Use of the land snail Helix aspersa for monitoring heavy metal soil contamination in Northeast Algeria

Article *in* Environmental Monitoring and Assessment · April 2014

DOI: 10.1007/s10661-014-3753-2 · Source: PubMed

2 authors:

Rabah Larba
Abbes Laghrour - Khenchela University

Noureddine Soltani
Badji Mokhtar - Annaba University

Accepted Version

Published in *Environmental Monitoring and Assessment*, 2014, 186 (8): 4987-4995.

Use of the land snail *Helix aspersa* for monitoring heavy metal soil contamination in Northeast Algeria

R. Larbaa - N. Soltani*

Laboratory of Applied Animal Biology, Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba, 23000-Annaba, Algeria

*Corresponding author: noureddine.soltani@univ-annaba.dz

Abstract

The objective of this study was to investigate the impact of anthropogenic activities on soil quality using the land snail *Helix aspersa* as a bioindicator. Soil samples and snails were collected from several sites in Northeast Algeria during the summer and winter of 2010. All of the sites were chosen due to their proximity to industrial factories – a potential source of soil pollution via heavy metal contamination. The concentration of heavy metals (Pb, Cd, Mn, and Fe) in soil samples was analyzed using atomic absorption spectrophotometry. Activity levels of glutathione S-transferase (GST) and acetylcholinesterase (AChE), indicators of oxidative stress and neurotoxicity respectively, were measured in snails collected from each site. GST and AChE activity were found to vary between sites and by season. The highest levels of GST activity were registered during the summer at sites closest to potential sources of pollution. AChE activity levels also peaked during the summer with the highest values recorded at the site in El Hadjar. These increased levels of bioindicative stress response correlated with increasing metal concentration in soil samples collected at each site.

Keywords: *Pollution, Soils, Heavy metals, Helix aspersa, Biomarkers*

Introduction

The city of Annaba, a major economic hub and tourism center located in East Algeria, has observed markedly increased levels of pollution in recent decades (Semadi and Deruelle 1978; Abdenour et al. 2000, 2004), most notably heavy metal contamination (Beldi et al. 2006; Maas et al. 2010). In an effort to understand the environmental impact of this contamination, several recent studies have examined the degradation of diflubenzuron, a commonly used insecticide, in saltwater (Soltani and Morsli 2003) and freshwater (Zaidi and Soltani 2013) and evaluated its ecotoxicological risks on non-target species such as shrimp (Morsli and Soltani 2003) and fish (Zaidi and Soltani 2010, 2011).

The regional coastal environments have been subjected to various forms of degradation, including chemical contaminants associated with densely populated urban areas via harbours and other industrial complexes (Semadi and Deruelle 1978; Abdenour et al. 2000; Beldi et al. 2006). In one study demonstrating the extent of this pollution, elevated levels of heavy metals (Cu, Zn, Pb, Cd) were detected in a locally prevalent edible mollusk *Donax trunculus* (Beldi et al. 2006). This species has been successfully used as a bioindicator of marine pollution (Verlecar et al. 2006; Sifi et al. 2007; Amira et al. 2011; Soltani et al. 2012) through the direct measurement of several biomarkers, such as malondialdehyde (MDA), lactate dehydrogenase (LDH), acetylcholinesterase (AChE), glutathione S-transferase (GST), and catalase (CAT) (Soltani et al. 2012).

Land snails have also been widely used as a sentinel species for the assessment of metallic pollution in terrestrial ecosystems (Gomot de Vaufleury and Pihan 2000; Regoli et al., 2006; Jordaens et al. 2006; Notten et al., 2006). *Helix aspersa* (Mollusca, Pulmonata, Helicidae; Müller, 1774) is a good bioindicator of metal and organic soil contamination (Scheifler et al. 2002; Gimbert et al. 2006) and is the most abundant and widespread gastropod species in Northeast Algeria (Larba and Soltani 2013). This pollution causes oxidative stresses which can induce the activity of antioxidants in local organisms (Kaloyianni et al. 2009; Tlili et al. 2010). Aerobic organisms have a variety of enzymatic and non-enzymatic antioxidant defenses that maintain endogenous reactive oxygen species (ROS) at relatively low levels and attenuate the damage related to their high reactivity. The enzymatic and non-enzymatic antioxidants are essential for the conversion of ROS to harmless substances and for maintenance of cellular metabolism and function (Mates 2000; Li et al. 2003; Zhang et al. 2008).

Enzymatic antioxidants such as superoxide dismutase (SOD), CAT, and GST as well as several low molecular weight antioxidants such as vitamins B, C, E, and glutathione (GSH) (Buttemer et al. 2010; Sussarellu et al. 2012) protect against oxidative damage by inhibiting reactive oxygen species formation. The activity of AChE has been used as a biomarker of exposure to various types of chemicals such as organophosphates, carbamate insecticides (Coeurdassier et al. 2001; Neuberger-Cywiak et al. 2007; Oliveira et al. 2007), metals, synthetic detergents, fuel oil components, and algal toxins (Amiard-Triquet et al. 1998; Guilhermino et al. 1998; Tim-Tim et al. 2009).

For human and ecological risk assessment, a growing body of evidence has shown the importance of determining the spatial distribution of pollutants (Maas et al. 2010). The main objective of the present study was to assess the utility of *H. aspersa* in environmental monitoring as a bioindicator of heavy metal contamination (Pb, Cd, Mn and Fe) in Northeast Algeria by measuring selected biomarkers (AChE and GST). Samples were collected during the summer and winter of 2010 from various sites located along a terrestrial soil pollution gradient according to their proximity to factories and other potential sources of pollution. The data collected allowed for the determination of a spatial mapping of contaminated soils and consequently to identify the location where remediation efforts should be focused.

