Nickel uptake in *Symbiodinium* spp. and host cnidarian - assessment using the model cnidarian *Exaiptasia pallida*

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**Abstract.** Nickel (Ni) is common marine pollutant and derived from discharge of industries, mining and agriculture. Elevated Ni concentrations, could be harmful the organisms in marine environment such as corals reef. The sea anemones *Exaiptasia pallida* has been widely used for toxicity tests in the laboratory because it is easy to maintain and culture in contained aquarium conditions. To understand the pathway of uptake and storage of elevated Ni between the sea anemone host and symbiotic algae, the model sea anemone *E. pallida* was exposed to three different Ni concentrations (500 μg/L, 1000 μg/L, 2000 μg/L) and control treatment over 9 days and 18 days. Ni accumulation was assessed in both the host anemone tissues and the *Symbiodinium* spp. The concentration of Ni was measured using Perkin Elmer NexION 300D ICPMS (Inductively Couple Plasma Mass Spectrophotometry) after sampel digestion. The statistical analysis was completed using R version 3.5.0 (R core team 2013) R Foundation for Statistical Computing, Vienne, Austria. The model was chosen by Akaike Information Criterion (AIC) after to comparing between model options. Shapiro-Wilks test was used to verify the normal distribution of the date set to be analysed. The overall result showed the accumulation of nickel was highest in *Symbiodinium* spp. (212.62 ± 50.12 mg/Kg) after a 9 days exposure period in concentration 2000 μg/L while 18 days exposure period was less (129.66 ± 31.94 mg/Kg) in concentration 2000 μg/L. The lower uptake of Ni in *Symbiodinium* spp. in the longer exposure time of 18 days compare to 9 days exposure might be due to the ability of *Symbiodinium* spp. to release the Ni through their metabolism or the Ni dose may have inhibited normal function of the algae, although the accumulation of both times period was significantly higher than the control treatments. Meanwhile, host anemones accumulated 77.43 ± 6.28 mg/Kg Ni over 9 days exposure period and the 2000 μg/L treatment and increased after 18 days exposure in concentration 2000 μg/L (45.54 ± 4.12 mg/Kg). The result of these finding highlight the importance of understanding the metal accumulation in host and cnidarian. This is particularly important if these species are used in biomonitoring studies where bleaching may occur due to stress, but may in effect be a depuration pathway for metal load, masking the biological uptake and interaction between metal and organisms.

**Key words:** nickel, *Exaiptasia pallida*, pollution
1. Introduction

Coral reef ecosystems face a wide and intensive range of threats, approximately 75 percent of coral reefs are threatened by thermal stress [1], anthropogenic disturbance [2] such as destructive fishing [3], trace metal pollution like mining[4,5]. A wide range of chemical pollution ended up in aquatic ecosystem [6]. Metal pollution enters the marine environment by atmospheric deposition or by weathering of the geological matrix and through anthropogenic sources such as agriculture waste, industrial discharge and mining waste [7]. Corals bleaching event occur annually in tropical and subtropical region [8]. Trace metals introduced to marine environment tend to accumulate in sediment and can be taken up and accumulated by marine organisms [9]. The toxicity of trace metal to marine organisms depends on the specific metal and concentration of that metal, and the species interest and how it interacts with the biotic and abiotic environment.

Trace element pollution is an environmental concern in marine ecosystem. The most common pollutants found in marine environment are lead, copper, zinc, cadmium and nickel. Those trace elements are present or introduced in the marine environment from point source and non-point sources including agriculture run off, industrial discharge, mining activities [4] and atmospheric deposition. The toxic effect of trace elements can be produced after a metal have been taken up by organisms and becomes available to interfere with to normal functioning resulting in sub-lethal deleterious effects or mortality [10, 11]. There are several kinds of mechanisms of uptake and depuration and storage metals by corals. For example, metal may be incorporate into crystal lattices of the skeleton, particulate of trace metal may have trapped within skeletal cavities by the uptake of trace metal from corals tissue or through feeding. Coral skeleton alone has very low levels of trace elements. Each coral species may exhibit a different response to trace of pollutants in marine organisms [10]. Importantly and unlike corals sea anemones do not have skeleton. Using anemones in uptake and depuration studies allows specific focus on the living components including both the host tissue and the symbiotic algae. This provides a step in unraveling the complexities associated with understanding uptake and depuration pathways. Knowing the effect of trace metal pollution in marine environment could help to allow realistic planning future industries and minimize the impact of man on the environment [12].

