EFFECTS OF POLLUTION ON THE ACTIVITY OF ACETYLCHOLINESTERASE IN THE BRAIN AND HEART OF BARBUS SETIVIMENSIS (FISH, CYPRINIDAE) IN EL HARRACH STREAM (NORTH OF ALGERIA)

Hamida FERGANI* & Abdeslem ARAB†

1 USTHB. Laboratory of Biodiversity and Dynamics, Faculty of Biological Sciences. BP 32, El Alia, Algiers, Algeria.
* Author for correspondence. Phone: 07.72.13.69.01. E-mail: ferganhamida@yahoo.fr

RESUME.— Effets de la pollution sur l’activité cérébrale et cardiaque de l’acétylcholinestérase chez Barbus setivimensis (Cyprinidé) de l’oued El Harrach (Nord de l’Algérie). — L’étude présente le suivi des niveaux cérébraux et cardiaques de l’activité de l’acétylcholinestérase (AChE) chez une espèce de poisson, Barbus setivimensis. Ce travail entre dans le cadre d’une approche de bioévaluation passive du bassin versant de l’oued El Harrach (Algérie). Mené durant 4 mois successifs au printemps 2013, il repose sur la comparaison d’une population dite de référence avec une population davantage exposée à la pression anthropique et ceci au regard des mesures d’un panel de facteurs physico-chimiques. L’analyse des résultats obtenus montre des niveaux moyens d’activité enzymatique plus élevés sur le site de référence que dans le site aval. Cette activité plus faible dans le site aval coïncide avec des changements physico-chimiques notables et notamment une augmentation des teneurs en matières en suspension et des sels nutritifs. L’activité enzymatique varie aussi en fonction du sexe, des paramètres intrinsèques liés aux poissons ainsi que des tissus étudiés. Les résultats obtenus confirment que ces variations sont un premier signe d’impact de la pollution issue essentiellement de l’activité humaine et agricole.

SUMMARY.— This study presents the monitoring of cerebral and cardiac activity levels of acetylcholinesterase (AChE) in a species of fish, Barbus setivimensis. This work falls within the framework of a passive bio-assessment approach of the watershed of the El Harrach stream (Algeria). Conducted during four successive months in spring 2013, it is based on the comparison of a population called reference with a population more exposed to human pressure and this in terms of measures of a panel of physico-chemical factors. The analysis of the results shows higher average levels of enzyme activity in the reference site than in the downstream site. This lower activity in the downstream site coincides with significant physico-chemical changes, including an increase in suspended matter and nutrients. The enzymatic activity also varies depending on sex, intrinsic parameters related to fish as well as studied tissues. These results confirm that these changes are a first sign of the impact of pollution by micropollutants mainly from human and agricultural activity.

Our contribution focuses on fish in the El Harrach stream. This site is subject to strong anthropogenic pressures due to major urban and agricultural activities. The aim of this study is to highlight the relationships between micropollutants in the environment and changes in the biochemical mechanisms of a particular population of fish, Barbus setivimensis (Valenciennes, 1842), a species endemic to North Africa (Arrignon, 1976; Bacha & Amara, 2007). We measured the activity of acetylcholinesterase (AChE), a target enzyme of the most neurotoxic pollutants. This enzyme (a neurotransmitter) ensures the transmission of nerve impulses at the level of the synapses of neuromuscular and inter-neural junctions (Toutant & Massouli, 1988; Jebali et al., 2013). Previous studies have reported that inhibition of the AChE activity in fish can be dangerous as it affects possibilities of feeding, swimming, avoidance of predator and spatial orientation of the species (Balint et al., 1995; Pan & Dutta, 1998; Beauvais et al., 2000, 2001, 2002).

