Supplementary information

Morphological parameters of hepatocytes in the European mole (*Talpa europaea*) and herb field mouse (*Sylvaemus uralensis*) under industrial pollution: Qualitative and quantitative assessment

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*The Little Mole (in Czech, Krteček) shown in the graphical abstract is the main character in a series of cartoons created by Czech animator Zdeněk Miler.

Parameter	Middle Ural Copper Smelter ¹	Karabashmed ²
	(MUCS)	(KaCS)
	Middle Ural	South Ural
	(56°50′N, 59°54′E)	(55°12′N, 60°12′E)
Copper production	95.233	40.000
(t/year), data of 2005 3		
Aerial emissions (t/year)	, data of 2005 3	
SO_2	24.300	38.100
CO	900	1.600
Dust	3.000	1.300
Cu	49	no data
Zn	255	no data
Pb	146	20
As	18	7

 Table S1 Characteristics of sources of environmental pollution

Heavy metals (movable forms) concentration ($\mu g/g$, dry weight, Mean \pm SE) and pH_{water} in birch forest litter of birch forests without ground vegetation in the background (above the line) and impact (below the line) zones

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Cu	$\frac{18.7 \pm 0.5}{3787.9 \pm 266.3}$	$\frac{40.6 \pm 2.3}{4251.9 \pm 247.0}$
Zn	$\frac{310.5 \pm 8.2}{1884.9 \pm 90.0}$	$\frac{410.3 \pm 7.3}{5102.4 \pm 343.3}$
Cd	$\frac{2.6 \pm 0.1}{38.2 \pm 1.8}$	$\frac{2.2 \pm 0.1}{24.3 \pm 0.8}$
Pb	$\frac{46.1 \pm 1.9}{2157.6 \pm 91.1}$	$\frac{136.2 \pm 5.4}{2447.1 \pm 197.9}$
pH _{water}	<u>5.84</u> 5.53	<u>5.84</u> 5.73
n	<u>30</u> 30	<u>15</u> 15
Data source	Belskaya and Vorobeichik, 2015 4	Own data ⁵

¹ UMMC. <u>https://ugmk.com/en</u> (accessed 20 October 2022).

² RCC. <u>https://rmk-group.ru/en/activities/enterprises/karabashmed</u> (accessed 20 October 2022).

³ Kozlov, M.V., Zvereva, E.L., Zverev V.E., 2009. Impacts of point polluters on terrestrial biota. New York, Springer. <u>https://doi.org/10.1007/978-90-481-2467-1</u>

⁴ Belskaya, E.A., Vorobeichik E.L., 2015. Changes in the trophic activity of leaf eating insects in birch along the pollution gradient near the Middle Ural copper smelter. Contemporary Problems of Ecology. 8 (3), 397–404. <u>https://doi.org/10.1134/S199542551503004X</u>

⁵ Unpublished.

Species	Pollution zone	Sex	Age group	Number of a	animals*
				"Normal"	"Pathological"
				liver	liver
Talpa	Background	Male	Young	3	0
europaea			Adult	0	3
		Female	Young	3	1
			Adult	2	1
	Impact	Male	Young	1	2
			Adult	2	1
		Female	Young	1	3
			Adult	0	3
	Total			12	14
Sylvaemus	Background	Male	Young	4	6
uralensis			Adult	3	0
		Female	Young	3	1
			Adult	0	1
	Impact	Male	Young	1	11
			Adult	1	0
		Female	Young	2	0
			Adult	2	1
	Total			16	20

Table S2 Sampling areas and sample structure and sizes

*Number of animals with "normal" / "pathological" liver



Fig. S1 Histological section of the M_3 root for *Talpa europaea* of different age: (a) 1.5 months and (b) 3 years: D – dentine, C – cementum, MZ – mesosteal zone bone, 1, 2 and 3 – dark incremental lines in cementum (Mayer's hematoxylin; scale bar 100 μ m)



Fig. S2 Histological structure of the normal and dystrophic liver in (a, b) *Talpa europaea* and (c, d) *Sylvaemus uralensis* (Mayer's hematoxylin–eosin; scale bar 50 μm)

