



Cadmium and Polyaromatic Hydrocarbons Exposure Changes the Condition Indices in *Dreissena polymorpha* (Pallas, 1771): A Case Study

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Abstract: Cadmium (Cd) and polyaromatic hydrocarbons (PAHs) are priority pollutants in surface waters according to Directive 2013/39/EC. They are toxic, persistent and tend to accumulate in high concentrations in aquatic organisms. In the present study, zebra mussel *Dreissena polymorpha* was confirmed as a bioindicator for contaminated freshwater ecosystems. We aimed to study the effects of short-term (96 h) and long-term (31 days) exposure to Cd and PAHs on the Condition index (CI) and Soft Tissue Wet Ratio (STWR) in zebra mussel. Overall, we found alterations in both CI and STWR (as compared to the control group) at all tested concentrations, including the one below the allowable concentration according to the EU legislation. These results demonstrated the toxicity of both contaminants. Furthermore, we confirmed that the studied condition indices could be successfully applied as biomarkers as they are fast and low-cost in future monitoring and risk assessment of polluted freshwater ecosystems.

Key words: Cd, PAHs, Zebra mussel, Biomarkers, Condition index, Soft tissue wet ratio

Introduction

Heavy metal pollution in aquatic systems, especially in freshwater systems, has become a serious environmental problem in many countries (HAN et al. 2016, ERK et al. 2018). For instance, Cd has become an increasingly severe environmental pollutant, in which human activities such as electroplating, chemical, electronics and nuclear industries increase its availability (LIAO et al. 2017, ESPOSITO et al. 2018). This toxic element, which can be found naturally in coastal environments or as a result of anthropogenic activities, is mainly accumulated by molluscs and crustaceans (EFSA 2012, MAX BLANC et al. 2018). Polycyclic aromatic hydrocarbons (PAHs) are normally a fixed core in the main lists of organic pol-

lutants measured worldwide (OSPAR 2010, VIÑAS et al. 2018) and cover a wide range of pollution sources including industrial, urban and traffic. In addition, while PAHs can also occur naturally, the main environmental input is anthropogenic. PAHs can enter the environment as products of incomplete combustion of fossil fuels (pyrolytic) or from petrogenic sources. PAHs are constituents of crude oil and are present in the marine environment as a result of natural seeps, oil spills, shipping movements and from activities associated with offshore oil and gas exploration and production. Furthermore, PAHs are of concern as metabolites of some of the high molecular weight PAHs, such as benzo[a]pyrene, as they are potent animal and human carcinogens (WEBSTER et al. 2018).

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To assess potential biological effects, biomarkers have been widely applied in aquatic toxicology. These include characteristics at the molecular, cellular, tissue or organism level that indicate exposure of the organism to pollutants (“exposure biomarkers”) and (or) the magnitude of the response to such exposures (“effect biomarkers”) (MCCARTHY & SHUGART 1990).

BROOKS & RUMSBY (1965) first found that bivalves have an extremely high tolerance to heavy metal exposure and a good ability to bioaccumulate heavy metals, especially Cd. The reasons may be that (1) bivalves commonly have low metabolism, high filter-feeding activity and longer life, which gives them the ability to tolerate chemical exposure and bioaccumulate pollutants (including organic and inorganic pollutants) (BONNAIL et al. 2016, AGUIRRE-RUBÍ et al. 2018), (2) bivalves are sedentary, contacting both water and sediment directly, to which their body contaminant burdens normally can be attributed (ZUYKOV et al. 2013), and (3) the large amount, wide distribution in the water system and suitable size of bivalves are appropriate for researchers to collect and assess the exact contamination situation in different water environments (ANACLETO et al. 2015). The concept of “mussel watch” was proposed by Goldberg (GOLDBERG et al. 1978, GOLDBERG 1986). Marine mussels as sentinel organisms have been successfully used to monitor seawater environment pollution (BESADA et al. 2014, KIBRIA et al. 2016). The zebra mussel *Dreissena polymorpha* is a highly invasive bivalve species, which is native for the Ponto-Caspian Region. This dreissenid species has successfully colonised freshwater systems in Western Europe and North America, engendering significant changes in ecosystems mainly by biofouling and impacting phytoplanktonic biomass (CUHEL & AGUILAR 2013, BÓDIS et al. 2014). Due to its ecological success, biological characteristics and high bioaccumulation potential, the zebra mussel constitutes an extensively used indicator species for biomonitoring of water pollution and laboratory experiments (FARIA et al. 2014, PAIN-DEVIN et al. 2014, PAROLINI & BINELLI 2014, POMA et al. 2014, CHÂTEL et al. 2015, PAROLINI et al. 2015, KERAMBRUN et al. 2016). It was recently described as the freshwater counterpart of the blue mussel for ecotoxicological studies (BINELLI et al. 2015) and we confirmed this in our previous studies (YANCHEVA et al. 2018, 2019).

