Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 21 | No. 3 | pp. 1217-1225 | 2022

Original Research Paper

https://doi.org/10.46488/NEPT.2022.v21i03.027

Open Access Journal

Study on the Establishment of the Gastropod *Lymnaea stagnalis* (Linné, 1758) as a Bio-sentinel to Monitor the Water Quality of North Algerian Rivers: Case of the El-Malah River

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Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 06-08-2021
Revised: 27-09-2021
Accepted: 24-10-2021

Key Words:
Gastropods
Sentinel species
River El-Malah
Biomarkers
Condition indices
Biometric parameters

ABSTRACT

Biomonitoring is a key solution for assessing the effects and risks of pollutants to preserve the most vulnerable ecosystems, including aquatic ecosystems. This study aims in establishing the gastropod *Lymnaea stagnalis,* as a sentinel species to assess the water quality of the El-Malah river in the Algerian North-West. Three sites were chosen along the river: upstream (US), midstream (MS), and downstream (DS). The responsiveness of the aquatic snails has been compared using physiological and biological markers: condition index (CI), volumetric condition index (VCI), acetylcholinesterase (AChE), glutathione s-transferase (GST), and catalase (CAT). Additionally, the occurrence of changes in biometric parameters of the specimens has also been treated: shell height (SH), shell thickness (ST) total weight (TW), and the ratio ST/SH. Snails from the site MS reacted in front to the water deterioration with low biometric values (ST 1.28 ± 0.17 cm; SH 1.83 ± 0.20 cm; TW 2.95 ± 0.69 cm), and condition indices values (CI 31.19 ± 3.58; VCI 2.09 ± 0.53 $g.cm^{-3}$), thereby signaling smaller individuals compared to the population of site US. Whereas, no specimen was recorded in the site submitted to wastewaters discharge (DS). This indicates that the degradation of the water quality affected the growth and the viability of the snails. Furthermore, a significant induction in the GST activity (88.98 ± 10.72 nmol $min^{-1}mg^{-1}$), and a significant inhibition in the CAT activity (82.85 ± 9.49 µmol $min^{-1}mg^{-1}$) were recorded in the site MS, whereas no statistically significant variation was observed in AChE activity. *L. stagnalis* demonstrated biological and physiological variability between the studied contrasting sites of the El-Malah River. These results allow us to propose this species as a model in the ecotoxicology of western Algerian freshwaters.

INTRODUCTION

Earth's freshwater surface accounts for only 3% of the total volume of terrestrial water. This surface supports life and other services that are vital to human well-being (Reddy et al. 2018), and are therefore important to survey and protect. In the North-Algerian fringe, rivers are vulnerable ecosystems because of the increase of anthropic pressure, i.e., water uses, mostly for irrigation, and industrial, agricultural, and domestic discharges. Thus, we need robust monitoring tools to survey the quality of these aquatic systems and protect their biodiversity.

Sentinel organisms can allow determine hot spots of pollution in rivers (Goncharov et al. 2020). But these organisms should have a wide geographical distribution and should be sensitive to environmental constraints. Currently, no sentinel organisms are known for monitoring western Algeria freshwaters.

Worldwide, the most used sentinel species for aquatic systems are Mollusks. For salt and estuarine waters, some researchers used clams to evaluate the water quality in Spain (*Ruditapes philippinarum*) (Maranho et al. 2015), or India (*Meretrix casta*) (Avelyno et al. 2018). In recent studies, we successfully used the filtering mussel *Mytilus galloprovincialis*, as a bioindicator for evaluating the state of the Algerian coasts (Benali et al. 2017). Regarding freshwaters, zebra mussels *Dreissena polymorpha* have been proposed as possible reference bioindicator organisms (Châtel et al. 2015). However, bivalve-filtering organisms, which could be used as sentinel organisms, have not yet been identified in freshwater biotas from Northwestern Algeria. It would be therefore important to test and select an available sentinel species that is endowed with specific reactivity to environmental stress.

Besides filtering organisms, gastropods are of particular interest for monitoring the pollution of aquatic areas. They can be found in all types of freshwater ecosystems (stagnant and current), where they constitute up to 50% of the benthic invertebrate biomass (Cummings et al. 2016). Indeed, gastropods have well-suited characteristics for ecotoxicology surveys: 1) they have limited migration patterns allowing for assessing site-specific impacts (Smitha & Mustak 2017); 2) they play a significant role as links in the food chain, as primary consumers (grazers and detritus feeders) (Côte et al. 2015); 3) as potential bio accumulators, they can give some indications on the behavior of persistent pollutants and their bioaccumulation potential. For all these reasons, it is interesting to test and evaluate gastropods as early warning sentinels of habitat deterioration in local freshwater biotopes.

A good candidate would be the gastropod *Lymnaea stagnalis* which is a fairly common species in ponds, lakes, and rivers across the northern continents of the world (Kemenes & Benjamin 2009), and the countries of northern Africa, such as Algeria. This snail has already been used to monitor the effects of organic and non-organic chemicals, and it was described as a model of choice for the study of human-induced ecotoxicological and evolutionary processes (Côte et al. 2015). Furthermore, this species was studied in the laboratory through bioassays tests using biological markers as pollution early-warning systems (Bouétard et al. 2013, Atli & Grosell 2016, Lance et al. 2016), but has not yet been used in biomonitoring studies conducted in the field. Through this study, we would like to demonstrate our interest in this species and establish it as a model for monitoring freshwater ecosystems in Algeria. Moreover, it is relevant and important to improve the knowledge of the sensitivity of biological responses of this organism, using ecotoxicological tools such as biomarkers, and to provide the first data on the health status of Algerian rivers through biomonitoring surveys.

