2021

Assessing the risk of sediment-associated nickel exposure to benthic marine biota in Southeast Asia and Melanesia

Megan Louise Gillmore

UNIVERSITY OF WOLLONGONG AUSTRALIA

Assessing the risk of sediment-associated nickel exposure to benthic marine biota in Southeast Asia and Melanesia

Megan Louise Gillmore
BEnvScAdv (Hons)

This thesis is presented as part of the requirement for the conferral of the degree:
Doctor of Philosophy

25th March 2021

The University of Wollongong,
School of Earth, Atmospheric and Life Sciences

This research has been conducted with the support of the Australian Government Research Training Program Scholarship

Supervisors:
Professor Dianne F. Jolley
University of Wollongong
Dr Lisa A. Golding
CSIRO Land and Water
Dr Jenny L. Stauber
CSIRO Land and Water

Abstract

The Southeast Asia and Melanesia (SEAM) region has extensive nickel-rich lateritic regoliths formed from the tropical weathering of ultramafic rocks. As the global demand for nickel continues to rise, these lateritic regoliths are increasingly being exploited for their economic benefit. Mining of these lateritic regoliths contributes to the enrichment of coastal sediments in trace metals, especially nickel. A review of tropical estuarine and marine ecotoxicity data for nickel highlighted the absence of sediment ecotoxicity data, benthic test species and the associated sediment toxicity test methods required to assess the potential impacts of sediment nickel exposure to benthic biota of SEAM. The aim of this thesis was to use a multiple lines of evidence approach to provide a robust evidence-base and risk assessment tools applicable for informing environmental risk assessment of nickel-rich estuarine and marine sediments within the SEAM region.

A whole sediment bioassay based on sub-lethal effects was adapted for use with the tropical benthic marine diatom *Ceratoneis closterium* (Chapter 3). Effects data relevant to the SEAM region were then derived for three nickel-spiked sediments and two field-contaminated sediments. No toxicity based on chlorophyll-*a* concentration was observed in the sediment with the highest total organic carbon content (5%) (72-h 10% effect concentration (EC10) >4,300 mg/kg dilute-acid extractable concentration of nickel). The sediments without significant total organic carbon content (≤1%) were toxic above 950 mg/kg dilute-acid extractable nickel (72-h EC10). The tropical benthic snail *Nassarius dorsatus* was also investigated for its suitability for developing a whole-sediment bioassay but was found to be relatively tolerant to dissolved nickel exposure (juvenile 21-d lowest observable effect concentration (LOEC) based on scavenging ability endpoint of 380 μg Ni/L). It was concluded that a species relevant to SEAM with greater sensitivity to nickel is required to provide greater confidence that using a whole-sediment bioassay as a line of evidence in sediment quality assessment will identify sediments likely to have adverse effects. This research highlighted the need for a more strategic approach for selecting species to develop new bioassays or alternative tools for investigating contaminated sediments in the absence of sensitive whole-sediment toxicity test methods relevant to the SEAM region.

The epibenthic marine temperate amphipod *Melita plumulosa* was used as a surrogate species to improve understanding of how sediment physicochemical properties relevant to the SEAM region influence nickel bioavailability and toxicity (Chapter 4). The current study was also used to investigate the suitability of the diffusive gradients in thin films (DGT) technique as an alternative tool for representing nickel bioavailability and predicting toxicity. The 10-d EC10 based on amphipod reproduction ranged from 280 to 690 mg/kg total recoverable nickel, 110 to 380 mg/kg dilute-acid extractable nickel, and 34 to 87 μg Ni/m^2/h DGT-labile nickel flux. Nickel bioavailability was lower in sediments with greater total organic carbon, clay content, and percentage of fine particles. DGT-labile

nickel flux measurements at the sediment-water interface integrated exposure to nickel from pore water, overlying water, and ingested sediment exposure pathways and were found to have the strongest relationship with the biological response. At most, there was a 29% reduction in 10-d *M. plumulosa* reproduction relative to the control when exposed to nickel from sediments collected from nickel laterite mining regions of New Caledonia. DGT was found to provide a complementary measure when compared to the traditional chemical measurements of nickel exposure and bioavailability in estuarine/marine sediments. Based on the combined dataset of the three nickel-spiked sediments, a DGT-labile nickel EC10 threshold of 50 (30-69) μg Ni/m^2/h was determined. The development of DGT-labile thresholds presents a promising research avenue for the SEAM region as a line of evidence given that there are no or few toxicity data available for determining biological effects on local species.

Given the lack of available whole sediment toxicity tests with which to assess nickel impacts in coastal waters, eDNA approaches to assess biodiversity were used as an additional line of evidence. High-throughput sequencing (metabarcoding) was used to determine changes in eukaryote (18S V7 rDNA and diatom specific subregion of the 18S V4 rDNA) and prokaryote (16S V4 rDNA) community compositions along a sediment nickel concentration gradient offshore from a large lateritised ultramafic regolith in New Caledonia (Vavouto Bay) (Chapter 5). This study allowed for the collective response, of a broad suite of indigenous species (including microbiota, meiofauna, and macrofauna) to be determined under ecologically relevant conditions. Significant changes in the eukaryote, diatom, and prokaryote community compositions were found along the nickel concentration gradient. Multivariate statistical analyses showed that these changes correlated most with the dilute-acid extractable concentration of nickel in the sediments, which explained 26, 23, and 19% of the variation for the eukaryote, diatom, and prokaryote community compositions, respectively. Univariate analyses showed that there was no consistent change in indices of biodiversity, evenness, or richness. Diatom richness and diversity did, however, decrease as the dilute-acid extractable nickel concentration of the sediment increased. Threshold indicator taxa analysis (TITAN) was conducted separately for each of the three targeted genes to detect changes in taxa whose occurrences decreased or increased along the dilute-acid extractable nickel concentration gradient. Based on these data, 46 mg/kg dilute-acid extractable nickel was determined as a threshold value where sensitive species began to disappear. In the case of the estuarine sediments offshore from lateritised ultramafic regolith in New Caledonia, this value is recommended as an interim threshold value until further lines of evidence can contribute to a region-specific nickel sediment quality guideline value. Putative nickel sensitive taxa that could be targeted for collection in SEAM and toxicity test development were also identified and included epibenthic and benthic species of Metazoans, including copepods, ostracods, polychaetes, and nematodes.

Within SEAM, pulsed suspended sediment events and sediment resuspension events provide an exposure pathway for particulate contaminants to suspension-feeding organisms such as corals and sponges. The risk of nickel-contaminated suspended sediment was investigated using the scleractinian

coral *Acropora muricata* when exposed to either dissolved nickel, clean suspended sediment or nickel-contaminated suspended sediment for seven days, followed by a seven-day recovery period (Chapter 6). Significant bleaching and nickel accumulation in coral tissue was observed only after exposure to high dissolved nickel concentrations and nickel-spiked suspended sediment. No effect on *A. muricata* was observed from exposure to a particulate-bound nickel concentration of 60 mg/kg acid-extractable nickel at a total suspended sediment concentration of 30 mg/L and provides a threshold above which further assessment of risk may be warranted. This study demonstrated that the bioavailability of nickel associated with suspended sediment exposure plays a key role in influencing nickel toxicity to corals.

Through this research, an evidence-base of ecotoxicity exposure and effects data and risk assessment tools that are applicable for informing environmental risk assessment of nickel-rich sediments of the SEAM region have been developed. Nickel exposure and effects data were developed for *C. closterium* and *M. plumulosa* for sediments with varying physicochemical properties. DGT was demonstrated as a suitable risk assessment tool for determining nickel exposure and predicting toxicity to aquatic organisms from nickel-contaminated sediments. Nickel threshold values for estuarine and marine sediments and suspended sediments were also derived, which, if exceeded, may indicate the potential for effects and should prompt further investigation of the risk to the environment. Overall, this body of research has addressed a knowledge deficit by providing timely information for progressing sediment risk assessment approaches within SEAM to reduce the potential impact that the intensification of nickel laterite mining may have on tropical coastal ecosystems.

Human Knowledge is never contained in one person. It grows from the relationships we create between each other and the world, and still, it is never complete.

– Paul Kalanithi

Acknowledgements

Akin to the quote on the previous page, the pursuit of scientific knowledge can rarely be achieved alone. I owe a great deal of gratitude to the many exceptional scientists that liberally provided me with their guidance, time, and support throughout my PhD. First and foremost, I would like to thank my supervisors, Lisa Golding (CSIRO), Jenny Stauber (CSIRO), and Dianne Jolley (UOW), for their unwavering support, particularly as the time taken to finish my PhD stretched out. Thank you for providing much-needed perspective and encouragement during my projects' low points and for being my biggest champions through the high points. I feel exceptionally privileged to have been mentored by three incredible women and have been greatly inspired by your passion for advancing the field of ecotoxicology and your dedication to your students. The guidance I received from all three of you has been instrumental to my development as an Ecotoxicologist and the career path I am now traversing.

Secondly, I would like to thank the many advisors and collaborators I was very fortunate to work with during my PhD. This multidisciplinary project would not have been possible without the expertise they have provided. I would like to thank Graeme Batley (CSIRO) and Stuart Simpson (CSIRO) for sharing their expertise in metal bioavailability and sediment quality assessment; Anthony Chariton (MQ), Sarah Stephenson (CSIRO), and Paul Green (CSIRO) for their expertise in metabarcoding, bioinformatics and statistics; Francesca Gissi (CSIRO), Merrin Adams (CSIRO), Amanda Reichelt-Brushett (SCU), Craig Humphrey (AIMS), and Andrea Severati (AIMS) for their expertise in marine research; Ross Smith (Hydrobiology) and Farid Juillot (IRD) for their expertise of the Southeast Asia and Melanesia region.

This project would also not have been possible without Chris Schlekat and Emily Garman (NiPERA). Thank you for allowing me the opportunity to contribute to this research project and join the strong, international and multidisciplinary research group you have built for progressing nickel research is Southeast Asia and Melanesia. I gratefully acknowledge the financial assistance provided by NiPERA, which allowed for the most incredible research opportunities to become a reality.

Thank you to all the wonderful people who helped me with various technical aspects of this project. In particular, I would like to thank Josh King and Chad Jarolimek (CSIRO) for their assistance with chemical analyses, David Spadaro (CSIRO) for providing training in the algal and amphipod bioassays, Darren Koppel (UOW) for DGT preparation and assistance with statistical analyses, Brian Jones (UOW) for assistance with particle size and XRD analyses, Zoe Lefebvre (SCU) for 3D scanning of the coral skeletons, Marc Long (UOW) for assistance with coral endpoint sampling, Tim Remaili (UOW), Jane Mellors (James Cook University), and Aleicia Holland (La Trobe) for assistance with sediment collection, Aleicia Holland for DOC analyses and providing field equipment, Captain Philippe Naudin (IRD) for guidance in the field, and Pierre Taberlet (UGA) for primer design. I also thank

Gwilym Price (UOW) for lending his talent to my project and his hard work performing the amphipod bioassays as part of his Honours degree.

I extend my thanks to all the incredible scientists at CSIRO Lucas Heights. Collectively you have created an environment that is enjoyable to work in and conductive for producing high-quality research. Attributable to the support and investment you generously provide to all the students that come through the lab. I also wish to thank the staff at the National Sea Simulator (AIMS), Vale Indonesia, and the National Centre for Technical Research, New Caledonia.

I extend so much appreciation to all the students I shared the PhD experience with. Tim Remaili, Darren Koppel, Francesca Gissi, Brett Knowles, Lakmini Egodawatta, Gaby Macoustra, Marc Long, Sarah Stone, Gwilym Price, Chloé Trinh, Shelby Lou Butz, and our cheerleader Kitty McKnight. It has been a privilege to be part of a group that is so supportive, that helps find humour in our mistakes and champions each other's success. You have made the whole PhD experience extremely enjoyable.

Lastly, I sincerely thank my family and friends. To my Mum Lexie and Partner Andrew, I cannot express how much your support and belief in me has helped me throughout my studies. You have shaped me into the person I am today, and I will be forever thankful for all that you have enabled me to achieve.

Research communication

Published manuscripts

Gillmore, M.L., Golding, L.A., Chariton, A.A., Stauber, J.L., Stephenson, S., Gissi, F., Greenfield, P., Juillot, F., Jolley, D.F. 2021. Metabarcoding reveals changes in benthic eukaryote and prokaryote community composition along a tropical sediment nickel gradient. *Environmental Toxicology and Chemistry*. https://doi.org/10.1002/etc.5039

Gillmore, M.L., Price, G.A.V., Golding, L.A., Chariton, A.A., Stauber, J.L., Adams, M.S., Simpson, S.L., Smith, R.E.W., Jolley, D.F. 2021. The diffusive gradients in thin films (DGT) technique predicts sediment nickel toxicity to the amphipod, *Melita plumulosa*. *Environmental Toxicology and Chemistry*. https://doi.org/10.1002/etc.4971

Garman, E.R., Schlekat, C.E., Middleton, E., Merrington, G., Peters, A., Smith, R., Stauber, J.L., Leung, K.M.Y., Gissi, F., Binet, M.T., Adams, M.S., **Gillmore, M.L.**, Golding, L.A., Jolley, D., Wang, Z., Reichelt-Brushett, A. 2021. Development of a bioavailability-based risk assessment framework for nickel in Southeast Asia and Melanesia. *Integrated Environmental Assessment and Management*. https://doi.org/10.1002/ieam.4384

Gillmore, M.L., Gissi, F., Golding, L.A., Stauber, J.L., Reichelt-Brushett, A.J., Severati, A., Humphrey, C.A., and Jolley, D.F. 2020. Effects of dissolved nickel and nickel-contaminated suspended sediment on the scleractinian coral, *Acropora muricata*. *Marine Pollution Bulletin*, 152:110886. https://doi.org/10.1016/j.marpolbul.2020.110886

Conference presentations

Gillmore, M.L., Stephenson, S., Gissi, F., Golding, L.A., Stauber, J.L., Juillot, F., Greenfield, P., Chariton, A.A., and Jolley, D.F. Metabarcoding reveals changes in benthic eukaryote and prokaryote community compositions along an estuarine sediment nickel gradient. Conference platform presentation. SETAC Australasia, 7-10 July 2019, Darwin, Northern Territory, Australia.

Gillmore, M.L., Price, G.A.V., Golding, L.A., Stauber, J.L., Adams, M.S., Simpson, S.L., and Jolley, D.F. The diffusive gradients in thin films (DGT) technique predicts toxicity of nickel-contaminated sediments to a marine amphipod. Conference poster presentation. SETAC Europe, 13-17 May 2018, Rome, Italy.

Gillmore, M.L., Golding, L.A., Stauber, J.L., Simpson, S.L., Chariton, A.A., Smith, R.E.W., Juillot, F., Schlekat, C.E., Garman, E.R., and Jolley, D.F. Determining the toxicity of nickel in whole-sediment to tropical marine biota: test development and sensitivity. Conference platform presentation. SETAC Australasia, 4-6 September 2017, Gold Coast, Queensland, Australia.

Gillmore, M.L., Golding, L.A., Stauber, J.L., Simpson, S.L., Smith, R.E.W., Schlekat, C.E., Garman, E.R., and Jolley, D.F. Bioavailability and toxicity of nickel in sediments to marine biota. Platform presentation. School of Chemistry Seminar Series, University of Wollongong, February 2017, Wollongong, Australia.

Gillmore, M.L., Golding, L.A., Stauber, J.L., Simpson, S.L., Smith, R.E.W., Schlekat, C.E., Garman, E.R., and Jolley, D.F. Toxicity of nickel in sediment to temperate and tropical biota. Conference poster presentation. SETAC Australasia, 4-7 October 2016, Hobart, Tasmania, Australia.

Gillmore, M.L., Golding, L.A., and Jolley, D.F. Enhancing tools for environmental risk assessment of metal contaminants in freshwater sediments. Conference platform presentation. School of Chemistry Annual Postgraduate Conference, University of Wollongong, 4-6 November 2015, Springwood, Australia.

Invited presentations

Gillmore, M.L., Golding, L.A., Stauber, J.L., Simpson, S.L., Smith, R.E.W., Schlekat, C.E., Garman, E.R., and Jolley, D.F. Bioavailability and toxicity of nickel in sediments to marine biota. Invited speaker. Centre National de Recherche Technologique (CNRT), Nickel et son Environnement, Seminar Day, 4 November 2016, Noumea, New Caledonia.

Gillmore, M.L., Gissi, F., Golding, L.A., Stauber, J.L., Reichelt-Brushett, A.J., Severati, A., Humphrey, C.A., and Jolley, D.F. The effects of dissolved nickel and nickel-contaminated suspended sediment on the branching coral, *Acropora muricata*. Invited speaker. SETAC Australasia, NSW regional meeting, 8 May 2018, Sydney, Australia.

Gillmore, M.L., Gissi, F., Golding, L.A., Stauber, J.L., Reichelt-Brushett, A.J., Severati, A., Humphrey, C.A., and Jolley, D.F. The effects of dissolved nickel and nickel-contaminated suspended sediment on the branching coral, *Acropora muricata*. Invited speaker. Laboratoire des Sciences de l'Environnement Marin, Université de Bretagne Occidentale, 22 May 2018, Plouzané, France.

Certification

I, Megan L. Gillmore, declare that this thesis, submitted in fulfilment of the requirements for the completion of the conferral of the degree of Doctor of Philosophy, from the University of Wollongong, is wholly my own work unless otherwise referenced of acknowledged. This document has not been submitted for qualifications at any other academic institution.

_____

Megan L. Gillmore

25th March 2021

What appears as a thoroughly systematic piece of scientific work is actually the final product: a cleanly washed offspring that tells us very little about the chaotic mess that fermented in the mental womb of its creator.

– Auner Treinin

Table of contents

List of tables

List of figures

Glossary of acronyms and common terms

Acute toxicity: An adverse lethal or sub-lethal effect from a toxicant that occurs as a result of an exposure period that is short relative to the organism's life span

AE-Metal: Dilute-acid extractable metal (1 M HCl). This is equivalent to simultaneously extracted metals (SEM)

AVS: Acid-volatile sulfide. The acid-soluble sulfide concentration of a sediment

Benthic organisms: Referring to biota living in or on the sediments of aquatic habitats

Bioavailable/ Bioavailability: Able to be taken up by organisms/ a relative measure of the extent to which a chemical can interact with an organism

Chronic toxicity: Adverse effects over a significant portion of the organism's life span

DGT: Diffusive gradients in thin films. A kinetic technique for passive sampling of labile metals

Dissolved: operationally defined by what will pass through a 0.45 μm sized filter

DOC: Dissolved organic carbon

DGV: Default guideline value. Indicates the concentrations below which there is a low risk of unacceptable effects occurring

DW: Dry weight

EC10/EC50: The concentration of a toxicant in solution that is estimated to be effective in producing a sublethal response in 10% or 50% of test organisms, respectively, under specified test conditions

GV-high: Upper guideline value. Indicates the concentrations at which you might already expect to observe toxicity-related adverse effects

Labile: A compound with metal-ligand bonds that may readily dissociate and re-associate depending on the physicochemical environment

LOEC: Lowest observable effect concentration. The lowest tested concentration of a toxicant at which organisms are adversely affected compared to control organisms

MOTU: Molecular operational taxonomic unit

NTU: Nephelometric turbidity units

Overlying water: The water above the sediment at a collection site or in a test chamber

PCA: Principal component analysis

Pelagic organism: Referring to biota living in the water column (generally free-swimming or floating)

PES: Polyethersulfone

PNEC: Predicted-no-effect concentration

Pore water: The water that occupies the space between and surrounds individual sediment particles in an aquatic sediment

PSU: Practical salinity units

SD: Standard deviation

SEAM: Southeast Asia and Melanesia

Speciation: Measurement of different chemical forms or species of an element in a solution or solid

SQGs: Sediment quality guidelines

SSD: Species sensitivity distribution

Sub-lethal: A toxic or deleterious effect below the level of exposure that causes death

TIC: Total inorganic carbon

TOC: Total organic carbon

TR-metal: Total recoverable metal

TSS: Total suspended solid

TWA: Time-weighted average

Whole sediment: The sediment and associated pore water

Definitions adapted from Simpson and Batley (2015)

Chapter 1

1 General introduction

1.1 Significance of the study

The Southeast Asia and Melanesia (SEAM) region (Figure 1.1) has unique and highly biodiverse coastal ecosystems with immense ecological, cultural, and economic significance (Hoeksema, 2007; Martínez et al., 2007). The region contains important mangrove forests, coral reefs, and seagrass beds that provide important ecosystem services (Burke et al., 2002; Peters et al., 1997). These habitats reside at the interface between terrestrial, estuarine, and coastal marine ecosystems and are increasingly under threat from natural influences and anthropogenic activity (Burke et al., 2002; Martínez et al., 2007; Peters et al., 1997; Todd et al., 2010; Zhang and Hou, 2020). Nickel mining and processing are growing steadily in tropical regions of the world due to the global shift in nickel production from the sulfide ore to the laterite ore (Dalvi et al., 2004; Mudd, 2010; Norgate and Jahanshahi, 2011). Nickel mining on small island nations of SEAM is expected to intensify to sustain global demand for nickel (Dalvi et al., 2004). This raises concern as to the impact potential increased nickel exposure may have on the fragile tropical coastal ecosystems of the SEAM region (Gissi et al., 2016).

Within SEAM, there are no defined risk assessment frameworks or guidelines for water or sediment quality assessments. A recent review of ecological risk assessment tools and ecotoxicological exposure and effects data for tropical estuarine and marine biota of SEAM highlighted the absence of nickel sediment toxicity data, benthic test species and the associated sediment toxicity test methods with which to investigate nickel bioavailability and toxicity to benthic estuarine and marine biota (Gissi et al., 2016). This leaves uncertainty in terms of the risk that nickel mining poses to benthic biota within the SEAM region. There are, however, commonalities between risk assessment frameworks and tools that exist for many other jurisdictions that could serve as a solid basis for evaluating the risk of sediment nickel exposure within the SEAM region. The appropriateness of these frameworks and tools for application in tropical SEAM should be determined. This will require evaluating what effect region-specific differences in sediment geochemistry and taxonomic composition of the SEAM region have on the bioavailability and toxicity of nickel within coastal ecosystems.

There is an urgent need to develop risk assessment tools and region-specific information so that the environmental risk associated with the development of laterite mining in SEAM can be appropriately represented and integrated into decision-making processes that also consider economic advancement, social progress, and cultural values. This is required to enhance the protection of coastal ecosystems of small island societies which rely heavily on these ecosystems to provide valuable food security, revenue streams (e.g. tourism and fishing), protection against physical forces (e.g. a physical buffer against extreme weather events), and for social recreation, traditional customs, or ceremonies.

Figure 1.1. A map outlining the Southeast Asia and Melanesia region (highlighted blue*). Map base from SketchBubble.com © 2020.*

1.2 Nickel in the aquatic environment

1.2.1 Sources and fate

Nickel is a naturally occurring transition metal that makes up approximately 1-3% of the composition of the Earth (Wells, 1943). While the majority of nickel is inaccessible in the Earth's core, some commercially exploitable nickel is broadly distributed through the Earth's crust in either laterite or sulfide deposits (Mudd, 2010). Laterite deposits form at the surface of the Earth from the intensive weathering of serpentinites under humid tropical or sub-tropical conditions whereas sulfide deposits are typically the result of volcanic or hydrothermal processes (Butt and Cluzel, 2013; Mudd, 2010). Historically, the sulfide ore has dominated global nickel production, owing to the more expensive and complex processing required by the laterite ore (Mudd, 2010). However, with 60% of world nickel resources being in laterite ore (USGS, 2020), their extraction is expected to dominate the long-term supply of nickel (Butt and Cluzel, 2013; Dalvi et al., 2004; Mudd, 2010; Norgate and Jahanshahi, 2011).

The majority of laterite deposits occur in tropical and subtropical regions, including New Caledonia (21%), Australia (20%), Philippines (17%), Indonesia (12%), Central and South America (9%), Africa (8%), Caribbean (7%), and other (6%) (Dalvi et al., 2004). A laterite deposit that contains an economically significant concentration of nickel in one or more horizons is defined commercially as a

"nickel laterite" (Butt and Cluzel, 2013). SEAM is a particular hotspot for nickel laterites, encompassing three of the world's major nickel producing countries (New Caledonia, the Philippines, and Indonesia). To sustain the global demand for nickel, the intensification of nickel mining within the SEAM region is expected, as future expansion in nickel production capacity will come from the processing of laterite ore (Dalvi et al., 2004).

Nickel is a significant metal for modern infrastructure development, manufacturing, and technological advancement. It is predominately used as an alloying metal in the production of stainless and alloy steel products, nonferrous alloys, and superalloys due to the unique physicochemical characteristics it imparts which include strength, corrosion resistance, stability at extreme high and low temperatures, malleability, ductility, and electrical conductivity properties (Mudd, 2010; Pyle and Couture, 2012). These alloys are utilised in applications that range from household appliances, medical equipment, and industrial machinery through to jet engines and aerospace structural materials (Geoscience Australia, 2018). The other main uses of nickel include electroplating (to form a protective coating on electronic components) and rechargeable battery production (especially Ni-Cd, Ni-metal hydride, and Li-ion batteries of electric vehicles) (Geoscience Australia, 2018; Nieminen et al., 2007). To satisfy consumer demand, nickel production experienced near exponential growth between the 1950s and 2000s (Mudd, 2010). World nickel production now exceeds 2 million metric tonnes annually (Figure 1.2) (USGS, 2020).

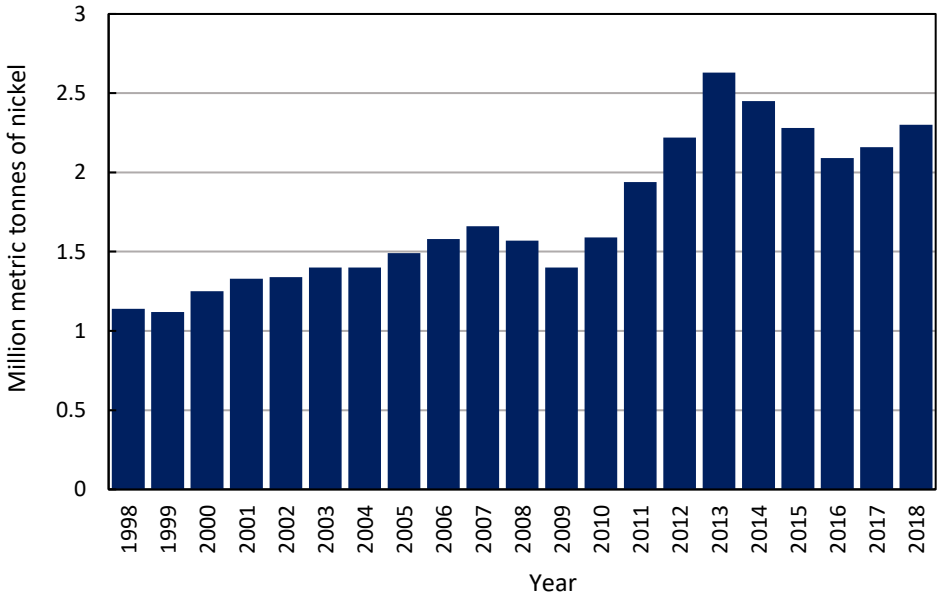


Figure 1.2. World nickel production between 1998 and 2018. Data compiled from the US Geological Survey, Mineral commodity summaries for nickel, 2000 to 2020 (e.g., USGS, 2020).

Nickel may enter the aquatic environment from natural or anthropogenic sources. Natural processes mobilising nickel include weathering of continental crust; atmospheric deposition of mineral dust, volcanic ash, and ash from forest fires; hydrothermal vent fluids; and surface runoff (Cempel and Nikel, 2006; Nieminen et al., 2007). Anthropogenic sources of nickel are from past and present industrial practices including mining, smelting, and refining; metal plating and manufacturing; battery disposal; and the combustion of oil for electricity and heat production (Cempel and Nikel, 2006; Nieminen et al., 2007).

Sediments are an important reservoir of metal contamination in SEAM resulting from erosion and sedimentation along the coastline which occur naturally but are also strongly enhanced by anthropogenic activities including urbanisation and mining. Nickel laterites are generally extracted by open-cast mining, leading to major landscape alteration, enhanced soil erosion rates, and the production of large volumes of waste material (Fernandez et al., 2006; Marchand et al., 2012; Noël et al., 2015). Tropical rainfall events can mobilise this material and transport associated particulate-bound metals to tropical coastal ecosystems, which can greatly increase turbidity, sedimentation and metal contaminant levels in estuarine and marine sediments (Clavier et al., 1995; Fernandez et al., 2017, 2006; Marchand et al., 2012). Within SEAM, nickel concentrations of sediments and pore waters within catchments receiving terrigenous inputs (Table 1.1) are significantly higher than in other similar environments of the Asia-Pacific without ultramafic rocks and lateritic soils (Table 1.2). Total sediment nickel concentrations higher than 100 mg/kg are not usually observed worldwide (Birch, 2017; Defew et al., 2005; Lewis et al., 2011; Morrison et al., 2010).

Within SEAM, dissolved nickel concentrations in freshwater can reach 50 μg/L (95^{th} percentile of data collated from monitoring sites, n = 483) and marine waters can reach 125 μg/L (95^{th} percentile of data collated from monitoring sites, n = 117) (Hydrobiology, 2016). For comparison, a background seawater concentration for nickel of 0.09 μg/L outside of the New Caledonia lagoon has been reported (Moreton et al., 2009). Vertical profiling of dissolved nickel in the open ocean has revealed that nickel typically occurs at very low concentrations at the ocean surface with concentrations increasing with depth over a range of 0.1 to 0.7 μg/L (Bruland, 1980; Sclater et al., 1976). This profile of surface depletion and deep-water maxima correlates with major nutrients such as silicon and phosphorous suggesting nickel is a micronutrient in the ocean (Sclater et al., 1976).

Nickel has been revealed to fulfil an important component for a variety of enzymes found in prokaryotes (eubacteria and archaebacteria) as well as fungi and plants, with critical roles in carbon and oxygen cycling, nitrogen fixation, and hydrogen metabolism (Chowdhury et al., 2008a; Zamble et al., 2017). Some examples of nickel-containing enzyme proteins include superoxide dismutase (protects photosynthetic organisms from harmful reactive superoxide radicals produced by their metabolism), ureases (used by algae and cyanobacteria to catalyses the hydrolysis of urea to ammonia and carbon

dioxide for nitrogen acquisition and by ammonia-oxidising microbes in the deep sea to generate their electron donor), and Ni-Fe hydrogenases (catalyses the reversible reaction between two protons and two electrons to generate hydrogen gas) (Zamble et al., 2017). The essentiality of nickel in higher-order aquatic organisms has not been elucidated (Chowdhury et al., 2008a; Muyssen et al., 2004). Preliminarily studies have provided indirect evidence that nickel is essential in fish (Chowdhury et al., 2008b; Leonard et al., 2009).

Table 1.1. Examples of nickel concentrations in surficial coastal sediments and pore waters within Southeast Asia and Melanesia (receiving inputs of terrigenous material from ultramafic rocks and lateritic soils)

Location	Sediment Ni* (mg/kg)	Pore water Ni (μg/L)	Reference
Southeast Asia and Melanesia			
Boulari Bay, New Caledonia	610 – 4,400	NR	Dalto et al. (2006)
	260 – 7,100[†]	NR	Fernandez et al. (2006)
Conception Bay[‡], New Caledonia	1.7 – 210[§]	10 – 110[§]	Marchand et al. (2011)
Dumbea Bay, New Caledonia	1,100 – 1,300	NR	Ambatsian et al. (1997)
	570 – 2,900	NR	Dalto et al. (2006)
	1,300 – 3,800[§]	≤1,800[§]	Marchand et al. (2012)
	2,900	≤42,000[§]	Noël et al. (2017)
Maa Bay[‡], New Caledonia	54 - 150	NR	Dalto et al. (2006)
Sainte Marie Bay, New Caledonia	170 – 370	NR	Fernandez et al. (2006)
	130 – 280	NR	Dalto et al. (2006)
Vavouto Bay, New Caledonia	800 – 5,200[§]	≤2,900[§]	Noël et al. (2015)
Philippine Sea, Philippines	13 – 260	NR	Li et al. (2019)

NR = Not reported.

* Where a vertical profile was provided, only values from depths <30 cm were included in the range provided

[†] Nickel concentration on suspended sediment.

[‡] Reference locations for the region that receive minimal terrigenous or anthropogenic inputs.

[§] Values originally expressed in μmol/g or μmol/L were converted to μg/g or μg/L by multiplication with molecular mass (Ni = 58.69 g/mol).

Table 1.2. Examples of nickel concentrations in surficial coastal sediments of the Asia-Pacific (not receiving inputs of terrigenous material from ultramafic rocks and lateritic soils). Pore water concentrations not reported within these articles

Location	Sediment Ni* (mg/kg)	Reference
Asia-Pacific		
Tutuila Island, American Samoa	1.2 – 67	Morrison et al. (2010)
Moreton Bay, Australia	<10 – 54	Morelli and Gasparon (2014)
	3.5 – 36	Coates-Marnane et al. (2016)
Sydney Coast, Australia	3 – 9	Schneider and Davey (1995)
Sydney Estuaries, Australia	6 – 26	Melville and Pulkownik (2007)
Jiulong River Estuary, China	11 – 16	Jingchun et al. (2006)
Great Astrolabe Lagoon, Fiji	4 – 25	Morrison et al. (1997)
Pago Bay, Guam	≤0.15 – 26	Denton and Morrison (2009)
New Territories, Hong Kong	0.9 – 64	Tam and Wong (2000)
East Coast, Malaysia	16 – 45	Ashraf et al. (2018)
West Coast Saipan, Marina Island	<0.2 – 12	Denton et al. (2009)
Philippine Sea, Philippines	13 – 260	Li et al. (2019)
North Coast, Singapore	7.4 – 12	Cuong et al. (2005)
Fanga'uta Lagoon, Tonga	11 – 14	Morrison and Brown (2003)
Sopu fringing reef, Tonga	<2	Morrison and Brown (2003)

* Where a vertical profile was provided, only values from depths <30 cm were included in the range provided.

1.2.2 Speciation and bioavailability in natural waters

The speciation of a metal in the aquatic environment is an important consideration as it strongly influences that metal's mobility and bioavailability to aquatic biota. It is widely regarded that the most bioavailable form of a metal is the freely dissolved ion (e.g. M^{2+}), followed by dissolved inorganic metal ion pairs (e.g. $M\text{-}OH^{+}$, $M\text{-}Cl^{+}$, $M\text{-}CO_3$), and organic forms (M-DOM) (Vink, 2009). The freely dissolved ion is often regarded as the most bioavailable form of a metal because it is thought to be more readily transported through, or able to interact with the external membranes of organisms (Di Toro et al., 2001).

Nickel can exist in any one of several oxidation states (including -1, 0, +1, +2, +3 and +4), however, in the aquatic environment, the 0 and +2 are the most important (Nieminen et al., 2007; Pyle and Couture, 2012). Many compounds can form complexes with the divalent nickel ion, precipitate it into insoluble compounds, or absorb it strongly to the surfaces of particulate material. Physicochemical properties that influence nickel speciation in the aquatic environment include pH, redox potential, temperature, ionic

strength, complexing and precipitating inorganic and organic ligands, other cations that compete for binding sites, and solid inorganic and organic material.

In freshwater, at pH values between 5 and 9, the freely dissolved divalent ion is the dominant form of nickel (occurring as the octahedral hexahydrate ion, $Ni[(H_2O)_6]^{2+}$) in the absence of dissolved organic carbon (DOC) (Nieminen et al., 2007; Pyle and Couture, 2012). In increasingly alkaline or calcareous waters nickel is stable as $NiHCO_3^+$, $NiCO_3^0$, and $Ni(OH)_2^0$, respectively (Nieminen et al., 2007). At lower pH, competition between Ni^{2+} and H^+ causes nickel dissociation from hydrous oxides (Pyle and Couture, 2012).

In seawater, nickel predominantly exists as the inorganic, Ni^{2+} cation (~50%) and to a lesser extent in chloride and sulfate complexes (Byrne, 2002). It is estimated that only a small portion (10-30%) of dissolved nickel in seawater is strongly bound to organic complexes (conditional stability constant $>10^{17}$) in contrast to freshwaters where 99% of nickel may be present as strongly complexed organic ligands (Pyle and Couture, 2012; Xue et al., 2001).

1.2.3 Speciation and bioavailability in sediment

The bioavailability of metals in sediments is controlled by the partitioning of metals between dissolved phases (in sediment pore waters and overlying waters) and solid phase particulate matter associated with the sediments (sulfide, complexed iron and manganese (oxy)hydroxides, and particulate organic matter) (Simpson and Batley, 2007). The sediment-water interface is the boundary between the bed sediment and the overlying water column and is where gradients in physical, chemical and biological parameters are greatest. Most bodies of water contain dissolved oxygen down to the sediment water interface, with an oxic-anoxic transition within the sediment (the exception is water bodies of high primary production or stagnant conditions which may shift this boundary upwards into the water column) (Batley and Simpson, 2015a). Sediment chemistry is strongly influenced by the oxygen content of the pore waters, which generally becomes depleted with sediment depth due to low oxygen penetration into sediments, and the utilisation of oxygen-rich compounds by the microbial community (Brune et al., 2000; Wenning et al., 2005). The oxic zone typically has a thickness of a few millimetres in silty sediments to several centimetres in coarser riverine and estuarine sands and is underlain by a sub-oxic and then an anoxic area (Simpson et al., 2015). The oxygen gradient leads to vertical stratification in sediments and pore waters of redox potential, pH, and various chemical species including lead, manganese, other trace metals, and sulfide (Wenning et al., 2005).

In oxidised sediments, organic material, carbonates, and hydrous oxides of iron and manganese (e.g. FeOOH and MnOOH) compete for the binding of metals (Landner and Reuther, 2004). In anoxic sediments, reduction rather than oxidation dominates chemical reactions and the primary control is the precipitation of metal sulfide (Di Toro et al., 1992, 1990; Landner and Reuther, 2004). Sulfide, produced by sulfate-reducing bacteria, can sequester many cationic metals from pore waters through

the formation of highly insoluble metal sulfide complexes that are not bioavailable (Di Toro et al., 1992, 1990; Landner and Reuther, 2004). The acid volatile sulfide (AVS) content of a sediment is considered to be the more reactive pool of solid-phase sulfide (major component being FeS) and is an operationally defined parameter indicating those sulfides that are readily extracted from sediments by cold dilute hydrochloric acid (Berry et al., 1996; Di Toro et al., 1990; Landner and Reuther, 2004). In general, appreciable concentrations of cadmium, copper, nickel, lead, and zinc will not be observed in pore waters until the reactive pool of AVS is exhausted, which is explained by Di Toro's equilibrium partitioning theory (Di Toro et al., 1992, 1990). The theoretical foundation for equilibrium partitioning theory is that the sulfides of cadmium, copper, nickel, lead, and zinc, all have a lower sulfide solubility product constant than do the sulfides of iron and manganese and as a result, these metals will displace iron and manganese whenever they are present together with iron and manganese monosulfides (Berry et al., 1996; Di Toro et al., 2005). Because the solubility product constants of these sulfides is small, sediment with an excess of AVS will have very low metal concentration in the pore waters and no toxicity due to these metals would be observed in the sediments; however, once the AVS binding potential is exhausted metals will be present in the pore water and toxicity is predicted (Berry et al., 1996; Di Toro et al., 2005).

In freshwater oxic surface sediments, the importance of iron and manganese over organic carbon for nickel binding and reducing metal bioavailability and toxicity has been demonstrated (Costello et al., 2016, 2011). In sub-oxic and anoxic freshwater sediments, AVS and iron have been shown to be the most important factors modifying nickel bioavailability and toxicity (Besser et al., 2013; Vangheluwe et al., 2013).

1.2.4 Nickel cycling in lateritic estuarine and marine sediments

There is a growing body of research investigating the influence of terrigenous inputs from open cast mining of lateritic horizons on sediment diagenesis within coastal environments, mainly from New Caledonia. These studies have shown that metals, including nickel, are mainly transported to the coast and deposited as oxides and (oxy)hydroxides (Ambatsian et al., 1997; Fernandez et al., 2006; Noël et al., 2014).

In the New Caledonian lagoon sediments, it has been shown that the major binding phase for nickel are iron bearing clay minerals (Merrot et al., 2019). In these sediments, evidence of the solubilisation and release of nickel after deposition has also been demonstrated (Ambatsian et al., 1997). However, sulfide does not contribute to nickel speciation at a detectable level, rather the speciation is attributed to low organic carbon content compared to the pool of iron in the sediment and the repeated re-oxidation of the upper layers of sediment from re-suspension due to the shallow and dynamic lagoon waters (Merrot et al., 2019). Within the lagoon, Fernandez et al. (2006) found that the nickel concentration of suspended matter reached as high as 7100 mg/kg.

Within the mangrove sediments of New Caledonia, it has been shown that following deposition, nickel undergoes transformation to organic and sulfide forms (Marchand et al., 2012; Noël et al., 2017, 2015, 2014). The transformation process involves the microbial decomposition of organic matter which leads to the release of soluble nickel from the oxide form to the dissolved phase which then precipitates with organic and sulfide compounds (Marchand et al., 2012). This process has been reported to release significant amounts of nickel, with dissolved nickel concentrations as high as 1,800 μg/L (Marchand et al., 2012), 2,900 μg/L (Noël et al., 2015), and 42,000 μg/L (Noël et al., 2017) being reported in pore waters of the organic-rich layer of mangrove sediments downstream of lateritic nickel deposits. It has been noted that further studies that investigate the export of dissolved trace metals from the sediments are required to understand the role of tropical coastal sediments as a sink or source for trace metals (Marchand et al., 2016).

Besides nickel, laterite deposits are typically enriched with other transitional metals including iron, manganese, cobalt, and chromium which also become enriched in the coastal sediments (Ambatsian et al., 1997; Dublet et al., 2012).

1.3 The response of aquatic organisms to nickel exposure

1.3.1 Organism exposure pathways

It is well established that different species of benthic organisms will differ in their sensitivity to sediment contaminants due to their physiology (e.g. internal regulation and detoxification) and differing exposure pathways (Rainbow, 2007). For benthic organisms, exposure to metals may occur from either dissolved exposure pathways (e.g. from the uptake of the free metal ion or weakly bound metal complexes over the body surface or respiratory organs, or via exposure to the interstitial pore water and overlying water) or dietary exposure pathways (e.g. from the ingestion of contaminated sediment particles, plants, algae, or prey) (Rainbow, 2007). The relative importance of these pathways will largely depend on the organisms feeding strategies and burrowing or irrigating behaviours (Rainbow, 2007). Pulsed suspended sediment events and sediment resuspension events provide an exposure pathway for particulate contaminants to suspension-feeding organisms such as corals and sponges (Jones et al., 2016). Sediment resuspension (and mobilisation of metals to the dissolved form) may occur from biotic activities, such as bioturbation and bioirrigation (Chapman et al., 1998) as well as abiotic activities, such as tidally-driven near-bottom currents and dredging (Jones et al., 2016). Within SEAM, a frequent cause of suspended sediment events is sediment resuspension and terrigenous input from run-off of particulate matter resulting from intense precipitation events (Clavier et al., 1995). According to Clavier et al., (1995) three-fold higher total suspended sediment concentrations and higher sedimentation rates occur during the wet season than during the dry season.

1.3.2 Mechanisms of nickel toxicity to aquatic organisms

Literature concerning the mechanisms of nickel toxicity to aquatic organisms have been thoroughly reviewed for several classes of organisms including freshwater microorganisms (Macomber and Hausinger, 2011), freshwater organisms (Brix et al., 2017), marine and estuarine invertebrates and fish (Blewett and Leonard, 2017). While the mechanisms of nickel toxicity to aquatic organisms have not been completely elucidated, these reviews have identified the following mechanisms as plausible based on evidence found in the literature: (i) ionoregulatory toxicity (disruption of Ca^{2+}, Mg^{2+}, or $Fe^{2+/3+}$ homeostasis); (ii) respiratory toxicity (allergic reaction at respiratory epithelia); and (iii) oxidative stress (generation of reactive oxygen species). There are however still several major uncertainties in the present understanding of the mechanisms of nickel toxicity to aquatic organisms.

1.3.3 Toxicity of nickel from sediment exposure

Over the past decade, significant effort has been channelled into developing a bioavailability-based risk assessment approach for nickel in temperate freshwater sediments (Schlekat et al., 2016; Vangheluwe et al., 2013). Bioavailability-based approaches take into account water and sediment chemistry (e.g. TOC, pH) and its influence on metal binding and uptake by organisms (Adams et al., 2020). These approaches allow for a bioavailability-based correction to be incorporated into environmental risk assessment for the protection of aquatic life, avoiding over conservative estimates. The research effort focused on temperate freshwater sediment involved developing robust nickel-spiking methodologies for sediments (Brumbaugh et al., 2013), extensive laboratory ecotoxicity testing (Besser et al., 2013; Custer et al., 2016a) and field validation of the importance of different sediment properties mediating nickel bioavailability and toxicity (Costello et al., 2012, 2011; Custer et al., 2016b; Nguyen et al., 2011). The ecotoxicological exposure and effects data were then compiled and used to develop a species sensitivity distribution (SSD) and evaluate the use of predictive models for bioavailability and chronic toxicity of nickel in freshwater sediments (Schlekat et al., 2016; Vangheluwe et al., 2013).

The first phase of ecotoxicological testing investigated chronic toxicity of two nickel-spiked sediments (of high and low nickel binding capacity based on concentrations of AVS and total organic carbon (TOC)) to eight taxa of benthic invertebrates using lethal and sub-lethal endpoints (Besser et al., 2013). The study found that the taxa most sensitive to sediment nickel exposure were amphipods, mayflies, and oligochaetes, whereas midges and mussels were relatively insensitive. This result is consistent with a previous study which had reported a similar ranking of sensitivity of sediment nickel exposure to amphipods, mayflies, and oligochaetes based on survival (Milani et al., 2003). The results of Milani et al. (2003) and many of the other early studies investigating the toxicity of nickel-spiked freshwater sediments (e.g. Borgmann, 2001; Vandegehuchte et al., 2007) are considered indicative only, due to insufficient equilibration time of the nickel-spiked sediments and exchange of overlying waters contributing to high dissolved nickel concentrations in the overlying water. Improved metal spiking

methods for sediments are now available (e.g. Brumbaugh et al., 2013; Hutchins et al., 2008; Simpson et al., 2004).

The second phase of ecotoxicological testing took the three most sensitive species (two amphipods, *Hyalella azteca* and *Gammarus pseudolimnaeus*, and the mayfly, *Hexagenia* sp.) and determined chronic nickel toxicity to six additional nickel-spiked sediments with a wide range of AVS and TOC concentrations (Besser et al., 2013). Toxicity from nickel exposure in the lowest binding capacity sediment was up to 11 times greater than in the highest nickel binding capacity sediment based on the total recoverable nickel concentration (e.g. for *H. azteca* the 28-d lethal concentration required to reduce survival by 20% (LC20), ranged from 317 to 3,480 mg/kg total recoverable nickel) demonstrating that sediment chemistry can greatly influence the ecotoxicological response (Besser et al., 2013). The significant modifying factors were concentrations of AVS, TOC, and iron and manganese in the sediment and DOC in the pore water.

Vangheluwe et al. (2013) used the phase one data from Besser et al. (2013) to develop an SSD based on 10% effective concentrations to derive a generic reasonable worst-case predicted-no-effect concentration (PNEC) for total recoverable nickel for application in sediment quality assessments where there is an absence of information on toxicity modifying physicochemical properties. A threshold value of 94 mg Ni/kg dry weight (DW) under reasonable worst-case conditions was derived. Vangheluwe et al. (2013) then used the phase two data to develop predictive bioavailability models based on relationships between sediment physicochemical properties and chronic toxicity thresholds (20% effect concentrations) to estimate sediment PNECs for total recoverable nickel to reflect bioavailability under site-specific conditions. This resulted in threshold values of 126 mg Ni/kg to 281 mg Ni/kg DW based on the AVS model, and 143 mg Ni/kg to 265 mg Ni/kg DW based on the iron model. After further toxicity testing to expand the database (Vangheluwe and Ngyuen, 2014), Schlekat et al., (2016) revised the SSD and derived a threshold of 136 mg Ni/kg which would be broadly protective for freshwater benthic communities and 437 mg Ni/kg for low nickel bioavailability sediments based on the AVS model (90^{th} percentile AVS). Field validation found the laboratory-based thresholds to be a conservative estimate of effects observed on a broad range of benthic species in natural freshwater environments (Costello et al., 2011; Schlekat et al., 2016).

Comparatively, far less attention has been given to developing ecotoxicological data for the effects of nickel-rich sediments on temperate estuarine or marine biota (Chandler et al., 2014). The risks posed to tropical benthic biota from nickel exposure, in both freshwater sediments (Binet et al., 2018) and estuarine or marine sediments (Gissi et al., 2016), is an area that is lacking quality effects data entirely. Likewise, the effect of particulate nickel, from suspended sediment exposure to nearshore coastal ecosystems, such as coral reefs, has not been investigated.

1.4 Sediment quality assessment

1.4.1 Risk assessment frameworks

Environmental risk assessment and management is a scientific process used by policymakers, governments, industry, and environmental agencies to identify and evaluate threats to the environment and allow for informed decision making within a regulatory framework. Specifically, sediment quality assessment is concerned with predicting the risk of adverse ecological effects from sediment-associated contaminants. Within SEAM, there are no defined risk assessment frameworks for water or sediment quality assessments. Other jurisdictions around the world have well-developed frameworks for the management of contaminants in the aquatic environment (e.g. Australia and New Zealand, Europe, Canada, and several states of the USA). In these jurisdictions, their frameworks for risk assessment have been refined over many years, and while they may differ slightly, some key elements have become consistent across the different frameworks (Merrington et al., 2014). For example, it is now widely regarded internationally that bioavailability-based approaches are best practice for conducting risk assessments (Schlekat et al., 2020). The integration of multiple lines of evidence, in a weight-of-evidence approach, has also gained strong international support (Batley and Simpson, 2015b; Chapman et al., 2002; Chapman and Anderson, 2005; Suter et al., 2017). Overall, investigations of sediment quality should be bioavailability-based and ideally integrate assessments of (i) sediment chemistry and potential contaminant bioavailability; (ii) toxicity; (iii) bioaccumulation; (iv) benthic ecology; and (v) other lines of evidence (Australian and New Zealand Governments, 2018; Batley and Simpson, 2015b). The weight-of-evidence approach then considers the quality, quantity, ecological relevance and congruence of each line of evidence and incorporates them for making a judgement on the potential for ecological impacts from contamination (Chapman et al., 2002).

1.4.2 Sediment chemistry

Chemical analyses of sediments are the most direct approach to reveal the metal contamination status in the aquatic environment and for this reason, often form the first step in the sediment quality assessment process. It is well recognised that the total concentrations of contaminants in sediments can be a poor indicator of the risk posed to benthic biota, as some of those contaminants may be present in less bioavailable forms (Besser et al., 2013; Simpson and Batley, 2007). While there is no direct way to measure the fraction of a contaminant that is bioavailable, the following measurements provide a good indication of the metal partitioning and risk posed by metal contaminants that are more relevant than the total metal concentration: (i) dilute-acid extractable metals; (ii) AVS; (iii) pore water metals and (iv) the labile-metal flux from the sediment (the latter is described in Section 1.4.2.2). Analytical measurements should also include pH, redox potential, moisture content, particle size distribution, TOC, AVS, particulate iron and manganese, and pore water constituents (e.g. iron, manganese,

ammonia, and sulfide) that can alter the bioavailability of contaminants in dissolved and particulate phases to aid in data interpretation (Batley and Simpson, 2015a).

1.4.2.1 Sediment quality guidelines

Generally, the derivation and application of sediment quality guidelines (SQGs) have become commonplace in sediment quality assessment frameworks to identify instances where further investigation or regulatory intervention is required based on the exceedance of the guideline value. A variety of approaches for developing SQGs have been investigated and can be broadly categorised as either empirically-based, mechanistically-based (e.g., equilibrium-partitioning), or consensus-based guidelines (Batley et al., 2005).

The empirically-based SQGs have typically been developed using large databases and are based on matching biological effects from toxicity testing with sediment chemistry of field-collected samples (Batley et al., 2005). The most widely applied SQGs internationally are based on the datasets of Long and Morgan, (1990), Long and MacDonald, (1992) Long et al., (1995), and MacDonald et al., (1996) following the empirical approach to guideline derivation. Long et al. (1995) generated two values, effects range low (ER-L) and effects range median (ER-M) from the ranking of both field ecological and laboratory ecotoxicity effects data compiled from studies conducted largely on temperate, estuarine and marine sediments of North America. The ER-L represents the 10^{th} percentile value of the data distribution and the ER-M represents the median value. The ER-L is indicative of concentrations below which adverse effects rarely occur, whilst the ER-M represents concentrations above which effects frequently occur in exposed macrofauna. Variants to this approach for setting SGQs include the threshold effects level (TEL), probable effects level (PEL) (Macdonald et al., 1996; Smith et al., 1996), and apparent effects threshold (AET) (Barrick et al., 1988). SQGs based on empirical approaches are being used in many jurisdictions internationally (Table 1.3).

Table 1.3. Sediment quality guidelines for nickel in use around the world (mg/kg dry weight)

SQG	Description	Ni (mg/kg DW)	Reference
North America			
ER-L	Concentration below which effects are observed infrequently	20.9	Buchman (2008); originally Long et al. (1995)
ER-M	Concentration above which effects are often observed	51.6	
TEL	Concentration below which effects are observed infrequently	15.9	Buchman (2008); originally MacDonald et al. (1996) and Smith et al. (1996)
PEL	Concentration above which effects are often observed	42.8	
TRV	Concentrations below which adverse effects are not expected to occur	20.9	US EPA (1999)
LEL	Most sensitive uses may be affected	16	NYDEC (1999); originally Long and Morgan (1990) and Persaud et al. (1993)
SEL	Majority of benthic organisms will be affected	50	
CB TEC	Concentration below which adverse effects are not expected to occur	22.7	MacDonald et al. (2000)
CB PEC	Concentration above which adverse effects are expected to occur often	48.6	
Australia and New Zealand			
DGV	Concentrations below which there is a low risk of unacceptable effects occurring	21	Australian and New Zealand Governments (2018); primarily adapted from Long et al. (1995) and MacDonald et al. (2000)
GV-High	Concentrations at which you might already expect to observe toxicity-related adverse effects	52	
Hong Kong			
ISQV-low	Concentration below which adverse biological effects are unlikely	40	Chapman et al. (1999); primarily adapted from Long et al. (1995)
ISQV-high	Concentrations above which severe adverse biological effects are very likely	-	

DW = Dry weight; ER-L = Effects range low; ER-M = Effects range median; TEL = Threshold effect level; PEL = Probable effect level; TRV = Toxicity reference value; LEL = Lowest effect level; SEL = Severe effect level; CB = Consensus based; TEC = Threshold effect concentration; PEC = Probable effect concentration
DGV = Default guideline value; GV-high = Upper guideline value; ISQV = Interim sediment quality value.

In Australia and New Zealand, interim SQG were introduced in 2000 (ANZECC/ARMCANZ, 2000) and rereleased in 2018 (Australian and New Zealand Governments, 2018) to predict the adverse biological effects caused by contaminated sediments. Based on the dataset of Long et al. (1995), the Australian and New Zealand guidelines took the form of a default guideline value (DGV) and an upper guideline value (GV-high) which are analogous to the ER-L and ER-M value, respectively. The DGV for nickel is 21 mg/kg and the GV-high is 52 mg/kg (Australian and New Zealand Governments, 2018). If the sediment quality guideline is exceeded, this would then trigger additional assessment steps to determine whether there is indeed a risk posed by the contaminant. Being derived from field data, a limitation of the empirically-based SQGs is that they implicitly deal with contaminant mixtures, and although the effects are assigned to a specific contaminant it is recognised that the measured biological effects actually reflect the cumulative interaction of all chemicals in the mixture. Thus, their reliability is likely to be poor, and it is acknowledged that on this basis they should only be used as a screening tool. Although their relevance for protecting temperate species within Australia has been demonstrated (McCready et al., 2006), their relevance for protecting tropical species is unknown.

The mechanistically based SQGs are derived from a theoretical understanding of the factors that govern bioavailability of sediment contaminants and known relationships between chemical exposure or uptake and toxicity (Batley et al., 2005). Equilibrium partitioning theory forms the basis for mechanistic guidelines. For metals, the theory is based on the assumption that the toxicity of a metal in sediments is proportional to its concentration in pore water, and that the concentration of a metal in pore water is related to that in sedimentary phases by appropriate equilibrium relationships (Berry et al., 1996; Di Toro et al., 1990). These guidelines attempt to account for bioavailability through normalisation to sediment characteristics that affect bioavailability. They have typically been developed and tested using laboratory-spiked sediments and compared to toxicity tests by using field-collected sediments. While many have focused on AVS as the primary factor controlling metal bioavailability in sediments (e.g. Ankley et al., 1996), the work of Vangheluwe et al., (2013) and Schlekat et al., (2016) which utilised a mechanistic-based approach to the derivation of a SQG for nickel in freshwater sediments (discussed in Section 1.3.3) attempts to account for the presence of metal binding phases beyond AVS (e.g. TOC, iron content, and percentage of fine particles) and to provide applicability to oxic sediments where AVS is not a factor. In contrast to empirically-based guidelines, mechanistic-based guidelines intend to assess the effects of only the contaminant for which the guideline is derived. They do not consider the potential additive or synergistic effects of other contaminants that may be present.

Consensus-based guidelines are based on the idea that if different methods for deriving SQGs result in a quantitatively similar concentration, then the validity of the result is greatly enhanced (Batley et al., 2005). If this requirement is satisfied, then SQGs from various sources can be combined into a single SQG or range of SQGs. This approach includes the threshold effect concentration (TEC) and probable effect concentration (PEC) for metals developed by MacDonald et al. (2000).

For many tropical regions, including SEAM, SQGs are not available. Information to assess whether risk assessment tools, such as water quality guidelines for temperate regions are applicable for tropical ecosystems, has gained attention in the last few years (Gissi et al., 2016; Kwok et al., 2007; Peters et al., 2019; Wang et al., 2014). Kwok et al. (2014) compared temperate and tropical datasets for freshwater organisms, while Wang et al. (2014) compared marine organisms and both studies found no consistent differences in temperate and tropical species acute sensitivity to chemicals. Both of these studies, however, found that temperate organisms were more acutely sensitive to nickel than tropical organisms. Peters et al. (2019) compared temperate and tropical freshwater organisms' chronic sensitivity to nickel and found that overall, organisms were responding to nickel at a similar range of concentrations. Gissi et al. (2016) similarly concluded that temperate and tropical marine organisms have similar chronic sensitivity to nickel but noted the limited data set for chronic nickel toxicity data to tropical species. Following recent investigations, it is recommended that derivation of protective guidelines for risk assessment should be based on the inclusion of as diverse a range of taxa as possible to ensure the protection of sensitive species in both temperate and tropical ecosystems (Gissi et al., 2020; Peters et al., 2019; Stauber et al., 2021).

A common concern for the application of SQGs to other jurisdictions is their inability to account for the unique biological characteristics of the region's biota or the site-specific environmental characteristics, leading to either inadequate or overly conservative risk management decisions (Wenning et al., 2005). In the context of protection of benthic aquatic species within SEAM, the inclusion of ecologically relevant species is an especially important consideration due to the high endemism of the region but also due to the higher background nickel concentrations within the region to which species may be adapted. Given the uncertainties associated with SQGs, using them as a screening tool and incorporating them into a weight-of-evidence approach is recommended. When SQGs are exceeded, having region-specific risk assessment tools that can provide additional lines of evidence are critically important to investigate the actual presence or absence of adverse effects from the sediment contaminants.

1.4.2.2 Diffusive gradients in thin films

Diffusive gradients in thin films (DGT) is now an established technique for the *in-situ* quantitative measurement of the concentration of labile metal complexes in waters, or their flux from sediments and soils into pore waters (Davison and Zhang, 2012, 1994; Zhang et al., 1995). DGT is a type of passive sampler, which are a class of chemical measurement tools that accumulate target analytes to a binding resin during their deployment in the environment. In waters, the advantage of DGT is low detection limits and time-averaged exposure concentrations of labile metal complexes, whereas in sediments DGT provides information about the supply of labile metal complexes from sediment to pore water. The DGT device most commonly consists of a selective ion-exchange binding resin and a hydrogel

diffusive gel separated from the solution by a filter membrane and plastic casing. A schematic of a DGT piston device is provided in Figure 1.3. By changing the binding resin and the diffusive gel pore size, different ions of interest can be measured (Davison and Zhang, 2012). Chelex-100 has proved suitable for many common metal contaminants including Cd, Co, Cr, Cu, Fe, Mn, Hg, Ni, Pb, and Zn (Peijnenburg et al., 2014).

The DGT technique is based on the controlled diffusion of dissolved ions across the diffusive layer of known thickness to the binding layer which selectively accumulates the ions of interest over a short, known period (usually 6-72 h for waters and 1-3 d in sediments) (Davison, 2016). When deployed in sediments, the removal of metals from pore waters causes the concentration to decline immediately adjacent to the device and the subsequent resupply of labile metals to the pore water. The resupply of metals adsorbed onto sediment particles to the pore water (known as the DGT-induced metal flux) is likely to be more rapid for sediments that contain labile forms of metals than for sediments that contain more refractory forms of metals. Hence differences in the DGT-induced metal fluxes can provide useful information on the potential bioavailability of the metals in sediments. The number of studies that have shown the DGT technique to be a suitable measure of the more bioavailable forms of metals in sediments and demonstrated links between DGT-labile metal flux and toxicity to benthic organisms is growing (Amato et al., 2015, 2014; He et al., 2018; Simpson et al., 2012b; Yin et al., 2014).

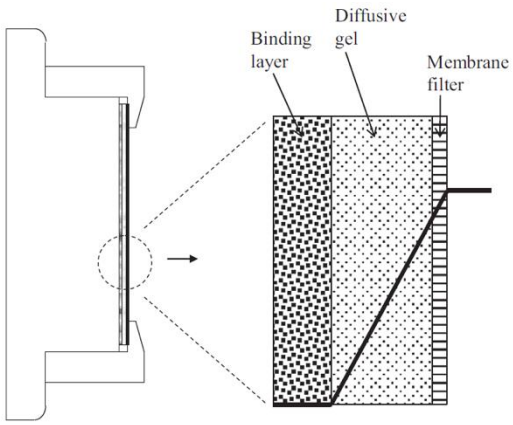


Figure 1.3. Schematic of a standard diffusive gradients in thin films (DGT) piston device adapted from Davison (2016). The bold black line represents the concentration gradient that is created when the target analytes pass from the aqueous environmental matrix through the membrane filter and the diffusive gel and are bound to the binding resin.