Nickel is a metal commonly thought as a ‘finite’ resource and therefore mining is intrinsically unsustainably [13]. Nickel is found in two types of deposits; nickel laterites and magmatic sulfide deposits. Nickel laterite make up 70% of the world’s Ni reserves, a few of them have become producing mines (https://www.geologyforinvestors.com/nickel-laterites/). Laterite is a type of soil that formed through prolonged chemical and mechanical weathering in wet, warm, tropical environments. Laterite rock and soil have become popular not only due to the scientific interest but also the economic significant of the laterite type nickel ore [14]. Lateralization processes involved the breakdown of primary minerals and release of their chemical components into groundwater, the leaching of component mobile, the residual concentration of immobile or insoluble components and the formation of new minerals which are stable in the weathering environment [15].

The objective of this study was to understand the nickel uptake in Symbiodinium spp. and host cnidarian assessment using the model cnidarian Exaiptasia pallida. This knowledge will contribute the understanding of bioaccumulation of metal in cnidarian for in situ biomonitoring study. Another section of your paper

2. Materials and Method

2.1. Maintenance of tested animal Exaiptasia pallida

Around 100 E. pallida were collected in March 2018 from the Seaworld oceanarium, Gold Coast (27°57'26.07"S and 153°25'31.81"E) , Queensland Australia and shipped to the marine aquaculture laboratory at Southern Cross University, Lismore. Upon arrival, the sea anemones (E. pallida) were immediately transferred into three different rearing tanks/aquaria that had been previously set up with unfiltered natural sea water. The rearing tank temperature was maintained at ± 23-25°C with a photoperiod of 12 : 12 hours and approximately photoperiod approximately (50-60 µE m²/s) using 30
--- W (white Sylvania Aqua star) blue (Coralstar). Unfiltered seawater exchanged of 25-50% were performed once every 2 weeks. Electrical conductivity and temperature were measured using Thermo Scientific Cond 6+, pH was measured by Thermo Scientific Cond 5+, and Dissolved Oxygen (DO) was measured using modified Winkler micro-titration method. Anemones were feed with frozen and thawed adult Artemia once in three weeks.

2.2. General experiment design

The experiment was conducted in an experimental laboratory at Southern Cross University for 18 days from 24th April until 11th May 2018, the marine ecology laboratory Southern Cross University. Two large big containers (90 cm length and 15 cm high) were set up as water baths to maintained temperature in the test using a water pump and heater. The temperature was maintained at around 23.5 ± 1.2 °C. A day before the nickel exposure commenced, the anemones from the rearing tank were acclimated with unfiltered seawater in 500 mL acid washed and seawater rinsed polyethylene containers. Two anemones, with a pedal disk diameter between 4-5 mm, were placed in each container. Four replicate containers of the three Ni concentration as well as four control replicate were made up using natural unfiltered sea water. The nominal dose concentrations of NiCl₂ were 500 µg/L, 1000 µg/L and 2000 µg/L. These concentrations were selected because previous study showed that the LC₅₀ for nickel effects on E. pallida after 3 days was 5751 µg/L [16] and since the intention of the present study was not to cause mortality a lower concentration range was used. The static-renewal experiment was maintained with renewal periods for each dose at 3, 6, 9, 12, 15, and 18 days. At the start and end of each renewal period representative water samples were collected from each dose, acidified and refrigerated for later analysis. At each time period of 9 and 18 days, duplicate whole anemones in test containers were collected, rinsed and frozen for later on nickel analysis in both tissue and Symbiodinium spp. after physical separation.

