

Impact of ageing and soil contaminants on telomere length in the land snail

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2 snail

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- 12
- 13 Keywords: *Cantareus aspersus*; bioindicators; cadmium; mercury; polycyclic aromatic
- 14 hydrocarbons
- 15

16 Highlights:

- Telomere length attrition (TLA) occurs between the juvenile and sub-adult stages.
- Exposure to chemical stress does not cause TLA in haemocytes of sub-adult snails.
- The health status of snails exposed to chemical stress was disrupted without TLA.

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29 ABSTRACT

Telomeres (TLs) are non-coding DNA sequences that are usually shortened with 30 ageing and/or chemical exposure. Bioindicators such as the land snail can be used to assess 31 32 the environmental risk of contaminated soils. As for most invertebrates, the evolution of TLs with ageing or exposure to contaminants is unknown in this mollusc. The aims of this study 33 were to explore the relationships between ageing, contaminant exposure, sublethal effects and 34 TL length in the terrestrial gastropod Cantareus aspersus. TL length was investigated in 35 haemocytes from five age classes of C. aspersus. The impact of contaminants on sub-adult 36 37 snails exposed to Cd, Hg or a mixture of polycyclic aromatic hydrocarbons (PAHs) in soils for one or two months was studied. Bioaccumulation, growth, sexual maturity and TLs were 38 39 measured. TL attrition was significant for the juvenile and sub-adult stages, but not later. Exposure to Cd increased the mortality (around 30%). Exposure to polluted soils inhibited 40 41 growth (19 to 40%) and sexual maturity (6 to 100%). Although the health of the snails exposed to Cd, Hg and PAHs was altered, TL length in haemocytes was not disturbed, 42 43 suggesting a high capacity of this snail species to maintain its TLs in haemocytes under chemical stress. These results first address TL length in snails and reveal that the relationship 44 commonly proposed for vertebrates between TL shortening and ageing or exposure to 45 contaminants cannot be generalized. 46

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70 INTRODUCTION

71 Telomeres (TLs) are highly conserved, repetitive and non-coding DNA sequences located at the ends of eukaryotic chromosomes (Blackburn, 1991; Traut et al., 2007). Their 72 73 main function is to protect the ends of chromosomes from loss during cell division and degradation (Blackburn, 2005; Oeseburg et al., 2009). Telomeric DNA is progressively lost in 74 75 most vertebrates during ageing due to the inability of linear chromosomes to be completely replicated by DNA polymerases (Blackburn, 1991). This loss can be accelerated by 76 endogenous and exogenous factors, such as infections (Giraudeau et al., 2019) or 77 78 environmental contaminants (metals, polycyclic aromatic hydrocarbons (PAHs), etc.) (Perera et al., 2018; Louzon et al., 2019; Matzenbacher et al., 2019). Indeed, contaminants can induce 79 80 TL alterations according to several mechanisms of action such as DNA strand breakage at the TL level, deficiencies in DNA repair machinery, oxidative stress, mutations in genes encoding 81 82 proteins related to TL maintenance and disturbances in the regulation of participants in TL maintenance (e.g., telomerase activity or alternative lengthening of TLs pathway) (Louzon et 83 al., 2019). 84

Data from longitudinal studies in vertebrates showed a TL loss within individuals with age 85 (Zeichner et al., 1999; Brümmendorf et al., 2002; Scott et al., 2006; Baerlocher et al., 2007) 86 with some exceptions (Ujvari and Madsen, 2009), although great variability exists depending 87 on the biological tissue (Parolini et al., 2019) and on TL length and loss between age-matched 88 individuals (Hall et al., 2004). The relationship between TL length and ageing is unknown for 89 most invertebrates as concluded following a comparative approach between biological groups 90 and the few studies on this topic have shown various patterns: either shortening, or no 91 variation or lengthening (Louzon et al., 2019). For example, for two species of *Daphnia*, two 92 93 different patterns have been reported: the attrition in TLs with ageing in D. pulicaria and no variation in TL length in D. pulex (Schumpert et al., 2015). These differences were related to 94 95 the high telomerase activity that maintains TLs during the life cycle of D. pulex (Schumpert et al., 2015). Surprisingly, for the gastropod of Baïkal Lake, Kobeltocochlea martensiana, which 96 97 has a lifespan of eight years (vs months for some Daphnia spp.), a lengthening of TLs with ageing was shown, probably due to the high activity of TL maintenance mechanisms 98 99 (Maximova et al., 2017).

For terrestrial snails, the relationships between TL dynamics and ageing are unknown,especially for the soil quality bioindicator, *Cantareus aspersus*, which have an average life of

five years and which belongs to various food chains at the air-soil-plant interface. It is consumed by predators and integrates various routes of exposure of contaminants in soils (digestive, cutaneous, respiratory) (Comfort, 1957; Scheifler et al., 2002; Scheifler et al., 2006). For these ecological reasons, this ubiquitous species is often used in environmental risk assessment of contaminated soils (Pauget and de Vaufleury, 2015) by combining assessments of bioavailability (Pauget et al., 2013) and health effects (Baurand et al., 2014).

108 Based on data showing that chemicals are able to induce premature ageing and decrease the 109 lifespan of humans (Chen et al., 2013; Puri et al., 2017), it is relevant to explore the 110 relationships among TL dynamics, health effects and contaminant exposure in C. aspersus. This could contribute to filling the gap between the molecular and individual effects of 111 112 contaminants. Considering this objective, the first aim is to describe how TL dynamics in haemocytes is affected by ageing in snails living in a safe environment. This study also aimed 113 114 to investigate the relationships between the exposure to contaminants in soils (cadmium, mercury or PAHs), the alteration in health status (by monitoring survival, growth and the 115 attainment of sexual maturity) and TL length in C. aspersus. 116

117 MATERIAL AND METHODS

118 SNAILS

Breeding snails from a snail farm (CFPPA of Châteaufarine, 25000 Besançon, France) 119 were used to produce a F1 laboratory generation. The species C. aspersus was verified by 120 DNA barcoding (Allgenetics, 15008 A Coruña, Spain). After incubating eggs, hatchlings were 121 reared in the laboratory under controlled conditions (20°C, 80% relative humidity, 122 photoperiod of 16 h of light and 8 h of dark) (Gomot-de Vaufleury and Pihan, 2000) for two 123 years. Snails reared of 1 month old were classified as juveniles, 3 months old as sub-adults, 4 124 months old as young adults, 5 months old as young reproducers and 2 years old as old 125 reproducers (Figure 1). Eight snails of each age class were selected to investigate the 126 127 relationships between ageing and TL length.

To study the effect of exposure to contaminants, only sub-adult snails were used to avoid confounding factors related to growth and reproduction (Gimbert et al., 2008). Two groups of sub-adults were exposed in quadruplicate to different contaminants for 1 and 2 months. Snails were fed with uncontaminated commercial food (**Table 1**) Helixal[®] (Berthon S.A., France) *ad libitum* during growth and exposure.

