

Assessment of soil metallic contamination in several sites from Northeast Algeria by use of terrestrial gastropod: *Cornu aspersum* (O.F. Müller, 1774) (Helicidae)

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ABSTRACT

The study aimed to determine concentrations of some trace elements (Fe, Mn, and Pb) in soils of five sites in Northeast Algeria (Sidi Ammar, Drean, Chatt, Besbes, and El-Kala) during the spring and autumn of 2019. All of the sites were chosen due to their proximity to industrial factories. In addition, the activity of glutathione S-transferase (GST) and acetylcholinesterase (AChE), indicators of oxidative stress and neurotoxicity respectively, were measured in land snail *Cornu aspersum* (O.F. Müller, 1774) (Mollusca Gastropoda Helicidae) collected from all studied sites. The concentration of heavy metals in these soils decreases as follows: Fe > Mn > Pb. GST and AChE activities were found to vary between sites and by season. The highest levels of GST activity were registered during the spring at sites closest to potential sources of pollution. AChE values showed inhibition in spring as compared to autumn. In addition, the highest inhibition values were recorded at the Sidi Ammar site. These increased levels of bioindication stress responses correlated significantly with increasing metal concentration in soil samples collected at each site. The differences recorded between the sites studied are related to their level of pollution, while the seasonal variations were due to the effect of heavy metals leaching in autumn.

KEY WORDS

Biomarkers; *Cornu aspersum*; Heavy metals; Northeast Algeria; Soils.

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INTRODUCTION

Environmental pollution soil by heavy metals is one of the most important issues in the world due to the non-biodegradability of the metals and their impact on living organisms (Naghipour et al., 2018). Particularly, long-range atmospheric trans-

port of heavy metals can lead to pollutant deposition even in supposedly pristine areas (De Vries et al., 2002). Heavy metals are the most common group of pollutants reducing the natural regeneration ability of the environment (Malik & Zeb, 2009; Mansouri et al., 2012). Many heavy metals are necessary for the proper functioning of living organ-

isms. Some others, however, exert a negative effect on their development (Chmielowska-Bąk et al., 2018; Murtaza et al., 2021) and it should be remembered that the toxicity of a given chemical compound is determined by the dose introduced into the organism in a unit of time (Dobrowolski & Otto, 2012). Soil is an integral element of the land ecosystem (Haslmayr et al., 2016) and is a non-renewable natural resource (Lal et al., 2015). It also plays several important roles in the cycling of elements, including heavy metals, in the terrestrial environment (Gorlach & Gambuś, 1991). Due to the constant contact with air and water (Zhang et al., 2015), soil is susceptible to excessive enrichment with trace elements as a consequence of human activity (Cabral-Oliveira et al., 2015; Puga et al., 2015; Rovira et al., 2015).

Land snails have also been widely used as a sentinel species for the assessment of pollution in terrestrial ecosystems (Larba & Soltani 2014; Hamdi-Ourfella & Soltani, 2014; De Vaufléury 2015; Bairi et al., 2018; Douafer et al., 2020). They accumulate various contaminants in their soft tissues, especially the digestive gland, as well as they are helpful species in monitoring exposure to trace metals, agrochemicals, urban pollution, and electromagnetic agents (Regoli et al., 2006).

Biochemical responses in organisms exposed to toxic contaminants have been used as biomarkers. *Cornu aspersum* (O.F. Müller, 1774) (Mollusca Gastropoda Helicidae) are among the most common land snails in the Mediterranean (Larba & Soltani 2013; Douafer & Soltani 2014; Belhjouani et al., 2019), West Europe, Northwest Africa, and eastern Asia, while they also appear in North and South America and Oceania. The wide range of its spread is related to human activities, as it has been introduced by humans in the continent and some islands. It is also a species with commercial value (Leomanni et al., 2015).

Several studies have investigated the soil quality and modulated the soil properties (soil pH, organic carbon, and iron oxides) of metal bioavailability to snails, and procedure for ranking field sites based on the evaluation of the metal transfer to the land snail *C. aspersum* (Pauget et al., 2013a, b; Larba & Soltani 2014; Mortensen et al., 2018; Douafer et al., 2020; Lozen et al., 2020). Therefore, the assessment of pollution effects on this organism could also be of concern for potential effects on human

health, due to the human consumption of this species. *Cornu aspersum* have shown to accumulate pollutants from contaminated soils (Bouriou et al., 2015). However, a limited number of studies exist supporting land snails as a potential bioindicator species for assessing the risk of environmental contamination (Abdel-Halim et al., 2013; Douafer et al., 2014; Itziou et al., 2018; Bairi et al., 2020; Baroudi et al., 2020; Louzon et al., 2020), and more specifically *C. aspersum* as a sentinel organism for both trace metals and organic pollution (Leomanni et al., 2015, 2016; Louzon et al., 2020). This land snail integrates three pathways of exposure: digestive (ingestion of soil particles, up to 40% to satisfy its physiological needs), cutaneous (transfer from the soil to the snail foot), and pulmonary (inhalation of soil particles) (de Vaufléury et al., 2006; Scheifler et al., 2006; Gimbert et al., 2008a; de Vaufléury, 2015). The use of oxidative stress biomarkers is of potential interest for the assessment of the pollutant's impact or seasonal variation in animals under field conditions (Regoli & Principato, 1995; Verlecar et al., 2008; Abdel-Halim et al., 2013; Larba & Soltani, 2014; Bairi et al., 2018; Douafer et al., 2020). Moreover, the interaction between xenobiotics and the components of the antioxidant defense systems plays an important role in the ecotoxicological response of an organism to its environment (Regoli et al., 2006).

The main objective of the present study was to assess the utility of *C. aspersum* in environmental monitoring as a bioindicator of heavy metals contamination (Fe, Mn, and Pb) in Northeast Algeria by measuring selected biomarkers (AChE and GST). Samples were collected during the spring and autumn of 2019 from various sites located along a terrestrial soil pollution gradient according to their proximity to factories and other potential sources of pollution.