The purpose of the present study was to further explore the negative effects of Cd and PAHs on zebra mussel. We have observed that very few data for

various biometric indices are available and, for the zebra mussel, such data are even scarcer. Therefore, we aimed to measure two indices, i.e. Condition Index (CI) and Soft Tissue Wet Ratio (STWR), in zebra mussels exposed to Cd and PAHs in short-term (96 hours) and long-term (31 days) experiments as well as to verify if they could be used as appropriate biomarkers.

Materials and Methods

The experimental design was previously explained in detail (YANCHEVA et al. 2018, 2019). Biometric analyses were made prior to the calculation of Condition (State) Index (further Condition Index I, CI I) and the Soft Tissue Wet Ratio Index (further Condition Index II, CI II) as described by GASMI et al. (2017). The whole weight (Ww) of each mussel was measured in g with an analytical scale (Kern, Germany) after cleaning the shell from epibionts and other debris. Then the mussels were carefully opened to separate the flesh from the shell with a stainless steel knife. The intervalvular liquid was removed. The soft tissues were drained on absorbent paper and wet-weighted. The shells were measured with callipers in mm to calculate the studied indices. They were calculated as follows:

$$\text{CI I: } [\text{soft tissue wet weight (g)}] \times [\text{shell length (mm)} \times 100]^{-1}$$

(see KAGLEY et al. 2003, GALVAO et al. 2015)

$$\text{CI II: } [\text{soft tissue wet weight (g)} \times 100] \times [\text{total animal fresh weight (g)}]^{-1}$$

(see MARQUES, 1998, GALVAO et al. 2015)

The statistical analyses were conducted using the PAST v. 3.20 (HAMMER et al. 2001). The raw data on indices were tested for normal distribution with the Shapiro-Wilk normality test and descriptive statistics (min, max, mean, median, standard deviation (SD) and standard error (SE)) were calculated. Since the data had no normal distribution, the Kruskal-Wallis test was applied to see if there was a significant difference for both indices in the control and the mussels exposed to different concentrations of Cd and PAHs on the 24th, 48th, 72nd and 96th hour as well as on the 31st day. Spearman's correlation analysis was applied when the data were not normally distributed in order to see if there was a linear correlation between the measured indices and the passed time (FOWLER et al. 1998). All statistical differences were accepted at the 0.05 level of significance.

Results

There are various methods in the literature (ENGLÉ 1949, LUCAS & BENINGER 1985, DAVENPORT & CHEN 1987, CROSBY & GALE 1990, LAGADE et al. 2015) for calculating the condition indices in bivalve molluscs. They are based on the ratio of the weight and volume of the shell; some formulas use fresh weight and others use dry weight. Furthermore, MANN (1978) included volumetric and gravimetric meat-to-shell ratios, biochemical and physiological indices and a comparison of biochemical and gravimetric indices. For example, some of the most widely applied indices are Meat Yield (MY), Body Condition Index (BCI), Hepatopancreas Index (HI), Gonadal Index (GI) and Shell Component Index (SCI). We also decided to use the wet tissue weight in our analyses. Descriptive statistics of CI I and CI II) in zebra mussels exposed to Cd and PAHs for 96 hours and 31 days, including control mussels are presented in Table 1.