1.4.3 Ecotoxicology

Toxicity data obtained from ecologically relevant, single-species laboratory toxicity tests, form an important component of sediment quality assessments for they directly examine cause-effect relationships between a contaminant and an organism. It is recommended that for ecotoxicological assessment of contaminated sediments a range of benthic organisms are used to capture the response of organisms with differing sensitivities to the contaminant due to differences in physiology and exposure pathways (Simpson and Kumar, 2015). Further consideration should be given to organism life-stage, tolerance to sediment physicochemical characteristics, test duration and endpoint, as these can all have a major influence on the test outcome and the inclusion of ecologically relevant species (Simpson and Kumar, 2015).

A recent review of tropical marine ecotoxicological data for nickel highlighted there is a paucity of sediment toxicity data for nickel and a lack of test species and associated sediment toxicity test methods with which to assess the potential impacts of sediment nickel exposure to benthic biota of SEAM (Gissi et al., 2016). The development of marine whole-sediment toxicity tests methods with species ecologically relevant to the SEAM region would allow for the generation of effects data for nickel which could be utilised by environmental regulators to derive SQGs and as an additional line-of-evidence in sediment quality assessments.

1.4.4 Bioaccumulation

Bioaccumulation refers to the gradual accumulation of contaminants in the tissues of organisms which occurs when an organism absorbs a contaminant at a rate faster than it is eliminated (Wang, 2016). The comparison of bioaccumulated concentration in sedentary organisms field-sampled or field-transplanted (caged) can be a useful line-of-evidence to indicate that organisms have been exposed to bioavailable contaminants (Maher et al., 2015). While high concentrations of bioaccumulated contaminants are not necessarily linked to toxic effects in an organism, bioaccumulation data can assist decision making around site prioritisation for where more detailed assessment may be warranted to determine if contaminant concentrations may have adverse effects on sediment ecosystem health (Maher et al., 2015). Within SEAM, the use of benthic sedentary organisms as bioindicators of mining contamination has been explored with a few species being identified as effective bioindicator species, including the brown alga *Lobophora variegate* (Hédouin et al., 2008) and the clam *Gafrarium pectinatum* (formerly *Gafrarium tumidum* Röding) (Hédouin et al., 2018, 2011).

1.4.5 Benthic ecology

Benthic communities are an important component of coastal ecosystems, providing many important ecological services and functions (e.g. transfer of energy to higher trophic levels and the physical and chemical alteration of sediments enhancing the availability of resources) (Chariton et al., 2015a). Proxies for the impact of contaminants on benthic communities have in the past been used, such as single-species laboratory toxicity tests, mesocosm experiments, or field biological surveys to look for the presence/absence of indicator species. Single-species laboratory ecotoxicological testing, while important for providing direct concentration-response relationships are limited in their ability to predict ecosystem effects as they cannot reflect the importance of biological interactions (e.g. population density effects, competition, or predator-prey relationships), biological attributes (e.g., the susceptibility of different life stages and life cycle variability) or environmental pressures in the natural environment (Simpson and Kumar, 2015). Traditional methods of performing biological surveys, such as invertebrate sorting, have been limited by factors such as time, region-specific taxonomic expertise, and low taxonomic resolution (Baird and Hajibabaei, 2012; Dafforn et al., 2014). With advances in DNA-based diversity methods, such as high-throughput sequencing (or metabarcoding) biological surveys of benthic communities can now be achieved more rapidly and with much higher taxonomic resolution than traditional invertebrate sorting techniques providing new insights into the biological structure, diversity and function of ecological communities (Baird and Hajibabaei, 2012; Taberlet et al., 2012)

Metabarcoding is a molecular technique that provides species composition information from the detection and identification of species using single or multiple genes typically from fragments of degraded DNA extracted from an environmental sample (e.g. sediment, water, or air) (Taberlet et al., 2012). Well-designed community-level metabarcoding studies of benthic communities have the potential to assess the collective response of a broad suite of indigenous species (including microbiota, meiofauna, and macrofauna) to contaminant metals under ecologically relevant conditions (Bohmann et al., 2014; Cordier et al., 2020; Dafforn et al., 2014).

1.5 Thesis aims and objectives

This research aims to investigate the risk posed by nickel-rich sediment to benthic estuarine and marine biota. Emphasis is placed on delivering an evidence-base applicable for informing environmental risk assessments of nickel-rich sediments of the SEAM region to provide for the responsible development of lateritic nickel deposits. A multiple lines of evidence approach was chosen to determine the effect of sediment nickel contamination on ecosystem health as this approach would provide both laboratory-based data (contaminant chemistry, bioavailability, and toxicity) and a field-based benthic community assessment. This research also sought to develop new tools (whole-sediment toxicity test methods with ecologically relevant species) and investigate emerging techniques (metabarcoding and passive samplers) to measure the risk of nickel-rich estuarine and marine sediments to benthic biota.

The principal objectives were as follows:

(I) Develop a whole sediment bioassay based on sub-lethal effects for a tropical benthic organism relevant to the SEAM region that can be used to generate ecotoxicological exposure and effects data applicable for estuarine and marine sediments (Chapter 3).

(II) Deliver an improved understanding of how sediment physicochemical properties influence nickel bioavailability and toxicity (Chapter 4).

(III) Determine benthic community composition along a concentration gradient of nickel in the field to explore the relationship between community composition and environmental variables using molecular-based techniques (metabarcoding) (Chapter 5).

(IV) Determine if sediment-associated nickel exacerbates the risk of suspended sediment exposures to coral (Chapter 6).

Together this research will build up a picture of the risk to benthic estuarine and marine biota from exposure to sediment-associated nickel and will advance risk assessment tools for tropical environments to support the responsible development of lateritic nickel mining in the SEAM region.

Chapter 2

2 General methods

This chapter provides a description of methods and materials that were common to the research conducted in more than one chapter. Methods specific to an individual chapter are described within that chapter.

2.1 General analytical

2.1.1 Laboratory equipment preparation

All glassware was cleaned using a laboratory-grade dishwasher (GW 3060, Gallay, Australia) on a three-phase cycle of phosphate-free detergent (Clean A Powder Gallay), 1% v/v HNO_3 (analytical reagent grade, Merck, Germany), and high purity water (18 MΩ.cm, Milli-Q, Millipore, Australia). Unless otherwise stated, all plastic-ware was acid-washed before use by soaking in 10% v/v HNO_3 (Tracepur, Merck, Germany) for ≥24 h, followed by thorough rinsing with high purity water. Plastic syringes (Terumo®, Japan) and 0.45 μm polyethersulfone (PES) filters (25 mm, Minisart® Syringe Filter, High Flow, Sartorius Stedium Biotech GmbH, Germany) used for the collection of dissolved (<0.45 μm) metal subsamples were acid-washed immediately before use by passing through 5 mL of 4% v/v HNO_3 (Tracepur) followed by 20 mL high purity water.

2.1.2 Seawater

Clean seawater was collected from either the South Coast or Cronulla, NSW, Australia. South Coast seawater was collected by a commercial company and delivered immediately to the laboratory where it was stored at ambient temperature in the dark and filtered (<1 μm) just before use. Cronulla seawater was collected from a rock platform (34°04’13.35” S, 151°09’25.69” E) using acid-washed high-density polyethylene carboys (5 and 10 L), filtered (0.45 μm Sartobran P MidiCaps, Sartorius) within 3 h and stored at 4°C in the dark until use. Trace metal background concentrations for these waters are provided in Appendix A1.

Deoxygenated seawater was prepared by bubbling the solution with high purity oxygen-free nitrogen gas overnight to give dissolved oxygen concentrations of <0.1 mg/L.

2.2 Analytical measurement of waters

2.2.1 Physicochemical analysis

In Chapters 3 and 4, measurements of the overlying water pH used an epoxy body Iodine/Iodide pH probe (Thermo Orion meter model 420, probe model ROSS 815600, Thermo Fisher Scientific) which was calibrated daily against pH 4.0, 7.0 and 10.0 buffers (Orion Pacific). Salinity and conductivity were measured using a SevenGo Duo meter (Mettler Toledo, United States) and dissolved oxygen was measured with a CellOx 325 oxygen electrode fitted to an Oxi 330 meter (Wissenschaftlich-Technische Werstätten, Germany) calibrated as per manufacturer's instructions. Dissolved ammonia was measured using a rapid test kit (API Aquarium Pharmaceuticals).

In Chapters 5 and 6, physicochemical parameters including temperature, pH, salinity, specific conductivity and dissolved oxygen were measured using a YSI Professional Plus meter calibrated as per manufacturer's instructions (YSI, Yellow Springs, United States).

2.2.2 Metals analysis

Samples for dissolved metal analysis were collected with an acid-washed plastic syringe and 0.45 μm PES filter after the initial filtrate was discarded to reduce the effect of metal adsorption onto the filter membrane and to remove residual high purity water from the rinse step to prevent metal dilution in the collected sample. Subsamples were collected in acid-washed 5-mL polypropylene vials (Technoplas, Australia), preserved by acidification (0.2% v/v HNO_3, Tracepur), and stored at 4°C until analysed.

Multiple metal concentrations were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES, Agilent Technologies 700 Series, United States) and then by inductively coupled plasma mass spectrometry (ICP-MS, Agilent Technologies 7500CE) if concentrations were ≤5 μg/L. The instruments were calibrated daily using matrix-matched multi-element calibration standards (QCS-27, High Purity Standards, United States). For measurement of dissolved metals in overlying water samples, calibration standards were prepared with filtered seawater (Cronulla) and preserved by acidification to 0.2% v/v HNO_3 (Tracepur). For measurement of metals in acid-digested samples (sediment, DGT, coral tissue or algal symbiont samples), calibration standards were prepared with high purity water and preserved by acidification to 2% v/v HNO_3 (Tracepur). A drift standard was run every 10 samples and an in-house independent standard was run with each batch of samples as part of the routine quality control procedures.

2.3 Sediment preparation and characterisation

2.3.1 Collection

Surface sediments were collected to a maximum depth of 10 cm using a clean plastic hand-trowel. The sediments used in Chapters 3 and 4 were gently sieved on site (6 mm plastic sieve) to remove coarse material (unwanted debris and biota). The sediments used in Chapter 6 were gently wet-sieved in the laboratory using seawater (South Coast) to remove particles >180 μm. The sediments were stored in polyethylene bags at 4°C in the dark.

2.3.2 Spiking procedure

For the sediments used in Chapters 3 and 4, the nickel-spiked sediment concentration series were prepared using a two-part spiking procedure based on the work of Hutchins et al. (2008) and Brumbaugh et al. (2013) which has been shown to improve the binding of nickel to solid sediment phases. The handling of sediments throughout the spiking procedure and equilibration period was carried out in a nitrogen-filled glove box. Initially, a stock solution was prepared using $NiCl_2.6H_2O$ (Chem Supply, Australia) dissolved in deoxygenated, filtered seawater (Cronulla). This was thoroughly mixed into each control sediment using a plastic spatula to prepare the super-spiked sediment (nominal concentration of 10,000 mg Ni/kg).

The pH of the sediment porewater was measured using a gel-filled electrode with a spearhead (FC200, Hanna Instruments, HI 98191) calibrated daily against pH 4.01, 7.01 and 10.01 buffers. The super-spiked sediments were immediately pH-adjusted to be similar to the original pH (pH 7.5 ± 0.5) by the addition of 1-M NaOH (Chem Supply) prepared in deoxygenated seawater (Cronulla). The super-spiked sediments were allowed to equilibrate at room temperature for 10 to 12 months with periodic rolling and further pH adjustment as necessary. The super-spiked sediments were then diluted with their respective control sediments to produce a concentration series (0-5,000 mg Ni/kg nominal) for toxicity testing and allowed to equilibrate for a further 2 to 4 weeks at room temperature. Previous work on nickel has demonstrated a minimum equilibration period of 10 weeks is required for nickel partitioning into various solid phases in both estuarine and marine sediments (Simpson et al., 2004) and freshwater sediments (Brumbaugh et al., 2013).

2.3.3 Characterisation

Total recoverable metals (TR-Metals) were determined using an aqua-regia digestion by adding a 3:1 ratio of concentrated HNO_3 and HCl (Tracepur, Merck) to a 50-mL centrifuge tube (Greiner Bio-One, Austria) containing 0.35 ± 0.05 g (mean ± standard deviation (SD)) of oven-dried (110°C for >24 h) sediment. Samples were heated in a microwave oven (CEM MARS) at 80°C for 1.5 h. Dilute-acid

extractable metals (AE-Metals) were determined by adding 25 mL of 1 M HCl to 0.50 ± 0.05 g wet sediment and mixing every 20 min for 1 h followed by filtration. Method blanks and certified reference material (AGAL-10; Hawkesbury River sediment, National Measurement Institute, Australia) were also processed as part of routine quality control procedures. Acceptance criteria for the certified reference material was between 70 and 130% recovery of the target analytes. AVS was determined colorimetrically by the reaction of small amounts of sediment with methylene blue reagent (Clines reagent) according to Simpson (2001). TR-Metal, AE-Metal, and AVS analyses were all performed in duplicate.

Total carbon was determined on dried (60°C for 24 h) and finely ground samples by high-temperature combustion in an atmosphere of oxygen and measurement of CO_2 by infrared detection using a Leco TruMAC instrument (LECO Corporation) as described by Matejovic (1997). Total inorganic carbon (TIC) was determined by reacting the sample (no more than 0.8 g $CaCO_3$ equivalent) with acid (8 mL of 3 M HCl and 3% $FeCl_2$) in a sealed container and measuring the pressure increase as described by Sherrod et al. (2002). Total organic carbon (TOC) was determined as the difference between total carbon and TIC measurements.

Sediment particle size was determined using laser diffraction techniques (Mastersizer 2000, Malvern Instruments Ltd). In this thesis, particle sizes of <4 mm diameter were considered clay, while 4 to 63 mm was silt and 63 to 180 mm was sand.

Sediment mineralogy was determined on dry and crushed sediment, using a quantitative x-ray powder diffractometer (XRD, Philips PW 1771/00) with Cu K_α radiation, X-ray-tube at 1 kW and a Spellman DF3 generator (40 kV and 30 mA, the angle of two theta ranged from 4-70^o at a step size of 0.02^o). The raw XRD profiles obtained from the diffractometer were analysed using the TRACES software for phase identification. The corrected profiles were then processed in SIROQUANT quantitative analysis software, which calculated the weight as a percentage of each mineral phase present (normalised chi-squared values (χ^2) for the analyses were between 2.84-3.29 and indicated acceptable matches).

Chapter 3

3 The development of sublethal whole-sediment toxicity tests for tropical benthic marine organisms endemic to Southeast Asia and Melanesia

3.1 Introduction

As the practice of lateritic nickel mining intensifies within the SEAM region there is a need to be able to assess the environmental risk associated with increased sediment nickel exposure to tropical benthic marine biota. This requires risk assessment and monitoring tools that are applicable to the region, to be able to confidently predict the risk of adverse effects on the environment and to make informed decisions regarding environmental management. In SEAM, and tropical regions more broadly, the application of whole-sediment toxicity tests for the assessment of contaminated sediments is not well established (Adams and Stauber, 2008; Binet et al., 2018; Gissi et al., 2016). However, increasing development and exploitation of natural resources in tropical regions have led to increasing demand for the development of whole-sediment toxicity testing methods with tropical species for risk assessment.

The development of tropical whole-sediment toxicity test methods must be focused on providing representation from a range of ecologically relevant taxa with differing potential exposure routes to sediment contaminants and the generation of sensitivity data for contaminants of interest (Spadaro and Simpson, 2015). For sediments which are the ultimate repository of many contaminants that enter the aquatic environment, it is important that test development also focuses on chronic endpoints (Adams and Stauber, 2008). An alternative to the development of novel whole-sediment toxicity test methods for tropical species is adapting testing protocols designed for use with temperate test species to accommodate tropical species. Adapting international protocols for use with local species has been the basis for the development of the majority of toxicity test protocols using Australian benthic species (e.g. King et al., 2004; Simpson and Spadaro, 2011).

Bioassays with chronic endpoints based on development, growth, and reproduction are most commonly utilised in ecotoxicological testing for their direct impact on an organism's life history and inferred population-level effects (an organism that can survive, grow, and reproduce is likely able to sustain its population) (Chapman, 1995). Disadvantages of these endpoints can, however, include long exposure times and a bias towards organisms that are easily reared in the laboratory. There is growing acceptance for endpoints that evaluate sublethal effects on behaviour and physiology for use in ecotoxicological assessments if their environmental relevance can be demonstrated. A recent review by Melvin and Wilson (2013), found behavioural endpoints to be comparatively more sensitive to contaminants over shorter exposure durations than traditional endpoints such as development and reproduction. The authors advocate that future studies on behavioural endpoints should aim to determine what organism

behaviours are most sensitive to various classes of contaminants and need to provide how the behaviours link to effects on organisms' population-level health and fitness.

A review of research on nickel toxicity to tropical marine pelagic species relevant to the SEAM region by Gissi et al. (2016), found echinoderms, anemones, crustaceans, gastropods, and polychaetes to be particularly sensitive to nickel. Their review identified a paucity of nickel ecotoxicity data for benthic species inhabiting tropical estuarine and marine sediments, attributed to a lack of tropical whole-sediment toxicity test methods. In the current study, the suitability of sublethal endpoints for the burrowing mud snail *Nassarius dorsatus* and the benthic diatom *Ceratoneis closterium* Ehrenberg (formerly *Nitzschia closterium* (Ehrenberg) W. Smith, a synonym of *Cylindrotheca closterium* (Ehrenberg) Reimann and J.C. Lewin) were investigated for developing whole-sediment chronic bioassay methods relevant for tropical marine ecotoxicity testing. These two species were chosen as they are both in direct contact with the sediment and have a distribution that covers tropical marine environments including recorded occurrences within northern Australia and parts of the SEAM region (Atlas of Living Australia, 2020a, 2020b).

For *N. dorsatus*, the current studies objectives were to compare the sensitivity of adult and juvenile snails, and the effectiveness of behavioural endpoints (e.g., mobility and feeding behaviour) for assessing the ecotoxicological effect of nickel exposure. Initial experiments involved dissolved nickel exposure with further development of a sediment test if the endpoints proved to be sensitive to nickel. Feeding behaviour (as a surrogate for chemoreception response) was investigated because these mud snails have a well-developed chemical sensory mechanism for quickly locating moribund and injured prey and retreating in response to damaged conspecifics (Morton and Britton, 2003; Rahman et al., 2000).

For C. *closterium*, the current studies objectives were to further develop a whole-sediment toxicity test previously proposed based on chlorophyll-*a* concentration (Strom, 2011; Watson, 2012) for use with this tropical strain of benthic algae. Chlorophyll-*a* concentration was chosen as a surrogate for growth due to the analytical difficulties in determining algal cell density in the presence of sediment particles. Effects thresholds for sediment nickel were determined using nickel-spiked sediments, prepared from uncontaminated field-collected sediments with varying physicochemical properties (referred to as nickel-spiked sediments). Sediments with elevated nickel concentrations, collected from nickel laterite mining regions of New Caledonia and Indonesia, were also tested (referred to as field-contaminated sediments).

3.2 Materials and methods

3.2.1 General analytical

General laboratory equipment was prepared as described in Section 2.1.1. Seawater was collected as per Section 2.1.2. For snail culture medium and snail bioassay solutions, South Coast seawater was used. For algal culture medium and algal bioassay solutions, Cronulla Seawater was used.

Nickel stock solutions were made using nickel (II) chloride hexahydrate salt ($NiCl_2.6H_2O$, analytical reagent grade, Chem Supply, Australia). Copper stock solutions were made using copper (II) sulfate pentahydrate salt ($CuSO_4.5H_2O$, analytical reagent grade, AJAX Chemicals, Australia). All metal stock solutions were prepared volumetrically with high purity water, acidified to 1% HCl (Tracepur, Merck) to ensure metals remained in the aqueous 2+ oxidation state and prevent loss from adsorption, and stored at 4°C in acid-washed high-density polyethylene bottles (Nalge Nunc International Corporation, United States).

3.2.2 Sediment preparation and characterisation

Sediment collection, characterisation, and nickel spiking were carried out as described in Section 2.3. Uncontaminated sediments were collected from coastal locations on the east coast of Australia (Table 3.1). The sediments were selected for their differing physicochemical properties (Table 3.2) that could alter nickel bioavailability and were described as Organic-silt, Sandy-silt, and Silty-sand. These sediments were used as controls and for preparing a nickel-spiked concentration series for establishing concentration-response relationships. Nickel-rich sediments were collected from coastal locations adjacent to nickel mining or processing facilities within New Caledonia (Site 1 and Site 2) and Indonesia (Site 3).

Table 3.1 Sediment collection locations

Name	Collection location	GPS Coordinates
Organic-silt	Bonnet Bay, NSW, Australia	34°00'25"S 151°03'24"E
Sandy-silt	Sturt Cove, QLD, Australia	16° 54' 07"S 145° 48' 59"E
Silty-sand	Snapper Island, QLD, Australia	16°17'19"S 145°29'31"E
High-sulfide	Grays Point, NSW, Australia	34°03'52"S 151°05'11"E
Site 1	Vavouto Bay, North Province, New Caledonia	20° 59' 50"S 164° 42' 19"E
Site 2	Dumbea Bay, South Province, New Caledonia	22° 10' 28"S 166° 26' 01"E
Site 3	South Sulawesi, Indonesia	02° 40' 09"S 121° 01' 20"E

Table 3.2. Physicochemical properties of the control sediments before the spiking process and field-contaminated test sediments

Sediment	Pore-water pH (n = 1)	Particle size (%, n = 5)			TOC (%, n = 1)	TIC (%, n = 1)	AVS (μmol/g, n =3)	Dilute-acid extractable metal (μg/g, n = 3)		
		<4	4-63	>63 (μm)				Fe	Mn	Ni
Organic-silt	7.1	11	83	6	5	0.05	0.9 ± 0.1	12,000	62	3
Sandy-silt	7.2	11	64	26	1	2	1.7 ± 0.2	3,500	570	2
Silty-sand	8.0	4	16	80	0.2	9	1.6 ± 0.1	1,600	140	1
Site 1	7.0	11	69	20	6	0.6	0.9 ± 0.3	11,000	250	120
Site 2	6.8	4	31	65	1	0.2	1.4 ± 0.9	3,400	100	84
Site 3	7.3	5	71	25	5	0.7	0.5 ± 0.2	8,300	300	47

TOC = Total organic carbon; TIC = Total inorganic carbon; AVS = Acid-volatile sulfide.

3.2.3 Development of a sub-lethal endpoint for tropical *N. dorsatus*

3.2.3.1 Snail collection and acclimation

Adult (21-28 mm shell length) and juvenile (12-19 mm shell length) *N. dorsatus* were field collected from a non-contaminated beach, approximately 20 km outside of Darwin Harbour (Northern Territory, Australia). They arrived in Sydney the following day and were transported to temperature-controlled facilities at CSIRO, Land and Water (Lucas Heights, NSW Australia). Here they were maintained in 32-L polypropylene tubs (Nally, Australia) fitted with a recirculating supply of filtered seawater (South Coast), air stones providing gentle aeration, and a 1 to 2 cm layer of sand as a burrowing substrate for the snails. Seawater was replaced on two occasions each week and maintained at a pH of 8.2 ± 0.1, a salinity of 30 ± 1 practical salinity units (PSU), and temperature of 28 ± 1 °C. A 12:12 h light/dark cycle was provided at 3.5 μmol photons/m^2/s. Snails were fed a mixture of Tropical Fish Flakes (TetraMin, Tetra Werke, Germany) and Sinking Wafers (Hikari, Japan) *ad libitum*. The snails were allowed to acclimate to laboratory conditions for at least two weeks before use.

3.2.3.2 Bioassay development and endpoint selection

The feasibility and sensitivity of potential endpoints for toxicity testing with the benthic *N. dorsatus* were assessed following exposure of either adult or juvenile snails to dissolved nickel for 21 days. The dissolved nickel exposures were performed in 1-L glass beakers with 800 mL of test solutions, gentle aeration, four snails per beaker, and two replicate beakers per treatment. A 1 cm layer of sieved sand (>180 μm) was provided as a burrowing substrate as initial experiments showed reduced survival when

no substrate was provided (Appendix B1). Adult snails were exposed to five dissolved nickel concentrations between 60 and 920 μg Ni/L, prepared by spiking the nickel stock solution into South Coast seawater, and a control (no added nickel). Juvenile snails were exposed to six dissolved nickel concentrations between 90 and 2,800 μg Ni/L (the concentration series was expanded to include higher dissolved nickel concentrations due to the tolerance demonstrated by the adult snails) and a control (no added nickel). The test beakers were set up 7 days in advance with sand and the nickel treatment solutions (or control). During this time the treatment solutions were renewed twice to replace nickel lost to binding sites of the glass test vessel and sand. Analysis of dissolved nickel concentrations during this pre-exposure equilibration period showed that this was sufficient to minimise loss of dissolved nickel between water renewals (data not shown).

The treatment water was renewed three times each week with sub-samples collected before and after each water change for the measurement of physicochemical parameters and dissolved metal analysis (as per Section 2.2). Before each water renewal, the snails were fed Tropical Fish Flakes (2 mg/snail/day). For the experiment with the juvenile stage, the snails were only fed twice each week and starved for five days before chemoreception endpoint determination.

For the experiments with either adult or juvenile snails, the endpoints of survival and time-to-flip (the time taken for the snail, when placed in an upside-down position (foot-up), to flip back to its normal position (onto its foot)) were measured and compared to determine which life stage was the most sensitive to dissolved nickel after 14 and 21 days of exposure. For the experiment with juvenile snails, the additional behavioural endpoints of scavenging ability (the time taken for a snail to reach food that was placed at a fixed distance of 15 cm away (Cheung et al., 2002)), and time to emerge from the sand given a food stimulus were measured after 21 days of exposure to determine which endpoint was the most sensitive. The endpoints of scavenging ability and emergence with a food stimulus were observed for 600 s and 300 s, respectively, with exceedances recorded if the endpoint was not achieved within the allocated time frame.

For the experiments with the adult snails, reproduction was considered as a potential endpoint, however, failure of the adults to reproduce under the experimental test control conditions meant this endpoint could not be pursued. Growth was also investigated as a potential endpoint, but no appreciable difference was observed over 21 days (data not shown) so was not pursued. Previous studies have utilised the pelagic initial life stage of *N. dorsatus* to investigate the toxicity of dissolved metals (Gissi et al., 2018; Trenfield et al., 2016). The current study planned to assess the use of *N. dorsatus* larvae that had just settled. However, this was not possible, as maintaining the pelagic larvae through to settlement was not achieved in this laboratory.

3.2.3.3 Statistical analyses

One-way analysis of variance (ANOVA) was applied to determine statistically significant differences between treatments. When differences were found, Dunnett's post hoc tests were applied to determine which nickel exposure treatments were significantly different from the control treatment. For all statistical tests, the significance level was set at $P<0.05$.

3.2.4 Development of a chronic whole-sediment toxicity test for tropical *C. closterium*.

3.2.4.1 Diatom cultures

The unicellular benthic marine diatom *C. closterium*, strains CS-111 and CS-114, were obtained from the CSIRO Australian National Algal Supply Service (Hobart, Australia). Both strains were isolated from the Coral Sea in 1981. Strain CS-111 was cultured in G/2 medium (half-strength G medium, Loeblich and Smith (1968)) while strain CS-114 was cultured in f/2 growth medium (half-strength f medium, Guillard and Ryther (1962)). Strain CS-111 was used during method optimisation experiments and initial range finder bioassays, while strain CS-114 was used for all definitive bioassays. The change in strains was due to a culture crash of the CS-111 strain and neither recovery nor resupply of this strain could be achieved within the necessary time frame. Cultures were maintained in temperature control rooms at 27 ± 2°C on a 12:12 h light/dark cycle at 70 μmol photos/m^2/s (Sylvania F20W/154-RS daylight fluorescent tubes).

*3.2.4.2 Whole-sediment chlorophyll-*a *extraction algal bioassay – optimisation and quality control*

The current study utilized the 72-h chlorophyll-*a* extraction bioassay for the temperate strain of *C. closterium* first proposed by Strom (2011) and further developed by Watson (2012). However initial experiments demonstrated the need for further method optimisation for the tropical strain of *C. closterium* due to the sub-optimal growth rate of the tropical algal strain over 72 h (<1 doubling/day) and the variation between replicates being unacceptably high (>20% coefficient of variation). The outcomes of the optimisation investigations (Section 3.3.2.1) highlighted the need to introduce more comprehensive quality assurance and control procedures to produce a robust standard method and to ensure the validity of the results. To address these deficiencies the following quality control procedures were introduced: (i) the use of sediment treatments without algae to determine background chlorophyll-*a* concentrations, (ii) having sacrificial control replicates to measure chlorophyll-*a* on days 0, 2, and 3 to ensure exponential growth is maintained over 72 h (acceptable growth was considered as an increase in chlorophyll-*a* concentration by a factor of at least eight over 72 h), (iii) performing a second extraction of chlorophyll-*a* to ensure acceptable recovery was achieved by the first extraction (acceptable recovery was considered as the measured chlorophyll-*a* concentration of the second extraction being ≤10% of the first extraction), and (iv) running a copper reference toxicity test to ensure

algal sensitivity was similar between tests (see Section 3.2.4.5). The final optimised test protocol for the tropical strain of *C. closterium* is provided in Section 3.2.4.3.

3.2.4.3 Whole-sediment chlorophyll-a extraction algal bioassay – final protocol

Sediment samples were frozen (-20°C) for 72 h to remove background algal and meiofauna populations and thawed just before use. Sediment samples were prepared the day before test commencement by weighing 2.00 ± 0.05 g of wet sediment into a new 50-mL centrifuge tube (Greiner Bio-One) and adding 20 mL of filtered seawater (Cronulla) enriched with nutrients (15 mg NO_3^-/L and 1.5 mg PO_4^{3-}/L, added as $NaNO_3$ and KH_2PO_4, respectively) then left to equilibrate overnight. The following day 15 mL of seawater was replaced with 22 mL of fresh nutrient-enriched filtered seawater (Cronulla) to remove the initial flux of dissolved nickel released from the sediments. Once the sediment had settled, 7 mL of overlying-seawater was removed from each replicate, passed through a 0.45 μm filter and 5 mL collected for dissolved metal analysis (as per Section 2.2.2).

Cells in an exponential growth phase (4 d old) were washed three times with filtered seawater (Cronulla) by centrifuging (2,500 rpm or approximately 700 x g, 7 min, spintron GT-175BR) to remove residual culture media, with cells gently homogenised between spins to reduce clumping. Tubes with sediment were inoculated with 2-4 x 10^4 cells/mL and placed randomly in a growth cabinet without their lids for 72 h at 27°C on a 12:12 h light/dark cycle (100-120 μmol photons/m^2/s, Phillips TLD 30W/33). Test tubes were given a gentle swirl and randomly re-positioned twice daily. Salinity was checked in a representative sub-set of the tubes daily and adjusted by the addition of high purity water (≤2 mL over the 72-h period) to maintain 35 ± 1 PSU. A summary of the test conditions and test protocol is provided in Table 3.3.

Physicochemical properties (pH, salinity, conductivity, and dissolved oxygen) were measured for each treatment in the bulk prepared bioassay solution at the time of test initiation and in a composite sample for each treatment at test termination. At test termination, subsamples were collected from each replicate for dissolved metal analysis (as per Section 2.2.2).

Each sediment treatment was prepared in quadruplicate for analysis at 72 h. For the controls, an additional four samples were prepared for analysis at 48 h to check that exponential cell growth was maintained over the 72-h exposure. For all sediment treatments, an additional two samples were prepared for pore water analyses at the end of the exposure and were prepared by doubling the previously described sediment weight and volume of solutions to ensure sufficient sample (to increase sample volume but keep the ratio of sediment to water the same). Sediment blanks, which consisted of sediment without the microalgae inoculum added, were prepared in quadruplicate for the control (two sets were prepared for analyses at the 48-h and 72-h time points) and the highest nickel-spiked treatment and the field-contaminated sediments (one set prepared for analyses at the 72-h time-point) to determine the background chlorophyll-*a* concentration in the sediment. The chlorophyll-*a* concentration was

determined in the spiked algae at 0 h, the control and sediment blank treatments at 48 h and all treatments at 72 h using a spectrophotometric method (Section 3.2.4.6). Bioassays for each sediment type were repeated.

*Table 3.3. Summary of test conditions and test protocol for the 72-h chlorophyll-*a *extraction algal bioassay with tropical* Ceratoneis closterium *in whole-sediment and water-only experiments*

Test parameter	Whole sediment	Water only
Test duration	72 h	72 h
Temperature	27 ± 1°C	27 ± 1°C
Salinity	35 ± 1 PSU	35 ± 1 PSU
pH	8.1 ± 0.1	8.1 ± 0.1
Dissolved Oxygen	≥80%	≥80%
Light intensity	100-120 μmol photons/m^2/s	100-120 μmol photons/m^2/s
Photoperiod	12-h light; 12-h dark	12-h light; 12-h dark
Replication	4	4
Age of organisms	4 days (in exponential cell growth)	4 days (in exponential cell growth)
Initial cell density	2-4 x 10^4 cells/mL	2-4 x 10^4 cells/mL
Test type	Static no renewal*	Static no renewal
Test chamber	50-mL conical bottom tube, without lids	250-mL glass conical flask with glass lids
Sediment weight	2.00 ± 0.05 g	Nil
Overlying seawater volume	20 mL†	50 mL†
Endpoint	Chlorophyll-*a* concentration (as a percentage of the control)	Chlorophyll-*a* concentration and algal growth rate inhibition (both as a percentage of control)
Test acceptability criteria	Chlorophyll-*a* concentration of the control increased by a factor of at least 8 over 72 h	Chlorophyll-*a* concentration of the control increased by a factor of at least 8 over 72 h
	Change in pH not more than 1.5 pH units	Change in pH not more than 1.5 pH units
	The control chlorophyll-*a* concentration coefficient of variation was <20%	The control chlorophyll-*a* concentration coefficient of variation was <20%
	The EC50 of the copper reference toxicant results should be within 2 standard deviations of the mean EC50	The EC50 of the copper reference toxicant results should be within 2 standard deviations of the mean EC50

* Topped up with high purity water (max 2 mL over 72 h) to maintain salinity at 35 PSU.

† Seawater filtered to <0.45 μm and enriched with nutrients (15 mg NO_3^-/L as $NaNO_3$, 1.5 mg PO_4^{3-}/L as KH_2PO_4).

3.2.4.4 Water-only exposures to dissolved nickel

Water-only exposures to dissolved nickel were performed to understand the contribution of dissolved nickel released to the overlying water of the sediment exposures to toxicity, and to compare the sensitivity of the chlorophyll-*a* endpoint to the population growth-rate inhibition endpoint. The water-only bioassays were carried out in acid-washed, 250-mL borosilicate glass Erlenmeyer flasks, coated with a silanising solution (Coatasil, Ajax Finechem, Australia) to reduce adsorption of nickel to the flask walls. Water-only experiments were trialled in plastic 50-mL conical tubes (in which the whole sediment bioassays were also performed) and 50-mL plastic flat bottom tubes but both these tests resulted in poor growth rates of the controls. For each bioassay, four different nickel treatments (625-10,000 μg Ni/L nominal concentration) and a control were prepared in bulk, enriched with nutrients (to give 15 mg NO_3^-/L as $NaNO_3$ and 1.5 mg PO_4^{3-}/L as KH_2PO_4) and 50-mL aliquots dispensed in quadruplicate. Algal inoculum and incubation followed the same procedure as the sediment-exposure bioassay. Chlorophyll-*a* determinations followed the same procedure as the sediment-exposure bioassay (see Section 3.2.4.6). Algal cell density was measured every 24 h for 72 h using flow cytometry (see Section 3.2.4.7). The water-only dissolved nickel exposure experiment was repeated.

3.2.4.5 Copper reference toxicant

Alongside all water-only bioassays and sediment bioassays, water-only 72-h population growth rate inhibition bioassays using the reference toxicant copper were performed to track the condition of the algae over time. Copper reference bioassays were carried out in 30-mL acid-washed and silanised glass-vials. For each bioassay, four different copper treatments (10-40 μg Cu/L nominal concentration) and a control (no added copper) were prepared in bulk, enriched with nutrients (to give 15 mg NO_3^-/L as $NaNO_3$ and 1.5 mg PO_4^{3-}/L as KH_2PO_4) and 6-mL aliquots were dispensed in duplicate. Subsamples were collected from each of the bulk prepared solutions for dissolved metal analysis (as per Section 2.2.2). Algal inoculum and incubation followed the same procedure as the sediment-exposure bioassay. Algal cell density was measured in the control at 0 h, and all treatments at 48 h and 72 h using flow cytometry (see Section 3.2.4.7). Tests were considered valid if population growth rates in the control treatment exceeded 1 doubling/day, if the percent coefficient of variation was less than 10% and if the change in pH was not more than 1.5 pH units over the 72-h incubation period (Franklin et al., 2005).

*3.2.4.6 Determination of chlorophyll-*a *(spectrophotometric method)*

The concentration of chlorophyll-*a* was used as a surrogate to estimate biomass and given that the volumes of sediment and water extracted were consistent, the chlorophyll-*a* concentration provides a relative measure between samples. Determination of the concentration of chlorophyll-*a* in the sediment samples was based on the method for waters described in the USEPA Method 446.0 (Arar, 1997). Samples were centrifuged at 700 g for either 5 min (water-only treatments) or 20 min (sediment

treatments) so that the algae and sediment (for the sediment treatments) would form a pellet at the bottom of the tube. An 18-mL aliquot of the overlying seawater was removed so that only 2 mL of solution remained at the bottom of the tube. From this 18-mL aliquot, subsamples were collected for dissolved metal analysis and measurement of physicochemical properties (as per Section 2.2). A 12-mL aliquot of 90% acetone (99.8% purity, Merck) buffered with a saturated solution of magnesium carbonate was added to the sediment pellet using a glass pipette (Livingstone International, Australia). The magnesium carbonate is added to prevent the extraction mixture from becoming acidic which may result in the decomposition of chlorophyll-*a* to give pheophytin degradation pigments (Watson, 2012). The tubes were immediately capped and shaken by hand to resuspend all the sediment, then wrapped in foil to prevent light exposure. The samples were stored at 4°C in the dark and shaken twice more during the 2-h extraction period (at 40-min intervals). Following the extraction period, the samples were centrifuged again for 5 or 20 min at 700 g. A 10-mL aliquot of the extracted sample was removed using a glass pipette and transferred to an acid-washed amber scintillation vial to prevent light exposure before spectrophotometric analysis. The extraction process was repeated for the control and highest treatment concentration to determine if acceptable recovery of chlorophyll-*a* was achieved (≤10% of the original chlorophyll-*a* extraction recovered in the second extraction).

For spectrophotometric analysis, the 10-mL aliquot of the extracted sample was transferred to a glass cuvette (4 cm path length) and the absorbance measured at 664 and 750 nm (before acidification) using an ultraviolet-visible light spectrophotometer (LKB Biochrome 4057, United Kingdom) zeroed with magnesium buffered acetone. The extract was then acidified with 0.2 mL of 0.1 M HCl (Tracepur), swirled, and a minimum of 10 min allowed for the reaction to take place. Acidification of the sample converts chlorophyll-*a* into phaeopigments via loss of a magnesium ion from the chlorophyll molecule. Measurements were taken at 665 and 750 nm (after acidification). The chlorophyll-*a* concentration in the extract was calculated using Equation 3-1 (Arar, 1997) to correct for turbidity and phaeopigment content. Absorbances measured at 750 nm were subtracted from measurements at 664 and 665 nm to correct for turbidity interferences. Absorbances measured at 665 nm were subtracted from measurements taken at 664 nm to correct for the presence of phaeopigments.

$$\textit{Chlorophyll-a (mg/L)} = 26.7 * (\textit{Abs 664} - \textit{Abs 665}) \qquad 3\text{-}1$$

Where: Abs 664 = Extract absorbance at 664 nm – absorbance at 750 nm (both measured before acidification); and Abs 665 = Extract absorbance at 665 nm – absorbance at 750 (both measured after acidification).

3.2.4.7 Determination of cell density (flow cytometric method)

Algal cell density was measured using a BD-FACSCalibur™ Flow Cytometer (BD Biosciences, United States) with an argon-ion laser providing an excitation wavelength of 488 nm. Chlorophyll-*a* or autofluorescence intensity was measured with a >650-nm long-pass filter band (FL3). Before sub-sampling, *C. closterium* cells were gently homogenized to reduce cell clumping. Data collection was performed at a high flow rate (60 µL/min), with an acquisition time of 120 s, and the collection of >1,000 bead events (Franklin et al., 2005). Absolute counts were determined using an internal reference fluorescent bead standard (DB Trucount™ tubes, BD Biosciences). Cell densities were calculated as per Franklin et al. (2005). The population growth rate (cell division rate) was calculated as the slope derived from a linear regression of $\log_{10}$ (cell density) versus time (h). Growth rates for treatment flasks (doublings/day) were expressed as a percentage of the control growth rate.

3.2.4.8 Statistical analyses

Results from the sediment and water-only toxicity tests were reported as a percentage of the control response (% control). The field-contaminated sediments were defined as toxic if there was more than 20% reduction in chlorophyll-*a* concentration when compared to the reference sediment (i.e., chlorophyll-*a* concentration <80% of the control sediment).

The concentration-response relationships for the different measures of nickel bioavailability were based on the arithmetic mean of the measured dissolved nickel concentrations taken at test initiation and test end, the initial TR-Ni concentrations and AE-Ni concentrations, and the dissolved nickel pore water concentrations at test end. For each of the water-only dissolved nickel exposures and the sediment exposures, data from the duplicate experiments were combined and analysed in the statistical analysis software R (4.0.2, 2020-06-22) (R Core Team, 2016) using the package 'Dose-Response Curve' (DRC) (Ritz et al., 2015). Log-logistic, Weibull I and Weibull II models were applied to the datasets and the model that best fitted the dataset was determined by comparing the Akaike information criterion (AIC) value (where a low value demonstrates a good fit) and by visual assessment of the curve (Pinheiro and Bates, 2000). For all models, the lower limit was fixed at 0% and the upper limit fixed to 100% (as the data were normalised to the control). The model of best fit was used to determine toxicity estimates (the nickel concentration to reduce reproduction by 10% (the EC10) and 50% (the EC50)) and 95% confidence intervals. Graphs of the concentration-response curves were created using the model of best fit in the R package 'ggplot2' (Wickham, 2016).

3.3 Results and discussion

3.3.1 A sub-lethal endpoint for tropical *N. dorsatus*

3.3.1.1 Comparing the sensitivity of adult and juvenile snails

No snail mortality occurred with either adult or juvenile snails over the 21-d exposures in the control treatments or the dissolved nickel concentrations <1,000 µg/L. Juvenile snails were exposed to additional dissolved nickel concentrations of 1,400 and 2,800 µg/L, which reduced survival to 88 ± 18% (mean ± SD, n = 2) and 63 ± 18%, respectively, after 21 d (Figure 3.1). The behavioural response of time-to-flip for both adult and juvenile snails became longer with both increasing dissolved nickel concentration and exposure duration (Figure 3.2). After 21-d of exposure, the time-to-flip for the juvenile snails was significantly longer at the highest dissolved nickel concentration tested (2,800 µg/L) when compared to the control (Dunnett's test: $P<0.05$). Increasing nickel concentration increased the variability in the adult snail's response time-to-flip, indicating that some individuals were starting to be adversely affected. While adult and juvenile life stages of *N. dorsatus* showed similar sensitivity to dissolved nickel, it was observed that there was less variation in results produced by the juvenile life stage and the adult life-stage failed to lay-eggs under test conditions, thus, it was decided that the juvenile life stage of *N. dorsatus* was more appropriate for continuing test endpoint development.

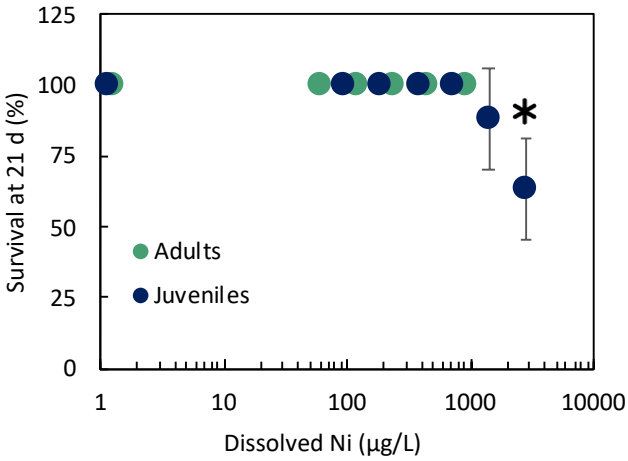


Figure 3.1. Percent survival of adult and juvenile Nassarius dorsatus *exposed to dissolved (filtered <0.45 µm) nickel for 21 days. Values are the mean ± standard deviation (vertical error bars), n = 2. (*) represents statistically significant difference relative to the control (*P*<0.05).*

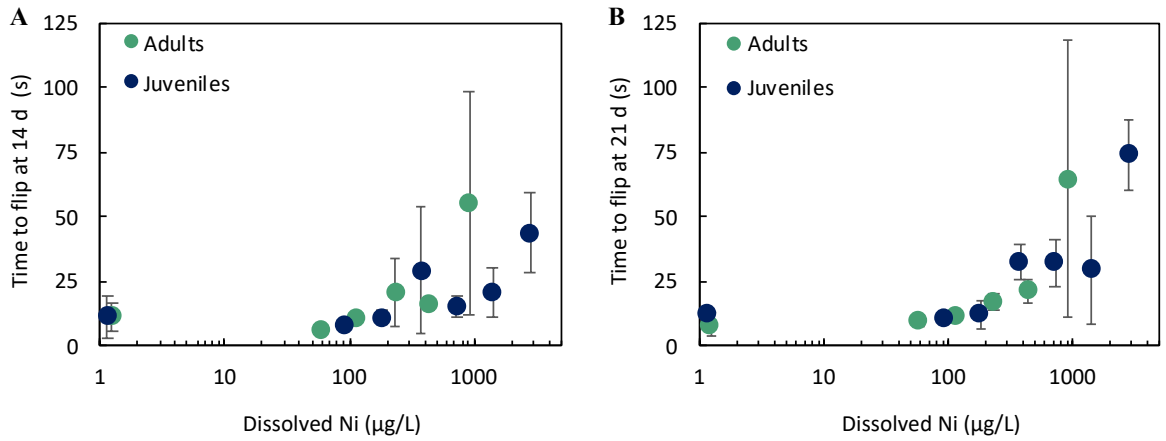


Figure 3.2. Effects of dissolved (filtered <0.45 µm) nickel on the time-to-flip of adult and juvenile Nassarius dorsatus *after exposure for: A) 14 days and B) 21 days. Values are the mean ± standard deviation (vertical error bars), n = 2. (*) represents statistically significant difference relative to the control (*P*<0.05).*

3.3.1.2 Comparing the sensitivity of behavioural endpoints of juvenile snails

The behavioural endpoints based on feeding behaviour and chemoreception were more sensitive indicators of nickel toxicity than the mobility endpoint of time-to-flip (Figure 3.3). After 21 d, the scavenging ability endpoint and the emergence time with a food stimulus endpoint were significantly longer at dissolved nickel concentrations of ≥380 µg/L and ≥730 µg/L, respectively, when compared to the control (Dunnett's test: *P*<0.05). The results show that among the three behavioural endpoints studied, scavenging ability (21-d lowest observable effect concentration (LOEC) of 380 µg Ni/L) was the most sensitive endpoint to nickel exposure followed by emergence time with a food stimulus (21-d LOEC of 730 µg Ni/L) and time-to-flip (21-d LOEC of 2,800 µg Ni/L) was the least sensitive.

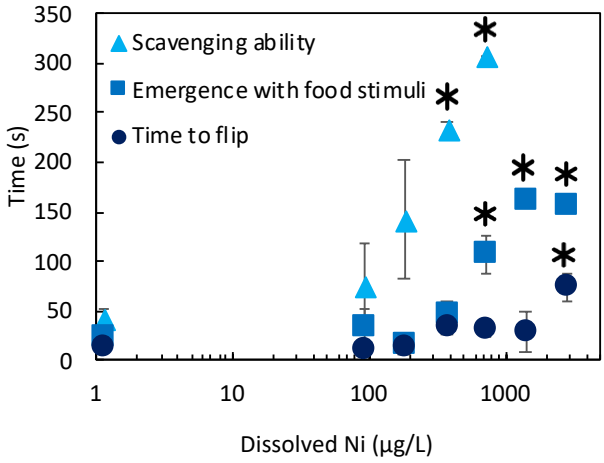


Figure 3.3. Behavioural responses of juvenile Nassarius dorsatus *exposed to dissolved (filtered <0.45 µm) nickel for 21 days. Values are the mean ± standard deviation (vertical error bars), n = 2. (*) represents statistically significant difference relative to the control (*P*<0.05).*

3.3.1.3 Evaluation of N. dorsatus *and behavioural endpoints for the development of a whole sediment toxicity test method*

The results highlight that the endpoints that included an assessment of feeding behaviour were more sensitive than the endpoint that assessed mobility alone. The impairment to feeding behaviour highlights that chemoreception of the mud snail *N. dorsatus* may be inhibited by nickel exposure. Previous studies on the effects of metals on chemoreception in gastropods are limited but have revealed that impairment to feeding behaviour seems to be both chemical- and organism-specific. Cheung et al. (2002) studied the dog whelk *Reticunassa festiva* (formerly *Nassarius festivus)* and found that the percentage of individuals observed to feed, and the time taken to reach food, was significantly affected by a 96-h water-only exposure to chromium (≥5 mg Cr/L) but not cadmium, copper, or zinc. Das and Khangarot (2011) found a reduction in food consumption by the pond snail *Lymnaea luteola* during seven week exposure to copper concentrations ≥56 µg Cu/L. Alonso and Valle-Torres (2018) found the percentage of actively feeding individuals of the mud snail *Potamopyrgus antipodarum* to be significantly reduced at cadmium concentrations ≥9 µg Cd/L in water-only exposures.

In gastropods, chemoreception has been suggested to be involved in several important navigational behaviours, among them are prey location, homing, avoiding or escaping predators, and recognising conspecifics for mate selection (Croll, 1983; Wyeth, 2019). Thus, chemoreception provides a fundamental link between an organism's physical and biological environments and plays a vital role in mediating species-specific responses that determine individual fitness (including foraging efficiency, predator evasion, and reproduction potential) (Kelley et al., 2018). Therefore, the behavioural endpoint of scavenging ability of *N. dorsatus* is ecologically relevant to species survival.

The current study has demonstrated the potential application of behavioural endpoints for ecotoxicity testing using juvenile *N. dorsatus* and dissolved nickel exposures. *N. dorsatus* is primarily a sediment-dwelling organism. They are most commonly found at the mid-tide level of sheltered intertidal mudflats where they emerge from their sediment burrows with the receding tide and quickly rebury upon the rising tide (McKillup and McKillup, 1997). In addition to being in direct contact with the sediment during normal behaviour, *N. dorsatus* fulfils many of the criteria for a suitable whole-sediment toxicity test species, exhibiting appropriate exposure pathways (both dissolved and dietary) for sediment contaminant assessment. Based on field studies of *N. dorsatus* in Central Queensland, Australia, it has been suggested that this species undergoes frequent recruitment (McKillup and McKillup, 1997) and is often described as the dominant scavenger on shores at low tide (Morton and Britton, 2003). Due to its presence and abundance on many coastal shores within the SEAM region (including Malaysia, Indonesia, Papua New Guinea, Fiji, and the Philippines) (Trenfield et al., 2016) and northern Australia (McKillup and McKillup, 1997; Morton and Britton, 2003) it can be readily collected from the field. It is also tolerant of laboratory holding conditions (Trenfield et al., 2016), however, due to their pelagic

life stage though, they are not able to be easily reared through their whole life cycle in the laboratory. Furthermore, there are many other gastropods of the genus *Nassarius* that are perhaps more abundant in some regions within the SEAM region that could also be utilised for region-specific assessments.

Proven sensitivity to the contaminant(s) of concern is also an important criterion for a suitable whole-sediment toxicity test species. In the current study, assessment of scavenging ability of juvenile *N. dorsatus* to dissolved nickel produced a 21-d LOEC of 380 μg Ni/L. The pelagic life stage of *N. dorsatus* has previously been utilised in the development of a chronic toxicity method for waters based on measuring growth (Trenfield et al., 2016). The larvae of *N. dorsatus* were shown to be sensitive to exposure to copper (96-h EC50 of 7.3 μg/L) and aluminium (96-h EC50 of 185 μg/L) but insensitive to exposure to gallium (96-h EC50 of 4,560 μg/L) and molybdenum (96-h no observable effect at ≤7,000 μg/L) (Trenfield et al., 2016). Gissi et al. (2018) utilised this method to determine the toxicity of dissolved nickel to larval *N. dorsatus* and two crustaceans, the barnacle *Amphibalanus amphitrite*, and the copepod *Acartia sinjiensis*. The copepod was the most sensitive species to nickel (~80-h EC10 of 5.5 μg/L and EC50 of 8.6 μg/L), while the gastropod (96-h EC10 of 64 μg/L and EC50 of 478 μg/L) and barnacle (96-h EC10 of 67 μg/L and EC50 of 171 μg/L) had similar sensitivities. The pelagic life stage is more sensitive to dissolved nickel than the juvenile or adult life stage investigated in the current study. Aside from the chronic toxicity data for the pelagic form of *N. dorsatus*, there are no other chronic nickel toxicity data available for marine gastropods (temperate or tropical) (Gissi et al., 2018). However, acute toxicity tests with different life-stages of the tropical marine snail *Babylonia areolate* exposed to dissolved nickel produced a 96-h LC50 of 200 μg Ni/L for the larval stage, 1,210 μg Ni/L for the juvenile stage, and 1,440 μg Ni/L for the adult stage (Vedamanikam and Hayimad, 2013); whereas another study reported the 96-h LC50 for adult *B. areolate* as 34,000 μg Ni/L (Hajimad and Vedamanikam, 2013). The toxicity of dissolved nickel to other tropical marine species has recently been reviewed and both acute and chronic toxicity data was found to be limited (Gissi et al., 2016). Based on acute endpoints, crustacea were the most sensitive taxa to dissolved nickel with the brine shrimp *Artemia urniana* and *Artemia franciscana* being the most sensitive with 24-h LC50 values of 7 μg Ni/L and 11 μg Ni/L, respectively (Asadpour et al., 2013). Based on chronic endpoints crustacea were also the most sensitive taxa to dissolved nickel with the tropical copepod, *Acartia sinjensis*, showing the highest sensitivity to nickel with an 80-h EC50 of 8.6 μg Ni/L (Gissi et al., 2018). Compared to other tropical marine invertebrates, *N. dorsatus* (juvenile and adult life-stage) is only moderately sensitive to dissolved nickel concentrations, with effect concentrations that are quite high in an environmental context. For example, a compilation of dissolved nickel concentrations in marine waters found that concentrations did not typically exceed 125 μg/L within the SEAM region (Hydrobiology, 2016) and 110 μg/L within industrialised estuaries around the world (Blewett and Leonard, 2017).

3.3.2 A chronic whole-sediment toxicity test for tropical *C. closterium*.

3.3.2.1 Optimisation

Investigations into the control response of *C. closterium* identified light limitation was the major factor influencing the sub-optimal growth rate of the alga over a 72-h exposure period. Common in algal bioassays is the provision of light from below, but in this instance, the light was obstructed by the presence of the sediment. The light limitation was addressed by removing the lids of the tubes and ensuring light was provided to each sample tube from above and the side. To compensate for evaporative loss and increase in salinity over 72 h, due to the sample lids being removed, salinity was checked in a representative subset of the tubes daily and adjusted to maintain 35 ± 1 PSU.

A high level of variation was observed between replicates in these tests, which was attributed to the incomplete extraction of chlorophyll-*a* and the incorrect determination of the proportion of pheophytin present. To address these issues, the current study investigated the processes involved in quantifying chlorophyll-*a* including the incubation periods for both acetone extraction and acidification. The results demonstrated that a 2-h extraction period was sufficient to maximise the amount of chlorophyll-*a* extracted if the sample tubes were shaken at the start of the incubation period and twice more during the incubation period (e.g. at 40-min intervals). Incorrect determination of the proportion of pheophytin present was attributed to insufficient acidification. Acidification is required for the conversion of chlorophyll-*a* to pheophytin by removing the magnesium ion from the chlorophyll molecules, converting the whole sample to pheophytin which is then used to correct the chlorophyll-*a* measurement by subtracting the pheophytin content. Test optimisation revealed that once acidified the extract required a minimum incubation period of 10 min for the chlorophyll-*a* to be entirely converted to pheophytin. Typically, 39 ± 1% (n = 8) of the measured chlorophyll-*a* content at 664 nm was pheophytin.

3.3.2.2 Quality control

The physicochemical properties of the overlying water remained within acceptable limits over the bioassay duration. Changes in pH were <1.5 units across all treatments and tests, salinity ranged from 34 to 37 PSU and dissolved oxygen was ≥80%. Diatom control growth rates of the water-only experiments were acceptable, with values of 1.3 ± 0.1 (arithmetic mean ± SD, n = 4) and 1.5 ± 0.05 doublings/day for the first and repeat experiments, respectively, and the percent coefficient of variation was ≤20% in both tests. The 72-h chlorophyll-*a* concentrations of the seawater controls in the two water-only experiments were 1.6 ± 0.2 and 1.4 ± 0.2 mg/L. The 72-h chlorophyll-*a* concentrations of the control treatments for the sediment experiments were comparable (ranging between 1.6-2.1 mg/L, n = 6) and the percent coefficient of variation was ≤20% in all tests (Table 3.4). This confirms the repeatability of the test.

Table 3.4 Results of quality control procedures in water-only and sediment exposure experiments

Experiment	Chlorophyll-*a* concentration (mg/L)*	Chlorophyll-*a* concentration (mg/L)*	Coefficient of variation (%)	Factor chlorophyll-*a* increased by over 72 h	Chlorophyll-*a* concentration (mg/L)*	Chlorophyll-*a* in 2nd extraction (% of first extraction)	Cu EC50 reference toxicant result (µg Cu/L) [§]
	Control (0 h)	Control (72 h)[†]		Control	Control blank (72 h)[‡]	Control (72 h)	
Water-only exposures							
Range finder	0.18 ± 0.02	1.6 ± 0.2	16%	9	NA	NA	15 (14-17)
Repeat	0.14 ± 0.02	1.4 ± 0.2	12%	10	NA	NA	17 (14-19)
Sediment exposures							
Organic-silt	0.07 ± 0.01	2.0 ± 0.3	14%	29	0.95 ± 0.08	5%	16 (13-19)
Repeat	0.06 ± 0.00	2.0 ± 0.3	13%	33	0.8 ± 0.3	4%	16 (14-18)
Sandy-silt	0.08 ± 0.01	2.1 ± 0.1	5%	26	0.18 ± 0.9	7%	41 (34-48)
Repeat	0.08 ± 0.01	2.0 ± 0.1	6%	25	0.21 ± 0.06	6%	48 (31-65)
Silty-sand	0.07 ± 0.02	1.7 ± 0.3	20%	24	0.04 ± 0.02	0%	23 (18-29)
Repeat	0.08 ± 0.01	1.6 ± 0.05	3%	20	0.04 ± 0.04	0%	21 (18-23)

NA = not applicable

EC50 = the concentration of Cu to cause a 50% effect on of growth of *C. closterium* (95% confidence interval shown in parentheses)

* Mean ± standard deviation (n = 4)

[†] Control after the no algae blank has been subtracted to remove background chlorophyll

[‡] Control sediment without algae added, used to determine background chlorophyll concentrations of the sediment

[§] Water-only copper exposure performed alongside each experiment, dissolved (<0.45 µm filtered) copper concentration

Additional quality control procedures were introduced to the method based on experimental observations (Table 3.4) and are summarised as follows. The background chlorophyll-*a* concentration of the sediments (control blanks, i.e. treatments without algae added) ranged between 0.04 mg/L in the Silty-sand treatment and 0.95 mg/L in the Organic-silt treatment. The initial (Day 0) chlorophyll-*a* concentration of the algae used for spiking the treatments ranged between 0.06 mg/L and 0.18 mg/L (n = 8) across the separate experiments. The chlorophyll-*a* concentration of the controls increased exponentially over the 72-h experiment, with the 72-h chlorophyll-*a* concentration being between 9 and 33 times greater than at 0 h. The second acetone extraction of chlorophyll-*a* demonstrated good recovery from the first extraction for all experiments, with the second acetone extraction chlorophyll-*a* concentrations ≤10% of the chlorophyll-*a* concentrations of the first extraction (values ranging between 0% in the Silty-sand treatment and 7% in the Sandy-silt treatment).

Algal sensitivity, as measured by the copper reference toxicant performed alongside each experiment, resulted in EC50s between 15 and 48 µg/L dissolved copper which was within 2 SD of the mean for all tests (25 (0-51) µg/L; mean (2 SD range), n = 8). The spread of the EC50 results is most likely due to the limited number of replicates and sample concentrations tested.

3.3.2.3 The response of algae exposed to dissolved nickel in water-only experiments and overlying water of sediment exposures

The sensitivity of *C. closterium* to water-only exposures of dissolved nickel was determined to understand the contribution of dissolved nickel dissociated from the sediment to the toxicity observed in the sediment exposures (Figure 3.4A). The dissolved nickel EC10 and EC50 values based on chlorophyll-*a* concentrations in the water-only test were 330 (140-510) µg/L (95% confidence interval) and 3,400 (2,700-4,000) µg/L, respectively. These effect concentrations are well above dissolved nickel concentrations reported within marine water of SEAM (125 µg Ni/L, Hydrobiology, 2016), and industrialised estuaries around the world (≤110 µg Ni/L, Blewett and Leonard, 2017).