MATERIAL AND METHODS

Study area

The sampling sites used in this study were uncultivated and located in Northeast Algeria between the east and west of the Annaba area. Sampling sites include Sidi Ammar, one of the most populated areas of this region, in addition to Drean, Chatt, and Bes-

bes. Each of these sites was chosen along a terrestrial soil pollution gradient according to its proximity to several types of factories, including those involved in the production of phosphoric fertilizers (Fertial), pesticides (Asmidal), steel products (Arcelor Mittal), and metallic construction (Ferovial). The sampling site at El Kala, located in a protected nature reserve, the National Park of El Kala, was used as a control site due to its location far from motorized traffic and other anthropogenic sources of metal contamination. The geographical positions of each site are listed in Fig. 1 and Table 1.

Metal analysis in soils

At the laboratory three subsamples of soil (~100 g) were randomly taken at a depth of 10 cm from

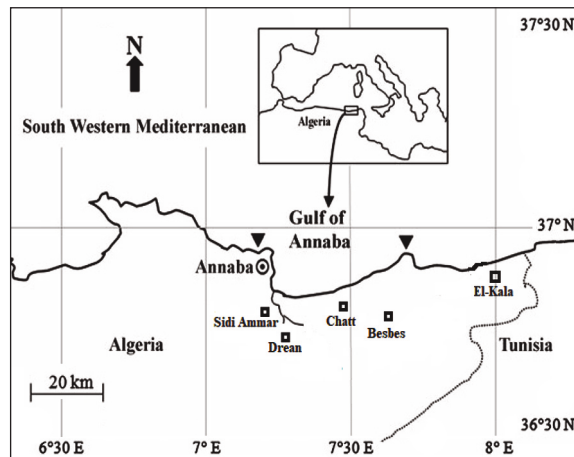


Figure 1. Geographical location of sampling sites (Sidi Ammar, Drean, Chatt, Besbes and El-Kala).

Sites	North	East
Sidi Ammar	36°48'00.36''	7°44'00.00''
Drean	36°46'02.06''	7°54'11.64''
Chatt	36°45'34.32''	7°58'22.59''
Besbes	36°46'57.16''	8°12'00.08''
El Kala	36°53'48.55''	8°26'36.80''

Table 1. Geographic position of sampling sites.

each site, the soil samples were dried at 105 °C for 24 h to a constant dry weight and sieved to 150 µm. Then, three 2.0 g replicates of each dried sample were digested in concentrated HCl and HNO₃ (Merck) solution at a 3:1 ratio. The mixture was heated (180 °C) in glass flasks for 30 min and then cooled for 30 min at an ambient temperature (25 °C). Each sample was filtered using filter paper (Whatman No. 1) and diluted with double deionized water in the approximate range of standard concentrations prepared from the stock standard solution of each metal (Merck). The Concentrations of Fe, Mn, and Pb in the extracts were evaluated using a flameless atomic absorption spectrophotometer (Shimadzu model AA6200) with air-acetylene flame equipped with a deuterium background corrector (Laib & Leghouchi, 2011). The values are expressed by the mean ± standard deviation ($m \pm SD$) in the analysis of three sub-samples for each soil sample. All metal samples were analyzed in duplicate and concentration was expressed in mg/Kg of dry mass.

Biomarker assays

The shells of five adult snails were dissected and samples were prepared for biomarker analyses. AChE activity was determined according to the procedure of Ellman et al. (1961), with the use of acetylthiocholine (ASCh) as a substrate. The activity was expressed in nMol/mn/mg of protein. GST activity was measured according to Habig et al., 1974, using 1-chloro 2,4-dinitrobenzene (CDNB) as substrate. The activity level was expressed in nMol/mn/mg of protein. AChE activity was determined according to the procedure of Ellman et al. (1961), with the use of acetylthiocholine (ASCh) as a substrate. The protein content was evaluated according to Bradford (1976) using serum albumin as a standard (BSA, Sigma).

Statistical analysis

The collected data were statistically analyzed regarding mean, standard deviation, and correlation, by using the Statistical Package for Social Sciences (STATISTICA) version 10. When testing null hypotheses, a probability level of $p \leq 0.05$ was set to be statistically significant. The correlation coefficients and linear regressions were calculated to detect the associations between the concentration of

heavy metals in soil and snail samples. A one-way and two-way ANOVA was used to analyze the difference in heavy metals concentration under different sampling locations.

RESULTS

Acetylcholinesterase and Glutathion S-transferase activities

The effect of pollution on AChE and GST activity in *C. aspersum* was evidenced by the variation in the ratios between different sites and seasons. Snails collected at all sites have the highest AChE activity compared to snails collected at the reference site (El-Kala). At all study sites, AChE activity levels were found to be higher during the spring compared to the autumn. During the spring season, the highest AChE activity was observed in El-Kala (34.7 ± 0.92 nMol/mn/mg protein) and the lowest activity was observed in Sidi Ammar (15.26 ± 0.98 nMol/mn/mg protein). During the autumn season, the highest AChE activity was observed in El-Kala (40.03 ± 0.53 nMol/mn/mg protein) and the lowest activity was observed in El Kala (20.88 ± 0.89 nMol/min/mg protein) (Fig. 2). The two-way ANOVA (site, season) of the AChE content a highly significant effect of both site ($F_{4,20} = 1700.26$, $p < 0.001$) and season ($F_{1,20} = 1058.07$, $p < 0.001$), since the site*season interaction was found significant ($F_{4,20} = 3.48$, $p < 0.05$). The highest GST activity was recorded in snails from the El-Kala control site (17.27 ± 0.67 nMol/mn/mg protein) during the autumn; on the other hand, the lowest GST activity was recorded in snails collected at the Sidi Ammar site (37.37 ± 0.84 nMol/mn/mg protein) during the spring. The use of two-way ANOVA test shows a highly significant effect site ($F_{1,20} = 1634.5$, $p < 0.001$) and season ($F_{1,20} = 418.4$, $p < 0.001$) and site*season interaction ($F_{4,20} = 7.4$, $p < 0.001$). The results of seasonal variations of GST activity in *C. aspersum* were represented in Fig. 3.

