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# **Effects of copper contamination on nitrogen cycling in a freshwater sediment**

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A thesis  
submitted in partial fulfilment  
of the requirements for the Degree of  
Master of Water Resource Management

at  
Lincoln University  
by  
Tomson Tomoiye

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Abstract of a thesis submitted in partial fulfilment of the  
requirements for the Degree of Master of Water Resource Management.

Effect of copper contamination on nitrogen cycling in a freshwater sediment

by

Tomson Tomoiye

The effects of trace elements on microbial populations and function can be wide-ranging and difficult to disentangle, hence only a few studies report specific effects on processes in elemental biogeochemical cycles. This study used a laboratory mesocosm experiment to test the effect of two levels of copper (Cu) contamination (65 mg/kg and 270 mg/kg) on nitrogen cycling in a freshwater sediment. The mobilisation of trace elements (copper, iron and manganese) and nitrogen (as nitrate and ammonium) in the sediments was analysed using diffusive gradients in thin films. A sequential extraction protocol was used to measure the partitioning of the introduced Cu into various geochemical fractions in the sediment. The release of nitrous oxide from the treated sediments was determined over an 82-h period. The results showed that, at these levels, Cu stimulated nitrate removal and increased the production of nitrous oxide ( $48 - 70 \mu\text{g}/\text{m}^2/\text{h}$ ) and accumulation of  $\text{NH}_4\text{-N}$  in the sediments. Most of the total extractable-Cu in the sediment (69 %) was bound to the organic matter fraction, while the rest was associated with the relatively inert residual fraction (>20 %). Only a small amount (~2%) was found exchangeable, carbonate, and Fe/Mn oxide fractions. This research concludes that Cu contamination can affect the nitrogen cycle in NZ freshwater sediments, which may have further implications on water quality and greenhouse gas inventories from freshwater environments.

**Keywords:** Trace elements, copper speciation, nitrous oxide, nitrate, ammonium, diffusive gradients in thin-films.

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# Table of Contents

<b>Abstract</b> .....	<b>ii</b>
<b>Acknowledgements</b> .....	<b>iii</b>
<b>Table of Contents</b> .....	<b>iv</b>
<b>List of Tables</b> .....	<b>vi</b>
<b>List of Figures</b> .....	<b>vii</b>
<b>Chapter 1 Introduction</b> .....	<b>1</b>
1.1 Background .....	1
1.1.1 Research aim and objective .....	2
<b>Chapter 2 Literature review</b> .....	<b>3</b>
2.1 Human intervention in the global nitrogen cycle .....	3
2.2 Respiration in river sediment.....	3
2.3 The nitrogen cycle in aquatic system.....	4
2.4 Trace elements in sediments .....	8
2.4.1 Accumulation of trace elements in sediments and their effects.....	8
2.4.2 Copper in sediments .....	9
2.4.2 Cu effects on the N-cycle .....	10
<b>Chapter 3 Material and Methods</b> .....	<b>12</b>
3.1 Sediment and water sampling .....	12
3.1.1 Sediment characterisation .....	13
3.2 Sediment treatment.....	13
3.3 The mesocosm experiment.....	14
3.4 Water physical and chemical characteristics .....	14
3.5 N <sub>2</sub> O sampling .....	15
3.6 DGT and DET preparation and measurements .....	16
3.7 Trace element speciation.....	19
3.8 Trace element and nutrient analyses and quality control procedures.....	21
3.9 Statistical analyses .....	21
<b>Chapter 4 Results</b> .....	<b>23</b>
4.1 Water physical and chemical characteristics.....	23
4.2 Sediment characteristics.....	24
4.3 Depth profiles of trace element concentrations .....	24
4.4 Cu Fractionation.....	27
4.5 Porewater NO <sub>3</sub> -N and NH <sub>4</sub> -N profiling.....	30
4.6 N <sub>2</sub> O release from sediments.....	32
<b>Chapter 5 Discussion</b> .....	<b>34</b>
5.1.1 Limitations of the experimental approach .....	37
5.2 Conclusion.....	38

5.3 Recommendation for further research.....	38
<b>References .....</b>	<b>39</b>

## List of Tables

Table 2-1 Generalized reactions in anaerobic respiration. Modified from Wang (2007) .....	4
Table 2-2 Nitrogen cycling reactions in river sediment .....	7
Table 3-1 Instrument Detection Limit and DET and DGT method detection limits for Cu, Fe, Mn and nitrogen DGT. Concentrations given as $\mu\text{g/L}$ .....	21
Table 4-1 Average pH, DO saturation, conductivity, and temperature over a period of 2 weeks prior to DGT, DET deployment and sediment core sampling. Averages with the same letter are not significantly different ( $P < 0.05$ ) .....	23
Table 4-2 Trace element concentrations (as $\mu\text{g/L}$ ) in overlying water during the day of DGT deployment.....	23
Table 4-3 Characteristics of the three treated sediment. The values show averages ( $\pm$ standard error; $n = 3$ ). Where the letters are the same, there was no significant difference between means ( $P < 0.05$ ).....	24

## List of Figures

Figure 2-1: A summary of the N cycle in various forms in sediment and water. Modified from (Potgieter-Vermaak, 2007)Potgieter-Vermaak, (2007). .....	5
Figure 2-2 The fate of Cu in river sediment. ....	10
Figure 3-1 Sampling location at Ararira/LII river near Lincoln township .....	13
Figure 3-2 Schematic of mesocosm with sediment and river water circulation system (not to scale). The mesocosm was sealed at the top and bottom using air-tight screw-on lids (not shown).....	15
Figure 3-3 Schematic representation of the DGT assembly (not to scale). Modified from Amato (December 2019) .....	18
Figure 3-4 The arrangement of DGT and DET probes during deployment in mesocosm. ....	20
Figure 4-1 Mean $C_{DGT-Cu}$ at 1 cm intervals below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).....	25
Figure 4-2 Mean $C_{DGT-Fe}$ depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).....	26
Figure 4-3 Mean $C_{DGT-Mn}$ depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).....	27
Figure 4-4 Mean exchangeable Cu concentration depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error. Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ). .....	28
Figure 4-5 Mean carbonate-bound Cu concentration depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error. Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ). .....	29
Figure 4-6. Average proportions of Cu bound to different sediment fractions in the three treated sediments across all depths ( $n = 27$ ). The values shown in the graphs show percentage of total. ....	30
Figure 4-7 Mean $C_{DGT-NO_3}$ depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 =	

average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).....31

Figure 4-8 Mean  $C_{DGT-NH_4}$  depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where the difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).....32

Figure 4-9 The mean  $N_2O$  release from each mesocosm over three consecutive 24 h periods. The error bars show standard error. Averages that share the same letter were not significantly different ( $P>0.05$ ) .....33

# Chapter 1

## Introduction

### 1.1 Background

Nitrogen (N) is an essential element to life because of its role in forming critical biomolecules, including proteins and nucleic acids. It is also a limiting factor for plant growth (Thomas & Walmsley, 2015). To satisfy crop demand and maintain productivity, farmers in New Zealand use N fertilisers to enhance plant growth (Ahmed et al., 2020; Stewart et al., 2011). However, the intensification of agriculture and increased use of N fertilizer and the waste from the livestock farming have increased N loads into freshwater (Shamrukh et al., 2001), resulting in declining water quality and issues such as eutrophication. In the environment N exists in a variety of organic and inorganic species. The conversion between different N species in the environment is facilitated by a range of micro-organisms whose activity can be affected by changes in environmental conditions or more direct effects, such as pollution.

Growing human populations, urban development and the associated release of hazardous substances into the environment are altering the freshwater ecosystem profoundly (Compton et al., 2011; Thomas et al., 2014). Sediment microorganisms are vulnerable to these changes. Runoff from urban and farming landscapes can deposit trace elements (mainly cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn)) into freshwater sediments where they often accumulate overtime due to their affinity to bind strongly with the particulate matter (Amoatey & Baawain, 2019). Upon entering freshwater sediments, they partition into a range of chemical species that can in turn regulate their availability to affect localized biological processes and mobilisation into the aquatic foodchain (Calmano et al., 1993; Chapman et al., 1998).

Due to the commonplace nature of trace element pollution, the effects trace element accumulation on the drivers of N-cycle in soil and river sediments has been of increasing interest (Li et al., 2019; Magalhães et al., 2007; Rother et al., 1982; Sakadevan et al., 1999; Sánchez-Andrea et al., 2011; Vázquez-Blanco et al., 2020). Certain microbial functions in river sediments can be greatly affected by trace element accumulation (Babich & Stotzky, 1985; Hoostal et al., 2008; Li et al., 2020) and some of the negative consequences of this include nutrient accumulation, eutrophication, and increased greenhouse gas accumulation (as nitrous oxide, N<sub>2</sub>O). Studies so far have focused on individual metals, such as zinc (De Brouwere et al., 2007) or metal mixtures (e.g.) Cd, Cu, Zn (Holtan-Hartwig et al., 2002). In New Zealand, copper is used as a fungicide on vineyards and orchards, added as fertilizer to low Cu soils (Morgan & Taylor, 2004) and in some areas as part of roofing (Wang et al., 2021) and it has been

shown to end up in freshwater courses (Pennington & Webster-Brown, 2008). The specific effects of Cu on the N-cycle in sediments are not well understood. The Australia and New Zealand Environment and Conservation (ANZECC) default guideline value (65 mg/kg) and the upper guideline value (270 mg/kg) for Cu concentrations in sediments are part of a suite of measures that aim to protect aquatic ecosystems, however, these values are only broadly indicative of potential for harm to ecosystems. The specific effects on sediment biogeochemical nutrient cycles at these levels have not been explored.

### **1.1.1 Research aim and objective**

The aim of this research project was to study the effects of Cu contamination at the ANZECC default guideline value and the upper guideline value on the N-cycle in a freshwater sediment.

To achieve the aim, the following objectives were set;

1. Measure the partitioning of Cu into different species when introduced into an otherwise uncontaminated sediment at two levels of Cu, following the guideline values set by ANZECC (65 and 270 mg/kg Cu).
2. Investigate the effects of introducing Cu on the mobilisation of two key N species: NO<sub>3</sub>-N and NH<sub>4</sub>-N in a controlled mesocosm experiment.
3. Measure the production rate of N<sub>2</sub>O gas from sediments subjected to the different Cu treatments.

## Chapter 2

### Literature review

#### 2.1 Human intervention in the global nitrogen cycle

Humans have approximately doubled the input of reactive nitrogen (N) to the earth's land surface via agricultural fertilization and burning of fossil fuels (Fog, 1988; Galloway et al., 2008; Gruber & Galloway, 2008; Lu et al., 2011; Schlesinger, 2009). In NZ, 40 % of farms are classified as 'sheep and beef' farms, 31 % classified as dairy farms, while the remaining 29 % is shared among horticulture, arable, and urban use (Pannell et al., 2021). Farmers employ an intense home-grown pasture for dairy (Pembleton et al., 2013; Vogeler & Cichota, 2017). This is driven by the use of nitrogen fertilizer and there are concerns of environmental sustainability of these farm systems (Clark et al., 2007; Vogeler & Cichota, 2017). The excess nitrate ( $\text{NO}_3^-$ ) from intensive farming practices and cow urine patches can leach into nearby fresh water and cause an overabundance of nutrients resulting in eutrophication. At anaerobic conditions, available  $\text{NO}_3^-$  can be removed through denitrification, producing  $\text{N}_2\text{O}$  which is a greenhouse gas. Eutrophication due to excess  $\text{NO}_3^-$  in aquatic systems and global acidification including stratospheric ozone loss due to productions of nitrous oxide are regarded as some of the negative consequences (Dalley et al., 2020; Gruber & Galloway, 2008).

#### 2.2 Respiration in river sediment

Under oxic conditions, oxygen ( $\text{O}_2$ ) is an important driver for microbial activity. Gibert et al. (1994) showed that sediment microbes preferentially use oxygen to decompose organic compounds using aerobic respiration to maximise energy. Aerobic respiration in sediments depends on  $\text{O}_2$  and, when the diffusion of the gas into sediment is impeded, respiration can deplete it with depth in the sediment (Mermillod-Blondin et al., 2005).

Under anaerobic conditions certain microorganisms can switch to using nitrate ( $\text{NO}_3^-$ ) as the electron acceptor when oxidizing organic matter, producing nitrous oxide ( $\text{N}_2\text{O}$ ) and dinitrogen gas ( $\text{N}_2$ ) or ammonium ( $\text{NH}_4^+$ ) and nitrite ( $\text{NO}_2^-$ ). The anoxic zone of freshwater sediments supports anaerobic oxidizing microorganisms that may be coupled to  $\text{NO}_3^-$ , iron (Fe), sulphate ( $\text{SO}_4^{2-}$ ), or manganese (Mn) reduction (Preisler et al., 2007). Facultative bacteria switch to using Fe (III) and Mn (IV) as electron acceptors (Nealson & Saffarini, 1994) when all the  $\text{NO}_3^-$  has been consumed. The microorganisms gain energy from by using Fe (III) and Mn (IV) to break down organic matter; however, the energy return from these processes decreases in the order  $\text{O}_2 > \text{NO}_3^- > \text{Mn(IV)} > \text{Fe(III)}$ . Reduced forms of the two metals ( $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$ ) are produced from these reactions (Equations 1 and 2, respectively).

Anaerobic bacteria continue to use Fe (III) and Mn (IV) until their easily available forms have been consumed, after which they switch to using sulfate ( $\text{SO}_4^{2-}$ ) as the electron acceptor, but at the cost of an even lower energy return (Equation 3). This process produces hydrogen sulfide ( $\text{H}_2\text{S}$ ) as an end product (Holmer & Storkholm, 2001). When this reaction happens in sediment, the toxic  $\text{H}_2\text{S}$  gas can affect ecosystem health (Dordević et al., 2021). When all the  $\text{SO}_4^{2-}$  has been consumed, the organic matter undergoes methanogenesis where methane ( $\text{CH}_4$ ) is produced (Rother, 2010)(Equation 4). Table 2.1 shows all the oxidation reactions that happens the river sediment.