The response of *C. closterium* to the dissolved nickel in the overlying water of nickel-spiked sediment treatments was dependent on the sediment type (Figure 3.4A). There were no adverse effects on algal growth (as measured by chlorophyll-*a*) in the Organic-silt sediment (highest dissolved nickel treatment concentration was 930 µg/L (mean, n = 4)), however, the Sandy-silt and Silty-sand treatments had EC10s based on dissolved nickel in the overlying water at 300 (220-380) µg/L and 160 (0-330) µg/L, respectively (Table 3.5). The absence of observed toxicity in the Organic-silt sediment treatment, despite exceedance of the dissolved nickel in water-only tests, suggests that the dissolved nickel was less bioavailable in the presence of highly organic sediments and may have been in a colloidal form. The EC10s of the Sandy-silt and Silty-sand treatments were lower (but within the confidence interval) than the EC10 for dissolved nickel exposure alone. The EC50 of the Silty-sand treatments was 620

(430-810) µg/L which is 5.5 times more toxic to *C. closterium* than can be attributed to dissolved nickel alone and suggests that toxicity was not solely due to dissolved nickel present in the overlying water.

The relationship between chlorophyll-*a* concentration (determined by spectrophotometric method) and cell density (determined by flow cytometric method) revealed a significant relationship under culturing conditions (R^2 = 0.99) and testing conditions, over 72-h dissolved nickel exposures (treatment concentrations of <1 to 9,800 µg Ni/L) (R^2 = 0.73) (Appendix B2). While chlorophyll-*a* concentration in periphyton and phytoplankton is widely used as a surrogate for algal cell density/biomass, some research has shown that there is not always a clear relationship between the two due to inter- and intra-species variation because of differences in chlorophyll-*a* content, size, and physiological condition (Bowles, 1982). However, the current study, which has utilised a single test species and uniform testing conditions has demonstrated that the chlorophyll-*a* concentration of *C. closterium* determined via the spectrophotometric method, is an appropriate surrogate for algal cell density/biomass which cannot be directly measured easily in whole-sediment toxicity tests.

Chlorophyll-*a* concentration was found to be a more sensitive measure of dissolved nickel effects on *C. closterium* than growth rate inhibition from the water-only exposure experiments (Figure 3.4). The dissolved nickel EC10 based on growth rate inhibition in the water-only test was 1,700 (1,200-2,200) mg/L, while the EC50 was greater than the highest concentration tested of 9,800 µg/L (mean, n = 4) (Figure 3.4B), compared to the EC10 and EC50 based on chlorophyll-*a* concentration of 330 (140-510) µg/L, and 3,400 (2,700-4,000) µg/L, respectively. Florence et al. (1994) have previously reported the tropical strain of *C. closterium* (CS-114, 27°C) to have a 72-h growth rate inhibition EC50 of >500 µg Ni/L. In the same study, the temperate strain of *C. closterium* (CS-5; 21°C) was more sensitive to nickel, having a 72-h EC50 of 250 µg/L. Gissi et al. (2016) also reported the tropical strain of *C. closterium* (CS-114, 27°C) to have a 72-h EC10 and EC50 of 2,900 (1,500-4,200) µg Ni/L and 7,600 (7,060-8,200) µg Ni/L, respectively. The difference between the EC10 and EC50 values derived in the current study and Gissi et al. (2016) is likely attributed to the initial cell density of *C. closterium* used which was 2-4 x 10^4 cells/mL in the current study and 1-3 x 10^3 cells/mL in Gissi et al. (2016). Previous studies have reported changes in algal sensitivity to metals based on initial algal cell density (Franklin et al., 2002; Gillmore et al., 2016; Moreno-Garrido et al., 2000). Low cell densities are usually preferred to avoid artefacts such as nutrient and dissolved metal depletion and more closely reflect cell densities found in aquatic ecosystems (Franklin et al., 2002). The current study utilised the higher initial cell density to improve the measurement of chlorophyll-*a* concentration via the spectrophotometric technique (which has higher detection limits than flow cytometry) to reliably detect a difference between treatments.

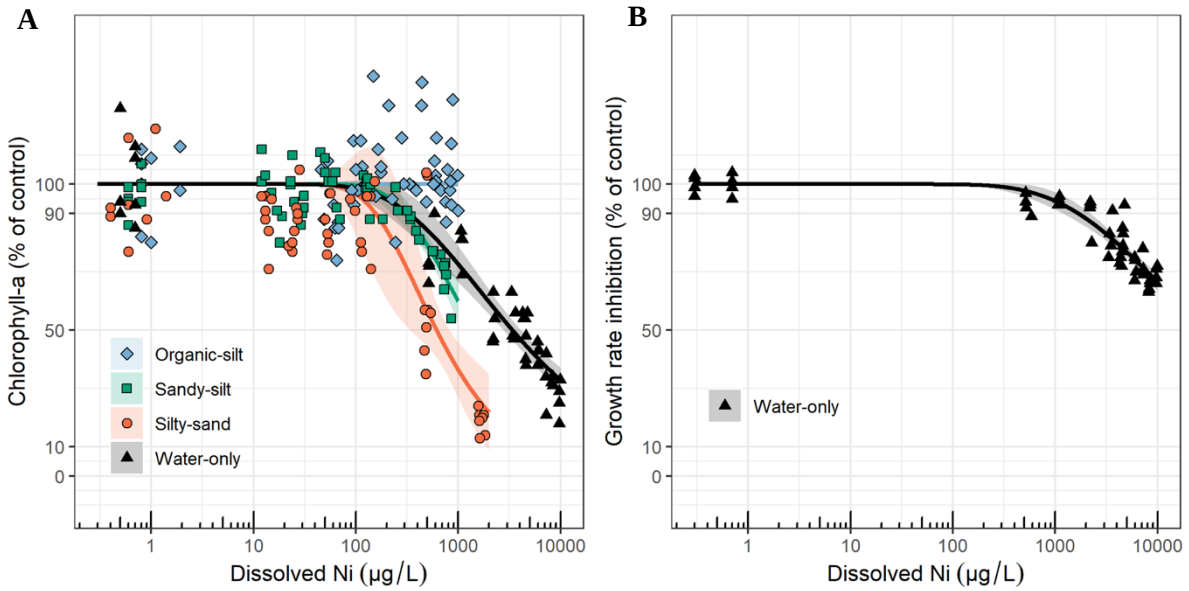


Figure 3.4. Relationships between dissolved nickel concentration in the overlying waters and effects on Ceratoneis closterium *following 72-h exposure, based on A) chlorophyll-*a *concentration and, B) growth rate inhibition. Data presented are for three sediment types over a nickel-spiked concentration series and a dissolved nickel concentration series in water-only experiments (data pooled from duplicate experiments). Each point represents one replicate. The shaded ribbons show the 95% confidence intervals.*

3.3.2.4 The response of algae exposed to nickel-spiked sediments

The toxicity of nickel-spiked sediment to *C. closterium* was dependent on the sediment type (Table 3.5). There were no adverse effects on chlorophyll-*a* concentration following exposure to the Organic-silt sediment, where the highest nickel spiked sediment treatment was 4,300 mg/kg AE-Ni (8,200 mg/kg TR-Ni). Measurement of TR-Ni and AE-Ni resulted in similar concentration-response relationships and demonstrated that the Silty-sand sediment treatment was more toxic to *C. closterium* compared to the Sandy-silt treatment (Figure 3.5A and B). The EC10 and EC50 values for the Silty-sand sediment treatment were 950 mg/kg AE-Ni (2,000 mg/kg TR-Ni) and 2,000 mg/kg AE-Ni (4,200 mg/kg TR-Ni), respectively, and for the Sandy-silt sediment treatment were 1,100 mg/kg AE-Ni (3,400 mg/kg TR-Ni) and >2,300 mg/kg AE-Ni (>6,700 mg/kg TR-Ni).

Similar to observations of the overlying water dissolved nickel concentrations, the toxicity observed in the Silty-sand treatment could not be due to effects of dissolved nickel in the pore water alone despite the higher concentrations, and conversely, the Organic-silt sediment led to less toxicity than would be otherwise expected from dissolved nickel exposure in the pore water (Figure 3.5C, Table 3.5). Potential stimulation of algal growth was observed in some replicates of the Organic-silt nickel-spiked sediment treatments and may have masked the toxic effects of nickel in the sediment. Stimulation was not observed in the other nickel-spiked sediments and is likely to be due to the enrichment of nutrients from the high organic content of the Organic-silt sediment. Similar stimulation of algae has been observed,

presumably in response to the release of nutrients such as ammonium, in elutriate testing (Hall et al., 1996) and for sediment toxicity testing where carbon assimilation rate was the endpoint (Munawar and Munawar, 1987). The sediment toxicity test for the temperate benthic marine microalga *Entomoneis cf punctulata* developed by Adams and Stauber (2004) demonstrated that the issue of algal stimulation could be overcome when the endpoint esterase activity was used.

While benthic diatoms do not ingest sediment particles, they are in direct contact with the sediments. It is hypothesised that the algae were responding to the flux of labile nickel from the sediment occurring at the sediment-water interface. This flux was determined in Chapter 4 using DGT technique and revealed that the Silty-sand and Sandy-silt sediments both had a greater flux of labile-nickel at the sediment water interface compared to the Organic-silt sediment treatment (Section 4.3.2). This is most likely attributed to the greater organic content and a greater proportion of <63 μm particles in the Organic-silt sediment compared to the other two sediments (Table 3.2) as nickel was predominately bound to the silty (<63 μm) component of the sediment (Section 4.3.1). Comparison between the algae and no algae treatments of the highest treatment tested for each sediment type revealed that the treatments without algae had a dissolved nickel concentration between 1.4 and 2.5 times greater than the treatments with algae. This result suggests that the algal cells were sequestering a large proportion of the dissolved nickel being released from the sediment to the overlying water.

While *C. closterium* is not sensitive to nickel, the results indicate that accumulation of nickel by the algal cells may represent a significant dietary source of nickel exposure to higher-level trophic organisms that graze on benthic algae. Previous research has shown that even low-level exposure of dissolved nickel to the marine diatom *Thalassiosira pseudonana* resulted in diet-borne-nickel toxicity to the marine copepod *Acartia tonsa* (LOEC was 7.6 μg/L dissolved nickel in the algal medium) (Bielmyer et al., 2006).

3.3.2.5 Application of the whole-sediment bioassay to assess the toxicity of field-contaminated sediment

No toxicity was observed from exposure of *C. closterium* to any of the field-contaminated sediments (Chlorophyll-*a* concentrations were all >80% of the control) (Figure 3.5). The three field-contaminated sediments had nickel concentrations (52-170 mg/kg AE-Ni) in the range of predicted no effects on chlorophyll-*a* concentration of *C. closterium* based on the nickel-spiked sediment treatments.

Table 3.5. Toxicity of nickel to Ceratoneis closterium *following 72-h exposure to nickel in three nickel-spiked sediments – effect on chlorophyll-*a *concentration*

Toxicity estimate*	**Sediment treatment**		
chlorophyll-*a*	**Organic-silt**	**Sandy-silt**	**Silty-sand**
Dissolved Ni in the overlying water (μg/L)			
EC10	>930	300 (220-380)	160 (0-330)
EC50	>930	>770	620 (430-810)
Total recoverable Ni (mg/kg)			
EC10	>8,200	3,400 (2,700-4,200)	2,000 (1,000-3,000)
EC50	>8,200	>6,700	4,200 (3,800-4,700)
Dilute-acid extractable Ni (mg/kg)			
EC10	>4,300	1,100 (880-1,300)	950 (490-1,400)
EC50	>4,300	>2,300	2,000 (1,800-2,200)
Pore water Ni (μg/L)			
EC10	>1,700	1,000 (550-1,500)	150 (0-310)
EC50	>1,700	>4,000	600 (410-790)

* EC10 or EC50 is the concentration of nickel to cause a 10% or 50% effect respectively, on the chlorophyll-*a* concentration of *C. closterium* (95% confidence interval shown in parentheses).

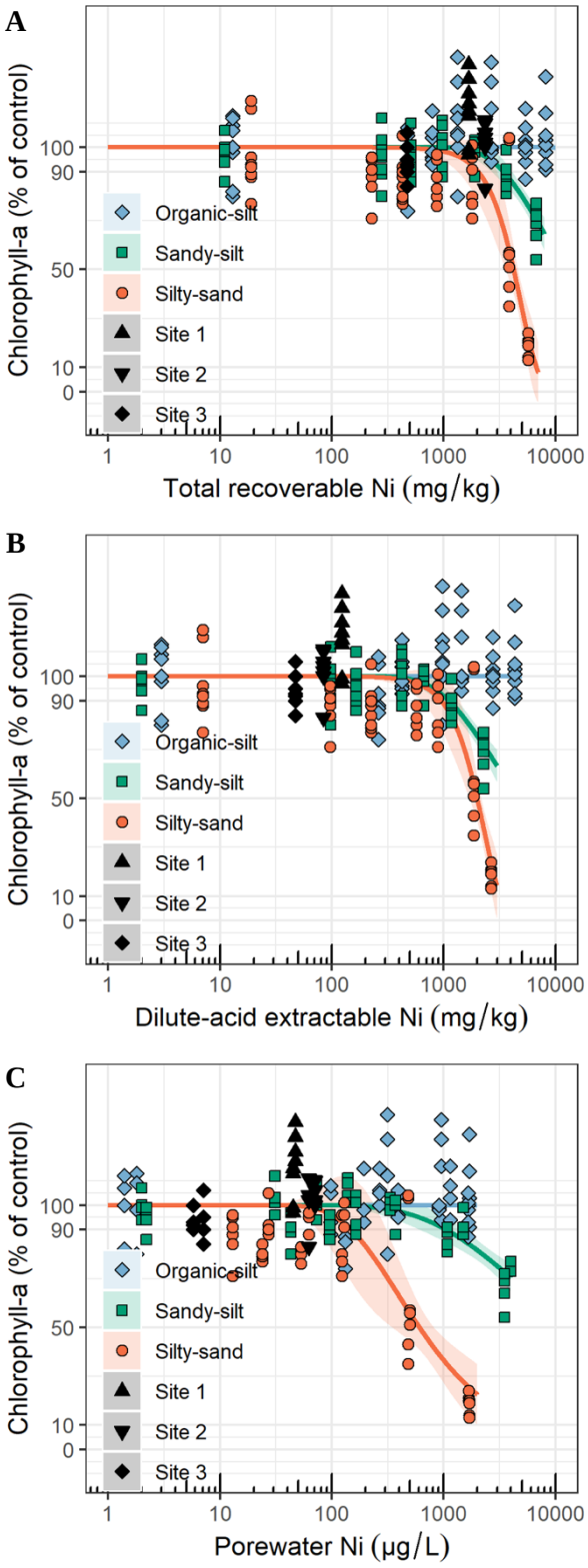


*Figure 3.5. Relationships between sediment nickel concentration and chlorophyll-*a *of* Ceratoneis closterium *following 72-h exposure, represented as: A) total recoverable nickel, B) dilute-acid extractable nickel, and C) pore water dissolved nickel concentration. Data are presented for five sediments including three nickel-spiked concentration series (Organic-silt, Sandy-silt and Silty-sand) and three field-contaminated sediments (Site 1, Site 2 and Site 3). Data pooled from duplicate experiments. Each point represents one replicate. The shaded ribbons show the 95% confidence intervals.*

3.4 Conclusions

The development of sensitive sub-lethal whole-sediment toxicity test methods with tropical marine species is important for risk assessment in tropical regions as they provide a link between exposure and effect in sediment quality assessments. These test methods ideally need to involve test species that are both ecologically relevant to the SEAM region and sensitive to nickel for identifying sediments likely to present a risk to benthic biota within the lateritic nickel mining regions of SEAM.

The physical attributes of *N. dorsatus* and its relative abundance and geographical distribution provided encouragement for the use of this species in the development of a whole-sediment toxicity test method for tropical marine environments. However, due to its relative insensitivity to nickel, other marine invertebrates should be considered for the development of a tropical marine whole-sediment bioassay for assessing the effects nickel-rich sediments may have on the benthic marine community.

The current study provided improvements to the whole-sediment toxicity test based on chlorophyll-*a* of temperate *C. closterium* proposed by Strom (2011) and Watson (2012) and demonstrated its applicability for use with the tropical strain of *C. closterium.* The current study has contributed sediment nickel toxicity data for tropical microalgae and in doing so demonstrated this species was insensitive to sediment-associated nickel. A species with greater sensitivity to nickel should be sought for the development of a whole-sediment bioassay to provide greater confidence that the toxicity test method will identify sediments likely to have adverse effects.

This work has highlighted the difficulties with finding appropriate test species for the development of whole-sediment toxicity tests. Future research could employ the use of genomic techniques to make more informed species selection choices. For example, DNA-based identification of species disappearance along a nickel-contamination gradient within a nickel impacted field-site could reveal potentially nickel sensitive species which could then be targeted for the development of whole-sediment toxicity tests (investigated in Chapter 5). Given the lack of nickel sediment ecotoxicity data and availability of whole-sediment toxicity tests for tropical estuarine and marine species and the significant effort that is required to make these available, alternative tools for risk assessment within SEAM should be explored. For example, the use of passive sampling techniques as a surrogate for toxicity testing (by identifying sediments with comparatively greater bioavailable nickel and thereby requiring further investigation) could be explored (investigated in Chapter 4).

Chapter 4

4 The bioavailability and toxicity of nickel-contaminated sediments of varying properties to the epibenthic amphipod, *Melita plumulosa*

This chapter has been redrafted from the published article: Gillmore, M.L., Price, G.A.V., Golding, L.A., Chariton, A.A., Stauber, J.L., Adams, M.S., Simpson, S.L., Smith, R.E.W., Jolley, D.F. 2021. The diffusive gradients in thin films (DGT) technique predicts sediment nickel toxicity to the amphipod, *Melita plumulosa*. *Environmental toxicology and Chemistry*. https://doi.org/10.1002/etc.4971

Co-author and Honours student G. Price and I performed the laboratory component of the current study. I processed all the data presented in this chapter and Gillmore et al. (2021b). G. Price also processed the data independently and his work is presented in his Honours thesis (Price, 2017). M. Gillmore wrote the original draft of this chapter/manuscript. All authors contributed to the experimental design and refining the manuscript before submission.

4.1 Introduction

It is well recognised that the total concentrations of contaminants in sediments can be a poor indicator of the risk posed to benthic biota, as some of those contaminants may be present in less bioavailable forms (Besser et al., 2013; Simpson and Batley, 2007). Metal bioavailability in sediments is influenced by factors such as pH, redox conditions (dissolved oxygen penetration), particle size, and binding with particulate sulfide, organic carbon, or iron oxy(hydroxide) phases. In freshwater sediments, AVS and iron are the most important factors modifying nickel bioavailability and toxicity (Besser et al., 2013; Vangheluwe et al., 2013). The importance of iron and manganese over organic carbon for nickel binding in oxic freshwater surface sediments and reducing metal bioavailability and toxicity has also been demonstrated (Costello et al., 2016, 2011). Research on nickel in sediments from lateritic nickel mining regions has shown that in the lagoon sediments, clay minerals and iron oxy(hydroxides) are the major binding phases for nickel (Ambatsian et al., 1997; Fernandez et al., 2006; Merrot et al., 2019), whereas, in mangrove sediments, the major binding phase is sulfide (Marchand et al., 2012; Noël et al., 2015). These studies have also highlighted that during sediment transformation processes, significant release of dissolved nickel to the overlying water can occur (Ambatsian et al., 1997; Marchand et al., 2012). Few studies, however, have investigated how these changes to nickel partitioning influence its bioavailability and toxicity to estuarine or marine organisms (Chandler et al., 2014).

The paucity of ecotoxicological exposure and effects data for sediment associated nickel to estuarine and marine biota is driven by the limited number of standardised temperate sediment toxicity test methods and the lack of tropical test species (Gissi et al., 2016). For this reason, and due to the relatively

high cost of ecotoxicological testing, chemical methods that can provide a more representative measure the bioavailable fraction of nickel in sediment are desirable.

Various methods for estimating the bioavailable concentration of metals in sediments have been proposed, including analysis of pore waters, using dilute-acid extraction of sediments, or by determining AVS or organic carbon-normalised concentrations (Di Toro et al., 2005). More recently, the DGT technique has proven useful for assessing the lability of metals in sediments. When placed in sediment, the DGT device accumulates only free metal ions and DGT-labile metal species (such as simple inorganic complexes and weak organic metal complexes which frequently dissociate/associate in the DGT gel domain) dissolved in the pore water and overlying water, which are more likely to be bioavailable (Davison, 2016). This accumulation results in a depletion of metals around the device and subsequently causes a release of weakly bound metals from the sediment particles to the pore water and overlying water, mimicking what would happen during organism uptake (Davison, 2016). There is a growing body of evidence demonstrating the potential of the DGT-labile metal flux at the sediment-water interface for predicting metal bioavailability and toxicity to benthic organisms (Simpson et al. 2012; Amato et al. 2014; Yin et al. 2014; Amato et al. 2015; He et al. 2018). In contrast, some studies have found that conventional chemical measures of bioavailability better predict metal bioaccumulation or toxicity compared to the DGT measure (Costello et al., 2012; Roulier et al., 2008). This includes the study by Costello et al. (2012) which found that organic matter normalised AVS-simultaneously extracted metals (SEM) measurements explained nickel toxicity from freshwater sediments to benthic macroinvertebrates better than DGT-labile nickel concentrations. Therefore, further research is required to assess the contaminants and organisms for which DGT measures of metal bioavailability are appropriate for predicting toxicity.

The current study aimed to determine how sediment physicochemical properties, relevant to the SEAM region, influence the bioavailability and toxicity of nickel to the amphipod *Melita plumulosa* by measuring reproductive output following a 10-d exposure to nickel-contaminated sediments. Effects thresholds for sediment nickel were determined using nickel-spiked sediments prepared from uncontaminated field-collected sediments with varying physicochemical properties (referred to as nickel-spiked sediments). Sediments with elevated nickel concentrations, collected from nickel laterite mining regions of New Caledonia and Indonesia, were also tested (referred to as field-contaminated sediments). Concentration-response relationships obtained using conventional measures of contaminant exposure and potential bioavailability were compared with the DGT-labile nickel flux to determine whether DGT can be used to predict nickel bioavailability and toxicity.

4.2 Materials and methods

4.2.1 General analytical

General laboratory equipment was prepared as described in Section 2.1.1. South Coast seawater was collected as per Section 2.1.2 and adjusted to test salinity (30 ± 1 PSU) using reverse osmosis water. Stock solutions for nickel were prepared as described in Section 3.2.1.

4.2.2 Sediment preparation and characterisation

Sediment collection, characterisation, and nickel spiking were carried out as described in Section 2.3. Uncontaminated sediments were collected from coastal locations on the east coast of Australia (Table 3.1). The sediments were selected for their differing physicochemical properties (Table 4.1) that could alter nickel bioavailability and were described as Organic-silt, Sandy-silt, and Silty-sand. These sediments were used as controls and for preparing a nickel-spiked concentration series for establishing concentration-response relationships. Nickel-rich sediments were collected from coastal locations adjacent to nickel mining or processing facilities within New Caledonia (Site 1 and Site 2) and Indonesia (Site 3). Trace metal concentrations of the control sediments and field-contaminated sediments are presented in Table 4.2.

Table 4.1 Physicochemical properties of the control sediments before the spiking process and field-contaminated test sediments

Sediment	**Pore-water pH (n = 1)**	**Particle size (%, n = 5)**			**TOC (%, n = 1)**	**TIC (%, n = 1)**	**AVS (μmol/g, n = 3)**	**Dilute-acid extractable metal (mg/kg, n = 3)**		
		<4	**4-63**	**>63 (μm)**				**Fe**	**Mn**	**Ni**
Organic-silt	7.1	11	83	6	5	0.05	0.9 ± 0.1	12,000	62	3
Sandy-silt	7.2	11	64	26	1	2	1.7 ± 0.2	3,500	570	2
Silty-sand	8.0	4	16	80	0.2	9	1.6 ± 0.1	1,600	140	1
High-sulfide	7.2	7	70	23	8	0.6	9 ± 2	4,800	66	3
Site 1	7.0	11	69	20	6	0.6	0.9 ± 0.3	14,000	280	170
Site 2	6.8	4	31	65	1	0.2	1.4 ± 0.9	8,600	150	150
Site 3	7.3	5	71	25	5	0.7	0.5 ± 0.2	8,700	290	52

TOC = Total organic carbon; TIC = Total inorganic carbon; AVS = Acid-volatile sulfide.

NB: The reported dilute-acid extractable metal concentrations presented here differ slightly from those presented in Table 3.2 for the field-contaminated sediments as they are the average of measurements taken immediately before and after the exposure bioassay.

Table 4.2 Sediment metal concentrations in control sediments and field-contaminated test sediments (n = 2)

Site	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Total recoverable metals* (mg/kg)												
Organic-silt	14,000	18	1	6	36	48	31,000	95	12	**76**	55	**240**
Sandy-silt	13,000	17	<1	7	15	5	19,000	650	13	22	34	35
Silty-sand	4,200	10	<1	2	5	1	5,500	210	**35**	4	10	10
High-sulfide	10,000	10	1	5	20	36	23,000	110	12	44	45	120
Site 1	7,200	10	<1	69	**570**	9	35,000	510	**1,300**	17	44	50
Site 2	4,000	3	<1	110	**940**	2	39,000	490	**2,000**	7	29	58
Site 3	9,600	10	1	32	**370**	23	37,000	510	**480**	18	29	44
Dilute-acid extractable metals† (mg/kg)												
Organic-silt	2,300	3	<1	2	3	16	9,800	47	3	**57**	29	170
Sandy-silt	1,500	4	<1	3	2	2	4,000	600	2	10	13	12
Silty-sand	530	2	<1	<1	1	<1	9,500	150	<1	1	3	4
High-sulfide	2,900	1	<1	1	<1	11	9,800	63	3	37	23	79
Site 1	1,200	<1	<1	16	25	2	14,000	280	**170**	<1	17	11
Site 2	650	<1	<1	30	17	1	8,600	150	**150**	1	5	16
Site 3	1,100	2	<1	9	21	5	8,700	290	**52**	8	11	10
Australian and New Zealand sediment quality guideline values‡ (mg/kg)												
DGV	-	20	1.5	-	80	65	-	-	21	50	-	200

* Total recoverable metals were determined by strong aqua-regia digestion at 80°C for 1.5 h.

† Dilute-acid extractable metals were determined by reacting with 1 M HCl for 1 h.

‡ Australian and New Zealand sediment quality default guideline value (DGV) (Australian and New Zealand Governments, 2018). Values in **bold** show an exceedance of the DGV.

4.2.3 Amphipod reproduction bioassay procedure

Melita plumulosa is a temperate, epibenthic, deposit-feeding amphipod, endemic to estuarine and marine sediments of the southeast coast of Australia (Hyne et al., 2005; King et al., 2006). It was cultured according to the method of Spadaro et al. (2008). The current study used the 10-d amphipod reproduction bioassay developed by Simpson and Spadaro (2011) adapted from the work of Mann et al. (2009), which measures the adverse effects of sediment contaminants on amphipod reproduction by counting the number of embryos and juveniles that are produced following exposure. For the sediment exposures, reproduction was represented as the sum of the number of embryos and juveniles at test end, divided by the original number of females (i.e. six) and expressed as a percentage of the control. The sensitivity of *M. plumulosa* to dissolved nickel was determined to understand the contribution of dissolved nickel released to the overlying water of the sediment exposures to toxicity. For the dissolved

nickel exposures, reproduction was represented as the sum of the number of embryos and juveniles at test end, divided by the number of surviving females at test end (due to the reduced survival rate of females under seawater-only experimental conditions) and expressed as a percentage of the control. A summary of the test conditions and test protocol is provided in Table 4.3.

For the nickel-contaminated sediment exposures, homogenised sediment (100 g) and clean filtered seawater (400 mL, South Coast) were added to 450-mL glass beakers and incubated at 21°C with aeration for an initial 10 to 15 days to allow the sediment to equilibrate under test conditions (equilibration period) before the addition of test organisms (exposure period). Overlying water was exchanged four times with clean seawater throughout the equilibration period including on the day of test commencement. Three replicates were used for biological response measurement and DGT-labile nickel flux measurement and one replicate (also containing biota) was collected and frozen (-20°C) for chemical analyses of AE-Metals and AVS on days 5 and 10. An aliquot of each sediment treatment was collected at the time the test beakers were set up for TR-Metal analyses. TR-Metals were analysed on the bulk sediment and the fine sediment fraction (<63 μm, wet press sieved). Experiments were performed once for each sediment type.

For the dissolved nickel exposures, clean sand that had minimal nickel-binding was provided as the amphipod will not reproduce consistently over 10 days in seawater-only exposures without a substrate. The sand was sieved (>180 μm) before use, to remove the fine ingestible fraction, to avoid dietary exposure via sediment ingestion. Therefore, even though sand was present, the dominant exposure pathway was via the dissolved nickel in the overlying water so this treatment is referred to as the dissolved nickel exposure from herein. The dissolved nickel treatments (0-1,000 μg Ni/L nominal concentration) were prepared in bulk by spiking the nickel stock solution into filtered seawater (South Coast) so that the metal stock accounted for less than 0.1% of the total volume. The sieved sand (40 g) and the dissolved nickel treatments (200 mL) were added to 250-mL glass beakers and incubated at 21°C for seven days (equilibration period) before the addition of test organisms (exposure period). Each treatment was prepared in triplicate. During the equilibration period, the treatment solutions (but not the sand) were renewed twice to replace dissolved nickel lost to binding sites of the glass beaker and sand. Since sand is difficult for the amphipods to burrow into, a 4×4-cm piece of 180-μm nylon mesh was also added to provide shelter for the amphipods, which was found to improve survival in trial experiments (data not shown). The dissolved nickel exposure was performed twice and the results pooled.

For both the dissolved nickel and nickel-contaminated sediment exposures, at test commencement (day 0), 6 male and 6 gravid females (≤2 days into gestation) were randomly assigned to each beaker and placed in an environmental chamber (21°C, 12:12 h light:dark cycle at 3.5 μmol photons/m^2/s). *Melita plumulosa* breed continuously, with fertilisation to the hatching of offspring being completed within

seven days (Mann and Hyne, 2008). Thus, during the 10-d exposure, females undergo two reproductive cycles, producing two separate broods. On day 5, the contents of each beaker were gently sieved (600-μm mesh size) to transfer the adults to new beakers leaving behind the first brood of juveniles conceived before the test exposure commenced. These beakers were prepared and equilibrated at the same time as the previous beakers used on day 0.

During the 10-d exposure period of the nickel-contaminated sediment exposures, the overlying water was replaced every day with clean seawater to minimise dissolved nickel concentrations in the overlying waters, as this was considered to better represent the likely dilution of released metals at field sites. The physicochemical properties of the overlying water (pH, salinity, conductivity, dissolved oxygen, and ammonia) were measured in a composite sample from each treatment every second day of the exposure period and from the clean replacement seawater every day (as per Section 2.2.1). Amphipods were fed (0.5 mg/amphipod, Sera Micron) on a limited number of days (day 0, 3, and 7 of the exposure period) to encourage foraging within the sediment.

During the 10-d exposure period of the dissolved nickel exposures, the nickel-treatment solutions were replaced on days 3, 5 and 7. The physicochemical properties of the overlying water (pH, salinity, conductivity, dissolved oxygen, and ammonia) were measured in a composite sample from each treatment before the renewal and in each of the bulk replacement nickel-treatment solutions before being dispensed into the test beakers. Amphipods were fed powdered fish food (1 mg/amphipod, Sera Micron, Sera) on days 0, 3, 5, and 7 of the exposure period after water renewals.

For both dissolved nickel and nickel-contaminated sediment exposures, dissolved metal subsamples were collected for metal analysis (as per Section 2.2.2) from every replicate before and after each overlying water replacement.

On day 10, the test was terminated. The contents of each beaker were gently sieved to separate the adults from the juveniles using the combination of 600- and 180-μm mesh sieves. Juveniles were collected in polycarbonate containers with reverse osmosis water, preserved (2 mL of 3.6-M formaldehyde), stained with rose bengal (4,5,6,7-tetrachloro-2’,4’,5’,7-tetraiodofluorescein) solution (2 mL, 0.001 M) and counted within 2 to 3 days. Embryos were counted on day 10 by microscopy (Leica MZ95 stereomicroscope with a Leica CLS 150X power source).

Table 4.3 Summary of test conditions and test protocol for the 10-d reproduction bioassay with the amphipod Melita plumulosa *in either nickel-contaminated sediment or dissolved nickel exposure experiments. Adapted from Spadaro and Simpson (2015)*

Test parameter	Nickel-contaminated sediment exposure	Dissolved nickel exposure
Test duration	10 days	10 days
Temperature	21 ± 1°C	21 ± 1°C
Salinity	30 ± 1 PSU	30 ± 1 PSU
pH	7.2-8.2	7.2-8.2
Ammonia	≤ 1 mg/L	≤ 1 mg/L
Light intensity	3.5 µmol photons/m^2/s	3.5 µmol photons/m^2/s
Photoperiod	12-h light:12-h dark	12-h light:12-h dark
Aeration	slow bubbling to maintain ≥ 85% dissolved oxygen saturation	slow bubbling to maintain ≥ 85% dissolved oxygen saturation
No. test organisms/beaker	12 (6 males and 6 gravid females)	12 (6 males and 6 gravid females)
Test type	Static renewal test. Daily overlying-water renewal. Sediment renewal on day 5.	Static renewal test. Overlying-water renewal on days 3, 5 and 7. Sand renewed on day 5.
Test chamber	450-mL glass beaker	250-mL glass beaker
Substrate weight	100 g (sediment, sieved <6 mm)	40 g (sand, sieved >180 µm)
Overlying seawater volume	400 mL	200 mL
No. of replicate beakers/treatment	4 (includes 1 chemistry beaker)	3
Feeding	0.5 mg of sera micron per amphipod on days 0, 3 and 7	1 mg of sera micron per amphipod on days 0, 3, 5 and 7
Standard endpoint	Reproduction at test end per original number of females	Reproduction at test end per surviving females at test end
Test acceptability criteria	8-19 juveniles per gravid female and ≥ 80% survival in the control*	8-19 juveniles per gravid female* and ≥ 50% survival in the control†

* Spadaro and Simpson (2015)

† A lower acceptable control survival in water-only experiments was due to increased competitive interactions as organisms sought refuge resulting in increased mortality

4.2.4 DGT preparation and deployment

Plastic DGT pistons (25 mm diameter), purchased from DGT Research Limited, comprising a backing disk, Chelex binding resin (0.4 mm gel thickness), polyacrylamide diffusive gel (0.8 mm thickness), a 0.45-µm polysulfone filter membrane, and a front window disk, were prepared following the procedures recommended by DGT Research Limited. Assembled DGT pistons were stored in 0.01 M $NaNO_3$ (prepared in high putity water) at 4°C until deployment. Following a thorough rinsing with high purity water, the DGT piston was inserted into the sediment so that the sediment-water interface aligned with the horizontal centre of the disk (semi-circle above and below sediment-water interface) on day 7 of the exposure period. Observation of *M. plumulosa* behaviour has demonstrated that these organisms typically spend most of their time at the sediment-water interface, and reside within the top 5 mm of the sediment. The pistons were removed after 48 h of deployment, thoroughly rinsed with high purity water, then stored in clean plastic bags at 4°C until disassembly. DGT deployment between days 7 and 9 was selected to provide a representative measure of the DGT-labile nickel flux during the period where the amphipod embryos, that would be counted for the reproduction endpoint, were developing. A deployment of 48 h was selected as it is commonly used to allow analyte measurement above analytical detections limits but not too long for DGT binding layer saturation or biofouling of the device to occur. Accumulated metals were eluted in 1 M HNO_3 (either Suprapur or distilled Tracepur grade) for 24 h with periodic shaking. The extracts were then diluted with high purity water and analysed for multiple metals (as per Section 2.2.2). Undeployed DGT pistons were analysed as laboratory controls. The DGT-induced nickel flux, which includes nickel present in pore water and labile forms released from sediment particles within the 48-h deployment period, was calculated as per Equation 4-1and 4-2 (Davison, 2016).

$$M = Ce(Vbl + Ve)/fe \qquad 4\text{-}1$$

$$J = M/AT \qquad 4\text{-}2$$

Where: M = Mass of metal on the binding layer; Ce = Measured concentration of metal in acid eluent; Vbl = Volume of binding layer; Ve = Volume of acid eluent; fe = Elution factor (0.8); J = Flux of solute; A = Exposed area; and T = Time.

4.2.5 Statistical analysis

Time-weighted averaged (TWA) dissolved nickel concentrations were calculated by averaging the concentration determined after each water renewal and just prior to the next water renewal (to account for either loss of dissolved nickel to binding sites during the dissolved nickel exposures, or increase in dissolved nickel from nickel being released from the sediment in the sediment exposures) assuming

linear change between the two time points, then taking the average of each set of water renewals. Analysis of all the dissolved metal subsamples from the first experiment revealed that results were comparable if TWA dissolved nickel concentrations were calculated from the entire data set or restricted to half the dataset (for every alternate water renewal). Therefore, in subsequent experiments, the dissolved metal subsamples were only measured for every alternate water renewal.

The concentration-response relationships for the different measures of potential nickel bioavailability were compared statistically and were based on the initial TR-Ni concentrations, the arithmetic mean of the day 5 and day 10 AE-Ni concentrations, the 48-h DGT-labile nickel flux between days 7 and 9, and the 10-d arithmetic mean of all measured dissolved nickel concentrations. All statistical analyses were performed in the statistical analysis software R (3.5.1, 2018-07-02) (R Core Team, 2016). For each sediment type and each measure of nickel bioavailability, EC10 and EC50 toxicity estimates, and 95% confidence intervals were calculated using a log-logistic four-parameter model with the lower limit fixed at 0% and the upper limit fixed to 100% (as the data were normalised to the control), using the R package 'Dose-Response Curve' (DRC) (Ritz et al., 2015). This model was found to be most suitable after the fit of different models applied were compared using the Akaike information criterion value and by visual assessment of the curve (Pinheiro and Bates, 2000). For the water-only experiments, the dissolved nickel toxicity estimates were derived using the R package 'Mixed Effect Dose-Response Modelling' (Gerhard et al., 2014) to account for the variation between and within repeated experiments, on the log-logistic four-parameter model with a fixed lower and upper limit (0 and 100%, respectively). Graphs of the concentration-response curves were created using the log-logistic four-parameter model with a fixed lower and upper limit (0 and 100%, respectively) in the R package 'ggplot2 (Wickham, 2016).

4.3 Results and discussion

4.3.1 Physicochemical properties of the sediments

Nickel-spiking of sediments achieved a TR-Ni concentration series of 330 to 4,400 mg/kg, 250 to 3,300 mg/kg, 480 to 3,600 mg/kg, and 250 to 5,600 mg/kg for the whole sediment fraction of the Organic-silt, Sandy-silt, Silty-sand, and High-sulfide sediment treatments, respectively. The AE-Ni concentrations of the same nickel-spiked treatments were comparable for the four different sediment types at 54 to 61%, 33 to 63%, 47 to 65% and 33 to 59% of the TR-Ni concentrations, respectively, indicating that a significant proportion of the nickel present was in a potentially bioavailable form.

The TR-Ni concentrations of the <63 µm sediment fraction were 0.9 to 1.3, 0.9 to 1.3, and 3.7 to 5.4 times the whole sediment fraction concentration for the Organic-silt (94% <63 µm), Sandy-silt (75% <63 µm), and Silty-sand (20% <63 µm) sediment treatments, respectively. This indicates that for all

three sediments, nickel was predominately bound to the silty (<63 μm) component of the sediment. For the Silty-sand sediment treatment, which had only 20% of particles <63 μm, there was a comparatively greater concentration of nickel on the silty sediment fraction compared to the other two sediment treatments, which had ≥75% of particles <63 μm particles. The silty sediment fraction was observed to have stratified to the top layer of the sediment at the sediment-water interface where the amphipods reside. The <63 μm fraction was not analysed for the high-sulfide sediment.

All three field-contaminated sediments had similar pH (6.8–7.3), low inorganic carbon (<0.7%), and low AVS (<1.4 μmol/g). Site 1 and Site 3 sediments were relatively silty (80 and 76% <63 μm fraction, respectively) with high TOC (6 and 5%, respectively), while Site 2 sediment was predominately sandy (35% <63 μm fraction) with low TOC (1%). Site 1, Site 2, and Site 3 field-contaminated sediments had AE-Ni concentrations 13, 7, and 11% of the TR-Ni concentrations, respectively, indicating that nickel present in the sediment was mostly in a mineralised form. The TR-Ni concentrations of the <63 μm sediment fraction were 0.8 and 0.6 times the whole sediment fraction for Site 1 and Site 2 sediments, respectively. This indicates that more nickel was within the >63 μm fraction of the sediment, particularly in the Site 2 sediment. The <63 μm fraction was not analysed for the Site 3 sediment.

All three field-contaminated sediments had TR-Ni (480-2,000 mg/kg) and AE-Ni (52-170 mg/kg) concentrations that exceeded the Australian and New Zealand sediment quality DGV for nickel of 21 mg/kg (Australian and New Zealand Governments, 2018) by 23 to 95 and 2 to 8 times, respectively (Table 4.2). The common co-occurring metals chromium and cobalt were also elevated in the field-contaminated sediments (Table 4.2). The AE-Cr and AE-Co concentrations were 2 to 6% and 23 to 28% of the TR-Cr and TR-Co concentrations, respectively, indicating that they were mostly present in a mineralised form and therefore potentially less bioavailable. The TR-Cr concentration exceeded the DGV for chromium of 80 mg/kg by 5 to 12 times, while the AE-Cr concentration was 0.2 to 0.3 times the DGV (Australian and New Zealand Governments, 2018). No guideline value was available for cobalt. For all other metals that had a DGV, there were no DGV exceedances (Table 4.2). Metal concentrations below the DGV indicate that there should be a low risk of adverse biological effects from the sediment-associated metal. The risk from chromium is likely to be low given that the AE-Cr did not exceed the DGV, while the risk of metals present for which there are no DGVs remains unknown.

XRD scans of the sediments indicated a similar composition of carbonate, sulfide, halide, and silicate mineralogy for both the nickel-spiked sediments and the field-contaminated sediments, but the ratios between each phase varied for the different sediments (Figure 4.1). For all sediments, silicate group minerals were the dominant mineral phase present, except for the Silty-sand sediment where carbonate group minerals dominated. Only minor changes in the proportion of each mineral group present occurred as a result of the nickel-spiking process (Figure 4.1).

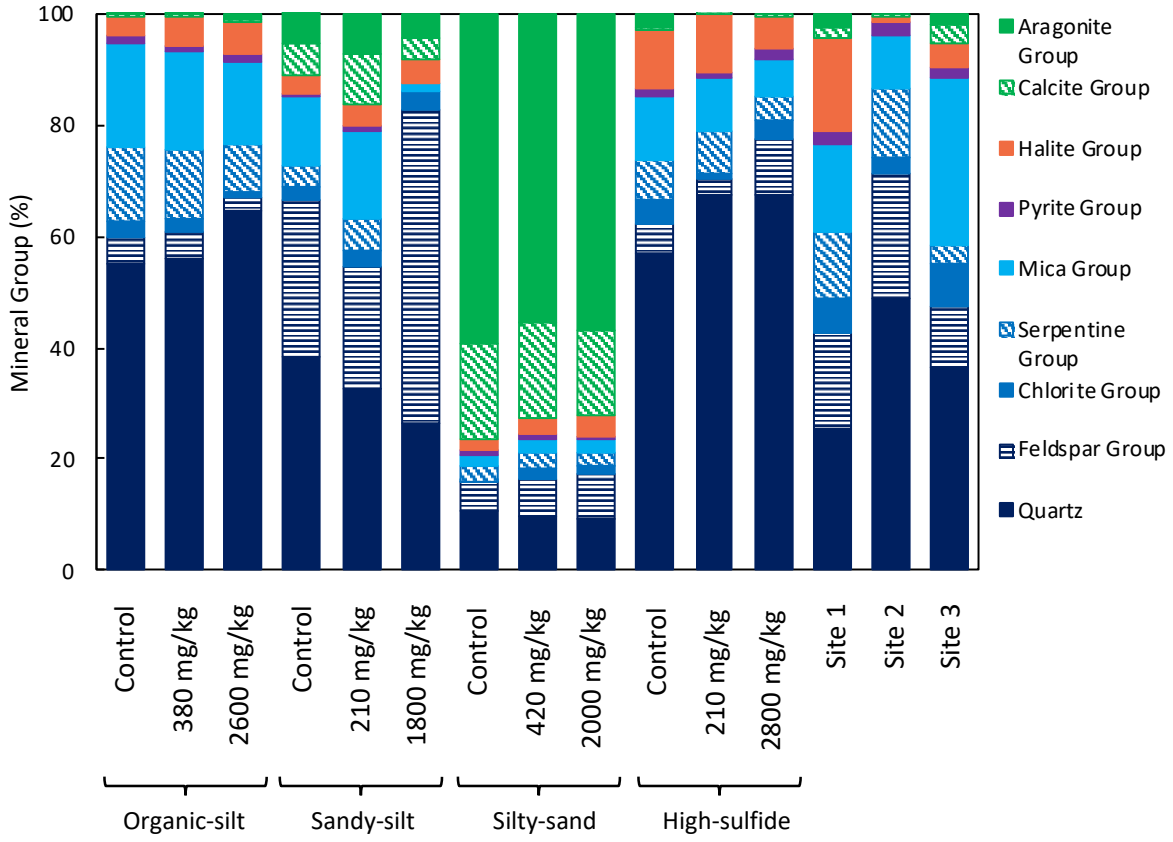


*Figure 4.1 Mineral groups from x-ray power diffraction (XRD) analyses. Dilute-acid extractable nickel concentrations reported on the x-axis. Colour indicates the mineral chemistry: carbonate minerals (*green*), halide minerals (*orange*), sulfide minerals (*purple*) and silicate minerals (*blue*).*

4.3.2 Dissolved nickel in the overlying water and the DGT-labile nickel flux at the sediment-water interface

The TWA concentration of dissolved nickel in the overlying water of the Organic-silt, Sandy-silt, Silty-sand, and High-sulfide sediment concentration series increased with increasing concentrations of particulate nickel and were in the range of <1 to 280, <1 to 310, <1 to 480, and <1 to 1500 µg/L, respectively (Figure 4.2A). The concentrations of dissolved nickel in the overlying water generally increased in the order Organic-silt < Silty-sand < Sandy-silt < High-sulfide at comparable AE-Ni concentrations (Figure 4.2A) indicating that nickel was most labile in the High-sulfide sediment and least labile in the Organic-silt sediment.

Analysis of the DGT-labile nickel flux at the sediment-water interface for the nickel-spiked sediments showed that there was a positive relationship with the AE-Ni concentration of the sediment (Figure 4.2B). For the Organic-silt, Sandy-silt, Silty-sand and High-sulfide sediment concentration series, the DGT-labile nickel fluxes at the sediment-water interface were in the range of 3.1 to 910, 1.8 to 1,700,

0.44 to 2,200 and 0.47 to 2,500 μg/m^2/h, respectively. A comparison of the DGT-labile nickel flux between the different nickel-spiked sediment types indicated that at comparable AE-Ni concentrations, the Organic-silt sediment had the lowest DGT-labile nickel flux and the High-sulfide sediment had the greatest DGT-labile nickel flux at the sediment-water interface (Figure 4.2B).

Overall, these results for the nickel-spiked sediments indicate that nickel was least labile in the Organic-silt sediment and most labile in the High-sulfide sediment at comparable AE-Ni concentrations. The lower lability of nickel in the Organic-silt sediment was likely due to the combination of high TOC, the high proportion of sediment particles being <63 μm (95%) and the significant contribution of clay minerals (which inherently have many metal-binding sites) to the overall composition of this sediment. Nickel was most labile in the High-sulfide sediment despite its relatively high content of AVS and TOC, potentially due to the oxidation of AVS phases occurring in response to a combination of the penetration of oxygenated overlying water and disturbance from the insertion of the DGT pistons through the sediment-water interface.

The TWA dissolved nickel concentrations of the overlying waters of the three field-contaminated sediments were 13, 30, and 2 μg/L for Sites 1, 2, and 3, respectively, and were comparable to the overlying water concentrations of the nickel-spiked sediments at comparable AE-Ni concentrations (Figure 4.2A). This demonstrated that the two-stage nickel-spiking process and extended equilibration period provided realistic nickel-contaminated sediments at the low end of the concentration range. As observed for the nickel-spiked sediments, the concentrations of the dissolved nickel in the overlying water were influenced by the sediment properties relevant to nickel binding. The two sediments that had the highest proportion of silt and TOC (Sites 1 and 3), also had the lowest dissolved nickel due to the binding properties of the TOC and large surface area of fine particles in the sediment.

The DGT-labile nickel flux at the sediment-water interface was similar for Site 1 and Site 2 sediments (96 and 36 μg Ni/m^2/h) and an order of magnitude lower in the Site 3 sediment (2.2 μg Ni/m^2/h). Accordingly, Site 3 also had the lowest dissolved nickel in the overlying water, and this is most likely due to the Site 3 sediment having the lowest bioavailable pool of nickel available of the three field-contaminated sediments (AE-Ni concentration of the Site 3 sediment was 0.3 and 0.4 times the AE-Ni concentration of the Site 1 and Site 2 sediments, respectively). The DGT-labile nickel flux of the field-contaminated sediments were similar to the DGT-labile nickel flux of the nickel-spiked sediments at comparable AE-Ni concentrations (Figure 4.2B).

Overall, the nickel-spiked sediments covered the range of total recoverable nickel concentrations that have been reported in estuarine and coastal marine sediments within New Caledonia (Ambatsian et al., 1997; Fernandez et al., 2006; Marchand et al., 2012; Noël et al., 2015) and were therefore of environmental relevance. They also provided differing physicochemical properties in terms of TOC, particle size and mineral composition to determine which properties were most influential to nickel

bioavailability. A key distinction between the nickel-spiked sediments and field-collected sediments was the proportion of weakly-bound nickel as determined from the AE-Ni as a percentage of the TR-Ni. Despite equilibration times of 10 to 12 months and pH adjustments, nickel was potentially less strongly bound to the nickel-spiked sediments than the field-contaminated sediments according to chemical methods of potential bioavailability. One hypothesis is that the iron oxyhydroxide binding sites were saturated in the nickel-spiked sediment. Whereas the DGT technique showed comparable DGT-labile nickel flux between the nickel-spiked sediments and field-contaminated sediments at comparable AE-Ni concentrations. The use of the DGT technique was important for capturing measurements of the more labile fraction of nickel at the sediment-water interface inhabited by the amphipods, also considered relevant to other benthic organisms for their sediment-burrow water interface.

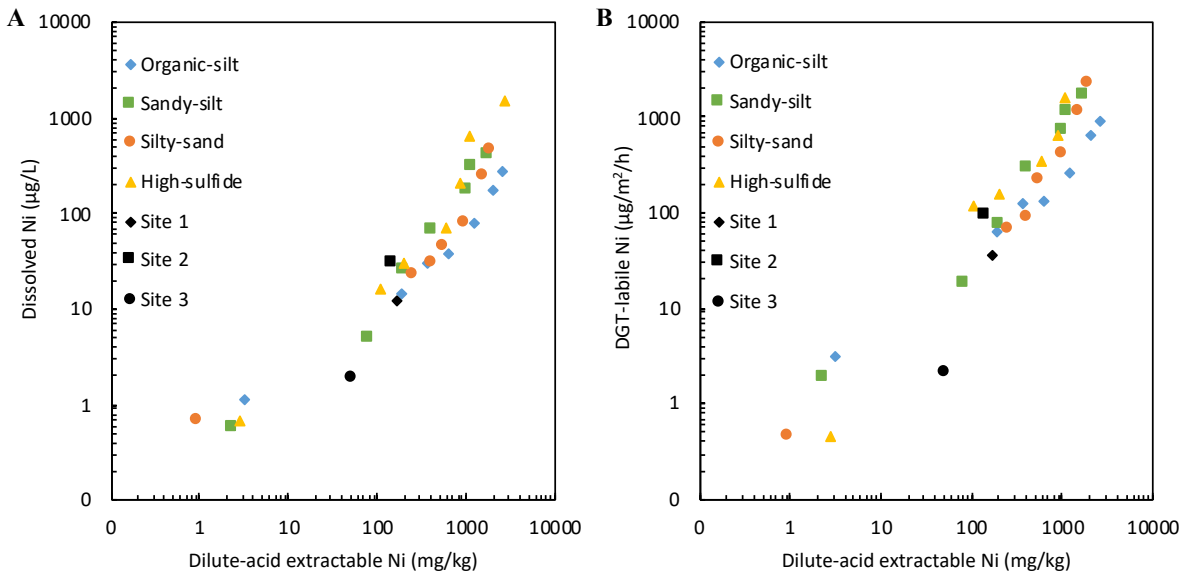


Figure 4.2 Relationship between dilute-acid extractable nickel concentration and A) dissolved nickel in the overlying water; and B) diffusive gradients in thin films (DGT)-labile nickel at the sediment-water interface. Data presented includes the combined dataset for the four nickel-spiked concentration series (Organic-silt, Sandy-silt, Silty-sand and High-sulfide) and three field-contaminated sediments (Sites 1, 2, and 3).

4.3.3 Amphipod reproduction bioassay, biological response

4.3.3.1 Quality assurance

Across all bioassays, physicochemical parameters of the overlying water remained within a narrow range throughout the tests and were within the physiological tolerance limits of the test organism (Appendix C1). In the controls, amphipod survival and reproduction test acceptability criteria (Table 4.3) were met for the water-only experiments and the sediment experiments except for the High-sulfide and the Site 3 sediment experiments (Table 4.4). Due to the toxicity tests with the High-sulfide and the Site 3 sediments not meeting quality assurance criteria, their results are presented in Appendix C2 but will not be discussed in detail.

Table 4.4 Survival and reproductive output of Melita plumulosa *for the control treatment following a 10-d test*

Experiment	**Control survival (%)***	**Control reproduction (no. of offspring per female) ***	**Test acceptability criteria**
Water only experiments			
Range finder	83 ± 0	8 ± 1	8-19 juveniles per gravid female[†] and ≥ 50% survival in the control[‡]
Repeat	58 ± 14	10 ± 4	
Sediment experiments			
Organic-silt (Site 1 and 2) [§]	94 ± 5	9 ± 1	8-19 juveniles per gravid female and ≥ 80% survival in the control[†]
Sandy-silt	89 ± 5	11 ± 1	
Silty-sand	92 ± 14	10 ± 1	
High-sulfide[‖]	78 ± 13	3 ± 1	
Organic-silt (Site 3) [§‖]	75 ± 29	7 ± 2	

* Mean ± standard error (n = 3)

[†] Spadaro and Simpson (2015)

[‡] A lower acceptable control survival in water-only experiments was due to increased competitive interactions for refuge resulting in increased mortality.

[§] The Organic-silt type sediment was used as the control for the field-contaminated sediments (Site 1, 2, and 3). Site 1 and Site 2 experiments were performed with the Organic-silt concentration series experiment while Site 3 was performed with the High-sulfide concentrations series.

[‖] Experiments where test acceptability criteria were not met.

4.3.3.2 *Sensitivity of* M. plumulosa *to dissolved nickel*

The sensitivity of *M. plumulosa* to water-only exposures of dissolved nickel was determined to understand the contribution of dissolved nickel released from the sediment to the toxicity observed in the sediment exposures (Figure 4.3). The TWA dissolved nickel EC10 and EC50 in the water-only test were calculated to be 42 (0–110) µg/L and 370 (150–590) µg/L (95% confidence interval), respectively. As a result, the TWA concentration of dissolved nickel in the overlying water of some of the higher concentration nickel-spiked sediment treatments exceeded the EC10 and EC50 values determined from the water-only dissolved nickel exposure, indicating that dissolved nickel may also contribute to the observed toxicity in the higher concentration nickel-spiked sediments. However, greater toxicity was observed for the nickel-spiked sediments than could be attributed to dissolved nickel exposure alone. The EC50 values based on dissolved nickel in the overlying waters of the nickel-spiked Organic-silt, Sandy-silt and Silty-sand sediments were 81 (65–97) µg/L, 75 (55–94) µg/L, and 47 (38–55) µg/L, respectively, which were significantly lower than the water-only EC50 value (Table 4.5). As a consequence of the presence of elevated concentrations of dissolved nickel in the overlying water of the sediment exposures, the results of the current study are likely to represent conservative estimates of toxicity, as nickel released to the overlying water in the marine environment would undergo substantial and rapid dilution.

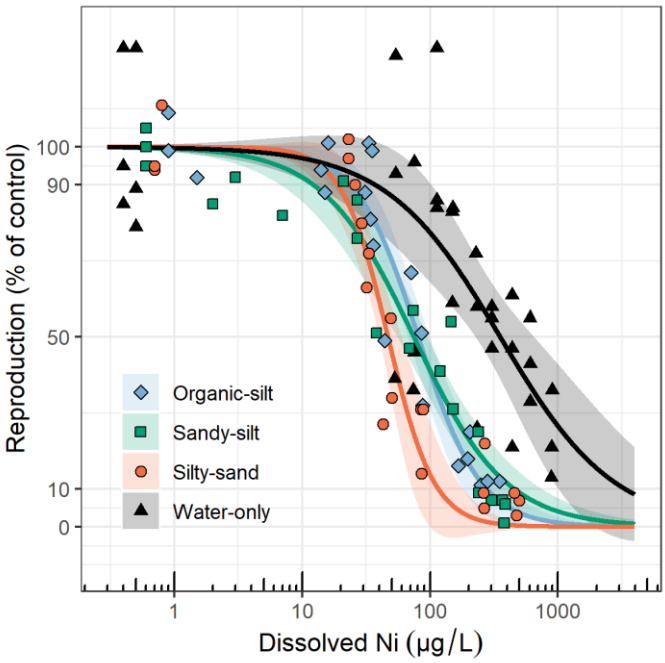


Figure 4.3 Relationships between amphipod reproduction and the time-weighted averaged dissolved nickel concentrations of the overlying waters. Data presented are for nickel-spiked sediment concentrations series with three sediment types (Organic-silt, Sandy-silt, and Silty-sand; single experiment per sediment type) and a dissolved nickel concentration series (water-only; data pooled from duplicate experiments). Each point represents one replicate. The shaded ribbons show the 95% confidence intervals.

Table 4.5 Toxicity of nickel to the reproductive output of Melita plumulosa *following a 10-d exposure to nickel in three nickel-spiked sediments*

Toxicity estimate*	Sediment treatment		
	Organic-silt	**Sandy-silt**	**Silty-sand**
Dissolved Ni in the overlying water (µg/L)			
EC10	24 (15 – 32)	12 (4 – 20)	19 (12 – 26)
EC50	81 (65 – 97)	75 (55 – 94)	47 (38 – 55)
Total recoverable Ni (mg/kg)			
EC10	690 (420 – 950)	280 (170 – 380)	420 (310 – 520)
EC50	2,000 (1,600 – 2,300)	1,000 (850 – 1,200)	1,100 (930 – 1,200)
Dilute-acid extractable Ni (mg/kg)			
EC10	380 (250 – 520)	110 (52 – 180)	220 (140 – 300)
EC50	1,100 (940 – 1,300)	510 (390 – 620)	570 (490 – 650)
DGT-labile Ni flux at the sediment-water interface (µg Ni/m^2/h)			
EC10	87 (51 – 120)	37 (3.6 – 70)	34 (9.7 – 59)
EC50	280 (220 – 340)	300 (200 – 400)	190 (130 – 250)

DGT = diffusive gradients in thin films.

* EC10/EC50 = the concentration or DGT-labile flux of nickel to cause a 10% or 50% effect respectively, on reproduction of *M. plumulosa* (95% confidence interval shown in parentheses).

4.3.3.3 Toxicity to M. plumulosa *based on AE-Ni and TR-Ni measured in sediments*

The relationships between amphipod reproduction and the different chemical measures of nickel exposure and potential bioavailability for the three nickel-spiked sediment types are shown in Figure 4.4. All three sediments spiked with nickel reduced amphipod reproduction relative to the control, regardless of the different sediment characteristics and the measurements of nickel bioavailability used. Measurement of TR-Ni and AE-Ni resulted in similar concentration-response relationships as shown by the overlapping 95% confidence limits of the model fit, with the Organic-silt sediment exhibiting less toxicity than the Sandy-silt and Silty-sand sediments at comparable TR-Ni and AE-Ni concentrations. The EC50 values based on TR-Ni concentrations were 2,000 (1,600-2,300), 1,000 (850-1,200), and 1,100 (930-1,200) mg/kg, for the Organic-silt, Sandy-silt, and Silty-sand concentrations series, respectively, while EC50 values based on AE-Ni concentrations were 1,100 (940-1,300), 510 (390-620), and 570 (490-650) mg/kg, respectively (Table 4.5). The large differences in the EC10 and EC50 values between the Organic-silt sediment and the other two sediments based on sediment nickel concentrations indicate that nickel bioavailability is being influenced (reduced) by the higher TOC, clay content and percentage of <63 µm particles of the Organic-silt sediment which all enhance the nickel binding capacity of this sediment.

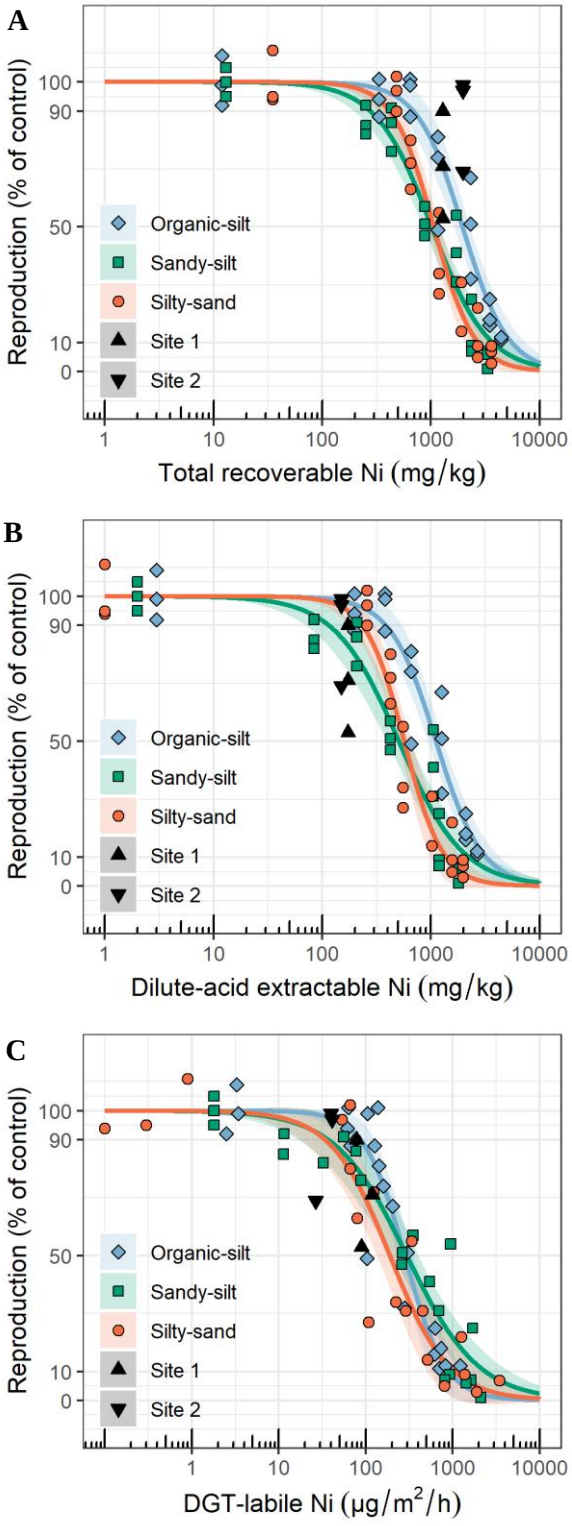


Figure 4.4 Relationships between amphipod reproduction and different chemical measures of nickel bioavailability following a 10-d exposure: A) total recoverable nickel; B) dilute-acid extractable nickel; and C) DGT-labile nickel flux at the sediment-water interface. Data are presented for five sediments including three nickel-spiked concentration series (Organic-silt, Sandy-silt, and Silty-sand) and two field-contaminated sediments (Sites 1 and 2). Each point represents one replicate. The shaded ribbons show the 95% confidence intervals.