Metal concentrations in soils

The results of the average heavy metals concentrations in the soils sampled at different sites in (Sidi Ammar, Drean, Chatt, Besbes and El-Kala) are presented in Table 2. Fe was found as an abundant metal in all sites (781.12 ± 19.65 g/kg dry mass)

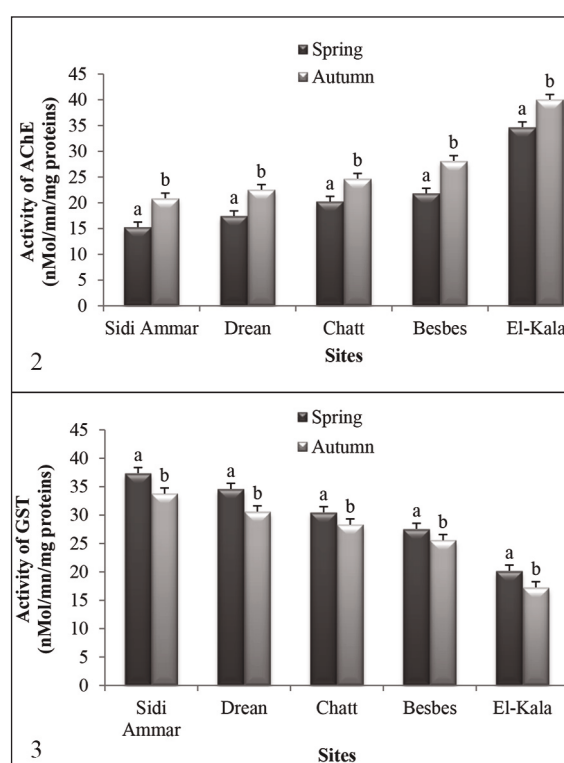


Figure 2. Results of seasonal variations of AChE activity in *C. aspersum*. Figure 3. Results of seasonal variations of GST activity in *C. aspersum*.

in Sidi Ammar site, along with low rate in El-Kala site (275.49 ± 19.78 g/kg dry mass). Furthermore, Mn (20.07 ± 0.87 g/kg dry mass), and Pb (0.13 ± 0.01 g/kg dry mass) level were found to be high in Sidi Ammar and low in El-Kala. On all sites, higher values were measured in samples taken in the autumn compared to samples taken in the spring. The average concentration of each metal recorded had the following decreasing order: Fe > Mn > Pb. The lowest concentrations of heavy metals were recorded at the El-Kala site (control site). For each metal, the use of the two-way ANOVA test (season, site) indicated a highly significant effect for Fe season ($F_{1,20} = 2291.0$, $p < 0.001$), site ($F_{4,20} = 9034.2$, $p < 0.001$) and site*season ($F_{4,20} = 26.6$, $p < 0.001$); for Mn, a significant effect due to season ($F_{1,20} = 581.78$, $p < 0.001$) and site ($F_{4,20} = 884.92$, $p < 0.001$) and no significant effect due to season*site interaction ($F_{4,20} = 1.96$, $p = 0.139$) and for Pd a significant effect due to to season ($F_{1,20} = 28.468$, $p < 0.001$), site ($F_{4,20} = 106.185$, $p < 0.001$), and site*season interaction ($F_{4,20} = 2.414$, $p < 0.01$).

The Tukey test showed a significant difference ($p < 0.05$) in metal concentration in all sites (Sidi Ammar, Drean, Chatt, Besbes and El-Kala) between spring and autumn (Table 2). The concentrations of each heavy metal varied from site to site.

Relation between the heavy metal contents in the soil with seasonal variation of biomarkers (GSH and AChE) in C. aspersum

Statistical analyses on the relationship between the content of heavy metal, and the variation of biomarkers (GSH, AChE), recorded during spring and autumn of 2019 (Table 3). During the spring season, we observed a highly significant negative correlation between AChE content and values of heavy metals Fe ($R = -0.971$, $p < 0.001$), Mn ($R = -0.968$, $p < 0.001$) and Pb ($R = -0.679$, $p < 0.05$) and a significant positive correlation between GST content

Site	Season	Fe	Mn	Pb
Sidi Ammar	Spring	717.95 ± 23.14 a A	17.51 ± 0.96 a A	2.61 ± 0.78 a A
	Autumn	781.12 ± 19.65 a B	20.07 ± 0.87 a B	2.20 ± 0.82 a B
Drean	Spring	666.24 ± 21.01 b A	15.79 ± 1.01 b A	1.91 ± 0.65 b A
	Autumn	747.90 ± 15.84 b B	18.47 ± 0.67 b B	1.48 ± 0.73 b B
Chatt	Spring	578.12 ± 22.45 c A	14.16 ± 0.92 c A	1.21 ± 0.55 c A
	Autumn	683.06 ± 18.62 c B	16.84 ± 0.73 c B	1.14 ± 0.91 c A
Besbes	Spring	475.17 ± 12.73 d A	12.63 ± 0.66 d A	2.21 ± 0.84 d A
	Autumn	566.96 ± 14.49 d B	14.45 ± 0.99 d B	1.89 ± 0.88 d B
El-Kala	Spring	275.49 ± 19.78 e A	8.81 ± 1.12 e A	1.13 ± 1.02 e A
	Autumn	334.47 ± 17.98 e B	11.25 ± 1.01 e B	1.02 ± 0.61 e B

Table 2. Soil heavy metals concentrations in all sites between spring and autumn.

Parameters	STATISTICAL Correlation (R)			
	Spring		Autumn	
	AChE	GST	AChE	GST
Fe	-0.971***	0.994***	-0.9920***	0.988***
Pb	-0.679**	0.995***	-0.9580***	0.982***
Mn	-0.968***	0.676**	-0.5916*	0.646**

Table 3. Statistical analyses on the relationship between the content of heavy metal, and the variation of biomarkers (GSH, AChE), recorded during spring and autumn of 2019. *** Correlation is significant at the level $p < 0.001$; ** Correlation is significant at the level $p < 0.01$; * Correlation is significant at the level $p < 0.05$

and Fe ($R = 0.994$, $p < 0.001$) and Mn ($R = 0.995$, $p < 0.001$) and Pb ($R = 0.676$, $p < 0.005$). During the autumn season, we noted a highly significant negative correlation between the AChE content and the heavy metal Fe ($R = -0.992$, $p < 0.001$), Mn ($R = -0.958$, $p < 0.001$) and Pb ($R = -0.591$, $p < 0.01$). However, a highly significant positive correlation between GST and heavy metal Fe ($R = 0.988$, $p < 0.001$), Mn ($R = 0.982$, $p < 0.001$) and Pb ($R = 0.646$, $p < 0.001$).

