizers, herbicides and growth regulators used in the agro-industry, these leading to increased environmental pollution (Mg'ong'o et al., 2021). Such anthropogenic activities cause the large-scale spread of metals into the environment that then accumulate in animal organs and circulate at various trophic levels (Marcheselli et al., 2010). However, although commercial orchards are an important field of agriculture, not all aspects of their ecology have attracted the attention of scientists, this true not only in Lithuania but also in other Baltic countries (Balčiauskas et al., 2019). We present the first investigation into the accumulation of chemical elements in small mammals inhabiting the territories of commercial orchards and berry plantations.

Small mammals are not only part of the food chain in the agro-ecosystem (Fischer et al., 2018), but are also pests of many crops (Fischer and Schröder, 2014; Hansen et al., 2016). Their negative effects are not limited to damage to agricultural crops, but also to their role in the distribution of weed seeds and to the fact that they are carriers of various pathogens (Luque-Larena et al., 2015; Balážová et al., 2021). It is also known that small terrestrial mammals are the most appropriate biological monitors for heavy metal pollution when studying environmental contamination (Petkovšek et al., 2014). The use of small mammals is particularly appropriate in the monitoring of heavy metals due to their high abundance, wide distribution, limited home range and short lifespan, as well as other factors such as their ease of collection (Marcheselli et al., 2010). Small mammals are more exposed to environmental pollutants than large mammals due to their small body size and rapid metabolism (Sánchez-Chardi et al. 2007; Levengood and Heske, 2008). Wild animals including insects (Aydogan et al., 2017), amphibians (Qureshi et al., 2015), fish (Yi et al., 2011), reptiles (Nasri et al., 2017), birds (Kral et al., 2017) and even large mammals (Lehel et al., 2016; Neila et al., 2017; Brand et al., 2020) are often used to monitor the environmental impact of heavy metal pollutants (Damek-Poprawa and Sawicka-Kapusta, 2003). However, there has been no research in commercial orchards or berry plantations, merely in studies of orchard soil contamination (Dong et al., 2021). An uncontaminated arable land ecosystem is a key requirement not only for food safety, but also for human health (Zeng et al., 2019). Heavy metals, especially toxic chemical elements including Pb, As, Cd, Hg and Cr, are considered to be major sources of soil contamination and have received considerable attention due to their persistence and strong toxicity (Singh and Kalamdhad, 2011). The uptake of heavy metals in plants and their further accumulation in the food chain can pose a significant risk to human and animal health. To reduce disease and increase yields, farmers use pesticides and fertilizers that contain heavy metals that directly affect the quality and safety of the fruit, which can pose a risk to human health and safety (Kılıçel and Dağ, 2006;Yan et al., 2018).

In Lithuania, there are only a few studies on the accumulation of heavy metals and trace elements in small mammals (Mažeikytė and Balčiauskas, 2003; Jasiulionis et al., 2018). The accumulation and content of heavy metals in small mammalian tissues in agricultural habitats in Lithuania is not known. Therefore, the main aim of this study was to collect the first data on the concentrations of various elements in small mammals from commercial orchards and berry plantations to use as reference for further investigations and for comparison of those from sites with high biological pollution with published results.

We hypothesized that if the concentration of chemical elements in the tissues of small mammals is related to fertilization and the use of pesticides in commercial orchards, then there should be differences in concentrations between crop areas and control habitats. Additionally, we tested whether concentrations differed depending on the small mammal species, this related to different functional groups (herbivores vs granivores), crop type, crop age and intensity of used agricultural measures (implying that concentrations should be higher in older habitats and habitats with higher intensity of treatment). For the dominant rodents, we also checked whether there were differences in concentrations by animal body mass, age or gender.

2. Material and methods

2.1. Study site

For this study, the small mammal sample was selected from nine study sites across Lithuania (northern Europe) in 2019. The investigated habitats included apple and plum orchards, currant, raspberry and highbush blueberry plantations, these further referred to as crop areas, along with control habitats (mowed meadow, unmoved meadow or forest ecotone) at nearby vicinities to each orchard. These sites were from northern (sites 1, 2) eastern (site 3), central (sites 4, 5), southern (site 6) and western (sites 7–9) Lithuania (Fig. 1).

Agricultural practices included grass mulching, mowing, soil scarification and application of plant protection agents and rodenticides. Crop age was defined as young, medium-aged or old, while the intensity of agricultural practices as high (frequent application of two or more of the above-listed measures, including rodenticides), medium (two listed measures during the crop season, once or twice during the season) or low (removal of grass only). Details may be found in Balčiauskas et al., 2019.

