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Biomarkers response in the snail *Cornu aspersum* (Gastropoda, Helicidae) used as bioindicator of soil pollution in extreme Northeast of Algeria

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ABSTRACT

Our study aims to use the snail *Cornu aspersum* as bioindicator species to biomonitoring soil quality in northeast of Algeria through measurement of the seasonal activities of three biomarkers, glutathione S-transferase, acetylcholinesterase and metallothioneins. *C. aspersum* were collected seasonally during the year 2016, at four sites located in the extreme northeast of Algeria. El Hadjar, site exposed to industrial wastes; the sites of El Tarf and Bouteldja subjected to urban and agricultural pollution; and El Kala, located in a protected park, chosen as a witness site. An inhibition of acetylcholinesterase and an increase of both glutathione S-transferase and metallothioneins were observed in samples collected from El Hadjar, El Tarf and Bouteldja as compared to El Kala. In addition, a significant seasonal effect was observed in spring and summer as compared to autumn and winter. This work reveal that *C. aspersum* can be used as a suitable bioindicator species for biomonitoring soil quality in northeast of Algeria who was related to sources of exposition to pollution.

Key words : *Snail, Cornu aspersum, Biomonitoring, Biomarker, Soil, Pollution*

Introduction

Intensive use of pesticides in agriculture, urban wastes, rapid industrialization and increasing vehicular traffic have produced an increase of soil pollution (Mleiki *et al.,* 2017). This pollution pose risks and hazards to ecosystems and human health through the contamination of ground water, as well as reducing the use of agricultural land (Roma *et al.,* 2017). That's why these last years, scientific research and international agencies increasing interest for soil pollution monitoring and assessment (Mariet *et al.*, 2017; Sturba *et al.*, 2018).

The extreme Northeast of Algeria is one of the most important urban, industrial, agricultural and tourist areas including El Tarf city, a very important agricultural area and Annaba city, the fourth greatest city in Algeria, shelter several major industries including those production phosphoric fertilizers (Fertial), pesticides (Asmidal), metallic construction (Ferovial) and one of the greatest steel complex of Africa (ArcelorMittal). Theses agricultural and industrial activities have generated an important contamination of aquatic and terrestrial areas especially metal contamination previously highlighted by other studies (Larba and Soltani, 2014; Belabed *et al.*, 2017; Drif *et al.,* 2019; Sifi and Soltani, 2019).

For terrestrial ecosystem, pulmonate gastropod mollusks, have been considered efficient sentinels to evaluate soil contamination (Kowalczyk-Pecka *et al.*, 2018). Among them, snail *Cornu aspersum* (syn. *Helix aspersa*) present several characteristics provide

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information on soil functions and translate disturbances caused by its pollution (Druart *et al.*, 2017). Indeed, this snail live in interface with soil, plant and air, so in direct contact with different ecosystems and have the capacity to accumulate several classes of chemicals (Mariet *et al.*, 2017). Thus, *C. aspersum*, has been commonly used as bioindicator species for monitoring metallic (Larba and Soltani, 2014), atmospheric (Regoli *et al.*, 2006) and agrochemical pollutions (Radwan and Mohamed, 2013).

Biomonitoring studies are usually based on biomarkers analysis measured in bioindicator species to provide information on ecosystem health (Viarengo *et al.*, 1997). Indeed, the induction and inhibition of biomarkers is an important tools to understand the possible harmful effects of pollutants on organisms (Boshoff *et al.*, 2015). Acetylcholinesterase (AChE) is an essential neurotransmitter of nervous system (Lionetto *et al.*, 2013). The inhibition of AChE has been widely used as biomarker of exposure to a neurotoxic pesticides (Radwan and Mohamed, 2013) and also other chemical compounds such as heavy metals and hydrocarbons (Zawisza-Raszka *et al.*, 2010). The glutathione S-transferase (GST), a phase II detoxifying enzymes, represents one of the most basic detoxification mechanisms in all organisms against several xenobiotics (Jakoby and Habig, 1980). It is also considered as antioxidant enzyme, since it can be eliminate products of reactive oxygen species (ROS) generated during an oxidative stress (Trevisan *et al.*, 2014). The induction of Metallothioneins (MTs) is widely used as biomarker of metallic contamination by binding and removing toxic metals (Pedrini-Martha *et al.*, 2017).

Snail physiological respons-es strongly depend on seasonal variations especially on humidity and temperature. Indeed, terrestrial snails are usually live in humid and semi-arid areas undergo annual cycles of aestivation and hibernation. These two physiological processes allow snails to cope with climate change in order to survive to the extreme conditions of cold and heat (Nowakowska *et al.*, 2014; Staikou *et al.*, 2017). The current research aims to biomonitoring soil quality in extreme northeast of Algeria by determining seasonal variations of some biomarkers (AChE, GST, MTs) in a land snail *C. aspersum*, chosen as bioindicator species and collected from unpolluted and polluted areas.