Due to the bioassay with the High-sulfide sediment treatment not meeting test acceptability criteria, the current study is unable to add definitive information about the influence of AVS on nickel toxicity in marine sediments. The results however suggest marked effects on 10-d *M. plumulosa* survival at AE-Ni concentrations above 210 mg/kg (Appendix C2), perhaps due to the low iron (oxyhydroxides) in this sediment or to oxidation of sulfide at the sediment-water interface. Besser et al. (2013) investigated the toxicity of nickel in eight different sediment types to eight species of freshwater invertebrates and found that the most important factor mitigating chronic nickel toxicity was AVS concentration. However, the mitigating effects of AVS were not consistent among the species, with AVS having less of a protective effect on species exhibiting burrowing behaviour and subsurface feeding leading to greater oxidation of AVS and nickel bioavailability. In previous studies, it has been observed that AVS lowers the bioavailability of copper and its toxicity to the survival of juvenile *M. plumulosa*, but AVS oxidation that occurs during tests increases the release and toxicity of copper to the benthic amphipod (Simpson et al. 2012). Chandler et al. (2014) similarly found that a bioturbating amphipod provided greater oxidation of AVS leading to greater nickel bioavailability in marine sediments over a less bioturbative copepod.

In the field-contaminated sediments, amphipod reproduction decreased to 71 ± 11% (mean ± standard error, n = 3), and 88 ± 10% of that in the control for Site 1 and Site 2 sediments, respectively (Table 4.6). This indicates significant toxicity was caused by the Site 1 sediment only (reproduction reduced to <80% of the control). In these sediments, dissolved nickel in the overlying water was 13 μg/L and 30 μg/L for Site 1 and Site 2 sediments, respectively which is below (but within the confidence interval of) the 10-d EC10 value of 42 (<1–110) μg/L for dissolved nickel determined in the water-only experiment. Therefore, most of the observed toxicity was associated with the sediment nickel rather than the overlying water, as the amphipod ingests a considerable amount of sediment while feeding and this has been previously observed to contribute to metal toxicity (Campana et al., 2012). The AE-Ni was 170 and 150 mg/kg for Site 1 and Site 2 sediments, respectively, which is within the range of the EC10 values found for the three nickel-spiked sediments based on dilute acid-extractable nickel of 110 to 380 mg/kg.

TR-Ni overestimated nickel ecotoxicity of the field-contaminated sediments (Figure 4.4A). AE-Ni provided a closer estimate of ecotoxicity with the field-contaminated sediments overlapping with the response of some of the nickel-spiked sediments (Figure 4.4B). These results support the usefulness of the AE-Metal concentration over the TR-Metal concentration as a screening tool in sediment quality assessments. Based on both these conventional measurements of potential nickel bioavailability, differences in toxicity are predicted based on sediment type.

Greater toxicity was observed in the Site 1 sediment compared to the Site 2 sediment and is most likely reflective of the Site 1 sediment having almost double the amount of potentially bioavailable nickel

present (the proportion of AE-Ni compared to TR-Ni were 13 and 7% for Sites 1 and 2, respectively). This is most likely due to the residence times of nickel in these field-collected sediments with Site 1 sediment being collected adjacent to a newly established and active nickel mine, whereas Site 2 sediments were collected adjacent to a historical nickel mine site with no active mining. This provides further support to the body of evidence that demonstrates that total contaminant concentrations are poor predictors for the risk posed by contaminants in sediments (Simpson and Batley, 2007), particularly when a large portion of the total metals are present in highly mineralised forms (Simpson and Spadaro, 2016).

Table 4.6 Toxicity of nickel to the reproductive output of Melita plumulosa *following a 10-d exposure to nickel in two field-contaminated sediments*

Sediment	Reproduction (% Control)*†	Reproduction (no. offspring per female)*	Total recoverable Ni (mg/kg)	Dilute-acid extractable Ni (mg/kg)	DGT-labile Ni flux (μg Ni/m^2/h)	Dissolved Ni in overlying water (μg/L)
Site 1	71 ± 11	7 ± 2	1,300	170	96	13
Site 2	88 ± 10	8 ± 2	2,000	150	36	30

DGT = diffusive gradients in thin films.

* Mean ± standard error (n = 3).

† The Organic-silt sediment, no added nickel, was used as the control sediment.

4.3.3.4 DGT-labile nickel flux and toxicity to M. plumulosa

In contrast to the conventional measurements of potential nickel bioavailability, plotting the amphipod response against the DGT-labile flux of nickel resulted in similar, overlapping concentration-response relationships and 95% confidence limits for all three nickel-spiked sediments despite large variations in sediment properties such as particle size and TOC (Figure 4.4C). The EC50 values calculated for *M. plumulosa* in the Organic-silt, Sandy-silt, and Silty-sand concentration series based on DGT-labile nickel were 280 (220-340), 300 (200-400), and 190 (130-250) μg Ni/m^2/h, respectively (Table 4.5). The strong relationship between amphipod response and the DGT-labile nickel flux across all nickel-spiked sediments suggests that the toxicity of nickel to *M. plumulosa* is largely due to the labile nickel released from the sediment to the pore water and overlying water which is well characterised by the DGT-labile flux of nickel measured at the sediment-water interface, which is the zone they inhabit as epibenthic amphipods. Based on the combined dataset of the three nickel-spiked sediments, a DGT-labile nickel 10% effect concentration threshold of 50 (30–69) μg Ni/m^2/h was determined (Figure 4.5).

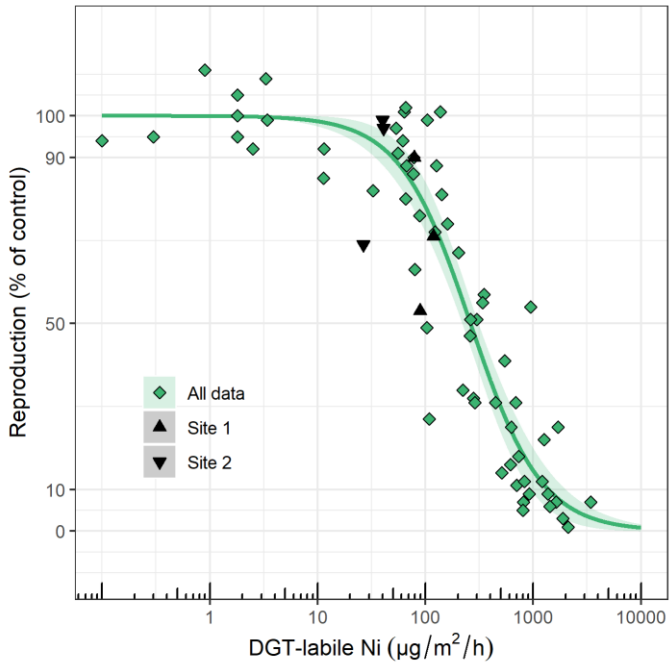


Figure 4.5 Relationships between amphipod reproduction and DGT-labile nickel flux at the sediment-water interface. Data presented includes the combined dataset for the three nickel-spiked concentration series (Organic-silt, Sandy-silt, and Silty-sand) and two field-contaminated sediments (Sites 1 and 2). Each point represents one replicate. The shaded ribbons show the 95% confidence intervals.

The response of the amphipods exposed to nickel in the field-contaminated sediments from Sites 1 and 2 strongly aligned with the response of the amphipods to the nickel-spiked sediments when expressed as the DGT-labile flux of nickel (Figure 4.4C). A 29 and 12% reduction in reproduction to *M. plumulosa* was observed for the Site 1 and Site 2 field-contaminated sediments, respectively. These two sediments had a DGT-labile nickel flux of 96 and 36 µg Ni/m^2/h, respectively. The DGT-labile nickel flux corresponding to a 10% impairment to reproduction from the nickel-spiked sediments was greater than or equal to 34 µg Ni/m^2/h (EC10 value for the Silty-sand treatment). This highlights the importance of the role of pore water nickel in the exposure pathway for this epibenthic amphipod that only the DGT measurement of nickel exposure and bioavailability was able to reveal.

This finding demonstrated that the DGT-labile flux of nickel measured at the sediment-water interface can effectively predict nickel bioavailability and therefore the toxic response of *M. plumulosa* to nickel-contaminated sediment despite the different sediment characteristics. The current study adds to the growing body of evidence demonstrating the usefulness of DGT-labile metal fluxes for predicting metal bioavailability and toxicity and that it can be a complementary approach to conventional metal bioavailability measurements in sediments (Simpson et al. 2012; Amato et al. 2014; Yin et al. 2014; Amato et al. 2015; Cleveland et al. 2017; He et al. 2018).

Many amphipod species ingest sediment particles and are therefore directly exposed to sediment-bound contaminants along with dissolved contaminants in pore waters and overlying water (Spadaro and Simpson, 2015). The importance of both dissolved and particulate copper from dietary exposure in contributing to toxicity in *M. plumulosa* has been demonstrated (Campana et al., 2012; Strom et al., 2011). Although the contribution of dietary nickel was not explicitly measured in the current study, the labile nickel flux measured by DGT may be indicative of a dietary contribution as it may include the labile fraction of the nickel that may have dissociated from ingestible particulate matter. Studies have demonstrated that the combination of sampling pore water metals, overlying water metals, and the labile metal species that desorb from solid phases during the DGT probe deployment provides a useful measure of the bioavailable metals potentially encountered by an organism across multiple exposure routes (Amato et al., 2015, 2014). The strong relationship between DGT-labile nickel and toxicity to *M. plumulosa* in the current study implies that there are multiple pathways of labile nickel exposure through pore water, overlying water and desorption from ingested particles that have been captured through the use of DGT that were not as evident when using AE-Ni or TR-Ni measurements alone.

In contrast to the results of the present study, Costello et al. (2012) investigated the use of the DGT technique for predicting nickel toxicity in nickel-spiked freshwater sediments and found that AVS-SEM measurements, when normalised for organic carbon, explained nickel toxicity to benthic macroinvertebrates better than DGT-labile nickel concentrations. The authors found that the DGT measurements overestimated nickel bioavailability and hypothesised that DGT induced the mobilisation of a solid-phase fraction of nickel that was not available to the invertebrates (Costello et al., 2012). However, other researchers have suggested that the discrepancy between the SEM-AVS and DGT results of Costello et al. (2012) may be more likely explained by the insertion of the DGT probe resulting in increased solubility of nickel in some sediments due to the perturbation and the resulting release of nickel from sulfide phases via oxidative release (Davison, 2016).

Rectangular flat DGT probes are most commonly used for soils and sediments as they enable sectioning and measurement of pore water concentrations at various depth profiles, whereas the circular DGT pistons are most commonly used for waters and soils. The current study has shown the usefulness of deploying the DGT pistons vertically into the sediment and aligned with the sediment-water interface for determining metal bioavailability. Vertical alignment is thus recommended over placing the DGT pistons horizontally face down onto the sediment surface which has been found to provide a poor relationship between metal bioavailability and toxicity observed (Zhang et al., 2019). This finding has the potential to reduce the cost of sediment assessments with the DGT technique if capturing metal flux information at the sediment-water interface using a piston (which is significantly cheaper than the probes) is sufficient for the purpose of the investigation.

4.3.4 Assessing impacts from nickel laterite mining on estuarine and marine benthic communities

Other studies that have focused on areas impacted by nickel laterite mining have predominantly been biomonitoring studies which provided a useful line of evidence to indicate that organisms have been exposed to bioavailable contaminants (e.g. Hédouin et al. 2008; Hédouin et al. 2011). Comparison of bioaccumulation concentrations in field-sampled or deployed, caged organisms collected from different sites can be used to identify areas where contaminants have greater bioavailability. This can assist decision making around site prioritisation for where more detailed assessment may be warranted to determine if contaminant concentrations may have adverse effects on sediment ecosystem health.

DGT-labile metal flux sampling offers a similar line of evidence to biomonitoring and has value in being able to identify locations with greater bioavailable metals and show how concentrations of these metals may change with time. However, unlike biomonitoring, DGT sampling is not subject to the availability of organisms and factors that influence metal uptake and depuration and has analytical quality control advantages. Additionally, further work in this area could lead to the derivation of DGT-based labile-metal flux threshold values that may be measured *in situ* during site-specific routine sediment quality assessments and used as a surrogate to ecotoxicological testing for prediction of effects from sediment exposure to benthic organisms. This is particularly relevant to the nickel laterite mining occurring in the SEAM region where there is a lack of standardised toxicity test protocols with local species and hence no sediment toxicity data available with which to derive sediment quality guideline values (Gissi et al., 2016).

4.4 Conclusions

The current study found that the sediment characteristics that had the greatest influence on reducing nickel bioavailability to *M. plumulosa* were TOC, clay content, and the proportion of <63 µm particle size. However, the current study was unable to add definitive information about the influence of AVS on nickel toxicity in estuarine and marine sediments, which should be investigated in the future. The DGT-labile nickel flux provided the most consistent chemical measurement of nickel ecotoxicity in the spiked sediments and demonstrated its ability to successfully predict toxicity in a limited number of nickel-rich field-collected sediments despite wide variations in nickel concentrations and sediment properties. The strength of the DGT measurement comes from its ability to capture the flux of nickel from the sediment to the pore water and overlying water at the sediment-water interface which is the zone inhabited by the amphipod *M. plumulosa*. The current study found that *M. plumulosa* was most likely responding to multiple pathways of labile nickel exposure via pore water, overlying water, and ingested sediment. The application of DGT resulted in the integration of the biological response to labile nickel across a range of sediment types. Based on the combined dataset of the three nickel-spiked

sediments a DGT-labile nickel 10% effect concentration threshold of 50 (30-69) µg $Ni/m^2/h$ was determined. The authors, therefore, consider that the DGT technique can be used to complement conventional chemical measurements of nickel bioavailability in estuarine and marine sediments. The DGT technique could be a particularly useful tool for industry and environmental regulators especially in regions where nickel laterite mining is occurring and there are no laboratory-based toxicity test methods available or ecotoxicological effects data to be able to assess potential effects.

Chapter 5

5 Benthic eukaryote and prokaryote community composition changes along a tropical sediment nickel gradient

This chapter has been redrafted from the manuscript: Gillmore, M.L., Golding, L.A., Chariton, A.A., Stauber, J.L., Stephenson, S., Gissi, F., Greenfield, P., Juillot, F., and Jolley, D.F. 2021. Metabarcoding reveals changes in benthic eukaryote and prokaryote community composition along a tropical marine sediment nickel gradient. *Environmental Toxicology and Chemistry*. https://doi.org/10.1002/etc.5039

Co-authors L. Golding, F. Juillot and I carried out the field component of the study. I performed the laboratory component of the study and the statistical analyses with technical guidance from A. Chariton, S. Stephenson, F. Gissi, and P. Greenfield. I wrote the original draft of the chapter/manuscript. All authors contributed to the experimental design and refining the manuscript before submission.

5.1 Introduction

Nickel mining is the main contributor to New Caledonia's economy and will remain so as the global demand for nickel continues to grow. Nickel products accounted for approximately 90% of the value of exports from New Caledonia, providing US$1.3 billion to the New Caledonian economy in 2017 (OEC, 2019). New Caledonia owes its extensive nickel deposits to the intensive weathering of ultramafic rocks under humid tropical conditions, which have resulted in the formation of lateritic regoliths (Chevillotte et al., 2006; Quesnel et al., 2017). These geological formations cover approximately 40% of the main island (Quesnel et al., 2017) and are enriched in transitional metals, mainly nickel, but also chromium, cobalt, iron, and manganese (Ambatsian et al., 1997; Dublet et al., 2012; Fandeur et al., 2009). Similar deposits exist throughout much of SEAM, encompassing three of the world's primary nickel producing countries: Indonesia (25% of global production in 2018), New Caledonia (9%), and the Philippines (14%) (USGS, 2020). In New Caledonia elevated total nickel concentrations, up to 7700 mg/kg, have been reported from coastal sediments adjacent to nickel mining and processing facilities (Fernandez et al., 2006; Merrot et al., 2019; Noël et al., 2015). This is significantly greater than typical coastal sediments which rarely have nickel concentrations above 100 mg/kg (Lewis et al., 2011).

New Caledonia's unique coastal ecosystem is recognised as a biodiversity hotspot with high endemism (Caesar et al., 2017). Mining in these environments may threaten these ecosystems from increased exposure to nickel if not appropriately managed. A review by Gissi et al. (2016) identified that there were no high-quality chronic nickel toxicity data for estuarine waters, estuarine sediments, or marine sediments. This hampers efforts to evaluate the ecological risk nickel exposure may pose to tropical ecosystems. Recent work has generated chronic nickel toxicity data for important classes of organisms

but is limited to the effect of dissolved nickel exposure to pelagic species (Gissi et al., 2018, 2017). Studies are needed to address the data gaps, such as the risk that nickel-rich sediments pose to tropical benthic marine organisms. However, at present, there are no standardised sediment toxicity tests for benthic tropical marine biota relevant to the SEAM region available for generating such data (Gissi et al., 2016).

Metabarcoding studies have proved to be extremely useful in assessing the effects of anthropogenic disturbances in aquatic ecosystems (Chariton et al., 2015b). Previous studies have, for example, demonstrated changes in benthic microbial communities along concentration gradients of metals, such as copper (Sutcliffe et al., 2018; Yang et al., 2018), uranium (Sutcliffe et al., 2017), and co-occurring chromium, manganese, and zinc (Yin et al., 2015). To the authors' knowledge, there are no gradient studies that have investigated the effect of a nickel concentration gradient related to lateritic nickel mining on benthic marine communities using DNA-based methods. A related study by Bordez et al. (2016) found for a terrestrial setting that changes in microbial community composition across an ultramafic ecosystem (Koniambo massif, New Caledonia) with a nickel, chromium, and cobalt concentration gradient were more likely associated with above-ground vegetation cover rather than the soil metal concentrations.

To investigate the risk posed by nickel to tropical estuarine and marine benthic communities, the current study determined the major environmental drivers of benthic community composition along a sediment nickel concentration gradient offshore from a large lateritic nickel deposit in New Caledonia (Vavouto Bay). The objectives of the study were to: (1) examine the influence of sediment nickel concentrations on benthic community composition; (2) identify associations between other key environmental variables and benthic community composition; (3) determine if protective thresholds for benthic taxa along a nickel-rich sediment gradient can be derived; and (4) identify potential taxa indicative of environmental impact. Collectively, the current study seeks to understand the role of nickel in influencing benthic community composition in tropical estuarine and marine sediments.

5.2 Materials and methods

5.2.1 Study area and sampling design

The current study focuses on Vavouto Bay (GPS co-ordinates: 20°59'45"S, 164°40'43"E), located on the north-west coast of Grande Terre, New Caledonia. Vavouto Bay is characterised by a semi-arid (~750 mm of rain/year) tropical climate. This site was chosen because previous studies have found enrichment of the coastal sediments in metals (including Ni, Cr, Co, Fe, and Mn) from the deposition of terrigenous materials from the mining of the nearby ultramafic Koniambo regolith (Merrot et al., 2019; Noël et al., 2015, 2014).

Sediment sampling was carried out at ten sites in Vavouto Bay over three days in July 2017 (Figure 5.1). Site selection was based on proximity to riverine inputs supplying terrigenous materials to the Bay from the nearby nickel mine to provide a sediment nickel concentration gradient. To minimise confounding effects from differences in the physical properties of the sediment, a visual inspection of the sediment was performed at each potential site and those sediments with dissimilar organic matter content or grain size were not sampled.

Sampling was conducted at a defined water depth (0.7 ± 0.1 m, mean ± SD, n = 10) to reduce the bias of tidal effects on benthic community composition. At each site, five replicate sediment samples were collected from a boat using a petite-ponar grab sampler (Wildco, Florida, United States) with a sampling area of 231 cm^2. Between sites the grab sampler was rinsed with 10% bleach and seawater collected from the new site to prevent cross-contamination of DNA. Subsamples for DNA were collected from the surficial layer (top 2 cm) from the centre of each grab into a sterile 50-mL centrifuge tube (Greiner Bio-One) using a sterile plastic spoon (sterilised by soaking in a 4% bleach solution, then rinsing five times with high purity water and UV-light treated). Remaining surficial sediment from the same grab sample was collected into two 50-mL tubes and purged with nitrogen gas for sediment chemical analyses. All samples were placed on ice immediately and frozen within 6 h of collection (-20°C) and thawed just before DNA extraction or chemical analyses. A field blank for DNA contamination was also collected at each site.

Environmental variables including temperature, pH, salinity, specific conductivity, dissolved oxygen, and turbidity of overlying water were measured *in-situ* at all sites at the time of sampling using a YSI Professional Plus Series Meter (YSI Inc., Yellow Springs, United States) and a Global Water turbidity meter (WQ770, Xylem Inc., Texas, United States) calibrated according to the manufacturer's instructions.

Sediment characterisation was performed as per Section 2.3.3. and included the determination of a suit of TR-Metals and AE-Metals, AVS, TOC, TIC, particle size, and mineralogy.

Figure 5.1. Aerial photograph showing the ten sampling locations in Vavouto Bay, New Caledonia. Labelling represents the dilute-acid extractable nickel concentration ranked from low (Ni1) to high (Ni10).

5.2.2 DNA extraction, amplification, and sequencing

Genomic DNA was extracted from a 10 g (wet weight) subsample of homogenised sediment using the DNeasy PowerMax Soil Kit (Qiagen, Germany) according to the manufacturer's instructions, with the following modifications: the incubations with the C2 and C3 buffers were extended to 30 min and an additional wash with buffer C5 was performed. The extracted DNA concentration and purity were quantified using the Nanodrop 2000 spectrophotometer (Thermo Scientific, United States) at 260 nm, and determined to be sufficient (sediment samples had 13-41 ng/µL DNA and a 260/280 ratio of 1.8-2.1, n = 50).

The genomic DNA was then amplified using polymerase chain reaction (PCR). Three different sets of primers were used to target different components of the benthic community composition. This approach was chosen to provide broad taxonomic coverage for eukaryotes and prokaryotes and more detailed taxonomic information on diatoms (microscopic unicellular eukaryotes with the defining feature of a cell wall made of silica). This latter group was specifically targeted as it contributes significantly to the primary productivity of benthic communities and responds directly and sensitively to changes in environmental conditions; hence diatoms have been found to serve as a robust indicator of change in ecological assessments (Desrosiers et al., 2013; Stevenson et al., 2010; Zimmermann et al., 2011). The eukaryotic community was determined by PCR of the 18S V7 rDNA gene, performed using the

universal primers All18SF (5'-3': TGGTGCATGGCCGTTCTTAGT) and All18SR (5'-3': CATCTAAGGGCATCACAGACC) (Hardy et al., 2010). The eukaryote community was further discriminated using diatom-specific primers by PCR of the SSU (18S V4) rDNA gene F-GCGGTAATTCCAGCTCCAATAG and R – CTCTGACAATGGAATACGAATA (Zimmermann et al., 2011). The prokaryotic community was determined by PCR of the 16S V4 rDNA gene, performed using 515f (5′-3': GTGYCAGCMGCCGCGGTAA) (Baker et al., 2003; Quince et al., 2011) and 806r (5′-3': GGACTACNVGGGTWTCTAAT) (Apprill et al., 2015).

For all PCRs, amplification was performed in a total volume of 50 µL with 25 µL of AmpliTaq® Gold 360 Master Mix (Applied Biosystems, United States), 18 to 21 µL Water (Molecular Biology Reagent, Sigma Aldrich, United States), 1 µL of 10 µM forward and reverse primers, and 2 to 5 µL of genomic DNA. A summary of the thermal cycling profiles for each primer set is provided in Appendix D1. The success of PCR amplification was confirmed using a microchip electrophoresis system (MultiNA, Shimadzu, Japan).

All PCRs were run with positive DNA controls: mussel (*Mytilus*) and polychaete (*Capitellidae*) DNA for eukaryotic 18S rDNA PCR, *Halothermothrix* for prokaryotic 16S rDNA PCR, and *Phaeodactylum* for the diatom specific PCR. All runs included a negative control of 2 to 5 µL of PCR grade water (Molecular Biology Reagent, Sigma Aldrich, United States).

PCR products were pooled into a single tube (separate tube for each set of primers) and purified using the QIAquick PCR purification kit (Qiagen). The concentration and purity of the purified PCR products were determined as reported above for DNA. Illumina MiSeq amplicon sequencing was performed by the Ramaciotti Centre for Genomics (UNSW, Australia). The three pooled samples of 18S rDNA, 16S rDNA, and diatom 18S v4 rDNA amplicons were prepared with the Illumina Tru-Seq PCR-free library preparation kit and sequenced over one 2x250 base-pair, paired-end Illumina MiSeq run. The raw sequence and filtered data sets are accessible via the CSIRO data portal at https://doi.org/10.25919/5da80af25e87c.

5.2.3 Bioinformatics

Sequenced data were processed using a custom amplicon clustering and classification pipeline (Greenfield Hybrid Analysis Pipeline, GHAP, V2.1) available at https://doi.org/10.4225/08/59f98560eba25. GHAP is built around tools from USEARCH (Edgar, 2013) and RDP (Cole et al., 2014), combined with locally written tools for demultiplexing and generating molecular operational taxonomic unit (MOTU) (Blaxter et al., 2005) tables complete with taxonomic classifications, species assignments, and their associated read counts for each sample.

In brief, GHAP first demultiplexes the sequence reads to produce a pair of files for each sample. The paired files are then trimmed, merged, de-replicated, and clustered at 97% similarity to generate MOTU sequences using the USearch tools (fastq_mergepairs, derep_fulllength and cluster_otus, (Edgar 2013)).

The 18S rDNA MOTU sequences were classified in two ways: first by using RDP classifier V2.10.2 (Cole et al. 2014) to determine a taxonomic classification for each sequence down to the level of genus where possible; and second by ublast to match a representative sequence from each MOTU against a curated set of 18S reference sequences derived from the SILVA V123 SSU reference set (Quast et al. 2013). This 18S rDNA reference set was built by taking all the eukaryote sequences from the SILVA V128 SSU dataset, and removing those sequences containing bacterial or chloroplast regions, as well as those with inconsistencies in their taxonomic lineages. A full description of this curation is provided in the GHAP documentation. The pipeline then used usearch_global to map the merged reads from each sample back onto the MOTU sequences to obtain accurate read counts for each MOTU/sample pairing.

The 16S rDNA MOTU sequences were classified in two ways: first by using RDP classifier V2.10.2 (Cole et al. 2014) to determine a taxonomic classification for each sequence down to the level of genus where possible; and second by using usearch_global to match the representative sequence from each MOTU against a 16S rRNA reference set built by merging the curated sequences from the RDP 16S training set (release 16) and the RefSeq 16S rRNA set.

The classified MOTUs and the counts for each sample were finally used to generate MOTU tables in both text and BIOM (V1) file formats, complete with taxonomic classifications, species assignments, and counts for each sample.

After processing, and before statistical analyses, the data were filtered to remove potentially erroneous sequences based on the proportion of contamination in the positive controls. For all data sets, the proportion of contamination in the positive controls was determined (the max read count that is not the positive control divided by the positive control read count). The proportion of contamination was low in all data sets (0.02, 0.2, and 0.02% for the eukaryotes, diatoms, and prokaryotes, respectively) and these values were set as the cut-off for filtering the dataset. The proportion of read counts for each MOTU in each sample was determined (the read count for each MOTU divided by the total read count for that sample). If the proportion of read counts for each MOTU per sample was less than the proportion of contamination then those reads were removed from the dataset. After quality control checks were complete, controls were removed from the dataset. Any MOTUs that appeared in less than two samples or had a match percent of <80 were removed. Appendix D2 shows the total number of reads and MOTUs before and after the data were filtered. The metagenomics visualisation tool Krona was used to generate taxonomic distribution plots (Ondov et al., 2011).

5.2.4 Statistical analyses

Changes in total MOTU richness, Pielou's evenness, and Shannon's diversity in the eukaryote, diatom, and prokaryote datasets between sites along the AE-Ni gradient were examined in NCSS V07.1.21 (Hintze, 2007). Analysis of variance (ANOVA) was used to determine significant differences between sites and linear regression to determine whether the relationship between site and species index had a slope significantly different from zero (i.e., a gradient in the biological response with increasing AE-Ni). The diversity metrics were obtained using Primer-E E7's Diverse function (Clarke and Gorley, 2015). Data sets were standardised beforehand to give the percentage of total abundance (over all species) that is accounted for by each species.

Patterns in sediment physicochemical properties and community composition were explored through multivariate analysis using Primer-E V7. Principal component analysis (PCA) was used to explore the spatial distribution of the system's measured abiotic variables, including confirming the presence of a particulate nickel gradient. Environmental variables were first explored with pairwise correlations (Draftsman Plot) to check for skewness and collinearity. Where variables were highly correlated ($r > 0.95$), a single variable was chosen as a representative for further analyses. In summary, sand (>63 μm) and AE-Ni were chosen to represent the correlated variables silt (4-63 μm) and AE-Fe, respectively. All metal concentration data were log-transformed, and all sediment variables were normalised (by subtracting the mean and dividing by the standard deviation) to allow comparison between variables with different units. All subsequent statistical analyses were performed using the AE-Metal concentrations (rather than the TR-Metal concentrations), which are more representative of bioavailable metals (Spadaro and Simpson, 2015). Before multivariate statistical analyses of the community composition data were undertaken, all species abundance data from the filtered datasets were Helligers transformed. The data were subsequently aggregated to family, genus, and species taxonomic level for the eukaryote, diatom, and prokaryote datasets, respectively.

Ordination of DNA compositional data was performed by non-metric multidimensional scaling (nMDS) using Bray-Curtis similarity matrices. For each gene, potential differences in the composition of biota between sampling locations were detected by permutational multivariate analysis of variance (PERMANOVA), with pairwise a posteriori tests based on 9999 random permutations used to identify similarities and differences among the treatment. Distance-based linear models (DistLM) (Legendre and Anderson, 1999) were used to examine the relationship between community composition and environmental predictor variables using a forward selection of selected environmental variables with the goodness of fit examined using the adjusted R^2 criterion. Distance-based redundancy analysis (dbRDA) was performed to visualise the influence of sediment predictor variables on community composition identified by the DistLM.

Threshold indicator taxa analysis (TITAN) was performed using the R package 'TITAN2' (King and Baker, 2010) to estimate how eukaryote, diatom, and prokaryote datasets responded along the most significant environmental gradient identified from the DistLM's sequential tests, which was the AE-Ni concentration. TITAN uses standardised z-scores to distinguish which MOTUs occurrences decreased (-z scores) or increased (+z scores) along the environmental gradient. Before running TITAN, all MOTUs which were observed in ≤5 samples or more than 95% of samples (i.e., >47 samples) were removed. Bootstrapping (999 replicates) was used to determine the purity and reliability of individual threshold indicator taxa to estimate the confidence limits of the change points. Only taxa that passed acceptability criteria (purity ≥0.95, and reliability ≥0.95, P<0.05, 999 permutations) are presented.

5.3 Results and discussion

5.3.1 Chemistry

The physicochemical properties of the overlying seawater were consistent across all sites (Table 5.1). In summary, temperatures were between 23 to 26°C (n = 10), pH 7.8 to 8.3, salinity 32 to 37 PSU, specific conductivity 49 to 64 mS/cm, dissolved oxygen 6.0 to 8.3 mg/L, and turbidity 6 to 29 (NTU).

Table 5.1. Physicochemical properties of the overlying seawater at each sampling site measured in situ (n = 1)

Site	Temperature (°C)	pH	Salinity (PSU)	Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	Turbidity (NTU)
Ni1	25	8.0	36	54	6.8	14
Ni2	25	8.3	37	64	8.3	6
Ni3	24	8.0	36	54	6.6	28
Ni4	25	7.8	37	57	6.7	9
Ni5	24	8.1	34	51	6.8	22
Ni6	23	8.1	35	53	6.0	18
Ni7	26	8.1	34	51	6.9	20
Ni8	23	7.9	34	51	6.5	20
Ni9	25	8.1	32	49	6.3	29
Ni10	26	8.1	33	50	7.1	18

There was a strong sediment nickel concentration gradient across the 10 sampling sites (Table 5.2, Figure 5.1). The highest nickel concentrations (120 and 1,500 mg/kg for AE-Ni and TR-Ni, respectively) were measured in the eastern-most portion of the Bay, close to the mouth of the Taleo-Coco River. The lowest nickel concentrations (24 and 250 mg/kg for AE-Ni and TR-Ni, respectively) were measured in the west, towards the open lagoon. The AE-Ni concentrations were 8 to 11% of the TR-Ni concentration, indicating that the nickel was mostly present in a mineralised form. In the absence of sediment quality guideline values for New Caledonia, or more broadly tropical-specific sediment quality guideline values, the measured AE-Metal concentrations have been compared to the Australian and New Zealand SQGs to indicate the relative ecological risk. The AE-Ni concentrations at all sites exceeded the DGV for nickel of 21 mg/kg (Australian and New Zealand Governments, 2018) by 1.1 to 6 fold.

The co-occurring metals chromium, cobalt, iron, and manganese were also elevated and showed a similar pattern of increase to the nickel gradient (Table 5.2). The AE-Cr and AE-Co concentrations were 4 to 19% and 18 to 35% of the TR-Cr and TR-Co concentrations, respectively, demonstrating that these trace metals were mostly present in a mineralised form. Although all TR-Cr concentrations exceeded the DGV for chromium of 80 mg/kg (Australian and New Zealand Governments, 2018), all AE-Cr concentrations were 0.2 to 0.4 times less. No DGV is available for cobalt for comparison due to the lack of an adequate toxicity dataset needed for its derivation. No DGV is available for iron or manganese due to their naturally high occurrence in sediments and well-recognised status as important metal-binding phases in oxic sediments. Although the TR-As concentrations for a few samples exceeded the DGV for arsenic of 20 mg/kg (Australian and New Zealand Governments, 2018), all AE-As concentrations were 0.1 to 0.3 times lower than the DGV. The TR-Metal and AE-Metal concentrations of the other trace metals (Cd, Cu, Pb, V, and Zn) were relatively low (≤60 mg/kg and ≤26 mg/kg, respectively) and were all below their respective DGVs (Table 5.2).

AVS concentrations were low (≤2 mmol/kg) in the surficial sediment across the nickel concentration gradient at all sites (Table 5.3). The physicochemical properties that varied the most across the nickel gradient were particle size, TOC, and TIC (Table 5.3). In summary, the proportion of particles classified as clay (<4 µm), silt (4-63 µm), and sand (>63 µm) ranged between 4 to 8%, 29 to 70%, and 23 to 67% (n = 50), respectively. TOC varied between 0.3 to 9%, and TIC between 0.1 to 8%.

Sites with the lowest nickel concentrations were separated from those with the highest nickel concentrations along the first PCA axis (Figure 5.2). This first PCA axis explained 61% of the variation across sites, and the top three variables this was related to were increasing AE-Zn and AE-Ni concentrations and decreasing particle size. The second PCA axis provided a small amount of additional separation, explaining 20% of the variation, and the top three variables this was related to were increasing AE-Mn and AE-Co concentrations and decreasing TOC.

Table 5.2. Sediment metal concentrations in Vavouto Bay, New Caledonia (n = 5)

Site	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Dilute-acid extractable metals* (mg/kg)												
Ni1	540	4	<1	4	18	1	3,300	100	**24**	2	7	6
Ni2	530	4	<1	7	16	1	4,200	150	**28**	2	8	6
Ni3	870	1	<1	11	14	1	4,700	85	**38**	2	4	7
Ni4	2,200	6	<1	6	15	3	6,500	74	**39**	4	26	9
Ni5	900	1	<1	14	15	3	8,200	150	**70**	3	10	8
Ni6	1,300	2	<1	15	24	5	9,500	140	**88**	5	23	15
Ni7	1,100	1	<1	20	18	4	8,200	160	**91**	3	9	11
Ni8	1,300	2	<1	27	26	3	10,000	250	**98**	3	12	11
Ni9	1,700	2	<1	19	31	5	11,000	190	**120**	5	23	16
Ni10	1,100	2	<1	20	23	4	11,000	200	**120**	4	14	11
Total recoverable metals† (mg/kg)												
Ni1	3,600	19	<1	14	**97**	2	15,000	160	**250**	2	21	15
Ni2	3,900	17	<1	24	**130**	3	20,000	230	**370**	3	18	20
Ni3	6,700	7	<1	33	**130**	6	23,000	230	**350**	3	19	35
Ni4	12,000	**29**	<1	28	**180**	12	29,000	180	**430**	5	46	35
Ni5	7,900	10	<1	57	**270**	11	38,000	350	**780**	3	30	48
Ni6	9,200	**31**	<1	75	**350**	16	54,000	300	**1,000**	10	42	48
Ni7	7,800	8	<1	62	**280**	12	40,000	350	**950**	6	29	49
Ni8	8,000	18	<1	77	**300**	9	43,000	430	**1,100**	5	32	46
Ni9	11,000	19	<1	83	**440**	19	57,000	400	**1,300**	9	53	60
Ni10	9,400	14	<1	110	**530**	14	60,000	450	**1,500**	7	40	55
Australian and New Zealand sediment quality guideline values‡ (mg/kg)												
DGV	-	20	1.5	-	80	65	-	-	21	50	-	200

* Dilute-acid extractable metals were determined by reacting with cold 1M HCl for 1 h.

† Total recoverable metals were determined by strong aqua-regia digestion at 80°C for 1.5 h.

‡ Australian and New Zealand sediment quality default guideline value (DGV) (Australian and New Zealand Governments, 2018). Values in **bold** show an exceedance of the DGV.

Table 5.3. Physicochemical properties of the sediment at each sampling site (mean ± standard deviation, n = 5)

Site	Moisture content (%)	AVS (mmol/kg)	Grain size (%) <4, 4–63, >63 μm	TOC (%)	TIC (%)
Ni1	43 ± 3	<0.5*	5, 47, 48	0.3 ± 0.4	8.1 ± 0.5
Ni2	49 ± 5	<0.5	5, 42, 54	1.5 ± 0.7	6.6 ± 0.7
Ni3	34 ± 1	<0.5	4, 29, 67	0.5 ± 0.1	0.3 ± 0.1
Ni4	71 ± 4	<0.5	6, 64, 30	9 ± 2	2.5 ± 0.4
Ni5	53 ± 2	0.9 ± 0.7	8, 59, 34	2.8 ± 0.4	0.4 ± 0.1
Ni6	71 ± 1	0.6 ± 0.2	5, 56, 39	9 ± 1	0.8 ± 0.3
Ni7	52 ± 2	<0.5	7, 70, 24	2.6 ± 0.1	0.1 ± 0.1
Ni8	54 ± 4	<0.5	7, 63, 30	2.3 ± 0.2	1.8 ± 0.2
Ni9	71 ± 1	0.7 ± 0.3	8, 69, 23	6.3 ± 0.4	0.6 ± 0.1
Ni10	64 ± 2	<0.5	6, 61, 32	6.2 ± 0.5	0.4 ± 0.1

AVS = Acid-volatile sulfide; TOC = Total organic carbon; TIC = Total inorganic carbon.

* Limit of detection = 0.5 mmol/kg

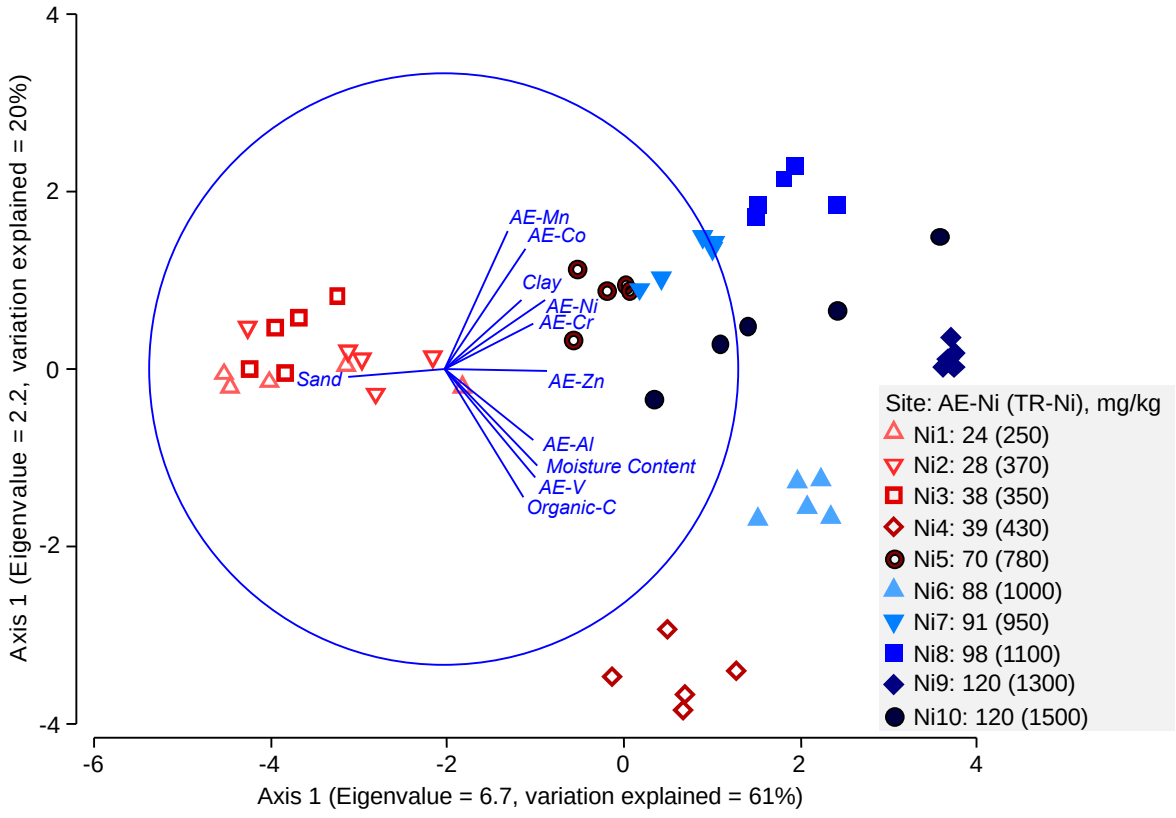


Figure 5.2. Principle components analysis of Vavouto Bay sampling sites. AE = dilute-acid extractable, TR = total recoverable, Sand = >63μm particle size, Clay = <4μm particle size.

X-ray powder diffraction patterns of the sediments collected along the nickel gradient indicated a similar composition of silicate, carbonate, sulfide, and halide species, but the ratios between each phase varied across the Bay (Figure 5.3). The ten sites could be grouped based on their similar mineralogical composition: sediments located outside of the Bay (Ni1, Ni2, and Ni4) had a higher proportion (40-70%) of carbonate minerals (e.g. aragonite and calcite group minerals), sediments located on the north side of the Bay (Ni3, Ni5, and Ni7) had a higher proportion (all approximately 90%) of silicate minerals (predominantly quartz and feldspar group minerals), and sediments located on the south and east sides of the Bay (Ni6, Ni8, Ni9, and Ni10) all had a large proportion (around 50%) of silicate minerals (predominantly quartz and feldspar group minerals) and (10-20%) carbonate minerals (e.g. aragonite and calcite group minerals).

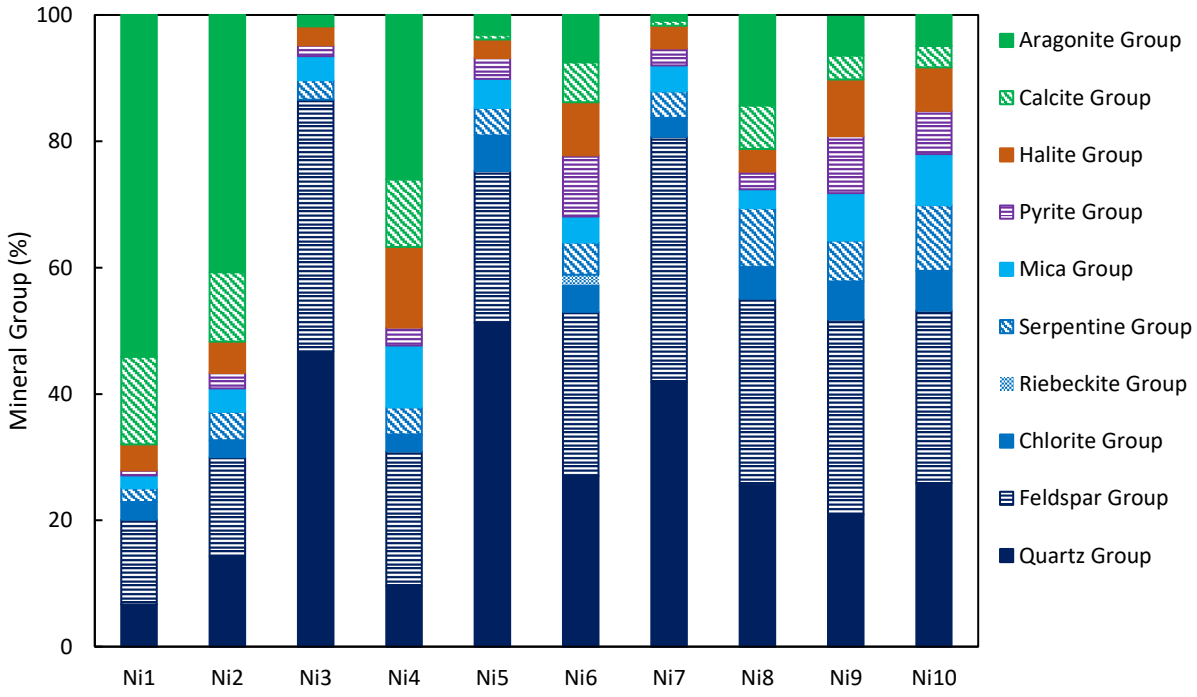


*Figure 5.3 Mineral groups from x-ray power diffraction (XRD) analyses of the 10 sampling sites. X axis labelling represents the dilute-acid extractable nickel concentration ranked from low (Ni1) to high (Ni10). Colour indicates the mineral chemistry: carbonate minerals (*green*), halide minerals (*orange*), sulfide minerals (*purple*), and silicate minerals (*blue*).*

5.3.2 Benthic community response along a sediment nickel concentration gradient

5.3.2.1 Eukaryotes

Eukaryotic 18S rDNA amplicon sequencing produced 2,573,808 reads and 1,149 MOTUs after the data were filtered (Appendix D2). MOTUs were present from 9 kingdoms, with most of the taxonomic richness of the samples coming from Metazoa (37%), followed by Stramenopiles (23%), Alveolata (14%), Rhizaria (12%), Fungi (4%), Protozoa (4%), Plantae (3%), Hacrobia (2%), and Excavata (<1%) (see Appendix D3 for the detailed taxonomic distribution to family level). Significant differences were observed in total MOTU richness (ANOVA: $F = 0.0023$, $P<0.05$), evenness (ANOVA: $F = 0.00085$, $P<0.05$), and diversity (ANOVA: $F = 0.0013$, $P<0.05$) with AE-Ni at each site, but there were no concentration-response relationships between increasing AE-Ni concentration and total MOTU richness (linear regression: $P = 0.59>0.05$, correlation coefficient = 0.088), evenness (linear regression: $P = 0.090>0.05$, correlation coefficient = -0.27), or diversity (linear regression: $P = 0.25>0.05$, correlation coefficient = -0.19) (Figure 5.4).

Ordination (nMDS) plots based on eukaryote community composition similarity showed strong grouping between the five replicates collected within a site and strong separation between sites (Figure 5.5A). There was a strong pattern of change in the eukaryotic community composition along the AE-Ni concentration gradient with sites with the lowest nickel concentrations on the opposite end of the x-axis compared to the sites with the highest nickel concentrations (Figure 5.5A). Significant differences in community composition between sites were confirmed by PERMANOVA (pseudo-F = 16.81, P = 0.001). *Post-hoc* pairwise comparisons of sampling sites revealed that all ten sites along the AE-Ni concentration gradient had a significantly different eukaryote community composition ($P<0.05$).

5.3.2.2 Diatoms

The targeted diatom amplicon sequencing produced 105,998 reads and 165 MOTUs after the data were filtered (Appendix D2). Diatoms predominately belonged to the class Bacillariophyceae (90%), with minor representation also from Mediophyceae (4%), Fragilariophyceae (3%), and Coscinodiscophyceae (2%) (see Appendix D3 for the detailed taxonomic distribution to genus level). There was a trend of gradual decline in the total MOTU richness (ANOVA: $F = 0.000$, $P<0.05$; linear regression: $P = 0.0001<0.05$, correlation coefficient = -0.62) and species diversity (ANOVA: $F = 0.000$, $P<0.05$; linear regression: $P = 0.0008<0.05$, correlation coefficient = -0.45) with increasing AE-Ni concentration (Figure 5.6A and C). Evenness differed with AE-Ni but there was no concentration-response relationship (ANOVA: $F = 0.000$, $P<0.05$; linear regression: $P = 0.097>0.05$, correlation coefficient = -0.23) (Figure 5.6B).

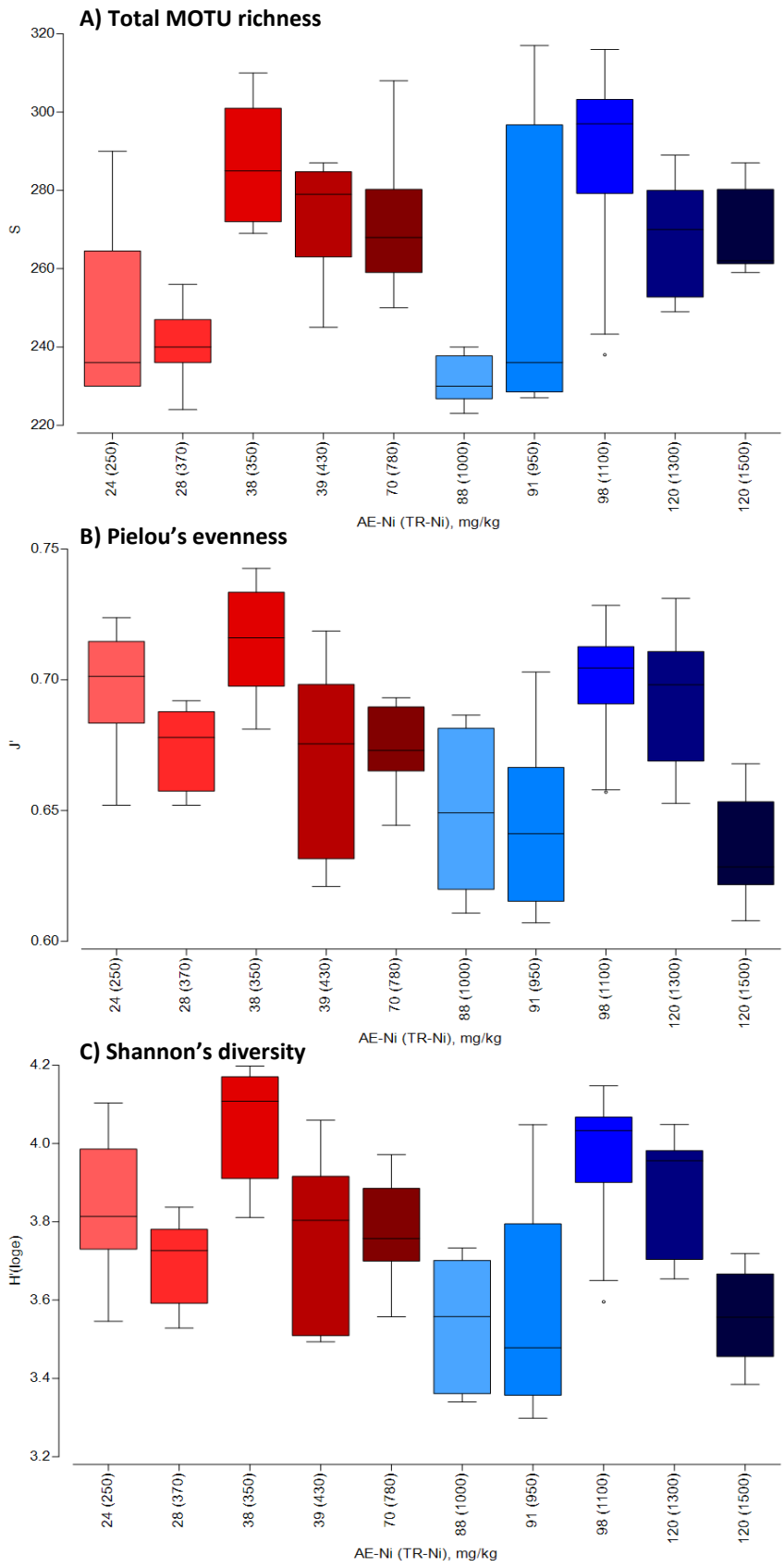


Figure 5.4. Univariate summary statistics for each site based on eukaryote 18S rDNA gene amplicon data: A) total MOTU richness; B) Pielou's evenness; and C) Shannon's diversity. Dataset standardised to 100 individuals before analyses.

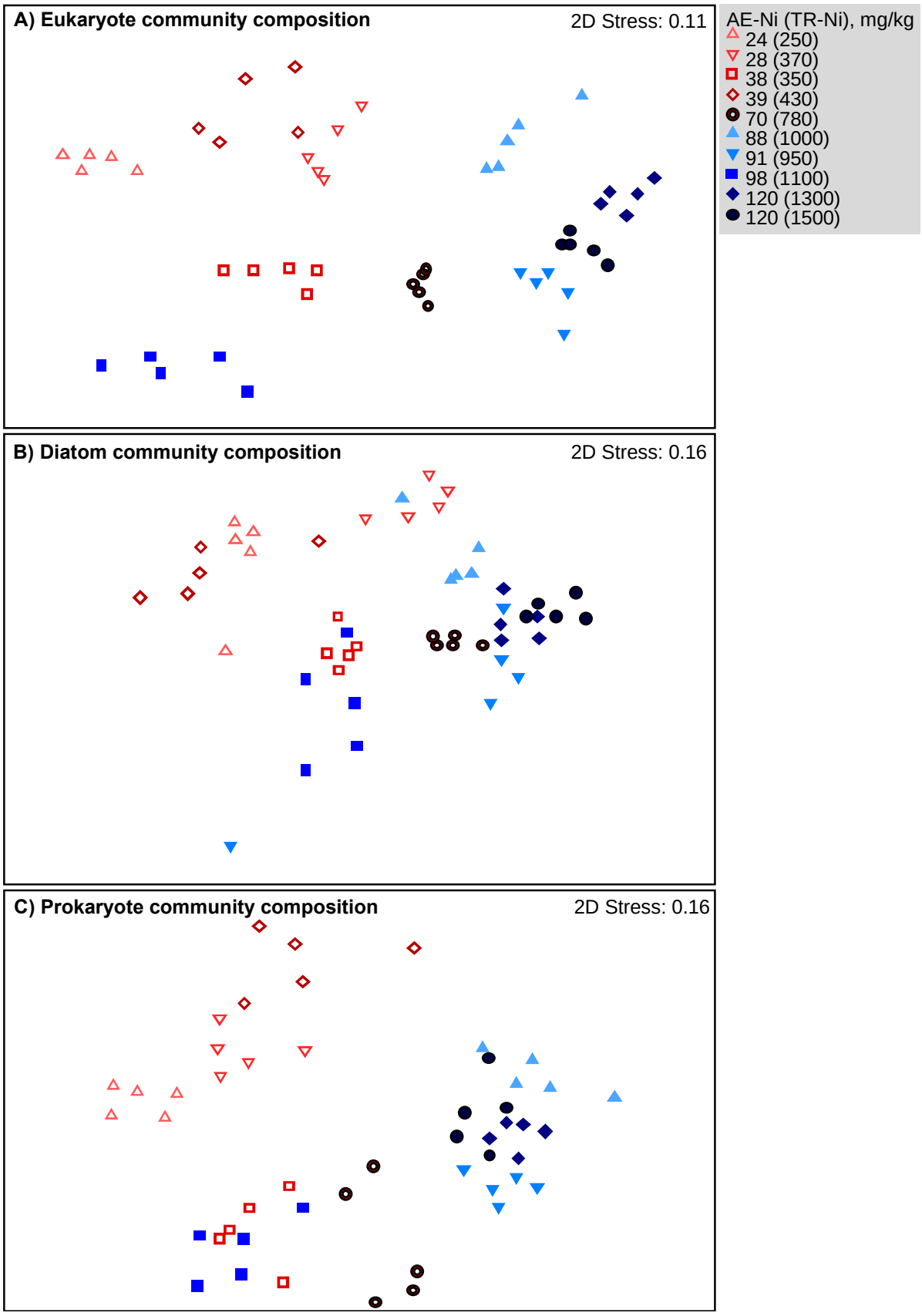


Figure 5.5. Non-metric multidimensional scaling (nMDS) plots illustrating the similarities and differences in benthic A) eukaryote, B) diatom, and C) prokaryote community compositions along a nickel concentration gradient in Vavouto Bay, New Caledonia. AE = Dilute-acid extractable and TR = Total recoverable.

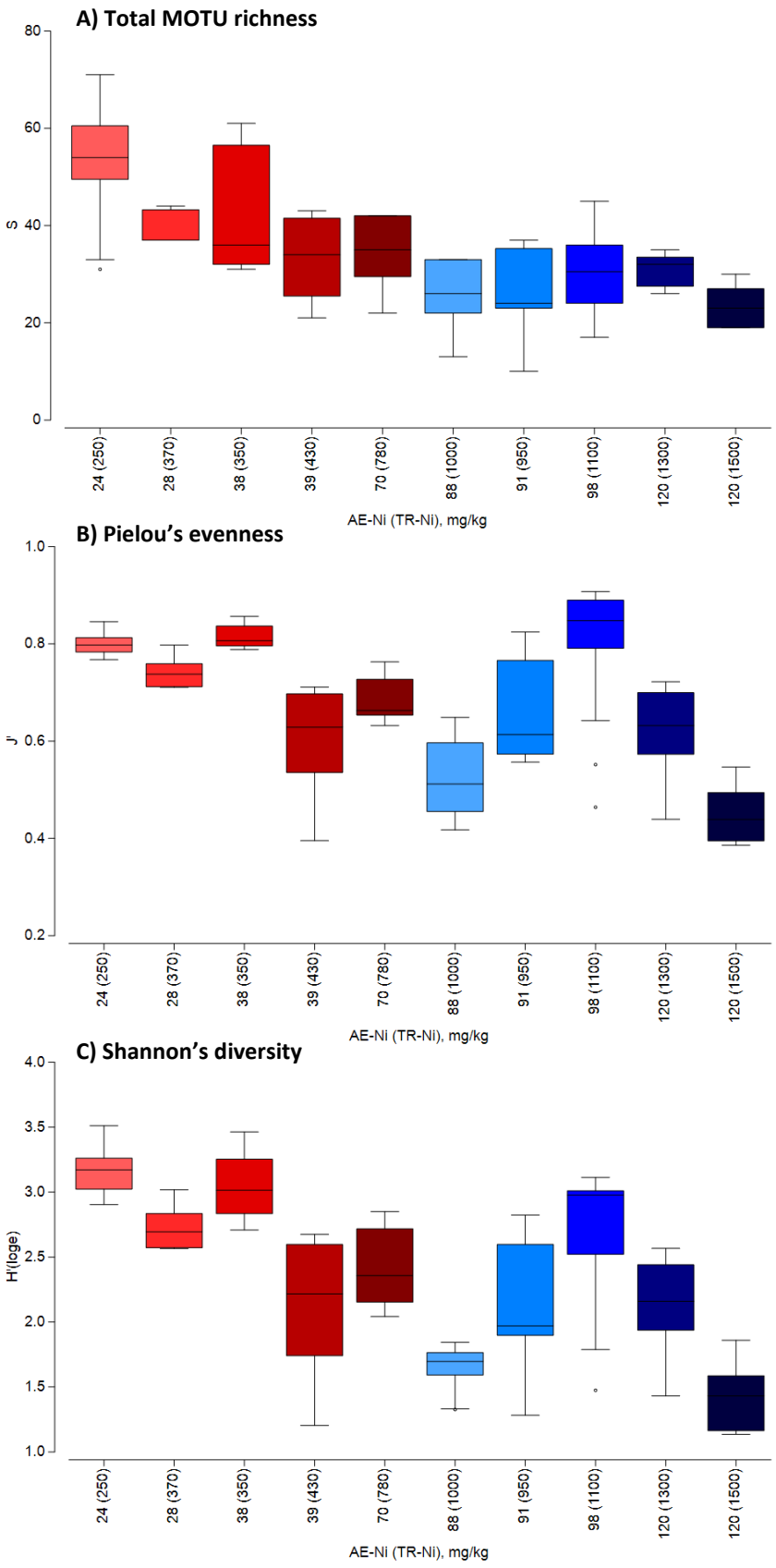


Figure 5.6. Univariate summary statistics for each site based on diatom 18S V4 rDNA gene amplicon data: A) total MOTU richness; B) Pielou's evenness; and C) Shannon's diversity. Dataset standardised to 100 individuals before analyses.

Ordination (nMDS) plots based on diatom community composition similarity showed less distinct grouping between replicates within sites than had been observed for the eukaryote community and greater overlap between different sites (Figure 5.5B). The pattern of change in the diatom community composition along the AE-Ni concentration gradient was similar to that observed for the eukaryote community, but not as defined (Figure 5.5B). Significant differences in community composition between sampling sites were confirmed by PERMANOVA (pseudo-F = 12.68, P = 0.001). *Post-hoc* pairwise comparisons of sampling sites identified that all ten sites had significantly different diatom community profiles ($P<0.05$).

