Nickel toxicity to cardinal tetra (*Paracheirodon axelrodi*) differs seasonally and among the black, white and clear river waters of the Amazon basin

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**Abstract**

This study investigated the acute toxicity of nickel (Ni) to cardinal tetra (*Paracheirodon axelrodi*), within the three main water types of the Amazon basin: black (Rio Negro), white (Rio Solimões) and clear (Rio Tapajós) during the wet and dry season at pH 7 (representative of white and clear rivers) and pH 4 (representative of black waters). The influence of dissolved organic carbon (DOC) quality on Ni toxicity within the three waters was also explored via the use of DOC isolates. Differences in water chemistry, DOC quality and ion concentrations were shown between waters and between seasons. Toxicity of Ni was shown to vary between river waters, seasons, and pHs. Ni was significantly less toxic during the dry season at pH 4 in all three river waters; for example, black water during the wet season had an LC50 of 9.72 mg Ni/L compared to 41.5 mg Ni/L during the dry season. At pH 7, contrasting effects in toxicity between seasons were shown between black and clear waters (black: wet = 28.9 mg/L, dry = 17.3 mg/L; clear: wet = 13.8 mg/L, dry = 24.1 mg/L). There were no significant differences in Ni toxicity for white waters at pH 7 (white: wet = 22.2 mg/L, dry = 21.8). Overall, Ni was shown to be more toxic at pH 7 than at pH 4 except in black water during the wet season. Toxicity of Ni at pH 4 was negatively related to DOC concentration and amount of humic-like and fulvic-like DOC and positively related to fluorescence index. Therefore, at pH 4, Ni is more toxic in waters containing more allochthonous DOC, consisting of higher amounts of humic-like and fulvic-like components. LC50 values for the different DOC concentrates at the same DOC concentration of 4.5 mg/L (black: 26.8 mg/L; white: 73.3 mg/L; clear: 49.2) support the river water findings at pH 4 (Ni more toxic in presence of black DOC) indicating that DOC quality alone can influence Ni toxicity at this pH.

**1. Introduction**

Metal contamination of freshwaters worldwide is increasing as urbanization, mining and climate change impacts increase (Rockström et al., 2014). Nickel (Ni) is one such metal of concern with increased contamination of freshwaters occurring globally (Pyle and Couture, 2012). Ni constitutes approximately 2% of the earth’s crust and reserves vary around the globe with Brazil containing the seventh highest reserves (Barrera, 2016). As anthropogenic processes such as mining encroach on many areas in Brazil, especially in the Brazilian Amazon, the relative risk that metals, such as Ni, pose to aquatic environments must be taken into consideration.

Metal bioavailability and toxicity to biota in aquatic systems are dependent on speciation of the metal in solution, uptake and binding to the target receptor, and subsequent depuration/detoxification processes. Speciation and bioavailability in turn depend on water quality characteristics, particularly pH, hardness, alkalinity,
temperature and concentration and quality of dissolved organic carbon (DOC). In nature, the quality and quantity of DOC differs between waterways and within them both spatially and seasonally (Fellman et al., 2010; Johansson et al., 2016). It has been widely shown that increased concentrations of DOC can significantly decrease toxicity of metals such as Ni (Hoang et al., 2004). Recent evidence also suggests that organic matter quality can lead to differences in toxicity of metals at fixed DOC concentrations (Al-Reasi et al., 2011, 2012; Wood et al., 2011), with most studies showing a protective effect of allochthonous aromatic optically dark DOC compared with autochthonous, optically light DOC.

The Amazon Basin in Brazil is characterised by three main water types: black, white and clear, which differ in physico-chemical properties such as pH, ionic composition, total suspended solids and DOC (Furch, 1984; Val and Almeida-Val, 1995). The black Rio Negro is acidic, low in ions and suspended solids, and contains high amounts of DOC leached from the surrounding vegetation, which stain the water a dark brown colour. White water rivers such as the Rio Solimões on the other hand are close to circumneutral pH and contain lower concentrations of DOC and higher amounts of ions and suspended solids which give the rivers a cloudy appearance. Clear water rivers are generally transparent, contain low amounts of ions and suspended solids, and vary in pH from acidic to circumneutral with DOC concentrations typically lower than 1 mg/l (Furch, 1984; Val and Almeida-Val, 1995). Marked seasonal differences in water quality between the wet and dry season have also been reported within each water type as a result of altered precipitation and flooding (Furch, 1984; Val and Almeida-Val, 1995).