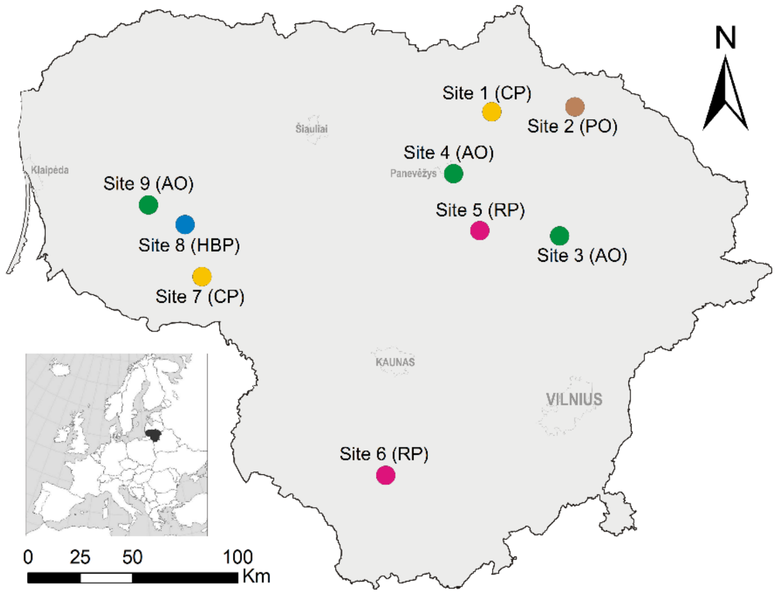

Fig. 1. Investigation sites and crops (AO – apple orchard, PO – plum orchard, RP – raspberry plantation, CP – current plantation, HBP – high blueberry plantation).

2.2. Small mammal sampling

Small mammals at each site were snap-trapped in the summer and autumn months with a total trapping effort of 4275 trap days. We trapped 167 individuals of eight small mammal species, the dominants being common vole (*Microtus arvalis*), yellow-necked mouse (*Apodemus flavicollis*) and striped field mouse (*Apodemus agrarius*), comprising 49.1%, 34.1% and 10.8% of all trapped individuals, respectively. Other species were not numerous, represented by one to four individuals. Trapping, species identification and aging details are presented in Balčiauskas et al., 2019.

2.3. Study of chemical elements

For the study of elemental composition, individuals were randomly selected from the total sample, this including all trapped house mice (*Mus musculus*), common shrew (*Sorex araneus*), water vole (*Arvicola amphibius*) and two out of four root voles (*Microtus oeconomus*). The most abundant species, *M. arvalis*, *A. flavicollis* and *A. agrarius,* were down-sampled (Table S1).

We used the skinned front leg (muscle and bones) to register the presence and concentration in ppb, particles per billion, of the following 21 elements in the body: Ca, Cd, Mo, Ni, Se, Ag, As, Be, Bi, Co, Cr, Cu, Fe, Ga, Mg, Mn, Pb, Sr, U, V and Zn. Before analysis, individual samples were placed in separate Eppendorph tubes, labelled and stored in a freezer at a temperature below –18 °C. All mouse legs were freeze dried and afterwards were mechanically grinded to a fine powder and dissolved following the standard procedure.

The homogenic powders of mouse legs were scaled to a 0.3±0.0001g and pored to high pressure quartz vials. 5 ml of nitric acid (Suprapur, 65%, Merck Germany) and 0.5 ml hydrochloric acid (37%, ROTI®Quant) were added for digestion process which took place in a tightly sealed pressure vials at the microwave oven of sample preparation (Anton Paar Multiwave). Digestion process lasted 35 min at 75 bars pressure and 300 °C temperature. Later, samples were diluted with ultrapure water. For ICP-MS calibration multi element standard solution MERCK VI was used (ICP Multi Element Standard Solution VI CertiPur). Measurements were carried out using double focusing high precision mass spectrometer ELEMENT2 (ThermoFisher, Germany), parameters presented in the Table S2.

2.4. Statistical analysis

We tested if concentrations were distributed normally using Kolmogorov-Smirnov's D test. Based on mixed conformity to normal distribution (concentrations in 13 out of 21 elements were distributed normally, see Table 1), parametric tests were further applied. Elemental concentrations in the body were presented as the central position (mean ± standard error) and range (minimum and maximum) without respect to the dominant species and habitat (Table 1).

To find the general pattern of elemental variability, we used generalized linear mixed model (GLMM) to find the influence of the crop (apple and plum orchard, raspberry and currant plantation) and species (*M. arvalis*, *A. flavicollis* and *A. agrarius*) as categorical factors on the dependent parameters (Ca, Cd, Mo, Ni, Ag, Co, Cr, Cu, Fe, Ga, Mg, Mn, Pb, Sr, U, Zn concentrations). Se, As, Be, Bi and V were not used due to insufficient sample size. To control spatial data variability, we used the part of the country (northern eastern, central, southern and western) as the continuous predictor. Wilks lambda was used to test the significance of the model and eta-squared for the influence of the categorical factor.

Species-related differences (between species, males and females and between age groups) were tested with parametric ANOVA, using Wilk's lambda test for significance and crop as a grouping factor. Additionally, we calculated correlations between concentrations and body mass of an individual using the Pearson correlation coefficient. Habitat-related differences (between crops, crop ages and intensities of agricultural practices) were tested using species as a grouping factor. Differences between groups were evaluated with post-hoc Tukey test and differences between pairs of variables with Student t-test. The confidence level was set as $p < 0.05$ (we interpret $p < 0.10$ as indicating the trend, which is non-significant).