Table 2-1 Generalized reactions in anaerobic respiration. Modified from Wang (2007)

$5\text{CH}_2\text{O} + 4\text{MNO}_4(\text{s}) + 12\text{H}^+ \rightarrow 4\text{Mn}^{2+}_{(\text{aq})} + 5\text{CO}_2 + 11\text{H}_2\text{O}$	Equation 1
$\text{CH}_2\text{O} + 4\text{FeOOH}(\text{s}) + 8\text{H}^+ \rightarrow 4\text{Fe}^{2+}_{(\text{aq})} + \text{CO}_2 + 7\text{H}_2\text{O}$	Equation 2
$2\text{CH}_2\text{O} + \text{SO}_4^{2-} + 2\text{H}^+ \rightarrow \text{H}_2\text{S} + 2\text{CO}_2 + 2\text{H}_2\text{O}$	Equation 3
$2\text{CH}_2\text{O} \rightarrow \text{CH}_4 + \text{CO}_2$	Equation 4

### 2.3 The nitrogen cycle in aquatic system

The aquatic N cycle circulates N in various forms in waters and sediments (Figure 2.1). Although N constitutes 78 % (by volume) of the atmosphere as the relatively unreactive dinitrogen gas,  $\text{N}_2$ , this abundant reservoir exists in a form unusable by most organisms. Nitrogen, as a building block in the structure of nucleic and amino acids, porphyrins, and amino sugars, has a central role in many biogeochemical cycles (Brandes et al., 2007). The interchange between  $\text{N}_2$  in the atmosphere and “reactive N” (i.e. N compounds that support, or are products of, cellular metabolism and growth) is entirely controlled by microbial processes (Stein & Klotz, 2016). However, these processes are strongly impacted by anthropogenic activities. Understanding how N-cycling microorganisms function in sediment from oxic to anoxic in aquatic environments is key to characterize and evaluate their activities under different environmental conditions (Taroncher-Oldenburg et al., 2003).

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[https://www.researchgate.net/figure/A-summary-of-the-major-processes-of-the-nitrogen-cycle-in-the-estuarine-environment\\_fig11\\_253328982](https://www.researchgate.net/figure/A-summary-of-the-major-processes-of-the-nitrogen-cycle-in-the-estuarine-environment_fig11_253328982)

Figure 2-1: A summary of the N cycle in various forms in sediment and water. Modified from Potgieter-Vermaak (2007).

Apart from the anthropogenic nitrogen input into freshwater sediment, previous study showed that nitrogen fixation favours the winter condition and contributes a certain amount of bioavailable N to the aquatic ecosystem (Yao et al., 2018). Nitrogen fixation rates are correlated to the abundance of *nifH* gene (Jabir et al., 2021). High levels of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in the overlying water reduces nitrogen fixation (Lin et al., 2017). Apart from the high N content in water, several other factors also affect nitrogen fixation, example, Qashqari et al. (2020) found that nitrogen fixation increased at 28 °C and reduces at 35 °C. Like any other sediment microorganisms, the nitrogen fixing microorganism is also affected by accumulation of trace elements (Li et al., 2020). Generally, the biological nitrogen fixation is the conversion of atmospheric dinitrogen ( $\text{N}_2$ ) gas to ammonium ( $\text{NH}_4^+$ ) or ammonia ( $\text{NH}_3$ ) (Equation 5; Table 2-2).

Ammonification is the release of ammonium ( $\text{NH}_4^+$ ) as a by-product of the breakdown of sedimented organic nitrogen by microorganisms. The released  $\text{NH}_4^+$  can then be integrated by benthic plants and the indigenous microflora (Herbert, 1999) or released to the overlying water (Tang et al., 2018). Ammonification is influenced by several environmental factors like dissolved oxygen (DO) and temperature, for instance, Lee et al. (2018) found that the higher  $\text{NH}_4^+$  and the lower  $\text{NO}_3^-$  in the

shallow sediment suggested that ammonification favours low sediment oxygen demand. The ammonium released from ammonification (Equation 6; Table 2-2) and from other anthropogenic sources that are not integrated by benthic plants undergo nitrification.

Nitrification is the oxidization of ammonia ( $\text{NH}_3$ ) to nitrite ( $\text{NO}_2^-$ ) and then further to nitrate ( $\text{NO}_3^-$ ) (Yilin et al., 2014). This process is favoured in aerobic conditions as oxygen is the key oxidizing agent. Certain types of bacteria are responsible for these two distinctive processes. Bacteria belonging to the genus *Nitrosomonas* convert  $\text{NH}_3$  to  $\text{NO}_2^-$  while those belonging to the genus *nitrobacter* oxidize  $\text{NO}_2^-$  to  $\text{NO}_3^-$  (Beeckman et al., 2018). The chemical equation for the first step is demonstrated in Equation 7 followed by the second step in Equation 8 (Table 2-2). Accumulation of trace elements can impact the nitrification process, although low concentration promotes certain bacterial activities, example, nitrate oxidizing bacteria promoted by low Cu concentration (Aslan & Sözüdoğru, 2017) and silver concentration at 2 mg/L stimulates coupled nitrification and denitrification by enhancing both nitrifying and denitrifying enzyme activities (Wang et al., 2020).

Nitrogen loss can also occur in the reduced oxygen zones of a sediment where both nitrification and denitrification coexist (Xia et al., 2017). River sediment with low carbon and nitrogen ratio have greater nitrogen removal capacity at low oxygen zones (Wu et al., 2021). In such low oxygen sediment environment, Nájera et al. (2021) found that nitrifiers were abundant in spring (14 °C) while denitrifiers were more abundant in summer with average temperature of 22 °C. From the oxygen low zone sediment down depth profile, nitrate is removed through denitrification, the process that produces nitrous oxides ( $\text{N}_2\text{O}$ ) as an intermediate gas and  $\text{N}_2$  as the product of this reaction (Equation 9; Table 2-2).

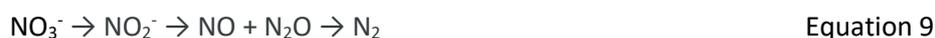
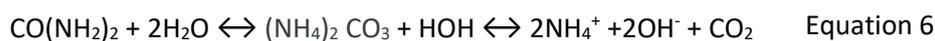
Recent research showed that denitrification rates in freshwater lakes are largely controlled by water physicochemical characteristics, for example excess nitrate with a low dissolved oxygen concentration can result in increased denitrification and reduced abundance of denitrifiers and submerged vegetation (Liu et al., 2018). In contrast, Xia et al. (2017) found that suspended sediment in freshwater aquatic system increased denitrification where the finer sediment accumulated. In such environments, the availability of  $\text{NO}_3^-$  is a major factor (electron acceptor) that controls the denitrification rate. Unda-Calvo et al. (2019) investigated the activities of both nitrate and nitrite reductase enzyme and concluded that an increase in nitrate concentration in overlying water increased nitrate reductase activities while organic matter was vital for nitrite reduction to take place. Temperature also affect denitrification. A recent study on nitrogen loss in anoxic condition focusing on denitrification and anammox indicated that denitrification favours the warm conditions (summer temperature) over anammox and leads to  $\text{N}_2\text{O}$  production (Tan et al., 2020). Nitrous oxide is of particular concern because

it contributes to the global warming, and is considered to have a global warming potential that is 300 times greater than carbon dioxide (Ramanathan, 1998).

Nitrate in anaerobic sediment can also be transformed to ammonium ( $\text{NH}_4^+$ ) through the dissimilatory nitrate reduction to ammonium (DNRA) process, where  $\text{NO}_2^-$  is an intermediate product (Equation 10; Table 2-2). Similar to denitrification, sediment microorganisms use nitrate as an electron acceptor for DNRA (Liu et al., 2020). DNRA retains  $\text{NH}_4^+$  back in the sediment. Kelso et al. (1997) showed that DNRA was the reason why  $\text{NH}_4^+$  increased in a base river sediment which later becomes the substrate for nitrification (oxic condition) and anammox (anoxic condition) (Jensen et al., 2011). Previous studies showed that sediment DNRA activities depended on several other environmental factors including organic matter, temperature, and pH variation. For example, Li et al. (2019) and Lai et al. (2021) showed that a temperature increase from 10 to 40 °C (tropical) increased DNRA in sediments and Zhang et al. (2015) found that the rate of DNRA was higher under alkaline pH increased, while Song et al. (2014) found that DNRA is dominant over denitrification in nitrate reduction in sediment that are rich in organic matter content.

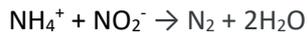
The available  $\text{NH}_4^+$  and  $\text{NO}_2^-$  produced in the anaerobic sediment column as a result of DNRA, denitrification, or anthropogenic input can go on to undergo anammox that produces  $\text{N}_2$  into the atmosphere (Equation 11; Table 2-2). In addition to denitrification, anammox is another pathway for N loss from sediments (Hu et al., 2012). Denitrification also produces  $\text{NO}_2^-$  in the process of reducing  $\text{NO}_3^-$ , providing more substrate for anammox bacteria to function (Wu et al., 2020). If denitrification is inhibited (e.g. by trace element accumulation) (Bollag & Barabasz, 1979; Kiskira et al., 2018), the anammox activity decreases due to the reduced production of the  $\text{NO}_2^-$  intermediate (Zhou et al., 2014). However, other research also suggest that high anammox activity was positively correlated to excess  $\text{NO}_3^-$  in the overlying water, suggesting a possibility of  $\text{NO}_2^-$  from nitrification (Equation 7; Table 2-2) facilitating anammox (Nicholls & Trimmer, 2009). Anammox in sediment occurs optimally when sediments are warm (25 °C), with low oxygen content and high  $\text{NH}_4\text{-N}$  and  $\text{NO}_x^-$  (Zhou et al., 2014).

Table 2-2 Nitrogen cycling reactions in river sediment





Equation 10



Equation 11

## 2.4 Trace elements in sediments

Freshwater river sediments have been exposed to trace element accumulation from anthropogenic activities such as industrialization and mining activities (Chaudhuri et al., 2014; Islam et al., 2017). The most common trace elements from those anthropogenic sources found in river sediments and have been studied widely are iron (Fe), zinc (Zn), lead (Pb), chromium (Cr), copper (Cu), and cadmium (Cd) (Abraham & Susan, 2017; Izydorczyk et al., 2021; Liu et al., 2018). Some rivers around the world have recorded very minimal trace elements (Fe, Zn, Pb, Cr, Cu, Cd) concentrations which have no significant effect on biota as in the case of Meghna river sediment in Bangladesh (Siddique et al., 2021), while some have reported very high concentration of Zn, Cu, Pb, Cd, and mercury (Hg), which were 15 – 17 times higher compared to their background value (Fuentes-Gandara et al., 2021). Previous research have shown that these trace elements can affect benthic microorganisms through to the highest tropic level in aquatic food chain (Kahlon et al., 2018).

### 2.4.1 Accumulation of trace elements in sediments and their effects.

Previous study in a contaminated sediment showed that trace element concentrations; V, Cr, Mn, Ni, Cu, Zn, As, Cd, Sb, Hg and Pb were 57.0, 62.0, 260, 110, 390, 410, 35.0, 3.0, 9.3, 0.41 and 400 mg kg<sup>-1</sup>, respectively and fall above the set sediment quality guidelines, presenting a threat to sediment microorganisms (Islam et al., 2017). Another study on sediment trace element risk assessment showed that sediment trace element concentrations (as mg/kg) were in the order of Cr (39 ± 15) > As (34 ± 15) > Cu (27 ± 11) > Ni (24 ± 9) > Se (17 ± 8), which also had adverse biological effect to benthic fauna (Sarkar et al., 2017). In such condition, monitoring and reporting of trace elements is critical to evaluate their status in the ecosystem and predict potential influences on other biogeochemical cycles. Benthic plants also uptake trace elements and store them in their tissues, Bonanno and Orlando-Bonaca (2017) found that seagrass accumulated As, Hg, and Pb in their roots while Cd, Cr, Cu, Mn, Ni, and Zn were accumulated in the leaves. If these seaweeds are consumed by other micro invertebrates, trace elements can easily pass up the food chain.

Some trace elements have been shown to affect the N cycle in sediments. Sakadevan et al. (1999) showed that Cu and Zn at a concentration of 100 mg/kg in a sediment increased N<sub>2</sub>O production by 170 ± 1.8 and 168 ± 2.7 mg N<sub>2</sub>O/kg sediment, respectively, while Cd at 100 mg/kg did not show any impact on. Others demonstrated that N fertilizer used in wheat farming exposed to trace elements (Ni,

Cu, Zn Cd, Pb) concentrations below 1 mg/kg reduced N<sub>2</sub>O production between 33 – 77% (Guangbin et al., 2021).

#### **2.4.2. Copper in sediments**

Main anthropogenic sources of Cu to freshwater sediments are from roads (car use), storm water, mining activities, and agricultural herbicide and fungicide use (Gillis & Birch, 2006; Jones et al., 2008; Teasdale et al., 2003; Willis et al., 2013). The fate of Cu (Figure 2.2) in sediment is determined by several processes including sorption/desorption, precipitation/dissolution, and complexation/decomplexation (Du Laing et al., 2009). Several studies showed that Cu binds strongly to organic matter, which has a significant influence on Cu distribution among various fractions (Joško, 2019; McLaren & Crawford, 2006; Song & Muller, 1995).