Element Zone		Species						
		Talpa europaea (n = 26)		Sylvaemus uralens $(n = 35)$	is			
		Young	Adult	Young	Adult			
Cu	Bg	34.0 (14.8) [7] 10.9–55.2	25.2 (4.8) [6] 17.3–29.7	14.4 (7.1) [14] ^A 8.2–20.2	26.9 (3.4) [4] ^B 22.2–30.3			
	Im	32.5 (17.9) [7] 12.8–65.0	39.7 (24.3) [6] 16.5–84.7	11.9 (4.8) [14] ^A 7.7–23.2	21.7 (1.8) [3] ^B 19.9–23.4			
Zn	Bg	161.9 (72.4) [7] 91.5–273.9	122.3 (21.5) [6] 98.0–149.7	102.9 (28.9) [14] ^a 67.2–157.3	108.5 (8.4) [4] ^a 100.0–117.9			
	Im	147.1 (41.7) [7] 107.5–206.1	143.5 (50.6) [6] 97.2–218.7	78.6 (17.3) [14] ^b 53.8–110.5	88.8 (13.3) [3] ^b 73.0–96.2			
Cd	Bg	20.5 (11.1) [7] ^{aA} 8.4–36.8	92.0 (22.6) [6] ^{aB} 71.5–127.3	0.6 (0.4) [14] 0.1–1.6	0.8 (0.6) [4] 0.2–1.3			
	Im	34.4 (29.2) [7] ^{bA} 10.6–98.9	232.7 (153.9) [6] ^{bB} 127.2–534.6	0.5 (0.2) [14] 0.1–0.8	1.2 (1.1) [3] 0.5–2.5			
Pb	Bg	3.0 (2.5) [7] 0.3–7.2	3.2 (2.2) [6] 1.1–7.6	1.9 (1.3) [14] <i>nd</i> –3.5	2.3 (2.8) [4] nd -6.0			
	Im	4.0 (3.0) [7] 0.8–8.6	3.2 (1.8) [6] 0.7–6.3	2.5 (1.9) [14] 0.6–4.3	4.0 (1.6) [3] 1.5–6.9			

Table S3 Heavy metals concentrations (μ g/g dry weight) in the liver of *Talpa europaea* and *Sylvaemus uralensis*

Above the line, arithmetic mean with standard deviation (in parentheses) and the number of samples [in brackets]; below the line, the minimum and maximum values; nd, the value below the detection limit. Different letters indicate statistically significant differences (P < 0.05; lowercase and uppercase letters are for pollution zones and age groups, respectively)

Table S4 Effect of different factors on heavy metals accumulation (log μ g/g) in the liver of *Talpa europaea* and *Sylvaemus uralensis*; results of randomization test of ANOVA, are given *F* values and *P*-levels (in parentheses)

Predictor	Element	Element								
	Cu	Zn	Cd	Pb						
	Talpa europaea	(<i>n</i> = 26)								
Age	0.002 (0.964)	1.0 (0.321)	76.5 (<0.001)	0.1 (0.757)						
Zone	0.3 (0.565)	0.1 (0.813)	8.3 (0.010)	0.3 (0.574)						
Age \times Zone	1.1 (0.314)	0.5 (0.500)	0.9 (0.361)	0.4 (0.558)						
	Sylvaemus urale	ensis $(n = 35)$								
Age	19.9 (<0.001)	1.1 (0.306)	3.3 (0.078)	0.8 (0.371)						
Zone	1.4 (0.241)	5.6 (0.024)	0.4 (0.518)	3.6 (0.066)						
Age \times Zone	0.03 (0.873)	0.1 (0.810)	0.4 (0.522)	0.01 (0.936)						

Values significant at P < 0.05 are boldfaced

Table S5 Effect of different factors on heavy metals accumulation (log μ g/g) in the liver of *Talpa europaea* and *Sylvaemus uralensis*; results of two-way ANOVA, are given *F* values and *P*-levels (in parentheses)

Predictor	Element								
	Cu	Zn	Cd	Pb					
	$Talpa \ europaea \ (n = 209)$								
Age	0.01 (0.944)	0.3 (0.574)	310.9 (<0.0001)	2.1 (0.147)					
Zone	1.8 (0.177)	24.3 (<0.0001)	87.4 (<0.0001)	9.7 (0.002)					
Age \times Zone	5.6 (0.019)	2.1 (0.147)	3.9 (0.051)	0.4 (0.511)					
	Sylvaemus uralen.	sis (n = 245)							
Age	0.1 (0.749)	0.3 (0.605)	2.7 (0.103)	0.1 (0.737)					
Zone	0.2 (0.654)	2.1 (0.154)	17.4 (<0.0001)	0.1 (0.775)					
Age \times Zone	0.8 (0.378)	0.03 (0.867)	2.1 (0.152)	1.3 (0.250)					