The electrical conductivity and water temperature in the test containers were measured every 24 hours using thermos scientific Eutech Cond 6+ meter. Salinity was maintained between 29-36 ppt and temperature between 25-26 °C. Photoperiod was provided with a 12 : 12 h at approximately (50-60 µE m²/s). Water samples were collected at the start and the end of each renewal period. Preserved with HNO₃ and analysed for nickel at the EAL (Environmental Analysis Laboratory) using a Perkin Elmer NexION 300D ICPMS (Inductively Couple Plasma Mass Spectrophotometry). Instrument conditions were 1.02 L/min nebuliser gas flow, 1.2L/min auxiliary gas flow, RF power set at 1450 watts. The instrument was calibrated using a three point curve with R2 coefficient of > 0.9999, independent standards were analysed to ensure the accuracy of the calibration. The detection limit was < 0.001 mg/L in water.

2.3. Homogenisation of Exaiptasia pallida

E. pallida (anemones) were thawed at room temperature. Samples were homogenised using a Omni Tissue Master 125 homogeniser. Between homogenisation of each sample, the shaft and mixing head was rinsed several times with 5 mL Milli-Q water and 10-20 second operation to ensure no tissue residue remained on the shaft. In this study counting individual Symbiodinium spp. was not completed due to the limit of time.

Tissues and Symbiodinium spp. were separated in the homogenate by centrifugation at 4000 rpm for 30 minutes. The products of each centrifuging were physically separated into two samples; the tissue supernatant and the Symbiodinium spp. pellet. Before the samples were transferred to digestion containers the containers were weighed. Symbiodinium spp. and tissue samples were dried in an oven at 40°C until dry and for up to 96 hours. Later, tissue and Symbiodinium spp. dry weight was calculated by subtracting the final weight from the empty digestion vial weight.

After samples were dried they were digested with 1 mL H₂O₂ for 18 hours. The digest of each sample was completed in water bath between 50-60 °C for one hour after the addition of 1 mL HNO₃. Digested samples were made up to 2 mL with distilled water. During the digest procedure adapted
from [17] digestions of three replicates of the certified reference material fish protein (DORM-4 CRM) were also completed to measure nickel recovery efficiency. Samples digestes were analysed by ICP-MS at the Southern Cross University and as describe above. The nickel concentration per mg/Kg dry weight was calculated.

2.4. Data analysis

The statistical analysis was completed using R version 3.5.0 (R core team 2013) R Foundation for Statistical Computing, Vienne, Austria. Model was chosen by Akaike Information Criterion (AIC) after to comparing between model options. Shapiro-Wilks test was used to verify the normal distribution of the data set to be analysed. Both test failed, and therefore data was transformed using Box Cox transformation and graphical analysis of the appropriate parameters (q-q-plot). Shapiro-Wilks test was used to verify the normal distribution of residual. Both test were failed, data was transformed using Box Cox transformation.

Since the data fitted the assumptions of homogeneity the Ni concentration in the separate anemones and zooxanthellae fraction as well as combines samples from different time and dose treatment were tested using ANOVA in R software. Post-hoc Tukey’s test was performed to make pairwise comparison of all factors and their interaction. The packages dplyr (version 0.7.5) and knitr (version 1.20) were used to prepare the data for analysis and reporting whereas MASS (7.3-49) was used for data transformation (BoxCox).

The data was analysed using an ANOVA including all three factors, their interaction between all as well as the interactions between nickel concentration and type and nickel concentration and duration. Ensuring that the assumptions of the ANOVA were met we transformed the measured nickel concentration using the Box Cox transformation. The assumptions were tested using Shapiro Wilks test for normality as well as graphical using the q-q-plot and the histogram of the residuals. Furthermore, we ran the Tuckey HSD post-hoc test. The AIC (Akaike Information Criterion) was used to select the most appropriate model for the best data set.