Guinoiseau et al. (2018) investigated the fate of particulate Cu and Zn using stable isotopes in two river sediments, one with high organic matter content ( $7.1 \pm 0.5$  mg/g) and the other with low organic matter content ( $3.5 \pm 0.1$  mg/g). Results show that the Cu isotope accumulated more in the sediment with the high organic matter content more than in the sediment with the low organic matter content. Sediment pH also plays a crucial role in determining which dissolve ligand or solid phase Cu must associate with. A study on the partitioning of Cu in a river sediment showed that at acidic pH (pH < 5), copper was in dissolved labile form and flowed freely but quickly shift to a non-labile form (solid phase enriched Cu) when the pH increases (pH > 6.5) (Montecinos et al., 2020).

Not all the Cu in the sediment will bind to organic matter fraction, some will be available in the exchangeable, Fe/Mn oxide, residual, and carbonate phase. These adsorption and precipitations reactions limits the amount of Cu that is available for uptake by aquatic biota including sediment microorganisms (“bioavailable Cu”) (Rader et al., 2019). However, Cu can be released and become bioavailable during the change in sediment redox reactions. For example, the oxidation of sulphides in the oxic layer of the sediment release available Cu (Rader et al., 2019), the reduction of Fe/Mn oxides can also release Cu to the sediment water interface (El Houssainy et al., 2020), and the reduction of organic matter also releases Cu to the sediment (Refaey et al., 2017).

Copper can be available in the oxic and anoxic layers a sediment or throughout the depth profile due to the redox reactions described earlier. A study identifying bioavailability of Cu and its toxicity using the diffusive gradient (DGT) technique identified Cu accumulation at the oxic and sub-oxic layer of the sediment were due to Fe/Mn reduction (Simpson et al., 2012). Sakellari et al. (2011) found Cu was mobilised at 8 cm below the sediment water interface, which was associated with organic matter decomposition at that depth.

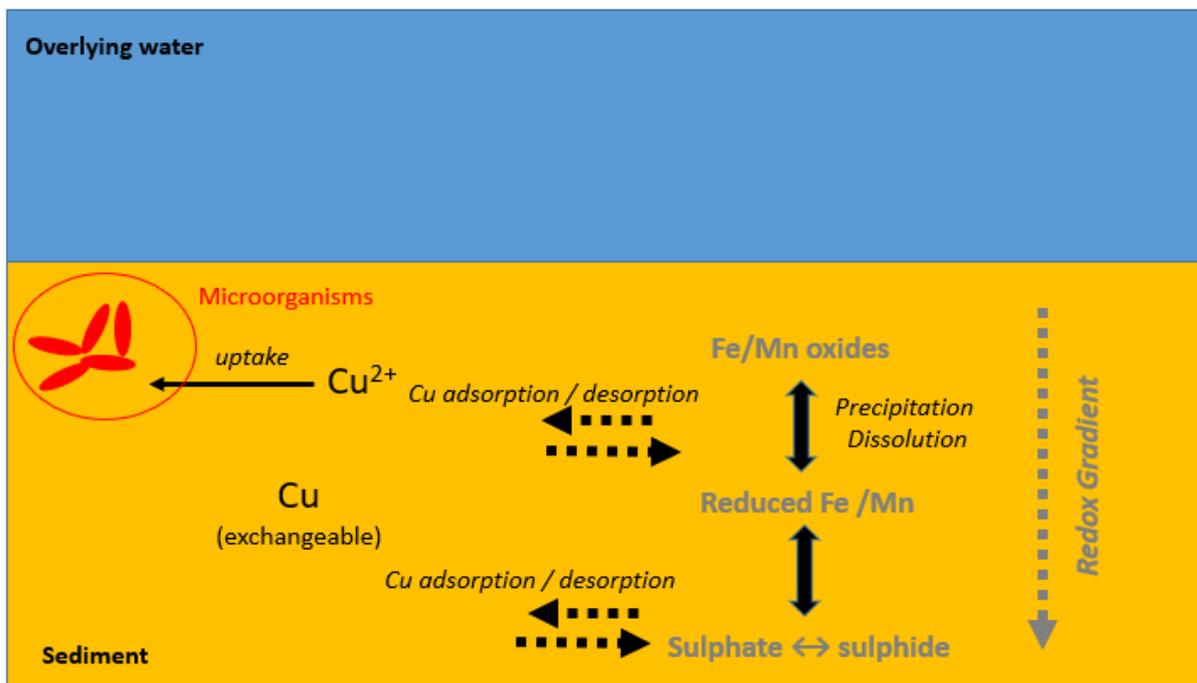


Figure 2-2 The fate of Cu in river sediment.

### Copper contamination in NZ freshwater sediments

Increased Cu concentration in New Zealand’s freshwater sediment indicates industrial use of copper roofing (Ashrafzadeh et al., 2018), fungicides for tomatoes (Wright et al., 2013), atmospheric deposition (Buteana et al., 2014), and agriculture where vineyards utilize copper, chromium, and arsenic treated post (Robinson et al., 2006). Copper (Cu) is found in Christchurch streams as anthropogenic input from the use of air conditioners and roof tops at levels close to 4 mg/kg (O’sullivan et al., 2012). Copper can also find its way into the sediment from ship repairs and shipwrecks. A study on sediment at Napier Harbor ship repair area found a wide range of Cu concentrations between 31 mg/kg to 435 mg/kg (Strong, 2005). About 40 % of these concentrations exceeded the default and high guidelines values for sediment quality (Anzecc, 2000). They attributed these concentrations to those that can negatively affect sediment microorganisms.

#### 2.4.2 Cu effects on the N-cycle

The effects of trace elements on microbial populations and function can be wide-ranging and difficult to disentangle, hence only a few studies report specific effects on processes in the biosphere overall. These are briefly reviewed below.

The negative effects of Cu were investigated focusing on the nitrogen microbial activities and found that Cu significantly reduced the microbial activities of both nitrification and denitrification at 100

mg/kg Cu, but at very low concentration 1 mg/kg Cu sediment, there was no effect (Simonin et al., 2018). Another research found that Cu concentration at  $10.5 \pm 0.3$  to  $18.7 \pm 0.3$  mg/kg slows down nitrification (Ali et al., 2021). Nitrifying bacteria are sensitive to the stress caused by Cu until they adapt to the stress (Broberg, 1984). Magalhães et al. (2007) explored the negative effects of Cu concentration (79 mg/kg) on denitrification in river sediment and found that denitrification was inhibited by 85 % and the production of nitrous oxide, a greenhouse gas was enhanced. Magalhaes also discovered that Cu (79 mg/kg) in subtidal muddy sediment did not affect denitrification, but sandy sites revealed high sensitivity to denitrification, which agrees with the earlier idea that sediment type has an important role in metal adsorption and its subsequent effect to sediment biota.

An increase in  $N_2O$  production was previously observed by Sakadevan et al. (1999) when 100 mg/kg Cu was added to the sediment. Another effect established in the same study by the authors was the accumulation of ammonium due to Cu contamination in depth profile, which can lead to eutrophication and nutrient accumulation, this effect was attributed to a stimulation of DNRA. The sediment microorganisms responsible for nitrification, denitrification, and DNRA have the ability to recover from Cu toxicity over time. Holtan-Hartwig et al. (2002) monitored the denitrification rate exposed to 80 mg/kg Cu sediment and observed immediate effect but took eight days to recover partly and a further 60 days to fully adapt to the Cu stress environment.

## **Chapter 3**

### **Material and Methods**

#### **3.1 Sediment and water sampling**

Sediment and water for the mesocosm experiment were collected from the LII river/Ararira in the Lincoln township, in the Canterbury Region (Figure 3.1). The LII river is primarily groundwater fed and part of a networks of drains that eventually feed into Te Waihora / Lake Ellesmere (Livingwater, 2015). The headwaters of the LII river are three groundwater springs that are confined within a larger catchment, which covers approx. 6,300 hectares (Doc, 2015). The land use surrounding this catchment is predominantly farming with increasing urbanization. Regular monitoring of the water quality in the LII river by Environment Canterbury (monitoring location: 43°41'42.08"S, 172°27'1.16"E), approximately 4.5 km downstream of the sediment sampling location, has found that the median total N concentration over the last five years in the river (3.55 mg/L) is in the worst 25<sup>th</sup> %ile in New Zealand (Lawa, 2020).

The sampling site (43°38'48.03"S, 172°29'49.55"E) was approximately 50 m from one of the groundwater springs within the Lincoln Township. The sediment was sampled using a plastic spade, targeting the top 10-30 cm of the sediment. Water samples were collected using a plastic 20 L container. Promptly after sampling, the sediments were sieved using a nylon sieve (2 mm mesh) to remove any particulate matter and most of the larger invertebrates. Subsets of 100 g of sediment and 30 mL of a filtered and acidified water sample were submitted for analysis to establish background physical and chemical characteristics (see below).



Figure 3-1 Sampling location at Ararira/LII river near Lincoln township

### **3.1.1 Sediment characterisation**

The sieved sediment was air dried and divided into subsamples for characterisation. Total C and N were analysed using a Vario-Max CN Elementar analyser (Elementar®, Germany). About 20 g of air-dried sample was submitted for organic matter analysis whereby the sample was dried at 105 °C for 24 h followed by 550 °C for 4 hours, the difference in weights were expressed in percentage. A subsample underwent digestion to enable measurement for total extractable elements. Briefly, 3 mL concentrated nitric acid (HNO<sub>3</sub>, trace element grade, 65%, Fisher Scientific, UK) and 3 mL hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%, Merck KGaA) were added to 0.5 g air-dried sediment and digested in a microwave digestion system (MARSXPRESS, CEM Corp), as established by Simmler et al. (2013). The digest was submitted for elemental analysis (See Section 3.8).

### **3.2 Sediment treatment**

The rest of the sediment was then divided to three equal parts and the Cu concentration was increased by introducing Cu (as CuCl<sub>2</sub>·2H<sub>2</sub>O, Scharlau AR Grade) to increase the sediment concentrations by 0 (high-purity water only), 65, and 270 mg/kg (expressed on an air-dried sediment basis), forthwith these amounts will be used to refer to the three Cu treatments (0 mg/kg, 65 mg/kg and 270 mg/kg, respectively). The sediments were then mixed thoroughly and left to equilibrate initially for two days under saturated conditions. Subsamples of the 65 mg/kg and 270 mg/kg-treated sediments were digested as before to determine the total extractable Cu concentrations.

### 3.3 The mesocosm experiment

After initial equilibration, the wet sediment was placed into cylindrical PVC mesocosms (internal diameter 110 mm, height: 350 mm) up to a height of 250 mm in 25 mm incremental stages to ensure constant density ( $0.8 \text{ g/cm}^3$ ) and porosity (0.7) throughout. The mesocosms were sealed at both ends (Figure 3.2.). The top cover was fitted with an air-tight water inlet and outlet to create a steady laminar flow of LII river water that circulated over the sediment-water interface and maintained a 5 cm water column over the sediment throughout the rest of the experiment (Figure 3.4). The mesocosm had a 5 cm high headspace. The circulating river water was aerated using a diffuser before entering the mesocosm to maintain a constant oxygen saturation in the water. The clear sides of the mesocosms were covered to keep the inside of the mesocosm dark and prevent biofilm growth. In total, nine mesocosms were established, with each of the three Cu treatments replicated thrice.

After establishment, the mesocosms were left for approximately six weeks to settle and to allow steady-state redox gradients to be established in the sediment. During this period, filtered ( $0.45 \mu\text{m}$ ) samples of overlying water, collected at the mesocosm water outlet, were tested for  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations for the seven weeks period prior to the final destructive sampling (see Section 3.8). The  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  concentrations were maintained at their natural river water levels using  $\text{NaNO}_3$  and  $\text{NH}_4\text{Cl}$  throughout the experiment.

### 3.4 Water physical and chemical characteristics

After the settlement period, the water pH, dissolved oxygen content, conductivity and temperature were measured daily at the mesocosm water outlet over two weeks using a calibrated multimeter (Hach HQ40D multiparameter two-channel). Immediately before the deployments commenced, water samples were collected and, in addition to the samples analysed for  $\text{N-NO}_3$  and  $\text{N-NH}_4$ , a further subset was filtered ( $0.45 \mu\text{m}$  pore), acidified using high purity  $\text{HNO}_3$  and analysed for Cu (see Section 3.8). Samples that could not be analysed on the day of sampling were stored at  $-20 \text{ }^\circ\text{C}$  until analysis.

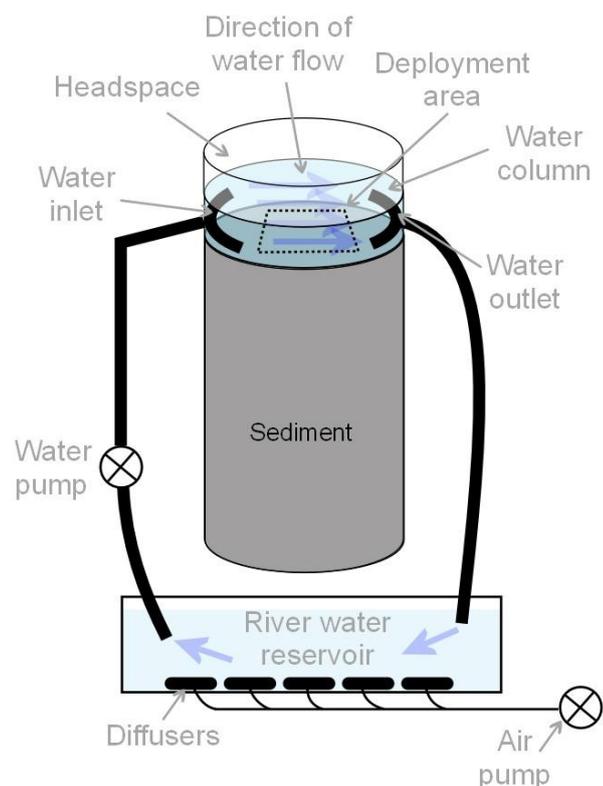


Figure 3-2 Schematic of mesocosm with sediment and river water circulation system (not to scale). The mesocosm was sealed at the top and bottom using air-tight screw-on lids (not shown).