5.3.2.3 Prokaryotes

Prokaryotic 16S amplicon sequencing produced 649,616 reads containing 2,602 MOTUs across all sediment samples after the data were filtered (Appendix D2). Representation was predominately from the bacterial domain (98%) with only minor representation from the archaeal domain (2%). MOTUs were present for 24 phyla groups (see Appendix D3 for the detailed taxonomic distribution to family level). The dominant phyla were Proteobacteria (47%); with Deltaproteobacteria, Gammaproteobacteria and Alphaproteobacteria, respectively, contributing most of the taxonomic richness across all samples. Other prevalent phyla included Bacteroidetes (13%), Acidobacteria (7%), Chloroflexi (7%), Planctomycetes (4%), Firmicutes (2%), Actinobacteria (2%), Verrucinucribia (2%), and Spirochaetes (1%). Of the bacterial taxa, 9% could not be classified beyond the domain. There were significant differences in 16S rDNA species evenness (ANOVA: $F = 0.000$, $P<0.05$) and diversity (ANOVA: $F = 0.000$, $P<0.05$) with AE-Ni but there were no concentration-response relationships between increasing AE-Ni concentration and total MOTU richness (linear regression: $P = 0.61>0.05$, correlation coefficient = -0.084), evenness (linear regression: $P = 0.35>0.05$, correlation coefficient = -0.15), or diversity (linear regression: $P = 0.41>0.05$, correlation coefficient = -0.13) (Figure 5.7).

Ordination (nMDS) plots based on prokaryote community composition similarity showed strong grouping between replicates collected within a site but some overlap between different sites (Figure 5.5C). The pattern of change in the prokaryote community composition along the AE-Ni concentration gradient was more significant than that observed for the diatom, but less marked than that observed for the eukaryote community (Figure 5.5C). Significant differences in community composition between sampling sites were confirmed by PERMANOVA (pseudo-F = 6.13, P = 0.001). *Post-hoc* pairwise comparisons of sampling sites revealed that all ten sites along the AE-Ni concentration gradient had significantly different prokaryote community composition ($P<0.05$).

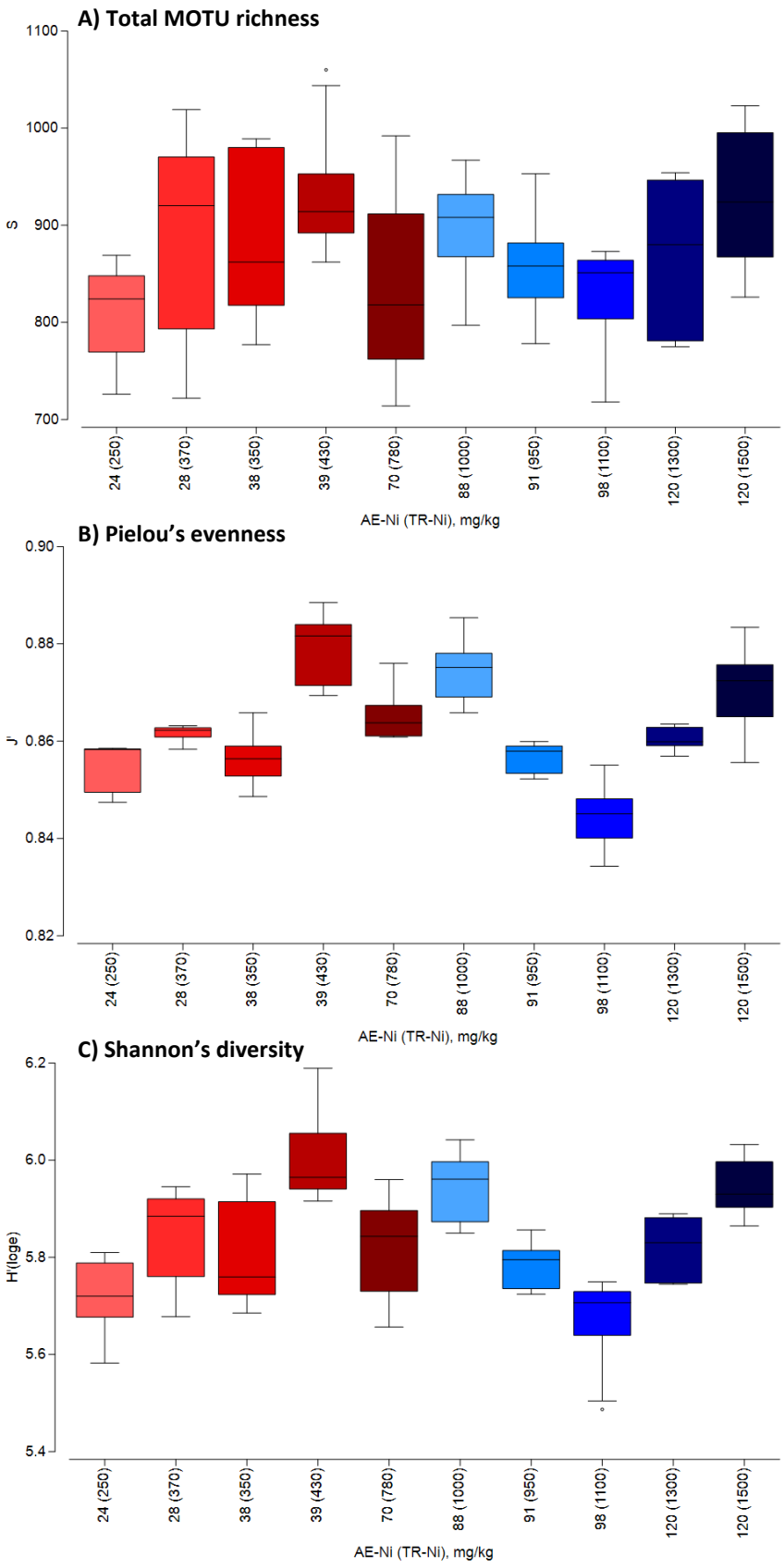


Figure 5.7. Univariate summary statistics for each site based on prokaryote 16S rDNA gene amplicon data: A) total MOTU richness; B) Pielou's evenness; and C) Shannon's diversity. Dataset standardised to 100 individuals before analyses.

5.3.2.4 Benthic community response summary

Considered together, the results of the univariate and multivariate statistical analyses suggest that species replacement may be occurring within both the eukaryote and prokaryote community compositions along the AE-Ni concentration gradient within Vavouto Bay. This finding is consistent with other metabarcoding studies that have revealed strong changes in community compositions in response to contaminant enrichment without discernible effects upon indices for richness, evenness, or diversity for both eukaryote (Chariton et al., 2010; DiBattista et al., 2020) and prokaryote (Misson et al., 2016; Yin et al., 2015) communities. Previous studies have suggested that resources liberated by the loss of contaminant-sensitive taxa become available for replacement by contaminant-tolerant species (e.g. Chariton et al., 2010; Johnston and Keough, 2005). In addition, the targeted diatom amplicon sequencing revealed a loss of diatom species richness and diversity suggesting that marine diatoms may be sensitive to the increasing AE-Ni gradient.

5.3.3 Influence of sediment predictor variables on benthic community response

5.3.3.1 Eukaryotes

DistLM analyses illustrated that the environmental predictor variables explained 66% of the total variation in eukaryote community composition (Appendix D4). The first dbRDA coordinate axis explained 31% of the total variation in the eukaryote community composition, with sites predominately separated along the increasing AE-Ni concentration gradient and to a lesser extent increasing AE-Zn concentration and moisture content (Figure 5.8). The second dbRDA coordinate axis explained a further 12% of the total variation in the eukaryote community compositions, with site separation along the vertical axis predominately associated with decreasing AE-Co concentration and to a lesser extent increasing TOC, moisture content, and AE-Cr concentration.

5.3.3.2 Diatoms

DistLM analyses illustrated that the environmental predictor variables explained 64% of the variation in diatom community composition (Appendix D4). The first dbRDA coordinate axis explained 31% of the total variation in the diatom community compositions with sites predominately separated along the increasing AE-Ni concentration gradient and to a lesser extent AE-Co concentration (Figure 5.8B). The second dbRDA coordinate axis explained a further 12% of the total variation in the diatom community compositions, with site separation along the vertical axis predominately associated with decreasing moisture content and to a lesser extent decreasing TOC and AE-V concentration and increasing AE-Co concentration.

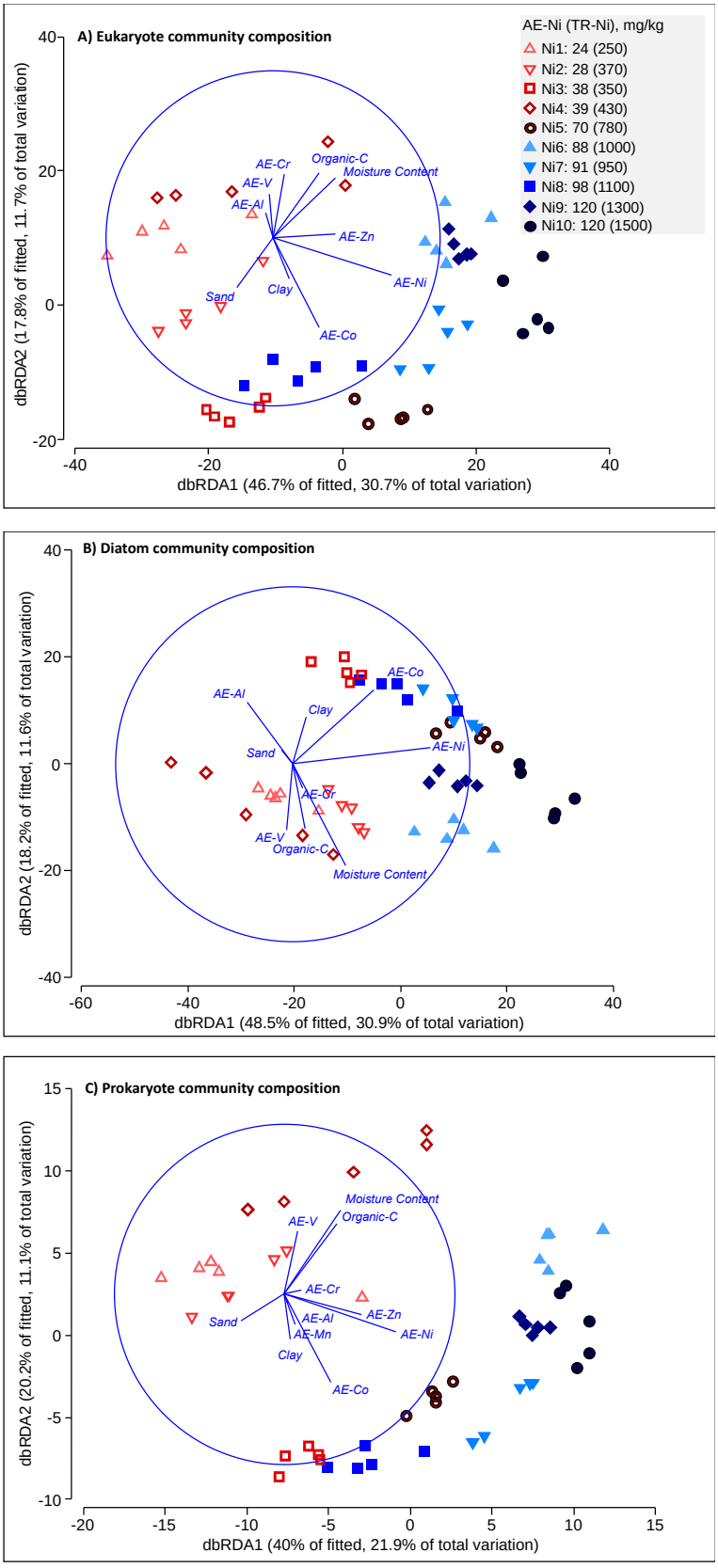


Figure 5.8. Distance-based linear modelling (DistLM) analysis (forward selection, adjusted R^2, 999 permutations) of selected sediment predictor variables and community composition: A) eukaryote; B) diatom, and C) prokaryote. AE = dilute-acid extractable, TR-Ni = total recoverable nickel, Sand = >63µm particle size, and Clay = <4 µm particle size. Variables that explained <1% of the variance not shown.

5.3.3.3 Prokaryotes

DistLM analyses illustrated that the sediment predictor variables explained 55% of the variation in prokaryote community composition (Appendix D4). The first dbRDA coordinate axis explained 22% of the total variation in the prokaryote community composition, with sites predominately separated along the increasing AE-Ni concentration gradient and to a lesser extent increasing AE-Zn concentration (Figure 5.8C). The second dbRDA coordinate axis explained a further 11.5% of the total variation in the prokaryote community compositions, with site separation along the vertical axis predominately associated with decreasing AE-Co concentration and to a lesser extent increasing moisture content and TOC.

5.3.3.4 Benthic community response summary

The metabarcoding techniques used in the current study were able to discriminate communities between marginal reference and nickel-rich locations. Multivariate statistical analyses, for all of the community profiles, showed that the most influential environmental predictor variable shaping the eukaryote, diatom, and prokaryote community compositions was AE-Ni concentrations, explaining 26%, 23%, and 19% of the variation, respectively (Appendix D4). Highly correlated variables (>0.95) were removed, so it is important to note that AE-Ni also represented AE-Fe in the statistical analyses. Iron is both ubiquitous and abundant in sediments, and under oxic conditions, iron bioavailability and toxicity are limited by its very low solubility which results in its precipitation and stability as iron oxyhydroxides (von der Heyden and Roychoudhury, 2015). Consequently, iron is not often considered as a sediment contaminant, and thus it is unlikely that AE-Fe is responsible for the changes in the community composition observed. After nickel, the co-occurring metals cobalt and chromium were identified as being the next most strongly correlated variables influencing eukaryote and prokaryote community composition. Compared to nickel, chromium, and cobalt varied over a narrow range (e.g. AE-Ni ranged between 24 to 120 mg/kg, whereas AE-Cr ranged between 14 to 31 mg/kg, and AE-Co ranged between 4 to 27 mg/kg) and AE-Cr was well below its DGV (Australian and New Zealand Governments, 2018).

The environmental variables measured in the current study accounted for between 55 to 66% of the total variation in community compositions. Future studies could include measurement of pore water metal concentrations or the flux of metal at the sediment-water interface using passive samplers such as DGT as additional lines of evidence (Gillmore et al., 2021b; Spadaro and Simpson, 2015). Variations in confounding environmental factors, such as water depth and sediment particle size, were minimised through the sampling design and site selection. However, biotic variables, such as predation, competition, and adaptation (particularly in the case of prokaryote taxa) cannot be excluded as factors that also may influence community composition.

Changes in community compositions alone, however, do not necessarily imply adverse ecological impact. The current study, using a natural *in situ* environmentally relevant concentration gradient, could

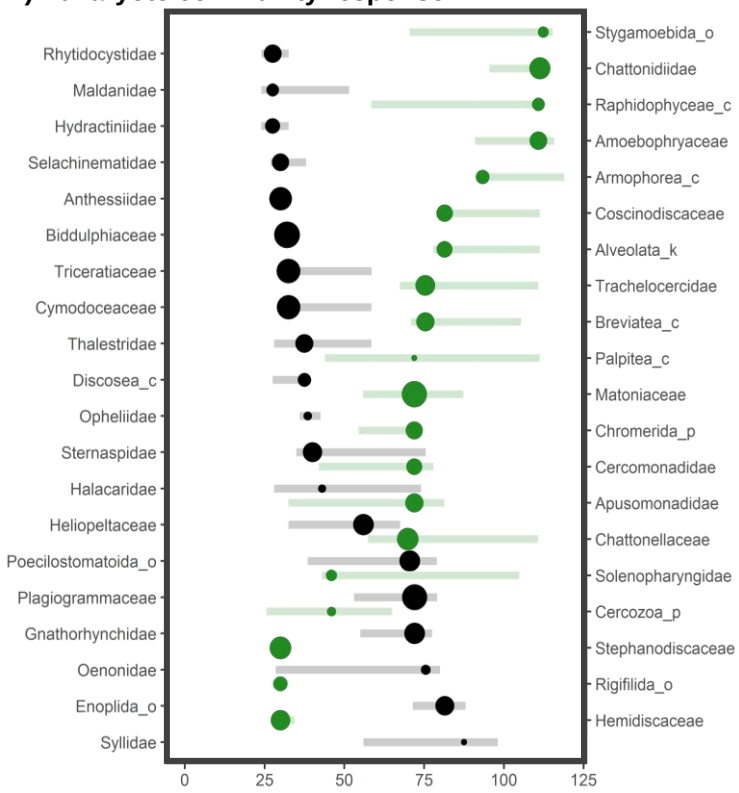
be further strengthened by the addition of complementary field or laboratory experiments using local macro and meiofaunal species that would investigate the various possible mechanisms that might be responsible for the observed ecological effects. In addition, characterising the functional and metabolic profile of the microbial community would provide important information about how ecological processes are being affected by the shift in community composition along the nickel concentration gradient (Zhang, 2019).

5.3.4 Taxon-specific patterns and identification of nickel-sensitive and nickel-tolerant taxa

5.3.4.1 Eukaryotes

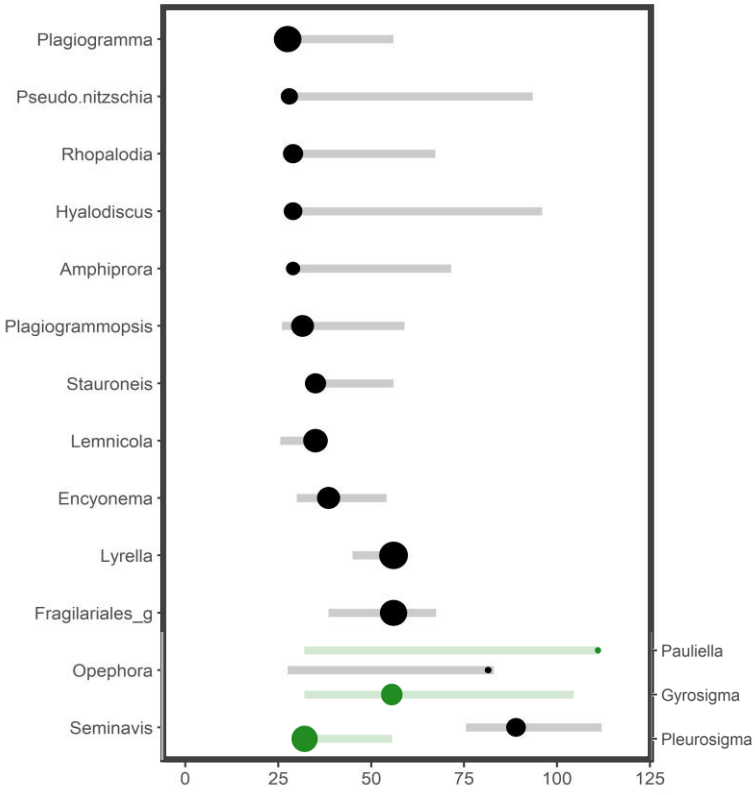
From the 18S rDNA eukaryote dataset, TITAN identified 145 taxa (family-level identification) that responded to changes in AE-Ni concentrations (Appendix D5). Fifty-five taxa (38%) responded negatively to increasing AE-Ni concentration. These putative nickel sensitive taxa were predominately from the phyla Arthropoda (n = 12), Annelida (n = 9), and Nematoda (n = 5) (kingdom Metazoa) and Bacillariophyta (n = 12 family groups) (kingdom Stramenophiles**)**. Positive responses to AE-Ni concentration were predominately from the phyla Ciliophora (n = 8) and Myzozoa (n = 8) (kingdom Alveolata), Bacillariophyta (n = 7) (kingdom Stramenophiles), Streptophyta (n = 6) (kingdom Plantae), Sulcozoa (n = 6) (kingdom Protozoa) and Cercozoa (n = 6) (kingdom Rhizaria). Taxa from the Kingdom Fungi (n = 11) were only represented in the group that responded positively to increasing AE-Ni concentration with taxa from Ascomycota (n = 4) and Chytridiomycota (n = 3) most represented. The top 20 taxa that responded negatively and positively to increasing AE-Ni concentration are presented in Figure 5.9A. The representation of taxonomic groups in the top 20 negatively responding taxa reflected the list above, but for the top 20 positively responding taxa, representation was dominated by taxa from the class Polychaeta (phylum Annelida), Maxillopoda (Arthropoda), and Mediophyceae (Bacillariophyta). The sum (-z) change point in the sensitive taxa (i.e. the sediment AE-Ni concentration where the most pronounced loss of taxa occurred) was at 46 (29-75) mg/kg AE-Ni (5-95th percentile). The sum (+z) change point in the tolerant taxa (i.e., the sediment AE-Ni concentration where the most pronounced increase of taxa occurred) was at 76 (56-112) mg/kg AE-Ni.

A) Eukaryote community response



Dilute-acid extractable nickel (mg/kg)

B) Diatom community response



Dilute-acid extractable nickel (mg/kg)

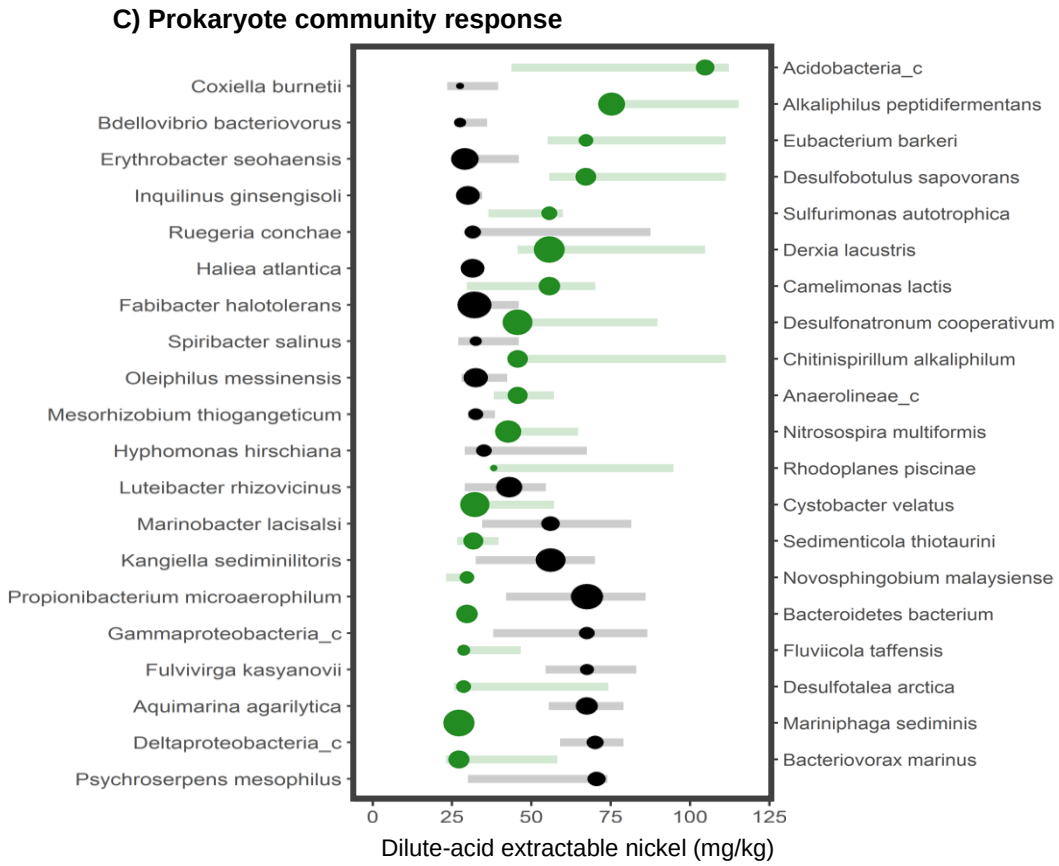


Figure 5.9. Threshold indicator taxa analysis (TITAN) results illustrating the change points and 95% confidence limits for the top 20 significant MOTUs that responded negatively (highest -z scores, left vertical axis, and black symbols) and positively (highest +z scores, right vertical axis, and green symbols) to the AE-Ni concentration gradient: A) eukaryote taxa; B) diatoms; and C) prokaryote taxa. The size of the change points is scaled to reflect the magnitude of their response (±z scores). Eukaryote taxa were identified to family level, diatoms to genus level, and prokaryote taxa to species level. Where family, genus, or species level identification could not be achieved, the lowest taxonomic level is stated and indicated by the following abbreviations: "_k" for kingdom, "_p" for phylum, "_c" for class, and "_o" for order.

The eukaryotic dataset largely revealed a shift from multicellular metazoans (especially copepods, ostracods, polychaetes, and nematodes) at low AE-Ni concentrations to fungi and unicellular eukaryotes (e.g. protozoa and protist lineages Alveolata and Rhizaria) at higher AE-Ni concentrations. This result may suggest that short-lived eukaryotes that grow and reproduce rapidly are occupying the space made vacant by the loss of metazoans. Of the putative nickel sensitive taxa identified here, epibenthic and benthic species of Metazoans, including copepods, ostracods, polychaetes, and nematodes could be targeted for collection in SEAM and toxicity test development. Previous testing with tropical pelagic copepods from SEAM has demonstrated they are particularly sensitive to chronic dissolved nickel exposure (Gissi et al., 2018).

5.3.4.2 Diatoms

TITAN identified 16 taxa (genus-level identification) from the SSU rDNA diatom dataset that responded significantly to the AE-Ni concentration gradient (Figure 5.9B and Appendix D5). Of these indicator taxa, 81% (n = 13) responded negatively to increasing AE-Ni concentration. These taxa were from four classes, including Bacillariophyceae (n = 8), Coscinodiscophyceae (n = 1), Fragilariophyceae (n = 2), and Mediophyceae (n = 2). All taxa that responded positively to increasing AE-Ni concentration were from the class Bacillariophyceae (n = 3). The 16 diatom taxa that responded negatively and positively to increasing AE-Ni concentration are presented in Figure 5.9B. The sum (-z) change point in the sensitive taxa occurred at 56 (28-59) mg/kg AE-Ni concentration. The sum (+z) change point in the tolerant taxa occurred at 56 (33-112) mg/kg.

The use of freshwater diatoms as indicators of water quality is well established (Morin et al., 2012; Stevenson et al., 2010). In comparison, the use of marine benthic diatoms as potential bioindicators of contamination is an emerging area of interest (Cunningham et al., 2005; Desrosiers et al., 2013). In that perspective, the results of the current study showed a loss of diatom species richness and diversity along the AE-Ni gradient and has highlighted that Coscinodiscophyceae, Fragilariophyceae, and Mediophyceae may represent potentially nickel-sensitive classes.

5.3.4.3 Prokaryotes

TITAN identified 152 taxa (species-level identification) from the prokaryote 16S dataset that responded significantly to the AE-Ni concentration gradient (Appendix D5). Ninety-four taxa (63%) showed a negative association with increasing AE-Ni concentration. The prokaryote phyla Proteobacteria and Bacteroidetes were heavily represented by taxa that responded both negatively (n = 51 and n = 19, respectively) and positively (n = 32 and n = 7, respectively) to increasing AE-Ni concentration. Of the Proteobacteria, taxa from the class Betaproteobacteria (n = 2) and Epsilonproteobacteria (n = 1) only appeared in the positively responding group. Alphaproteobacteria and Gammaproteobacteria appeared in both groups, but with higher representation in the negatively responding group (n = 27 and n = 19, respectively) than in the positively responding group (n = 7 and n = 9, respectively), whereas the opposite was observed for Deltaproteobacteria (n = 4 and n = 12 negatively and positively responding taxa, respectively). From Bacteroidetes, taxa from the class Cytophagia (n = 7) and Sphingobacteriia (n = 3) only appeared in the negatively responding group, and Flavobacteriia also had higher representation in the negatively responding group (n = 7) than the positively responding group (n = 2). Of the other phylum present in the sediment, Chloroflexi (n = 3) and Firmicutes (n = 3) were only represented in the group that responded positively to increasing AE-Ni concentration, while Actinobacteria (n = 9), Verrucomicrobia (n = 3), and Chlamydiae (n = 1) were only represented in the negatively responding group. The top 20 taxa that responded negatively and positively to increasing AE-Ni concentration are presented in Figure 5.9C. The representation of taxonomic groups in these top

20 responding taxa generally reflected the list above with taxa from Alphaproteobacteria and Gammaproteoacteria most represented in the positively responding taxa and Deltaproteobacteria most represented in the negatively responding taxa. The sum (-z) change point in the sensitive taxa occurred at 56 (35-68) mg/kg AE-Ni. The sum (+z) change point in the tolerant taxa occurred at 46 (31-112) mg/kg.

Similar changes in the bacterial community have been observed in the literature. In response to metal contamination of the sediment of the Xiangjiang River, China, taxa from the phylum Firmicutes, Chloroflexi, and Crenarchaeota were found to be highly tolerant to metals while taxa from Proteobacteria and Actinobacteria were less tolerant (Yin et al., 2015; Zhu et al., 2013). Sun et al. (2013) found trace metals as the most influential variable shaping sediment microbial community composition within estuaries receiving anthropogenic inputs in Australia. These authors identified taxa within the groups Gammaproteobacteria, Alphaproteobacteria, and Deltaproteobacteria as potentially contaminant sensitive, while taxa from Chloroflexi and Deltaproteobacteria were considered as potentially metal tolerant.

5.3.4.4 Benthic community response summary

Of all the prokaryote taxa present in the Vavouto Bay sediments identified from metabarcoding, only 6% were identified by TITAN as responding significantly, either negatively or positively, to the AE-Ni concentration gradient. This may suggest that the environmental conditions, which include high background concentrations of trace metals, has established a benthic microbial community that is tolerant to metal exposure. Previous research has demonstrated that the microbial communities within New Caledonian sediments can maintain activity (oxygen consumption) under metal spiking conditions that are known to be inhibitory for non-metal tolerant bacteria (Pringault et al., 2008; Viret et al., 2006). The benthic microbial community has also been shown to play an important role in the removal of dissolved nickel from the water column (Pringault et al., 2010).

Of all eukaryote and diatom taxa present in the Vavouto Bay sediments, similarly, only a small portion, 13% and 10% of taxa respectively, were identified as responding significantly, either positively or negatively, to the AE-Ni concentration gradient and may also indicate a benthic eukaryotic community that is largely tolerant to metal exposure.

It is important to highlight that the current study has captured the response of the benthic community at a single point in time during the dry season. It would be ideal to repeat this study during the wet season to determine whether the benthic community is influenced similarly or differently by the increased supply of terrigenous material from the nearby ultramafic Koniambo regolith and the potential change in sediment composition (e.g., metal concentration and grain size). Previous research has suggested that benthic bacterial community structure in coastal lagoon sediments, however, exhibit a relative temporal

stability and are only weakly influenced by seasonal variations in environmental conditions (Pereira et al., 2006; Pringault et al., 2008).

TITAN identified putative nickel-sensitive and nickel-tolerant taxa, which may also include species increasing or decreasing in occurrence due to reduced competition or predation. Targeting the eukaryote and prokaryote community assemblages allowed for shifts in taxa composition to be observed that would not have been evidenced through traditional benthic ecology surveys such as invertebrate sorting techniques which focus on macro and sometimes meiofaunal assemblages. While metabarcoding generally allows for the inclusion of a broader range of taxa to be investigated, taxonomic resolution can be limited by the quality and availability of annotated sequences retained within on-line genomic databases such as RDP Classifier (Cole et al., 2014) and SILVA (Quast et al., 2013) as used in the current study. The large number of unclassified sequences obtained in the eukaryote amplicon dataset restricted the taxonomic analysis to the family level. This was most likely due to the high endemism of New Caledonia's fauna and the lack of DNA-based work that has been performed within the region. The taxonomic resolution was improved with the use of diatom-specific primers, confidently categorising taxa down to genus level identification. This supports the composite multi-gene approach, which includes targeting genes with broad taxonomic coverage and genes that can provide more detailed information on key taxa of interest for environmental monitoring studies (Stat et al., 2017).

5.3.5 Deriving environmentally relevant thresholds of effects from benthic community responses

The use of an environmental gradient of increasing sediment nickel concentrations allowed for the determination of community change points for the benthic eukaryote, diatom, and prokaryote communities. Considered together, there is a community change point (threshold) around 46-76 mg/kg for this ecosystem. Based on the disappearance of sensitive taxa, a value of 46 mg AE-Ni/kg is thus recommended as a conservative threshold concentration. The ability to identify at what metal concentrations community changes appear can be considered to be a *de facto* guideline value. In the case of the estuarine and marine sediments in New Caledonia, it is recommended that 46 mg AE-Ni/kg be treated as an interim threshold value until further lines of evidence can contribute to a site-specific nickel sediment quality guideline value.

For comparison to the Australian and New Zealand SQGs, the DGV for nickel is 21 mg/kg and the GV-high is 52 mg/kg (Australian and New Zealand Governments, 2018). The DGV is indicative of concentrations below which adverse effects rarely occur, whilst the GV-high represents concentrations above which effects frequently occur in exposed macrofauna (Australian and New Zealand Governments, 2018). It is important to note that these SQGs were derived using a ranking of both field ecological and laboratory acute ecotoxicological-effects data from mixed metal exposures to North American species (Long et al., 1995). Their reliability is likely to be poor, and it is acknowledged that

on this basis, it is difficult to define thresholds between effects and no effects. In addition, their derivation is confounded by the influence of co-occurring contaminants on the observed effects. Although their relevance for protecting temperate species within Australia has been demonstrated (McCready et al., 2006), their application to tropical species or species endemic to the SEAM region is unknown. As the guidelines were developed using macrofauna data only, their relevance for protecting benthic bacterial communities is also unknown. Given this general uncertainty, it is recommended that the Australian and New Zealand SQGs be used as screening tools and to trigger further investigation if exceeded (Simpson and Batley, 2015). The application of more robust methods for the derivation of SQGs for trace metals (e.g. through the use of SSDs applied to data from multiple toxicity tests) has only been demonstrated for copper (with temperate marine species) and nickel (with temperate freshwater species) (Schlekat et al., 2016; Simpson et al., 2011; Vangheluwe et al., 2013). The derivation of community change points, as demonstrated here using metabarcoding, represents an important line of evidence that could be used in the development of more robust sediment quality guideline for nickel in estuarine and marine sediments.

Background estuarine and marine sediment nickel concentrations in New Caledonia are significantly higher than those occurring in other estuarine and mangrove sediments worldwide due to the geology of the region. Previous work has demonstrated that the primary source of trace metals to the sediments of Vavouto Bay is from the erosion of lateritised ultramafic outcrops of the Koniambo regolith, enhanced by open-cast mining for nickel (Merrot et al., 2019; Noël et al., 2015, 2014). Due to this metal-rich environmental context, it is possible that local species may have adapted to and be more tolerant than those in other regions. Due to the possibility of evolved tolerance, to further evaluate if a risk to ecosystem health exists, toxicity testing with local species identified in the TITAN analyses are necessary to investigate cause and effect relationships as another line of evidence in deriving a region-specific nickel sediment quality guideline value.

In assessing the risks of sediment nickel contamination, a weight of evidence approach is now universally recommended (Australian and New Zealand Governments, 2018). Lines of evidence will typically include chemistry, toxicity, bioaccumulation, and ecology. The interim threshold value derived in the current study is a starting point for assessing the effect of sediment chemistry on communities, that can then be combined with other evidence, to determine whether there is a risk to ecosystem health. In addition, the current study has also established a base-line of the benthic community response along the nickel gradient that could be used to monitor possible ecological changes over time using the same metabarcoding approach.

5.4 Conclusions

The current study found that changes in benthic eukaryote, diatom, and prokaryote community composition within Vavouto Bay, New Caledonia, was significantly correlated with increasing AE-Ni concentrations. Changes in community composition alone, however, do not necessarily indicate adverse effects on ecosystem health. Multiple lines of evidence from a combination of chemical, biological, and ecological studies are required to assess the risk associated with nickel-contaminated sediments. Future research will need to investigate cause-effect relationships, as at present there is an absence of this type of ecotoxicological data (Gissi et al., 2016). The current study has identified potential sensitive taxa that could be used for developing single-species toxicity tests to investigate nickel toxicity and for use as bio-indicators of field-based contamination. TITAN along the nickel concentration gradient found that thresholds of change in the benthic eukaryote, diatom, and prokaryote community composition occurred between 46 to 76 mg AE-Ni/kg. Based on these data, a threshold value of 46 mg AE-Ni/kg was selected as a point where sensitive species began to be affected. The current study represents a first example where metabarcoding has been used to derive an interim threshold value for nickel. This is a strong improvement on existing sediment quality guideline values for temperate regions (which are based on a ranking of largely acute toxicity effects from mixed metal exposures) and for the SEAM region where no guidelines exist, and is thus an essential contribution in a weight of evidence approach to sediment risk assessment.

Chapter 6

6 The physiological effects of nickel-contaminated suspended sediment exposure to the scleractinian coral, *Acropora muricata*

This chapter has been redrafted from the published article: Gillmore, M.L., Gissi, F., Golding, L.A., Stauber, J.L., Reichelt-Brushett, A.J., Severati, A., Humphrey, C.A., and Jolley, D.F. 2020. Effects of dissolved nickel and nickel-contaminated suspended sediment on the scleractinian coral, *Acropora muricata*. *Marine Pollution Bulletin*, **152**. https://doi.org/10.1016/j.marpolbul.2020.110886.

Co-author and fellow PhD student, F. Gissi, and I designed and performed the experiment together with assistance from the other co-authors. I collected and processed all the data presented in this chapter and Gillmore et al. (2020). F. Gissi used the experiment to investigate how the coral microbiome was affected by nickel-contaminated suspended sediment exposure and this work is presented in her PhD thesis (Gissi, 2019). M. Gillmore wrote the original draft of this chapter/manuscript. All authors contributed to refining the manuscript before submission.

6.1 Introduction

The SEAM region has unique and highly biodiverse coastal ecosystems with immense ecological and economical significance. It has nearly 100,000 km^2 of coral reefs in Southeast Asia alone, and the highest level of coral richness and biodiversity in the world (Burke et al., 2002). The projected intensification of lateritic nickel mining in SEAM has raised concerns as to the potential risk imposed by increased sediment loads and nickel contamination to these vital and fragile tropical marine coastal ecosystems (Gissi et al., 2016). Within the SEAM region, background total suspended solid (TSS) concentrations in coastal marine waters are typically low (ca. 1-5 mg/L) (Browne et al., 2015b; Jouon et al., 2008). However, suspended sediment events can peak at over 100 mg/L TSS in near-shore coastal waters and can sustain moderate concentrations (10-50 mg/L TSS) for several days (e.g. Browne et al., 2015a; Hoitink, 2004; Wolanski et al., 2003). Nickel concentrations as high as 7,100 mg/kg have been reported in suspended sediment in the New Caledonia lagoon (Fernandez et al., 2006).

Limited ecotoxicity data exist for assessing the risk of the adverse impacts of dissolved nickel on coral ecology. Effects data are largely restricted to a few studies that have investigated the effect of nickel on the fertilisation success of scleractinian corals (Gissi et al., 2017; Reichelt-Brushett and Hudspith, 2016; Reichelt-Brushett and Harrison, 2005). The studies of scleractinian corals have shown that the larval life stage exhibits a broad range of sensitivities to dissolved nickel, but overall hard corals are relatively insensitive to nickel compared to other trace metals such as copper (Gissi et al., 2017). More recent work by Gissi et al. (2019), investigated the effects of dissolved nickel on adult corals and their microbiome, and reported toxicity to the coral only at very high dissolved nickel concentrations (LOEC

of 470 μg Ni/L) after a 96-h exposure. Additional data are required to quantify corals' interspecies variability in sensitivity to dissolved nickel. Other work has focused on the physiological response of adult scleractinian coral to nickel enrichment, identifying a beneficial effect at low nickel concentrations (3.5 μg Ni/L), through stimulating the use of urea as a carbon and nitrogen source, and as a consequence, enhancing both photosynthesis and calcification processes (Biscéré et al., 2018, 2017).

The impact of sediments on corals has been extensively reviewed (e.g., Browne et al., 2015a; Erftemeijer et al., 2012; Fabricius, 2005; Jones et al., 2016; Rogers, 1990). Suspended sediment may impact coral by limiting the quantity and quality of light available for photosynthesis in the algal symbionts (*Symbiodinium* sp.) (Anthony and Fabricius, 2000); by sedimentation of the coral surface inhibiting feeding, increasing respiration and leading to a decline in net photosynthetic yield (Junjie et al., 2014; Philipp and Fabricius, 2003); and by impairing recruitment by reducing fertilisation success, larval survival in the water column and larval settlement (Ricardo et al., 2018). Specifically, a decline in photosynthetic productivity of the *Symbiodinium* sp. can lead to coral bleaching (death or loss of the *Symbiodinium* sp. and their associated pigments) (Brown, 1997). Corals have a range of active sediment-clearance mechanisms for dealing with the effects of suspended sediment exposure (e.g. polyp inflation, tentacle action, polyp movement, and mucus production) (Stafford-Smith and Ormond, 1992). However, in doing so, they incur an energetic cost, which can lead to sub-lethal effects such as reduced growth, lower calcification rates, reduced productivity, and increased susceptibility to diseases (Erftemeijer et al., 2012). A review of over 30 studies by Browne et al. (2015a) found that tolerance thresholds for coral species to suspended sediment exposure vary widely, over a range of timeframes (hours to one year) and suspended sediment concentrations (10-1000 mg/L TSS).

Few studies have determined the effects of metal-contaminated suspended sediment on corals and none have conducted controlled concentration-response studies and subsequently examined recovery of the coral from environmentally relevant exposures to nickel-contaminated sediments. The current study examined the physiological response of the branching coral, *Acropora muricata* (formerly *A. formosa*), following a 7-d continuous exposure to either dissolved nickel; clean suspended sediment; or nickel-contaminated suspended sediment. A laboratory spiked nickel-contaminated sediment and a nickel-rich field-collected sediment were tested to provide different nickel bioavailability scenarios (referred to as the Added-Ni and Field-Ni sediment treatments, respectively). The response of the coral was determined by quantifying *Symbiodinium sp.* cell densities and loss of colour intensity (i.e. bleaching), as well as measuring nickel concentrations in the coral tissues and algal symbionts. These same endpoints were used to examine the recovery of the coral 7-d post-exposure termination. This controlled study seeks to determine if there was any additional impact from the nickel associated with the suspended sediments and assists in assessing the potential impacts of the anticipated increased exploitation of lateritic nickel ores in the SEAM region.

6.2 Materials and methods

6.2.1 Coral collection and acclimation

A. muricata is a dominant reef-building species, common to tropical coral reefs throughout the world, including the SEAM region. Large coral fragments were collected from six colonies of *A. muricata* on the 14 to 16th of June 2017 from Trunk Reef (Queensland, Australia) at 3 to 5 m depth (GBRMPA permit number G12/35236.1). The fragments were further cut into nubbins (4-10 cm length) each including one axial corallite, glued (XTRA Loctite super glue, Loctite, Australia) to aragonite plugs and placed in 60-L tanks supplied with flowing seawater (5 L/min) for transport to the laboratory. The coral nubbins were transferred to 200-L indoor flow-through tanks at the National Sea Simulator (Australian Institute of Marine Science (AIMS), Cape Ferguson, near Townsville, Queensland, Australia) for seven weeks of acclimation. During the acclimation phase, the tanks were supplied with ultra-filtered (<0.04 μm) seawater (temperature 27°C, salinity 35 ppt, and pH 8.1). A 12:12 h light/dark photoperiod was provided, which consisted of a 2-h period of gradually increasing light in the morning, 8 h of continuous illumination at 100-150 μmol photons/m^2/s, a 2-h period of decreasing light in the afternoon, followed by 12 h of darkness. Corals were fed daily, a combination of newly hatched Artemia nauplii and a mixture of microalgae (*Tisochrysis lutea*, *Pavlova lutheri*, *Dunaliella* sp., *Nannochloropsis oceania*, *Chaetoceros muelleri* and *Chaetoceros calciltrans* at 5 x 10^6 cells/mL). Only coral nubbins that were in a healthy condition (i.e., having produced new growth during acclimation) were selected for use in the experiment.

6.2.2 Sediment preparation

To mimic the type of suspended sediment that would be present in a pulse event within the mining impacted SEAM region, fine-grained, organic sediment from two tropical locations in Queensland, Australia were collected as per Section 2.3.1. Nickel-rich sediment was collected near Saunders Beach (Yabulu, 19°10'14.5"S 146°37'25.4"E), adjacent to a nickel refinery. Uncontaminated sediment was collected from Hartleys Creek (Wangetti, 16°39'17.3"S 145°34'04.6"E) and was used as a control, as well as for the spiked, high nickel treatments. Sediment characterisation was performed as per Section 2.3.3. The physicochemical properties of the sediments before sieving are provided in Table 6.1.

The high nickel treatment was prepared using a two-part nickel-spiking procedure (Hutchins et al., 2008) with the sieved Hartleys Creek sediment. Initially, a super-spike sediment was prepared (10,000 mg Ni/kg nominal concentration) then diluted with clean sediment to achieve the treatment concentration (6,000 mg Ni/kg nominal concentration). The treatment concentration was selected to be representative of the high concentrations of nickel that have been reported in coastal sediments near mining activities in the SEAM region (Fernandez et al., 2006; Noël et al., 2015). To prepare the super-spiked sediment, $NiCl_2.6H_2O$ (analytical reagent grade) was dissolved in clean filtered seawater

(Cronulla) and thoroughly mixed into the sediment (200 mL at 171 g Ni/L:3.4 kg sediment DW) by hand and allowed to equilibrate at room temperature for 10 weeks, with twice weekly mixing by hand. The super-spiked sediment was then diluted with clean sediment to achieve the desired treatment concentration and allowed to equilibrate for a further two weeks at 4°C.

6.2.3 Experimental design and conditions

The study consisted of exposing coral nubbins to nine treatments: a water-only control and two dissolved nickel concentrations (0, 200, and 400 µg Ni/L nominal concentration); and six suspended sediment treatments of clean, nickel-spiked, and nickel-rich field-collected sediment, each at 5 and 30 mg/L TSS. Exposure duration was 7 d, with coral physiological response variables measured on days 4 and 7. Post-exposure, there was an additional 7-d recovery period where corals were exposed to non-contaminated seawater and coral response variables were measured again on day 14. Corals were not fed over the 14 days. The water-only and suspended sediment treatments had two and three replicate tanks per treatment, respectively. One day prior to exposure, 16 to 20 individual coral nubbins were placed on a suspended plastic grating (false bottom floor), 25 cm below the water surface in each replicate tank. First, a unique identification number was randomly assigned to 6 or 9 of the coral nubbins per tank for the water-only and suspended sediment, respectively, by attaching a small piece of paper to the aragonite plug with glue (XTRA Loctite super glue). These select coral nubbins were photographed before being placed in the tank and again when removed (Section 6.2.4.1).

The study was carried out in a controlled environment room at the National Sea Simulator (AIMS), with a sediment dosing and suspension system set-up similar to that of Bessel-Brown et al. (2017). The experiment was carried out in 24 square, clear polyvinylchloride tanks (115-L capacity each) with an inverted pyramid at the base. Each tank was supplied with a constant flow (400 ml/min) of ultra-filtered (<0.04 µm) seawater (temperature 27°C, salinity 35 ppt, and pH 8.1) to provide six complete turnovers of water per day. Above each tank was a custom-built LED lighting panel, providing even illumination with a mixture of blue and white chips, with a spectral frequency around 460 nm. A 12:12 h light/dark photoperiod was provided, which consisted of a 2-h period of gradually increasing light in the morning, 8 h of continuous illumination at 100-150 µmol photons/m^2/s, a 2-h period of decreasing light in the afternoon, followed by 12 h of darkness.

Table 6.1. Characteristics of tropical sediments collected from Hartleys Creek and Saunders Beach, Queensland, Australia

Sediment	Particle size (%, n = 5)			Total organic carbon	Dilute-acid extractable metals (mg/kg, n = 3)			Total recoverable metals (mg/kg, n = 3)		
	<4 µm	4-63 µm	>63 µm	(%, n = 1)	Ni	Fe	Mn	Ni	Fe	Mn
Hartleys Creek	5	81	14	3.6	1	500	6	7	11,000	97
Saunders Beach	6	65	29	9.8	81	22,000	86	300	34,000	140

Table 6.2. Background concentrations of metals in tropical sediments collected from Hartleys Creek (HC) and Saunders Beach (SB), Queensland, Australia and the sediments used as suspended sediment treatments

	Dilute-acid extractable metals (mg/kg, n = 3)									Total recoverable metals (mg/kg, n = 3)								
	Al	As	Cd	Co	Cr	Cu	Pb	V	Zn	Al	As	Cd	Co	Cr	Cu	Pb	V	Zn
Field collected sediments																		
Hartleys Creek	700	3	0	1	0	1	6	8	9	6,500	12	0	5	10	10	11	12	30
Saunders Beach	1,200	6	0	1	7	3	11	26	16	12,000	15	0	11	98	11	13	32	40
Suspended sediment treatments (sieved to <180 µm)																		
Clean (HC)	880	2	0	0	0	1	7	9	10	8,400	12	0	6	13	12	13	15	38
Added Ni (HC)	800	2	0	0	0	3	6	9	10	8,200	13	0	6	12	13	15	15	39
Field Ni (SB)	1,500	6	0	0	5	3	10	22	14	12,000	9	1	5	100	11	11	34	40

The three suspended sediment treatments (clean, nickel-spiked, and field-contaminated sediment) were stored in separate 70-L fiberglass tanks and kept in suspension through recirculation at high velocity (3 m/sec) in closed loops around the experimental room by air-operated diaphragm pumps (Sand piper S30). Each tank was managed as a completely independent system, being equipped with a magnetic drive pump (Iwaki MD-70) providing constant uplifting flow through the port placed at the vertex of the inverted pyramid. The uplifting flow provided kept the flow of sediment in suspension, while the additional movement pump (VorTech MP10, EcoTech Marine, United States) position at the level of the coral samples provided accessory water flow needed for coral husbandry requirements.

A programmable logic controller (Siemens S7-1500) and custom model predictive control logic was used to control the dosing of the tanks and to achieve the desired TSS concentration by opening and closing pivoting solenoid valves connected to the sediment treatment tanks via the high-velocity loop. Turbidity within each experimental tank was monitored with nephelometers (Turbimax CUS31, Endress and Hauser) positioned in the line of the recirculating uplifting pump. The custom model predictive control logic was designed to manage potentially inconsistent aliquots (both for volume and concentration) of sediment through a self-learning process, where the system kept acquiring in real time data on the effectiveness of a train of doses, as well as the rate of sediment depletion due to settlement and dilution; this strategy gave the dosing logic both stability and the ability to rapidly reach the turbidity set value without overshooting.

For quality control, the TSS concentration was also determined gravimetrically at five time points (days 0, 1, 3, 5, and 6) in duplicate for every suspended sediment exposure tank by passing a known volume of treatment water (0.5-1 L) through pre-weighed 47-mm diameter polycarbonate filters (0.45 μm pore size), rinsed with high purity water then dried at 60°C for ≥24 h and weighed to five decimal places. Linear relationships were established between turbidity and TSS concentration to enable the conversion of nephelometric turbidity units (NTUs) to mg/L to allow for the sediment dosing system to be adjusted as needed during the exposure.

Concentrated stocks of dissolved nickel (0.5 and 1.0 g Ni/L) were prepared by dissolving $NiCl_2.6H_2O$ (analytical reagent grade) in filtered (<0.04 μm) seawater. Dissolved nickel was then introduced to each of the nickel treatment tanks at a rate of 0.3 mL/min by peristaltic pump to give nominal concentrations of either 200 or 400 μg Ni/L. These concentrations were chosen as they occur within the range where measurable effects were observed from dissolved nickel exposures in previous work (Gissi et al., 2019) and to enable comparison with likely nickel concentrations released from the nickel-spiked sediments into the overlying water.

Experimental conditions including water temperature, turbidity, and light intensity were continuously monitored, logged, and controlled throughout the entire experiment by the programmable logic controller.

Water temperature was measured in each of the experimental tanks by temperature sensors (TC Direct, FEP Insulated RTD Pt100 Sensors coupled with a Pt100 Transmitter), and the readings provided feedback for the temperature control, which was actuated by solenoid valves that allowed flow of refrigerated water into titanium coils used as in-tank heat exchangers. Light intensity was measured at the depth of the coral using PAR transmitters (Skye PAR Quantum sensor SKP-215) connected to the programmable logic controller. Water quality parameters (including temperature, pH, salinity, specific conductivity, and dissolved oxygen) were also measured manually (see Section 2.2.1) in every tank, every second day (days 0, 2, 4, 6, 8, 10, 12, and 14).

Sub-samples for dissolved metal analyses were collected from each tank every day during the exposure (days 0 to 7) and every second day during the recovery period (days 8, 10, 12, and 14) and analysed as per Section 2.2.2.

Sub-samples for DOC were collected from 1 replicate tank per treatment on days 0 and 6. Samples were passed through an acid-washed filter (0.45 μm PES) and collected in a glass vial with 2 mL of concentrated H_2SO_4. DOC was measured using a Shimadzu TOC-VCSH/CSN, TOC/TN analyser according to Holland et al. (2018).

6.2.4 Coral response variables

Coral response variables were measured on days 4, 7 (at the end of the exposure period) and 14 (at the end of the recovery period). Visual observations of coral behaviour (e.g., polyp activity and mucus production) were also recorded throughout the experiment.

6.2.4.1 Loss of coral tissue colour intensity (i.e. bleaching)

Coral nubbins were individually removed from the tanks and rinsed with clean filtered (<0.02 μm) seawater. They were immediately imaged using a high-resolution digital camera (Olympus tough TG4). The coral nubbins were randomly selected from the numbered subset that were imaged pre-exposure so that before and after exposure comparisons could be made. The camera settings and distance of the coral fragment to the camera were standardised throughout the duration of the experiment. The coral images were analysed for colour intensity using the image analysis software ImageJ (Schneider et al., 2012). The method was based on that described by Bessell-Browne et al. (2017) with modification. The outline of the coral nubbins was carefully, digitally traced then the histogram function was used to display the traced area colour intensity in greyscale (black = 0 and white = 255), and the arithmetic mean of the pixel values was recorded. Differences in ambient lighting across images were normalised by analysis of a standard black and white square present in every image collected.

6.2.4.2 Separating skeleton, tissue and algal symbionts

Following imaging, coral tissue and algal symbionts were removed from the coral skeleton by air-blasting into a clean zip-lock bag (Deschaseaux et al., 2013). The skeleton was placed into a separate zip-lock bag and frozen (-20°C) for surface area quantification (Section 6.2.4.3). Seawater (5 mL) was added to the bag of tissue and algae, and the slurry homogenised by hand massaging of the bag. A 2-mL aliquot of this homogenate was transferred to a 2-mL tube (VWR International, United States) and fixed with 1% v/v glutaraldehyde (25% in H_2O, Sigma-Aldrich, Germany) for quantifying *Symbiodinium* sp. density (Section 6.2.4.3). The remaining homogenate was transferred to a pre-weighed, acid-washed 10-mL centrifuge tube and centrifuged at 1000 g for 10 min to separate the denser algal symbionts from the tissue. The tissue solution was pipetted off the top and transferred to a pre-weighed, acid-washed 50-mL centrifuge tube. Water was removed from the algal symbiont pellet by oven drying at 60°C for ≥24 h and from the tissue solution by freeze-drying (0.28 mbar for 6 h then 0.0010 mbar for ≥6 h). Dried samples of coral tissue and algal symbionts were acid digested to determine metal content (Section 6.2.4.4).

6.2.4.3 Algal symbionts cell density quantification

The cell density of the algae symbionts was determined using a flow cytometer following the procedure previously described in Section 3.2.4.7 for *C. closterium*. Samples were gently homogenised immediately prior to analysis using a tissue grinder and where required, diluted with clean filtered seawater (Cronulla).

Flow cytometry identified two distinct populations of algae isolated from the homogenate. Microscopy revealed that there was likely two genera of algae present, *Symbiodinium* sp. and possibly a *Prorocentrum*–like dinoflagellate (Appendix E1). Both algal populations were individually quantified by flow cytometry and in the context of cell density measurements will be distinguished hereinafter as *Symbiodinium sp.* and unidentified alga, respectively.

Cell density of the two algal populations was also measured using a haemocytometer for quality control and validation of the flow cytometer values at a frequency of 1 in every 6 samples (n = 25). Cell density values obtained by flow-cytometry and haemocytometer were in good agreement (R^2 = 0.96 and R^2 = 0.95 for *Symbiodinium* sp. and the unidentified alga, respectively) and neither method was found to consistently produce higher or lower densities (Appendix E2). Values obtained from the flow cytometer were converted from cells/mL to cells/cm^2 using the surface area of the specific coral skeleton from which the algae were extracted.

The surface area of each coral skeleton was determined by 3D scanning. The set-up included an LED projector (ACER), camera (DAVID-CAM-4-M) and powered by the computer software HD 3D Scan 5. A total of 16 scans (22.5° rotation between scans) were collected for each skeleton with the scanning

settings as follows: quality control 0.4 to 0.6, outlier removal 0.3 to 1%, close holes 100%, and fusion resolution 500. The visualisation software Autodesk Netfabb was then used to obtain surface area and volume measurements for each coral skeleton.

6.2.4.4 Nickel accumulation in coral tissue and algal symbionts

The dried algal symbionts and coral tissue samples were pre-digested in a 2:1 mixture of concentrated nitric acid (Merck, Tracepur) and hydrogen peroxide (Merck, Emsure) at room temperature for ≥18 h. The samples were then microwave heated (CEM MARS) at 60°C for 1 h. After digestion samples were diluted to a nitric acid concentration of 6% (v/v with high purity water). Blanks and certified reference material (0.01 g or 0.10 g to match the sample weights of the algae and tissue, respectively, Dorm-3 Fish protein, National Research Council, Canada) were included with each sample batch to ensure consistency in results achieved (recovery of 89 ± 33% and 65 ± 11% for 0.01 g and 0.10 g samples, respectively). Nickel concentrations in the algae symbionts and coral tissue will need to be considered alongside these recoveries; however, the authors note that the algal symbionts and coral tissue were completely dissolved by the digestion process whereas the reference material was not. Baseline concentrations of metals in the coral tissue and algal symbionts were determined from six individual replicate coral nubbins collected prior to the treatment exposures. The distribution of cellular nickel (inside the algal cells and coral tissue or adsorbed to the outside of the cells/tissue) was not considered within the scope of this study.

The results of the nickel accumulation in the algal symbionts needs to be considered in the context of the presence of both the *Symbiodinium* sp. and the unidentified alga. The authors note, however, that the unidentified alga may have been introduced to the sample from the addition of 5 mL of seawater to produce the homogenate and therefore may not be associated with the coral or may not have been present during the nickel exposures. Its presence is therefore, if anything, likely to result in a slight underestimation of the nickel concentration in the algal symbionts.

6.2.5 Statistical analysis

Statistical analyses were carried out in NCSS (NCSS Statistical Software 2007, v07.1.21, United States). The repeated measures ANOVA function was used to assess: 1) if there was an effect of nickel on coral physiological response variables (bleaching and *Symbiodinium* sp. density) by detecting significant differences between the control and each treatment at key time points; and 2) if there was an effect of nickel on bioaccumulation in either the tissue or algal symbionts over time (compared to the baseline measurement) by detecting significant differences within each treatment. The within-group variable was time point (baseline, days 4, 7, and 14), and the between-group variable was the experimental treatments. Data were tested for sphericity using Mauchly's Test of Sphericity and where the homogeneity of variance assumption was violated, data were adjusted using the Greenhouse Geisser

adjustment. Post hoc analysis with the Dunnett's two-sided multiple-comparison test with the control was used to indicate at which time points and in which treatments coral responses were significantly different. An alpha level of 0.05 was used for all statistical tests.

6.3 Results

6.3.1 Sediment characterisation

The physicochemical properties of the sieved suspended sediment treatments are presented in Table 6.3 and additional chemical properties are provided in Table 6.2. The particle size composition of all treatments was relatively uniform and predominately silty (82% 4-63 µm, mean particle size 19-20 µm). TOC concentrations were moderate to high (3.5-7.7%). The Hartleys Creek sediment (used as the control) had low concentrations of metals, well below sediment quality guideline DGVs (Australian and New Zealand Governments, 2018). The Saunders Beach sediment TR-Ni and TR-Cr concentrations exceeded their respective DGVs (21 mg/kg and 80 mg/kg) by 11 and 1.3 times, respectively. The AE-Ni and AE-Cr concentrations were 25 and 5% of the TR-M concentrations, respectively, demonstrating that they were mostly present in a mineralised form. Spiking of the sieved (<180 µm) Hartley Creek sediment produced a TR-Ni concentration of 6,300 mg/kg and an AE-Ni concentration 6,100 mg/kg, close to the target nominal concentration.

Both sediments had a similar mineral composition with the main mineral phases present being silicate group minerals followed by halide, carbonate and sulfide group minerals (Figure 6.1).

6.3.2 Experimental conditions

Water quality was consistent throughout the experiment and across all individual tanks; temperature was 26.8 ± 0.1°C (mean ± SD, n = 192), pH 8.05 ± 0.05, salinity 35.9 ± 0.3 PSU, specific conductivity 54.4 ± 0.5 mS/cm and dissolved oxygen 6.3 ± 0.4 mg/L. DOC ranged from 1.1 to 1.4 mg/L across all treatment tanks (Table 6.4).

The time-averaged dissolved nickel concentrations in the Dissolved-Ni treatments during the exposure period were equivalent to the target nominal concentrations (Table 6.4). During the recovery period, dissolved nickel decreased to concentrations close to the detection limit (1.1 µg/L).

Dissolved nickel was below the detection limit in the Clean and the Field-Ni (5 and 30 mg/L TSS) treatments during both the exposure and recovery periods. Dissolved nickel concentrations in the Added-Ni TSS treatments were relatively high (70 ± 30 µg/L and 300 ± 100 µg/L, for the 5 and 30 mg/L TSS treatments, respectively) during the exposure period and were highest towards the end of the exposure period. In Added-Ni TSS treatments the dissolved nickel was much lower (3 ± 3 µg/L and 10

± 20 µg/L, respectively) during the recovery period, when the corals were exposed to clean seawater for 7 days.