DISCUSSION

Anthropogenic activities are at the origin of 2.5 million polluted fields in the European Union (EEA, 2019). Contaminated soils are still a subject of concern as they can cause health problems (Eeva & Lehtikoinen, 2000), notable cancers for humans (Nawrot et al., 2006) and the collapse of animal populations (Laskowski & Hopkin, 1996) and disturb community structures (Migliorini et al., 2004; Cébron et al., 2011).

Biomarkers are becoming an integral part of soil ecosystem health assessment (Asensio et al., 2013; Rodriguez-Ruiz et al., 2014). Particularly, oxidative stress biomarkers are used to measure the toxicologic effect of soil ecosystem pollution, including

Acetylcholinesterase (AChE) (Mleiki et al., 2017; Douafer et al., 2020). Antioxidant enzymes, including glutathione-S-transferase (GST) (Itziou & Dimitriadis, 2011). In terrestrial gastropods, there is increasing interest in oxidative stress biomarkers in response to pollutants, as these biomarkers can be integrated into a battery for biological effect assessment in soil pollution monitoring (Regoli et al., 2006; El-Gendy et al., 2009; Radwan et al., 2010a, 2010b; Itziou & Dimitriadis, 2011; Douafer et al., 2020). The overall results revealed a marked reference zone which confirmed *C. aspersum* as a suitable bioindicator for these environmental pollutants (Beeby & Richmond, 2002; Larba & Soltani 2014; Bairi et al., 2020). Most of the examined metals were observed at high levels according to whether animals were near or far from the selected location of brick factories, traffic, and chemical factories.

In the present study, a higher GST activity was recorded in *C. aspersum* from sample sites exposed to several sources of pollution compared to samples taken from the reference site, El Kala. This increase in the GST is probably due to the activation of the natural detoxification process of xenobiotics and also a pro-oxidant defense system (Elia et al., 2007; Radwan et al., 2010). Snails collected from six sites exhibited significant inhibition of GST, a multigene family that catalyzes both detoxification of organic compounds and antioxidant reactions through the reduction of hydroperoxides. *C. aspersum* showed elevated basal levels for these enzymes at least an order of magnitude above those commonly measured in the digestive gland of *Mytilus galloprovincialis* Lamarck, 1819 (Bivalvia Mytilidae) (Regoli & Principato, 1995). GST activity was also found to vary seasonally with higher induction of GST activity in the summer compared to winter. This may be due to spring rains which promote the leaching of soil pollutants. Variation in GST levels between sites may be due to increasing or decreasing proximity to pollution sources (Abdel-Halim et al., 2013; Mleiki et al., 2017; Douafer et al., 2020; Bairi et al., 2020).

AChE activity, a neurotoxicity biomarker in soil invertebrates (Rorke & Gardner, 1974; Engenheiro et al., 2005), was significantly inhibited in tissues of *C. aspersum* from all sites, compared to the reference site. Our study revealed decreased levels of AChE activity in snails collected in spring compared to autumn. This decrease is potentially due to the effect of heavy metal pollutant leaching. As with

GST, variation in AChE levels between sites may be due to increasing or decreasing proximity to pollution sources (Abdel-Halim et al., 2013; Mleiki et al., 2017; Douafer et al., 2020; Bairi et al., 2020). In the present study, soil samples from six sites were collected in 2019 and analyzed by a method commonly used for several years and applied for the determination of heavy metal concentrations in various media (Leghouchi et al., 2009; Laib & Leghouchi, 2011). The highest concentrations of Fr, Mn, and Pb are recorded in the soil collected at Sidi Ammar, indicating that this industrial area is a major source of waste contamination from the soil. The concentrations decreased in the other sites compared to their distance from the source of pollution such as metallurgical industries, the industrial area of Sidi Ammar, the Arcelor Mittal steel complex, and Ferroval (Kluge & Wessolek, 2012; Modrzewska & Wyszowski, 2014). The most basic heavy metal concentration values were recorded in El Kala, a protected area used as a control site. Pb concentrations in soils collected near the road to Sidi Ammar and Besbes are significantly high compared to other sites may be due to contamination by metals from anthropogenic sources, for example from automobile transport (Johansson et al., 2009; Helmreich et al., 2010; Duong & Lee 2011; Liu et al., 2012). The difference in heavy metals between seasons can also be influenced by weather conditions such as wind (Piron-Frenet et al., 1994), and precipitation (Garcia & Millan, 1998). This study indicates that all heavy metal contents in the soil have a significant negative correlation with AChE and a positive correlation with GST during spring and autumn from different sites located in the Northeast Algeria were below the standard limits (AFNOR 1996) (Table 4).

CONCLUSIONS

In conclusion, the obtained results may provide baseline data for future human impact assessment and risk programs concerning heavy metals pollution in industrial areas. *C. aspersus* is considered a good tool for biomonitoring ecotoxicological effects. In addition, antioxidant defense components are sensitive parameters that could be useful as oxidative stress biomarkers in animals exposed to metals in their environment. More studies must be

Heavy metals	Concentrations
Cd	2
Cr	150
Cu	100
Pd	100
Zn	300
Fe	na
Mn	na

Table 4. Heavy metal concentrations in the soil from different sites located in the Northeast Algeria

done in this region to complete the information about the deleterious risk and hazards impacted on humans and terrestrials. The results show a decreasing gradient of pollution from west to east correlating to the proximity of the pollution sources.

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REFERENCES

- Abdel-Halim K.Y., Abo El-Saad A.M., Talha A.A., Hussein M.M. & Bakry N.M., 2013. Oxidative stress on land snail *Helix aspersa* as a sentinel organism for ecotoxicological effects of urban pollution with heavy metals. *Chemosphere*, 93: 1131–1138. <https://doi.org/10.1016/j.chemosphere.2013.06.042>
- Agence Française de Normalisation (AFNOR), (1996). Qualité des sols. Recueil des normes françaises. AFNOR, Paris.