Among other parameters, biomarkers are reliable and valuable indicators of toxic effects on bio-sentinel organisms (Valavanidis et al. 2006). They are considered the most promising tool in environmental health surveys. The primary goal of using biomarkers is to allow the early detection of pressure due to pollution (Nickolson & Lam 2005) at the biochemical, physiological, and behavioral levels before a dysfunction is visible at the community and ecosystem levels (Amiard et al. 1998). Physiological indices have also shown to be suitable indicators of chemical stress (Guerlet 2007). They serve to provide information on the physiological state and growth of organisms (Andral et al. 2004) and indicate physiological disturbances between populations (Benali et al. 2017). By combining physiological and biochemical measurements (biomarkers), we can estimate the degree of chemical stress the organism was exposed to and therefore determine hot spots of pollution (Sharifinia et al. 2016). Furthermore, the quality of the habitat's water can be determined by the measurements of physico-chemical parameters. A relationship between the biotope's abiotic factors and the dynamics of gastropod populations (growth, survival) has already been demonstrated (Smitha & Mustak 2017).

Three biomarkers were chosen because of their biological involvement and their usual use in ecotoxicology surveys. 1) Catalase (CAT) is an intracellular antioxidant enzyme involved in defense systems against radicals: reactive oxygen species (ROS), produced by oxidizing ambient pollutants (Benali et al. 2015). CAT is essential for the degradation of hydrogen peroxide (H_2O_2). H_2O_2 is a precursor of the hydroxyl radical and is a species that is reactive with O_2 causing DNA damage (Halliwell & Gutteridge 1999). 2) GST is an enzyme family involved in the metabolism of glutathione which belongs to non-enzymatic antioxidant systems (Valavanidis et al. 2006). GST is also known to be involved in the biotransformation mechanism of organic compounds in freshwater *Mollusks* (Lance et al. 2016). 3) Acetylcholinesterase (AChE) is a neurotoxicity biomarker. They participate in the functioning of the central and peripheral nervous systems. AChE is commonly used to detect the toxicity of organophosphate and carbamate pesticides (Tufi et al. 2016), but this enzyme is also sensitive to heavy metals (Mora et al. 1999) and hydrocarbons in *Mollusks* (Kopecka et al. 2006) and even plant oils (Lopez et al. 2015) and the temperature (Bocquené & Galgani 1998, Benali et al. 2015) can influence its activity.

The main goal of this study is to demonstrate the sensitivity and the usefulness of aquatic gastropod *Lymnaea stagnalis* as an ecological descriptor, of water quality in freshwater ecosystems from North-Western Algeria. This was done using morphometric parameters and measuring physiological and biochemical markers in individuals collected from different sites of the El-Malah river. We have also measured the water physico-chemical parameters of the study sites.

MATERIALS AND METHODS

Sampling Sites and Strategy

The El-Malah River has located 65 km from the city of Oran, which is the main city in western Algeria. This river is among the most important rivers in terms of flow and volume of water in western Algeria and is characterized by brackish water. Because of the urbanization, the river carries domestic wastewater from several municipalities and as well from an industrial detergents fabricant. Moreover, an important agricultural area borders the river.

Three contrasting locations in El-Malah River were considered: upstream (US), midstream (MS), and downstream (DS) (Fig. 1). Into the river, we sampled *L. stagnalis* in two locations: US and MS, during the winter season, 2017. At the DS site, no specimen was noticed.

- The upstream side (US) (35°20'47.34''N, 0°59' 06.62''W). This site seemed like a relatively preserved area regarding the clearness of the water and the biodiversity of species. Near this location, there is a freshwater source about 200m from the sampling point. In this study, the US is considered as a less impacted area in the river (reference site).
- The midstream side (DS) (35°21'03.83'' N, 0°59'42.53'' W). At this site, the water is muddy and the specimens are less developed morphologically than in the US. This place is characterized by a very important agricultural area with many cultures, and a part of the irrigation water is pumped directly from this site. We also noted the presence of metal and plastic wastes.
- The downstream side (DS) (35°24'57.76'' N, 1°4'50.86'' W). This side of the river is subject to a flow of wastewaters from two neighboring localities (3830 $m^3.d^{-1}$), and an industry of detergent. As specified before, no specimen of the species *L. stagnalis* was observed in the river after the discharge of the sewage.

Samples of water were collected on the surface using polyethylene bottles, previously washed with distilled water. They were transported and frozen at -20°C in plastic bottles until physico-chemical analyses.

Twenty specimens of pond snail *L. stagnalis* (1.6±0.2 cm), were collected from each of the sampling areas. The specimens were immediately transported to the laboratory, where the morphometric measurements were done for the calculation of condition indices and the ratio. After a stabling period of 48 H, the whole soft tissue of the rest of the organisms was dissected and then stored at –80 °C for the biochemical measurements.

Hydrological Parameters

Collected water samples in the three stations mentioned above were tested for different physico-chemical parameters. The water temperature was measured *in situ* and the other parameters: pH, electrical conductivity (EC), suspender matter, total phosphate, and orthophosphate were performed in the water company of Oran SEOR. The pH was measured with a

