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ASSESSMENT OF HEAVY METAL SOIL CONTAMINATION IN SOME NORTHEASTERN ALGERIAN BIOTOPES BY USING THE TERRESTRIAL SNAIL, *HELIX ASPERSA*

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Abstract: The study was aimed to assess the ecotoxicological impact of anthropogenic activities on soil quality by using the land snail *Helix aspersa* as a bioindicator. Soil samples and snails were collected from several sites of northeast Algeria during spring and winter, 2017. All sites were chosen in this study for the reason of their proximity to industrial factories as a potential source of heavy metal soil contamination. The concentration of heavy metals in soil samples was analyzed using the X-ray Fluorescence (XRF) spectrometer (Thermo Scientific Model Niton FXL 950) since the three metals of the highest levels in soil samples were examined in *Helix aspersa* hepatopancreas and feet by means of the atomic absorption spectrophotometry. Also, the highest levels of heavy metals were noticed during spring in *Helix aspersa* of the closest sites to the potential sources of pollution. These results are correlated with the physico-chemical characteristics of soil (texture, organic matter, pH Water, conductivity, limestone, and porosity) in each site.

Keywords: Contamination, Heavy metals, *Helix aspersa*, North-East Algeria, Biotope.

INTRODUCTION

Soil acts as vital resource for human societies and ecosystems, and hence it needs to be against increased degradation process, mainly due to population growth or pollution. The monitoring and the implementation of soil protection and management are preferably focused on using the biological indicators to identify and to qualify the impact of disturbances and changes in the soil and ecosystems (Larba et al., 2014). In this regard, many human activities are resulted in producing various pollutant substances, in particular, heavy metals inducing serious effects on human and ecosystems health (Carpenter, 2001). The northeast Mediterranean coastal biotope of Algeria is characterized by a rich variety of flora and fauna (Boudechiche, 2007). Recently, great attention has been paid to the use of invertebrates in the assessment of ecosystem quality in aquatic and terrestrial environments (Phillips, 1977) and the fauna, including snails (mollusks, gastropods, and pulmonic) (Eijasackers, 1983). As previously reported (Bergy et al., 1993 and Cortet et al., 1999), the mollusks are relevant environmental indicators due to their ability to accumulate trace elements, especially Fe, Cd, Pb, and Zn, making thus the snails as valuable bio-indicators of metal contamination (Viard et al., 2004a and Notten et al.,2005).

The snails, *Helix aspersa* is identified as model of choice due mainly to its worldwide distribution, reflecting the adaptation to habitats, soil, and varied climate and ease of farming (Gomot et al., 1987). Moreover, the digestive gland (or hepatopancreas) usually contains the highest concentrations of Cd, Pb, and Zn (Cooke et al., 1979), since cadmium is mainly stored in the digestive tract of snails. Noteworthy, the high capacity of trace element accumulations in snails are related to the effectiveness of detoxification systems, involving not only sequestration structures and intracellular compartments, but also their limited ability to excrete some metals conditioned by the need to avoid excessive loss of water (Dallinger et al., 2001).

This study was devoted to evaluate the metal soil contamination of some biotope of North-eastern Algeria by using the *Helix aspersa* snail as a bioindicator.

MATERIALS AND METHODS

Study Area and Sampling Sites

The study was performed in six sites of the Northeast Algeria; three sites in Annaba city (site 1, Sidi Amar 36° 49 'North, 007° 43 'East, site 2, El – Hadjar 36° 48 'North, 007° 44 'East, and site 3, Seraidi 36° 55 'North, 007° 40 'East), two sites in Souk-Ahras city (site 4, Taoura 36° 10 'North, 008° 02 'East, and site 5, Chaabani 36° 17 'North, 007° 57 'East) and one site in El-Taref city (site 6, Drean 36° 41 'North, 007° 45 'East). Each of these sites was selected along a contamination gradient in terrestrial soil close to major soil polluting industries, such as those producing phosphoric fertilizers (Fertial), and steel products (Arcelor Mittal). The geographical positions of each site are listed in Figure 1 and Table 1.