3. Results

The central positions of the elemental concentrations of the most abundant small mammal species, *M. arvalis*, *A. flavicollis* and *A. agrarius*, are presented in Table 1. For species with insufficient sample size, namely *A. amphibius*, *M. oeconomus*, *M. musculus* and *S. araneus*, the ranges of the elemental concentrations are given in Table S3. The dispersion of the concentrations of all elements was high, the maximum recorded concentrations exceeding the minimum ones by 1.7–7.7 times in Mg, Zn, Cu, Ga, Ni and Ca, and 11.0–23.2 times in Mn, Be, Mo, Co, Sr, V, Pb and As. In the rest of the elements, the dispersion of the registered concentrations was even higher (Table 1).

Element	N[a]	Concentration, ppb		Normality	
		Mean±SE	Min–Max	D	p<
Ca	85	633112.8±28611.2	328907.1–2532581.9	0.226	0.001
Cd	54	3.4±0.3	0.1–13.1	0.141	NS
Mo	80	127.1±8.3	31.6–456.6	0.065	NS
Ni	85	5329.7±116.9	2436.4–7889.8	0.065	NS
Se	30	7427.2±1798.1	320.6–50562.8	0.266	0.05
Ag	50	23454.7±20430.9	0.6–1023403.2	0.484	0.001
As	27	502.8±74.9	60.9–1415.7	0.172	NS
Be	20	0.7±0.1	0.1–1.6	0.196	NS
Bi	31	0.8±0.1	0.0–3.1	0.208	NS
Co	86	30.9±2.5	10.1–154.3	0.244	0.001
Cr	86	333.3±26.6	5.6–1594.9	0.118	NS
Cu	86	6328.4±112.3	4174.4–8814.8	0.060	NS
Fe	86	59766.9±29394.2	9021.1–1850951.2	0.488	0.001
Ga	86	473.3±11.9	275.7–808.5	0.076	NS

Mg	86	1787466.4±17644.7	1376650.1–2292156.2	0.062	NS
Mn	86	1813.1±131.4	676.6–7472.1	0.185	0.01
Pb	86	250.4±19.6	60.5–1215.5	0.131	NS
Sr	86	27836.6±2374.5	9257.2–178287.0	0.231	0.001
U	81	7.7±2.5	0.1–179.5	0.364	0.001
V	5	1050.3±520.9	149.7–2960.9	0.300	NS
Zn	86	7165.5±100.3	5445.7–11066.1	0.130	NS

Table 1. Central position (mean±SE, range) and conformity to the normal distribution (Kolmogorov-Smirnov's D) of elemental concentrations (ppb) in the body of the most abundant small mammals from commercial orchards irrespective of species and habitat.

[a] – in cases where N < 86, the missing values were lower than the LOD (lowest level that can be detected)

Spatially controlled GLMM confirmed the cumulative influence of species (Wilks lambda, $\lambda = 0.089$, $F_{32,40} = 2.95$, $p < 0.001$) and crop ($\lambda = 0.082$, $F_{48,60} = 1.66$, $p < 0.05$), but no influence of part of the country ($\lambda = 0.410$, $F_{16,20} = 1.80$, $p = 0.11$). The cumulative influence of the species and crop was significant and explained 30–80% of the distribution of several elements: Ca ($F_{6,35} = 3.88$, $p < 0.004$, $R^2 = 0.40$), Ni ($F = 3.88$, $p < 0.004$, $R^2 = 0.40$), Co ($F = 3.15$, $p < 0.02$, $R^2 = 0.35$), Cu ($F = 23.01$, $p < 0.001$, $R^2 = 0.80$), Ga ($F = 3.65$, $p < 0.01$, $R^2 = 0.38$), Mn ($F = 5.10$, $p < 0.001$, $R^2 = 0.47$), Pb ($F = 2.98$, $p < 0.02$, $R^2 = 0.34$) and U ($F = 2.47$, $p < 0.05$, $R^2 = 0.30$). A trend was found for Sr ($F = 2.11$, $p = 0.08$, $R^2 = 0.27$).

3.1. Species-related aspects of elemental concentrations

Depending on the crop, a significant effect of species ($\lambda = 0.119$, $F_{32,36} = 2.14$, $p < 0.02$) and age of the individual ($\lambda = 0.142$, $F_{32,36} = 1.86$, $p < 0.05$) on the variance of elemental concentrations was found, while that of the gender was not expressed ($\lambda = 0.482$, $F_{16,18} = 1.21$, NS). The cumulative influence of species, age and gender was significant for Ni ($F_{8,33} = 4.94$, $p < 0.001$, $R^2 = 0.55$), Co ($F = 2.26$, $p < 0.051$, $R^2 = 0.35$), Cu ($F = 15.24$, $p < 0.001$, $R^2 = 0.79$), Fe ($F = 2.81$, $p < 0.02$, $R^2 = 0.41$), Ga ($F = 3.67$, $p < 0.005$, $R^2 = 0.47$), Mn ($F = 3.82$, $p < 0.01$, $R^2 = 0.48$) and Pb ($F = 2.25$, $p < 0.05$, $R^2 = 0.35$).