Materials and methods

Study Area and Sampling Sites

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 El Hadjar, one of the most populated areas of this region, in addition to Ben M'Hidi, Sidi Kaci, Bouteldja, and El Tarf. 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 (ArcelorMittal), 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 Figure 1 and Table 1.

Animal Biomarker Assay

In this study, five adult specimens of *Helix aspersa* (weight: 12.4 ± 0.5 gm; shell diameter: 29.09 ± 0.5 mm) were collected during the summer and winter of 2010 at each sampling site, (including the control site), transferred to the laboratory, and dissected the same day. The animals were sacrificed and the head and hepatopancreas were sampled. AChE and GST were individually analyzed as previously described (Zaidi and Soltani 2010, 2011). AChE activity was measured in the brain (Ellman et al. 1961). The heads were homogenized in a 1 ml solution composed of 38 mg ethylene glycol tetracetic acid (EGTA), 1 ml Triton X-100%, 5.845 g NaCl and 80 ml Tris buffer (0.01 M, pH 7). After centrifugation (5000 rpm, 5 min), AChE activity was measured in aliquots (100 µl) of supernatant added to 100 µl of 5-5'dithiobis-2-nitrobenzoic acid (DNTB) and 1 ml of Tris buffer (0.1 M, pH 7). After 5 min, 100 µl of acetylthiocholine was added. Measurements were conducted at 412 nm every 4 min for a period of 20 min.

GST activity was determined in hepatopancreas according to Habig et al. (1974) with use of GSH (5 mM) and 1-chloro-2-4-dinitrobenzoic acid (CDNB, 1 mM). Hepatopancreas were individually homogenized in 1 ml of buffer phosphate (0.1 M, pH 6). The homogenate was centrifuged (1300 rpm for 30 min) and the supernatant was used for the enzymatic assay. Hereto, 200 µl of the supernatant was added to 1.2 ml of the mixture GSH-CDNB in phosphate buffer (0.1 M, pH 7). Changes in absorbance were measured at 340 nm every minute for a period of 5 min. All enzymatic activities were expressed as nM/mn/mg protein. Hereto, the protein concentrations in the total homogenate were quantified according to Bradford (1976) with Coomassie brilliant blue G250 as a reagent and bovine serum albumin (BSA) as a standard. The absorbance was read in a spectrophotometer at 595 nm.

Soils Sampling and Heavy Metal Extraction and Analysis

Three subsamples of soil (~100 gm) were randomly taken at a depth of 10 cm from each site, placed in acid-washed polyethylene bags, and homogenized. Soil analyses were performed by the Laboratory of Pharmacology and Phytochemistry (courtesy of Pr. E. Leghouchi, Jijel University, Algeria) according to the procedure of Laib and Leghouchi (2011). In brief, the extraction was made with the following steps. All reagents were obtained from Merck and were of analytical grade. Each sample was dried at 105°C for 24 hours 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 HNO_3 (Merck) solution at a 3:1 ratio. The mixture was heated (180°C) in glass flasks for 30 minutes and then cooled for 30 minutes at an ambient temperature (25°C). Each sample was filtered using filter paper (Whatman No. 1) and diluted with double deionised water in the approximate range of standard concentrations prepared from stock standard solution of each metal (Merck). Concentrations of Cd, Fe, Pb, and Mn 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 and Leghouchi 2011). The values are expressed by the mean ± standard deviation (m ± 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.

Statistical analysis

The normality of data was verified using the Kolmogorov-Smirnov test, and the homogeneity of variances was checked by Levene's test. Data are expressed by the mean ± standard deviation (m ± SD) and were subjected to two way analysis of variance (ANOVA). Differences between sites were determined by Tukey's test. All statistical analyses were performed using Minitab Software (Version 15, Penn State College, PA, USA) with $p< 0.05$ considered as a statistically significant difference.

Results

Glutathione S-transferase and acetylcholinesterase activities

Data on AChE and GST activity in snails collected from the six study sites are presented in Figures 2 and 3. At sites located near pollution sources, snails showed higher GST activity compared to snails from the control reference site El-Kala (14.18 ± 1.78 nM/min/mg). The highest GST activity was found in El Hadjar (30.57 ± 1.30 nM/min/mg). In addition, higher levels of GST activity were found in samples taken during the summer months compared to winter at all study sites. Two way ANOVA revealed significant effects of both seasons ($F_{1, 24} = 411.63$; $p < 0.001$) and site ($F_{5, 24} = 138.67$; $p < 0.001$), and no significant season-site interaction ($F_{5, 24} = 1.82$; $p= 0.231$).

AChE activity was significantly lower in snails collected from all sampling sites compared to snails from the reference site. During the winter season, the lowest AChE activity was observed in El Hadjar (25.57 ± 1.39 nM/min/mg) and the highest activity was observed in El Kala (40.472 ± 1.236 nM/min/mg protein). At all study sites, AChE activity levels were found to be lower during the summer compared to the winter. Two way ANOVA revealed significant effects of both season ($F_{1, 24}= 2332.79$; $p<0.001$), site ($F_{5, 24}= 277.18$; $p<0.001$) and season-site interaction ($F_{5, 24}= 5.62$; $p= 0.001$).

