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Light-stick: A problem of marine pollution in Brazil

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ABSTRACT

Light-sticks are used as bait in surface long-line fishing, to capture swordfish and other large pelagic predators. When discharged in the ocean, it may reach the beaches. The traditional Brazilian community of Costa dos Coqueiros, Bahia, use light-sticks as a medicine for rheumatism, vitiligo and mycoses. It may affect the marine life when its content leak in the open ocean. This work evaluated and identified the acute and chronic toxicity of the light-stick. A high acute toxicity was observed in the mobility/mortality of *Artemia* sp.; in the fertilization of sea urchin eggs, and a high chronic toxicity in the development of the pluteus larvae of the same sea urchin. The main compounds that probably caused toxicity were the volatiles such as the fluorescent PAH and oxidants such as the hydrogen peroxide. Its disposal in the open ocean is a potential threat for marine life.

1. Introduction

Global garbage widely affects the marine fauna. A list of 267 species of marine animals all over the world, suffer with the presence of solid waste in the ocean and at the beaches (Mascarenhas et al., 2004), from which 86% of all species of marine turtles, 44% of the marine birds, 43% of marine mammals, and many fish species and crustaceans are included.

Only recently solid wastes in marine environment were recognized as an environmental problem. Until de 80′s, waste management was only a municipal policy, and it was believed that the debris discharged in the sea would easily disappear. To the public, plastic (and other products like nylon and polystyrene) was not considered as one of the most important environmental pollutants of the XXI century (Ivar do Sul and Costa, 2007).

The Non-Governmental Organization (NGO) Global Garbage develops environmental and social activities such as marine debris removal in Costa dos Coqueiros (the coconut coast), Bahia state, Brazil. The focus of such activities is to collect and classify the international garbage found at the beaches. Possibly, it arrives in Brazilian coast through the Brazilian current. International debris is identified by the bar code which is specific for each country. Among the sampled debris, it's possible to find materials of high environmental impact. Plastic pellets, for example, are studied by different groups around the world (e.g. Turra et al., 2014), not only for their PCB, DDT and PAH adsorption capacity, but also because of the risk of being ingested by marine birds (Mato et al., 2001; Endo et al., 2005; Takada, 2006; Karapanagioti and Klontza, 2007). Other important materials collected were the light-sticks, object of this study.

The stick emits light, result of a chemiluminescent reaction between two compounds separated by a glass ampoule. When the tube is bent, the glass ampoule breaks, mixing an ester-oxalate (trichlorosalicylate derivative) with hydrogen peroxide. The light shines for about 48 h. This process is catalyzed by fluorescent polycyclic aromatic hydrocarbons (9,10-diphenylanthracene, perylene, rubrene) and the chemical reaction takes place in a highly viscous solvent (generally di-*n*-butyl phthalate) (Stevani and Baader, 1999).

The light-sticks are used as bait for long-line surface fishing and can lead to the accidental capture of marine turtles that are attracted by the emitted light (Wang et al., 2007). Besides, the light-sticks discharged in

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the oceans may continue to circulate as a solid residue that can be eaten by sea birds or even fishes, creating a gastrointestinal obstruction and leading to hormonal and reproductive complications. (Shaw and Mapes, 1979; Wehle and Coleman, 1983; Furness, 1985; Azzarello and Vleet, 1987).

Traditional, low educated communities may use the light-sticks found on the shore as a medicine. The tube content is used as suntan lotion or to cure diseases such as rheumatism, vitiligo and mycoses (Cesar-Ribeiro and Palanch-Hans, 2010). High temperatures and solar irradiation after drifting to the shore indicated increased cyto- and genotoxic potential in human cells forming mutagenic lesions (Oliveira et al., 2014).

The light-sticks often have its pack broken in the ocean and its toxic chemicals leak directly in the seawater. The toxicity of the internal solution have been previously tested on Winstar rats (Ivar Do Sul et al., 2007), and in cytotoxicity tests (Bagattini et al., 2006). The acute toxicity and the hatchability of *Artemia* sp. cysts were evaluated as well (Pinho et al., 2009), while the chronic toxicity of the supernatant extracted of the light-stick was tested on the pluteus larvae of *Echinometra lucunter* and *Lytechinus variegatus* (Cesar-Ribeiro and Palanch-Hans, 2010). These authors discovered a high toxicity of the light-stick in the sea urchins larvae development.

The objective of this work was to develop methodologies for toxicity tests with light-sticks using the supernatant fraction and/or an organic solvent (ethanol); to evaluate the acute and chronic toxicity of light-sticks to different marine organisms and to identify the main chemical compounds that cause deleterious effects.

2. Material and methods

2.1. Study area

With the support of the German NGO Global Garbage, a scientific hike was undertaken from 14 to 31 of July 2007, along almost 200 km of the Costa dos Coqueiros beaches – Bahia, Brazil. A total of 2554 tubes of luminous attractors were collected, of which 34% were opened and 63% were still closed. These tubes were opened and used to prepare the stock solution to be used in the toxicity tests.

2.2. Supernatant extraction

The light-stick internal solution has greater density than water and low solubility, due to hydrophobic substances, mainly the solvent di-n-butyl phthalate. The preparation of the stock solution intended to simulate the hydrophilic compounds arrangement in the water column. Therefore, a homogenized oil was mixed with sea water (salinity 35), in the proportion 1:1. Clear, oceanic seawater was collected in Laje de Santos, São Paulo – Brazil. The mixture was centrifuged for 1 min, resulting in a aqueous fraction that was in contact with the liquid inside the light-stick. Because of the light-stick high density, the aqueous fraction remained in the supernatant parcel. This fraction corresponds to the stock solution 100% (SS 100%) (Cesar-Ribeiro and Palanch-Hans, 2010).

2.3. Extraction using solvent

The immiscible compounds were extracted dissolving 0.1 mL of light-stick liquid in 100 mL of filtered seawater (salinity 35) and ethanol 0.5% (v/v) as a solvent. This solution was named stock solution ethanol (SSE 0.1%). Dilutions were prepared for the chronic toxicity test in the development of sea urchin (*L. variegatus*) embryos. The ethanol was also tested to understand the toxicity of the solvent and to interpret the real deleterious effect caused by the light-stick. Then dilutions were prepared with ethanol in these concentrations: 0.1; 0.25 and 0.5%.

2.4. Toxicity tests

2.4.1. Artemia sp. - acute test (mortality/immobility)

The acute toxicity test with *Artemia* sp. was based on Vanhaecke and Persoone (1984). The developmental stages used in toxicity tests with *Artemia* sp. were nauplii II and III. In these stages begins the filtration activity that allows the contact of the digestive tract epithelium with the external environment, increasing organisms sensibility and reducing the test variability (Sorgeloos et al., 1978). The cysts of *Artemia* sp. were free from significant levels of contaminants and came from a salt industry, in Macau, Rio Grande do Norte – Brazil. The hatchability rate was higher than 70%.

The definitive concentrations for the tests were obtained after preliminary standardization and therefore chosen as: 0.1; 0.2; 0.3; 0.5; 0.7 and 2%. Quadruplicates were used for each concentration. The test tubes were prepared with 10 mL of water in the control or in the specific concentrations; ten nauplii were added. The tubes were maintained for 48 h in 25 ± 1 °C in the dark.

The observed effects were mortality or immobility of the individuals after 24 and 48 h of exposure. The lethal concentration to 50% of the organisms (LC50) – 24 and 48 h were calculated.

2.4.2. Acute toxicity test (fertilization of Lytechinus variegatus eggs)

The acute toxicity test with eggs from the sea urchin *L. variegatus*, was performed according to Nipper et al. (1993), by adding 100 μL of a spermatic solution. The sea urchin was collected at Ilha das Palmas, São Paulo coast – Brazil. The organisms were fed with the alga *Ulva lactuca* and the following water parameters were evaluated: salinity, dissolved oxygen, ammonium and temperature.

To stimulate the release of gametes, an electric shock (35 V) was applied to the aboral portion of the sea urchins. In each of the test tubes (10 mL), 100 μL of a spermatic solution was added, starting the sperms exposition period under different concentration. After 20 min, 2000 ovules were added on each test tube. The tubes were mixed to ease the fecundation. The test ended after transferring the contents of each replica to tubes identified by the test number with 100 μL of buffered formalin (final concentration of 4%).

The concentrations used in the test with the extracted supernatant were established in preliminary tests, and defined as 0.002, 0.003, 0.005, 0.01, 0.02 and 0.1%.

2.4.3. Lytechinus variegatus – chronic test (embryo development)

To evaluate the chronic toxicity, short time tests with sea urchin *L. variegatus* embryos were developed. The method was adapted from Nipper et al. (1993), Rumbold and Snedaker (1997) and USEPA (2002).

Fertilization of the ovules was necessary prior to the exposition period. Approximately 400 eggs were added per test tube (10 mL). The embryos remained in light-stick supernatant dilutions for 24 h, necessary time for the embryos to reach the pluteus larvae stage. The test was conducted at a constant temperature of 25 ± 2 °C and a 12L/12D photoperiod. The concentrations used in the chronic test were 0.0005, 0.001, 0.002, 0.007, 0.02, 0.05, 0.1, 0.5 and 1.0% of SS, increasing the number of concentrations tested by Cesar-Ribeiro and Palanch-Hans (2010), in order to determinate the effective concentration to 50% of the organisms (EC50) with more accuracy. The same test was performed for the SSE, but the concentrations were: 0.0001, 0.00025, 0.0005, 0.00075, 0.001, 0.005% of light-stick diluted in ethanol.

Abnormalities in the development of the pluteus larvae were observed. Therefore, the embryos that showed a growth delay or morphological alterations were considered not developed.

2.4.4. Toxicity Identification Evaluation

The TIE (Toxicity Identification Evaluation) aims to identify the toxic compounds responsible for the toxicity of effluents and environmental samples as part of toxicities reduction protocols (Costa et al., 2008).