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Identification of snails

A random sampling of snails was performed from various sites, between the periods from winter to spring, 2017. The live mollusks were collected depending on the climatic conditions (rainfall, humidity), as well as the snail species were identified following the color and the number of spiral bands in the shells (Charrier, 1981 and Bonnet et al., 1990).

Physico-chemical properties of soils

The distribution and the growth of snail species are strongly related to the physico-chemical properties of the soil ecosystem. Thus the quality of soil containing snails focuses on the determination of major physico-chemical parameters, including texture, water pH (Gaucher, 1968), organic substances (Rouiller et al., 1994), electrical conductivity and porosity (Delaunois, 1976), and active and total limestone were determined (Duchaufour, 1970).

Soils Sampling and Heavy Metal Extraction and Analysis

The analysis of samples was performed through, a dry step in an oven at 60 ° C up to obtain constant weight, and a milling and sieving (140µm) step to achieve at least ten grams of powder. For the X-Ray Fluorescence (XRF) analysis, we needed to use 8 grams of sample for the test, otherwise, it would be possible to use boric acid to increase the pellet under the sample of which, we would not have a sufficient quantity. As a result, the sample can be prepared as a pellet through centrifugation (speed =40 rpm). The samples were then placed in an autosampler of the XRF spectrometer (Thermo Scientific Model Niton FXL 950). The analyses were conducted at the Research Unit level, Materials, Nanomaterials, and Ecosystems. Faculty of Sciences of Bizerte Tunisia.

The XRF method requires the placement of samples under an X-ray beam. The energy spectrum of the fluorescent X-rays reveals the characteristic peaks of the elements present in samples, i.e the peaks show the nature of the element since the height of the peaks explains the element quantity.

Analysis of heavy metals in hepatopancreas and feet of snails by atomic absorption spectrophotometry

The organ fragments are placed separately in screw tubes and then dried in an oven (50 ° C) between 48 and 72 hours (Coeurdassier, 2001). After weighing the dried fragments, 4 ml of 50% nitric acid were added to each tube, which afterward was sealed with screw caps and, kept in an oven at 60 ° C until the digestion of the tissues under pressure (about 72 hours) is completed. Then each sample was made up to 19 ml with distilled water and stored at 4 ° C until analysis (Coeurdassier, 2001). The metal concentrations in different samples were measured by atomic absorption spectrophotometry (ICP-AES).

Fig. 1. Geographical location of the sampling sites (www.google.fr).

Table 1

Geographic position of sampling sites

Sites	North	East
Site1	36° 49′ 04″	7° 43′ 05″
Site2	36° 48′ 00″	7° 44′ 00″
Site3	36° 55′ 00″	7° 40′ 00″
Site4	36° 10′ 12″	8° 02′ 34″
Site5	36° 17′ 15″	7° 57′ 15″
Site6	36° 41′ 00″	7° 45′ 00″

Statistical analysis

Data are displayed as mean ± SD standard deviation. Statistical significance was tested by analysis of variance (ANOVA). The statistical test was performed using MINITAB software (version 18, Penn State College, PA, USA) (Dagnelie, 2001).

RESULTS AND DISCUSSION

Physico-chemical characteristics of soils in different study sites

The results of the physico-chemical parameters of the soil in the different study sites are displayed in table 2, showing that the studied soils are alkaline (pH>7.5). Also, soil moisture values vary between 43.01 and 65.29%, which explains therefore the reason why soils are clay-silty in site 2, site 3, site 4, site 5, site 6; and silty clay in site 2 (43.01%). In addition, the soil conductivity varies between 0.07 and 0.58, and as it is less than 0.6 ms/cm, it indicates that the soil is not salted. The two types of limestone measured at the study sites indicate that the soils have active limestone-type varied between 16.25 and 27.75%, in site 2, site 3, site 4, site 5, site 6, in addition to total limestone ranged from 15 to 30% in the six sites, in which the limestone varies between 19.95 and 27 %. Moreover, the studied soils exhibit a percentage varies between 2.7 and 18.8% in organic matter, explaining therefore why the rate is very high, so they are very rich in organic matter, except in the site 2, 5 and 6 where the soil is less rich in organic matter (2.7%, 4.57%). On top of that, the lowest porosity (less than 10%) was noticed at the sites 5 (9.2%) and 6 (8.07%), since the other sites showed a fairly low porosity (10-20%), and the values are 11.74% and 18.38% (Table 2). ANOVA analysis test revealed a highly significant effect of site ($p < 0,001$) regarding organic matter ($F5, 12 = 974.38$; $p = 0.000$), soil texture ($F5, 12 = 18.26$; $p = 0.000$), porosity ($F5, 12 = 73.21$; $p = 0.000$) and active limestone ($F5, 12 = 23.85$; $p = 0.000$), significant effect of site ($p < 0.05$) regarding conductivity ($F5, 12 = 4.20$; $p = 0.019$), and no significant effect of site ($p > 0.05$) regarding pH water ($F5, 12 = 2.38$; $p = 0.101$) and total limestone ($F5, 12 = 2.23$; $p = 0.118$).

Table 2

Physico-chemical characteristics of soils from the six studied sites

Parameters/ Sites	Site1	Site2	Site3	Site4	Site5	Site6
pH water	7.30 ± 0.17	7.82± 0.03	8.18 ± 1.09	8.28± 0.97	7.98± 0.01	7.63 ± 0.08
Texture (%)	43.01 ± 3.62	65.26 ± 4.48	55.96 ± 0.15	55.33± 4.51	49.35±1.033	49.66 ± 1.52
Conductivity (mS/cm)	0.17 ± 0.01	0.58 ± 0.04	0.50 ± 0.36	0.07± 0.02	0.28±0.14	0.38 ± 0.02
Organic matter (%)	5.86 ± 0.32	2.7 ± 0.59	18.38 ± 0.04	18.8± 0.20	4.53±0.49	4.57 ± 0.51
Active limestone (%)	31 ± 3.61	16.25 ± 0.21	28.75 ± 2.86	24.38 ± 0.07	20.06 ± 0.50	27.66 ± 1.52
Total limestone (%)	24.90 ± 4.06	23.00 ± 1.73	21.41 ± 0.67	19.8 ± 1.44	26.99 ± 4.20	23.67 ± 3.51
Porosity (%)	11.74 ± 0.30	12.12 ± 0.09	12.1 ± 0.18	12.30 ± 0.10	9.2 ± 0.79	8.07 ± 0.18

(m ± SD, n = 3)

Soil heavy metal concentrations

The concentrations of the most important heavy metals (Fe, Mn, Pb) were measured in soil samples of each site (Tables 3 and 4, Figs 2, 3, and 4). The mean concentration of each metal noticed in this study provides the following decreasing order: Fe, Mn, Pb. The higher concentration values of metals in samples collected from all sites during season 2 were measured and compared with those found during season 1. Additionally, the concentrations of each heavy metal were found to be varied between sites. Notably, site 6 exhibiting increased lead (Pb) levels during sampling is located close to the major road networks, known as potential sources of Pb pollution exposure from highway traffic, while the highest levels of Fe and Mn were found in soil samples adjacent to the waste dump site 2 and 1. Whilst the lowest heavy metal concentrations were noticed in the sites 3 and 4, selected as control sites.

Two-way ANOVA test (season, site) indicated a number of effects of each heavy metal. Fe levels found during sampling showed a significant effect due to site ($F5,5 = 7.70$; $p=0.021$), and a highly significant effect due to season ($F1,5 = 50.31$; $p= 0.001$) . Mn levels in samples showed a highly significant effect due to site ($F5,5 = 25.34$; $p=0.001$) and a very significant effect due to season ($F1,5 = 21.34$; $p=0.006$), since lead (Pb) levels showed a very significant effect due to site ($F5,5 = 12.59$; $p=0.007$) and no significant effect due to season ($F1,5 = 5.13$; $p= 0.073$).

The concentration of heavy metal in snail hepatopancreas and feet

The analysis of heavy metal levels in *Helix aspersa* hepatopancreas and feet shows a marked variation in the contents of the three studied metals. Also, the average concentration of heavy metals found in viscera is higher than that found in feet for all study sites. In fact, the highest concentration is that of iron (2795.78 µg/g in hepatopancreas and 1807.88 µg/g in feet), followed by Mn (22.22 µg/g in hepatopancreas, and 18.75 µg/g in feet), since the concentration of Pb was found to be much lower than the others (13.00 µg/g in hepatopancreas, and 11.80 µg/g in feet). The higher values were measured in the samples collected in season 2 and then were compared with those collected in season 1 (Table 5, Figs 5, 6, 7, 8, 9, 10).

Two-way ANOVA (season, site) performed for each heavy metal and each organ revealed a number of effects. Fe levels obtained during sampling showed a no- significant effect due to season ($F_{1,5} = 2.61$; $p=0.167$), a significant effect due to site ($F_{5,5} = 9.71$; $p= 0.013$) in hepatopancreas, a no- significant effect due to season ($F_{1,5} = 3.31$; $p=0.129$) and a very significant effect due to site ($F_{5,5} = 20.10$; $p= 0.003$) in feet. Whilst Mn level in samples showed a significant effect due to season ($F_{1,5}=7.52$; $p=0.041$), a highly significant effect due to site ($F_{5,5}=285.49$; $p=0.000$) in hepatopancreas, a no-significant effect due to season ($F_{1,5} = 3.15$; $p=0.136$) and a highly significant effect due to site ($F_{5,5} = 39.91$; $p= 0.000$) in feet . Lead (Pb) level showed a no- significant effect due to season ($F_{1,5} = 6.27$; $p=0.054$), a highly significant effect due to site ($F_{5,5} = 31.48$; $p=0.001$) in hepatopancreas, a no- significant effect due to season ($F_{1,5} = 0.44$; $p=0.536$) and a no- significant effect due to site ($F_{5,5} = 2.56$; $p= 0.163$) in feet.

Table 3

Composition in ppm, of soil heavy metals in different sites during the season 1

Site	Fe	Mn	Pb
Site 1	25453.6	390	24.6
Site 2	26090.3	720	27.4
Site 3	20100.0	178.4	09.1
Site 4	22172.7	210	10
Site 5	24738.0	380	20
Site 6	24183.0	350	31.3

Table 4

Composition in ppm, of soil heavy metals in different sites during the season 2

Site	Fe	Mn	Pb
Site 1	33540	677.1	30
Site 2	42889	998	32
Site 3	22580	291	9.9
Site 4	29280	310	15
Site 5	33220	539.5	23.2
Site 6	31360	454.4	50

Table 5

The concentration of heavy metals (µg/g) in hepatopancreas and feet of Snail, *Helix aspersa* in different sites during the season 1 and the season 2

Heavy metals	Season	Organs	Heavy metals concentrations					
			Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Fe	**Season1**	**Hepatopancreas**	897.07	1379.72	1.89	7.86	91.03	22.48
		Feet	579.55	1372.80	1.70	6.33	41.36	15.65
	Season2	**Hepatopancreas**	1320.31	2795.78	1.91	14.79	391.90	39.53
		Feet	1305.20	1807.88	1.79	10.99	191.55	28.50
Mn	**Season1**	**Hepatopancreas**	11.11	21.28	0.88	1.07	7.28	1.56
		Feet	10.71	13.35	0.40	0.74	5.63	1.24
	Season2	**Hepatopancreas**	13.33	22.22	0.97	1.18	8.15	3.81
		Feet	11.23	18.75	0.47	1.08	7.37	1.92
Pb	**Season1**	**Hepatopancreas**	6.44	7.59	0.32	2.57	5.74	9.46
		Feet	5.46	6.62	0.22	1.96	4.37	7.08
	Season2	**Hepatopancreas**	6.66	7.47	0.49	3.49	6.20	13.00
		Feet	5.30	6.10	0.30	3.10	4.80	11.80