The inhibition of cholinesterases affects a currently widely used bio-indicator and constitutes an early bio-marker of exposure to organic substances (Coppage & Breidecht, 1976; Olson & Christensen, 1980; Zinckel et al., 1987, 1991; Pavlov et al., 1990; Galgani et al., 1991, 1992; Payne et al., 1994, 1996; Dutta et al., 1992; Dutta & Arends, 2003; Archana et al., 2011; Kamel et al., 2012; Jebali et al., 2013). The presence of pollutants such as heavy metals could enhance the inhibitory effects of organophosphates (OP) and carbamates (C) (Bocquené et al., 1990, 1995,
1997; Jehali et al., 2013). Inhibition of AChE in the brain of fish has been suggested as a variable that could indicate exposure to organophosphates (Sturm et al., 1999; Dembele et al., 2000; De La Torre et al., 2002).

In the present study, two sampling sites were selected, the first station considered as control, is located upstream of all discharge points and receives water from three mountain tributaries not exposed to any pollution. The second station is located downstream of two villages; it receives all the wastes from the agricultural and urban land. Monthly, during spring 2013, using gillnets, a total of 160 individuals were caught. An analysis of the physico-chemical parameters of the water and a comparison of the enzymatic activity measured in the brain and heart of fish have been made during the entire sampling period.

MATERIALS AND METHODS

El Harrach stream starts 60 km from Algiers, runs from north to south about 51 km and from east to west about 31 km. It reaches the Mediterranean Sea after a course of 37.5 km (A.N.R.H, 2014). Two sampling sites were selected, the first at an altitude of 273 m (36° 27’ 46’’ N, 03° 01’ 46’’ E), upstream of all release points of micropollutants; this station collects the waters from three water courses (the Lakhra stream, the Laach stream and the Boumaane stream). The second site is at an altitude of 136 m (36° 29’ 27’’ N, 03° 02’ 31’’ E), and is located downstream of two villages. This site receives the leachates of agricultural land as well as of domestic and urban wastes.

Regarding fish fauna, samples of barbel (B. setivimensis) were collected from March to August 2013, the period of reproduction of this species (Aberkane & Iguer-Ouada, 2011; Djoudad-Kadjji et al., 2012) using gillnets of different mesh gaps (20, 25 and 30 mm) according to the method of Bouhbouh (2002). A total of 160 fish were sampled with 81 individuals (44 males and 37 females) at the station I and 79 individuals (26 males and 53 females) at the station II. Many biometric variables were studied to characterize the barbel population of El Harrach stream. Fish lengths were measured using an ichtyometer graduated in mm. Weights (in grams) were measured with precision scales. The total weight of the fish, gutted weight and organ weights were obtained by the method described by Ameur et al. (2003). Organs (brain and heart) were collected immediately after fishing through a ventral incision using the technique of Ameur et al. (2003) and Marcano et al. (1996) and stored at -80°C until used for biochemical measurements. For enzymatic analyses, samples of the brain and heart were homogenized with a grinder and centrifuged. The AChE activity of the extracts was measured by the colorimetric method of Ellman (Ellman et al., 1961; Bocquené, 1996).

Monitoring of cardiac and cerebral levels of AChE activity was performed in males and females of B. setivimensis.

The analysis of water quality was conducted according to the methods described by Rodier (1984, 1996, 2005). Eleven physico-chemical parameters were measured, three in situ with a multiparameter as probe (water temperature, pH, dissolved oxygen (DO)), oxygen saturation, level of electricity conductivity) and eight in the laboratory: nitrates (NO3), nitrates (NO3), magnesium (Mg), phosphates (PO4), suspended matter, calcium (Ca), carbonates (CO3) and chlorides (Cl).

Statistics were carried out by using nonparametric tests (Mann-Whitney, Kruskal-Wallis, Spearman correlation). Principal component analysis (PCA) is a multivariate statistical method, essentially descriptive, transforming correlated variables into new uncorrelated ones named "main components", or main roads. In our case, PCA was applied to represent the biological parameters (size and body mass of fish), the biochemical parameters (AChE activity) and the physico-chemical parameters of water. All statistical analyses were performed using the R language /Cran R, 2016.