- Alloway BJ., 1995. Heavy Metals in Soils. 2nd ed., Blackie Academic and Professional, London, 7-39. <https://doi.org/10.1007/978-94-011-1344-1>
- Bairi Y., Sifi K. & Kilani-Morakchi S., 2020. Biomarkers response in the snail *Cornu aspersum* (Gastropoda, Helicidae) used as bioindicator of soil pollution in extreme Northeast of Algeria. Ecology, Environment and Conservation, 26: 47–54.
- Bargagli R., Monaci F., Borghini F., Bravi F. & Agnorelli C., 2002. Mosses and lichens as biomonitors of trace metals. A comparison study on *Hypnum cupressiforme* and *Parmelia caperata* in a former mining district in Italy. Environmental Pollution, 116: 279–287. [https://doi.org/10.1016/s0269-7491\(01\)00125-7](https://doi.org/10.1016/s0269-7491(01)00125-7)
- Bairi Y., Sifi K. & Soltani N., 2018. Growth and Responses of Biomarkers in the Snail *Helix aspersa* (Mollusca, Gastropoda) Used as Bioindicator of Soil Pollution in Northeast of Algeria. Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions, pp. 339–341. <https://doi.org/10.1007/978-3-319-70548-4-107>.
- Bargagli R., 1998. Trace Elements in Terrestrial Plants: An Ecophysiological Approach to Biomonitoring and Biorecovery. Berlin: Springer Verlag.
- Baroudi F., Al-Alam J., Fajloun Z. & Millet M., 2020. Snail as sentinel organism for monitoring the environmental pollution; a review. Ecological Indicators, 113: 106240. <https://doi.org/10.1016/j.ecolind.2020.106240>
- Beeby A. & Richmond L., 2002. Evaluating *Helix aspersa* as a sentinel for mapping metal pollution. Ecological Indicators, 1: 261–270. [https://doi.org/10.1016/51470-160x\(02\)00022-5](https://doi.org/10.1016/51470-160x(02)00022-5)
- Belhieuani H., El-Okki M.H., Afri-Mehennaoui, F.Z. & Sahli L., 2019. Terrestrial gastropod diversity, distribution and abundance in areas with and without anthropogenic disturbances, Northeast Algeria. Biodiversitas, 20: 243–249. <https://doi.org/10.13057/biodiv/d200128>
- Bourioung M., Gimbert F., Alaoui-Sehmer L., Benbrahim M., Aleya L. & Alaoui-Sossé B., 2015. Sewage sludge application in a plantation: Effets on trace metal transfer in soil-plant-snail continuum. Science of the Total Environment, 502: 309–314. <https://doi.org/10.106/j.scitotenv.2014.09.022>
- Bradford M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical Biochemistry, 72: 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Cabral-Oliveira J., Pratas J., Mendes S. & Pardal M.A., 2015. Trace elements in edible rocky shore species: Effect of sewage discharges and human health risk implications. Human and Ecological Risk Assessment, 21: 135–145. <https://doi.org/10.1080/10807039.2014.890480>
- Cébron A., Cortet J., Criquet S., Biaz A., Calvert V., Caupert C., Pernin C. & Leyval C., 2011. Biological functioning of PAH-polluted and thermal desorption-treated soils assessed by fauna and microbial bioindicators. Research in Microbiologie, 162: 896–907. <https://doi.org/10.1016/j.resmic.2011.02.011>
- Chmielowska-Bąk J., Izbiańska K., Ekner-Grzyb A., Bayar M. & Deckert, J., 2018. Cadmium stress leads to rapid increase in RNA oxidative modifications in soybean seedlings. Frontiers in plant science, 8: 19–28. <https://doi.org/10.3389/fpls.2017.02219>
- Coskun M., Steinnes E., Viladimirovna Frontasyeva M., Eidhammer Sjobakk T. & Demkina S., 2006. Heavy metal pollution surface soil in the thrace region, Turkey. Environmental Monitoring and Assessment, 119: 545–556. <https://doi.org/10.1007/s10661-005-9042-3>
- Dallinger R., 1994. Invertebrate organisms as biological indicators of heavy metal pollution. Applied Biochemistry and Biotechnology, 48: 27–31. <https://doi.org/10.1007/BF02825356>
- De Vaufléury A., 2015. Land snail for Ecotoxicological assessment of chemicals and soil contamination, chapter 23: 365–366 In: Armon RH, Hänninen O (Eds), Environ Indicators (Springer) pp 345–391.
- De Vaufléury A., Coeurdassier M., Pandard P., Scheiffler R., Lovy C., Crini N. & Badot P.M., 2006. How terrestrial snails can be used in risk assessment of soils. Environmental and Toxicology Chemistry, 25: 797–806. <https://doi.org/10.1897/04-560R.1>
- De Vries W., Vel E., Reinds G.J., Deelstra H., Klap J.M., Leeters E.E.J.M., Hendriks C.M.A., Kerkvoorden M., Landmann G., Herkendell J., Haussmann T. & Erisman J.W., 2002. Intensive monitoring of forest ecosystems in Europe 1. Objectives, set-up and evaluation strategy. Forest Ecology and Management, 189: 1–19. [https://doi.org/10.1016/S0304-3820\(02\)00029-4](https://doi.org/10.1016/S0304-3820(02)00029-4)
- Dijkstra E.A., 1998. Micromorphological study on the development of humus profiles in heavy metal polluted and non-polluted forest soils under Scots pine. Geoderma, 82: 341–358. [https://doi.org/10.1016/S0016-7061\(97\)00114-6](https://doi.org/10.1016/S0016-7061(97)00114-6)
- Dobrowolski R. & Otto M., 2012. Determination of nickel and cobalt in reference plant materials by carbon slurry sampling GFAAS technique after their simultaneous preconcentration onto modified activated carbon. Journal of food composition and analysis, 26: 58–65. <https://doi.org/10.1016/j.jfca.2012.03.002>
- Douafer L. & Soltani. N., 2014. Inventory of land snails in some Sites in the Northeast Algeria: correlation with soil characteristics. Advanced Environmental Biology, 8: 236–243.