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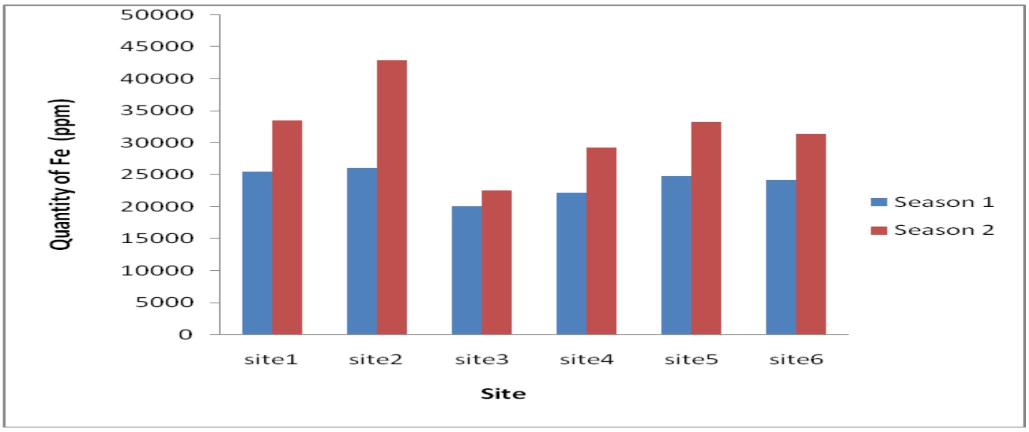


Fig. 2. Quantity of Fe in different sites during season 1 and season 2.

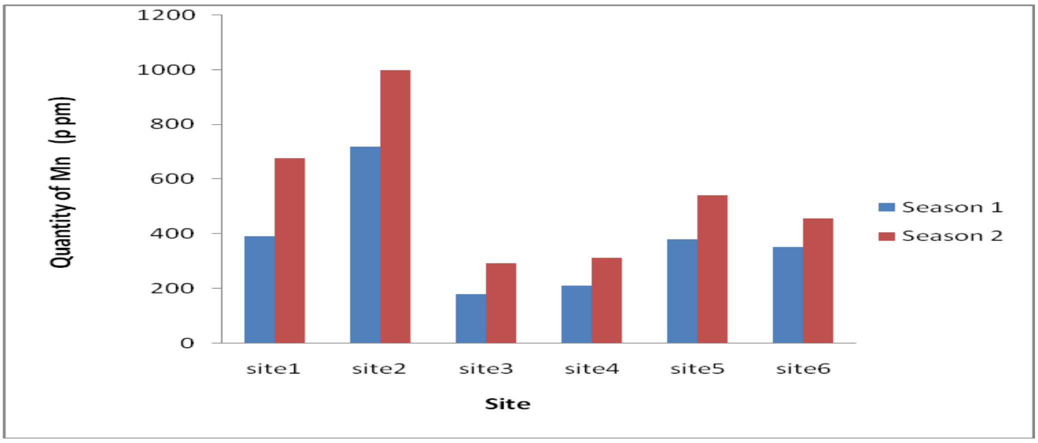


Fig. 3. Quantity of Mn in different sites during season 1 and season 2.