### 3.5 N<sub>2</sub>O sampling

Eight weeks after the water circulation was started, N<sub>2</sub>O release from the sediments was measured. For this, the water circulation was stopped for 24 h and the mesocosm was sealed. During this time N<sub>2</sub>O was allowed to accumulate within the water column and headspace of each mesocosm. The N<sub>2</sub>O concentration in the water column and the aerated water were then analysed, using the protocol outlined in Clough et al. (2007). Briefly, triplicate water samples from each mesocosm were collected using 100 mL sterile syringes and purged into 100 mL evacuated serum bottles at atmospheric pressure. They were then equilibrated by purging 80 cc N<sub>2</sub> gas and kept on a shaker for 1 h. The N<sub>2</sub>O concentration in the headspace of the serum bottles was then measured using a gas chromatograph, as described by Clough et al. (2006). Atmospheric pressure at the time of sampling was recorded using a precision pressure indicator (GE Sensing, Druck DPI 150, USA). The amount of N<sub>2</sub>O in the stationary water column was used to estimate the amount of gas in the headspace, using Henry's Law. Briefly, the amount of N<sub>2</sub>O in the headspace was calculated and expressed as the proportional to its partial pressure of the N<sub>2</sub>O in the liquid phase in the serum bottle (Equation 12).

$$P_{gas} = C_{eq}/(P_{bar} \times B) \quad (\text{Equation 12})$$

where  $C_{eq}$  is the conc of  $N_2O$  in the water (mol),  $P_{gas}$  is the partial pressure of  $N_2O$  in the headspace (atm/atm),  $P_{bar}$  is the barometric pressure during the sampling (atm),  $B$  is the Bunsen coefficient ( $2.480 \times 10^{-2}$  mol/(L  $\times$  atm) ), according to Weiss and Price (1980)

The  $N_2O$  concentration in the water column was then used to estimate its concentration in the headspace of the mesocosm, assuming that the dissolved gas was at equilibrium with the headspace. Briefly, the  $P_{gas}$  was used to calculate the mass of  $N_2O$  in the headspace ( $M$ ), using the ideal gas equation (Equation 13):

$$M = MW_{N_2O} \times P_{gas} \times V/(R \times T) \quad (\text{Equation 13})$$

where  $MW_{N_2O}$  is the molecular mass of  $N_2O$  (44.013),  $V$  is the volume of the headspace (L),  $R$  is the ideal gas constant (0.0821 L.atm/mol.K) and  $T$  is the temperature (in °K)

The difference between the background (aerated water) and the total amount of gas in the headspace and water column was used to calculate the total release over the 24 h period. These measurements were repeated over three consecutive 24 h periods, between which the water in the column was refreshed with aerated water for six hours.

### 3.6 DGT and DET preparation and measurements

Diffusive gradients in thin-films (DGT) and diffusive equilibrium in thin-films (DET) probes for nitrogen species measurements in sediments were prepared as described by Huang et al. (2019). Further DGT probes for trace element measurements in sediments were prepared as described by (Jolley et al., 2016). The two techniques (DET and DGT) were used to provide a measure the potentially available solute for micro-organisms to take up (i.e. “bioavailable”) at different depths below the sediment-water interface (SWI). The steps for preparing the different types are described briefly below.

All DGT probes used a 0.8 mm thick diffusion layer made of a polyacrylamide hydrogel (acrylamide cross-linked with an agarose derivative) and a 0.14 mm thick filter membrane (0.45  $\mu$ m pore size). The binding layers in the DGT probes used to measure nitrogen species were AMI-7001 anion exchange membrane for  $NO_3$ -N DGT and CMI 7000 cation exchange membrane for  $NH_4$ -N DGT (both supplied by Membranes International Inc., USA). The DGT probes used to measure trace elements used Chelex-100 resin beads (200-400 mesh, Bio-Rad) embedded in a polyacrylamide gel as the binding layer. The DET probes only had a filter membrane (as before) and a 1.2 mm-thick material diffusive layer made from ultrapure agarose (molecular grade, Bioline reagent, UK) (Huang et al., 2016). The DGT and DET probes were assembled within protective pre-cleaned polycarbonate housings designed for sediment

deployments, supplied by DGT Research Ltd. (UK)(Figure 3-3). These housings feature a top retaining plate that holds the gel flat against the backing plate and has a  $1.8 \times 20$  cm sampling window that allows solute to diffuse from the sediment through the filter and into the hydrogel layer(s) inside the housing.

Prior to deployment, the assembled probes were deoxygenated in two separate sealed 0.01 M NaCl baths by bubbling  $N_2$  gas through the water overnight. This was done to minimise oxygen contamination of the anoxic parts of the sediment by the probes during deployment. One bath was dedicated to trace element DGT probes, while the other was reserved for N species DGT and DET probes. All probes were deployed into the sediments directly from the oxygen-free baths within a pre-designated “area of deployment” at the centre of the mesocosm (Figure 3.4) within 5 minutes of each other. The probes were spaced 2-3 cm apart and faced the direction of water flow. The water temperature was recorded at the start of the deployment as 25 °C.

The DET probes were harvested after 7 h of deployment, at which point the water temperature was recorded again. At harvest, the SWI was marked on the DET probe, and the probe was then promptly rinsed with a gentle jet of deionised water. The filter membrane and agarose diffusive layer were cut out from within the probe window immediately and the filter membrane was discarded. The remaining diffusive layer was promptly cut into ten 10 mm sections (final dimensions  $1.8 \text{ cm} \times 1 \text{ cm}$ ), starting from the gel that was level with the SWI (depth interval of 0 -10 mm) and then the gels deeper (depth intervals of 10 – 100 mm). The sections were transferred into 30 mL centrifuge vials containing 5 mL high purity water and left on a rocker overnight. The gels were then removed, and the vials stored at -20 °C prior to analysis for  $NO_3$  and  $NH_4^+$  concentrations ( $C_{DET-NO_3}$  and  $C_{DET-NH_4^+}$ , respectively) (Section 3.8).

The DGT probes were harvested 24 h after deployment and the water temperature was recorded. The probes were cleaned as before, before the gel area within the probe sample window was excised. The filter and diffusive gels were discarded, and the binding layers were sectioned into ten 10 mm sections, starting from the sediment water interface, and then collecting the sections that experienced solute fluxes from the sediment. The AMI-7001 anion exchange membrane and CMI 7000 cation exchange membrane sections were eluted in 10 mL of 2 M NaCl solution for at least 24 h (actual time recorded) after which the membranes were removed, and the eluent was analysed for

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[https://www.ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20\(TIMES\)/TIMES62.pdf](https://www.ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20(TIMES)/TIMES62.pdf)

Figure 3-3 Schematic representation of the DGT assembly (not to scale). Modified from Amato (December 2019)

$\text{NO}_3^-$  and  $\text{NH}_4^+$  (Section 3.8). The trace element binding gels were eluted in 1 mL 1 M  $\text{HNO}_3$  for 24 h, after which the eluent was analysed for Cu, Fe and Mn (see Section 3.8)

The eluent concentrations were used to calculate the total mass of solute bound by a given section of the resin gel ( $M$ ), using Equation 14.

$$M = C_{el}(V_{acid} + V_{gel})/f_e \quad (\text{Equation 14})$$

where  $C_{el}$  ( $\mu\text{g/L}$ ) is the concentration of solute in the eluent,  $V_{acid}$  (mL) is the volume of the eluent,  $V_{gel}$  (mL) is the volume of the binding layer section and  $f_e$  is the elution factor. The elution factors used for trace elements were those reported by Garmo et al. (2003) (0.8 for Cu, Fe, and Mn). The elution factors for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were 0.87 and 1, respectively as reported by Huang et al. (2017).

The mass of solute bound was then used to calculate the average concentration of the given analyte at the DGT probe-sediment interface over the duration of the deployment,  $C_{DGT}$ , using equation 15

$$C_{DGT-analyte} = \frac{M \times \delta^{MDL}}{D_{gel} \times A_p \times t} \quad (\text{Equation 15})$$

where,  $\delta^{MDL}$  is the thickness of the material diffusion layer and comprises the filter and diffusive hydrogel (0.94 mm),  $A_p$  is the physical surface area of sampled gel section (1.8 cm<sup>2</sup>),  $t$  is the deployment time,  $D_{gel}$  is the temperature-specific diffusion coefficient of the solute in question in the hydrogel. Forthwith,  $C_{DGT-Cu}$ ,  $C_{DGT-Fe}$ ,  $C_{DGT-Mn}$ ,  $C_{DGT-NO_3}$  and  $C_{DGT-NH_4}$  will be used to refer to the DGT-determined concentrations of Cu, Fe, Mn, NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>, respectively.

### 3.7 Trace element speciation

After the DGT probes were harvested, a 110 mm deep sediment core (diameter 30 mm) was collected from each mesocosm using an acid washed cylindrical polypropylene corer. Upon retrieval, the base of the corer was immediately sealed off using a clean plastic membrane and the cores were stored at -20 °C for up to 5 days. The cores were extruded in batches in an oxygen-free cabinet purged with N<sub>2</sub> gas in 10 mm sections into 50 mL acid-washed centrifuge tubes. The exposure of the bottom 10 mm sediment in the core to air during retrieval could not be avoided, so they were discarded.

The extruded sections underwent the sequential extraction procedure first described by Tessier et al. (1979). Briefly, each section was extracted in order, as follows:

- (i) The exchangeable fraction was extracted using 1 M MgCl<sub>2</sub> at pH 7.0;
- (ii) The carbonate-bound fraction was extracted using 1 M sodium acetate, adjusted to pH 5.0 using acetic acid;
- (iii) The fraction bound to (Fe and Mn) oxides was extracted with 0.04 M NH<sub>2</sub>OH.HCl adjusted to pH 2 using 25 % acetic acid;
- (iv) The organic matter-bound fraction was extracted using a combination of 0.02 M HNO<sub>3</sub> and 30% H<sub>2</sub>O<sub>2</sub>, adjusted to pH 2 with HNO<sub>3</sub> and heated to 85 °C. Subsequent addition of 30% acidified H<sub>2</sub>O<sub>2</sub> and 3.2 M NH<sub>4</sub>OAc were used to ensure recovery and re-adsorption of trace elements;
- (v) The residual fraction was dried at 80°C and then underwent microwave digestion using 10 mL HNO<sub>3</sub>.
- (vi) The (pseudo) total fraction was calculated from the total amount of Cu recovered from fractions i – v.



Figure 3-4 The arrangement of DGT and DET probes during deployment in mesocosm.

The exchangeable and carbonate fractions were extruded under anoxic conditions and the extracts were analysed individually for each mesocosm (see section 3.8). Oxide, and organic matter-bound fractions were then extracted outside of the anoxic cabinet where the first three 10 mm segments, down to 30 mm below the sediment-water interface, were analysed individually. Extract from the lower depths were combined to provide a composite sample to represent the average concentrations for those fractions between 30 – 100 mm below the SWI.

### 3.8 Trace element and nutrient analyses and quality control procedures

To ensure quality, all materials used for analysis of trace elements were acid-washed first for at least 8 h in 10 % HCl, followed by another >8 h wash in 10 % HNO<sub>3</sub> (AG grade; Fisher scientific, UK) and finally rinsed with double deionised water (ASTM Type I water, resistivity 18.2 MΩ cm<sup>-1</sup>) and stored in polythene bags. Materials for nutrient DGT and DET preparations were acid washed in a separate 10% HCl baths (AR grade; Fisher scientific, UK) to avoid cross-contamination.

Standard reference material, sample blanks, solution blanks, method blanks, and triplicate samples were treated identically to the samples. Sediment trace element digestion (Cu, Fe, Mn) used a certified reference material (Montana 2710, NIST<sup>®</sup> SRM<sup>®</sup>, New Zealand), to ensure the validation of data and the accuracy of the analytical method. All the other analyses were done in triplicates where the relative errors were determined to be within 10 %. Copper, Fe and Mn were analysed using ICP OES (720 series, Agilent, USA), while the N<sub>2</sub>O was measured using gas chromatography (SRI 8610C ECD detector gas chromatograph, USA). All nitrogen analyses were done using Alpkem 2 channel FIA 3000 series. Table 3.1 shows method detection limit (MDL) for DGT (trace elements, NH<sub>4</sub>-N, NO<sub>3</sub>-N) analysis.

Table 3-1 Instrument Detection Limit and DET and DGT method detection limits for Cu, Fe, Mn and nitrogen DGT. Concentrations given as µg/L.

	<b>Cu</b>	<b>Fe</b>	<b>Mn</b>	<b>NO<sub>3</sub>-N</b>	<b>NH<sub>4</sub>-N</b>
Instrument Detection Limit	0.34	3.4	0.23	100	100
DET method detection limit*	NA	NA	NA	2315	2312
DGT method detection limit <sup>@</sup>	0.15	1.6	0.11	2315	2312

\* Estimated as the instrument detection limit ( ) × dilution factor (23.1)

<sup>@</sup> estimated as 3 × S.D. of replicate blank DGT gel segments (*n* = 5) and expressed as a 24 h deployment equivalent.

NA – not analysed.

### 3.9 Statistical analyses

The data sets were tested for normality using Shapiro-Wilks' test and log-transformed where necessary (exchangeable and carbonate Cu fractions only). Levene's test was used to confirm homogeneity of variance. The data were then subject to one-way analysis of variance (ANOVA) to test for differences between treatments. Where differences were significant (*P*<0.05), Fisher's protected Least Squared Difference (l.s.d.) post-hoc test ( $\alpha$  = 0.05) was used to identify differences between means. All tests

were carried out using Genstat (20<sup>th</sup> edition, VSN International Ltd.), significance was assumed at the 5% level, unless stated otherwise.