RESULTS

Results on physico-chemical and the biological parameters (average / standard deviation) and average comparison tests are summarized in table I and illustrated in figures 1-4.

* During the four months of sampling the net reduction of enzyme activity in the brain and the heart recorded at Station II (Fig. 1) coincides with the disruption of physico-chemical parameters of water such as:
  - raising the average temperature of the water ranges from 21.9°C in station I to 25.9°C in station II (Fig. 1a).
  - very strong mineralization due to the increase in electrical conductivity that increases during the months of March, April and May in the station II (Fig. 1c).
TABLE I
Results of the biological parameters (mean and standard deviation) and the average comparison tests
AChE.Br: acetylcholinesterase activity in the Brain; AChE.He: acetylcholinesterase activity in the Heart; Lt: total length; Pt: total weight of *B. setivimensis* (El Harrach stream, measured in 2013).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Station</th>
<th>Months</th>
<th>n</th>
<th>Parameters</th>
<th>Lt(cm)</th>
<th>Pt(g)</th>
<th>AChE Br(nmol/g/min)</th>
<th>AChE He(nmol/g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Station I</td>
<td>March</td>
<td>17</td>
<td>mean</td>
<td>11.429</td>
<td>19.803</td>
<td>37.31</td>
<td>19.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>3.08</td>
<td>7.378</td>
<td>24.00</td>
<td>10.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>April</td>
<td>9</td>
<td>mean</td>
<td>12</td>
<td>20.954</td>
<td>31.03</td>
<td>18.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>3.62</td>
<td>7.378</td>
<td>11.01</td>
<td>15.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May</td>
<td>7</td>
<td>mean</td>
<td>16.667</td>
<td>57.653</td>
<td>33.68</td>
<td>13.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>1.528</td>
<td>16.803</td>
<td>23.24</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>11</td>
<td>mean</td>
<td>14.618</td>
<td>39.586</td>
<td>21.82</td>
<td>10.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>1.57</td>
<td>14.322</td>
<td>11.02</td>
<td>4.28</td>
</tr>
<tr>
<td>Female</td>
<td>Station I</td>
<td>March</td>
<td>3</td>
<td>mean</td>
<td>12.664</td>
<td>25.532</td>
<td>10.12</td>
<td>5.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>1.922</td>
<td>10.809</td>
<td>2.16</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>April</td>
<td>11</td>
<td>mean</td>
<td>10.36</td>
<td>17.952</td>
<td>14.99</td>
<td>9.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>4.513</td>
<td>7.913</td>
<td>6.36</td>
<td>4.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May</td>
<td>13</td>
<td>mean</td>
<td>13.9</td>
<td>32.792</td>
<td>9.06</td>
<td>13.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>1.558</td>
<td>7.141</td>
<td>6.05</td>
<td>10.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>10</td>
<td>mean</td>
<td>13.413</td>
<td>23.23</td>
<td>9.92</td>
<td>2.70</td>
</tr>
<tr>
<td>Female</td>
<td>Station II</td>
<td>March</td>
<td>3</td>
<td>mean</td>
<td>12.664</td>
<td>25.532</td>
<td>10.12</td>
<td>5.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>1.922</td>
<td>10.809</td>
<td>2.16</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>April</td>
<td>11</td>
<td>mean</td>
<td>10.36</td>
<td>17.952</td>
<td>14.99</td>
<td>9.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>4.513</td>
<td>7.913</td>
<td>6.36</td>
<td>4.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May</td>
<td>13</td>
<td>mean</td>
<td>13.9</td>
<td>32.792</td>
<td>9.06</td>
<td>13.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sd</td>
<td>1.558</td>
<td>7.141</td>
<td>6.05</td>
<td>10.59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>June</td>
<td>10</td>
<td>mean</td>
<td>13.413</td>
<td>23.23</td>
<td>9.92</td>
<td>2.70</td>
</tr>
</tbody>
</table>