Materials and Methods

Study area and sampling sites

The sampling sites were located in extreme northeast of Algeria, including Annaba and El Tarf cities, which are one of the largest cities in Algeria in terms of demography and famed for their very important industrial and agricultural areas. The site of El Kala (36°53′44′′ N; 8°26′36′′ E), chosen as a reference site is located in very extreme northeast of Algeria close to the Tunisian border, it is located in a national protected park, distant from any source of pollution. The sites of El Tarf (36°46′2′′ N; 8°18′50′′ E) and Bouteldja (36°30′10′′ N; 8°0617′′ E), are located around urban and agricultural activities. Finally, the site of El Hadjar (36°48′0′′ N; 7°43′60′′ E), is submitted to a metallic contamination because it is located near one of the greatest steel complex in Africa which generates an important industrial pollution (Fig. 1).

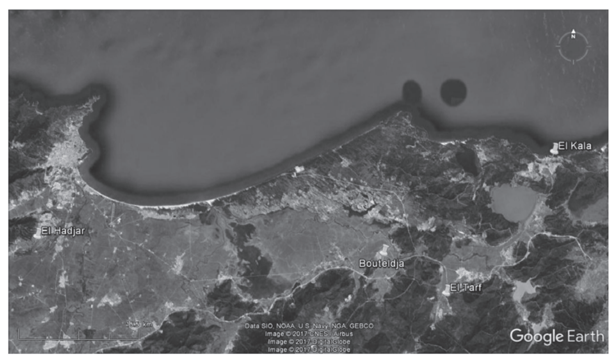

Fig. 1. Location of sampling sites of *Cornu aspersum* in the extreme Northeast of Algeria: El Kala, El Tarf, Bouteldja and El Hadjar (Google Earth Pro).

Species sampling

Adult of *C. aspersum* were collected seasonally (spring, summer, autumn, winter) during 2016 year from the selected sites. After sampling, *C. aspersum* were transported to the laboratory in plastic transparent boxes whose bottom is covered with a layer of moist soil and in above with tulle tissue to allow the aeration and avoid the escape of snails. Before the beginning of experimentation, snails were submitted to acclimation period of two (02) hours in laboratory and 15 specimens of standardized weight (10 ± 1 g) and shell diameter (30 ± 1 mm) were dissected and the head and hepatopancreas were removed for biomarkers analysis.

Acetylcholinesterase analysis

The specific activity of AChE was measured in head of *C. aspersum* using the method described by of Ellman *et al.* (1961). The AChE reacts with a specific substrate acetylthiocholine using the 5,52-dithio-bis-2-nitrobenzoic acid (DTNB) as reagent. The absorbances were read at a wavelength of 412 nm. Results were expressed as millimoles per minute per milligram of protein ($mmol.min^{-1}.mg^{-1}$ protein).

Glutathione S-transferase analysis

The GST activity was determined in hepatopancreas of *C. aspersum* according to the method of Habig *et al.* (1974). The GST catalyze the conjugation reaction of reduced glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) used as substrate. The absorbances were recorded at 340 nm and the enzyme activity was expressed in millimoles per minute per milligram of protein ($mmol.min^{-1}.mg^{-1}$ protein).

Metallothionein analysis

The quantification of metallothioneins was performed in hepatopancreas of *C. aspersum* as described by Viarengo *et al.* (1997). The protocol is based on a spectrophotometric Ellman's method by quantification of SH-residue contents using a glutathione as a standard. Absorbances were red at 412 nm wavelength and he results were expressed as micrograms per milligram of protein ($\mu g.\ mg^{-1}$ protein).

Protein quantification

The biomarkers analyzed in this study were expressed according to a quantities of proteins quantified in supernatant using Bradford (1976) method, with bovine serum albumin (Sigma) as standard and measured at 595 nm.

Statistical analysis

The results were represented by mean ± standard deviation (SD) and statically analyzed with Graph Pad. Prism.v6. The two-way ANOVA analysis and Tukey's post-hoc test were applied to compare each parameter among sites and between seasons at $p< 0.05$ as significant level.

