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# An aquarium hobbyist poisoning: Identification of new palytoxins in *Palythoa* cf. *toxica* and complete detoxification of the aquarium water by activated carbon

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ABSTRACT

Palytoxin (PLTX) is a lethal natural toxin often found in *Palythoa* zoantharians that, together with its congeners, may induce adverse effects in humans after inhalation of toxic aerosols both in open-air and domestic environments, namely in the vicinity of public and private aquaria. In this study, we describe a poisoning of an aquarium hobbyist who was hospitalized after handling a PLTXs-containing zoantharian hexacoral. Furthermore, we provide evidence for water detoxification. The zoantharian was morphologically and genetically identified as *Palythoa* cf. *toxica* (Cnidaria: Anthozoa). Palytoxin itself and two new PLTX congeners, a hydroxyPLTX and a deoxyPLTX, were detected and structurally identified by liquid chromatography high resolution multiple stage mass spectrometry (LC-HRMS$^n$, n = 1, 2). Total and individual toxins were quantified by LC-HRMS and sandwich ELISA both in the zoantharian (93.4 and 96.80 μg/g, respectively) and in the transport water (48.3 and 42.56 μg/mL, respectively), with an excellent mean bias of 1.3% between the techniques. Activated carbon adsorbed 99.7% of PLTXs contained in the seawater and this represents a good strategy for preventing aquarium hobbyist poisonings.


## 1. Introduction

Palytoxin (PLTX) (Fig. 1) is a lethal natural toxin originally discovered in the zoantharian *Palythoa toxica* (Cnidaria: Anthozoa: Hexacorallia: Zoantharia) from Hawaii (Moore and Scheuer, 1971). Subsequently, PLTX and/or its analogues have been detected in other *Palythoa* and *Zoanthus* species (Uemura et al., 1985) as well as in other marine organisms, such as dinoflagellates of the genus *Ostreopsis* (Ciminiello et al., 2014a), cyanobacteria of the genus *Trichodesmium* (Kerbrat et al., 2011), and in other various invertebrate and vertebrate species (Aligizaki et al., 2011; Birè et al., 2013).

Besides concerns regarding oral toxicity of PLTXs (Munday, 2011), there is growing evidence that PLTXs exert adverse effects through inhalation of toxic aerosols both in open-air and domestic environments as well as through cutaneous and/or ocular exposure to zoantharians or aquaria waters (Deeds and Schwartz, 2010; Tubaro et al., 2011; Ciminiello et al., 2014b; Pelin et al., 2016a; Tartaglione et al., 2016). In this frame, a number of case reports on human poisonings following manipulation of PLTX-contaminated zoantharians, widely used as aquaria decorative elements, have been reported, with a total of 53 people poisoned (Tubaro et al., 2011; Pelin et al., 2016a; Tartaglione et al., 2016). In most cases, exposure occurred through inhalation of steam generated during installation or cleaning of home aquaria containing

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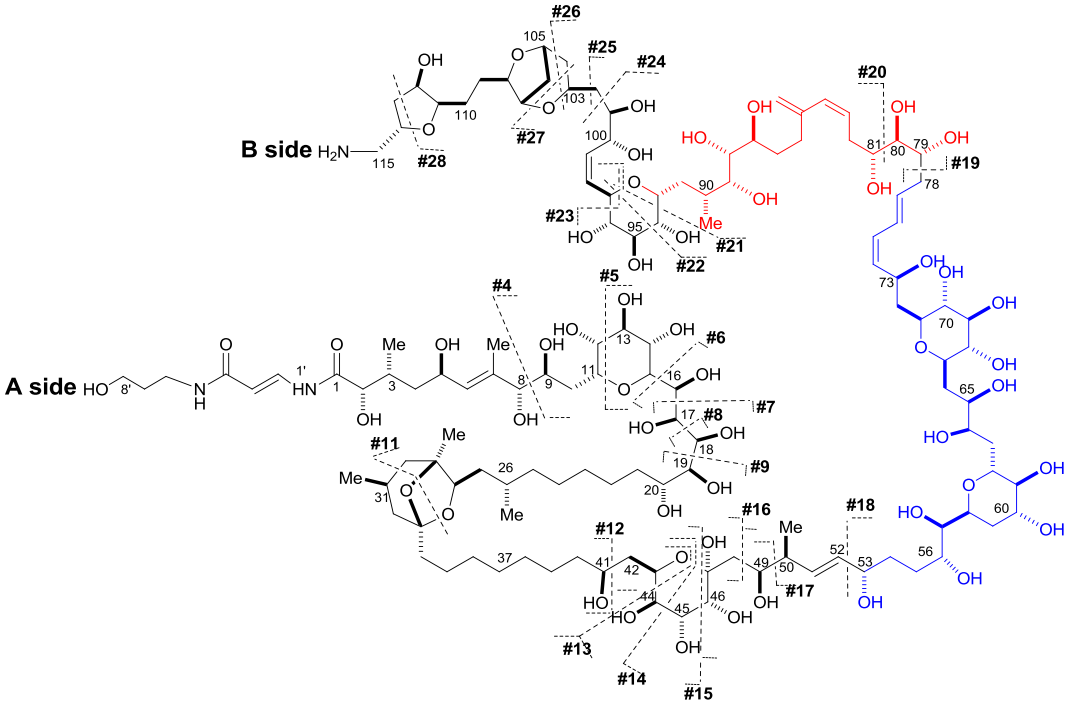


**Fig. 1.** Structure of palytoxin (PLTX) numbered following the convention of Uemura et al. (1985). HydroxyPLTX has an additional O atom in region C-79 to C-93 (highlighted in red) while deoxyPLTX lacks one O atom in region C-53 to C-78 (highlighted in blue). Cleavages resulting from HR CID $MS^2$ experiments are reported. Relevant ion fragments are reported in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*Palythoa* spp. (Moore et al., 1982; Majlesi et al., 2008; Deeds and Schwartz, 2010; Bernasconi et al., 2012; Dijkman and de Vries, 2012; Snoeks and Veenstra, 2012; Sud et al., 2013; Rumore and Houst, 2014; Wieringa et al., 2014; Hall et al., 2015; Hamade et al., 2015; Tartaglione et al., 2016). and only in a few cases through ocular exposure (Moshirfar et al., 2010; Ruiz et al., 2015) or skin contact (Moore et al., 1982; Hoffmann et al., 2008; Deeds and Schwartz, 2010; Nordt et al., 2011; Dijkman and de Vries, 2012) with zoantharians. The main signs and symptoms of inhalatory poisonings involved the respiratory tract (dyspnea, rhinorrhea, cough, sore throat), the skeletomuscular apparatus (myalgia, weakness, spasms, huge increase in creatine phosphokinase), the cardiovascular system (tachycardia), the gastrointestinal apparatus (dysgeusia, as bitter metallic taste, nausea and/or vomiting, diarrhea) and/or the nervous system (dizziness, paresthesia, ataxia, numbness, tremors). Fever $\geq$38 °C was observed in all cases (Tartaglione et al., 2016). It should be noted that for most reported poisonings, the involvement of PLTXs was only postulated (Majlesi et al., 2008; Nordt et al., 2011; Bernasconi et al., 2012; Dijkman and de Vries, 2012; Snoeks and Veenstra, 2012; Sud et al., 2013; Rumore and Houst, 2014; Hall et al., 2015; Ruiz et al., 2015) based on the symptomatology and on the assumption that some *Palythoa* spp. contain PLTXs, and not confirmed by chemical or biological means. In only five case-reports following cutaneous (Moore et al., 1982; Hoffmann et al., 2008) or inhalational exposure (Deeds and Schwartz, 2010; Wieringa et al., 2014; Hamade et al., 2015) to PLTXs-containing zoantharians or steamed water from aquaria were PLTXs confirmed to be the causative agents by hemolytic assay and/or liquid chromatography coupled with ultraviolet (LC-UV) or mass spectrometry (LC-MS) detection.

Aquarium hobbyist poisonings may be largely underestimated considering that *Palythoa heliodiscus* and related species' colonies containing high levels of PLTX and deoxyPLTX are commonly sold in the home aquarium trade (Deeds et al., 2011). In this study, we describe the first case in Italy of an aquarium hobbyist, who was hospitalized after handling a PLTXs-containing zoantharian that was morphologically and genetically identified as *Palythoa* cf. *toxica* (Cnidaria: Anthozoa: Hexacorallia). High Resolution LC-MS and a sandwich ELISA detected high amounts of PLTX and of new PLTX congeners, namely a hydroxyPLTX and a deoxyPLTX, both in the zoantharian and in the surrounding water. As well, the ability of activated carbon to detoxify the aquarium water was investigated through chemical means.

## 2. Materials and methods

### 2.1. Sample collection

A colony of a green-brown *Palythoa* species growing in the home aquarium of the poisoned patient was collected (See Supplementary material, Fig. S1-A). The polyps were readily contractile and their maximum oral disc diameter, without tentacles, was 15 mm. The colony morphologically resembled that of *Palythoa* sp. VAZOA responsible for a severe respiratory reaction in an aquarium hobbyist in Virginia (USA) in 2008 (Deeds et al., 2011). After photographic documentation (See Supplementary material, Fig. S1-B), 3 polyps were fixed in 96% ethanol for DNA analyses. A small colony with supporting substrate (approximately 10 individual polyps) was designated as specimen "NMS1" while a control specimen from a *Palythoa* colony of the Museum of Nature South Tyrol aquarium (See Supplementary material, Fig. S1-C) was sampled and designated as 'NMS2'. Both NMS1 and NMS2 samples were shipped alive in aquarium water for toxin analyses.

### 2.2. Species identification

High-resolution images of specimens NMS1 (See Supplementary material, Fig. S1) were examined, noting the following characters: colony and polyp form ('immersae', 'intermediae' or 'liberae'; see Pax, 1910), external coloration, oral disk coloration and