Concentrations of Mo, Be, Co, Cu and Mn in *A. flavicollis* were significantly (Tukey HSD, $p < 0.05$) lower than those in *M. arvalis*, while Mn, Cu and Pb were lower than those in *A. agrarius*. *A. flavicollis* was characterized by the highest concentrations of Ga, while *A. agrarius* by the smallest concentrations of Mg (Fig. 2).

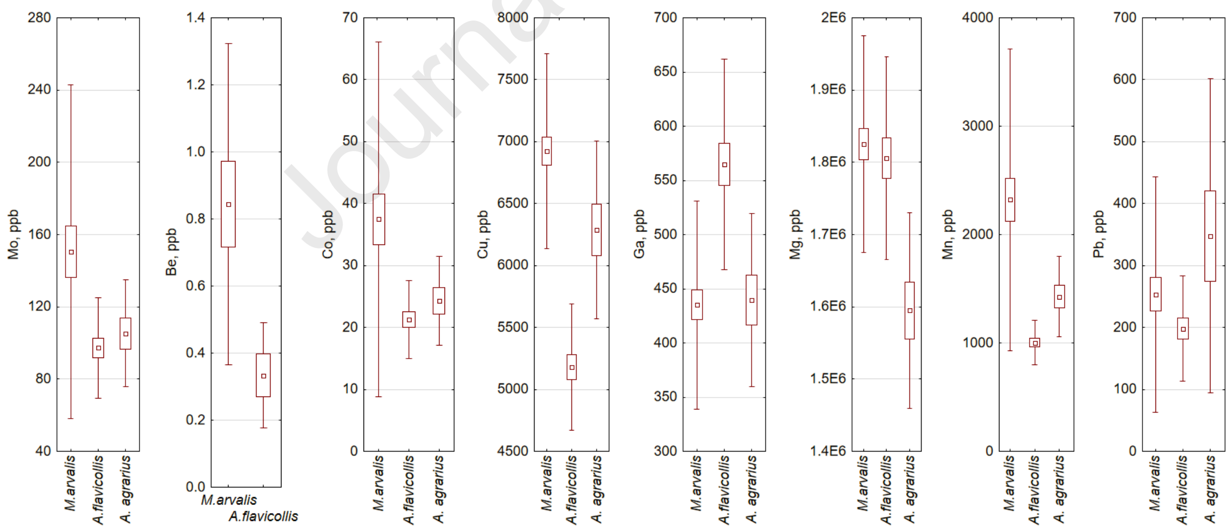

Fig. 2. Differences in elemental concentrations in the most abundant small mammal species. Central point shows average values, box – SE, whiskers – SD.

In *M. arvalis*, the concentrations of Ni and Mg were significantly (HSD, $p < 0.002$) higher in adult animals; adults of *A. flavicolis* had the highest concentrations of Fe (Fig. 3a). At a trend level (HSD, $p < 0.10$), the concentration of Sr in adults and subadults of *A. flavicollis* was higher than that in juveniles. Juveniles of *A. agrarius* were characterized by a trend of higher Cu concentration.

The only significant difference according to gender was in *A. flavicolis*, where the concentration on Ni in females exceeded (HSD, $p < 0.01$) that in males (Fig. 3a). At a trend level (HSD, $p < 0.10$), females of *M. arvalis* had higher concentrations of Mg and females of *A. flavicolis* had higher concentrations of Mo, Co and Mn, while females of *A. agrarius* had higher concentration of Cu. In all other elements, the concentrations in males and females did not differ.

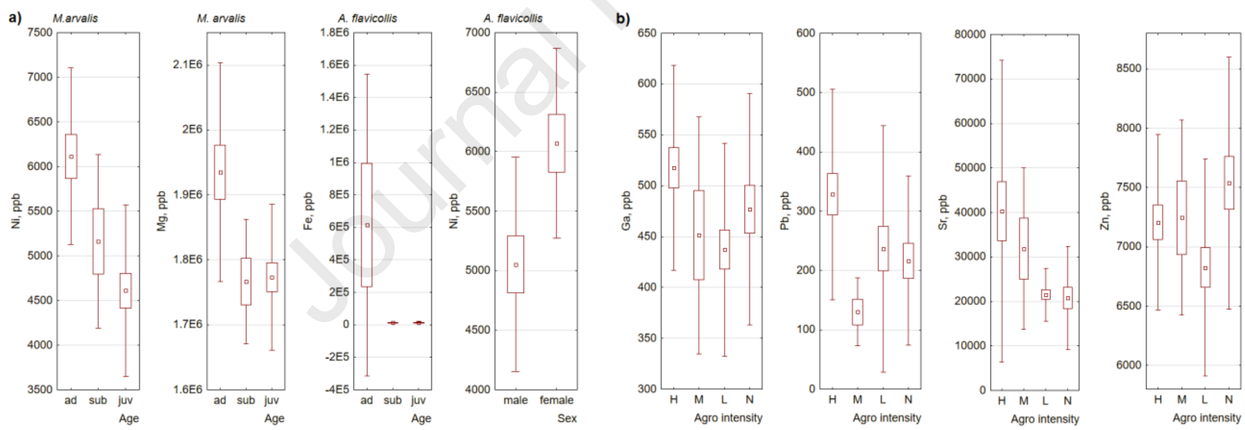

Fig. 3. Differences in elemental concentrations depending a) on the age and gender of the most abundant small mammal species, and b) on the intensity of agricultural practices (H – high, M – medium, L – low intensity, N – none, the control habitat). Central point shows average values, box – SE, whiskers – SD.