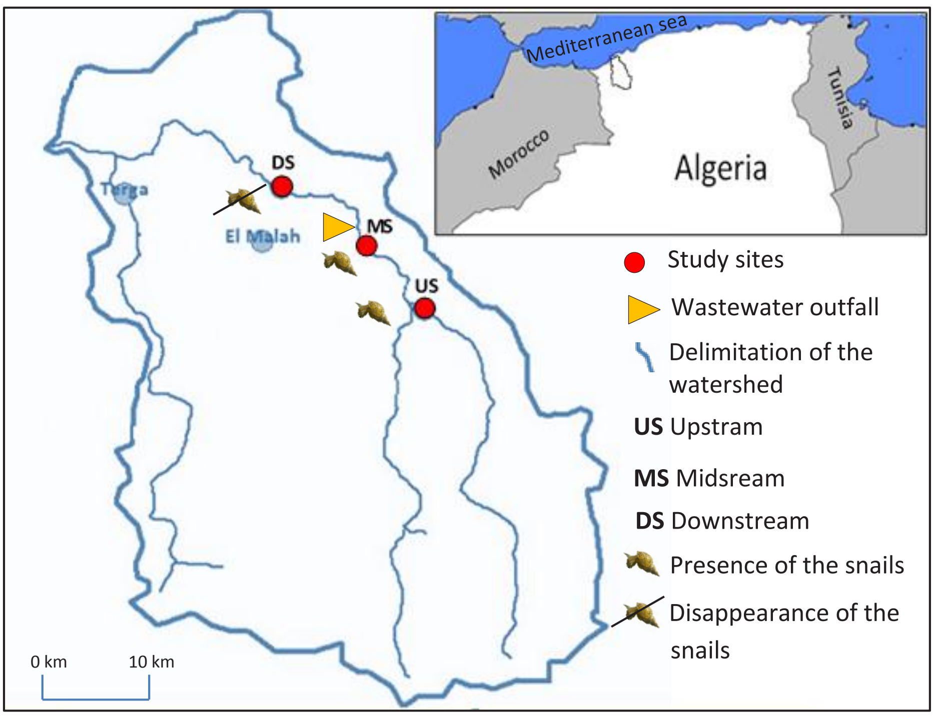

Fig. 1: Schematic locations of the study sites.

pH meter WTW *inoLab® Cond 7310* fitted with an electrode previously calibrated. The conductivity is measured with an electrical conductivity meter type WTW *inoLab® pH 7310* which directly measures the conductivity of the sample in ms/cm or µs/cm. Suspended matters were determined by filtering a volume of water on the cellulosic filter (0.45-micron meter). Total phosphate and orthophosphates were determined by colorimetric determination by a spectrophotometer (Rodier 2009). For the site DS, the physico-chemical data are those obtained in 2019 during the same season (winter).

Biological Analyses

Biometric Parameters and Physiological Condition Indices: Morphometric parameters of fifteen specimens were considered in this study, including the shell high (SH), the thickness of the shell (ST) the total weight of the specimen (TW) (Fig. 2), and the ST/SH ratio (Zbikowska & Zbikowski 2005). We also calculated two condition indices to determine the physiological condition of the specimens: 1) CI: condition index, based on the wet chair. It is calculated using the ratio of (weight of soft tissues/total weight of specimens) multiplied by 100 (AFNOR 1985). 2) VCI: Volumic condition index, based on the shell volume. It is calculated according to the equation: 3×weight of soft tissue/(π(ST), assimilated to the diameter of the cone basic disk)/2)^2× SH) (g.cm^{-3}) (Guerlet 2007).

Biochemical Markers

Three biomarkers were analyzed in the snails (AChE: acetylcholinesterase, CAT: catalase, and GST: glutathione S-transferase). Before the analyses, the whole soft tissues of five snails were dissected. For measuring AChE, the tissues were ground and homogenized in Tris–HCl buffer (0.1 M, pH 7.5) (1:4 w/v), while for the measures of CAT and GST, the tissues were ground in phosphate buffer (100 mM, pH 6.5) (1:5 w/v). The homogenates were centrifuged at 9000 g for 20 min at 4°C (Benali et al. 2017). Aliquots of the supernatant

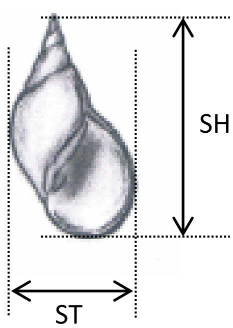

Fig. 2: Schematic representation of the shell height measurement (SH), and the thickness of the shell (ST).

(S9 fraction), containing the cytosol, endoplasmic reticulum, Golgi apparatus, and cytosolic proteins, were frozen at -80 °C until analysis.

The AChE activity was assayed by the method of Ellman et al. (1961). Acetylthiocholine is hydrolyzed by AChE to acetic acid and thiocholine. According to this method, the catalytic activity is measured by the increase of the yellow anion, 5-thio-2-nitrobenzoate (TNB), which absorbs at 412 nm; TNB is produced from thiocholine when it reacts with 5, 50-dithio-bis-2-nitro benzoic acid (DTNB). The enzyme kinetics reaction is measured for 20 min at 25°C using a spectrophotometer. For this method, 50 µL of S9 was added to a reaction mixture containing 850 µL Tris–HCl (0.1 M pH 7.5) and 50 µL (1875 mM) DTNB. After preincubation, the reaction begins with the addition of 50 µL of 8.25 mM acetylthiocholine. AChE activity was expressed in nmoles of thiocholine produced/min/mg protein.

The reaction mixture for the determination of GST activity consists of 20 mM of 1-chloro-2,4-dinitro-benzene (CDNB; Sigma) dissolved in 100 mM phosphate buffer at pH 6.5 with 100 mM of reduced glutathione and 10 µL of the supernatant fraction S9. GST activity is determined at 340 nm at 25 °C for the 30s according to Habig et al. (1974). The results are calculated as nmol of substrate hydrolyzed min^{-1} mg^{-1} relative to the protein in the sample.

CAT activity was determined following the method described by Claiborne (1985), i.e., through the measurement of the rate of enzymatic decomposition of H_2O_2 as a substrate at 240 nm for 30 s at 25 °C. In this application, 750 µL of phosphate buffer (100 mM, pH 7.4) was added to 200 mL of H_2O_2 (500 mM) in a spectrophotometer tank. The enzymatic reaction starts by adding fraction S9. Enzyme activity was expressed in µmole of H_2O_2 hydrolyzed/min/mg protein.