Little information, however, is currently available on the toxicity of metals such as Ni within the different waters of the Amazon basin and whether such physico-chemical differences among rivers and between seasons (wet and dry) lead to significant differences in toxicity. It has been reported that Ni toxicity is influenced by pH, hardness, DOC concentrations and total suspended solids (TSS) (Hoang et al., 2004; Pyle and Couture, 2012; Pyle et al., 2002), all of which differ among the different waters and seasons of the Amazon basin (Sioli, 1984). It is therefore predicted that Ni toxicity will vary greatly among the three natural water types, as well as with season.

Cardinal Tetra (Paracheirodon axelrodi) are important ecologically and commercially to the Amazon basin (de Oliveira et al., 2008; Crémazé et al., 2016). Cardinal are endemic to Brazil and the Amazon region and are the most common fish species in the Rio Negro (de Oliveira et al., 2008). Cardinal are also the most exported ornamental fish from the Amazon region, representing 80% of fish exported (de Oliveira et al., 2008; Crémazé et al., 2016). Its small size makes it easy to use in laboratory trials and it has been previously used in studies with metals such as copper (Duarte et al., 2009; Crémazé et al., 2016).

This study therefore aimed to investigate the acute toxicity of nickel to cardinal tetra (P. axelrodi), as assessed by classic 96 h LC50 tests, within the black (Rio Negro), white (Rio Solimões) and clear (Rio Tapajós) river waters of the Amazon Basin during the wet and dry season as well as a groundwater (INPA well water) low in ions and DOC which served as a reference water. Tests were performed at both pH 7.0 and pH 4.0 in all water types to capture the range of pHs seen in the natural waters (black water as low as pH 4, white water and clear water as high as pH 7). Investigations into the influence of DOC quality in determining toxicity were also conducted using the different DOC isolates from these rivers obtained using reverse osmosis (RO). Water chemistry in each of the test conditions was thoroughly characterized, with a particular emphasis on DOC quantity and quality, the latter assessed by a range of optical parameters. Our goal was to link any differences in toxicity with key water quality parameters and/or differences in DOC quality.

2. Materials and methods

2.1. Water chemistry

Water was collected from the black (S03 05.780, W60 21.352), white (S03 15.335, W60 14.861) and clear water rivers (S2 23 50.6869, W54 47 51.3569), both during the wet season (May–July 2015) and the dry season (October to November 2015) for use in the experimental trials. INPA well water was also collected and used in the dry season. Samples for determination of ionic composition and metal suites were collected from each river water before the start of experiments. Ionic compositions (Na+, Ca2+, Mg2+, K+) of waters (n = 3) were analysed using flame atomic absorption spectroscopy (Perkin-Elmer model 3100: Perkin-Elmer Inc, USA). Cl was measured using the colorimetric method described by Clarke (1950) (n = 3). Hardness was calculated from the Ca2+ and Mg2+ concentrations and alkalinity was measured using the method APHA Standard Methods for Examination of Water and Wastewater method 2320 (1992). Analyses for a suite of metals (Al, Cu, Pb, Fe and Ni) were conducted on the waters (black, white, and clear) collected during the wet season via inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700x: Agilent Technologies, US), while graphite furnace atomic absorption spectroscopy (GFAAS) (Perkin-Elmer model 3100: Perkin-Elmer Inc, USA) was used for dry season samples (n = 3). To ensure results were comparable between instruments certified standards were used to develop calibration curves for both machines and the same quality control procedures conducted. This included metal analysis of method blanks, laboratory duplicates, and matrix spikes using certified standards. Percent recoveries ranged from 98 to 110%. Turbidity was determined using the EPA method 180.1: Determination of turbidity by Nephelometry and TSS was determined using Gravimetric analysis as outlined in the EPA Method 160.2.

