WESTERN SYDNEY
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Ecological responses to the discharge of polluted coal mine wastewater

Thesis presented by Benjamin Simpson Green

Supervisor: Dr Ian Alexander Wright

School of Science and Health

Western Sydney University

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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge, original except where acknowledged in the text. I hereby declare that I have not previously submitted this material, either in whole or in part, for a degree at this or any other institution.

Signature: [redacted]

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Abbreviations

Abbreviation	**Full Term**
AMD	Acid Mine Drainage
ANZECC	Australian and New Zealand Environment and Conservation Council
DGV	Default Guideline Values
DO	Dissolved Oxygen
EC	Electrical Conductivity
EPA	Environmental Protection Agency
EPL	Environmental Protection Licence
EPT	Trichoptera, Ephemeroptera, and Plecoptera
NATA	National Association of Testing Authorities
NSW	New South Wales
NTU	Nephelometric Turbidity Units
OEH	Office of Environment and Heritage (now
One-way ANOVA	One-way analysis of variance (one-way ANOVA)
POEO Act	Protection of the Environment Operations Act 1997
µg/L	Micrograms per litre
PPM	Parts Per Million

Abstract

The discharge of coal mine generated wastewater has degraded the Georges River downstream of the discharge point. This study measured the water quality, sediment quality and macroinvertebrate community of the upper Georges River to determine the impact of the influx of wastewater from an underground coal mine. The investigation revealed that the wastewater discharge from the West Cliff Colliery into the Georges River was highly polluted. The pollution of the river is characterised by increases in; electrical conductivity, pH levels and a suite of metals including; antimony, aluminium, arsenic, barium, boron, cobalt, lithium, molybdenum, nickel, strontium, titanium, uranium and zinc. The elevated concentrations of metals downstream of the wastewater discharge point has caused a suite of metals including; aluminium, barium, chromium, cobalt, copper, iron, lead, lithium, manganese, nickel, strontium, titanium, uranium and zinc to accumulate within the sediment. The altered water chemistry downstream of the wastewater discharge point is expected to be the salient factor in the significant loss of biodiversity within the macroinvertebrate community. The aquatic macroinvertebrate community downstream of the wastewater discharge is characterised by a loss of pollution sensitive taxa and an increase in pollution tolerant taxa. A laboratory experiment has identified that a suite of metals including; aluminium, boron, barium, cobalt, copper, iron, lithium, manganese, molybdenum, nickel, strontium and uranium present in the coal mine wastewater discharge are able to bioaccumulate within plants that are exposed to the wastewater. This research has demonstrated that the current implementation of environmental protection legislation has failed to protect one of Sydney's major waterways from the effects of coal mining. Consequently, it is essential that we better manage our aquatic ecosystems to avoid further instances of degradation and to preserve ecosystem functions and services.

Keywords

Conservation, Waterway Management, Water Quality, Sediment, Macroinvertebrate Community Structure, Metal Accumulation in Plants, Georges River, Environmental Regulation

1. Introduction

In New South Wales (NSW) there is a serious disconnect between the value that we place on our natural environment and the implementation of the measures that we have constructed to protect it. This issue is evident within the underground coalmining sector and the resulting discharge of wastewater into the receiving waterway which often results in the degradation of the aquatic system (see; Wright and Burgin 2009; Belmer et al. 2014; NSW OEH 2015; Wright et al. 2017; Xingli et al. 2018). The NSW Environmental Protection Agency (NSW EPA) is the primary body that regulates the discharge of wastewater from underground coalmines in NSW, legislated by the Protection of the Environment Operations Act 1997 (POEO Act).

Underground coalmining frequently results in a variety of environmental impacts, including; aquatic ecosystem degradation, noise pollution, air pollution, subsidence, land clearing, increased bushfire risk and a reduction in aesthetic qualities. Of all these aspects, this literature focusses on the problem of aquatic ecosystem degradation caused by the discharge of wastewater generated by the coalmine. Wastewater is often generated by the colliery through; the dewatering of underground workings and the washing of coal. Surface runoff, stormwater and the discharge of effluent are from the colliery are usually of minor consequence when compared to the overall discharge. The pollutants that are present in the discharge reflect the local geology where the mining is occurring (Younger 1997). As surface water and groundwater infiltrate the underground mine workings it contacts exposed coal seams and other geological layers that which undergo dissolution, transferring minerals into the water. To avoid flooding, the water is then discharged from the mine (Younger 2004; Wright et al. 2018).

The discharge of wastewater from underground coal mining often results in the degradation of the receiving waterway. This degradation is often characterised by reduced water quality, altered sediment geochemistry and a loss of instream biodiversity. Changes in aquatic biodiversity are often measured through the assessment of the instream macroinvertebrate community (Wright et al. 1997). Some studies have also explored the uptake of metals by plants that inhabit the riparian zone downstream of coal mine wastewater discharge (Belmer et al. 2018). It is emerging that some of the pollutants present in the mine wastewater discharge are bioaccumulating and or biomagnifying in the plant tissue. This represents an avenue of ecosystem pollution of which is less frequently studied and represents a significant knowledge gap. It is important to understand how pollutants present in coal mine wastewater discharge persist and impact the environment; in the water column, in sediment and in riparian vegetation. Increased understanding will enable scientists, regulatory authorities and the miner to better protect waterways now and into the future. This research will address the following questions to determine how pollutants present in coal mine wastewater persist and impact the environment:

1. What are the key water quality changes (particularly changes to ionic and metal load) to the waterway downstream of the mine discharge?
2. What metals accumulate in the benthic sediment downstream of coal mine wastewater discharge?
3. How do stream macroinvertebrates respond to coal mine wastewater discharge?

4. Do plants exposed to coal mine wastewater accumulate metals in their biological tissue?

To answer these questions, the Georges River was examined. The Georges River flows from its head waters near the NSW township of Appin before discharging into Botany Bay. The catchment covers an area of approximately 96000 hectares. Approximately 45% of this area is natural bushland with urban, agriculture and industrial use accounting for the majority of remaining catchment area (Tippler et al. 2012). The Georges River is of ecological significance as it serves as a natural refuge, housing numerous endangered species of flora and fauna (Wales & Cavanagh 2018). The river also holds great cultural significance for the Indigenous Australians both past and present and to other inhabitants of the region for which the Georges River is regarded as iconic. The Georges River is exposed to wastewater pollution from an underground coal mine (Green et al. 2018). The Georges River is significant as it serves as a natural refuge, housing numerous endangered species of flora and fauna including the critically endangered Shale Sandstone Transition Forest ecological community (Wales & Cavanagh 2018).

Water quality

A waterway that is exposed to coal mine wastewater discharge commonly experiences a variety of issues that stem from; the influx of polluted water, an increase in flow volume and geological subsidence. These changes to water quality and hydrology often lead to a loss in ecosystem function and a corresponding reduction in ecosystem services. The discharge of wastewater from underground coal mining operations has long been associated with the pollution and hydrological alterations of aquatic ecosystems (see Banks et al. 1997; Brake et al. 2001; Younger 2004). The wastewater from coal mining operations is often characterised by an increase in electrical conductivity, modified stream ionic composition, an alteration of pH and increased concentrations of metals and metalloids (see Younger 1997; Johnson 2003; Capcott et al. 2015; Green et al. 2018). Metals are transported into the mine wastewater through a process known as 'acid mine drainage' (AMD). AMD occurs when pyrite (FeS_2) is exposed to water and oxygen which then dissolves, forming sulphuric acid (Grey 1996). The formation of sulphuric acid results in a reduction in pH of the water within the mine. This acidic environment causes metals to change from solid state to a soluble state, a mobilisation process that moves metals from the geology into the water column. Heavy metals such as: iron, cobalt, nickel, copper, zinc, arsenic, selenium, tin are often associated these various forms of pollution from coal mine wastewater discharge. The discharge of polluted water from a coal mine commonly results in a corresponding degradation of the sediment (see Cohen 2002). When acid mine wastewater is discharged, many of the metals are still in an ionic, dissolved state. Downstream, as the waterway gradually returns to a more neutral pH, metal ions that were in solution begin to precipitate out. Over time, metals accumulate in the sediment often at greater concentrations than are present in the water column. This amplification of pollutant concentrations within the sediment can result in significant 'legacy' pollution that can impact aquatic systems for decades (see Ali et al. 2018; Belmer and Wright 2019b.

In NSW, the NSW Environmental Protection Agency (NSW EPA) is the primary body that regulates the discharge of wastewater from underground coal mines, legislated in the *Protection of the*

Environment Operations Act 1997 (POEO Act). The POEO Act requires West Cliff Colliery to obtain an Environmental Protection Licence (EPL) from the NSW EPA for the scheduled activities; Coal Works, Mining for Coal and Waste Disposal that it conducts. In relation to the discharge of wastewater, the EPL governs the pollutants and the concentration of the pollutants that are permitted to be discharged. If a pollutant is not listed in the EPL it is not permitted to be discharged. Extract from EPL 2504, paragraph L2.3:

"To avoid any doubt, this condition does not authorise the pollution of waters by any pollutant other than those specified"

The West Cliff colliery discharges wastewater to the environment under specified conditions contained in the EPL issued to Endeavour Coal (EPL 2504; Table 1). Of particular concern is the wastewater discharge from the coal mine (of about two to three ML/day) to Brennans Creek, a small tributary of the Georges River (South32, 2018; Figure 1). The flow in dry weather in Brennans Creek is attributed to the colliery waste discharge. It has been estimated that the wastewater flows from the mine generally contribute about 90% of the flow of the upper Georges River (Price & Wright 2016).

The wastewater discharged by West Cliff Colliery has previously been shown to impact the Georges River in the following three ways:

1. Reduced water quality (Wright 2012; Price and Wright 2016; Green et al. 2018; Belmer and Wright 2019a).
2. Increased metal load in the sediment (Ali et al. 2018; Belmer and Wright 2019b).
3. Reduced aquatic biodiversity within the macroinvertebrate community (Belmer and Wright 2019c).

Previous studies (Wright 2012; Price and Wright 2016; Green et al. 2018; Belmer and Wright 2019a) that have examined the impact of the West Cliff Colliery's wastewater discharge on the Georges River have found that the parameters listed on the EPL are generally below the concentration limits imposed. However, the concentration limits dictated in the EPL have been shown to be at such elevated levels that ecological damage is occurring (Belmer and Wright 2019c). The studies recommended that the EPL conditions of pollutants be revised to reflect background conditions and the ANZECC 2000 water quality guidelines. These studies have also shown that the West Cliff Colliery is discharging other pollutants that are not listed in the EPL. The extent of this pollution as found by both the author and others is discussed below.

The journal article published in *Water, Air and Soil Pollution* titled; *Water Quality Impact from the Discharge of Coal Mine Wastes to Receiving Streams: Comparison of Impacts from an Active Mine with a Closed Mine* by Price and Wright (2016) found that the discharge of wastewater had modified the water chemistry of the Georges River. The study monitored three sites on three occasions in 2014. These sites were; the discharge from West Cliff Colliery, upstream of the discharge and downstream of the discharge. These sites correspond to, and are hereafter referred to as BC, GR1 and GR2. The study found that the following physio-chemical parameters; pH, electrical conductivity and temperature were elevated and statically dissimilar downstream at GR2 compared to GR1. The study found that the following anions and cations; carbonate, bicarbonate, chloride, sulphate, calcium, magnesium and sodium were all elevated and statically dissimilar at

GR2 compared to GR1. The study also identified the following metals; aluminium, arsenic, barium, cadmium, copper, lead, nickel, strontium and zinc were all elevated and statically dissimilar at GR2 compared to GR1.

The paper by the author (Green et al. 2018) and others; presented and published as part of the proceedings of the 9th Australian Stream Management Conference acted as a 'pilot study' for this thesis. The paper by Green et al. (2016) compared the water quality of the upper Georges River and of the Brennans Creek discharge with data collected in 2012 with data collected in 2018. The Green et al. (2018) paper had similar findings to Price and Wright (2016). The paper by Green et al. (2018) found that the magnitude of the pollution had changed from 2012 to 2018. The introduction of a revised EPL in 2013 did slightly reduce the concentration of some pollutants present within the discharge. However, the drought conditions experienced in 2018 resulted in diminished flow volumes from the Georges River above the West Cliff wastewater discharge. As the volume of the discharge from Brennans Creek remained similar in 2018 compared to 2012 the upstream dilution factor was greatly reduced. The lack of dilution caused an increase in the downstream plume of the pollution with pollutants remaining elevated at GR3, approximately 22km downstream of the discharge. Green et al. (2018) concluded that the Georges River was still degraded in. To address this degradation the authors recommended that the EPL should reflect the local reference conditions and the ANZECC 2000 water quality guidelines. The authors also recommended that the EPL needs to account for low flow conditions where the upper Georges River is not able to sufficiently dilute the wastewater discharged from West Cliff Colliery via Brennans Creek.

The article published in the *Water and Environment Journal* titled; *The regulation and impact of eight Australian coal mine waste water discharges on downstream river water quality: a regional comparison of active versus closed mines* by Belmer and Wright (2019a) found that the discharge of wastewater continued to modify the water chemistry of the Georges River. The study monitored three sites on four occasions between 2015 and 2017. These sites were the same as the Price and Wright (2016) study which sampled; the discharge from West Cliff Colliery, upstream of the discharge and downstream of the discharge. The study found that the following physio-chemical parameters; pH and electrical conductivity were elevated downstream at GR2 compared to GR1. Comparing the results at GR2 from Belmer and Wright (2019a) to the results from Price and Wright (2016) pH was elevated in the 2017 period while electrical conductivity was reduced. The study found that the following anions and cations; carbonate, bicarbonate, chloride and sulphate were all elevated and statically dissimilar at GR2 compared to GR1. Comparing the results at GR2 from Belmer and Wright (2019a) to the results from Price and Wright (2016) carbonate was elevated in the 2017 period while chloride, sulphate and bicarbonate were reduced. The study also identified the following metals; aluminium, copper, lead, nickel, uranium and zinc were all elevated at GR2 compared to GR1. Comparing the results at GR2 from Belmer and Wright (2019a) to the results from Price and Wright (2016) aluminium was elevated in the 2017 period while copper, lead, nickel and zinc were reduced.

Table 1: South 32 EPL 2504 issued by the NSW EPA. Water quality parameters are presented for the Brennans Creek wastewater discharge from West Cliff Colliery along with the ANZECC 2000 water quality guidelines.

Pollutant	Current discharge limits (EPL 2504)	ANZECC (2000) water quality guidelines (95% species protection)
Chemical oxygen demand (mg/L)	50	-
Electrical conductivity (µS/cm)	3570 (Low water) 2500 (High water)	350
Oil & Grease (mg/L)	10	-
pH (pH units)	6.5 - 9.3	6.5 - 7.5
Total dissolved solids (mg/L)	2500	-
Total suspended solids (mg/L)	50	-
Turbidity (NTU)	-	2 – 25
Aluminium (µg/L)	1000	55
Arsenic (µg/L)	19	Arsenic (As III): 24 Arsenic (AsV):13
Barium (µg/L)	-	-
Cadmium (µg/L)	-	0.2
Chromium (µg/L)	-	Chromium (CrVI): 1.0
Cobalt (µg/L)	-	-
Copper (µg/L)	8	1.4
Iron (µg/L)	-	-
Lead (µg/L)	6	3.4
Lithium (µg/L)	-	-
Manganese (µg/L)	102	1900
Molybdenum (µg/L)	-	-
Nickel (µg/L)	200	11
Strontium (µg/L)	-	-
Titanium (µg/L)	-	-
Uranium (µg/L)	-	-
Zinc (µg/L)	84	8

Sediment metal composition

Benthic sediment downstream of coal mine wastewater discharge is often enriched with similar pollutants present in the wastewater (Mishra et al. 2008). Metals present in the wastewater accumulate in the sediment primarily through the process of precipitation then sedimentation (Sheoran and Sheoran 2006). Due to the constant discharge of wastewater and corresponding precipitation, metal concentrations in the sediment can persist at elevated concentrations that those present in the water column. This accumulation of metals in the sediment can pose a significant threat to biodiversity as the concentration of contaminants can build up to ecologically hazardous levels. Sediment contamination also presents a long-term threat to the ecosystem where the metals can leach out into the water column, acting as source of pollution that may persist for decades (Neff 1984; Wang and Rainbow 2008). Evidence of this legacy pollution can be found all around the globe (Mishra et al. 2008, Bazrafshan et al 2016, Ali et al. 2017).

The article published in the journal *American Journal of Science of the Total Environment* titled; *River sediment quality assessment using sediment quality indices for the Sydney basin, Australia affected by coal and coal seam gas mining* by Ali et al. (2017) found that elevated concentrations of metals are present within the Georges River. Sediment samples were collected upstream of the Brennans Creek discharge point on the Georges River and downstream of the discharge point and at the discharge point. Three replicate samples were collected on a single occasion during in the 2014 – 2015 period. The study found elevated levels of the metals; aluminium, barium, chromium, cobalt, lead, manganese, nickel, titanium, uranium and zinc at the Brennans Creek discharge point. Below the discharge point on the Georges River the following metals remained elevated compared to upstream; aluminium, iron, lead, manganese, titanium and zinc.

The article published in the journal *American Journal of Water Science and Engineering* titled; *Regional Comparison of Impacts from Seven Australian Coal Mine Wastewater Discharges on Downstream River Sediment Chemistry, Sydney Basin, New South Wales Australia* by Belmer and Wright (2019b) also found that elevated concentrations of metals are present within the Georges River sediment. Sediment samples were collected upstream of the Brennans Creek discharge point on the Georges River and downstream of the discharge point. Three replicate samples were collected on a single occasion during the same time period as the related study; Belmer and Wright (2019a). The study found that elevated concentrations of the metals; barium, copper, lithium, manganese, strontium and zinc were elevated both upstream (GR1) and downstream (GR2) of the discharge. The elevated metal concentrations present in the sediment at GR1 as indicate substantial legacy pollution within the sediment. With this result and the knowledge that Appin East Colliery (South 32 EPL monitoring data) has previously discharged wastewater and continues to discharge small volumes above GR1 a more suitable reference site was needed. To remedy this, another site (GRU), above Appin East Colliery on the Georges River was identified a more appropriate site that would determine the natural background.

Macroinvertebrate community

The presence of pollutants within the water column and the sediment typically result in a degradation of river health. River health is commonly assessed by examining the macroinvertebrate community. Waterways that experience coal mine wastewater discharge often show a reduction in pollution sensitive taxa both in total abundance and taxonomic richness. This loss of pollution sensitive macroinvertebrates is contrasted to increases in pollution tolerant animals in both total abundance and taxonomic richness. The most common measures used to compare the macroinvertebrate community between waterways that experience coal mine wastewater discharge and the reference site are; community richness and abundance and the Ephemeroptera, Plecoptera and Trichoptera (EPT) community structure.

The article published in the journal *American Journal of Water Science and Engineering* titled; *Regional Comparison of Impacts to Stream Macroinvertebrates from Active and Inactive Coal Mine Wastewater Discharges* by Belmer and Wright (2019c) found that the macroinvertebrate community structure differed downstream of the discharge compared to upstream. The study sampled the macroinvertebrate community at sites equivalent to GR1 and GR2. The macroinvertebrate community was sampled on one occasion during the same time period as the related studies; Belmer and Wright (2019a) and Belmer and Wright (2019b) with five replicate samples collected at each site. The traditional 'Kick sampling technique' where a 30cm by 30cm quadrat is disturbed immediately upstream of a macroinvertebrate net was used by Belmer and Wright (2019c) to collect samples. The study found that abundance was elevated at statically significant levels below the mine wastewater discharge at GR2. The study also found that while family richness was greater below the mine wastewater discharge, the percentage of pollution sensitive species (EPT%) was reduced. With the knowledge that water quality and sediment quality is impaired above the mine wastewater discharge at GR1 (Price and Wright 2016; Belmer and Wright 2019a; Belmer and Wright 2019b). This impairment is likely a result of previous wastewater discharge from Appin East Colliery. To remedy this, another site (GRU), above Appin East Colliery on the Georges River was identified a more appropriate site that would determine the natural macroinvertebrate community structure.

Bioaccumulation of metals in plant tissue

The study of bioaccumulation of metals in riparian plants exposed to coal mine wastewater is an emerging area of study, both internationally and in Australia. Numerous observational studies conducted in the field have explored this issue (see Karathanasis and Johnson 2003, Sheoran and Sheoran 2006, Belmer and Wright 2018). Fewer laboratory studies have been conducted with the aim of measuring heavy metal uptake by plants grown in coal mine wastewater. One such study has identified the uptake of heavy metals from coal mine wastewater discharge by macrophytes (see Mishra et al. 2008). The study by Karathanasis and Johnson 2003 examined the uptake of metals; aluminium, iron and manganese in wetland plant tissue exposed to coal mine wastewater. The study found that all three metals accumulated in the plant tissue. The greatest uptake of metals aluminium and iron was found in the root tissue, while manganese concentration was similar in root and leaf tissue.

An Australian study by Belmer and Wright (2018) examined the uptake of metals by the riparian plant *Acacia rubida* growing in coal mine generated wastewater. Ten metals; Aluminium, Arsenic, Beryllium, Cadmium, Cobalt, Molybdenum, Nickel, Strontium, Thallium and Zinc were identified as being above background concentrations downstream of the wastewater discharge. All metals with the exception of arsenic were found to be elevated in *Acacia rubida* growing downstream of the wastewater discharge point compared to the same species growing upstream of the wastewater discharge.

Figure 1: Photo of O'Hares Creek, one of two reference sites used in the study. (Benjamin Green 2019).

2. Research aims, objectives, questions and hypothesis

Aims

This research is aimed to determine the downstream implications of coal mine wastewater discharge on the receiving waterways ecosystem. To assess change, the water and sediment quality are monitored along with the aquatic macroinvertebrate community. This research also aims to determine if metals which are traditionally associated with coal mine wastewater discharge have the potential to accumulate in plant tissue.

Key objectives

1. To investigate the impact of the discharge of coal mine wastewater on the receiving waterway in terms of; water quality, sediment composition and the aquatic macroinvertebrate community.
2. To investigate the potential for metal accumulation within plants exposed to coal mine wastewater.

Research question

1. What are the key water quality changes (particularly changes to ionic and metal load) to the waterway downstream of the mine discharge?
2. What metals accumulate in the benthic sediment downstream of coal mine wastewater discharge?
3. How do stream macroinvertebrates respond to coal mine wastewater discharge?
4. Do plants exposed to coal mine wastewater accumulate metals in their biological tissue?

Hypotheses

1. The inflow of coal mine wastewater will modify the ionic and metal composition of the waterway.
2. The benthic sediment downstream of the coal mine wastewater discharge will have a different metal composition to sediment collected at reference sites.
3. The aquatic macroinvertebrate community structure will be different downstream of the inflow of coal mine wastewater.
4. Metal composition in plant tissue will differ between plants exposed to mine wastewater compared to plants exposed to water collected from reference sites.

3. Methods

Coal mine wastewater discharge location

The West Cliff Colliery is an underground coal mine that has operated since 1976. It is located south-west of Sydney in south-eastern Australia on the outskirts of the small township of Appin, about 15 km inland from the coast (Price and Wright, 2016). The mine operation has surface workings that include buildings and a coal washery and it generates wastewater (comprising both mine drainage and washery wastes) that is treated and then disposed into the upper reaches of the Georges River via a small tributary (Brennans Creek) at an elevation of 240 m above sea level. It has been estimated that the wastewater flows from the mine generally contribute about 90% of the flow of the upper Georges River (Price & Wright, 2016). There is a second, smaller wastewater discharge point on the Georges River above the Brennans Creek discharge point (Figure 3). This discharge infrequently discharges wastewater from Appin East Colliery into the Georges River at low volumes. This discharge from this point did occur on multiple occasions during the study period (South 32 EPL monitoring report, personal observation and personal communication with South 32). The NSW Environment Protection Authority (NSW EPA) regulates the colliery wastewater discharge using an *environmental protection licence* (EPL 2504; Table 1) that enforces discharge limits for pollutants in the wastewater.

Study sites

The study area includes the Georges River and two tributaries (Brennans Creek and O’Hares Creek) in the upper Georges River sub-catchment (Figure 4). Brennans Creek is a very small tributary that contains the West Cliff colliery wastewater. In normal weather, the flow in Brennans Creek is attributed to the colliery waste discharge. Two reference sites were used in the study. One was placed on the upper Georges River (GRU) about 1.3km upstream of Brennans Creek. The second reference site was O’Hares Creek (Figure 1). O’Hares Creek flows into the Georges River about 22 km downstream (Figure 2) from the Brennans Creek inflow and its catchment is mostly undisturbed and naturally vegetated. Both reference sites represent background water quality in the most undisturbed waterway reaches available in the area. The next site below GRU; GR1 is not considered as pristine as it has previously received small volumes of wastewater discharge from a second colliery, Appin East Colliery, and urban runoff from the Appin township. However, it acts as an upstream comparison to determine the impact that the Brennans creek discharge has on the Georges River (Figure 4).

Figure 2: Photo of the confluence of O'Hares Creek and the Georges River (Benjamin Green 2018).

Uses of the upper Georges River waterways include heritage protection, conservation and public recreation. A large percentage of the upper Georges River catchment is protected and valued for conservation purposes (flora and fauna, endangered species and cultural heritage) as National Park reserves (Tippler et al. 2012) and Commonwealth Defence land. The Georges River is also a known habitat for an isolated population of Platypus (Patricia Durman 2017). The lower reaches of the Georges River are part of south western Sydney and it is one of the most urbanised catchments in Australia and it is reported to house approximately 1.2 million people (Tippler et al. 2012). Water quality of the urbanised sections of the Georges River catchment is degraded in proportion with the intensity of urban development (Tippler et al. 2014). This makes the relatively undisturbed upper Georges catchment, in the environs of the study area, of additional value.

Table 2: Site details; code, type, distance from wastewater discharges and location.

Site Name	Waterway name	Site Type	Distance (Km) downstream from mine wastewater discharges (West Cliff), Appin East}	Latitude and longitude (degrees)	Elevation above sea level (m)
GRU	Georges River	Reference; natural background	(NA), {NA}	-34.215837, 150.79784	240
GR1	Georges River	Reference upstream of West Cliff Colliery discharge at Brennans Creek	(NA), {0.75}	-34.205514, 150.799177	230
GR2	Georges River	0.3km Below wastewater discharge	(0.3), {1.0}	-34.204259, 150.798474	220
GR3	Georges River	22km Below wastewater discharge	(23), {24}	-34.094426, 150.833785	60
BC	Brennans Creek	West Cliff Colliery wastewater discharge	(0.2), {NA}	-34.204305, 150.799845	225
OH	O'Hares Creek	Reference; natural background	(NA), {NA}	-34.095581, 150.836778	65

Figure 3: Photos from all six sample sites. From left to right; GRU, GR1, BC, GR2, GR3, OH (Benjamin Green 2018)

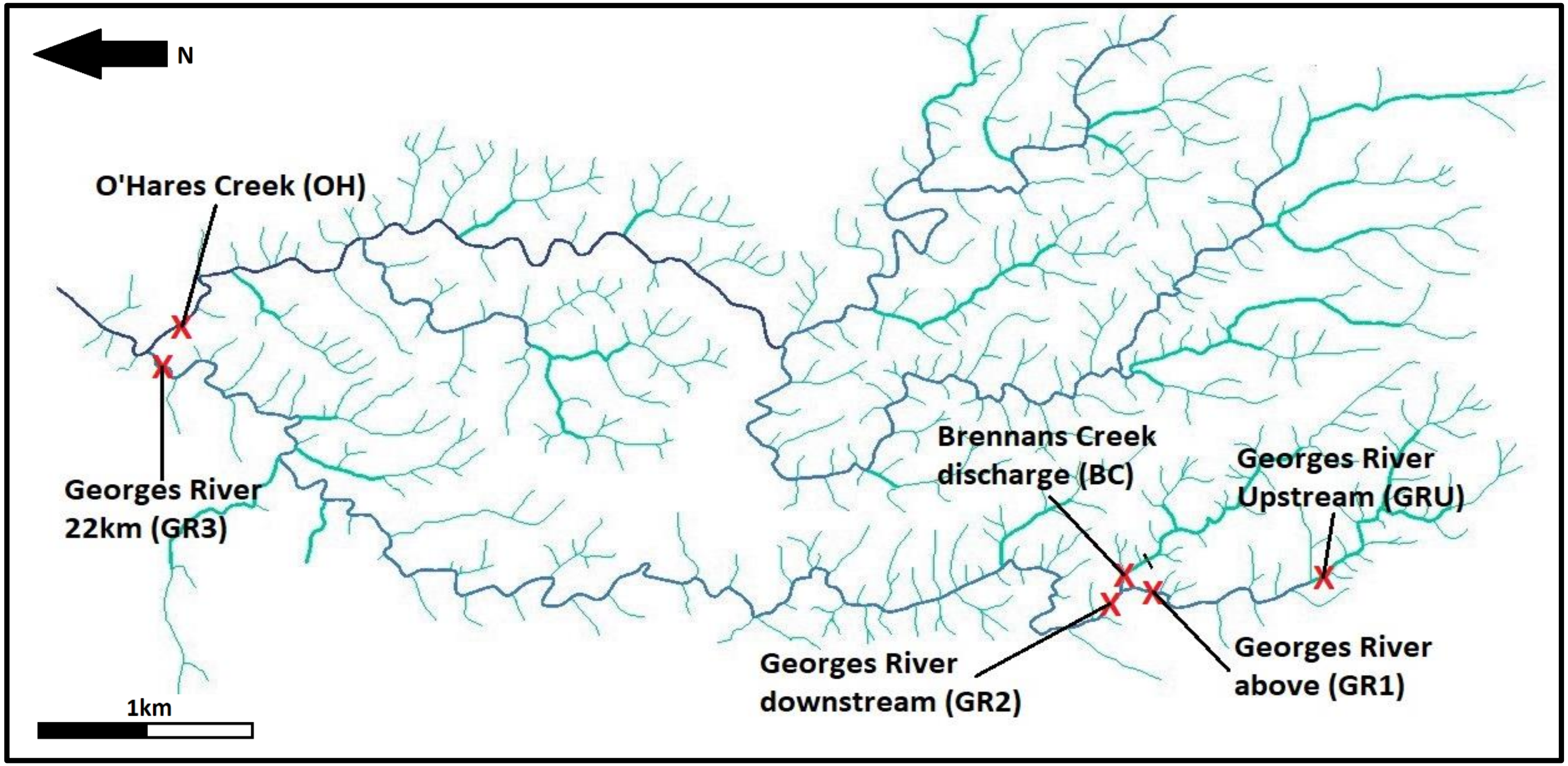

Figure 4: Map of the upper Georges River Catchment showing all waterways and sample sites.

Water quality sampling

Water was collected from five sites GR1, BC, GR2, GR3 and OH (Figure 3 and Figure 4) over a seventeen-month period from April 2018 to September 2019. During this period there were six sampling events with two sample containers collected at each site. An additional site; GRU was sampled twice July 2019 and September 2019 to provide a greater understanding of the reference conditions experienced above the impact zone of the mine (Figure 3 and Figure 4). The water samples were collected from a flowing section of the waterway at a depth of approximately 0.1m where the water was deemed to be thoroughly mixed. The sample containers that were tested for total metals were clean, sterile and contained a small amount of nitric acid to act as a preservative. The sample containers that were tested for major anions and cations were clean, sterile and did not contain any preservatives. After collection, samples were appropriately labelled and placed in a cooled container. The samples were then refrigerated and transferred to the laboratory within the required holding time.

Figure 5: Benjamin Green recording water physio-chemical data at sites GR2 (left) and OH (right) in 2019 (Ian Wright 2019).

Water samples were later tested by the Envirolab laboratory and total metals were determined using ICP-MS. The laboratory is a National Association of Testing Authorities (NATA) endorsed commercial laboratory for analysis of water chemistry samples. The laboratory has NATA accreditation based on using appropriate sample analysis methods within a quality assured analytical chemistry environment. The samples were tested for a suite of 23 metals/metalloids (Aluminium, antimony, arsenic, boron, barium, beryllium, cadmium, chromium, cobalt, copper, iron, mercury, lithium, manganese, molybdenum, nickel, selenium, tin, strontium, titanium, uranium and zinc). Major anion and cation testing was also conducted by the two laboratories using methods approved by NATA.

At each sampling site, field meter results were obtained for stream pH, electrical conductivity, dissolved oxygen and turbidity. pH and EC were measured using a TPS Aqua-CP/A meter and turbidity was measured using a HACH 2100P portable turbidity meter. At each site the meter was allowed to equilibrate before recording five replicate measurements. Weather conditions were assessed prior to collecting water samples to avoid periods of heavy rain, which could have caused confounded results.

At each site, on each sampling occasion a field meter was used to assess physiochemical water quality attributes. These attributes were; electrical conductivity, dissolved oxygen, pH, turbidity and water temperature. Once the reading on the field meter had stabilised, five replicate readings were taken (Figure 5). The field meter used was the TPS WP-82Y data logger with the appropriate sensor (Figure 6). On the day of sampling, the field meters were calibrated following standard methods. Electrical conductivity was calibrated in the air when dry to obtain a zero reading then in standards of 150 µS/cm,1413 µS/cm and 2760 µS/cm. pH was calibrated in standards of 4.01 pH units and 7.00 pH units and turbidity was calibrated using standards of 0.20 NTU and 20.00 NTU. Calibration for dissolved oxygen was performed in air to determine maximum saturation. At each site on each occasion, the field meters were checked against the standard solutions to confirm calibration.

Figure 6: Photos showing part of the calibration process for field meters (Benjamin Green 2019).

Sediment sampling

Sediment samples were collected on two occasions (Table 2 and Table 3) at all six sampling sites (Figure 4). Two replicate samples per site were collected in June 2019 and four replicates per site were collected in September 2019. Samples were collected by extracting sediment in-stream from the benthic layer. Samples were then placed in clean sterile glass jars and stored in the same manner as the water quality samples. The laboratory; Envirolab is a National Association of Testing Authorities (NATA) endorsed commercial laboratory for analysis of sediment samples. It has an NATA accreditation based on using appropriate sample analysis methods within a quality assured analytical chemistry environment. The samples were tested for a suite of 23 metals/metalloids (Aluminium, antimony, arsenic, boron, barium, beryllium, cadmium, chromium, cobalt, copper, iron, mercury, lithium, manganese, molybdenum, nickel, selenium, tin, strontium, titanium, uranium and zinc).

Figure 7: Typical photo of sediment collected (left) with water and sediment containers pictured (right) (Benjamin Green 2018).

Macroinvertebrate Sampling

Macroinvertebrate sampling was conducted on two occasions, once in September 2018 and again in September 2019 (Figure 3 and Figure 4). Macroinvertebrate sampling was conducted at the reference site GRU and below the mine wastewater discharge point at GR2 (Figure 3 and Figure 4). These two sites were selected to determine the impact of coalmine wastewater discharge on the macroinvertebrate community by comparing the community upstream of the mine discharge with the community below the discharge. Sample conditions were similar as both sampling events; however there did appear to be slightly elevated flow at GRU in 2019 compared with 2018. While the two sites are in close geographically proximity and share many common physical features the flow volume and velocity are greater in GR2 than in GRU (Personal observation; Figure 3). This increase in flow is attributed to the mine wastewater discharge. As different macroinvertebrate taxa have different flow velocity tolerances it is expected that some of the differences in community structure may be due to the altered flow regime at GR2.

Figure 8: Sorting and macroinvertebrate identification using microscopes. Photos of macroinvertebrates were taken using the righthand microscope (Benjamin Green 2019).

The samples were collected from similar habitats which featured; riffle zones, bedrock and aquatic vegetation. The type of habitat sampled was located in flowing sections of the streams where depth was no greater than 0.2m. At both sites, the benthos was comprised of small to medium sandstone rocks (cobbles), sand and small amounts of detritus located on sandstone bedrock. The samples were obtained by disturbing a 900cm^2 area of the stream immediately upstream of a 250μm mesh net using the 'kick sampling' technique (Rosenberg & Resh 1993). The disturbance of the substrate lasted for a period of 30 seconds with the same effort applied at both sites. The disruption of the benthic layer caused debris and any macroinvertebrates to become dislodged and washed into the net. The contents of the net, including sediment and detritus were emptied into labelled sample containers and preserved in methylated spirits. Five replicate samples were taken at both sites in 2018 while three replicates were collected at each site in 2019. The identification and count of the macroinvertebrates was undertaken at the Western Sydney University laboratory (Figure 8 and Figure 9). The content of each sample was sorted under a dissecting microscope (X10 to X60) where the macroinvertebrates were separated from the mix of sediment, rocks and detritus (Figure 8). The macroinvertebrates were identified to the family taxonomic level primarily using the guide by Hawking (2000). In the event where identification was uncertain, the macroinvertebrates were subject to confirmation identification by expert freshwater ecologists, namely; Dr Ian Wright and Nakia Belmer.

Figure 9: Photo of selected macroinvertebrates taken with the microscope (Benjamin Green 2018).

Willow laboratory experiment

A laboratory experiment was designed to determine the bioavailability of metals present in the mine wastewater. The experiment would determine if metals present in the mine wastewater would accumulate in the tissue of willow (*Salix nigra*) leaves and roots. Groups of willow cuttings were exposed to six treatments in two categories; reference water and mine wastewater.

Willow cutting collection

Willow cuttings were collected on the 12th of June 2019 from a mature *Salix nigra* specimen. Approximately 140 cuttings were made in live wood. The cuttings were approximately 0.2m long and between 5mm and 10mm in diameter. At the laboratory at Western Sydney University, the cuttings were trimmed to approximately equal lengths of 0.2m with any cuttings that were deformed or deemed unsuitable were discarded. The base of the willow stalk was then cut in a diagonal direction to enhance root development. The cuttings were then washed using distilled water to remove any foreign material.

Water collection

Water for the experiment was collected one day before the experiment commenced on the 11/06/2019. Water was collected at five sites to act as five treatments; OH, GRU, GR1, BC and GR2 immediately after water quality and in-situ sampling was conducted. The water was collected in clean 10L drums which were rinsed with water from the site prior to collection (Figure 10).

Experiment configuration

At the Western Sydney University laboratory 125, 500mL beakers were washed and cleaned prior to the experiment. 300mL of water from the respective treatment (location) was poured into each of twenty-five, 500mL beakers. Plastic wrap was then used to cover the mouth of the beaker to lessen the evaporation rate. Twenty-five willow cuttings were then randomly selected and placed into the beakers. This procedure was carried out for the four other treatments. The laboratory was kept at consistent conditions through-out the experiment. Light was maintained by four fluorescent lights and a stable temperature range (21°C to 23°C) and relative humidity (23% to 39%) was achieved by an automated air conditioning system (Figure 10).

Figure 10: Collection of water (left) and 1st day of the willow experiment (right) (Benjamin Green 2019)

Experiment maintenance and monitoring

The experiment was conducted over a period of 61 days from the 12th of June 2019 to the 12th of August 2019. Every three days the experiment was inspected. At these times, de-ionised water was added to the beakers to compensate for transpiration and evaporation loss. Every three weeks electrical conductivity and pH was measured to ensure that the treatments remained within 10% of the recorded value when the samples were collected (Figure 11 and Figure 12).

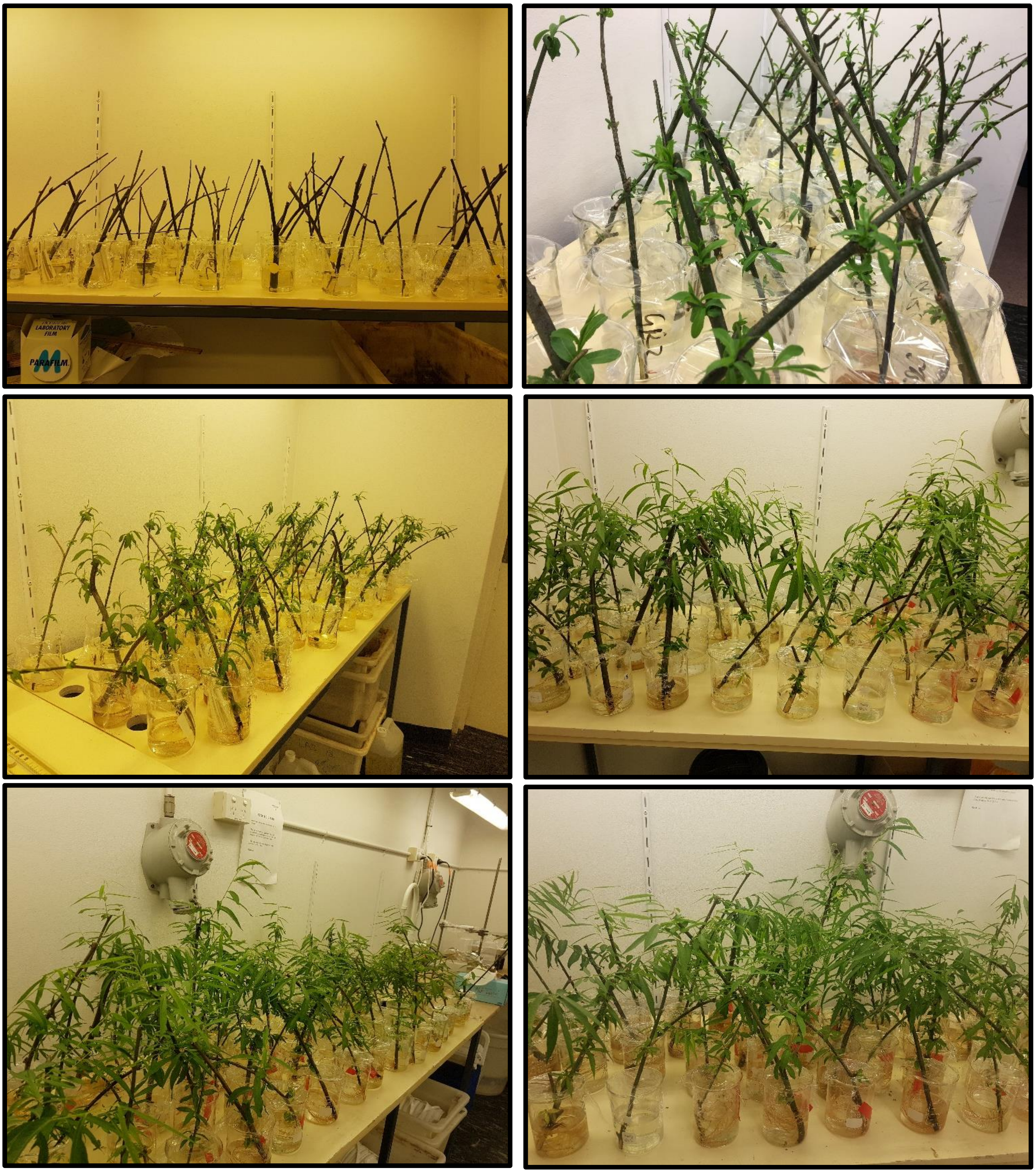

Figure 11: Willow growth throughout the experiment. Photos placed in chronological order from the start of the experiment to the end of the experiment (Benjamin Green 2019)

Harvesting

The willow cuttings were harvested after 61 days of growth (Figure 11 and Figure 12) on the 12th of August 2019. The willow cuttings were removed from the beakers and thoroughly washed using distilled water to remove any residue or sedimentation. Each individual willow cutting had its leaves and roots separated and placed into an individually labelled paper envelope that was then sealed. The envelopes and willow tissue were then placed in a fan forced drying oven that maintained a temperature between 59°C and 62°C for a seven-day period to extract moisture from the tissue samples. The samples were then weighed and ground into a fine powder using a mortar and pestle that was cleaned after the grinding of each sample. To further eliminate the chance of sample cross-contamination, the plant tissue from the reference treatments (GRU, GR1 and OH) was ground before the mine wastewater treatments (GR2 and BC). For analysis the laboratory needed a minimum sample mass of 0.2g and a recommended mass of <0.5g. To achieve this requirement composite samples were created. To form the composite samples, each individual willow foliage sample was randomly selected. A total of 82 willow leaf samples were collected from the five treatments; 15 from GRU, 18 from GR1, 20 from OH, 15 from GR2 and 14 from BC. A total of 21 willow root samples were collected from the five treatments; four from GRU, five from GR1, five from OH, four from GR2 and three from BC.