Results

Acetylcholinesterase activity

The results of seasonal variations of AChE activity in *C. aspersum* were represented in Table 1 and Figure 2. A significant inhibition ($p< 0.001$) of AChE was recorded in all seasons at El Hadjar, Bouteldja and El Tarf as compared to the site of El Kala. This inhibition of AChE was significantly higher in summer with 0.09 ± 0.007 $mm.min^{''1}.mg^{''1}$ protein in El Hadjar, against 1.88 ± 0.10 $mm.min^{''1}.mg^{''1}$ protein at winter in El Kala (Fig. 2). Indeed, both effects of site and season were significantly showed ($p< 0.001$) by ANOVA test. The comparison between seasons by Tukey's post-hoc test showed a significant ($p< 0.05$) inhibition of AChE in summer and spring as compared to winter and autumn (Table 1).

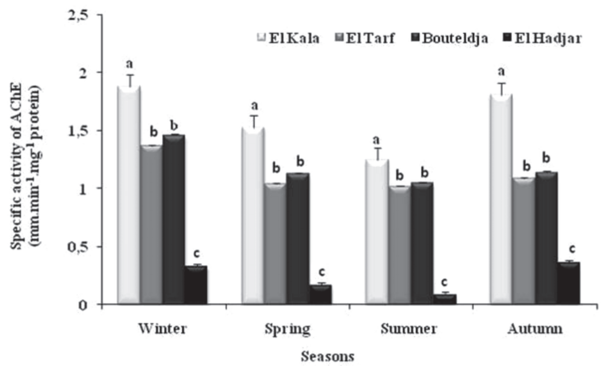

Fig. 2. Seasonal specific activity of Acetylcholinesterase ($mm.min^{-1}.mg^{-1}$ protein) measured in the head of *Cornu aspersum* sampled in four sites of extreme northeast of Algeria (El Kala, Bouteldja, El Tarf, El Hadjar), during 2016 (mean ± SD; n = 15). Means followed by the same small case letters are not significantly different at $p> 0.05$ between sites in each season (Tukey's post hoc test, $p< 0.05$).

Glutathione S-transferase activity

The seasonal activities of GST (Table 1; Fig. 3) recorded in hepatopancreas of *C. aspersum*, were increased significantly ($p< 0.001$) in polluted site of El Hadjar, Bouteldja and El Tarf as compared with reference site of El Kala. The activities of GST varied from minimum of 0.12 ± 0.01 $mm.min^{-1}.mg^{-1}$ protein in El Kala to maximum of 0.82 ± 0.03 $mm.min^{-1}.mg^{-1}$ protein in El Hadjar, both at autumn (Fig. 3). The comparison of seasonal effects revealed a significant increase ($p< 0.01$) of GST activities more marked in summer and autumn than other seasons (Table 1). Indeed, application of ANOVA tow-way test was reveal a significant effects ($p< 0.001$) of site and season.

Metallothioneins rates

The MTs rates determined in hepatopancreas of *C.*

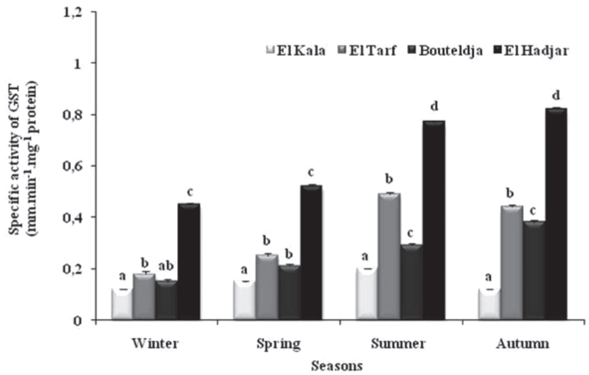

Fig. 3. Seasonal specific activity of Glutathione S-transferase (mm.min^{-1}.mg^{-1} protein) measured in the hepatopancreas of *Cornu aspersum* sampled in four sites of extreme northeast of Algeria (El Kala, Bouteldja, El Tarf, El Hadjar), during 2016 (mean ± SD; n = 15). Means followed by the same small case letters are not significantly different at $p > 0.05$ between sites in each season (Tukey's post hoc test, $p < 0.05$).

aspersum individuals were showed in Table 1 and Figure 4. The rates of MTs recorded in El Hadjar, Bouteldja and El Tarf were significantly increased ($p < 0.001$) as compared of those of El Kala. This increased of rate was more observed in summer with 1.73 ± 0.57 µg.mg^{-1} protein against 0.16 ± 0.02 µg.mg^{-1} protein recorded in winter at El Kala (Fig. 4). A significant effects of site and season ($p < 0.001$) were performed after a two-way ANOVA analysis. The effect of season was more pronounced in sum-