133 **SOILS**

Snails were exposed to four different soil conditions (ISO, Cd, Hg and PAHs). An 134 artificial ISO soil was prepared according to the OECD (1984) guidelines (mixture of 70%) 135 fine sand, 20% kaolin clay, and 10% coarse ground sphagnum peat, and the pH was adjusted 136 137 to 6.7 ± 0.1 with powdered calcium carbonate, 99.5%, Sigma Aldrich, Lyon, France). The cation exchange capacity (CEC) was 13.5 cmol kg⁻¹, and the organic matter content 138 (estimated on the basis of the loss of ignition) was 12%. The ISO substrate was spiked at 139 13 000 mg.kg Hg.kg⁻¹ dry weight (dw) with a mercury chloride solution (HgCl₂, 99.5%, 140 Sigma Aldrich) or at 1500 mg.kg⁻¹ dw with a cadmium chloride solution (CdCl₂, 99.9%, 141 Sigma Aldrich), in both cases diluted in a volume of osmotic water needed to achieve 50% of 142 the soil water holding capacity (Pauget et al., 2012). The soils were then allowed to stabilize 143 for 5 days prior to the experiment. An anthropogenic French soil (Vieux Charmont, France) 144 contaminated with PAHs due to past industrial activities of the automobile sector (BASOL 145 25.0020, 2015) was used under the same preparation conditions (except spiking). 146 Concentrations were measured by ICP-MS for Cd and Hg (Mariet et al., 2017) and by GC-147 MS/MS for the 16 PAHs listed by the US EPA (Morin-Crini et al., 2014) (Table 1). 148

149 EXPOSURE MODALITIES

150 Sub-adult snails (3-months old snails) were exposed to Cd or Hg contaminated substrate, PAHs contaminated soils and or uncontaminated ISO substrate for 1 and 2 months. For each 151 of the 4 substrates/soils, six snails were exposed in quadruplicate in transparent polystyrene 152 containers (4032 cm³) (Gomot-de Vaufleury, 2000). Two snails per replicate (n=8 individuals 153 per exposure conditions) were sampled randomly after 1 and 2 months for analysis of the 154 visceral concentrations of contaminants and assessment of TL length. Briefly, Helixal® food 155 was provided in a petri dish left on the bottom of the container, the day/night cycle 156 photoperiod was 18/6 h, the temperature was $20^{\circ}C \pm 1$, and the air relative humidity was $80 \pm 10^{\circ}C$ 157 10%. Three times a week, the containers were cleaned, the faeces were removed, survival was 158 monitored, and the food was renewed. At the beginning of exposure (t0), sub-adult snails 159 weighed 5.22 g \pm 0.58, and the internal concentrations of Cd, Hg and PAHs were measured in 160 161 the visceral mass from six sub-adult snails (**Table 1**).

162 GROWTH AND ATTAINMENT OF SEXUAL MATURITY ASSESSMENTS

163 Growth was assessed monthly by weighing the fresh mass (whole body) of snails. The 164 growth inhibition (GI) was calculated as follows (after validation of the absence of abnormal

mortality of snails exposed to the ISO soil): GI = 100-(mean mass of exposed snails / mean 165 mass of snails exposed to uncontaminated ISO soil)*100. Sexual maturity was assessed 166 monthly by counting the snails exhibiting a shell with a curled edge, which can be used as an 167 indicator of the attainment of sexual maturity in snails (Lucarz and Gomot, 1985; Gimbert et 168 al., 2008; Gimbert et al., 2016). The inhibition of the attainment of sexual maturity was 169 calculated by the following formula: 100-(number of exposed snails having acquired sexual 170 maturity/number of snails exposed to uncontaminated ISO substrate having acquired sexual 171 172 maturity)*100.

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BIOACCUMULATION MEASUREMENT

Internal concentrations of Cd and Hg were measured according to the 174 methodology detailed in Pauget and de Vaufleury (2015). Snails were frozen at -80°C. After 175 176 thawing, the visceral mass was dissected for each snail and lyophilized for two days before Cd and Hg analyses. Viscera were digested between 47°C and 98°C for 265 min (DigiPREP 177 MS, SCP Science, Courtaboeuf, France) in 7 mL of nitric acid (HNO₃, 65%, ultratraces 178 Optima[®], FisherScientific, Illkirch, France) that was diluted with 43 mL of Milli-Q water. 179 Then, samples were filtered through a 1 µm filter and diluted as a function of concentration 180 between 10 to 100x for Cd and 10 to 10000x for Hg analyses in ICP-MS (Thermo Scientific 181 X Series II, Courtaboeuf, France). Analyses were validated with a certified material (TORT2, 182 lobster hepatopancreas, Canada). The recovery rates were 98% and 116% for Cd and Hg, 183 respectively. For the PAHs analysis, two fresh visceral masses per replicate were dissected 184 and pooled. Then, pooled samples were mashed with 25 ceramic sphere mills (FastPrep, MP 185 186 Biomedicals, Illkirch, France) for two cycles (4.0 m/s, 20 s) interrupted by a pause time of 180 s. PAHs were extracted with QuECHERs methodology from RESTEK (Lisse, France), 187 188 and concentrations of the 16 PAHs (Sun et al., 1998) were measured by GC-MS/MS (7890A GC system, 7000 GC triple quadrupole mass spectrometer, HP5MS column, Agilent, Massy, 189 190 France). The bioaccumulated concentrations in the snails and the soil concentrations were 191 used to calculate the bioaccumulation factors (BAF).

192 **DNA EXTRACTION FROM HAEMOCYTES**

193 The haemolymph of snails was collected by individually puncturing the heart with a needle (18 G) and was frozen at -20°C in sterile 1.5 mL microtubes (DNase, RNase, 194 endotoxin free, VWR). After quick thawing at 4°C, DNA extractions were performed using a 195 preliminary optimized method to isolate DNA with a sufficient amount and quality from 196

haemolymph. Salting out was the selected methodology (Gentra Puregene Blood Kit, Qiagen,
Courtaboeuf, France). The concentration and purity of extracted DNA were checked with a
NanoDrop 1000 spectrophotometer (ThermoScientific, Illkirch, France) and by fluorescence
(Quantifluor[®] ONE dsDNA System, Promega, Charbonnière-les-Bains, France). The quality
and the integrity of DNA were confirmed by electrophoresis on 1% agarose gels stained with
ethidium bromide.