Table 6.3. Characteristics of the tropical sediments used as suspended sediment treatments (sieved to <180 µm), collected from Hartleys Creek (HC) and Saunders Beach (SB), Queensland, Australia

Sediment	Particle size (%, n = 5)			Total organic carbon (%, n = 1)	Dilute-acid extractable metal (mg/kg, n = 3)			Total recoverable metal (mg/kg, n = 3)		
	<4 µm	4-63 µm	>63 µm		Ni	Fe	Mn	Ni	Fe	Mn
Clean (HC)	6	82	12	3.5	1	650	7	9	14,000	120
Added-Ni (HC)	6	82	12	3.5	6,100	540	6	6,300	15,000	120
Field-Ni (SB)	7	82	11	7.7	60	11,000	33	240	23,000	100

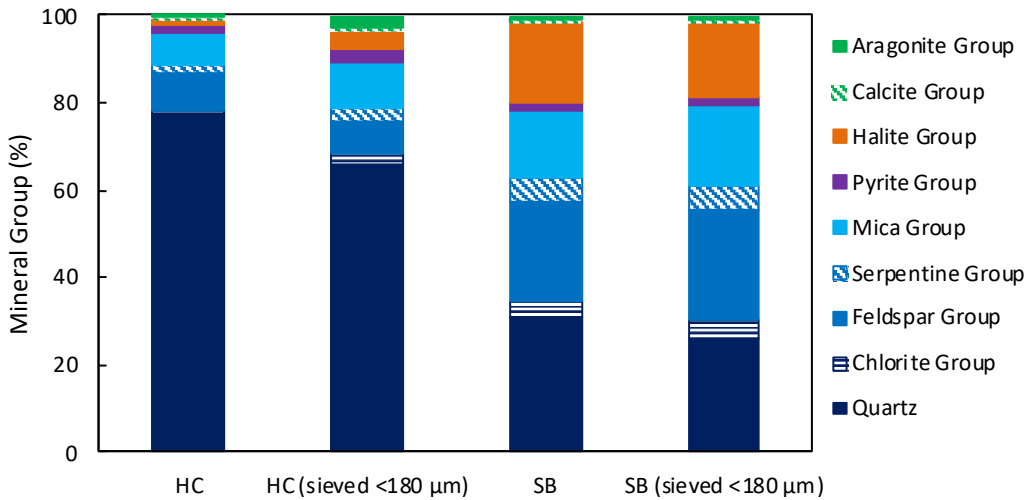


*Figure 6.1. Mineral groups from x-ray powder diffraction (XRD) analyses of the Hartleys Creek (HC) and Saunders Beach (SB) sediments before and after sieving to remove particles >180 µm. Colour indicates the mineral chemistry: carbonate minerals (*green*), halide minerals (*orange*), sulfide minerals (*purple*) and silicate minerals (*blue*).*

All other dissolved metal concentrations were low across all treatments and time points (As, Cd, Co, Cr, Cu, Fe, Mn, V, and Zn were ≤1.1 µg/L, and Al was ≤4.4 µg/L, n = 300, data not shown).

The time-averaged TSS concentrations were close to the nominal values for all treatments: 5 mg/L (4.3-5.1 mg/L) and 30 mg/L (27-28 mg/L) (Table 6.4). Sediment particles were observed to attach to some of the internal surfaces of the tank walls and the false bottom plastic grating, despite the action of the water circulation pumps. No coral nubbins were observed to have considerable sediment deposited on their surface, apart from on those that experienced significant bleaching and polyp retraction and could no longer actively remove the settled particles. Algal growth was observed on the plugs of some coral nubbins across all treatments.

Daytime light levels over the 7-d exposure period in the suspended sediment treatments were consistent between treatments, with the time-averaged light level between 120 to 136 µmol/m^2/s (Table 6.4).

6.3.3 Physiological response of coral to nickel and suspended sediment exposure

All coral nubbins from the control treatments survived the 7-day exposure and 7-day recovery period. Only one fragment across the entire experiment (from the Added-Ni, 30 mg/L TSS treatment) experienced complete tissue necrosis and subsequent sloughing of the tissue from the skeleton. This fragment was not representative of the treatment and so was not included in the analyses.

6.3.3.1 Loss of coral tissue colour intensity (i.e. bleaching)

Significant bleaching of coral tissue was observed in the Dissolved-Ni treatments and Added-Ni TSS treatments, but not in the Field-Ni TSS treatments (Figure 6.2 and Figure 6.3A). A small loss of tissue colour intensity (<10%) was observed in the Control on day 14, however, this change was not significant and visual inspection indicated that coral nubbins in the control remained in a healthy condition throughout the experiment (Figure 6.2). Significant effects of coral bleaching in the Dissolved-Ni treatments were first observed in the 200 µg Ni/L treatment on day 7 and in both treatments on day 14. Significantly reduced colour was observed in the Added-Ni 5 mg/L TSS treatment on day 7 and 14 and in Added-Ni 30 mg/L TSS treatment on day 7.

Table 6.4. Summary of water quality parameters in treatment tanks during the exposure (Days 0-7) and recovery (Days 8-14) periods

Treatment (nominal concentrations)	**Time-averaged dissolved Ni (<0.45 µm filtered) (µg/L)**		**Dissolved organic carbon (mg/L)**	**Total suspended solids (mg/L)**	**Daytime light intensity (µmol/m^2/s)**	
	Exposure	**Recovery**	**Exposure**	**Exposure**	**Exposure**	**Recovery**
Water-only treatments	n = 16	n = 8	n = 2			
Control	<1.1*	<1.1	1.10 ± 0.04	NM	NM	NM
Dissolved-Ni (200 µg/L)	200 ± 70	2 ± 2	1.2 ± 0.2	NM	NM	NM
Dissolved-Ni (400 µg/L)	400 ±100	3 ± 4	1.16 ± 0.01	NM	NM	NM
Suspended sediment treatments (sieved to <180 µm)	n = 24	n = 12	n = 2	n = 22	(Continuously logged)	
Clean (5 mg/L TSS)	<1.1	<1.1	1.3 ± 0.3	4.4 ± 0.7	136 ± 5	140 ± 6
Clean (30 mg/L TSS)	<1.1	<1.1	1.2 ± 0.2	27 ± 4	128 ± 5	130 ± 9
Added-Ni (5 mg/L TSS)	70 ± 30	3 ± 3	1.22 ± 0.05	4.3 ± 0.7	120 ± 20	132 ± 9
Added-Ni (30 mg/L TSS)	300 ± 100	10 ± 20	1.2 ± 0.2	28 ± 3	130 ± 4	133 ± 7
Field-Ni (5 mg/L TSS)	<1.1	<1.1	1.35 ± 0.05	5.1 ± 0.7	120 ± 20	130 ± 9
Field-Ni (30 mg/L TSS)	1.4 ± 0.9	<1.1	1.3 ± 0.1	28 ± 3	128 ± 5	130 ± 9

NM = Not measured. Values are the mean ± standard deviation

* Value less than the limit of detection (0.6-1.9 µg Ni/L, n = 6)

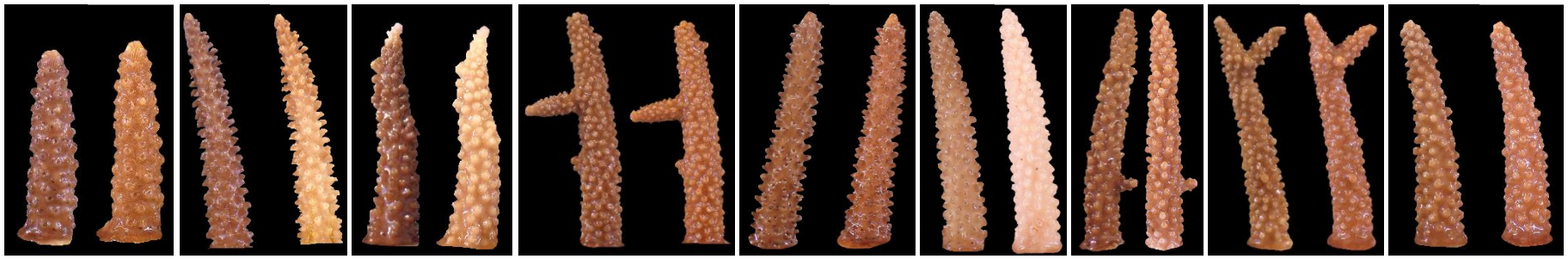


Figure 6.2. Representative images of coral nubbins taken from each treatment at different times. Each pair of images within a treatment is the same coral fragment imaged prior to exposure (left image) and on Day 14 (right image) following a 7-d treatment and a 7-d recovery period. TSS = total suspended solids, nominal values.

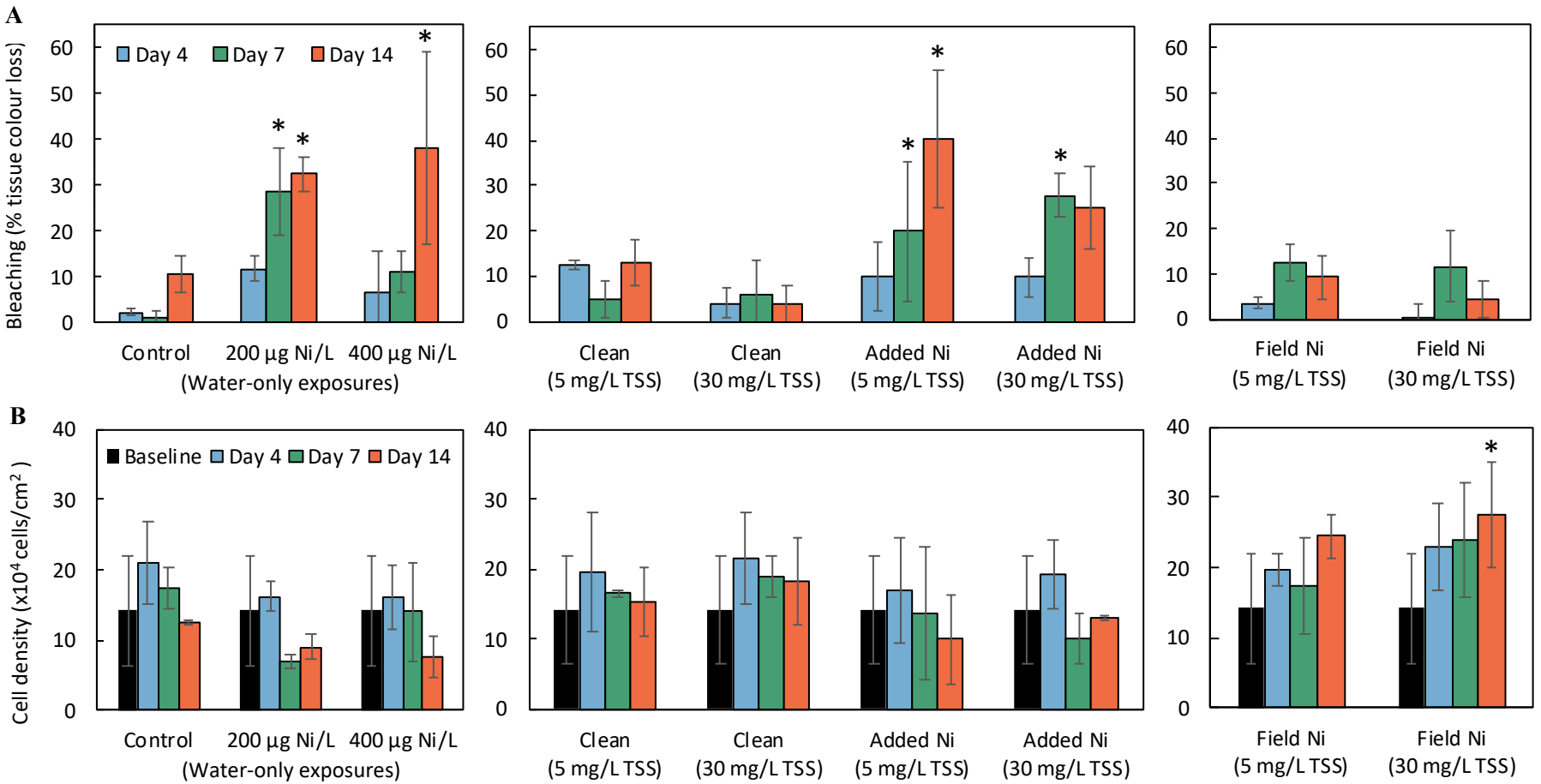


Figure 6.3. The influence of treatment condition on A) bleaching and B) Symbiodinium *sp. density, before exposure (Baseline), during the exposure (Days 4 and 7) and following a 7-d recovery period (Day 14):* Left = *Dissolved-Ni (0, 200 and 400 µg Ni/L);* Middle = *suspended sediment without Ni (Clean) and spiked with nickel (Added-Ni, 6300 mg total Ni/kg); and* Right = *field-collected nickel-contaminated suspended sediment (Field-Ni, 240 mg total Ni/kg). Values presented are the mean ± standard deviation (*vertical error bars*), n = 6 (baseline), n = 2 (water-only exposure) or n = 3 (suspended sediment exposures). Significant (*$P<0.05$*) differences from the control at the corresponding time point are indicated with an asterisk. TSS = total suspended solids, nominal values.*

6.3.3.2 Symbiodinium sp. density

Baseline measurement of *Symbiodinium* sp. density showed a large range (5-27 x 10^4 cell/cm^2, n = 6) for visibly healthy corals. There was no consistent or statistically significant effect on *Symbiodinium* sp. density over the exposure or recovery period in the Dissolved-Ni treatments, Clean TSS or the Added-Ni TSS treatments (Figure 6.3B). Only in the Field-Ni TSS treatments was there a trend of increasing *Symbiodinium* sp. density over time observed, with the highest cell density observed in the Field-Ni 30 mg/L TSS treatment after 14 days being significantly greater than the Control (Figure 6.3B)

The presence of the unidentified alga's was highly variable and showed no consistent pattern (Appendix E3). There was also no relationship between the presence of *Symbiodinium* sp. and the unidentified alga (R^2 = 0.0054) (Appendix E4).

6.3.4 Nickel accumulation in coral tissue and algal symbionts

6.3.4.1 Algal symbionts

The algal symbionts accumulated nickel from the water-only Dissolved-Ni treatments to a maximum of 17 ± 5 µg Ni/g DW (Figure 6.4), which was 6.8 times greater than the maximum concentration of nickel accumulated by coral tissue in the same treatments (Figure 6.5). The algal symbionts accumulated nickel from the Dissolved-Ni treatment as measured on days 4 and 7 in the 200 µg Ni/L and day 4 in the 400 µg Ni/L treatment. There was large variability in the nickel accumulation in the algae in the 400 µg Ni/L treatment after 7 days of exposure. Following the recovery period, the concentration of nickel in the algae in the Dissolved-Ni treatments had decreased and was not significantly different to the baseline concentration (2 ± 1 µg/g DW) pre-exposure.

The nickel concentrations measured in the algal samples from the suspended sediment treatments were confounded by the presence of sediment particles, which also concentrated in the algal cell pellet (Appendix E5). Thus, the current study was unable to confirm whether or not nickel can be transferred from the sediment and bioaccumulated by the algal symbionts.

6.3.4.2 Coral tissue

Significant accumulation of nickel in the tissue of *A. muricata* was observed in the Dissolved-Ni treatments and the Added-Ni 30 mg/L TSS treatment (Figure 6.5). The tissue of *A. muricata* had significantly accumulated nickel from the Dissolved-Ni treatment on day 4 in the 200 µg Ni/L and days 4 and 7 of the 400 µg Ni/L treatment. In both these treatments, the nickel concentrations were highest on day 4 (2 ± 1 µg/g DW and 3 ± 1 µg/L DW, respectively) then decreased at day 14. For both Dissolved-Ni treatments, the nickel concentrations had returned to baseline concentrations (0.3 ± 0.2 µg/g DW, n = 6) following the recovery period. An increase in nickel concentrations in the Added-Ni

TSS treatments was observed, but this was only significantly greater than baseline for the Added-Ni 30 mg/L TSS treatment at day 4 and 7. No nickel was accumulated above the baseline concentration in the tissue of *A. muricata* exposed to the Control, Clean TSS or the Field-Ni TSS treatments.

No changes in the concentrations of other metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, V, and Zn) compared to the baseline were observed over time (data not shown).

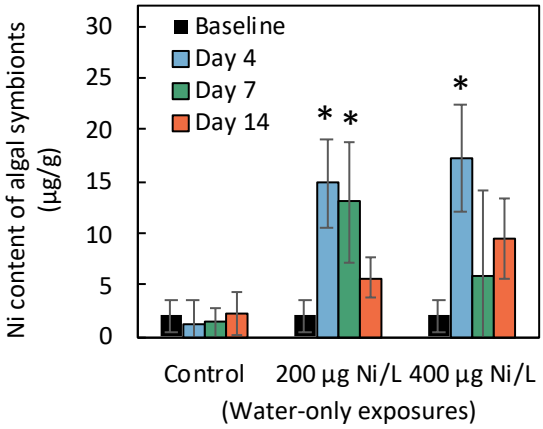


Figure 6.4. Nickel concentrations (µg/g dry weight) in the algal symbionts of Acropora muricata *exposed to Dissolved-Ni (0, 200, and 400 µg Ni/L). Values presented are the mean ± standard deviation (*vertical error bars*), n = 2. Significant difference (*P*<0.05) from the baseline is indicated with an asterisk.*

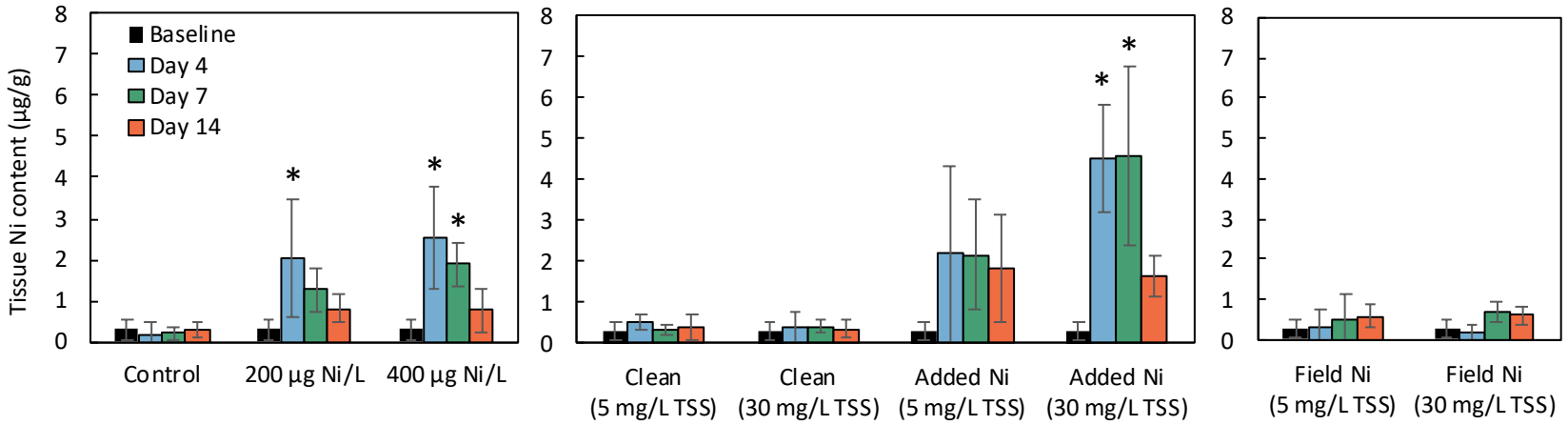


Figure 6.5. Nickel concentrations (µg/g dry weight) in coral tissue of Acropora muricata *exposed to:* Left = *Dissolved-Ni (0, 200, and 400 µg Ni/L);* Middle = *suspended sediment without Ni (Clean) and spiked with nickel (Added-Ni, 6300 mg total Ni/kg); and* Right = *field-collected nickel-contaminated suspended sediment (Field-Ni, 240 mg total Ni/kg). Values presented are the mean ± standard deviation (*vertical error bars*), n = 2 (water-only exposure) or n = 3 (suspended sediment exposures). Significant difference (*P*<0.05) from the baseline is indicated with an asterisk. TSS = total suspended solids, nominal values.*

6.4 Discussion

6.4.1 Physiological response of coral to nickel and suspended sediment exposure

Changes in coral tissue colour and *Symbiodinium* sp. density were quantified as a measure of coral stress. Bleaching was observed in response to high Dissolved-Ni exposure (200 µg/L and 400 µg/L) in the water-only treatments. These results were comparable to a recent study by Gissi et al. (2019), which observed no effect of dissolved nickel on coral health at 90 µg Ni/L and bleaching at 470 µg Ni/L, suggesting that the effects from a short-nickel exposure (4-7 d) occur between 90 to 200 µg Ni/L. This result is, however, caveated by the observation that concentrations of nickel greater than 90 µg/L are unlikely to occur in the environment except perhaps for poorly flushed estuarine environments heavily impacted by industrial activities (Angel et al., 2010; de Melo Gurgel et al., 2016; Ezekwe and Edoghotu, 2015; Knauer, 1977).

No bleaching of coral nubbins was observed in *A. muricata* from the Clean TSS treatments. This result is consistent with other studies that have reported tolerance of scleractinian corals to short durations of exposure to TSS ≤30 mg/L without significant light limitation or sediment deposition (Browne et al., 2015a; Flores et al., 2012). Bleaching was observed in the directly comparable Added-Ni TSS treatments indicating that a stress response was induced by the presence of the high-concentration nickel-spiked sediment. In the 30 mg/L TSS treatment however, the dissolved nickel (300 ± 100 µg/L) was above the concentration where effects were observed for dissolved nickel alone (200 µg/L), so it was not possible to determine if the particulate nickel also contributed to the bleaching response. Considering the results of Gissi et al. (2019) who found no bleaching of coral tissue at a dissolved nickel concentration of 90 µg Ni/L following a 4-d exposure, dissolved nickel leached from the sediment (70 µg/L) in the 5 mg/L TSS treatment may be below the concentration for effects and the results of the current study provide some evidence that the bleaching effect could be due to a combination of dissolved nickel in the overlying water and particulate-bound nickel on the sediment. Cloran et al. (2010) similarly found reduced survival of the freshwater crustacean *Daphnia magna* exposed to suspensions of nickel-spiked sediment than when exposed to either dissolved nickel or suspended sediment alone. These authors suggested that either the dissolved nickel and suspended sediment acted synergistically to reduce survival or that the test organisms may have also ingested particle-associated nickel (Cloran et al., 2010).

No bleaching of coral tissue was observed in the Field-Ni TSS treatments. Nickel associated with the Field-Ni treatment was highly mineralised (25% acid extractable nickel) and consequently very little nickel was released into solution. Dissolved nickel concentrations in this treatment were close to the natural background concentrations in coastal marine waters (<1.1 µg/L and 1.4 ± 0.9 µg/L for the 5

mg/L and 30 mg/L TSS treatments, respectively) during the exposure period. Therefore, a 7-d continuous exposure of nickel-contaminated suspended sediment (≤30 mg/L) at ≤60 µg AE-Ni/kg (≤240 µg TR-Ni/kg) was below the concentration to produce effects from nickel for adult *A. muricata*.

During the 7-d recovery period, bleaching continued to occur in the Dissolved-Ni 400 µg/L and Added-Ni 5 mg/L TSS treatments, demonstrating there can be a latent physiological response of the coral following a period of stress. Previous studies have noted that the capacity for corals to recover from bleaching and be repopulated with *Symbiodinium* sp. is dependent on the duration and severity of the stress (Hoegh-Guldberg, 1999). It has been reported that recovery and repopulation of bleached coral tissue by the *Symbiodinium* sp. can take from weeks to several months depending on the extent of tissue damage incurred during the bleaching event (Cunning et al., 2016). Furthermore, it has been demonstrated that corals exposed periodically to short-duration turbidity events have a greater capacity for recovery from higher levels of sediment exposure and longer durations than corals that infrequently experience suspended sediment events (Nyström et al., 2000) or corals that are constantly exposed to suspended sediment over an extended period of time (such as during dredging) (Browne et al., 2015a).

Changes in coral tissue colour were a more sensitive indicator of coral stress than *Symbiodinium* sp. density. This was most likely due to the naturally high variability in the density of *Symbiodinium* sp. in healthy coral tissue on Day 0. Little to no change in *Symbiodinium* sp. density was observed for the Dissolved-Ni, Clean TSS or Added-Ni TSS treatments. Interestingly, *Symbiodinium* sp. density in the Field-Ni TSS treatment increased over the duration of the experiment (although there was high variability). This result may suggest that the *Symbiodinium* sp. were responding positively to the suspended sediment exposure. The Field-Ni treatments had a relatively high organic carbon content. It has been reported that *Symbiodinium* sp. density and the concentration of photosynthetic pigments may increase in response to enrichment by particulate and dissolved nutrient loads (Tomascik and Sander, 1985). However, such a stimulatory response is not likely to occur at higher TSS concentrations which may cause polyps to retract and reduce their feeding rate (Anthony, 2000).

The mechanism of nickel toxicity to corals and their symbionts is unknown. Limited work on other metals suggests that the symbiotic relationship in adult corals may be disrupted by oxidative stress (Kuzminov et al., 2013). Kuzminov et al. (2013) examined the toxic effect of Cu, Zn, Cd, and Pb on *Symbiodinium* spp. and found that the primary targets of metal toxicity were secondary photosynthetic reactions, including electron transport between PSII and PSI and Rubisco activity in the Calvin-Benson cycle.

6.4.2 Nickel accumulation in coral tissue and algal symbionts

The pre-exposure, baseline nickel concentration in the coral tissue (0.3 ± 0.2 µg/g DW) was similar to that reported for other scleractinian corals from the Great Barrier Reef (Denton and Burdon-Jones,

1986; Reichelt-Brushett and McOrist, 2003). The pre-exposure, baseline nickel concentration in the algae was an order of magnitude higher (2 ± 1 μg/g DW) than for coral tissue, but lower than previously reported concentrations of 5 to 14 μg/g in Reichelt-Brushett and McOrist (2003).

In the Dissolved-Ni treatments, the algal symbionts had about 7-fold higher nickel content than the coral tissue. This result is consistent with other studies that have reported greater concentrations of metals in the symbionts than the tissues of corals (Ranjbar Jafarabadi et al., 2018; Reichelt-Brushett and McOrist, 2003) and the high metal accumulation efficiency of isolated coral symbionts (Hédouin et al., 2016; Metian et al., 2015). It has been suggested that *Symbiodinum* sp. may play an important role in trace metal regulation by sequestering metals more rapidly than the coral tissue, reducing toxic effects to the invertebrate host (Harland and Nganro, 1990). When under high metal stress the corals may then expel their *Symbiodinium* sp. as a possible mechanism to reduce accumulated metal (Hardefeldt and Reichelt-Brushett, 2015; Harland and Brown, 1989; Harland and Nganro, 1990; Peters et al., 1997).

Following the initial increase in accumulated nickel in coral tissues on day 4, continuing nickel exposure did not lead to any further uptake on day 7 (although there was high variability). These results suggest that following an initial uptake of nickel, the corals were able to better regulate the excess nickel in attempting to reach a steady state. Retraction of coral polyps and the production of mucus were both observed responses of the coral in the current study to nickel exposure and may have played a role in limiting metal accumulation. Previous studies have identified that retraction of coral polyps in response to metal stress, exposes the coral skeleton which can then directly adsorb metals which are later incorporated into the newly formed skeleton through calcification (Brown et al., 1991). The production of mucus has also been shown to effectively bind metals and thus may be involved in metal regulation (Reichelt-Brushett and McOrist, 2003; Stafford-Smith and Ormond, 1992). In the current study, the coral skeletons were not analysed for nickel accumulation due to the short exposure duration and noting that previous work has shown that nickel is only bioaccumulated into newly formed skeleton (Hédouin et al., 2016). Furthermore, the living component of coral (host tissue and algal symbionts) have been shown to display a greater bioaccumulation of metals compared to the skeleton (Hédouin et al., 2016; Metian et al., 2015; Ranjbar Jafarabadi et al., 2018; Reichelt-Brushett and McOrist, 2003).

Following the recovery period, nickel concentrations in coral tissue and the algal symbionts returned to baseline concentrations by day 14. These results demonstrate the ability of the corals and algae to depurate nickel during a recovery period. The low retention of nickel in the tissue and algae has been demonstrated in other scleractinian coral species (Hédouin et al., 2016). Using ^{63}Ni, Hédouin et al. (2016) observed a biological half-life of 6.5 days for accumulated nickel following a 14-day exposure.

The amount of nickel accumulated by the coral tissue was greater in the Added-Ni TSS treatment where there was a combined exposure to particulate-bound nickel and dissolved nickel compared to the Field-

Ni TSS treatments where exposure was solely from particulate-bound nickel. While the particulate nickel concentration of the nickel-spiked sediment was two orders of magnitude greater than the field-collected sediment, the most likely explanation for the differences in nickel accumulation observed is the difference in the bioavailability of nickel in the nickel-spiked sediment (97% of TR-Ni was AE-Ni) compared to the field-collected nickel sediment (25% of TR-Ni was AE-Ni) leading to much greater nickel lability and dissolved nickel concentrations in the nickel-spiked sediment treatment. While the greater lability of nickel in the nickel-spiked sediment is most likely due to this sediment being artificially amended with nickel, the greater concentration of iron and TOC in the field-collected nickel-contaminated sediment compared to the nickel-spiked sediment may have also played a role in reducing the bioavailability of nickel in the nickel-rich field-collected sediment (Besser et al., 2013; Costello et al., 2016, 2011). A 12-week equilibration period for the spiked sediments was chosen based on previous work, which reported an equilibration period of ≥10 weeks is required for nickel partitioning into various solid phases in both marine (Simpson et al., 2004) and freshwater sediments (Brumbaugh et al., 2013). Additionally, the indirect spiking method was used as this has been shown to more rapidly achieve equilibrium of metals in sediments (Hutchins et al., 2008). However, metal equilibration in sediments can be affected by several aspects of the spiking methodology, most notably control of pH and oxidation-reduction potential. The high proportion of total nickel present as AE-Ni in the current study demonstrates the need for further research into laboratory nickel spiking procedures. Field-collected contaminated sediments allow for equilibration of metals with the different sediment binding phases over long periods of time, and generally these better reflect metal bioavailability in mine waste over nickel-spiked sediments. This was confirmed in the current study, where no adverse effects on corals or their associated algae were found after exposure to nickel-rich field-collected sediments for 7 days.

The amount of nickel accumulated by the coral tissue was greater in the Added-Ni TSS treatments where there was a combined exposure to particulate nickel and dissolved nickel compared to the water-only Dissolved-Ni exposure, so it is likely that both water-borne exposure and TSS uptake via ingestion contributed to the nickel accumulated in the coral tissue. Anthony and Fabricius (2000) found that when light was limited, some corals can switch from being primarily autotrophic to heterotrophic (suspension/detritus). Ingestion of sediment by corals is widely considered to be part of a normal feeding mechanism with corals being capable of assimilating and obtaining nutritional benefits from the associated organic matter (Jones et al., 2016).

6.4.3 Environmental relevance and implications for risk assessment of nickel in SEAM

As the SEAM region develops additional nickel ore extraction and processing capabilities, specific information is needed to effectively understand the risk posed to important tropical ecosystems such as coral reefs. Reef-building corals, such as *A. muricata*, are important foundation species in tropical

environments, providing the structural framework that serves as a habitat for a plethora of other reef-organisms (Peters et al., 1997). Thus, understanding the effects of dissolved and particulate-bound sources of nickel on scleractinian corals is important because changes to their distribution or abundance will have consequences for the stability of entire reef systems and associated importance of these for food safety and food security of local communities. Scleractinian corals and particularly those of the genus *Acropora* have been identified as particularly sensitive to changes in water quality and chemical contaminants making them a suitable first representative tropical test species for use in investigating potential risks from contamination to coral reefs (Peters et al., 1997). It is important to note however, that different coral species and even individuals of the same species that have been exposed to slightly different environmental conditions can have different sensitivities to the same stressor (Anthony and Larcombe, 2000).

Tropical climates are characterised by high seasonal variability, intense storms and high precipitation, which may episodically lead to large volumes of terrestrially derived material or the resuspension of deposited sediment being transported to coral reef ecosystems. In the current study the intensity and duration of the suspended sediment exposure was comparable to low (5 mg/L) and moderate (30 mg/L) suspended sediment concentrations that corals could episodically experience in the SEAM region (Browne et al., 2015b; Hoitink, 2004; Jouon et al., 2008; Wolanski et al., 2003). During the experiment, no deposition of suspended sediment on the coral nubbins was observed, apart from on those that experienced significant bleaching and polyp retraction and could no longer actively remove the settled particles, and the light levels were the same in all treatments, so the experimental conditions did not confound the effects of the dissolved and particulate nickel. However, in a natural system lack of light and sediment deposition may be co-occurring stressors that may exacerbate the adverse effect of nickel on coral health. For example, Jones et al. (2016) reported that at suspended sediment concentrations comparable to the current study (30 mg/L), nearly all light would be attenuated at 5 m depth. Sediment deposition is generally considered more detrimental to corals than turbidity alone because there is an energetic cost for corals to regulate particulate intake, which may lead to down-regulation of photosynthesis, and increased rates of respiration and mucus production (Fabricius, 2005; Philipp and Fabricius, 2003). It is therefore recommended that when using the results of the current study, consideration be given to other environmental stressors, both natural and anthropogenic, that may amplify any detrimental effects that may occur and lower coral tolerance to metals.

The mineral composition, particle size and organic content of the sediments used in the experimental suspended sediment treatments were consistent with those reported in suspended sediment measured nearshore from the SEAM region (Fernandez et al., 2017; Ouillon et al., 2010). Previous work has found these finer-grained, organic rich sediments may pose a greater risk to corals than coarser-grained, lighter siliceous and carbonate sediments (Storlazzi et al., 2015; Weber et al., 2006). The nickel concentration of the spiked sediment was chosen to reflect the upper concentrations of nickel reported

in sediment in the mining-impacted SEAM region (Ambatsian et al., 1997; Fernandez et al., 2006; Marchand et al., 2012; Noël et al., 2015). Elevated concentrations of dissolved nickel have been reported in near-shore coastal marine ecosystems adjacent to mining or industrial activities (Angel et al., 2010; Fernandez et al., 2017; Knauer, 1977). The current study has demonstrated the importance of considering nickel associated with both particulate and dissolved phases in determining the potential for risk to corals.

6.5 Conclusions

At present there are very few nickel sediment toxicity data or whole sediment toxicity tests for tropical marine and estuarine benthic biota (Gissi et al., 2016). The current study found that nickel-contaminated sediment may present a risk above that of uncontaminated suspended sediment when the nickel is present in a more bioavailable form. For a water-only exposure, the concentration for bleaching to occur was estimated to be between 90 and 200 µg Ni/L considering the results of the current study and Gissi et al. (2019). A 7-d exposure of 30 mg/L TSS at 60 mg/kg AE-Ni had no effect on adult *A. muricata*. This information will assist in assessing the potential impacts of the anticipated increased exploitation of lateritic nickel ores in the SEAM region.

Chapter 7

7 General discussion

This body of research aimed to develop risk assessment tools and ecotoxicological exposure and effects data that could be used by environmental managers and regulators when evaluating the risk of nickel-rich estuarine and marine sediments associated with lateritic nickel mining within SEAM. The development of these tools and their application for generating ecotoxicity data to inform risk assessment is an important research avenue given the projected expansion of nickel laterite mining within the SEAM region to satisfy the global demand for nickel. Preserving sediment quality is both an ecological, social, and economic issue. Thus, risk assessment tools and the generation of ecotoxicity data are vital to assist environmental managers and regulators to make informed decisions, including around the development of a natural resource, for navigating dredging projects, or for contaminated site remediation or ecosystem restoration.

Given the paucity of whole sediment toxicity test methods and nickel ecotoxicity data for tropical marine biota at the start of the study (Gissi et al., 2016), a multiple-lines-of-evidence approach was adopted so that information could be generated that would cover important habitats, ecosystems, and organism groups. Within SEAM, mangroves and fringing coral reefs are especially important habitats which form a protective boundary between the land and the lagoon, and between the lagoon and open ocean, respectively. In the process of fulfilling the primary objectives of the study, several important insights for advancing risk assessment tools for tropical estuarine and marine sediments were learned. These insights, along with the experimental limitations and recommended future research directions are discussed below.

7.1 Lines of evidence for advancing sediment quality assessment within SEAM

7.1.1 Toxicity: ecotoxicological effects data and species representation

One of the objectives of my research was to develop whole-sediment toxicity test methods with estuarine or marine species ecologically relevant to the SEAM region in order to generate ecotoxicity effects data for sediment-associated nickel exposure. In Chapter 3, a whole-sediment toxicity test for the tropical marine benthic diatom *C. closterium* was adapted and utilised to generate nickel ecotoxicity effects data for sediments with different physicochemical properties. This diatom was, however, found to be relatively tolerant to sediment nickel exposure. No toxicity based on chlorophyll-*a* concentration was observed in the sediment with the highest TOC content (5%) (72-h EC10 >4,300 mg/kg AE-Ni). In the sediments with low TOC content (≤1%), toxicity was only observed above 950 mg/kg AE-Ni (72-h EC10). The development of a whole-sediment toxicity test for the tropical snail *N. dorsatus* was

also attempted but initial experiments exposing this species to dissolved nickel indicated that the juvenile and adult life stages of this species were also relatively tolerant to nickel (juvenile 21-d LOEC based on scavenging ability endpoint of 380 μg Ni/L).

There remains a need to find test species that are both sensitive to nickel and ecologically relevant to the SEAM region that are suitable for the development of whole-sediment toxicity test methods. Local representation of species from SEAM is important for a number of reasons, which include i) the great species diversity and richness of the region means there may be a greater likelihood of finding very sensitive and very tolerant species; ii) the high degree of endemism and small spatial distribution of the biota may mean that tolerance to environmental change is reduced, and iii) local species may have evolved different levels of contaminant sensitivity due to the influence of higher temperature and rainfall on contaminant bioavailability within tropical ecoregions. Recent investigations comparing dissolved nickel toxicity to pelagic temperate and tropical species have recommended the inclusion of as diverse a range of taxa as possible to ensure protection of sensitive species in both temperate and tropical ecosystems (Gissi et al., 2020; Peters et al., 2019; Stauber et al., 2021).

Ecotoxicology has an important role in site-specific sediment quality assessments, providing a direct link between contaminant exposure and toxicity. The development of standardised test methods for use in regulatory programs in SEAM would provide increased confidence in the reliability of ecotoxicity effects data used for environmental decision making. It is generally agreed that a suite of toxicity tests from different trophic levels is required to characterise the potential for toxicity due to different organism sensitivities and exposure pathways (Simpson and Kumar, 2015). The absence of ecologically relevant test species and whole-sediment toxicity testing methods for the SEAM region also creates a barrier to the generation of ecotoxicological effects data for establishing region appropriate SQGs. For example, the use of probabilistic tools like the SSD for deriving thresholds, from which SQGs can be derived (Schlekat et al., 2016; Simpson et al., 2011; Vangheluwe et al., 2013) is not possible for nickel in tropical estuarine and marine sediments. For waters in Australia and New Zealand, biological effects data for at least five species that belong to at least four taxonomic groups is required in an SSD for the development of water quality guideline values (Warne et al., 2018). However, Warne et al. (2018) recommend using biological effects data from at least eight species, with more than 15 species considered optimal. For sediments there is no guidance available on the number of species and taxonomic groups that are needed to represent an adequate database of benthic species for the determination of reliable sediment effects thresholds. However emphasis on the collection of chronic data is recommended as these are most appropriate for deriving guideline values which seek to protect against harmful effects from long-term exposure (Warne et al., 2018). Schlekat et al. (2016) applied the SSD approach to chronic nickel effects data for eight benthic freshwater species belonging to four different phyla (i.e., oligochaetes, molluscs, crustaceans, and insects) with different feeding habitats and ecological niches. The result was a threshold value of 136 mg Ni/kg which was considered to be

broadly protective for freshwater benthic communities based on the comparison to effects measured in benthic communities in the field (Costello et al., 2011; Schlekat et al., 2016). While there are few examples of the application of the SSD approach for SQG derivation for metals (Schlekat et al., 2016; Simpson et al., 2011; Vangheluwe et al., 2013), generally the approach has not been feasible due to the limited range of species available for toxicity tests and ecotoxicological effects data for sediment contaminants more broadly (Batley et al., 2005).

Finding tropical test species that are sensitive to nickel and amendable to handling for the development of chronic whole-sediment toxicity tests requires a significant input of resources which can be both practically and financially hindering. A more strategic approach to the selection of potential whole-sediment toxicity test species is thus required. In Chapter 6 the potential usefulness of the metabarcoding technique for informing species selection was demonstrated. The technique allowed for both broad taxonomic coverage and high taxonomic resolution for the investigation of potentially sensitive taxa. Putative nickel sensitive organisms within estuarine and marine sediments of Vavouto Bay (New Caledonia) were identified and this has the potential to provide more informed species selection for test development. Effort could be made to isolate some of these organisms from control sites within the SEAM region and to investigate their ease of handling, ability to be cultured in the laboratory and sensitivity to nickel.

7.1.2 Chemistry: the role of passive sampling for the advancement of chemical exposure assessment

The passive sampling technique of DGT has been gaining popularity over the past decade as a time-integrated *in situ* tool for evaluating potentially bioavailable chemical exposures via diffusive (both gradient and biologically mediated) and advective fluxes suspected of contributing to biological effects (Amato et al., 2015, 2014; He et al., 2018; Simpson et al., 2012b; Yin et al., 2014). Given the paucity of whole-sediment toxicity test methods and ecotoxicity data for the SEAM region (Gissi et al., 2016) the suitability of DGT as a surrogate technique for determining exposure and uptake of nickel from estuarine and marine sediments to aquatic organisms was explored.

Environmentally relevant species are the preferred choice for elucidating the link between exposure and effects, however, when not available, the use of surrogate test species with appropriate contaminant exposure pathways may be necessary. In Chapter 4, the temperate epibenthic estuarine/marine amphipod *M. plumulosa* was utilised for investigating DGT as an alternative tool for sediment quality assessment and investigating the role of sediment properties in influencing nickel bioavailability and toxicity to aquatic organisms. *M. plumulosa* was an appropriate choice for this research as it has a known sensitivity to metal contaminants (Simpson and Spadaro, 2011) and covers the important exposure pathways for nickel-contaminated sediments. The literature suggested that a potentially important exposure pathway for benthic estuarine and marine biota inhabiting nickel-rich sediments

offshore from lateritised ultramafic regoliths is via benthic fluxes from the desorption or dissolution of nickel from the solid phase into a more mobile and bioavailable dissolved phase (Ambatsian et al., 1997; Marchand et al., 2012; Noël et al., 2017, 2015). *M. plumulosa* resides at the sediment-water interface and is subsequently exposed to contaminants in the surficial sediments as well as those transferred to the dissolved phase in the pore water and overlying water. For this reason, the DGT piston was placed so that the centre of the disc aligned with the sediment-water interface. It would be appropriate for others to consider the same approach if their study is targeted at epibenthic species, or to push the piston deeper into the sediment so that the top of the disc aligns with the sediment-water interface if their study is targeted at sediment dwelling invertebrates.

Although a previous body of research has investigated the effect of sediment properties on nickel bioavailability with benthic organisms, the focus had been on freshwater species and environments (Custer, 2012). The current body of work aimed to examine the influence of sediment properties relevant to the SEAM region on nickel bioavailability and toxicity to benthic estuarine and marine organisms. For this purpose, highly organic sediments representative of the mangrove habitat through to highly carbonate-based sediments representative of the lagoon habitat were selected for nickel spiking, as well as nickel-rich lateritic sediments from the SEAM region.

My research found that nickel bioavailability was lower in sediments with greater TOC, iron and manganese hydrous oxides, clay content and percentage of fine particles. This work confirms the importance of considering iron and manganese hydrous oxides for controlling the bioavailability of nickel which dominate at the sediment-water interface in oxic waters (Costello et al., 2011). The work also demonstrated the dissolution of nickel from solid phases at the sediment-water interface and its role in influencing toxicity to *M. plumulosa*. In particular, while nickel in the field-collected sediments collected offshore from lateritised ultramafic regoliths within mangrove habitat were predominately in the mineralised form, the DGT technique demonstrated the release of dissolved nickel to the pore water and overlying water confirming the importance of nickel dissolution from sediments in the SEAM region as an exposure pathway to benthic organisms.

Overall, the suitability of DGT as a surrogate technique for determining nickel exposure and predicting toxicity to aquatic organisms from contaminated sediments was confirmed. The passive sampling technique has the potential to improve the mechanistic-based approach to sediment quality guideline derivation which is based on partitioning between solid and liquid phases (i.e., sediment particles and pore waters). The DGT technique accounts for the flux of metal at an oxic boundary (e.g., at the sediment-water interface and what would occur at the boundary of a burrow wall). It goes beyond the partitioning between pore waters because DGT actively captures the labile forms of metal that desorbs from the sediment. My research contributes to the growing body of research that suggests DGT could integrate exposure from dissolved and particulate phases (Amato et al., 2015, 2014; Simpson et al., 2012b) which would bridge the limitation of the current approach based on pore water partitioning.

Furthermore, the approach does not require normalising to organic carbon as the DGT can only absorb labile metal complexes. Based on the combined dataset of the three nickel-spiked sediments a DGT-labile nickel 10% effect concentration threshold of 50 (30-69) µg Ni/m^2/h was determined. The development of DGT-labile thresholds presents a promising research avenue for the SEAM region as a line of evidence given that there are no or few toxicity data available for determining biological effects on local species.

This body of research identified that DGT has great potential for measuring nickel bioavailability and identifying potential toxicity which could improve sediment quality assessments when regionally-relevant test species and whole-sediment toxicity test methods are absent. It should however be recognised that tools measuring exposure do not, by themselves, indicate effects. Ideally the application of the DGT technique should be assessed with more than one species covering different potential exposure pathways. Initial experiments were also performed with the temperate estuarine/marine benthic copepod *Nitocra spinipes* but due to poor culture health throughout the time period available for this phase of research, this species could not be pursued (data not reported as it did not meet quality control criteria). Further investigation with the use of additional benthic test species, with different exposure pathways, would assist in demonstrating the applicability of the DGT-technique and for refining the DGT-labile thresholds for sediment quality assessment by encompassing differences in species sensitivities to nickel exposure. For example, an aspirational target would be to combine the individual thresholds into an SSD to generate a hazard concentration for 5% of species and see how it compares to conventional derived SSDs. Field validation of the use of the DGT pistons rather than the DGT probes as an assessment tool for nickel availability at the sediment-water interface should also be undertaken and for sediments with a wider range of physicochemical properties and nickel enrichment concentrations.

In lateritic sediments, nickel enrichment rarely occurs alone but generally co-occurs with other metals including iron, manganese, cobalt, and chromium (Ambatsian et al., 1997; Dublet et al., 2012). In this thesis, the use of nickel-spiked sediments allowed for the isolation of nickel from other potential contaminants of concern. A limited number of field-collected nickel-contaminated sediments were also assessed and generally showed the co-occurring potential contaminants of concern, namely chromium and cobalt, to be less bioavailable based on chemical measures of bioavailability. The potential risk of metal exposure from nickel laterite mining to benthic marine biota, however, would benefit from laboratory-based manipulative experiments of other metals enriched in lateritised sediments, especially cobalt which has seldom been the focus of ecotoxicological studies, and their interactive effect with nickel. This would assist in interpreting the impacts of co-occurring contaminants to benthic organisms and determining causality to guide specific management actions.

7.1.3 Ecology: the role of field-based benthic community assessment

Laboratory-based manipulative experiments play an important role in sediment quality assessment, providing the ability to control environmental factors to facilitate the identification of cause and effect relationships. The limitations however may include the removal of natural conditions and integrity of the sediment leading to subsequent changes in contaminant dynamics at the sediment-water interface due to sediment sampling and or manipulation. Field-based experiments provide the most realistic contaminant dynamics and exposure conditions but at the sacrifice of experimental control and consequently the ability to identify causality is limited. Therefore, both types of assessment generally have a role to play in sediment quality assessment investigations.

Ecological assessment allows for the investigation of sediment contamination among the more complex dynamic ecological patterns and processes occurring in the natural environment, for example capturing the influence of trophic transfer of contaminants through predator-prey interactions or the avoidance of sediment contaminants by some benthic organisms. In Chapter 5, metabarcoding and field-based testing allowed the collective response of a broad suite of indigenous biota (including microbiota, microfauna and meiofauna) to contaminant metal exposure under ecologically relevant conditions. Multivariate statistical analyses were used to infer the role of measured environmental factors in influencing changes in community composition along the nickel concentration gradient. The use of the *in situ* nickel-concentration gradient means that there is the possibility that co-occurring contaminants or environmental factors may be contributing to the observed response. However, this is also true of the empirically-based SQGs (Long and Morgan, 1990; Long et al., 1995; Long and MacDonald, 1992; Macdonald et al., 1996) which are based on field ecological and laboratory ecotoxicological-effects data from mixed metal-exposures and currently used in several countries (Chapter 1, Table 1.3). In Chapter 5 significant changes in the eukaryote, diatom, and prokaryote community compositions were found to correlate most with the dilute-acid extractable concentration of nickel (AE-Ni) in the sediment. A threshold of change in the benthic eukaryote, diatom, and prokaryote community composition between 46 to 76 mg AE-Ni/kg was identified. Further investigation is required to discern whether the changes in community composition are linked to adverse outcomes for the benthic community at the individual and population level and provide validation for this threshold. Functional analysis of the prokaryote community dataset from this study also would provide insight into what impact the change in community composition may have on overall ecosystem functioning and health.

Chapter 5 has demonstrated the utility of the metabarcoding approach as a promising tool for providing broad scale taxonomic information on changes in community composition and biodiversity to assess effects of sediment contaminants along a nickel concentration gradient. Bridging the gap between the observed/measurable changes in community composition and effects on ecosystem functioning will go a long way to determining whether metabarcoding derived threshold values would be effective as

guideline values. However, there needs to be consideration of the theoretical level of protection that is used to generate guideline values using metabarcoding techniques. Conventional approaches to guideline development, such as the SSD, generally protect the hypothetical 1st or 5th percentile of species as generally there is only data for a handful of species available to generate the SSD. The metabarcoding derived threshold value presented in this thesis however encompasses a far greater portion of the population, capturing typical eukaryotic species that have been the focus of SSDs but also capturing species not previously considered for protection depending on the primers selected such as microbiota and meiofauna. As interest in this emerging technique grows, it is therefore important that further consideration is given to how the information can be applied to environmental management. In the interim the use of the threshold value to indicate where further investigation may be warranted is suggested.

7.1.4 Other lines of evidence: assessment of stressor related exposure

Suspended sediment exposure is a specific concern for the SEAM region that could have impacts on ecosystem health. In Chapter 6, a laboratory-based manipulative experiment was designed to address the specific concern of the potential risk of nickel-contaminated suspended sediment exposure on important coral reef habitat. Sediment-associated nickel was found to only present an increased risk above clean suspended sediment exposures when nickel was in a highly labile form (i.e. readily dissociated from the particulate phase to the dissolved phase). Nickel in lateritic sediments of the SEAM region is generally in a highly mineralised form and is therefore less likely to be bioavailable. Regardless, careful observation and monitoring regarding the nature and extent of suspended sediment events is considered important as suspended sediment itself can influence the physical and community structure of coral reefs (Browne et al., 2015a).

7.2 Application of these lines of evidence for sediment quality assessment in SEAM

This body of research has employed a diverse range of experimental and statistical methods to quantify the potential for adverse biological effects from sediment nickel exposure. While each of the different approaches provided some information on the relationship between sediment-associated nickel exposure and biological effects, as discussed above, none of the approaches alone were entirely satisfactory. Together however, these individual lines of evidence build up an understanding of the physical and biological condition of nickel-rich estuarine and coastal marine sediments. Integrative consideration of these multiple lines of evidence such as in a weight-of-evidence approach has been proven to be useful for the evaluation of risks posed by sediment contaminants (Chapman et al., 2002).

Within SEAM there are no defined risk assessment frameworks or guidelines for sediment quality assessment. This body of work has confirmed that a bioavailability-based approach should be employed for risk assessment of nickel-rich sediments of the SEAM region. High concentrations of total recoverable nickel have been reported in the estuarine and coastal marine sediments of SEAM (Table 1.1). Based on the chemical extraction of field contaminated sediments, a high proportion of this nickel may be in a highly mineralised form, with as little as 10% in a potentially more labile form (Chapter 4). It was also demonstrated that nickel bioavailability is likely to be greater in the lagoon sediments than the mangrove sediments. It is therefore recommended that the chemistry line of evidence for sediment quality assessment in SEAM should ideally investigate the bioavailability of sediment contaminants (e.g., the dilute-acid extractable metal concentration) and this value, rather than total metals, be used for screening assessments.

The Australian and New Zealand DGV and GV-high for nickel (equivalent to the ER-L and ER-M values used in other jurisdictions) in freshwater and estuarine/marine sediments is 21 mg/kg and 52 mg/kg, respectively (Australian and New Zealand Governments, 2018). Work by Schlekat et al. (2016) identified this empirically-based guideline value to be conservative for the protection of temperate freshwater benthic communities based on a comparison to the mechanistic-based guideline they derived for sediment-associated nickel. The relevance of screening criteria used in other jurisdictions for protecting estuarine and marine benthic communities in the SEAM region had not been previously explored. In the case of estuarine and marine sediments offshore from lateritised ultramafic regolith in New Caledonia, this body of research identified significant changes of the benthic community composition occurred between 46 and 76 mg AE-Ni/kg (Chapter 5). My work showed only a small percentage of the benthic community responded either positively or negatively along the nickel gradient which may suggest that the micro- and macro-biota of the SEAM region may be well adapted to the presence of nickel-rich sediments. However, despite the uncertainties involved in the derivation of the community change point threshold, the data suggests that there are sensitive species within the benthic community that responded within a similar nickel concentration range to those of the temperate environment derived using empirical SQGs. It is therefore recommended that 46 mg AE-Ni/kg be applied as an interim threshold value for estuarine and marine sediments offshore from lateritised ultramafic until further lines of evidence can contribute to a region-specific nickel-sediment quality guideline value.

This thesis has identified potentially nickel sensitive groups of organisms from the SEAM region that could be the focus of further investigation for the development of a whole-sediment toxicity test method (Chapter 5). It is considered important that this work is continued to assist in building an evidence base of nickel effects data for evaluating the suitability of the different threshold values presented in this thesis. It would be ideal to have an ecologically relevant test species and standardised whole-sediment toxicity test method that could be utilised for a toxicity line of evidence for sediment quality assessment

in the SEAM region. However, as I learned during this thesis and many researchers have learned before me, even when there is a significant investment of resources into the development of a region-specific robust test species and test method it does not always lead to success. Noting the difficulty and significant investment of resources it takes to produce new and robust whole sediment toxicity test methods, in the interim the use of standard temperature test species in sediment quality assessments within SEAM is warranted and investment into the verification of promising novel techniques such as DGT and DNA metabarcoding could prove to be the way forward.

The DGT technique was demonstrated to be a promising tool for the assessment of nickel bioavailability for sediment-associated nickel exposure in the SEAM region and is recommended as a suitable surrogate assessment tool. The DGT-labile threshold value derived with *M. plumulosa* (50 µg Ni/m^2/h) presents a starting point for indicating the potential for risk from sediment nickel exposure to benthic estuarine and marine biota, which can be refined through further species representation. Perhaps the greatest advantage of the DGT technique and reason to direct research efforts down this path, is its ability to detect locations with bioavailable contaminants without the need for the use of living organisms once thresholds have been established.

Given the climatic regime of the SEAM region, exposure of the benthic community to nickel-contaminated suspended sediment within the lagoon is an important consideration. Bioavailability was also found to be an important consideration for assessing the risk from nickel-contaminated suspended sediment exposure to coral (Chapter 6). A threshold value for particulate-bound nickel concentration of 60 mg/kg AE-Ni at a suspended sediment concentration of 30 mg/L was derived. It is recommended that exceedance of this value triggers further investigation of the risk to the benthic community or environmental managers and regulators consider management controls that can be applied to their operations to limit the removal and transport of material from their sites to the coastal environment.

7.3 Further opportunities for enhancing resource management in SEAM

It is important to acknowledge that scientific knowledge is not the only knowledge system that can be utilised when informing environmental risk assessment, decision making and policy formulation for natural resource management and sustainable development. Indigenous and local knowledge are also knowledge systems that should be integrated into such practices. Indigenous and local knowledge systems recognise important social-ecological connections and interrelations and encompass local natural resource management, historical and contemporary experiences, social norms, sociocultural governance structures and spiritual beliefs (Williams et al., 2020). The inclusion of indigenous and local knowledge into landscape affecting projects can support more equitable and sustainable use of land and ecosystem services (Williams et al., 2020). For example, local and indigenous knowledge may

encompass critical breeding areas for valuable fish stocks and other marine species, migration pathways of species, and the ability to provide early indication of significant environmental change.

In a region where the nickel mining and processing industry exerts particularly strong economic and political influence (Clarke and David, 2011), the inclusion of indigenous and local knowledge into environmental risk assessment within SEAM presents the opportunity to provide integrated management of natural resources to the satisfaction of the local communities, while also ensuring the protection of the outstanding natural heritage values of the mangrove and coral reef ecosystems of the region.

Chapter 8

8 General conclusions

This research aimed to provide risk assessment tools and an evidence-base applicable for sediment quality assessments of nickel-rich estuarine and marine sediments within the SEAM region. The outcomes of this research program with respect to the principal objectives of the study are outlined below:

(I) A whole sediment bioassay based on sub-lethal effects was adapted for use with the tropical benthic marine diatom *C. closterium* (Chapter 3). Ecotoxicity effects data relevant to the SEAM region was then derived for three nickel-spiked sediments and two nickel-rich field-collected sediments and the species was found to be relatively tolerant to nickel. A species relevant to SEAM with greater sensitivity to nickel is required to provide greater confidence that the use of a whole-sediment bioassay as a line of evidence in sediment quality assessment will identify sediments likely to have adverse effects. This research highlighted the need for a more strategic approach for selecting species for the development of new bioassays or alternative tools for investigating contaminated sediments in the absence of sensitive whole-sediment toxicity test methods relevant to the SEAM region.

(II) The epibenthic marine temperate amphipod *M. plumulosa* was used as a surrogate species to improve understanding of how sediment physicochemical properties relevant to the SEAM region influence nickel bioavailability and toxicity (Chapter 4). The suitability of DGT as a surrogate technique for determining nickel exposure and predicting toxicity to aquatic organisms from contaminated sediments was demonstrated. The development of DGT-labile thresholds presents a promising research avenue for the SEAM region as a line of evidence given there are no or few toxicity data available for determining biological effects on local species.

(III) Benthic community composition was determined in estuarine sediments offshore from a lateritised ultramafic regolith in New Caledonia using environmental DNA and metabarcoding. and was found to significantly correlate with the potentially bioavailable nickel (AE-Ni) present in the sediment (Chapter 5). The use of metabarcoding along a nickel concentration gradient was found to be a useful approach for the derivation of an environmental threshold value for nickel for the SEAM region based on changes in benthic community composition. The identification of putative nickel sensitive species also provides a strategic approach to selecting species for the development of whole sediment toxicity test methods.

(IV) The physiological response of the scleractinian coral, *A. muricata,* to 7-day exposure to either dissolved nickel, clean suspended sediment, or nickel-contaminated suspended sediment for seven days, followed by a seven-day recovery period was investigated (Chapter 6). Sediment-associated nickel was found to only present an increased risk above clean suspended sediment exposure when the sediment-associated nickel was highly labile (i.e., readily dissociated from

the particulate phase to the dissolved phase). This information will help identify when management controls may be required to limit the generation of suspended nickel-contaminated material.

The development of region-specific risk assessment tools and ecotoxicological exposure and effects data is required to provide environmental managers and regulators, who hold the responsibility for the management of mining activities, with a suitable evidence base to make informed decisions. This is vital for the responsible development of lateritic nickel deposits and effective implementation of environmental controls. Through this research I have established a number of threshold values, which if exceeded, may indicate the potential for adverse effects and should prompt further investigation of the risk to the environment. Overall, this body of research provides timely information for progressing sediment risk assessment approaches within SEAM to reduce the potential impact that the intensification of nickel laterite mining may have on tropical coastal ecosystems. The knowledge presented in this thesis should be used in combination with indigenous and local knowledge as only through integrated efforts will responsible economic efficiency, social equity and ecological sustainability be achieved.

References

Adams, M.S., Stauber, J.L., 2008. Marine whole sediment toxicity tests for use in temperate and tropical australian environments: current status. *Australas. J. Ecotoxicol.* 14, 155.

Adams, M.S., Stauber, J.L., 2004. Development of a whole-sediment toxicity test using a benthic marine microalga. *Environ. Toxicol. Chem*. 23, 1957–1968.

Adams, W., Blust, R., Dwyer, R., Mount, D., Nordheim, E., Rodriguez, P.H., Spry, D., 2020. Bioavailability assessment of metals in freshwater environments: a historical review. *Environ. Toxicol. Chem.* 39, 48–59. https://doi.org/10.1002/etc.4558

Alonso, Á., Valle-Torres, G., 2018. Feeding behavior of an aquatic snail as a simple endpoint to assess the exposure to cadmium. *Bull. Environ. Contam. Toxicol.* N. Y. 100, 82–88. http://dx.doi.org.ezproxy.uow.edu.au/10.1007/s00128-017-2230-3

Amato, E.D., Simpson, S.L., Belzunce-Segarra, M.J., Jarolimek, C.V., Jolley, D.F., 2015. Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates. *Environ. Sci. Technol.* 49, 14204–14212. https://doi.org/10.1021/acs.est.5b03655

Amato, E.D., Simpson, S.L., Jarolimek, C.V., Jolley, D.F., 2014. Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments. *Environ. Sci. Technol.* 48, 4485–4494. https://doi.org/10.1021/es404850f

Ambatsian, P., Fernex, F., Bernat, M., Parron, C., Lecolle, J., 1997. High metal inputs to closed seas: the New Caledonian lagoon. *J. Geochem. Explor.* 59, 59–74. https://doi.org/10.1016/S0375-6742(96)00020-9

Angel, B.M., Hales, L.T., Simpson, S.L., Apte, S.C., Chariton, A.A., Shearer, D.A., Jolley, D.F., 2010. Spatial variability of cadmium, copper, manganese, nickel and zinc in the Port Curtis Estuary, Queensland, Australia. *Mar. Freshw. Res.* 61, 170–183. https://doi.org/10.1071/MF09046

Ankley, G.T., Di Toro, D.M., Hansen, D.J., Berry, W.J., 1996. Technical basis and proposal for deriving sediment quality criteria for metals. *Environ. Toxicol. Chem.* 15, 2056–2066. https://doi.org/10.1002/etc.5620151202

Anthony, K.R.N., 2000. Enhanced particle-feeding capacity of corals on turbid reefs (Great Barrier Reef, Australia). *Coral Reefs 19*, 59–67. https://doi.org/10.1007/s003380050227

Anthony, K.R.N., Fabricius, K.E., 2000. Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *J. Exp. Mar. Biol. Ecol.* 252, 221–253. https://doi.org/10.1016/S0022-0981(00)00237-9

Anthony, K.R.N., Larcombe, P., 2000. Coral reefs in turbid waters: sediment-induced stresses in corals and likely mechanisms of adaptation. Proc. 9th Int. Coral Reef Symp. Bali Indones. 23–27.