3. Results and Discussion

3.1. Comparison between Ni uptake in host anemones and Symbiodinium spp. at different exposure concentration

Background pre-exposure concentration of Ni in anemones samples taken before experimental were 109.96 mg/Kg (average) in Symbiodinium spp. Both Symbiodinium spp. and anemones accumulated Ni. However, the accumulation of Ni varied between the doses. The Ni accumulation in the Symbiodinium spp. was influence by different doses and duration of exposure to Ni. In summary, the accumulation of nickel shows a general pattern of increase of Ni dose and duration of exposure (Figure 1). The highest accumulation of Ni in Symbiodinium spp. occurred in 9 days exposure in concentration 2.115 mg/L (average Ni 212.61± 50.12 mg/Kg), meanwhile, 18 days exposure slightly lower in concentration 2.115 mg/L (average 129.66 ± 31.94 mg/Kg). The lower concentration occurred in control for both 9 and 18 days exposure 22.73 ± 4.61 mg/Kg and 11.51 ± 5.17 mg/Kg respectively.
Figure 1. Average concentration measured of Ni in Symbiodinium spp. in 9 and 18 days exposure

Control concentration of Ni in anemones samples taken before experimental dosing were 15.93 mg/Kg (average) less than accumulation of Ni in Symbiodinium spp. (Figure 2). The highest accumulation of Ni in anemone occurred after 9 days exposure to Ni concentration 2.115 mg/L (average 77.43 ± 4.12 mg/Kg) while the Ni measured in 18 days exposure slightly lower (average 45.54 ± 6.28 mg/Kg).

Figure 2. Average concentration measured of Ni in host (anemones) spp. in 9 and 18 days exposure
3.2 Ni uptake between the host (anemone) and Symbiodinium spp. concerns for biomonitoring using cnidarian

The present study assess Ni uptake between *Symbiodinium* spp. and the host anemone of *E. pallida* over duration of exposure up to 18 days. The result describe that the *Symbiodinium* spp. had a greater concentration of Ni than the host after all exposure time and doses. *Symbiodinium* spp. had a higher concentration of Ni compare with the host after the 9 days exposure to 2.115 mg/L, Ni was (212.60 ± 50.12 mg/Kg) while the host accumulated 45.54 ± 6.28 mg/Kg Ni. This study determined *Symbiodinium* spp. had a greater capacity for uptake the uptake of dissolved Ni than the host.

For over a decade, there were several studies have been conduct to understand the role of *Symbiodinium* spp. and the host accumulation of trace metal in cnidarian (Howard & Brown, 1987) [18] found that the concentration of trace metal significantly higher in tissue compare with skeleton of *Pachyseris damicornis* (p < 0.05), the Ni concentration in the tissues (*Symbiodinium* spp. and host coral tissue combined) was 44.3 mg/Kg dry weight (dw) and in skeleton Ni was absent. Another study investigated the uptake of zinc (Zn) and cadmium (Ca) in *Anemon viridis* and reported a higher level of metal in *Symbiodinium* spp. compare to the host [19].

The preferential uptake of different metal zinc (Zn) in *E. pallida* examined by [17] shown that the greatest accumulation of Zn occurred in *Symbiodinium* spp. after 32 days exposure to concentration 0.10 mg/L with average Zn accumulation 295 mg/Kg, while in the tissue the average accumulation of Zn was 80 mg/Kg. Overall, significantly more Zn accumulated in *Symbiodinium* spp. compare with the host over duration of time with (P=< 0.05).

Esslemont (1999) [20], reported that the in skeleton of some species of coral such as *Goniastera aspera* and *Acropora nobilis* from Herond Island, Great Barrier Reef, Australia contained low concentration of Ni (i.e. *A. nobilis* (0.45 ± 0.21 mg/Kg, dw, n=10) and *G. aspera* (1.62 ± 0.75, n=10)). There were no available data regarding the accumulation of Ni in living tissues associated with this study. According to the study, the accumulation of Ni in A nobilis skeleton was greater than *G.aspera*. The concentration of trace metal in corals studied around Darwin Harbour and Nort Shell Island, Australia showed skeleton concentration including; *G. aspera* (0.35 ± 0.11), *M. annuligra* (< 0.47), East Point *M. annuligera* (1.97 ± 0.24), Weed Reef *G. aspera* (1.73 ± 0.77) and *M. annuligra* (0.99 ± 0.65), Darwin *G. aspera* (1.10 ± 0.91), *M. annuligra* (1.39 ± 0.33) [20]. The inter-site comparison showed the significant difference between North Shell Island and Weed Reef. Ni in living tissues associated with this study assessed both the tissue of the coral host and the *Symbiodinium* spp. combined. The combined tissue samples generally had 1-2 orders of magnitude high Ni than the skeleton [20].