Values significant at P < 0.05 are boldfaced

Predictor	β	b	t	Р	–95% CI	+95% CI
	Cu					
b_0		1.25	39.0	<0.001	1.18	1.31
Age	0.48	0.12	3.7	0.001	0.21	0.75
Liver dystrophy	-0.35	-0.07	-2.1	0.042	-0.68	-0.01
Zone	-0.07	-0.01	-0.4	0.672	-0.41	0.27
	Zn					
b_0		1.96	80.2	<0.001	1.91	2.01
Zone	-0.45	-0.05	-2.0	0.050	-0.89	0.00
Liver dystrophy	-0.29	-0.03	-1.3	0.191	-0.73	0.15
Age	0.09	0.01	0.5	0.603	-0.27	0.45

Table S6 Dependence of the copper and zinc accumulation (log μ g/g) on different factors in *Sylvaemus uralensis*, n = 35 (results of linear regression analysis)

 b_0 is the reference group of young animals with the normal liver from the background zone. Predictors for each species are ranked by the value of standardized regression coefficient β . Values significant at *P* < 0.05 are boldfaced

	Aniso- karyosis	$S_{cell}(\mu m^2)$	Aniso- cytosis	N/C	Cell packing density, cells/10 ⁵ μm ²	Proportion of binucleate cells (%)
$N(\mu m^2)$	<u>0.34</u> 0.90	<u>0.62</u> 0.46	<u>0.04</u> 0.73	<u>0.70</u> 0.71	-0.32 -0.47	$-\underline{0.03}$ -0.23
Anisokaryosis		$\frac{0.40}{0.31}$	<u>0.37</u> 0.63	<u>0.08</u> 0.70 *	- <u>0.31</u> -0.35	- <u>0.12</u> -0.30
$S_{cell}(\mu m^2)$			<u>0.70</u> 0.75	- <u>0.12</u> -0.28	- <u>0.78</u> -0.71	<u>0.03</u> -0.07
Anisocytosis				- <u>0.55</u> 0.22	- <u>0.62</u> -0.76	<u>0.12</u> -0.10
N/C					<u>0.34</u> -0.01	- <u>0.03</u> -0.20
Cell packing						<u>0.19</u>
density, cells/ $10^5 \mu m^2$						-0.03

Table S7 Correlations (*r*) between morphometric parameters of hepatocytes of *Talpa europaea*, n = 26 (above the line) and *Sylvaemus uralensis*, n = 36 (below the line)

Values significant at P < 0.05 are boldfaced

Table	S8 – Di	ifferences	in r	norphometric	features	of	Talpa	europaea	and	Sylvaemus	uralensis
hepato	cytes in	"normal"	and	"pathological"	' liver (M	lan	n–Whi	itney U-tes	t)		

Parameter	Species									
	$Talpa \ europ$ $(n = 12 / 14)$	0aea)*	Sylvaemus uralens $(n = 16 / 20)$							
	U	Р	U	Р						
$N(\mu m^2)$	58.0	0.657	82.0	0.118						
Anisokaryosis	63.0	0.847	94.0	0.216						
$S_{cell}(\mu m^2)$	27.0	0.041	115.0	0.690						
Anisocytosis	35.0	0.106	142.0	≈ 1						
N/C	71.0	≈ 1	50.0	0.008						
Cell packing density, cells/ $10^5 \mu m^2$	42.0	0.186	159.0	≈ 1						
Proportion of binucleate cells (%)	50.5	0.223	155.0	≈ 1						

*Number of animals with "normal" / "pathological" liver. Values significant at P < 0.05 are boldfaced

Table S9 Correlations (*r*) of morphometric parameters of hepatocytes and individual levels of heavy metals accumulation in the liver *Talpa europaea*, n = 26 (above) and *Sylvaemus uralensis*, n = 35 (below); in parentheses are given FDR-adjusted *P*-values

Parameter	Element			
	Cu	Zn	Cd	Pb
$N(\mu m^2)$	$0.23 (\approx 1)$	0.20 (≈ 1)	-0.04 (≈ 1)	0.11 (≈ 1)
	0.43 (0.082)	0.20 (≈ 1)	0.44 (0.127)	-0.13 (≈ 1)
Anisokaryosis	0.42 (0.188)	-0.28 (0.771)	-0.06 (≈ 1)	-0.30 (≈ 1)
	0.41 (0.090)	0.15 (≈ 1)	0.34 (0.272)	-0.07 (≈ 1)
$S_{cell}(\mu m^2)$	0.17 (≈ 1)	-0.25 (0.810)	$0.18 (\approx 1)$	-0.10 (≈ 1)
	-0.26 (0.408)	-0.32 (0.535)	0.32 (0.272)	-0.01 (≈ 1)
Anisocytosis	0.49 (0.188)	-0.41 (0.230)	0.11 (≈ 1)	-0.21 (≈ 1)
	0.30 (0.354)	0.05 (≈ 1)	0.25 (0.519)	-0.20 (≈ 1)
N/C	0.44 (0.188)	0.49 (0.181)	-0.23 (≈ 1)	0.21 (≈ 1)
	0.68 (0.002)	0.43 (0.163)	0.19 (0.811)	-0.14 (≈ 1)
Cell packing density, cells/ $10^5 \mu m^2$	0.27 (0.858)	0.43 (0.230)	-0.44 (0.472)	-0.04 (≈ 1)
	-0.26 (0.408)	0.21 (≈ 1)	-0.32 (0.272)	0.28 (≈ 1)
Proportion of binuclear cells (%)	$0.15 (\approx 1)$	0.01 (≈ 1)	-0.25 (≈ 1)	0.14 (≈ 1)
	$0.10 (\approx 1)$	0.11 (≈ 1)	0.04 (≈ 1)	-0.31 (≈ 1)