203 TELOMERE LENGTH MEASUREMENT

For quickness and robustness (Nussey et al., 2014), TLs were measured by quantitative 204 real-time PCR amplification (qPCR) (Cawthon, 2002) using the method described by 205 Criscuolo et al. (2009) and adapted to land snails. This method amplified telomeric DNA and 206 207 expressed its quantity as a T/S ratio, where T was the quantity of telomeric DNA and S was 208 the quantity of a gene used as a reference (gene that is non-variable in copy number in the study species) (Reichert et al., 2017). Primers used for the telomeric DNA of snails were the 209 5'-210 same as those used for humans (forward: CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTT-3', 5'-211 reverse: GGCTTGCCTTACCCTTACCCTTACCCTTACCCT-3') 212 because the TL sequences of C. aspersus are the same as those of humans (Vitturi et al., 2005). To identify 213 the most appropriate reference gene, we conducted preliminary analyses considering a variety 214 of candidate reference genes and primer pairs. The most consistent amplification profile and 215 cleanest melting curve was obtained using Eurofins Genomics primers targeting the β-actin 216 which selected reference (forward: 5'-217 gene, we as our gene 218 GCTGGTCGTGACCTCACAGAT-3', reverse: 5'-AGCTTCTCCTTGATGTCACGAAC-3'). The TL and β -actin primers were used at a concentration of 4 pmol for a final volume of 10 219 µL containing 5 µL of GoTaq[®] qPCR master mix (Promega, Charbonnière-les-Bains, France). 220 Real-time amplification of TL and β-actin sequences was performed in separate 384 well 221 222 plates filled by an automated liquid handling workstation (epmotion, Eppendorf, Montesson, France). The TL thermal profile was 2 min at 95°C, followed by 27 cycles of 15 s at 95°C, 30 223 224 s at 56°C and 30 s at 72°C. The β-actin thermal profile was 2 min at 95°, followed by 40 cycles of 30 s at 95°C, 30 s at 60°C and 1 min at 72°C. Both assays were followed by melting 225 226 curve analysis (60-95°C; 1°C/5 s ramp). Due to the different thermal profiles between TL and β -actin, two 384 well plates were used in our study to measure TL length of all our samples. 227 The first is for the amplification of TL and the second is for the amplification of the β -actin 228 gene. Each DNA extract was tested in duplicate due to the use of the calibrated automated 229

liquid handling workstation and to the fact that mean coefficient of variation intra-run were 230 $0.51\% \pm 0.05$ and $1.07\% \pm 0.09$ for Cq- β -actin and Cq-TL, respectively. Moreover, the 231 intraclass correlation coefficient intra plate for the T/S ratio was ranked excellent, 0.949 232 (Cicchetti, 1994; Nettle et al., 2019). Relative TL length (RTL) was calculated for each 233 sample by the ratio of T/S. DNA from the same reference snail was used as a golden sample 234 and to generate a reference curve to control for the amplifying efficiency of the qPCR 235 between various 384 well plates (based on a standard range of 7 points, the amplification 236 efficiencies for TL and β -actin amplification were 99.3% and 99.7%, respectively). 237

238 DATA ANALYSIS

All statistical analyses were performed with R (version 3.4.2.). Normality and 239 homogeneity of variances were checked with Shapiro-Wilk and Bartlett tests, respectively. 240 241 The relationship between ageing and TL length was fitted with a non-linear model using the nlme (nonlinear mixed effect) procedure (Lindstrom and Bates, 1990) (package nlme). The 242 adequacy and quality of the model were compared (package pgirmess) according to the 243 Akaike information criterion (AICc) (Akaike, 1998) to select the best model. Then, 244 differences in TL length between age classes or control (ISO) vs contaminated soils were 245 assessed with a non-parametric Kruskal-Wallis test followed by the multiple pairwise 246 comparison test (Magniez et al., 2018). The Wilcoxon test was used to distinguish significant 247 248 differences of mortality, growth inhibition and the inhibition of the attainment of the sexual maturity between the different soils (control ISO vs contaminated) after transforming data 249 with $log_{10}(x+1)$. 250

251 **RESULTS AND DISCUSSION**

252 RELATIONSHIPS BETWEEN AGEING AND TELOMERE LENGTH

253 TLs were studied in the haemocytes of a *C. aspersus* population because they are involved in immune functions of gastropods (Pila et al., 2016) and haemolymph sampling is a non-254 lethal way in snails. The relationship between TL length in haemocytes and ageing in C. 255 256 *aspersus* was studied for five age classes, from juveniles to old adults (**Figure 1**). The attrition in TLs in haemocytes (p-value < 0.001, r^2 adjusted = 0.364) (Figure 2a) was significant 257 258 between juveniles and all other stages (p-values < 0.05) (Figure 2b). Indeed, TL length decreased rapidly in C. aspersus haemocytes during the first phase of growth before the 259 260 maturation of the genital tract (Gimbert et al., 2008) and then, was stable from sub-adults to 261 old reproducers (Figure 2b). TL attrition is one of the nine hallmarks of ageing identified in

most organisms (Lopez-Otin et al., 2013) because chromosomes lose telomeric patterns 262 (Harley et al., 1992) at each cell division. This attrition in TLs was detected at a different 263 magnitude in the foot of the freshwater mollusc Benedictia fragilis, in which TL length 264 decreased progressively during approximately 0 to 5 years for males (studied over 8 years) 265 (Maximova et al., 2017). However, for two other long-lived freshwater molluscs studied from 266 0 to 8 years, Benedictia baicalensis and K. martensiana, the patterns were different and sex-267 specific, with an increase in TL length in the foot with ageing (Maximova et al., 2017). 268 Notably, the influence of gender is irrelevant to study for C. aspersus due to its protandrous 269 270 hermaphroditic reproductive system (Bride and Gomot, 1991). These various patterns can possibly be explained by a higher telomerase activity in tissues that maintain TL length 271 (Greider and Blackburn, 1985; Greider and Blackburn, 1989) during ageing (Goyns and 272 Lavery, 2000; Shay, 2016), as demonstrated by Schumpert et al. (2015) for the aquatic 273 274 arthropod Daphnia pulex and for the marine bivalve Arctica islandica (Gruber et al., 2014). Although rapid attrition during growth seems not to be a general rule for invertebrates 275 276 excepted for some organisms such as the Sydney rock oyster (Saccostrea glomerata) (Godwin et al., 2012), this pattern is often found in vertebrate species (Angelier et al., 2018; Dugdale et 277 278 al., 2018) (e.g., Acrocephalus sechellensis, Spurgin et al. (2018)). The stability of TL length after the initial growth of land snails is similar to the TL dynamics described in invertebrates 279 that have a long lifespan and high telomerase activity, such as lobster (Klapper et al., 1998). 280 281 This finding is also consistent with the hypothesis of Schumpert et al. (2015) who assumed 282 that the differences in TL length dynamics with ageing between the short and long lifespans of Daphnia spp. were associated with an enhanced ability to protect individuals from 283 proteotoxicity with chaperone HSP70 (Schumpert et al., 2014). 284

