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The effects of heavy metal concentration on bio-accumulation, productivity and pigment content of two species of marine macro algae

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Abstract Environmental pollution due to heavy metals is a world-wide problem in estuarine, coastal and marine waters. Metal pollution affects different organisms in different ways and the degree of the impact is site-specific. Aim of the present study was to determine the effects of heavy metals *viz.*, Cu, Cd and Pb exposure on tissue accumulation, pigmentation and productivity of two marine macro algae, *Fucus vesiculosus* and *Ulva lactuca* under controlled laboratory conditions. Algae were collected from a reference location, Wemeldinge in the Eastern Scheldet Estuary in Netherlands. Metal concentrations were determined using ICP-MS. Productivity of algae was measured using Winkler method and the results were expressed as carbon equivalent. Pigment profiles of two species were analyzed by spectral absorbance over 250 -1100 nm range. The results revealed that the metal accumulation in tissues significantly increased with increasing exposure to metal concentration whereas, pigmentation, and photosynthetic productivity decreased with increasing metal concentration. Hence, *F. vesiculosus* and *U. lactuca* could be used as bio-indicators to determine metal pollution in coastal waters.

Keywords: heavy metal pollution, *Fucus vesiculosus*, *Ulva lactuca*, bio-indicator

INTRODUCTION

Most countries in the world are presently facing chemical pollution problem severely with increasing industrial activities as well as rapid population growth and daily human activities. Metal pollution in the marine ecosystem due to anthropogenic activities is well documented (De Kock and Kramer 1994; Galloway et al. 2002). Hence, chemical pollutants such as heavy metals and their impacts on the aquatic environment will always be a matter of concern.

Biological monitoring or bio-monitoring is defined as the systematic use of biological responses to evaluate changes in the environment, with a view to establishing a quality control programme (Cairns and van der Schalie 1980). Such bio-monitors should involve relatively inexpensive equipment and methodology that are easy and fast to perform rather than introducing very sophisticated analytical methods using advance technologies and instrumentation that cannot be affordable by most developing countries. Therefore, such an approach may be an alternative method to monitor pollution status of developing countries.

Environmental pollutants originating from diverse anthropogenic sources have been known to possess adverse effects capable of degrading the ecological integrity of marine environment (Torres et al. 2008). In many situations, the consequences of anthropogenic contamination of marine environments have been ignored or poorly identified (Cairns 1982). Monitoring the impact of pollutants on aquatic life forms is challenging due to the differential sensitivities of organisms to a given pollutant, and the inability to assess the long-term effects of persistent pollutants on the ecosystem as they are bio-accumulated at every trophic level (Torres et al. 2008).

Aquatic environments are more susceptible to the harmful effects of heavy metal pollution because aquatic organisms are in close and prolonged contact with the soluble metals (Torres

et al. 2008). Marine macro algae are in particular potential indicator species for organic and inorganic pollutants since they are comparatively more abundant life forms in aquatic environments and occupy the base of the food chain (Torres et al. 2008).

A good choice of a bio-monitor species is an integral part of a successful bio-monitoring programme. Therefore, it is important to select a good candidate with relevant qualities that suit the best general situation of the study environment. For organic xenobiotics, algae play an important role in the dispersal (Wang et al. 1998; Kowalewska 1999), chemical transformation and bioaccumulation of many toxic compounds (Okay et al. 2000; Lei et al. 2002, 2007; Murray et al. 2003).There are special advantages of using marine macro algae as 'sentinel' or 'indicator' organisms in environmental programmes throughout the world.

Many studies have reported uptake of heavy metals by different macro algae (Bryan 1969; Phillips 1990; Leal et al. 1997). Further, macro-algae (seaweeds) have increasingly been used as bio-detectors to monitor xenobiotics in marine environments (Levine, 1984; Stewart, 1995; Whitton and Kelly, 1995). Use of macro algae in bio monitoring studies was limited in 1980s although marine macro algae have been shown to be good bio-indicators of heavy metal contamination in seawater (Bryan 1983; Soderlund et al. 1988). However, use of marine algae as test organisms in laboratory studies of marine pollution has gradually been increased (Fletcher 1991). Heavy metals may also lead to a decrease in primary productivity of macro-algae.

Therefore, this study was carried out to examine to what extent does the heavy metal exposure impacts on bio-accumulation, primary productivity and pigment content of two species of marine macro-algae.