The quantity of proteins present in the S9 fraction was determined via the Coomassie blue method using bovine serum albumin (BSA) as the standard. The absorbance of samples was measured at 595 nm according to Bradford (1976).

Statistical Analyses

Twenty specimens of *L. stagnalis* were collected per station for the calculation of physiological indices and biochemical measurements, which represent a total of 40 gastropods. Data are expressed as the mean ± standard deviation. The data were tested for homogeneity of variance through a Levene test. Significant variations of biochemical markers and physiological indices recorded between the different study sites were tested with a one-way analysis of variance (ANOVA) at $p<0.05$. Post-hoc comparisons were made using the significant honest difference (HSD) Duncan test ($p<0.05$, $p<0.001$), to discriminate between sites. Statistical analyses were performed using the STATISTICA software program (v. 6.1.478.0 Statsoft).

RESULTS AND DISCUSSION

The general objective of the study was to evaluate if *L. stagnalis* could be sensitive to environmental disturbances and therefore used as sentinel species. This was done by studying their physiological and biochemical changes in different parts of the river, two sites presumed polluted (mid and downstream) in comparison with another site ostensibly less polluted (upstream). We first analyzed the physical appearance of the sampling sites.

Physico-Chemical Parameters

The physico-chemical water quality of the El-Malah River has been determined in the three study sites: US, MS, and DS. The recorded analysis results shown in Table 1 have been compared to international standards of the World Health Organization (WHO 2006).

The results indicate that the values of the different parameters vary generally between the sites. Temperatures of the river water are higher in the site US (14°C), in comparison with the site MS (12°C), and the site DM (12°C), because of the naturally warm groundwater source that joins the river not far from the site US and warms this place. The pH of the water is higher in the site submitted to the flow of wastewaters (DS) (8.2), compared to US (7.21), and MS (6.98). The suspended matters (SM) are higher in the site MS (90 $mg.L^{-1}$), and the site DS (30 $mg.L^{-1}$), compared to the reference site (US), exceeding the threshold tolerable by WHO (30 $mg.L^{-1}$). The elevation of suspended matters might be related to anthropic releases (wastewaters), and the clay nature of the floor. In the same line, we observe high concentrations of PO_4^{-3} and total phosphates in the impacted sites MS (0.071 $mg.L^{-1}$ and 0.227 $mg.L^{-1}$, respectively), and DS (1.73 mg/l and 2.95 mg/l, respectively), compared to the reference site the US, with an exceedance of WHO permissible values in the site DS. These results indicate a longitudinal degradation of the water from the site US to the site DS, particularly linked to agriculture runoff containing fertilizer and wastewater outlets. Indeed, various authors described that the increase in the values of orthophosphates is a sign of urban influence (Peric et al. 2018), such as agriculture and wastewaters (Sharma et al. 2016, Sunantha & Vasudevan 2016). The electrical conductivity values (EC) are higher in all the studied sites: (8400 $\mu s.cm^{-1}$); MS (8750 $\mu s.cm^{-1}$); DS (5750 $\mu s.cm^{-1}$). The high conductivity of El Malah river water is normal due to its brackish nature. However, the low value of the conductivity in the site DS could be linked to the significant inflows of wastewater which would dilute the water.

Growth and Physiological Condition

The mean values of morphometric parameters (shell height, shell thickness, and total weight), and biometric ratio (ST/SH) for *L. stagnalis* are presented in Table 2. The general observation of the results shows significantly lower values ($p<0.05$) of all morphometric parameters (ST, SH, and TW) in the population collected midstream the river (MS) compared to the reference population collected upstream (US). Whereas no specimens were recorded in the site DS, which is subjected to a flow of wastewaters. The obtained results in the site MS suggest that the specimens sampled at the site exposed to human activity are smaller in size and therefore less developed than those from the reference site. This could indicate that degradation of river water quality affects snail growth. The shell morphological variability of *Lymnaea* has already been described as the result of an environmental influence (Zbikowska & Zbikowski, 2005), and a relationship was established between abiotic factors and the dynamics of gastropods (growth and survival) (Smitha & Mustak 2017). Moreover, the reduction of shell growth could be also related to the presence of pollutants such as trace metals, in particular, Zinc. Indeed, Soto et al. (2000)

Table 1: Quality physico-chemical parameters of water in the different sampling stations and comparison of values with the WHO standards

Sites/season Parameters	**US site** Winter 2017	**MS site** Winter 2017	**DS site** Winter 2019	**WHO standards**
T [°C]	14	12	12	15-21
pH	7.21	6.98	8.2	6.5-8.5
Conductivity [$\mu s.cm^{-1}$]	8400*	8750*	5750*	1500
Suspender matter SM [$mg.L^{-1}$]	20	90*	32*	30
Total phosphate {$mg.L^{-1}$]	0.048	0.071	1.73	0.5
Orthophosphate PO^{3-}_4 [$mg.L^{-1}$]	0.150	0.227	2.95	1

* Exceeding the WHO standards; - No threshold values.

summarized in their work that the reduced growth of flesh and shell of *Mollusks* (in their study case, mussels), was related to the bioavailability of Zinc in the environment. As described above, the midstream side of the river could be exposed to pesticides, containing Zinc, from agricultural lands near the sampling point. Alternatively, reduced shell growth could be related to other natural factors such as seasonal variations and food availability. In view of our results, we can suggest the use of the shell size of specimens as an indicator of habitat deterioration. The ratio (ST/SH) gave similar results for both populations indicating a similar shape of the shells. According to Zbikowska & Zbikowski (2005), this index indicates a slender shell shape when values are low and a large shell shape when values are high. Our results demonstrated that the water degradation of the river did not affect the morphological shape of specimens. Other studies on the subject could indicate if it might be a difference between populations of snails from other rivers. The disappearance of snails in the part of the river subjected to wastewater (DS) could indicate that pollution by domestic and/or industrial effluents has a strong impact on our species of snails.

Fig. 3A and 3B present the comparison of the results of physiological indices (CI and VCI) of the populations from the sites US and MS. The digital data of analyses are given in the Supporting Information. Here too, we have recorded the same general trend. The lower values were found in the population MS compared to the reference population US, with significant differences for CI ($p<0.05$). Condition indices express the soft-tissue mass in relation to the total mass or measurements of the shell. Generally, we record high values of condition indices in the presence of organic matter of natural origin (algae, phytoplankton bloom) (Romeo et al. 2003) or anthropogenic origin (municipal waste) (Schiedek et al. 2006). In our case, the high values of CI and VCI at the site US can be related to the high biomass of algae *Cladophora sp.*, that adult snails are very fond of. Indeed, as it was mentioned above, the site US seemed relatively preserved and characterized by the biodiversity of species. However, the significant low values of the condition indices in the site MS might reflect the consequences of pollution, as this site is exposed to strong agricultural activity. Indeed, Guerlet (2007), described limitation of the growth has already been demonstrated, in the *L. stagnalis* exposed to high concentrations of herbicides (Diquat). In the same study, a negative correlation between ICV and the degree of contamination by heavy metals and HAPs in the Zebra mussels *Dreissena polymorpha* was demonstrated.

Biochemical Markers

Fig. 4 compares the measured biomarker variations (GST,

Table 2: Means ± standard deviation of biometric parameters, biometric ratios, and physiological indices of *L. stagnalis* from the upstream river site (US) and the downstream river site (DS).

	ST [cm] (Mean ± SD)	SH [cm] (Mean ± SD)	TW [g] (Mean ± SD)	ST/SH (Mean ± SD)
US site	0.62 ± 0.08	1.77 ± 0.22	1.07 ± 0.20	0.35 ± 0.05
MS site	0.54* ± 0.08	1.56* ± 0.14	0.78* ± 0.17	0.35 ± 0.04
DS site	-	-	-	-

SD: standard deviation; * Significance set (bold*) at $p< 0.05$; – No specimens

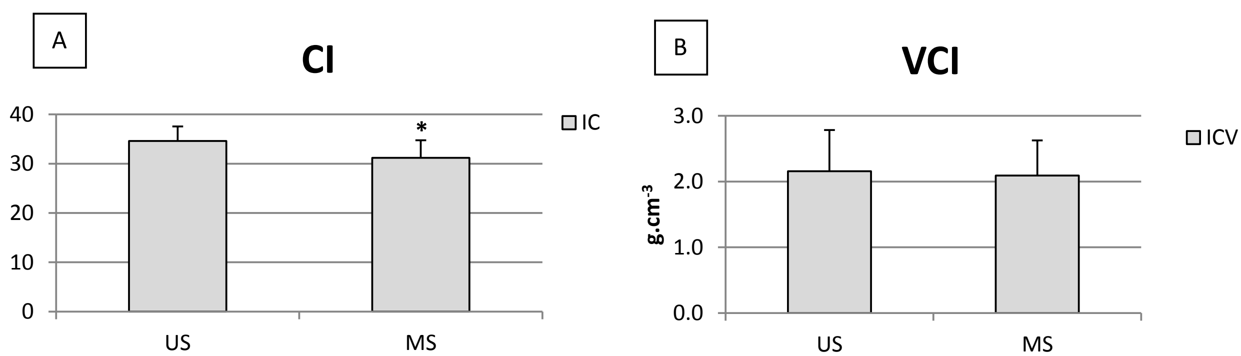

Fig. 3: Condition indices (n = 15, mean ± SD); A) condition index (CI), B) volumic condition index (VCI) ($g.cm^{-3}$) of *L. stagnalis* collected from Upstream (US) and Midstream (MS) of the River. * Indicates the difference between sites is significant at $p<0.05$.

CAT, and AChE) between the populations of snails collected from the site US and the site MS. Fig. 4A is a comparison of GST activity between the studied sites and shows a highly significant difference (*p<0.05*), between the two populations of snails, with high values in the population of site MS. This induction of this enzymatic activity could be due to the presence of stressors such as pollutants of organic, biological or chemical nature. For example, a strong induction of GST activity has been demonstrated by exposing *L. stagnalis* to herbicides: glyphosate (Lance et al. 2016), diquat and aural (Guerlet 2007), copper sulfate contained in fungicides and molluscicides (Côte et al. 2015), and even during exposure to secondary metabolites of cyanobacteria (Lance et al. 2016). It has also been shown the role of GSTs in the detoxification of organic compounds such as organochlorines, in other mollusks, such as bivalves (Damiens et al. 2007). Moreover, in the presence of metal excess, gastropods may respond through mechanism adjustments, such as the biotransformation system (detoxification) (Côte et al. 2015), to which GSTs enzymes belong.

Fig. 4B shows a significant variation (*p*<0.05) in CAT activity between the different sites, with the lowest value in the site MS, which is in contrast to the results of GST activity. As cited above, these enzymes participate in the anti-oxidant defense systems against radicals. The decrease in CAT activity in the site MS might suggest that the enzymes have been submerged by the production of ROS upon exposure to oxidative stressors, such as pesticides, heavy metals, complex effluents, etc. (Guerlet 2007). Indeed, a collapse of the antioxidant defense mechanism, via the CAT activity, has already been demonstrated in several studies. Several authors have reported that long periods of exposure to contaminants, such as metals (Stohs et al. 2000, Duarte et al. 2011) or cyanobacteria toxins (Lance et al. 2006), can invade the antioxidant defense in the Mollusks. For instance, in the case of mussels, authors refer to a ''biochemical adaptation'' in the CAT responses (Lionetto et al. 2003), and their induction/inhibition depending on pollutant concentrations (Atli & Grosell 2016), the exposure time (Duarte et al. 2011), tissue or species (Atli & Grosell 2016).

No variation was statistically highlighted for the AChE activity between the studied sites (Fig. 4C). The values are substantially similar, to the last significant digit. Several hypotheses can be suggested to interpret our results. This could indicate that 1) there is no neurotoxic compound that may affect AChE activity in the presumed affected site DS; 2) pollutants are not present at a toxic dose; 3) snails have not been exposed to pollutants long enough for the toxic

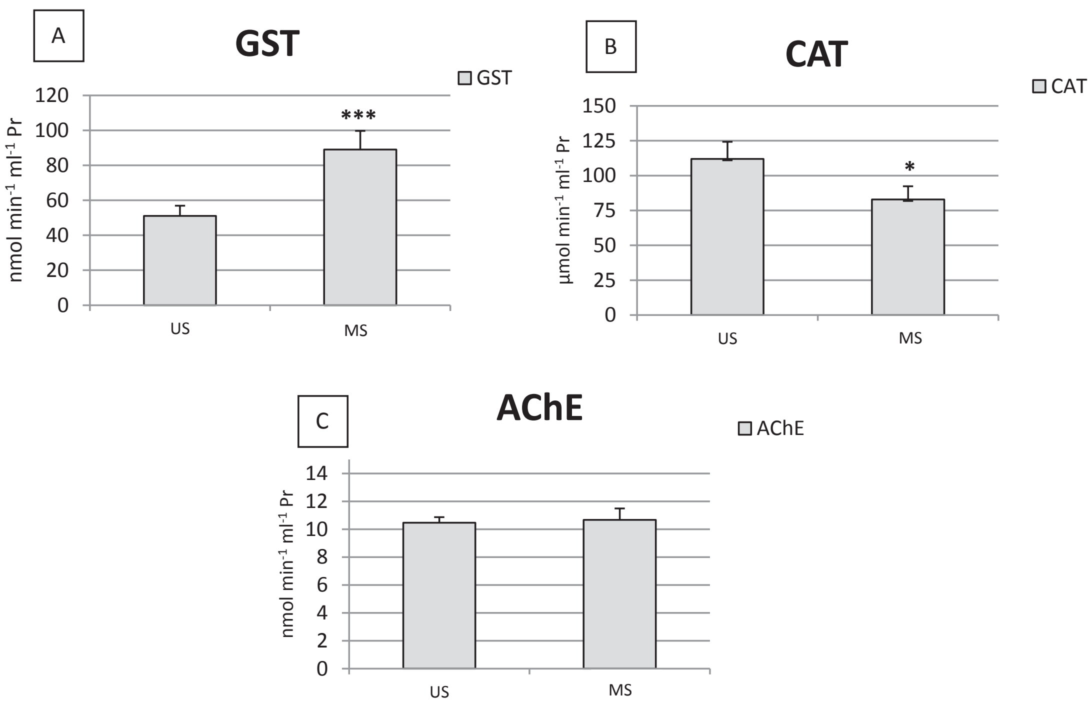

Fig. 4: Biomarker activity (n = 5, mean ± SD) ; A) gluthation-s-transferase (GST), B) catalase (CAT) and C) acetylcholinesterase (AChE) in *L. Stagnalis* collected from Upstream (US) and Downstream (DS) of El-Malah River. * Indicates the difference between sites is significant at p<0.05 and *** significant at *p*<0.001.

effect to be expressed on cholinesterase activity; 4) both populations are prone to neurotoxic damage because of the presence of neurotoxic compounds. Comparing the level of AChE activity obtained for our species (09.86-11.87 nmol. $min^{-1}mg^{-1}$) with the literature, we can suggest that our last hypothesis might be the most plausible. Indeed, Gagnaire et al. (2008) have recorder values of 11.4-4.9 nmolmin^{1}mg^{-1} in freshwater gastropods *Potamopyrgus antipodarum* exposed to an organophosphate and Schiedek et al. (2006) have reported low values of AChE activity, between 25-40 nmol min^{1} mg^{-1}, in mussels at a site characterized by agricultural dominance. A decrease in the cholinesterase activity was extensively described (Tufi et al. 2016) depending on the time and dose of pollutants, such as insecticides (Lionetto et al. 2003, Gagnaire et al. 2008). However, the low values could be also related to the presence of heavy metals (Mora et al. 1999), hydrocarbons (Kopecka et al. 2004), and even the temperature can influence the enzyme activity (Bocquené & Galgani 1998, Benali et al. 2015).

CONCLUSION

The presented pilot study aimed to propose the freshwater invertebrate *Lymnaea stagnalis*, as a model in ecotoxicology in west Algerian rivers. Our observations and results on the El Malah river revealed a longitudinal decrease in water quality, reflected by higher values of MS and nutrients (phosphates), and the presence of wastewater discharges. The physiological indicators measured on the *L. Stagnalis* individuals showed that the snail's growth was affected by river quality decrease and that a high level of pollution, led to the disappearance of the species. The antioxidant biomarkers showed significant variations in GST and CAT activities between populations, but no significant variability was observed for AChE activity.

This study allowed establishing a link between the absence of *Lymnaea stagnalis* and urban wastewater discharges and highlights the toxic effects of pollutants in the sites situated within the agricultural region. Considering the observed variability in the physiological and biochemical comportment of the snails *L. stagnalis* in the contrasted habitats, we suggest that freshwater species would be a suitable bioindicator of contaminant distribution within the river.

ACKNOWLEDGMENTS

The authors are grateful to the Institute of Earth Surface Dynamics (IDYST), the University of Lausanne for its welcome.

REFERENCES

AFNOR 1985. Cupped oysters. Names and classification, NF V 45-056. pp. 5.

Amiard, J.C. Gefard, A. and Amiard-Triquet, C. 1998. Metallothionein in the mussel Mytilus edulis as a biomarker of metal pollution: variability between sites, seasons, and organs. J. Rech. Oceanogr. 23: 25-30.

Andral, B., Stanisiere, J.Y., Sauzade, D., Damier, E., Thebault, H., Galgani, F. and Boissery, P. 2004. Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. Mar. Pollut. Bull., 49: 704-712.

Atli, G. and Grosell, M. 2016. Characterization and response of antioxidant systems in the tissues of the freshwater pond snail (*Lymnaeas stagnalis*) during acute copper exposure. Aqua. Toxicol., 176: 38-44.

Avelyno, H.D., Shyama, S.K. and Praveen Kumar, M.K. 2018. The Backwater Clam (*Meretrix casta*) is a bioindicator species for monitoring the pollution of an estuarine environment by genotoxic agents Swizzle Furtado. Mutat. Res. Gen. Tox. En., 825: 8-14.

Benali, I., Boutiba, Z., Grandjean, D., De Alencastro, L.F., Rouane-Hacène, O. and Chèvre, N. 2017. Spatial distribution and biological effects of trace metals (Cu, Zn, Pb, Cd) and organic micropollutants (PCBs, PAHs) in mussels *Mytilus galloprovincialis*along the Algerian west coast. Mar. Pollut. Bull., 115: 539-550.

Benali, I., Boutiba, Z., Merabet, A. and Chèvre, N. 2015. Integrated use of biomarkers and condition indices in mussels (*Mytilus galloprovincialis*) for monitoring pollution and development of biomarker index to assess the potential toxicity of coastal sites. Mar. Pollut. Bull., 95: 385-394.

Bocquené, G. and Galgani, F. 1998. Biological effects of contaminants: Cholinesterases inhibition by organophosphate and carbamate compounds. Proc. ICES Tech. Mar. Environ. Sci., 22: 1-13.

Bouétard, A., Besnard, A.L., Vassaux, D., Lagadic, L. and Coutellec, M.A. 2013. Impact of the redox-cycling herbicide diquat on transcript expression and antioxidant enzymatic activities of the freshwater snail *Lymnaea stagnalis*. Aqua. Toxicol., 126: 256-265.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 772: 248-264.

Châtel, A., Faucet-Marquis, V., Gourlay-Francé, C., Pfohl-Leszkowicz, A. and Vincent-Hubert, F. 2015. Genotoxicity and activation of cellular defenses in transplanted zebra mussels *Dreissenapoly morphs* along the Seine river. Ecotoxicol. Environ. Safety, 114: 241-249.

Claiborne, A. 1985. Catalase Activity. In: Greenwald, R.A. (Ed), Handbook of Methods for Oxygene Radical Research. CRC Press, Boca Raton, FL, pp. 283-284.

Côte, J., Bouétard, A., Pronost, Y., Besnard, A.L., Coke, M., Piquet, F., Caquet, T. and Coutellec, M.A. 2015. Genetic variation of Lymnaea stagnalis tolerance to copper: A test of selection hypotheses and its relevance for ecological risk assessment. Environ. Pollut., 205: 209-217.

Cummings, K.S., Jones, H.A. and Lopes-Lima, M. 2016. Rapid Bioassessment Methods for Freshwater Molluscs. 185-207. In T.H. Larsen (ed.), Core standardized methods for rapid biological field assessment. Conservation International, Arlington, VA, pp. 209.

Damiens, G., Gnassia-Barelli, M., Loquès, F., Roméo, M. and Salbert, V. 2007. Integrated biomarker response index as a useful tool for environmental assessment evaluated using transplanted mussels. Chemosphere, 66: 574-583.

Duarte, C.A., Giarratano, E., Amin, O.A. and Comoglio, L.I. 2011. Heavy metal concentrations and biomarkers of oxidative stress in native mussels (*Mytilus edulis chilensis*) from Beagle Channel Coast (Tierra del Fuego, Argentina). Mar. Pollut. Bull., 62: 1895-1904.

Ellman, G.L., Courtney, K.D., Andres, V. and Featherstone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol, 7: 88-95.

Gagnaire, B., Geffard, O., Xuereb, B., Margoum, C. and Garric, J. 2008. Cholinesterase activities as potential biomarkers: Characterization in two freshwater snails, *Potamopyrgus antipodarum* (*Mollusca*, Hydrobiidae, Smith 1889) and *Valvata piscinalis* (*Mollusca*, Valvatidae, Müller 1774). Chemosphere, 71(3): 553-560.

Goncharov, A.V., Baturina, N.S., Maryinsky, V.V., Kaus, A.K. and Chalov, S.R. 2020. Ecological assessment of the Selenga River basin, the main tributary of Lake Baikal, using aquatic macroinvertebrate communities as bioindicators. J. Great Lakes Res., 46: 53-61.

Guerlet, E. 2007. Use of cellular biomarkers in several species of invertebrates for the assessment of contamination in freshwater environments. Ecol. Environ. Univ. Metz. 13: 317.

Habig, W.H., Pabst, M.J. and Jakoby, W.B. 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

Halliwell, B. and Gutteridge, J.M.C. 1999. The Chemistry of Free Radicals and Related ''Reactive Species''. In Free Radicals in Biology and Medicine, Third ed. Oxford University Press, Oxford, pp. 36-104.

Kemenes, G. and Benjamin, P.R. 2009. Lymnaea. Curr. Biol., 19(1): 9-11.

Kopecka, J., Lehtonen, K.K., Barsiené, J., Broeg, K., Vuorinen, P.J., Gercken, J. Pempkowiak, J. 2006. Measurements of biomarker levels in flounder (*Platichthys flesus*) and blue mussel (*Mytilus Baltic*). Mar. Pollut. Bull., 53: 406-421.

Lance, E., Desprat, J., Holbech, B.F., Gérard, C., Bormans, M., Lawton, L.A., Edwards, C. and Wiegand, C. 2016. Accumulation and detoxication responses of the gastropod *Lymnaeas stagnalis* to single and combined exposures to natural (cyanobacteria) and anthropogenic (the herbicide RoundUp®Flash) stressors. Aquatic Toxicology 177: 116-124.

Lionetto, M.G., Caricato, R., Giordano, M.E., Pascariello, M.F., MarinoscI, L. and Schettino, T. 2003. Integrated use of biomarkers (acetylcholinesterase and antioxidant enzymes activities) in *Mytilus galloprovincialis* and *Mullus barbatus* in an Italian coastal marine area. Mar. Pollut. Bull., 46: 324-330.

Lopez, M.D., Campoy, F.J., Pascual-Villalobos, M.J., Muñoz-Delgado, E. and Vidal, C.J. 2015. Acetylcholinesterase activity of electric eel is increased or decreased by selected monoterpenoids and phenylpropanoids in a concentration-dependent manner. Chem. Biol. Interact., 229: 36-43.

Maranho, L.A., Andre, C., Del Valls, T.A., Gagné, F. and Martín-Díaz, M.L. 2015. Adverse effects of wastewater discharge in reproduction, energy budget, neuroendocrine, and inflammation processes observed in marine clams *Ruditapes philippinarum*. Estuar. Coast. Shelf Sci., 164: 324-334.

Mora, P., Fournier, D. and Narbonne, J.F. 1999. Cholinesterases from the marine mussels *Mytilus galloprovincialis* LmK. and *Mytillus edulis* from the freshwater bivalve *Corbicula fluminea* Muller. Comp. Biochem. Physiol. C, 122: 353-361.

Nickolson, S. and Lam, P.K.S. 2005. Pollution monitoring in Southeast Asia using biomarkers in the *Mytilidae* mussel *Perna viridis* (Mytilidae: Bivalvia). Environ. Int., 31: 121-132.

Peric, M.S., Kepčija, R.M., Miliša, M., Gottstein, S., Lajtner, J., Dragun, Z., Marijić, V.F., Krasnići, N. and Ivanković, D. Erk, M. 2018. Benthos-drift relationships as proxies for the detection of the most suitable bioindicator taxa in flowing waters - a pilot study within a Mediterranean karst river. Ecotoxicol. Environ. Safety 163: 125-135.

Reddy, M.T., Sivaraj, N., Kamala, V., Pandravada, S.R., Sunil, N. and Dikshit, N. 2018. Classification, characterization, and comparison of aquatic ecosystems in the landscape of Adilabad District, Telangana, Deccan Region, India. Open Access Lib. J., 5: 4459.

Rodier J. (ed) 2009. Water Analysis. Dunod, pp. 1600.

Romeo, M., Hoarau, P., Garello, G., Gnassia-Barelli, M. and Girard, J.P. 2003. Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. Environ. Pollut., 122: 369-378.

Schiedek, D., Broeg, K., Barsiene, J., Lehtonen, K.K., Gercken, J., Pfeifer, S., Vuontisjärvi, H., Vuorinen, P.J., De donyte, V., Koehler, A., Balk, L. and Schneider, R. 2006. Biomarker responses as indication of contaminant effects in blue mussel (*Mytilus edulis*) and female eelpout (*Zoarces viviparus*) from the Southwestern Baltic sea. Mar. Pollut. Bull., 53: 387-405.

Sharifinia, M., Mahmoudifard, A., Imanpour Namin, J., Ramezanpour, Z. and Yap, C.K. 2016. Pollution evaluation in the Shahrood River: Do physico-chemical and macroinvertebrate-based indices indicate the same responses to anthropogenic activities? Chemosphere, 159: 584-594.

Sharma, R.C., Singh, N. and Chauhan, A. 2016. The influence of physico-chemical parameters on phytoplankton distribution in a headwater stream of Garhwal Himalayas: A case study. Egypt. J. Aqua. Res., 42: 11-21.

Smitha, N. and Mustak, M.S., 2017. Gastropod diversity with physico-chemical characteristics of water and soil in selected areas of Dakshina Kannada district of Karnataka, India. Int. J. Fauna Biol. Stud., 4(1): 15-21.

Soto, M., Ireland, M.P. and Marigomez, I. 2000. Changes in mussel biometry on exposure to metals: implications in the estimation of metal bioavailability in 'Mussel-Watch' programs. The Science of the Total Environment 247: 175-187.

Stohs, S., Bagchi, D., Hassoun, E. and Bagchi, E. 2000. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol., 19: 201-213.

Sunantha, G. and Vasudevan, N. 2016. Assessment of bacterial indicators and physico-chemical parameters in Tiruppur, Erode, and Chennai, Tamil Nadu (India). Environ. Nanotechnology, Monitoring & Management, 6: 219-260.

Tufi, S., Wassenaar, P.N.H., Osorio, V., de Boer, J., Leonards, P.E.G. and Lamoree, M.H. 2016. Pesticide mixture toxicity in surface water extracts in snails (*Lymnaea stagnalis*) by an in vitro acetylcholinesterase inhibition assay and metabolomics. Environ. Sci. Technol., 50(7): 3937-3944.

Valavanidis, A., Vlachogianni, T., Dassenakis, M. and Scoullos, M. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Safe. 64: 178-189.

WHO, 2006. A proposal for Updating Lebanese Norm of Drinking Water (1999) based on WHO Guidelines. WHO, Washington DC.

Żbikowska, E. and Żbikowski, J. 2005. Differences in shell shape of naturally infected Lymnaea stagnalis (L.) individuals as the effect of the activity of digenetic trematode larvae. J. of Parasitology, 91(5): 1046-1051.