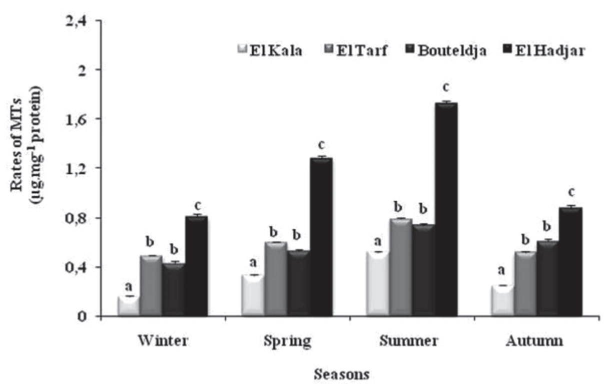

Fig. 4. Seasonal Metallothioneins rates (µg. mg^{-1} protein) measured in the hepatopancreas of *Cornu aspersum* sampled in four sites of extreme northeast of Algeria (El Kala, Bouteldja, El Tarf, El Hadjar), during 2016 (mean ± SD; n = 15). Means followed by the same small case letters are not significantly different at $p > 0.05$ between sites in each season (Tukey's post hoc test, $p < 0.05$).

Table 1. Seasonal specific activity of Acetylcholinesterase and Glutathione S-transferase (mm.min^{-1}.mg^{-1} protein), and rates of Metallothioneins (µg. mg^{-1} protein) measured in *Cornu aspersum* sampled in four sites of extreme northeast of Algeria (El Kala, Bouteldja, El Tarf, El Hadjar), during 2016 (mean ± SD; n = 15).

Sites/Seasons	AChE				GST				MTs			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
El Kala	1.88 ± 0.10^{a}	1.53 ± 0.10^{b}	1.25 ± 0.05^{c}	1.81 ± 0.27^{a}	0.12 ± 0.02^{a}	0.15 ± 0.003^{ab}	0.20 ± 0.01^{b}	0.12 ± 0.01^{a}	0.16 ± 0.02^{a}	0.33 ± 0.06 b	0.52 ± 0.07^{c}	0.25 ± 0.04^{ab}
El Tarf	1.37 ± 0.08^{a}	1.04 ± 0.02^{b}	1.02 ± 0.06^{b}	1.09 ± 0.08^{b}	0.18 ± 0.07^{a}	0.25 ± 0.03^{b}	0.49 ± 0.05^{c}	0.44 ± 0.04 c	0.49 ± 0.01^{a}	0.60 ± 0.08^{a}	0.79 ± 0.11^{b}	0.52 ± 0.01^{a}
Bouteldja	1.46 ± 0.05^{a}	1.13 ± 0.05^{b}	1.05 ± 0.04^{b}	1.14 ± 0.09^{b}	0.15 ± 0.04^{a}	0.21 ± 0.008^{b}	0.29 ± 0.03^{c}	0.38 ± 0.04^{d}	0.43 ± 0.02^{a}	0.53 ± 0.05^{ac}	0.74 ± 0.02^{bc}	0.61 ± 0.06^{c}
El Hadjar	0.33 ± 0.08^{a}	0.17 ± 0.08^{b}	0.09 ± 0.007^{b}	0.36 ± 0.18^{a}	0.45 ± 0.07^{a}	0.52 ± 0.05^{b}	0.77 ± 0.12^{c}	0.82 ± 0.03^{d}	0.81 ± 0.08^{a}	1.28 ± 0.15^{b}	1.73 ± 0.57^{c}	0.88 ± 0.10^{a}

Means followed by the same small case letters are not significantly different at $p > 0.05$ between seasons in each site (Tukey's post hoc test, $p < 0.05$).

mer and spring than winter and autumn (Table 1).

Discussion

Our research showed an inhibition of AChE activity in *C. aspersum* from polluted sites El Hadjar, Bouteldja and El Tarf as compared to reference site El Kala. These results were agree with study of Larba and Soltani (2014) in the same areas which highlighted a decrease in AChE activities in *C. aspersum* due to the metal pollution with high values of Fe, Mn, Pb and Cd. Indeed, the site of El Hadjar is submitted to metallic pollution derived from steelworks factory and also from a complex that produces pesticides and phosphoric fertilizers. Furthermore, the sites of Bouteldja and El Tarf are located near farmlands who generate pesticides contamination and also near highways which brings deposited atmospheric contamination. Grara *et al.* (2012) have reported an inhibition of AChE in *C. aspersum* after treatment with metal dust (Cu, Zn, Pb, Cr, Ni, Mn, Fe) collected from the site of El Hadjar. An inhibition of AChE as biomarker of metallic neurotoxicity was reported in *Cantareus apertus* exposed to several metals (Mleiki *et al.*, 2015, 2017). Leomanni *et al.* (2016) have been also demonstrated in their studies on *C. apertus* exposed to mercury a decrease of AChE. The neurotoxic action of pesticides through inhibition of AChE was reported in *C. aspersum* after treatment with imidacloprid (Radwan and Mohamed, 2013), dimethoate (Coeurdassier *et al.*, 2002) and carbamat molluscicides methomyl and methiocarb (Essawy *et al.*, 2009); and also in *C. apertus* exposed to Carbaryl (Leomanni *et al.*, 2015).