DOC was analysed at the beginning of each trial using a total carbon analyzer (Apollo 9000 combustion TOC analyzer; Teledyne Tekmar, Mason, USA) (n = 3). The TOC machine was calibrated as per manufacturer’s instructions, using primary standard grade potassium hydrogen phthalate (KHP). QA/QC KHP standards were run every 20–30 samples. DOC quality was measured using a range of different absorbance and fluorescence measurements. River water samples and concentrations were adjusted to approximately pH 7 using 0.1 M NaOH. All absorbance and fluorescence scans were performed using a 1 cm quartz cuvette in a SpectraMax® M2e (VWR, Pennsylvania) spectrophotometer and replicated three times. SAC340 (2.303 × absorbance at 340 nm/DOC) was used to give an indication of aromaticity (Curtis and Schindler, 1997), fluorescence Index (FI) provided an indicator of DOC source (McKnight et al., 2001), while molecular weight was indicated by the absorbance ratio Abs254/365 (Dahlen et al., 1996). SUVA254 served as a measure of the UV-absorbing moieties (abs254/DOC). Fluorescence excitation emission scans (FEEM) were conducted on all samples at emission wavelengths of 250–600 nm using steps of 10 nm excitation wavelengths between 250 and 450 nm. The excitation emission matrixes were then analyzed in MATLAB R2014b (MathWorks, Inc. 1994–2016) to produce three-dimensional FEEMs and contour plots. Rayleigh scatter was removed and replaced with "not a number" values (NaN). The FEEMs were then modelled using parallel factor analysis (PARAFAC) based on the a priori assumption of 4 components (PLS-toolbox in MATLAB: Eigenvectors Research Inc, WA, USA) (Al-Reasi et al., 2012; Johansson et al., 2016). Tyrosine and tryptophan spectra were also included within the PARAFAC analysis to weigh the resolved components towards the a priori assumption that the DOC included only humic, fulvic and proteinaceous fluorophores (i.e, tyrosine and tryptophan-like
2.2. Toxicity trials

Cardinal tetras (Paracheirodon axelrodi) that had been collected from black water in the wild were purchased from a supplier in Manaus, and ranged in size from 1.5 to 2.5 cm and weighed between 0.1 and 0.2 g. Fish were acclimated in 300-L tanks containing INPA well water for at least one month before use in experimental trials. Fish were fed TetraMin tropical fish flakes daily until satiation up until 48 h before the beginning of the experiment. Ni concentration within the food provided to the fish before exposure was not determined. All experimental work was approved by the Ethics Committee on Animal Experiments of INPA under registration number 026/2015, and conformed to national animal care regulations.

96 h static renewal acute tests were conducted in 2-L plastic containers, with each container, containing 1 L of test solution and two fish. All containers and lids were previously acid washed using 10% nitric acid before the test. Eighty percent of the treatment water was renewed every 24 h. Each treatment in each trial consisted of two replicates. Fish were exposed to five Ni (NiCl₂·6H₂O: Sigma Aldrich) concentrations ranging from 7.3 mg/L-240 mg/L Ni plus the control (treatment water with no added Ni) at pH 4 and pH 7. Ni concentrations were chosen based on preliminary tests to ensure at least one concentration recorded minimum 90% survival and 100% mortality after 96 h. pH was adjusted when needed using 1 M KOH or HNO₃. Measured concentrations of dissolved and total Ni recorded during each trial are provided in Supplementary Table 1. A natural light and temperature regime was used for the wet season experiments which represented a 12 h light: 12 h dark photoperiod and average temperature within test chambers of 27 °C, with conditions altered in the dry season experiments using a chill to maintain exposure temperature within test chambers to average of 27 °C to mimic the same conditions experienced during the wet season, with a natural light regime also employed.

Containers were continuously supplied with aeration. Lids were placed on top of the test chambers to prevent contamination and the escape of test subjects. Fish were monitored every 24 h for signs of morbidity or mortality. Fish displaying signs of persistent loss of equilibrium were removed from the test chambers to average of 27 °C after water change, every 24 h and analysed using GFAAS (Perkin-Elmer model 3100: Perkin-Elmer Inc. USA).