Negative correlations of Cu concentration and body mass confirm depletion of this element, significant in all three of the most abundant rodent species (Table 2). By contrast, the concentrations of Ni were higher in the heavier individuals, namely adults (Table 2, Fig. 3a). The same trend was found in Fe concentration, this increasing in adults and best expressed in *A. flavicollis.* Zn and Sr concentrations in *A. agrarius*, Ca concentrations in both Apodemus species and Cd, Mo and Mg concentrations in *M. arvalis* were also higher in adults.

Element	*M. arvalis*	*A. flavicollis*	*A. agrarius*
Ca		0.41*	0.72**
Cd	0.37*		
Mo	0.37*		
Ni	0.65***		0.65*
Se	-0.36^		
Cr		0.38^	
Cu	-0.47**	-0.75***	-0.76**
Fe		0.45*	
Mg	0.46**		
Mn		-0.39^	
Sr			0.77**
U		-0.41^	
Zn			0.70*

Table 2. Correlations (Pearson's r) of elemental concentrations and body mass in the three most abundant small mammal species. * denotes $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^ denotes trend with $p < 0.1$, non-significant correlations are not shown.

3.2. Habitat-related aspects of elemental concentrations

With species as a grouping factor, significant effects of crop ($\lambda = 0.294$, $F_{16,19} = 2.85$, $p < 0.02$) and intensity of agricultural practices ($\lambda = 0.328$, $F_{16,19} = 2.43$, $p < 0.05$) on the variance of elemental concentrations were found, while that of crop age was not expressed. The cumulative influence of these habitat-related factors was significant for Ca ($F_{7,34} = 5.39$, $p < 0.001$, $R^2 = 0.53$), Ni ($F = 3.14$, $p < 0.02$, $R^2 = 0.39$), Co ($F = 2.65$, $p < 0.03$, $R^2 = 0.35$), Cu ($F = 19.80$, $p < 0.001$, $R^2 = 0.80$), Ga ($F = 13.18$, $p < 0.02$, $R^2 = 0.40$), Mn ($F = 4.25$, $p < 0.002$, $R^2 = 0.47$) and Pb ($F = 4.01$, $p < 0.003$, $R^2 = 0.45$), while a trend was found for U ($F = 2.07$, $p < 0.08$, $R^2 = 0.30$).

Comparing the elemental concentrations in the three most abundant rodents trapped in crops with those trapped in control areas, we did not find unambiquous trends (Table 3). In *M. arvalis,* 10 elements were found in greater concentration in the rodents from crop areas: Cu and Mn being significantly higher, Bi, Co, Cr and Fe higher at a trend level and concentrations of five more elements at least 1.5 times higher. Bi and V were better represented in the rodents from the control meadows at a trend level, while concentrations of Zn differed significantly.

In the crop-trapped *A. agrarius,* the concentrations of Ni were higher significantly, while those of Sr were higher at a trend level, Bi and Pb being at least 1.7 times higher. By contrast, the concentrations of Ag and As were higher in the control-trapped *A. agrarius*.

In the crop-trapped *A. flavicollis*, the concentrations of Ag and Sr were considerably higher, while that of Pb was significantly higher. The concentrations of Se, As, Bi, Fe, Ga and V were considerably higher, while that of U was significantly higher in the control-trapped individuals (Table 3).

Element	*M. arvalis*		*A. flavicollis*		*A. agrarius*	
	Crop	Control	Crop	Control	Crop	Control
Ca	667684.6±59486.6	493394.4±31296.1	653956.1±20450.5	675846.4±32119.5	607519.4±56732.8	553002.0±19786.9
Cd	4.3±0.6	1.9±1.3	2.4±0.3	2.2±0.5	3.8±1.0	3.4±1.4
Mo	146.6±16.1	167.4±28.9	98.9±6.4	91.9±11.6	80.7±10.5	117.7±9.2
Ni	5125.5±191.8	5525.1±331.7	5435.5±222.4	5705.6±449.1	5854.1±519.3	5359.6±255.7*
Se	6266.9±1351.0	4206.0±2606.9	4066.2±1839.7	23097.1±14339.7	2941.0	4351.2±3094.5
Ag	42561.1±37764.3	409.9	1312.0±676.8	690.9±619.4	2.9	38.6
As	540.4±99.9	843.0±317.2	146.9±4.9	303.2±133.5		598.1±246.6
Be	0.9±0.1	0.3	0.2±0.1	0.4±0.1		
Bi	0.9±0.2	2.6^	0.3±0.1	0.6±0.3	0.9	0.5
Co	41.1±4.8	21.3±2.2^	22.1±1.3	18.5±3.1	23.2±3.0	24.9±2.8
Cr	407.1±34.0	268.7±64.4^	271.4±77.0	292.7±76.3	198.1±48.4	281.9±86.6
Cu	7031.1±122.9	6451.2±229.6*	5148.5±115.9	5279.0±232.2	6169.5±446.5	6345.8±237.5
Fe	21682.7±1597.2	14884.0±1108.6^	107133.5±92879.8	318358.9±306518.9	16321.7±597.3	15963.6±1313.4