## Chapter 4

### Results

#### 4.1 Water physical and chemical characteristics

The average results for water physical and chemical characteristics measured over 2 weeks prior to DET and DGT deployments are shown in Table 4.1. The pH and dissolved oxygen (DO) saturation of the mesocosms were consistent throughout at 7.66 and 96 %, respectively. Temperatures were relatively stable at *ca.* 25 °C. The average conductivity in the water of the 270 mg/kg mesocosm was 280  $\mu\text{S}/\text{cm}$ , which was significantly higher ( $p < 0.05$ ) than the average of 250  $\mu\text{S}/\text{cm}$  in the sediment spiked with 65 mg/kg Cu. The average conductivities of the water circulating the 0 and 65 mg/kg sediment mesocosms were 269.1 and 250.1, respectively ( $P > 0.05$ ), and both were significantly lower than the conductivity of the water circulating in the 270 mg/kg mesocosm. There was no invertebrate activity observed (e.g. burrows or casts on the SWI or deeper in the sediment) observed in any of the three treatments.

Table 4-1 Average pH, DO saturation, conductivity, and temperature over a period of 2 weeks prior to DGT, DET deployment and sediment core sampling. Averages with the same letter are not significantly different ( $P < 0.05$ )

Mesocosm Cu concentration (mg/kg)	pH	DO Saturation (%)	Conductivity ( $\mu\text{S}/\text{cm}$ )	Temperature ( $^{\circ}\text{C}$ )
0	7.66 $\pm$ 0.46 <sup>a</sup>	95.9 $\pm$ 1.81 <sup>a</sup>	269.1 $\pm$ 10.13 <sup>b</sup>	24.93 $\pm$ 0.42 <sup>a</sup>
65	7.66 $\pm$ 0.29 <sup>a</sup>	96.4 $\pm$ 2.55 <sup>a</sup>	250.1 $\pm$ 13.3 <sup>a</sup>	24.75 $\pm$ 0.72 <sup>a</sup>
270	7.65 $\pm$ 0.33 <sup>a</sup>	97.4 $\pm$ 2.19 <sup>a</sup>	283.4 $\pm$ 33.9 <sup>b</sup>	24.77 $\pm$ 0.89 <sup>a</sup>

Trace element concentrations in overlying water, sampled at the time of deployment showed higher Cu concentration (2.82  $\mu\text{g}/\text{L}$ ) in mesocosm treated with 270 mg/kg Cu compared to other treatments (Table 4.2). Iron (Fe) and manganese (Mn) results were below and close to the detection limits, respectively for all mesocosms. The concentration of  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  were measured at 3740  $\pm$  20 and 60  $\pm$  10  $\mu\text{g}/\text{L}$  respectively, reflecting the natural water.

Table 4-2 Trace element concentrations (as  $\mu\text{g}/\text{L}$ ) in overlying water during the day of DGT deployment.

Sediment treatment	Cu	Fe	Mn
0 mg/kg	1.33	<MDL	0.91
65 mg/kg	<MDL	<MDL	0.30
270 mg/kg	2.82	<MDL	0.52

\*<MDL below detection limit (Cu: 0.34  $\mu\text{g}/\text{L}$ ; Fe 3.4  $\mu\text{g}/\text{L}$ ; Mn 0.23  $\mu\text{g}/\text{L}$ )

The average NO<sub>3</sub>-N and NH<sub>4</sub>-N in the overlying water measured over 30 days were within 5% of their values in the river water at the day of sampling. The average NH<sub>4</sub>-N concentration was 70 ± 20 µg/L NH<sub>4</sub>-N between all mesocosms. The average NO<sub>3</sub>-N measurement in the water circulating in the 0 mg/kg sediment was 3,410 ± 460 µg/L. The water circulating in treated sediments (65 and 270 mg/kg Cu) showed 3,340 ± 400 and 3,480 ± 600 µg/L NO<sub>3</sub>-N, respectively. There were no significant differences in the overlying water for both NH<sub>4</sub>-N and NO<sub>3</sub>-N concentrations (p > 0.05).

## 4.2 Sediment characteristics

The pH of the 0 mg/kg sediment was 6.67 ± 0.02 (Table 4.3). During the spiking process, the Cu added to the sediment lowered the pH in both treatments (pH 6.43 ± 0.03). Organic matter and total carbon were 1.03 ± 1.18 % and 0.60 ± 0.69 %, respectively. The background value for Cu in the sediment was 6.22 ± 0.26 mg/kg. The total extractable Cu concentrations in the two treated sediments were 51 % and 44 % of the total added concentrations of 65 mg/kg and 270 mg/kg, respectively. Sediment concentrations ranged from 13 000 mg Fe/kg sediment and from 150 – 153 mg Mn/kg sediment across all mesocosms.

Table 4-3 Characteristics of the three treated sediment. The values show averages (± standard error; n = 3). Where the letters are the same, there was no significant difference between means (P<0.05)

	Sediment Treatment		
	0 mg/kg	65 mg/kg	270 mg/kg
pH	6.67 ± 0.02 <sup>b</sup>	6.43 ± 0.03 <sup>a</sup>	6.43 ± 0.04 <sup>a</sup>
Moisture (%)	40 ± 0		
Organic matter (%)	1.03 ± 1.18		
Total carbon (%)	0.60 ± 0.69		
Total nitrogen (mg/kg)	4.1 ± 0.10		
Cu (mg/kg)	6.22 ± 0.26 <sup>a</sup>	33.14 ± 1.35 <sup>b</sup>	119.66 ± 1.40 <sup>c</sup>
Fe (mg/kg)	13 000 ± 300 <sup>a</sup>	13 000 ± 500 <sup>a</sup>	13 000 ± 85 <sup>a</sup>
Mn (mg/kg)	153.78 ± 4.10 <sup>a</sup>	151.18 ± 0.17 <sup>a</sup>	150 ± 3.20 <sup>a</sup>

## 4.3 Depth profiles of trace element concentrations

The average DGT-measured Cu concentration (C<sub>DGT-Cu</sub>) depth profile for each treatment showed a trend where more Cu available to the DGT was present close to the SWI (Figure 4.1). Overall, the available Cu concentrations varied among mesocosms depending on the amount of Cu received. The concentrations immediately below the SWI (0 – 1 cm depth) were 3.44 µg/L and 3.00 µg/L for 65 mg/kg and 270 mg/kg sediment, respectively. Copper concentrations below 1 cm depth were close to or below the DGT detection limit (0.15 µg/L) in all the sediments. The C<sub>DGT-Cu</sub> in the 270 mg/kg sediment was generally high with a peak concentration between 6 cm and 9 cm. Across mesocosms, significant differences in C<sub>DGT-Cu</sub> concentrations were identified between the 270 mg/kg sediment and the two

other treatments ( $p < 0.05$ ). The difference between 0 mg/kg and 65 mg/kg treatments was only significant at the 0 – 1 cm depth interval.

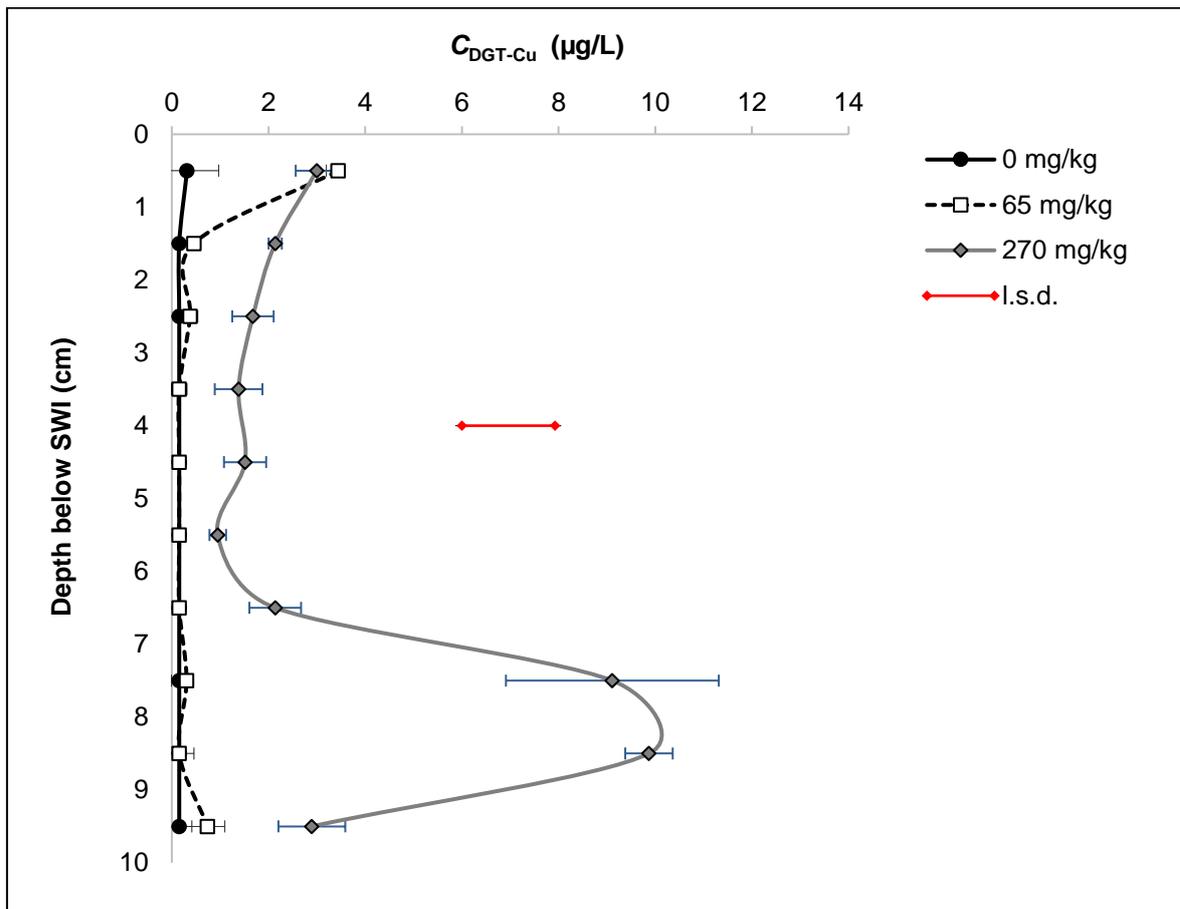


Figure 4-1 Mean  $C_{DGT-Cu}$  at 1 cm intervals below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).

Iron results showed trends where the  $C_{DGT-Fe}$  increased with depth. The Fe depth profile observed (Figure 4.2) varied depending on the Cu treatments. The concentrations close to the SWI for the 0 mg/kg sediment and 65 mg/kg sediment were lower than the concentration in 270 mg/kg sediment. The highest  $C_{DGT-Fe}$  was observed throughout the 270 mg/kg sediment, followed by 65 mg/kg sediment, and the 0 mg/kg sediment. Differences in  $C_{DGT-Fe}$  concentrations between the three treatments were generally significant at every depth ( $p < 0.05$ ). A unique feature in the 65 and 270 mg/kg treated sediments was observed at 6.5 cm depth (270 mg/kg sediment) and 8.5 cm (65 mg/kg sediment), where Fe concentration decreased suddenly.

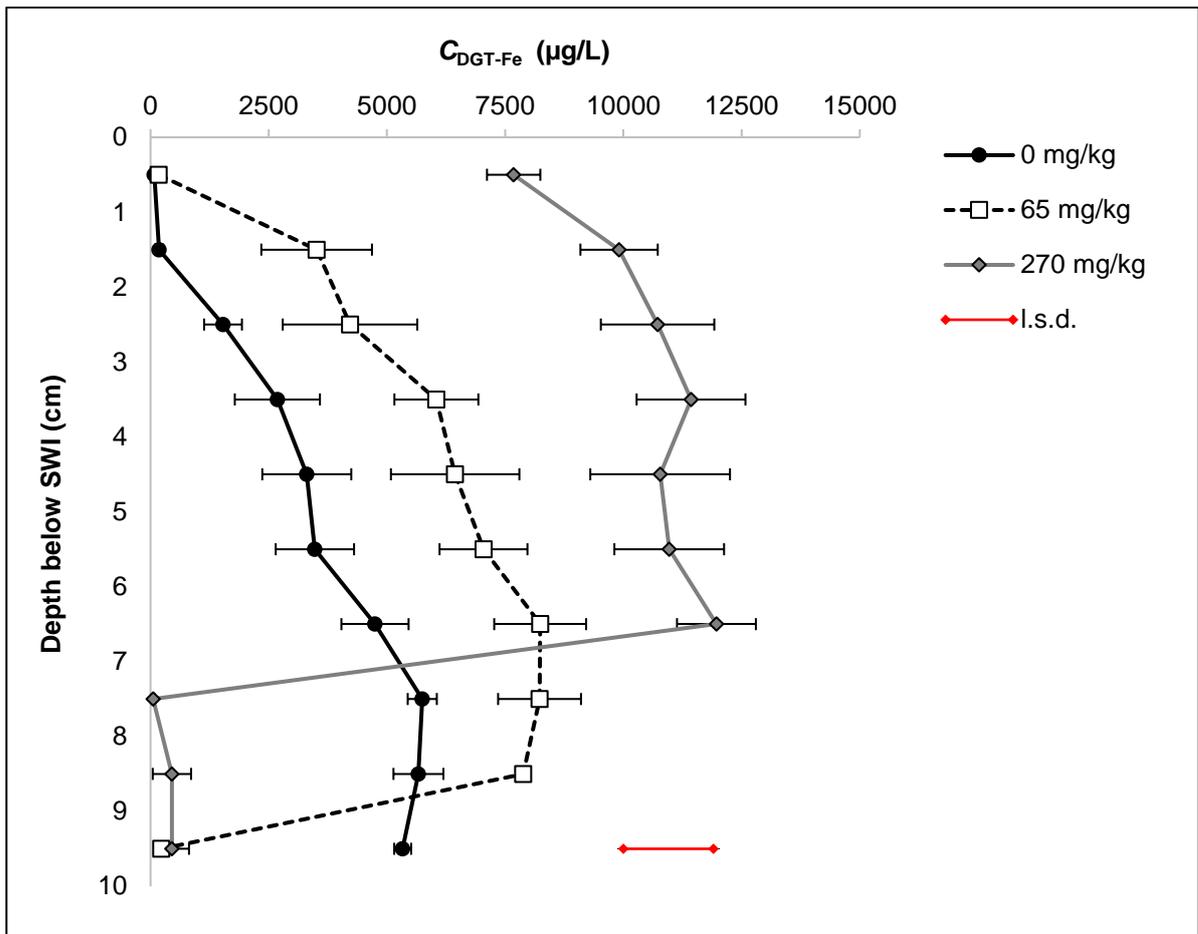


Figure 4-2 Mean  $C_{DGT-Fe}$  depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).