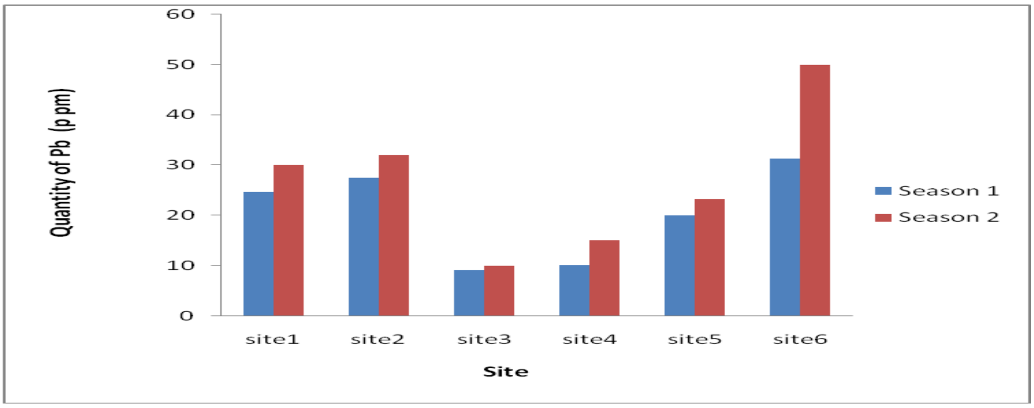
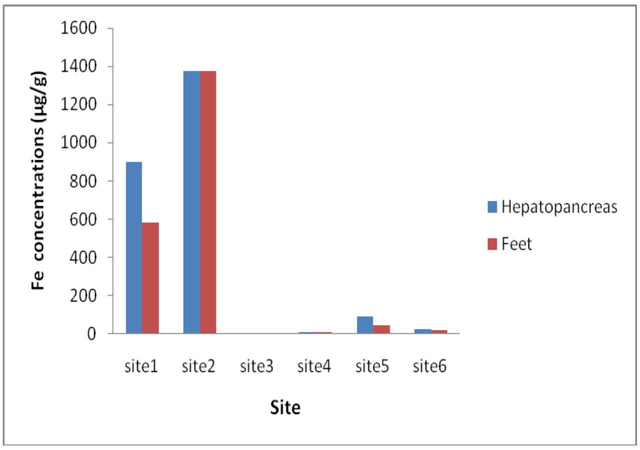


Fig. 4. Quantity of Pb in different sites during season 1 and season 2.



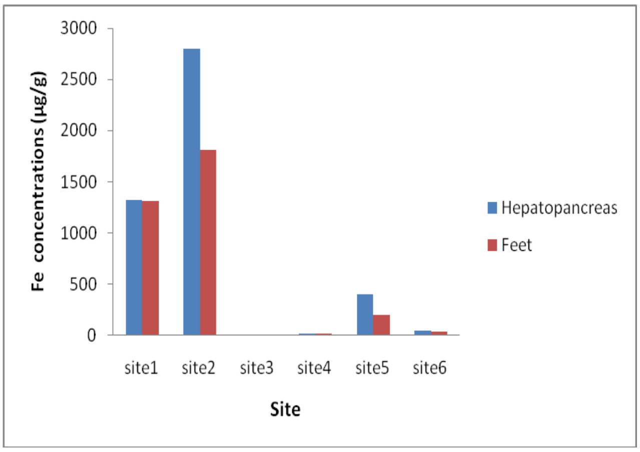
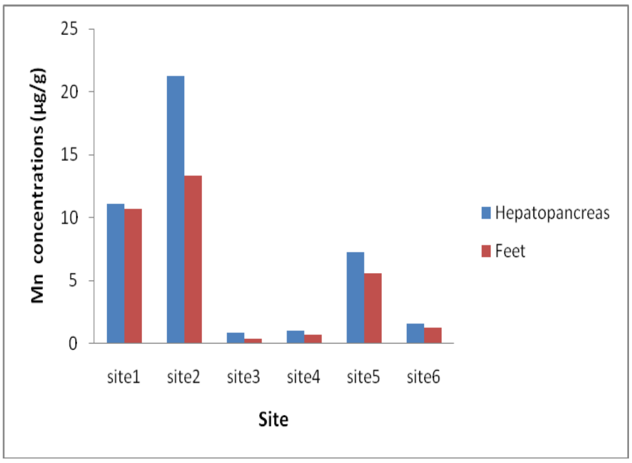


Fig. 5. (Left) Concentration of Fe (µg/g) in Snail hepatopancreas and feet in different sites during season 1.
Fig. 6. (Right) Concentration of Fe (µg/g) in Snail hepatopancreas and feet in different sites during season 2.