Ga	432.4±16.2	446.2±21.3	558.1±18.3	585.4±59.9	459.9±41.8	429.8±29.0
Mg	1821243.9±25306.9	1842457.5±32947.8	1797662.3±30849.7	1832646.9±68237.5	1663270.1±72324.2	1560711.0±44431.0
Mn	2511.4±232.0	1474.6±113.9*	1014.0±52.2	965.8±53.8	1378.2±107.5	1452.8±156.1
Pb	255.1±31.6	244.7±49.6	227.6±17.3	105.1±11.4***	505.9±181.9	268.8±53.7
Sr	33620.8±4533.5	27888.3±4601.1	23969.3±2746.4	15613.9±3404.3	28764.2±8495.9	16745.6±2431.4^
U	13.8±4.8	1.4±0.4	1.1±0.1	2.4±0.5**	2.3±0.5	3.2±2.3
V	713.6±323.0	2960.9^		149.7		
Zn	6860.3±133.4	7735.6±492.4*	7237.3±154.6	7518.7±330.5	7739.0±603.7	7327.8±248.7

Table 3. Elemental concentrations (mean±SE, in ppb) in the most abundant small mammals trapped in crop areas and respective control habitats. Differences according Student's t: * denotes $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ^ denotes trend with $p < 0.1$.

Small mammals trapped in currant plantations had the highest concentrations of Co, Cu, Mn and U, while the concentrations of Ga and Zn were the lowest (Fig. 4). Co and Cu concentrations in individuals trapped in raspberry plantations and control habitats were significantly lower than those in currant plantations (HSD, $p < 0.05$ and $p < 0.01$, respectively). The concentration of Ga was significantly higher in individuals from apple orchards, raspberry plantations and control habitats. As for Mn, high concentrations were found in the rodents from apple orchards and currant plantations, while concentrations were low in other crops and control habitats. The concentration of Sr was significantly highest in rodents from apple orchards, exceeding that in all other crops and control habitats (Fig. 4). Between-habitat differences for concentrations of all other elements were not significant.

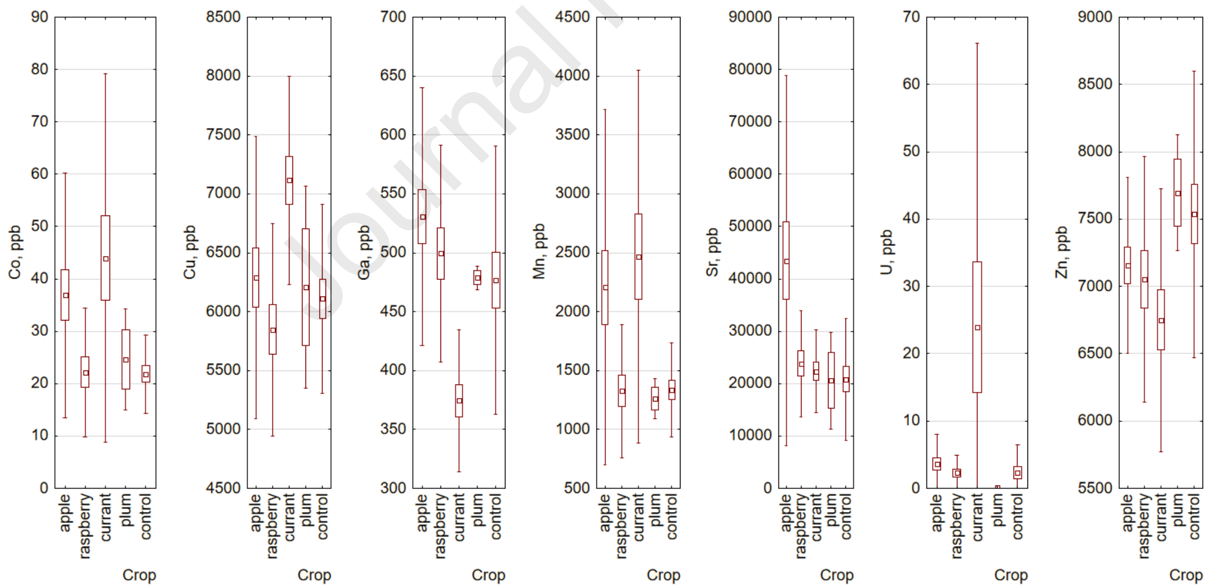

Fig. 4. Differences in elemental concentrations in the most abundant small mammals depending on the habitat. Central point shows average values, box – SE, whiskers – SD.

