



Regulated Coal Mine Wastewater Contaminants Accumulating in an Aquatic Predatory Beetle (*Macrogyrus rivularis*): Wollangambe River, Blue Mountains New South Wales Australia

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Abstract: This study investigates contaminants from a single coal mine wastewater discharge released to the Wollangambe River accumulating in an aquatic predatory beetle (*Macrogyrus rivularis*). The study was undertaken within the Wollangambe River and its surrounding tributaries. The coal mine wastewater discharge is regulated by the New South Wales Environment Protection Authority and the regulation of the pollutants only concentrates on water column concentrations. The majority of the Wollangambe River flows within the World Heritage Greater Blue Mountains National Park and is protected through many layers of legislation from state to federal and international (Threatened Species Conservation Act 2005, Environment Protection and Biodiversity Conservation ACT 1999, United Nations Educational, Scientific and Cultural Organization 2000). Results show that many contaminants are at statistically higher concentrations within the water column, stream sediment and beetles sampled when compared between reference and impacted sample locations. Analysis of Similarity (ANOSIM) found significant differences for contaminants in beetles sampled at impacted sites compared to reference sites with no significant difference recorded between reference sites. Biota and/or Environmental matching (Best) found Manganese, Cobalt, Nickel and Zinc as the factors which have the greatest influence in differences. The implications that contaminants from the regulated wastewater being discharged may be accumulating within aquatic biota is of major concern as the regulation of the wastewater only concentrates on water column pollutants and is not taking into account the greater environmental ramifications of the pollution.

Keywords: Water Pollution, Bioaccumulation, Heavy Metals, Macroinvertebrates, Freshwater Ecology, Coal Mine Pollution, Regulated Water Pollution

1. Introduction

In recent years anthropogenic activities and the subsequent contamination from pollutants of global ecosystems has reached unparalleled heights. Exponentially increasing industrial and mining activities have led to further environmental pollution through wastes produced by these activities. A host of pollutants from mining and industrial activities, when released to the environment, have the

potential to accumulate within biota at toxic concentrations and cause chronic ecological impacts in ecosystem food chains [1-4]. Metal pollution is a global environmental issue and has been for many decades. In many cases metal pollutants are directly discharged into waterways from anthropogenic activities. Many of these metal pollutants become absorbed to suspended particulates within the water column. Once absorbed to these suspended particulates the metal contaminated suspended particulates eventually deposit

into waterway sediments often remaining indefinitely [5, 6, 7].

Aquatic ecosystems are often more highly sensitive to contamination from anthropogenic activities, especially as waterways are often used as sources of discharge for many industrial wastes [8]. Heavy metal contamination within aquatic environments can persist much longer than terrestrial organic pollutants. This is due to the lack of a "biodegradation function" of heavy metals in aquatic ecosystems in comparison to a terrestrial ecosystem [9, 3]. Bioaccumulation of contaminants within fish species have been recorded worldwide as a result of metal and coal mining activities [3, 10-13] along with other aquatic fauna and flora [14, 15].

One study found bioaccumulation of arsenic and selenium within caddisflies of coal ash polluted sites [16] whilst increases in the levels of pollutants were also recorded for turtles, crayfish, tadpoles and varying fish species by [17]. Bioaccumulation of wastewater contaminants within macroinvertebrates from metal mining activities have been reported in Canada, Australia and North America [18-23].

The pH of water is identified as an important factor directly linked to the speciation and bioaccumulation of metals and metalloids. pH will affect the bioavailability of metals and metalloids by influencing their solubility and subsequent ability of bioaccumulate to a medium [17, 24]. The individual constituents of coal can also influence metal bioavailability [17]. Atkinson et al. found that lower water pH allowed for greater bioavailability and sequestration of heavy metals in biota. They found that iron and manganese oxidatively precipitated increasingly as pH decreased. This decreasing pH had a secondary effect which allowed a greater sequestration rate of lead and zinc [25].

Coal mining practices are well documented to contribute to an array of differing environmental problems including air pollution, fire hazards, ground subsidence or deformation, surface and or ground water pollution. Surface water pollution is a major environmental problem associated with coal mining and it occurs through the discharge of mine wastewaters that are contaminated by various disturbances associated with mining practices [26-30]. Water pollution from coal mining occurs as large volumes of surface and groundwater are required to be removed from most underground coal mines. This is generally through the pumping of the wastewater to the surface. Without this, groundwater would flood most sections of the underground mining operation [27, 31]. Coal mine wastewater will often be contaminated due to the disturbance of the local geology associated with mining activities. The exact nature of the water contamination will vary depending on local factors such as groundwater geochemistry, hydrology and mineralogy of the local strata. In addition to the physical activity of the mining operation and the removal of the wastewater, other activities will also often contaminate water used throughout a mining plant which can include; coal washing and the inclusion of other

wastes generated by the surface operation at the mine such as sewage wastes [31].

Coal mine wastewater discharges in New South Wales, Australia are regulated by the New South Wales Environmental Protection Authority (NSW EPA) and environmental protection of receiving waterways is implemented through Environmental Protection Licenses (EPL's), under the Protection of the Environment Operations Act 1997 (POEO Act 1997) [34]. EPL's set discharge limits for water quality and chemical properties in which coal mine wastewaters that are discharged to the environment must adhere to [32, 33]. In many cases the EPL's for coal mine wastewater discharges are failing to protect the receiving waterways ecosystems by failing to identify ecologically hazardous chemicals in the waste discharges that may accumulate or become persistent in the receiving waterways ecosystem. This non-wholistic regulation is failing to protect the aquatic environment of these receiving waterways by not taking into account legacy pollutants. Studies have been conducted on the impacts of metal bioaccumulation from coal mining activities on terrestrial and aquatic plant and fish tissue with none investigating the bioaccumulation of pollutants from actively licenced and regulated coal mine wastewater discharges.

This study investigates if the contaminants from a single licenced and regulated coal mine wastewater discharge is accumulating in the ambient environment. More specifically within stream sediments and a single species of aquatic predatory beetle (*Macrogyrus rivularis*) from the family Gyrinidae within the high conservation Wollangambe River. Macroinvertebrates are widely used as ecological indicators of water quality due to well-established methods and ease of sampling [34]. Macroinvertebrates are regarded as being effective indicators as they have relatively long-life cycles and different taxonomic groups have differing sensitivities to disturbance and water pollution [34, 35]. There is a large amount of well-established literature on freshwater macroinvertebrates and their applications to assess various human-induced anthropogenic disturbances including a broad variety of pollution types [35] including coal mine drainage [27, 30, 36, 37]. The beetle species (*Macrogyrus rivularis*) was used as it was identified as a reliable source of sampling, its predacious habit allowing it to consume other aquatic fauna which may be contaminated and due to its long-life cycle (2 years) allowing for a prolonged period in which it can accumulate contaminants from the wastewater [38]. This stream also allows for a great test case as other than the coal mine wastewater the stream resides within an untouched pristine catchment with the two reference locations having no anthropogenic influence on water quality or chemistry [33, 39].