The mean  $C_{DGT-Mn}$  concentrations increased with depth in the 0 mg/kg sediment and the 65 mg/kg sediment (Figure 4.3). However, the opposite was observed in the 270 mg/kg sediment where the  $C_{DGT-Mn}$  decreased with depth. In the layers closest to the SWI (0 – 2 cm) the average  $C_{DGT-Mn}$  was significantly higher in the 270 mg/kg treatment, than the other two sediments.

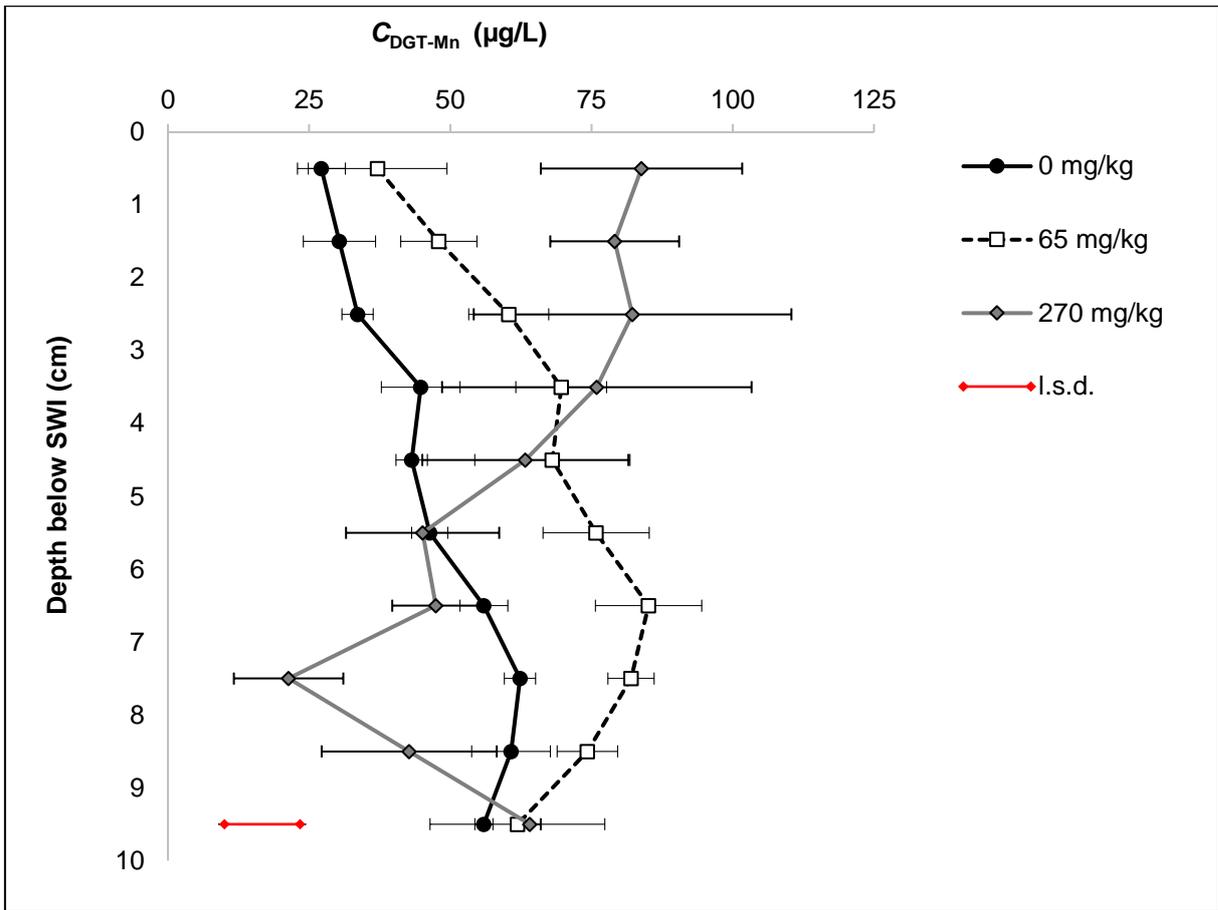


Figure 4-3 Mean  $C_{DGT-Mn}$  depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).

#### 4.4 Cu Fractionation.

The concentration of exchangeable Cu was significantly greater in the 270 mg/kg Cu sediment compared to the 65 mg/kg Cu sediment and the 0 mg/kg sediment at all depths (Figure 4.4). The difference in exchangeable Cu between 0 mg/kg and 65 mg/kg sediments was not significant. The concentration of the exchangeable Cu fraction did not vary with depth greatly, with the biggest variation seen in the 270 mg/mg, where the relative standard deviation between different depths was 20 %. The concentration of carbonate-bound Cu was generally highest in the 270 mg/kg sediment, but generally there were few significant differences between treatments (Figure 4-5).

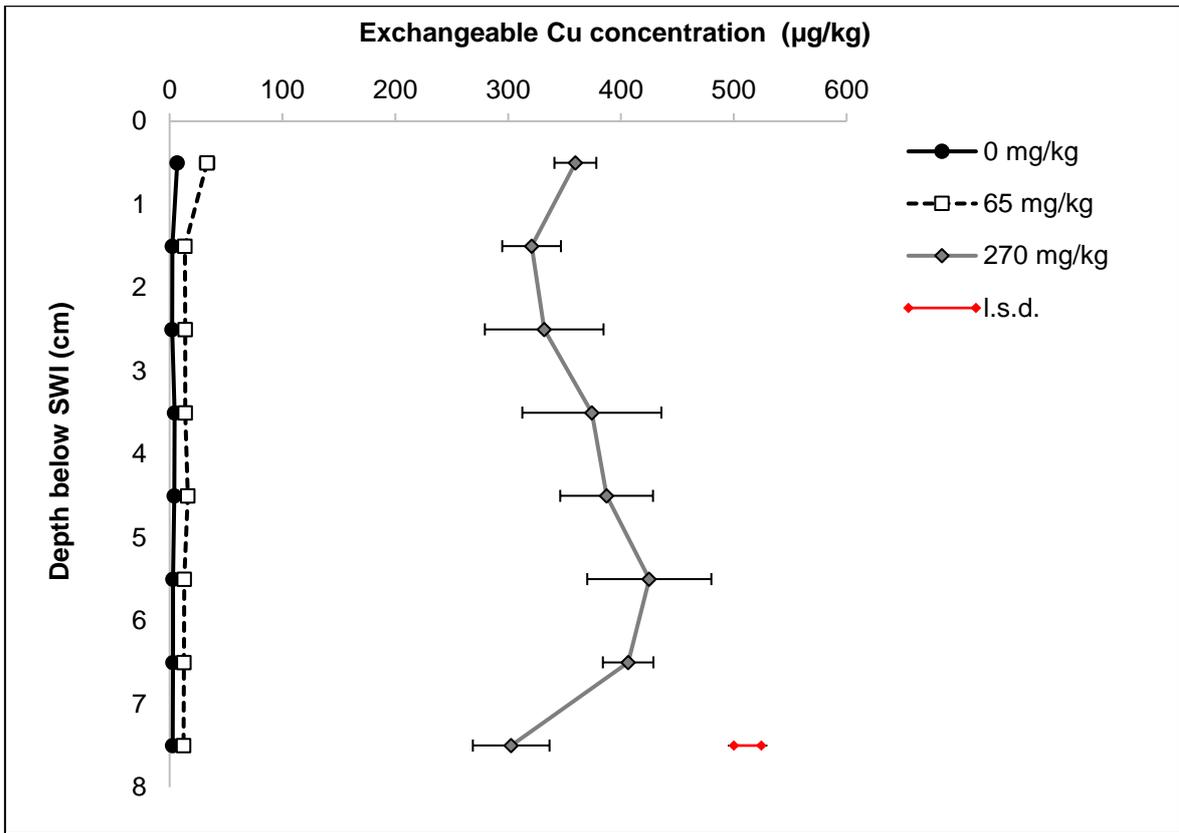


Figure 4-4 Mean exchangeable Cu concentration depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error. Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P < 0.05$ ).

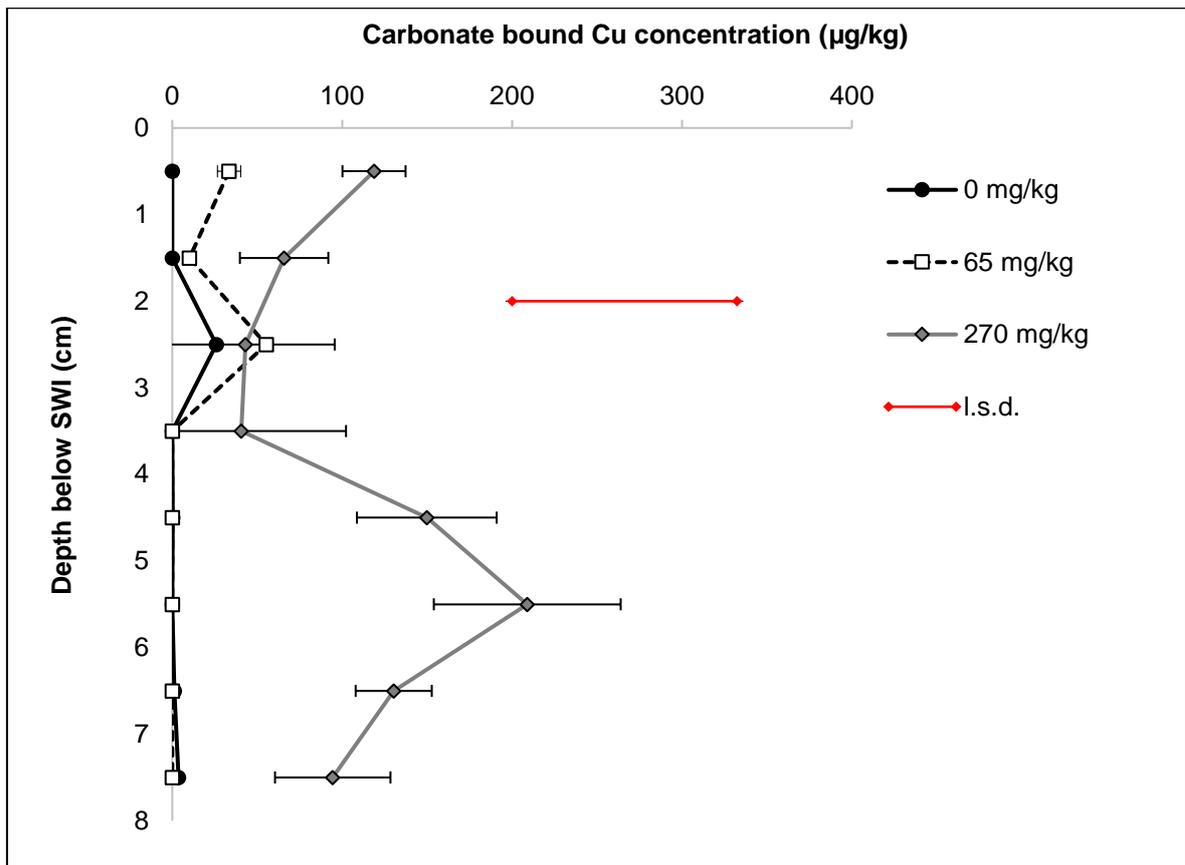


Figure 4-5 Mean carbonate-bound Cu concentration depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error. Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P < 0.05$ ).

Between the various fractions analysed, on average the highest concentration of Cu was found in the organic matter phase, which amounted to between 78% and 69 % of the total Cu in the three treatments where the proportion decreased with increasing Cu treatment. Another 20 – 30% of the Cu was found in the residual fraction. Generally, less than 2 % of the remaining Cu concentration were shared between the Fe/Mn oxide fraction, the exchangeable fractions, and the carbonate fractions (Figure 4.6).