285 LETHAL AND SUBLETHAL EFFECTS OF CONTAMINANTS

The mortality rates we measured in the snails exposed to the uncontaminated ISO 286 287 substrate, 4.25% and 8.50% after one and two months of experiment, respectively, were 288 closed to that reported for snails reared on uncontaminated ISO substrates for 168 days (i.e., 289 4%) in the study of Gimbert et al. (2016). For the soils contaminated with Hg and PAHs (**Table 1**), the survival rates were similar to those of the ISO groups (p-values > 0.05), while 290 291 it was lower for Cd-exposed snails compared to controls (p-value = 0.011), reaching 20.5% after two months of exposure (Figure 3a). This high mortality of Cd exposed snails is in 292 accordance with the literature, notably after Cd ingestion (Gomot, 1997). Snail growth was 293 inhibited after one and two months of exposure to all of the contaminated soils, with 294

inhibitions of $34.4\% \pm 10.5$, $39.3\% \pm 4.95$, and $18.5\% \pm 5.04$ for snails in Cd, Hg and PAHs 295 substrates/soils, respectively (Figure 3b). Such inhibition in the sub-adults was in the same 296 range as that reported for juvenile C. aspersus (Coeurdassier et al., 2002; Gimbert et al., 297 298 2016). Some contaminants, such as Cd or Hg, are also known to cause reproductive disorders in snails (de Vaufleury and Kerhoas, 2000; Gimbert et al., 2016), especially Cd, which is an 299 endocrine disruptor (Sabir et al., 2019) for pulmonate molluscs (Lagadic et al., 2007). All the 300 studied contaminants inhibited sexual maturity after one and two months of exposure, 301 whereas 100% of snails were sexually mature after one month of exposure on the control 302 303 substrate (ISO). No snail exposed to Cd or Hg was mature after one month of exposure, while only a few individuals matured (12.5%) on Cd substrate after two months of exposure (Figure 304 305 3c). In PAHs soil, maturity was also strongly inhibited after one and two months of exposure but was significantly lower than the inhibition of maturity in the snails exposed to the Cd and 306 307 Hg soils (Figure 3c). The disruption of growth and sexual maturity can be the result of the reallocation of energy to excretion and repair systems, a decrease in metabolic performance, 308 309 behaviour alterations for food intake and neuro-endocrine disruption (Gomot et al., 1992; Gomot and Gomot, 1995; Matthiessen and Gibbs, 1998; Lagadic et al., 2007; Druart et al., 310 311 2017; Sabir et al., 2019). Because the concentration of Hg-contaminated substrate reflects 312 those of contaminated soils found in anthropic areas as the mining district of Almaden (Spain) (Molina et al., 2006), the inhibition of growth and sexual maturity can impact the snail 313 population size and dynamic. The sub-lethal effects we measured showed that the health 314 315 status of snails exposed to contaminated substrates/soils in this study was strongly altered.

316 **BIOACCUMULATION**

Bioaccumulation measurements at 0, 1 and 2 months are presented in Table 1. The 317 results showed high internal concentrations of Cd, Hg and PAHs after exposure to 318 contaminated substrates/soils, while they remained low in the control snails (Table 1). 319 320 Bioaccumulation factors (BAFs) at 1 and 2 months for Cd- and Hg-contaminated soils were 321 0.434, 0.518, 0.159, and 0.183, respectively. These results were in accordance with those obtained by Coeurdassier et al. (2002) after exposing juvenile snails to 1000 mg kg⁻¹ Cd for 322 28 days in artificial ISO substrate (BAF = 0.290). BAFs were lower for PAHs (for one and 323 324 two months: 0.004 and 0.003, respectively) than for Cd and Hg, probably due to the low contamination of the soil used (9.36 mg kg⁻¹) and possible metabolization of PAHs (Ismert et 325 al., 2002; Sverdrup et al., 2006). Measured internal concentrations may result of the digestive 326 327 and cutaneous exposure of snails to metals and PAHs in soils (Gomot et al., 1989; Gomot-de Vaufleury and Pihan, 2000; de Vaufleury, 2015; Bamze Attoumani et al., 2020). The results
reveal bioaccumulation of the studied compounds associated to damage on some
physiological endpoints (survival, growth).

331 EFFECTS OF CONTAMINANTS ON TELOMERE LENGTH IN HAEMOCYTES

Based on the bioaccumulation level and the sub-lethal effects we measured, the health 332 status of snails exposed to contaminated substrates/soils was altered. Additionally, some 333 molecular markers can be used as early indicators of the alteration in health status (Mleiki et 334 al., 2018), TLs are commonly proposed as good candidates to reveal exposure and deleterious 335 effects of environmental contaminants on individuals and their populations (Louzon et al., 336 2019). However, no significant difference (p-value > 0.05, Figure 4) in RTLs was detected 337 between the snails exposed to contaminated substrates/soils and the snails exposed to 338 339 uncontaminated substrates for one or two months. Snails have been previously described as sensitive to oxidative stress and have exhibited genotoxic damages notably on haemocytes 340 when exposed to soils contaminated with inorganic and organic compounds, such as Cd, 341 glyphosate, and PAHs (Regoli et al., 2006; Itziou et al., 2011; Abdel-Halim et al., 2013; 342 Angeletti et al., 2013; Baurand et al., 2014; Baurand et al., 2015). However, C. aspersus 343 seems to have a strong ability to maintain its TL length in haemolymph even under chemical 344 stress. This can possibly be linked to telomerase activity and alternative lengthening of TLs 345 pathway activities or to the induction of other mechanisms to protect and repair TLs, such as 346 DNA repair mechanisms. In any case, the mechanisms underlying sublethal effects observed 347 on the growth or sexual maturation of snails exposed to Cd, Hg and PAHs (at least at the 348 349 tested concentrations and durations of exposure) do not indicate TL alteration in haemocytes of surviving snails. Moreover, although the lifespan and TL length of vertebrates can be 350 351 modified (shortened or lengthened) by chemicals (Louzon et al., 2019), some studies have 352 shown that TL length is not altered by pollutants, despite cytotoxic and genotoxic damages 353 occurred, notably for soybean farmers exposed to various pesticides (de Oliveira et al., 2019). 354 Nevertheless, as it has been shown that TL length varied across somatic tissues suggesting independent TL dynamics (Parolini et al., 2019), further measurements of TLs on other 355 biological tissues may be considered in the future. 356

357 CONCLUSIONS

This study provides the first results on the TL length dynamic in terrestrial gastropods according to ageing or chemical exposure to contaminated soils. TL attrition was observed only during the first phase of growth suggesting that TL length in the haemocytes of *C. aspersus* is not a specific marker of ageing for this invertebrate. The health of the snails exposed to Cd, Hg or PAHs was altered (survival, growth and sexual maturity) but TL length in haemocytes was not changed, suggesting the high capacity of this snail species to maintain its TLs in haemocytes under chemical stress. Present results first address TL length in land snails and reveal that the relationship proposed for vertebrates between TL shortening and ageing or exposure to contaminants cannot be generalized to the terrestrial gastropods.