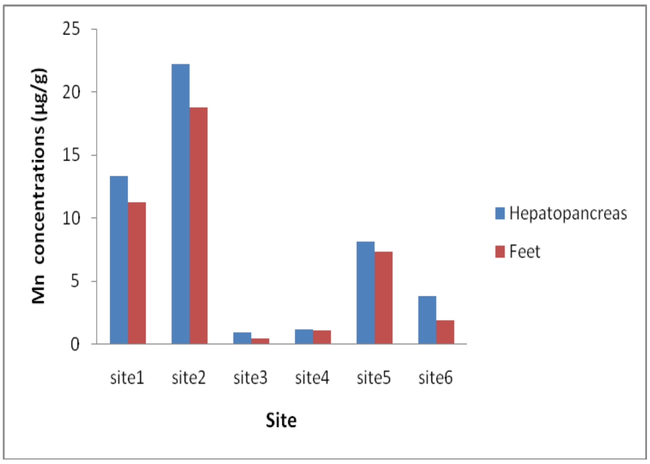
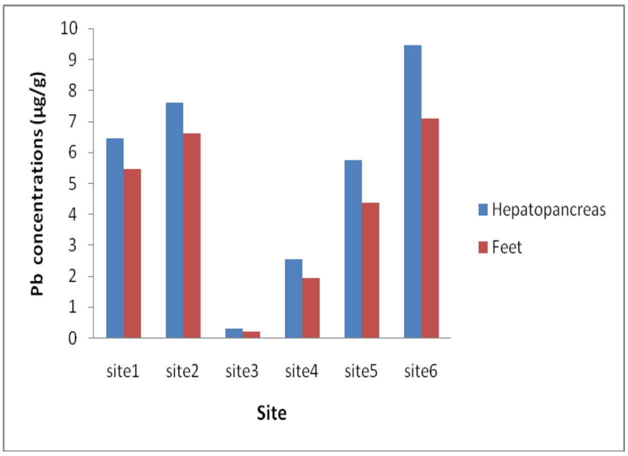


Fig. 7. (Left) Concentration of Mn (µg/g) in Snail hepatopancreas and feet in different sites during season 1.
Fig. 8. (Right) Concentration of Mn (µg/g) in Snail hepatopancreas and feet in different sites during season 2.



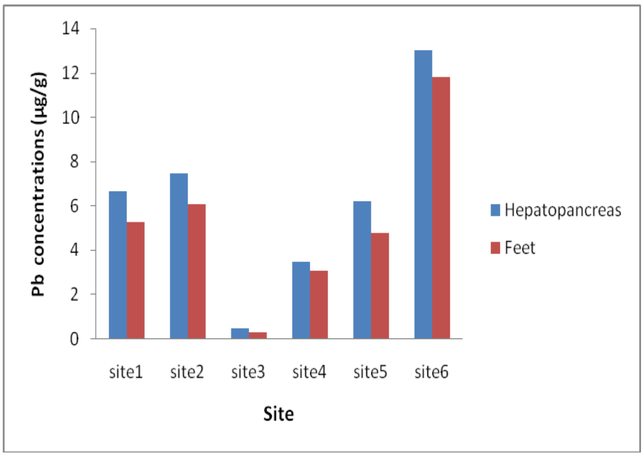


Fig. 9. (Left) Concentration of Pb (µg/g) in Snail hepatopancreas and feet in different sites during season 1.
Fig. 10. (Right) Concentration of Pb (µg/g) in Snail hepatopancreas and feet in different sites during season 2.

Nowadays, heavy metal soil contamination due to increased industrialization and urbanization becomes one of the environmental-relevant subjects for several researchers. The levels of heavy metals in the environment are strongly related to both natural and anthropogenic factors. Anthropogenic processes are main sources of heavy metal soil contamination, as well as utilization of agricultural chemicals containing metals (e.g, fertilizers and pesticides), in addition to vehicle traffic, combustion of petroleum fuels containing metal additives (Reichman, 2010), and surface runoff produced by atmospheric pollutants

(Agarwal, 2009 and Ryu et al., 2010). Heavy metals are harmful to environmental health due to their bioaccumulation in terrestrial ecosystems and the negative effect on food quality and safety.