It is hypothesised that the contaminants from the single coal mine wastewater are accumulating within a single species of predacious aquatic beetle found within the Wollangambe River downstream of the coal mine wastewater discharge.

2. Methods

This study was conducted at two upland streams found within the Blue Mountains area of Sydney, Australia. One stream being the Wollangambe River, the other Bell Creek which is a tributary of the Wollangambe River. Both of which flow mostly within the Blue Mountains World Heritage National Park Estate and the majority of their catchments are naturally vegetated (Figure 1). Four sample sites were used in total for this study, Wollangambe 1 (W1) and Bell Creek (Bell) both as reference sites and Wollangambe 3 (W3) and Wollanagmbe 5 (W5) as impact sites. The reference site (W1) is located approximately 200m above the discharge point and is a first order stream [40], whilst (W3) is approximately

500m downstream and is a second order stream [40]. Both sample locations share similar altitudes between 1025m and 960m above sea level (Figure 1 and Table 1). Two sample locations were located approximately 18km downstream of the coal mine. One being the reference site (Bell Creek) and is a naturally vegetated tributary of the Wollangambe River and is a first order stream [40]. Bell Creeks paired impact site (W5) was located on the Wollangambe River and was approximately 200m downstream of the confluence of Bell Creek and the Wollangambe River and is a second order stream [40]. Both lower sample sites share similar altitudes between 760m and 740m above sea level (Figure 1 and Table 1). Stream order level was determined by the Strahler 1952 method [40].

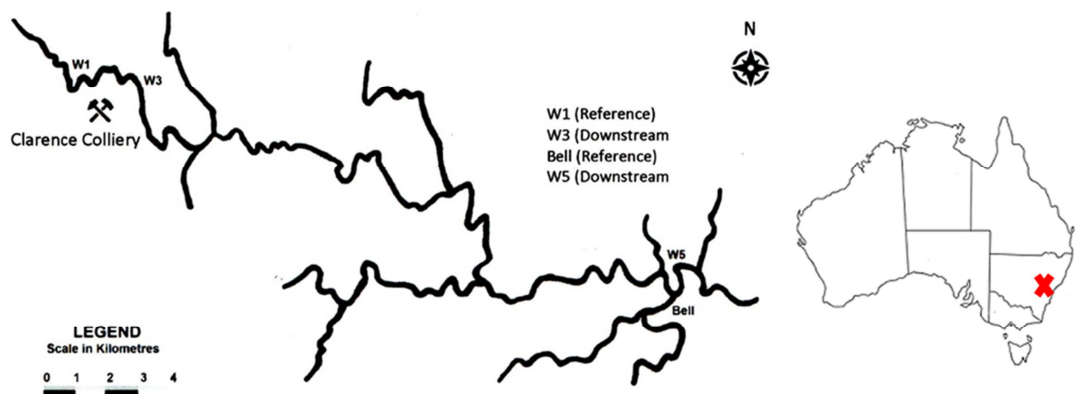


Figure 1. Map of Australia and sample locations and Clarence colliery. Red X depicts approximate location of study area. W1 (reference) sample location is located approximately 200m above the coal mine wastewater discharge and its paired (impact) sample location W3 is located approximately 200m downstream of the wastewater inflow. W5 (impact) sample location is located on the Wollangambe River approximately 18m downstream of the wastewater inflow and its paired (reference) sample location Bell Creek (Bell) is a clean tributary of the Wollangambe River and is also located approximately 18km downstream of the coal mine wastewater discharge just upstream of W5.

Table 1. Sample location name, longitude and latitude, altitude (Metres above sea level) and stream order of the four sample locations used in this study. Stream order is derived from the Strahler method (Strahler 1952).

Sample location	longitude	latitude	Altitude (ASL)	Stream order
W1 (reference)	150.249101 E	-33.455964 S	1025 m	1
Bell Creek (reference)	150.353770 E	-33.490335 S	760 m	1
W3 (downstream)	150.257359 E	-33.455673 S	960 m	2
W5 (downstream)	150.355208 E	-33.487474 S	740 m	2

Five replicated water samples (grab samples) and insitu field water quality parameters were sampled and measured at the same time as sediment and beetle sampling occurred from the same four sample locations above. Field meters used include a TPS WP-82Y meter with a YSI dissolved oxygen probe for Dissolved Oxygen, TPS WP-88 Turbidity meter with a TPS turbidity sensor for Turbidity and a TPS WP-81 Conductivity, pH and Temperature meter with TPS Conductivity and Temperature probe and a TPS submersible k407 pH sensor. Five field grab samples were collected in commercial preserved sample containers provided by a commercial laboratory (EnviroLab) (to allow for quality control of sampled water) at each site (20 total) and analysed using standard methods (APHA 1998) by Envirolab (Chatswood, NSW) a National Associations of Testing Authorities accredited laboratory for fifteen metals (Aluminium, Barium, Boron, Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Manganese, Molybdenum, Nickel,

Strontium, Uranium and Zinc).

Stream sediments were sampled at the same time as water and beetle samples were collected from the same four sample locations. This was achieved by sampling stream sediments within unused commercial laboratory sample containers provided by (EnviroLab) (to allow for quality control of sampled sediments) in a zone of accumulated sediment, following standard methods recommended by the Victorian Environmental Protection Authority [41]. Samples were placed into sealed glass sampling jars supplied by a commercial laboratory and stored. Five samples were collected at each sample location (20 total) and analysed using standard methods (APHA 1998) by Envirolab (Chatswood, NSW) a National Associations of Testing Authorities accredited laboratory for the same fifteen metals analysed for water samples (Aluminium, Barium, Boron, Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Manganese, Molybdenum, Nickel, Strontium, Uranium and

Zinc).

Six replicated predacious aquatic beetle samples (*Macrogyrus rivularis*) from the family Gyrinidae were collected on one occasion at all four sample sites (24 total beetles from 4 sites). The beetle species was used as it was identified as a reliable source of sampling, its predacious habit allowing it to consume other aquatic fauna which may be contaminated and due to its long-life cycle (2 years) [38]. Sampling was achieved by identifying and catching beetles from the surface or mid water column as they retreated using a macroinvertebrate sampling 'kick' net with a frame of 30 x 30 cm and 250 µm mesh. Beetles were caught and stored in deionised water within individual sample location sample containers. These containers were sealed and labelled on collection and dispatched to the commercial laboratory (EnviroLab) and analysed for 21 metals (Aluminium, Arsenic, Barium, Beryllium, Boron, Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Manganese, Molybdenum, Nickel, Selenium, Silver, Strontium, Titanium, Uranium, Vanadium and Zinc).