Soil Heavy Metal Concentrations

In order to determine the level of correlation between land snail biomarker responses and heavy metal contamination of soils, the concentrations of the most important heavy metals (Fe, Mn, Pb and Cd) were measured in soil samples at each site (Tables 2 and 3). Globally, the average concentration of each metal recorded exhibited the following decreasing order: Fe, Mn, Pb, and Cd. At all sites, higher values were measured in samples collected in the summer compared to samples collected in the winter. Moreover, the concentrations of each heavy metal varied between sites. Notably, Sidi Kaci, Bouteldja and El Tarf, sites which showed increased Pb levels during sampling, are all located near major road networks that are potential sources of Pb pollution exposure from highway traffic. The lowest heavy metal concentrations were registered in the site of El Kala, which validates its selection as a control site.

For each heavy metal, two-way ANOVA (season, site) indicated a number of effects. Fe levels obtained during sampling showed a significant effect due to season ($F_{1,24}= 5.5\ 10^4$; $p<0.001$), site ($F_{5,24}= 3.1\ 10^4$; $p<0.001$) and season-site interaction ($F_{5,24}= 1131.86$; $p<0.001$); for Mn a significant effect due to season ($F_{1,24}= 5311.60$; $p<0.001$), site ($F_{5,24}= 660.65$; $p<0.001$), and season-site interaction ($F_{5,24}= 33.54$; $p<0.001$); for Pb a significant effect due to season ($F_{1,24}= 547.98$; $p<0.001$), site ($F_{5,24}= 191.07$; $p<0.001$), and season-site interaction ($F_{5,24}= 5.52$; $p=0.002$); and for Cd a significant effect due to season ($F_{1,24}= 422.57$; $p<0.001$) and site ($F_{5,24}= 140.02$; $p<0.001$) and no significant effect due to season-site interaction ($F_{5,24}= 1.42$; $p=0.231$).

Discussion

In recent years, considerable concern has mounted over the problem of soil contamination with heavy metals due to industrialization and urbanization. Environmental concentrations of heavy metals depend on both natural and anthropogenic factors. Anthropogenic processes recognized as potential sources of heavy metal soil contamination predominately consist of agricultural utilization of metal-containing fertilizers and pesticides, vehicle traffic, combustion of petroleum fuels containing metal additives (Reichman 2010), and surface runoff produced by atmospheric pollutants (Calvet and Barriuso 1994; Fernández et al. 2006). Heavy metals can cause serious threats to environmental health due to their bioaccumulation in terrestrial ecosystems and affect food quality and safety (Agarwal 2009; Ryu et al. 2010).

Biomarkers are now becoming an integral part of ecosystem health assessment and management in addition to more traditional water chemical analysis (Lam 2009; Boyd 2010). The antioxidant defense system is being increasingly studied because of its potential utility to provide biochemical biomarkers that can be used in environmental monitoring systems (Ballesteros et al. 2009). They serve as important biological defense against environmental oxidative stress at a cellular level (Van der Oost et al. 2003; Pandey et al. 2003). The antioxidant defence system consists of both enzymatic and non-enzymatic systems. Enzymatic system includes enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), catalase, and glutathione S-transferase (GST).

Many studies confirm that the antioxidant defense system can be markedly induced under certain levels of stress at a given time. However, with increasing exposure time and concentration of pollutants, they show a decrease tendency (Hao and Chen 2012). GSTs, a family of dimeric multifunctional enzymes, have been shown to be involved in detoxification of xenobiotics, protection from oxidative damage, and the intracellular transport of hormones, endogenous metabolites, and exogenous chemicals in diverse organisms (Zhou et al. 2009). Pollution by heavy metals plays an important role in increasing the rate of GST activity (Hamed et al. 2003).

In this study, GST activity was measured in hepatopancreas samples of *H. aspersa*. The hepatopancreas was chosen as a target organ for biochemical assessment because it plays an integral role in detoxification processes resulting in heavy metal deposition (Nowakowska et al. 2012). GST activity was found to be significantly higher in the snails from sample sites adjacent to potential sources of pollution (factories, harbours, etc.) compared to samples taken from the reference control site, El Kala. The induction of GST activity indicates an adaptation of the organism to enhanced pollution stress (Astani et al. 2012). In addition, increased GST activity suggests that the detoxification process against pro-oxidation forces, which are mediated by this enzyme, is induced (Elia et al. 2007, Radwan et al. 2010). 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 winter rains which promote leaching of soil pollutants. Indeed, the climate of the study areas is Mediterranean, with an average annual temperature of 18 °C and an annual rainfall ranging from 650 to 1000 mm with peak rainfall in winter and deficits occurring typically during summer (Debieche 2002). Variation in GST levels between sites may be due to increasing or decreasing proximity to pollution sources (ArcelorMittal, Fertial).

AChE activity has commonly been used as a biomarker of exposure to several chemicals such as neurotoxic pesticides (Oliveira et al. 2007) and heavy metals (Amiard-Triquet et al. 1998; Lam 2009). Our study revealed decreased levels of AChE activity in snails collected in summer compared to winter. 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 (ArcelorMittal, Fertial).

Flame atomic absorption spectrometry is one of the most reliable techniques for the evaluation of metal ions content but has some limitations such as low concentrations of metal ions in environmental samples and complicated matrix interferences (Ghaedi et al. 2013c). Solid-phase extraction has currently become the most common technique for environmental sample pre-treatment of trace metals from matrices because of advantages such as high recovery, short extraction time, high enrichment factor, lower cost and consumption of organic solvents over liquid–liquid extraction. Thus, the efficiency and utility of novel sorbents for removal and recoveries of heavy metal ions by solid-phase extraction were recently investigated (Ghaedi et al. 2013a; 2013b, 2013d).