MATERIALS AND METHODS

Source of the plants

Two species of macro-algae, *Ulva lactuca* (a green alga) and *Ficus vesiculosus* (a brown alga) were collected from Wemeldinge in the Eastern Scheldt estuary in Netherlands (Fig. 1) and were transported to the laboratory in sea water. In the laboratory, they were further cleaned using clean seawater to remove any sand or other epiphytes and acclimatized for the laboratory conditions for 72 hours.

Exposure medium

The seawater used in the experiments was prepared from hw Sea Salt by dissolving 35 g/L sea salt in deionised water (Wiegandt GmbH, Germany). This artificial sea water was then enriched with nutrients and filtered before use (pore size 0.45µm filter paper).

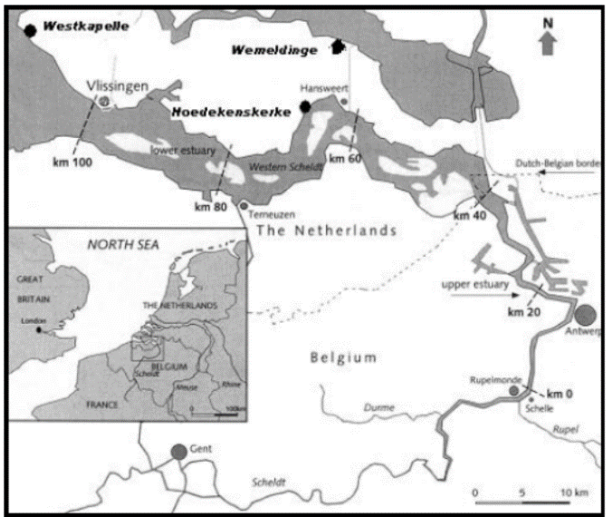

Fig. 1 Sampling location (Wemeldinge) in the Scheldt estuary in the Netherlands.

Metal concentrations

Test metals mixture (100 µM stock solution) was prepared in the form of high grade chloride salts (for Cu and Cd) and a nitrate salt (for Pb) of the metals. These heavy metals were specifically selected in order to have representative metal, Cu among essential elements whereas Cd and Pb represented non-essential elements. To make the metal mixture (100µM stock solution), $CuCl_2$, $CdCl_2.H_2O$ and $Pb(NO_3)$ with the weights of 17.048 mg, 20.132 mg and 33.12 mg, respectively were dissolved in 1 L of artificial sea water. From this stock solution, the dilutions series of 0.01, 0.1, 1.0 and 10.0 µM were prepared (Final concentrations were measured using ICP-MS) by mixing suitable amounts of artificial sea water to give different exposure levels for the organisms during lab experiment.

Experimental setup

The experiment was conducted in a climate chamber. Three individual plants from each algal species were placed in each exposure concentrations (0.01, 0.1, 1.0 and 10 μmol l^{-1}) as well as in clean seawater as control (0 μmol l^{-1}), resulting in a total of five treatments for each algae species. Water temperature in all the tanks was maintained at 16(±0.5)°C. Light was from fluorescence tubes (Philips TLD 38W/840). These light and temperature conditions were chosen as they allowed the most stable conditions in culture for the algae (unpublished data). Also water was constantly aerated with filtered air. After 48 hours first sub sampling was done from each exposure medium as three pieces of each species (one piece from one plant) in order to use in different analyzing procedures i.e. (i) material for metal analysis which was approximately 0.1 g, (ii) material for productivity measurements, approximately 1 g and (iii) for pigment analysis, approximately 0.01 g. This was repeated after 98 hours and 504 hours for each species.

Primary productivity analysis in marine plants

Primary productivity of algae (piece) was measured using light and dark bottle method (Gaitán-Espitia 2011) in three replicates. The amount of oxygen produced was then converted into the equivalent carbon that the algae incorporated over the same period.

Pigment concentrations analysis in marine plants

Concentrations of pigments were determined using spectrometric measurements. Firstly, tissue homogenation in liquid nitrogen followed by pigment extractions in acetone. The samples then scanned for spectral absorbance over the range of 350–750 nm wavelengths (the absorbance was read in a spectrophotometer at every 1 nm or 20 nm) with respect to a blank sample (i.e. only acetone) that was used to extract the pigments.

Metal analysis in marine plants

Fifteen replicates for each species were inserted into certified trace metal-free tubes and oven dried at 60ºC for 72 hours. After drying, the tissues were weighed to obtain dry tissue weight and digested in concentrated (99%) HNO_3 for at least 12 hours. Samples were then digested by heating in a microwave oven during four consecutive steps i.e. for 8 minutes. Samples of reference materials of algae tissue (BCR-279, *Ulva lactuca*) were always included with each batch of samples for verification of the measurements. Reagent blanks were also included. After digestion, sample volumes were made up to 12 ml with Milli-Q water.

Metal concentrations in the samples were determined using Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Varian, Australia) with certified reference samples (BCR-279).

Statistical Analysis

GraphPad Prism Ver. 5 software was used to make graphs and all statistical analyses. Kruskal-Wallis test was performed for comparisons and to confirm whether the observed result was significant followed by Dunn's Multiple Comparison Post-hoc Test.

RESULTS

Metal concentrations in both algae species after 48h, 96h and 504h of exposure to a wide range of concentrations (0.01 – 10 μM) are shown in Figure 2.

As shown in Figure 2, metal accumulation only appeared to increase at exposure concentration above 1 μM of metal. Results shown that metal accumulation in the highest exposure (10 μM) was significantly higher for both species and for all the three metals studied.

The data was also tested to see if there were differences in accumulations among the three sampling times (48h, 96 and 540h) and the results revealed the levels of metal accumulations at high exposure time were not high enough to be significantly different from the earlier sampling times.

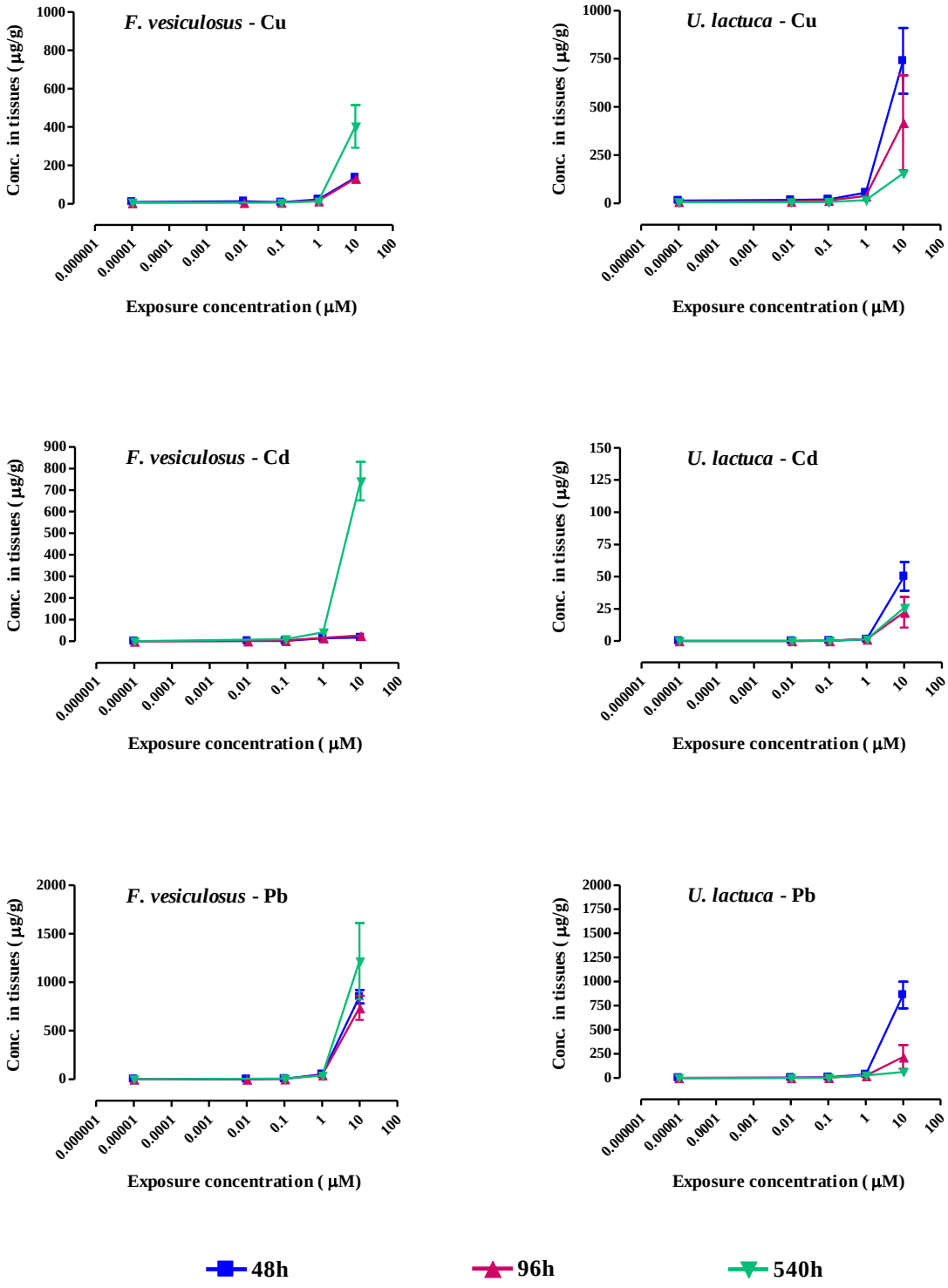

Fig. 2 Net metal accumulations (mean ±SD) in *F. versiculasis* and *U. lactuca* at the range of exposure concentrations for 48 h, 96 h and 540 h.

Figure 3 shows the mean spectral absorbance between 350 nm and 750 nm wavelength for *U. lactuca* and *F. vesiculosus* at three different sampling time periods. Here, the area of the peak Chlorophyll a, b and c pigment profiles of *F. vesiculosus* and *U. lactuca* are known to contain these pigments between 600 – 740 nm.

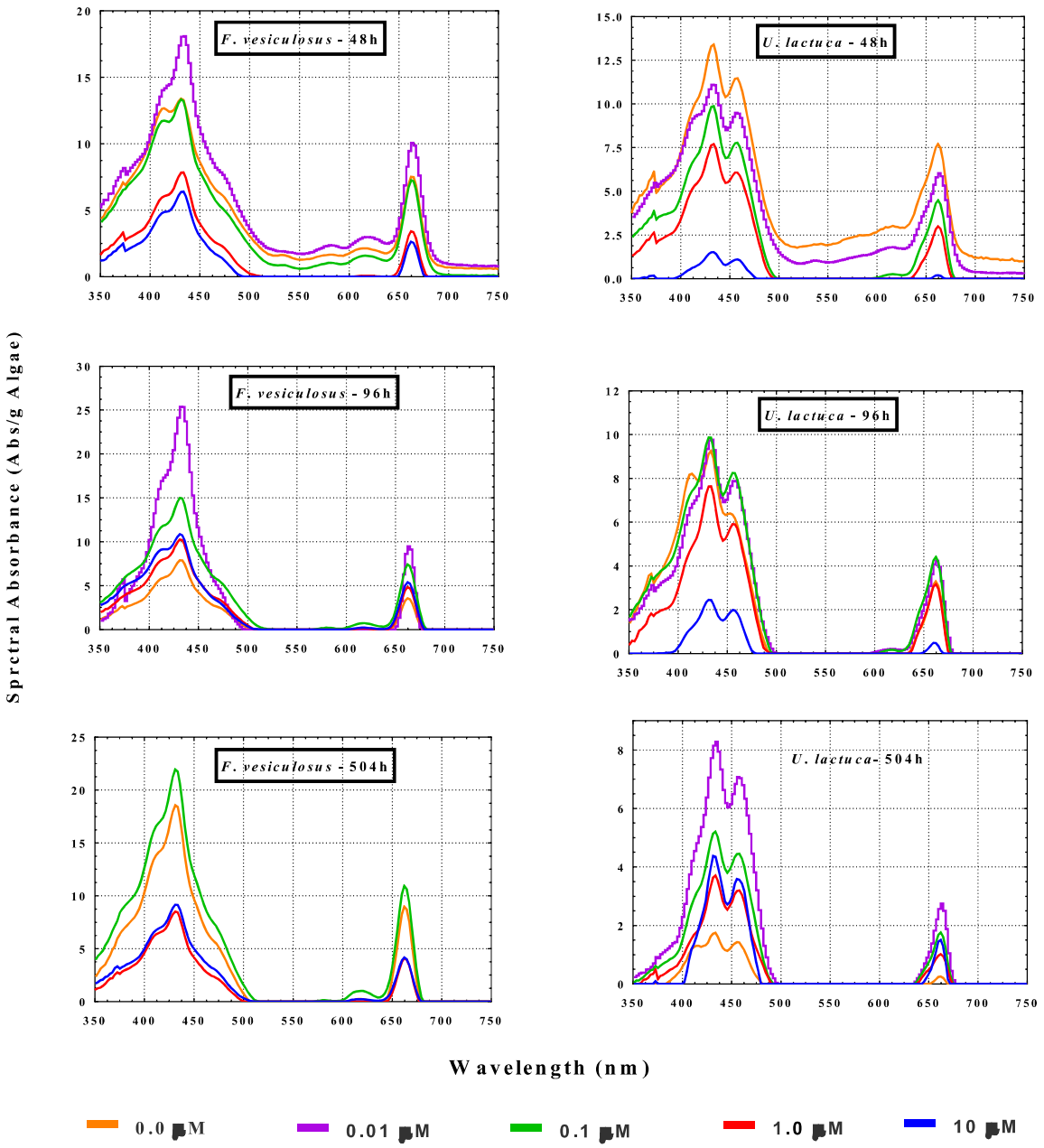

Fig. 3 Differences in pigmentation of U. *lactuca* and F. *vesiculosus* after 48h, 96h and 504h respectively.

The effects of metal exposure (Cu, Cd and Pb mixture) and time (48h, 96h, and 504h) on primary productivity of *F. vesiculosus* and *U. lactuca* are shown in Figures 4 and 5 respectively. In all exposure concentrations *F. vesiculosus* showed low amount of net productivity than *U. lactuca.*

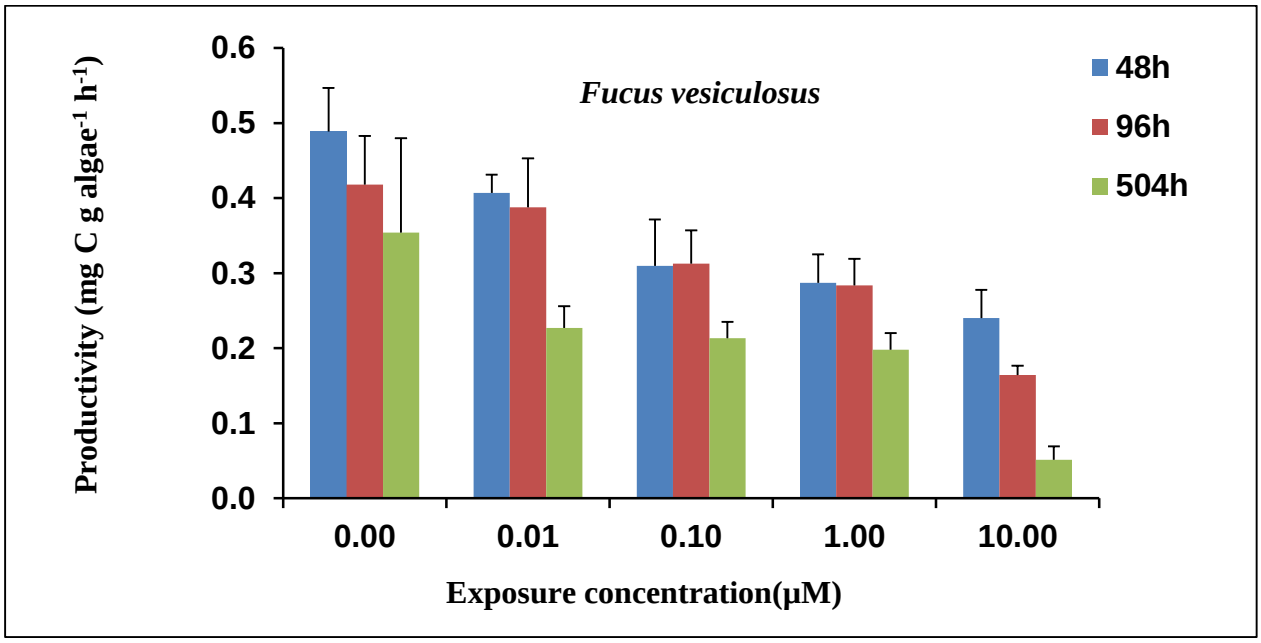

Fig. 4 Productivity (mean±SD in mg C/g algae/h) of *F. vesiculosus* at different exposure concentrations (µM) and exposure time (hrs)

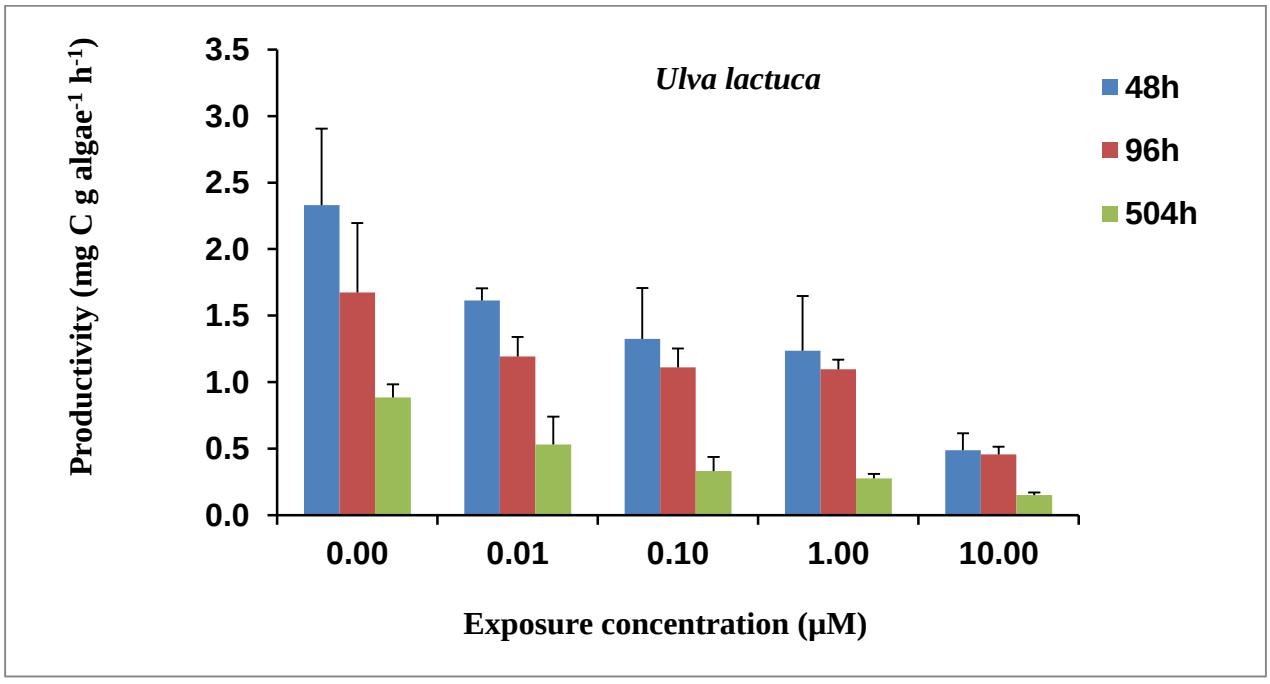

Fig. 5 Productivity (mean±SD in mg C/g algae/h) of U. *lactuca* at different exposure concentrations (µM) and exposure time (hrs)

Kruskal-Wallis test showed that the observed accumulation was significant for all the three metals and also for both species as well as for exposure concentrations. However the results of Post-hoc test (Dunn's Multiple Comparison) showed the metal accumulation was significantly high for both species and for all the three metals at the highest exposure (10 μM). Accordingly in order to obtain significant values it is necessary to expose them to the concentrations above 1μM.

DISCUSSION

In order to use pigmentation as a good or potential indicator of environmental pollution, there should a clear indication (by color changing) of deviation of the natural status when it is exposed to metal contaminants. As it can be seen in Figure 3, the pigment profiles are exactly the same but the heights of peaks are proportionately reducing when increasing exposure concentration.

According to Prasad and Strzalka (1999), certain pollutants, such as heavy metals, reduce photosynthesis by affecting the light harvesting complex, oxygen evolution complex, cytochrome complex, plastoquinone, plastocyanin, ferrodoxin and $NADP^+$. Some heavy metals, such as Hg^{2+}, Cu^{2+}, Cd^{2+}, $Zn2^+$ and Ni^{2+}, are known to substitute the central Mg^{2+} atom in the chlorophyll molecule, a process that lowers the fluorescence quantum yield and results in a shift in the fluorescence spectrum (Küpper et al. 1996). As heavy metal toxicity is generally considered to be dose-dependent, it could be assumed that exposure to higher concentrations or over longer periods should result in higher toxicity as stated by Baumann et al. (2009). Eklund and Kautsky (2003) and Baumann et al. (2009) have also reported that the effects of metal exposure on algae are species-specific and metal-specific, and that in some cases there can be seen an obvious relationship between internal metal contents and effects on chlorophyll fluorescence.

According to results of the present study, exposure to heavy metal ions has effects on pigmentation of different algae Therefore, from the results of this laboratory study, it can be concluded that both *Ulva lactuca* and *Fucus vesiculosus* are potential biomarkers as pigment changes are easy to detect.

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