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368 **Disclosures**

369 The authors declare that they have no competing financial interests.

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378 **References**

Abdel-Halim, K.Y., Abo El-Saad, A., Talha, M., Hussein, A., Bakry, N., 2013. Oxidative
stress on land snail *Helix aspersa* as a sentinel organism for ecotoxicological effects of urban
pollution with heavy metals. Chemosphere 93(6), 1131-1138.
https://doi.org/10.1016/j.chemosphere.2013.06.042

Akaike, H., 1998. Information theory and an extension of the maximum likelihood
principle. In: Akaike H (ed). Selected papers of Hirotugu Akaike, Springer Series in Statistics
(Perspectives in Statistics), Springer, Dordrecht, 199-213. https://doi.org/10.1007/978-14612-1694-0_15

Angeletti, D., Sebbio, C., Carere, C., Cimmaruta, R., Nascetti, G., Pepe, G., Mosesso, P.,
2016. Terrestrial Gastropods (*Helix Spp*) as sentinels of primary DNA damage for
biomonitoring purposes: A validation study. Environ. Mol. Mutagen. 54(3), 204–212.
https://doi.org/10.1002/em.21766

Angelier, F., Costantini, D., Blévin, P., Chastel, O., 2018. Do gluococorticoids mediate
the link between environmental conditions and telomere dynamic in wild vertebrates? A
review. Gen. Comp. Endocrinol. 256, 99-111. https://doi.org/10.1016/j.ygcen.2017.07.007

Bamze Attoumani, R., de Vaufleury, A., Crini, N., Fatin-Rouge, N., 2020. Assessing
natural clays of contaminated site to stabilize and reduce the ecotoxicity of a coal tar.
Ecotoxicol. Environ. Safe. 190, 110081. https://doi.org/10.1016/j.ecoenv.2019.110081

Baerlocher, G.M., Rice, K., Vulto, I., Lansdorp, P.M., 2007. Longitudinal data on
telomere length in leukocytes from newborn baboons support a marked drop in stem cell
turnover around 1 year of age. Aging cell 6(1), 121-123. https://doi.org/10.1111/j.14749726.2006.00254.x

401 BASOL 25.0020, 2015. Fiche 25 - Vieux-Charmont – IPM FRANCE (ex Burgess
 402 Norton). https://basol.developpement-durable.gouv.fr/fiche.php?page=1&index_sp=25.0020

Baurand, P.E., Capelli, N., Scheifler, R., de Vaufleury, A., 2014. An assessment of the
embryotoxicity of cadmium in the terrestrial mollusk *Cantareus aspersus*: From
bioaccumulation to impacts at different levels of biological organization. Ecotoxicol. Environ.
Safe. 110, 89-94. https://doi.org/10.1016/j.ecoenv.2014.08.017

Baurand, P.E., Capelli, N., de Vaufleury, A., 2015. Genotoxicity assessment of pesticides
on terrestrial snail embryos by analysis of random amplified polymorphic DNA profiles. J.
Hazard. Mater. 298, 320-327. https://doi.org/10.1016/j.jhazmat.2015.05.051

Blackburn, E., 1991. Structure and function of telomeres. Nature 350, 569-573.
https://doi.org/10.1038/350569a0

Blackburn, E.H., 2005. Telomeres and telomerase: their mechanisms of action and the
effects of altering their functions. FEBS Lett. 579, 859-862.
https://doi.org/10.1016/j.febslet.2004.11.036

Bride, J., Gomot, L., 1991. Asynchronous development of the genital tract during growth
and reproduction in the snail, *Helix aspersa*. Reprod. Nutr. Dev. 31, 81-96.

Brümmendorf, T., Mak, J., Sabo, K.M., Baerlocher, G.M., Dietz, K., Abkowitz, J.L.,
Lansdorp, P.M., 2002. Longitudinal studies of telomere length in feline blood cells:
implications for hematopoietic stem cell turnover in vivo. Exp. Hematol. 30(10), 1147-1152.
https://doi.org/10.1016/S0301-472X(02)00888-3

de Vaufleury, A., Kerhoas, I., 2000. Effects of cadmium on the reproductive system of
the land snail *Helix aspersa*. Bull. Environ. Contam. Toxicol. 64, 434-442.
https://doi.org/10.1007/s001280000019

de Vaufleury, A., 2015. Landsnail for ecotoxicological assessment of chemicals and soil
contamination. In Armon, R.H Hänninen, O. (Ed.), Environmental Indicators. Springer,
Dordrecht, 345-391. https://doi.org/10.1007/978-94-017-9499-2_23

427 Cawthon, R.M., 2002. Telomere measurement by quantitative PCR. Nucleic. Acids. Res.
428 30(10), 1-6. https://doi.org/10.1093/nar/30.10.e47

Chen, Y., Ebenstein, A., Greenstone, M., Li, H., 2013. Evidence of the impact of
sustained exposure to air pollution on life expectancy from China's Huai River policy. Proc.
Natl. Acad. Sci. U.S.A. 110(32), 12936-12941. https://doi.org/10.1073/pnas.1300018110

432 Cicchetti, D.V., 1994. Guidelines, criteria, and rules of thumb for evaluating normed and
433 standardized assessment instruments in psychology. Psychol. Assess. 6(4), 284-290.
434 https://doi.org/10.1037/1040-3590.6.4.284

Cœurdassier, M., de Vaufleury, A., Lovy, C., Badot, P.M., 2002. Is the cadmium uptake
from soil important in bioaccumulation and toxic effects for snails? Ecotoxicol. Environ. Safe.
53(3), 425-431. https://doi.org/10.1016/S0147-6513(02)00004-0

- 438 Comfort, A., 1957. The duration of life in molluscs. J. Moll. Stud. 32(6), 219-241.
 439 https://doi.org/10.1093/oxfordjournals.mollus.a064785
- Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N., Foote, C., Griffiths, K., Gault, E.,
 Monaghan, P., 2009. Real-time quantitative PCR assay for measurement of avian telomeres.
 J. Avian. Biol. 40, 342-347. https://doi.org/10.1111/j.1600-048X.2008.04623.x
- de Oliveira, A.F.B., de Souza, M.R., Benedetti, D., Scotti, A.S., Piazza, L.S., Garcia,
 A.L.H., Dias, J.F., Niekraszewicz, L.A.B., Duarte, A., Bauer, D., Amaral, L., Bassi Branco,
 C.L., de Melo Reis, E., da Silva, J., 2019. Investigation of pesticide exposure by
 genotoxicological, biochemical, genetic polymorphic and *in silico* analysis. Ecotoxicol.
 Environ. Safe. 179, 135-142. https://doi.org/10.1016/j.ecoenv.2019.04.023
- Druart, C., Gimbert, F., Scheifler, R., de Vaufleury, A., 2017. A full life-cycle bioassay
 with *Cantareus aspersus* shows reproductive effects of a glyphosate-based herbicide
 suggesting potential endocrine disruption. Environ. Pollut. 226, 240-249.
 https://doi.org/10.1016/j.envpol.2017.03.061
- 452 Dugdale, H.L., Richardson, D.S., 2018. Heritability of telomere variation: it is all about
 453 the environment! Philos. Trans. R. Soc. Lond. B Biol. Sci. 373(1741), 20160450.
 454 https://doi.org/10.1098/rstb.2016.0450
- Gimbert, F., Perrier, F., Caire, A., de Vaufleury, A., 2016. Mercury toxicity to terrestrial
 snails in a partial life cycle experiment. Environ. Sci. Pollut. Res. 23(4), 3165-3175.
 https://doi.org/10.1007/s11356-015-5632-y
- Gimbert, F., de Vaufleury, A., Douay, F., Coeurdassier, M., Scheifler, R., Badot, P.M.,
 2008. Long-term responses of snails exposed to cadmium-contaminated soils in a partial lifecycle experiment. Ecotox. Environ. Safe. 70, 138-146.
 https://doi.org/10.1016/j.ecoenv.2007.05.014
- Giraudeau, M., Heidinger, B., Bonneaud, C., Sepp, T., 2019. Telomere shortening as a
 mechanism of long-term cost of infectious diseases in natural animal populations. Biol. Lett.
 15(5), 20190190. https://doi.org/10.1098/rsbl.2019.0190