In the present study, soil samples from six sites were collected in 2010 and analyzed by a method commonly used for several years and applied for determination of heavy metal concentrations in various media (Leghouchi et al. 2009; Laib and Leghouchi 2011). The lowest heavy metal concentrations were registered in El Kala, a protected area used as a control site. In contrast, the soil collected from the other five sample sites showed higher concentrations of Fe, Cd, Pb, and Mn, indicating the presence of metallic pollution. The highest levels of Fe, Mn, and Cd were found in soil samples adjacent to the waste dumpsite of El-Hadjar, indicating that the waste produced by this industrial area is a potential major source of soil contamination. Pb levels were significantly higher at Sidi Kaci, Bouteldja, and El-Tarf. This may be linked to the increased traffic congestion in these sites in accordance with previous studies (Ho and Tai 1988, Garcia and Millan 1998). Average metal concentrations across the six sites varied in a similar manner with Fe being the most significant pollutant, followed by Mn, Pb, and Cd in decreasing order. Metal concentration in soil samples seems to decrease with increasing distance from highways and other pollution sources such as the industrial area of El Hadjar and the ArcelorMittal steel complex. The dispersion of contaminants may also be influenced by meteorological conditions such as wind (Piron-Frenet et al 1994), rainfall, or motorized traffic intensity (Garcia and Millan 1998). According to Debieche (2002), the dominant wind in the studied area comes from the North and Northeast and, to a lesser extent, from the North and the West. Recently, the concentrations of some heavy metals were determined in soils from Annaba and its surroundings (Maas et al. 2010). The average concentrations reported of Cd, Cr, Cu, Pb, Zn, Fe and Mn were 0.30, 28.3, 23.8, 42.3, 64.7, 25240 and 405.9 mg/Kg, respectively. Moreover, the spatial distribution of heavy metals observed in the Annaba area was explained by various anthropogenic activities including urban traffic (Pb and Zn), local industrial contamination (Cd and Cr), and agriculture (Cu). In the present study, the concentration of heavy metals determined in soils from six sites located in Northeast Algeria were below the standard limits (Table 4) (AFNOR 1996) and are in agreement with natural background values (Möller at al. 2005). Moreover, Alloway (1995) indicated that Cd concentrations do not exceed 1.0 mg/ kg for most of surface soils worldwide.

Conclusion

In conclusion, the results show a decreasing gradient of pollution from west to east correlating to the proximity of the pollution sources. The highest levels of contamination were observed at El Hadjar near the ArcelorMittal steel complex. *H. aspersa*, a common species of land snail present in the area, was tested as a bioindicator of metal contamination. GST and AChE activities measured from each animal served as sensitive parameters measuring exposure to pollution and the induction of an environmentally mediated biochemical stress response. Overall results show that *H. aspersa* is a useful bioindicator for assessing heavy metals from atmospheric pollution, several industrial factories, and vehicle traffic. Moreover, AChE and GST are reliable biomarkers of environmental stress.

.

Acknowledgements

The authors wish to thank Pr. E. Leghouchi (Laboratory of Pharmacology and Phytochemistry, Faculty of Sciences, Jijel University, Algeria) and Pr. M. Lahouel (Laboratory of Molecular Toxicology, Faculty of Sciences, Jijel University, Algeria) for heavy metal analysis. This study was financed by the Algerian Fund for Scientific Research and by the Ministry of High Education and Scientific Research of Algeria (CNEPRU and PNR projects, Pr. N. Soltani).

References

Abdennour, C., Khelili, K., Boulakoud, M.S., & Rainbow, P.S. (2000). Trace metals in marine, brackish and freshwater prawns (Crustacea, Decapoda) from North-East Algeria. *Hydrobiologia*, *432*, 217-227.

Abdennour, C., Smith, B.D., Boulakoud, M.S., Samraoui, B., & Rainbow, P.S. (2004). Trace metals in marine, brackish and freshwater prawns (Crustacea, Decapoda) from North-East Algeria. *Hydrobiologia, 432*, 217-227.

Agence Française de NORmalisation (AFNOR), (1996). Qualité des sols. Recueil des normes françaises 1996. AFNOR, Paris.

Agarwal, S.K. (2009). Heavy metal pollution. New Delhi: APH Publishing Corporation, pp. 1-89.

Alloway, B.J. (1995). Cadmium. In: Alloway, B.J. (Ed.), Heavy Metals in Soils, second ed. Blackie Academic and Professional, Glasgow, pp. 122-151

Amiard-Triquet, C., Altmann, S., Amiard, J.C., Ballan-Dufrançais, C., Baumard, P., Budzinski, H., Crouzet, C., Garrigues, P., His, E., Jeantet, A.-Y., Menasria, R., Mora, P., Mouneyrac, C., Narbonne, J.F., & Pavillon, J.F. (1998). Fate and effects of micropollutants in the Gironde estuary, France: a multidisplinary approach. *Hydrobiologia*, *373/374*, 259–279.

Amira, A., Sifi, K., & Soltani, N. (2011). Measure of environmental stress biomarkers in *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba (Algeria). *European Journal of Experimental Biology, 1* (2), 7-16.

Astani, M., Vosoughi, A.R., Salimi, L., & Ebrahimi, M. (2012). Comparative study of heavy metal (Cd, Fe, Mn, and Ni) concentrations in soft tissue of gastropod *Thais mutabilis* and sediments from intertidal zone of Bandar Abbas. *Advances in Environmental Biology, 6*, 319-326.

Ballesteros, M.L., Wunderlin, D.A., & Bistoni, M.A. (2009). Oxidative stress responses in different organs of *Jenynsia multidentata* exposed to endosulfan. *Ecotoxicology & Environmental Safety, 72,* 199-205.