Figure 12: Photo of willow root growth during the experiment (Benjamin Green 2019).

Willow sample analysis

The laboratory; Envirolab is a National Association of Testing Authorities (NATA) endorsed commercial laboratory for analysis of vegetation tissue samples. It has a NATA accreditation based on using appropriate sample analysis methods within a quality assured analytical chemistry environment. The samples were tested for a suite of 23 metals/metalloids (Aluminium, antimony, arsenic, boron, barium, beryllium, cadmium, chromium, cobalt, copper, iron, mercury, lithium, manganese, molybdenum, nickel, selenium, tin, strontium, titanium, uranium and zinc).

Data analysis

To determine whether a mine impacted site or treatment exhibited a different result to the reference conditions a statistical test was performed. The statistical test used was a one-way analysis of variance (one-way ANOVA). To perform the test, data from the two reference sites was grouped to better approximate the true background conditions. This block of reference data was compared using the one-way ANOVA to each individual site or treatment for each parameter. The limit of statistical significance was where the *p*-value was greater than 0.05. *p*-values were reported as; <0.05, <0.01 and <0.001. This method was used to analyse water quality data, sediment data, willow tissue data and the macroinvertebrate community structure. The macroinvertebrate community structure was also examined by grouping sensitive macroinvertebrate taxa belonging to the families Ephemeroptera, Plecoptera and Trichoptera (EPT). The Shannon-Wiener biodiversity index was also used to examine differences in macroinvertebrate community structure. The Shannon-Wiener biodiversity index is a common biodiversity measure that reflects taxon richness and evenness of the abundances of taxa (Krebs 1289).

Table 3: Dates of sampling events and willow experiment. NT indicates not tested.

	Reference	**Upstream**		**Discharge**	**Downstream**	
Site Name	**O'Hares**	**GRU**	**GR1**	**BC**	**GR2**	**GR3**
Water Quality (in-situ)	06/04/2018 20/04/2018 11/05/2018 19/09/2018 11/06/2019 06/09/2019	NT NT NT 19/09/2018 11/06/2019 06/09/2019	06/04/2018 20/04/2018 11/05/2018 19/09/2018 11/06/2019 06/09/2019	06/04/2018 20/04/2018 11/05/2018 19/09/2018 11/06/2019 06/09/2019	06/04/2018 20/04/2018 11/05/2018 19/09/2018 11/06/2019 06/09/2019	06/04/2018 20/04/2018 11/05/2018 19/09/2018 11/06/2019 06/09/2019
Water Quality (samples)	06/04/2018 20/04/2018 11/05/2018 11/06/2019 06/09/2019	NT NT NT 11/06/2019 06/09/2019	06/04/2018 20/04/2018 11/05/2018 11/06/2019 06/09/2019	06/04/2018 20/04/2018 11/05/2018 11/06/2019 06/09/2019	06/04/2018 20/04/2018 11/05/2018 11/06/2019 06/09/2019	06/04/2018 20/04/2018 11/05/2018 11/06/2019 06/09/2019
Sediment	11/06/2019 06/09/2019	11/06/2019 06/09/2019	11/06/2019 06/09/2019	11/06/2019 06/09/2019	11/06/2019 06/09/2019	11/06/2019 06/09/2019
Macroinvertebrate	NT	19/09/2018 06/09/2019	NT	NT	19/09/2018 06/09/2019	NT
Willow Experiment (start date – end date)	12/06/2019 - 12/08/2019	12/06/2019 - 12/08/2019	12/06/2019 - 12/08/2019	12/06/2019 - 12/08/2019	12/06/2019 - 12/08/2019	NT

4. Results

Water physiochemical properties

The wastewater discharge from the West Cliff Colliery into Brennans Creek (BC) has caused extensive changes to the chemistry of the Georges River. This alteration of water chemistry in the Georges River was still evident at the furthest downstream sample site (GR3), 22km below the inflow of Brennans Creek.

The physiochemical properties of water differed significantly between sites that were located downstream of mine wastewater discharge and the reference sites. Mean Electrical conductivity, pH and turbidity was elevated at the sites downstream of mine wastewater discharges compared to the dilute conditions present at the reference sites (Table 4).

Electrical conductivity at the two 'pristine' reference sites was dilute (Table 4, Figure 14). Mean electrical conductivity at OH was 181 µS/cm, with a median value of 178 µS/cm and a range of 11.4 µS/cm from a minimum value of 176.7 µS/cm to a maximum value of 188.1 µS/cm. Mean electrical conductivity at the most upstream site on the Georges River (GRU) was 186 µS/cm, with a median value of 186 µS/cm and a range of 30.2 µS/cm from a minimum value of 170.6 µS/cm to a maximum value of 200.8 µS/cm.

Above West cliff Colliery's discharge point but below the decommissioned discharge point at Appin East Colliery the Georges River at GR1 experienced elevated electrical conductivity compared to the two reference sites (Table 4, Figure 14). Mean elevated electrical conductivity at GR1 was 456 µS/cm a statistically significant difference compared to the reference sites (*p*<0.001). Median electrical conductivity was 426 µS/cm and a range of 503 µS/cm from a minimum value of 245 µS/cm to a maximum value of 748 µS/cm.

The discharge of wastewater from Brennans Creek was very saline (Table 4, Figure 14). Mean electrical conductivity at BC was 1787 µS/cm, a statistically significant difference compared to the reference sites (*p*<0.001). The median value of 1784 µS/cm and a range of 855 µS/cm from a minimum

Figure 13: In-stream rocks with suspected salt deposits at GR2 (Benjamin Green 2018).

value of 1366 µS/cm to a maximum value of 2221 µS/cm showed elevated electrical conductivity levels over the entire sampling period.

Brennans Creek is the main contributor to flow in the upper Georges River (personal observation) and the electrical conductivity concentration at GR2 supports this observation (Table 4, Figure 14). Mean electrical conductivity at GR2 was 1677µS/cm, a statistically significant difference compared to the reference sites (*p*<0.001). The median value of 1728 µS/cm and a range of 869 µS/cm from a minimum value of 1173 µS/cm to a maximum value of 2042 µS/cm showed elevated electrical conductivity levels over the entire sampling period. At both BC and GR2 there was suspected salt deposits on in-stream rocks (Figure 13).

The Georges River remained saline at the furthest downstream site at GR3; a highly statistically significant difference (*p*<0.001) compared to the two reference sites. Mean electrical conductivity at GR3 was 1102 µS/cm, with a median value of 1314 µS/cm and a range of 1233 µS/cm from a minimum value of 394 µS/cm to a maximum value of 1627 µS/cm (Table 4, Figure 14).

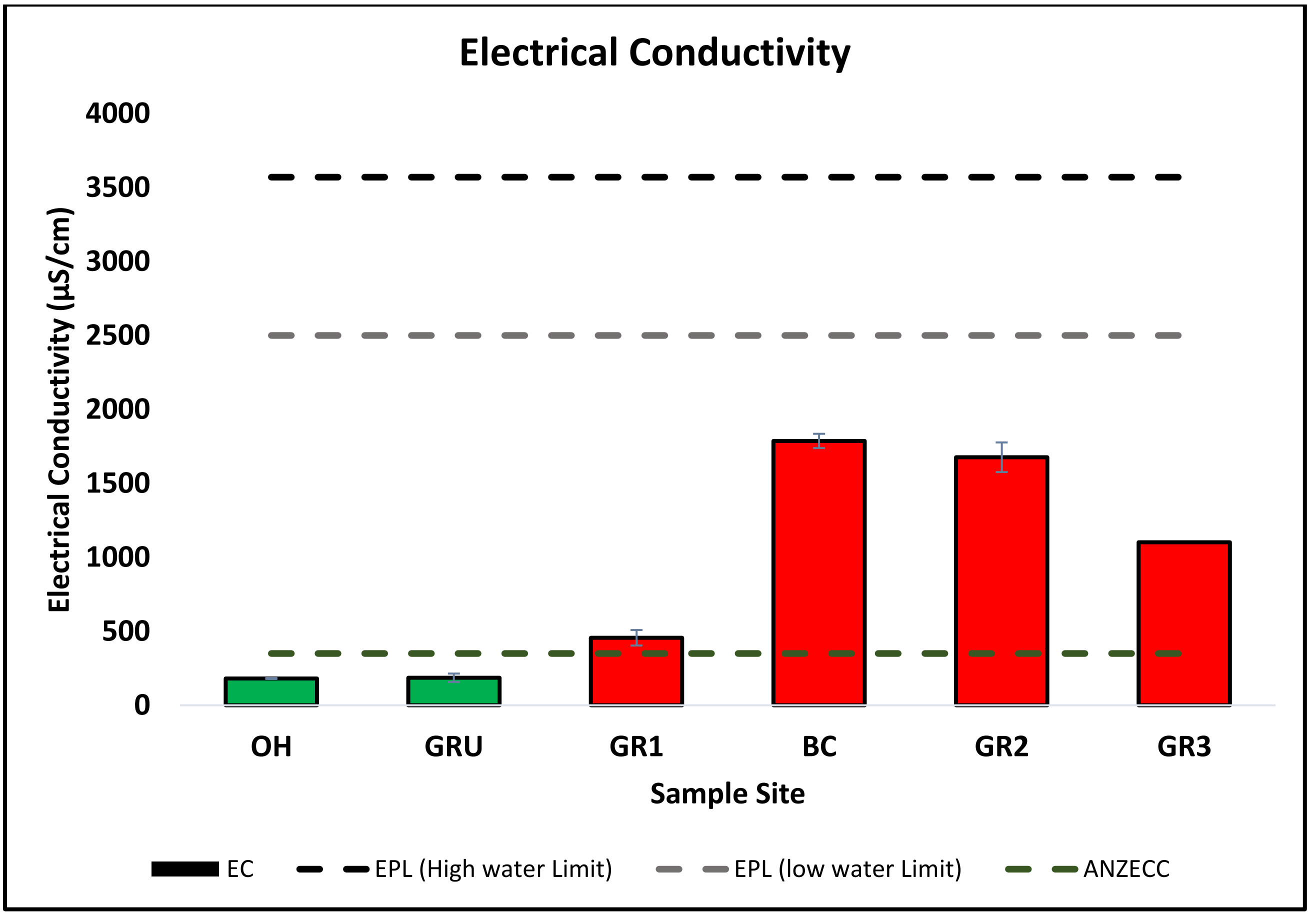

Figure 14: Graph of mean electrical conductivity with standard error bars at the six sample sites. Green fill indicates mean concentration below the ANZECC 2000 water quality guideline limit indicated by the green dashed line. The EPL limit is indicated by black and grey dashed lines.

pH levels followed a similar trend to electrical conductivity. At the two reference sites, pH was in the acidic to neutral range (Table 4, Figure 15). Mean pH at OH was 6.78 pH units, with a median value of 6.97 pH units and a range of 1.12 pH units from a minimum value of 6.23 pH units to a maximum value of 7.35 pH units. Mean pH at the most upstream site on the Georges River (GRU) was 5.83 pH units, with a median value of 5.78 pH units and a range of 0.38 pH units from a minimum value of 5.64 pH units to a maximum value of 6.02 pH units. Downstream from GRU at GR1, pH was elevated and statistically dissimilar to the reference sites (p<0.001). Mean pH at GR1 was 6.94 pH units, with a median value of 6.92 pH units and a range of 0.72 pH units from a minimum value of 6.57 pH units to a maximum value of 7.29 pH units (Table 4, Figure 15).

The Westcliff Colliery discharge at Brennans Creek was highly alkaline and statistically dissimilar to the reference sites (p<0.001). Mean pH at BC was 8.82 pH units, with a median value of 8.82 pH units and a range of 1.01 pH units from a minimum value of 8.27 pH units to a maximum value of 9.28 pH units (Table 4, Figure 15).

Downstream of the Brennans Creek discharge point at GR2, pH was highly alkaline and statistically dissimilar to the reference sites (p<0.001). Mean pH was also 2.06 pH units higher than immediately upstream of the discharge at GR1. Mean pH at GR2 was 9.00 pH units, with a median value of 8.91 pH units and a range of 0.75 pH units from a minimum value of 8.75 pH units to a maximum value of 9.5 pH units (Table 4, Figure 15).

22km downstream at GR3, pH was alkaline and statistically dissimilar to the reference sites (p<0.001). Mean pH at GR3 was 8.50 pH units, with a median value of 8.48 pH units and a range of 0.71 pH units from a minimum value of 8.12 pH units to a maximum value of 8.83 pH units (Table 4, Figure 15).

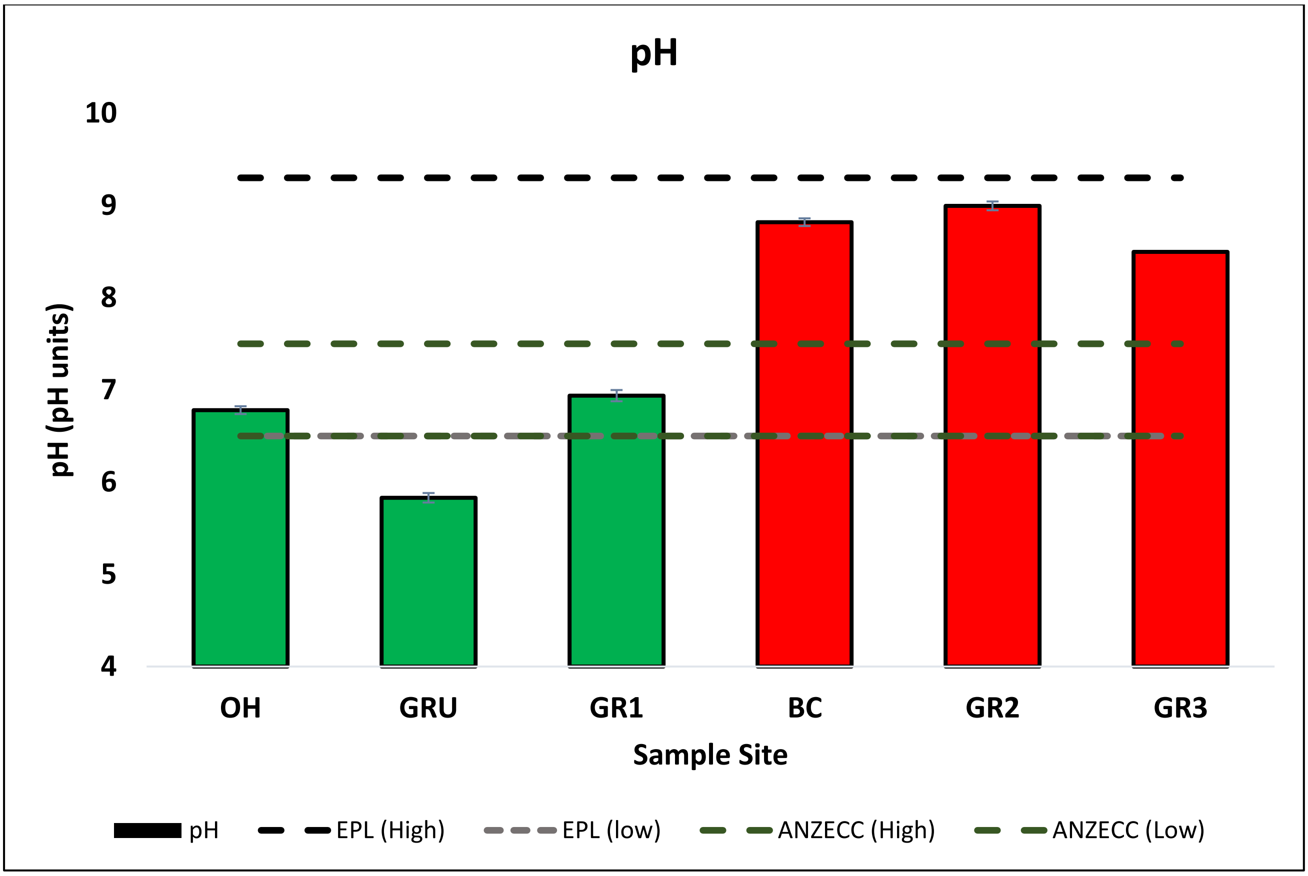

Figure 15: Graph of mean pH with standard error bars at the six sample sites. Green fill indicates mean concentration below the ANZECC 2000 water quality guideline limit indicated by the green dashed line. The EPL limits are indicated by black and grey dashed lines.

Turbidity at the reference sites and at GR1 was low compared to the wastewater discharge at Brennans Creek and downstream of the discharge at GR2 and GR3 (Table 4, Figure 16). At OH, mean turbidity was

1.4 NTU, with a median of 1.1 NTU and a range of 1.2 NTU from a minimum of 0.7 NTU to a maximum of 2.4 NTU. At GRU, mean turbidity was 3.2 NTU, with a median of 2.4 NTU and a range of 3.4 NTU from a minimum of 2.3 NTU to a maximum of 5.7 NTU (Table 4, Figure 16).

Turbidity at GR1 was statistically similar to the reference sites. Mean turbidity was 2.3 NTU, with a median of 1.8 NTU and a range of 2.4 from a minimum value of 1.5 to a maximum value of 4.0 (Table 4, Figure 16).

The West Cliff Colliery discharge had a ‘cloudy’ appearance (personal observation), which was reflected in elevated turbidity levels. Turbidity was elevated and statistically dissimilar (p<0.001) to the reference sites. Mean turbidity was 7.8 NTU, with a median of 7.3 NTU and a range of 7.4 from a minimum value of 4.4 to a maximum value of 11.8 (Table 4, Figure 16).

Downstream of the Brennans Creek discharge point at GR2 the water also appeared ‘cloudy’ (personal observation). Turbidity was elevated and statistically dissimilar (p<0.001) to the reference sites. Mean turbidity was 8.5 NTU, with a median of 8.0 NTU and a range of 12.1 from a minimum value of 3.84 to a maximum value of 15.9. 22km downstream at GR3, turbidity was statistically similar to the reference sites. Mean turbidity at GR3 was 4.8 NTU, with a median of 3.0 NTU and a range of 9.7 NTU from a minimum of 2.5 NTU to a maximum of 12.2 NTU (Table 4, Figure 16).

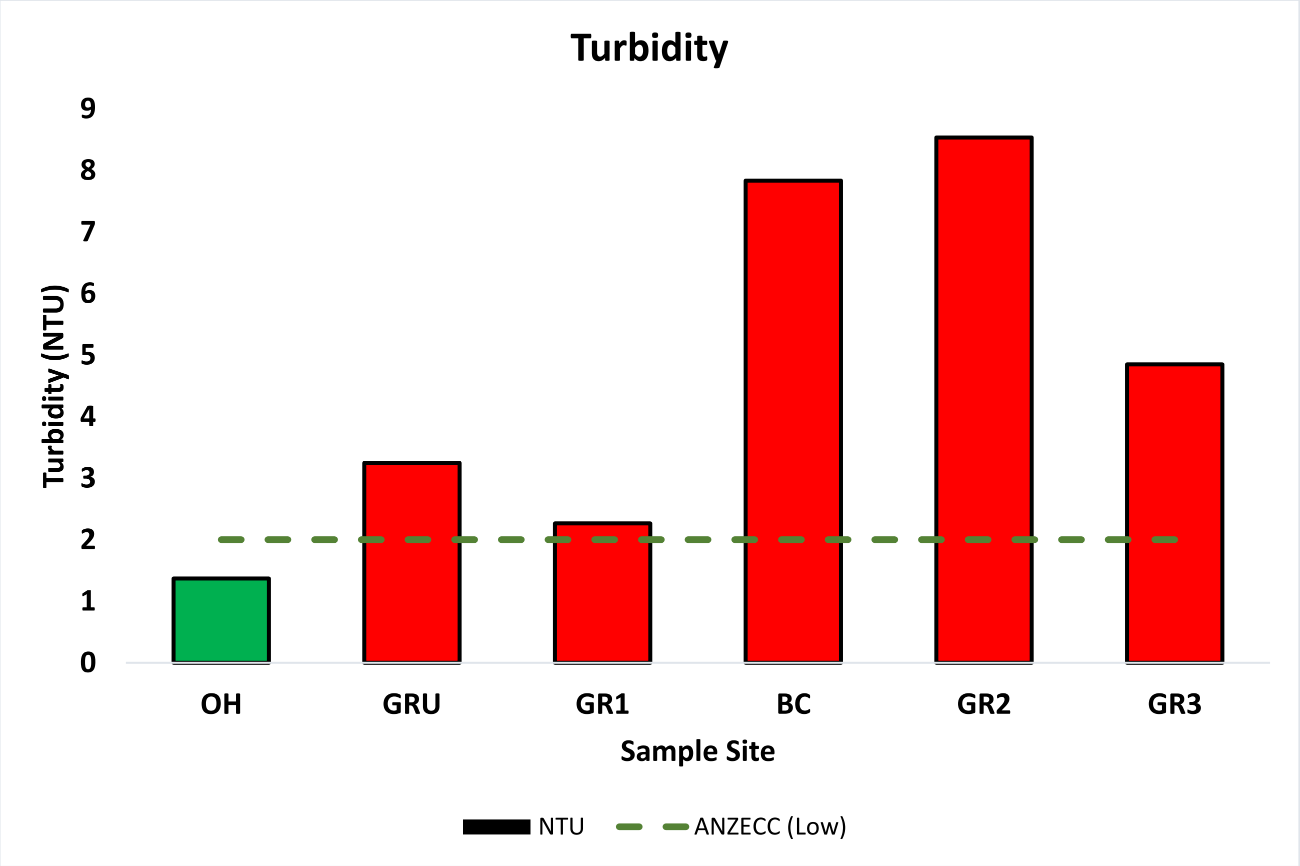

*Figure 16:*Graph of mean turbidity with standard error bars at the six sample sites. Green fill indicates mean concentration below the ANZECC 2000 water quality guideline limit indicated by the green dashed line.

Table 4: Summary table of in~situ water quality data at all sites. Mean, median and p-values are shown. NS indicates not statically significant.

	Reference				Upstream			Discharge			Downstream					
Site Name	**O'Hares**		**GRU**		**GR1**			**BC**			**GR2**			**GR3**		
Variables (units)	Mean	Med.	Mean	Med.	Mean	Med.	p -value	Mean	Med.	p -value	Mean	Med.	p -value	Mean	Med.	p -value
Electrical conductivity (µS/cm)	181	178	186	186	456	426	<0.001	1787	1784	<0.001	1677	1728	<0.001	1102	1314	<0.001
pH (pH units)	6.78	6.97	5.83	5.78	6.94	6.92	<0.001	8.82	8.82	<0.001	9.00	8.91	<0.001	8.50	8.48	<0.001
Turbidity (NTU)	1.4	1.1	3.2	2.4	2.3	1.8	NS	7.8	7.3	<0.001	8.5	8.0	<0.001	4.8	3.0	NS
Dissolved Oxygen (%)	89.6	93.3	72.8	69.8	72.3	72.5	<0.001	72.5	74.1	<0.001	85.3	81.6	NS	91.8	92.9	<0.001

Water ionic composition

The ionic composition of the waterways downstream of mine wastewater discharge was altered compared to the reference sites (Table 5). The reference sites; OH and GRU recorded the lowest mean concentrations of dissolved anions and cations with the exception of hydroxide alkalinity which was below detection limits at all locations. While no mine wastewater was released at GR1 during the study period, it is expected that the altered ionic composition of the water at the site is due to a combination of mine legacy pollution and small amounts of urban run-off.

Dissolved calcium concentrations were lowest at the two reference sites, while the concentrations at the mine affected sites were all statistically significantly different ($p<0.001$). O'Hares Creek had a mean concentration of 2.8 mg/L and GRU had a mean concentration of 1.2 mg/L. GR1 had the highest mean dissolved calcium concentration of 21.4 mg/L. The West Cliff Colliery discharge at BC had an elevated mean dissolved calcium concentration of 8.3 mg/L while GR2 and GR3 recorded means of 8.8 mg/L and 7.5 mg/L respectively (Table 5).

Mean dissolved potassium was elevated and statistically significantly different ($p<0.001$) at sites GR1, BC, GR2 and GR3 to the two reference sites. O'Hares Creek had a mean concentration of 1.2 mg/L and GRU had a mean concentration of 1.0 mg/L. GR1 mean dissolved potassium concentration of 3.1 mg/L. The West Cliff Colliery discharge at BC had a mean dissolved potassium concentration of 3.8 mg/L while GR2 and GR3 recorded means of 3.6 mg/L and 3.0 mg/L respectively (Table 5).

Mean dissolved sodium was elevated and statistically significantly different ($p<0.001$) at sites GR1, BC, GR2 and GR3 to the two reference sites. O'Hares Creek had a mean concentration of 22.2 mg/L and GRU had a mean concentration of 23.3 mg/L. GR1 mean dissolved sodium concentration of 51.9 mg/L. The West Cliff Colliery discharge at BC had a mean dissolved sodium concentration of 491.0 mg/L which is more than twenty times greater than the mean reference conditions. GR2 and GR3 recorded means of 442.0 mg/L and 316.6 mg/L respectively (Table 5).

Mean dissolved magnesium concentration was highest at GR1 (8.1 mg/l), a statistically dissimilar result ($p<0.05$) to the reference sites. O'Hares Creek had a mean dissolved magnesium concentration of 4.0 mg/L. GR3 had the next highest mean concentration of 4.3 mg/L, a result statistically similar to the reference site. Both BC and GR2 had mean concentrations of 3.4 mg/L and 3.3 mg/L respectively which were statistically dissimilar ($p<0.05$) to the reference site. All results for hydroxide alkalinity were below detection limits (Table 5).

Dissolved bicarbonate alkalinity was statistically dissimilar ($p<0.001$) to the reference sites at all other sites. Mean dissolved bicarbonate alkalinity was lowest at the two reference sites O'Hares (10.2 mg/L) and GRU (5.3 mg/L). GR1 had an increased mean of 39.6 mg/L which was approximately fifteen times less than the mean at GR2; 607.0 mg/L. This increase is due to the inflow of wastewater from Brennans Creek which had a mean concentration of 686.0 mg/L. Dissolved bicarbonate alkalinity remained elevated at GR3 which had a mean of 449 mg/L (Table 5, Figure 17).

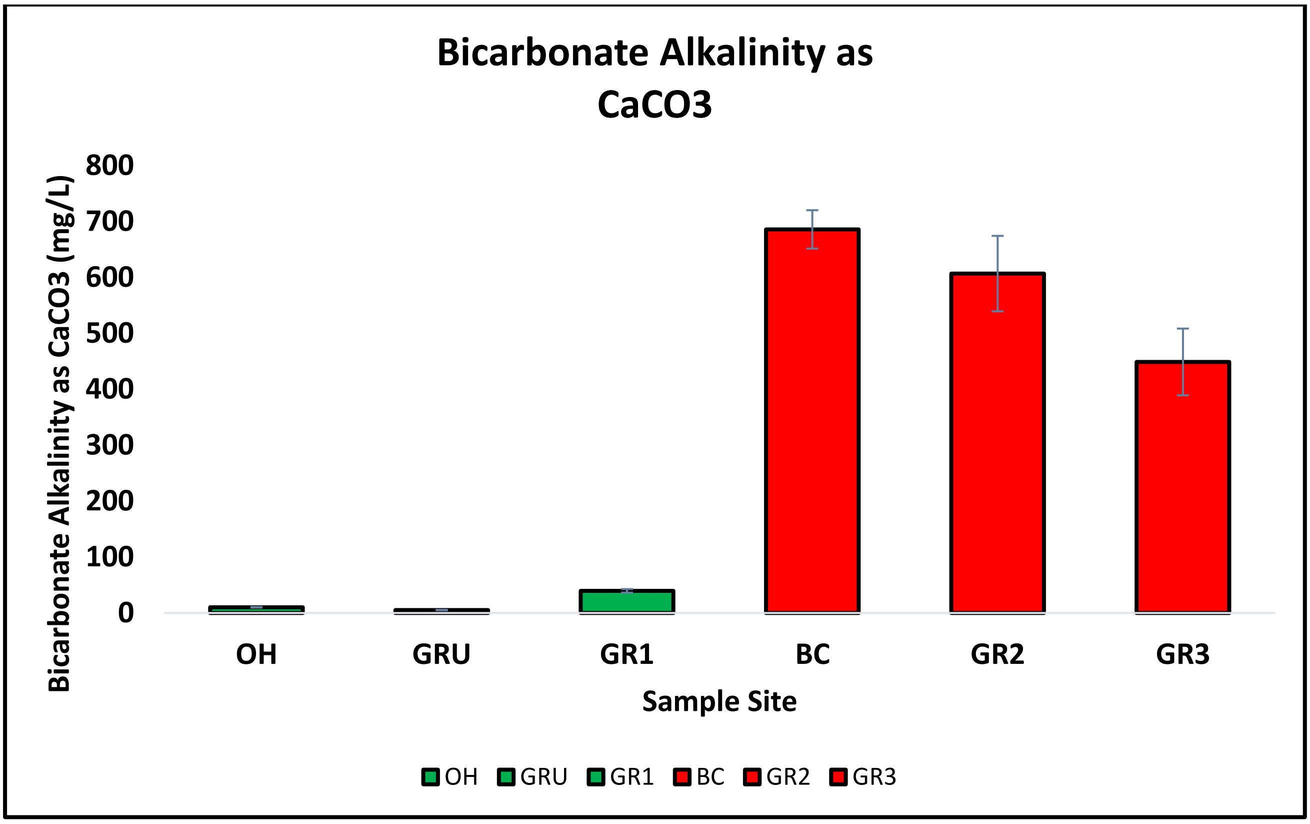

Figure 17: Graph showing mean bicarbonate alkalinity in mg/L with standard error bars at all six sample sites.

Dissolved carbonate alkalinity was statistically dissimilar (*p*<0.001) to the reference sites BC, GR2 and GR3. Mean dissolved carbonate alkalinity was below detection limits at the two reference sites O'Hares Creek, GRU and at GR1. GR2 has a mean concentration of 89.0 mg/L due to the influx of wastewater from Brennans Creek which had a mean concentration of 86.6 mg/L. Dissolved carbonate alkalinity remained elevated at GR3 which had a mean of 19.5 mg/L (Table 5, Figure 18).

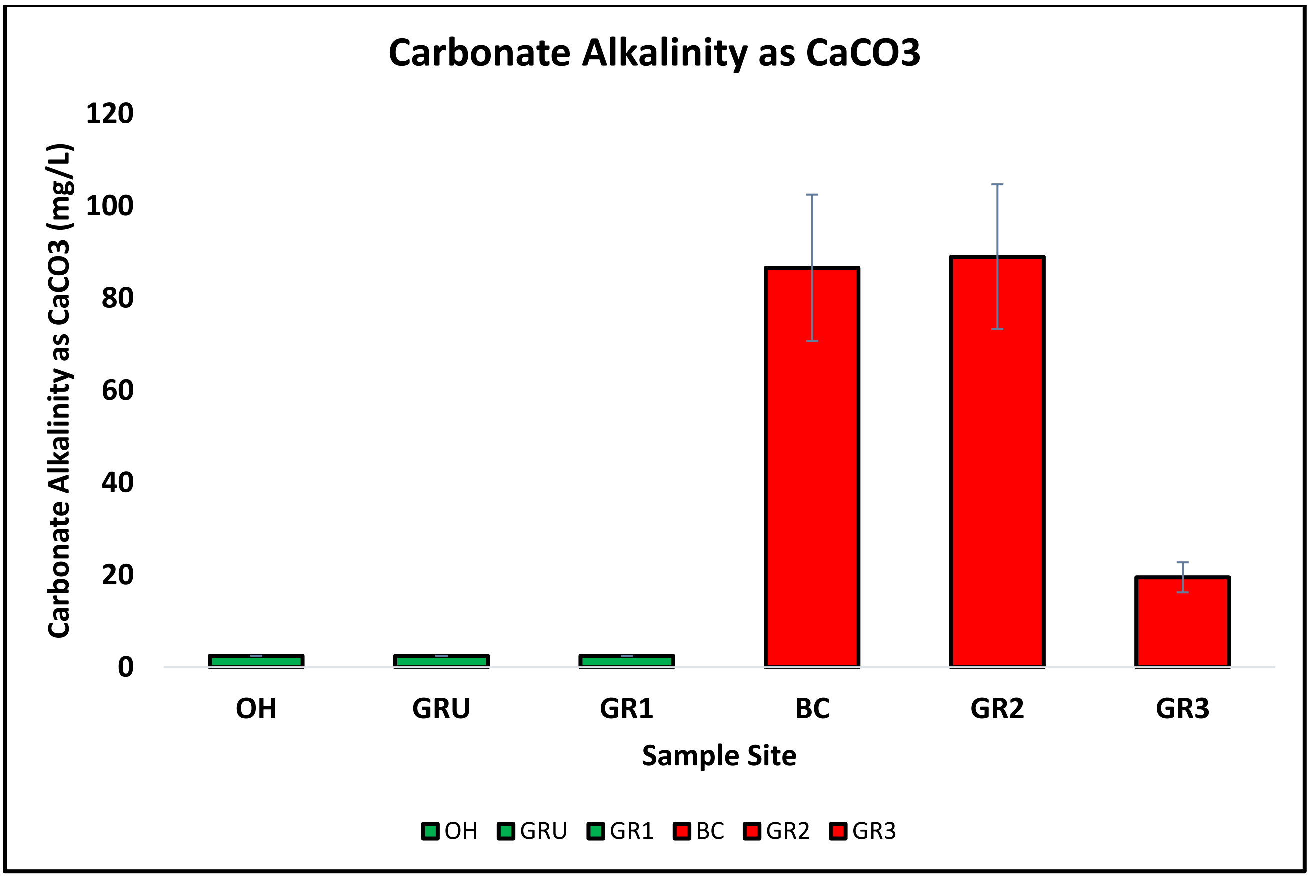

Figure 18: Graph showing mean carbonate alkalinity in mg/L with standard error bars at all six sample sites.

Total alkalinity was statistically dissimilar (*p*<0.001) to the reference sites at all other sites. Mean dissolved bicarbonate alkalinity was lowest at the two reference sites O'Hares (10.2 mg/L) and GRU (5.3 mg/L). GR1 had an increased mean of 39.6 mg/L which was approximately twenty times less than the mean at GR2; 772.0 mg/L. This increase is due to the inflow of wastewater from Brennans Creek which had a mean concentration of 697.0 mg/L. Dissolved bicarbonate alkalinity remained elevated at GR3 which had a mean of 467 mg/L (Table 5, Figure 19).

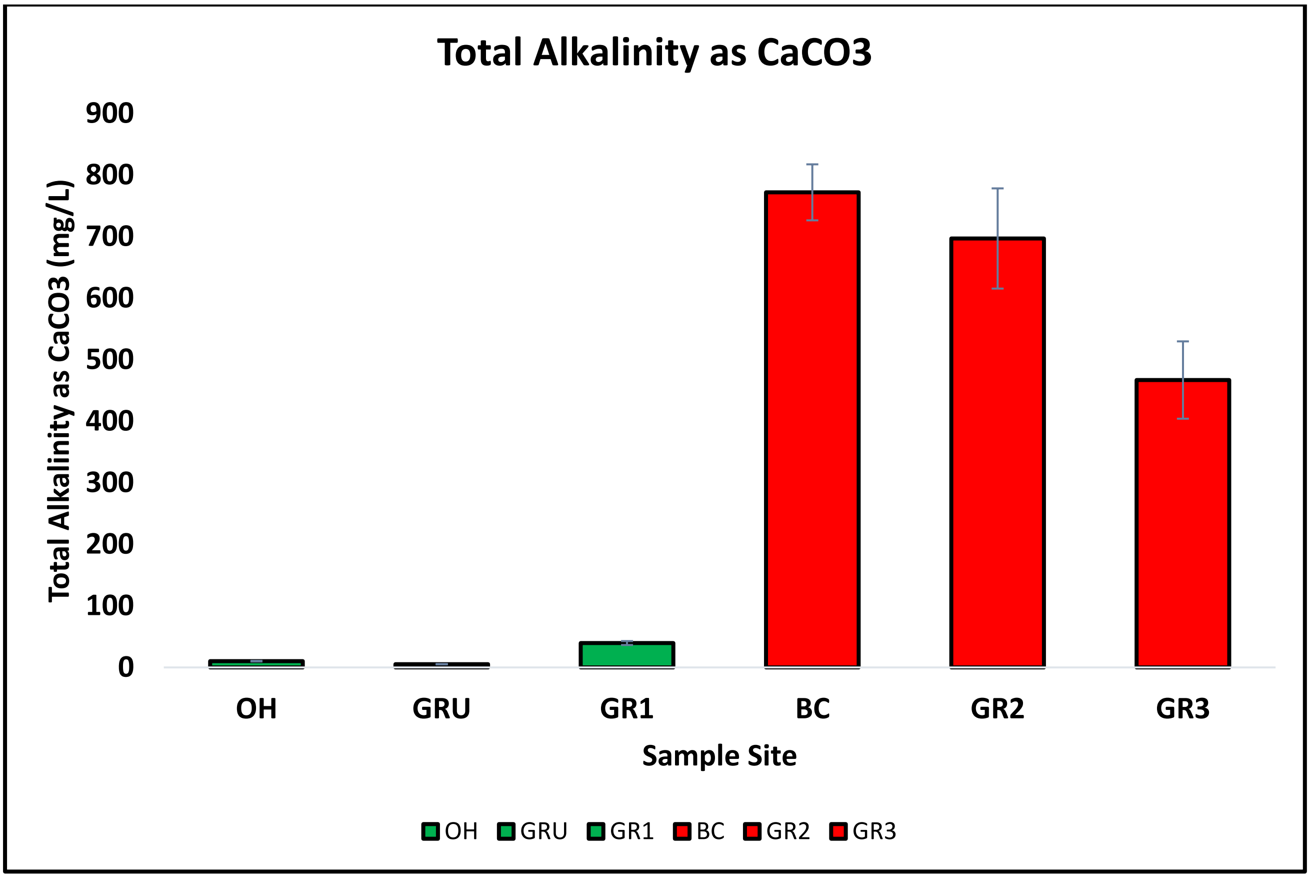

Figure 19:Graph showing mean total alkalinity in mg/L with standard error bars at all six sample sites.

Sulphate concentration was statistically dissimilar (*p*<0.001) to the reference sites at all other sites. Mean sulphate concentration was lowest at the two reference sites O'Hares (9.5 mg/L) and GRU (7.5 mg/L). GR1 had an increased mean of 13.6 mg/L which was less than the mean at GR2; 22.2 mg/L. This increase is due to the inflow of wastewater from Brennans Creek which had a mean concentration of 24.4 mg/L. Sulphate concentrations remained elevated at GR3 which had a mean of 14.9 mg/L (Table 5).

Chloride concentration was statistically dissimilar (*p*<0.001) to the reference sites at all other sites. Mean Chloride concentration was lowest at the two reference sites O'Hares (37.5 mg/L) and GRU (39.8 mg/L). GR1 had an increased mean of 91.9 mg/L which was less than the mean at GR2; 120.7 mg/L. This increase is due to the inflow of wastewater from Brennans Creek which had a mean concentration of 124.1 mg/L. Chloride concentrations remained elevated at GR3 which had a mean of 102.8 mg/L (Table 5).

Table 5: Summary table of quality data (anions and cations) at all sites. Mean, median and p-values are shown.

	Reference				**Upstream**			**Discharge**			**Downstream**					
Site Name	**O'Hares**		**GRU**		**GR1**			**BC**			**GR2**			**GR3**		
Variables mg/L	Mean	Med.	Mean	Med.	Mean	Med.	p - value	Mean	Med.	p - value	Mean	Med.	p - value	Mean	Med.	p - value
Calcium	2.8	2.8	1.2	1.2	21.4	22.0	<0.001	8.3	7.3	<0.001	8.8	7.0	<0.001	7.5	7.1	<0.001
Potassium	1.2	1.2	1.0	1.0	3.1	3.0	<0.001	3.8	3.7	<0.001	3.6	3.6	<0.001	3.0	3.3	<0.001
Sodium	22.2	23.5	23.3	23.5	51.9	49.5	<0.001	491.0	505.0	<0.001	442.0	495.0	<0.001	316.6	360.0	<0.001
Magnesium	4.0	4.0	3.9	4.0	8.1	8.6	<0.001	3.4	2.9	<0.05	3.3	2.8	<0.05	4.3	4.6	NS
Hydroxide Alkalinity	2.5	2.5	2.5	2.5	2.5	2.5	NS	2.5	2.5	NS	2.5	2.5	NS	2.5	2.5	NS
Bicarbonate Alkalinity	10.2	10.0	5.3	5.0	39.6	36.0	<0.001	686.0	620.0	<0.001	607.0	625.0	<0.001	449.0	480.0	<0.001
Carbonate Alkalinity	2.5	2.5	2.5	2.5	2.5	2.5	NS	86.6	99.5	<0.001	89.0	98.0	<0.001	19.5	21.5	<0.001
Total Alkalinity	10.2	10.0	5.3	5.0	39.6	36.0	<0.001	772.0	730.0	<0.001	697.0	740.0	<0.001	467.0	500.0	<0.001
Sulphate	9.5	10.0	7.5	7.5	13.6	12.0	<0.001	24.4	24.5	<0.001	22.2	25.0	<0.001	14.9	15.0	<0.01
Chloride	37.5	36.0	39.8	39.0	91.9	91.0	<0.001	124.1	120.0	<0.001	120.7	120.0	<0.001	102.8	110.0	<0.001
Calcium	2.8	2.8	1.2	1.2	21.4	22.0	<0.001	8.3	7.3	<0.001	8.8	7.0	<0.001	7.5	7.1	<0.001
Potassium	1.2	1.2	1.0	1.0	3.1	3.0	<0.001	3.8	3.7	<0.001	3.6	3.6	<0.001	3.0	3.3	<0.001

Metals concentration in water

The wastewater discharge into Brennans Creek had elevated concentrations of a suite of metals; antimony, aluminium, arsenic, barium, boron, cobalt, lithium, molybdenum, nickel, strontium and uranium, titanium and zinc that were statistically different compared to the reference sites (Table 6). The inflow of wastewater from Brennans Creek into the Georges River caused elevated concentrations of metals; antimony, aluminium, arsenic, barium, boron, cobalt, copper lithium, molybdenum, nickel, strontium, uranium, titanium and zinc that were statistically different at GR2 compared with to the reference sites. 22 km downstream from the West Cliff Colliery's discharge, concentrations of metals remained elevated at statistically significantly levels on the Georges River at GR3. The metals at elevated concentrations were; aluminium, arsenic, barium, boron, lithium, molybdenum, nickel, strontium, titanium and uranium. The reference sites; O'Hares and GRU metal load were dominated iron and characterised be low concentrations of other metals. GR1 had a suite of metals (aluminium, barium, boron, copper, lithium, nickel, strontium, titanium and zinc) that were present at elevated concentrations that were statistically dissimilar to the reference site. Metals beryllium, cadmium, chromium, mercury, selenium and tin were below laboratory detection limits at all sites on all occasions. The metals present in GR1 are likely due to legacy pollution and small volumes of discharge from the Appin East Colliery and potentially from the small amounts of urban runoff that GR1 receives.

Total antimony concentrations were below detection limits (<1 µg/L) at O'Hares Creek, GRU and GR1. While sites BC and GR2 had increased concentrations of antimony that were statistically dissimilar to the reference sites with *p*-values that were <0.001 and <0.05 respectively. 22 km downstream at GR3 antimony was below detection limits. Mean antimony concentration at the West Cliff Colliery discharge into Brennans Creek was 1.5 µg/L. The wastewater discharge caused an increase in mean antimony concentration in the Georges River at GR2 to 1.3 µg/L.

Total aluminium concentrations at the reference sites were 21 µg/L at O'Hares and 30 µg/L at GRU (Table 6, Figure 20). All other sites recorded aluminium concentrations that were elevated and statistically dissimilar (*p*<0.001) to the two reference sites. Mean aluminium concentration at GR1 was 76 µg/L. Mean aluminium concentration at the West Cliff Colliery discharge into Brennans Creek was 595 µg/L. The wastewater discharge caused an increase in mean aluminium concentration in the Georges River at GR2 to 609 µg/L. 22 km downstream at GR3 the mean aluminium concentration was 170 µg/L.

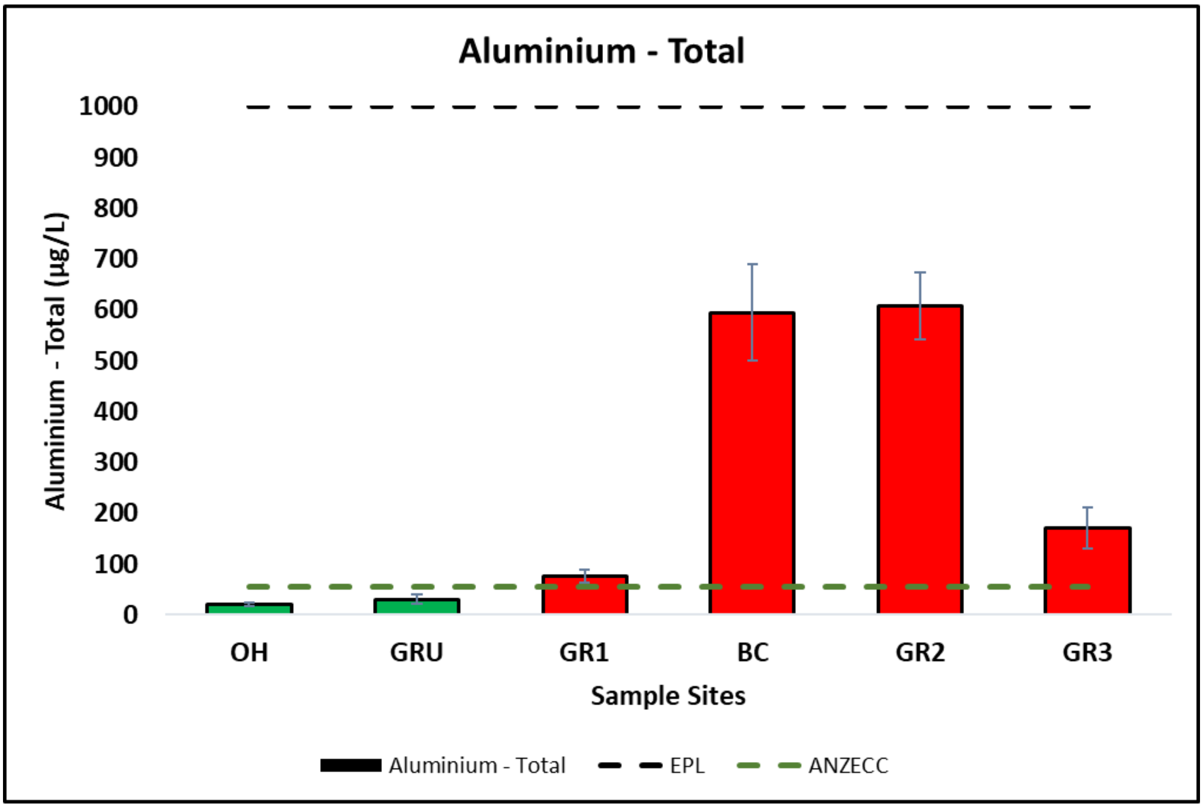

Figure 20: Graph of total aluminium *(*µg/L) with error bars at all six sample sites. EPL limit is shown by the grey dotted line and the ANZECC 2000 guideline is indicated by the green dashed line.

Total arsenic concentrations were below detection limits (<1 µg/L) at O'Hares Creek, GRU and GR1 (Table 6, Figure 21). While sites BC, GR2 and GR3 had increased concentrations of arsenic that were statistically dissimilar to the reference sites with *p*-values that were <0.001. Mean arsenic concentration at the West Cliff Colliery discharge into Brennans Creek was 8.2 µg/L. The wastewater discharge caused an increase in mean arsenic concentration in the Georges River at GR2 to 7.9 µg/L. 22 km downstream at GR3 the mean arsenic concentration was 1.3 µg/L.

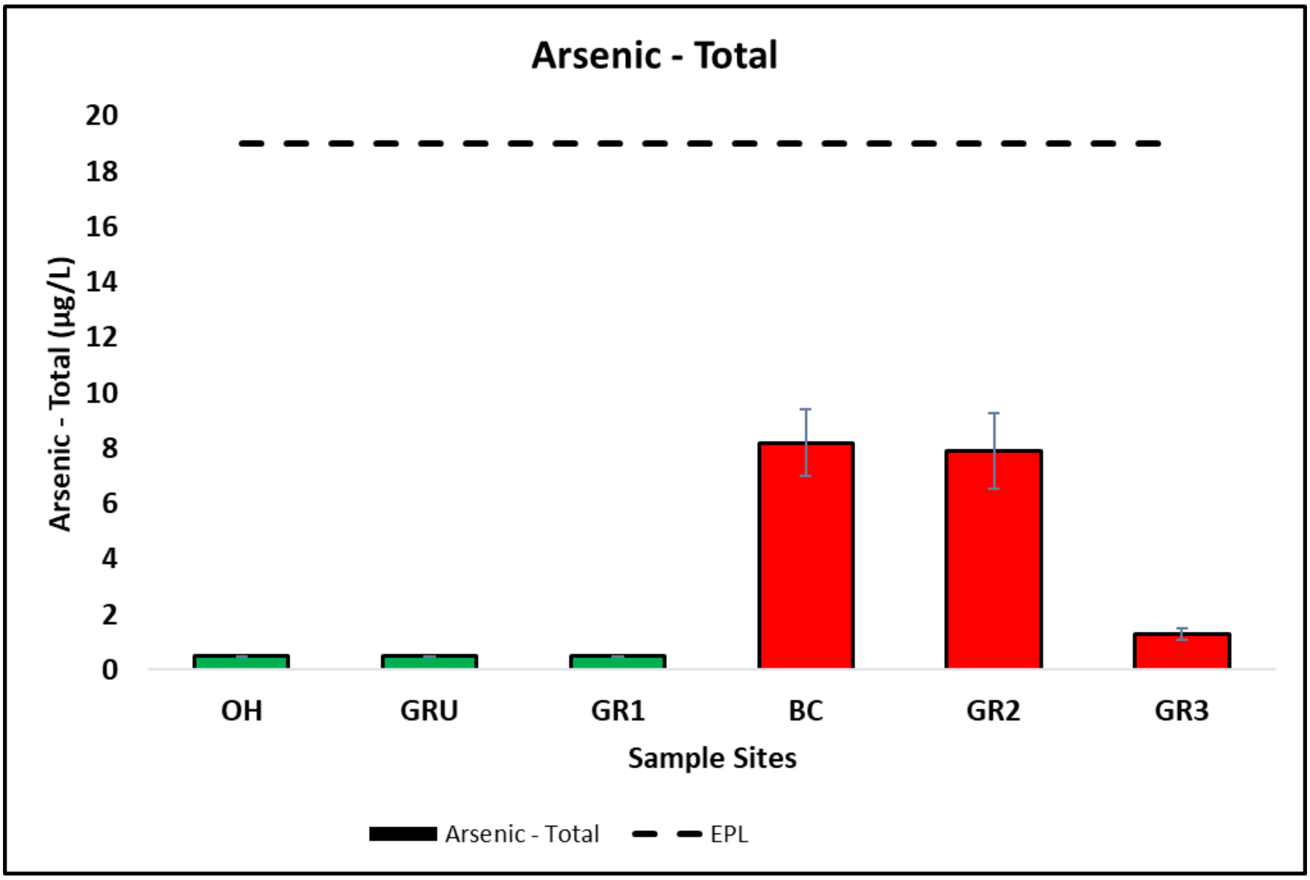

Figure 21: Graph of total arsenic (µg/L) with error bars at all six sample sites. EPL limit is shown by the grey dotted line.

Total barium concentrations at the reference sites were 21 µg/L at O'Hares and 9.0 µg/L at GRU (Table 6, Figure 22). All other sites recorded barium concentrations that were elevated and statistically dissimilar (*p*<0.001) to the two reference sites. Mean barium concentration at GR1 was 117. Mean barium concentration at the West Cliff Colliery discharge into Brennans Creek was 304 µg/L. The wastewater discharge caused an increase in mean barium concentration in the Georges River at GR2 to 255 µg/L. 22 km downstream at GR3 the mean barium concentration was 74 µg/L.

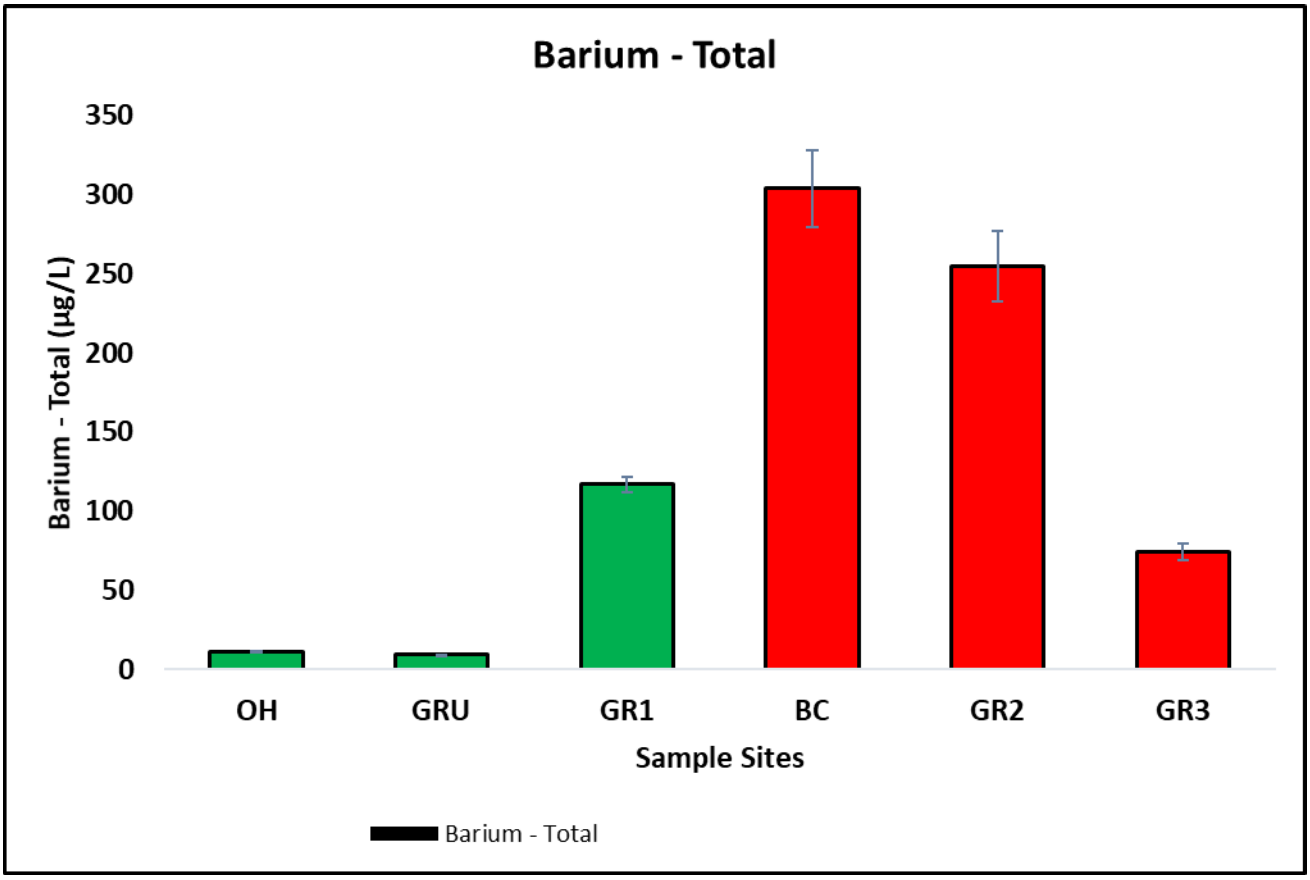

Figure 22: Graph of total barium (µg/L) with error bars at all six sample sites.

Total boron concentrations at the reference sites; O'Hares and GRU were below detection limit of 20 µg/L (Table 6). All other sites recorded boron concentrations that were elevated and statistically dissimilar ($p<0.001$) to the two reference sites. Mean boron concentration at GR1 was 23 µg/L. Mean boron concentration at the West Cliff Colliery discharge into Brennans Creek was 48 µg/L. The wastewater discharge caused an increase in mean boron concentration in the Georges River at GR2 to 40 µg/L. 22 km downstream at GR3 the mean boron concentration was 25 µg/L.

Total cobalt concentrations were below detection limits (<1 µg/L) at O'Hares Creek, GRU and GR1 (Table 6). While sites BC and GR2 had increased concentrations of cobalt that were statistically dissimilar to the reference sites with p-values of <0.001. 22 km downstream at GR3 cobalt was below detection limits. Mean cobalt concentration at the West Cliff Colliery discharge into Brennans Creek was 1.5 µg/L. The wastewater discharge caused an increase in mean cobalt concentration in the Georges River at GR2 to 1.4 µg/L.

Total copper concentrations were lowest at the two reference sites (Table 6). O'Hares Creek had a mean copper concentration of 1.0 and copper concentrations were below detection limits at GRU. GR1 had the highest mean copper concentration of 2.2 µg/L and were statistically significantly different (p-value <0.01) to the reference sites. BC had a mean concentration of 1.2 µg/L and was not statistically significantly different to the reference sites. GR2 had a mean concentration of 1.4 µg/L and was statistically significantly different (p-value <0.01) to the reference sites. 22 km downstream at GR3 the mean concentration of copper was 1.1 µg/L and was not statistically significantly different to the reference sites.

Mean total iron concentrations were highest at the West Cliff Colliery discharge into Brennans Creek; BC with a mean of 560 µg/L followed by GRU (488 µg/L), GR3 (297 µg/L), GR2 (247 µg/L), O'Hares (201 µg/L) with the lowest mean concentration observed at GR1 (83 µg/L). GR1 was the only site that was statistically significantly different (*p*-value <0.01) to the reference sites (Table 6).

Total lead concentrations at O'Hares and GRU were below detection limits on all occasions. Lead concentrations were not statistically significantly different at GR1, BC, GR2 and GR3 although elevated mean concentrations were observed. GR1 had a mean concentration of 0.7 µg/L, BC, GR2 and GR3 had mean lead concentrations of 0.6 µg/L. Manganese concentrations were not statistically significantly different at sites BC, GR1, GR2 and GR3 compared to the reference sites (Table 6).

Total lithium concentrations were lowest at O'Hares and GRU while all other sites had elevated concentrations that were statistically significantly different (*p*-value <0.001) to the reference sites. The wastewater discharge from West Cliff Colliery at BC was the highest, recording a mean concentration of 317 µg/L. The inflow of the discharge caused the lithium concentration in the Georges River to rise from 15.6 µg/L at GR1 to 280.5 µg/L at GR2. 22 km downstream at GR3 the concentration of lithium remained elevated with a mean of 155.3 µg/L (Table 6, Figure 23).

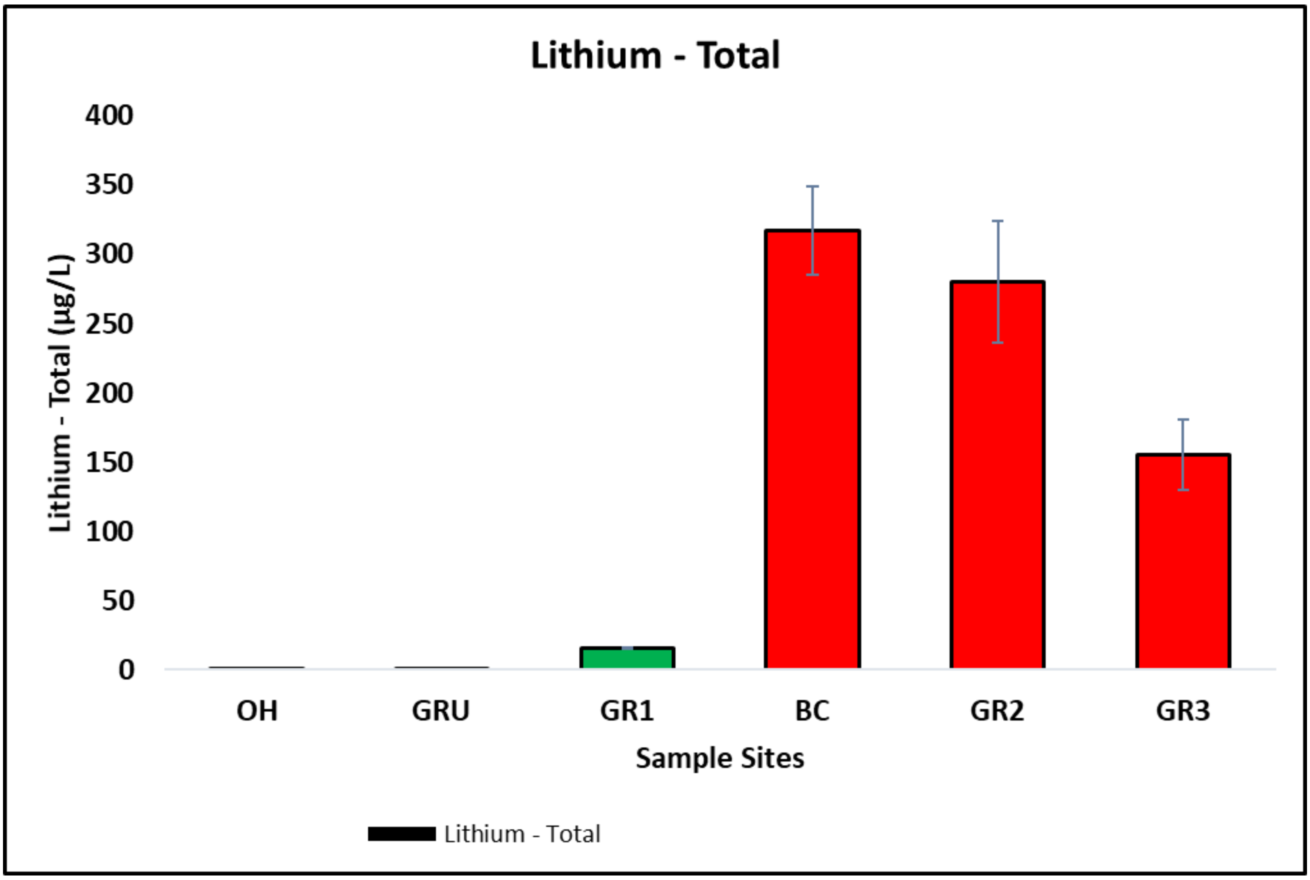

Figure 23: Graph of total lithium (µg/L) with error bars at all six sample sites.

Total molybdenum concentrations were below detection limit of 1 µg/L at O'Hares and at GRU (Table 6, Figure 24). On two occasions molybdenum concentration at GR1 was above the detection limit of 1 µg/L, at 1 µg/L. These two events gave GR1 a mean concentration of 0.6 µg/L. The inflow of wastewater from West Cliff Colliery into Brennans Creek with a mean molybdenum concentration of 29.2 µg/L caused mean molybdenum to rise to 27.5 µg/L. 22 km downstream at GR3 the concentration of molybdenum remained elevated with a mean of 15.7 µg/L. Concentrations at BC, GR2 and GR3 were statistically significantly different (*p*-value <0.001) compared to the reference sites.

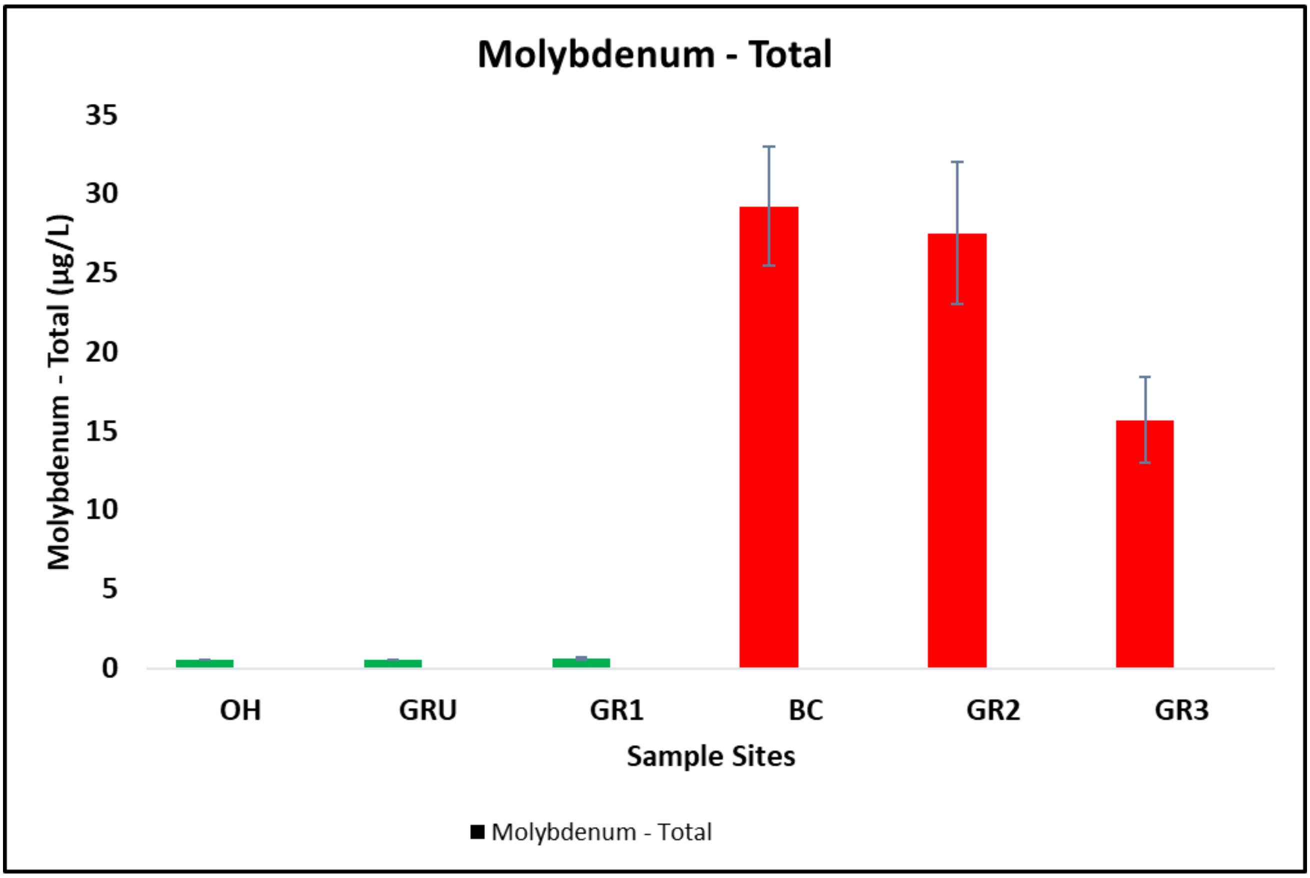

Figure 24: Graph of total molybdenum (µg/L) with error bars at all six sample sites.

Total nickel concentrations were elevated and statistically significantly different (*p*-value <0.001) compared to the reference sites (Table 6, Figure 25). Nickel was below detection limits (1 µg/L) at O'Hares and GRU. The wastewater discharge from West Cliff Colliery at BC had the highest concentration, recording a mean of 83.8 µg/L. The inflow of the discharge caused the nickel concentration in the Georges River to rise from 3.8 µg/L at GR1 to 76.6 µg/L at GR2. 22 km downstream at GR3 the concentration of nickel remained elevated with a mean of 54.6 µg/L.

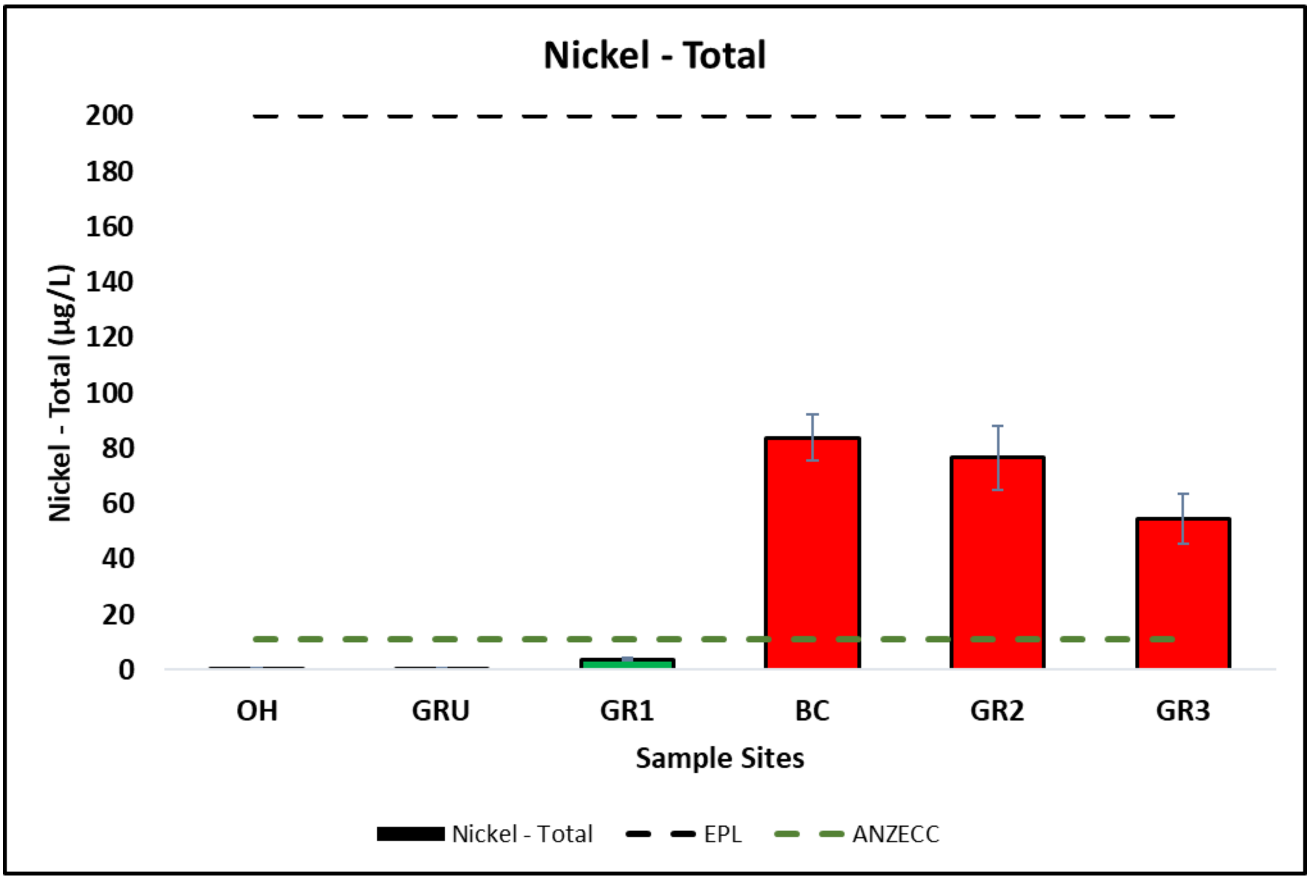

Figure 25: Graph of total nickel (µg/L) with error bars at all six sample sites. EPL limit is shown by the grey dotted line and the ANZECC 2000 guideline is indicated by the green dashed line.

Total strontium concentrations were elevated and statistically significantly different (*p*-value <0.001) compared to the reference sites (Table 6, Figure 26). Mean strontium was 21 µg/L at O'Hares and 10 µg/L at GRU. The wastewater discharge from West Cliff Colliery at BC had the highest concentration, recording a mean of 188 µg/L. The inflow of the discharge caused the strontium concentration in the Georges River to rise from 129 µg/L at GR1 to 168 µg/L at GR2. 22 km downstream at GR3 the concentration of strontium remained elevated with a mean of 91 µg/L.

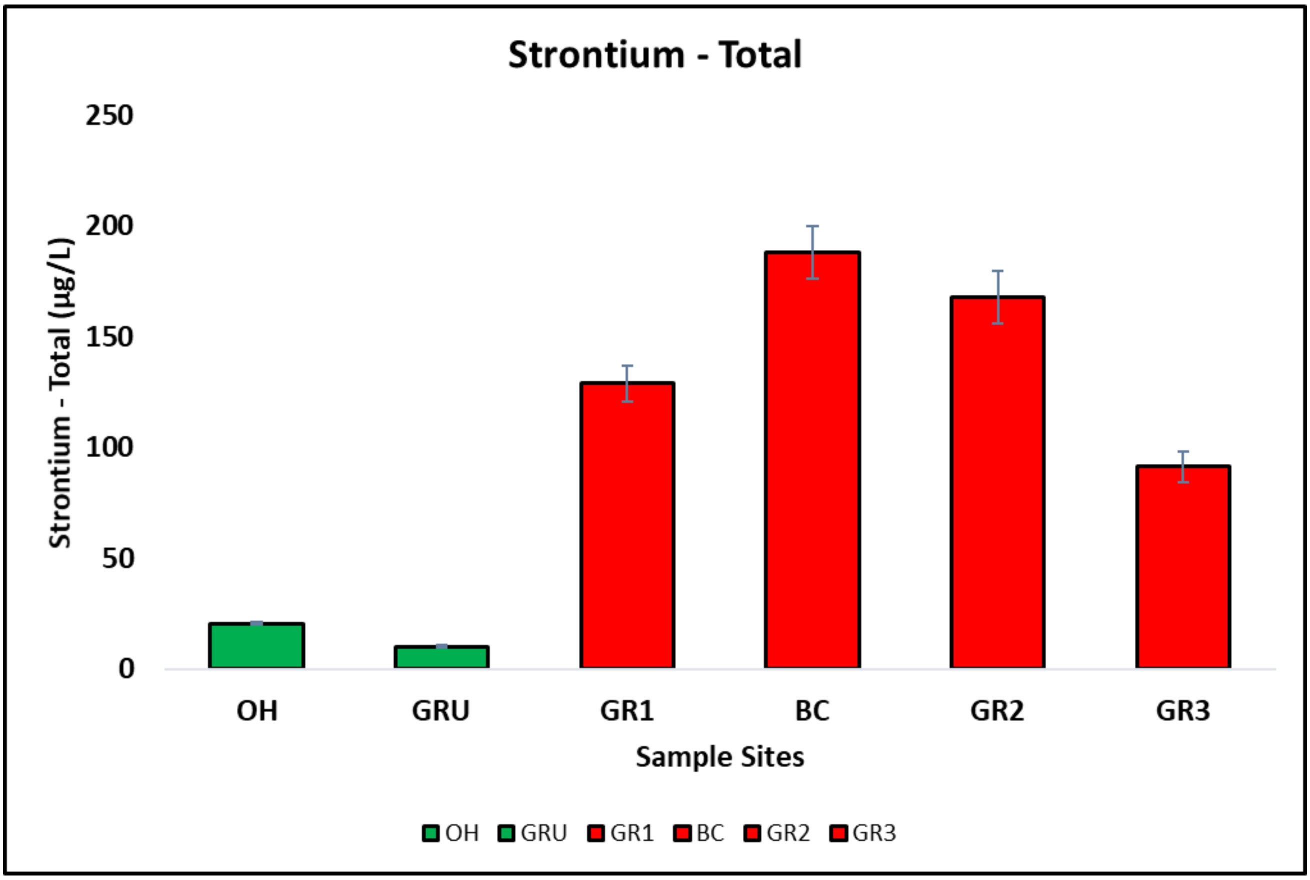

Figure 26: Graph of total strontium (µg/L) with error bars at all six sample sites.

Total titanium concentrations were elevated and statistically significantly different (*p*-value <0.01) compared to the reference sites (Table 6, Figure 27). Mean titanium was 0.9 µg/L at O'Hares and 0.7 µg/L at GRU. The wastewater discharge from West Cliff Colliery at BC had mean of 4.4 µg/L. The inflow of the discharge caused the titanium concentration in the Georges River to rise from 1.9 µg/L at GR1 to 5.9 µg/L at GR2. 22 km downstream at GR3 the concentration of titanium remained elevated with a mean of 3.1 µg/L.

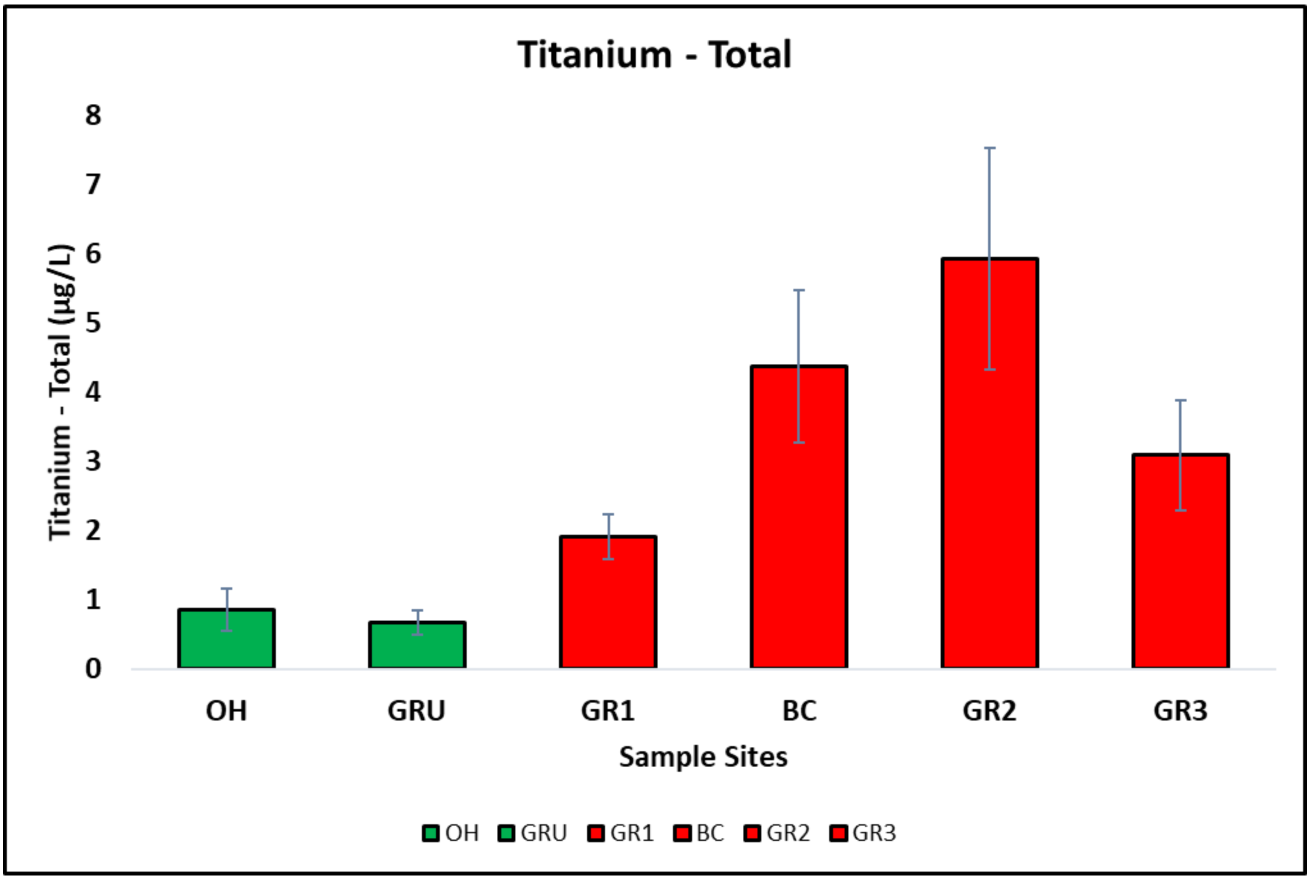

Figure 27: Graph of total titanium (µg/L) with error bars at all six sample sites.

Total uranium concentrations were elevated and statistically significantly different (*p*-value <0.001) compared to the reference sites (Table 6, Figure 28). Uranium concentration was below the laboratory detection limit of 0.5 µg/L on all occasions at sites O'Hares, GRU and at GR1. The wastewater discharge from West Cliff Colliery at BC had mean of 7.8 µg/L. The inflow of the discharge caused the uranium concentration in the Georges River to 7.0 µg/L at GR2. 22 km downstream at GR3 the concentration of uranium remained elevated with a mean of 3.0 µg/L.

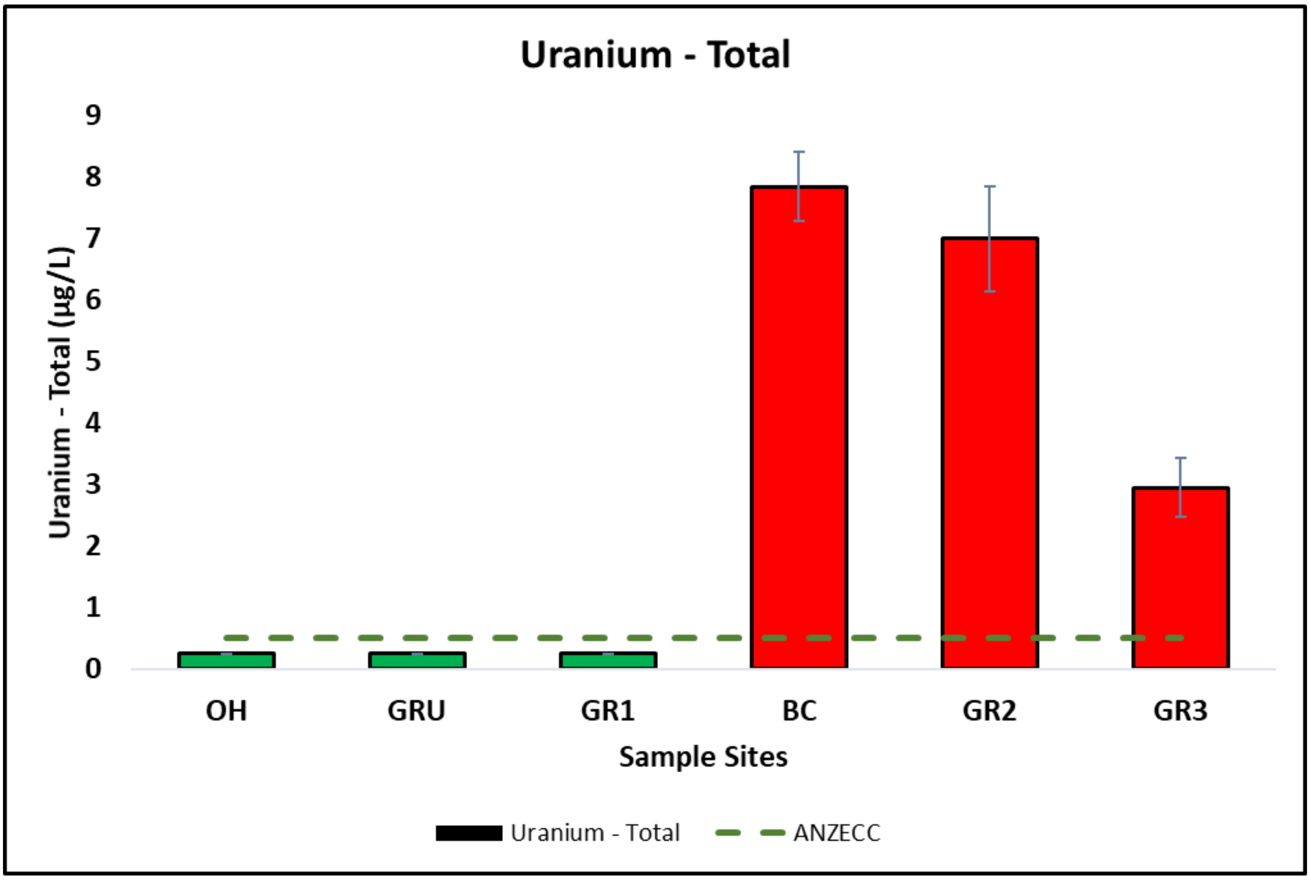

Figure 28: Graph of total uranium (µg/L) with error bars at all six sample sites. ANZECC 2000 guideline is indicated by the green dashed line.

Total zinc concentration was lowest at site GR3 which had a mean of 3.7 µg/L (Table 6, Figure 29). This result was statistically similar to the reference site O'Hares and GRU which had means of 5.1 µg/L and 5.0 µg/L respectively. Sites GR1, BC and GR2 experienced elevated concentrations of zinc at statistically significantly different (*p*-value <0.001) levels. The wastewater discharge from West Cliff Colliery at BC had mean of 10.3 µg/L with GR1 and GR2 recording means of 16.5 µg/L and 10.9 µg/L respectively.

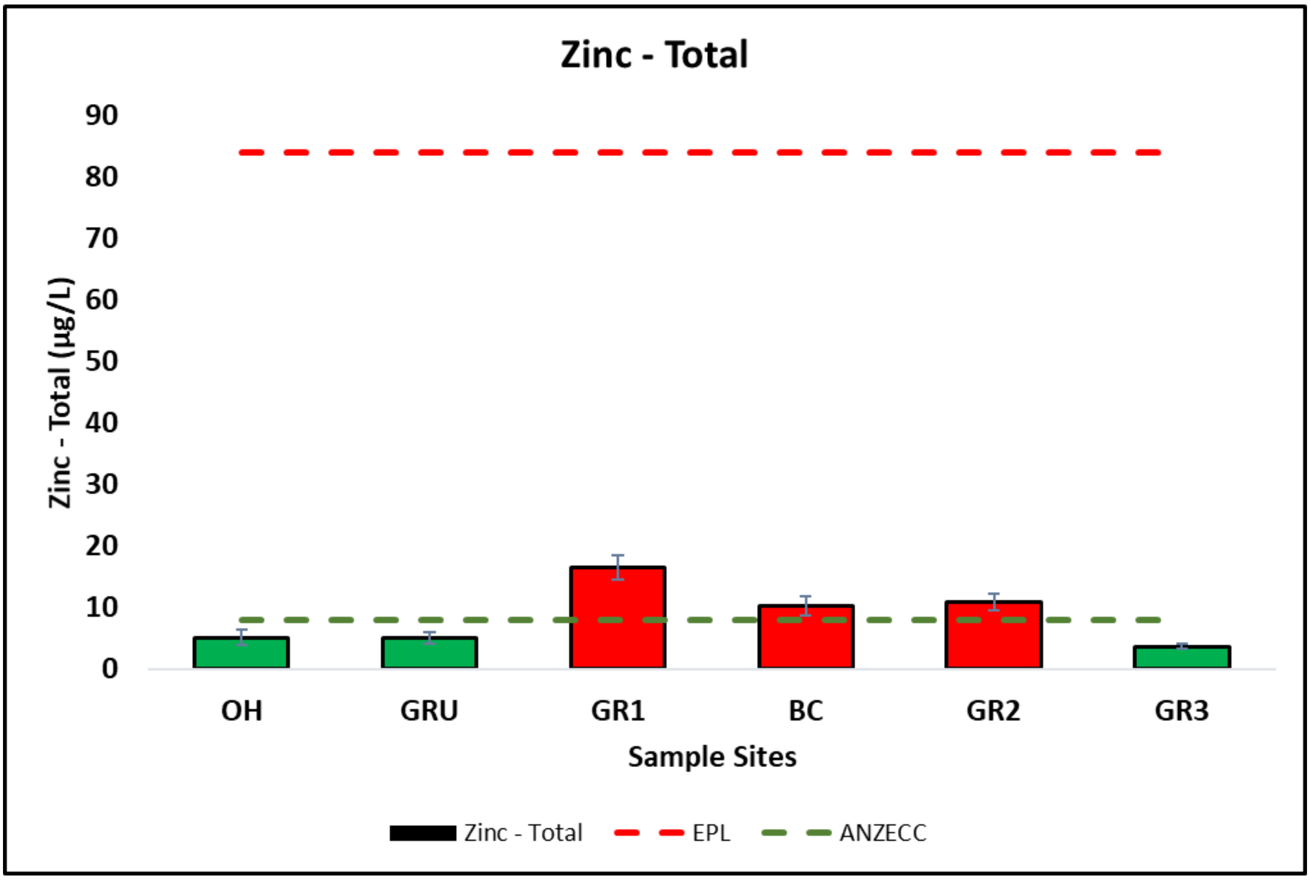

Figure 29: Graph of total zinc (µg/L) with error bars at all six sample sites. EPL limit is shown by the red dotted line and the ANZECC 2000 guideline is indicated by the green dashed line.

Table 6: Summary table of metals in water samples at all sites. Mean, median and p-values are shown, NS indicates that no statically significance difference was observed.

	Reference				Upstream			Discharge			Downstream					
Site Name	**O'Hares**		**GRU**		**GR1**			**BC**			**GR2**			**GR3**		
Variables µg/L	Mean	Med.	Mean	Med.	Mean	Med.	p - value	Mean	Med.	p - value	Mean	Med.	p - value	Mean	Med.	p - value
Antimony	0.5	0.5	0.5	0.5	0.5	0.5	NS	1.5	1.5	<0.001	1.3	1.3	<0.05	0.5	0.5	NS
Aluminium	21	20	30	30	76	90	<0.001	595	490	<0.001	609	530	<0.001	170	85	<0.001
Arsenic	0.5	0.5	0.5	0.5	0.5	0.5	NS	8.2	9.0	<0.001	7.9	9.0	<0.001	1.3	1.0	<0.001
Barium	12	12	9	9	117	115	<0.001	304	320	<0.001	255	230	<0.001	74	82	<0.001
Beryllium	0.3	0.3	0.3	0.3	0.3	0.3	NS	0.3	0.3	NS	0.3	0.3	NS	0.3	0.3	NS
Boron	10	10	10	10	23	20	<0.001	48	45	<0.001	40	40	<0.001	25	25	<0.001
Cadmium	0.1	0.1	0.1	0.1	0.1	0.1	NS	0.1	0.1	NS	0.1	0.1	NS	0.1	0.1	NS
Chromium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.6	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Cobalt	0.5	0.5	0.5	0.5	0.5	0.5	NS	1.5	2.0	<0.001	1.4	1.5	<0.001	0.5	0.5	NS
Copper	1.0	0.5	0.5	0.5	2.2	2.0	<0.01	1.2	1.0	NS	1.4	1.5	<0.01	1.1	1.0	NS
Iron	201	225	488	460	83	79	<0.01	560	145	NS	247	140	NS	297	300	NS
Lead	0.5	0.5	0.5	0.5	0.7	0.5	NS	0.6	0.5	NS	0.6	0.5	NS	0.6	0.5	NS
Lithium	0.5	0.5	0.6	0.5	15.6	15.5	<0.001	317.0	320.0	<0.001	280.5	265.0	<0.001	155.3	180.0	<0.001
Manganese	8	8	35	35	14	10	NS	33	11	NS	9	8	NS	22	23	NS
Mercury	0.0	0.0	0.0	0.0	0.0	0.0	NS	0.0	0.0	NS	0.0	0.0	NS	0.0	0.0	NS
Molybdenum	0.5	0.5	0.5	0.5	0.6	0.5	NS	29.2	32.5	<0.001	27.5	31.5	<0.001	15.7	18.0	<0.001
Nickel	0.5	0.5	0.5	0.5	3.8	3.0	<0.001	83.8	87.5	<0.001	76.6	85.5	<0.001	54.6	64.5	<0.001
Selenium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Stomium	21	21	10	11	129	145	<0.001	188	200	<0.001	168	150	<0.001	91	100	<0.001
Tin	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Titanium	0.9	0.5	0.7	0.5	1.9	1.8	<0.01	4.4	3.2	<0.01	5.9	2.8	<0.01	3.1	1.9	<0.01
Uranium	0.3	0.3	0.3	0.3	0.3	0.3	NS	7.8	7.4	<0.001	7.0	7.4	<0.001	3.0	3.5	<0.001
Zinc	5.1	3.5	5.0	5.0	16.5	17.0	<0.001	10.3	10.0	<0.01	10.9	11.0	<0.001	3.7	3.0	NS

Macroinvertebrate community structure

Thirty-one different macroinvertebrate taxa were identified in the study at the two sites; GRU and GR2, with 7003 individual macroinvertebrates counted (Table 7). Thirty of these taxa were identified to the family taxonomic level while some members from the taxonomic order Cladocera were unable to be identified to family level. Twenty-six different macroinvertebrate taxa were found at GRU in total while 18 different macroinvertebrate taxa were found at GR2. Thirteen taxonomic families were found only at GRU while five taxonomic families were exclusively found at GR2. This presence verses absence comparison indicates what species are unable to survive the polluted nature of the Georges River downstream of the West Cliff Colliery discharge (Table 8). The macroinvertebrate community was severely impacted in the Georges River downstream of the West Cliff Colliery wastewater discharge point. The reference site at GRU was characterised by a diverse macroinvertebrate community which include the presence of animals that are highly sensitive to water pollution. At GR2 the macroinvertebrate community was less diverse, with a higher number of taxa that are recognised to be pollution tolerant. The parameters; abundance, taxa richness, Shannon-Wiener index EPT count, EPT richness and EPT % were all statistically dissimilar between GRU and GR2.

Mean abundance at the reference site GRU was 88 macroinvertebrates, with a median of 77 and a range of 91 from a minimum of 61 to a maximum of 152. Abundance at GR2 was statistically significantly different ($p<0.05$) with a mean of 788 macroinvertebrates. The median number of macroinvertebrates at GR2 was 383, with a range of 1744 from a minimum of 301 to a maximum of 2045 (Table 7, Figure 30).

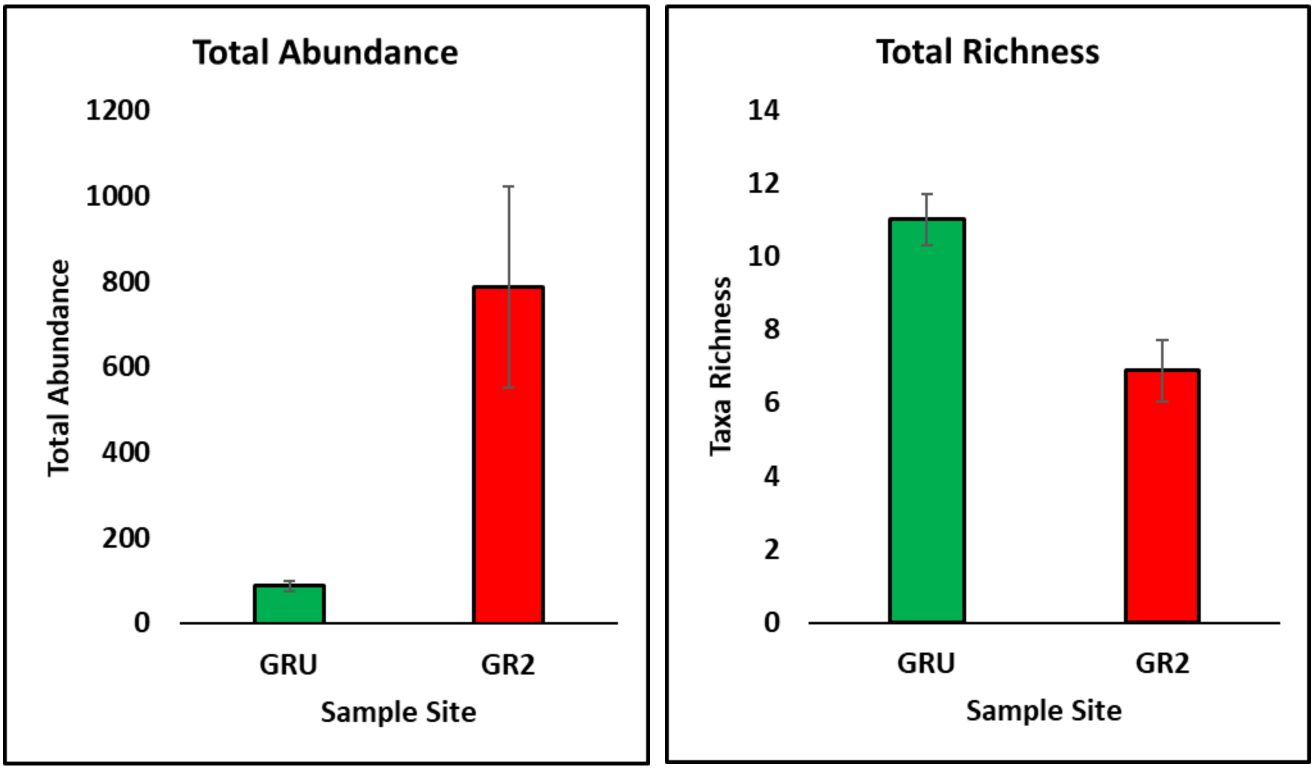

Figure 30:(Left) Mean total abundance of macroinvertebrates with standard error at GRU and GR2. (Left) Mean total taxa richness with standard error at GRU and GR2.

Mean richness at the reference site GRU was 11 taxa, with a median of 12 and a range of 5 from a minimum of 8 to a maximum of 13. Richness at GR2 was statistically significantly different (*p*<0.05) with a mean of 7 taxa. The median number of macroinvertebrate taxa at GR2 was 7, with a range of 7 from a minimum of 5 to a maximum of 12 (Table 7, Figure 30).

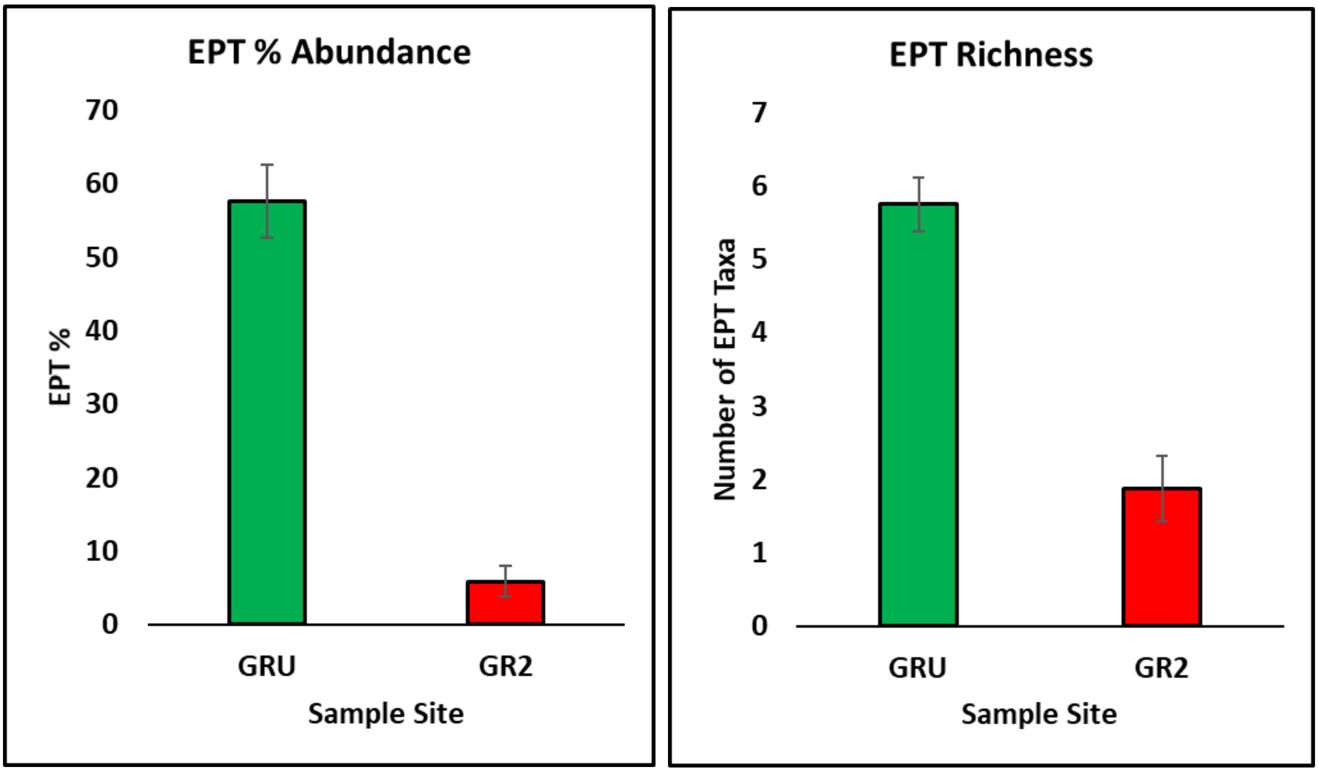

Figure 31:(Left) Mean EPT abundance of macroinvertebrates with standard error at GRU and GR2. (Left) Mean EPT taxa richness with standard error at GRU and GR2.

Mean EPT abundance at the reference site GRU was 53 macroinvertebrates, with a median of 38 and a range of 89 from a minimum of 29 to a maximum of 118. EPT abundance at GR2 was statistically significantly different (*p*<0.05) with a mean of 21 macroinvertebrates. The median number of EPT macroinvertebrates at GR2 was 19, with a range of 54 from a minimum of zero to a maximum of 54 (Table 7).

Mean EPT richness at the reference site GRU was 6 taxa, with a median of 6 and a range of 3 from a minimum of 4 to a maximum of 7. Richness at GR2 was statistically significantly different (*p*<0.001) with a mean of 2 taxa. The median number of macroinvertebrate taxa at GR2 was 2, with a range of 4 from a minimum of zero to a maximum of 4 (Table 7, Figure 31).

EPT percentage of abundance was statistically different between the two sites (*p*<0.001). The reference site GRU had a mean EPT % of 58%, a median of 59%, a range of 40% from a minimum value of 38% to a maximum value of 78%. Downstream of the wastewater discharge at GR2 EPT% was reduced. Mean EPT% was 6% with a median of 5%, a range of 17% from a minimum of zero percent to a maximum of 17% (Table 7, Figure 31).

The difference in the macroinvertebrate community structure was further demonstrated by through the use of the Shannon-Weiner Index. Shannon-Weiner Index scores were statistically significantly different (*p*<0.001) between GRU and GR2. The mean Shannon-Weiner Index score at GRU was 1.8, with a median of 1.7, a range of 0.6 from a minimum value of 1.5 to a maximum value of 2.2. The mean Shannon-Weiner Index score at GR2 was 0.8, with a median of 0.7, a range of 0.4 from a minimum value of 0.6 to a maximum value of 1.0.

Table 7:Macroinvertebrate summary data with mean, median and p-value shown.

Macroinvertebrate	**Reference**		**Downstream**		
Site Name	GRU		GR2		
Variables	Mean	Med.	Mean	Med.	p - value
Abundance	88	77	788	383	<0.05
Richness	11	12	7	7	<0.05
EPT count	53	38	21	19	<0.05
EPT richness	6	6	2	2	<0.001
EPT % abundance	58	59	6	5	<0.001
Shannon-Wiener index	1.8	1.7	0.8	0.7	<0.001

The presence and absence of macroinvertebrate taxa is shown by Table 8. A total of 31 taxonomic families were recorded in the study. There were 13 taxonomic families that were only found at the reference site (GRU) while five taxonomic families were unique to the site downstream of the wastewater discharge (GR2). Four of the families found exclusively at GRU belonged to the Tricoptera taxonomic order; Philopotamidae, Philorheithridae, Hydroptlidae and Helicopsychidae. The single Plecoptera family; Eustheniidae was found at exclusively at GRU along with the families; Arrenuridae, Branchipodidae, Daphniidae, Elmidae, Atyidae, Baetidae, Notonectidae, and Corduliidae. The following five families were found exclusively downstream of the wastewater discharge at GR2; Ceratopogonidae, Psychodidae, Corixidae, Veliidae and Leptoceridae.

Table 8: Total presence (marked by 'X') and absence (marked by '-') of macroinvertebrate taxa over the study period at the reference site (GRU) and the site (GR2) receiving coal mine wastewater.

Order	Family	GRU	GR2
Acarina	Arrenuridae	X	-
Anostraca	Branchipodidae	X	-
Cladocera	Daphniidae	X	-
Cladocera	Cladocera	X	X
Coleoptera	Dytiscidae	X	X
Coleoptera	Elmidae	X	-
Coleoptera	Hydrophilidae	X	X
Decapoda	Atyidae	X	-
Diptera	Ceratopogonidae	-	X
Diptera	Chironomidae	X	X
Diptera	Tabanidae	X	X
Diptera	Psychodidae	-	X
Diptera	Simuliidae	X	X
Ephemeroptera	Baetidae	X	-
Ephemeroptera	Caenidae	X	X
Ephemeroptera	Leptophlebiidae	X	X
Hemiptera	Corixidae	-	X
Hemiptera	Veliidae	-	X
Hemiptera	Notonectidae	X	-
Megaloptera	Corydalidae	X	X
Odonata	Aeshnidae	X	X
Odonata	Corduliidae	X	-
Plecoptera	Eustheniidae	X	-
Trichoptera	Ecnomidae	X	X
Trichoptera	Helicopsychidae	X	-
Trichoptera	Hydropsychidae	X	X
Trichoptera	Leptoceridae	-	X
Trichoptera	Odontoceridae	X	X
Trichoptera	Philopotamidae	X	-
Trichoptera	Philorheithridae	X	-
Trichoptera	Hydroptilidae	X	-

Metal concentration in the sediment

The results from sediment testing show substantial metal pollution of benthic sediment (Table 9). The West Cliff Colliery discharge site at Brennans Creek (BC) had increased levels of thirteen metals at statistically significantly levels. These metals at elevated concentrations were; aluminium, barium, chromium, cobalt, copper, iron, lead, lithium, manganese, nickel, strontium, uranium and zinc. GR1 experienced elevated concentrations of the same metals at statistically significantly levels as BC, with the omission of chromium and the addition of titanium. GR2 experienced elevated levels of the same suite of metals with the addition of titanium at statistically significantly levels. 22km downstream on the Georges River, GR3 recorded six metals (barium, copper, lithium, strontium, titanium and zinc) in the sediment that were present at statistically significantly levels. The metals; antimony, beryllium, cadmium, mercury and selenium were below laboratory detection limits at all sites on all occasions. Additionally, tin, molybdenum, boron and arsenic were at concentrations below detection limits or approaching this limit, with no statistically significant difference between sites.

Aluminium concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.01), GR2 (*p*-value <0.05) with no significant difference at GR3. Mean aluminium concentration at the two reference sites; O'Hares was 633 mg/kg and 1330 mg/kg at GRU. Mean aluminium concentration at GR1 was 4433 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean aluminium concentration was 2350 mg/kg. Below the BC discharge at GR2 mean aluminium concentration in the sediment was 2200 mg/kg. 22 km downstream at GR3 the mean concentration of aluminium was 773 mg/kg (Table 9).

Barium concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001), GR2 (*p*-value <0.01) and at GR3 (*p*-value <0.05). Mean barium concentration at the two reference sites; O'Hares was 3.7 mg/kg and 6.8 mg/kg at GRU. Above the West Cliff Colliery discharge point mean barium concentration was 48.3 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean barium concentration was 206.7 mg/kg. Below the BC discharge at GR2 mean barium concentration in the sediment increased from GR1 to 2200 mg/kg. 22 km downstream at GR3 the mean concentration of barium was 11.7 mg/kg (Table 9, Figure 32).

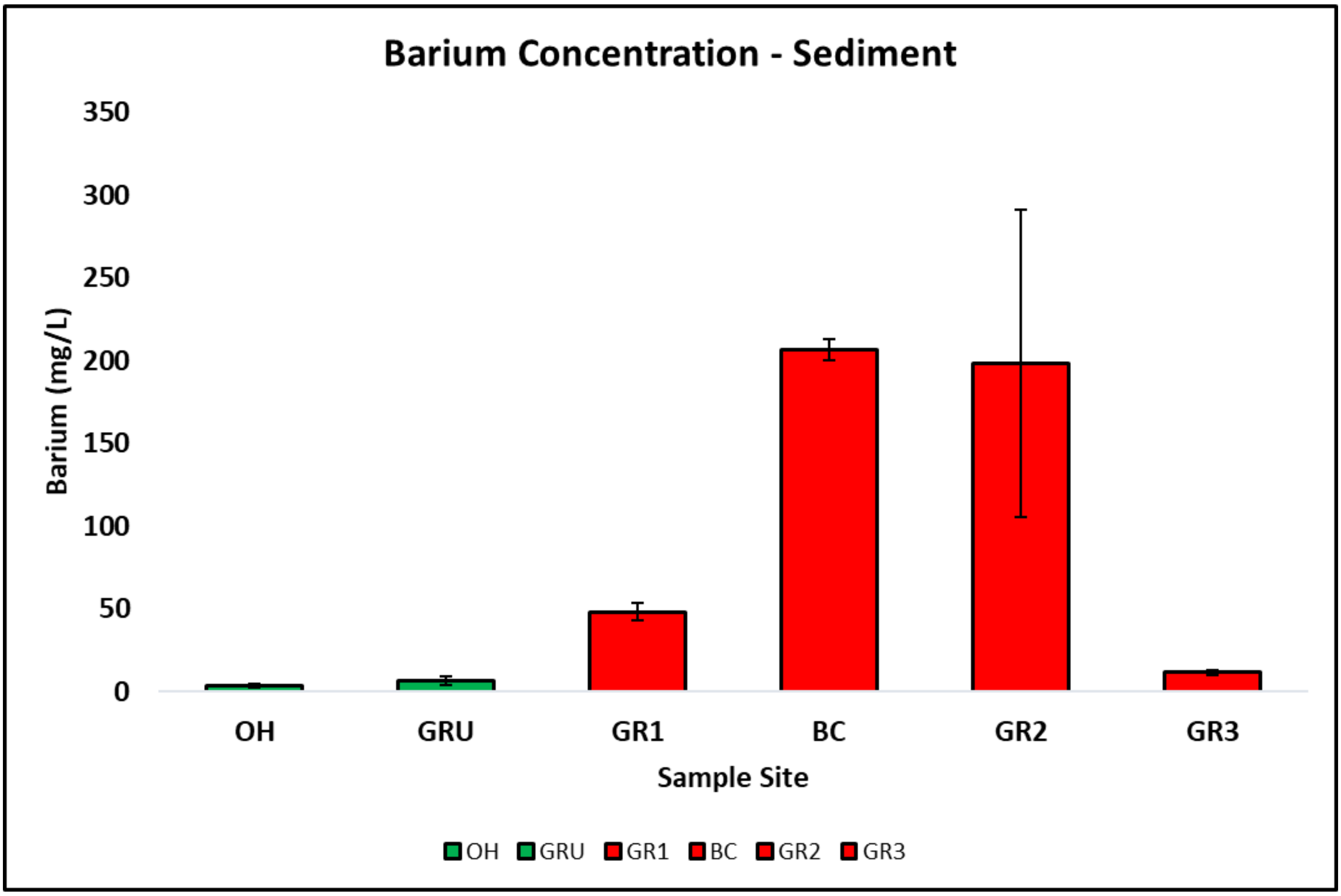

Figure 32: Mean barium concentration (mg/L) and standard error at all sites.

Chromium concentration in the sediment were elevated at statistically dissimilar concentrations at BC (*p*-value <0.05) and GR2 (*p*-value <0.01) while there was no statistically difference at GR1 and GR3 compared to the reference sites. Mean chromium concentration at the two reference sites; O'Hares was 1.7 mg/kg and 1.4 mg/kg at GRU. Above the West Cliff Colliery discharge point mean at GR1 cobalt concentration was 3.3 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean cobalt concentration was 3.2 mg/kg. Below the BC discharge at GR2 mean cobalt concentration in the sediment increased from GR1 to 5.8 mg/kg. 22 km downstream at GR3 the mean concentration of cobalt was 2.1 mg/kg.

Cobalt concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001) and GR2 (*p*-value <0.05) there was no statistically difference at GR3 compared to the reference sites. Mean cobalt concentration at O'Hares was below the limit of detection (1.0 mg/kg) while at GRU one sample recorded a concentration of 2 mg/kg with all other samples were below the limit of detection, giving GRU a mean concentration of 0.8 mg/kg. Above the West Cliff Colliery discharge point mean at GR1 cobalt concentration was 2.0 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean cobalt concentration was 1.8 mg/kg. Below the BC discharge mean cobalt concentration in the sediment increased from GR1 to 2.2 mg/kg at GR2. 22 km downstream at GR3 the mean concentration of cobalt was 0.7 mg/kg (Table 9).

Copper concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001), GR2 (*p*-value <0.001) and at GR3 (*p*-value <0.01). Mean copper concentration at O'Hares was below the limit of detection (1.0 mg/kg) while at GRU one sample recorded a concentration of 1 mg/kg with all other samples were below the limit of detection, giving GRU a mean concentration of 0.6 mg/kg. Above the West Cliff Colliery discharge point at GR1 mean copper concentration was 3.2 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean copper concentration was 2.8 mg/kg. Below the BC discharge at GR2 mean copper concentration in the sediment increased from GR1 to 5.5 mg/kg. 22 km downstream at GR3 the mean concentration of copper was 1.3 mg/kg (Table 9).

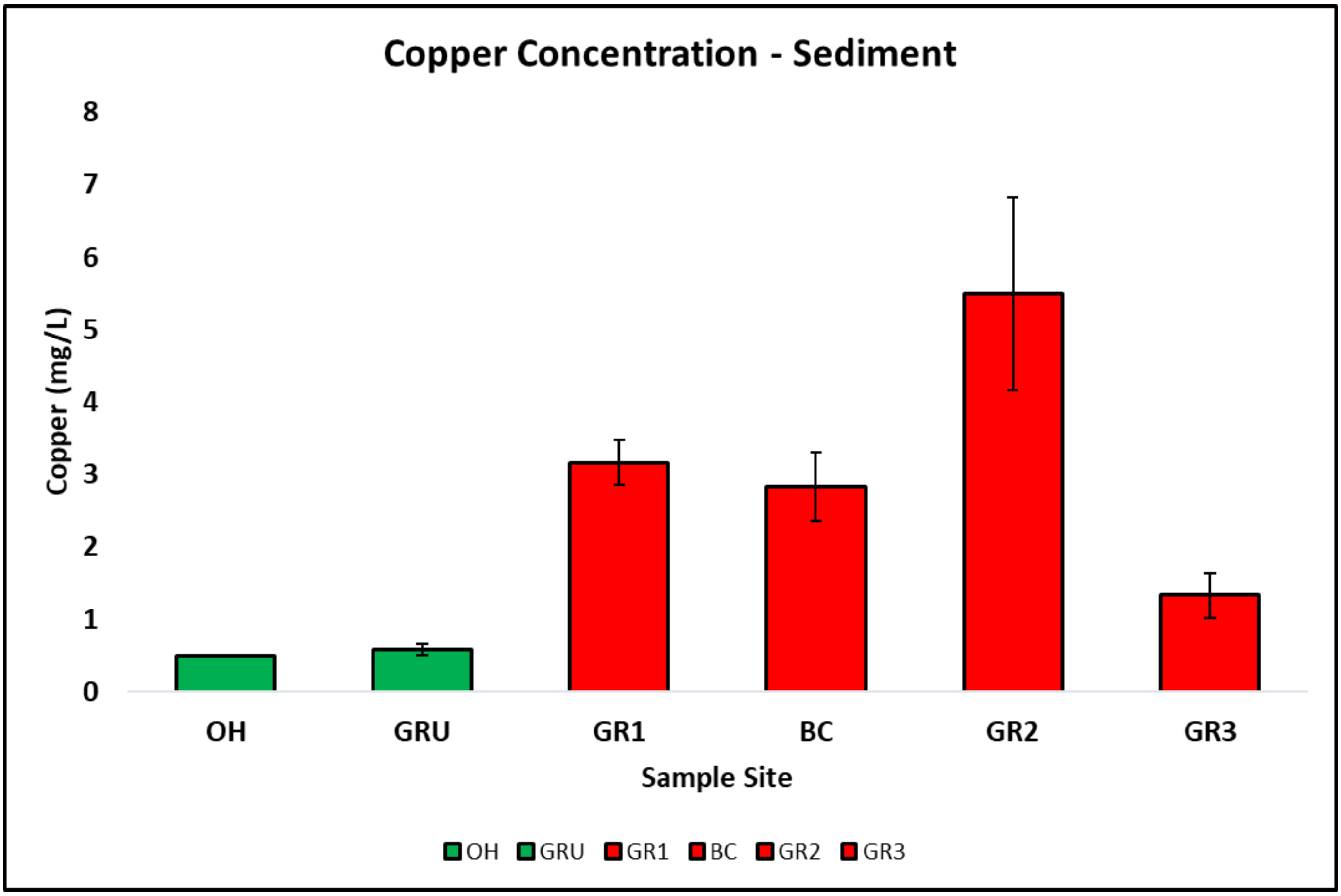

Figure 33: Mean copper concentration (mg/L) and standard error at all sites.

Iron concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.05), BC (*p*-value <0.05), GR2 (*p*-value <0.01) with no significant difference at GR3. Mean iron concentration at the two reference sites; O'Hares was 3317 mg/kg and 2572 mg/kg at GRU. Mean iron concentration at GR1 was 7317 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean iron concentration was 6283 mg/kg. Below the BC discharge at GR2 mean iron concentration in the sediment was 8700 mg/kg. 22 km downstream at GR3 the mean concentration of iron was 3600 mg/kg (Table 9).

Lead concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.05), BC (*p*-value <0.001), GR2 (*p*-value <0.01) with no significant difference at GR3. Mean lead concentration at the two reference sites; O'Hares was 1.3 mg/kg and 2.7 mg/kg at GRU.

Mean lead concentration at GR1 was 4.8 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean lead concentration was 6.5 mg/kg. Below the BC discharge at GR2 mean lead concentration in the sediment was 7.3 mg/kg, an increase compared to GR1. 22 km downstream at GR3 the mean concentration of lead was 2.7 mg/kg (Table 9, Figure 34).

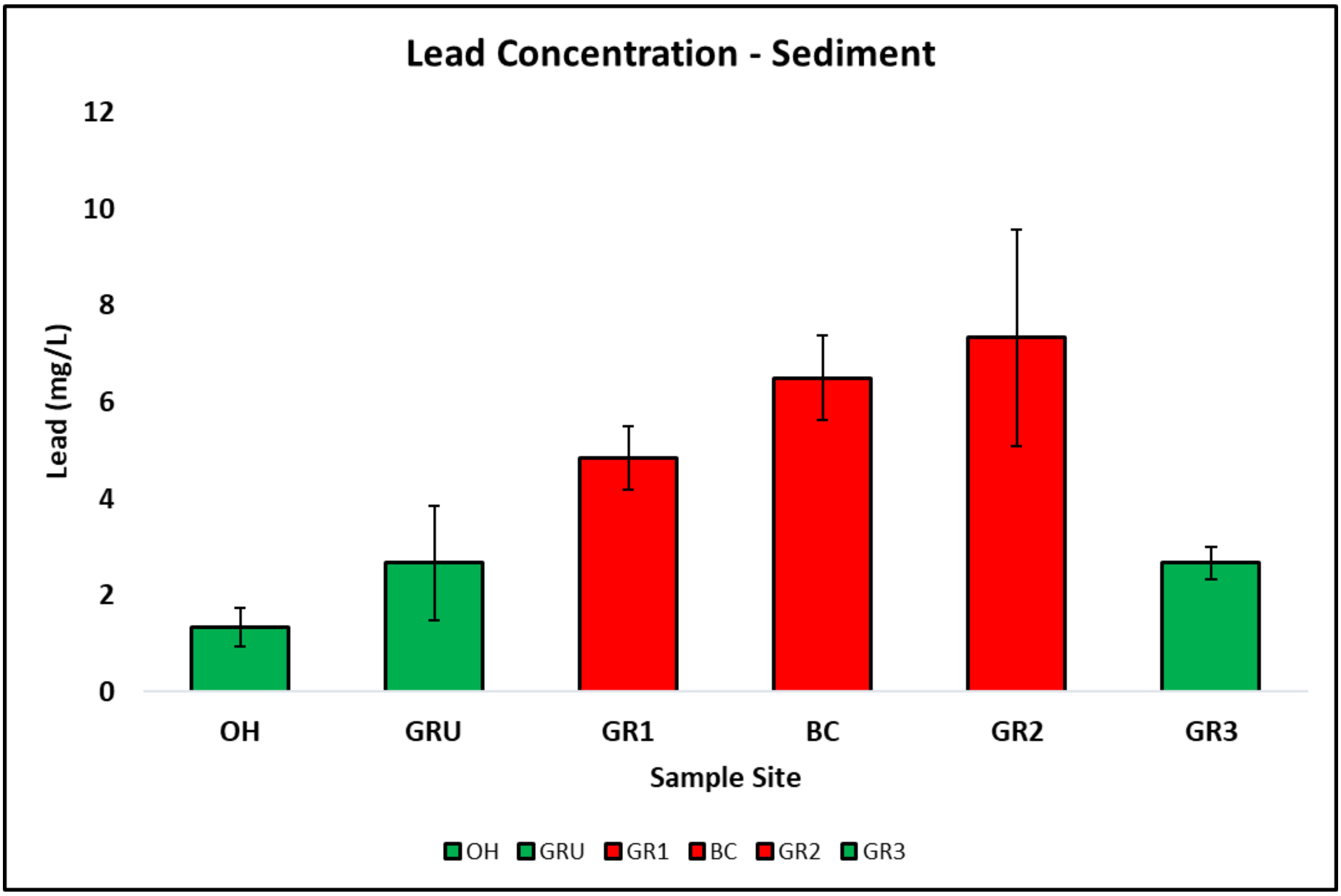

Figure 34: Mean lead concentration (mg/L) and standard error at all sites.

Lithium concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001), GR2 (*p*-value <0.001) and at GR3 (*p*-value <0.001) compared to the reference sites. Lithium concentration at O'Hares and GRU was below the limit of detection (1.0 mg/kg). Above the West Cliff Colliery discharge point at GR1 mean lithium concentration was 6.2 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean lithium concentration was 12.3 mg/kg. Below the BC discharge at GR2 mean lithium concentration in the sediment increased from GR1 to 11.3 mg/kg. 22 km downstream at GR3 the mean concentration of lithium was 3.0 mg/kg (Table 9; Figure 35).

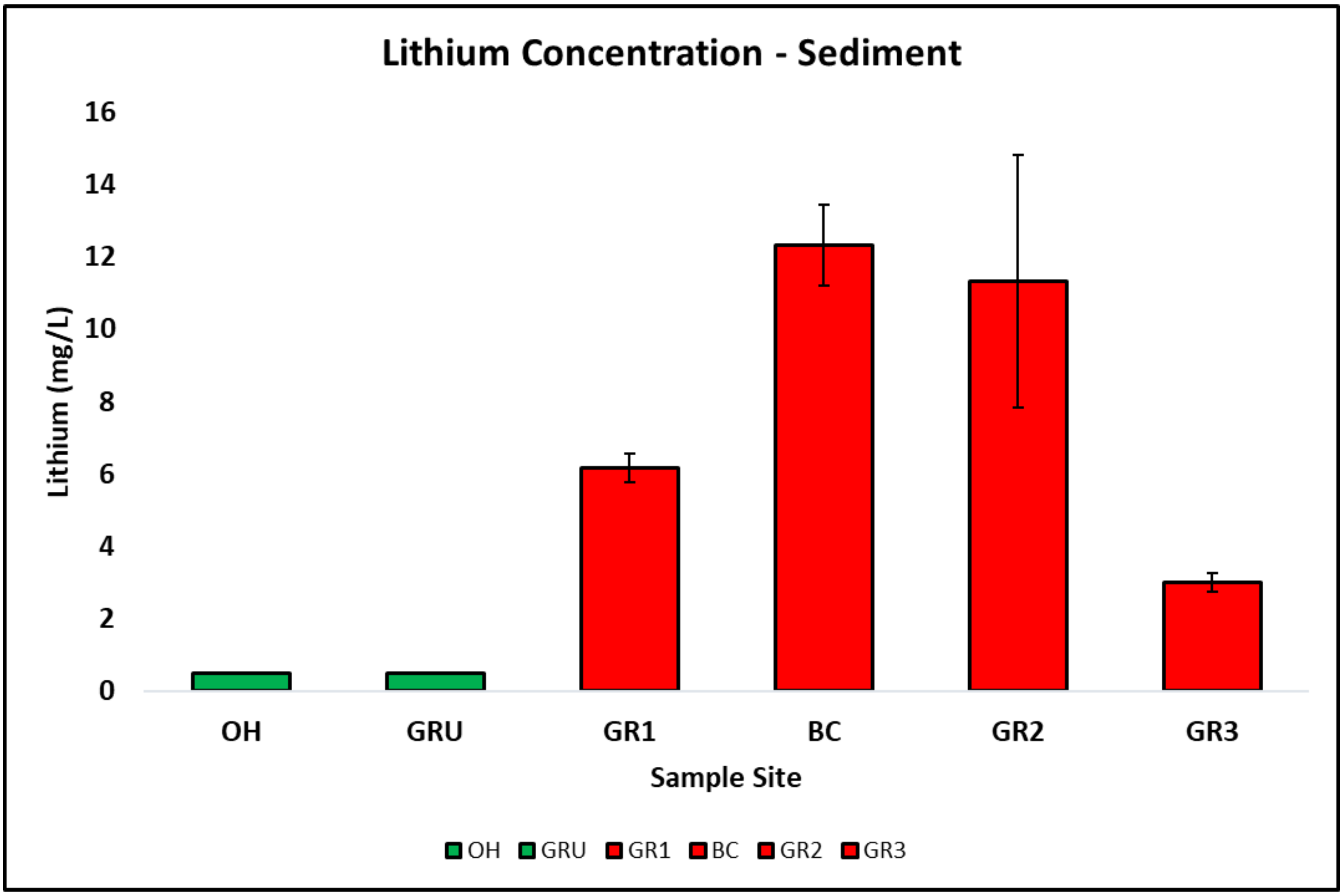

Figure 35: Mean lithium concentration (mg/L) and standard error at all sites.

Manganese concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001), GR2 (*p*-value <0.001). Manganese concentrations were statistically similar to the reference site at GR3. Mean manganese concentration at the two reference sites; O'Hares was 7.7 mg/kg and 27.5 mg/kg at GRU. Above the West Cliff Colliery discharge point at GR1 mean manganese concentration was 236.7 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean manganese concentration was 66.8 mg/kg. Below the BC discharge at GR2 mean manganese concentration in the sediment increased from GR1 to 131.7 mg/kg. 22 km downstream at GR3 the mean concentration of manganese was 25.0 mg/kg (Table 9; Figure 36).

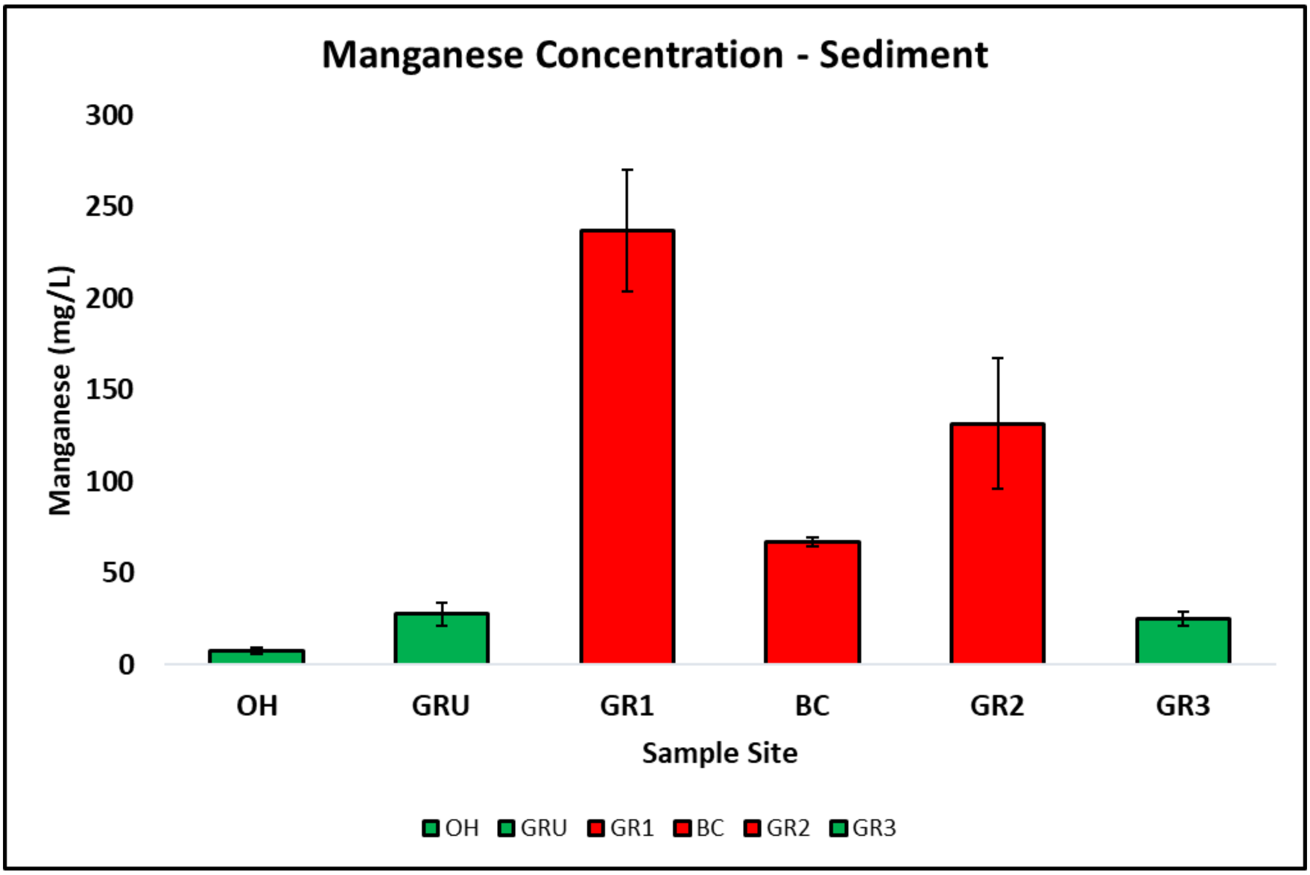

Figure 36: Mean manganese concentration (mg/L) and standard error at all sites.

Nickel concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001) and GR2 (*p*-value <0.01) there was no statistically difference at GR3 compared to the reference sites. Mean nickel concentration at O'Hares was below the limit of detection (1.0 mg/kg) while at GRU one sample recorded a concentration of 2 mg/kg with all other samples were below the limit of detection, giving GRU a mean concentration of 0.8 mg/kg. Above the West Cliff Colliery discharge point mean at GR1 nickel concentration was 3.0 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean nickel concentration was 1.8 mg/kg. Below the BC discharge mean nickel concentration in the sediment was 3.0 mg/kg at GR2. 22 km downstream at GR3 the mean concentration of nickel was 0.7 mg/kg (Table 9; Figure 37).

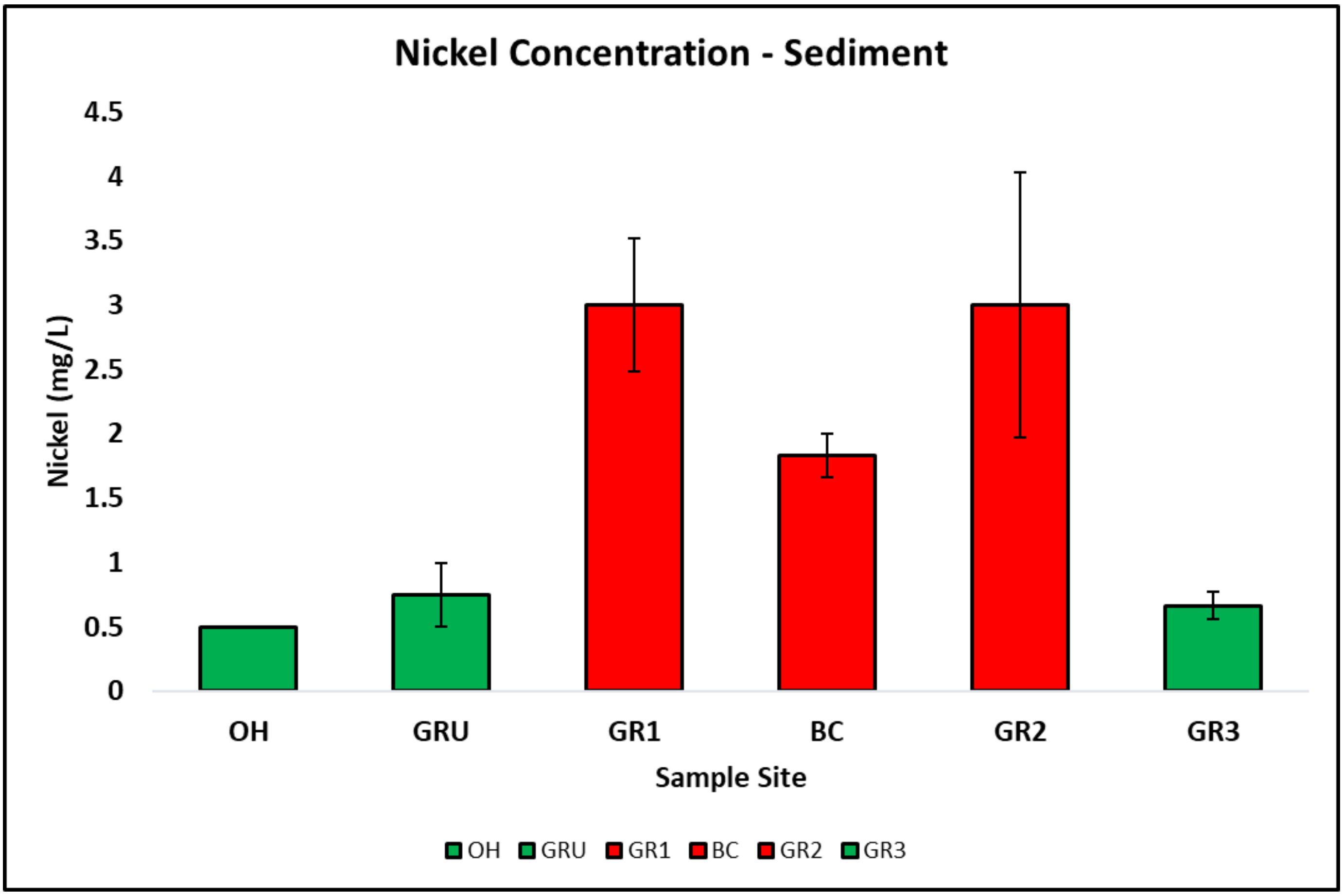

Figure 37: Mean nickel concentration (mg/L) and standard error at all sites.

Strontium concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001), GR2 (*p*-value <0.01) and at GR3 (*p*-value <0.001) compared to the reference sites. Mean strontium concentration at the two reference sites; O'Hares was 0.8 mg/kg and 1.6 mg/kg at GRU. Above the West Cliff Colliery discharge point at GR1 mean strontium concentration was 15.0 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean strontium concentration was 70.2 mg/kg. Below the BC discharge at GR2 mean strontium concentration in the sediment increased from GR1 to 64.5 mg/kg. 22 km downstream at GR3 the mean concentration of strontium was 3.8 mg/kg (Table 9; Figure 38).

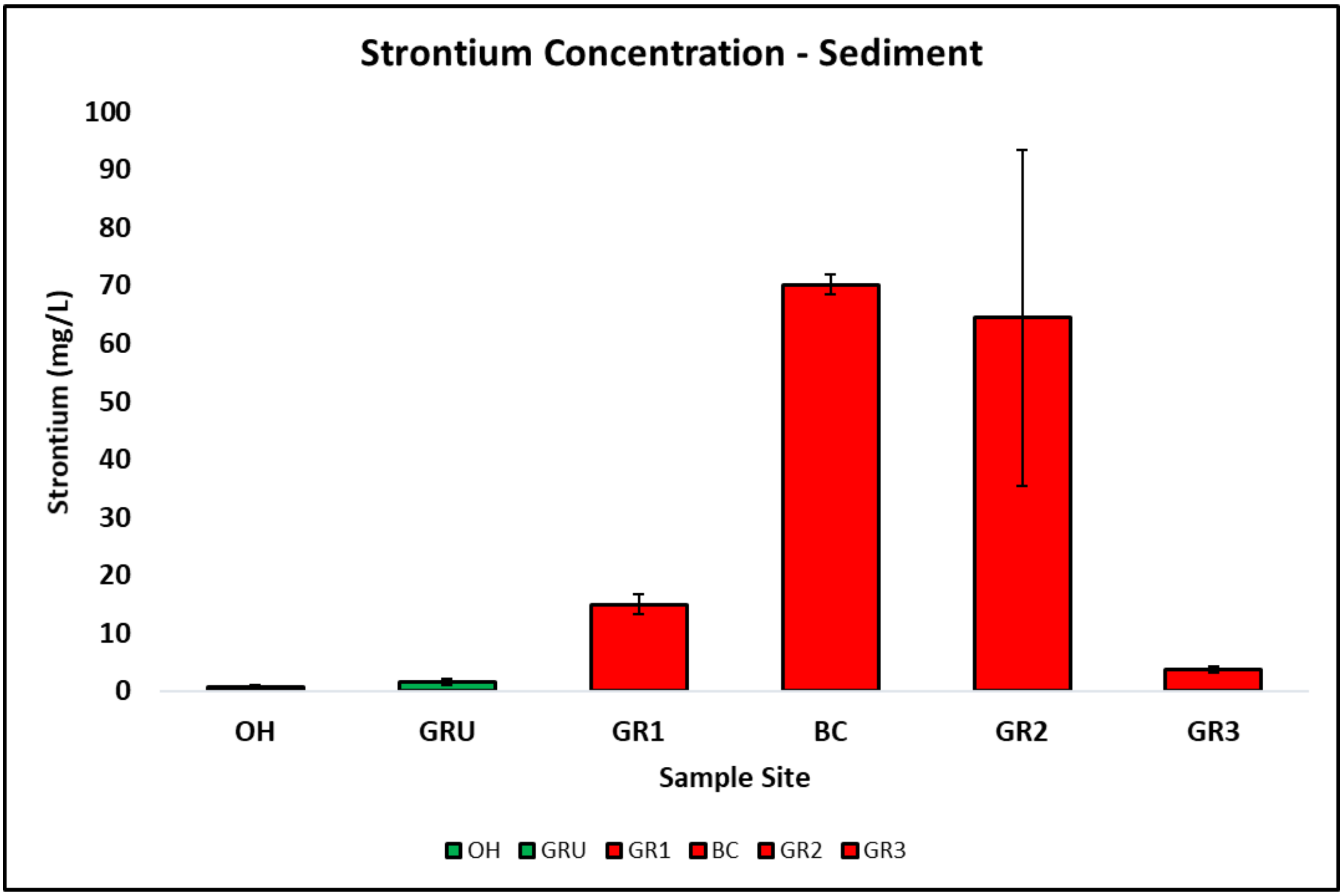

Figure 38: Mean strontium concentration (mg/L) and standard error at all sites.

Titanium concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), GR2 (*p*-value <0.001) and at GR3 (*p*-value <0.001) compared to the reference sites. There was no statistically similar difference in concentration at BC where titanium was above the limit of detection (<1.0 mg/kg) on one occasion at 1.0 mg/kg. Titanium concentration at O'Hares and GRU was below the limit of detection. Above the West Cliff Colliery discharge point at GR1 mean titanium concentration was 56.8 mg/kg. Below the BC discharge at GR2 mean titanium concentration in the sediment decreased from GR1 to 5.7 mg/kg. 22 km downstream at GR3 the mean concentration of titanium was 1.1 mg/kg (Table 9).

Uranium concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001) and GR2 (*p*-value <0.05) there was no statistically difference at GR3 compared to the reference sites. Mean uranium concentration at O'Hares Creek and GRU was below the limit of detection (<0.1 mg/kg). Above the West Cliff Colliery discharge point mean at GR1 uranium concentration was 0.55 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean uranium concentration was 0.4 mg/kg. Below the BC discharge mean uranium concentration in the was 0.35 mg/kg at GR2. 22 km downstream at GR3 the mean concentration of uranium was 0.1 mg/kg (Table 9; Figure 39).

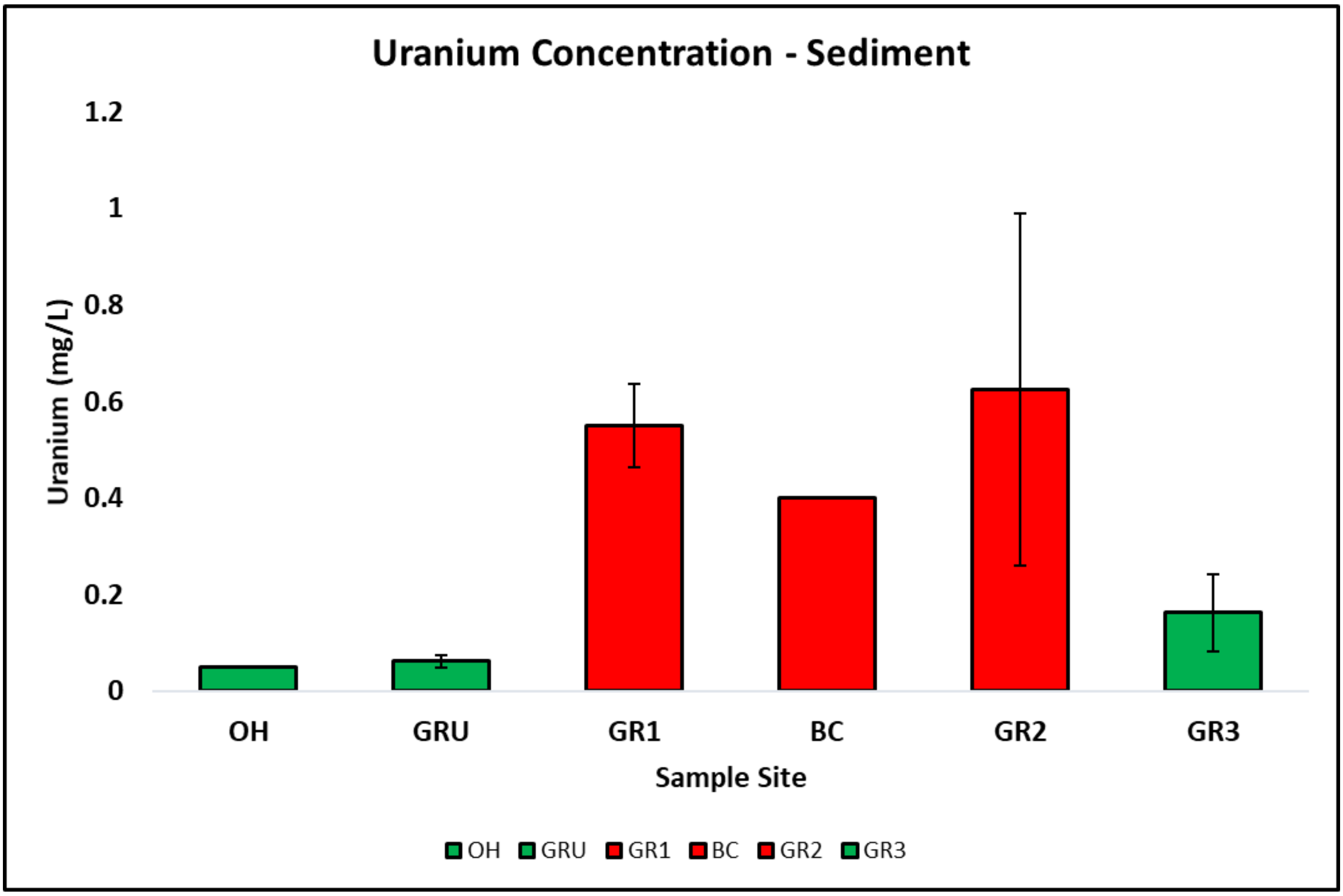

Figure 39: Mean uranium concentration (mg/L) and standard error at all sites.

Zinc concentration in the sediment were elevated at statistically dissimilar concentrations at GR1 (*p*-value <0.001), BC (*p*-value <0.001), GR2 (*p*-value <0.01) and at GR3 (*p*-value <0.05). Mean zinc concentration at the two reference sites; O'Hares was 4.0 mg/kg and 5.3 mg/kg at GRU. Above the West Cliff Colliery discharge point mean zinc concentration was 38.3 mg/kg. At BC where the West Cliff Colliery discharge wastewater mean zinc concentration was 12.5 mg/kg. Below the BC discharge at GR2 mean zinc concentration in the sediment was 18.3 mg/kg. 22 km downstream at GR3 the mean concentration of zinc was 15.8 mg/kg (Table 9; Figure 40).

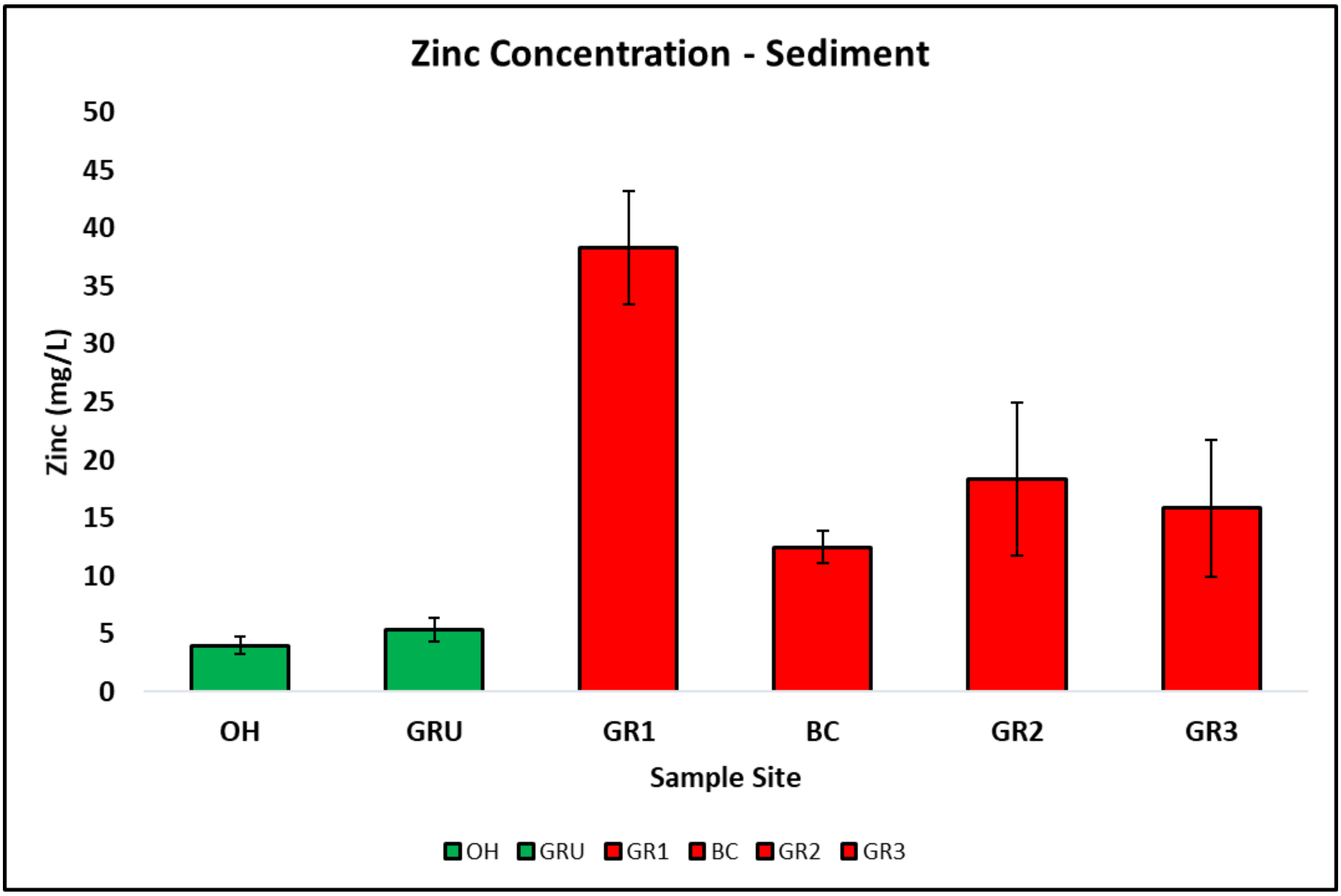

Figure 40: Mean zinc concentration (mg/L) and standard error at all sites.

Table 9: Summary table of metals in sediment samples at all sites. Mean, median and p-values are shown. NS indicates that no statically significance difference was observed.

	Reference				Upstream			Discharge			Downstream					
Site Name	**O'Hares**		**GRU**		**GR1**			**BC**			**GR2**			**GR3**		
Metals (mg/kg)	Mean	Med.	Mean	Med.	Mean	Med.	p - value	Mean	Med.	p - value	Mean	Med.	p - value	Mean	Med.	p - value
Antimony	3.5	3.5	3.5	3.5	3.5	3.5	NS	3.5	3.5	NS	3.5	3.5	NS	3.5	3.5	NS
Aluminium	633	430	1330	890	4433	4100	<0.001	2350	2450	<0.01	2200	2000	<0.05	773	755	NS
Arsenic	2.0	2.0	2.0	2.0	2.3	2.0	NS	2.0	2.0	NS	3.0	2.0	NS	2.0	2.0	NS
Barium	3.7	3.0	6.8	5.0	48.3	44.5	<0.001	206.7	210.0	<0.001	198.3	120.0	<0.01	11.7	12.5	<0.05
Beryllium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Boron	1.5	1.5	1.5	1.5	1.5	1.5	NS	1.5	1.5	NS	2.3	1.5	NS	1.5	1.5	NS
Cadmium	0.2	0.2	0.2	0.2	0.2	0.2	NS	0.2	0.2	NS	0.2	0.2	NS	0.2	0.2	NS
Chromium	1.7	0.5	1.4	1.3	3.3	3.0	NS	3.2	3.5	<0.05	5.8	5.0	<0.01	2.1	1.5	NS
Cobalt	0.5	0.5	0.8	0.5	2.0	2.0	<0.001	1.8	2.0	<0.001	2.2	1.0	<0.05	0.7	0.5	NS
Copper	0.5	0.5	0.6	0.5	3.2	3.0	<0.001	2.8	3.0	<0.001	5.5	4.0	<0.001	1.3	1.5	<0.01
Iron	3317	1850	2572	2150	7317	6550	<0.05	6283	6450	<0.05	8700	8400	<0.01	3600	3050	NS
Lead	1.3	1.0	2.7	1.5	4.8	5.0	<0.05	6.5	7.0	<0.001	7.3	6.0	<0.01	2.7	2.5	NS
Lithium	0.5	0.5	0.5	0.5	6.2	6.0	<0.001	12.3	13.5	<0.001	11.3	8.0	<0.001	3.0	3.0	<0.001
Manganese	7.7	6.0	27.5	24.0	236.7	215.0	<0.001	66.8	68.5	<0.001	131.7	124.0	<0.001	25.0	27.5	NS
Mercury	0.1	0.1	0.1	0.1	0.1	0.1	NS	0.1	0.1	NS	0.1	0.1	NS	0.1	0.1	NS
Molybdenum	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.8	0.5	NS	0.9	0.5	NS
Nickel	0.5	0.5	0.8	0.5	3.0	3.5	<0.001	1.8	2.0	<0.001	3.0	2.0	<0.01	0.7	0.5	NS
Selenium	1.0	1.0	1.0	1.0	1.0	1.0	NS	1.0	1.0	NS	1.0	1.0	NS	1.0	1.0	NS
Strontium	0.8	0.5	1.6	1.3	15.0	14.0	<0.001	70.2	69.5	<0.001	64.5	42.5	<0.01	3.8	4.0	<0.001
Tin	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	1.3	0.5	NS	0.6	0.5	NS
Titanium	0.5	0.5	0.5	0.5	56.8	59.0	<0.001	0.6	0.5	NS	5.7	5.5	<0.001	1.1	0.8	<0.05
Uranium	0.1	0.1	0.1	0.1	0.6	0.5	<0.001	0.4	0.4	<0.001	0.6	0.4	<0.05	0.2	0.1	NS
Zinc	4.0	4.0	5.3	4.5	38.3	41.0	<0.001	12.5	14.0	<0.001	18.3	12.5	<0.01	15.8	11.0	<0.05

Metal concentration in willow leaf tissue

The accumulation of metals in the willow tissue differed between the leaves and roots of the plants. Within the leaf tissue there were five metals for which concentration was elevated at statistically significant levels in willows grown in water from BC, GR1 and GR2 compared to the reference sites. Strontium was elevated in willow leaves grown in water from BC, GR1 and GR2. Boron and lithium were elevated at BC and GRU while barium and manganese were elevated at statistically significant levels in willow leaves grown in water from GR1 and BC respectively. Manganese in willow leaf tissue was elevated at statically significant concentrations at BC. The following metals were below detection limits in willow leaves grown in water from sites; arsenic, beryllium, cadmium, chromium, cobalt, mercury, nickel, lead, antimony, selenium, titanium and uranium. There was also no at statistically significant difference between sites; BC, GR1 and GR2 compared to the reference sites for the following metals; aluminium, iron, molybdenum and zinc (Table 10).

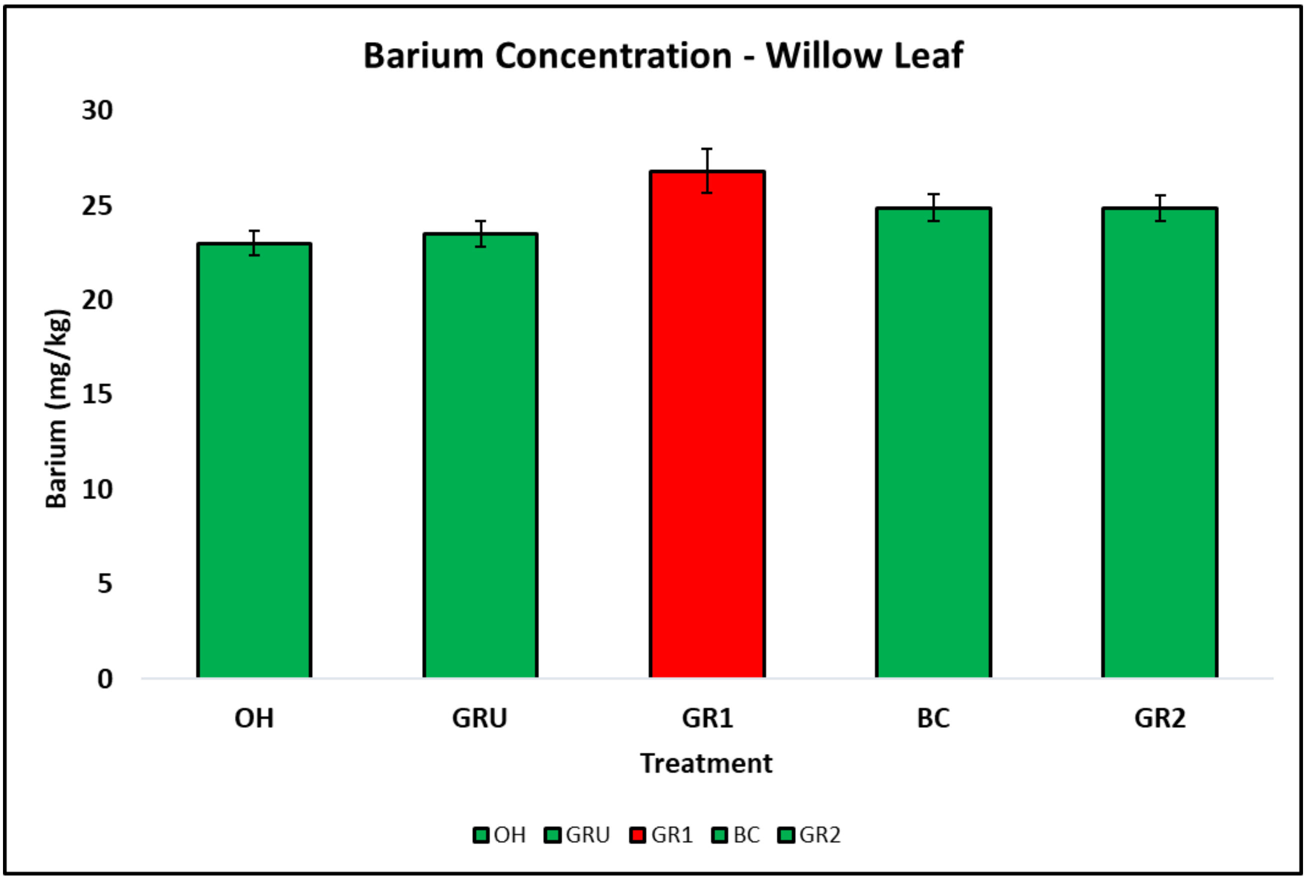

Figure 41: Mean barium concentration (mg/L) and standard error in willow leaf tissue at all treatments.

Barium concentration in the willow leaf tissue was elevated at statistically significantly levels at treatment GR1 (*p*-value <0.01) compared to the two reference sites. Mean concentration of barium in the willow leaf tissue with water from the two reference sites; O'Hares and GRU was 23.5 mg/kg and 23.0 mg/kg respectively. Mean barium concentration at the willows grown in water from GR1 was 26.8 mg/kg. Mean barium concentration in willow leaf tissue grown in water from BC was 24.9 mg/kg and 24.9 mg/kg in willow tissue grown in water from GR2 (Table 10; Figure 41).

Manganese concentration in the willow leaf tissue was elevated at statistically significantly levels at BC (*p*-value <0.05) compared to the two reference sites. Mean concentration of manganese in the willow leaf tissue with water from the two reference sites; O'Hares and GRU was 108 mg/kg and 109 mg/kg respectively. Mean manganese concentration at the willows grown in water from GR1 was 111 mg/kg. Mean manganese concentration in willow leaf tissue grown in water from BC was 118 mg/kg and 115 mg/kg in willow tissue grown in water from GR2 (Table 10; Figure 42).

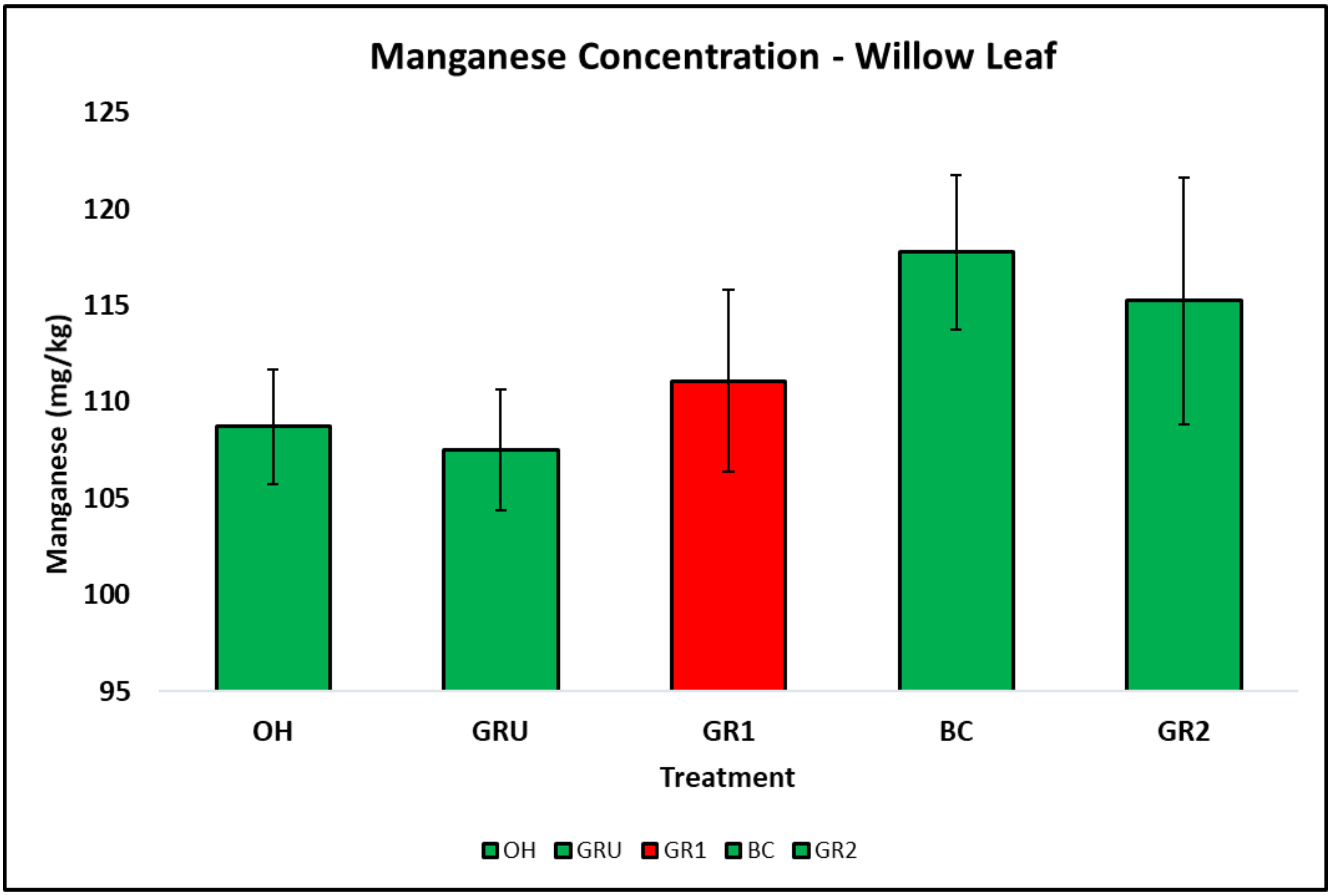

Figure 42: Mean manganese concentration (mg/L) and standard error in willow leaf tissue at all treatments.

Boron concentration in the willow leaf tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Mean concentration of boron in the willow leaf tissue with water from the two reference sites; O'Hares and GRU was 31.5 mg/kg and 28.5 mg/kg respectively. Mean boron concentration at the willows grown in water from GR1 was 27.3 mg/kg. Mean boron concentration was elevated in willow leaf tissue grown in water from BC (41.7 mg/kg) and from GR2 (41.5 mg/kg) (Table 10; Figure 43).

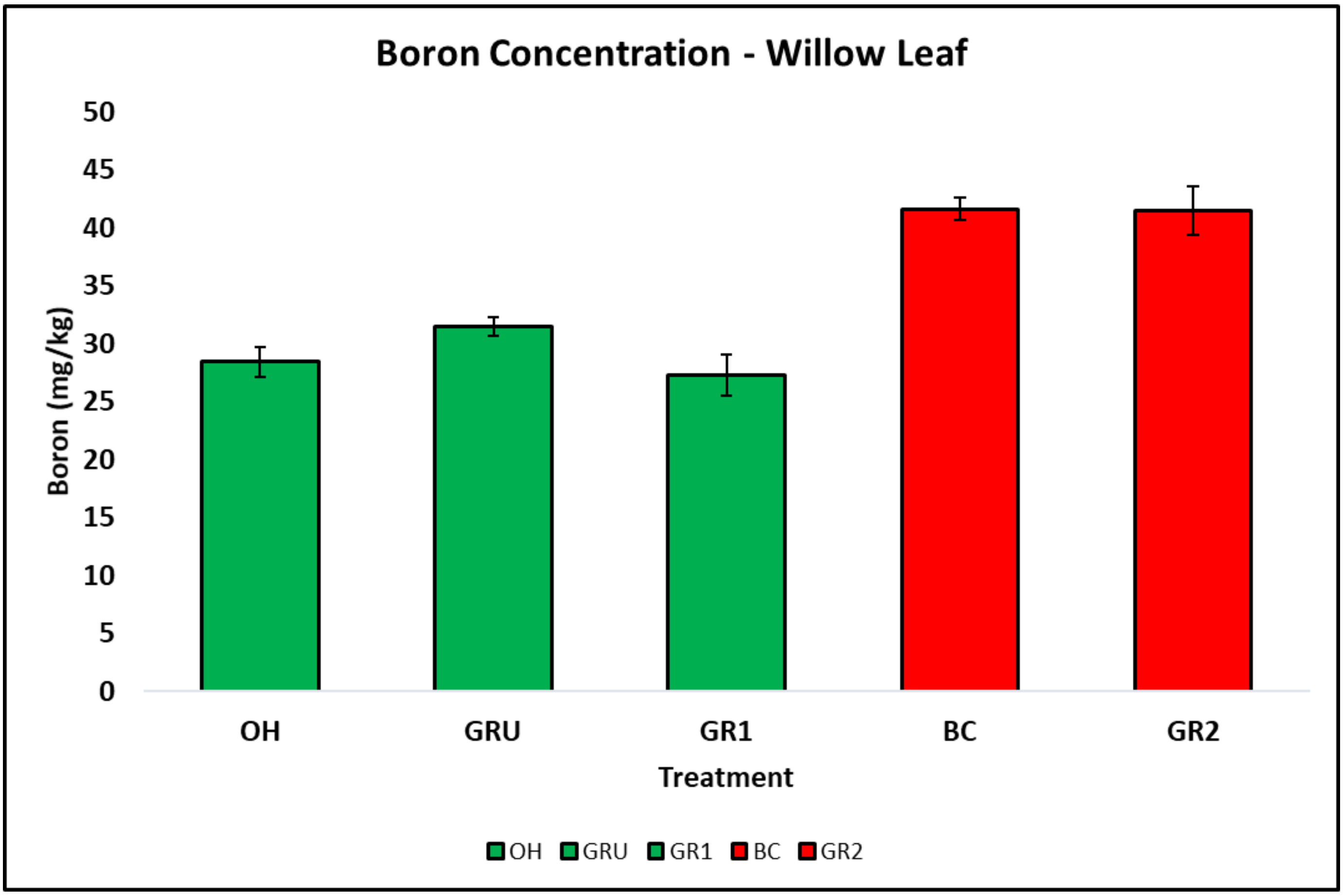

Figure 43: Mean boron concentration (mg/L) and standard error in willow leaf tissue at all treatments.

Lithium concentration in the willow leaf tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Concentration of lithium in the willow leaf tissue with water from the two reference sites; O'Hares and GRU was below the laboratory detection limit of 1.0 mg/kg. Lithium concentration at the willows grown in water from GR1 was also below the detection limit. Mean lithium concentration was elevated in willow leaf tissue grown in water from BC (mean of 15.2 mg/kg) and from GR2 (mean of 18.3 mg/kg) (Table 10; Figure 44).

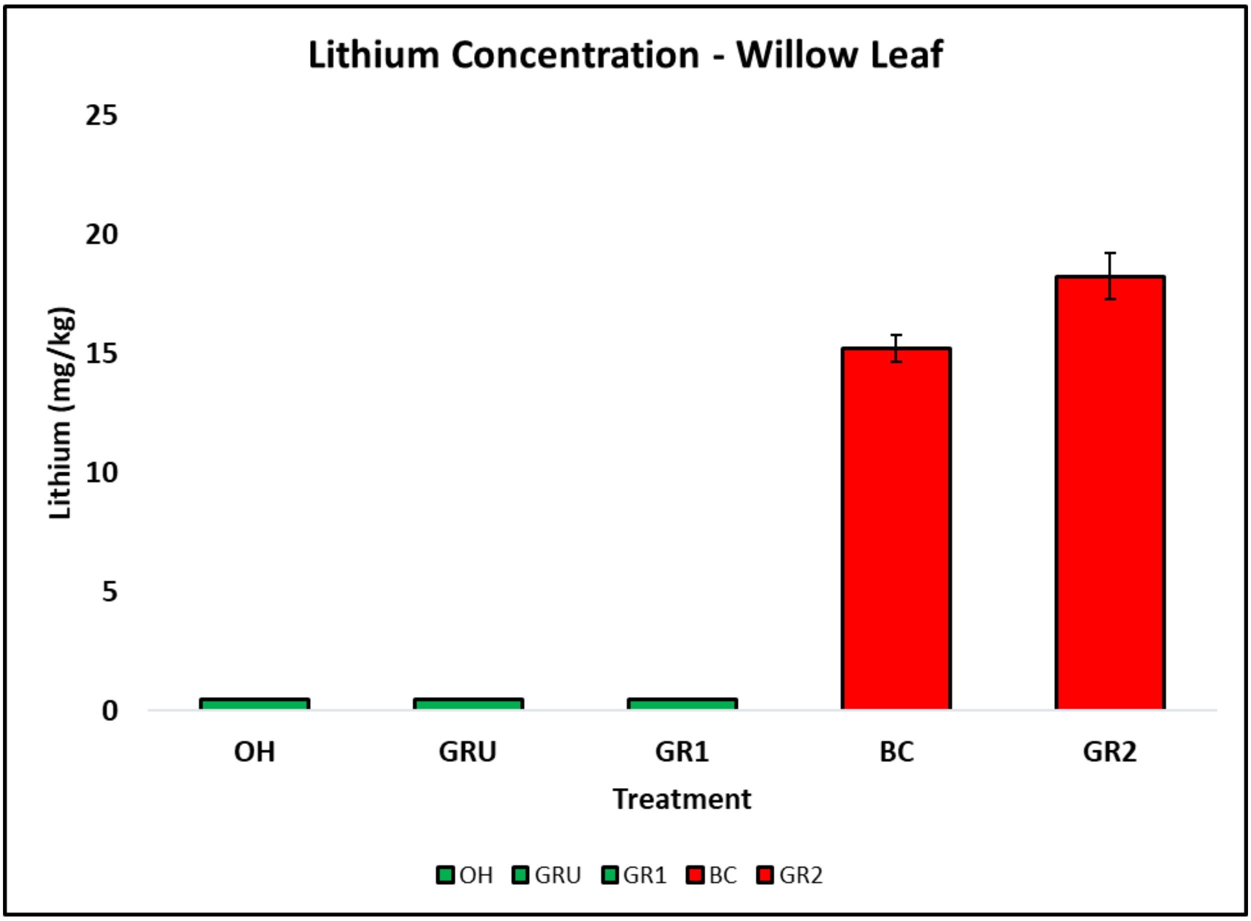

Figure 44: Mean lithium concentration (mg/L) and standard error in willow leaf tissue at all treatments.

Strontium concentration in the willow leaf tissue was elevated at statistically significantly levels at BC (*p*-value <0.01), GR2 (*p*-value <0.05) and GR1 (*p*-value <0.01) compared to the two reference sites. Mean concentration of strontium in the willow leaf tissue with water from the two reference sites; O'Hares and GRU was 34.0 mg/kg and 33.9 mg/kg respectively. Strontium concentration at the willows grown in water from GR1 was 38.5 mg/kg. Mean strontium concentration was elevated in willow leaf tissue grown in water from BC (mean of 37.6 mg/kg) and from GR2 (mean of 37.9 mg/kg) compared with the reference sites (Table 10; Figure 45).

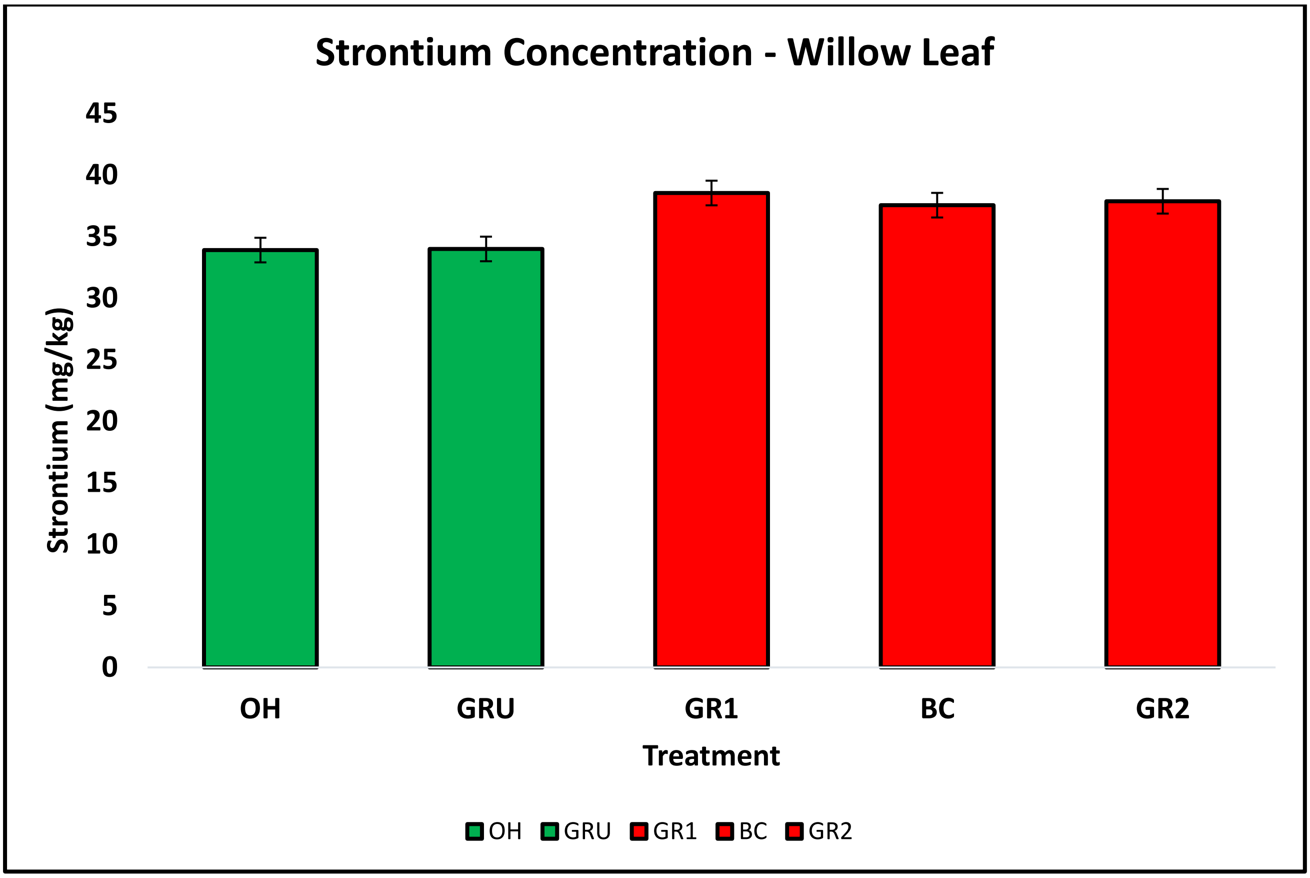

Figure 45: Mean strontium concentration (mg/L) and standard error in willow leaf tissue at all treatments.

Table 10: Summary table of metals in willow leaf tissue at all treatments. Mean, median and p-values are shown. NS indicates that no statically significance difference was observed.

Willow Leaf	**Reference**				**Upstream**			**Discharge**			**Downstream**		
Site Name	**O'Hares**		**GRU**		**GR1**			**BC**			**GR2**		
Variables Metals (mg/kg)	Mean	Med.	Mean	Med.	Mean	Med.	*p* -value	Mean	Med.	*p* -value	Mean	Med.	*p* -value
Aluminium	2.6	2.5	2	2	2	2	NS	2.1	2	NS	2	2	NS
Arsenic	2	2	2	2	2	2	NS	2	2	NS	2	2	NS
Boron	31.5	30	28.5	30	27.3	30	NS	41.7	42	<0.001	41.5	43	<0.001
Barium	23.5	23	23	23	26.8	27	<0.01	24.9	25	NS	24.9	25	NS
Beryllium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Cadmium	0.2	0.2	0.2	0.2	0.2	0.2	NS	0.2	0.2	NS	0.2	0.2	NS
Chromium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Cobalt	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Copper	9.6	9.5	8.5	10	8.8	9	NS	6.2	6	<0.01	7.3	7.5	NS
Iron	49.9	47.5	44.4	45	38.1	37	NS	43.1	43	NS	40.5	41	NS
Mercury	0.1	0.1	0.1	0.1	0.1	0.1	NS	0.1	0.1	NS	0.1	0.1	NS
Lithium	0.5	0.5	0.5	0.5	0.5	0.5	NS	15.2	15	<0.001	18.3	17	<0.001
Manganese	108	105	109	110	111	110	NS	118	120	<0.05	115	110	NS
Molybdenum	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.6	0.5	NS
Nickel	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Lead	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Antimony	3.5	3.5	3.5	3.5	3.5	3.5	NS	3.5	3.5	NS	3.5	3.5	NS
Selenium	1	1	1	1	1	1	NS	1	1	NS	1	1	NS
Tin	0.5	0.5	0.6	0.5	0.5	0.5	NS	0.6	0.5	NS	0.5	0.5	NS
Strontium	34	34	33.9	33	38.5	38	<0.01	37.6	38	<0.05	37.9	37.5	<0.01
Titanium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Uranium	0.1	0.1	0.1	0.1	0.1	0.1	NS	0.1	0.1	NS	0.1	0.1	NS
Zinc	67.9	68	56.1	53	53.6	51	NS	63.4	62	NS	66.6	65	NS

Metal concentration in willow root tissue

There was greater uptake of metals within the willow roots. The metals; barium and strontium were elevated at statistically significant levels in treatments GR1, BC and GR2. The metals; aluminium, boron, lithium, manganese, molybdenum, nickel and uranium were elevated at statistically significant levels in treatments BC and GR2. The metals copper and iron were elevated at statistically significant levels in treatment GR2. The following metals were below detection limits in all treatments; arsenic, beryllium, chromium, mercury, antimony, selenium, tin and titanium. Additionally, there was no statistically significant difference between the following treatments for the metals; cadmium, lead and zinc from the reference sites and GR1, GR2, GR3 and BC (Table 11).

Aluminium concentration in the willow root tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Mean concentration of aluminium in the willow root tissue with water from the two reference sites; O'Hares and GRU was 14.5 mg/kg and 13.4 mg/kg respectively. Mean aluminium concentration at the willows grown in water from GR1 was 15.0 mg/kg. Mean aluminium concentration was elevated in willow root tissue grown in water from BC (186.7 mg/kg) and from GR2 (207.5 mg/kg) (Table 11; Figure 46).

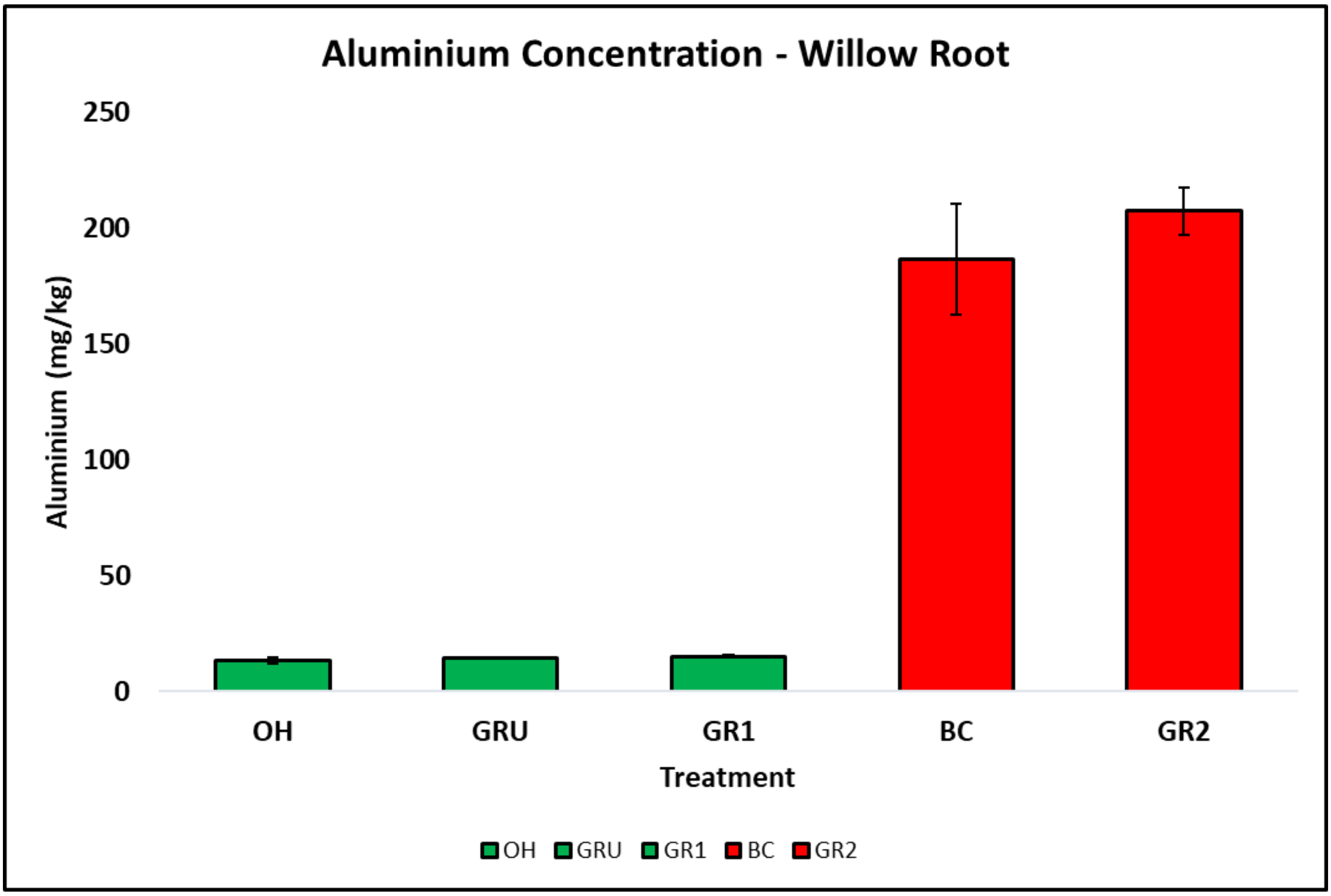

Figure 46: Mean aluminium concentration (mg/L) and standard error in willow root tissue at all treatments.

Boron concentration in the willow root tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Mean concentration of boron in the willow root tissue with water from the two reference sites; O'Hares and GRU was 12.5 mg/kg and 10.0 mg/kg respectively. Mean boron concentration at the willows grown in water from GR1 was 10.0 mg/kg. Mean boron concentration was elevated in willow root tissue grown in water from BC (26.7 mg/kg) and from GR2 (20.0 mg/kg) (Table 11; Figure 47).

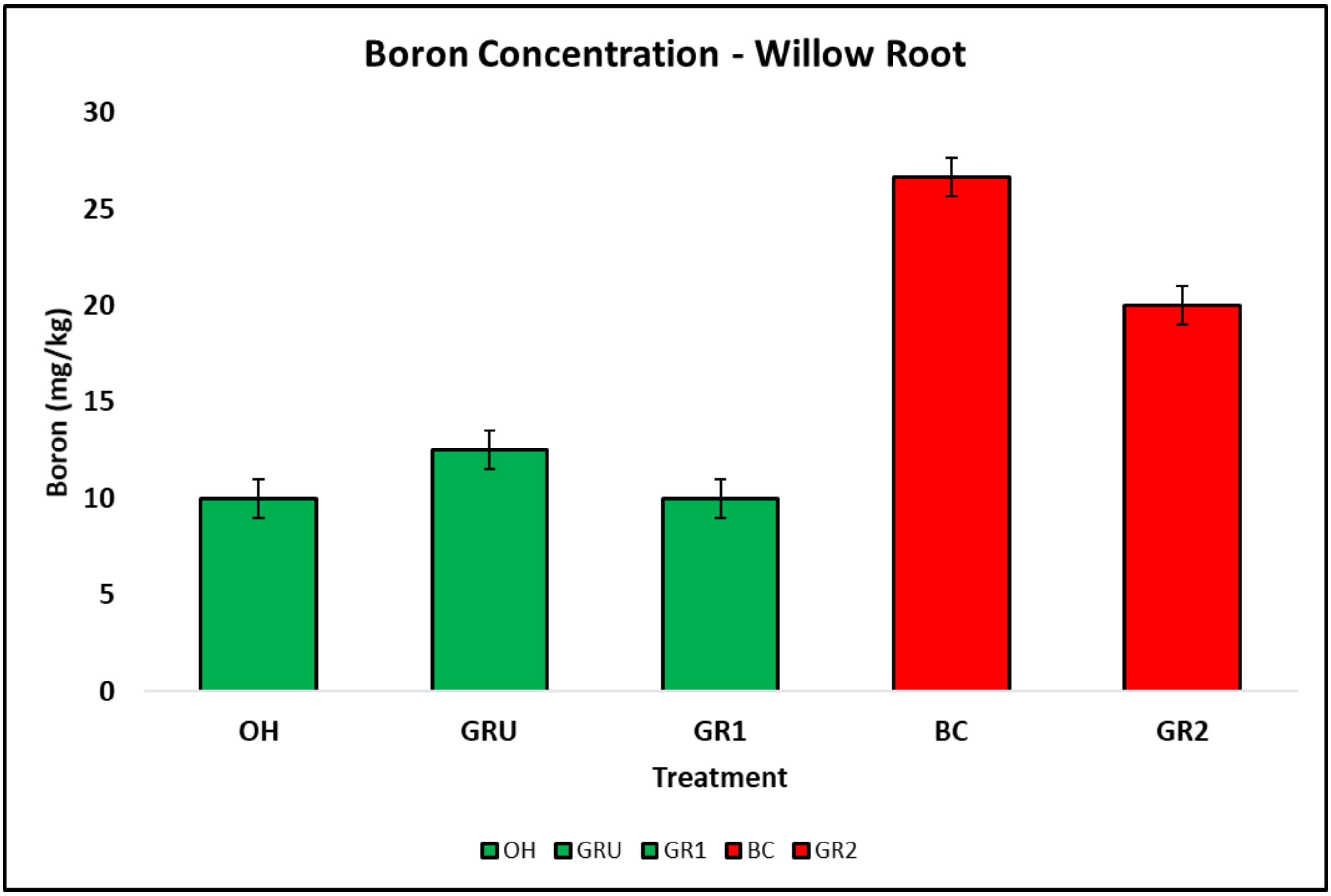

Figure 47: Mean boron concentration (mg/L) and standard error in willow root tissue at all treatments.

Barium concentration in the willow root tissue was elevated at statistically significantly levels at treatments GR1, BC and GR2 (*p*-value <0.01) compared to the two reference sites. Mean concentration of barium in the willow root tissue with water from the two reference sites; O'Hares and GRU was 11.3 mg/kg and 10.6 mg/kg respectively. Mean barium concentration at the willows grown in water from GR1 was 20.4 mg/kg. Mean barium concentration in willow root tissue grown in water from BC was 193.3 mg/kg and 155.0 mg/kg in willow tissue grown in water from GR2 (Table 11; Figure 48).

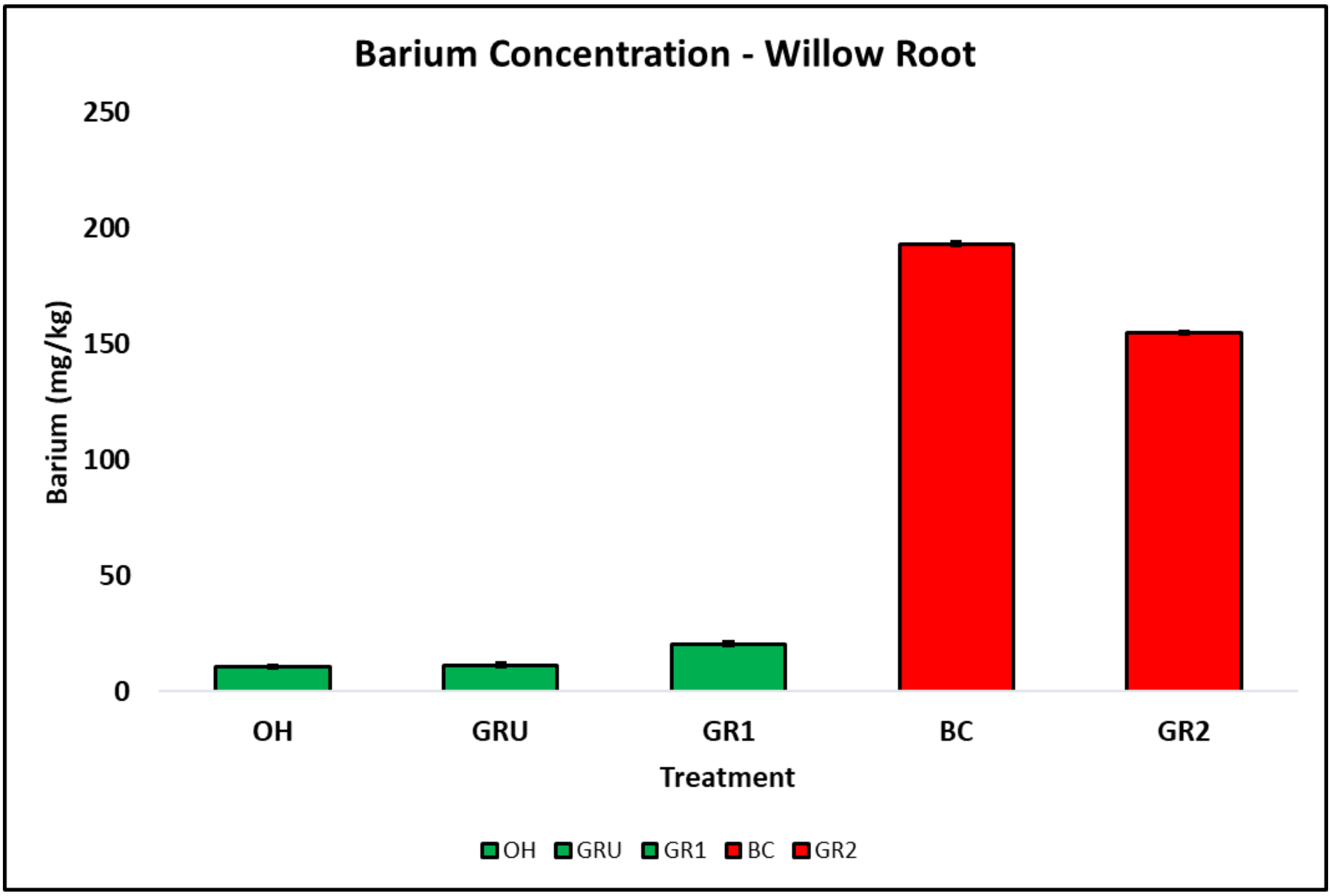

Figure 48: Mean barium concentration (mg/L) and standard error in willow root tissue at all treatments.

Copper concentration in the willow root tissue was elevated at statistically significantly levels at treatment GR2 (*p*-value <0.01) compared to the two reference sites. Mean concentration of copper in the willow root tissue with water from the two reference sites; O'Hares and GRU was 25.5 mg/kg and 24.8 mg/kg respectively. Mean copper concentration at the willows grown in water from GR1 was 28.2 mg/kg and 34.0 mg/kg in treatment BC. Copper concentration was elevated at treatment GR2 with a mean of 44.5 mg/kg (Table 11; Figure 49).

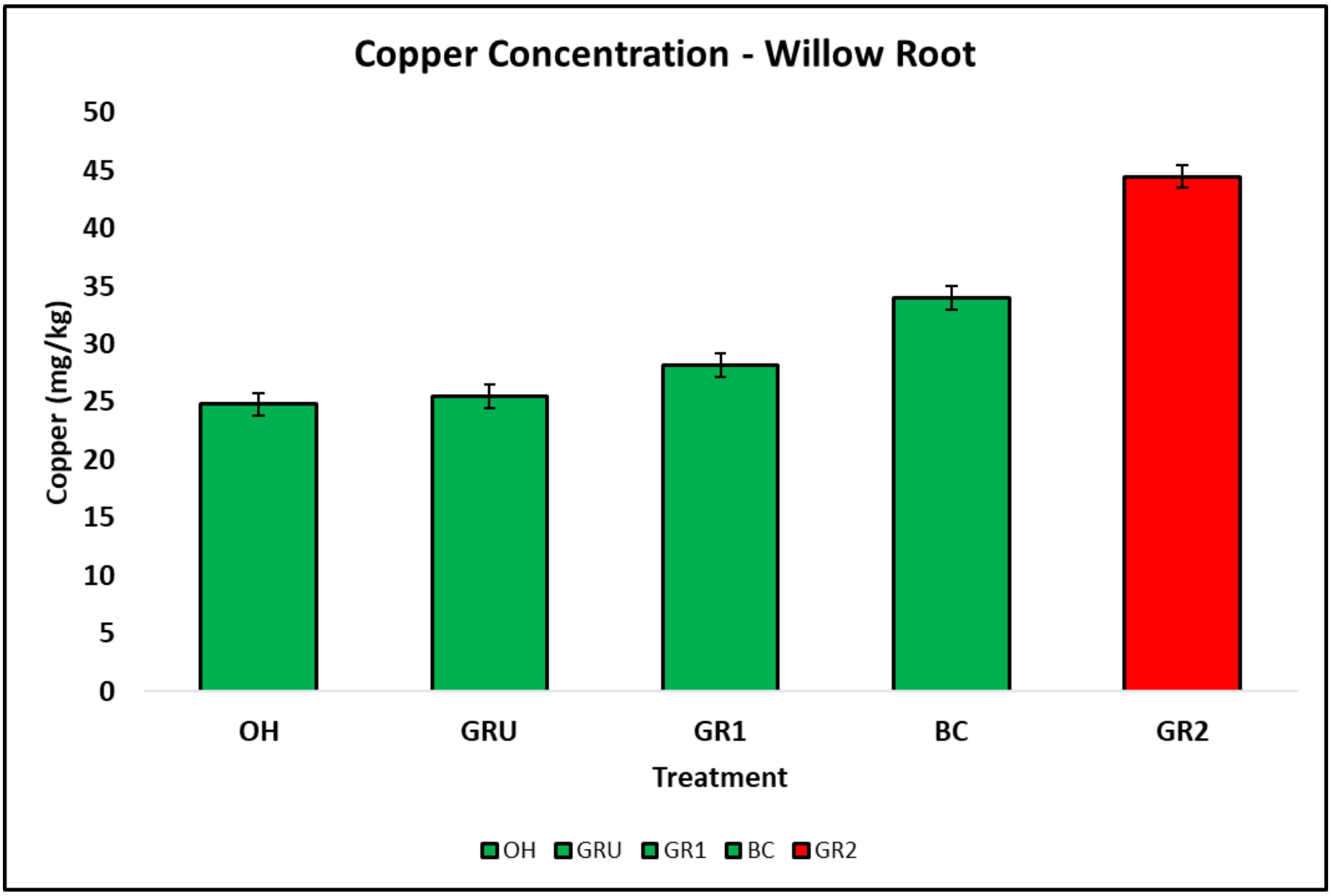

Figure 49: Mean copper concentration (mg/L) and standard error in willow root tissue at all treatments.

Iron concentration in the willow root tissue was elevated at statistically significantly levels at treatment GR2 (*p*-value <0.01) and statistically significantly lower at GR1 compared to the two reference sites. Mean concentration of iron in the willow root tissue with water from the two reference sites; O'Hares and GRU was 157.5 mg/kg and 101.6 mg/kg respectively. Mean iron concentration at the willows grown in water from GR1 was lower at 66.2 mg/kg. Mean iron concentration at the willows grown in treatment BC was 150.0 mg/kg. Iron concentration was elevated at treatment GR2 with a mean of 262.5 mg/kg (Table 11).

Lithium concentration in the willow root tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Concentration of lithium in the willow root tissue with water from the two reference sites; O'Hares and GRU were below the laboratory detection limit of 1 mg/kg. Lithium concentrations in the root tissue of treatment GR1 was also below the laboratory detection limit Mean lithium concentration was elevated in willow root tissue grown in water from BC (8.7 mg/kg) and from GR2 (9.5 mg/kg) (Table 11; Figure 50).

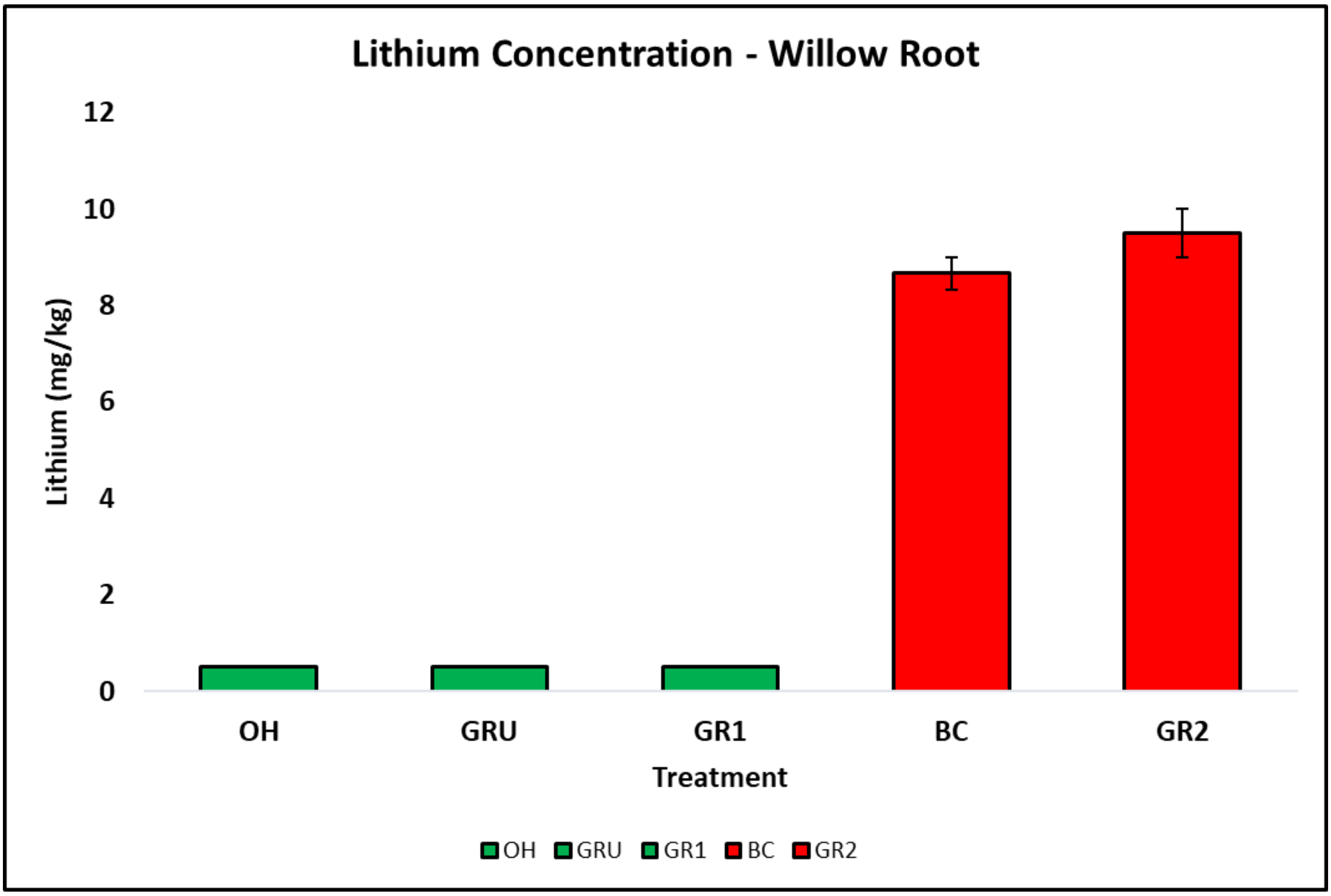

Figure 50: Mean lithium concentration (mg/L) and standard error in willow root tissue at all treatments.

Manganese concentration in the willow root tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Mean concentration of manganese in the willow root tissue with water from the two reference sites; O'Hares and GRU was 17.8 mg/kg and 12.6 mg/kg respectively. Mean manganese concentration at the willows grown in water from GR1 was 13.8 mg/kg. Mean manganese concentration was elevated in willow root tissue grown in water from BC (25.3 mg/kg) and from GR2 (30.8 mg/kg) (Table 11; Figure 51).

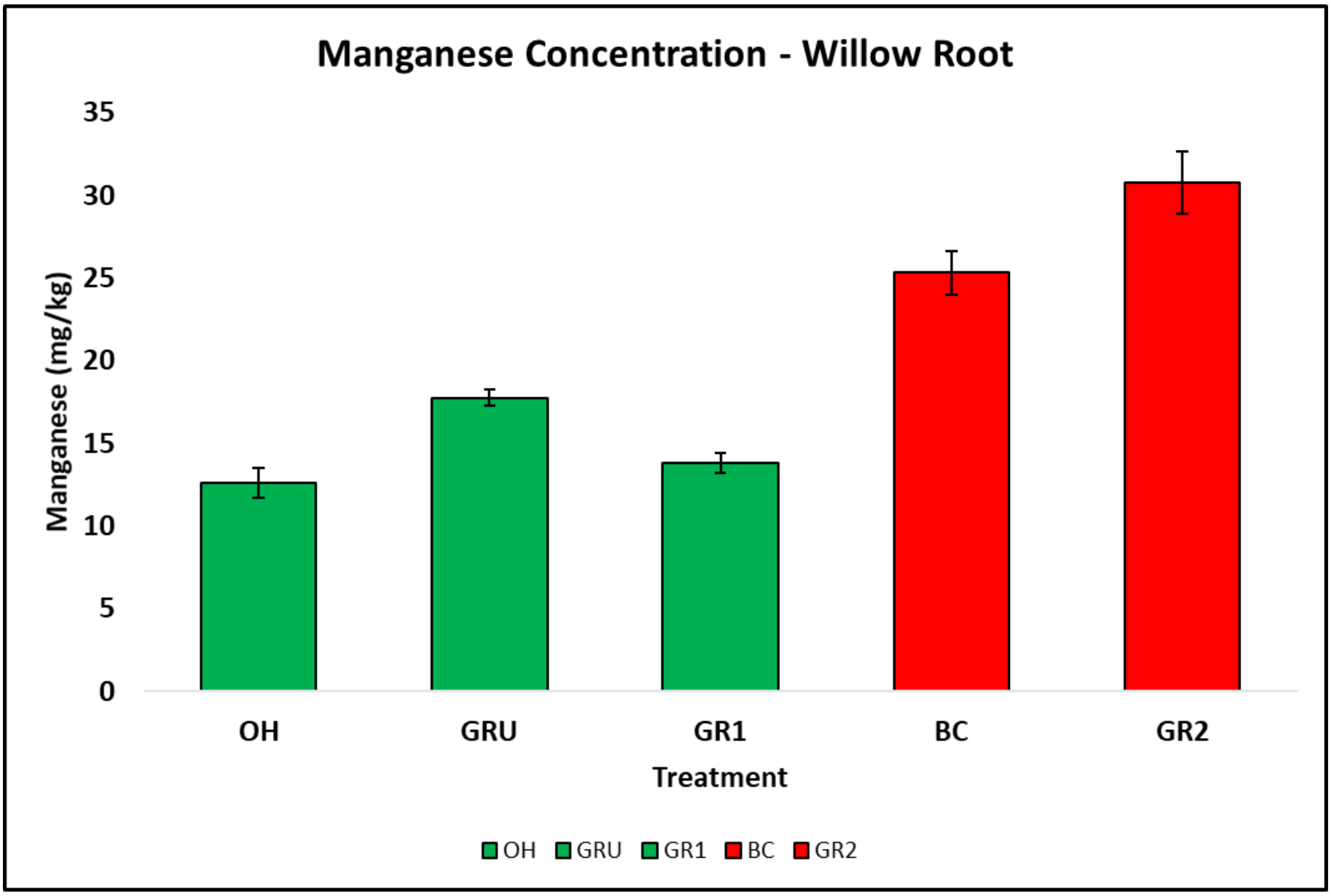

Figure 51: Mean manganese concentration (mg/L) and standard error in willow root tissue at all treatments.

Molybdenum concentration in the willow root tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Concentration of molybdenum in the willow root tissue with water from the two reference sites; O'Hares and GRU were below the laboratory detection limit of 1 mg/kg. Molybdenum concentrations in the root tissue of treatment GR1 was also below the laboratory detection limit. Mean molybdenum concentration was elevated in willow root tissue grown in water from BC (13.7 mg/kg) and from GR2 (13.8 mg/kg) (Table 11; Figure 52).

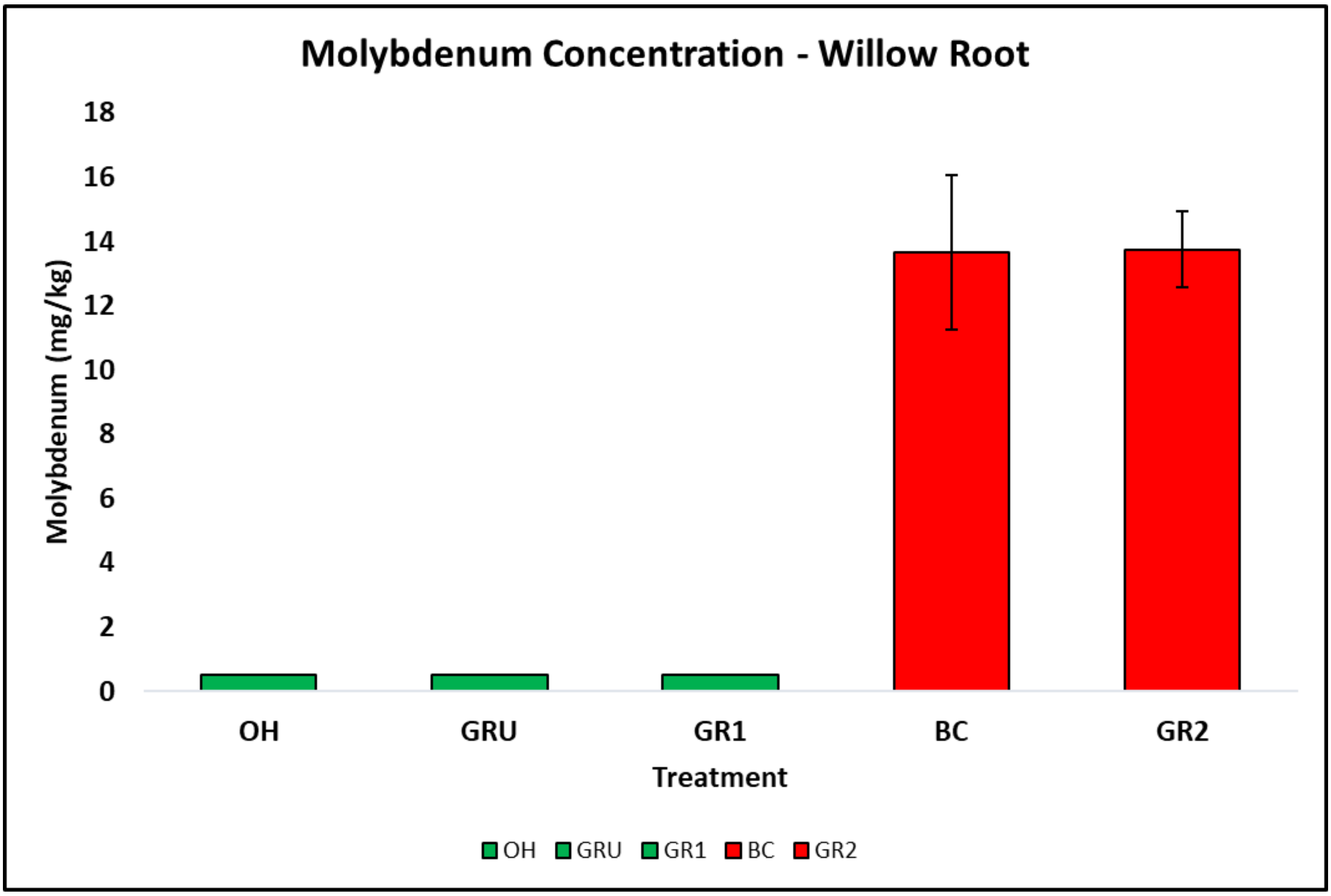

Figure 52: Mean molybdenum concentration (mg/L) and standard error in willow root tissue at all treatments.

Nickel concentration in the willow root tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Mean concentration of nickel in the willow root tissue with water from the two reference sites; O'Hares and GRU was 0.8 mg/kg and 0.7 mg/kg respectively. Two replicates from each treatment; O'Hares and GRU were at the laboratory detection limit of 1 mg/kg. Mean nickel concentration at the willows grown in water from GR1 was 1.1 mg/kg. Mean nickel concentration was elevated in willow root tissue grown in water from BC (2.7 mg/kg) and from GR2 (3.0 mg/kg) (Table 11; Figure 53).

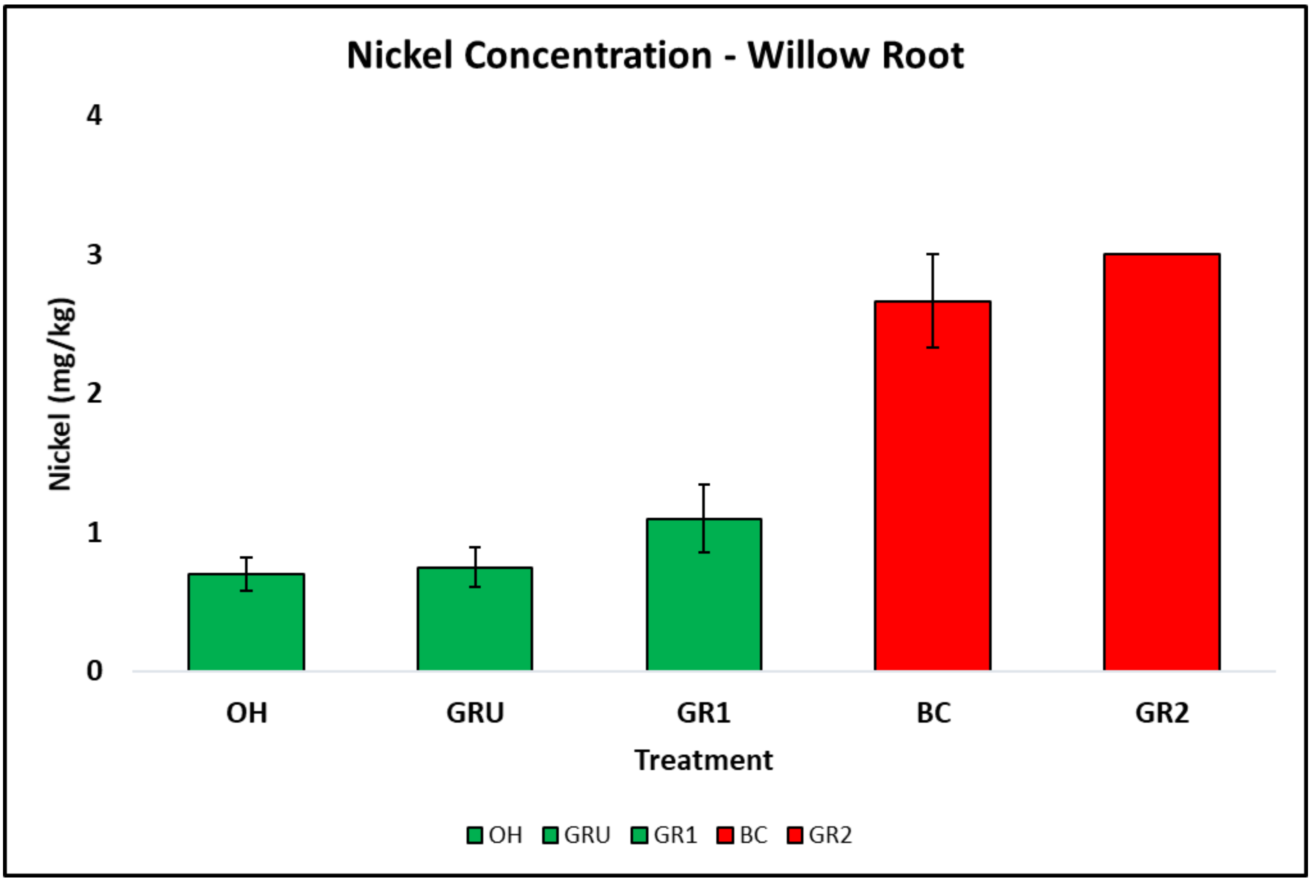

Figure 53: Mean nickel concentration (mg/L) and standard error in willow root tissue at all treatments.

Strontium concentration in the willow root tissue was elevated at statistically significantly levels at treatments GR1, BC and GR2 (*p*-value <0.01) compared to the two reference sites. Mean concentration of strontium in the willow root tissue with water from the two reference sites; O'Hares and GRU was 13.0 mg/kg and 11.8 mg/kg respectively. Mean strontium concentration at the willows grown in water from GR1 was 17.8 mg/kg. Mean strontium concentration in willow root tissue grown in water from BC was 90.7 mg/kg and 89.0 mg/kg in willow tissue grown in water from GR2 (Table 11; Figure 54).

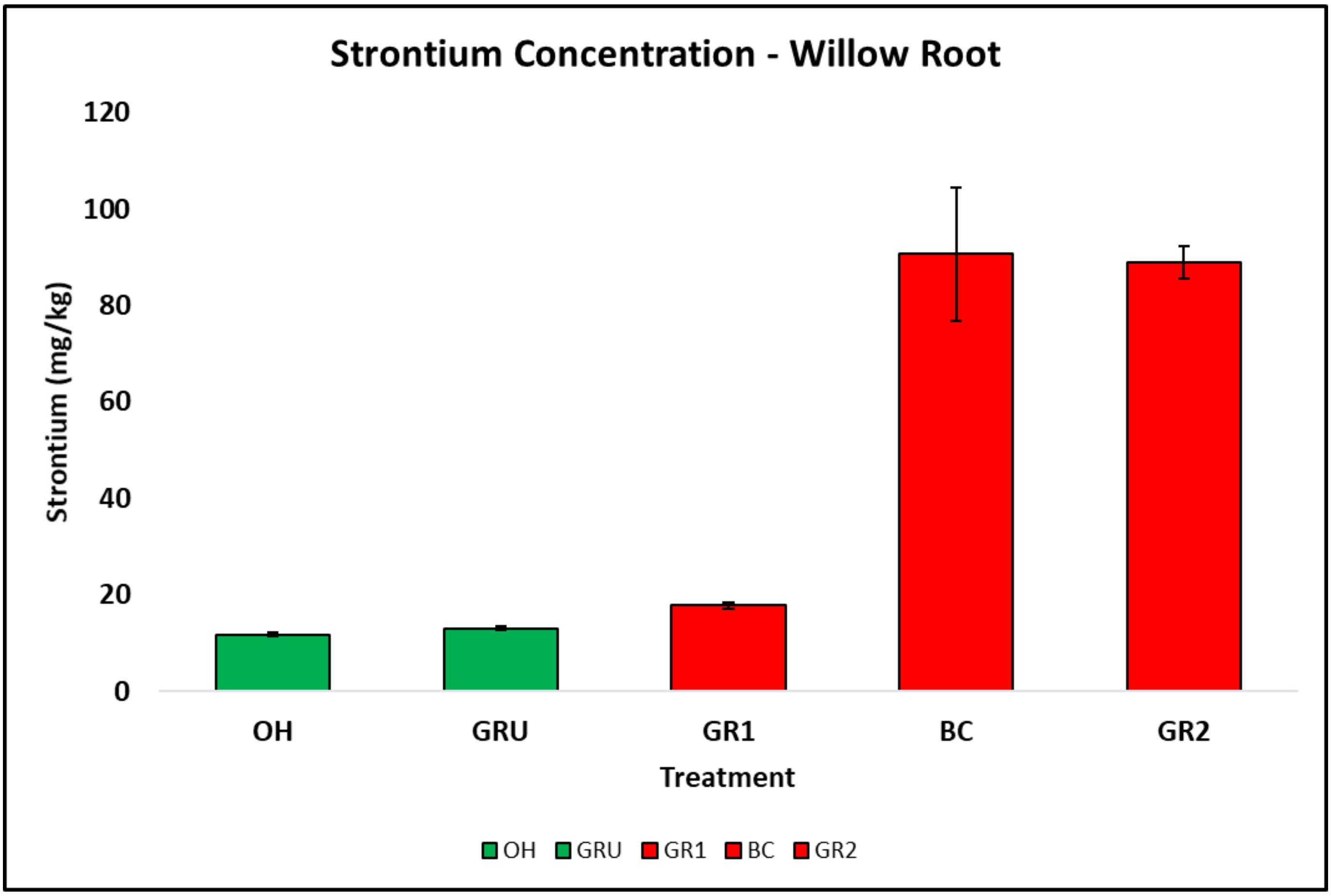

Figure 54: Mean strontium concentration (mg/L) and standard error in willow root tissue at all treatments.

Uranium concentration in the willow root tissue was elevated at statistically significantly levels at BC (*p*-value <0.001) and at GR2 (*p*-value <0.001) compared to the two reference sites. Concentration of uranium in the willow root tissue with water from the two reference sites; O'Hares and GRU were below the laboratory detection limit of 0.1 mg/kg. Uranium concentrations in the root tissue of treatment GR1 was also below the laboratory detection limit. Mean uranium concentration was elevated in willow root tissue grown in water from BC (3.6 mg/kg) and from GR2 (3.0 mg/kg) (Table 11; Figure 55).

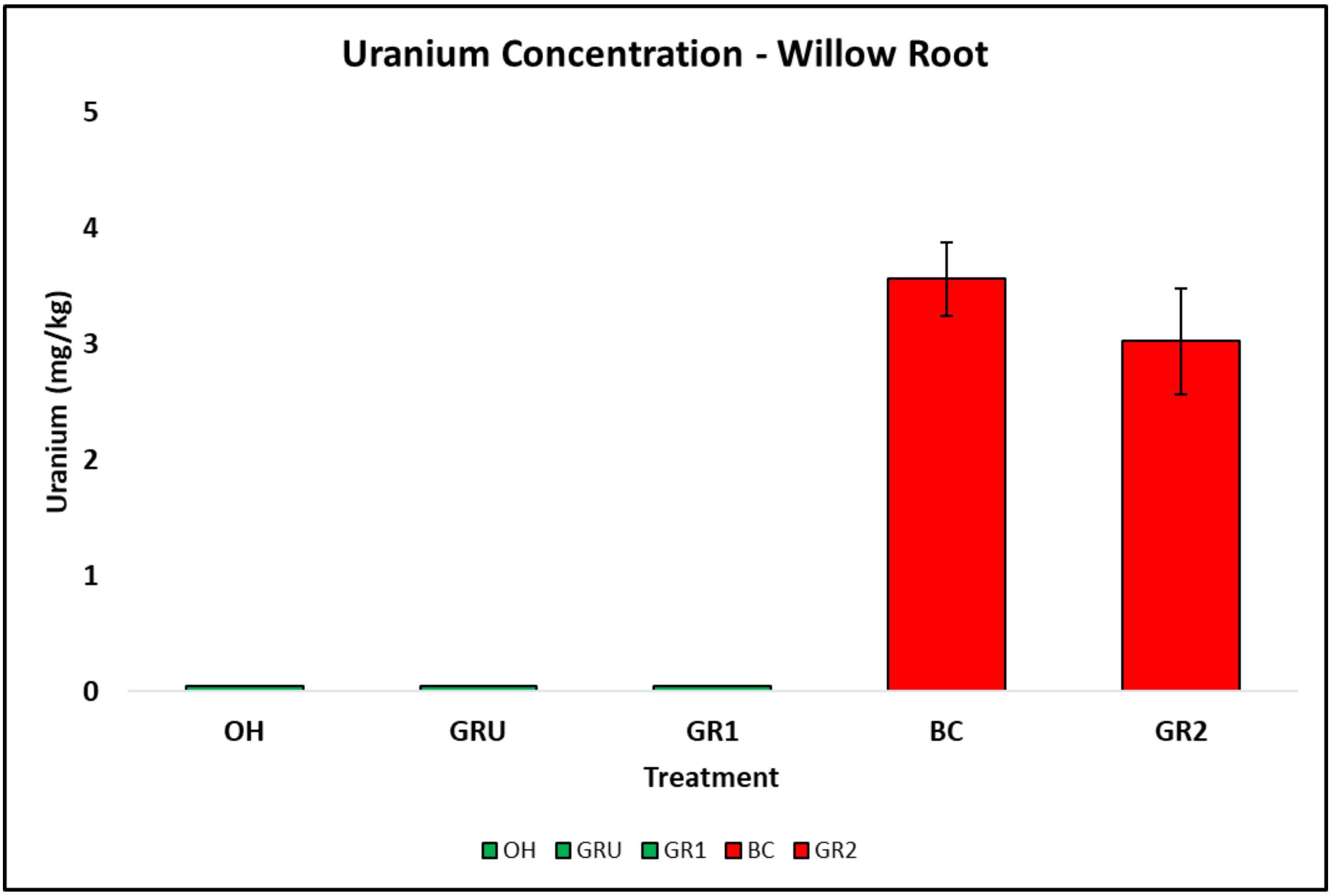

Figure 55: Mean uranium concentration (mg/L) and standard error in willow root tissue at all treatments.

Table 11: Summary table of metals in willow root tissue at all treatments. Mean, median and p-values are shown. NS indicates that no statically significance difference was observed.

Willow Root	**Reference**				**Upstream**			**Discharge**			**Downstream**		
Site Name	**O'Hares**		**GRU**		**GR1**			**BC**			**GR2**		
Metals (mg/kg)	Mean	Med.	Mean	Med.	Mean	Med.	p -value	Mean	Med.	p -value	Mean	Med.	p -value
Aluminium	14.5	14.5	13.4	13.0	15.0	15.0	NS	186.7	200.0	<0.001	207.5	205.0	<0.001
Arsenic	2.0	2.0	2.0	2.0	2.0	2.0	NS	2.0	2.0	NS	2.0	2.0	NS
Boron	12.5	10.0	10.0	10.0	10.0	10.0	NS	26.7	30.0	<0.001	20.0	20.0	<0.001
Barium	11.3	11.5	10.6	11.0	20.4	20.0	<0.001	193.3	200.0	<0.001	155.0	155.0	<0.001
Beryllium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Cadmium	1.5	1.5	1.3	0.4	1.3	0.8	NS	1.0	0.7	NS	2.0	2.0	NS
Chromium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Cobalt	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.9	1.0	<0.001
Copper	25.5	27.5	24.8	24.0	28.2	26.0	NS	34.0	31.0	NS	44.5	43.5	<0.001
Iron	157.5	160.0	101.6	88.0	66.2	66.0	<0.01	150.0	160.0	NS	262.5	260.0	<0.001
Mercury	0.1	0.1	0.1	0.1	0.1	0.1	NS	0.1	0.1	NS	0.1	0.1	NS
Lithium	0.5	0.5	0.5	0.5	0.5	0.5	NS	8.7	9.0	<0.001	9.5	9.0	<0.001
Manganese	17.8	17.5	12.6	13.0	13.8	13.0	NS	25.3	24.0	<0.001	30.8	31.0	<0.001
Molybdenum	0.5	0.5	0.5	0.5	0.5	0.5	NS	13.7	15.0	<0.001	13.8	13.0	<0.001
Nickel	0.8	0.8	0.7	0.5	1.1	1.0	NS	2.7	3.0	<0.001	3.0	3.0	<0.001
Lead	2.0	1.5	2.4	2.0	3.0	3.0	NS	1.0	1.0	NS	1.6	2.0	NS
Antimony	3.5	3.5	3.5	3.5	3.5	3.5	NS	3.5	3.5	NS	3.5	3.5	NS
Selenium	1.0	1.0	1.0	1.0	1.0	1.0	NS	1.0	1.0	NS	1.0	1.0	NS
Tin	0.5	0.5	0.5	0.5	0.7	0.5	<0.05	0.5	0.5	NS	0.6	0.5	NS
Strontium	13.0	13.0	11.8	12.0	17.8	18.0	<0.001	90.7	98.0	<0.001	89.0	91.5	<0.001
Titanium	0.5	0.5	0.5	0.5	0.5	0.5	NS	0.5	0.5	NS	0.5	0.5	NS
Uranium	0.1	0.1	0.1	0.1	0.1	0.1	NS	3.6	3.3	<0.001	3.0	3.1	<0.001
Zinc	100.5	100.0	86.2	84.0	75.2	74.0	NS	85.3	86.0	NS	88.0	90.0	NS

Magnification of metals

Biomagnification of metals was most apparent with metals; barium, boron, copper, lithium, molybdenum, strontium and uranium which were found to be occurring at multiple levels in the ecosystem. Typically, the highest concentrations of metals were found in plant tissue, followed by the sediment then the water column. Typically, the root tissue had increased metal concentration concentrations than those observed in the leaf tissue with the exceptions of boron, lithium and manganese. All values have been converted into parts per million (ppm) for easier comparison.

Barium bioaccumulated from the water column to the sediment and from the water column to plant tissue. Mean barium concentration was highest at the two sites and corresponding treatments; BC and GR2 exposed to wastewater from West Cliff Colliery. The concentration of barium at BC increased from water (0.304 ppm) by approximately 680 times to sediment (206 ppm). This magnification was also present in willow tissue. The barium concentration in treatment BC increased by a factor of 82 times from water to leaf tissue (25 ppm) and a by a factor of 636 times from water to root tissue (193 ppm). A similar result was observed at GR2. The concentration of barium at GR2 increased from water (0.255 ppm) by approximately 778 times to sediment (198 ppm). This magnification was also present in willow tissue. The barium concentration in treatment GR2 increased by a factor of 98 times from water to leaf tissue (25 ppm) and a by a factor of 608 times from water to root tissue (155 ppm) (Figure 56).

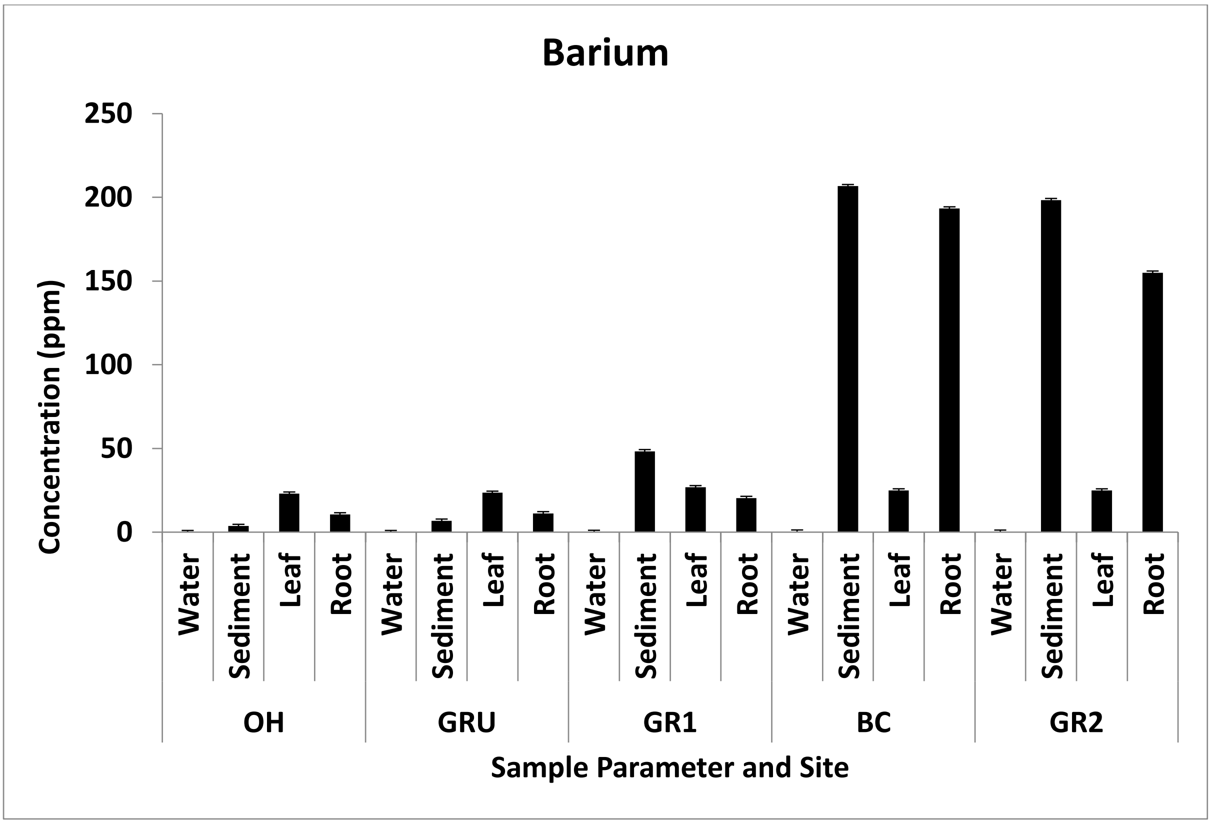

Figure 56: Mean barium concentration (ppm) at all sites and respective treatments.

Boron bioaccumulated from the water column to plant tissue. Mean Boron concentration was highest at the two sites and corresponding treatments; BC and GR2 exposed to wastewater from West Cliff Colliery. The Boron concentration in treatment BC increased by a factor of 877 times from water (0.048 ppm) to leaf tissue (42 ppm) and a by a factor of 561 times from water to root tissue (27 ppm). A similar result was observed at GR2. The Boron concentration in treatment GR2 increased by a factor of 1038 times from water (0.04 ppm) to leaf tissue (42 ppm) and a by a factor of 500 times from water to root tissue (20 ppm) (Figure 57).

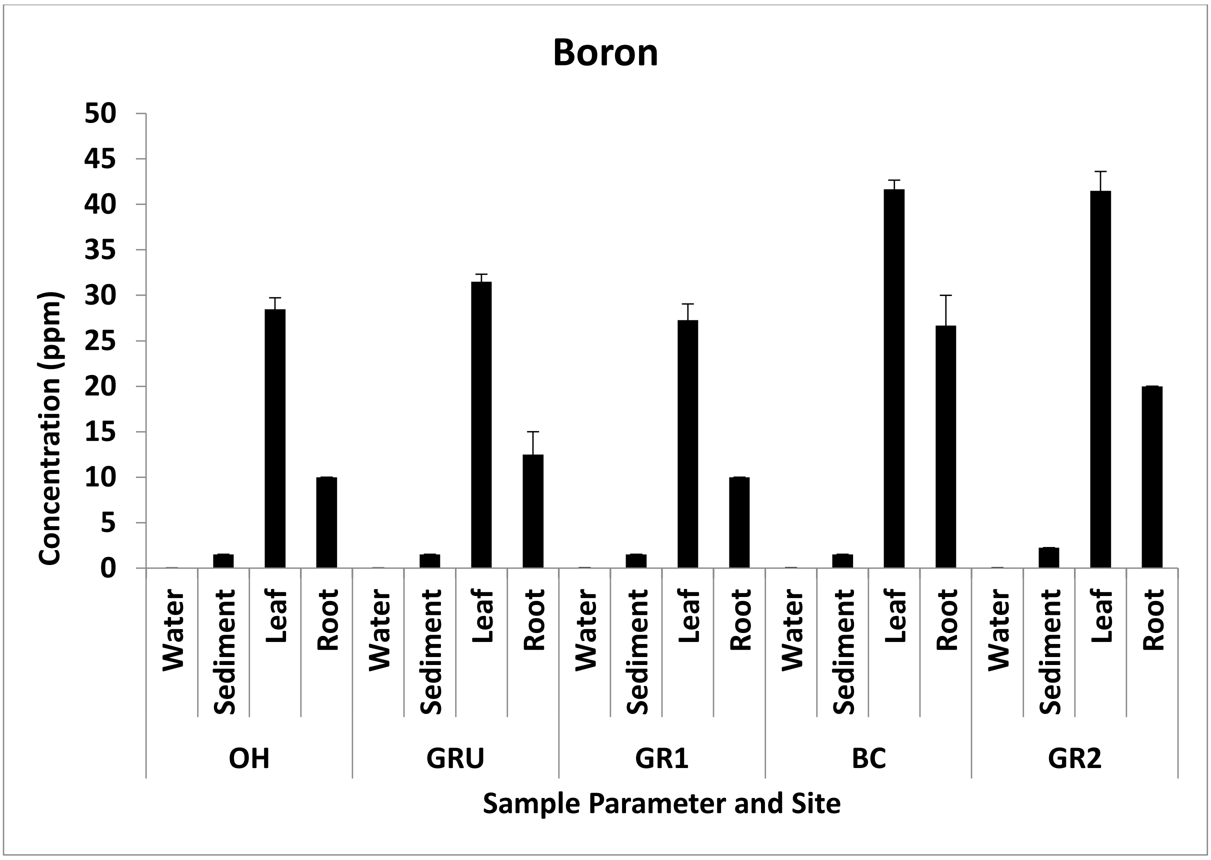

Figure 57: Mean boron concentration (ppm) at all sites and respective treatments.

Copper bioaccumulated from the water column to the sediment and from the water column to plant tissue. Mean copper concentration was in the willow root tissue was highest at the two treatments; BC and GR2 exposed to wastewater from West Cliff Colliery. The concentration of copper at BC increased from water (0.0012 ppm) by approximately 2361 times to sediment (2.8 ppm). This magnification was also present in willow tissue. The copper concentration in treatment BC increased by a factor of 5185 times from water to leaf tissue (6.2 ppm) and a by a factor of 28333 times from water to root tissue (34 ppm). A similar result was observed at GR2. The concentration of copper at GR2 increased from water (0.0014 ppm) by approximately 3929 times to sediment (5.5 ppm). This magnification was also present in willow tissue. The copper concentration in treatment GR2 increased by a factor of 5179 times from water to leaf tissue (7.25 ppm) and a by a factor of 31786 times from water to root tissue (44.5 ppm) (Figure 58).

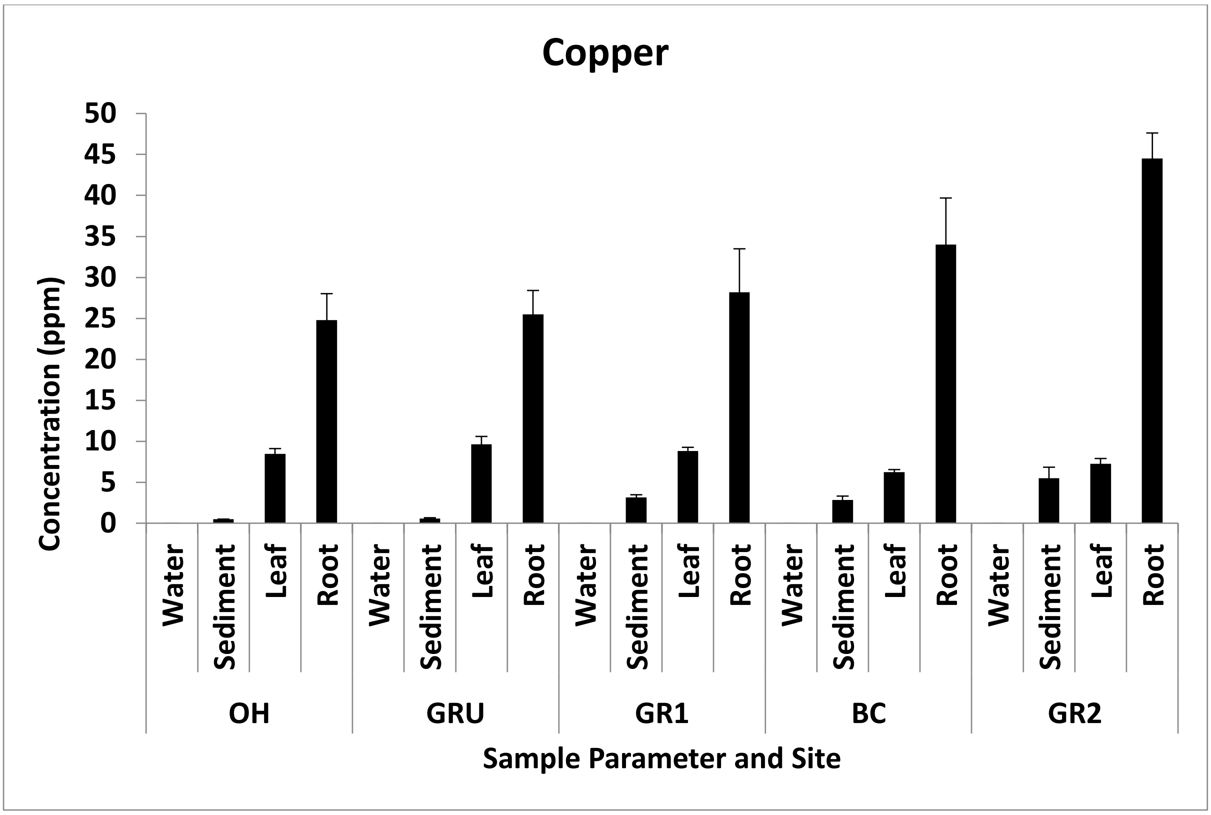

Figure 58: Mean copper concentration (ppm) at all sites and respective treatments.

Lithium bioaccumulated from the water column to the sediment and from the water column to plant tissue. Mean lithium concentration was highest at the two sites and corresponding treatments; BC and GR2 exposed to wastewater from West Cliff Colliery. The concentration of lithium at BC increased from water (0.317 ppm) by approximately 39 times to sediment (12 ppm). This magnification was also present in willow tissue. The lithium concentration in treatment BC increased by a factor of 48 times from water to leaf tissue (15 ppm) and a by a factor of 27 times from water to root tissue (8.6 ppm). A similar result was observed at GR2. The concentration of lithium at GR2 increased from water (0.28 ppm) by approximately 40 times to sediment (11 ppm). This magnification was also present in willow tissue. The lithium concentration in treatment GR2 increased by a factor of 65 times from water to leaf tissue (18 ppm) and a by a factor of 34 times from water to root tissue (9.5 ppm) (Figure 59).

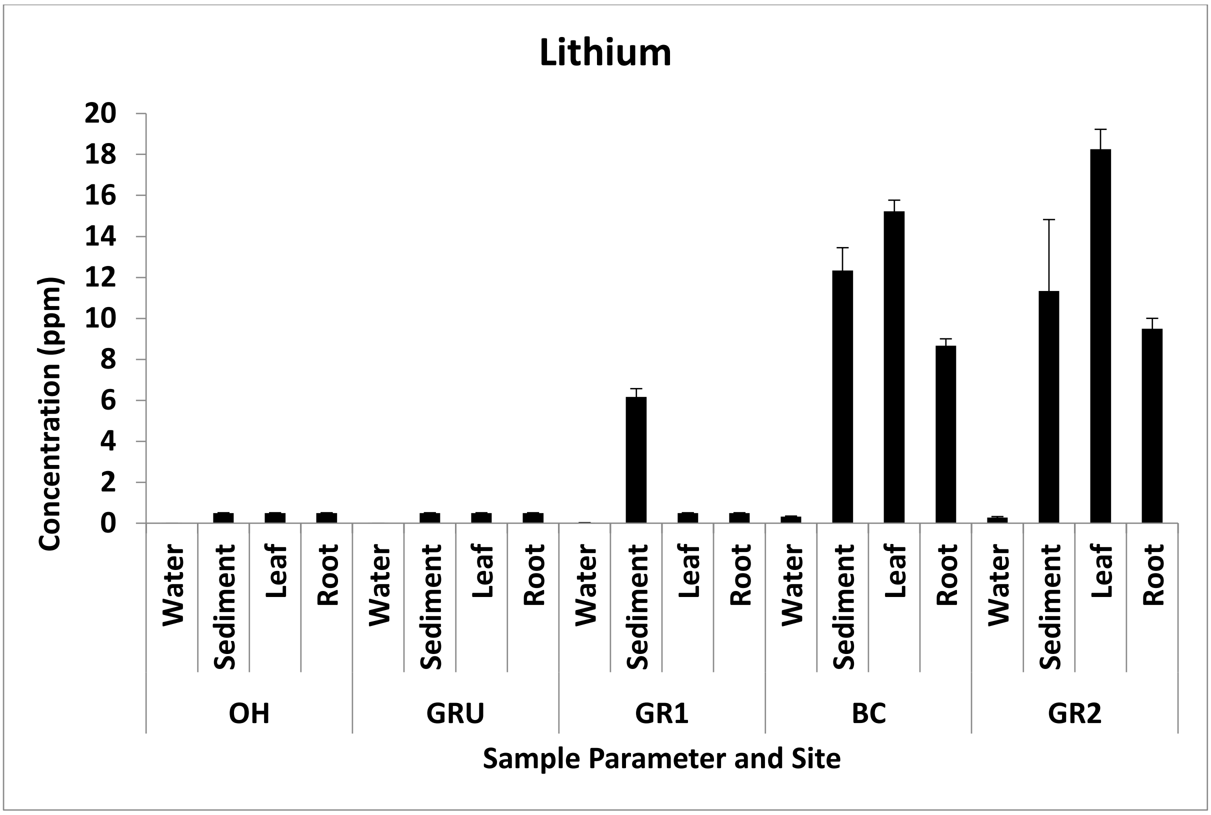

Figure 59: Mean lithium concentration (ppm) at all sites and respective treatments.

Molybdenum bioaccumulated from the water column to plant tissue. Mean molybdenum concentration in willow root tissue was highest at the two treatments; BC and GR2 exposed to wastewater from West Cliff Colliery. The concentration of molybdenum at BC increased from water (0.0292 ppm) by approximately 468 times root tissue (13.7 ppm). A similar result was observed at GR2. The concentration of molybdenum at GR2 increased from water (0.0275 ppm) by approximately 500 times from water to root tissue (13.8 ppm) (Figure 60).

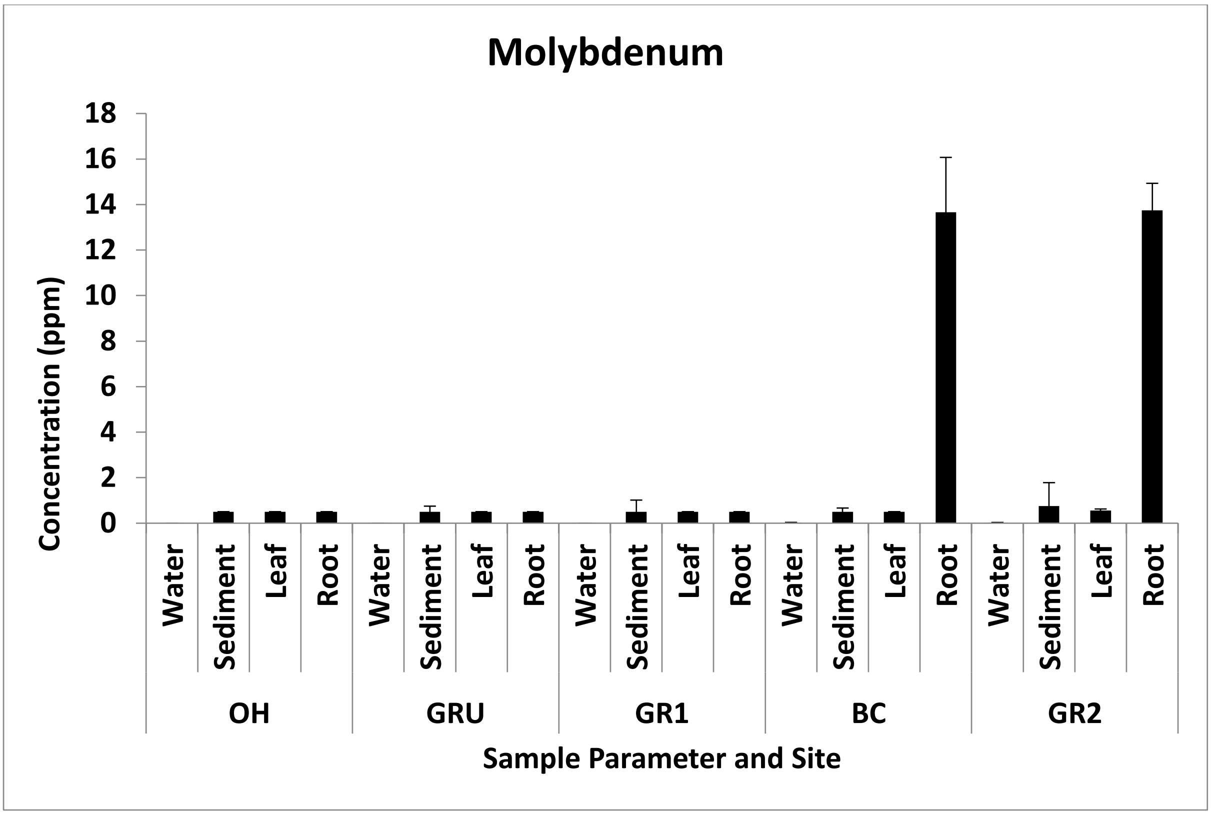

Figure 60: Mean molybdenum concentration (ppm) at all sites and respective treatments.

Strontium bioaccumulated from the water column to the sediment and from the water column to plant tissue. Mean strontium concentration in the sediment and willow root tissue was highest at the two sites and corresponding treatments; BC and GR2 exposed to wastewater from West Cliff Colliery. The concentration of strontium at BC increased from water (0.188 ppm) by approximately 373 times to sediment (70 ppm). This magnification was also present in willow tissue. The strontium concentration in treatment BC increased by a factor of 200 times from water to leaf tissue (38 ppm) and a by a factor of 482 times from water to root tissue (91 ppm). A similar result was observed at GR2. The concentration of strontium at GR2 increased from water (0.168 ppm) by approximately 384 times to sediment (64.5 ppm). This magnification was also present in willow tissue. The strontium concentration in treatment GR2 increased by a factor of 225 times from water to leaf tissue (38 ppm) and a by a factor of 530 times from water to root tissue (89 ppm) (Figure 61).

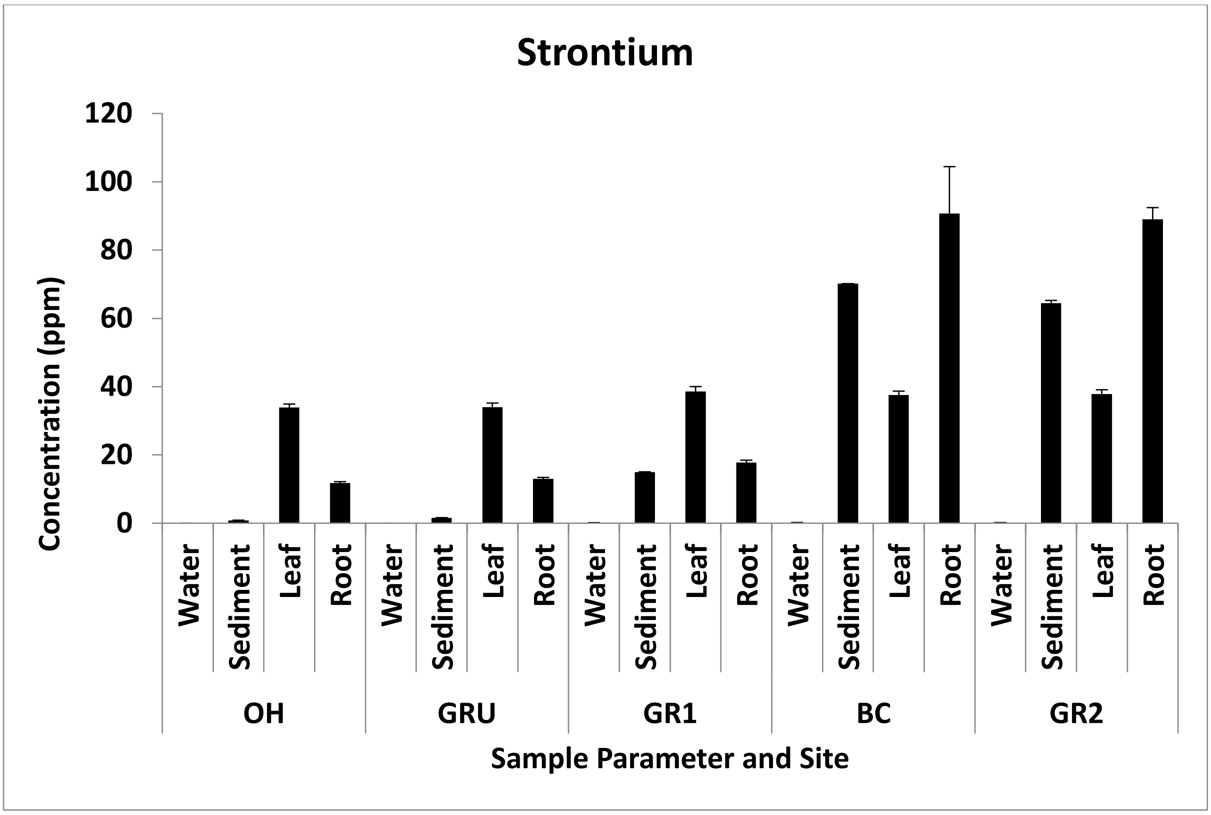

Figure 61: Mean strontium concentration (ppm) at all sites and respective treatments.

Uranium bioaccumulated from the water column to the sediment and from the water column to plant tissue. Mean uranium concentration was highest at the two sites and corresponding treatments; BC and GR2 exposed to wastewater from West Cliff Colliery. The concentration of uranium at BC increased from water (0.008 ppm) by approximately 51 times to sediment (0.4 ppm). This magnification was much greater in willow tissue. The uranium concentration in treatment BC increased by a factor of 455 times from water to root tissue (3.6 ppm). A similar result was observed at GR2. The concentration of uranium at GR2 increased from water (0.007 ppm) by approximately 89 times to sediment (0.6 ppm). This magnification was also present in willow tissue. The uranium concentration in treatment GR2 increased by a factor of 433 times from water to root tissue (3.0 ppm) (Figure 62).

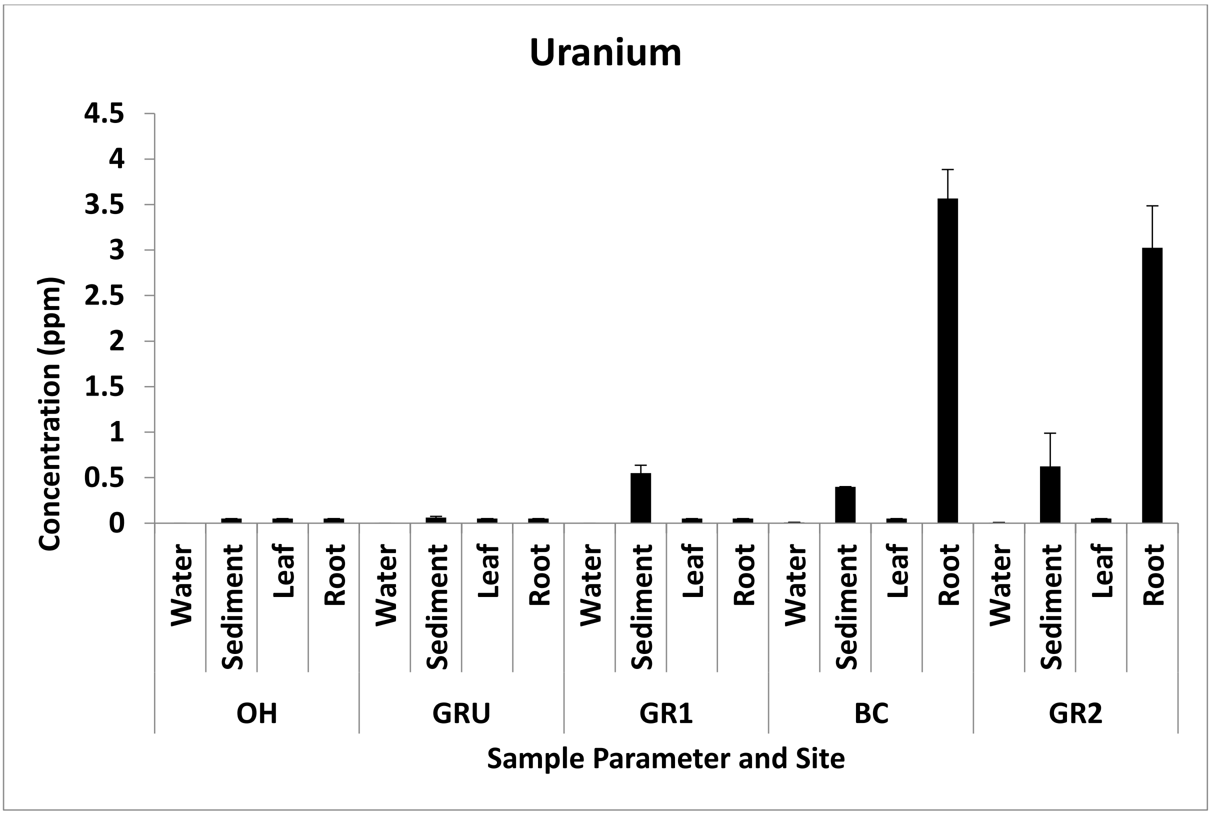

Figure 62: Mean uranium concentration (ppm) at all sites and respective treatments.

Discussion

The discharge of highly polluted wastewater from the West Cliff Colliery into Brennans Creek has caused ecological degradation of the Georges River. The wastewater was characterised by high concentrations of anions, cations and metals at levels that often exceed the recommended guidelines for the protection of 95% of species in upland waterways (ANZECC 2000). The inflow of wastewater into the Georges River has resulted in statically significant increases in; electrical conductivity, pH levels and a suite of metals including; antimony, aluminium, arsenic, barium, boron, cobalt, lithium, molybdenum, nickel, strontium, titanium, uranium and zinc. This study has identified several pollutants in both the sediment and in the water column that continue to persist 22km downstream of the discharge point, at concentrations that are significantly different to the reference sites. The extent of the pollution plume is an important factor when determining the effect of coal mine wastewater discharge on a receiving waterway. This is especially important when the pollution source is located at the headwaters of the catchment, as is the case with the Georges River. Legacy pollution was observed above the inflow of wastewater from West Cliff Colliery at GR1. GR1 received small volumes of wastewater discharge on a sporadic basis from Appin East Colliery prior to and during the study period. This legacy pollution is most apparent when the sediment composition is examined, which reveals the presence of metals at elevated concentrations compared with other sites (GRU and OH) that lack any major forms of catchment disturbance. It was concluded that GR1 would act as a comparison of the impact of the West Cliff Colliery discharge on the Georges River and not the total impact to the Georges River from underground coal mining.

The sediment downstream of the mine wastewater discharge had elevated concentrations of most metals that were present in the wastewater discharge compared to reference conditions. The metals that were present in the sediment at statically significantly elevated concentrations were; aluminium, barium, chromium, cobalt, copper, iron, lead, lithium, manganese, nickel, strontium, titanium, uranium and zinc. There was significant degree of accumulation of metals in the sediment where the concentration of metals in the sediment was far greater than concentrations found in the water column. This accumulation of metals in the sediment presents an environmental pollution issue that has the potential to last for decades (see Mays and Edwards 2001; Mishra et al. 2007; Ali et al. 2018; Belmer and Wright 2019b).

The altered water chemistry downstream of the wastewater discharge point is expected to be the salient factor in the significant loss of biodiversity within the waterway. The aquatic macroinvertebrate community downstream of the wastewater discharge is characterised by a loss of pollution sensitive taxa and an increase in pollution tolerant taxa. This change in community composition is most apparent when the parameters; taxonomic richness, EPT % and presence / absence data is examined.

A laboratory experiment has identified that a suite of metals including; aluminium, boron, barium, cobalt, copper, iron, lithium, manganese, molybdenum, nickel, strontium and uranium present in the coal mine wastewater discharge and in the Georges River downstream of the discharge are able to bioaccumulate within plants that are exposed to the wastewater. The metals listed above were found in plant tissue at concentrations that were greater by orders of magnitude than the concentrations observed in the water column. The following metals; boron, copper, lithium, molybdenum, strontium and uranium were recorded at concentrations greater than was observed in both the water column and within the sediment. Findings from this research has identified that metals tend to accumulate at different concentrations in different parts of the plant. This study found that most metals in the mine wastewater treatments (BC and GR2) accumulated at greater concentrations in the root tissue rather than leaf tissue. Exceptions to this were the metals; boron and lithium which accumulated at greater concentrations in the leaf tissue.

This research has demonstrated that the current implementation of environmental protection legislation has failed to protect one of Sydney's major waterways from the effects of coal mining. This study, along with other research has determined that the Georges River is not alone in experiencing degradation as a result of coal mine wastewater discharge at a local and global context (Brake et al. 2001; Younger et al. 2004; Belmer et al. 2019a). Currently, the EPL that imposes limits of the pollutants and concentration of the pollutants that may be discharged is inadequate in preventing the degradation of the river. The concentrations of pollutants permitted to be discharge as per the EPL are not reflective of the local natural background conditions or reflect the recommended trigger values listed in the ANZECC (2000) water quality guidelines.

Water quality

The generation of contaminated wastewater from underground coal mining is an inherent consequence of the activity (Grey 1997; Cohen 2002; Younger; 2004; Clapcott et al. 2015; Wright and Belmer 2018). The corresponding discharge of this contaminated water into a river system is well known to affect the waterways geochemical composition (see Banks et al. 1997; Johnson 2003; Price and Wright 2016; Green et al. 2018). This study found conclusive evidence that supports hypotheses number one, that; *The inflow of coal mine wastewater will modify the ionic and metal composition of the waterway*.

The inflow of coal mine wastewater modified the electrical conductivity of the Georges River. This modification was evident immediately after the inflow of wastewater from Brennans Creek and with electrical conductivity remaining elevated at the furthers downstream site; 22km below the Brennans Creek junction. Mean electrical conductivity of water released from West Cliff Colliery at BC was 1787 µS/cm. This result is well within the EPL limit of 2500 – 3570 µS/cm imposed by the NSW EPA. However, the EPL limit does not reflect the recommended ANZECC (2000) trigger value guideline for slightly disturbed ecosystems (NSW upland rivers) which is 350 µS/cm. Nor does the EPL reflect the background electrical conductivity range identified by this study. The failure of the EPL to reflect the dilute nature of the upper Georges River in the limit imposed have resulted in an increase in electrical conductivity downstream of the discharge. The magnitude of the increase in electrical conductivity was by a factor of nine downstream of the discharge point at GR2 compared with the mean of the two reference sites; GRU and OH. Mean electrical conductivity at GR2 was 1677µS/cm (Table 4, Figure 14) compared to mean electrical conductivity at the two reference sites; OH and GRU of 181 µS/cm and 186 µS/cm. 22km downstream at GR3 Mean electrical conductivity was still elevated at 1102 µS/cm. This sustained elevation of electrical conductivity demonstrates how the plume of pollution from coal mine wastewater discharge can extend along way downstream, likely presenting numerous ecological impacts. The salinity of the West Cliff Colliery is elevated compared to other collieries in the southern coal fields. Approximately 20km south west of the study area is the township of Tahmoor and the Tahmoor Colliery which also mines the Bulli seam. The wastewater discharge from this colliery was found to result in the mean electrical conductivity rising from 206 µS/cm above the discharge point to 1011 µS/cm below the discharge point (Belmer and Wright 2019a). Another study by Wright et al. (2018) examined the closed Medway Colliery, also in the southern coal fields and 60km south west of Appin. The continued wastewater discharge from this mine was lower than the West Cliff Colliery. The mine wastewater discharge had a mean electrical conductivity of 1000 µS/cm.

The pH of the wastewater discharge had a mean of 8.82 pH units. Again, this result is well within the current EPL discharge limits of between 6.5 and 9.3 pH units. However, the EPL limit does not reflect the recommended ANZECC (2000) trigger value guideline for slightly disturbed ecosystems (NSW upland rivers) which is 6.0 – 7.5 pH units. Nor does the EPL reflect the background electrical conductivity range identified by this study. The failure of the EPL to reflect the mildly acidic nature of the upper Georges River in the limit imposed have resulted in a mean increase of 2.7 pH units downstream of the discharge compared to the mean of the reference sites. Mean pH at GR2 was 9.00 pH units (Table 4, Figure 15) compared to mean pH at the two reference sites; OH and GRU of 6.78 and 5.83 pH units. 22km downstream at GR3 Mean pH was still elevated at 8.50 pH units. This sustained elevation of pH demonstrates how the plume of pollution from coal mine wastewater discharge can extend along way downstream, likely presenting numerous ecological impacts. pH was observed in the West Cliff Colliery discharge in Brennans Creek was similar to the Tahmoor Colliery discharge which had a mean pH of 8.60 pH units.

The ionic composition of the reference waterways was typical of upland, east flowing rivers that are minimally disturbed (ANZECC 2000; Tippler et al. 2012; Green et al. 2018). These rivers are broadly characterised as being dilute with pH in the acidic to neutral range, dominated by the ions sodium and chloride. The geochemical ionic structure within the water column changed in the Georges River as a result of the inflow of mine wastewater. At the reference sites GRU and OH, the cation dominance from lowest concentration to highest concentration was; potassium < calcium < magnesium < sodium. A similar study by Price and Wright (2016) also found that that sodium was the dominant cation below the West Cliff Colliery discharge. However, the remaining sequence has changed since then with the next most dominant cation being calcium, followed by potassium then magnesium. Downstream of the Brennans Creek discharge at GR2 this order of dominance changed to magnesium < potassium < calcium < sodium. The mean sodium concentration increased by a factor of approximately nineteen between the reference sites and immediately downstream on the Georges River at GR2 (table 5). A similar trend was observed with total alkalinity where total alkalinity increased by a factor of approximately 10 at GR2 compared to the reference sites (Table 5, Figure 19). The anionic composition was altered downstream of the discharge at Brennans Creek compared to the reference sites. The anionic dominance from smallest concentration to lowest concentration at OH was sulphate < total alkalinity as $CaCO_3$ < chloride and at GRU; total alkalinity as $CaCO_3$ < sulphate < chloride. At GR2 this relationship changed to sulphate < chloride < total alkalinity as $CaCO_3$. The study by Price and Wright (2016) and Green et al. (2018) recorded the same order of anionic dominance as this study. South West of Appin at the Tahmoor Colliery, bicarbonate was the dominant anion in the Bargo River downstream of the Tahmoor Colliery wastewater discharge. The next highest concentration of anionic species was chloride, followed by carbonate and sulphate (Wright et al. 2015). The difference in results within the same coal field is likely due to local variations in geology and or different wastewater treatment methods. The EPL does not list any anions or cations as pollutants that are permitted to be discharged. Therefore, there is a need to determine whether the West Cliff Colliery wastewater discharge in in breach of its EPL and the POEO Act (1997). The ANZECC (2000) water quality guidelines do not state any recommendations or trigger values for the ionic composition of waterways. However, the ANZECC (2000) water quality guidelines emphasis the importance of deriving locally applicable guidelines which is especially important where no default guideline exists.

The discharge of coal mine generated wastewater has servery altered the presence and concentration of metals within the receiving waterway; the Georges River (Table 6). The wastewater discharge into Brennans Creek had elevated concentrations of a suite of metals; antimony, aluminium, arsenic, barium, boron, cobalt, lithium, molybdenum, nickel, strontium and uranium, titanium and zinc that were statistically different compared to the reference sites. Of the metals listed in the EPL (Table 1); aluminium, arsenic, copper, lead, manganese, nickel and zinc mean concentrations were below the prescribed limits in the West Cliff Colliery wastewater discharge (Table 1 and Table 6). The metals listed in the EPL reflect the metals listed in the ANZECC (2000) water quality guidelines. However, the concentration limits in the EPL bear little resemblance to the ANZECC (2000) water quality guidelines (Table 1). For example, the EPL limit for aluminium is 1000 µg/L while the ANZECC (2000) guideline is 55 µg/L. This represents a disparity where the EPL limit is eighteen times greater than the ANZECC guideline. The EPL limit for; copper is approximately 5 times greater, nickel which is also eighteen times greater and the EPL limit for zinc which is approximately 10 times greater than the ANZECC (2000) guideline. The combination of the elevated metal concentrations present in the wastewater discharge and the lack of significant flow from upstream has caused elevated levels of a suite of metals in the Georges River. The metals at elevated, statically significantly different concentrations to the reference sites were: antimony, aluminium, arsenic, barium, boron, cobalt, copper lithium, molybdenum, nickel, strontium, uranium, titanium and zinc (Table 6). Of particular concern is the high mean aluminium concentration at GR2 (609 µg/L). A literature review by Gensemer and Playle (1999) found a range of biological impacts can occur at similar aluminium concentrations to those present at GR2. Their research uncovered a literature that documented loss of biomass and or death of aquatic macrophytes, toxicity to fish and impairment of the macroinvertebrate community. However, in the case of mine wastewater, where there is a wide variety of pollutants the exact toxicity of a particular element is difficult to identify. This is shown by Havas (1985) which found that the macroinvertebrate family Daphnia experienced increased mortality at 200 µg/L, but when calcium ions were added mortality reduced. This study found concentrations of uranium, arsenic, lithium in the Georges River below the wastewater discharge that were above other mines in the Sydney basin (Ali et al. 2017; Belmer and Wright 2019a). There appears to be limited research on underground coal mines that are discharging wastewater containing uranium and lithium. Whether this is due to the metals being undetected in the waterways or a lack of testing for the metals is unknown. 22 km downstream from the West Cliff Colliery's discharge, concentrations of metals remained elevated at statistically significantly levels on the Georges River at GR3. The metals at elevated concentrations were; aluminium, arsenic, barium, boron, lithium, molybdenum, nickel, strontium, titanium and uranium (Table 6). The reference sites; OH and GRU metal load was dominated iron and characterised be low concentrations of other metals. GR1 had a suite of metals (aluminium, barium, boron, copper, lithium, nickel, strontium, titanium and zinc) that were present at elevated concentrations that were statistically dissimilar to the reference site. Metals beryllium, cadmium, chromium, mercury, selenium and tin were below laboratory detection limits at all sites on all occasions (Table 6). The metals present in GR1 are likely due to legacy pollution and small volumes of discharge from the Appin East Colliery and potentially from the small amounts of urban runoff that GR1 receives.

Macroinvertebrate community

As discussed above, the individual contaminants present in coal mine wastewater and their impact of aquatic systems are difficult to separate. Even if this was possible, the sum of the contaminants will give a better understanding of the impact to the waste receiving waterway. To determine the total impact that the coal mine wastewater discharge was having on the Georges River the following question was asked; *How do stream macroinvertebrates respond to coal mine wastewater discharge?* To which the hypothesis; *The aquatic macroinvertebrate community structure will be different downstream of the inflow of coal mine wastewater* was tested.

The macroinvertebrate community was assessed to quantify the ecological impacts of the wastewater discharge on the Georges River. Above the wastewater discharge at GRU, mean abundance was 88 animals compared to a mean abundance of 788 animals downstream of the wastewater discharge (Table 7). This increase in abundance below the coal mine is not consistent with other observations of coal mining effects at nearby locations (see Wright at al. 2015 and Wright et al. 2018. However, it is consistent with another macroinvertebrate study conducted on the Georges River by Belmer and Wright (2019c). It is likely that this increase in abundance is being driven by an increase in nutrient concentrations (see Wright and Burgin 2009) present in the wastewater discharge that other studies have previously identified (Georges Riverkeeper 2018). This increase in abundance was largely driven by an increase in the *Chironomidae* and *Simuliidae* populations, both of which are known indicators of nutrient pollution (Wright and Burgin 2009). Taxonomic family richness was reduced downstream of the wastewater discharge point compared with the upstream GRU site (Table 7; Table 8). Mean richness at GR2 was 7 taxa compared to 11 taxa at GRU. This result is similar to other studies that document the wastewater impacts on the aquatic macroinvertebrate community. Studies both local to the Sydney basin and internationally found reduced taxonomic richness and a corresponding reduction in EPT invertebrates in coal mine impacted waterways (Greenfield and Ireland 1978; Wright and Burgin 2009; Bott et al. 2012; Wright et al 2015; Capcott et al. 2015; Xingli et al 2015).

The impaired water quality in the Georges River resulted in a decrease of sensitive ‘EPT’ organisms (Table 7; Table 8). This change was measured as EPT % of total abundance which decreased by a factor of approximately 10 after the inflow of wastewater. The downstream site was also characterised by the complete absence of several pollution sensitive taxa (Chessman 1995) from the families; *Elmidae*, Baetidae, Notonectidae, Corduliidae, *Eustheniidae*, *Helicopsychidae, Philopotamidae, Philorheithridae* and *Hydroptlidae* (Table 7; Table 8). Other local studies that examined the effect of coal mine wastewater discharge on the macroinvertebrate community recorded a similar drop in EPT%. A study by Wright et al. (2018) observed a percentage EPT reduction of 90% in the Wingecarribee River after the inflow of coal mine wastewater. At another location (Tahmoor, NSW) in close proximity to the study area an EPT reduction of 32% was observed after the influx of coal mine wastewater (Wright et al. 2015). A study by Wright et al. (2017) also reported an EPT reduction of 72% below the inflow of Clarence Colliery’s wastewater discharge, a coal mine that is located approximately 80km NW of the upper Georges River in the Western Coalfields of NSW.

Sediment

The results from sediment testing show substantial metal pollution of benthic sediment (Table 9). Confirming the hypotheses: *The benthic sediment downstream of the coal mine wastewater discharge will have a different metal composition to sediment collected at reference sites.* The West Cliff Colliery discharge site at Brennans Creek (BC) had increased levels of thirteen metals at statistically significantly levels. These metals at elevated concentrations were; aluminium, barium, chromium, cobalt, copper, iron, lead, lithium, manganese, nickel, strontium, uranium and zinc. The biggest increase in metal concentration at the wastewater discharge point relative to the mean of the two reference sites was strontium, which was 59 times greater. Strontium was followed by barium which was 39 times greater, lithium (25 times greater), copper (5 times greater), uranium (4 times greater) manganese (4 times greater), nickel, lead and zinc which were three times greater. Downstream on the Georges River at GR2 the same suite of metals was elevated in the sediment with the addition of titanium. The biggest increase in metal concentration at the downstream of the wastewater discharge point at GR2 relative to the mean of the two reference sites was strontium, which was 54 times greater. Strontium was followed by barium which was 38 times greater, lithium (23 times greater), titanium (11 times greater), copper (10 times greater), manganese (7 times greater), uranium (6 times greater), chromium, lead, zinc which were four times greater. 22km downstream on the Georges River, GR3 recorded six metals (barium, copper, lithium, strontium, titanium and zinc) in the sediment that were present at statistically significantly levels. Lithium recorded the biggest increase in metal concentration at GR3, being six times greater than the mean of the reference sites. Strontium and zinc were present at three times greater concentration while barium, copper and titanium were approximately twice the concentration of the reference sites. The metals; antimony, beryllium, cadmium, mercury and selenium were below laboratory detection limits at all sites on all occasions. Additionally, tin, molybdenum, boron and arsenic were at concentrations below detection limits or approaching this limit, with no statistically significant difference between sites.

The ANZECC (2000) guidelines stipulate 'default guideline values' (DGV) for ten metals and metalloids (antimony, cadmium, chromium, copper, lead, mercury, nickel, silver, zinc and arsenic). No metals found in the sediment at any of the sites in this study were above the DGV.

A similar study by Belmer and Wright (2019b) that examined the metal concentrations in the sediment above and below the West Cliff Colliery. This study used an upstream reference which corresponds to site GR1 in this study. At this site the Belmer and Wright (2019b) study found metal concentrations were typically greater immediately above the West Cliff Colliery wastewater discharge point than below. As already discussed, this is likely to be legacy pollution from when Appin East Colliery was discharging wastewater. This study also reported typically lower metal concentrations downstream of the discharge point than data presented in this report. Another study that examined the metal concentrations in the sediment above and below the West Cliff Colliery by Ali et al. (2018) found elevated concentrations of metals than the Belmer and Wright (2019b) study, recording similar concentrations to data presented here. While all three studies reported slightly different concentrations, the overall conclusion was that metal enrichment was present in the study area. Enrichment of metals in the sediment occurred at immediately above the discharge (GR1) representing legacy pollution from Appin East Colliery and within and downstream of the wastewater discharge from West Cliff Colliery.

Bioaccumulation

The links between waterway pollution and the impact on the surrounding riparian vegetation are not fully understood. In a recent study by Belmer and Wright (2018) the uptake of heavy metals by riparian vegetation was studied at a location that receives coal mine wastewater. Their study found that downstream of the coal mine wastewater discharge a suite of metals; aluminum, beryllium, cadmium, cobalt, molybdenum, strontium, thallium, zinc and nickel had bioaccumulated in riparian plant tissue. Similarities in the Belmer and Wright (2018) study to the data presented here show accumulation of aluminum, nickel, molybdenum and strontium occurred in both studies. Differences in relative increase can be attributed to several factors; most notably the study conducted by the author was a laboratory experiment and thus not exposed to other environmental factors. Also, the plants sampled by Belmer and Wright (2018) are expected to have had a longer growth period and exposed to chemically different mine wastewater.

The accumulation of metals in the willow tissue differed between the leaves and roots of the plants. This relationship with between the uptake of metals associated with coal mining by different parts of plants has been documented by Mays and Edwards (2001) and Deo et al. (2011). The study by Deo et al. (2011) found that in plants grown on coal mine spoils, copper accumulated at greater concentrations in the stem and leaves of plants. Mays and Edwards (2001) found metal accumulation in wetland plants exposed to acid mine drainage. Plants in this study exhibited a similar trend to this study where metals accumulated in greater concentration in plant root tissue. The study by Mays and Edwards (2001) identified one metal; boron that accumulated in greater concentration in the plant leaf tissue than the plant root, reflecting the result found in this study.

A laboratory experiment has identified that a suite of metals including; aluminium, boron, barium, cobalt, copper, iron, lithium, manganese, molybdenum, nickel, strontium and uranium present in the coal mine wastewater discharge and in the Georges River downstream of the discharge are able to bioaccumulate within plants that are exposed to the wastewater. These findings confirmed the hypotheses: *Metal composition in plant tissue will differ between plants exposed to mine wastewater compared to plants exposed to water collected from reference sites.* The metals listed above were found in plant tissue at concentrations that were greater by orders of magnitude than the concentrations observed in the water column. The following metals; boron, copper, lithium, molybdenum, strontium and uranium were recorded at concentrations greater than was observed in both the water column and within the sediment. Findings from this research has identified that metals tend to accumulate at different concentrations in different parts of the plant. This study found that most metals in the mine wastewater treatments (BC and GR2) accumulated at greater concentrations in the root tissue rather than leaf tissue. Exceptions to this were the metals; boron and lithium which accumulated at greater concentrations in the leaf tissue.

Within the leaf tissue there were six metals for which concentration was elevated at statistically significant levels in willows grown in water from BC, GR1 and GR2 compared to the reference sites. Strontium was elevated in willow leaves grown in water from BC, GR1 and GR2. Boron and lithium were elevated at BC and GRU while barium and manganese were elevated at statistically significant levels in willow leaves grown in water from GR1 and BC respectively. Manganese in willow leaf tissue was elevated at statically significant concentrations at BC. Both sites BC and GR2 had similar increases in metal accumulation within the leaves. Mean lithium concentration was 30 times higher at BC and 37 times higher at GR2 compared with the mean of the reference sites. Boron had the next highest accumulation factor of 1.4 times greater at BC and GR2 than mean of the reference sites, a result substantially lower than the accumulation of lithium. Barium, manganese and strontium accumulated by a factor of 1.1 times greater at BC and GR2 than mean of the reference sites. The following metals were below detection limits in willow leaves grown in water from sites; arsenic, beryllium, cadmium, chromium, cobalt, mercury, nickel, lead, antimony, selenium, titanium and uranium. There was also no at statistically significant difference between sites; BC, GR1 and GR2 compared to the reference sites for the following metals; aluminium, iron, molybdenum and zinc (Table 10). The concentration of these metals in the willow leaf tissue were many times higher than the concentration observed in the water column.

There was greater uptake of metals within the willow roots. The metals; barium and strontium were elevated at statistically significant levels in treatments GR1, BC and GR2. The metals; aluminium, boron, lithium, manganese, molybdenum and nickel were elevated at statistically significant levels in treatments BC and GR2. The metals copper and iron were elevated at statistically significant levels in treatment GR2. Uranium showed the biggest increase in concentration at BC and GR2 by a factor of 33 and 30 times respectively compared to the mean of the reference sites. Molybdenum increased by a factor of 30 times at BC and 28 times at GR2. Lithium concentrations in root tissue experienced the next biggest increase in relative concentration by a factor of 18 and 19 at BC and GR2. Barium had a similar increase in relative concentration to lithium where barium was 18 and 14 times higher in root tissue in treatments BC and GR2. Aluminium concentrations in root tissue increased by a factor of 14 at BC and 15 at GR2, followed by strontium (increased by factors of 8 and 7 in BC and GR2). Nickel increased by a factor of four in BC and GR2 while boron, manganese, copper and iron increased by factors of between 2.7 times and 1.2 times in root tissue in treatments BC and GR2. The following metals were below detection limits in all treatments; arsenic, beryllium, chromium, mercury, antimony, selenium, tin and titanium. Additionally, there was no statistically significant difference between the following treatments for the metals; cadmium, lead and zinc from the reference sites and GR1, GR2, GR3 and BC (Table 11). The concentration of these metals in the willow root tissue were many times higher than the concentration observed in the water column.

Conclusion

Through the analysis of water quality on the Georges River, this study has determined that the release of coal mine wastewater has degraded the ecological condition of the river. This impact was observed in reduced water quality, metal enrichment in the sediment and a loss of sensitive macroinvertebrate taxa downstream of the wastewater discharge point. This research has also shown that a suite of the metals that are present in, and downstream of the coal mine wastewater discharge have the potential to bioaccumulate in plant tissue. A magnification effect where the metals; aluminium, boron, lithium, manganese, molybdenum, nickel and strontium were found in greater concentration in the plant tissue that in the water column.

Though the administration of the POEO Act (1997) by the issuing of an EPL by the NSW EPA it is expected that the natural environment will not suffer significant ecological impacts from controlled activities. This has not been the case with the Georges River which has suffered degradation due to the inflow of coal mine wastewater. Many other regulated coal mine wastewater discharges within the Sydney Basin have caused degradation of the receiving waterway. The broad nature of river degradation points to a systemic failure of stakeholders to prevent pollutants associated with coal mining from entering the environment at levels which impair river ecology.

To attempt to rectify this issue it is essential that the concentrations of pollutants that are permitted to be discharged reflect the natural background conditions and the ANZECC (2000) water quality guidelines. A long-term management plan should be adopted to ensure that when underground coal mining ceases, continued degradation does not occur. This unfortunate consequence of continued degradation after closure has been shown to occur locally within the Sydney basin and internationally (see Younger 1777; Johnson 2003, Price and Wright 2016; Belmer and Wright 2019a,b,c). Consequently, it is essential that we better manage our aquatic ecosystems in the context of underground coal mining to avoid further instances of degradation and to preserve ecosystem functions and services.

References

Ali, A, Strezov, V, Davies, P, & Wright, I 2018, ‘River sediment quality assessment using sediment quality indices for the Sydney basin, Australia affected by coal and coal seam gas mining’, *Science of the Total Environment,* vol. *616*, pp. 695-702.

ANZECC (Australian and New Zealand Environment and Conservation Council) (2000). *Australian and New Zealand guidelines for fresh and marine waters. National Water Quality Management Strategy Paper No. 4.* Australian and New Zealand Environment and Conservation Council, Canberra.

Banks, D, Younger, PL, Arnesen, RT, Iversen, ER & Banks, SB 1997, ‘Mine-water chemistry: the good, the bad and the ugly’, *Environmental Geology,* vol. 32 pp. *157–174.*

Bazrafshan, E, Mostafapour, FK, Esmaelnejad, M, Ebrahimzadeh, GR & Mahvi, AH 2016, ‘Concentration of heavy metals in surface water and sediments of Chah Nimeh water reservoir in Sistan and Baluchestan province, Iran’, *Desalination and Water Treatment*, vol. 57, no. 20, pp. 9332-9342.

Belmer, N & Wright, IA 2019a, ‘The regulation and impact of eight Australian coal mine waste water discharges on downstream river water quality: a regional comparison of active versus closed mines’, *Water and Environment Journal*.

Belmer, N & Wright, IA 2019b, ‘Regional comparison of impacts from seven Australian coal mine wastewater discharges on downstream river sediment chemistry, Sydney Basin, New south Wales Australia’, *American Journal of Water Science and Engineering,* vol. 5, no. 2, pp. 37-46.

Belmer, N & Wright, IA 2019c, ‘Regional comparison of impacts to stream macroinvertebrates from active and inactive coal mine wastewater discharges, Sydney Basin, New South Wales Australia’, *American Journal of Water Science and Engineering,* vol. 5, no. 2, pp. 62-75.

Belmer, N, & Wright, IA 2018, ‘Heavy metal contamination of water column from a coal mine waste water discharge resulting in mobilisation of metal contaminants to riparian vegetation. Wollangambe River, Blue Mountains Australia, In Carr Vietz, G.J. & Rutherfurd, ID (ed.s), *Proceedings of the 9th Australian Stream Management Conference*, 12 – 15 August 2018, Hobart, Tasmania, River Basin Management Society, Queensland, pp. 484-492.

Belmer, N, Tippler, C, Davies, PJ & Wright, IA 2014, ‘Impact of a coal mine waste discharge on water quality and aquatic ecosystems in the Blue Mountains World Heritage Area’, In G Viets, ID, Rutherfurd & R Hughes (ed.s), *Proceedings of the 7th Australian stream management conference.* 27-30 July, Townsville, River Basin Management Society, Queensland, pp. 385–391.

Bott, T, Jackson, J, McTammany, M, Newbold, J, Rier, S, Sweeney, B, & Battle, J 2012, ‘Abandoned coal mine drainage and its remediation: Impacts on stream ecosystem structure and function’, *Ecological Applications: A Publication of the Ecological Society of America,* vol. 22, no. 8, pp. 2144-2163.

Brake, SS, Connors, KA & Romberger, SB 2001, ‘A river runs through it: impact of acid mine drainage on the geochemistry of West Little Sugar Creek pre- and post-reclamation at the Green Valley coal mine, Indiana, USA’, *Environmental Geology*, vol. 40, no. 11, pp. 1471-1481.

Chessman, BC 1995, ‘Rapid assessment of rivers using macroinvertebrates: a procedure based on habitat-specific sampling, family level identification and a biotic index’, *Australian Journal of Ecology*, vol. 20, pp. 122–129.

Clapcott, JE, Goodwin, E & Harding, J 2015, ‘Identifying catchment-scale predictors of coal mining impacts on New Zealand stream communities’, *Environmental Management*, vol. 16 pp. 711-721.

Cohen, D 2002, ‘Best practice mine water management at a coal mine operation in the Blue Mountains, Masters thesis, University of Western Sydney, Penrith.

Deo, B, Nahak, G & Sahu, RK 2011, ‘Studies on the uptake of heavy metals by selected plant species growing on coal mine spoils in sub-tropical regions of India’, *Journal of American Science,* vol. 7, no. 1, pp. 26-34.

Dillon, PJ, Yan, ND, Harvey, HH & Schindler DW 1984, ‘Acidic deposition: Effects on aquatic ecosystems’, *CRC Critical Reviews in Environmental Control*, vol. 13, no. 3, pp. 167-194.

EPL 2504 (2019) Environment Protection Licence # 2504. Endeavour Coal Pty Limited. West Cliff and North Cliff Collieries. Environment Protection Authority. Available at: http://app.epa.nsw.gov.au/prpoeoapp/Detail.aspx?instid=2504&id=2504&option=licence&searchrange=licence&range=POEO%20licence&prp=no&status=Issued

Gensemer, R, & Playle, R 1999, ‘The bioavailability and toxicity of aluminum in aquatic environments’, *Critical Reviews in Environmental Science and Technology,* vol. *29,* no. 4, pp. 315-450.

Gensemer, RW & Playle, RC 1999, ‘The bioavailability and toxicity of aluminum in aquatic environments’, *Critical Reviews in Environmental Science and Technology*, vol. 29, no. 4, pp. 315–450.

Gray, NF 1997, ‘Environmental impact and remediation of acid mine drainage: a management problem, *Environmental Geology,* vol. 30, pp. 62-71.

Green, BS, Carroll, R, Morrison, KG, Reynolds, JK & Wright, IA 2018, ‘Community triggers EPA action on coal mine river pollution’, In Carr Vietz, G.J. & Rutherfurd, ID (ed.s), *Proceedings of the 9th Australian Stream Management Conference,* 12 – 15 August 2018, Hobart, Tasmania, River Basin Management Society, Queensland, pp. 681-688.

Greenfield, J, & Ireland, M 1978, ‘A survey of the macrofauna of a coal-waste polluted lancashire fluvial system, ‘*Environmental Pollution, vol. 16*, no. 2, pp. 105-122.

Havas, M 1985, ‘Aluminium bioaccumulation and toxicity to Daphnia magna in soft water at low pH’, *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 42, no. 11, pp. 1741–1748.

Johnson, DB 2003, ‘Chemical and microbiological characteristics of mineral spoils and drainage waters at abandoned coal and metal mines’, *Water, Air, and Soil Pollution*, vol. 3, pp. 47-66.

Karathanasis, AD & Johnson, CM 2003, ‘Metal Removal Potential by Three Aquatic Plants in an Acid Mine Drainage Wetland’, *Mine Water and the Environment*, vol. 22 no. 1, pp. 22–30.

Krebs, CJ, 1989. *Ecological methodology*, New York: Harper & Row.

Mays, P, and Edwards, G 2001, ‘Comparison of heavy metal accumulation in a natural wetland and constructed wetlands receiving acid mine drainage’, *Ecological Engineering,* vol.16, no. 4, pp. 487-500.

Mishra, V, Upadhyaya, A, Pandey, S, & Tripathi, B 2008, ‘Heavy metal pollution induced due to coal mining effluent on surrounding aquatic ecosystem and its management through naturally occurring aquatic macrophytes’, *Bioresource Technology,* vol. 99, no. 5, pp. 930-936.

Neff, JM 1984, ‘Bioaccumulation of organic micro pollutants from sediments and suspended particulates by aquatic animals’, *Fresenius' Zeitschrift für analytische Chemie*, vol. 319, no. 2. pp 132-136.

NSW OEH (NSW Office of Environment and Heritage) 2015, *Clarence Colliery discharge investigation*. Office of Environment and Heritage: 59 Goulburn Street Sydney NSW 2000. http://www.epa.nsw.gov.au/resources/licensing/150171-clarence-colliery-discharge-investigation.pdf.

POEO Act (2019) *Protection of the Environment Operations* Act (1997) NSW. Available at: https://www.legislation.nsw.gov.au/#/view/act/1997/156.

Price, P & Wright, IA 2016, ‘Water quality impact from the discharge of coal mine wastes to receiving streams: comparison of impacts from an active mine with a closed mine’, *Water, Air, and Soil Pollution*, vol. 227, pp. 155.

Rosenberg, DM, & Resh, VH 1993, *Freshwater biomonitoring and benthic macroinvertebrates*. New York: Chapman & Hall.

Sheoran, AS & Sheoran, V 2006, ‘Heavy metal removal mechanism of acid mine drainage in wetlands: A critical review’, *Minerals Engineering,* vol. 19, no. 2, pp. 105-116.

South32 (2019) Illawarra Coal Documents. Bulli Seam Operations 14-day Monitoring Report. Available at https://www.south32.net/what-we-do/places-we-work/illawarra-metallurgical-coal/documents

Tippler, C, Wright, IA & Hanlon A 2012, ‘Is catchment imperviousness a keystone factor degrading urban waterways? A case study from a partly urbanised catchment (Georges River, south-eastern Australia)’, *Water, Air, and Soil Pollution*, vol. 223, no. 8, pp. 5331-5344.

Wales, AC & Cavanagh V 2018, ‘Georges Riverkeeper Aboriginal Riverkeeper Team: a model for successful engagement of Aboriginal communities in natural resource management of urban areas’, In Carr Vietz, G.J. & Rutherfurd, ID (ed.s), *Proceedings of the 9th Australian Stream Management Conference*, 12 – 15 August 2018, Hobart, Tasmania, River Basin Management Society, Queensland, pp. 342-349.

Wang, XW, & Rainbow, PS 2008, ‘Comparative approaches to understand metal bioaccumulation in aquatic animals’, *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, vol. 148, no. 4. pp 315-323.

Wright, IA, Chessman, BC, Fairweather, PG & Benson, LJ 1995, ‘Measuring the impact of sewage effluent on the macroinvertebrate community of an upland stream: The effect of different levels of taxonomic resolution and quantification’, *Australian Journal of Ecology*, vol. 20, pp. 142-149.

Wright, IA & Burgin, S 2009, ‘Comparison of sewage and coal-mine wastes on stream macroinvertebrates within an otherwise clean upland catchment, southeastern Australia’, *Water, Air, and Soil Pollution*, vol. 204, pp. 227.

Wright, IA 2012, Submission to EPA regarding review of EPL 2504. Investigation of water quality and ecosystem health in the upper Georges River: focus on the influence of West Cliff wastewater discharge and recommendations for new discharge conditions for EPL 2504. Available at: (https://www.epa.nsw.gov.au/-/media/04F6DEE98AF644D5921134775DFC1FB9.ashx?la=en)

Wright, IA, McCarthy, B, Belmer, N & Price, P 2015, ‘Subsidence from an underground coal mine and mine wastewater discharge causing water pollution and degradation of aquatic ecosystems’, *Water, Air, & Soil Pollution,* vol. *226* no. 10, pp. 1-14.

Wright, IA, Belmer, N & Davies, P 2017, ‘Coal mine water pollution and ecological impairment of one of Australia’s most ‘protected’ high conservation-value rivers’, *Water, Air,and Soil Pollution*, vol. 228, no. 3, pp. 1-18.

Wright IA, & Belmer, N 2018, ‘Increased water pollution after closure of Australia's longest operating underground coal mine: A 13-month study of mine drainage, water chemistry and river ecology’, *Water, Air, & Soil Pollution*, vol. 229, no. 3, pp. 1-20.

Wright, IA, Paciuszkiewicz, K & Belmer N 2018, ‘Increased water pollution after closure of Australia’s longest operating underground coal mine: a 13-month study of mine drainage, water chemistry and river ecology’, *Water, Air, and Soil Pollution*, vol. 229, pp. 55.

Xingli, G, Olden, JD & Simberloff, D 2018, ‘Impact of coal mining on stream biodiversity in the US and its regulatory implications’, *Nature Sustainability*, vol. 1, pp. 176-183.

Younger, PL 1997, ‘The longevity of minewater pollution: a basis for decision-making’, *The Science of the Total Environment*, vol.194, pp. 457-466.

Younger, PL 2004, ‘Environmental impacts of coal mining and associated wastes: a geochemical perspective’, *Geological Society*, London, Special Publication, vol. 236, pp. 169-209.