- Douafer L., Zaidi N. & Soltani N., 2020. Seasonal variation of biomarker responses in *Cantareus asperses* and physic-chemical properties of soils from North-east Algeria. *Environmental Science and pollution research*, 26.
<https://doi.org/10.1007/s11356-020-08694-4>
- Duong T.T. & Lee B., 2011. Determining contamination level of heavy metals in road dust from busy traffic areas with different characteristics. *Journal of Environmental Management*, 92: 554–562.
<https://doi.org/10.1016/j.jenvman.2010.09.010>
- EEA, 2019. Progress in Management of Contaminated Sites. European Environment Agency, Copenhagen
<https://www.eea.europa.eu/data-and-maps/indicators/progress-in-management-of-contaminated-sites-3/assessment>
- Eeva T. & Lehtikoinen E., 2000. Recovery of breeding success in wild birds. *Nature*, 403: 851–852.
<https://doi.org/10.1038/35002672>
- El-Gendy K.S., Radwan M.A. & Gad A.F., 2009. In vivo evaluation of oxidative stress biomarkers in the land snail, *Theba pisana* exposed to copper-based pesticides. *Chemosphere*, 77: 339–344.
<https://doi.org/10.1016/j.chemosphere.2009.07.015>
- El-Gendy K.S., Radwan M.A., & Gad A.F., 2011. Feeding and Growth Responses of the Snail *Theba pisana* to Dietary Metal Exposure. *Archives of Environmental Contamination and Toxicology*, 60: 272–280.
<https://doi.org/10.1007/s00244-010-9542-1>
- Elia A.C., Galarini R., Dorr A.J.M. & Taticchi, M.I., 2007. Heavy metal contamination and antioxidant response of a freshwater bryozoan (*Lophopus crystallinus* Pall., Phylactolaemata). *Ecotoxicology and Environmental Safety*, 66: 188–194.
<https://doi.org/10.1016/j.ecoenv.2005.12.004>
- Ellman G.L., Courtney K.D., Andres V. & Featherstone R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Reviews of Physiology, Biochemistry and Pharmacology*, 38: 84–90.
[https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
- Engenheiro E.L., Hankard P.K., Sousa J.P., Lemos M.F., Weeks J.M. & Soares A.M.V.M., 2005. Influence of dimethoate on acetylcholinesterase activity and locomotor function in terrestrial isopods. *Environmental and Toxicology Chemistry*, 24: 603–609.
<https://doi.org/10.1897/04-131r.1>
- Faiz Y., Tufail M., Tayyeb Javed M., Chaudhry M.M. & Siddique, N., 2009. Road dust pollution of Cd, Cu, Ni, Pb and Zn along Islamabad Expressway, Pakistan. *Microchemical Journal*, 92: 186–192.
<https://doi.org/10.1016/j.microc.2009.03.009>
- Gimbert F., Mench M., Coeurdassier M., Badot P.M. & de Vaulfleur A., 2008a. Kinetic and dynamic aspects of soil-plant-snail transfer of cadmium in the field. *Environmental Pollution*, 152: 736–745.
<https://doi.org/10.1016/j.envpol.2007.06.044>
- Gorlach E. & Gambuś F., 1991. Desorption and phytotoxicity of heavy metals depending on soil properties. *Soil Science Annual*, 3: 207–214.
- Hamdi-Ourfella A.N. & Soltani N., 2014. Qualité des sols dans deux sites du Nord-Est Algérien: utilisation d'une espèce bioindicatrice de la pollution des sols (Soil quality of two sites in Northeast Algeria: use of a bioindicator species of soil pollution). *Journal of Materials and Environmental Science*, 5(S2): 2527–2533.
<http://www.jmaterenvironsci.com/Journal/vol5-S2.html>
- Haslmayr H.P., Geitner C., Sutor G., Knoll A. & Baumgarten A., 2016. Soil function evaluation in Austria – Development, concepts and examples. *Geoderma*, 264, B: 379–387.
<https://dx.doi.org/10.1016/j.geoderma.2015.09.023>
- Helmreich B., Hilliges R., Schriewer R. & Horn H., 2010. Runoff pollutants of a highly trafficked Urban road - Correlation analysis and seasonal influences. *Chemosphere*, 80: 991–997.
<http://dx.doi.org/10.1016/j.geoderma.2015.09.023>
- Ibanez-Asensio S., Marques-Mateu A., Moreno-Ramon H. & Balasch S., 2013. Statistical relationships between soil colour and soil attributes in searid areas. *Biosystems Engineering*, 116: 120–129.
<https://doi.org/10.106/j.biosystemseng.2013.07.013>
- Itziou A. & Dimitriadis V.K., 2009. The Potential Role of cAMP as a Pollution of Terrestrial Environments Using the Land Snail *Eobania vermiculata*: Correlation with Lysosomal Membrane Stability. *Chemosphere*, 76: 1315–1322.
<https://doi.org/10.1016/j.chemosphere.2009.06.032>
- Itziou A. & Dimitriadis V.K., 2011. Introduction of the land snail, *Eobania vermiculata*, as a bioindicator organism of terrestrial pollution using a battery of biomarkers. *Science of the total Environment*, 409: 1181–1192. <https://doi.org/10.1016/j.scitotenv.2010>
- Itziou A., Patsis P.A. & Dimitriadis V.K., 2018. Introduction of the land snail *Cornu aspersum* as a bioindicator organism of terrestrial pollution with the use of a suite of biomarkers. *Toxicological and Environmental Chemistry*, 100: 8–10, 717–736.
<https://doi.org/10.1080/02772248.2019.1588274>
- Johansson C., Norma M. & Burman L., 2009. Road traffic emission factors for heavy metals. *Atmospheric Environment*, 43: 4681–4688.
<https://doi.org/10.1016/j.atmosenv.2008.10.024>
- Kluge B. & Wessolek G., 2012. Heavy metal pattern and solute concentration in soils along the oldest highway of the world-the AVUS Autobahn. *Environmental Monitoring and Assessment*, 184: 6469–6481.
<https://doi.org/10.1007/s10661-011-2433-8>

- Laib E. & Leghouchi E., 2011. Cd, Cr, Cu, Pb, and Zn concentrations in *Ulva lactuca*, *Codium fragile*, *Jania rubens*, and *Dictyota dichotoma* from Rabta Bay, Jijel (Algeria). Environmental Monitoring and Assessment, 184: 1711–1718.
<https://doi.org/10.1007/s10661-011-2072-0>
- Lal R., 2015. Restoring soil quality to mitigate soil degradation. Sustainability, 7: 5875–5895.
<http://dx.doi.org/10.3390/su7055875>
- Larba R. & Soltani N., 2013. Diversity of the terrestrial gastropods in the Northeast Algeria: spatial and temporal distribution. European Journal of Experimental Biology, 3: 209–215.
- Larba R. & Soltani N., 2014. Use of the land snail *Helix aspersa* for monitoring heavy metal soil contamination in Northeast Algeria. Environmental Monitoring Assessment, 186: 4987–4995.
<https://doi.org/10.1007/s10661-014-3753-2>
- Laskowski R. & Hopkin S.P., 1996. Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): implications for predators. Environmental and pollution, 91: 289–297.
[https://doi.org/10.1016/0269-7491\(95\)00070-4](https://doi.org/10.1016/0269-7491(95)00070-4)
- Leghouchi L., Laib E. & Guerbet M., 2009. Evaluation of chromium contamination in water, sediment and vegetation caused by the tannery of Jijel (Algeria): a case study. Environmental monitoring and assessment, 153: 111–117.
<https://doi.org/10.1007/s10661-0080341-3>
- Leomanni A., Schettino T., Calisi A. & Lionetto M.G., 2016. Mercury Induced Haemocyte Alterations in the Terrestrial Snail *Cantareus apertus* as Novel Biomarker. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology, 183–184: 20–27.
<https://doi.org/10.1016/j.cbpc.2016.01.004>
- Leomanni A., Schettino T., Calisi A., Gorbi S., Mezzelani M., Regoli F. & Lionetto M.G., 2015. Antioxidant and oxidative stress related responses in the mediterranean land snail *Cantareus apertus* exposed to the carbamate pesticide carbaryl. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology, 168: 20–27.
<https://doi.org/10.1016/j.cbpc.2014.11.003>
- Liu Q., Liu Y. & Zhang M., 2012. Mercury and cadmium contamination in traffic soil of Beijing, China. Bulletin of Environmental Contamination and Toxicology, 88: 154–157.
<https://doi.org/10.1007/s00128-011-0441-6>
- Louzon M., Pauget B., Gimbert F., Morin-Crini N. & de Vaufléury A., 2020. Ex situ environmental risk assessment of polluted soils using threshold guide values for the land snail *Cantareus aspersus*. Science of the Total Environment? 15: 721, 137789.
<https://doi.org/10.1016/j.scitotenv.2020.137789>
- Louzon M., Pelfrène A., Pauget B., Gimbert F., Morin-Crini N., Douay F. & de Vaufléury A., 2020. Bioaccessibility of metal (loid)s in soils to humans and their bioavailability to snails: A way to associate human health and ecotoxicological risk assessment?; Journal of Hazardous Materials, 15: 384.
<https://doi.org/10.1016/j.jhazmat.2019.121432>
- Malik R.N. Zeb N., 2009: Assessment of environmental contamination using feathers of *Bubulcus ibis* L., as a biomonitor of heavy metal pollution, Pakistan. Ecotoxicology, 18: 522–536.
<https://doi.org/10.1007/s10646-009-03109>
- Mansouri B., Pourkhabbaz A., Babaei H., Hoshyari E., Khodaparast S.H. & Mirzajani A., 2012. Assessment of trace-metal concentrations in Western Reef heron (*Egretta gularis*) and Siberian gull (*Larus heuglini*) from southern Iran. Archives of Environmental Contamination and Toxicology, 63: 280–287.
<https://doi.org/10.1007/s00244-012-97627>
- Migliorini M., Pigino G., Bianchi N., Bernini F. & Leonzio C., 2004. The effects of heavy metal contamination on the soil arthropod community of a shooting range. Environmental and Pollution, 129, 331–340.
<https://doi.org/10.1016/j.envpol.2003.09.025>
- Modrzejewska B. & Wyszowski M., 2014. Trace metals content in soils along the state road 51 (northeastern Poland). Environmental Monitoring Assessment, 186: 2589–2597.
<https://doi.org/10.1007/s10661-013-3562>
- Möller A., Müller H.W., Abdullah A., Abdelgawad G. & Utermann J. 2005. Urban soil pollution in Damascus, Syria: concentrations and patterns of heavy metals in the soils of the Damascus Ghouta. Geoderma, 124: 63–71. <https://doi.org/10.1016/j.geoderma.2004.04.003>
- Mortensen L.H., Rønn R. & Vestergård M., 2018. Bioaccumulation of cadmium in soil organisms-With focus on wood ash application. Ecotoxicol and Environmental Safety, 156: 452–462.
<https://doi.org/10.1016/j.ecoenv.2018.03.018>
- Murtaza G., Ditta A., Ullah N., Usman M. & Ahmed Z., 2021. Biochar for the management of nutrient impoverished and metal contaminated soils: Preparation, applications, and prospects. Journal of Soil Science and Plant Nutrition, 21: 2191–2213.
<https://doi.org/10.1007/s42729-021-00514-z>
- Naghipour D., Ashrafi S.D. & Taghavi K., 2018. Data of heavy metals in soil and groundwater at Kiwi gardens of Amlash in Guilan Province, Iran. Data Brief, 18: 1556–1561. <https://doi.org/10.1016/j.dib.2018.04.046>
- Nawrot T., Plusquin M., Hogervorst J., Roels H.A., Celis H., Thijs L., Vangronsveld J., Van Hecke E. & Staessen J.A., 2006. Environmental exposure to cadmium and risk of cancer: a prospective population-based study. The Lancet Oncology, 7: 119–126.
[https://doi.org/10.1016/S1470-2045\(06\)70545-9](https://doi.org/10.1016/S1470-2045(06)70545-9)

- Pauget B., Gimbert F., Coeurdassier M., Crini N., Pérès G., Faure O., Douay F., Richard A., Grand C. & de Vaufleury A., 2013a. Assessing the *in situ* bioavailability of trace elements to snails using accumulation kinetics. *Ecological Indicators*, 34: 126–135. <https://doi.org/10.1016/j.ecolind.2013.04.018>.
- Pauget B., Gimbert F., Coeurdassier M., Crini N., Pérès G., Faure O., Douay F., Hitmie A., Beguiristain T., Alaphilippe A., Guernionb M., Houoth S., Legrasi M., Vian J.F., Heddek M., Bispo A., Grand C. & De Vaufleury A., 2013b. Ranking field site management priorities according to their metal transfer to snails. *Ecological Indicators*, 29: 445–454. <https://doi.org/10.1016/j.ecolind.2013.01.012>.
- Pauget B., Villeneuve A., Redon P.O., Cuvier A. & de Vaufleury A., 2017. Assessment of the bioavailability and depuration of uranium, cesium and thorium in snails (*Cantareus aspersus*) using kinetics models. *Journal of Hazardous Materials*, 335: 75–83. <https://doi.org/10.1016/j.jhazmat.2017.03.056>.
- Piron-Frenet M., Bureau F. & Pineau R., 1994. Lead accumulation in surface roadside soil: its relationships to traffic density and meteorological parameters. *Science of the Total Environment*, 144: 297–304. [https://doi.org/10.1016/0048-9697\(94\)90449-9](https://doi.org/10.1016/0048-9697(94)90449-9).
- Puga A.P., Abreu C.A., Melo L.C.A. & Beesley L., 2015. Biochar application to a contaminated soil reduces the availability and plant uptake of zinc, lead and cadmium. *Journal of Environment Management*, 159: 86–93. <http://dx.doi.org/10.1016/j.jenvman.2015.05.036>.
- Radwan M.A., El-Gendy K.S. & Gad A.F., 2010a. Biomarkers of oxidative stress in the land snail, *Theba pisana* for assessing ecotoxicological effects of urban metal pollution. *Chemosphere*, 79: 40–46. <http://dx.doi.org/10.1016/j.chemosphere.2010.01.056>.
- Radwan M.A., El-Gendy K.S. & Gad A.F., 2010b. Oxidative stress biomarkers in the digestive gland of *Theba pisana* exposed to heavy metals. *Archives of Environmental Contamination and Toxicology*, 58: 828–835. <https://doi.org/10.1007/s00244-009-9380-1>.
- Regoli F. & Principato G., 1995. Glutathione, glutathione-dependent and antioxidant enzymes in mussel, *Mytilus galloprovincialis*, exposed to metals under field and laboratory conditions: implications for the use of biochemical biomarkers. *Aquatic Toxicology*, 31:143–164. [https://doi.org/10.1016/0166-445X\(94\)00064-W](https://doi.org/10.1016/0166-445X(94)00064-W).
- 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* sentinel organism for monitoring ecotoxicologic effects of urban pollution: an integrated approach. *Environmental Health Perspectives*, 114: 63–69. <https://doi.org/10.1289/ehp.8397>.
- Rorke M.A. & Gardner D.R., 1974. Lethality and behavioural symptoms produced by some organophosphorous compounds in the snail *Helix aspersa*. *Bulletin of Environmental Contamination and Toxicology*, 11: 417–424. <https://doi.org/10.1007/BF01685297>.
- Rovira J., Nadal M., Schuhmacher M. & Domingo J.L., 2015. Human exposure to trace elements through the skin by direct contact with clothing: Risk assessment. *Environmental Research*, 140: 308–316. <http://dx.doi.org/10.1016/j.envres.2015.03.032>.
- 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. *Environmental Toxicology Chemistry*, 25: 815–822. <https://doi.org/10.1897/04-675R.1>.
- Sheppard D.S., Claridge G.G.C. & Campbell I.B., 2000. Metal contamination of soils at Scott base, Antarctica. *Applied Geochemistry*, 15: 513–530. [https://doi.org/10.1016/S0883-2927\(99\)00055-4](https://doi.org/10.1016/S0883-2927(99)00055-4).
- Wolterbeek B. 2000. Biomonitoring of trace element air pollution: principles, possibilities and perspectives. *Environmental Pollution*, 120: 11–21. [https://doi.org/10.1016/S269-7491\(02\)00124-0](https://doi.org/10.1016/S269-7491(02)00124-0).
- Zaidi N. & Soltani N. 2010. Chronic toxicity of flucyclohexuron in the mosquitofish, *Gambusia affinis*: acetylcholinesterase and catalase activities and pattern of recovery. *Annals of Biological Research*, 1: 210–217.
- Zaidi N. & Soltani N., 2011. Environmental risks of two chitin synthesis inhibitors on *Gambusia affinis*: chronic effects on growth and recovery of biological responses. *Biological Control*, 59: 106–113. <https://doi.org/10.1016/j.biocontrol.2011.04.001>.
- Zhang X., Zhong T., Liu L. & Ouyang X., 2015. Impact of soil heavy metal pollution on food safety in China. *PLOS One*, 10: 1–14. <http://dx.doi.org/10.1371/journal.pone.0135182>.

Figure. 2 Specific activity of AChE (nMol/mn/mg proteins) in head of *C. aspersus* collected from the different sites in spring and autumn of 2019 ($m \pm SD$; $n = 5$; for each site, mean values followed by the same letter are not significantly different at $p > 0.05$).

Figure. 3 Specific activity of GST (nMol/mn/mg proteins) in digestive gland of *C. aspersus* collected from the different sites in spring and autumn of 2019 ($m \pm SD$; $n = 5$; for each site, mean values followed by the same letter are not significantly different at $p > 0.05$).

Table 2. Soil concentrations of heavy metals (at a depth of 10 cm in mg/Kg dry mass) in the different sites during the spring and autumn of 2019 ($m \pm SD$; $n = 3$; for each metal mean values from the same season followed by different small letters are significantly different at $p < 0.05$; while for each metal mean values from the same site followed by different capital letters are significantly different at $p < 0.05$).

Table 3. Relation between the heavy metal contents in the soil with seasonal variation of biomarkers (GSH and AChE) in *C. aspersus* (R = coefficient of correlation, p = significance level).

Table 4. Regulatory limits of heavy metal concentrations (g/Kg) in soils according to AFNOR U44- 041 (na: not available).