Godwin, R., Brown, I., Montgomery, S., Frusher S., Green, T., Ovenden, J. 2012.
Telomere dynamics in the Sydney rock oyster (*Saccostrea glomerata*): an investigation into

the effects of age, tissue type, location and time of sampling. Mar. Biol. 159, 77-86.
https://doi.org/10.1007/s00227-011-1791-7

Gomot, A., Gomot, L., Boukraa, S., Bruckert, S., 1989. Influence of soils on the growth
of the land snail *Helix aspersa*. An experimental study on the absorption route for the
stimulating factors. J. Mollusc. Stud. 55(1), 1-7. https://doi.org/10.1093/mollus/55.1.1-a

Gomot, A., Gomot, L., Marchand, C., Colard, C., Bride, J., 1992. Immunocytochemical
localization of insulin-related peptide(s) in the central nervous system of the snail *Helix aspersa* Müller: involvement in growth control. Cell. Mol. Neurobiol. 12(1), 21-32.
https://doi.org/10.1007/BF00711636

Gomot, A., Gomot, L., 1995. Brain grafts of cerebral ganglia have effectiveness in
growth restoration of damaged *Helix aspersa* mesocerebrum. Brain Res. 682, 127-132.
https://doi.org/10.1016/0006-8993(95)00339-R

Gomot, A., 1997. Dose-dependent effects of cadmium on the growth of snails in toxicity
bioassays. Arch. Environ. Contam. Toxicol. 33, 209-216.
https://doi.org/10.1007/s002449900245

482 Gomot-de Vaufleury, A., 2000. Standardized growth toxicity testing (Cu, Zn, Pb and
483 pentachlorophenol) with *Helix aspersa*. Ecotoxicol. Environ. Safe. 46, 41-50.
484 https://doi.org/10.1006/eesa.1999.1872

Gomot-de Vaufleury, A., Pihan, F., 2000. Growing snails used as sentinels to evaluate
terrestrial environment contamination by trace elements. Chemosphere 40, 275-283.
https://doi.org/10.1016/S0045-6535(99)00246-5

488 Goyns, M.H., Lavery, W.L., 2000. Telomerase and mammalian ageing: a critical 489 appraisal. Mech. Ageing Dev. 114(2), 69-77. https://doi.org/10.1016/S0047-6374(00)00095-6

Greider, C.W., Blackburn, E.H., 1985. Identification of a specific telomere terminal
transferase activity in Tetrahymena extracts. Cell 43, 405-413. https://doi.org/10.1016/00928674(85)90170-9

Greider, C.W., Blackburn, E.H., 1989. A telomeric sequence in the RNA of Tetrahymena
telomerase required for telomere repeat synthesis. Nature 337, 331-337.
https://doi.org/10.1038/337331a0

Gruber, H., Schaible, R., Ridgway, I.D., Chow, T.T., Held, C., Philipp. E.E.R., 2014.
Telomere-independent ageing in the longest-lived non-colonial animal, *Arctica islandica*.
Exp. Gerontol. 51, 38-45. https://doi.org/10.1016/j.exger.2013.12.014

Hall, M.E., Nasir, L., Daunt, F., Gault, E.A., Croxall, J.P., Wanless, S., Monaghan, P.,
2004. Telomere loss in relation to age and early environment in long-lived birds. Proc. R. Soc.
Lond. 271, 1571-1576. https://doi.org/10.1098/rspb.2004.2768

Harley, C.B., Vaziri, H., Counter, C.M., Allsopp, R.C., 1992. The telomere hypothesis of
cellular aging. 27(4), 375-382. https://doi.org/10.1016/0531-5565(92)90068-B

Ismert, M., Oster, T., Bagrel, D., 2002. Effects of atmospheric exposure to naphthalene
on xenobiotic-metabolising enzymes in the snail *Helix aspersa*. Chemosphere 46(2), 273-280.
https://doi.org/10.1016/S0045-6535(01)00124-2

Itziou, A., Kaloyianni, M., Dimitriadis, V., 2011. Effect of organic contaminants in
reactive oxygen species, protein carbonylation and DNA damage on digestive gland and
haemolymph of land snails. Chemosphere 85, 1101-1107.
https://doi.org/10.1016/j.chemosphere.2011.07.043

Itziou, A., Patsis, P., Dimitriadis, V., 2018. Introduction of the land snail *Cornu aspersum*as a bioindicator organism of terrestrial pollution with the use of a suite of biomarkers.
Toxicol. Environ. Chem. 100, 717-736. https://doi.org/10.1080/02772248.2019.1588274

Klapper, W., Kühne, K., Singh, K.K., Heidorn, K., Parwaresch, R., Krupp, G., 1998.
Longevity of lobsters is linked to ubiquitous telomerase expression. FEBS Lett. 439, 143-146.
https://doi.org/10.1016/S0014-5793(98)01357-X

Lagadic, L., Coutellec, M., Caquet, T., 2007. Endocrine disruption in aquatic pulmonate
molluscs: few evidences, many challenges. Ecotoxicology 16(1), 45-59.
https://doi.org/10.1007/s10646-006-0114-0

Lindstrom, M.J., Bates, D.M., 1990. Nonlinear mixed effects models for repeated measures data. Biometrics 46(3), 673-687. https://doi.org/10.2307/2532087

López-Otín, C., Blasco, M., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks
of aging. Cell 153, 1194-1217. https://doi.org/10.1016/j.cell.2013.05.039

Louzon, M., Coeurdassier, M., Gimbert, F., Pauget, B., de Vaufleury, A., 2019. Telomere
dynamic in humans and animals: review and perspectives in environmental toxicology.
Environ. Int. 131, 105025. https://doi.org/10.1016/j.envint.2019.105025

Lucarz, A., Gomot, L., 1985. Influence de la densité de population sur la croissance
diamétrale et pondérale de l'escargot *Helix aspersa* Müller dans différentes conditions
d'élevage. J. Moll. Stud. 51, 105-115. https://doi.org/10.1093/oxfordjournals.mollus.a065892

Magniez, G., Franco, A., Geffard, A., Rioult, D., Bonnard, I., Delahaut, L., Joachim, S.,
Daniele, G., Vulliet, E., Porcher, J.M, Bonnard, M., 2018. Determination of a new index of
sexual maturity (ISM) in zebra mussel using flow cytometry: interest in ecotoxicology.
Environ. Sci. Pollut. Res. 25, 11252-11263. https://doi.org/10.1007/s11356-017-9256-2

Mariet, A.L., Pauget, B., de Vaufleury, A., Bégeot, C., Walter-Simonnet, A.V., Gimbert,
F., 2017. Using bioindicators to assess the environmental risk of past mining activities in the
Vosges Mountains (France). Ecol. Indic. 75, 17-26.
https://doi.org/10.1016/j.ecolind.2016.11.042

Matthiessen, P., Gibbs, P.E., 1998. Critical appraisal of the evidence for tributyltinmediated endocrine disruption in mollusks. Environ. Toxicol. Chem. 17(1), 37-43.
https://doi.org/10.1002/etc.5620170106

Matzenbacher, C.A., Da Silva, J., Garcia, A.L.H., Cappetta, M., de Freitas, T.R.O., 2019.
Anthropogenic effects on natural mammalian populations: correlation between telomere
length and coal exposure. Sci. Rep. 6325. https://doi.org/10.1038/s41598-019-42804-8

Maximova, N., Koroleva, A., Sitnikova, T., Khanaev, I., Bukin, Y., Kirilchik, S., 2017.
Age dynamics of telomere length of Baikal gastropods is sex-specific and multidirectional.
Folia Biol. 65(4), 187-197. https://doi.org/10.3409/fb65_4.187

Mleiki, A., Zaldibar, B., Izagirre, U., El Menif, N.T., Marigomez, I., 2018. Effects of
dietary Pb and Cd and their combination on lysosomal and tissue-level biomarkers and
histopathology in digestive gland of the land snail, *Cantareus apertus* (Born, 1778).
Ecotoxicol. Environ. Safe. 156, 301-310. https://doi.org/10.1016/j.ecoenv.2018.02.079

Molina, J., Oyarzun, R., Esbrí, J., Higueras, P., 2006. Mercury accumulation in soils and
plants in the Almaden mining district, Spain: one of the most contaminated sites on Earth.
Environ. Geochem. Health 28, 487-498. https://doi.org/10.1007/s10653-006-9058-9

Morin-Crini, N., Druart, C., Amiot, C., Gimbert, F., Chanez, E., Crini, G., 2014. Towards 554 a convenient procedure to characterize polycylic aromatic hydrocarbons in sediments 555 Pollut. Cont. 1000128. 556 receiving industrial effluents. J. Eff. 3(1), 557 https://doi.org/10.4172/2375-4397.1000128

558 Muraki, K., Nyhan, K., Han, L., Murnane, J.P., 2012. Mechanisms of telomere loss and 559 their consequences for chromosome instability. Front. Oncol. 135, 2. 560 https://doi.org/10.3389/fonc.2012.00135

Nettle, D., Seeker, L., Nussey, D., Froy, H., Bateson, M., 2019. Consequences of
measurement error in qPCR telomere data: A simulation study. PLoS ONE 14(5), e0216118.
https://doi.org/10.1371/journal.pone.0216118

Nussey, D.H., Baird, D., Barrett, E., Boner, W., Fairlie, J., Gemmell, N., Hartmann, N.,
Horn, T., Haussmann, M., Olsson, M., Turbill, C., Verhulst, S., Zahn, S., Monaghan, P., 2014
Measuring telomere length and telomere dynamics in evolutionary biology and ecology.
Methods. Ecol. Evol. 5, 299-310. https://doi.org/10.1111/2041-210X.12161

568 OECD, 1984. Test no. 207: earthworm acute toxicity tests. In: Organisation for Economic
569 Co-operation and Development (ed). Guidelines for the testing of chemicals 1, 1-9.
570 https://doi.org/10.1787/9789264070042-en

571 Oeseburg, H., de Boer, R., van Gilst, W., van der Harst, P., 2009. Telomere biology in
572 healthy aging and disease. Eur. J. Physiol. 459, 259-268. https://doi.org/10.1007/s00424-009573 0728-1

Parolini, M., Possenti, C.D., Caprioli, M., Rubolini, D., Romano, A., Saino, N., 2019.
Egg testosterone differentially affects telomere length in somatic tissues of yellow-legged gull
embryos. Physiol. Biochem. Zool. 92(5), 459-462. https://doi.org/10.1086/705037

Pauget, B., Gimbert, F., Scheifler, R., Coeurdassier, M., de Vaufleury, A., 2012. Soil
parameters are key factors to predict metal bioavailability to snails based on chemical

 579
 extractant
 data.
 Sci.
 Total
 Environ.
 431,
 413-425.

 580
 https://doi.org/10.1016/j.scitotenv.2012.05.048
 6
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Pauget, B., Gimbert, F., Coeurdassier, M., Crini, N., Pérès, G., Faure, O., Douay, F.,
Richard, A., Grand, C., de Vaufleury, A., 2013. Assessing the in situ bioavailability of trace
elements to snails using accumulation kinetics. Ecol. Indic. 34, 126-135.
https://doi.org/10.1016/j.ecolind.2013.04.018

Pauget, B., de Vaufleury, A., 2015. The SET and ERITME indices: Integrative tools for
the management of polluted sites. Ecol. Indic. 53, 206-210.
https://doi.org/10.1016/j.ecolind.2015.01.037

Perera, F., Lin, C.J., Qu, L., Tang, T., 2018. Shorter telomere length in cord blood
associated with prenatal air pollution exposure: benefits of intervention. Environ. Int. 113,
335-340. https://doi.org/10.1016/j.envint.2018.01.005

Pila, E.A., Sullivan, J.T., Wu, X.Z., Fang, J., Rudko, S.P., Gordy, M.A., Hanington, P.C.,
2016. Haematopoiesis in molluscs: A review of haemocyte development and function in
gastropods, cephalopods and bivalves. Dev. Comp. Immunol. 58, 119-128.
https://doi.org/10.1016/j.dci.2015.11.010

Puri, P., Nandar, S., Kathuria, S., Ramesh, V., 2017. Effects of air pollution on the skin:
A review. Indian J. Dermatol. Venereol. Leprol. 83(4), 415-423. https://doi.org/10.4103/03786323.199579

Regoli, F., Gorbi, S., Fattorini, D., Tedesco, S., Notti, A., Machella, N., Bocchetti, R.,
Benedetti, M., Piva, F., 2006. Use of the land snail *Helix aspersa* as sentinel organism for
monitoring ecotoxicological effects of urban pollution: an integrated approach. Environ.
Health Perspect. 114, 63-69. https://doi.org/10.1289/ehp.8397

Reichert, S., Froy, H., Boner, W., Burg, T., Daunt, F., Gillespie, R., Griffiths, K., Lewis,
S., Phillips, R., Nussey, D.H., Monaghan, P., 2017. Telomere length measurement by qPCR
in birds is affected by storage method of blood samples. Oecologia 184, 341-350.
https://doi.org/10.1007/s00442-017-3887-3

Sabir, S., Sajid, M., Akash, H., Fiayyaz, F., Saleem, U., Mehmood, M.H., Rehman, K.,
2019. Role of cadmium and arsenic as endocrine disruptors in the metabolism of
carbohydrates: inserting the association into perspectives. Biomed. Pharmacother. 114,
108802. https://doi.org/10.1016/j.biopha.2019.108802

Salomons, H.M., Mulder, G.A., van de Zande, L., Haussmann, M.F., Linskens, M.H.K.,
Verhulst, S., 2009 Telomere shortening and survival in free-living corvids. Proc. R. Soc.
Lond. 276. https://doi.org/10.1098/rspb.2009.0517

Scheifler, R., de Vaufleury, A., Coeurdassier, M., Crini, N., Badot, P.M., 2006.Transfer
of Cd, Cu, Ni, Pb and Zn in a soil-plant-invertebrate food chain: a microcosm study. Environ.
Toxicol. Chem. 25(3), 815-822. https://doi.org/10.1897/04-675R.1

616 Scheifler, R., Gomot-de Vaufleury, A., Toussaint, M., Badot, P.M., 2002. Transfer and 617 effects of cadmium in an experimental food chain involving the snail *Helix aspersa* and the 618 predatory carabid beetle *Chrysocarabus splendens*. Chemosphere 48, 571-579.
619 https://doi.org/10.1016/S0045-6535(02)00116-9

Schumpert, C., Handy, I., Dudycha, J.L., Patel, R.C., 2014. Relationship between heat
shock protein 70 expression and life span in Daphnia. Mech. Ageing Dev. 139, 1-10.
https://doi.org/10.1016/j.mad.2014.04.001

Schumpert, C., Nelson, J., Kim, E., Dudycha, J., Patel, R., 2015. Telomerase activity and
telomere length in daphnia. PLoS One 10, e0127196.
https://doi.org/10.1371/journal.pone.0127196

Scott, N.M Haussmann, M.F Elsey, R.M Trosclair, P.L Vleck, C.M., 2006. Telomere
length shortens with body length in *Alligator mississippiensis*. Southeast. Nat. 5, 685-692.
https://doi.org/10.1656/1528-7092(2006)5[685:TLSWBL]2.0.CO;2

Shay, J.W., 2016. Role of telomeres and telomerase in aging and cancer. Cancer Discov.
6(6), 584-593. https://doi.org/10.1158/2159-8290.CD-16-0062

Spurgin, L.G., Bebbington, K., Fairfield, E.A., Hammers, M., Komdeur, J., Burke, T.,
Dugdale, H.L., Richardson, D.S., 2018. Spatio-temporal variation in lifelong telomere
dynamics in a long-term ecological study. J. Anim. Ecol. 87(1), 187-198.
https://doi.org/10.1111/1365-2656.12741

Sun, F., Littlejohn, D., Gibson, M.D., 1998. Ultrasonication extraction and solid phase
extraction clean-up of US EPA 16 priority pollutant polycyclic aromatic hydrocarbons in soils
by reversed-phase liquid chromatography ultraviolet absorption detection. Anal. Chim. Act.
364(1-3), 1-11. https://doi.org/10.1016/S0003-2670(98)00186-X

Sverdrup, L., de Vaufleury, A., Hartnik, T., Hagen, S., Loibner, A., Jensen, J., 2006.
Effects and uptake of polycyclic aromatic compounds in snails (*Helix aspersa*). Environ.
Toxicol. Chem.. 25(7), 1941-1945. https://doi.org/10.1897/05-620R1.1

Traut, W., Szczepanowski, M., Vítková, M., Opitz, C., Marec, F., Zrzavý, J., 2007. The
telomere repeat motif of basal Metazoa. Chromosome Res. 15, 371-382.
https://doi.org/10.1007/s10577-007-1132-3

Ujvari, B., Madsen, T., 2009. Short telomeres in hatchling snakes: erythrocyte telomere
dynamics and longevity in tropical pythons. PLoS One 4, e7493.
https://doi.org/10.1371/journal.pone.0007493

Vitturi, R., Libertini, A., Sineo, L., Sparacio, I., Lannino, A., Gregorini, A., Colomba, M.,
2005. Cytogenetics of the land snails *Cantareus aspersus* and *C. mazzullii* (Mollusca:
Gastropoda: Pulmonata). Micron 36, 351-357. https://doi.org/10.1016/j.micron.2004.12.010

Zeichner, S.L., Palumbo, P., Feng, Y., Xiao, X., Gee, D., Sleasman, J., Goodenow, M.,
Biggar, R., Dimitrov, D., 1999. Rapid telomere shortening in children. Blood 93(9), 28242830. https://doi.org/10.1182/blood.V93.9.2824

Table of figures

Figure 1: The investigated *C. aspersus* age classes.

Figure 2: A: Relationship between the relative telomere length (RTL) in haemocytes and age of *C. aspersus* (n=8 snails per age class represented by black dots); **B:** RTL in haemocytes of *C. aspersus* of different age classes (statistically significant differences are indicated by letters when p-values < 0.05).

Figure 3: A: Percentage of mortality of snails (mean and standard deviation), **B:** percentage of growth and **C:** sexual maturity inhibition (mean and standard deviation) after exposure to contaminated (Cd, Hg, PAHs) or uncontaminated soils (ISO) for 1 and 2 months (statistically significant differences are indicated by letters with p-value < 0.05).

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Juveniles	Sub-adults (t0)	Young adults (t1)	Young reproducers (t2)	Old reproducers
~1 g – 1 month	~5 g – 3 months	~9g – 4 months	~11 g – 5 months	~14 g – 24 months



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Juveniles	Sub-adults (t0)	Young adults (t1)	Young reproducers (t2)	Old reproducers
~1 g – 1 month	~5 g – 3 months	~9 g – 4 months	~11 g – 5 months	~14 g – 24 months







Table 1: Concentration of Cd, Hg and sum of the 16 PAHs in uncontaminated commercial food (Helixal[®]), soils and snails before exposure (t0) and after 1 month (t1) and 2 months (t2) exposure to Cd, Hg or PAHs in soils (mean and standard deviation for Cd, Hg and PAHs concentrations, expressed in mg kg⁻¹ dry weight (dw) for Cd or Hg in the soils, food and snails; while PAHs are expressed in mg kg⁻¹ fresh weight (fw) of snails and dw of the food and soils).

			Cd (mg kg ⁻¹ dw)	Hg (mg kg ⁻¹ dw)	ΣPAHs (mg kg ⁻¹)
Food	He	lixal®	0.430	0.490	< 1.00
	ISO soil		0.315	0.029	0.044
Soil	Cd soil		1508	-	-
	Hg soil		-	13410	-
	PAHs soil		-	-	9.36
Snails	T0	t0	3.57 ± 4.83	0.017 ± 0.014	0.016 ± 0.003
	ISO	t1	1.84 ± 0.021	0.009 ± 0.000	0.017 ± 0.002
		t2	4.08 ± 1.72	0.009 ± 0.000	0.017 ± 0.002
	Cd	t1	654 ± 275	-	-
		t2	781 ± 243	-	-
	Hg	t1	-	2136 ± 754	-
		t2	-	2458 ± 714	-
	PAHs	t1	-	-	0.035 ± 0.021
		t2	-	-	0.026 ± 0.011

