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FULL LENGTH ARTICLE

Bioaccumulation of gasoline in brackish green algae and popular clams

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Abstract The green algae (*Ulva lactuca* and *Enteromorpha clathrata*) and the clams (*Tapes decussates* and *Venerupis aurea*) grow together in Timsah Lake, Suez Canal, Egypt. Our ultimate goal is to validate the bioaccumulation of gasoline in the marine organisms and their behavior after exposure to the pollutant, experimentally. These species were treated with a serial treatment of gasoline (1000, 4000, 16,000 and 64,000 μl) in aquaria with brackish sea-water for 72 h. The tested green algae and clams were taken for an analysis of total hydrocarbon accumulation daily. The statistical analysis showed significant differences between the four species and also between the duration of exposure. The accumulation of gasoline in *U. lactuca* and *E. clathrata* reached their maximum after 48 h at 1000 and 4000 μl. The highest absorption was registered after 24 h only at 16,000 and at 64,000 μl. *U. lactuca* recorded complete mortality in 64,000 μl at 72 h whereas *E. clathrata* registered death at 48 h and 72 h in the same treatment. *V. aurea* was more sensitive than *T. decussates*. The accumulation of gasoline reached its maximum in *V. aurea* after only 24 h in the first treatment while it retarded to 48 h in *T. decussates* with a lesser accumulation. However, both clam species accumulated the highest amount of petroleum hydrocarbons during the first hour of exposure at the first treatment. In the third and fourth treatments, clams did not accumulate gasoline but began to dispose it from their tissues till it became less than that in the control. Mortality gradually increased with time in each treatment except the last one (64,000 μl) in which 100% death of the specimens was observed. In general, the bioaccumulation of gasoline level was in a descending order as follows: *U. lactuca* > *E. clathrata* > *V. aurea* > *T. decussates*. Their behavior changed from accumulation to detoxification with time and with the increase in pollutant concentration. Generally, these marine biota are sensitive to hydrocarbons and can be used as biomonitors to contaminants in aquatic environments except *T. decussates* that we recommend the possibility of using it as a good biomonitor in the sediment rather than in the water column.

Introduction

Oil hydrocarbons, such as gasoline, are the most ubiquitous organic contaminants in the marine environment, often at a

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high level in areas submitted to intense ship traffic (National Research Council, 2003 and Chen et al., 2008). Gasoline is a light volatile oil that makes up about 20% of crude oil (Paixao et al., 2007). The presence of saturated bonds only makes reactions with alkanes difficult; hence they are very resistant to degradation. Beaching of gasoline may occur if a spill occurs near the shore and the wind in the right direction (Lobban and Harrison, 1994). Gasoline may adhere to rocks, plants and animals, or be penetrated into the sediments if a dispersant is used. Generally, it affects directly and indirectly aquatic organisms that live in the upper water column and also that live on the bottom having great gasoline varying from species to species within a particular taxonomic group; it is recognized that some groups are more sensitive than others. Seaweeds and bivalves were of interest because of implications to the marine environment and human health (Zhou et al., 1996 and Allen et al., 2002). They accumulated pollutants in their tissues and hence are considered to be good bioindicators of contamination (Favero et al., 1996; Callow and Callow, 2000 and Carro et al., 2005). Nechev et al. (2002) also used the green algae and the bivalves as biomonitors of diesel fuel pollution.

Timsah Lake has an economic importance to Ismailia city. It is exposed to human and anthropogenic processes as fisheries and industrial activities, ship traffic discharges, untreated sewage and waste water discharges. It received high inputs of organic matter, in addition to chemical pollutants mainly of polycyclic aromatic hydrocarbons (PAH) and heavy metals (Mostafa, 2002). *Enteromorpha clathrata* and *Ulva lactuca* were visible as predominant lush mats of green seaweeds in Timsah Lake annually and these macroalgal blooms appeared during spring season (El Shoubaky and Hamed, 2006 and El Shoubaky, 2015). These are among the world's most common fouling algae (Callow and Callow, 2000). Several *Ulva* and *Enteromorpha* species are used as bio indicators of pollution (Favero et al., 1996), due to eutrophication and harmful effects to the environment (Blomster et al., 2002).

The assessment of oil impact on biological community ranges from minimal damage to severe destruction of exposed floras. Csaba and Csaba (2011) mentioned that aquatic algae and seaweed respond variably to oil, and oil spills may result in die-offs for some species. They also revealed that oil can prevent the germination and growth of aquatic plants. The green algae *U. lactuca*, *Chaetomorpha aerea* and *Enteromorpha intestinalis* showed damage with the effects being most severe on the highest occurring *Enteromorpha* sp. (Foster et al., 1970,1971).

On the other hand, many studies reported the occurrence of petroleum hydrocarbons in bivalve molluscs taken from the environment (Veerasingam et al., 2011). The uptake of petroleum hydrocarbons by the bivalve had also been shown to occur under laboratory conditions (Neff, 2002). Ganning et al. (2003), Boehm and Quinn (2004) and Marigomez et al. (2006) also assessed the accumulation and retention of petroleum hydrocarbons by bivalves experimentally. Among bivalves, clams are common in most of the Mediterranean and Red seas coastal areas. *Tapes decussates* and *Venerupis aurea* are one of the most abundant species in macro-benthic communities with respect to both density and biomass in Timsah Lake. These edible clams are distributed from intertidal zones to a water depth of approximately 2 m.

Clams usually live in the top 10 cm of the sediments and extend their siphons to take in water containing organic materials, including plankton, for food (Kasai et al., 2004). Due to the ability of clams to accumulate pollutants, they were often used as an indicator of contamination levels in situ (Luisa et al., 2007 and Qiao et al., 2011). Neff (2002) documented that the bivalves, which filter large volumes of water while feeding, can take up and concentrate petroleum hydrocarbons from the water, either from solution or adsorbed to suspended particles. The present work aimed to evaluate: (1) the effect of gasoline on the marine organisms (green algae and bivalves) as in their bioaccumulation (2) the possibility of their use as biomonitors of gasoline pollution (3) the change in their behavior after their exposure to this pollutant, experimentally.

Materials and methods

Specimens collected location

Specimens of two green algae, *U. lactuca* (Linnaeus, 1753) and *E. clathrata* (Greville, 1830) as well as two clams (*Tapes decussatus* (Linnaeus, 1758) and *V. aurea* (Gmelin, 1791)) were used for the experiment. The algae and clams were collected in April 2014 from El Taween Beach, southern part of Timsah Lake (30°33′ and 30°35′ N latitude and 30°16′ and 32°19′ E longitude), Suez Canal, Egypt (Fig. 1).

Experimental technique

The samples of algae and clams were transported to the laboratory, and then transferred directly to aquaria 48 h prior to starting the assay. After this acclimation period, groups of 500 g of each alga and clam species were maintained together in 20 L glass aquaria containing 10 L sea-water. The experiment included 5 aquaria in duplicates and we calculated the mean of results. The control (without any treatment) and four different aquaria were exposed to gasoline concentrations in ascending series (1000, 4000, 16,000 and 64,000 µl). Dead clams were removed and counted for mortality estimation. The survival samples of algae and clams were periodically taken for analysis after 24 h for three days. All aquaria were continuously aerated under laboratory temperature of 25 ± 2 °C with light/dark cycle (12:12).

Extraction and determination of gasoline from algal and bivalve tissues

10–20 g of freeze biota samples was extracted in a soxhlet (Electromantle ME, England) with 250 ml methylene chloride. The siphon cycle was carried out by Rotary evaporator (Heidolph VV2000, Germany) for 20–30 min and it was repeated at least 10 times. After complete extraction the solvent was evaporated over low heat (35 °C) to a volume of less than 20 ml. The extract was transferred to a 25 ml measuring flask. The soxhlet extraction flask was rinsed with methylene chloride and the rinse was used to make the volume up to 25 ml. Three ml of extract was transferred to a 10 ml measuring flask and the volume was increased to 10 ml mark with *n*-hexane. Clean-up is made to remove non-petroleum materials which may interfere in the analysis. An appropriate size