Biodiversity Journal, 2023,14 (2): 373–383 https://doi.org/10.31396/Biodiv.Jour.2023.14.2.373.383

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.

Received 16.11.2022; accepted 01.06.2023; published online 26.06.2023

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 transport 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 organisms. 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 Vaufleury 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; Belhiouani 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 (Bourioug 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 Vaufleury et al., 2006; Scheifler et al., 2006; Gimbert et al., 2008a; de Vaufleury, 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 Besbes. 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 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 ± 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.

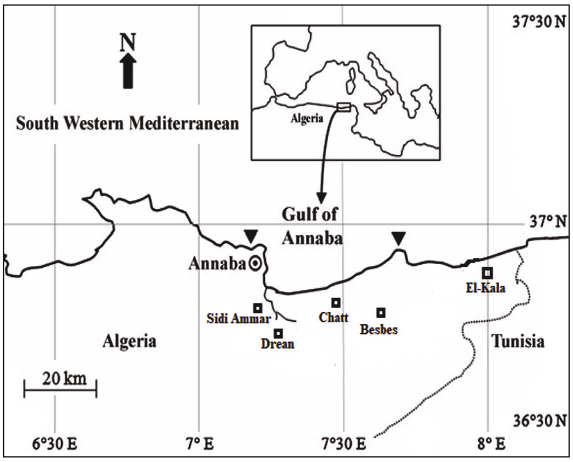


Figure 1. Geographical location of sampling sites (Sidi Ammar, Drean, Chatt, Besbes and El-Kala).

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.

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).

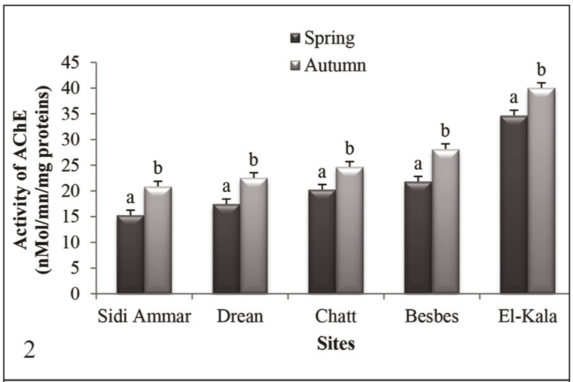
Statistical analysis

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 Glutation S-tranferase 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.



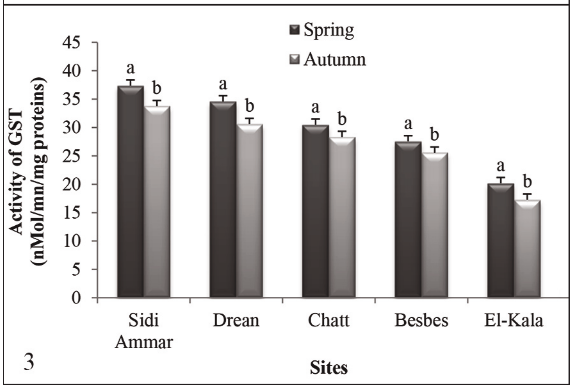


Figure 2. Results of seasonal variations of AChE activity in *C. aspersum.* Figure 3. Results of seasonal variations of GST activity in *C. aspersum.*

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) 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 & Lehikoinen, 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 Ferrovial (Kluge & Wessolek, 2012; Modrzewska & Wyszkowski, 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).

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

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 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.

ACKNOWLEDGEMENTS

Our thanks go to Pr. M. Lahouel (Laboratory of Molecular Toxicologie, Faculté of Sciences, Jijel University, Algeria) and Pr. E. Leghouchi (Laboratory of Pharmacology and Phytochemistry, Faculté of sciences, Jijel University, Algeria) for heavy metal analysis. This study was financed by the Algerian Fund for Scientific Research of Algeria (Laboratory Applied Animal Biology to Pr. N. Soltani) and by the Ministry of High Education and Scientific Research of Algeria.

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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 ± 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 ± 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 ± 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 sccording to AFNOR U44- 041 (na: not vailable).