The results on CI I and CI II showed that the values fluctuated during the study period. In addition, they fluctuated between the tested hours but were similar for each hour between the different applied concentrations. Overall, both indices were lower for the mussels treated with Cd compared to PAHs (Table 1). Exceptions were the results for CI II measured for Cd 1 µg on the 24th hour, CI I measured for Cd 1 and 4 µg and CI II measured for Cd 4 µg on the 72nd hour, CI I measured for Cd 1 and 4 µg and CI I measured for Cd 4 µg on the 96th hour as well as CI I measured for Cd 2 and 4 µg and CI II measured for Cd 1, 2 and 4 µg on the 31st day. In addition, we found statistically significant differences between the control and all three tested concentration for Cd and PAHs for the short- and long-term experiments as shown in Table 1. Furthermore, we found strong negative correlation between the past time in the short-term exposure and both indices for the mussels treated with different Cd and PAHs concentrations, although they were not statistically significant ($P > 0.05$).

Discussion

Some authors have shown that the fluctuation of indices can be affected by pollution (ALMEIDA et al. 2011, ROUANE-HACENE et al. 2015). We agree with YAP & AL-BARWANI (2012) that these indices are an indirect estimate of the health condition of the organism when it is under environmental stress. In addition, as stated by WIDDOWS (1985), these indices are simple and reflect changes in the nutri-

ent state of mussels, such as stored energy reserves and metabolic responses to environmental stress. Furthermore, according to WYATT et al. (2014), while loss of weight can be the first sign of environmental or physiological stress in mussels, other methods may be used to support these observations. Our results are in close agreement with this opinion and our previous studies on the effects of Cd and PAHs on zebra mussel (YANCHEVA et al. 2018, 2019). Lower CI values in molluscs collected from metal-contaminated sites were also previously reported by LARES & ORIANS (1997) and LEUNG & FURNESS (2001). ROBELO et al. (2005) demonstrated that in the oyster *Crassostrea rhizophorae* (Guilding, 1828) this index was negatively correlated with the concentration of Cd in the sediment. The same correlation was demonstrated in the grooved carpet shell *Tapes decussatus* (Linnaeus, 1758) in the Gulf of Gabès (southern Tunisia) (SMAOUI-DAMAK et al. 2006). We also found a negative correlation between the studied indices in zebra mussel and toxicant concentrations in the water and the applied Cd and PAHs concentrations.

Conclusions

We can recommend both CI I and CI II as easily applied and representative biomarkers to determine the effects of heavy metals and organic contaminants on bivalves. Additionally, we agree with GALVAO et al. (2015) that these indices are not restricted only to aquaculture, but they can also be widely used as biological tools in studies of environmental contamination and to assess the relation between the contaminants and the mussel health. However, we suggest that other indices, such as Meat Yield (MY), Body Condition Index (BCI), Hepatopancreas Index (HI), Gonadal Index (GI) and Shell Component Index (SCI) are also included in future bioaccumulation studies in order to deepen our knowledge in this particular field.

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Table 1. Condition (State) Index (CI I) and Soft Tissue Wet Ration Index (STWR, CI II) in zebra mussel exposed to Cd and PAHs for 96 hours and 31 days.

Index	CI I							STWR, CI II							
Groups	Control	PAHs 1µg	PAHs 2µg	PAHs 3µg	Cd 1µg	Cd 2µg	Cd 4µg	Control	PAHs 1µg	PAHs 2µg	PAHs 3µg	Cd 1µg	Cd 2µg	Cd 4µg	
24 hours															
Min	0.038	0.141	0.230	0.200	0.161	0.131	0.156	0.056	0.013	0.307	0.250	0.258	0.162	0.141	
Max	0.078	0.218	0.350	0.305	0.196	0.170	0.211	0.250	0.378	0.554	0.712	0.339	0.350	0.562	
Mean	0.063	0.187	0.293	0.264	0.179	0.157	0.188	0.164	0.252	0.419	0.524	0.301	0.285	0.364	
Median	0.066	0.204	0.296	0.268	0.179	0.161	0.189	0.171	0.279	0.453	0.567	0.314	0.289	0.370	
Std. error	0.007	0.014	0.019	0.018	0.006	0.007	0.010	0.034	0.062	0.048	0.080	0.018	0.034	0.067	
Std. dev.	0.016	0.031	0.043	0.040	0.013	0.015	0.022	0.077	0.139	0.108	0.178	0.040	0.077	0.149	
Kruskal-Wallis	H=15.42, p=0.001				H=13.81, p=0.003			H=11.61, p=0.009				H=9.24, p=0.026			
48 hours															
Min	0.038	0.091	0.081	0.070	0.060	0.055	0.050	0.056	0.120	0.146	0.125	0.081	0.086	0.118	
Max	0.078	0.120	0.157	0.091	0.100	0.087	0.091	0.250	0.198	0.166	0.181	0.145	0.194	0.181	
Mean	0.063	0.101	0.107	0.080	0.079	0.072	0.072	0.164	0.158	0.156	0.152	0.121	0.132	0.147	
Median	0.066	0.095	0.093	0.081	0.070	0.071	0.074	0.171	0.162	0.158	0.154	0.130	0.131	0.136	
Std. error	0.007	0.005	0.014	0.004	0.008	0.005	0.007	0.034	0.013	0.004	0.011	0.012	0.018	0.012	
Std. dev.	0.016	0.012	0.031	0.008	0.018	0.012	0.015	0.077	0.030	0.009	0.025	0.026	0.040	0.026	
Kruskal-Wallis	H=13.66, p=0.003				H=1.27, p=0.733			H=0.24, p=0.969				H=1.80, p=0.614			
72 hours															
Min	0.038	0.047	0.031	0.038	0.055	0.026	0.057	0.056	0.089	0.006	0.051	0.074	0.043	0.108	
Max	0.078	0.089	0.080	0.081	0.080	0.087	0.114	0.250	0.200	0.162	0.145	0.175	0.119	0.141	
Mean	0.063	0.064	0.057	0.060	0.069	0.062	0.086	0.164	0.128	0.096	0.092	0.125	0.090	0.130	
Median	0.066	0.064	0.052	0.068	0.066	0.063	0.087	0.171	0.108	0.105	0.086	0.104	0.100	0.135	
Std. error	0.007	0.007	0.009	0.009	0.005	0.010	0.009	0.034	0.020	0.026	0.017	0.020	0.013	0.006	
Stand. dev.	0.016	0.017	0.019	0.019	0.011	0.023	0.021	0.077	0.045	0.059	0.038	0.045	0.030	0.013	
Kruskal-Wallis	H=0.31, p=0.958				H=4.07, p=0.251			H=3.67, p=0.297				H=5.65, p=0.130			
96 hours															
Min	0.038	0.060	0.058	0.059	0.060	0.057	0.083	0.056	0.112	0.098	0.095	0.056	0.097	0.148	
Max	0.078	0.093	0.106	0.085	0.140	0.070	0.100	0.250	0.172	0.166	0.128	0.090	0.151	0.170	
Mean	0.063	0.071	0.073	0.069	0.087	0.064	0.091	0.164	0.131	0.115	0.111	0.069	0.115	0.157	
Median	0.066	0.068	0.069	0.060	0.072	0.065	0.091	0.171	0.127	0.104	0.108	0.062	0.112	0.151	
Std. error	0.007	0.006	0.009	0.006	0.014	0.002	0.004	0.034	0.011	0.013	0.006	0.007	0.010	0.004	
Std. dev.	0.016	0.013	0.020	0.013	0.032	0.005	0.009	0.077	0.024	0.029	0.013	0.015	0.022	0.010	
Kruskal-Wallis	H=0.72, p=0.867				H=9.88, p=0.019			H=4.86, p=0.182				H=10.25, p=0.016			
31 days															
Min	0.038	0.040	0.026	0.033	0.033	0.050	0.070	0.056	0.067	0.004	0.043	0.056	0.097	0.148	
Max	0.078	0.057	0.040	0.055	0.047	0.083	0.100	0.250	0.087	0.082	0.132	0.090	0.151	0.170	
Mean	0.063	0.046	0.033	0.044	0.041	0.058	0.084	0.164	0.077	0.050	0.098	0.069	0.115	0.157	
Median	0.066	0.042	0.033	0.042	0.043	0.052	0.081	0.171	0.076	0.054	0.109	0.062	0.112	0.151	
Std. error	0.007	0.003	0.003	0.004	0.002	0.006	0.006	0.034	0.003	0.013	0.015	0.007	0.010	0.004	
Std. dev.	0.016	0.007	0.007	0.008	0.005	0.014	0.012	0.077	0.007	0.028	0.034	0.015	0.022	0.010	
Kruskal-Wallis	H=9.77, p=0.019				H=12.12, p=0.007			H=8.69, p=0.034				H=10.25, p=0.016			

***Bold** – significant differences (P < 0.05)

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