Sex/ Mann-Whitney Test (p-value) 0.96 0.96 0.0000001238
Station/ Mann-Whitney Test (p-value) 0.13 0.16 0.00002135
Months/Kruskal-Wallis Test (p-value) 0.01 0.02 0.00000166
Spearman correlations Test (r- p-value)
<table>
<thead>
<tr>
<th>Lt(cm)</th>
<th>AChE Br(nmol/g/min)</th>
<th>AChE He(nmol/g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5471(0.0008)</td>
<td>0.5463(0.0008)</td>
</tr>
<tr>
<td>Pt(g)</td>
<td>0.5484(0.0089)</td>
<td>0.4626(0.0039)</td>
</tr>
</tbody>
</table>

Figure 1.— Spatiotemporal variations of physico-chemical parameters and activity of AChE in the Brain (AChE Br) and in the Heart (AChE He) of *B. setivimensis* (El Harrach stream, measured in 2013).

- High levels of Mg, PO4 and nutrients NO2 and NO3 (Fig. 1g, h, i, j) especially at station II that receives all urban and agricultural discharges.
- The percentage saturation of dissolved oxygen in station II decreases due to the presence of organic matter (dump); registered average rates around 64.51 % against 80.34 % in station I (Fig. 1e).

Figure 2.— Spatial analysis of the AChE activity at the level of the Brain (AChE Br.) and the Heart (AChE He.), as a function of the biological and physico-chemical parameters (PCA) among males (M) and females (F) of B. setivimensis, and according to month and sampling station (El Harrach stream, measured in 2013).

The principal component analysis shown in figure 2 has been established to complement and to better understand the links that can exist between descriptors characterizing enzymatic activity, biological parameters and water physico-chemical factors of El Harrach stream (Fig. 2A) according to month and sampling station (Fig. 2B). The plane of the axes F1x F2 holds 66.02 % of the total inertia.

Along the axis F1, which holds the maximum information with inertia ratio of 46.62%, we note an opposition effect between two groups:
- Factors LtM, Lt.F, Wt.M, Wt.F, Ca, Cond, MES, WaterT, Mg and No2 have the same grouping in the positive values of F1 and are positively correlated with factor AChE.He.F.
- The negative part of the F1 axis opposes AChE.Br.M, AChE.Br. F, AChE.He.M in the presence of Cl, Co3 and O2d, to the previous group.

AChE activity in the brain in both sexes, and the activity at the heart of males are negatively correlated with biological parameters and pollutant abiotic factors.

According to the F2 axis which has a rate of inertia of 16.40 %, our data show again the presence of two groups: Po4, So4 and O2d are located in the positive part of this axis and are negatively correlated with the pH and No3.

The PCA also shows variations between stations and according to the month of sampling, thus at both stations samples of May and June are in the positive part of the axis F1 whereas March and April samples are in the negative part. Axis 2 opposes May and June at station I (positive values) to May and June (negative values) at station II, and opposes March (positive value) at station II to March and April at station I and April at station II (negative values).

* The values obtained show a clear change in AChE activity during the months of sampling. In the brain values ranged from 17.448 nmole/gr/mn in March to 12.762 nmole/gr/mn in June in Station I, as opposed to values ranging from 11.870 nmole/gr/mn to 10.772 nmole/gr/mn from March to June in Station II. The graph shown in figure 3a reflects this variation which is very significant (p-value = 0.006688 / Kruskal-Wallis Test).

In the heart, a reduction is noted with values ranging from 33.233 nmole/gr/mn in March to 15.547 nmole/gr/mn in June in Station I, compared to 17.384 nmole/gr/mn in March to 16.020 nmole/gr/mn in June in Station II. The graph shown in figure 3b indicates that this change is significant (p-value= 0.08166 / Kruskal-Wallis Test).
Variation of the acetylcholinesterase activity in the Brain (AChE, Br) and the Heart (AChE, He) of *B. setivimensis* as a function of months-stations (a & b), and of the sexes (c & d) and of the organ (e). (El Harrach stream, measured in 2013).

