Effects of phthalates on the biology of the mussel *Mytilus galloprovincialis* (from the Atlantic Coast of El Jadida, Morocco)

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Introduction

Phthalates are found in many consumer products (can coating, shampoos, food containers, *etc*.). Di-2-ethylhexyl phthalate (DEHP) is widely used in the medical field.[1] DEHP represents about 50% of the 18 esters marketed as phthalates. DEHP and n-dibutyl phthalate (DBP) are the most frequent components found in collected environmental samples.[2] There are little scientific data about phthalates in marine animals, including bivalves. These organisms are used to estimate the quality of coastal ecosystems in several biomonitoring programs. In the Moroccan Atlantic area of El Jadida, the bioaccumulation of metals and induction of metallothionein was revealed by several authors.[3-5] This research is an important component for our research team, to assess the health of the El Jadida coastal region. Our aim is to estimate the effect of potassium hydrogen phthalate (KHP) on growth, mortality and degree of filling of the mussel *Mytilus galloprovincialis*.

Materials and Methods

The mussels of the species *M. galloprovincialis* were collected during low tide at the wild mussel cluster of beach of El Jadida. They were stored in the laboratory in containing continuously oxygenated seawater (n=50), with acclimatization periods of 36 h. For this experiment, sizes of 1, 2 and 3 cm were chosen for the mussels, and the contamination doses for the potassium hydrogen phthalate [C8H5KO4 (KHP)] were of 250 mg and 500 mg/kg of mussel for the first treatment period (2 and 4 mg/L for small; 20 and 40 mg/L for medium; 50 and 100 mg/L for large size mussels); and of 750 mg and 1000 mg/kg for the second treatment period (6 and 8 mg/L for small; 60 and 80 mg/L for medium; 150 and 200 mg/L for large size mussels). Each treatment period was 3-week long. The seawater (2 L/animal groups) is changed each day for theses animals. Control animals (three sizes) were set in the same conditions but without any contamination. Mortality, growth and condition index were the parameters studied in all groups (experimental and control): i) the mortality rate was assessed by inventorying deceased animals every 24 h, for 6 weeks. It is expressed in percentage (%). Dead animals were removed; ii) the study of the growth levels is based on the measurement of the length of the shell of the contaminated mussels; once a week during the six weeks of exposure to the KHP; using calipers. Five individual mussels were randomly selected from each tray for this purpose; iii) the condition index is a biometric index that reflects the degree of filling of the mussel shell. It is calculated through the following formula (AFNOR index):

$$CI = (\text{soft tissue weight} / \text{total weight}) \times 100. \quad (1)$$

The analysis of variance (ANOVA) and Student's *t* test were used to assess the results (TGV Biosta software).

Results and Discussion

Mortality study

Following the contamination of the mussels at 250 mg/kg and 500 mg/kg of KHP, for small and medium-size individuals (respectively 1 and 2 cm), we did not observe any deaths during the first three weeks. The mortality rate reached a maximum of 10% for small mussels on the 5th week. The 3 cm mussels represented 7% of the mortality during the 3rd week period, versus 2% in the control group. During the 2nd stage when the KHP doses were increased, the mortality rate increased significantly to 14% at 6 weeks of exposure at 750 mg/kg of KHP and to 21% on the 5th week of exposure at the 1000 mg/kg levels.

Study of the growth

The first result to note is the KHP negative effect on the growth of *M. galloprovincialis*. This effect is more significant for large mussels (3 cm) that were exposed to 750 and 1000 mg/kg of KHP (Figure 1).

Study of the condition index

A slight difference in condition index (CI) is noticed between mussels exposed to KHP and the control groups. The highest and significant value was 17.54 recorded in control animals versus 15.66 for those of 3 cm in size exposed to 500 mg/kg of KHP (first treatment period). The other significant difference of the CI is noticed in mussel of the same size are 13.86 of control versus 12.86 and 12.30 exposed to 750 and 1 000 mg/kg of phthalate respectively (second treatment period).

Conclusions

In the light of the results, we can conclude that the mussel *M. galloprovincialis* can survive high levels of phthalate exposure, at least, during the 6 weeks. Disturbances are notably found on the growth rate across the length of the shell and its degree of filling. According to data from the literature, the phthalate DEHP in rats has a very low acute toxicity as the lethal dose required to reach 50% mortality (LD 50) is greater than 25 g/kg when administered orally and 200 mg/kg when administered by parenteral way. As well, the observed effect is mostly physiological. In fact, after administration to rabbits, by *forced feeding*, increasing doses of di-n-butyl phthalate at 250, 500, 750 mg/kg/day for 4 weeks, had no impact on mortality, but blood-toxic, hepatotoxic, reprotoxic effects together with effects on kidneys were observed.[6,7] Genital malformations were also observed in male rats under the influence of phtalates.[8] This study should be supplemented by the extraction of phthalates (*via* Soxhlet) and phthalate detection achieved by gas chromatography or mass spectrometry. Subsequently a physiology disturbance study should be conducted.[9]

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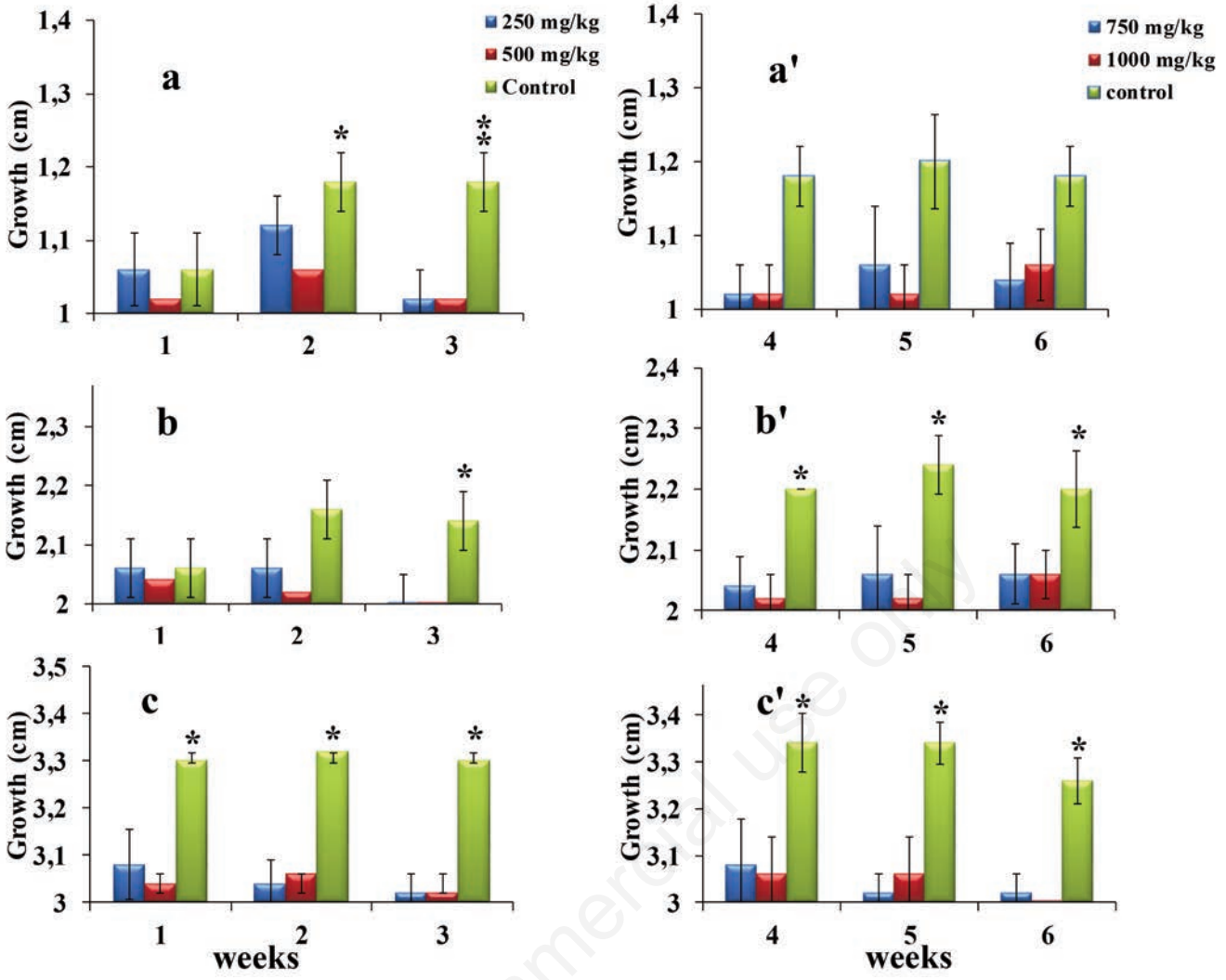

Figure 1. Evaluation of growth in the mussel Mytilus galloprovincialis of small (a, a'), medium (b, b') and large (c, c') size, exposed to potassium hydrogen phthalate. (*) Meaning P<0.05 between control and [KHP]; (**) significance P<0.05 between control and [KHPs].

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