The intensity of agricultural practices had a significant effect on the concentrations of Ga, Sr, Pb and Zn in small mammals trapped in the respective areas (Fig. 3b). The concentrations of Ga, Pb and Sr in the rodents from crops with a high intensity of agricultural practices significantly exceeded those in crops with less intensive care and control habitats (HSD, $p < 0.05$). As for Zn, the highest concentration in rodents from control areas significantly exceeded only that in rodents from crops with low intensity of agricultural practices ($p < 0.03$). Between-intensity differences for concentrations of all other elements were not significant.

4. Discussion

Humans release chemical elements into the environment every year, many of these being related to agricultural activities (Chitra and Priya, 2020). As the response of mammals to contaminants is mediated spatially, temporally and across media, the element concentrations in their bodies is a realistic indicator of exposure to chemicals (Wren, 1986; Talmage and Walton, 1991; Sánchez-Chardi et al., 2007; Marcheselli et al., 2010; Petkovšek et al., 2014). The exposure to chemicals differs among consumers (e.g., herbivores, omnivores, carnivores) due to the specific ways in which contaminants move through food webs (Smith et al., 2002; Ziętara et al., 2019).

The accumulation of heavy metals and some other chemical elements in small mammals has been well studied in contaminated habitats, mainly in the environment of various mines (Smith and Rongstad, 1982; Erry et al., 2000; Milton et al., 2003; Pereira et al., 2006; Ivanter and Medvedev, 2015; Bortey-Sam et al., 2016; Khazaee et al., 2016; Camizuli et al., 2018; Demir and Yavuz, 2020), but also paper mills (Gajdoš and Janiga, 2015), disposal facilities and dumps (Mertens et al., 2001; Andras et al., 2006), power stations (Jančová et al., 2006; Minami et al., 2006, 2009; Martiniaková et al., 2010, 2012), urbanized sites (Way and Schroder, 1982;

Tifarouine et al., 2021), roadsides (Scanlon, 1987), sites of petrochemical industries, thermal power plants (Ieradi et al., 2003; Petkovšek et al., 2014), smelters (Beernaert et al., 2007; Rogival et al., 2007; Petkovšek et al., 2014; Dimitrov et al., 2016; Kovalchuk et al., 2017), sites of oil extraction (Rodríguez-Estival and Smits, 2016) and cities (Komarnicki, 2000).

A few investigations present data of elemental concentrations in small mammals from natural environments such as wetlands (Sawicka-Kapusta et al., 1990; Torres and Johnson, 2001) and forests (Mažeikytė and Balčiauskas, 2003; Topolska et al., 2004; Beernaert et al., 2007; González et al., 2008) and nationals parks (Zakrzewska et al., 1993). Agricultural areas are insufficiently covered (Marcheselli et al., 2010). Therefore, the values we obtained for the elemental concentrations in the three dominant species of rodents from the commercial orchards are not directly comparable with results published elsewhere, as there are no published data on the elemental concentrations in small mammals from similar habitats. Our study is only one covering the accumulation of chemical elements in small mammals inhabiting such habitats.

A comparison between the elemental concentrations in the bodies of *A. flavicollis* in orchards and a cormorant colony in Lithuania, this being a most heavily biologically polluted site (Jasiulionis et al., 2018), revealed that the concentrations of eight out of nine elements differed significantly (Fig. S1). Specifically, the concentrations of Mg, Ni, Cu, Sr and Pb were higher in commercial orchards and their controls, while those of Ca, Zn and Mo were higher in the cormorant colony and its surroundings. While the higher concentration of Mg and Cu are explained as the effect of fertilizing, the higher presence of Ni, Sr and Pb is related to the use of herbicides and pesticides (He et al., 2005; Rubio-Armendáriz et al., 2021, etc.). As we noted earlier (Balčiauskas et al., 2019), the use of various chemicals was common in the orchards with a high intensity of agricultural measures.

Checking the ranges of elemental concentrations in various habitats irrespective of the country (Table S4) proved that: (i) the variability of elemental concentrations is very high, with the registered minimum and maximum values being up to hundreds or thousand of times different, (ii) there are species-based differences, and (iii) elemental concentrations also depend on the source of pollution, the leading one being industrial (Damek-Poprawa and Sawicka-Kapusta, 2003; Ieradi et al., 2003; Andras et al., 2006; Jančová et al., 2006; Martiniaková et al., 2010, 2011, 2012, 2015; Blagojević et al., 2012; Petkovšek et al., 2014; Dimitrov et al., 2016; Jasiulionis et al, 2018). Summarizing Table S4, the concentrations of Pb, Cu and Mn in rodents from the agricultural areas (commercial orchards and berry plantations) were at the same level, while the concentrations of Cd, Fe and Zn were lower as those in the reference areas. Compared to those in industrial areas, the concentrations of Pb, Cu, Cd, Fe, Zn, Mn, Co and Ni in rodents from the agricultural areas were lower.