Beldi, H., Gimbert, F., Maas, S., Scheifler, R., & Soltani, N. (2006). Seasonal variations of Cd, Cu, Pb and Zn in the edible mollusc *Donax trunculus* (Mollusca, Bivalvia) from the gulf of Annaba, Algeria. *African Journal of Agricultural Research*, *1*(4), 85-90.

Broutin, N. (2009). L'économie du nord-est algérien. Missions Economiques. Fiches de Synthèse. Ministère de l'Economie, des Finances et de l'Emploi. Etat Français.

Boyd, R.S. (2010). Heavy metal pollutants and chemical ecology: exploring new frontiers. *Journal of Chemical Ecology*, *36*, 46-58.

Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgramquantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, *72*, 248 – 254.

Calvet, R., & Barriuso, E. (1994). Retention and bioavailability of pesticides on soil. In: Environmental Behaviour of Pesticides and Regulatory Aspects, A. Copin, G. Houins, L. Pussemier, J.F. Salembier, Eds. Cost. European Study Service, Rixensart, Belgique, 63-71.

Coeurdassier, M., Saint-Denis, M., Gomot-de Vaufleury, A., Ribera, D., & Badot, P.M. (2001). The garden snail (*Helix aspersa*.) as bioindicator of organophosphorous exposure: effects of dimethoate on survival, growth and acetylcholinesterases activity. *Environmental Toxicology and Chemistry, 20*, 1951-1957.

Debieche, T.H. (2002). Evolution de la qualité des eaux (salinité, azote et métaux lourds) sous l'effet de la pollution saline, agricole et industrielle - Application à la basse plaine de la Seybouse (Nord-Est Algérien). Thèse d'Etat, Faculté des Sciences et Techniques, Université de Franche-Comté.

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.

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.

Fernandez, M.D., Vega, M.M., & Tarazona, J.V. (2006). Risk-based ecological soil quality criteria for the characterization of contaminated soils. Combination of chemical and biological tools. *Science of The Total Environment*, *366* (3), 466-484.

Garcia, R., & Millan, E. (1998). Assessment of Cd, Pb and Zn contamination in roadside soils and grasses from Gipuzkoa (Spain). *Chemosphere*, *37*(8), 1615-1625.

Ghaedi, M., Montazerozohori, M., Nazari, E., & Nejabat, R. (2013a). Functionalization of multiwalled carbon nanotubes for the solid-phase extraction of silver, cadmium, palladium, zinc, manganese and copper by flame atomic absorption spectrometry. *Human and Experimental Toxicology, 32* (7), 687-697.

Ghaedi, M., Montazerozohori, M., Hekmati, A., & Roosta, M. (2013b). Solid phase extraction of heavy metals on chemically modified silica-gel with 2-(3-silylpropylimino) methyl)-5-bromophenol in food samples. *International Journal of Environmental Analytical Chemistry*, 93 (8), 843-857.

Ghaedi, M., Montazerozohori, M., Sajedi, M., Roosta, M., Nickoosiar Jahromi, M., & Asghari, A. (2013c). Comparison of novel sorbents for preconcentration of metal ions prior to their flame atomic absorption spectrometry determination. *Journal of Industrial and Engineering Chemistry, 19* (6), 1781-1787.

Ghaedi, M., Niknam, K., Zamani, S., Abasi Larki, H., Roosta, M., & Soylak, M. (2013d). Silica chemically bonded N-propyl kriptofix 21 and 22 with immobilized palladium nanoparticles for solid phase extraction and preconcentration of some metal ions. *Materials Science and Engineering, 33* (6), 3180-3189.

Gimbert, F., de Vaufleury, A., Douay, F., Coeurdassier, M., Scheifler, R., & Badot, P.M. (2006). Modelling chronicexposure to contaminated soil: a toxicokinetic approach with the terrestrial snail *Helix aspersa*. *Environment International, 32*, 866-875.

Gomot, A., de Vaufleury, A., & Pihan, F. (2000). Growing snails used as sentinels to evaluate terrestrial environment contaminaton by trace elements. *Chemosphere, 40*, 275-284.

Guilhermino, L., Barros, L., Silva, M.C., & Soares, A.M. (1998**)**. Should the use of inhibition of cholinesterases as a specific biomarker for organophosphate and carbamate pesticides. *Biomarkers, 3*, 157-164.

Habig, W.H., Jakobby, W.B., & Ketly, J.N. (1965). Glutathione S-transferase (rat and humain). *Methods in Enzymology*, *77*, 218-231.

Hamed, R.R., Farid, N.M., Elowa, S.E., & Asdalla, A.M. (2003). Glutathione related enzyme levels of freshwater fish as bioindicators of pollution. *Environmentalist, 23*, 313-322.

Hao, L., & Chen, L. (2012). Oxidative stress responses indifferent organs of carp (*Cyprinus carpio*) with exposure to ZnO nanoparticles. *Ecotoxicology and Environmental Safety, 80*, 103-110

Ho, Y.B., & Tai, K.M. (1988). Elevated levels of lead and other metals in roadside soil and grass and their use to monitor aerial metal depositions in Hong Kong. *Environmental Pollution, 49*, 37-51.

Jordaens, K., De Wolf, H., Vandecastelle, B., Blust, R., & Backeljau, T. (2006). Associations between shell strength, shell morphology and heavy metals in the land snail *Cepaea nemoralis*. *Science of the Total Environment*, *363*, 285–293.