Soil is the habitat for a multitude of a permanent living being (Davet, 1996), due to its typical physicochemical properties providing a good habitat for snail survival and biological activities. Among soil invertebrates, pulmonate gastropods are commonly described as bioindicators for the assessment of pollution by metallic elements and pesticides in terrestrial ecosystems (Gimbert, 2006, Salama et al., 2005). Several studies have reported that soil quality parameters, including pH, organic matter contents, and moisture strongly affect the bioavailability of metals in soil (Portail, 2005 and Scheifler, 2002). Moreover, the accumulation of Fe, Mn, Pb, Cd, Cu, and Zn by terrestrial gastropod mollusks is related to their environmental concentrations (Coeurdassier, 2001), since the concentrations of these elements in *Helix aspersa* viscera are significantly correlated with total soil concentrations (Viard et al., 2004). The soil heavy metals content varies according to the exposure to various pollution sources (Modrzewska et al., 2014). In our study, the highest values of heavy metals were measured in soils collected from all sites in spring and then were compared to those collected in winter. This is likely due to the leaching of pollutants by rain (Piron-Frenet et al., 1994). Additionally, the average metal concentrations at these sites vary in the same way, with Fe being the most important pollutant, followed by Mn, Pb in descending order. Also, the snails collected in the sites of El-Hadjar and Sidi-Amar exhibit the highest concentrations of heavy metals (Fe, Mn) in spring compared to those collected in winter. These two sites are characterized by a strong industrial, automotive, and railway activities, such as the El-Hadjar industrial zone and the Arcelor Mittal steel complex, which are considered as contamination sources of heavy metals.

In winter, the unfavorable environmental factors put the snail under the environmental stress, and subsequently, they go into hibernation, meanwhile, their physiological activities are reduced and their metabolism is slowed. Hence, the snails are not in continuous contact with their environment, i.e a reduction in exposure periods to contaminants in the environment leads to low accumulation of xenobiotics in snails. On the other hand, environmental factors would be favorable in spring to resume physiological activities. Long periods of exposure favor an important accumulation of pollutants by the snail. Our findings showed that Pb levels were significantly higher at Drean resulting from the increase in road congestion at this site (Ho and Tai1988, Garcia and Millan 1998). The lowest concentrations of heavy metals were noticed in the sites; Taoura and Séraidi, and this is likely due to the fact that these sites are located in uninhabited areas characterized by the absence of any industrial activity, automobile, and any agricultural practice. Viard (2004) has reported that the removal of contamination sources (highways, factories, etc.) reduces the risk of soil contamination by heavy metals. Our results are in line with those previously reported (Viard, 2004; Belabed, 2010; Fritsch, 2010; Bourbia-ait hamlet, 2013 and Larba, 2014). Overall, terrestrial snails potentially accumulate metals by storing a large quantity in their organs (Berger et al., 1993).

Interestingly, the accumulation of heavy metals by invertebrate organisms is the result of the absorption, distribution, and storage processes. When metals are absorbed, terrestrial gastropods have a strategy of non-regulation (Notten et al., 2005). According to the results of our study, heavy metals are stored mainly in the viscera. Indeed, the studies of Gomot (1998) carried out on the same species indicated that the main accumulator organ of heavy metals is hepatopancreas. The snail integrates pollutants, especially heavy metals via different exposure routes: digestive by ingestion of food (plants, soil particles) (Gomot et al., 1989) and cutaneous by diffusion of the pollutant from the soil through the epithelium of the foot (Coeurdassier et al., 2002).

CONCLUSIONS

In conclusion, the results of the present investigation show a decreasing gradient of contamination correlating to the proximity of the pollution sources. The highest levels of contamination were observed at El Hadjar near the Arcelor Mittal steel complex. *H. aspersa*, a common species of land snail present in the soil area was tested as a bioindicator of metal contamination. Overall, the results show that *H. aspersa* is an efficient bioindicator to evaluate the heavy metal atmospheric pollution, due to several industrial factories, and vehicle traffic.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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