The concentration of Ni measured by Neutron Activation Analysis (NAA) in host *Acropora tenuis* and associated *Symbiodinium* spp. Magnetic Island (an inshore reef of the Great Barrier Reef, Australia) and One Tree Island (an offshore reef of the Great Barrier Reef, Australia) showed that *Symbiodinium* spp. were consistently higher in Ni (5.4-14.4 mg/Kg ) compared to coral tissue (0.11-1.67 mg/Kg) and skeleton (0.3-1.1 mg/Kg) [21]. These results are from corals samples from natural exposure condition but highlight that similar to the finding of the present study, *Symbiodinium* spp. accumulates Ni more effectively than the host cnidarian (coral or anemone).

Rainbow (2002) [22] asserted that all invertebrates accumulate and take up both trace metal (essential or non-essential) such as Ni could be content requirement. There are several pathways for the accumulation trace metals in marine invertebrate and generally it occurs, through water, sediment and/or diet. Once in the body it may be used metal in metabolically (depending on the species), maybe store and depurated. We can consider that the metal excretion and detoxification would also vary. In the present study, results from all the Ni treatments and control showed that *Symbiodinium* spp. has a greater accumulation potential for Ni compared to the host anemone. Ni concentration in the host and *Symbiodinium* spp. were lower after a long term exposure period of 18 days compared to 9 days. However, the concentration of Ni in *Symbiodinium* spp. were consistently higher compared to those...
measured in the host anemone and suggest that there are separate or different uptake pathways of Ni for the host and the Symbiodinium spp. of the trace metal such as Ni than the host [21].

This study showed the concentration of Ni higher in Symbiodinium spp. compared to the host anemone tissue. Past studies that have attempted to use corals as bio-monitors have not considered this different accumulation between the host anemone and Symbiodinium spp.

It well understood that bleaching is a stress response species that contain Symbiodinium spp. and trace metal are known to invoke such a response [23]. Bleaching is physiological change to cnidarian (corals and anemones) causing the symbiont leaving the host [24]. This has been measured both in the laboratory and in situ, for example; Pacillopora domicornis and Montipora verrucosa discoloured during exposure 10 µg/L copper [25]. Since the present study and other studies [17, 21] have identified a different accumulation rate of metals in Symbiodinium spp. compare to the host, biomonitoring studies must consider the abundance of Symbiodinium spp. in the tissue associated with the measured metal concentration. Stress and bleaching will reduce the abundance of Symbiodinium spp. in the host, providing a depuration pathway for metals complicating the interpretation of metal uptake and accumulation. Knowing the mechanism of trace metal effects on relationship between anemone or coral host and Symbiodinium spp. could contribute a better understanding of the risks associated with metal exposure to anemone.

Marshall (2002) [26] suggested that the bioaccumulation of trace metal of Symbiodinium spp. and the subsequent expulsion of Symbiodinium spp. may be a means of regulating metal uptake in corals. Reichelt-Brushett and McOrist (2003) [21] indicate that the Symposium spp. have an important role in trace metal regulation in corals, when the corals are stressed the Symbiodinium spp. are expelled. The concentration of trace metal found in Symbiodinium spp. vary of each different species of corals but are commonly higher in the Symbiodinium spp. e.g studied by [27] that differential metal bioaccumulation was found in scleretinian corals and their alga symbiont.

4. Conclusion
Assessment of the uptake of Ni in the host anemone and Symbiodinium spp. were completed. The present study highlighted the role of Symbiodinium spp. in accumulate and depuration of Ni. The result showed that accumulation of Ni in E. pallida was influenced by concentration of dose and duration of exposure. Symbiodinium spp. accumulated higher concentration of Ni over time compare with the host. The different accumulation of Ni by both Symbiodinium spp. and the host indicate the different of mechanisms of uptake for Ni and presumably they have different metabolic requirement. As Symbiodinium spp. make a large contribution to the whole E. pallida metal concentration, its important to include an understanding of Symbiodinium spp. abundance when using these species as biomonitors. Further research is required to aid in understanding of direct and indirect pathways of uptake between the aquatic system, the anemone and the Symbiodinium spp.

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