*Results on the activity of acetylcholinesterase according to the station and month of sampling show that:

- concerning the brain, the measurements recorded show a strong inhibitory AChE activity in Station II. The average values are 22.169 nmole/gr/mn and 14.354 nmole/gr/mn respectively in Stations I and II. The results presented in figure 3a show that this variation is significant (p-value = 0.006042 / Mann-Whitney Test).

- concerning the heart, there is a decrease of AChE activity in Station II with a mean value of 10.504 nmole/gr/mn compared to 15.201 nmole/gr/mn in Station I. The graph in figure 3b shows that this variation is very significant (p-value = 0.0002935 / Mann Whitney Test).

* We also note that enzyme activity differs according to the sex of fish. The average reported values show a clear decrease in female fish. The average values in the brain are 30.96 nmole/gr/mn (Station I), 20.95 nmole/gr/mn (Station II) in male and 10.82 nmole/gr/mn (station I), 9.46 nmole/gr/mn (Station II) in females. The plots shown in figure 3c indicate that this variation is very significant (p-value < 2.2e-16 / Mann Whitney Test).

For the heart one observes in females of Station I values of 15.53 nmole/gr/mn and 12.57 nmole/gr/mn in males of Station II against 10.87 nmole/gr/mn for males in Station I and 10.50 nmole/gr/mn for females in Station II. The plots shown in Figure 3d indicate that this variation is very significant (p-value = 0.001238 / Mann Whitney Test).

* We also find that the enzymatic inhibition varies according to the tissues studied. Results show that the activity of AChE in the brain is greater than in the heart, average values recorded at
the station I are respectively in the range of 20.89 n mole/gr/mn and 13.20 n mole/gr/mn. The net reduction in activity in the polluted station was observed for the two bodies with average values of 18.33 n mole/gr/mn and 11.53 n mole/gr/mn respectively in the brain and heart tissue. The graphs shown in figure 3e testify to this very significant variation (p = 0.0004744 / Mann-Whitney Test).

* Results also show that the levels of the AChE also vary according to intrinsic parameters including the size and body mass of fish. Figure 4a & b clearly shows that enzymatic activity varies proportionally to the total length of individuals.

![Graphs showing enzymatic activity](image)

Figure 4. — Evolution of the acetylcholinesterase activity in the Brain (AChE. Br) and the Heart (AChE. He) of *B. setivimensis* as a function of the total length (a & b) and of the total weight (c & d). (El Harrach stream, measured in 2013).

These results are statistically very significant (p-value = 0.0008 / brain; 0.0008 / heart; / Spearman correlation Test). The figures 4c and 4d show clearly that the enzyme activity varies in proportion to the total weight of individuals. These results are statistically very significant (p-value = 0.0089 / brain; 0.0059 / heart; / Spearman correlation Test).

DISCUSSION

The analyses of the results show a clear decrease in enzymatic activity in Station II. The physico-chemical characteristics of the waters of this resort reflect a critical situation under the influence of anthropogenic impacts. The fluctuations are due to increased concentrations of nitrates, nitrites and phosphates, in connection with the leaching farmland. The concentration of nitrite is considered toxic to fish as of 0.03mg / l according to Beard *et al.*, (1990). The increase in temperature causes an increase in salinity and electrical conductivity (Arrignon, 1976; Marcano *et
al., 1996). These three specific parameters are proportional to each other. The excessive increase in conductivity causes a disturbance of the environment; it influences the atmospheric pressure causing metabolic problems in these sensitive aquatic organisms (Gaujous, 1995, 1998). An adaptation to high salinity conditions has however been reported for this species (Kraiem & Pattee, 1988).

The decrease in the amount of dissolved oxygen recorded in Station II is an important index related to the biological activity of the environment. The solubility of oxygen in water decreases with increasing temperature (Blifert & Perraud, 2001).

Together, these results show how environmental factors such as fluctuating physico-chemical parameters associated with the presence of pollutants can affect these cholinesterase activities. The results obtained by Jebali et al. (2013) lead to similar conclusions. These authors evaluated the potential use of cholinesterase activity in the common sole, inhabiting the coast of Tunisia, and subjected to various stress conditions, such as exposure to various contaminants.

The analysis of the results obtained shows that the levels of cholinesterase activity in the brain and heart of B. setivimensis especially in station II are lower than control individuals caught in resort I. Indeed, there is a correlation between acetylcholinesterase activity studied in fish tissue and contamination of the global environment by urban and agricultural waste. Levels of this enzyme activity are inversely proportional to the intensity of the pollution.

The inhibition varies, depending on the organs of the fish, as demonstrated by Chuiiko (2000), Chuiko et al. (2002, 2003) and Dutta & Arends (2003), who observed that the activity of brain AChE varied depending on the presence of fish and that cyprinids had higher activity than other fish families (Pavlov, 1992; Pavlov et al., 1994). In plasma the activity was on average 100 times lower than in the brain. Furthermore, when fingerlings Cyprinus carpio were exposed to different doses of Karate (Pesticide), the results showed that the maximum reduction of AChE activity was observed in the brain, followed by muscular tissue and then by hepatic tissue (Bibi et al., 2013).

The activity of AChE and swimming activity were studied in juvenile Carassius auratus exposed to carbofuran, a carbamate insecticide. This compound induced a significant inhibition of AChE activity in the brain after 2h of exposure, and significantly altered the fish swimming activity (Bretraud et al., 2001).

In addition, the presence of pollutants may enhance the inhibitory effects (Bocquené et al., 1995, 1997; Kamel et al, 2012). Other studies (Bouachrine, 1996; Bennasser, 1997; Maarouf et al, 1994) even revealed the presence of high levels of heavy metals in the estuary of Sebou. This may also explain fluctuations in AChE activity observed between standard fish. The inhibition of the enzyme by pollutants was also demonstrated in carp (Bertrand et al., 1998).

It has been emphasized that the inhibition of the enzyme can be quickly detected after the beginning of exposure to these toxic compounds and that the recovery time to normal activity may take several weeks (Morgan et al., 1990; Sturm et al., 1999, 2000). The pattern of recovery was described by Ferrari et al. (2004, 2007) who reported that it took two weeks for the enzymatic activity to return to normal.

The sex of the fish and the intrinsic biological parameter settings can have a major effect on the levels of AChE: the same conclusion is reported by Burgeot et al. (1996) and Pathiratne et al. (2008).

CONCLUSION

This study shows that the cholinesterase activity in the brain and heart in Barbus setivimensis in station II is lower than that of control individuals caught in Station I. In fact, there is a correlation between the acetylcholinesterase activity in the tissues studied of the fish and the
overall environmental contamination. Activity levels of this enzyme are inversely proportional to the intensity of the pollution.

All these measures were designed to present biological meaning to the presence of contaminants in the habitat and thus help diagnose the health of organisms.

Our results could be useful for better management of our water bodies and above all for the sustainable development and conservation of our species subjected to continuous and increasing stocking. At least, these preliminary data could open new horizons in bio-ecological studies of the genus *Barbus* as a bio-indicator of pollution.

ACKNOWLEDGMENTS

We thank the members of the Laboratory "Dynamics and Biodiversity", Faculty of Sciences and Biology at the University of Sciences and Technology of Houari Boumediene for their contribution to the realization of this article. We are also grateful to the anonymous referees who commented on previous versions of our text.

REFERENCES