Kaloyianni, M., Dailianis, S., Chrisikopoulou, E., Zannou, A., Koutsogiannaki, S., Koliakos, G., Dimitriadis, V.K., & Alamdari, D.H. (2009). Oxidative effects of inorganic and organic contaminants on haemolymph of mussels. *Comparative Biochemistry and Physiology, 149*, 631–639.

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* (3), 1711-1718.

Larbaa, R., & Soltani, N. (2013). Diversity of the terrestrial gastropods in the Northeast Algeria: Spatial and temporal distribution. *European Journal of Experimental Biology*, *3*(4), 209-215.

Leghouchi, E., 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.

Lam, PKS. (2009). Use of biomarkers in environmental monitoring. *Ocean and Coastal Management, 52*, 348-354.

Li, X.Y., Liu, Y.D., Song, L.R., & Liu, H.T. (2003). Responses of antioxidant systems in the hepatocytes of common carp (*Cyprinus carpio* L.) to the toxicity of microcystin-LR. *Toxicon*, *42*, 85-89.

Maas, S., Scheifler, R., Benslama, M., Crini, N., Lucot E., Brahmia, Z., Benyacoub, S., & Giraudoux, P. (2010). Spatial distribution of heavy metal concentrations in urban suburban and agricultural soils in a Mediterranean city of Algeria. *Environmental Pollution, 158*, 2294-2301.

Mates, J.M. (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, *153*, 83-104.

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.

Morsli, S.M., & Soltani, N. (2003). Effets d’un insecticide inhibiteur de la synthèse de la chitine, le diflubenzuron, sur la cuticule de la crevette *Penaeus kerathurus. Journal de Recherche Océanographique, 28*, 85-88.

Neuberger-Cywiak, L., Achituv, Y., & Garcia, E.M. (2007). Effects of sublethal Zn^{++} and Cd^{++} concentration of filtration rate, absorption efficiency and scope for growth in *Donax trinculus* (bivalvia, donacidae). *Bulletin of Environnemental Contamination and Toxicology, 79*, 622-627.

Notten, M.J.M., Oosthoek, A.J.P., Rozema, J., & Aerts, R. (2006). Heavy metal pollution affects consumption and reproduction of land snail *Capaea nemoralis* fed on naturally polluted *Urtica dioica* leaves. *Ecotoxicology, 15*, 295–304.

Nowakowska, A., Łaciak, T., & Caputa, M. (2012). Organ Profiles of the Antioxidant Defence System and Accumulation of Metals in Helix aspersa Snails. *Polish Journal of Environmental Studies, 21* (5), 1369-1375.

Oliveira, M.M., Silva Filho, M.V., Fernandes, F.C., & Cunha Bastos, J. (2007). Brain acetylcholinesterase as a marine pesticide biomarker using Brazilian fishes. *Marine Environmental Research, 63*, 303-309.

Pandey, S., Parvez, S., Sayeedl, I., Haque, R., Bin-Hafeez, B., & Raisuddin, S. (2003). Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu. Science of the Total Environment, 309*, 105-115.

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.

Radwan, M.A., El-Gendy, K.S., & Gad, A.F. (2010). Biomarkers of oxidative stress in the land snail, *Theba pisana* for assessing ecotoxicological effects of urban metal pollution. *Chemosphere, 79,* (1), 40-46.

Reichman, S. M. (2010). The Responses of Plants to Metal Toxicity: A Review Focusing on Copper, Manganese and Zinc. *Australian Minerals & Energy Environment Foundation, 14,* 612-621.

Regoli, F., Gorbi, S., Fattorini, D., Tedesco, S., Notti, A., Machella, N., Bocchetti, R., Benedetti, M., & Piva, F. (2006). Use of the land snail *Helix aspersa* as sentinel organism for monitoring ecotoxicologic effects of urban pollution: An integrated approach. *Environmental Health Perspectives. 114,* 63-69.

Ryu, H., Chung, J.S., Nam, T., Moon, H.S., & K. Nam. (2010). Incorporation of heavy metals bioavailability into risk characterization. *Clean, 38,* 812-815.

Scheifler, R., Gomot, A., de Vauflenry, A., & Badot, P.M. (2002). Transfer of cadmium from plant leaves and vegetable flour to the snail *Helix aspersa*: bioaccumulation and effects. *Ecotoxicology and Environmental Safety, 53,* 148–153.

Semadi, A., & Deruelle, S. (1978). Lead pollution monitoring by transplanted lichens in Annaba area (Algeria). *Review of Pollution Atmospheric,* (Oct-Dec), 86-102.

shrimps and sediments from Algerian water. *Journal Metal Catalysis and Environment, 3,* 9-12.

Sifi, K., Chouahda, S., & Soltani, N. (2007). Biosurveillance de l'environnement par la mesure de biomarqueurs chez *Donax trunculus* dans le golfe d'Annaba. *Mésogée, 63,* 11-18.

Soltani, N., & Morsli, S.M. (2003). Quantification du Dimilin^R par chromatographie liquide haute performance : étude de la dégradation dans l'eau de mer. *Journal de Recherche Océanographique, 28,* 118-120.

Soltani, N., Amira, A., Sifi, K., & Beldi, H. (2012). Environmental monitoring of the Annaba gulf (Algeria): measurement of biomarkers in *Donax trunculus* and metallic pollution. *Bulletin de la Société zoologique de France, 137*(1-4), 47-56.

Sussarellu, R., Dudognon, T., Fabioux, C., Soudant, P., Moraga, D., & Kraffe, E. (2013). Rapid mitochondrial adjustments in response to short-term hypoxia andreoxygenation in the Pacific oyster *Crassostrea gigas. Journal of experimental Biology, 216,* 1561-1569.