ANZECC/ARMCANZ, 2000. Australian and New Zealand guidelines for fresh and marine water quality. Australian and New Zealand Environment and Conservation Council/ Agriculture and Resource Management Council of Australia and New Zealand, Canberra, ACT.

Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. https://doi.org/10.3354/ame01753

Arar, E.J., 1997. Method 446.0: in vitro determination of chlorophylls a, b, c + c and pheopigments in marine and freshwater algae by visible spectrophotometry. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-15/005.

Asadpour, Y.A., Manavi, P.N., Baniamam, M., 2013. Evaluating the bioaccumulation of nickel and vanadium and their effects on the growth of *Artemia urmiana* and *A. franciscana*. *Iran. J. Fish. Sci.* 10. http://jifro.ir/article-1-881-en.html

Ashraf, A., Saion, E., Gharibshahi, E., Yap, C.K., Kamari, H.M., Elias, M.S., Rahman, S.A., 2018. Distribution of heavy metals in core marine sediments of coastal east Malaysia by instrumental neutron activation analysis and inductively coupled plasma spectroscopy. *Appl. Radiat. Isot.* 132, 222–231. https://doi.org/10.1016/j.apradiso.2017.11.012

Atlas of Living Australia, 2020a. Species: *Nassarius* (Zeuxis) *dorsatus*. (Channelled Dog Whelk). Occurrence records map. Cited 2020 July 24, Available at https://bie.ala.org.au/species/urn:lsid:biodiversity.org.au:afd.taxon:2c16f4e9-4e18-4c0b-978d-4e1c7ff3371d

Atlas of Living Australia, 2020b. Species: *Nitzschia closterium* (Ehrenberg) W.Smith. Occurrence records map. Cited 2020 July 24, Available at https://bie.ala.org.au/species/NZOR-6-113261

Australian and New Zealand Governments, 2018. Australian and New Zealand guidelines for fresh and marine water quality. Australian and New Zealand Governments and Australian state and territory governments, Canberra ACT, Australia. Available at www.waterquality.gov.au/anz-guidelines.

Baird, D.J., Hajibabaei, M., 2012. Biomonitoring 2.0: a new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. *Mol. Ecol.* 21, 2039–2044. https://doi.org/10.1111/j.1365-294X.2012.05519.x

Baker, G.C., Smith, J.J., Cowan, D.A., 2003. Review and re-analysis of domain-specific 16S primers. *J. Microbiol. Methods* 55, 541–555. https://doi.org/10.1016/j.mimet.2003.08.009

Barrick, R., Becker, S., Brown, L., Beller, H., Pastorok, R., 1988. Sediment-quality-values refinement. Volume 1. 1988 update and evaluation of Puget Sound AET (Apparent Effects Threshold). Final report (No. PB-89-200398/XAB). PTI Environmental Services, Bellevue, WA (USA).

Batley, G.E., Simpson, S.L., 2015a. Chapter 2 - Sediment sampling, sample preparation and general analysis, in: Simpson, S.L., Batley, G.E. (Eds.), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic.

Batley, G.E., Simpson, S.L., 2015b. Chapter 1 - Introduction, in: Simpson, S.L., Batley, G.E. (Eds.), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic.

Batley, G.E., Stahl, R.G., Babut, M.P., Bott, T.L., Clark, J.R., Field, L.J., Ho, K.T., Mount, D.R., Swartz, R.C., Tessier, A., 2005. Chapter 3 - Scientific underpinnings of sediment quality quidelines, in: Wenning, R.J., Batley, G.E., Ingersoll, C.G., Moore, D.W. (Eds.), Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments. Presented at the Pellston Workshop on Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments, SETAC Press, Pensacola, FL.

Berry, W. j., Hansen, D. j., Boothman, W. s., Mahony, J. d., Robson, D. l., Di Toro, D. m., Shipley, B. p., Rogers, B., Corbin, J. m., 1996. Predicting the toxicity of metal-spiked laboratory sediments using acid-volatile sulfide and interstitial water normalizations. *Environ. Toxicol. Chem.* 15, 2067–2079. https://doi.org/10.1002/etc.5620151203

Bessell-Browne, P., Negri, A.P., Fisher, R., Clode, P.L., Duckworth, A., Jones, R., 2017. Impacts of turbidity on corals: the relative importance of light limitation and suspended sediments. *Mar. Pollut. Bull.* 117, 161–170. https://doi.org/10.1016/j.marpolbul.2017.01.050

Besser, J.M., Brumbaugh, W.G., Ingersoll, C.G., Ivey, C.D., Kunz, J.L., Kemble, N.E., Schlekat, C.E., Rogevich Garman, E., 2013. Chronic toxicity of nickel-spiked freshwater sediments: variation in toxicity among eight invertebrate taxa and eight sediments. *Environ. Toxicol. Chem.* 32, 2495–2506. https://doi.org/10.1002/etc.2271

Bielmyer, G.K., Grosell, M., Brix, K.V., 2006. Toxicity of silver, zinc, copper, and nickel to the copepod *acartia tonsa* exposed via a phytoplankton diet. *Environ. Sci. Technol.* 40, 2063–2068. https://doi.org/10.1021/es051589a

Binet, M.T., Adams, M.S., Gissi, F., Golding, L.A., Schlekat, C.E., Garman, E.R., Merrington, G., Stauber, J.L., 2018. Toxicity of nickel to tropical freshwater and sediment biota: a critical literature review and gap analysis. *Environ. Toxicol. Chem.* 37, 293–317. https://doi.org/10.1002/etc.3988

Birch, G.F., 2017. Determination of sediment metal background concentrations and enrichment in marine environments – A critical review. *Sci. Total Environ.* 580, 813–831. https://doi.org/10.1016/j.scitotenv.2016.12.028

Biscéré, T., Ferrier-Pagès, C., Grover, R., Gilbert, A., Rottier, C., Wright, A., Payri, C., Houlbrèque, F., 2018. Enhancement of coral calcification via the interplay of nickel and urease. *Aquat. Toxicol.* 200, 247–256. https://doi.org/10.1016/j.aquatox.2018.05.013

Biscéré, T., Lorrain, A., Rodolfo-Metalpa, R., Gilbert, A., Wright, A., Devissi, C., Peignon, C., Farman, R., Duvieilbourg, E., Payri, C., Houlbrèque, F., 2017. Nickel and ocean warming affect scleractinian coral growth. *Mar. Pollut. Bull.* 120, 250–258. https://doi.org/10.1016/j.marpolbul.2017.05.025

Blaxter, M., Mann, J., Chapman, T., Thomas, F., Whitton, C., Floyd, R., Abebe, E., 2005. Defining operational taxonomic units using DNA barcode data. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 360, 1935–1943. https://doi.org/10.1098/rstb.2005.1725

Blewett, T.A., Leonard, E.M., 2017. Mechanisms of nickel toxicity to fish and invertebrates in marine and estuarine waters. *Environ. Pollut.* 223, 311–322. https://doi.org/10.1016/j.envpol.2017.01.028

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W., de Bruyn, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol. Evol.* 29, 358–367. https://doi.org/10.1016/j.tree.2014.04.003

Bordez, L., Jourand, P., Ducousso, M., Carriconde, F., Cavaloc, Y., Santini, S., Claverie, J.M., Wantiez, L., Leveau, A., Amir, H., 2016. Distribution patterns of microbial communities in ultramafic landscape: a metagenetic approach highlights the strong relationships between diversity and environmental traits. *Mol. Ecol.* 25, 2258–2272. https://doi.org/10.1111/mec.13621

Borgmann, U., 2001. Quantification of bioavailable nickel in sediments and toxic thresholds to H*yalella azteca*. Environ. Pollut. https://doi.org/10.1016/s0269-7491(00)00076-2

Bowles, B., A., 1982. Nutrient criteria for inland waters. Environ. Stud. Ser. 394 Minist. Conserv. Melb. Vic.

Brix, K.V., Schlekat, C.E., Garman, E.R., 2017. The mechanisms of nickel toxicity in aquatic environments: An adverse outcome pathway analysis. *Environ. Toxicol. Chem.* 36, 1128–1137. https://doi.org/10.1002/etc.3706

Brown, B.E., 1997. Coral bleaching: causes and consequences. *Coral Reefs* 16, S129–S138. https://doi.org/10.1007/s003380050249

Brown, B.E., Tudhope, A.W., Le Tissier, M.D.A., Scoffin, T.P., 1991. A novel mechanism for iron incorporation into coral skeletons. *Coral Reefs* 10, 211–215. https://doi.org/10.1007/BF00336776

Browne, N.K., Tay, J., Todd, P.A., 2015a. Recreating pulsed turbidity events to determine coral–sediment thresholds for active management. *J. Exp. Mar. Biol. Ecol.* 466, 98–109. https://doi.org/10.1016/j.jembe.2015.02.010

Browne, N.K., Tay, J.K.L., Low, J., Larson, O., Todd, P.A., 2015b. Fluctuations in coral health of four common inshore reef corals in response to seasonal and anthropogenic changes in water quality. *Mar. Environ. Res.* 105, 39–52. https://doi.org/10.1016/j.marenvres.2015.02.002

Bruland, K.W., 1980. Oceanographic distributions of cadmium, zinc, nickel, and copper in the North Pacific. *Earth Planet. Sci. Lett.* 47, 176–198. https://doi.org/10.1016/0012-821X(80)90035-7

Brumbaugh, W.G., Besser, J.M., Ingersoll, C.G., May, T.W., Ivey, C.D., Schlekat, C.E., Garman, E.R., 2013. Preparation and characterization of nickel-spiked freshwater sediments for toxicity tests: toward more environmentally realistic nickel partitioning. *Environ. Toxicol. Chem.* 32, 2482–2494. https://doi.org/10.1002/etc.2272

Brune, A., Frenzel, P., Cypionka, H., 2000. Life at the oxic–anoxic interface: microbial activities and adaptations. *FEMS Microbiol. Rev.* 24, 691–710. https://doi.org/10.1111/j.1574-6976.2000.tb00567.x

Buchman, M.F., 2008. NOAA screening quick reference tables, NOAA OR&R Report 08-1. Seattle WA, Office of Response and Restoriation Division, National Oceanic and Atmospheric Admininstration, 34 pages.

Burke, L., Selig, E., Spalding, M., 2002. Reefs at risk in Southeast Asia. World Resources Institute, USA.

Butt, C.R.M., Cluzel, D., 2013. Nickel laterite ore deposits: weathered serpentinites. *Elements* 9, 123–128. https://doi.org/10.2113/gselements.9.2.123

Byrne, R.H., 2002. Inorganic speciation of dissolved elements in seawater: the influence of pH on concentration ratios. *Geochem. Trans.* 3, 11–16. https://doi.org/10.1039/b109732f

Caesar, M., Grandcolas, P., Pellens, R., 2017. Outstanding micro-endemism in New Caledonia: more than one out of ten animal species have a very restricted distribution range. *PLOS ONE* 12, e0181437. https://doi.org/10.1371/journal.pone.0181437

Campana, O., Simpson, S.L., Spadaro, D.A., Blasco, J., 2012. Sub-lethal effects of copper to benthic invertebrates explained by sediment properties and dietary exposure. *Environ. Sci. Technol.* 46, 6835–6842. https://doi.org/10.1021/es2045844

Cempel, M., Nikel, G., 2006. Nickel: A review of its sources and environmental toxicology. *Pol. J. Environ. Stud.* 15, 375–382.

Chandler, G.T., Schlekat, C.E., Garman, E.R., He, L., Washburn, K.M., Stewart, E.R., Ferry, J.L., 2014. Sediment nickel bioavailability and toxicity to estuarine crustaceans of contrasting bioturbative behaviors – an evaluation of the SEM-AVS paradigm. *Environ. Sci. Technol.* 48, 12893–12901. https://doi.org/10.1021/es5025977

Chapman, P.M., 1997. Acid volatile sulfides, equilibrium partitioning, and hazardous waste site sediments. *Environ. Manage.* 21, 197–201. https://doi.org/10.1007/s002679900018

Chapman, P.M., 1995. Ecotoxicology and pollution - Key issues. Mar. Pollut. Bull., Selected Papers from the International Conference on Marine Pollution and Ecotoxicology 31, 167–177. https://doi.org/10.1016/0025-326X(95)00101-R

Chapman, P.M., Allard, P.J., Vigers, G.A., 1999. Development of sediment quality values for Hong Kong special administrative region: a possible model for other jurisdictions. *Mar. Pollut. Bull.* 38, 161–169. https://doi.org/10.1016/S0025-326X(98)00162-3

Chapman, P.M., Anderson, J., 2005. A decision-making framework for sediment contamination. Integr. Environ. *Integr. Environ. Assess. Manag.* 1, 163–173.

Chapman, P.M., McDonald, B.G., Lawrence, G.S., 2002. Weight-of-evidence issues and frameworks for sediment quality (and other) assessments. *Hum. Ecol. Risk Assess. Int. J.* 8, 1489–1515. https://doi.org/10.1080/20028091057457

Chapman, P.M., Wang, F., Janssen, C., Persoone, G., Allen, H.E., 1998. Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. *Can. J. Fish. Aquat. Sci.* 55, 2221–2243. https://doi.org/10.1139/f98-145

Chariton, A.A., Court, L.N., Hartley, D.M., Colloff, M.J., Hardy, C.M., 2010. Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. *Front. Ecol. Environ.* 8, 233–238. https://doi.org/10.1890/090115

Chariton, A.A., Pettigrove, V.J., Baird, D.J., 2015a. Chapter 7 - Ecological assessment, in: Simpson, S.L., Batley, G.E. (Eds.), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic.

Chariton, A.A., Stephenson, S., Morgan, M.J., Steven, A.D.L., Colloff, M.J., Court, L.N., Hardy, C.M., 2015b. Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. *Environ. Pollut.* 203, 165–174. https://doi.org/10.1016/j.envpol.2015.03.047

Cheung, S.G., Tai, K.K., Leung, C.K., Siu, Y.M., 2002. Effects of heavy metals on the survival and feeding behaviour of the sandy shore scavenging gastropod *Nassarius festivus* (Powys). *Mar. Pollut. Bull.* 45, 107–113. https://doi.org/10.1016/S0025-326X(01)00324-1

Chevillotte, V., Chardon, D., Beauvais, A., Maurizot, P., Colin, F., 2006. Long-term tropical morphogenesis of New Caledonia (Southwest Pacific): importance of positive epeirogeny and climate change. *Geomorphology* 81, 361–375. https://doi.org/10.1016/j.geomorph.2006.04.020

Chowdhury, M.J., Bucking, C., Wood, C.M., 2008a. Is nickel an essential metal for aquatic animals? *Integr. Environ. Assess. Manag.* 4, 266–267. https://doi.org/10.1002/ieam.5630040219

Chowdhury, M.J., Bucking, C., Wood, C.M., 2008b. Pre-exposure to waterborne nickel downregulates gastrointestinal nickel uptake in rainbow trout: indirect evidence for nickel essentiality. *Environ. Sci. Technol.* 42, 1359–1364. https://doi.org/10.1021/es071889n

Clarke, K.R., Gorley, R.N., 2015. PRIMER v7: User Manual/Tutorial PRIMER-E: Plymouth, UK.

Clarke, P., David, C., 2011. New provincial environmental legislation in New Caledonia: continuity and reform in environmental governance in a French Pacific Territory. *Asia Pac. J. Environ. Law*. https://hal.archives-ouvertes.fr/hal-02117052

Clavier, J., Chardy, P., Chevillon, C., 1995. Sedimentation of particulate matter in the south-west lagoon of New Caledonia: spatial and temporal patterns. *Estuar. Coast. Shelf Sci.* 40, 281–294. https://doi.org/10.1016/S0272-7714(05)80011-3

Cleveland, D., Brumbaugh, W.G., MacDonald, D.D., 2017. A comparison of four porewater sampling methods for metal mixtures and dissolved organic carbon and the implications for sediment toxicity evaluations. *Environ. Toxicol. Chem.* 36, 2906–2915. https://doi.org/10.1002/etc.3884

Cloran, C.E., Burton, G.A., Hammerschmidt, C.R., Taulbee, W.K., Custer, K.W., Bowman, K.L., 2010. Effects of suspended solids and dissolved organic carbon on nickel toxicity. *Environ. Toxicol. Chem.* 29, 1781–1787. https://doi.org/10.1002/etc.226

Coates-Marnane, J., Olley, J., Burton, J., Grinham, A., 2016. The impact of a high magnitude flood on metal pollution in a shallow subtropical estuarine embayment. *Sci. Total Environ.* 569–570, 716–731. https://doi.org/10.1016/j.scitotenv.2016.06.193

Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A., Kuske, C.R., Tiedje, J.M., 2014. Ribosomal database project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* 42, D633–D642. https://doi.org/10.1093/nar/gkt1244

Cordier, T., Alonso-Sáez, L., Apothéloz-Perret-Gentil, L., Aylagas, E., Bohan, D.A., Bouchez, A., Chariton, A., Creer, S., Frühe, L., Keck, F., Keeley, N., Laroche, O., Leese, F., Pochon, X., Stoeck, T., Pawlowski, J., Lanzén, A., 2020. Ecosystems monitoring powered by environmental genomics: a review of current strategies with an implementation roadmap. *Mol. Ecol.* 15472. https://doi.org/10.1111/mec.15472

Costello, D.M., Burton, G.A., Hammerschmidt, C.R., Rogevich, E.C., Schlekat, C.E., 2011. Nickel phase partitioning and toxicity in field-deployed sediments. *Environ. Sci. Technol.* 45, 5798–5805. https://doi.org/10.1021/es104373h

Costello, D.M., Burton, G.A., Hammerschmidt, C.R., Taulbee, W.K., 2012. Evaluating the performance of diffusive gradients in thin films for predicting Ni sediment toxicity. *Environ. Sci. Technol.* 46, 10239–10246. https://doi.org/10.1021/es302390m

Costello, D.M., Hammerschmidt, C.R., Burton, G.A., 2016. Nickel partitioning and toxicity in sediment during aging: variation in toxicity related to stability of metal partitioning. *Environ. Sci. Technol.* 50, 11337–11345. https://doi.org/10.1021/acs.est.6b04033

Croll, R.P., 1983. Gastropod chemoreception. *Biol. Rev.* 58, 293–319. https://doi.org/10.1111/j.1469-185X.1983.tb00391.x

Cunning, R., Ritson-Williams, R., Gates, R.D., 2016. Patterns of bleaching and recovery of *Montipora capitata* in Kāneʻohe Bay, Hawaiʻi, USA. *Mar. Ecol. Prog. Ser.* 551, 131–139. https://doi.org/10.3354/meps11733

Cunningham, L., Raymond, B., Snape, I., Riddle, M.J., 2005. Benthic diatom communities as indicators of anthropogenic metal contamination at Casey Station, Antarctica. *J. Paleolimnol.* 33, 499–513. https://doi.org/10.1007/s10933-005-0814-0

Cuong, D.T., Bayen, S., Wurl, O., Subramanian, K., Shing Wong, K.K., Sivasothi, N., Obbard, J.P., 2005. Heavy metal contamination in mangrove habitats of Singapore. *Mar. Pollut. Bull.* 50, 1732–1738. https://doi.org/10.1016/j.marpolbul.2005.09.008

Custer, K.W., 2012. Factors controlling nickel bioavailability and effects on benthic invertebrates in hardwater freshwater streams. Doctor of Philosophy Thesis, Wright State University, Dayton, Unites States of America.

Custer, K.W., Hammerschmidt, C.R., Burton Jr., G.A., 2016a. Nickel toxicity to benthic organisms: The role of dissolved organic carbon, suspended solids, and route of exposure. *Environ. Pollut.* 208, Part B, 309–317. https://doi.org/10.1016/j.envpol.2015.09.045

Custer, K.W., Kochersberger, J.P., Anderson, P.D., Fetters, K.J., Hummel, S., Burton, G.A., 2016b. Macroinvertebrate responses to nickel in multisystem exposures. *Environ. Toxicol. Chem.* 35, 101–114. https://doi.org/10.1002/etc.3157

Dafforn, K.A., Baird, D.J., Chariton, A.A., Sun, M.Y., Brown, M.V., Simpson, S.L., Kelaher, B.P., Johnston, E.L., 2014. Faster, higher and stronger? The pros and cons of molecular faunal data for assessing ecosystem condition, in: Advances in Ecological Research. Elsevier, pp. 1–40. https://doi.org/10.1016/B978-0-08-099970-8.00003-8

Dalto, A.G., Grémare, A., Dinet, A., Fichet, D., 2006. Muddy-bottom meiofauna responses to metal concentrations and organic enrichment in New Caledonia South-West Lagoon. *Estuar. Coast. Shelf Sci.* 67, 629–644. https://doi.org/10.1016/j.ecss.2006.01.002

Dalvi, A.D., Bacon, W.G., Osborne, R.C., 2004. The past and the future of nickel laterites, in: PDAC 2004 International Convention, Trade Show & Investors Exchange. Toronto: The prospectors and Developers Association of Canada, pp. 1–27.

Das, S., Khangarot, B.S., 2011. Bioaccumulation of copper and toxic effects on feeding, growth, fecundity and development of pond snail *Lymnaea luteola* L. *J. Hazard. Mater.* 185, 295–305. https://doi.org/10.1016/j.jhazmat.2010.09.033

Davison, W., 2016. Diffusive gradients in thin-films for environmental measurements. Cambridge University Press, Cambridge, UK.

Davison, W., Zhang, H., 2012. Progress in understanding the use of diffusive gradients in thin films (DGT) – back to basics. *Environ. Chem.* 9, 1–13. https://doi.org/10.1071/EN11084

Davison, W., Zhang, H., 1994. In situ speciation measurements of trace components in natural waters using thin-film gels. *Nature* 367, 546–548. https://doi.org/10.1038/367546a0

de Melo Gurgel, P., Navoni, J.A., de Morais Ferreira, D., do Amaral, V.S., 2016. Ecotoxicological water assessment of an estuarine river from the Brazilian Northeast, potentially affected by industrial wastewater discharge. *Sci. Total Environ.* 572, 324–332. https://doi.org/10.1016/j.scitotenv.2016.08.002

Defew, L.H., Mair, J.M., Guzman, H.M., 2005. An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. *Mar. Pollut. Bull.* 50, 547–552. https://doi.org/10.1016/j.marpolbul.2004.11.047

Denton, G.R.W., Burdon-Jones, C., 1986. Trace metals in corals from the Great Barrier Reef. *Mar. Pollut. Bull.* 17, 209–213. https://doi.org/10.1016/0025-326X(86)90602-8

Denton, G.R.W., Morrison, R.J., 2009. The impact of a rudimentary landfill on the trace metal status of Pago Bay, Guam. *Mar. Pollut. Bull.* 58, 150–162. https://doi.org/10.1016/j.marpolbul.2008.09.015

Denton, G.R.W., Morrison, R.J., Bearden, B.G., Houk, P., Starmer, J.A., Wood, H.R., 2009. Impact of a coastal dump in a tropical lagoon on trace metal concentrations in surrounding marine biota: a case study from Saipan, Commonwealth of the Northern Mariana Islands (CNMI). *Mar. Pollut. Bull.* 58, 424–431. https://doi.org/10.1016/j.marpolbul.2008.11.029

Deschaseaux, E.S.M., Deseo, M.A., Shepherd, K.M., Jones, G.B., Harrison, P.L., 2013. Air blasting as the optimal approach for the extraction of antioxidants in coral tissue. *J. Exp. Mar. Biol. Ecol.* 448, 146–148. https://doi.org/10.1016/j.jembe.2013.07.002

Desrosiers, C., Leflaive, J., Eulin, A., Ten-Hage, L., 2013. Bioindicators in marine waters: benthic diatoms as a tool to assess water quality from eutrophic to oligotrophic coastal ecosystems. *Ecol. Indic*. 32, 25–34. https://doi.org/10.1016/j.ecolind.2013.02.021

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical Basis. *Environ. Toxicol. Chem.* 20, 2383–2396. https://doi.org/10.1002/etc.5620201034

Di Toro, D.M., Mahony, J.D., Hansen, D.J., Scott, K.J., Carlson, A.R., Ankley, G.T., 1992. Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. *Environ. Sci. Technol.* 26, 96–101. https://doi.org/10.1021/es00025a009

Di Toro, D.M., Mahony, J.D., Hansen, D.J., Scott, K.J., Hicks, M.B., Mayr, S.M., Redmond, M.S., 1990. Toxicity of cadmium in sediments: the role of acid volatile sulfide. *Environ. Toxicol. Chem.* 9, 1487–1502. https://doi.org/10.1002/etc.5620091208

Di Toro, D.M., McGrath, J.A., Hansen, D.J., Berry, W.J., Paquin, P.R., Mathew, R., Wu, K.B., Santore, R.C., 2005. Predicting sediment metal toxicity using a sediment biotic ligand model: methodology and initial application. *Environ. Toxicol. Chem.*. 24, 2410–27.

DiBattista, J.D., Reimer, J.D., Stat, M., Masucci, G.D., Biondi, P., De Brauwer, M., Wilkinson, S.P., Chariton, A.A., Bunce, M., 2020. Environmental DNA can act as a biodiversity barometer of anthropogenic pressures in coastal ecosystems. *Sci. Rep.* 10, 8365. https://doi.org/10.1038/s41598-020-64858-9

Dublet, G., Juillot, F., Morin, G., Fritsch, E., Fandeur, D., Ona-Nguema, G., Brown, G.E., 2012. Ni speciation in a New Caledonian lateritic regolith: a quantitative X-ray absorption spectroscopy investigation. *Geochim. Cosmochim. Acta* 95, 119–133. https://doi.org/10.1016/j.gca.2012.07.030

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* 10, 996. https://doi.org/10.1038/NMETH.2604

Erftemeijer, P.L.A., Riegl, B., Hoeksema, B.W., Todd, P.A., 2012. Environmental impacts of dredging and other sediment disturbances on corals: a review. *Mar. Pollut. Bull.* 64, 1737–1765. https://doi.org/10.1016/j.marpolbul.2012.05.008

Ezekwe, C.I., Edoghotu, M.I., 2015. Water quality and environmental health indicators in the Andoni River estuary, Eastern Niger Delta of Nigeria. Environ. *Earth Sci.* 74, 6123–6136. https://doi.org/10.1007/s12665-015-4635-9

Fabricius, K.E., 2005. Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. *Mar. Pollut. Bull.* 50, 125–146. https://doi.org/10.1016/j.marpolbul.2004.11.028

Fandeur, D., Juillot, F., Morin, G., Olivi, L., Cognigni, A., Webb, S.M., Ambrosi, J.-P., Fritsch, E., Guyot, F., Brown, Jr., Gordon E., 2009. XANES evidence for oxidation of Cr(III) to Cr(VI) by Mn-oxides in a lateritic regolith developed on serpentinized ultramafic rocks of New Caledonia. *Environ. Sci. Technol.* 43, 7384–7390. https://doi.org/10.1021/es900498r

Fernandez, J.-M., Meunier, J.-D., Ouillon, S., Moreton, B., Douillet, P., Grauby, O., 2017. Dynamics of suspended sediments during a dry season and their consequences on metal transportation in a coral reef lagoon impacted by mining activities, New Caledonia. *Water* 9, 338. https://doi.org/10.3390/w9050338

Fernandez, J.-M., Ouillon, S., Chevillon, C., Douillet, P., Fichez, R., Gendre, R.L., 2006. A combined modelling and geochemical study of the fate of terrigenous inputs from mixed natural and mining sources in a coral reef lagoon (New Caledonia). *Mar. Pollut. Bull.* 52, 320–331. https://doi.org/10.1016/j.marpolbul.2005.09.010

Florence, T.M., Stauber, J.L., Ahsanullah, M., 1994. Toxicity of nickel ores to marine organisms. *Sci. Total Environ*. 148, 139–155. https://doi.org/10.1016/0048-9697(94)90391-3

Flores, F., Hoogenboom, M.O., Smith, L.D., Cooper, T.F., Abrego, D., Negri, A.P., 2012. Chronic exposure of corals to fine sediments: lethal and sub-lethal impacts. *PLOS ONE* 7, 1–12. https://doi.org/10.1371/journal.pone.0037795

Franklin, N.M., Adams, M.S., Stauber, J.L., 2005. Improved methods of conducting microalgal bioassays using flow cytometry, in: Techniques in Aquatic Toxicology, Volume 2. CRC Press.

Franklin, N.M., Stauber, J.L., Apte, S.C., Lim, R.P., 2002. Effect of initial cell density on the bioavailability and toxicity of copper in microalgal bioassays. *Environ. Toxicol. Chem*. 21, 742–751. https://doi.org/10.1002/etc.5620210409

Geoscience Australia, 2018. Australian mineral facts - nickel. URL https://www.ga.gov.au/education/classroom-resources/minerals-energy/australian-mineral-facts/nickel (accessed 8.15.20).

Gerhard, D., Bremer, M., Ritz, C., 2014. Estimating marginal properties of quantitative real-time PCR data using nonlinear mixed models. *Biometrics* 70, 247–254. https://doi.org/10.1111/biom.12124

Gillmore, M.L., Gissi, F., Golding, L.A., Stauber, J.L., Reichelt-Brushett, A.J., Severati, A., Humphrey, C.A., Jolley, D.F., 2020. Effects of dissolved nickel and nickel-contaminated suspended sediment on the scleractinian coral, *Acropora muricata*. *Mar. Pollut. Bull.* 152, 110886. https://doi.org/10.1016/j.marpolbul.2020.110886

Gillmore, M.L., Golding, L.A., Angel, B.M., Adams, M.S., Jolley, D.F., 2016. Toxicity of dissolved and precipitated aluminium to marine diatoms. *Aquat. Toxicol.* 174, 82–91. https://doi.org/10.1016/j.aquatox.2016.02.004

Gillmore, M.L., Golding, L.A., Chariton, A.A., Stauber, J.L., Stephenson, S., Gissi, F., Greenfield, P., Juillot, F., Jolley, D.F., 2021a. Metabarcoding reveals changes in benthic eukaryote and prokaryote community composition along a tropical marine sediment nickel gradient. *Environ. Toxicol. Chem*. https://doi.org/10.1002/etc.5039

Gillmore, M.L., Price, G.A.V., Golding, L.A., Stauber, J.L., Adams, M.S., Simpson, S.L., Smith, R.E.W., Jolley, D.F., 2021b. The diffusive gradients in thin films technique predicts sediment nickel toxicity to the amphipod *Melita plumulosa*. *Environ. Toxicol. Chem.* https://doi.org/10.1002/etc.4971

Gissi, F., 2019. Biological effects of nickel on tropical marine biota to underpin the development of water quality guidelines for metals. Doctor of Philosophy Thesis, University of Wollongong, Wollongong, Australia.

Gissi, F., Reichelt-Brushett, A.J., Chariton, A.A., Stauber, J.L., Greenfield, P., Humphrey, C., Salmon, M., Stephenson, S.A., Cresswell, T., Jolley, D.F., 2019. The effect of dissolved nickel and copper on the adult coral *Acropora muricata* and its microbiome. *Environ. Pollut.* 250, 792–806. https://doi.org/10.1016/j.envpol.2019.04.030

Gissi, F., Stauber, J., Reichelt-Brushett, A., Harrison, P.L., Jolley, D.F., 2017. Inhibition in fertilisation of coral gametes following exposure to nickel and copper. *Ecotoxicol. Environ. Saf.* 145, 32–41. https://doi.org/10.1016/j.ecoenv.2017.07.009

Gissi, F., Stauber, J.L., Binet, M.T., Golding, L.A., Adams, M.S., Schlekat, C.E., Garman, E.R., Jolley, D.F., 2016. A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. *Environ. Pollut.* 218, 1308–1323. https://doi.org/10.1016/j.envpol.2016.08.089

Gissi, F., Stauber, J.L., Binet, M.T., Trenfield, M.A., Van Dam, J.W., Jolley, D.F., 2018. Assessing the chronic toxicity of nickel to a tropical marine gastropod and two crustaceans. *Ecotoxicol. Environ. Saf.* 159, 284–292. https://doi.org/10.1016/j.ecoenv.2018.05.010

Gissi, F., Wang, Z., Batley, G.E., Leung, K.M.Y., Schlekat, C.E., Garman, E.R., Stauber, J.L., 2020. Deriving a chronic guideline value for nickel in tropical and temperate marine waters. *Environ. Toxicol. Chem.* 39, 2540–2551. https://doi.org/10.1002/etc.4880

Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms. *Can. J. Microbiol*. 8, 229–239. https://doi.org/10.1016/0011-7471(62)90036-0

Hajimad, T., Vedamanikam, V.J., 2013. Temperature effects on the toxicity of four trace metals to adult spotted Babylonia snails (*Babylonia areolata*). *Toxicol. Environ. Chem.* 95, 1380–1387. https://doi.org/10.1080/02772248.2013.864450

Hall, N.E., Fairchild, J.F., La Point, T.W., Heine, P.R., Ruessler, D.S., Ingersoll, C.G., 1996. Problems and recommendations in using algal toxicity testing to evaluate contaminated sediments. *J. Gt. Lakes Res.* 22, 545–556. https://doi.org/10.1016/S0380-1330(96)70979-6

Hardefeldt, J.M., Reichelt-Brushett, A.J., 2015. Unravelling the role of zooxanthellae in the uptake and depuration of an essential metal in *Exaiptasia pallida*; an experiment using a model cnidarian. *Mar. Pollut. Bull.* 96, 294–303. https://doi.org/10.1016/j.marpolbul.2015.04.055

Hardy, C.M., Krull, E.S., Hartley, D.M., Oliver, R.L., 2010. Carbon source accounting for fish using combined DNA and stable isotope analyses in a regulated lowland river weir pool. *Mol. Ecol.* 19, 197–212. https://doi.org/10.1111/j.1365-294X.2009.04411.x

Harland, A.D., Brown, B.E., 1989. Metal tolerance in the scleractinian coral *Porites lutea*. *Mar. Pollut. Bull.*, Pollution in the Far East 20, 353–357. https://doi.org/10.1016/0025-326X(89)90159-8

Harland, A.D., Nganro, N.R., 1990. Copper uptake by the sea anemone *Anemonia viridis* and the role of zooxanthellae in metal regulation. *Mar. Biol.* 104, 297–301. https://doi.org/10.1007/BF01313271

He, Y., Guo, C., Lv, J., Hou, S., Zhang, Yan, Zhang, Yuan, Xu, J., 2018. Predicting trace metal bioavailability to chironomids in sediments by diffusive gradients in thin films. *Sci. Total Environ.* 636, 134–141. https://doi.org/10.1016/j.scitotenv.2018.04.285

Hédouin, L., Bustamante, P., Fichez, R., Warnau, M., 2008. The tropical brown alga *Lobophora variegata* as a bioindicator of mining contamination in the New Caledonia lagoon: a field transplantation study. *Mar. Environ. Res.* 66, 438–444. https://doi.org/10.1016/j.marenvres.2008.07.005

Hédouin, L., Metian, M., Teyssié, J.-L., Fichez, R., Warnau, M., 2018. High contribution of the particulate uptake pathway to metal bioaccumulation in the tropical marine clam *Gafrarium pectinatum*. *Environ. Sci. Pollut. Res.* 25, 11206–11218. https://doi.org/10.1007/s11356-017-8562-z

Hédouin, L., Metian, M., Teyssié, J.-L., Oberhänsli, F., Ferrier-Pagès, C., Warnau, M., 2016. Bioaccumulation of ^{63}Ni in the scleractinian coral *Stylophora pistillata* and isolated *Symbiodinium* using radiotracer techniques. *Chemosphere* 156, 420–427. https://doi.org/10.1016/j.chemosphere.2016.04.097

Hédouin, L., Pringault, O., Bustamante, P., Fichez, R., Warnau, M., 2011. Validation of two tropical marine bivalves as bioindicators of mining contamination in the New Caledonia lagoon: field transplantation experiments. *Water Res.* 45, 483–496. https://doi.org/10.1016/j.watres.2010.09.002

Hintze, J., 2007. NCSS V07.1.21. NCSS LLC Kaysville Utah USA. www.ncss.com.

Hoegh-Guldberg, O., 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res*. 50, 839. https://doi.org/10.1071/MF99078

Hoeksema, B.W., 2007. Chapter 5 - Delineation of the Indo-Malayan centre of maximum marine biodiversity: the coral triangle, in: Renema, W. (Ed.), Biogeography, Time, and Place: Distributions, Barriers, and Islands, Topics In Geobiology. Springer Netherlands, Dordrecht, pp. 117–178. https://doi.org/10.1007/978-1-4020-6374-9_5

Hoitink, A.J.F., 2004. Tidally-induced clouds of suspended sediment connected to shallow-water coral reefs. *Mar. Geol.* 208, 13–31. https://doi.org/10.1016/j.margeo.2004.04.021

Holland, A., Stauber, J., Wood, C.M., Trenfield, M., Jolley, D.F., 2018. Dissolved organic matter signatures vary between naturally acidic, circumneutral and groundwater-fed freshwaters in Australia. *Water Res.* 137, 184–192. https://doi.org/10.1016/j.watres.2018.02.043

Hutchins, C.M., Teasdale, P.R., Lee, S.Y., Simpson, S.L., 2008. Cu and Zn concentration gradients created by dilution of pH neutral metal-spiked marine sediment: a comparison of sediment geochemistry with direct methods of metal addition. *Environ. Sci. Technol.* 42, 2912–2918. https://doi.org/10.1021/es702673w

Hydrobiology, 2016. NiPERA regional geochemical data: data collection and collation. Hydrobiology, Auchenflower Australia, Brisbane, QLD.

Hyne, R.V., Gale, S.A., King, C.K., 2005. Laboratory culture and life-cycle experiments with the benthic amphipod *Melita plumulosa* (Zeidler). *Environ. Toxicol. Chem.* 24, 2065–2073.

Jingchun, L., Chongling, Y., Macnair, M.R., Jun, H., Yuhong, L., 2006. Distribution and speciation of some metals in mangrove sediments from Jiulong river estuary, People's Republic of China. *Bull. Environ. Contam. Toxicol. N. Y.* 76, 815–22. http://dx.doi.org.ezproxy.uow.edu.au/10.1007/s00128-006-0992-0

Johnston, E.L., Keough, M.J., 2005. Reduction of pollution impacts through the control of toxicant release rate must be site- and season-specific. *J. Exp. Mar. Biol. Ecol.* 320, 9–33. https://doi.org/10.1016/j.jembe.2004.12.024

Jones, R., Bessell-Browne, P., Fisher, R., Klonowski, W., Slivkoff, M., 2016. Assessing the impacts of sediments from dredging on corals. *Mar. Pollut. Bull.* 102, 9–29. https://doi.org/10.1016/j.marpolbul.2015.10.049

Jouon, A., Ouillon, S., Douillet, P., Lefebvre, J.P., Fernandez, J.M., Mari, X., Froidefond, J.-M., 2008. Spatio-temporal variability in suspended particulate matter concentration and the role of aggregation on size distribution in a coral reef lagoon. *Mar. Geol.* 256, 36–48. https://doi.org/10.1016/j.margeo.2008.09.008

Junjie, R.K., Browne, N.K., Erftemeijer, P.L.A., Todd, P.A., 2014. Impacts of sediments on coral energetics: partitioning the effects of turbidity and settling particles. *PLOS ONE* 9. https://doi.org/10.1371/journal.pone.0107195

Kelley, J.L., Chapuis, L., Davies, W.I.L., Collin, S.P., 2018. Sensory system responses to human-induced environmental change. *Front. Ecol. Evol.* 6. https://doi.org/10.3389/fevo.2018.00095

King, C.K., Dowse, M.C., Simpson, S.L., Jolley, D.F., 2004. An assessment of five Australian polychaetes and bivalves for use in whole-sediment toxicity tests: toxicity and accumulation of copper and zinc from water and sediment. *Arch. Environ. Contam. Toxicol.* 47, 314–323. https://doi.org/10.1007/s00244-004-3122-1

King, C.K., Gale, S.A., Hyne, R.V., Stauber, J.L., Simpson, S.L., Hickey, C.W., 2006. Sensitivities of Australian and New Zealand amphipods to copper and zinc in waters and metal-spiked sediments. *Chemosphere* 63, 1466–1476. https://doi.org/10.1016/j.chemosphere.2005.09.020

King, R.S., Baker, M.E., 2010. Considerations for analyzing ecological community thresholds in response to anthropogenic environmental gradients. *J. North Am. Benthol. Soc.* 29, 998–1008. https://doi.org/10.1899/09-144.1

Knauer, G.A., 1977. Immediate industrial effects on sediment metals in a clean coastal environment. *Mar. Pollut. Bull.* 8, 249–254. https://doi.org/10.1016/0025-326X(77)90322-8

Kuzminov, F.I., Brown, C.M., Fadeev, V.V., Gorbunov, M.Y., 2013. Effects of metal toxicity on photosynthetic processes in coral symbionts, *Symbiodinium spp. J. Exp. Mar. Biol. Ecol.* 446, 216–227. https://doi.org/10.1016/j.jembe.2013.05.017

Kwok, K.W., Batley, G.E., Wenning, R.J., Zhu, L., Vangheluwe, M., Lee, S., 2014. Sediment quality guidelines: challenges and opportunities for improving sediment management. *Environ. Sci. Pollut. Res. Int.* 21, 17–27. http://dx.doi.org/10.1007/s11356-013-1778-7

Kwok, K.W.H., Leung, K.M.Y., Lui, G.S.G., Chu, V.K.H., Larn, P.K.S., Morritt, D., Maltby, L., Brock, T.C.M., Van Den Brink, P.J., Warne, M.S.J., Crane, M., 2007. Comparison of tropical and temperate freshwater animal species' acute sensitivities to chemicals: implications for deriving safe extrapolation factors. *Integr. Environ. Assess. Manag.* 3, 49–67. https://doi.org/10.1002/ieam.5630030105

Landner, L., Reuther, R., 2004. Metals in society and in the environment: a critical review of current knowledge on fluxes, speciation, bioavailability and risk for adverse effects of copper, chromium, nickel and zinc. Springer Science & Business Media, Springer-Verl. N. Y.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecol. Monogr.* 69, 1. https://doi.org/10.2307/2657192

Leonard, E.M., Nadella, S.R., and, C.B., Wood, C.M., 2009. Characterization of dietary Ni uptake in the rainbow trout, *Oncorhynchus mykiss*. *Aquat. Toxicol.* 93, 205–216. https://doi.org/10.1016/j.aquatox.2009.05.002

Lewis, M., Pryor, R., Wilking, L., 2011. Fate and effects of anthropogenic chemicals in mangrove ecosystems: a review. *Environ. Pollut.* 159, 2328–2346. https://doi.org/10.1016/j.envpol.2011.04.027

Li, T., Li, X., Luo, W., Cai, G., 2019. Combined classification and source apportionment analysis for trace elements in western Philippine Sea sediments. *Sci. Total Environ.* 675, 408–419. https://doi.org/10.1016/j.scitotenv.2019.04.236

Loeblich, A., Smith, V., 1968. Chloroplast pigments of the marine dinoflagellate *Gyrodinium resplendens*. *Lipids* 3, 5. https://doi.org/10.1007/BF02530961

Long, E., Morgan, L.G., 1990. The potential for biological effects of sediment-sorbed contaminants tested in the national status and trends program. NOAA Technical Memo NOS OMA 52, National Oceanic and Atmospheric Administration, Seattle.

Long, E.R., MacDonald, D.D., 1992. Chapter 14 - National status and trends program approach., in: Sediment Classification Methods Compendium. Washington DC: US Environmental Protection Agency, EPA 823-R-92-006.

Long, E.R., Macdonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Manage.* 19, 81–97. https://doi.org/10.1007/BF02472006

Macdonald, D.D., Carr, R.S., Calder, F.D., Long, E.R., Ingersoll, C.G., 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. *Ecotoxicology* 5, 253–278. https://doi.org/10.1007/BF00118995

MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Arch. Environ. Contam. Toxicol.* 39, 20–31. https://doi.org/10.1007/s002440010075

Macomber, L., Hausinger, R.P., 2011. Mechanisms of nickel toxicity in microorganisms. *Metallomics* 3, 1153. https://doi.org/10.1039/c1mt00063b

Maher, W.A., Taylor, A.M., Batley, G. E., Simpson, S. L., 2015. Chapter 5 - Bioaccumulation, in: Simpson, Stuart L., Batley, Graeme E. (Eds.), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic.

Mann, R.M., Hyne, R.V., 2008. Embryological development of the Australian amphipod, *melita plumulosa* zeidler, 1989 (amphipoda, gammaridea, melitidae). *Crustaceana* 81, 57–66. https://doi.org/10.1897/08-346.1

Mann, R.M., Hyne, R.V., Spadaro, D.A., Simpson, S.L., 2009. Development and application of a rapid amphipod reproduction test for sediment-quality assessment. *Environ. Toxicol. Chem.* 28, 1244–1254. https://doi.org/10.1897/08-346.1

Marchand, C., Allenbach, M., Lallier-Vergès, E., 2011. Relationships between heavy metals distribution and organic matter cycling in mangrove sediments (Conception Bay, New Caledonia). *Geoderma* 160, 444–456. https://doi.org/10.1016/j.geoderma.2010.10.015

Marchand, C., Fernandez, J.-M., Moreton, B., 2016. Trace metal geochemistry in mangrove sediments and their transfer to mangrove plants (New Caledonia). *Sci. Total Environ.* 562, 216–227. https://doi.org/10.1016/j.scitotenv.2016.03.206

Marchand, C., Fernandez, J.-M., Moreton, B., Landi, L., Lallier-Vergès, E., Baltzer, F., 2012. The partitioning of transitional metals (Fe, Mn, Ni, Cr) in mangrove sediments downstream of a ferralitized ultramafic watershed (New Caledonia). *Chem. Geol.* 300–301, 70–80. https://doi.org/10.1016/j.chemgeo.2012.01.018

Martínez, M.L., Intralawan, A., Vázquez, G., Pérez-Maqueo, O., Sutton, P., Landgrave, R., 2007. The coasts of our world: ecological, economic and social importance. *Ecol. Econ.* 63, 254–272. https://doi.org/10.1016/j.ecolecon.2006.10.022

Matejovic, I., 1997. Determination of carbon and nitrogen in samples of various soils by the dry combustion. *Commun. Soil Sci. Plant Anal.* 28, 1499–1511. https://doi.org/10.1080/00103629709369892

McCready, S., Birch, G.F., Long, E.R., Spyrakis, G., Greely, C.R., 2006. An evaluation of Australian sediment quality guidelines. *Arch. Environ. Contam. Toxicol.* 50, 306–15. https://doi.org/10.1007/s00244-004-0233-7

McKillup, S.C., McKillup, R.V., 1997. Effect of food supplementation on the growth of an intertidal scavenger. *Mar. Ecol. Prog. Ser.* 148, 109–114. https://doi.org/10.3354/meps148109

Melville, F., Pulkownik, A., 2007. Investigation of mangrove macroalgae as biomonitors of estuarine metal contamination. *Sci. Total Environ.* 387, 301–309. https://doi.org/10.1016/j.scitotenv.2007.06.036

Melvin, S.D., Wilson, S.P., 2013. The utility of behavioral studies for aquatic toxicology testing: a meta-analysis. *Chemosphere* 93, 2217–2223. https://doi.org/10.1016/j.chemosphere.2013.07.036

Merrington, G., An, Y.-J., Grist, E., Jeong, S.-W., Rattikansukha, C., Roe, S., Schneider, U., Sthiannopkao, S., Suter, G., Dam, R., Sprang, P., Wang, J.-Y., Warne, M., Yillia, P., Zhang, X.-W., Leung, K., 2014. Water quality guidelines for chemicals: learning lessons to deliver meaningful environmental metrics. *Environ. Sci. Pollut. Res.* 21, 6–16. https://doi.org/10.1007/s11356-013-1732-8

Merrot, P., Juillot, F., Noël, V., Lefebvre, P., Brest, J., Menguy, N., Guigner, J.-M., Blondeau, M., Viollier, E., Fernandez, J.-M., Moreton, B., Bargar, J.R., Morin, G., 2019. Nickel and iron partitioning between clay minerals, Fe-oxides and Fe-sulfides in lagoon sediments from New Caledonia. *Sci. Total Environ.* 689, 1212–1227. https://doi.org/10.1016/j.scitotenv.2019.06.274

Metian, M., Hédouin, L., Ferrier-Pagès, C., Teyssié, J.-L., Oberhansli, F., Buschiazzo, E., Warnau, M., 2015. Metal bioconcentration in the scleractinian coral *Stylophora pistillata*: investigating the role of different components of the holobiont using radiotracers. *Environ. Monit. Assess*. 187, 1–10. https://doi.org/10.1007/s10661-015-4383-z

Milani, D., Reynoldson, T.B., Borgmann, U., Kolasa, J., 2003. The relative sensitivity of four benthic invertebrates to metals in spiked-sediment exposures and application to contaminated field sediment. *Environ. Toxicol. Chem*. 22, 845–854.

Misson, B., Garnier, C., Lauga, B., Dang, D.H., Ghiglione, J.-F., Mullot, J.-U., Duran, R., Pringault, O., 2016. Chemical multi-contamination drives benthic prokaryotic diversity in the anthropized Toulon Bay. *Sci. Total Environ.* 556, 319–329. https://doi.org/10.1016/j.scitotenv.2016.02.038

Morelli, G., Gasparon, M., 2014. Metal contamination of estuarine intertidal sediments of Moreton Bay, Australia. *Mar. Pollut. Bull.* 89, 435–443. https://doi.org/10.1016/j.marpolbul.2014.10.002

Moreno-Garrido, I., Lubián, L.M., Soares, A.M.V.M., 2000. Influence of cellular density on determination of EC50 in microalgal growth inhibition tests. *Ecotoxicol. Environ. Saf.* 47, 112–116. https://doi.org/10.1006/eesa.2000.1953

Moreton, B.M., Fernandez, J.-M., Dolbecq, M.B.D., 2009. Development of a field preconcentration/elution unit for routine determination of dissolved metal concentrations by ICP-OES in marine waters: application for monitoring of the New Caledonia Lagoon. Geostand. *Geoanalytical Res.* 33, 205–218. https://doi.org/10.1111/j.1751-908X.2009.00899.x

Morin, S., Cordonier, A., Lavoie, I., Arini, A., Blanco, S., Duong, T.T., Tornes, E., Bonet, B., Corcoll, N., Faggiano, L., Laviale, M., Pérès, F., Becares, E., Coste, M., Feurtet Mazel, A., Fortin, C., Guasch, H., Sabater, S. Consistency in diatom response to metal-contaminated environments, in Guasch, H., Ginebreda, A., Geiszinger, A. (Eds.), Emerging and priority pollutants in rivers: bringing science into river management plans, Springer, pp.117-146, 2012, The Handbook of Environmental Chemistry 19, 978-3-642-25721-6. ⟨10.1007/978-3-642-25722-3_5⟩. ⟨hal-02596950⟩

Morrison, R.J., Brown, P.L., 2003. Trace metals in Fanga'uta Lagoon, Kingdom of Tonga. *Mar. Pollut. Bull*. 46, 146-152. https://doi.org/10.1016/S0025-326X(96)00147-6

Morrison, R.J., Gangaiya, P., Naqasima, M.R., Naidu, R., 1997. Trace metal studies in the Great Astrolabe Lagoon, Fiji, a pristine marine environment. *Mar. Pollut. Bull.* 34, 353–356. https://doi.org/10.1016/S0025-326X(96)00147-6

Morrison, R.J., Peshut, P.J., Lasorsa, B.K., 2010. Elemental composition and mineralogical characteristics of coastal marine sediments of Tutuila, American Samoa. *Mar. Pollut. Bull.* 60, 925–930. https://doi.org/10.1016/j.marpolbul.2010.04.006

Morton, B., Britton, J., 2003. The behaviour and feeding ecology of a suite of gastropod scavengers at Watering Cove, Burrup Peninsula, Western Australia, in Wells, F.E., Walker, Diana, Jones, D.S., Walker, D.I. (Eds.), The Marine Flora and Fauna of Dampier, Western Australia. Western Australian Museum, Perth, WA.

Mudd, G.M., 2010. Global trends and environmental issues in nickel mining: sulfides versus laterites. *Ore Geol. Rev.* 38, 9–26. https://doi.org/10.1016/j.oregeorev.2010.05.003

Munawar, M., Munawar, I.F., 1987. Phytoplankton bioassays for evaluating toxicity of in situ sediment contaminants. *Hydrobiologia* 149, 87–105. https://doi.org/10.1007/BF00048650

Muyssen, B.T.A., Brix, K.V., DeForest, D.K., Janssen, C.R., 2004. Nickel essentiality and homeostasis in aquatic organisms. *Environ. Rev.* 12, 113–131. https://doi.org/10.1139/a04-004

Nguyen, L.T.H., Burton, G.A., Schlekat, C.E., Janssen, C.R., 2011. Field measurement of nickel sediment toxicity: role of acid volatile sulfide. *Environ. Toxicol. Chem.* 30, 162–172. https://doi.org/10.1002/etc.358

Nieminen, T.M., Ukonmaanaho, L., Rausch, N., Shotyk, W., 2007. Chapter 1 - Biogeochemistry of nickel and its release into the environment, in: Sigel, A., Sigel, H., Sigel, R.K.O. (Eds.), Nickel and Its Surprising Impact in Nature. John Wiley & Sons, U.K.

Noël, V., Juillot, F., Morin, G., Marchand, C., Ona-Nguema, G., Viollier, E., Prévot, F., Dublet, G., Maillot, F., Delbes, L., Marakovic, G., Bargar, J.R., Brown, G.E., 2017. Oxidation of Ni-rich mangrove sediments after isolation from the sea (Dumbea Bay, New Caledonia): Fe and Ni behavior and environmental implications. *ACS Earth Space Chem.* 1, 455–464. https://doi.org/10.1021/acsearthspacechem.7b00005

Noël, V., Marchand, C., Juillot, F., Ona-Nguema, G., Viollier, E., Marakovic, G., Olivi, L., Delbes, L., Gelebart, F., Morin, G., 2014. EXAFS analysis of iron cycling in mangrove sediments downstream a lateritized ultramafic watershed (Vavouto Bay, New Caledonia). *Geochim. Cosmochim. Acta* 136, 211–228. https://doi.org/10.1016/j.gca.2014.03.019

Noël, V., Morin, G., Juillot, F., Marchand, C., Brest, J., Bargar, J.R., Muñoz, M., Marakovic, G., Ardo, S., Brown Jr., G.E., 2015. Ni cycling in mangrove sediments from New Caledonia. *Geochim. Cosmochim. Acta* 169, 82–98. https://doi.org/10.1016/j.gca.2015.07.024

Norgate, T., Jahanshahi, S., 2011. Assessing the energy and greenhouse gas footprints of nickel laterite processing. *Miner. Eng.* 24, 698–707. https://doi.org/10.1016/j.mineng.2010.10.002

NYSDEC, 1999. Technical Guidance for Screening Contaminated Sediments. New York State Department of Environmental Conservation, Division of Fish and Wildlife, Division of Marine Resources, Albany.

Nyström, M., Folke, C., Moberg, F., 2000. Coral reef disturbance and resilience in a human-dominated environment. *Trends Ecol. Evol.* 15, 413–417. https://doi.org/10.1016/S0169-5347(00)01948-0

OEC, 2019. New Caledonia (NCL) Exports, Imports, and Trade Partners. URL https://oec.world/en/profile/country/ncl/ (accessed 10.20.19).

Ondov, B.D., Bergman, N.H., Phillippy, A.M., 2011. Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics* 12, 385. https://doi.org/10.1186/1471-2105-12-385

Ouillon, S., Douillet, P., Lefebvre, J.P., Le Gendre, R., Jouon, A., Bonneton, P., Fernandez, J.M., Chevillon, C., Magand, O., Lefèvre, J., Le Hir, P., Laganier, R., Dumas, F., Marchesiello, P., Bel Madani, A., Andréfouët, S., Panché, J.Y., Fichez, R., 2010. Circulation and suspended sediment transport in a coral reef lagoon: the south-west lagoon of New Caledonia. *Mar. Pollut. Bull.* 61, 269–296. https://doi.org/10.1016/j.marpolbul.2010.06.023

Peijnenburg, W.J., Teasdale, P.R., Reible, D., Mondon, J., Bennett, W.W., Campbell, P.G., 2014. Passive sampling methods for contaminated sediments: state of the science for metals. *Integr. Environ. Assess. Manag.* 10, 179–196. https://doi.org/10.1002/ieam.1502

Pereira, M.G., Latchford, J.W., Mudge, S.M., 2006. The use of terminal restriction fragment length polymorphism (T-RFLP) for the characterisation of microbial communities in marine sediments. *Geomicrobiol. J.* 23, 247–251. https://doi.org/10.1080/01490450600760617

Persaud, D., Jaaguagi, R., Hayton, A., 1993. Guidelines for the protection and management of aquatic sediment quality in Ontario. Ontario Ministry of the Environment, Toronto.

Peters, A., Merrington, G., Leverett, D., Wilson, I., Schlekat, C., Garman, E., 2019. Comparison of the chronic toxicity of nickel to temperate and tropical freshwater species. *Environ. Toxicol. Chem.* 38, 1211–1220. https://doi.org/10.1002/etc.4384

Peters, E.C., Gassman, N.J., Firman, J.C., Richmond, R.H., Power, E.A., 1997. Ecotoxicology of tropical marine ecosystems. *Environ. Toxicol. Chem*. 16, 12–40. https://doi.org/10.1002/etc.5620160103

Philipp, E., Fabricius, K., 2003. Photophysiological stress in scleractinian corals in response to short-term sedimentation. *J. Exp. Mar. Biol. Ecol.* 287, 57–78. https://doi.org/10.1016/S0022-0981(02)00495-1

Pinheiro, J.C., Bates, D.M., 2000. Mixed-effects models in S and S-PLUS. Springer Science & Business Media, Springer-Verl. N. Y.

Price, G.A.V., 2017. Can metal fluxes from sediments and soils predict metal toxicity to organisms in temperate and polar systems? Honours Thesis, University of Wollongong, Wollongong, Australia.

Pringault, O., Duran, R., Jacquet, S., Torréton, J.-P., 2008. Temporal variations of microbial activity and diversity in marine tropical sediments (New Caledonia lagoon). *Microb. Ecol.* 55, 247–258. https://doi.org/10.1007/s00248-007-9272-8

Pringault, O., Viret, H., Duran, R., 2010. Influence of microorganisms on the removal of nickel in tropical marine sediments (New Caledonia). *Mar. Pollut. Bull.* 61, 530–541. https://doi.org/10.1016/j.marpolbul.2010.06.038

Pyle, G., Couture, P., 2012. Chapter 5 - Nickel, in: Wood, C.M., Farrell, A.P., Brauner, C.J. (Eds.), Homeostasis and Toxicology of Essential Metals, Fish Physiology. Academic Press, Amsterdam ; New York.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. https://doi.org/10.1093/nar/gks1219

Quesnel, B., de Veslud, C.L.C., Boulvais, P., Gautier, P., Cathelineau, M., Drouillet, M., 2017. 3D modeling of the laterites on top of the Koniambo Massif, New Caledonia: refinement of the per descensum lateritic model for nickel mineralization. *Miner. Deposita* 52, 961–978. https://doi.org/10.1007/s00126-017-0712-1

Quince, C., Lanzen, A., Davenport, R.J., Turnbaugh, P.J., 2011. Removing noise from pyrosequenced amplicons. *BMC Bioinformatics* 12, 38. https://doi.org/10.1186/1471-2105-12-38

R Core Team, 2016. R: a language and environment for statistical computing. R Foundation for statistical Computing, Vienna, Austria.

Rahman, Y.J., Forward, R.B., Rittschof, D., 2000. Responses of mud snails and periwinkles to environmental odors and disaccharide mimics of fish odor. *J. Chem. Ecol.* 26, 679–696.

Rainbow, P.S., 2007. Trace metal bioaccumulation: models, metabolic availability and toxicity. *Environ. Int.* 33, 576–582. https://doi.org/10.1016/j.envint.2006.05.007

Ranjbar Jafarabadi, A., Riyahi Bakhtiari, A., Maisano, M., Pereira, P., Cappello, T., 2018. First record of bioaccumulation and bioconcentration of metals in Scleractinian corals and their algal symbionts from Kharg and Lark coral reefs (Persian Gulf, Iran). *Sci. Total Environ.* 640–641, 1500–1511. https://doi.org/10.1016/j.scitotenv.2018.06.029

Reichelt-Brushett, A., Hudspith, M., 2016. The effects of metals of emerging concern on the fertilization success of gametes of the tropical scleractinian coral *Platygyra daedalea*. *Chemosphere* 150, 398–406. https://doi.org/10.1016/j.chemosphere.2016.02.048

Reichelt-Brushett, A.J., Harrison, P.L., 2005. The effect of selected trace metals on the fertilization success of several scleractinian coral species. *Coral Reefs* 24, 524–534. https://doi.org/10.1007/s00338-005-0013-5

Reichelt-Brushett, A.J., McOrist, G., 2003. Trace metals in the living and nonliving components of scleractinian corals. *Mar. Pollut. Bull*. 46, 1573–1582. https://doi.org/10.1016/S0025-326X(03)00323-0

Ricardo, G.F., Jones, R.J., Clode, P.L., Humanes, A., Giofre, N., Negri, A.P., 2018. Sediment characteristics influence the fertilisation success of the corals *Acropora tenuis* and *Acropora millepora*. *Mar. Pollut. Bull*. 135, 941–953. https://doi.org/10.1016/j.marpolbul.2018.08.001

Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. *PLOS ONE* 10, e0146021. https://doi.org/10.1371/journal.pone.0146021

Rogers, C., 1990. Responses of coral reefs and reef organisms to sedimentation. *Mar. Ecol. Prog. Ser.* 62, 185–202. https://doi.org/10.3354/meps062185

Roulier, J.L., Tusseau-Vuillemin, M.H., Coquery, M., Geffard, O., Garric, J., 2008. Measurement of dynamic mobilization of trace metals in sediments using DGT and comparison with bioaccumulation in *Chironomus riparius*: first results of an experimental study. *Chemosphere* 70, 925–932. https://doi.org/10.1016/j.chemosphere.2007.06.061

Schlekat, C., Stubblefield, W., Gallagher, K., 2020. State of the science on metal bioavailability modeling: introduction to the outcome of a society of environmental toxicology and chemistry technical workshop. *Environ. Toxicol. Chem.* 39, 42–47. https://doi.org/10.1002/etc.4561

Schlekat, C.E., Garman, E.R., Vangheluwe, M.L., Burton, G.A., 2016. Development of a bioavailability-based risk assessment approach for nickel in freshwater sediments. *Integr. Environ. Assess. Manag*. 12, 735–746. https://doi.org/10.1002/ieam.1720

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to ImageJ: 25 years of image analysis. *Nat. Methods* 671–675. https://doi.org/10.1038/nmeth.2089

Schneider, P.M., Davey, S.B., 1995. Sediment contaminants off the coast of Sydney, Australia: a model for their distribution. *Mar. Pollut. Bull.* 31, 262–272. https://doi.org/10.1016/0025-326X(95)00176-N

Sclater, F.R., Boyle, E., Edmond, J.M., 1976. On the marine geochemistry of nickel. *Earth Planet. Sci. Lett.* 31, 119–128. https://doi.org/10.1016/0012-821X(76)90103-5

Sherrod, L.A., Dunn, G., Peterson, G.A., Kolberg, R.L., 2002. Inorganic carbon analysis by modified pressure-calcimeter method. *Soil Sci. Soc. Am. J.* 66, 299–305. https://doi.org/10.2136/sssaj2002.2990

Simpson, S. L., Kumar, A., 2015. Chapter 4 - Sediment ecotoxicology, in: Simpson, Stuart L., Batley, G.E. (Eds.), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic.