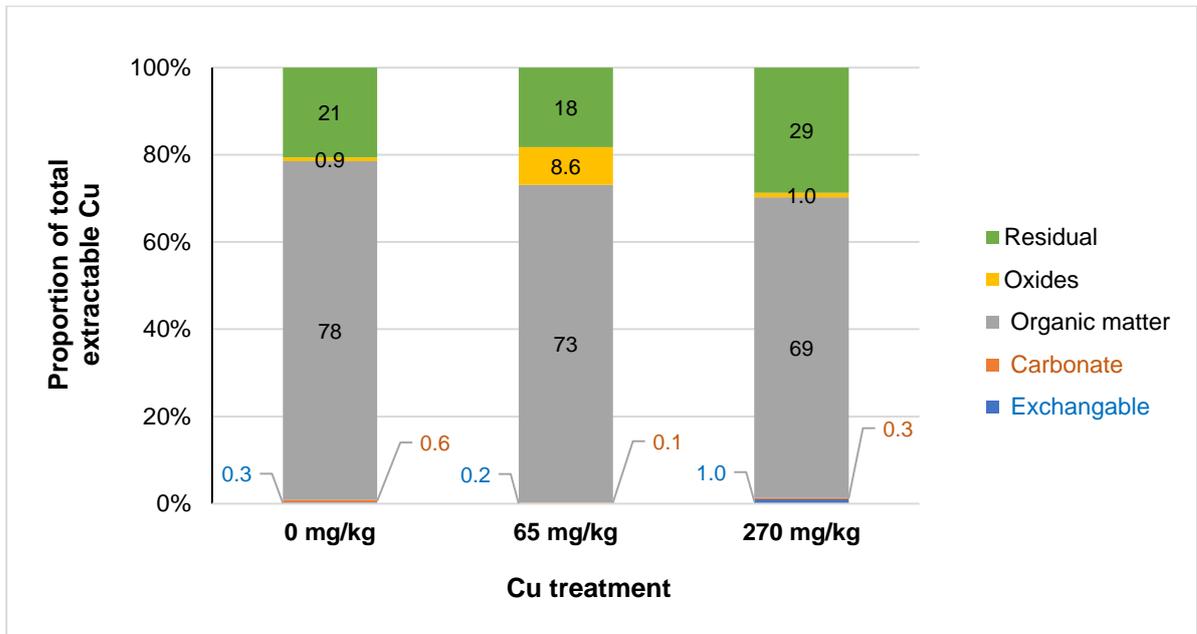


Figure 4-6. Average proportions of Cu bound to different sediment fractions in the three treated sediments across all depths ( $n = 27$ ). The values shown in the graphs show percentage of total.

#### 4.5 Porewater $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ profiling.

The  $C_{\text{DET-NO}_3}$  and  $C_{\text{DET-NH}_4}$  concentrations were all below the MDL throughout the depth profiles (not shown). The  $C_{\text{DGT-NO}_3}$  varied significantly between treatments in the 0 - 1 cm depth interval (Figure 4-7). The highest mean  $C_{\text{DGT-NO}_3}$  at this depth interval was observed in the 0 mg/kg sediment (278  $\mu\text{g/L}$ ), whereas the lowest was observed in the 270 mg/kg Cu sediment (< 22.8  $\mu\text{g/L}$ ). Below 1 cm, the  $C_{\text{DGT-NO}_3}$  fell below the detection limit in all but the 0 mg/kg sediment and the differences between treatments were no longer significant.

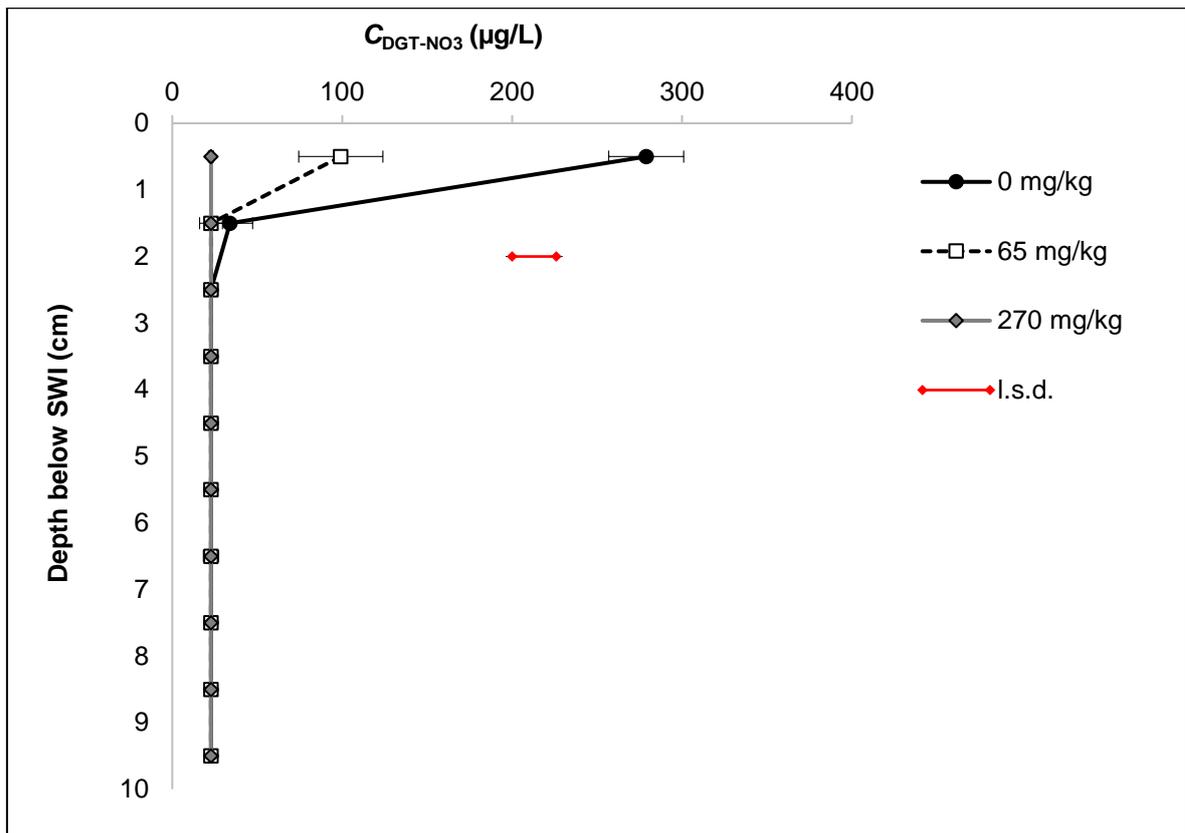


Figure 4-7 Mean  $C_{DGT-NO_3}$  depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where a difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).

Generally, the  $C_{DGT-NH_4}$  was highest in the top 1 cm in the sediment in all treatments and similar across them ( $P>0.05$ ); however, the differences between treatments were seen deeper in the sediments (Figure 4-8). The  $C_{DGT-NH_4}$  in the 0 mg/kg sediment decreased with depth to 20 µg/L in the 2-3 cm depth interval, while in the 65 mg/kg and 270 mg/kg treatments the  $C_{DGT-NH_4}$  remained relatively constant (89 - 108 µg/L) down to the 2-3 cm and 4-5 cm depth intervals, respectively, and then rapidly decreased to below the MDL. Below these minima, the  $C_{DGT-NH_4}$  then increased linearly with depth, reaching a concentration of 56 – 86 mg/kg at the 9-10 cm depth interval where the concentration was significantly higher in the 0 mg/kg sediment than the other two sediments.

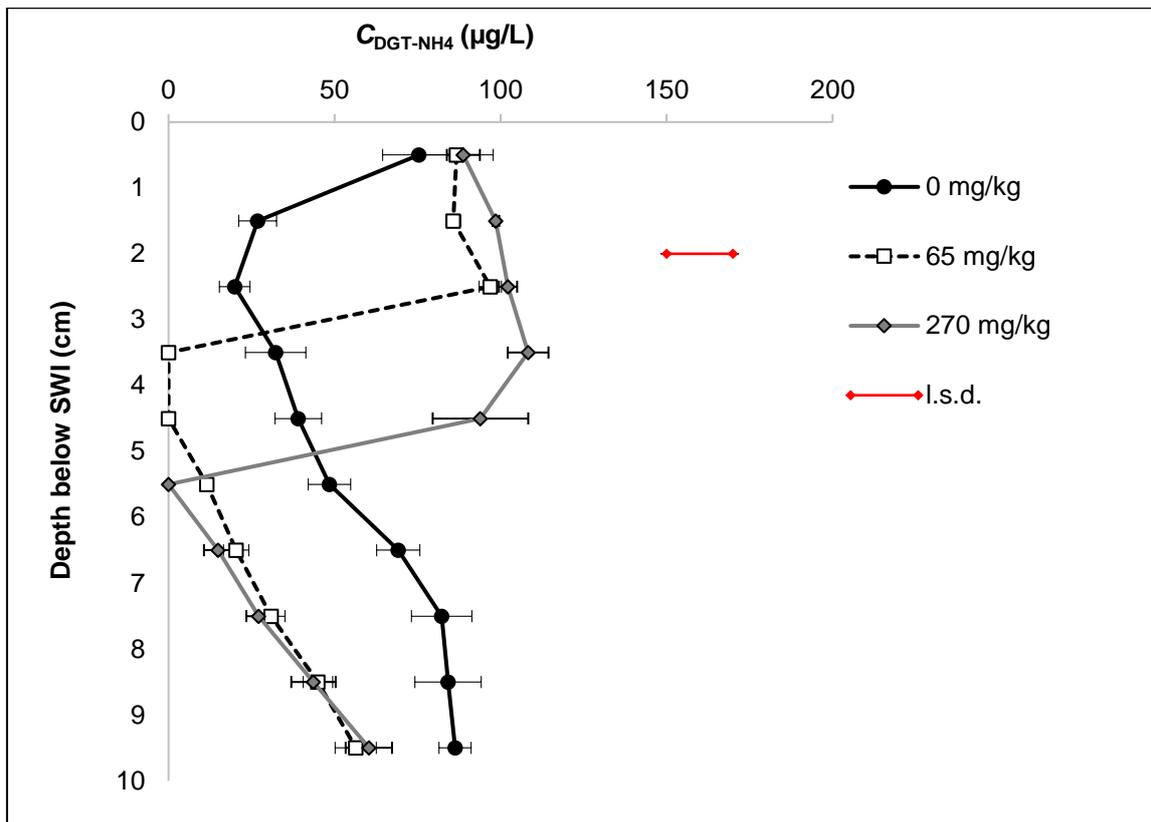


Figure 4-8 Mean  $C_{DGT-NH_4}$  depth profiles below the sediment-water interface (SWI) in the three treatments (0, 65 and 270 mg/kg Cu). The half-centimetre depths on the y-axis represent the average concentration for the corresponding centimetre interval (0.5 = average for 0 - 1 cm, 1.5 = average for 1 – 2 cm, etc.). The error bars show standard error ( $n=3$ ). Where the difference between two means for a given depth is greater than the least squared difference (l.s.d.), a significant treatment effect was assumed ( $P<0.05$ ).

#### 4.6 $N_2O$ release from sediments.

The average daily release of  $N_2O$  from the sediments ranged from a minimum of  $48 \mu\text{g}/\text{m}^2/\text{h}$  (0 mg/kg sediment) to a maximum of  $75 \mu\text{g}/\text{m}^2/\text{h}$  (270 mg/kg sediment) (Figure 4-9). The average daily release over the three day period was significantly higher in the two sediments where Cu was added, than in the 0 mg/kg sediment, but the difference between the 65 mg/kg and 270 mg/kg sediments was not significant.

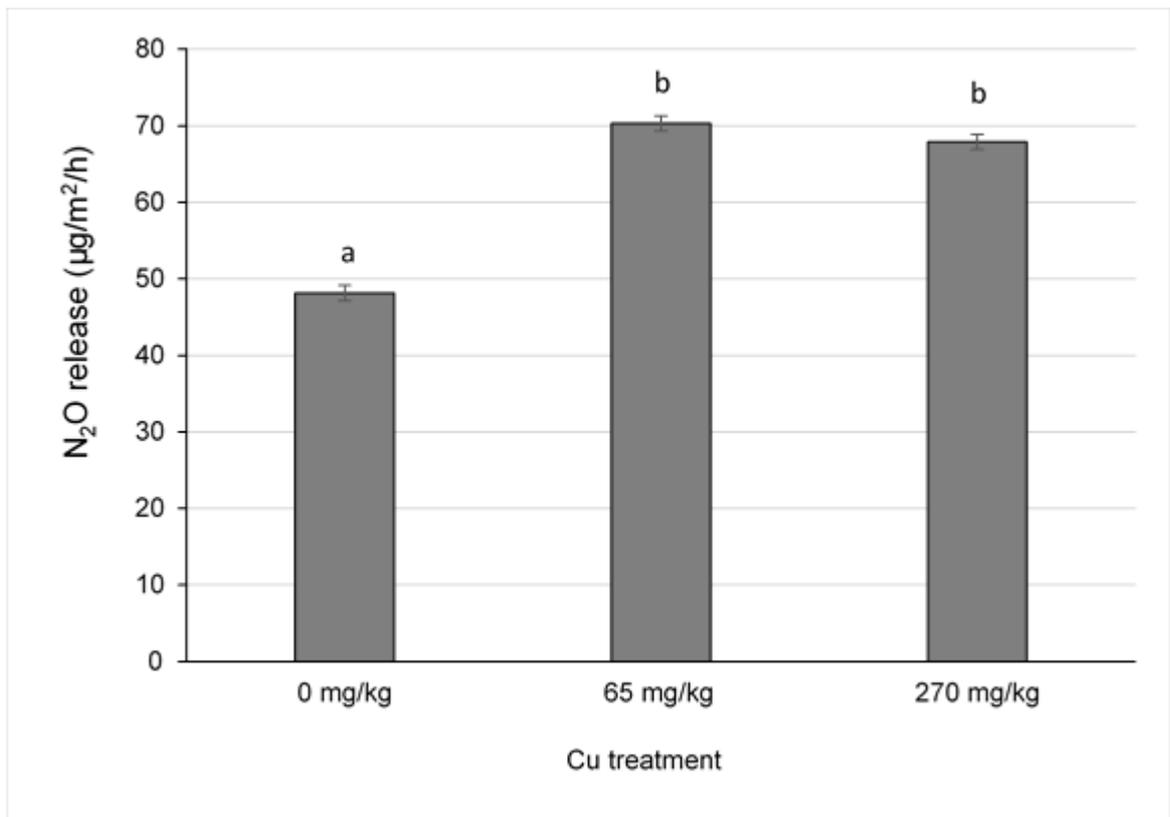


Figure 4-9 The mean N<sub>2</sub>O release from each mesocosm over three consecutive 24 h periods. The error bars show standard error. Averages that share the same letter were not significantly different (P>0.05)

## Chapter 5

### Discussion

The sediment in this study had relatively low concentration of organic matter and total carbon (Table 4-3), suggesting that the Cu in parts of the sediment may have been relatively bioavailable (Magalhães et al., 2007). This was confirmed by the  $C_{DGT-Cu}$  and exchangeable Cu results that showed an elevated amount of bioavailable Cu near the SWI, with greater amounts in the 270 mg/kg than in the 65 and 0 mg/kg Cu sediments, respectively (Figure 4.1).

The effect of the Cu treatment on the sediment N cycle can be seen in the top 1 cm of the  $C_{DGT-NO_3}$  profile (Figure 4-7). The ratio of DGT-measured concentrations to porewater solute concentrations,  $R$  (in this case  $R = C_{DGT-NO_3} : C_{DET-NO_3}$ ) has been used to estimate the rate of supply from the sediments, where ratios above 0.1 are indicative that supply to the DGT is by other means than diffusion alone (e.g. buffering by the solid-phases) (Harper et al., 1998; Lehto, 2016; Zhang et al., 1995). However, the very high method detection limit for the DET-measurements (Table 3-1) meant that  $R$  could not be used to estimate the extent of the solute supply accurately here. The differences in  $C_{DGT-NO_3}$  show that the supply of  $NO_3^-$  to the DGT decreased with Cu concentration in the order  $0 > 65 > 270$  mg/kg treatments ( $P < 0.05$ ). Nitrate does not bind to most sediment solid phases and the rates of diffusion in the sediments and the N concentrations in the overlying water were likely to be very similar, so the difference in  $NO_3^-$  supply between treatments was likely caused by an effect of Cu on the microbial processes that affect N cycling directly or indirectly. Previous studies have also shown the effects of trace elements on the N-cycle in natural waters (Broberg, 1984; Rother et al., 1982; Sakadevan et al., 1999). This is the first time this effect has been shown directly to affect in-situ  $NO_3^-$  mobilisation in freshwater sediments.

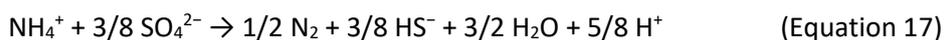
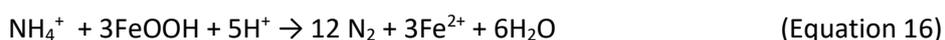
The rates of  $N_2O$  production measured here (48 – 70  $\mu g/m^2/h$ ; Figure 4-9) are within the range of 3 – 87  $\mu g/m^2/h$  measured by (Kelliher, 2013) *in-situ* in sediments of the Waikato River. Differences in  $N_2O$  production between Cu treatments suggest that increased rates of denitrification may be an important driver for the reduced  $NO_3^-$  concentrations in the sediments that were treated with Cu. The  $C_{DGT-Fe}$  profiles, that are mainly thought to be driven by  $Fe^{2+}$  (Zhang & Davison, 1995), support this theory. The high  $C_{DGT-Fe}$  very close to the SWI suggest that the highest rates of reductive dissolution of Fe-oxide minerals occurred in the 270 mg/kg treatment, where  $NO_3^-$  would have been consumed beforehand although it is possible that the elevated Cu concentrations may have also caused some desorption of  $Fe^{2+}$  from organic matter and solid particles into the sediment porewater.

The effects of Cu on  $\text{N}_2\text{O}$  production and  $\text{NO}_3^-$  depletion agree with findings by Magalhães et al. (2007) and Sakadevan et al. (1999) where Cu concentration at 79 mg/kg and 100 mg/kg stimulated  $\text{NO}_3^-$ -N reduction and  $\text{N}_2\text{O}$  production. This contradicts some studies that have suggested that Cu concentrations as low as 2.6 mg/kg (Feng et al., 2013) and as high as 500 mg/kg (Zhao et al., 2020) can repress microbial activities responsible for  $\text{N}_2\text{O}$  production. However, Sakadevan et al. (1999) and Zou et al. (2015) proposed that the microorganisms responsible for  $\text{N}_2\text{O}$  production develop metal tolerance. The speed at which this tolerance is restored may be between 8 – 60 days (Holtan-Hartwig et al., 2002) and could have occurred during the 42 day experiment reported here .

Dissimilatory nitrate reduction to ammonium (DNRA) (Dong et al., 2011; Kelso et al., 1997) may also have suppressed the supply of  $\text{NO}_3^-$  to the nitrate DGT, while increasing  $\text{NH}_4^+$  supply to the ammonium DGT. The higher  $C_{\text{DGT-NH}_4}$  in the top 3 cm of the 65 mg/kg sediment and 4 cm of the 270 mg/kg sediment (Figure 4-8) suggest that in these sediments the increased Cu may have promoted DNRA and thus reduced the available  $\text{NO}_3^-$ , in addition to denitrification. Furthermore, Rother et al. (1982), suggested that trace elements (including Cu) can slow down nitrification and others have shown that lower pH can also negatively affect the process (Prosser, 1990; Suzuki et al., 1974), which could have contributed to the lower  $\text{NO}_3^-$  concentration in the Cu-treated sediments.

*Biotic* anaerobic ammonia oxidation (Anammox) may be important for explaining differences in the  $C_{\text{DGT-NH}_4}$  concentration profiles. Hu et al. (2012) and Thamdrup (2012) have suggested that  $\text{NH}_4\text{-N}$  can be oxidized to  $\text{N}_2$  under anoxic conditions by anaerobic sediment microorganisms. Mak et al. (2018) studied the effects of nine trace elements, including Cu and Fe, on anammox activity in N-rich wastewater. They found that Cu had the strongest inhibitory effect out of the metals studied. Others found that that it took up to 50 days for the micro-organisms responsible for anammox activity to recover from exposure to metal contamination (Zhang et al., 2013). While similar effects have not been shown in sediments, with relatively low-N concentrations, the inhibition of anammox micro-organisms by Cu may help explain why  $C_{\text{DGT-NH}_4}$  concentrations were generally higher in the Cu-treated sediments.

Ammonium concentrations can also be affected by *abiotic* processes, such as anaerobic ammonium oxidation to where the reduction of metal oxide phases, such as Fe(III) (Clément et al., 2005) facilitates the process. Along with reduced rates of respiration and ammonification in the anoxic part of the sediment, such processes may explain the decrease in  $C_{\text{DGT-NH}_4}$  below 1 cm depth. The increasing  $C_{\text{DGT-Fe}}$  with depth (Figure 4-2) and the lower pH in the Cu treated sediments (Table 4-3) also suggest that this may be relevant here, although thermodynamic calculations by Thamdrup (2012) suggest that this is unlikely to as important above pH 4 as direct reduction to  $\text{N}_2$  with FeOOH or sulphate, as shown in Equations 16 and 17, respectively.



The production of sulphide ( $\text{HS}^-$ ) below 6 cm through this process and through sulphate reduction at this depth could also promote precipitation of  $\text{FeS}$  and  $\text{FeS}_2$ , thus reducing  $\text{Fe}^{2+}$  concentrations (Morse & Luther lii, 1999) and causing the sudden reduction in  $C_{\text{DGT-Fe}}$  below this depth in the 65 and 270 mg/kg treatments. The pronounced mobilisation of Cu at -7 - 8 cm in the 270 mg/kg treated sediment (Figure 4.1) is linked to a marked decrease in Mn and Fe release at the same depth (Figures 4.2 and 4.3). The reasons for this are not immediately obvious and may be a function of Fe precipitating as sulphide minerals (e.g.  $\text{FeS}$ ,  $\text{FeS}_2$ ) (Morse & Luther lii, 1999). There may be a localized pH decrease at these depths due to pyritization of Fe to  $\text{FeS}$  (Equation 18), respiration and ammonia oxidation with sulphate (Equation 17), which could in turn mobilise sorbed or precipitated Cu, but this would need further measurements of redox profiles and sulphide release to prove.



While the general  $C_{\text{DGT-Mn}}$  results indicate reductive dissolution of Mn oxide minerals and, thus, support the finding that the sediment became anoxic within the top 1-2 cm below the SWI (Du Laing et al., 2007), these results should be interpreted with caution. The binding of Mn by the Chelex-100 resin is relatively weak and Mn may have been outcompeted by the high Cu and soluble Fe concentrations seen the Cu-treated sediments (Tankéré-Muller et al., 2012).

Apart from sediment properties that were discussed earlier, the bioavailability of metal (Cu) is also influenced by sediment microbial function (Chapman et al., 1999; He et al., 2018; Simpson & Batley, 2007). The reductive dissolution of solid Fe (III)/Mn (IV) oxide phases can result in the release of metals (including Cu) into the sediment porewater and become potentially toxic to the sediment microorganisms (He et al., 2018). However, this was not clearly reflected by the  $C_{\text{DGT-Cu}}$  or exchangeable Cu results (Figures 4-1 and 4-4, respectively). The sequential extraction results suggest that a vast majority of the Cu was bound to the organic matter fraction, with over 69% of the total Cu bond in that fraction (Figure 4.6). These findings were similar to those reported in Cu contaminated sediment where Cu strongly bound to organic matter (Gonzaga et al., 2020; Hickey & Kittrick, 1984; McLaren & Crawford, 1973). The rest of the Cu was bound in the relatively inert residual fraction (>20 %), with only a small amount (~2%) left in the exchangeable, carbonate, and Fe/Mn oxides fractions. These results were similar to the findings of Fontes and Dias (2003) where they discovered that Cu was largely bound to organic matter fraction and only a small fraction retained as oxides, residual, carbonates, and exchangeable and helps to explain why the  $C_{\text{DGT-Cu}}$  depth profiles did not vary very much between treatments or with depth. A relatively large proportion (44 – 55 %) of the introduced copper was not

recovered in the sediment digestion, which suggests that the size of the residual pool was underestimated here; however, the relevance of that unrecovered fraction on microbial functioning or the cycling of N is unlikely to be important.

The results here show that Cu concentrations of 65 and 270 mg/kg, although accepted as guideline limits for regulation, can have consequences for ecosystem health. At these guideline values, Cu may stimulate nitrate reduction and increase N<sub>2</sub>O production, while the accumulation of NH<sub>4</sub>-N in depth profile in Cu-contaminated sediment may serve as a longer term source of the nutrient and thus stimulate eutrophication in affected water bodies. Macrophytes and phytoplankton will favour the nutrient rich sediments thus causing further water quality issues. The wider consequences of Cu contamination at, and around these ANZECC guidelines, may present issues at the local and national (affecting recreational waterways) and global scale (production of the greenhouse gas) that go beyond the movement of the metal up the trophic levels in ecosystems.

### **5.1.1 Limitations of the experimental approach**

The experimental approach used here was chosen to establish an experimental system that could be replicated and carefully controlled to test the effect of Cu contamination systematically. However, this approach has numerous limitations that should be recognized. The extensive disturbance of the sediment during sampling and processing prior to the start of the equilibration is likely to have disrupted many of the natural microbiological processes and redox gradients in the sediment. Others have found that equilibration times of 3 weeks are sufficient for helping to restore steady-state gradients in sediment (Lehto et al., 2014); this was partly confirmed by monitoring the water quality variables in the circulating water. Moreover, the exclusion of invertebrates and plants will have negated the possible effects that they are believed to have on various elemental cycles in freshwater sediments, including N (Nogaro & Burgin, 2014). The temperature of the sediment (~25 °C) was higher than would be encountered in many natural watercourses in the temperate regions and this is likely to increase rates of microbial processes and the rates of diffusion of dissolved solute (including O<sub>2</sub> and NO<sub>3</sub><sup>-</sup>) and this may have amplified or suppressed some of the effects that would be otherwise seen in New Zealand. For example, the effects of elevated temperature on the balance between denitrification and anammox (Tan et al., 2020) may have been important; however, due to the complexity of the processes involved, the extent of the effect of the higher temperature is difficult to quantify. The tolerance of different microbial populations to trace element contamination may also vary between populations and sediments, hence these results are only an indication of the short-term effects of Cu contamination. However, for the purposes of an initial mechanistic study into the effects of Cu on an NZ freshwater sediment, the methodology was appropriate.

## 5.2 Conclusion

This study showed that Cu partitioned strongly to the organic matter and relatively inert phases of residual fraction upon introduction to this sediment here. However, despite the relatively low bioavailable concentrations, the work reported here showed for the first time in New Zealand that introduction of the metal resulted in the increased production of nitrous oxide and the accumulation of ammonium in the sediments. These effects were believed to have arisen due to the strong effect that Cu has on the microbial processes that drive the cycling of N in the sediment. However, the specific microbial processes could not be conclusively identified, due to complexity of the biogeochemical cycles in this environment.

While the ANZECC guideline values for Cu (65 and 270 mg/kg) are intended for the protection of ecosystems, there are implications for water quality arising from Cu contamination and its effects on the N cycle that now should be investigated. While the mesocosm approach used here served as a useful means by which to carry out a replicated experiment in a controlled environment, these findings should now be tested under in-situ conditions.

## 5.3 Recommendation for further research

Some of the limitation of this research provided the basis for recommendations for future studies as follows:

- Test the effects of Cu contamination on N cycling in natural river mesocosms across New Zealand to show differences arising from different sediment types and river environments.
- Examine specific mechanisms through the use of stable N isotopes to identify which processes in the N cycle are most affected and to what extent. The use of oxygen and pH micro electrodes would further support interpretation of sediment profiles of Cu, Fe, Mn, and NH<sub>4</sub> dynamics.

Sample microbial populations and measure their functioning through molecular biology techniques to further elucidate specific effects of trace elements on them.

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