In the laboratory Ultra High Purity (UHP) water was used to wash the beetles and remove any potentially contaminated residues from their outer surface (precipitates). They were then kept in individual sealed plastic containers. The samples were dried in a 60-degree Celsius oven for 48 hours prior to being ground using a clean pestle and mortar. Approximately 0.05 grams of the ground samples were weighed into plastic digestion tubes. High purity Nitric and Hydrochloric acid was added to the tubes and the samples were digested at 98 Degrees Celsius for 1.5 hours. The samples were then made up to 20mL with UHP water. These samples were analysed by Inductively Coupled Mass Spectrometry (ICP-MS). Quality control measures were used to ensure the integrity of the results including blanks and spiked laboratory control samples (LCS).

Multivariate analysis was performed using Single Factor ANOVA and the software package Primer 6 for analysis of similarity (ANOSIM) to test for differences between combined reference sites (W1 and Bell) and both individual impact sites (W3 and W5). Water quality and chemistry, stream sediment chemistry and aquatic predacious beetle chemistry individually. Primer 6 was also used to identify which water quality and chemistry parameters influenced the dissimilarity in beetle contaminants through a BIOENV test. Percentage increases from water column concentrations (µg/L) to aquatic beetle concentrations (µg/kg) were calculated to assess potential uptake differences between reference water chemistry to impacted water chemistry.

3. Results

Mean water column concentrations of Barium, Boron, Cobalt, Iron, Manganese, Nickel, Strontium and Zinc were found to be statistically different from reference to impacted sample sites (Table 2). Reference sites recorded mean barium of 9.5 and 8.6 µg/L whilst in comparison downstream sites recorded mean barium of 20.8 and 16.0 µg/L. Reference sites

recorded mean boron of 8.0 µg/L in comparison downstream sites had mean boron of 9.1 and 9.5 µg/L. Reference site Cobalt concentrations were below laboratory detectable limits. Whilst in comparison downstream sites recorded mean cobalt of 26.5 and 1.7 µg/L. Mean iron concentrations at reference sites was 300 and 381.7 µg/L whilst in comparison downstream iron was of 134.3 and 66.8 µg/L. Reference sites recorded mean manganese concentrations of 22.8 and 35.2 increasing downstream to a mean of 178.5 and 29.2 µg/L. Reference sites recorded mean nickel of 0.5 µg/L just above the laboratory threshold. Whilst in comparison downstream mean nickel was 76.7 and 17.7 µg/L. Reference strontium concentrations were recorded a mean of 3.6 and 5.2 µg/increasing downstream to 59 and 36.2 µg/L. Reference sites recorded mean zinc of 2.4 and 3.7 µg/L and downstream sites a mean of 105 and 20.8 µg/L. Cadmium, chromium, copper, lead, molybdenum and uranium were all at concentrations within water that were below laboratory detectable limits (Table 2).

Mean stream sediment concentrations of Aluminium, Barium, Cadmium, Chromium, Cobalt, Copper, Iron, Manganese, Molybdenum, Nickel, Strontium, Uranium and Zinc were found to be statistically different from reference to impacted sample sites (Table 3). Bell Creek (reference) recorded the lowest mean aluminium in stream sediment of 233.3 mg/kg, whilst in comparison W1 (reference site), W3 and W5 (impacted sites) had mean aluminium of 1248, 5083 and 1034 mg/kg respectively. Reference sites recorded mean barium of 4.75 and 18.0 mg/kg whilst downstream recorded mean barium of 101.6 and 7.5 mg/kg. Both reference sites Bell Creek and W1 as well as the downstream site W5 all recorded cadmium concentrations lower than laboratory detectable limits. W3 recorded mean cadmium of 0.47 mg/kg. Bell Creek, W1 and W5 all recorded replicates which were below laboratory detectable concentrations for chromium. W1 and W5 both recorded mean chromium of 1 mg/kg whilst Bell Creek recorded a mean of 0.25 mg/kg. In comparison W3 recorded chromium concentrations between 2-8 mg/kg and a mean of 4 mg/kg. The reference site Bell Creek recorded below detectable laboratory concentrations of cobalt for all replicates sampled. W1 ranged between below laboratory concentrations and 5 mg/kg, whilst in comparison W5 was found to have mean cobalt of 6 mg/kg and W3 recorded mean concentrations of 552 mg/kg. The reference sites Bell Creek recorded below detectable laboratory concentrations of copper for all replicates sampled and W1 recorded a mean of 3.5 mg/kg. Whilst the downstream sites W3 and W5 recorded mean copper of 17.0 and 2.4 mg/kg respectively (Table 3).

Bell Creek and W1 recorded mean iron of 1443 and 4000 mg/kg whilst in comparison W3 and W5 recorded mean iron of 9950 and 1982 mg/kg. Manganese recorded at the reference sites (Bell and W1) was mean 13.3 and 127.5 mg/kg in comparison W3 and W5 recorded mean manganese of 5474 and 76 mg/kg respectively. Bell Creek, W1 (reference sites) and W5 (impacted site) samples recorded concentrations of molybdenum within sediment below laboratory detectable limits. Whilst in comparison W3 (downstream site) had mean molybdenum of 4

mg/kg. W3 recorded one replicate below laboratory detectable limits for molybdenum. Bell Creek recorded mean nickel below laboratory detectable limits whilst W1 (reference sites) recorded mean nickel of 2 mg/kg. In comparison W3 and W5 (downstream sites) recorded mean nickel of 606.7 and 19 mg/kg with all samples recording detectable concentrations. Reference site mean strontium was found to be 1.25 and 5 mg/kg and ranged from below laboratory detectable limits and 9 mg/kg across both sites. Whilst in comparison W3 and W5 (downstream sites) recorded mean strontium of 12.3 and 2.8 mg/kg respectively. All sample locations recorded low concentrations of uranium in sediment with the reference stream

Bell Creek recording all replicates at concentrations below laboratory detectable limits. W1 was recorded between below laboratory limits and 0.2 mg/kg with a mean of 0.06 mg/kg. W5 was similar to both reference uranium concentrations measuring between below laboratory detectable limits and 0.3 mg/kg with a mean of 0.08 mg/kg. Whilst in comparison W3 mean uranium of 0.53 mg/kg and ranged between 0.1-1.4 mg/kg. The reference site Bell Creek recorded all replicates at concentrations below laboratory detectable limits for Zinc. W1 was found to have a mean of 1.75 mg/kg. Whilst in comparison W3 and W5 (downstream sites) had mean zinc of 734 and 25 mg/kg respectively (Table 3).

Table 2. Water quality and water chemistry parameters, sample locations, Single Factor ANOVA, F value, p value and degrees of freedom (df) between groups indicates statistical difference between merged upstream (reference) and individual downstream (W3 and W5) sample locations, range and mean for water chemistry for all four sample locations. All water chemistry data is measured in µg/L, pH in pH units, Electrical Conductivity in µs/cm, Dissolved Oxygen in % saturation, Turbidity in Nephelometric Turbidity Units and Temperature in degrees Celsius. BD = below laboratory detectable limits, n/a = not any.

Water	Statistics			Reference Sites					
Parameter/Sample location	F value	p value	df	W1 Range	W1 Mean	W1 Median	Bell Range	Bell Mean	Bell Median
pH	1544.1	4.8E-40	2	5.04-5.06	5.05	5.04	5.17-5.20	5.18	5.20
Electrical Conductivity	11232.8	4.9E-58	2	25.7-25.7	25.7	25.7	27.7-27.7	27.7	27.7
Dissolved Oxygen	0.28	0.75	2	90.4-90.4	90.4	90.4	89.1-89.9	89.6	89.6
Turbidity	6.29	0.004	2	0.1-1.3	0.5	2.2	0.1-1.2	0.6	0.9
Water Temperature	10.7	0.0002	2	11.2-11.2	11.2	11.2	10.2-10.2	10.2	10.2
Aluminium	4.05	0.03	2	30-330	178.3	175	40-70	54	50
Barium	65.3	3.4E-12	2	8-11	9.5	9.5	8-9	8.6	9
Boron	31.6	1.09E-07	2	7-9	8.0	8	8-8	8.0	8
Cadmium	n/a	n/a	2	BD	BD	BD	BD	BD	BD
Chromium	n/a	n/a	2	BD	BD	BD	BD	BD	BD
Cobalt	5.57	0.008	2	BD	BD	BD	BD	BD	BD
Copper	n/a	n/a	2	BD	BD	BD	BD	BD	BD
Iron	18.7	3.75E-06	2	210-550	381.7	390	140-410	300	375
Lead	n/a	n/a	2	BD	BD	BD	BD	BD	BD
Manganese	4.24	0.02	2	14-56	35.2	35	20-24	22.8	23
Molybdenum	n/a	n/a	2	BD	BD	BD	BD	BD	BD
Nickel	11.3	0.0002	2	BD-1	0.5	0.5	BD	BD	BD
Strontium	88.6	5.38E-14	2	4.3-6.3	5.22	5.1	3.4-3.8	3.58	3.6
Uranium	n/a	n/a	2	BD	BD	BD	BD	BD	BD
Zinc	7.50	0.002	2	1-6	3.67	3.5	2-3	2.4	2

Table 2. Continued.

Water	Downstream Mine Sites					
Parameter/Sample location	W3 Range	W3 Mean	W3 Median	W5 Range	W5 Mean	W5 Median
pH	7.27-7.37	7.29	7.35	6.97-6.98	6.97	6.97
Electrical Conductivity	316-316	316	316	217.8-217.9	217.9	217.9
Dissolved Oxygen	92.4-92.5	92.4	91.2	89.6-90.1	89.9	89.8
Turbidity	0.5-0.7	0.62	3.4	0.4-1.2	0.78	0.9
Water Temperature	12.4-12.4	12.4	12.4	10.8-10.8	10.8	10.8
Aluminium	BD-210	71.7	50	BD-40	21.7	25
Barium	19-23	20.8	20.5	14-19	16	15
Boron	1-9	9.5	9.5	9-10	9.17	9
Cadmium	BD	BD	BD	BD	BD	BD
Chromium	BD	BD	BD	BD	BD	BD
Cobalt	3-47	26.5	28	BD-3	1.67	2
Copper	BD	BD	BD	BD	BD	BD
Iron	38-420	134.3	87.5	42-82	66.8	77
Lead	BD	BD	BD	BD	BD	BD
Manganese	43-430	178.5	200	22-34	29.2	22
Molybdenum	BD	BD	BD	BD	BD	BD
Nickel	24-130	76.7	70	8-25	17.7	20
Strontium	45-68	59	59	28-47	36.2	34
Uranium	BD	BD	BD	BD	BD	BD
Zinc	21-190	105	99	8-31	20.8	24.5

Table 3. Sediment chemistry parameters, sample locations, Single Factor ANOVA, F value, p value and degrees of freedom (df) between groups indicates statistical difference between merged upstream (reference) and individual downstream (W3 and W5) sample locations range and mean for river sediment for all four sample locations. All river sediment chemistry data is measured in mg/kg. BD = below laboratory detectable limits.

Sediment Parameter/Sample location	Statistics			Reference Sites					
	F value	p value	df	W1 Range	W1 Mean	W1 Median	Bell Range	Bell Mean	Bell Median
Aluminium	9.52	0.003	2	790-2200	1247.5	1000	190-280	233.3	230
Barium	16.1	0.0002	2	10-33	18	14.5	3-8	4.75	4
Boron	103.9	6.03E-07	2	BD	BD	BD	BD	BD	BD
Cadmium	10.2	0.003	2	BD	BD	BD	BD	BD	BD
Chromium	9.99	0.003	2	BD-2	1.0	1	BD-1	0.25	BD
Cobalt	18.4	0.0002	2	BD-5	2.0	1.5	BD	BD	BD
Copper	9.59	0.003	2	2-6	3.5	3	BD	BD	BD
Iron	8.39	0.005	2	2300-7800	4000	2950	670-2500	1442.5	1300
Lead	3.02	0.09	2	3-8	4.5	3.5	BD-2	0.5	BD
Manganese	36.4	4.7E-06	2	38-300	127.5	86	7-22	13.3	12
Molybdenum	10.6	0.002	2	BD	BD	BD	BD	BD	BD
Nickel	20.8	0.0002	2	1-4	2	1.5	BD	BD	BD
Strontium	1.19	0.3	2	3-9	5.0	4	BD-4	1.25	0.5
Uranium	19.7	0.002	2	BD-0.2	0.06	BD	BD	BD	BD
Zinc	27.5	3.32E-05	2	1-3	1.75	1.5	BD	BD	BD

Table 3. Continued.

Sediment Parameter/Sample location	Downstream Mine Sites					
	W3 Range	W3 Mean	W3 Median	W5 Range	W5 Mean	W5 Median
Aluminium	1500-8700	5083.3	5200	230-2200	1034	310
Barium	37-170	101.6	88	2-22	7.5	3
Boron	BD-6	2	BD	BD	BD	BD
Cadmium	BD-1	0.47	0.5	BD	BD	BD
Chromium	2-8	4.0	2	BD-3	1.0	BD
Cobalt	61-1000	551.8	635	BD-23	6.0	0.5
Copper	8-29	17.0	15.5	BD-7	2.4	BD
Iron	2400-19000	9950	10350	490-3900	1982	1100
Lead	5-17	8.4	6	BD-6	2.0	BD
Manganese	270-9300	5474	6900	3-220	76.0	11
Molybdenum	BD-9	4	4	BD	BD	BD
Nickel	160-1100	606.7	630	2-67	19.0	2
Strontium	3-27	12.3	9	BD-8	2.8	0.5
Uranium	0.1-1.4	0.53	0.4	BD-0.3	0.08	BD
Zinc	300-1100	734	760	2-97	25.0	2

Table 4. Beetle chemistry parameters, sample locations, Single Factor ANOVA, F value, p value and degrees of freedom (df) between groups indicates statistical difference between merged upstream (reference) and individual downstream (W3 and W5) sample locations, range, mean for all four sample locations. Percentage increase from W3 water chemistry contaminants to W3 beetle chemistry contaminants are offered in the last column. All beetle data is measured at µg/Kg. BD = below laboratory detectable limits, n/a = not any.

Beetle Parameter Sample location	Statistics			Reference Sites					
	F value	p value	df	W1 Range	W1 Mean	W1 Median	Bell Range	Bell Mean	Bell Median
Aluminium	3.04	0.07	2	3713-8184	6023.3	6496	3963.4-7349.8	4990.8	4578
Arsenic	1.56	0.23	2	177-496.2	501.2	514	260-745.9	318.8	326.1
Barium	0.99	0.38	2	177.8-908.2	479.9	394.4	205.8-724.2	405.9	339.7
Beryllium	9.87	0.00009	2	0.3-2.74	1.3	1.36	0.65-2.83	1.4	1.21
Boron	1.23	0.31	2	947.6-1573	1250.9	1309	1039.9-4473.9	1869.2	1494
Cadmium	1.50	0.24	2	6.4-62.1	19.3	12.7	13.0-142.8	59.5	33.7
Chromium	0.16	0.85	2	31.7-258.7	118.1	88.9	42.8-171.4	94.9	70.5
Cobalt	4.31	7.44E-10	2	49.45-176.9	87.2	75.2	83.88-492.7	209.6	147.1
Copper	0.66	0.53	2	13791-29689	23279.8	25755	12563-102888	17147.9	17810
Iron	5.21	0.01	2	32925-59215	45530.3	47658	34133-71457	43373.4	37886
Lead	1.16	0.33	2	12.9-48.5	30.8	30.1	18.3-43.4	32.5	34.2
Manganese	19.5	0.00001	2	17998-35040	26850.7	27718	27758-55660	43243.1	43229
Molybdenum	10.1	0.0008	2	46.61-64.66	53.4	49.4	40.49-61.75	49.8	48.5
Nickel	30.2	4.97E-07	2	16.45-97.61	62.1	73.4	12.27-106.1	40.9	27.5
Selenium	14.8	8.33E-05	2	918-1855	1376.1	1274	1339-2019	1726.1	1732
Silver	1.78	0.19	2	21.5-70.4	47.7	52.3	15.9-50.8	31.0	31.7
Strontium	6.17	0.007	2	2285-4543	3445.5	3376	3260-6176	4730.3	4642
Titanium	0.48	0.62	2	109.9-270.3	189.7	189.6	178.8-203.8	189.0	189.1

Beetle	Statistics			Reference Sites					
Parameter Sample location	F value	p value	df	W1 Range	W1 Mean	W1 Median	Bell Range	Bell Mean	Bell Median
Uranium	6.14	0.008	2	0.45-1.46	0.9	0.97	0.64-2.25	1.2	1.11
Vanadium	1.79	0.19	2	30.6-85.1	64.8	73.6	55.0-75.6	64.7	63.1
Zinc	5.22	0.01	2	52037-96949	75489.9	73689	76576-14418	103241.4	99791

Table 4. Continued.

Beetle	Downstream Mine Sites						Mean % Increase
Parameter Sample location	W3 Range	W3 Mean	W3 Median	W5 Range	W5 Mean	W3 Median	
Aluminium	5624.9-63126	18167.3	11582	5003.3-14091	9227.8	8976	253.4
Arsenic	346.7-584	474.0	486.5	453.2-867.3	541.0	485.3	n/a
Barium	292.7-844.0	623.1	684.3	276.8-1427.9	565.3	399.5	29.9
Beryllium	5.06-26.8	10.3	5.31	1.83-4.1	2.7	2.39	n/a
Boron	671.2-1473.5	1065.2	1024	1018.8-14	1230.8	1206	112.1
Cadmium	35.9-373.4	103.5	53.9	14.9-195.9	58.9	33.4	n/a
Chromium	30.2-868.9	124.1	103.4	44.4-156.9	113.2	126	n/a
Cobalt	2930-8959	6270.5	6310	577.7-2513	1089.8	856.2	236.6
Copper	9619-55561	24945.9	18892	1852-44140	25828.3	19838	n/a
Iron	45220-114968	72586.4	64092	40420-109613	68016.2	64954	540.5
Lead	94.1-294.2	42.0	26.8	30.0-257.2	42.9	39.2	n/a
Manganese	79352-184237	117147.9	96872	39751-132119	70644.1	63304	656.3
Molybdenum	52.42-160.9	100.7	81.2	59.13-123.9	86.2	83.9	2013.7
Nickel	1918-7780	4491.1	3419.2	585.7-1497	932.9	857	58.6
Selenium	2092-3578	2767.4	2864	1673-3178	2106.4	1709	n/a
Silver	20.2-140.8	67.5	53.7	20.4-83.4	46.5	38.8	n/a
Strontium	2232-2973	2490.0	2426	2743-5376	3502.3	3075	42.2
Titanium	128.5-285.3	207.2	206	170.7-284.6	203.8	190.2	n/a
Uranium	1.98-13.53	4.7	2.88	1.04-3.16	2.3	2.53	94.6
Vanadium	41.8-124.1	71.7	62.1	66.3-126.9	84.5	78.8	n/a
Zinc	67701-121424	96659.4	101064	96772-200559	132707.2	129659	920.6

Mean aquatic beetle concentrations of Arsenic, Beryllium, Cobalt, Iron, Manganese, Molybdenum, Nickel, Selenium, Strontium, Uranium and Zinc were found to be highly statistically different between reference and coal mine impacted samples (Table 4). When analysed by Analysis of Similarity (ANOSIM) significant differences were found between W1 and W3 (R statistic 0.963), W1 and W5 (R statistic 0.639), Bell and W3 (R statistic 0.924) and Bell and W5 (R statistic 0.523) whilst no significant difference was recorded for W1 and Bell (R statistic 0.123). Biota and/or Environmental matching (BEST) results show that Manganese, Cobalt, Nickel and Zinc have the strongest influence in the differences recorded across beetle sample locations (best results, Corr = 0.944), (method, BIOENV).

Reference site beetles recorded mean arsenic concentrations of 318.8 and 501.2 µg/kg increasing slightly at both downstream sites W3 and W5 (mean 474 and 541 µg/kg). Mean Beryllium at reference sites was recorded at 1.42 and 1.3 µg/kg. The downstream sites W3 and W5 recorded mean Beryllium results of 10.3 and 2.7 µg/kg. Reference sites recorded mean cobalt concentrations of 209.6 and 87.2 µg/kg, in comparison W3 and W5 results showed mean cobalt of 6270.5 and 1089.8 µg/kg. Iron at reference sites was recorded at 43373 and 45530 µg/kg. Whilst in comparison W3 and W5 recorded mean iron

results of 72586 and 68016 µg/kg. Reference site results show mean manganese concentrations of 43243 and 45530 µg/kg. The downstream sites W3 and W5 recorded mean manganese of 117148 and 70644 µg/kg. Beetles sampled at both reference sites recorded mean molybdenum of 49.8 and 53.4 µg/kg. In comparison W3 and W5 (downstream sites) recorded mean molybdenum of 100.7 and 86.2 µg/kg. Nickel concentrations from both reference sites was 40.9 and 62.1 µg/kg (mean), whilst in comparison mean nickel at the downstream sites (W3 and W5) was recorded at 4491.1 and 932.9 µg/kg (Table 4).

Reference site selenium concentrations were recorded at 1726 and 1376 µg/kg respectively. In comparison W3 and W5 recorded mean selenium results was 2767 and 2106 µg/kg. Mean strontium concentrations were recorded at reference sites as 4730.3 and 3445.5 µg/kg with the downstream sites (W3 and W5) recording mean strontium of 2490.0 and 3502.3 µg/kg. Reference sites mean uranium was recorded at 1.2 and 0.9 µg/kg respectively. In comparison W3 and W5 (downstream sites) recorded mean uranium results of 4.7 and 2.3 µg/kg. Mean zinc results for both reference sites recorded concentrations of 103241 (Bell) and 75490 (W1) µg/kg respectively, increasing downstream of each impacted sample locations paired reference site to mean zinc concentrations of 132707 (W5) and 96659 (W3) µg/kg (Table 4).

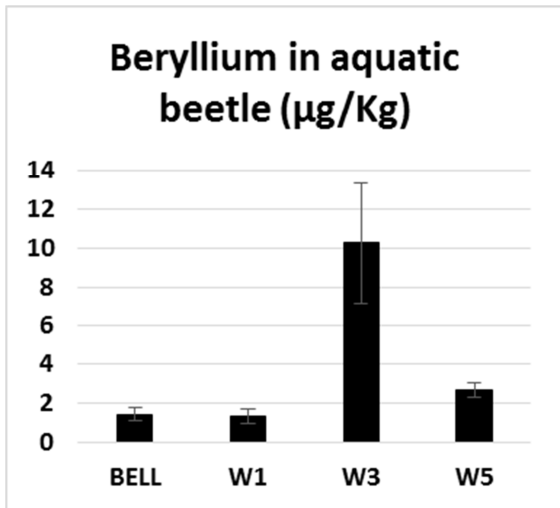


Figure 2. Mean beryllium with standard error bars for aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

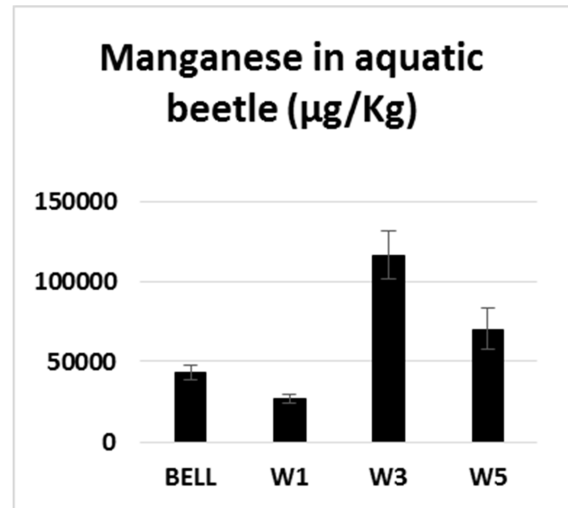


Figure 5. Mean Manganese with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

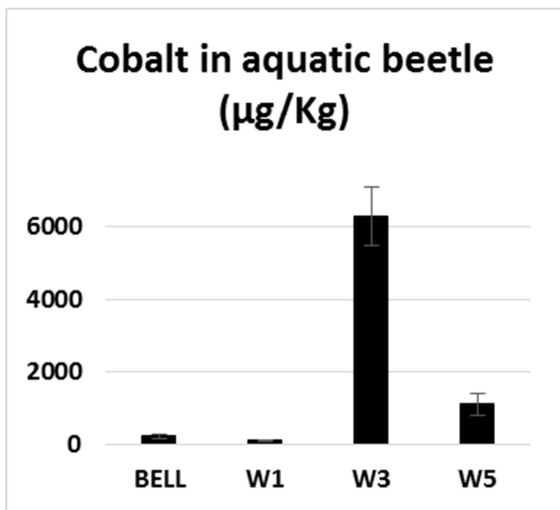


Figure 3. Mean Cobalt with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

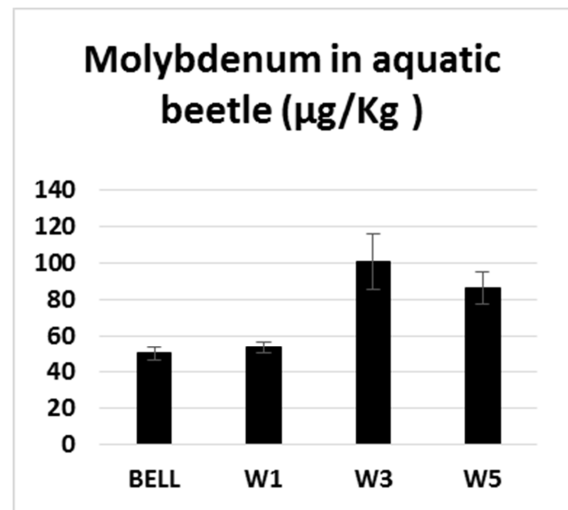


Figure 6. Mean Molybdenum with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

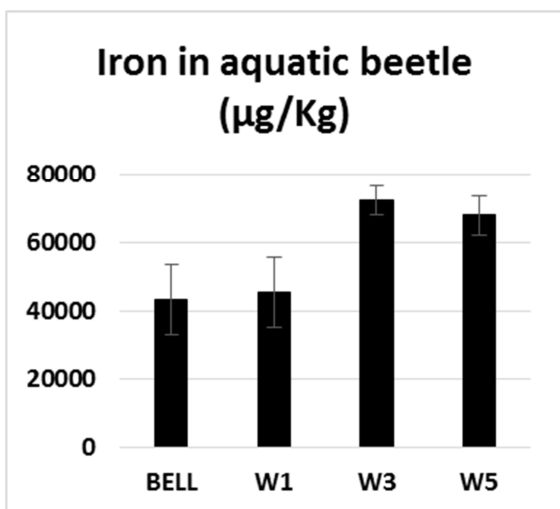


Figure 4. Mean Iron with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

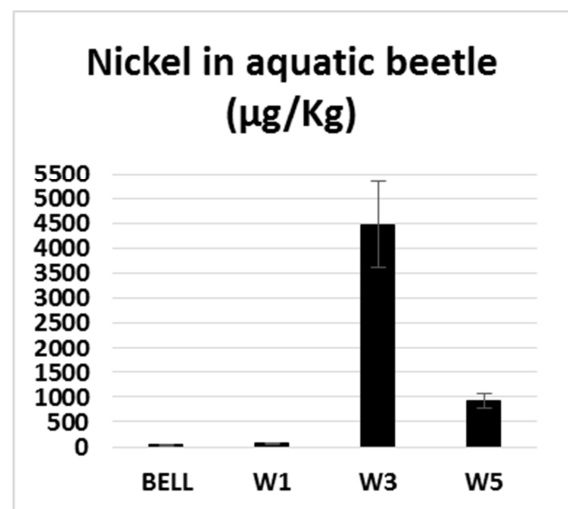


Figure 7. Mean Nickel with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

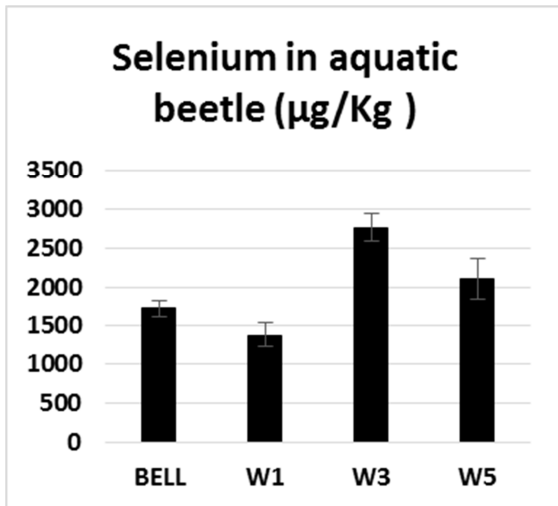


Figure 8. Mean Selenium with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

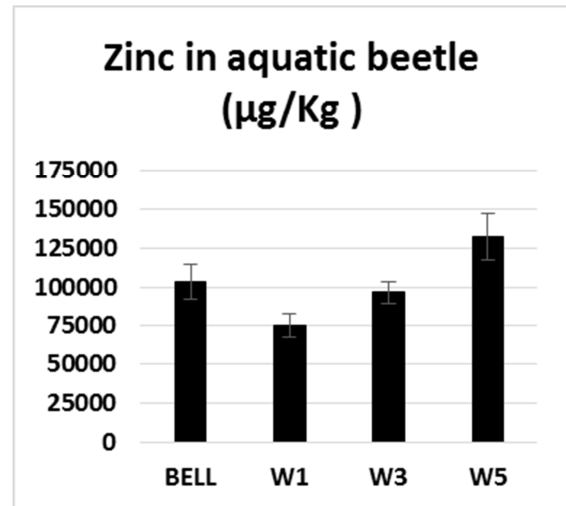


Figure 11. Mean Zinc with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

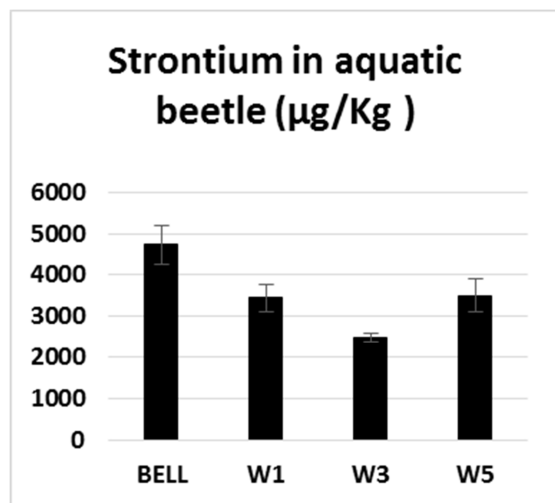


Figure 9. Mean Strontium with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

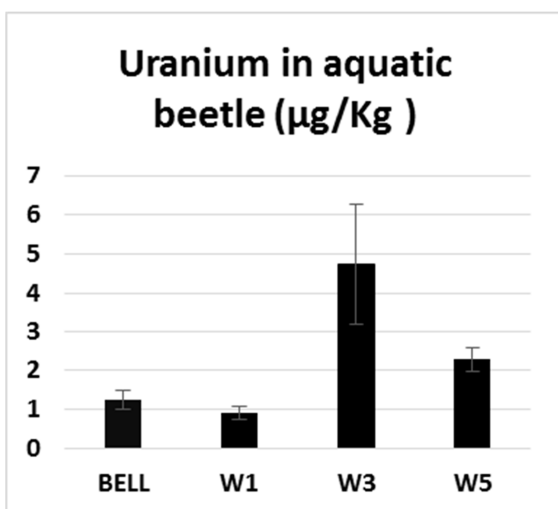


Figure 10. Mean Uranium with standard error bars in aquatic predacious beetles (Gyrinidae) measured in µg/Kg. Bell and W1 are reference samples whilst W3 and W5 are coal mine impacted samples.

4. Discussion

This study may be the first to investigate the bioaccumulation of contaminants from a licenced and regulated coal mine wastewater discharge on an aquatic predacious beetle (*Macrogyrus rivularis*).

Results show that the one species of aquatic predacious beetle has increased concentrations of contaminants at impacted sample locations when compared to non-impacted reference sites. Many of the contaminants can be directly linked to the coal mine wastewater discharge as shown by water column and stream sediment results. In its crux, this one aquatic predacious beetle is at the lower trophic level of the food chain and is a food source for many other aquatic and terrestrial species. The implications of concentrating large amounts of contaminants within lower trophic order species, whom are predated on by aquatic and terrestrial species may allow for a link to continue the biomagnification of the contaminants found within the impacted sampled beetles. The ability for these aquatic beetles to be pray for terrestrial species is also of major concern as this may also allow for the mobilisation of these contaminants from the aquatic ecosystem to the terrestrial environment.

There are many studies investigating water column pollution from metal contamination from a broad range of mining activities. Many investigate the links between metal mining impacts on water chemistry, stream sediments, aquatic flora, aquatic fauna, including; fish, turtles to an array of macroinvertebrates [1, 3, 5, 6, 14, 15]. Only a few studies have investigated the bioaccumulation of metals from coal mining activities within macroinvertebrates. Most often these impacts are from waste coal ash dams or coal fine spills but not discharged, licenced and regulated coal mining wastewaters.

Miller et al. 2013 studied the bioaccumulation of the metal selenium from abandoned coal mine pit lakes within two fish species. It was reported that selenium was bioaccumulating

within the fish tissue to levels above USA EPA tissue guidelines and concluded that the current reclamation practices implemented in the abandoned coal mine pit lakes were failing and that significant risk to wildlife and human health was of concern [11]. Within Australia, Telford et al. 2009 and Jasonsmith et al. 2008 have conducted studies assessing the bioaccumulation of metals from mining activities. Telford et al. 2009 investigated bioaccumulation of metals in aquatic gastropods from antimony and arsenic mines and found that concentrations of arsenic were statistically higher in mining impacted samples [21]. Jasonsmith et al. 2008 studied the bioaccumulation of selenium in water, sediment, zooplankton, benthic material, benthic algae, oligochaetes, gastropods, crustaceans, insects and fish residing in a coal power stations cooling reservoir in Lithgow, NSW close (within 15km) to the vicinity of this study. It was found that selenium was found in low concentrations within oligochaetes, gastropods, bivalves and crustaceans sampled and in contrast insects sampled recorded concentrations some 1000 times higher [23]. Jasonsmith et al 2008 concluded that the detritus invertebrates bioaccumulated selenium in much greater concentrations than oligochaetes, gastropods, bivalves and crustaceans they sampled [23].

Swansburg et al. 2002 found many metal concentrations below laboratory detectable limits within water samples in their study whilst recording detectable (in some cases statistically different) limits of the same heavy metals in chironomid tissue. Their study investigated metal mining impacts on clean dilute ($EC < 100 \mu\text{S/cm}$), circumneutral (pH 6.5-7.5) streams in New Brunswick, Canada. Cadmium, Chromium, Cobalt, Copper, Lead and Nickel all recording detectable limits within chironomid tissue whilst only Molybdenum still recorded below detectable limits. Significant differences between reference and mine impacted chironomid samples were recorded for copper, cadmium and zinc only [22]. This is similar to this study with many contaminants within the water column recording below laboratory detectable limits at both reference and impacted sample locations, becoming more detectable within stream sediment and in turn sampled beetles at impacted sites.

Swansburg et al 2002 found increases between 3 and 16 times were recorded across the five streams for zinc in chironomid tissue. The greatest increase being the stream impacted by the metal mine facility "Caribou" was reported to increase some 30 times from a reference mean of 113000 mg/kg and an impacted mean of 1813000 mg/kg [22]. This current study found zinc in beetles to be statistically significant recording increases at lower concentrations close to the vicinity of 1.5 times greater at impacted sites and increasing from water column concentrations to beetle concentrations in the magnitude of 77000 times. Cadmium was found by Swansburg et al 2002 to be nearly 65 times higher in impacted chironomid tissue samples at the same mining site increasing from 600 to 37200 mg/kg [22]. This study found cadmium to increase two-fold between the reference site W1 and the impacted site W3 but was not

statistically significant. Copper was recorded by Swansburg et al 2002 to increase 11 and 12 times at two impacted sites from 13mg/kg upstream to 153mg/kg downstream and 10mg/kg upstream to 115 mg/kg downstream. Copper in this current study was not statistically significantly different with W1, W3 and W5 all recording similar copper concentrations with Bell Creek recording copper concentrations approximately 0.15 times less.

Swansburg et al 2002 found mine effected chironomids to have deformed mentums. It was also reported increases in cobalt for four of their five sample streams [22]. The highest increase from reference to mine effected cobalt in chironomid tissue was 600 (reference) to 3200 (mine impacted) $\mu\text{g/kg}$ just over five times higher. This study found cobalt increased from 87.2 $\mu\text{g/kg}$ at the reference site W1 to 6271 $\mu\text{g/kg}$ directly below the coal mine wastewater inflow at site W3 which is over 70 times higher than W1. Water column concentrations of cobalt in this current study increased some 50000 times from water column concentrations to beetle concentrations.

Otter et al. 2012 found Selenium and Arsenic bioaccumulated at differing concentrations within two fish species they studied impacted by a wet coal ash spill [17]. Statistical differences were recorded for both metal concentrations when compared between reference and impacted samples. Otter et al. 2012 concluded that the difference in bioaccumulated selenium and arsenic across species in impacted sites was due to the differing stomach pH of the species [17]. In this current study, the reference sites used may have a greater functional ability to sequester and bioaccumulate heavy metals due to their naturally mildly acid pH in comparison to the impacted sites whom have a "treated" alkaline pH [17, 24, 25]. This is of major concern as impacted sites beetles in this study have significantly higher concentrations of heavy metals whilst having a much lower ability to bioaccumulate these contaminants.

This raises questions as to what will occur after mining activities are no longer undertaken at this colliery as when mining ceases so does the water treatment process. At present pH is increased during the treatment process and water currently being discharged may have a much lower rate of bioavailability of some contaminants for aquatic biota as described by Atkinson et al. 2007 [25]. Once treatment ceases and the Wollangambe Rivers pH will naturally reduce to a background pH which is mildly acidic. This decrease in pH may open a new avenue for increased bioaccumulation from the legacy pollutants within the impacted streams sediments. The findings of this research evoke concerns over the validity of water column pollutant limits if they are in fact allowing for legacy pollutants to bioaccumulate and or magnify within the receiving waterways aquatic ecosystem.

5. Conclusions

Long term legacy pollutants are of great concern worldwide. Sericano et al. 1995 and Ashraf 2011 suggest heavy metals have the potential to accumulate within biota at

toxic concentrations and have chronic ecological impacts within ecosystem food chains [1, 3]. Wang and Rainbow 2008 raised concerns over the longevity of heavy metal pollutants in aquatic environments due to their ability to deposit into waterway sediments which can potentially remain indefinitely [6]. Kolaříková *et al.* 2012 found that bioaccumulation of pollutants within four macroinvertebrate species within the Elbe and Vltava Rivers in the Czech Republic were still persistent after water quality improvements were implemented. It was reported that these heavy metals concentrations were consistent over their 12-year study and they recorded no significant reduction in heavy metal concentrations other than Hg [7].

The implications that contaminants from the licenced and regulated wastewater being discharged may be accumulating within aquatic biota is of major concern. The implications that this regulated water column pollution is accumulating at magnified rates shows a major floor in water column pollution licensing. It is recommended that further research should be undertaken by the New South Wales Environmental Protection Authority to better assess these broader implications of legacy contaminants from licenced and regulated coal mine wastewater discharges are having on the aquatic ecosystem of EPL protected waterways.

6. Recommendations

It is recommended that further research should be undertaken by the New South Wales Environmental Protection Authority to better assess the implications of coal mine wastewater contaminants bioaccumulation and or biomagnification in EPL protected waterways stream sediments and their biota. If in fact the contaminants are leaving the water column and bioaccumulating within the aquatic biota of their receiving waterways, serious long-term legacy pollutant impacts may persist. Of equal concern is if in fact these pollutants are biomagnifying within the aquatic biota there is feasibility that this may transpose to the terrestrial environment and the extent of the contamination may be more far spreading than this study has found.

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