Simpson, S.L., 2001. A rapid screening method for acid-volatile sulfide in sediments. *Environ. Toxicol. Chem.* 20, 2657–2661. https://doi.org/10.1002/etc.5620201201

Simpson, S.L., Angel, B.M., Jolley, D.F., 2004. Metal equilibration in laboratory-contaminated (spiked) sediments used for the development of whole-sediment toxicity tests. *Chemosphere* 54, 597–609. https://doi.org/10.1016/j.chemosphere.2003.08.007

Simpson, S.L., Batley, G.E., 2015. Sediment quality assessment: a practical guide. CSIRO Publishing, Clayton, Vic.

Simpson, S.L., Batley, G.E., 2007. Predicting metal toxicity in sediments: a critique of current approaches. *Integr. Environ. Assess. Manag.* 3, 18–31. https://doi.org/10.1002/ieam.5630030103

Simpson, S.L., Batley, G.E., Hamilton, I.L., Spadaro, D.A., 2011. Guidelines for copper in sediments with varying properties. *Chemosphere* 85, 1487–1495. https://doi.org/10.1016/j.chemosphere.2011.08.044

Simpson, S.L., Batley, G.E., Maher, W.A., 2015. Chapter 3 - Chemistry of sediment contaminants, in: Simpson, S.L., Batley, G.E. (Eds.), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic.

Simpson, S.L., Spadaro, D.A., 2016. Bioavailability and chronic toxicity of metal sulfide minerals to benthic marine invertebrates: implications for deep sea exploration, mining and tailings disposal. *Environ. Sci. Technol*. 50, 4061–4070. https://doi.org/10.1021/acs.est.6b00203

Simpson, S.L., Spadaro, D.A., 2011. Performance and sensitivity of rapid sublethal sediment toxicity tests with the amphipod Melita plumulosa and copepod *Nitocra spinipes*. *Environ. Toxicol. Chem*. 30, 2326–2334. https://doi.org/10.1002/etc.633

Simpson, S.L., Ward, D., Strom, D., Jolley, D.F., 2012a. Oxidation of acid-volatile sulfide in surface sediments increases the release and toxicity of copper to the benthic amphipod *Melita plumulosa*. *Chemosphere* 88, 953–961. https://doi.org/10.1016/j.chemosphere.2012.03.026

Simpson, S.L., Yverneau, H., Cremazy, A., Jarolimek, C.V., Price, H.L., Jolley, D.F., 2012b. DGT-induced copper flux predicts bioaccumulation and toxicity to bivalves in sediments with varying properties. *Environ. Sci. Technol.* 46, 9038–9046. https://doi.org/10.1021/es301225d

Smith, S.L., MacDonald, D.D., Keenleyside, K.A., Ingersoll, C.G., Jay Field, L., 1996. A preliminary evaluation of sediment quality assessment values for freshwater ecosystems. *J. Gt. Lakes Res.* 22, 624–638. https://doi.org/10.1016/S0380-1330(96)70985-1

Spadaro, D.A., Micevska, T., Simpson, S.L., 2008. Effect of nutrition on toxicity of contaminants to the epibenthic amphipod *Melita plumulosa*. *Arch. Environ. Contam. Toxicol.* 55, 593–602. https://doi.org/10.1007/s00244-008-9153-2

Spadaro, D.A., Simpson, S.L., 2015. Appendix E: Protocol for 10-day whole-sediment sub-lethal (reproducion) and acute toxicity tests using the epibenthic amphipod *Melita plumulosa*, in: Simpson, S.L., Batley, G.E. (Eds.), Sediment Quality Assessment: A Practical Guide. CSIRO Publishing, Clayton, Vic.

Stafford-Smith, M., Ormond, R., 1992. Sediment-rejection mechanisms of 42 species of Australian scleractinian corals. *Mar. Freshw. Res.* 43, 683. https://doi.org/10.1071/MF9920683

Stat, M., Huggett, M.J., Bernasconi, R., DiBattista, J.D., Berry, T.E., Newman, S.J., Harvey, E.S., Bunce, M., 2017. Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. *Sci. Rep.* 7, 12240. https://doi.org/10.1038/s41598-017-12501-5

Stauber, J., Golding, L., Peters, A., Merrington, G., Adams, M., Binet, M., Batley, G., Gissi, F., McKnight, K., Garman, E., Middleton, E., Gadd, J., Schlekat, C., 2021. Application of bioavailability models to derive chronic guideline values for nickel in freshwaters of Australia and New Zealand. *Environ. Toxicol. Chem*. 40, 100–112. https://doi.org/10.1002/etc.4885

Stevenson, R.J., Pan, Y., Van Dam, H., 2010. Assessing environmental conditions in rivers and streams with diatoms, in: Smol, J., Stoermer, E. (Eds.), The Diatoms: Applications for the Environmental and Earth Sciences. Cambridge: Cambridge University Press., pp. 57–85. https://doi.org/10.1017/CBO9780511763175.005

Storlazzi, C.D., Norris, B.K., Rosenberger, K.J., 2015. The influence of grain size, grain color, and suspended-sediment concentration on light attenuation: Why fine-grained terrestrial sediment is bad for coral reef ecosystems. *Coral Reefs* 34, 967–975. https://doi.org/10.1007/s00338-015-1268-0

Strom, D., 2011. Development of a robust sediment quality guideline for copper. Doctor of Philosophy Thesis, University of Wollongong, Wollongong, Australia.

Strom, D., Simpson, S.L., Batley, G.E., Jolley, D.F., 2011. The influence of sediment particle size and organic carbon on toxicity of copper to benthic invertebrates in oxic/suboxic surface sediments. *Environ. Toxicol. Chem*. 30, 1599–1610. https://doi.org/10.1002/etc.531

Sun, M.Y., Dafforn, K.A., Johnston, E.L., Brown, M.V., 2013. Core sediment bacteria drive community response to anthropogenic contamination over multiple environmental gradients. *Environ. Microbiol.* 15, 2517–2531. https://doi.org/10.1111/1462-2920.12133

Sutcliffe, B., Chariton, A.A., Harford, A.J., Hose, G.C., Greenfield, P., Elbourne, L.D.H., Oytam, Y., Stephenson, S., Midgley, D.J., Paulsen, I.T., 2017. Effects of uranium concentration on microbial community structure and functional potential. E*nviron. Microbiol*. 19, 3323–3341. https://doi.org/10.1111/1462-2920.13839

Sutcliffe, B., Chariton, A.A., Harford, A.J., Hose, G.C., Greenfield, P., Midgley, D.J., Paulsen, I.T., 2018. Diverse fungal lineages in subtropical ponds are altered by sediment-bound copper. *Fungal Ecol.* 34, 28–42. https://doi.org/10.1016/j.funeco.2018.03.003

Suter, G., Cormier, S., Barron, M., 2017. A weight of evidence framework for environmental assessments: Inferring qualities. *Integr. Environ. Assess. Manag*. 13, 1038–1044. https://doi.org/10.1002/ieam.1954

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. *Mol. Ecol.* 21, 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x

Tam, N.F.Y., Wong, Y.S., 2000. Spatial variation of heavy metals in surface sediments of Hong Kong mangrove swamps. *Environ. Pollut*. 110, 195–205. https://doi.org/10.1016/S0269-7491(99)00310-3

Todd, P.A., Ong, X., Chou, L.M., 2010. Impacts of pollution on marine life in Southeast Asia. *Biodivers. Conserv*. 19, 1063–1082. http://dx.doi.org/10.1007/s10531-010-9778-0

Tomascik, T., Sander, F., 1985. Effects of eutrophication on reef-building corals. *Mar. Biol*. 87, 143–155. https://doi.org/10.1007/BF00539422

Trenfield, M.A., van Dam, J.W., Harford, A.J., Parry, D., Streten, C., Gibb, K., van Dam, R.A., 2016. A chronic toxicity test for the tropical marine snail *Nassarius dorsatus* to assess the toxicity of copper, aluminium, gallium, and molybdenum: metal toxicity to a tropical marine snail. *Environ. Toxicol. Chem*. 35, 1788–1795. https://doi.org/10.1002/etc.3331

US EPA, 1999. Screening level ecological risk assessment protocol. Appendix E: toxicity reference values. U.S. Environmental Protection Agency, Region 6 Multimedia Planning and Permitting Division, Washington, DC,.

USGS, 2020. Mineral commodity summaries 2020: U.S. Geological Survey 116–117. https://doi.org/10.3133/mcs2020

Vandegehuchte, M.B., Roman, Y.E., Nguyen, L.T.H., Janssen, C.R., De Schamphelaere, K.A.C., 2007. Toxicological availability of nickel to the benthic oligochaete *Lumbriculus variegatus*. *Environ. Int*. 33, 736–742. https://doi.org/10.1016/j.envint.2007.02.006

Vangheluwe, M.L., Ngyuen, L., 2014. Advanced research on nickel toxicity in sediments: additional species, bioavailability, and diet-borne toxicity. Part 1: additional species and bioavailability models. Final Report. Durham (NC), Nickel Produces Environmental Research Association.

Vangheluwe, M.L.U., Verdonck, F.A.M., Besser, J.M., Brumbaugh, W.G., Ingersoll, C.G., Schlekat, C.E., Garman, E.R., 2013. Improving sediment-quality guidelines for nickel: development and application of predictive bioavailability models to assess chronic toxicity of nickel in freshwater sediments. *Environ. Toxicol. Chem*. 32, 2507–2519. https://doi.org/10.1002/etc.2373

Vedamanikam, V.J., Hayimad, T., 2013. Effect of mixtures of metals on the spotted Babylon snail (*Babylonia areolata*) under different temperature conditions. *Toxicol. Environ. Chem*. 95, 1388–1394. https://doi.org/10.1080/02772248.2014.881077

Vink, J.P.M., 2009. The origin of speciation: trace metal kinetics over natural water/sediment interfaces and the consequences for bioaccumulation. *Environ. Pollut*. 157, 519–527. https://doi.org/10.1016/j.envpol.2008.09.037

Viret, H., Pringault, O., Duran, R., 2006. Impact of zinc and nickel on oxygen consumption of benthic microbial communities assessed with microsensors. *Sci. Total Environ*. 367, 302–311. https://doi.org/10.1016/j.scitotenv.2005.11.017

von der Heyden, B.P., Roychoudhury, A.N., 2015. Application, chemical interaction and fate of iron minerals in polluted sediment and soils. *Curr. Pollut. Rep*. 1, 265–279. https://doi.org/10.1007/s40726-015-0020-2

Wang, W.-X., 2016. Chapter 4 - Bioaccumulation and Biomonitoring, in: Blasco, J., Chapman, P.M., Campana, O., Hampel, M. (Eds.), Marine Ecotoxicology. *Academic Press*, pp. 99–119. https://doi.org/10.1016/B978-0-12-803371-5.00004-7

Wang, Z., Kwok, K.W.H., Lui, G.C.S., Zhou, G.-J., Lee, J.-S., Lam, M.H.W., Leung, K.M.Y., 2014. The difference between temperate and tropical saltwater species' acute sensitivity to chemicals

is relatively small. *Chemosphere* 105, 31–43. https://doi.org/10.1016/j.chemosphere.2013.10.066

Warne, M.S.J., Batley, G.E., van Dam, R.A., Chapman, J.C., Fox, D.R., Hickey, C.W., Stauber, J.L., 2018. Revised method for deriving Australian and New Zealand water quality guideline values for toxicants - update of 2015 version. Prepared for the revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Governments and Australian state and territory governments, Canberra, ACT, Australia.

Watson, C.E., 2012. Development of a chronic microalgal bioassay to assess contaminated sediment. Honours Thesis, University of Wollongong, Wollongong, Australia.

Weber, M., Lott, C., Fabricius, K.E., 2006. Sedimentation stress in a scleractinian coral exposed to terrestrial and marine sediments with contrasting physical, organic and geochemical properties. *J. Exp. Mar. Biol. Eco*l. 336, 18–32. https://doi.org/10.1016/j.jembe.2006.04.007

Wells, R.C., 1943. Relative abundance of nickel in the earth's crust (USGS Numbered Series No. 205-A), Professional Paper.

Wenning, R.J., Batley, G.E., Ingersoll, C.G., Moore, D.W. (Eds.), 2005. Use of sediment quality guidelines and related tools for the assessment of contaminated sediments. Presented at the Pellston Workshop on Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments, SETAC Press, Pensacola, FL.

Wickham, H., 2016. ggplot2: elegant graphics for data analysis. *J. Stat. Softw.* Springer-Verl. N. Y. 77. https://doi.org/10.18637/jss.v077.b02

Williams, P.A., Sikutshwa, L., Shackleton, S., 2020. Acknowledging indigenous and local knowledge to facilitate collaboration in landscape approaches—lessons from a systematic review. *Land* 9, 331. https://doi.org/10.3390/land9090331

Wolanski, E., Richmond, R.H., Davis, G., Bonito, V., 2003. Water and fine sediment dynamics in transient river plumes in a small, reef-fringed bay, Guam. *Estuar. Coast. Shelf Sci*. 56, 1029–1040. https://doi.org/10.1016/S0272-7714(02)00321-9

Wyeth, R.C., 2019. Olfactory navigation in aquatic gastropods. *J. Exp. Biol*. 222. https://doi.org/10.1242/jeb.185843

Xue, H.B., Jansen, S., Prasch, A., Sigg, L., 2001. Nickel speciation and complexation kinetics in freshwater by ligand exchange and DPCSV. *Environ. Sci. Technol*. 35, 539–546. https://doi.org/10.1021/es0014638

Yang, J., Xie, Y., Jeppe, K., Long, S., Pettigrove, V., Zhang, X., 2018. Sensitive community responses of microbiota to copper in sediment toxicity test. *Environ. Toxicol. Chem*. 37, 599–608. https://doi.org/10.1002/etc.3980

Yin, H., Cai, Y., Duan, H., Gao, J., Fan, C., 2014. Use of DGT and conventional methods to predict sediment metal bioavailability to a field inhabitant freshwater snail (*Bellamya aeruginosa*) from Chinese eutrophic lakes. *J. Hazard. Mater*. 264, 184–194. https://doi.org/10.1016/j.jhazmat.2013.11.030

Yin, H., Niu, J., Ren, Y., Cong, J., Zhang, X., Fan, F., Xiao, Y., Zhang, X., Deng, J., Xie, M., He, Z., Zhou, J., Liang, Y., Liu, X., 2015. An integrated insight into the response of sedimentary microbial communities to heavy metal contamination. *Sci. Rep*. 5. https://doi.org/10.1038/srep14266

Zamble, D., Rowińska-Żyrek, M., Kozlowski, H., 2017. The Biological Chemistry of Nickel. Royal Society of Chemistry, Croydon, U.K.

Zhang, H., Davison, W., Miller, S., Tych, W., 1995. In situ high resolution measurements of fluxes of Ni, Cu, Fe, and Mn and concentrations of Zn and Cd in porewaters by DGT. *Geochim. Cosmochim*. Acta 59, 4181–4192. https://doi.org/10.1016/0016-7037(95)00293-9

Zhang, X., 2019. Environmental DNA shaping a new era of ecotoxicological research. *Environ. Sci. Technol*. 53, 5605–5612. https://doi.org/10.1021/acs.est.8b06631

Zhang, Y., Hou, X., 2020. Characteristics of coastline changes on Southeast Asia islands from 2000 to 2015. *Remote Sens*. 12, 519. https://doi.org/10.3390/rs12030519

Zhang, Y., Yang, J., Simpson, S.L., Wang, Y., Zhu, L., 2019. Application of diffusive gradients in thin films (DGT) and simultaneously extracted metals (SEM) for evaluating bioavailability of metal contaminants in the sediments of Taihu Lake, China. *Ecotoxicol. Environ. Saf*. 184, 109627. https://doi.org/10.1016/j.ecoenv.2019.109627

Zhu, J., Zhang, J., Li, Q., Han, T., Xie, J., Hu, Y., Chai, L., 2013. Phylogenetic analysis of bacterial community composition in sediment contaminated with multiple heavy metals from the Xiangjiang River in China. *Mar. Pollut. Bull.* 70, 134–139. https://doi.org/10.1016/j.marpolbul.2013.02.023

Zimmermann, J., Jahn, R., Gemeinholzer, B., 2011. Barcoding diatoms: evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols. *Org. Divers. Evol.* 11, 173. https://doi.org/10.1007/s13127-011-0050-6

Appendices

Appendix A – Appendix to Chapter 2

A1. Background concentrations of metals in filtered seawater

Analysed by ICP-AES. Values less than the limit of detection (LOD) were substituted as half the LOD.

Metal	Typical LOD for waters* (µg/L)	Cronulla seawater† (filtered <0.45 µm)	South Coast seawater‡ (filtered <1 µm)
Aluminium	0.2	0.3	9.5
Arsenic	1.3	1.9	<1.3
Cadmium	0.3	<0.3	<0.3
Chromium	0.4	<0.4	<0.4
Cobalt	2.1	<2.1	<2.1
Copper	0.4	<0.4	0.5
Iron	0.8	<0.8	<0.8
Lead	1.6	<1.6	NR
Manganese	0.07	0.1	<0.07
Nickel	0.6	<0.6	<0.6
Zinc	0.2	0.2	1.8

NR = Not reported

* The LOD was calculated for each batch of analysis as three times the standard deviation of the method blanks (n = 4)

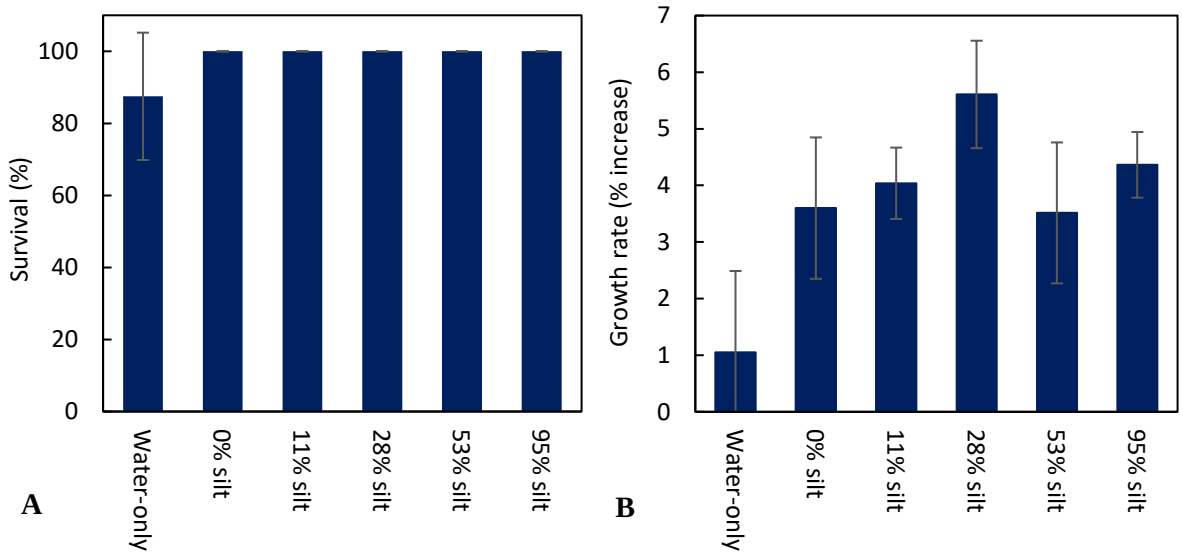
† Median concentration of seawater batches stored for use in 2018 (n = 51)

‡ Median concentration of seawater batches stored for use in 2017 (n = 2)

Appendix B – Appendix to Chapter 3

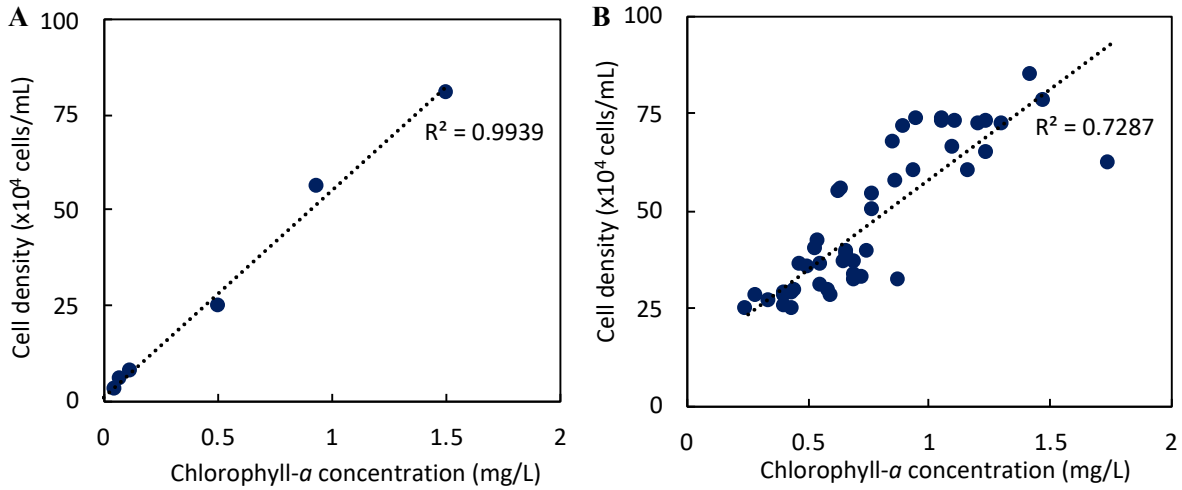
B1. The response of *Nassarius dorsatus* to testing conditions with and without substratum

Responses measured were A) survival and B) growth rate, following exposure to testing conditions for 28 days with either no substratum provided (water-only) or substratum with different proportions of particle size <63 µm. The substratum was prepared with clean sand (sieved to >180 µm to remove the fine fraction) diluted with different amounts of Organic-silt sediment (95% <63 µm size) to prepare substratum with a varied proportion of fine particles present. Treatments are present as the percentage of silt (<63 µm) particles present.



B2. Relationship between growth-rate and Chlorophyll-*a* concentration of *Ceratoneis closterium*

Relationship between growth-rate and Chlorophyll-*a* concentration of *Ceratoneis closterium*: A) standard curve; and B) following 72-h exposure to a concentration series of dissolved nickel (treatment concentrations <1-9,800 µg Ni/L). The standard curve was produced by preparing a dilution series of the cultured algae during exponential phase growth. Each point represents one replicate. Figure B, data pooled from duplicate experiments.



Appendix C – Appendix to Chapter 4

C1. Physicochemical properties of the overlying seawater in *Melita plumulosa* bioassays for water only experiments and sediment experiments

Measurements were taken on a composite sample of the overlying water for all replicates of a single treatment before every water renewal (overlying water experiments) or every second water renewal (sediment experiments) and of the clean seawater before each water renewal. For each individual experiment, all measurements were pooled and presented as the min and/or the maximum for the exposure period.

Experiment	pH	Salinity (PSU)	Conductivity (mS/cm)	Dissolved Oxygen (%)	Ammonia (mg/L)
Water only experiments					
Range finder	7.9 – 8.1	29 – 31	44 – 48	≥82	≤1
Repeat	8.0 – 8.2	29 – 32	45 – 48	≥84	≤1
Sediment experiments					
Organic-silt	8.0 – 8.2	31 - 35	48 - 55	≥90	≤0.5
Sandy-silt	7.7 – 8.2	30 - 32	47 - 50	≥84	≤1
Silty-sand	8.0 – 8.3	31 - 35	48 - 55	≥90	≤0.5
High-sulfide	7.8 – 8.5	30 - 34	45 - 52	≥87	≤0.5
Site 1	8.0 – 8.1	31 - 35	50 - 54	≥90	≤1
Site 2	8.0 – 8.1	31 - 35	50 - 54	≥91	≤0.5
Site 3	8.1 – 8.3	30 - 34	46 - 52	≥91	≤0.5

C2. Survival and reproductive output of *Melita plumulosa* for the High-sulfide and Site 3 sediments following a 10-d test which did not meet test acceptability criteria

Sediment Treatment	Survival (%)*	Reproduction (no. of offspring per female)*	Total recoverable Ni (mg/kg)	Dilute-acid extractable Ni (mg/kg)	DGT-labile Ni (µg Ni/m²/h)	Dissolved Ni (µg/L)
High-sulfide						
Control	78 ± 13	3 ± 1	12	3	0.005	0.7
Ni spike 1	83 ± 8	5 ± 0	250	110	1.2	19
Ni spike 2	83 ± 0	5 ± 2	460	210	1.5	35
Ni spike 3	69 ± 21	4 ± 0	1,000	600	3.4	79
Ni spike 4	64 ± 5	3 ± 1	1,700	900	6.6	240
Ni spike 5	11 ± 5	0	3,400	1100	16	690
Ni spike 6	0	0	5,600	2800	25	1,600
Organic-silt[†]	75 ± 29	7 ± 2	12	3	0.07	0.7
Site 3	69 ± 5	5 ± 2	480	52	0.02	2

DGT = diffusive gradients in thin films.

* Mean ± standard error (n = 3)

[†] Organic-silt sediment, no added nickel, was used as the control sediment for Site 3

Appendix D – Appendix to Chapter 5

D1. PCR conditions for amplification of DNA using various primers

Primer set region	PCR conditions			
	Denature (°C, min)	Annealing (°C, s)		Extension (°C, min)
18S V7 rDNA (eukaryotes)	95, 2	40 cycles:	95, 30 58, 30 72, 60	72, 7
18S V4 rDNA (diatoms)	95, 2	40 cycles:	95, 30 58, 30 72, 90	72, 5
16S V4 rDNA (prokaryotes)	94, 3	35 cycles:	94, 45 50, 60 72, 90	

D2. Summary of the total number of reads and molecular operational taxonomic units before and after data was filtered in preparation for statistical analysis.

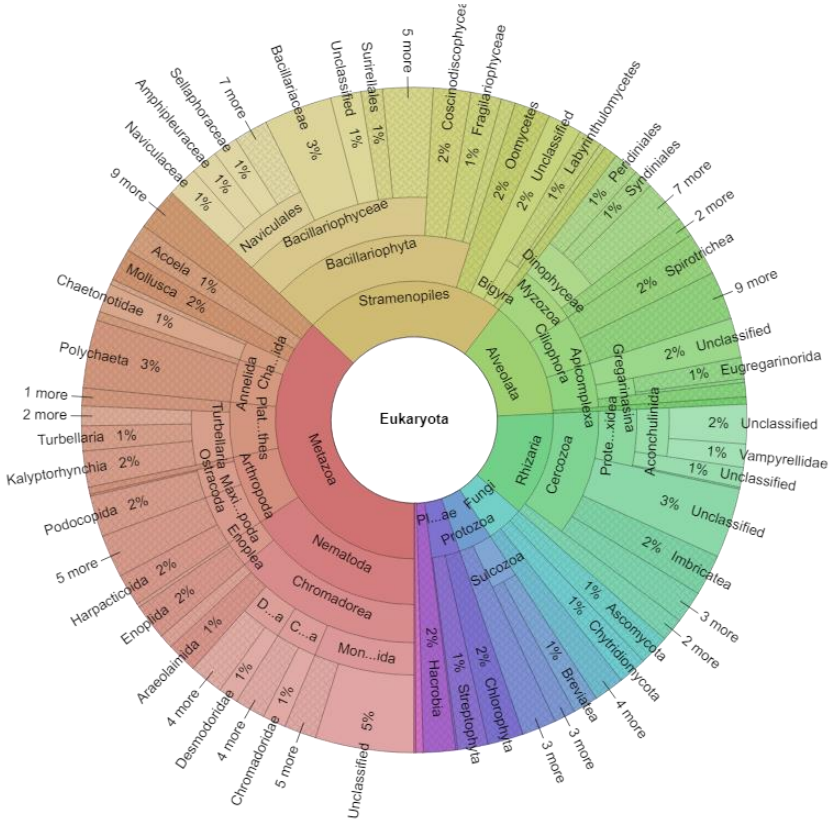
Primer set region	Number of reads		Removed (%)	Number of MOTUs		Removed (%)
	Before filtering	After filtering		Before filtering	After filtering	
18S V7 rDNA (eukaryotes)	2,776,810	2,573,808	7	3,268	1,149	65
18S V4 rDNA (diatoms)	115,937	105,998	9	380	165	57
16S V4 rDNA (prokaryotes)	834,330	649,616	22	4,697	2,602	45

D3. Taxonomic distribution of the benthic community composition of Vavouto Bay sediments.

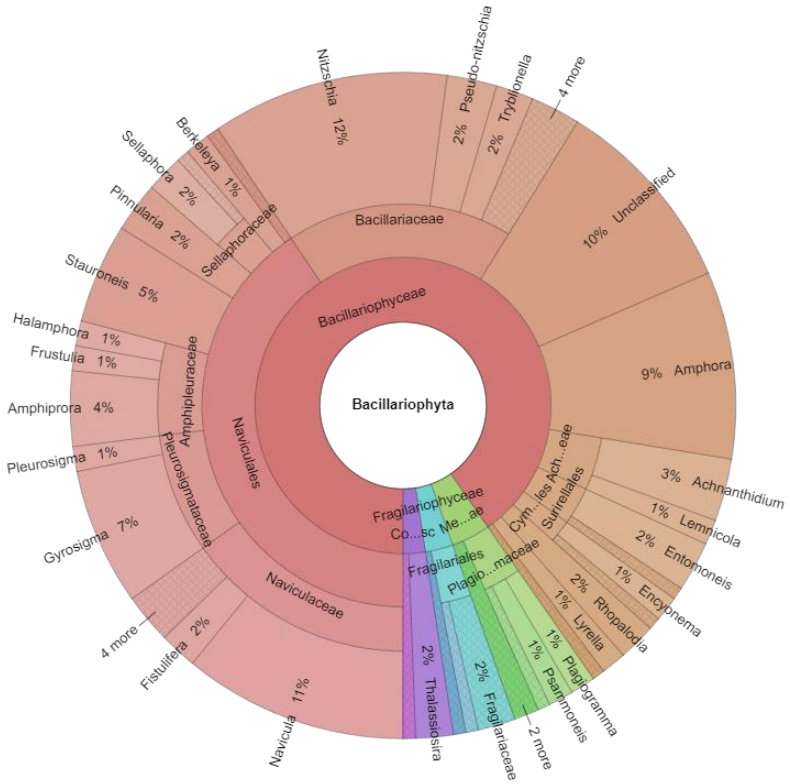
Taxonomic distribution of the benthic A) eukaryote, B) diatom, and C) prokaryote community compositions of Vavouto Bay sediments. Eukaryote and prokaryote taxonomic assignment is provided to family level and the diatoms to genus level.

Interactive versions of the Krona charts are available as supplementary material to Gillmore et al. (2021a). https://doi-org.ezproxy.uow.edu.au/10.1002/etc.5039

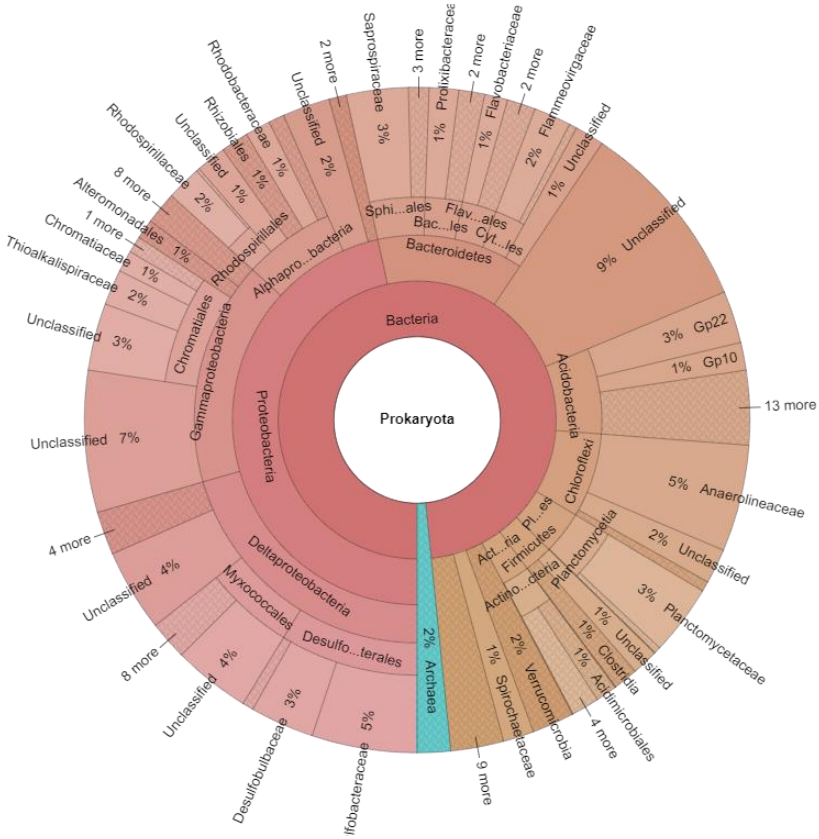
A) Eukaryote taxonomic distribution



B) Diatom taxonomic distribution



C) Prokaryote taxonomic distribution



D4. The relationship between community composition and sediment predictor variables

Distance-based linear modelling (DistLM) results for exploring the relationship between community composition and sediment predictor variables (forward selection, adjusted R^2, 999 permutations).

Variable	Forward selection sequential tests			
	Pseudo-F	P	Variance explained (%)	Cumulative variation explained (%)
Eukaryote				
AE-Ni	16.658	**0.001**	26	26
AE-Co	7.869	**0.001**	11	36
AE-Cr	5.696	**0.001**	7	43
AE-Al	5.200	**0.001**	6	49
AE-V	3.865	**0.001**	4	53
Sand (>63µm)	4.026	**0.003**	4	57
MC	3.162	**0.003**	3	60
Clay (<4µm)	2.776	**0.009**	3	63
AE-Zn	1.837	0.053	2	64
Organic-C	1.471	0.135	1	66
AE-Mn	0.887	0.511	<1	67
Diatom				
AE-Ni	13.996	**0.001**	23	23
AE-Al	8.791	**0.001**	12	35
MC	9.710	**0.001**	11	46
AE-Co	4.000	**0.002**	4	51
AE-Cr	3.545	**0.003**	4	54
Sand (>63µm)	3.35	**0.002**	3	58
AE-V	3.344	**0.004**	3	61
Organic-C	2.007	**0.049**	2	63
Clay (<4µm)	1.286	0.213	1	64
AE-Zn	0.976	0.432	<1	65
AE-Mn	0.471	0.885	<1	65
Prokaryote				
AE-Ni	10.899	**0.001**	19	19
AE-Co	6.859	**0.001**	10	29
AE-Cr	3.323	**0.001**	5	34
Organic-C	2.854	**0.001**	4	38
AE-Al	2.484	**0.002**	3	41
MC	2.232	**0.004**	3	44
Sand (>63µm)	1.980	**0.009**	3	46
Clay (<4µm)	2.329	**0.001**	3	49
AE-Zn	1.946	**0.005**	2	52
AE-V	1.351	0.096	2	53
AE-Mn	1.201	0.203	1	55

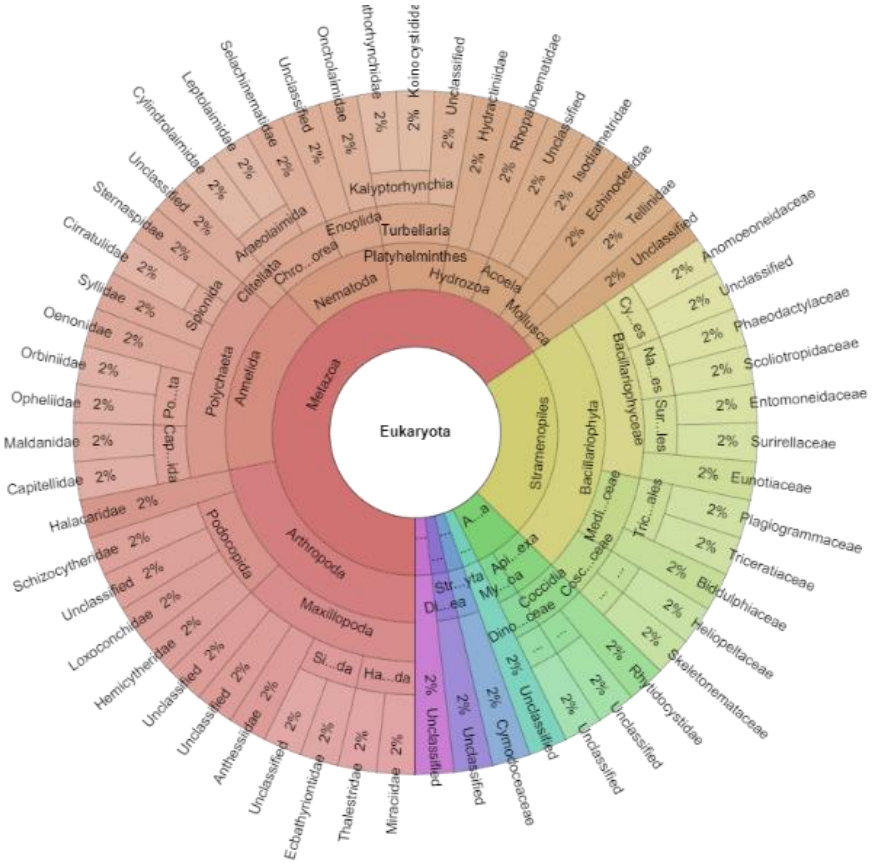
AE = Dilute-acid extractable; MC = Moisture content.

D5. Taxonomic distribution of the benthic community that responded to increasing dilute-acid extractable nickel concentration

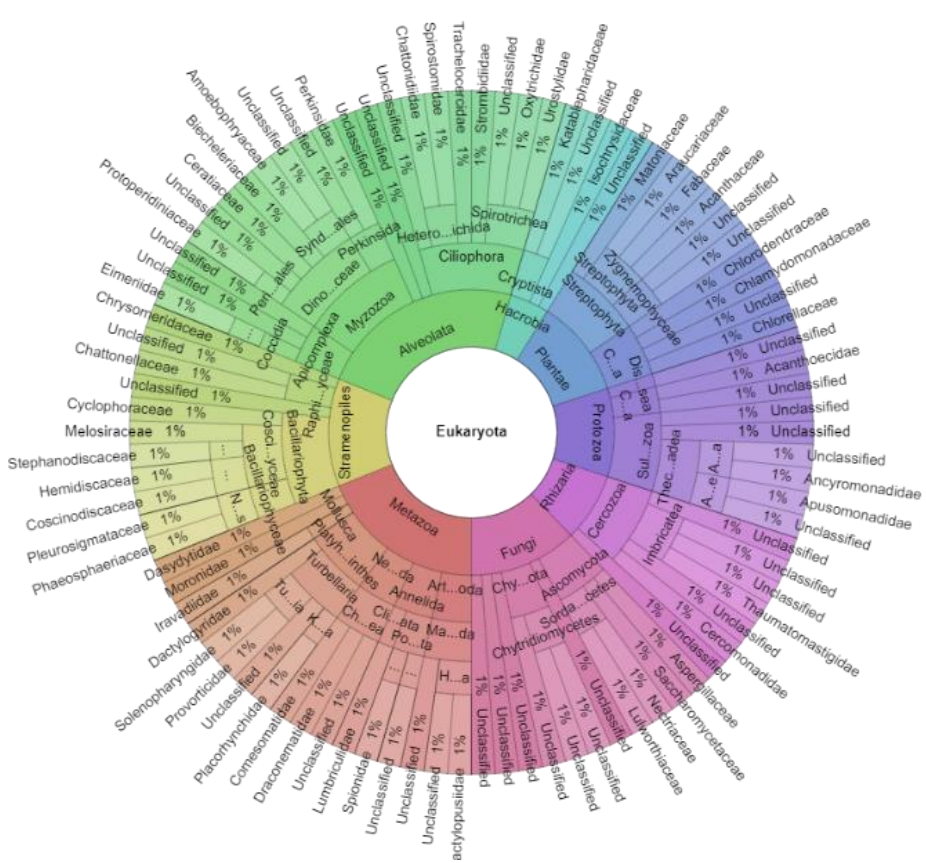
Taxonomic distribution of the benthic eukaryote community that responded A) negatively and B) positively to increasing dilute-acid extractable nickel concentration. Taxonomic assignment is provided to family level.

Interactive versions of the Krona charts are available as supplementary material to Gillmore et al. (2021a). https://doi-org.ezproxy.uow.edu.au/10.1002/etc.5039

A) Negatively responding

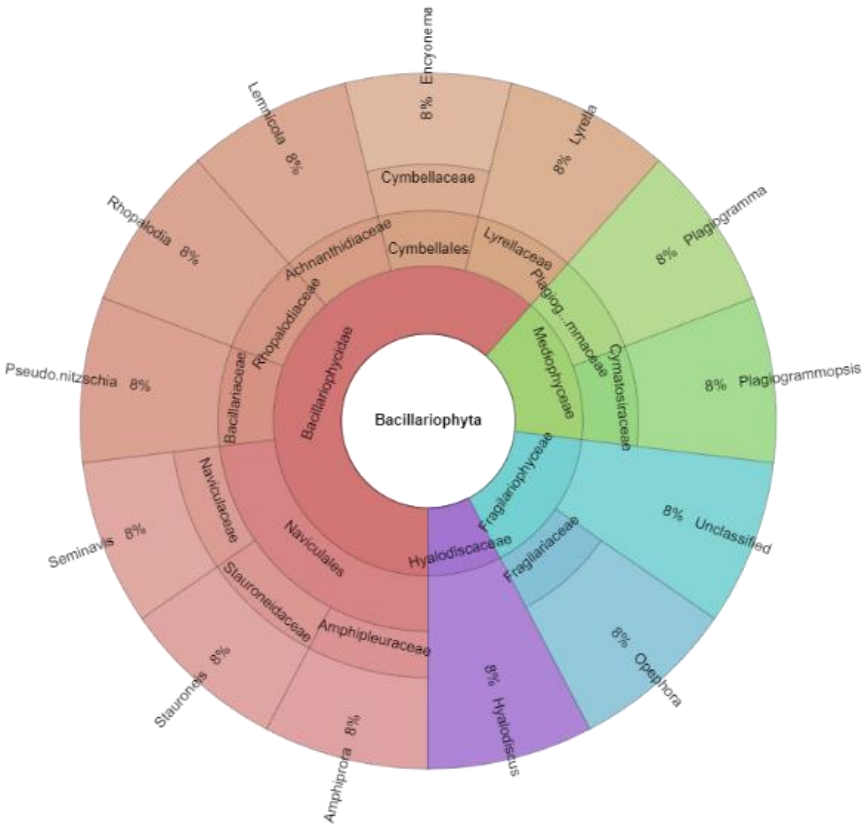


B) Positively responding

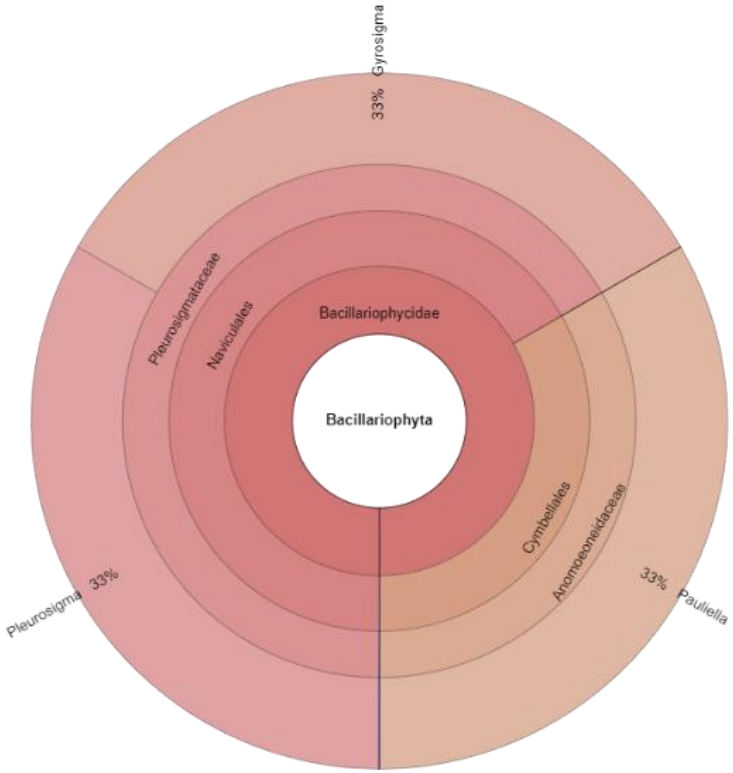


Taxonomic distribution of the benthic diatom community that responded C) negatively and D) positively to increasing dilute-acid extractable nickel concentration. Taxonomic assignment is provided to genus level.

C) Negatively responding

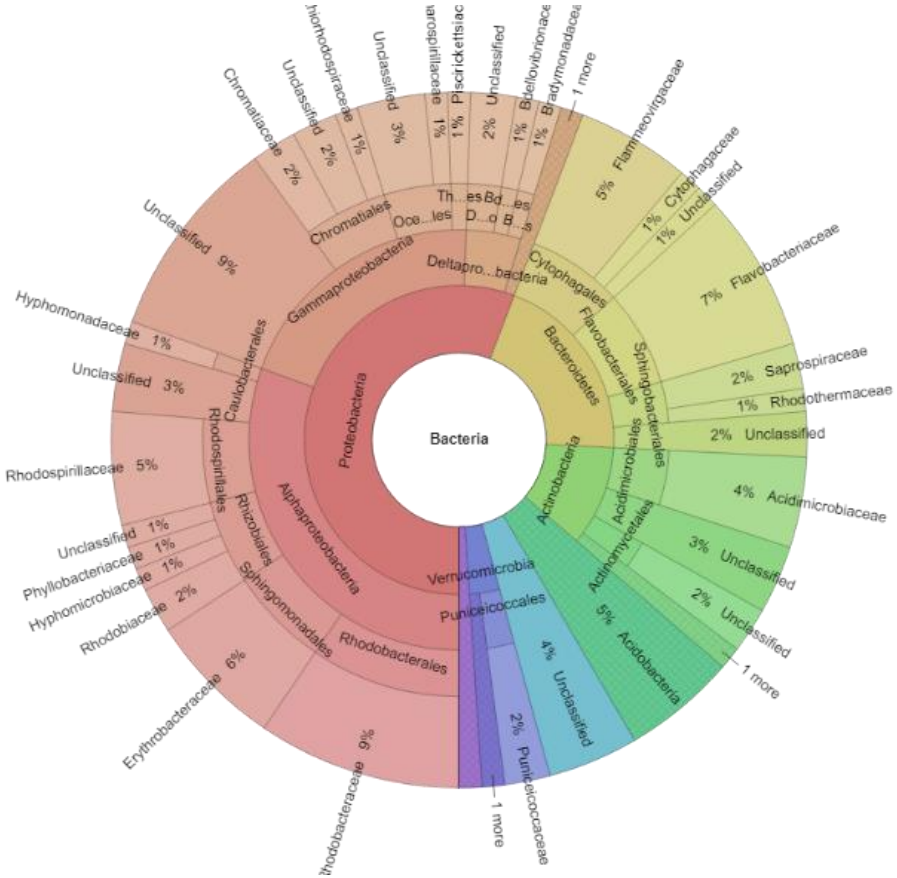


D) Positively responding

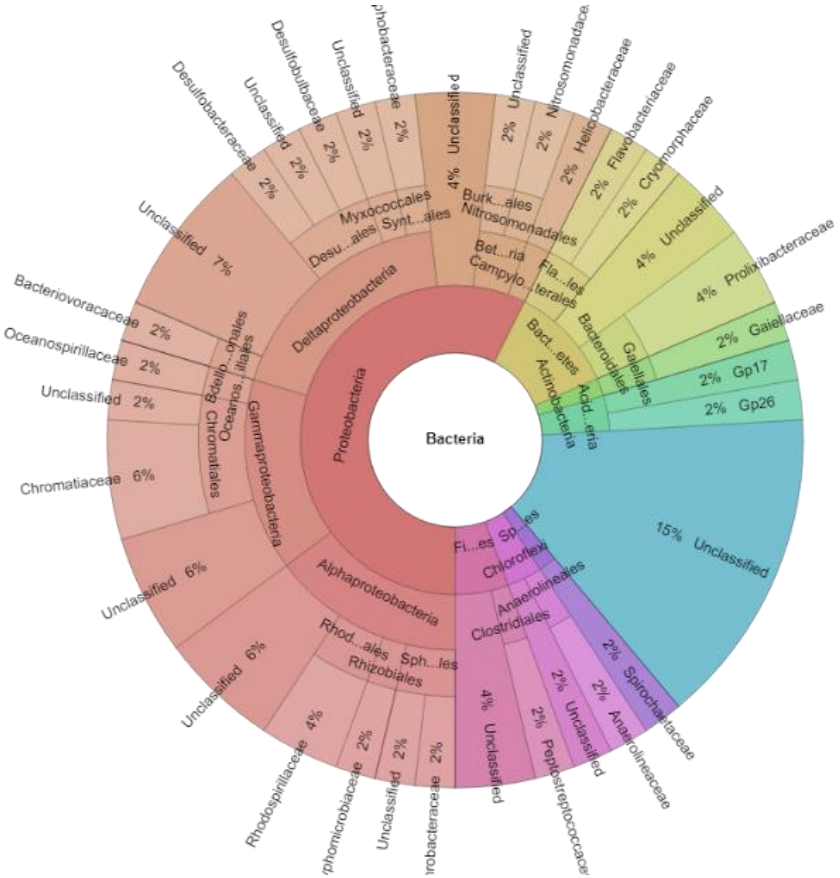


Taxonomic distribution of the benthic prokaryote community that responded E) negatively and F) positively to increasing dilute-acid extractable nickel concentration. Taxonomic assignment is provided to family level.

E) Negatively responding

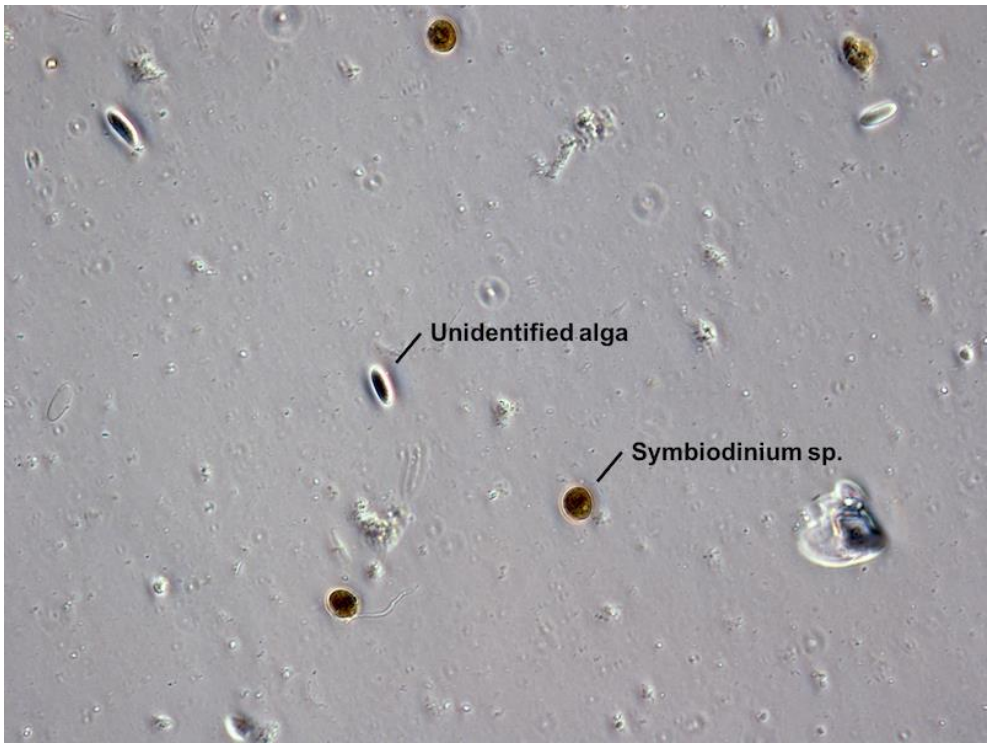


F) Positively responding



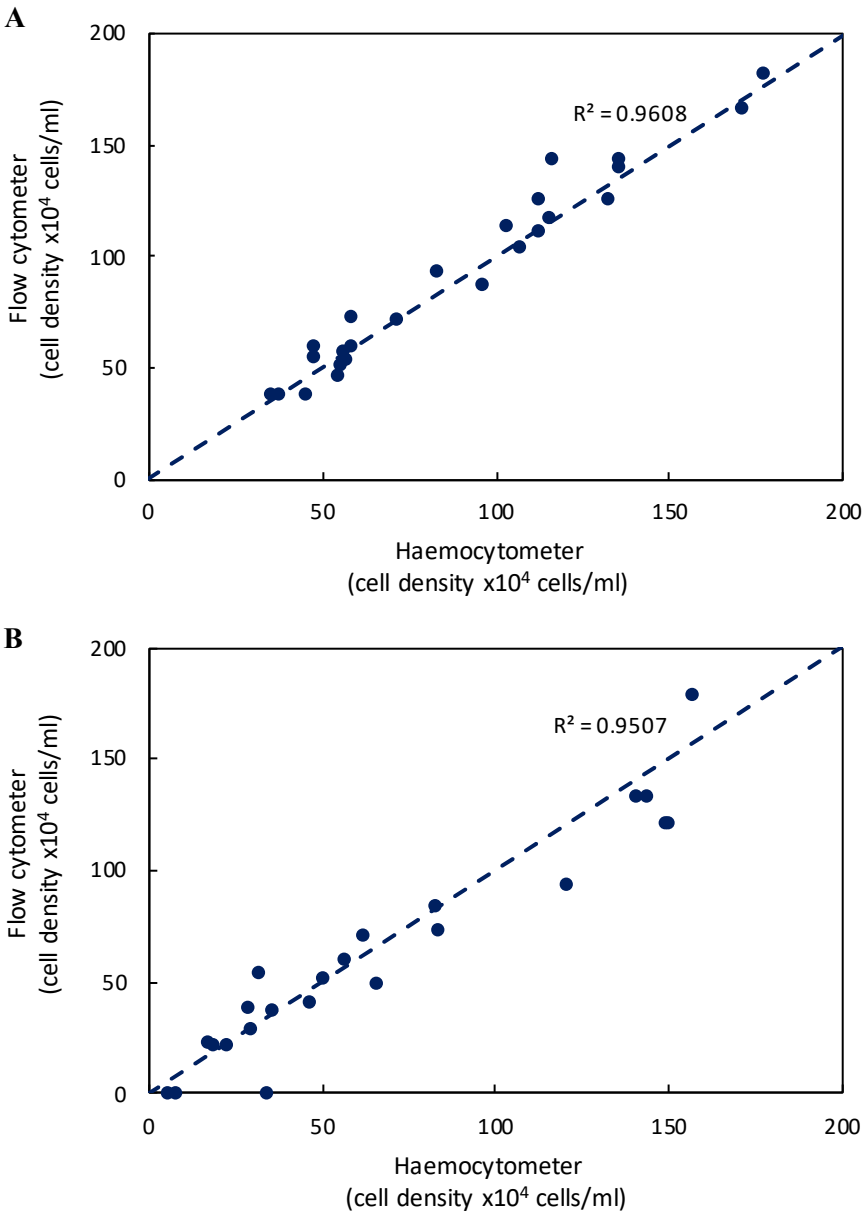
Appendix E – Appendix to Chapter 6

E1. Example of the *Symbiodinium* sp. and unidentified alga isolated from the coral tissue



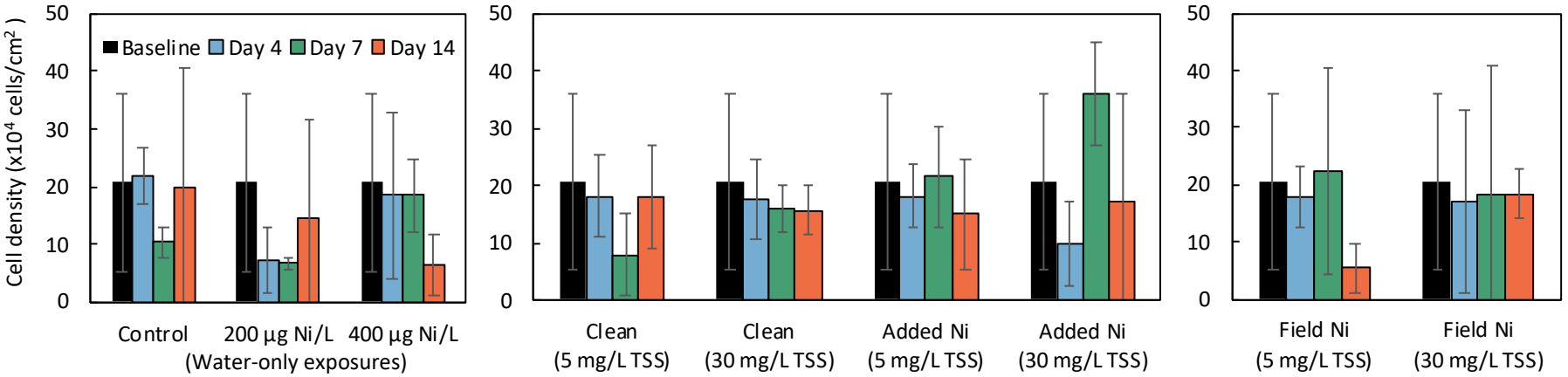
E2. Comparison of cell density counts measured by flow cytometry and using a haemocytometer

Comparison of cell density counts measured by flow cytometry and using a haemocytometer viewed under a microscope (n = 18) of A) *Symbiodinium sp*. and B) unidentified alga. The dashed line (x = y) represents a 1:1 relationship between cell density counts using the two methods. Each point represents one replicate.



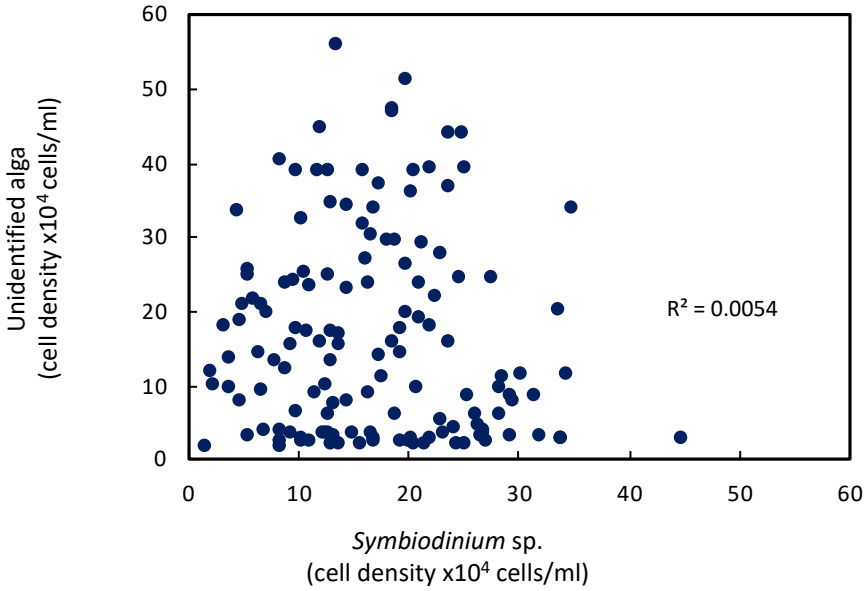
E3. The influence of treatment condition on the unidentified alga's density

The influence of treatment condition on unidentified alga's density, before exposure (Baseline), during the exposure (Days 4 and 7) and following a 7-d recovery period (Day 14): *Left* = water-only dissolved (<0.45 µm) nickel (0, 200 and 400 µg Ni/L); *Middle* = suspended sediment without Ni (Clean) and spiked with nickel (Added Ni, 6300 mg total Ni/kg); and *Right* = field-collected nickel-contaminated suspended sediment (Field Ni, 240 mg total Ni/kg). Values presented are the mean ± standard deviation (*vertical error bars*), n = 6 (baseline), n = 2 (water-only exposure) or n = 3 (suspended sediment exposures). TSS = total suspended solids, nominal values.



E4. Relationship between the quantity of *Symbiodinium* sp. and the unidentified alga

Relationship between the quantity of *Symbiodinium* sp. present and the quantity of the unidentified alga present in each sample. The dotted line represents the plotted linear trendline.



E5. Nickel concentrations in the algal symbionts of *Acropora muricata*

Nickel concentrations (µg/g DW) in the algal symbionts of *Acropora muricata* exposed to: *Left* = suspended sediment without Ni (Clean) and spiked with nickel (Added Ni, 6300 mg total Ni/kg); and *Right* = field-collected nickel-contaminated suspended sediment (Field Ni, 240 mg total Ni/kg). Values presented are the mean ± standard deviation (*vertical error bars*), n = 3. The baseline concentration of nickel in the algal symbionts was 2 ± 1 µg/g DW. The concentration of nickel in the algal symbionts in the Clean 5 mg/L TSS and 30 mg/L TSS treatments were 1.8 to 4.2 µg/g DW.