Tim-Tim, A.L.S., Morgado, F., Moreira, S., Rangel, R., Nogueira, A.J.A., Soares, A.M., & Guilhermino, L. (2009). Cholinesterase and glutathione S-transferase activities of three mollusc species from the NW Portuguese coast in relation to the Prestige oil spill. *Chemosphere, 77,* 1465-1472.

Tlili, N., Saadaoui, E., Sakouhi, F., Elfalleh, W., El Gazzah, M., Triki, S., & Khaldi, A. (2010). Morphology and chemical composition of Tunisian caper seeds: variability and population profiling. *African Journal of Biotechnology, 10* (10), 2112-2118.

Van der Oost, R., Beyer, J., & Vermeulen, N.P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology, 13,* 57-149.

Verlecar, X.N., Jena, K.B., & Chainy, G.B. (2006). Modulation of antioxidant defences in digestive gland of *Perna viridis* , on mercury exposures. *Chemosphere, 71,* 1977-1985.

Zaidi, N., & Soltani, N. (2010). Chronic toxicity of flucycloxuron in the mosquitofish, *Gambusia affinis*: acetylcholinesterase and catalase activities and pattern of recovery. *Annals of Biological Research, 1*(4), 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(2),* 106-113.

Zaidi, N., Farine, JP., & Soltani, N. (2013). Experimental Study on Diflubenzuron: Degradation in Freshwater and Bioconcentration in Mosquitofish Following Chronic Exposure. *Journal of Environmental Protection, 4* (2), 188-194.

Zhang, X., Yang, F., Zhang, X., Xu, Y., Liao, T., Song, S., & Wang, H., 2008. Induction of hepatic enzymes and oxidative stress in Chinese rare minnow (*Gobiocypris rarus*) exposed to waterborne hexabromocyclododecane (HBCDD). *Aquatic Toxicology, 86,* 4-11.

Zhou, J., Wang, W.N., Wang, A.L., He, W.Y., Zhou, Q.T., Liu, Y., & Xu, J. (2009). Glutathione S-transferase in the white shrimp Litopenaeus vannamei: characterization and regulation under pH stress. *Comparative Biochemistry and Physiology, 150* (2), 224-230.

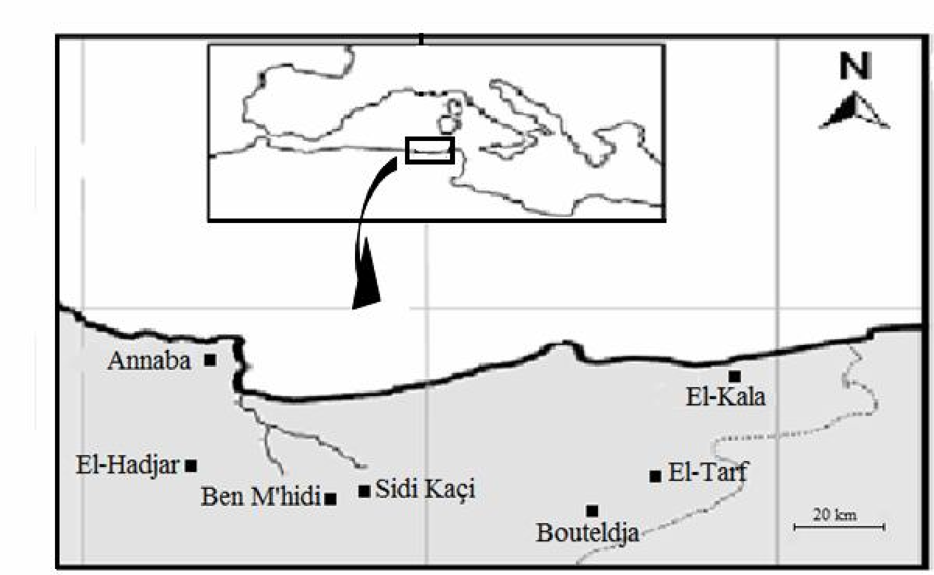

Fig. 1 Geographical location of sampling sites (El-Hadjar, Ben M'hidi, Sidi Kaci, Bouteldja, El Tarf, El Kala)

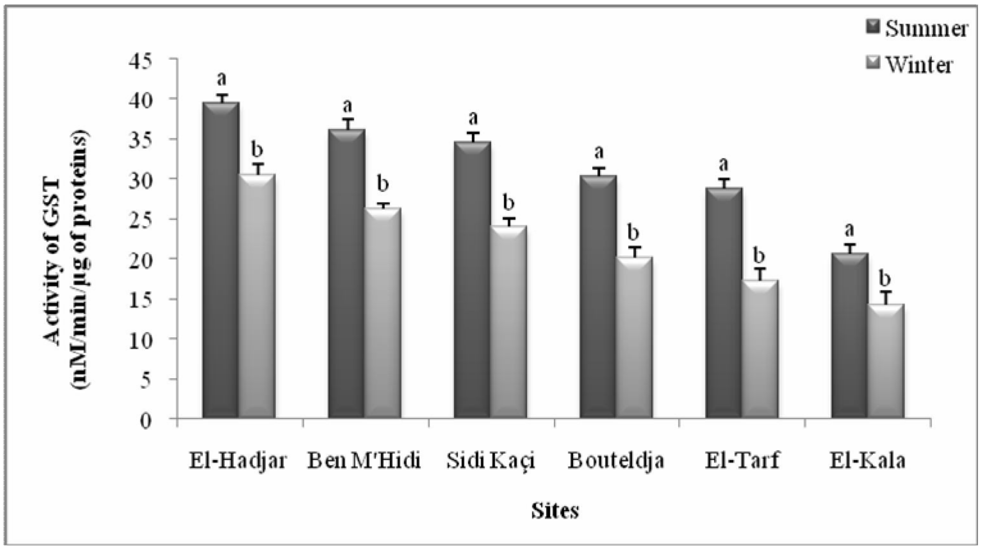

Fig. 2 Specific activity of GST (nM/mn/mg proteins) in hepatopancreas of *H. aspersa* collected from the different sites in summer and winter of 2010 (m ± SD; n = 5; for each site, mean values followed by the same letter are not significantly different at $p > 0.05$)