To try to determine mechanisms behind any differences in toxicity between the waters additional dilution and DOC concentrate experiments were conducted. To determine the effects of ions and/or DOC concentration on Ni toxicity at pH 7 and 4 in black and white river water, dilution experiments were conducted. Black and white river waters were diluted by 50% with INPA well water during the dry season. INPA well water contains a similar ionic composition to the black water with little to no DOC (Table 1), making it the perfect diluent to explore the role that DOC concentration and ions play in determining metal toxicity within the black and white waters. Fish were exposed to the same Ni concentrations, experimental setup and conditions as outlined above. Trials were conducted at the same time as the river water dry season experiments. To determine the influence of DOC quality alone on the river water toxicity results, DOC concentrates were isolated from the black, white and clear river water using reverse osmosis (Vontron®ULP21-4021 polyamide membrane, Permution, model PEOS-0001, Curitiba, Brazil) following the recommendations of Serkiz and Perdue (1990). DOC concentrates were resinated to remove metals and cations using Amberlite IR-118 hydrogen form. DOC concentrates (black, white and clear) were added to INPA well water (which is virtually DOC-free, <0.04 mg/L DOC) to give a DOC concentration of 4.5 ± 0.1 mg/L INPA well water was used as a control. Fish were exposed to the same Ni concentrations, experimental set-up and conditions as outlined for the river water trials. Trials were conducted during September 2015.

2.3. Statistical analysis

Dose-response curves (log-logistic) using the average dissolved Ni values for each Ni treatment were conducted on morbidity data to determine the concentration which is lethal to 50% (LC₅₀) of the fish using the ‘drc‘ package in R (Ritz et al., 2015). 95% confidence intervals were determined using the delta method. Statistical calculations to determine differences between LC₅₀ values were conducted as outlined by Litchfield and Wilcoxon (1949). Linear regressions were used to determine and visualise significant relationships between water quality/chemistry and/or DOC quality.

**Table 1**

<table>
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<th>Season/experiment</th>
<th>DOC</th>
<th>Na</th>
<th>Ca</th>
<th>Mg</th>
<th>K</th>
<th>Cl</th>
<th>Al</th>
<th>Fe</th>
<th>Cu</th>
<th>Pb</th>
<th>Alkalinity (CaCO₃)</th>
<th>Hardness (CaCO₃)</th>
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<td>0.52</td>
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variables and LC\textsubscript{50} values using SigmaPlot 13.

3. Results

3.1. Water quality and chemistry

The three river types were characterised by different water quality/chemistry (Table 1) and quality of DOC (Table 2). The white water contains higher concentrations of major ions and suspended solids (TSS) compared to the other two waters (black and clear) (Table 1). Similar ionic compositions (extremely low levels of all major ions) were observed between the black and clear river waters and INPA well water (Table 1). The black water contains the highest amounts of DOC and Al followed by the white water, than the clear and well water which both contained little to no DOC ($\leq 1 \text{ mg/L}$) (Table 1). Differences in water chemistry between wet and dry seasons were also shown with slight changes in ion concentrations and decreases in DOC and Al between the wet and dry seasons (Table 1). Diluting the black water by 50% with well water led to nearly a 50% decrease in DOC concentrations (Table 1); however, since the well water has similar ionic composition to the black water, ion concentrations remained relatively the same (Table 1). Dilution of the white water also led to a decrease in DOC concentration, but also close to a 50% decrease in ion concentrations (Table 1).

3.2. DOC quality

Four fluorescent DOC components were determined by PARAFAC and accounted for 97.4% of the variability (Fig. 1). These include a humic-like (component 1), representative of high molecular weight, aromatic humic acid commonly associated with wetlands, flooded forests, and forested environments; fulvic-like (component 2), low molecular weight fulvic acid; and tryptophan-like and tyrosine-like (components 3 and 4 respectively), representative of protein compounds, free or bound amino acids (Fig. 1 and Table 2). The four components were determined based on descriptions provided in Fellman et al. (2010), Al-Reasi et al. (2012) and Johannsson et al. (2016).

DOC quality, differed among the river waters and INPA well water (Table 2). The DOC within the black water is allochthonous in nature (FI: 1.4). Aromatic (high SAC\textsubscript{340} values: 44–67 cm\textsuperscript{2} mg\textsuperscript{-1}), and of a higher molecular weight indicated by the lower Abs\textsubscript{254/365} ratios: Rio Solimões: 33–85 cm\textsuperscript{2} mg\textsuperscript{-1}; Rio Tapajós: 30–58 cm\textsuperscript{2} mg\textsuperscript{-1}), and is likely to be of similar molecular weights (Abs\textsubscript{254/365} ratios: Rio

