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# **sSnail as Sentinel Organism for Monitoring the environmental Pollution; A Review**

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## **Abstract**

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

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

## 1. Introduction

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

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

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

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

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

Representative species of the environment must be studied to assess the effects of pollutants in a matrix. In fact, a biomonitor can be identified as an organism or group of organisms that are important for qualitative and quantitative environmental pollution determination (Fränze, 2006).

Many studies have shown that gastropod mollusks including terrestrial snails are pollution bioindicators, including metals, pesticides, PAHs, PCBs and PBDEs (Beeby and Richmond, 2002; Fu et al., 2011; De Vaufleury and Pihan, 2000; Silva et al., 2019). Such species occupy an

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

## **2.1. Characteristics of snails**

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

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

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



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

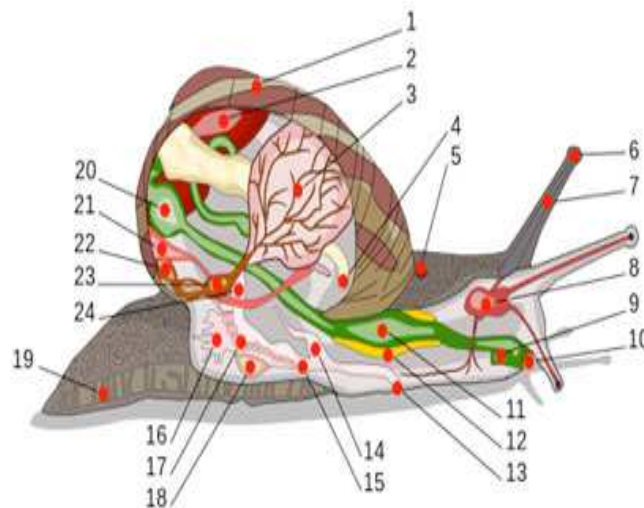
In common with other invertebrates, mollusks are known by their innate immunity composed of both cellular and humoral elements consider as the first line of defense that reflects the immune response against foreign particles and organisms by the phagocytic cells, anatomic barriers and physiological components.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

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

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

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

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



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

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

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

### 2.3. Methods of pollutants extraction and analysis

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

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

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> using	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 <sub>3</sub> 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 <sub>3</sub> 75% for 24 h and warmed for 30 min for HNO <sub>3</sub> 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 <sub>3</sub> and H <sub>2</sub> O <sub>2</sub>	-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 <sub>2</sub> ) and lead nitrate (PbNO <sub>3</sub> )	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 <sub>3</sub> and H <sub>2</sub> O <sub>2</sub>	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 <sub>3</sub> and H <sub>2</sub> O <sub>2</sub>	Inductively coupled plasma Emission spectrometer	(Rybak et al., 2012)

**Table1:** Summary of the use of snails as biomonitors.

As shown in table 1, snails are widely used as environmental detectors for heavy metals, glufosinate, glyphosate, PAHs, PBDEs, PCBs and pesticides including OCPs. Several methods for extraction of organic pollutants and heavy metals from snails have been published in the 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 ( $\text{HNO}_3$ ), deionized water  
231 ( $\text{H}_2\text{O}_2$ ), perchloric acid ( $\text{HClO}_4$ ), chloride ( $\text{CdCl}_2$ ), lead nitrate ( $\text{PbNO}_3$ ) using microwave-  
232 assisted acid digestion technology (Abdel Gawad, 2018; Dummee 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).

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

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

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

Metal trace elements (MTEs) are part of trace elements family and constitute only 0.6% of the total elements. Their denomination is due to their low concentration, which usually does not exceed 1000 mg kg<sup>-1</sup> naturally in soils (Crémazy et al., 2019; Emilia et al., 2016). Among these MTEs, zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb) and chromium (Cr) are classified in the metal class while arsenic (As) and antimony (Sb) are among the metalloid group. Due to their harmful effects on the environment and their classification as carcinogenic or dangerous to human health, their presence in the environment and soil is a significant source of concern. However, three of these metals (mercury (Hg), cadmium (Cd) and lead (Pb)) are listed as priority dangerous substances in Decision 2455/2001/EC of the European Council (Cheng and Yap, 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 aspersa* snails ' soft tissue, shells, and feces that were analyzed by flame atomic absorption  
272 spectrometry after mineralization and extraction using concentrated HNO<sub>3</sub>. 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<sup>-1</sup>, 33.93-148.40 mg kg<sup>-1</sup>

312 and 94 mg kg<sup>-1</sup> 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 Vaufléury  
332 and Pihan, 2000).

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

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

In general, digestion with  $\text{HNO}_3$  and  $\text{H}_2\text{O}_2$  mixture (Abdel Gawad, 2018; Emilia et al., 2016; Dummee et al., 2012; Rybak et al., 2012; Santos et al., 2009) followed by the inductively coupled plasma mass spectrometer (ICP-MS) (Mleiki et al., 2016; Yin et al., 2014; Rybak et al., 2012; Cui et al., 2012; Santos et al., 2009) was the main multi-residue extraction and analytical procedure of heavy metals from the different snail species used.

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

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

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



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

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

#### **4. Conclusion**

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

chosen as sentinels due to their limited toxic response or little ability to control their tissue levels. Studying the impact of metals and other pollutants on organism physiology contributes to the development of many toxic studies which can be used as an environmental evaluation tool.

For all these reasons, the use of snails as matrix is highly recommended in order to monitor a wide class of environmental pollutants that might be present in the environment.

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Graphical abstract:

