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Assessing the chronic toxicity of nickel to a tropical marine gastropod and two crustaceans

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ABSTRACT

The mining and processing of nickel ores from tropical regions contributes 40% of the global supply. The potential impact of these activities on tropical marine ecosystems is poorly understood. Due to the lack of ecotoxicity data for tropical marine species, there is currently no available water quality guideline value for nickel that is specific to tropical species.

In this study, we investigated the toxicity of nickel to three tropical marine invertebrates, the gastropod *Nassarius dorsatus*, the barnacle *Amphibalanus amphitrite,* and the copepod *Acartia sinjiensis*. All toxicity tests used chronic endpoints, namely larval growth, metamorphosis (transition from nauplii to cyprid larvae) and larval development for the snail, barnacle and copepod respectively. Toxicity tests were carried out under environmentally relevant conditions (i.e. 27–30°C, salinity 34–36‰, pH 8.1–8.4). Copper was also tested for quality assurance purposes and to allow for comparisons with previous studies.

The copepod was the most sensitive species to nickel, with development inhibited by 10% (EC10) at 5.5 (5.0–6.0) µg Ni/L (95% confidence limits (CL)). Based on EC10 values, the gastropod and barnacle showed similar sensitivities to nickel with growth and metamorphosis inhibited by 10% at 64 (37 − 91) µg Ni/L and 67 (53 − 80) µg Ni/L, respectively. Based on existing data available in the literature, the copepod *A. sinjiensis* is so far the most sensitive tropical marine species to nickel. This study has provided high quality data which will contribute to the development of a water quality guideline value for nickel in tropical marine waters. A species sensitivity distribution of chronic nickel toxicity used the data generated in this paper supplemented by available literature data, comprising 12 species representing 6 taxonomic groups. A 5% hazard concentration (HC5) was determined as 8.2 µg/L Ni.

1. Introduction

Within the tropics, unique ecosystems harbouring rich biodiversity are juxtaposed with highly dense coastal urban populations which have the potential to impact the valuable coastal marine environment. Many countries within the tropics, particularly within the Asia-Pacific region, are extremely dependent on their coastal ecosystems for food, income (through tourism and fishing), and spiritual and cultural values. Most countries within the Asia-Pacific region are economically classified as developing, and, as a consequence, their limited environmental regulatory frameworks may hinder the implementation of environmental protection measures (Reichelt-Brushett, 2012).

The mining and production of nickel has recently intensified in the Asia-Pacific region (USGS, 2016). Approximately 70% of the world's nickel reserves are found in the tropics, contributing about 40% of the global supply (Van der Ent et al., 2013). Currently, there is limited research into the potential impacts of these activities on the coastal marine environment and also a lack of ecologically relevant risk assessment tools. These tools include bioavailability-based toxicity tests and water quality guideline values (WQGVs, i.e. a numerical threshold below which risks to aquatic ecosystems are not expected) that can be used by government and industry to contribute to the continued management of contaminants in aquatic environments and protection of local aquatic biota (Wang et al., 2014). In general, GVs for metals in

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tropical regions have been based on temperate data due to the limited availability of ecotoxicity data for tropical species (Howe et al., 2014; Van Dam et al., 2008). In areas of intensified nickel mining activity, it would be beneficial to have a regionally-specific GV for nickel.

Water quality GVs are increasingly being derived using species sensitivity distributions (SSDs), which estimate a protective concentration (PC), usually for 95% of species (known as the PC95 value), which corresponds to the 5% hazardous concentration (HC5). The guidance around SSDs and WQGV derivation is jurisdiction-dependent. In Europe, 10–15 species are recommended for input into SSDs (ECHA, 2008; OECD, 2011); in North America the recommendation is at least 15 species (USEPA, 2005). In Australia and New Zealand, at least eight species representing four taxonomic groups is preferred (ANZECC/ARMCANZ, 2000; Warne et al., 2015). The underlying principle in all jurisdictions is that the greater the number of species (data) used in an SSD, the more likely the PC95 is to be protective of a broad range of species in the ecosystem.

A recent review by Gissi et al. (2016) compiled and quality-checked available nickel toxicity data for tropical marine species. Using only high quality (e.g. reported measured metal concentrations in chronic toxicity tests), a total of six data points representing four taxonomic groups was found and this was insufficient to derive a GV (Gissi et al., 2016). Key data gaps identified in the review included cnidarians, molluscs, crustaceans, echinoderms, macroalgae and fish. To address this data gap, nickel toxicity data have recently been published for three different species of corals (cnidarians), based on fertilisation success (Gissi et al., 2017). In the present study, we aimed to further contribute to the body of chronic nickel toxicity data for tropical marine species, specifically for molluscs (gastropods) and crustaceans. These taxonomic groups have high ecological importance in tropical regions because of the important role they play in the food web as well as species richness and biodiversity which is highest in the tropics (Bouchet et al., 2002; Humes, 1994; Roberts et al., 2002). Additionally crustaceans and molluscs are among the most sensitive species to nickel exposure in temperate systems (Bielmyer et al., 2006; Deforest and Schlekat, 2012; Niyogi et al., 2014).

We have investigated the effects of nickel on a gastropod, the channelled dog whelk *Nassarius dorsatus,* and two crustaceans, the purple-acorn barnacle *Amphibalanus amphitrite* and the copepod *Acartia sinjiensis.* All three species tested in this study are ecologically relevant, and are found in tropical marine environments of the Indo-Pacific region. *Nassarius dorsatus* is most common in tropical North Australia, and has also been reported in coastal waters off Malaysia, Indonesia, Papua New Guinea, Fiji and the Philippines (Trenfield et al., 2016). *Amphibalanus amphitrite* is a common biofouling organism, widely distributed across tropical to temperate waters, in the mid to low intertidal zone (Desai et al., 2006; van Dam et al., 2016). This species has been widely used as a model species in biofouling and ecotoxicity tests (Rittschof et al., 1992; van Dam et al., 2016). *Acartia sinjiensis* is found in tropical and sub-tropical brackish to marine waters in Australia and some other locations within the Asia-Pacific (Gissi et al., 2013). This species of copepod is a common food source for many higher trophic organisms (Camus and Zeng, 2008).

The objectives of this study were to assess larval growth rate for the snail, metamorphosis (successful transition from nauplii to cyprid larvae) for the barnacle and larval development for the copepod, to determine the chronic toxicity of nickel to each of the three species. In addition, copper, a commonly-used reference toxicant, was tested for quality assurance purposes, enabling comparisons with previous studies and also because copper is a widespread anthropogenic contaminant in marine environments (Levy et al., 2007). The toxicity data presented here will contribute to the further development of reliable WQGVs for nickel in tropical marine waters.

2. Materials and methods

2.1. General laboratory techniques and reagents

Snail and barnacle toxicity tests were conducted at the Australian Institute of Marine Science (AIMS, Darwin, Northern Territory, Australia). One rangefinding and four definitive tests were conducted each for the snail and barnacle. Three definitive copepod tests were carried out at the CSIRO Land and Water laboratories in Sydney, NSW, Australia.

All glassware and plastic containers used in the tests were acid-washed in 10% (v/v) nitric acid (Merck) and thoroughly rinsed with demineralised water, followed by Milli-Q® water (MQ, 18.2 MΩ/cm; Merck). Glass funnels used in barnacle tests were silanized (Coatasil, Ajax, Finechem), approximately 2 weeks prior to testing and thoroughly rinsed with demineralised water, soaked in 10% nitric acid for 24 h, and finally rinsed again with demineralised water, followed by MQ water. All metal stock solutions were made volumetrically using MQ water. Copper stock solutions of 5 and 100 mg Cu/L were prepared using copper (II) sulfate salt (A.R. grade, AJAX Chemicals, Australia). Nickel stock solutions of 10 mg/L and 100 mg/L were made using nickel (II) chloride hexahydrate salt (A.R. grade, Chem Supply, Australia). All metal stocks were acidified to 0.1% HCl (Tracepur; Merck) to ensure metals did not precipitate.

For the snail and barnacle tests, water quality parameters including dissolved oxygen (DO), pH, conductivity and salinity were measured using a multi-probe (Hach multiprobe HQ40d), which was calibrated each day according to the manufacturer's instructions. For snail tests, measurements were taken on Day 0 and every 24 h. For barnacle tests, measurements were taken on Days 0 and 4. In all copepod tests, salinity measurements were taken using a YSI salinity and conductivity meter (model30/10FT, YSI, Ohio, USA). The pH was measured using a Thermo Orion pH meter with an epoxy body probe (meter model 420, probe model ROSS815600, Thermo Fisher Scientific, USA) which was calibrated daily. Dissolved oxygen (%) was measured using an Oximeter (Oxi330WTW, Weilheim, Germany), which was calibrated immediately prior to use. Water quality parameters were measured on Day 0, Day 2 before and after renewal and on Day 3. Temperature was recorded in all toxicity tests throughout the exposure.

2.2. Toxicity tests with the snail Nassarius dorsatus

Culturing, larval hatching and toxicity testing with *N. dorsatus* followed the methods described in Trenfield et al. (2016), and utilised the same broodstock of snails. In brief, egg batches were laid on polystyrene tubes positioned in the broodstock tank. Prior to the eggs hatching, tubes were transferred to a clean container with filtered seawater. The egg batches were maintained at 29°C and after ~5 days larvae hatched, then were fed once per day with a mixture of the microalgae *Chaetoceros muelleri* and *Rhodomonas salina* for 2 days.

Seawater for snail tests was collected in 20-L polyethylene containers from Nightcliff Jetty, Northern Territory, Australia (12°22′59″S, 130°50′56″E)), at high tide and filtered (0.45-μm filter, Quickfilter groundwater cartridge; Thermofisher Scientific) immediately upon return to the laboratory. Filtered seawater was stored at 4°C in the dark.

Treatment solutions were prepared using seawater in 2-L high density polyethylene (HDPE) bottles 24 h prior to test commencement. Across four individual toxicity tests, nominal nickel concentrations were in the range of 50 to 1500 μg Ni/L. In the single copper test, nominal concentrations were 2, 4, 8, 12 and 20 μg Cu/L. On Day 0, unwashed axenic cultures of the microalgae *C. muelleri* and *R. salina* were added to each test container to give a final concentration of 1×10^4 cells/mL of each species, then 100 mL of each treatment solution was dispensed into the relevant test container, and 10 larvae (2-day old) were gently added using a wide-mouthed glass pipette. Test containers were placed in a temperature controlled cabinet set to 28°C