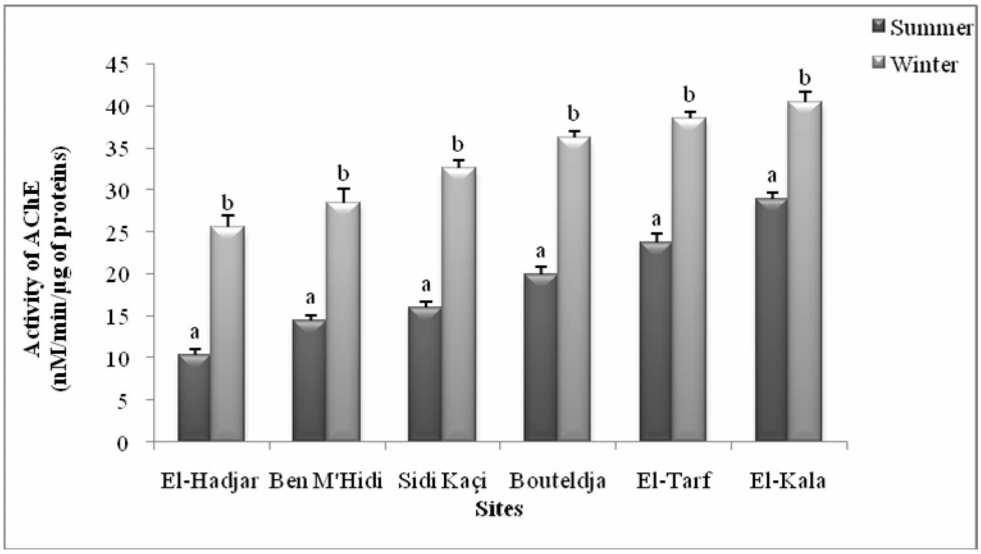

Fig. 3 Specific activity of AChE (nM/mn/mg proteins) in head of *H. aspersa* collected from from the different sites in summer and winter of 2010 (m ± SD; n = 5; for each site, mean values followed by the same letter are not significantly different at p> 0.05)

Table 1. Geographic position of sampling sites.

Sites	North	East
El Hadjar	36°48’00.36’’	7°44’00.00’
Ben M’hidi	36°46’02.06’’	7°54’11.64’
Sidi Kaçi	36°45’34.32’’	7°58’22.59’
Bouteldja	36°46’57.16’’	8°12’00.08’
El Tarf	36°46’01.58’’	8°19’01.84’’
El Kala	36°53’48.55’’	8°26’36.80’’

Table 2 Soil concentrations of heavy metals (at a depth of 10 cm in mg/Kg dry mass) in the different sites during the summer of 2010 (m ± SD; n= 3; for each metal mean values followed by different letters in minuscule are significantly different at p< 0.05)

Site	Fe	Mn	Pb	Cd
El Hadjar	886.99 ± 2.96 a	20.55 ± 0.38 a	1.63 ± 0.04 a	0.15 ± 0.00 a
Ben M’hidi	683.44 ± 5.89 b	15.76 ± 0.46 b	1.17 ± 0.08 b	0.12 ± 0.00 b
Sidi Kaci	620.42 ± 1.45 b	13.66 ± 0.08 c	1.26 ± 0.07 b	0.11 ± 0.00 b
Bouteldja	493.52 ± 1.46 c	14.08 ± 0.25 c	1.89 ± 0.05 c	0.08 ± 0.00 c
El Tarf	412.67 ±52.67c	13.98 ± 0.27 c	1.95 ± 0.03 d	0.07 ± 0.00 c
El Kala	330.70 ± 0.65 c	10.76 ± 0.41 d	1.06 ± 0.06 e	0.06 ± 0.00 c

Table 3 Soil concentrations of heavy metals (at a depth of 10 cm in mg/Kg dry mass) in the different sites during the winter of 2010 (m ± SD; n= 3; for each metal mean values followed by different letters in minuscule are significantly different at $p < 0.05$)

Site	Fe	Mn	Pb	Cd
El Hadjar	586.94 ± 3.92 a	11.39 ± 0.39 a	1.14 ± 0.05 a	0.09 ± 0.00 a
Ben M'hidi	490.29 ± 0.53 b	8.67 ± 0.21 b	0.85 ± 0.05 b	0.07 ± 0.00 b
Sidi Kaci	412.55 ± 0.52 b	8.23 ± 0.10 b	1.24 ± 0.04 c	0.06 ± 0.00 b
Bouteldja	332.46 ± 1.52 c	8.35 ± 0.05 b	1.24 ± 0.04 c	0.04 ± 0.00 c
El Tarf	295.87 ± 1.16 c	10.56 ± 0.26 c	1.39 ± 0.05 d	0.03 ± 0.00 c
El Kala	192.75 ± 0.78 d	3.20 ± 0.07 d	0.38 ± 0.00 e	0.01 ± 0.00 c

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

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