Values significant at P < 0.05 are boldfaced

Table	S10 –	Factor	loadings	of	two	principal	components	(PC	1,	PC	2)	calculated	by
morpho	ometric	paramet	ers of hep	atoc	ytes	of <i>Talpa el</i>	uropaea and S	Sylvae	emu	s urc	ilen	sis.	

Parameter	Species					
	Talpa europaea		Sylvaemus uralensis			
	PC 1	PC 2	PC 1	PC 2		
$N(\mu m^2)$	-0.47	-0.85	-0.93	-0.27		
Anisokaryosis	-0.57	-0.26	-0.86	-0.40		
S_{cell} (µm ²)	-0.94	-0.13	-0.65	0.68		
Anisocytosis	-0.82	0.42	-0.90	0.30		
N/C	0.25	-0.95	-0.50	-0.80		
Cell packing density, cells/ $10^5 \mu m^2$	0.87	-0.12	0.71	-0.53		
Proportion of binuclear cells (%)	0.06	0.11	0.26	0.35		
Eigenvalue	2.91	1.92	3.66	1.83		
Total variance (%)	41.6	27.4	52.3	26.1		

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Predictor	β	b	t	Р	–95% CI	+95% CI	
	Talpa europaea						
b_0		392.9	24.3	<0.001	359.3	426.5	
Age × Zone	0.50	57.7	3.6	0.002	0.21	0.79	
Zone	-0.41	-47.1	-3.0	0.007	-0.70	-1.12	
Age	-0.35	-40.1	-2.5	0.021	-0.64	-0.06	
	Sylvaemus uralensis						
b_0		231.3	21.4	<0.001	209.3	253.3	
Age	-0.52	-37.8	-3.5	0.001	-0.83	-0.22	
Age × Zone	0.08	4.9	0.5	0.653	-0.28	0.45	
Zone	-0.03	-1.9	-0.18	0.862	-0.40	0.33	

Table S11 Dependence of hepatocyte packing density on different factors in *Talpa europaea* and
 Sylvaemus uralensis (results of linear regression analysis)

 b_0 is the reference group of young animals from the background zone. Predictors for each species are ranked by the value of standardized regression coefficient β . Values significant at P < 0.05 are boldfaced

Table S12 Factor coordinates of the variables (F 1, F 2), based on correlations active and supplementary* variables of *Talpa europaea* and *Sylvaemus uralensis*

Parameter	Species					
	Talpa eu	ropaea	Sylvaemu	Sylvaemus uralensis		
	F 1	F 2	F 1	F 2		
Liver dystrophy	-0.79	0.45	0.84	-0.002		
N/C	0.85 -0.15		-0.64	-0.65		
Cell packing density, cells/ $10^5 \mu m^2$	0.62	0.77	-0.54	0.76		
Zone*	-0.41	0.12	0.27	0.13		
Age*	-0.37	-0.04	-0.01	-0.76		
Log Cu*	0.38	0.27	-0.43	-0.60		
Log Zn*	0.48	0.28	-0.49	-0.11		
Log Cd*	-0.45	0.05	0.14	-0.37		
Log Pb*	0.12	0.13	0.12	0.31		
Eigenvalue	1.72	0.82	1.41	1.00		
Total variance (%)	57.4	27.3	47.0	33.5		

Model	C _p	k- predic- tors	Zone	Age	Liver dystrophy	Zone × Age	Zone × Liver dystrophy	Age × Liver dystrophy
1	7.6	3	-0.41	-0.35	_	0.50	_	-
2	9.8	3	_	_	-0.47	_	_	0.43
3	10.3	3	_	_	-0.38	0.44	_	_

Table S13 Standardized β -regression coefficients and selection of optimal ($C_p = \min$) models for describing hepatocytes (cell packing density) variability of *T. europaea*