![Fig. 1. The four components of dissolved organic carbon (DOC) determined by PARAFAC analysis. Component 1 — humic-like, component 2 — fulvic-like, component 3 — tryptophan-like and component 4 — tyrosine-like. These four components explained 97.4% of the variability in the excitation emission scans.](image-url)
Solimões: 3.5–4.6; Rio Tapajós: 4.0) (Table 2). INPA well water DOC is autochthonous in nature (FI: 1.6), consisting of mostly protein-like (tyrosine and tryptophan) DOC of low aromaticity (SAC340: 6.91 cm² mg⁻¹), and low molecular weight (high Abs254/365 ratio: 7.00) (Table 2). Difference in DOC quality was also shown among seasons. Specifically, higher SAC340, SUVA254, and Abs254/365 values were recorded during the dry season for the black and white waters and lower values were recorded in the clear compared with those recorded in the wet season (Table 2).

Differences in DOC characteristics were also shown among concentrates, with SAC340 values highest in the black (45 cm² mg⁻¹) and lowest in the white (24 cm² mg⁻¹). FI and Abs254/365 lowest in the black (FI: 1.41 and Abs254/365: 4.11) and highest in the white (FI: 1.55 and Abs254/365: 4.69) (Table 2). Dilution of the concentrates with well water led to increases in protein-like DOC (higher amounts of tryptophan and tyrosine-like DOC) and fulvic-like DOC and decreases in humic-like components compared with the river water from which the concentrate was isolated from (wet season black and white water and dry season clear) (Table 2). However, the ratio of the components remained similar with humic-like components still dominating within the black water DOC and fulvic-like components within the white and clear.

3.3. Toxicity of nickel

3.3.1. River waters

Negligible differences were shown between the total and dissolved Ni in treatment waters and among tests (Supplementary Table 1). Negligible differences were shown across trials in regards to water quality measured every 24 h (Supplementary Tables 2 and 3). pH in the pH 4 treatments varied from 3.9 to 4.4 within all trials and from 6.9 to 7.4 in the pH 7 treatment. Dissolved oxygen (% sat) varied from 50 to 80% and temperature ranged from 25 to 30 °C for all experiments. Conductivity varied between waters and between Nickel treatments. Toxicity of Ni within the waters of the Amazon basin varied depending on the pH, season, and river water. This is explored in the following sections.

3.3.1.1. Between pHs (4 and 7). Ni was significantly less toxic at pH 4 than at pH 7, as indicated by higher LC50 values, with the single exception of the black water during the wet season (9.72 mg/L Ni). LC50 values in waters from the other sites at pH 4 ranged between 27.83 (clear, wet) to 90.33 mg/L (well water) (Fig. 2a), while at pH 7 they ranged from 13.45 (50% black water) to 28.87 mg/L (black water, wet) (Fig. 2b).

3.3.1.2. Between wet and dry seasons. Significant differences were shown between the toxicity of Ni in black and clear water between the wet and the dry season at both pHs (Fig. 2a and b). However, no significant difference was shown between the seasons with regard to Ni toxicity within the white water at pH 7 (Fig. 2b). Ni at pH 4 was consistently more toxic in all river waters collected during the wet compared with the dry season (Fig. 2a). At pH 7 Ni was more toxic in black water collected during the dry and in clear water during the wet season (Fig. 2b).

3.3.1.3. Among waters. Significant differences in Ni toxicity among waters were also recorded at pH 4. Ni was significantly more toxic in black water (LC50 values: 9.71 mg/L Ni (wet season) and 41.46 (dry season)), compared with the other two waters during both the wet and dry seasons and well water (Fig. 2a). Toxicity of Ni was lowest in the well water (LC50: 90.33 mg/L Ni) (Fig. 2a). Ni toxicity at pH 4 was significantly related to DOC concentration (p = 0.02; r² = −0.31) and DOC quality (FI (p = 0.01; r² = 0.34), amount of humic-like DOC (p = 0.01; r² = −0.30) and fulvic-like DOC (mg/L C) (p = 0.02; r = −0.31)) (Fig. 3).

At pH 7 during the wet season the clear water recorded a significantly lower LC50 value in comparison to the black and white waters. The black water exhibited the highest LC50 value, followed by the white water and the clear (Fig. 2). In contrast the opposite occurred during the dry season with Ni at pH 7 in clear water less toxic than in the white water and most toxic in the black water; however, no significant difference in LC50 values was determined (Fig. 2).