We compared the concentrations of As (Elfving et al., 1979, names adapted to current classification) in Chihuahua voles (*Microtus pennsylvanicus*), woodland voles (*Microtus pinetorum*) and white-footed mice (*Peromyscus leucopus*) in orchards and control areas in North America with our data (Table S5). While the values were of the same order in the orchards, the rodents from the control areas in our study were characterized by higher As concentrations. Apart from the different geographic location of the compared samples, this trend may depend on the proximity of the reference areas to the orchards and to the different time period of sampling.

The above mentioned negative correlations of Cu concentrations with the body mass of all three of the most abundant rodent species was in line with the low concentration of this element in the orchards in comparison with other habitats. In our study, the ranges of the Cu concentrations in the rodents were lower than shown by other authors, including those in the reference areas (Table

S4). Cu depletion with animal age has been shown for various mammal species (Bakka and Webb, 1981), decreasing in first three weeks *post partum*. Cu depletion in the rodents from the orchards is interesting in connection with the confirmed Cu deficiency that occurs in rats in connection to the high fructose levels in their diet (Fields et al., 1984). For the orchards, this requires further investigations of rodent diet in addition to that found earlier (Balčiauskas et al., 2021 a, b, c), specifically determining the proportions of fruits and berries eaten. Moreover, the deficiency of Cu in various mammals is related to impaired immune response (Minatel and Carfagnini, 2000).

An ambiquity regarding the influence of compounding biological factors, such as gender and age, on the concentrations of various elements has been known for decades (see Wren, 1986). Thus, our similar findings, such as the absence of gender-related and few age-related differences in elemental concentrations, are in line with published results (Mažeikytė and Balčiauskas, 2003; Fritsch et al., 2010; Blagojević et al., 2012; Jasiulionis et al., 2018; etc.).

5. Conclusions

Analyzing 21 elemental concentrations in small mammals trapped in commercial gardens, berry plantations and near-lying meadows as control habitat, we found the main sources of variability in elemental concentration were animal species and age, crop and intensity of agricultural practices, while location, animal gender and crop age were out of importance. GLMM explained 30–80% of the distribution of Ca, Ni, Co, Cu, Ga, Mn, Pb and U.

Higher concentrations of Cu, Mn, Bi, Co, Cr, Fe, Ni, Sr and Pb in the muscle and bones of the dominant species of rodents from the crop areas in comparison to those in control habitats confirmed the hypothesis that fertilization and the use of pesticides in commercial orchards should induce differences in the elemental concentrations accumulated by muscle and bones of small mammals.

Compared to published data on elemental concentrations in rodents from territories with industrial, agricultural and biological pollution and sites free from pollution, we confirm that commercial orchards are cleaner than industrially heavily polluted sites.

CRediT author statement

Linas Balčiauskas: Investigation, Statistical analysis, Writing – review & editing. Žilvinas Ežerinskis: Methodology, Investigation, Formal analysis. Vitalijus Stirkė: Data curation, Investigation. Laima Balčiauskienė: Data curation, Investigation, Writing – review & editing. Andrius Garbaras: Conceptualization. Vidmantas Remeikis: Project administration, Resources. All authors participated in Writing – original draft and agreed to final version.

Institutional Review Board Statement

The study was conducted in accordance with Lithuanian (the Republic of Lithuania Law on the Welfare and Protection of Animals No. XI-2271) and European legislation (Directive 2010/63/EU) on the protection of animals and approved by the Animal Welfare Committee of the Nature Research Centre, protocol No GGT-7. Snap trapping was justifiable as we studied reproduction parameters and collected tissues and internal organs for analysis of pathogens and stable isotopes (not covered in this publication).

Informed Consent Statement: Not applicable.

Data Availability Statement: this is ongoing research, therefore data are available from the corresponding author upon request.

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Figure captions

Fig. 1. Investigation sites and crops (AO – apple orchard, PO – plum orchard, RP – raspberry plantation, CP – currant plantation, HBP – high blueberry plantation).

Fig. 2. Differences in elemental concentrations in the most abundant small mammal species. Central point shows average values, box – SE, whiskers – SD.

Fig. 3. Differences in elemental concentrations depending a) on the age and gender of the most abundant small mammal species, and b) on the intensity of agricultural practices (H – high, M – medium, L – low intensity, N – none, the control habitat). Central point shows average values, box – SE, whiskers – SD.

Fig. 4. Differences in elemental concentrations in the most abundant small mammals depending on the habitat. Central point shows average values, box – SE, whiskers – SD.

Highlights

- 21 chemical elements studied in 3 rodent species dominating commercial orchards
- Main sources of elemental variability: crop, agro-intensity, species and animal age
- Depletion of Cu with increased body mass was significant in all studied species
- The values of 9 elements in rodents in orchards exceeded those in control areas Rodents indicate orchards being cleaner than heavy polluted areas
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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: