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Snail as sentinel organism for monitoring the environmental pollution; a review

Firas Baroudi, Josephine Al Alam, Ziad Fajloun, Maurice Millet

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21 **Abstract**

22 Environmental pollution, one of the most serious problems facing human health, ecosystems and
23 biodiversity, is defined as the contamination of the physical and biological components of the
24 atmosphere system which has harmful consequences for normal environmental processes.
25 Animals, such as snails used as environmental pollution biomonitors, show multiple
26 physiological mechanisms to counteract the effects of toxins in the environment due to their
27 sensitivity to various contaminants and their ability to accumulate them through their tissues. The
28 objective of this review is to explore the possibility of using different types of snails as potential
29 and ideal monitoring matrices to assess air pollution and to detect heavy metal and POPs
30 concentration by different extraction techniques including Soxhlet extraction, Accelerated
31 Solvent Extraction, Solid Phase Extraction, Microwave-Assisted Extraction, Pressurized Hot
32 Water extraction and Microwave Acid Digestion.

33 **Keywords :** Air Pollution, Bioindicator, Snails, Metallic trace elements, Organic pollutants.

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40 **1. Introduction**

41 The World Health Organization (WHO) remains strictly bound by the principles set out in the
42 constitution preface that described health as “a complete state of physical, mental and social
43 well-being and not just the absence of disease or infirmity” (Ortmann et al., 2016). Several
44 factors, such as demographic, socio-economic, environmental geographic, climatic and
45 meteorological factors, affect directly or indirectly the health of individuals and populations. In
46 fact, the contribution of all these factors is of paramount importance on health sector (Ebi et al.,
47 2017).

48 Various human activities, industrial and other anthropogenic waste, concentrated around the
49 world, exceed the critical levels set by the European Union and other environmental
50 governments leading to serious problems (Guerreiro et al., 2014). Indeed, air pollution’s
51 exposure is characterized by a person's exposure to pollutant concentration over a time period
52 that depends on the concentrations of air pollutants present at the sites through which the person
53 moves as well as the time spent at each site causing respiratory, cardiovascular and cancer
54 diseases problems at excessive levels (Reames and Bravo, 2019; Lee et al., 2014; Le Tertre et al.,
55 2002; Stieb et al., 2002). The existence of a pollutant in the soil is therefore not a direct risk, but
56 the danger occurs as soon as this pollutant can be activated and acts on the atmosphere or on
57 humans (Tchounwou et al., 2012). By definition, a complete risk model, consisting of five
58 components (sources, transport, exposure, dose and effect), is used as a method of adjusting the
59 concentration of a toxic pollutant to estimate the variance in health risk (Laumbach et al., 2015;
60 Muralikrishna and Manickam, 2017).

61 Therefore, monitoring and predicting occurring changes in the environment as well as the effects
62 of air pollution is critical. Such monitoring stations evaluate long-term environmental changes
63 but are complicated to implement and maintain. Thus, biomonitoring can provide alternative
64 method for assessing air pollution (Marshall et al., 2019). In recent years, the expansion of
65 passive samplers has been one of the significant developments in air sampling technology.
66 Palmes and Gunnison first applied air sampling to the safety and health field in 1973 (Palmes
67 and Gunnison, 1973). As the applications of this technology have developed and changed
68 recently, the number and different kinds of passive samplers are increasing rapidly and become a
69 crucial element in the range of air sampling equipment (Amato et al., 2018; Osytek et al., 2008).

70 Among the very well-known passive samplers, figure the biomonitors. In fact, a biomonitor,
71 described as a species of animals, plants or fungi providing information on the quality of the
72 environment in which it resides, is usually characterized by its sedentary lifestyle, wide
73 distribution and ease of identification and collection (Parmar et al., 2016; Kovalchuk and
74 Kovalchuk, 2008). Invertebrate species, such as snails are recognized as appropriate biological
75 indicators due to their potential accumulation of persistent organic pollutants (POPs) and
76 metallic trace elements and are also used as a research species to evaluate the effect of these
77 pollutants on their development (Cossi et al., 2018; Krupnova et al., 2018; Hodgkinson and
78 Jackson, 2005).

79 For instance, terrestrial snails (*Helix aspersa*, *Cepaea nemoralis*, *Theba pisana* and *Eobania*
80 *vermiculata*) are excellent biomonitors of environmental contamination due to their easy
81 sampling, wide distribution, high tolerance to stress and their ability to accumulate diverse

82 contaminants in the air, soil and flora (Soltani, 2013; Itziou and Dimitriadis, 2011; De Vaufleury
83 and Pihan, 2000).

84 The purpose of this study is to assess the feasibility to use naturally distributed snails to control
85 the concentration of contaminants throughout the ecosystem. The theory is that snails feeding on
86 species that are directly exposed to dry and wet pollutant deposition and inhaling major urban air
87 pollutants can be an important biomonitor sentinel for determining pollutants and heavy metals'
88 spatial distribution and bioavailability. This review sheds light on some parameters including
89 production of nitric oxide, phenol oxidase, and lysozymes that are associated with the immune
90 response that is resulting from the interaction between the snail and the particles and organisms.
91 The quality of the growth of some snails gives indices of degree of soil pollution by pesticides or
92 some metal trace elements. It allows for example the evaluation of the bioassimilable chromium
93 content of a soil for example or of organophosphorus pesticides or even to study the
94 bioaccumulation of heavy metals.

95

96 **2. Snails as atmospheric pollution biomonitoring tools**

97 Representative species of the environment must be studied to assess the effects of pollutants in a
98 matrix. In fact, a biomonitor can be identified as an organism or group of organisms that are
99 important for qualitative and quantitative environmental pollution determination (Fränze, 2006).
100 Many studies have shown that gastropod mollusks including terrestrial snails are pollution
101 bioindicators, including metals, pesticides, PAHs, PCBs and PBDEs (Beeby and Richmond,
102 2002; Fu et al., 2011; De Vaufleury and Pihan, 2000; Silva et al., 2019). Such species occupy an

103 important place in the soil-air-vegetation interface of the environment and incorporate many
104 pollution sources (soil, atmosphere, plants) through the digestive, respiratory and/or cutaneous
105 tracts. Multiple species of snails have been recorded, for example *Papillifera papillaris* (Emilia
106 et al., 2016), *Indothais gradata* (Proum et al., 2016), *Pomacea canaliculata* (Ramli et al., 2019;
107 Dumme et al., 2012), *Cantareus apertus* (Mleiki et al., 2016), *Helix aspersa* (Viard et al., 2004;
108 Abdel-Halim et al., 2013), *Eobania vermiculata* (El-Shenawy et al., 2012; Itziou and Dimitriadis,
109 2011; Itziou et al., 2011), *Cepaea nemoralis* (Boshoff et al., 2015), *Bellamya aeruginosa* (Yin et
110 al., 2014), *Theodoxus niloticus* (Abdel Gawad, 2018) and *Achatina fulica* (Cho et al., 2019).
111 Such snails colonize most environments and frequent the periphery of agricultural and forest
112 areas. In contrast, several species have shown their ability to resist and accumulate contaminants
113 (Notten et al., 2005; Radwan et al., 2010; Scheifler et al., 2002). For this reason, snails can be
114 useful biomonitoring organisms commonly used as effective indicators in recent studies to
115 determine the effects of pollutant mixtures on environments in space and time, based on the
116 species collected (Cœurdassier et al., 2002; Druart et al., 2011; Gimbert et al., 2006).

117 **2.1. Characteristics of snails**

118 Snails have long been used to study pollutant accumulation. The assessment of the accumulation
119 of contaminants such as metals is dependent on the assessment of internal concentrations after a
120 specified exposure period and authorize the possibility to assess the accumulation capacity of
121 snails, their bioavailability and the intensity of the transfer of contaminants from the environment
122 (food and / or soil) (Gimbert et al., 2006). Invertebrate species accumulate pollutants as a
123 consequence of absorption-assimilation, storage, transmission and excretion processes (Regoli et

124 al., 2006). Once pollutants are absorbed, terrestrial snails have a non-regulation strategy that is
125 influenced by calcium metabolism (Notten et al., 2005), and the need to prevent excessive water
126 losses (Dallinger et al., 2001). In fact, there is two great parts to be considered: the foot and the
127 viscera. The viscera refer to the shell's organs and includes the kidney, hepatopancreas, heart and
128 part of the genital system that also extends into the foot. The foot essentially comprises the
129 anterior part of the digestive tract and the nervous system. There is a dose-dependent increase in
130 the concentration of pollutants in both organs analyzed (hepatopancreas and kidney) and their
131 accumulation is related to their organism's bioavailability and environmental concentrations
132 (Cœurdassier et al., 2002). Due to the open circulatory system of snails, there is no distinct
133 difference between the blood and the lymph. As a consequence, the circulatory fluid is generally
134 known to as a haemolymph, instead of blood. The organic and inorganic composition of the
135 haemolymph is variable, and factors such as temperature, photoperiod, activity, hibernation,
136 hydration and feeding, affect the composition of the haemolymph. However, shell size and age,
137 influences the haemolymph pollutants accumulation (Pagano et al., 2017). Moreover, Itziou et
138 al., in 2011 showed the use of land snails as biomarkers for early detection of organic pollutants
139 in the environment, since significant alterations have been reported in haemolymph and digestive
140 gland of snails exposed to organic contaminants (Itziou et al., 2011).

141 For instance, a high accumulation capacity of heavy metals, particularly of Cu, Zn, Cd and Pb,
142 was shown in gastropod mollusks in 2004, as indicated by Viard et al. (Viard et al., 2004).
143 Additionally, particularly in the Mediterranean and oceanic regions, snails are widespread, and
144 they colonize anthropogenic environments and are also found in woods, rocks and areas of
145 agriculture. They mainly feed on plants, lichens and fungi and adapt to the plants that colonize

146 the ecosystem (Chevalier et al., 2001; Guiller et al., 2012). Therefore, the soil is also a part of the
147 diet of snails and can affect their growth, so a low calcium soil could be a restricting factor in the
148 development of snails (Dallinger et al., 2001). In addition, snails exposed to terrestrial
149 environment, are subjects to frequent and seasonal fluctuations in temperature. In order to resist
150 to these fluctuations such as heat and aridity over long periods of estivation, snails are able to
151 form an epiphragm by their dried mucous. Such snails normally resume activities in the early
152 spring when temperatures are around 12-14 °C (Arad and Heller, 2009; Eugene Havel et al.,
153 2014; Gaitán-Espitia et al., 2013).

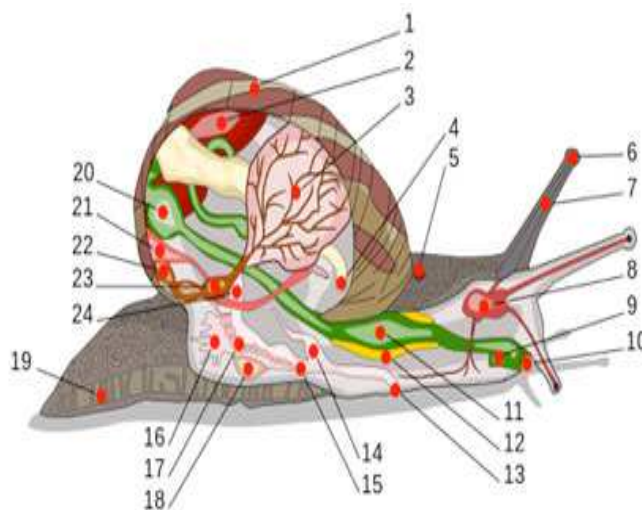
154 In common with other invertebrates, mollusks are known by their innate immunity composed of
155 both cellular and humoral elements consider as the first line of defense that reflects the immune
156 response against foreign particles and organisms by the phagocytic cells, anatomic barriers and
157 physiological components. Nitric oxide, phenyloxidase system, lysozyme activity and lectins are
158 the humoral components, while the circulating phagocytic hemocytes are the cellular
159 components of snail immunity. Small invaders are eliminated by phagocytic hemocytes, while
160 large invaders are eliminated by encapsulation. The pathogens and foreign invaders are then
161 hemolyzed by the action of certain toxic enzymes that catalyze oxidative burst reactions (Wang
162 et al., 2018).

163 **2.2. Snails, matrix used for biomonitoring**

164 Owing to their wide distribution, snails can be used to evaluate soil contaminant bioavailability
165 by measuring soil-snail or soil-plant-snail transmission and to analyze the bioavailability of
166 environmental pollutants (soil, plants air) by measuring their concentration in caged snails during

167 a specified time period. Snails may reflect the quality of their atmosphere by accumulating some
168 toxic pollutants present in their biotope in their twist or visceral mass and shell. Their mucus
169 protects them from external threats and from the infections of bacteria and fungi (De Vaufleury
170 and Gimbert, 2009).

171 Ecotoxicology is important for assessing the value of ecosystems and biomes: it is particularly
172 interesting in interactions between environmental chemicals and biotope, covering several areas
173 including bioindicator development. The snails can be easily collected, raised, identified and
174 found nearly anywhere. In addition, snails are in contact with different pollutants that they
175 consume transcutaneous, digestively and respiratory from water, plants and contact with the
176 ground soil (De Vaufleury and Pihan, 2000).



177

178 *Figure 1: Sectional diagram of the anatomy of a snail (Winkelmann A, 2007).*

179

180 1: Shell. 2: Liver. 3: Lung. 4: Anus. 5: Respiratory pore. 6: Eye. 7: Tentacle. 8: Brain. 9: Salivary duct. 10: Mouth.
181 11: Panse. 12: Salivary gland. 13: Genital opening. 14: Penis. 15: Vagina. 16: mucous gland. 17: Oviduct. 18: Bag
182 of darts. 19: Foot. 20: Stomach. 21: Kidney. 22: Coat. 23: Heart. 24: vas deferens.

183

184 The existence of pollutants has shown that snails are more likely to accumulate in the viscera
185 than in the foot, which indicates the appropriateness of the viscera to expose the pollutant
186 bioavailability in the ecosystem and illustrating the utility of separately analyzing foot and
187 viscera (De Vaufleury and Pihan, 2000). Bioaccumulation of pollutant in snails depends on the
188 duration of exposure, which implies that wild snails are highly contaminated due to their lifetime
189 exposure to environmental pollutants. The exposure period of several weeks reflects the harmful
190 effects and demonstrates that bioaccumulation is modulated by pollution type compared to short
191 exposures that may be sufficient to detect bioaccumulation (Eeva et al., 2010; Nica et al., 2013).

192 In fact, snails can accumulate in their shell toxic minerals and metals (Beeby and Richmond,
193 2011). Studying toxic accumulation in snails is allowed by modifying two parameters:

- 194 • Biological parameters expressed by the organ growth and weight
- 195 • Chemical parameters presented through the phase of bioaccumulation (Abdel-Halim et
196 al., 2013; Gimbert et al., 2008).

197 Several snails are herbivorous with lungs that provide information on air, soil and flora quality.
198 They absorb air through breathing to measure the atmospheric pollution and have the capacity to
199 accumulate large quantities of pollutants in their body without any hurtful effects on their life
200 cycle (De Vaufleury and Gimbert, 2009). Furthermore, snails have the particularity of
201 concentrating the chemical substances present in the soil, the air and the plants of their

202 environment in their tissues and can be used both as test organisms for the study the toxicity of
 203 metals and as bioindicators of terrestrial pollution (Druart et al., 2012; Kramarz et al., 2009). The
 204 pollution state of a soil observed by the analysis of what is accumulated in the gastropod
 205 organism, could be estimated as well as the amount of pollutants and their evaluation, such as
 206 pesticides likely to disperse in nature and contaminate living things (Scheifler et al., 2003;
 207 Gimbert et al., 2006).

208 **2.3. Methods of pollutants extraction and analysis**

209 Various snail species have been studied, such as *Achatina fulica* (Cho et al., 2019), *Indothais*
 210 *gradata* (Proum et al., 2016), *Helix aspersa* (Abdel-Halim et al., 2013; Viard et al., 2004),
 211 *Papillifera papillaris* (Emilia et al., 2016), *Eobania vermiculata* (El-Shenawy et al., 2012; Itziou
 212 and Dimitriadis, 2011), *Cantareus apertus* (Mleiki et al., 2016), and *Pomacea canaliculata*
 213 (Ramli et al., 2019 ; Wu et al., 2019; Dumme et al., 2012). Among these species, *Helix aspera*
 214 was used to investigate fungicides (tebuconazole, folpet, pyraclostrobin and cymoxanil) and
 215 herbicides (glufosinate and glyphosate) contamination using fluorescence detection GC-MS and
 216 HPLC respectively (Druart et al., 2011) while analysis of polycyclic aromatic compounds was
 217 done using liquid chromatography coupled to a fluorimetric detector (Sverdrup et al., 2006).

218 Table 1 summarizes some snail matrices commonly used in studies on biomonitoring, the aim of
 219 the study and the target analytes and their extraction methods and analysis.

220

Matrix	Aim of the study	Analytes	Extraction and clean up	Analytical method	Ref.
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<i>Helix aspersa</i>	Effects and uptake of polycyclic of aromatic compounds <i>Helix aspersa</i>	Polycyclic aromatic compounds	Accelerated solvent extraction	Liquid chromatography coupled to a fluorimetric detector	(Sverdrup et al., 2006)
<i>Buccinum spp. and N. lyrata</i>	Analysis of pyrene metabolites in marine snails	Polycyclic aromatic hydrocarbons: pyrene	Solid phase extraction	Liquid chromatography using fluorescence and mass spectrometry detection	(Beach et al., 2009)
<i>Achatina fulica</i>	Optimization and characterization of polysaccharides extraction <i>Achatina fulica</i> causing	Polysaccharides	Pressurized hot water extraction (PHWE)	-High-performance liquid chromatography (HPLC) -Gel permeation chromatography (GPC)	(Cho et al., 2019)
<i>Pomacea canaliculata</i>	Optimization of saponin extracts using microwave-assisted extraction as a sustainable biopesticide to reduce <i>Pomacea canaliculata</i> population in paddy cultivation	Saponin	Microwave-assisted extraction	-HPLC-UV -UV/Vis spectrophotometric	(Ramli et al., 2019)
<i>Ampullariidae</i>	Spatial distribution of	-Polychlorinated biphenyls (PCBs)	Isotope dilution methods	Gas chromatograph coupled with high-	(Fu et al., 2011)

	polychlorinated biphenyls and polybrominated biphenyl ethers in China	-Polybrominated biphenyl ethers (PBDEs)		resolution mass spectrometer	
<i>Helix aspersa</i>	Measure the exposure, the transfer and the effects of pesticides on a non-target soil invertebrate, the land snail <i>Helix aspersa</i>	-Herbicides (glyphosate and glufosinate) -Fungicides (cymoxanil, folpet, tebuconazole and pyraclostrobin)	Liquid–liquid extraction	-Herbicides : HPLC -Fongicides : GC-MS	(Druart et al., 2011)
<i>Cepaea nemoralis</i>	Trace metal transfer in a soil–plant–snail microcosm field experiment and biomarker responses in snails	As, Ni, Pb and Zn	Digestion with a mixed solution of nitric acid and hydrochloric acid	Inductively Coupled Plasma Mass Spectrometer (ICP-MS)	(Boshoff et al., 2015)
<i>Hexaplex trunculus</i>	Levels of polychlorinated biphenyls in <i>Hexaplex trunculus</i>	Polychlorinated biphenyls	Soxhlet	Thermo Trace GC connected with a Thermo PolarisQ MS operated in electron impact ionization	(Storelli et al., 2014)
<i>Theodoxus niloticus</i>	Acute toxicity of some heavy metals to the snail, <i>Theodoxus niloticus</i>	Zn, Fe and Pb	Digestion with acidified water	Atomic Absorption Reader	(Abdel Gawad, 2018)

			samples, HNO ₃ and perchloric acid		
<i>Cipangopaludina chinensis</i>	Effect of periphyton community structure on heavy metal accumulation <i>Cipangopaludina chinensis</i>	V, Cr, Co, Ni, Cu, Cd, and Pb	Microwave-Assisted Acid Digestion System	ICP-MS	(Cui et al., 2012)
<i>Pomacea canaliculata</i>	Bioaccumulation of heavy metals in water, sediments, aquatic plant and histopathological effects on the golden apple snail	Cu, Mn, Fe, Zn, Pb and Cd	Digestion with a mixed solution of nitric acid-perchloric acid and concentrated hydrochloric acid	Flame atomic absorption spectrophotometer	(Dumme et al., 2012)
<i>Eobania vermiculata</i>	Using the enzymatic and non-enzymatic antioxidant defense system of <i>Eobania vermiculata</i> as biomarkers of terrestrial heavy metal pollution	Cd, Cu, Fe, Ca, Pb and Zn	Digestion with concentrated nitric acid in a microwave digester using microwave digestion program	Inductively coupled plasma optical emission spectrometry (ICPOES)	(El-Shenawy et al., 2012)
<i>Eobania vermiculata</i>	Enrich the group of sentinel organisms of terrestrial pollution biomonitoring, by investigating the efficacy of the land snail <i>Eobania vermiculata</i>	Cadmium, lead and copper	Digestion with HNO ₃ 75% for 24 h and warmed for 30 min for HNO ₃ evaporation	Flame Atomic Absorption	(Itziou and Dimitriadis, 2011)

<i>Papillifera papillaris</i>	<i>Papillifera papillaris</i> , as indicator of metal deposition and bioavailability in urban environments	Al, Cd, Cr, Ni, Pb, Hg, Cu, Fe, Mn and Zn	Digestion in Teflon containers with HNO ₃ and H ₂ O ₂	-Atomic absorption spectrometry with graphite furnace for Al, Cd, Cr, Ni and Pb. -Inductively coupled plasma emission spectrometry for Cu, Fe, Mn and Zn.	(Emilia et al., 2016)
<i>Cepaea nemoralis</i>	Associations between shell strength, shell morphology and heavy metals in <i>Cepaea nemoralis</i>	Ca, Cd, Cr, Pb and Zn	Digestion in nitric acid using the microwave digestion procedure	Inductively coupled plasma-atomic emission spectrophotometry (ICP-AES)	(Jordaens et al., 2006)
<i>Theba pisana</i>	Biomarkers of oxidative stress in <i>Theba pisana</i> for assessing ecotoxicological effects of urban metal pollution	Zn, Cu, Pb and Cd	Digestion in concentrated nitric acid and deionized water	Atomic absorption spectrophotometry (AAS)	(Radwan et al., 2010)
<i>Bellamya aeruginosa</i>	Use of DGT and conventional methods to predict sediment metal bioavailability to a field inhabitant freshwater snail	Cr, Ni, Cu, Zn, Cd and Pb	Digestion with concentrated ultrapure nitric acid in a microwave digestion system	ICP-MS	(Yin et al., 2014)

<i>Cantareus apertus</i>	Bioaccumulation and tissue distribution of Pb and Cd and growth effects in <i>Cantareus apertus</i> after dietary exposure to the metals alone and in combination	Pb and Cd	Digestion with cadmium chloride (CdCl ₂) and lead nitrate (PbNO ₃)	Inductively coupled plasma mass spectrometry (ICP-MS)	(Mleiki et al., 2016)
<i>Nassarius reticulatus</i>	Biomonitoring of metal contamination in <i>Nassarius reticulatus</i>	Cu, Zn, Cd, Hg and Pb	Digestion with HNO ₃ and H ₂ O ₂	Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)	(Santos et al., 2009)
<i>Ulva thalli</i>	Freshwater Ulva as a bioaccumulator of selected heavy metals and alkaline earth metals	Cd, Ni, Pb, Ca and Mg	Digestion with a mixture of HNO ₃ and H ₂ O ₂	Inductively coupled plasma Emission spectrometer	(Rybak et al., 2012)

221 **Table 1:** Summary of the use of snails as biomonitors.

222

223 As shown in table 1, snails are widely used as environmental detectors for heavy metals,
224 glufosinate, glyphosate, PAHs, PBDEs, PCBs and pesticides including OCPs. Several methods
225 for extraction of organic pollutants and heavy metals from snails have been published in the
226 literature (Wu et al., 2019). For instance, organic pollutants were mainly extracted by

227 Accelerated Solvent Extraction (ASE) (Sverdrup et al., 2006), Solid Phase Extraction (SPE)
228 (Beach et al., 2009), Pressurized Hot Water Extraction (PHWE) (Cho et al., 2019) and
229 Microwave-Assisted Extraction (MAE) (Ramli et al., 2019). However, the extraction of heavy
230 metals was especially done by digestion with a mixture of nitric acid (HNO_3), deionized water
231 (H_2O_2), perchloric acid (HClO_4), chloride (CdCl_2), lead nitrate (PbNO_3) using microwave-
232 assisted acid digestion technology (Abdel Gawad, 2018; Dumme et al., 2012; Emilia et al.,
233 2016). However, a purification step may be required by simple centrifugation or filtration, SPE
234 or chromatography technique.

235 Furthermore, there are many separation and detection techniques available for the study of
236 emerging pollutants from such species such as liquid chromatography (LC) (Cho et al., 2019;
237 Ramli et al., 2019; Druart et al., 2011; Beach et al., 2009; Sverdrup et al., 2006), Gas
238 chromatography (GC) (Fu et al., 2011), Atomic Absorption Spectrophotometry (AAS) (Abdel
239 Gawad, 2018; Emilia et al., 2016; Radwan et al., 2010) and Inductively Coupled Plasma Mass
240 Spectrometer (ICP-MS) (Emilia et al., 2016; Boshoff et al., 2015; Cui et al., 2012). Moreover, it
241 was shown that LC was the main process of analyzing POPs in combination with a fluorometric
242 detector to analyze polycyclic aromatic hydrocarbons (Sverdrup et al., 2006; Beach et al., 2009)
243 and to mass spectrometry to analyze polychlorinated biphenyls (Storelli et al., 2014). However,
244 GC-MS could also be used for the assessment of several organic pollutants in snails (Druart et
245 al., 2011). On the other side, the AAS, ICP-AES and ICP-MS are the most widely used methods
246 for the assessment of heavy metals in environmental samples (Sastre et al., 2002).

247 In addition, it has been confirmed that the apple snail “*Pomacea canaliculata*” is identified as
248 bioindicators for many environmental pollutants, indicating both the level and the profile of
249 pollutants, as well as for persistent organic pollutants (Harmon and Wiley, 2010; Fu et al., 2011)
250 as for metals and organometallic compounds (Giraud-Billoud et al., 2018; Cueto et al., 2013;
251 Campoy-Diaz et al., 2018). Their ecology and biology can be classified as having the main
252 properties of an ideal indicator species to evaluate the pollution of the environment, such as the
253 capacity for bioaccumulation, short lifetime, limited movement range, wide distribution and ease
254 of collection compared to other animals like fish or birds (Tanabe and Subramanian, 2006;
255 Gerlach et al., 2013).

256 **3. Presence and detection of pollutants in snails**

257 **3.1. Snail pollution by metallic trace elements (MTE)**

258 Metal trace elements (MTEs) are part of trace elements family and constitute only 0.6% of the
259 total elements. Their denomination is due to their low concentration, which usually does not
260 exceed 1000 mg kg⁻¹ naturally in soils (Crémazy et al., 2019; Emilia et al., 2016). Among these
261 MTEs, zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb) and chromium (Cr) are classified in the
262 metal class while arsenic (As) and antimony (Sb) are among the metalloid group. Due to their
263 harmful effects on the environment and their classification as carcinogenic or dangerous to
264 human health, their presence in the environment and soil is a significant source of concern.
265 However, three of these metals (mercury (Hg), cadmium (Cd) and lead (Pb)) are listed as priority
266 dangerous substances in Decision 2455/2001/EC of the European Council (Cheng and Yap,
267 2015; Emilia et al., 2016).

268 Snails are well known for the bioaccumulation of these pollutants (Viard et al., 2004;
269 Cœurdassier et al., 2002). In fact, it has been observed that about 68% of the cadmium (Cd), 90%
270 of the copper (Cu), 43% of the lead (Pb), and 60% of the zinc (Zn) ingested were accumulated in
271 *Helix aspera* snails ' soft tissue, shells, and feces that were analyzed by flame atomic absorption
272 spectrometry after mineralization and extraction using concentrated HNO₃. Snails seem to be
273 more significant pathways for transport along the Cu and Cd food chains than Zn and Pb and are
274 not able of depositing large amounts of metals into their shells. Resistance to the appearance of
275 effects associated with the accumulation of metals by the snails may cause their predators to be
276 contaminated (Laskowski and Hopkin, 1996). However, snails have many predators, including
277 vertebrates, such as birds, small mammals, reptiles and invertebrates, such as carabids (Liew and
278 Schilthuizen, 2014). Staikou and Lazaridou emphasize the role of snails in the transfer of
279 material and energy from producers to higher trophic levels (Staikou and Lazaridou, 2013),
280 suggesting their potential involvement in the transfer of metallic pollutants along trophic chains
281 (Dar et al., 2019; Hispard et al., 2008). Human consumption of the *Helix aspersa* snail is also
282 small (15 tons of canned food in France in 2008) relative to the Bourgogne *Helix pomatia* snail
283 (876 tons of canned food in France in 2008) (Druart et al., 2011).

284 In addition, mosses absorb metals in deposition of wet and dry atmosphere via passive cation
285 exchange mechanisms and the detection of airborne particles (Bargagli, 2016), while metal
286 absorption may occur via various processes in snails, like inhalation, contact with the surfaces of
287 the walls and ingestion of lichens, herbs, algae, soil particles and mosses. Metabolism and
288 detoxification processes of cadmium (Cd), zinc (Zn), chromium (Cr), mercury (Hg), copper
289 (Cu), and lead (Pb) are conducted in digestive gland, and their bioaccumulation is mainly due to

290 the compartmentalization in the granules inside the cells or vesicles in the digestive gland or
291 other tissues like foot (Boshoff et al., 2013; Regoli et al., 2006). Soft snail tissues purged of their
292 intestine content have lower metals levels and their composition may provide an accurate image
293 of emissions from environmental metals. For example, lead (Pb) concentrations in Italian urban
294 areas can be detected in very high concentrations in street dust suspended by a vehicle, and even
295 in low concentrations that have relatively small impact on the chains of foods (Barca et al.,
296 2014).

297 Moreover, the adsorption and precipitation of trace metals increase under alkaline soils. A higher
298 percentage of metals in their ionic state with a lower pH level. In order to succeed in binding to
299 the exchange sites, such metal ions should interact with specific cations including magnesium
300 (Mg^{2+}), calcium (Ca^{2+}), iron (Fe^{2+}), aluminum (Al^{3+}) and hydrogen (H^+) (Bakircioglu et al.,
301 2011). Metals can thus be more attainable for plant absorption at lower pH levels (Bakircioglu et
302 al., 2011). In fact, in their study, Pauget et al. in 2012, demonstrated an important correlation
303 between *Cantareus aspersus* metal accumulation and soil physicochemical properties such as
304 cation exchange capacity and pH (Pauget et al., 2012). Nonetheless, based on the maximum
305 residue limit (MLR), it was not reasonable to deduce with confidence that physicochemical
306 properties of the soil and metals contributed to the cumulation of metal in the digestive gland,
307 although the physicochemical characteristics of the soil might have indirectly affected the
308 metal's accumulation in snails (*Cepaea nemoralis*) and determined the accumulation of metals in
309 plants (*Urtica dioica*) as well as snails by their digestive exposure (Gimbert et al., 2006;
310 Cœurdassier et al., 2002). Moreover, Boshoff et al., in 2013 and 2014 and Notten et al., in 2006,
311 have reported differing levels of cadmium (Cd) such as 60-150 mg kg⁻¹, 33.93-148.40 mg kg⁻¹

312 and 94 mg kg⁻¹ in several studies in *Cepaea nemoralis*, respectively (Boshoff et al., 2015;
313 Boshoff et al., 2013; Notten et al., 2006). The absorption of cadmium (Cd) as a non-essential
314 element is not controlled at an accurate level or is less efficiently regulated than fundamental
315 elements (Tchounwou et al., 2012). Higher concentrations are detected in the *helix aspersa* snail
316 that fed a cadmium-rich diet (Cd) (Scheifler et al., 2002) by binding to metallothionein-like
317 proteins present in the digestive gland that accumulate metal at higher levels without severe
318 effects (Nica et al., 2013; Gimbert et al., 2006; Manzl et al., 2004). For snail physiological
319 functioning, copper (Cu) and zinc (Zn) are required and their absorption is controlled till it
320 exceeds the threshold rate (Nica et al., 2012). Specifically, snails require large amounts of copper
321 as an element of hemocyanin that is converted and absorbed after accumulation (Manzl et al.,
322 2004). Snails can maintain zinc in tissues for essential functions at low levels and can affect the
323 feeding and development at high concentrations (Swaileh and Ezzughayyar, 2001).

324 Moreover, snails living in areas highly contaminated with metals also exhibit physiological and
325 morphological modifications due to higher energy costs correlated with process of excretion and
326 detoxification (Radwan et al., 2010; Regoli et al., 2006). As the strength of the shell increases
327 slightly, there was no significant change in the thickness or strength of the shell that can be
328 correlated with the site and time interaction even when exposed to contaminated soil. Similarly,
329 there are no detected effects of metal toxicity such as lead (Pb) and cadmium (Cd) on the weight
330 or size of the snail shell (Mourier et al., 2011; Jordaens et al., 2006). Although adult snails have a
331 completely formed shell, the morphology of wild population shells may change (De Vaufleury
332 and Pihan, 2000).

333 Furthermore, snails have mechanisms for metabolizing, exporting storing and excreting metals,
334 and these mechanisms are triggered by the acclimatization process, such as transfer to new
335 environment, physiological changes resulting from experimental stressors or response to stress
336 due to the current microcosm conditions (Bighiu et al., 2017). Cumulative metals will be stable
337 after a specified exposure time and therefore cannot interact with biochemical reactions
338 (Tchounwou et al., 2012; Rainbow, 2007).

339 Some studies have shown that high doses and duration of metal exposure cause more serious
340 effects, such as kidney failure caused by the combination of inorganic arsenic and cadmium,
341 compared to low doses and individual exposure to various elements (Wang and Fowler, 2008;
342 Nordberg et al., 2005).

343 In general, digestion with HNO₃ and H₂O₂ mixture (Abdel Gawad, 2018; Emilia et al., 2016;
344 Dumme et al., 2012; Rybak et al., 2012; Santos et al., 2009) followed by the inductively
345 coupled plasma mass spectrometer (ICP-MS) (Mleiki et al., 2016; Yin et al., 2014; Rybak et al.,
346 2012; Cui et al., 2012; Santos et al., 2009) was the main multi-residue extraction and analytical
347 procedure of heavy metals from the different snail species used.

348 **3.2. Snail pollution by organic pollutants**

349 The high use of pesticides as a result of the increasing world population growth and plant
350 protection policies as well, led to an increase of the invertebrates' contamination by these
351 pollutants. They are scarcely used separately in agriculture and can be used in association during
352 crop production at specific times (Ngowi et al., 2007). Due to their usage and application, the
353 presence of pesticides overlaps in space and time (Smiley et al., 2014). Snails are widely used in

354 ecotoxicological studies to investigate pesticide exposure effects that observed in response to
355 each of the individual and combined pesticides exposures (Hock and Poulin, 2012; Mora et al.,
356 2011). The effects of pesticides and their accumulation via different routes on snails that
357 affecting their response, are influenced by several factors, such as, the sensitivity of species to
358 pesticide (Lushchak et al., 2018), the chemical mode of action (Staley et al., 2015) and their
359 exposure period (Damalas and Eleftherohorinos, 2011). Exposure can have direct effects on
360 biological organization at all levels, while toxicant mode of action mainly defines the class of
361 organisms are affected. Several characteristics of snails such as, fertility (Coutellec et al., 2008),
362 survival (Qiu et al., 2011), and movement (Perez et al., 2009) can be quantified to estimate the
363 fitness of snails which decrease in response to stress associated with the environment (Coutellec
364 et al., 2008), including pesticides contamination (Coutellec and Lagadic, 2006) and
365 physicochemical parameters (Elias and Bernot, 2017). In addition, the effects of pesticides on
366 snails can either directly affect their egestion and movement or indirectly affect food chains and
367 predator interactions. Lower snail egestion levels once exposed to pesticides may limit the
368 availability of carbon and nitrogen that affect biomass algae and nutrient flows (Fink and Elert,
369 2006). In addition to the pesticide mode of action, it is necessary to consider the sensitivity of
370 organisms to pesticides in combination with different exposures periods to comprehend the
371 potential adverse environmental impacts of co-occurring pesticides on biodiversity. The ecology
372 and biology of snails, such as large distribution, bioaccumulation capacity and ease of sampling
373 reflect most of the essential characteristics of an ideal bioindicator (Hall et al., 2009), and these
374 advantages make the snail field an effective bioindicator for toxins and other natural substances
375 such as *Pomacea canaliculata* (Martín et al., 2019; Koch et al., 2013; Fu et al., 2011).

376 It is also noted that snails are involved in multiple food chains: carabids, amphibians, mammals,
377 birds, other gastropods and humans. Consequently, the contamination of snails by pesticides can
378 be transferred for consumers leading to a risk of secondary poisoning. The pesticide
379 concentrations determined in snails exposed exceed the MRLs (Maximum Residue Limit) set for
380 a type of animal food (Glyphosate: Animal product MRL = 0.1 mg kg⁻¹, Snail MRL 6 mg kg⁻¹)
381 (Druart et al., 2011).

382 However, the effects of pesticides on the snails are limited to few studies. Among the available
383 data, it appears that carbamates and organophosphorus insecticides cause low mortality in snails
384 feeding for 10 days with contaminated food (Schuytema et al., 1994). The insecticide
385 pentachlorophenol is not fatal for snails at concentrations of 1000 mg kg⁻¹ in food (De Vaufleury,
386 2000). Moreover, Coeurdassier et al., in 2002 studied the effects on survival, growth, and
387 acetylcholinesterase (AChE) of an organophosphorus insecticide, dimethoate, and demonstrated
388 growth inhibition in relation to its accumulation. These authors also observed that snails are
389 more sensitive to pesticides contained in the soil than in food (Cœurdassier et al., 2002).

390

391 **4. Conclusion**

392 Snails used as sensitive environmental pollution indicators has taken considerable importance
393 and been applied for incorporate the pollution signal over a period of time or an area. Their use
394 in toxicity bioassays is an effective process as snails are easy to adapt and manipulate in the
395 laboratory and it can be treated with the desired amounts of contaminants on specific diets and
396 react quickly to pollutant contamination in the sublethal dosage range. Such organisms are also

397 chosen as sentinels due to their limited toxic response or little ability to control their tissue
398 levels. Studying the impact of metals and other pollutants on organism physiology contributes to
399 the development of many toxic studies which can be used as an environmental evaluation tool.
400 For all these reasons, the use of snails as matrix is highly recommended in order to monitor a
401 wide class of environmental pollutants that might be present in the environment.

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409

410

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Graphical abstract:

