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Biomarkers and contaminants in the bivalve *Perna perna* caged in coastal sites in Senegal

Fatou TABANE [1*], Diabou DIAGNE[2], Doudou Georges NIANG[3], Fabrice CAZIER[4], Dorothée DEWAÉLÉ[4], Nafissatou LÈYE[2], Ketil HYLLAND[5], Cheikh DIOP[6], Mathilde CABRAL[6,] Cheikh TIDIANE BA[7] and Mamadou FALL[6]

[1] *Fondation Centre Régional de Recherche en Écotoxicologie et Sécurité Environnementale (CERES-Locustox), Unité Biologie, Km 15 Route de Rufisque, BP 11705, CP 13 000, Peytavin Dakar, Sénégal.*

[2] *Institut de Recherche en Santé, de Surveillance Épidémiologique et de Formations (IRESSEF), 4 Rue 2 D1 Pôle urbain diamniado BP 7325, Dakar, Senegal.*

[3] *Unité d'Immunologie, Faculté de Médecine Pharmacie et Odonto-Stomatologie, Université Cheikh Anta Diop de Dakar , BP 5005 Dakar-Fann, Sénégal.*

[4] *Centre Commun de Mesures, Université du Littoral Côte d'Opale, MREI 1 145 avenue Maurice Schumann 59140 Dunkerque.*

[5] *Department of Biosciences, University of Oslo, phone : +4722857315, Room : 4503, Kristine Bonnevies hus _ Institute for Marine Research, Bergen, Nordnesgaten 50, 5005 Bergen, Norway.*

[6] *Laboratoire de Toxicologie et d'Hydrologie, Faculté de Médecine Pharmacie et Odonto-Stomatologie Université Cheikh Anta Diop Dakar, BP 5005 Dakar-Fann, Sénégal.*

[7] *Laboratoire d'Écologie et Biologie évolutive, écologie et gestion des écosystèmes, Faculté des Sciences et Techniques, Université Cheikh Anta Diop Dakar, BP 5005 Dakar-Fann, Sénégal.*

[*] *Corresponding author; E-mail: tabanefatou@yahoo.fr, Telephone: +221774136345.*

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ABSTRACT

There is a scarcity of model species for contaminant monitoring in Africa meanwhile several studies have reported cases of pollution by numerous chemical contaminants. The aim of this study was to evaluate the use of one possible model species, the filter-feeding coastal bivalve *Perna perna*, for contaminant monitoring. *P. perna* collected from an unpolluted area of the Senegalese coast were transplanted to the Dakar harbour and to a reference location in the open coast in the vicinity of Dakar. Mussels were retrieved after four weeks' deployment and either analysed immediately or allowed to depurate for another four weeks. Mussels were analysed for biomarkers (selected transcripts and cellular responses) in gills and digestive gland (hepatopancreas), as well as for whole body residues of polycyclic aromatic hydrocarbons (PAHs) and metals. Mussels held in the harbour had accumulated both PAHs and the metals Pb, Cu and Se. Transcripts of biotransformation enzymes were downregulated whereas transcripts for antioxidant enzymes were upregulated in the gills compared to those of mussels held at the reference location. Digestive gland acetylcholinesterase and lactate dehydrogenase enzymatic activities were decreased compared to mussels from the reference location. Following depuration, gill transcripts had

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returned to baseline level except for gill glutathione *S*-transferase and lactate dehydrogenase activity, which decreased. Acetylcholinesterase inhibition was less prominent following depuration, but still significantly inhibited. The results suggest that caging of *P. perna* and measurement of selected biomarkers can be used to monitor effects of coastal environmental contamination.

Keywords : Gene Expression, Enymatic Activity, Polycyclic Aromatic Hydrocarbons, Trace Elements.

INTRODUCTION

The Senegal coast is 700 km long and almost 65% of the country population lives along the coast. Activities and increasing urbanization and industrialization along the coastline contribute to organic and inorganic pollution, through a range of processes, including discharge of effluents, coastal run-off and deposition of waste containing e.g. polycyclic aromatic hydrocarbons (PAHs) and trace elements (TEs). Studies carried out along the Senegalese coast has confirmed that sediment, mussels and fish are contamination by PAHs and TEs in some areas (Ndiaye et al., 2012 ; Diagne et al., 2013 ; Touré et al., 2016; Amara et al., 2019).

Exposure to environmental contaminants may lead to responses in organisms that start at the sub-cellular level and may progress to effects on the individual. In all higher organisms, PAHs are to some extent, biotransformed through two phases that lead to their transformation to more soluble and excretable metabolites.

The first phase reactions, in vertebrates primarily cytochrome P450s (CYPs), result in an intermediate metabolite that may be or may not be water soluble. Expression of some of the genes associated with phase one metabolism is regulated via the aryl hydrocarbon receptor (AhR), which binds planar compounds including PAHs. This receptor will initiate the transcription of a range of genes, including some glutathione *S*-transferases (GST). The role of AhR in *Perna perna* has been suggested to be analogous to signalling in vertebrates (Guerreiro et al., 2020). They observed that both *AhR*-like and *CYP1A2*-like mRNA levels were up-regulated in the mussel *P. perna* when exposed to chlorothalonil. The second biotransformation phase of organic contaminants results in a polar metabolite by adding endogenous hydrophilic groups to form water-soluble metabolites that can be excreted. The main enzyme families involved in the second phase in vertebrates and some invertebrates are glutathione *S*-transferases (GSTs), UDP-glucuronosyl transferases (UDP-GT) and sulfotransferases (SULT).

Many TEs act as pro-oxidant stressors, increasing the intracellular generation of reactive oxygen species (ROS), thereby causing oxidative stress. Essential TEs such as iron (Fe) or copper (Cu) can also induce ROS. Increased intracellular levels of reactive oxygen species (ROS) may lead to oxidative stress through the Fenton reaction (Aljohani et al., 2023). Cellular protection against deleterious effects of ROS is maintained by antioxidant defense systems. Enzymes involved in this defense include superoxide dismutases (SOD), metalloenzymes that catalyse the dismutation of superoxide anion ($O_2^{\cdot-}$) into molecular oxygen and hydrogen peroxide (H_2O_2), which may be subsequently converted into water (H_2O) by catalase and glutathione peroxidase (Kim and al., 2003). There are several SODs characterized by their redox-active metals at the catalytic sites. In mussel, Cu/Zn–SOD in gills and digestive gland has been shown to be induced by pollutants (Manduzio et al., 2003). GSTs are also involved in the antioxidant defense system, through the conjugation of ROS with reduced glutathione (Boutet et al., 2004).

Exposure to contaminants have been shown to modulate lactate dehydrogenase (LDH) and acetylcholinesterase (AChE) in bivalves (Dos Santos et al., 2022). LDH is a metabolic enzyme, responsible for the regeneration of nicotinamide adenine dinucleotide (NAD), necessary in the

metabolism of glucose and for the subsequent production of ATP from NADH for glycolysis. AChE is involved in neurotransmission in vertebrates and some invertebrates. In mussels it is probably an indicator of contaminant-related metabolic stress rather than neurotoxicity as it is difficult to link AChE inhibition with the adverse neurotoxicity in sessile organisms (Rickwood and Galloway, 2004).

Studies about enzyme activitiies modulation in fishes exposed to contaminants in West Africa could be found (Kantati et al., 2013), but very few have been done for mussels. The present study aimed to test the appplicability of the mussel caging using *Perna perna* for pollution monitoring in west African and other tropical and subtropical coastal areas, including the analysis of a suite of biomakers.

MATERIALS AND METHODS

Study area and site descriptions

The study area was in Dakar, the Cape Verde peninsula, in Senegal (Figure 1). The sea temperature along this coast varied from 18°C in February to 32°C in October. The tide varied between 0.5 and 1.6 m, but with strong diurnal and interannual variation (Mbaye et al., 2015).

Three sites, with quite similar ecological and oceanographic conditions, were chosen during the present study. The first location was the Yoff Tonghor Island (14°46'09.4"N 17°28'37.1"W), a little frequented place considered as sacred. The rocky substrate was suitable to macrophytes and sessile organisms (Guèye et al., 2014). Mussels to be used in the caging experiments were collected at this location. The two other locations were the reference site and the contaminated site. The reference site, the Almadies Tip (14°74'41.50" N 17°52'93.96" W), was situated close to the beach of the former Mediterranean Club of Dakar, on the westernmost point of Africa. There was no access for the general public at the location. The contaminated site was located between the terminal for fertilizers and cement and that of oil, in the harbour of Dakar, PAD (14°41'07.34" N North 17°25'19.93" W). The area was the site for facilities of several companies active in the transport, distribution, refining and processing of hydrocarbons and chemicals. Previous studies have shown that PAH concentrations in PAD water was the highest of the three coastal sites of the Dakar area (Ndiaye et al., 2012).

Mussel collection, acclimatisation and experimental design

Adult brown mussels, *Perna perna* (n ≈ 1500 ; 6.5–7.5 mm shell length), were used for the experiment (Gardner et al. 2016). Mussels were collected at low tide in February 2021 from Yoff Tonhor Island. They were transferred from the collection site to the Almadies Tip in polyethylene basins half-filled with seawater and aerated using an air-bubbler. Transportation was completed within 1 hour. Mussels were maintained in Almadies Tip water for 5 weeks for acclimatation before being deployed. One week before the experiment started, they were measured and individuals larger than 7.5 cm and smaller than 6.5 cm were excluded. Seventy individuals were placed in each cage and left to further acclimatize. Eight nets were deployed at each site (PAD and Almadies) for four weeks of exposure, from March 29 to April 26, 2021. The nets were placed 2.5 m below sea level in the intertidal at both sites. Following the four-week exposure period, half of the nets at PAD were transferred to Almadies for depuration for another four weeks. The depuration period ran from April 26, 2021 to May 24, 2021. Two nets were lost at PAD.

Sample collection and treatment

Around 30 mussels were sampled before and after the experiment began, from each site at 7, 14, 21, 28 days after the exposure started, and at 14 and 28 days after the depuration started. Ten liters of seawater were taken from each site on each sampling day, for the purposes of the depuration of mussels intended for contaminants analysis. The time series samples were used as replicates for each experiment. Three groups of samples were obtained : the before samples, the exposure samples and depuration samples.

After retrieval of cages, mussels were transferred alive to the laboratory. Fouling and byssus threads were removed, following which gills and digestive glands were rapidly excised from twelve individuals. Each tissue sample was transferred to a 1.5 mL cryotube and frozen in liquid nitrogen before being kept at -80°C until further processing. Ten to twelve mussels intended for PAHs and TEs analysis were placed into 10 L of seawater (previously filtered and aerated) for 24 hours, in order to allow them to clear their gut contents. They were dissected and whole soft tissues were pooled in two samples, of three replicates. One sample was intended for PAHs and the other, for TEs analyses. They were stored at -20°C until analysis.

Chemicals analysis

PAHs and TEs analysis

The 16 USEPA PAHs were extracted from homogenized whole mussel tissues using a modified QuEChERS method (QuEChERS AOAC) (Pule and al., 2012). The extract was analyzed by Gas Chromatography coupled to Mass Spectrometry (GS/MS) (Varian, model 1200). The chromatography column (DB-5MS) specifications were as follows : 30 m x 0.25 mm x 0.25 µm. A spitless mode was applied: injection 1 µL. The injection and source temperatures, were set at 295 and 280°C respectively. The scan was set at 50/500 m/z. Helium was used as carrier gas (flow rate : 1 mL/min). Selective Reaction Monitoring (SRM) method was used for PAHs quantification. All the regents and standards used were purchased from Sigma-Aldrich.

The TEs included both essential and non-essential elements : chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), vanadium (V), selenium (Se), arsenic (As), cadmium (Cd), mercury (Hg), tin (Sn), Antimony (Sb) and iron (Fe), were analyzed in mussels dried soft tissues using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Thermo ICAP 6300 DUO) with an automatic sampler by method described previously (Diop et al., 2015). Radial mode was selected for quantification.

Quality assurance and quality control

Three test samples were taken in the crushed reference mussels to measure the PAHs. One aliquot of crushed tissues was spiked with 1 µL of PAH standard at 2000 µg/mL (16 EPA PAHs) meanwhile the other two were not spiked. The limit of detection (lod) of PAH was set to 40 ng/g. For TEs, a multi-element calibration was carried out, using a single-element standard solution (SCP Sciences). Standards were injected after every 9 th sample in the analyzer (Diop et al., 2015).

Gene expression analysis

The mRNA extraction from gills was performed using the TRIzol® Reagent (Invitrogen), following the protocol described by Guerreiro et al. (2020). Immediately after extraction, the synthesis of cDNA was conducted using 1 µg of total RNA per sample. Real time quantitative PCR (RT-qPCR) were performed with primers shown in Table 1 using an Applied Biosystems 7500 and 7500 FAST Real-Time PCR System and a QuantStudio 5 Real-Time PCR Detection System. RT-PCR reactions were conducted in duplicate using NEB Luna Universal One-Step RT-qPCR Kit (Biolabs), optimized for dye-based real-time quantitation of target RNA sequences via the SYBR®/FAM fluorescence channel of most real-time instruments. Reverse transcription step was performed during 10 min at 55°C. The PCR reaction parameters included an initial denaturation step for 1 min at 95°C, followed by 40 cycles of denaturation at 95°C for 10 s and then, of extension at 60°C for 60 s. The melt curve was programmed to run from 65°C to 95°C. Gene expression was quantified using the 2^{-ddCt} method (Livak and Schmittgen, 2001).

Enzymatic activities

Assay kits for glutathione S-transferase (GST) (Catalog Number CS0410), acetylcholinesterase (AChE) (Catalog Number MAK119) and lactate dehydrogenase (LDH) (Catalog Number MAK066) were purchased from Sigma-Aldrich. The reaction media were prepared according to the manufacturer's

instructions. Briefly, tissues samples (100 mg) were homogenized in the corresponding assay buffer (500 µL). After centrifugation at 10,000 x g for GST and LDH analysis, and 14000 x g for AChE, for 15 minutes at 4°C, the resulting S9 fractions were used for the assays. Enzyme kinetics were determined in duplicate using a Thermo Scientific Multiskan microplate reader. GST activity was measured in digestive glands using the protocol described by Sturve et al. (2017). AChE activity was measured at 405 nm in digestive glands based on the Ellman method (Dos Santos et al., 2022). LDH activity was measured at 450 nm in gills and digestive glands according to the protocol of Olivia et al. (2012). Results were expressed in milliunit/mg (mU/mg) of tissue wet weight.

Statistical analysis

Results were expressed as mean ± S.E. For statistical analysis, "not detected" concentrations of contaminant were assigned as half of the corresponding detection limit. Data were tested for normality and homogeneity of variance using Shapiro-Wilk and the Levene tests, respectively. TEs concentrations were firstly grouped according to their biological role, into essential TEs and non-essential TEs and then considered individually. Depending on whether the distribution of PAH and TE concentrations allowed the use of parametric analyses or not, two-way ANOVA or Kruskal Wallis test were used, followed by post hoc Tukey HSD or Dunn test after Bonferroni correction, respectively. Spearman correlation test was used to compare gene expression and enzymes activity. The differences of all the tests were considered significant when the p-value was less than 0.05. Integrated biological responses were examined by Principal Component Analysis (PCA). Pairwise correlations intensity with p-values, was checked using Spearman's rank coefficient ($\boldsymbol{\rho}$). Correlations with $\boldsymbol{\rho} \geq 0.5$ were considered as strong (Dobal and al., 2022).

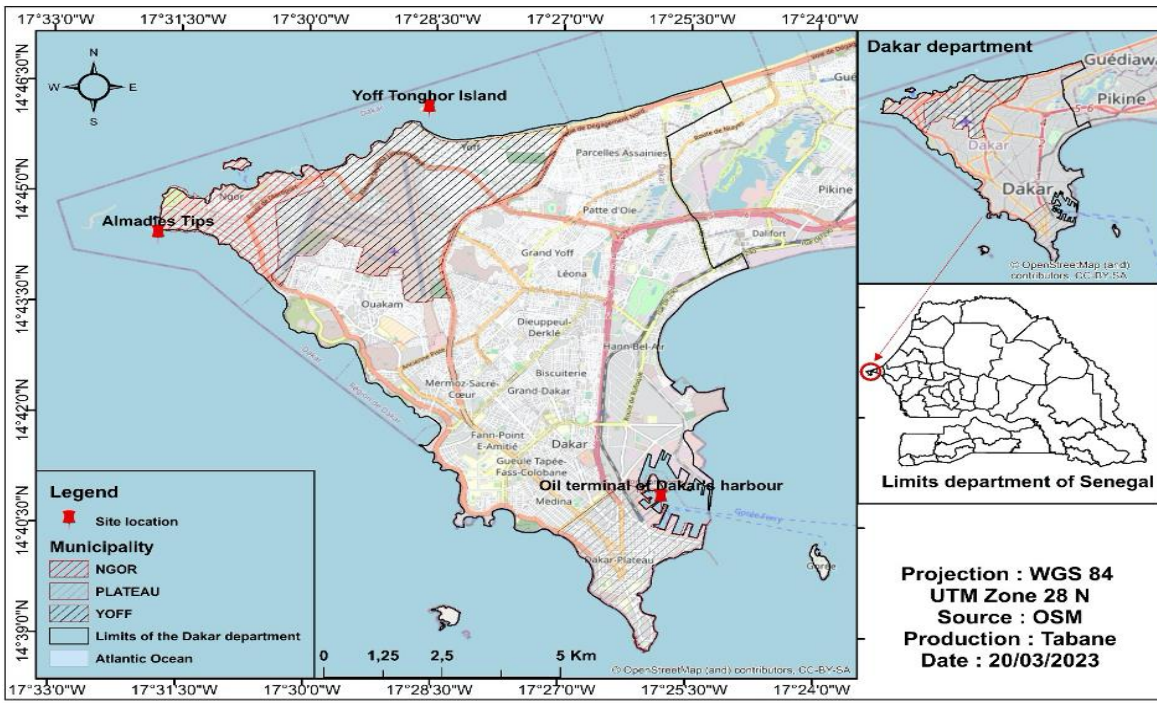

Figure 1 : Map indicating the location of the study area and sites : Yoff Tonghor Island, Almadies Tip (Almadies) and Dakar Harbour (PAD) in Dakar, the Cape Verde peninsula, In Senegal.

Table 1: Primer sequences used for evaluation of genes expression by Real-Time PCR.

Genes	Primer sequences
Actin-like	F : 5'- CAGGATCTGGCGACATGGTT - 3' R : 5'- CAGGCTTGTGGTCCTGAACT - 3'
AhR-like	F : 5'- GTACTGGGCCACGACAATCA - 3' R : 5'- AGGACAAAGGACCGAATGGAC - 3'
CYP1A2-like	F : 5'- AATCTTCCCCCAGGACCAAA - 3' R : 5'- CGCCATATTCTTTCCACCACC - 3'
SULT1A1-like	F : 5'- GCTTAGAGGCTCTCCTTCTCCT - 3' R : 5'- ATAGTTCCACCAGCCTCCGT - 3'
MGST-like	F : 5'- CGGAATGGTCTGGCTACTTG - 3' R : 5'- GCAAACGCTCCTCTCATTCT - 3'
GSTO-like	F : 5'- AGGTTCCGAGTGTCCGCCATT - 3' R : 5'-AGTCGTGTTCGTTGAGCGTATGG - 3'
SOD-like	F : 5'- CCCTCCGCGGTGAGAACTCATT - 3' R : 5'-GCA TGC AAC TCT TCC GCC AGC -3'

RESULTS

Mussel PAH and metal body burden

The recovery of PAHs in spiked mussels varied from 83 to 128%. PAHs were below detection limits in mussels before the experiment and in mussels from the reference site (Almadies) at the end of the exposure and depuration times (Table 2). Only mussels exposed at the contaminated site (PAD) had detectable concentration of PAHs, comprising three and four-rings congeners. The non-essential elements Hg, Sn and Sb were not detected in any sample. Following exposure, mussels from PAD had significantly elevated concentrations of Pb, Cu and Se compared to reference mussels. After transfer of mussels from PAD to Almadies for depuration, a decrease of Cu, Pb and Se concentrations was apparent.

Gene expression

After mussels were exposed by caging in PAD, the expression of *AhR*-like, *CYP1A2*-like, *GSTO*-like and *SULT1A1*-like genes decreased significantly in gills while that of *SOD*-like genes was up-regulated (Figure 2).

Following depuration, the transcripts of most biotransformation genes and *SOD*-like were back to baseline expression in mussels transferred from PAD to Almadies.

Cellular biomarkers

Following exposure at PAD, digestive gland GST activity in mussels was not significantly different to the same activity in mussels caged at Almadies **(**Figure 3**)**. In contrast, digestive gland AChE and LDH activities in mussels held at PAD were significantly lower than in those held at the reference site. However, the LDH activity in the gills of PAD mussels was higher than in mussels from the reference location, but not compared to baseline level.

Following depuration, AChE inhibition decreased in mussels originally held at PAD, whereas digestive gland LDH activity returned to baseline level. For the other biomarkers there was no change.

Multivariate analysis

The first two axes of the PCA, including all measured genes and biomarkers, expressed 72% of the variability (Figure 4). The PCA discriminated between the reference mussels and those caged at PAD as well as between exposure and depuration. According to the PCA three groups of mussels could be identified, regarding the pollutants they bioaccumulated. First, mussels with high amounts of the PAHs, Cu, Pb Se had high expression levels of *SOD*-like and *MGST*-like genes and important GST and gills LDH activities. However, their expressions of *AhR*-like, *CYP1A2*-like, *GSTO*-like and *SULT1A1*-like genes, the digestive glands LDH and AChE activities were weak. In contrary to the first group, the second group was characterised by mussels with higher tissue levels of Ni, V, As and Cd with higher expression of *AhR*-like, *CYP1A2*-like, *GSTO*-like and *SULT1A1*-like genes and high AChE and digestive gland LDH activity. Nevertheless, these individuals, had lower GST activity and *SOD*-like and *MGST*-like expression levels. In the last group, Fe, Zn and Cr were the major TEs bioaccumulated leading to high digestive glands LDH activity and low expressions of all the six studied genes and GST activity.

The Spearman rank's test supported some of these correlations through negative and weak coefficients ($\rho < 0.5$) between *AhR*-like, *CYP1A2*-like, *GSTO*-like, *SULT1A1*-like, GST, AChE and LDH (in gills and digestive glands) activity and PAHs tissue residues (p-values > 0.05). Some exceptions were noticed for fluorene correlations. It was weakly but positively correlated to *AhR*-like, *GSTO*-like and *SULT1A1*-like (p-values > 0.05) and negativeley but strongly correletd to LDH activity in digestive glands ($\rho = -0.5$) (p-value > 0.05). In contrary to the previous biomarkers, *MGST*-like and *SOD*-like were positively and strongly correlated to PAHs. *SOD*-like correlations to anthracene ($\rho = 0.8$), fluoranthene ($\rho = 0.8$), pyrene ($\rho = 0.8$) and chrysene ($\rho = 0.8$) were all significant (p-values < 0.05). Positive Spearman coefficients for the correlations of biomarkers to TEs, but non-significances (with all p-values > 0.05), were found between Cd and *AhR*-like, *CYP1A2*-like, *GSTO*-like, LDH in gills and AChE (all the ρ coefficients are equal to 0.6) ; Ni and *CYP1A2*-like ($\rho = 0.6$), LDH in gills ($\rho = 0.7$), AChE ($\rho = 0.8$) ; Se and *MGST*-like ($\rho = 0.6$) ; Pb and *MGST*-like ($\rho = 0.6$) and As and LDH in gills ($\rho = 0.6$). The most significant and positive correlation was found between V and LDH gills (0.8) (p-value < 0.05). Negative and strong correlations, though non-significant, was found between Pb and *AhR*-like ($\rho = -0.6$), *CYP1A2*-like ($\rho = -0.6$) and *GSTO*-like ($\rho = -0;6$) ; Cr and *CYP1A2*-like ($\rho = -0.6$) and AChE ($\rho = -0.7$) ; Zn and *SOD*-like ($\rho = -0.6$) ; V and *SOD*-like ($\rho = -0.5$) ; As and *SOD*-like ($\rho = -0.8$) ; Cd and *SOD*-like ($\rho = -0.6$) ; As and *MGST*-like ($\rho = -0.5$) ; Fe and *CYP1A2*-like ($\rho = -0.5$), Fe and AChE ($\rho = -0.6$) and Se and AChE ($\rho = -0.6$).

Regarding gene expression inter-correlations, *AhR*-like wa strongly, positively and significantly correlated to *CYP1A2*-like, *GSTO-like* and *SULT1A1*-like ; *CYP1A2*-like was strongly, positively and significantly correlated to *GSTO-like* and the last one was strongly and posifitively correlated to *SULT1A1*-like. *SOD*-like and *MGST*-like were weakly linked to the other genes (all the ρ co-efficients < 0.3).

Enzymes activities were in general, positively, but weakly and not significantly inter-correlated. The exceptions were from GST and AChE, whose correlation was negative ($\rho = -0.4$) and LDH in gills and AChE whose one was strong ($\rho = 0.7$).

Regarding the correlations between the genes and the enzymes, *AhR*-like was strongly and negatively correlated to GST ($\rho = -0.6$) and LDH in digestive glands ($\rho = -0.6$) but with AChE, its correlation was strong and positive ($\rho = 0.7$). AChE was strongly correlated to *CYP1A2*-like ($\rho = 0.8$), *GSTO*-like ($\rho = 0.7$) and *SULT1A1*-like ($\rho = 0.6$) and its correlation to *CYP1A2*-like was significant. The only significant gene-enzyme correlation, was that of *CYP1A2*-like and AChE. The Spearman correlation table is given in supplementary material (Table 3).

Table 2 : PAHs and TEs concentrations in mussel samples, given as means ± standard errors. Statistical significance codes are displayed as a series of stars: '***' for p-value < 0.001, '**' for p-value < 0.01, '*' for p-value < 0.5.

Contaminants		Before	Exposure		Depuration	
			Almadies	PAD	Almadies	PAD
PAHs (ng/g ww)	Naph	< lod	< lod	< lod	< lod	< lod
	Acene	< lod	< lod	< lod	< lod	< lod
	Aceny	< lod	< lod	< lod	< lod	< lod
	Fluo	< lod	< lod	68.33±3.05	< lod	< lod
	Phen	< lod	< lod	263.33±69.49**	< lod	< lod
	Anthra	< lod	< lod	79.33±14.57	< lod	< lod
	Fluora	< lod	< lod	88.67±10.68	< lod	< lod
	Pyr	< lod	< lod	256.33±43.25**	< lod	< lod
	BaA	< lod	< lod	< lod	< lod	< lod
	Chrys	< lod	< lod	126.66±26.10	< lod	< lod
	BbF	< lod	< lod	< lod	< lod	< lod
	BkF	< lod	< lod	< lod	< lod	< lod
	BaP	< lod	< lod	< lod	< lod	< lod
	IP	< lod	< lod	< lod	< lod	< lod
	DbA	< lod	< lod	< lod	< lod	< lod
	BP	< lod	< lod	< lod	< lod	< lod
	∑PAH	-	-	776.2±165.69	-	-
Non-essential trace elements (mg/kg dw)	Cd	2.50±0.34	2.56±0.51	2.07±0.59	3.12±1.85	2.69±1.12
	Ni	5.12±0.76	6.89±2.734	5.29±1.80	10.03±2.29	5.41±1.86
	Pb	0.12±0.17	0.34±0.168	0.64±0.184*	0.41±0.04	0.35±0.30
	As	10.45±1.61	10.14±1.56	8.85±1.00	11.01±1.51	9.78±0.75
	Hg	< lod	< lod	< lod	< lod	< lod
	Sn	< lod	< lod	< lod	< lod	< lod
	Sb	< lod	< lod	< lod	< lod	< lod
Essential trace elements (mg/kg dw)	Cr	0.43±0.02	0.28±0.169	0.40±0.22	0.34±0.10	0.51±0.21
	Cu	3.75±0.04	4.54±1.015	7.16±1.09**	5.33±0.61	5.30±1.37
	Zn	76.85±0.63	120.87±26.99	129.05±26.37	155.40±74.97	136.03±63.52
	V	1.43±0.14	1.50±0.51	1.41±0.31	2.05±0.06	1.49±0.08
	Se	1.72±0.08	1.81±0.28	2.53±0.51*	1.79±0.18	1.91±0.41
	Fe	90.45±22.98	119.11±39.14	141.62±42.27	194.43±89.78	159.23±105.18

Acene = Acenaphthene ; Aceny=Acenapthylene ; Anthra = Anthracene ; As=Arsenic ; BaA = Benzo(a)anthracene ; BaP = Benzo(a)pyrene ; BbF = Benzo(b)fluoranthene ; BkF = Benzo(k)fluoranthene ; BP= Benzo(ghi)perylene ; Cd=Cadmium ; Chrys = Chrysene ; Cr = Chromium ; Cu = Copper ; DbA = Dibenzo(ah)anthracene ; dw=dry weight ; Fe=Iron ; Fluora = Fluoranthene ; Fluo = Fluorene ; Hg= Mercury ; IP = Indeno(1,2,3,cd)pyrene ; lod= limit of detection ; Napth = Naphthalene ; Ni= Nickel ; Pb=Lead ; Phen = Phenanthrene ; Pyr = Pyrene ; Se=Selenium ; Sb= Antimony ; Sn = Tin ; V= Vanadium ; Zn=Zinc ; ww = wet weight ; ∑PAH = total PAHs concentration.

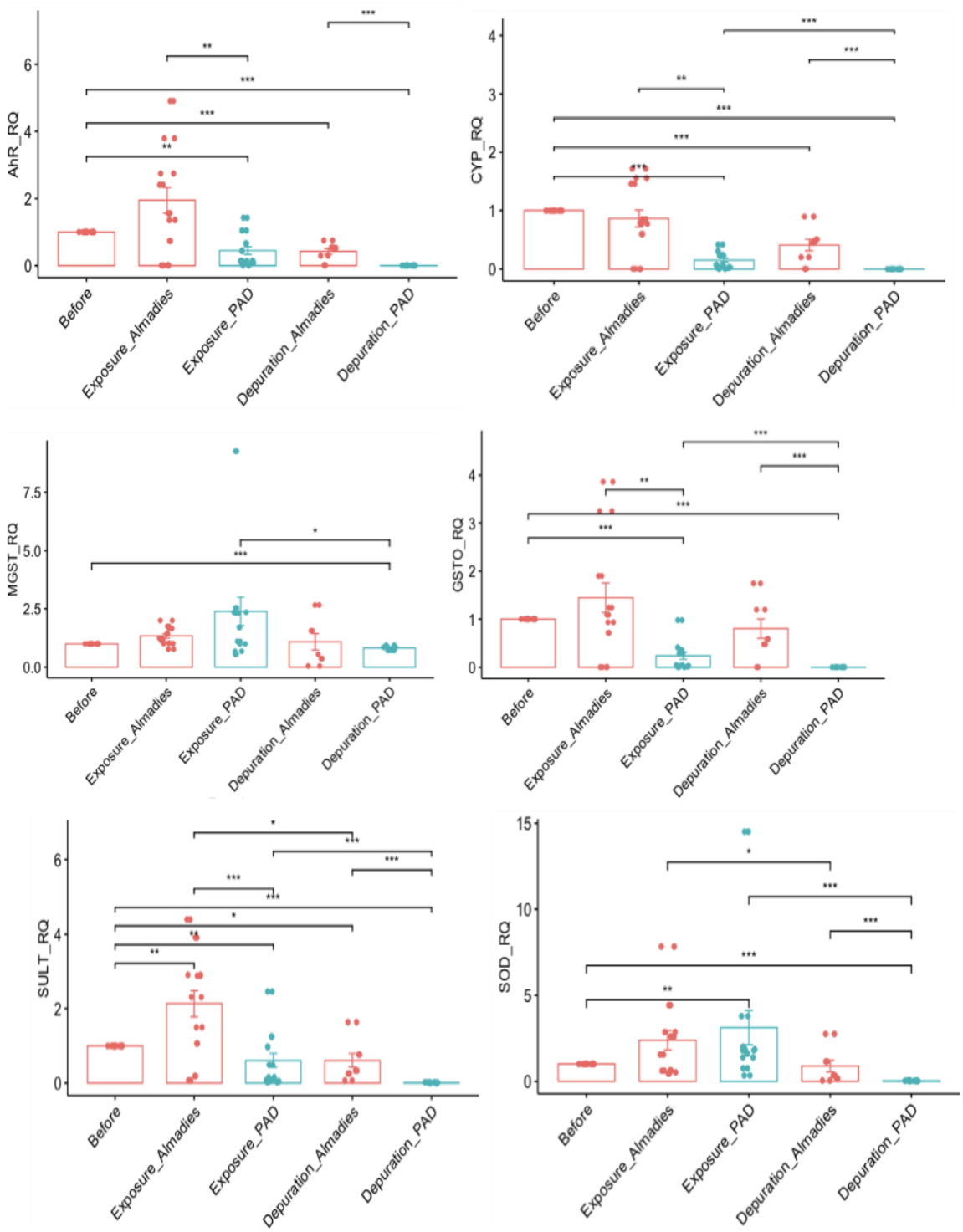

Figure 2 : Transcriptional responses of *AhR*-like, *CYP*-like, *MGST*-like, *GSTO*-like, *SULT1A1*-like and *SOD*-like genes in *P. perna* gills before and after exposure and after depuration from PAHs and TEs pollution. Values were normalized to baseline levels, recorded before the experiment. Statistical significance tests are represented with horizontal lines and the significance codes are displayed as a series of stars: '***' for p-value < 0.001, '**' for p-value < 0.01, '*' for p-value < 0.5.

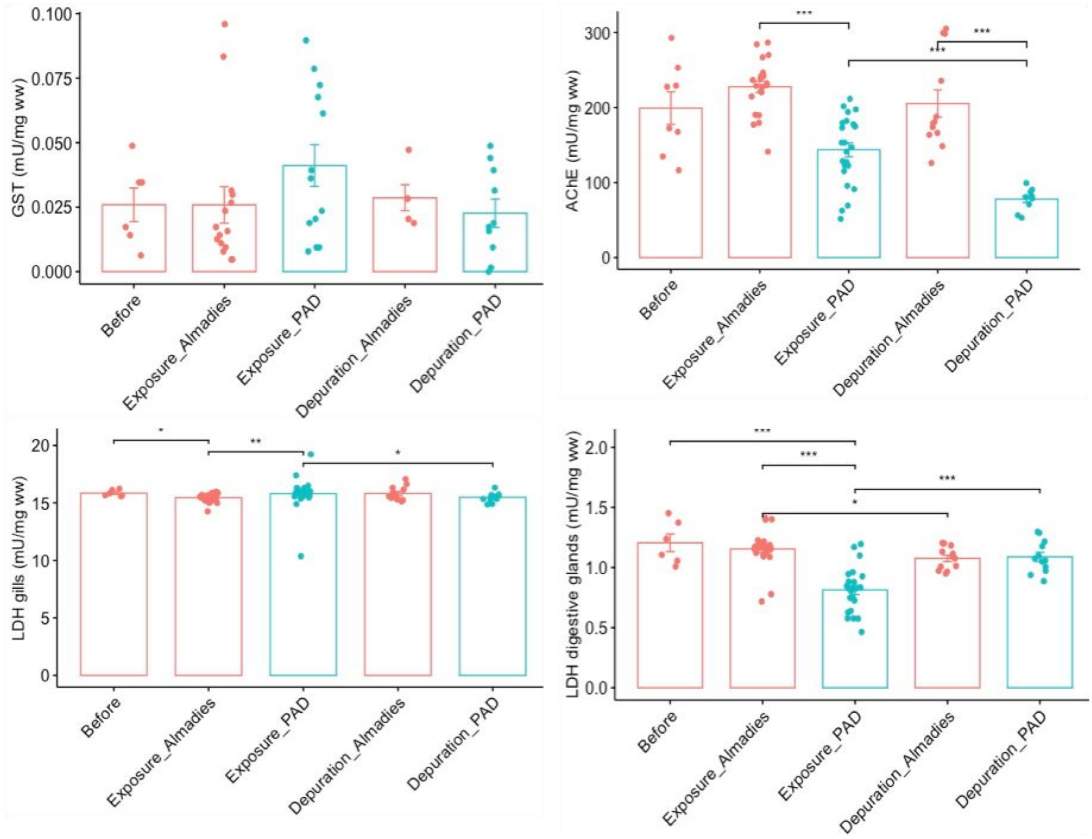

Figure 3 : GST, AChE and LDH activities in *P. perna* before and after exposure and after depuration from PAHs and TEs pollution. Statistical significance tests are represented with horizontal lines and the significance codes are displayed as a series of stars : '***' for p-value < 0.001, '**' for p-value < 0.01, '*' for p-value < 0.5.

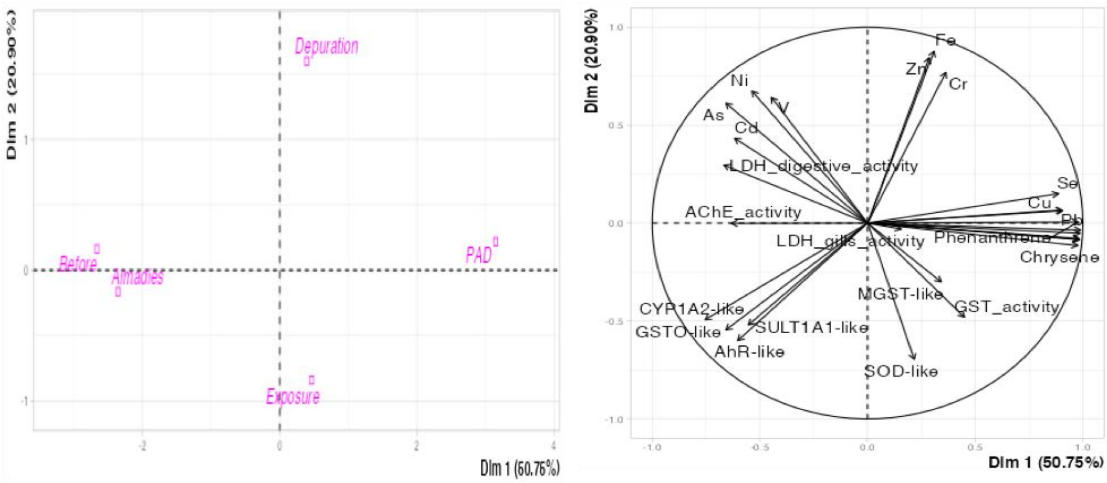

Figure 4: PCA graph of individuals and variables, summarizing data from *P. perna* exposure to PAHs and TEs pollution, pooled by site and experiment. The first two dimensions express 71.65% of the total dataset inertia. The first plan (Dim 1) separates sites and expresses 50.75% of the dataset and the second plan (Dim 2), separating experiments, expresses, 20.90% of the dataset.

Table 3: Spearman correlations coefficients among the chemicals (PAHs and TEs), the genes expressions (*AhR-like*, *CYP1A2-like*, *GSTO-like*, *SULT1A1-like*, *MGST-like* and *SOD-like*) and the enzymes activities (GST, AChE and LDH in gills and digestive glands) tested across sites. (n = 2).

	∑PAH	**Fluo**	**Phen**	**Anthra**	**Fluora**	**Pyr**	**Chrys**
AhR-like	-0.273	0.030	-0.216	-0.273	-0.273	-0.273	-0.273
CYP1A2-like	-0.395	-0.213	-0.370	-0.395	-0.395	-0.395	-0.395
GSTO-like	-0.273	0.030	-0.216	-0.273	-0.273	-0.273	-0.273
SULT1A1-like	-0.091	0.152	-0.062	-0.091	-0.091	-0.091	-0.091
MGST-like	0.759	0.759	0.741	0.759	0.759	0.759	0.759
SOD-like	***0.820****	0.516	0.772	***0.820****	***0.820****	***0.820****	***0.820****
GST	-0.092	-0.092	-0.141	-0.092	-0.092	-0.092	-0.092
LDH_gills	-0.273	-0.152	-0.309	-0.273	-0.273	-0.273	-0.273
LDH_dg*	-0.273	-0.577	-0.339	-0.273	-0.273	-0.273	-0.273
AChE	-0.334	-0.334	-0.370	-0.334	-0.334	-0.334	-0.334

	Cr	Cu	Ni	Pb	Zn	V	Se	As	Cd	Fe
AhR-like	-0.429	-0.086	0.429	-0.609	-0.143	0.257	-0.257	0.314	0.600	-0.371
CYP1A2-like	-0.657	-0.257	0.657	-0.696	-0.086	0.314	-0.486	0.371	0.657	-0.543
GSTO-like	-0.429	-0.086	0.429	-0.609	-0.143	0.257	-0.257	0.314	0.600	-0.371
SULT1A1-like	-0.371	-0.029	0.314	-0.377	-0.371	0.143	-0.143	0.086	0.371	-0.429
MGST-like	0.314	0.600	-0.486	0.696	-0.429	-0.143	0.657	-0.543	-0.486	0.029
SOD-like	-0.200	0.371	-0.371	0.493	-0.600	-0.543	0.314	-0.829	-0.657	-0.371
GST	0.493	0.058	-0.029	0.456	0.348	0.406	0.203	0.232	-0.058	0.406
LDH_gills	-0.314	0.029	0.714	-0.145	0.371	***0.886****	-0.200	0.600	0.657	-0.257
LDH_dg	-0.200	-0.314	0.314	-0.029	0.429	0.143	-0.371	0.200	0.029	-0.029
AChE	-0.771	-0.257	0.829	-0.493	0.029	0.486	-0.543	0.371	0.600	-0.657

	AhR-like	*CYP1A2-like*	*GSTO-like*	*MGST-like*	*SOD-like*	*SULT1A1-like*	GST	LDH_gills	LDH_dg
CYP1A2-like	***0.943****								
GSTO-like	***1.000****	***0.943****							
SULT1A1-like	***0.943****	0.829	***0.943****	0.371	0.257				
MGST-like	0.086	-0.143	0.086						
SOD-like	0.086	0.029	0.086	0.657					
GST	-0.638	-0.638	-0.638	0.029	-0.522	-0.551			
LDH_gills	0.429	0.543	0.429	-0.029	-0.257	0.371	0.203		
LDH_dg	-0.600	-0.314	-0.600	-0.714	-0.371	-0.771	0.348	0.086	
AChE	0.714	***0.886****	0.714	-0.200	0.029	0.600	-0.406	0.771	0.029

Numbers in bold indicate significant correlations. Statistical significance codes are displayed as a series of stars : '***' for p-value=0.001 , '**' for p-value=0.01 and '*', for p-value=0.05 ; Acene = Acenaphthene ; Aceny=Acenapthylene ; Anthra = Anthracene ; As=Arsenic ; BaA = Benzo(a)anthracene ; BaP = Benzo(a)pyrene ; BbF = Benzo(b)fluoranthene ; BkF = Benzo(k)fluoranthene ; BP= Benzo(ghi)perylene ; Cd=Cadmium ; Chrys = Chrysene ; Cr = Chromium ; Cu = Copper ; DbA = Dibenzo(ah)anthracene ; dw=dry weight ; Fe=Iron ; Fluora = Fluoranthene ; Fluo = Fluorene ; Hg= Mercury ; IP = Indeno(1,2,3,cd)pyrene ; LDH_DG = LDH activity in digestive glands ; Napth = Naphthalene ; Ni= Nickel ; Pb=Lead ; Phen = Phenanthrene ; Pyr = Pyrene ; Se=Selenium ; Sb= Antimony ; Sn = Tin ; V= Vanadium ; Zn=Zinc ; ∑PAH = total PAHs concentration.

DISCUSSION

Genes expression modulation

Biotransformation genes

Down-regulation of *CYP1A2*-like genes in mussels following caging at PAD could suggest exposure to potential CYP inhibitors like fluorene, anthracene and phenanthrene, in higher proportions than inducers such as benzo(a)pyrene (Hylland, 2006). Prooxidant metals such as Pb could affect *CYP1A2*-like gene expression (Risso-de Faverney et al., 2000). It may act by suppressesing the inducibility of this biotransformation pathway through a post-transcriptional mechanisms which cause reduced availability of heme groups the prosthetic moiety of several vital hemoproteins such as CYP 450.

PAHs biotransformation phase I reactions introduce groups into PAHs molecules to provide sites allowing phase II conjugation reactions. Modulation of phase I activity (*AhR*-like and *CYP1A2*-like) may lead to the disruption of the phase II biotransformation reactions (*GSTO-like and SULT-like*). It has been reported that *AhR*-like genes may control the transcription of phase II genes from the *GST*-like family (Rolland et al., 2014). The correlation between *AhR*-like and *GSTO*-like could by seen as a support for this, including the interdependance of phase II (*GSTO*-like, *SULT1A1*-like) and phase I genes (*AhR*-like and *CYP1A2*-like).

The continued downregulation of genes observed at the end of the depuration period was possibly the consequence of a depletion of oxidative stress capacity in the mussel tissues (Liu, 2020).

Oxidative stress genes

Increased expression of *SOD*-like genes following caging of mussels at the PAD site may indicate a defense response to cellular oxidative stress induced by ROS from both prooxidant metals such as Pb and Cu and PAHs photoactiviation. Pb act by inhibiting the δ-amino-levulinic acid dehydratase (δ-ALAD) which lead to superoxide anion production and then, to *SOD-like* induction (Salazar-Flores, 2019). Regarding their significant relationships with *SOD-like* gene, PAHs are probably the main responsible for its induction through photooxidation and oxidative stress (Ertl et al., 2016).

A reduced expression of the *SOD*-like gene observed at the end of a depuration period has earlier been observed by Manduzio et al. (2003) with mussels transferred from a contaminated to a clean site.

The correlation of *SOD*-like to *MGST*-like and *GSTO*-like gene expression could suggest that the upregulation of *MGST*-like genes observed after caging of mussels at PAD may be related to its protective role against the oxidative stress induced by ROS generated by PAHs, Pb and Cu (Aljohani et al., 2023).

At the end of the depuration, *MGST*-like were the only genes to be increased in gills of mussels transferred from PAD to Almadies. The lack of increase of the *MGST*-like gene could be a compensation for the low expression of *SOD*-like gene as concluded by Manduzio et al. (2004) concerning a switch in the modulation of SOD and GST in *Mytilus edulis* gills.

Enzymatic biomarkers

Oxidative stress enzyme

The present study does not suggest that GST plays an important role in the cellular defense against oxidative stress. This is in line with the findings of Fernández et al. (2010), arguing that in some field studies, a clear response of GST in mussels exposed to pollutants was observed while in others, little or no response was reported. The lack of correlation between GST and *GSTO*-like and *MGST*-like joins the point of view of Letelier et al. (2010), who argued that GST constitute many isoenzymes.

Metabolic-related enzymes

The 20% inhibition of AChE enzyme activity recorded in digestive glands after mussels were were caged at PAD, is indicative of the presence of contaminants. This level of AChE depression found in this study is lower than those found by Bocquené et al. (2004) in mussels gills (30%) in comparison with nearby reference sites. They also found negative correlations between AChE and PAHs. Selenium (Se) and to a lesser extent, Pb are likely among the TEs that induced the

depression. From a toxicological perspective, Se can become extremely toxic at concentrations slightly above its nutritional levels (Kim and Kang, 2015). Pb-induced ROS cause central nervous system cell signaling deregulation and neurotransmission impairment (Dos Santos et al., 2022).

The 60% inhibition of AChE observed at the end of the depuration time in mussels transferred from PAD to Almadies is above the sublethal impact threshold of the Oslo-Paris (OSPAR) Commission (between 20 and 50%) (OSPAR, 2013). The OSPAR Convention is the legal instrument guiding international cooperation on the protection of the marine environment of the North-East Atlantic (NEA). It is the European equivalent of the Abidjan Convention which govers international coorperation on the protection of the marine environnement of South Atlantic.

LDH activity in gills of mussels caged at PAD remained unchanged in relation to pre-exposure levels meanwhile these tissues are major site of uptake of waterborne chemicals. LDH decrease in digestive glands observed is in line with results found by Georgieva et al. (2022). The latters reported significant decreases of the activity after sub-chronic exposure of encaged mussels to anthropogenic pollution indicating disturbances in the metabolism linked chemcials such as PAHs.

At the end of depuration, LDH activity decreased in gills whereas it increased in digestive glands. The decrease in gills could be due to a decrease in overall biosynthetic activities (Schaefer et al., 2021).

Conclusion

This pilot study is a first step in establishing protocols to monitor contaminants in coastal ecosystems of Senegal the western zone of the Abidjan Convention. It is the first one of this kind in Senegal. The results suggest that *Perna perna* can be a useful bioindicator for environmental contamination. The analyses demonstrated the possibility of including genes expression and enzymatic activities as biomarkers.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

FT : Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original draft and editing, Visualization, Funding acquisition, Project administration. DD : Conceptualization, Methodology, Formal analysis, Investigation, Resources. DGN : Conceptualization, Methodology, Formal analysis, Investigation, Resources, Validation. FC : Conceptualization, Methodology, Formal analysis, Investigation, Resources, Validation. DDe : Conceptualization, Methodology, Formal analysis, Investigation, Resources, Validation. NL : Conceptualization, Methodology, Investigation, Resources, Validation. KH : Methodology, Investigation, Writing – Original draft and editing, Validation. CD : Conceptualization, Methodology, Investigation, Resources. MC : Conceptualization, Methodology, Investigation, Resources. CTB : Conceptualization, Methodology, Investigation, Resources, Validation. MF : Conceptualization, Methodology, Investigation, Resources, Validation.

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