In 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 of the GST is probably due to the activation of the natural detoxification process of xenobiotics and also a pro-oxidant defense system. This induction of GST was probably induced by presence of several chemical contaminants like heavy metals and pesticides results from industries and agriculture activities in the polluted sites. Larba and Soltani (2014) have reported higher level of GST in this species from same sites exposed to metallic pollution. An activation of GST have been also reported in *C. aspersum* exposed to imidacloprid (Radwan and Mohamed, 2013) and to nickel and diazinon pesticide (Zawisza-Raszka *et al.*, 2010). An induction of GST was also observed in the same species collected from sites contaminated with coal (de Souza *et al.*, 2015). Moreover, an induction of GST has been reported in other several snails species exposed to various pollutants like *Theba pisana* treated with sub lethal doses of some metals (Cu, Pb, Zn) (Radwan *et al.*, 2010) and copper-based pesticide (El-Gendy *et al.*, 2009); *Cepaea nemoralis* exposed to soil contaminated with trace metals (Boshoff *et al.*, 2015); *Eobania vermiculata* collected from metal and agricultural contaminated sites (El-Shenawy *et al.*, 2012); *Biomphalaria alexandrina* exposed to inorganic fertilizers (El-Deeb *et al.*, 2017) and *C. apertus* exposed to metal trace elements (Mleiki *et al.*, 2017).

Our study showed an increase of MTs rates in *C. aspersum* samples from polluted sites of El Hadjar, El Tarf and Bouteldja compared to clean site of El Kala, proving a metal contamination. Grara *et al.* (2012) and Larba and Soltani (2014) previously demonstrated a metal contamination in these areas due to industrial activities especially metallic production. An increase of MTs after metallic contamination have been demonstrated by Sturba *et al.* (2018) in *C. aspersum* exposed to chlorure of cadmium ($CdCl_2$), Hispard *et al.* (2008) after exposed to cadmium and by Abdel-Halim *et al.* (2013) in the same species exposed to urban pollution. An increase of MTs has been reported in terrestrial snails *C. nemoralis* and *E. vermiculata* from metallic polluted sites as compared to reference site (Boshoff *et al.*, 2015; Iziou and Dimitiadis, 2011; El-Shenawy *et al.*, 2012). Other studies have showed an increase of MTs rates in snails after exposure to metallic pollution like *C. apertus* exposed to cadmium (Dallinger *et al.*, 2004) and mercury (Leomanni *et al.*, 2016).

Studies have previously shown that snails can modulate their defense system seasonally in response to biotic and abiotic conditions, such as low and high temperature and humidity (Nowakowska *et al.*, 2010; Staikou *et al.*, 2016). Our results showed an inhibition of AChE and increase of GST and MTs higher in summer followed by spring and autumn than in winter, this may be explained by the meteorological conditions and the activity of the aestivation and hibernation of the snails. We know that elevated temperature actively participates in the concentration of pollutants in environment (Staiko *et al.*, 2017). Larba and Soltani (2014) demonstrated a lower AChE and higher GST activities in *C. aspersum* in summer than in winter. Furthermore, Nowakowska *et al.* (2014) have demonstrated that *C. aspersum* increased its GST activity in response to arousal aestivation. Also, the CdMT gene expression in *Helix pomatia* is influenced by biotic and abiotic conditions as oxygen depletion and starvation (Pedrini-Martha *et al.,* 2017). Moreover, some authors have reported an increased of antioxidant enzymes during aestivation in the land snail *C. aspersum*, and in freshwater snail *Biomphalaria tenagophila* (Ramos-Vasconcelos and Hermes-Lima, 2003; Ferreira *et al.,* 2003). Also, Staikou *et al.* (2016) reported that a decrease of metabolism in snail *Cattania trizona olympica* are more observed at low temperature in winter.

Conclusion

In conclusion the biomarkers responses recorded in *C. aspersum* collected from different sites located in the extreme northeast of Algeria, is related to the level and sources of exposition to pollution mainly industrial and agricultural. As well, our investigation suggest that terrestrial snail *C. aspersum* could be used in biomonitoring of soil pollution as a suitable bioindicator species.

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