3.3.1.4. Within the same water. Dilution of white water with well water significantly increased toxicity of Ni (LC50 100%: 71.3 mg/L and 50%: 47.6 mg/L) at pH 4 (Fig. 2a), however had little effect at pH 7 (Fig. 2b). Within the black water on the other hand no significant difference was shown between the 100% and 50% dilutions (Fig. 2a). At pH 7, however, dilution by 50% led to a significant increase in toxicity from 17.29 mg/L Ni to 21.46 mg/L Ni (Fig. 2b).

3.3.2. DOC concentrates

Significant differences in Ni toxicity were shown in the presence of different DOC concentrates at pH 4 (Fig. 4), with toxicity greatest...
in presence of black water DOC. Ni was significantly less toxic in presence of well water (no DOC) than in the presence of black and clear water concentrates, indicating that the presence of these two DOC increases toxicity at pH 4. No significant differences among DOC concentrates were shown at pH 7; however, toxicity of Ni was significantly reduced in the presence of all three concentrates compared to well water which contained no added DOC (Fig. 3).

4. Discussion

4.1. Water quality and chemistry

Measured water chemistry within each of the three river waters was similar to previous published work (Furch, 1984; Val and Almeida-Val, 1995). The white water in comparison to the other two river waters (black and clear) contained approximately between 2 and 13 times greater ion concentrations and suspended solids. The black water on the other hand was characterised by the highest DOC concentrations during both the wet and dry season (approximately 2.3 (white) – 15 times (clear)). The black water also contained DOC of a more terrestrially derived nature (FI: 1.4) compared to the other waters (FI: 1.5–1.6). The black water DOC was also characterized predominately by humic-like components (60–64%) compared with the other two river waters which were dominated by fulvic-like components (white: 47–48% and clear: 41–46%), and well water which was dominated by protein-like DOC (66%).

SAC340 values for the black water (wet: 44 cm² mg⁻¹ and dry season: 67 cm² mg⁻¹) and clear (wet season: 58 cm² mg⁻¹) were within readings previously reported for the black water(44–73 cm² mg⁻¹) (Duarte et al., 2016; Johannsson et al., 2016) and higher than values reported in the literature for waters from other sites such as Luther Marsh, a peat bog (39 cm² mg⁻¹). This indicates they were characterised by highly aromatic DOC (Richards et al., 2001; Al-Reasi et al., 2011; 2012). Fl scores for the three rivers and well water (Fl: 1.4–1.6) also fall among those reported from rivers in the US receiving terrestrial inputs of DOC and Antarctic lakes containing only autochthonous DOC (McKnight et al., 2001). Abs₂₅₄/₃₆₅ values for the white (wet), clear and well waters (3.7–15.7) which serve as an indicator of molecular weight, were within ranges previously reported for other freshwater DOC (Al-Reasi et al., 2012). Black and white waters during the dry season recorded slightly lower Abs₂₅₄/₃₆₅ (3.4–3.6) than previously reported indicating the DOC is likely to be of a higher molecular weight (Al-Reasi et al., 2012; Johannsson et al., 2016).
Differences in ionic composition, DOC concentration and quality were shown among seasons. Increased levels of DOC during the wet season compared with those recorded during the dry season are likely to reflect increased flushing of the surrounding soils and forest inputs (Sousa et al., 2011) and have also been reported from other rivers within the Amazon basin and the Congo River basin (Sousa et al., 2011; Bouillon et al., 2014). Differences in DOC quality were also shown among the different seasons with higher SAC340, SUVA254, Abs254/365 values recorded during the dry season for the black and white waters and lower values in the clear. This result is unexpected as it was assumed that higher SAC340, SUVA254, Abs254/365 values associated with more aromatic and higher molecular weight DOC would be associated with the wet season due to flooding of the forests, however the opposite appears true for the black and white waters. Teixeira et al. (2011) also recorded higher SUVA254 values for DOC from lakes along the Upper Paraná River floodplain during the dry season compared with the wet and suggested this may be due to increased primary production in the wet season as indicated by higher chlorophyll-a levels. Our results indicate that DOC quality differs between the wet and dry seasons within the Amazon basin and this must be considered when determining the quality of DOC in this region.

### 4.2. Nickel toxicity

LC50 values for Ni at pH 7 and pH 4 range between (13–29 mg/L) and (28–90 mg/L), respectively. Adult cardinal tetra used in this study appear to be tolerant to Ni at pH 7, when compared to other fish species exposed to Ni in soft waters and at a similar pH. 96 h LC50 value of 4 mg/L for adult fathead minnows (Birge and Black, 1980) and 0.5 mg/L for <24 h old larve (Pyle et al., 2002), 10 mg/L for adult goldfish (Birge and Black, 1980) 8–9 mg/L for Arctic graylings, 17–18 mg/L for Coho salmon, 8–25 mg/L for aleven and juvenile rainbow trout, respectively (Buhl and Hamilton, 1991) have been previously reported in the literature. Currently no data exists for Ni toxicity to fish at a pH as low as 4, making it hard to determine whether or not cardinal are also a tolerant species at this pH. Ni is considerably less toxic (approximately 1000 times) to cardinal tetra compared with Cu (LC50 value of 46 and 56 μg/L) in well water used in this study, approximately 10–40 times less toxic in black water (LC50 value of 1090 μg/L) and approximately 100–400 times less toxic in white water (LC50 value of 194 μg/L) (Duarte et al., 2009; Crémazy et al., 2016).

Differences in water chemistry, DOC concentration and quality among rivers and seasons significantly influence toxicity of Ni within the Amazon basin, with toxicity of Ni shown to differ among other rivers within the Amazon basin and this must be considered when determining the quality of DOC in this region. Such differences in water chemistry parameters appear to affect the toxicity of Ni within each water type differently. Within the black water at pH 7, dilution of this water by 50% led to a significant increase in Ni toxicity. This appears to be linked with the associated decrease in DOC concentration. This along with the significant difference between the well water (no DOC) and the three DOC
concentrates at pH 7 supports previous studies which have shown that toxicity of Ni decreases with increases in DOC at circumneutral pH (Doig and Liber, 2006; Cloran et al., 2010; Custer et al., 2016). This is the first study to show contrasting effects between pH 4 and pH 7 in regards to the effect of DOC concentration on metal toxicity. This double edged sword of DOC, where at high pH’s, DOC is protective and at low pH’s DOC increases toxicity has also previously been shown for acidity alone, where increased DOC concentrations protected fish against respiratory stress at pH’s above 4 and caused increased mortality at pH’s of 4 and below (Holland et al., 2014).

In contrast to the black water, no significant difference was shown between the 100 and 50% dilutions within the white water at pH 7. However, at pH 4 dilution of the white water caused a significant increase in toxicity of Ni. The reduction in ions is likely to be responsible for the increased Ni toxicity within the 50% dilution water compared with the 100% white water. It is well established that increases in the amount of ions decreases toxicity of metals including Ni (Hall and Anderson, 1995; Hoang et al., 2004; Meyer et al., 1999). This is not surprising since ions such as Na⁺, Ca²⁺, and Mg²⁺ will compete with Ni for binding sites on the target animal (in this case tetras), leading to decreased binding of Ni and lower toxicity (Blewett and Wood, 2015; Hoang et al., 2004).

Therefore, increases in this ion may also limit toxicity of Ni. The Ni concentrations reflected in this study does not reflect environmentally relevant concentrations within the Amazon basin, however reflect concentrations previously reported in mine wastewaters from Brazil (Wildemann, 2004). Further research investigating the chronic toxicity of Ni to fish within the different waters of the Amazon Basin, at environmental relevant concentrations of Ni is needed to improve our understanding of Ni toxicity within this region. Although the direct physiological and/or chemical mechanisms behind why Ni toxicity differs between pHs, seasons and between waters of the Amazon Basin could not be determined from these experiments, results suggest that DOC concentrations and its quality along with ion concentrations may play a role.

5. Conclusion

- Toxicity and accumulation of Ni within the different waters of the Amazon basin was shown to vary depending on river water, season, pH, DOC and ion concentrations and DOC quality.
- At pH 4, contrasts in Ni toxicity among rivers and between seasons were linked with changes in DOC concentration and quality.
- These results have important implications for metal risk assessment and development of appropriate water quality guidelines, as results from bioassays in water collected during one season (dry or wet) may not be representative of that waterway during the other season. Results may also not be representative of other waterways within that region.
- Seasonal and spatial variability in DOC concentration and quality thus must be taken into consideration when analysing the toxicity and bioavailability of metals in freshwaters especially in regions were both circumneutral and naturally acidic waters such as the black waters within the Amazon basin coincide.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.06.044.

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