Impact of Heavy Metal Contamination on Aquatic Species Richness at Ashfield Flat Reserves, Western Australia

Introduction

Ashfield Flats Reserve, situated in Perth, Western Australia, comprises wetlands and salt marshes that hold significant ecological value and has been included in the Directory of Important Wetlands in Australia (Department of Agriculture Water and the Environment, 2021). However, the reserve faces challenges due to changes in hydrology resulting from groundwater usage and multiple drains discharging polluted stormwater into the reserve (Kellenberger, 1998; Rate & McGrath, 2022). Heavy metals are a concerning group of pollutants due to their toxic effects on the aquatic ecosystem, including poisoning and impaired reproductive capabilities in marine organisms (Authman et al., 2015). Therefore, it is imperative to assess the impacts of heavy metals on the marine ecosystem at Ashfield Flats, in order to implement effective measures that minimize their detrimental effects.

Water samples were collected from drains and wetlands throughout the riparian reserve to measure the concentration of heavy metals, with their locations depicted in *figure 1*. Additionally, eDNA samples were collected around the same area to measure the number of aquatic species present. The objective of this report is to evaluate the effect of heavy metals on the ecosystem of Ashfield Flats Reserve. To accomplish this, we will: 1) compare the concentration of heavy metals with guideline values from ANZECC & ARMCANZ (2000), 2) analyse the spatial patterns of heavy metals across the site and 3) analyse the relationship between heavy metal concentrations and species richness using correlation and regression.

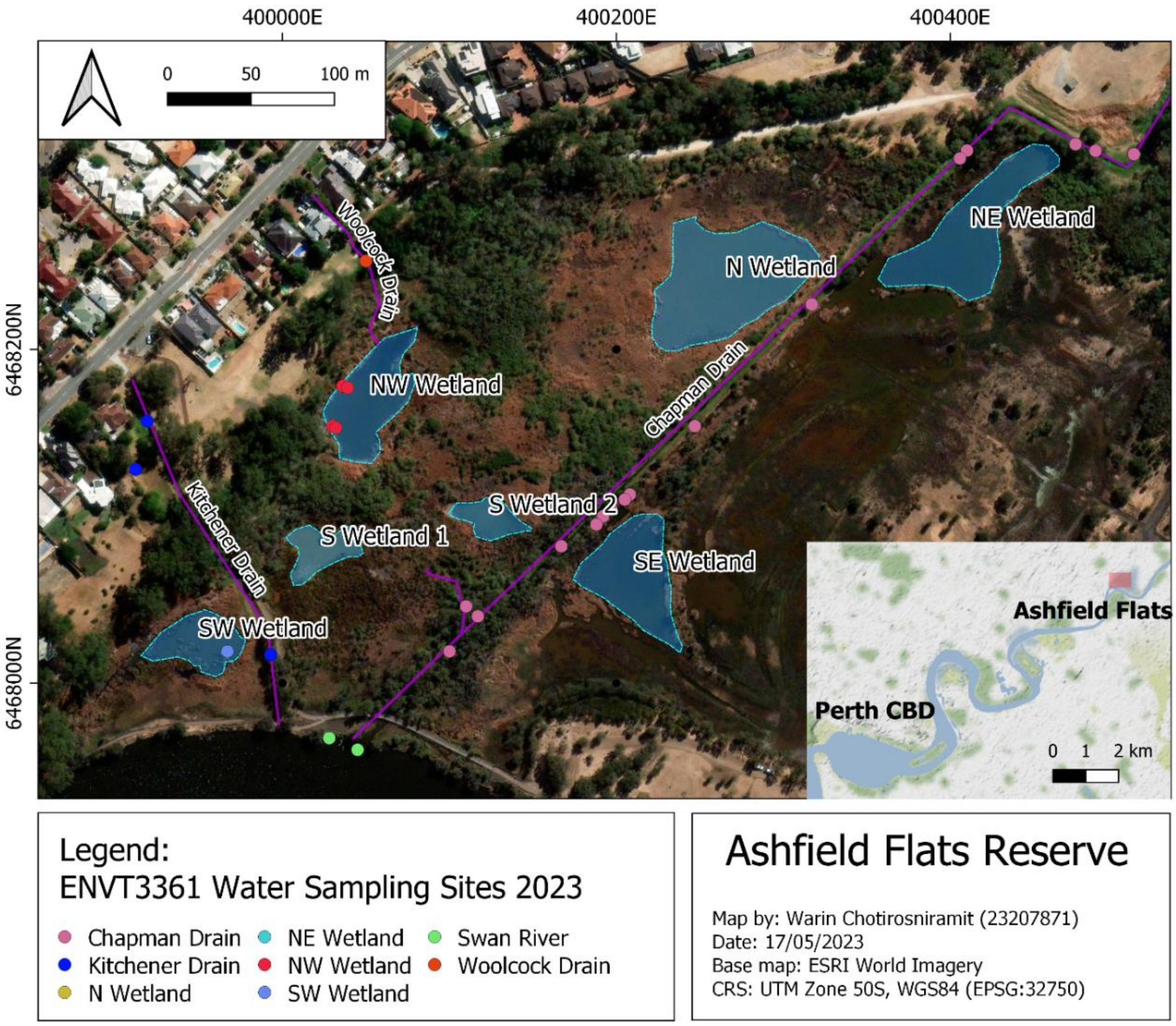

Figure 1: Map of Ashfield Flats Reserve showing the drains, the wetlands, and the water sampling sites

Results

Comparison with toxicant guideline values

From our water analysis, only aluminium, chromium, manganese, nickel and zinc had enough observations above the detection limit for further analysis, as shown in *Table 1*. Visualizations in *figure 2,3,4,5* and *6* show the distribution of data for the five toxicants with the guideline thresholds given by ANZECC & ARMCANZ (2000). Outliers above the upper boundary were observed for all elements except chromium. All toxicants, except nickel, had concentrations exceeding at least one of the thresholds.

Table 1: Summary table of mean statistics of the concentration of each metal toxicant in mg/L

Toxicant	Mean	Median	Lower quartile	Upper quartile	Minimum value	Maximum value	Number of observations above detection limit
Aluminium (Al)	0.0963	0.05	0.04	0.0825	0.03	0.51	24
Chromium (Cr)	0.00609	0.008	0.002	0.009	0.001	0.01	35
Manganese (Mn)	0.2	0.18	0.09	0.24	0.02	1.01	35
Nickel (Ni)	0.00171	0.001	0.001	0.002	0	0.008	28
Zinc (Zn)	0.452	0.01	0.01	0.03	0	3.76	17

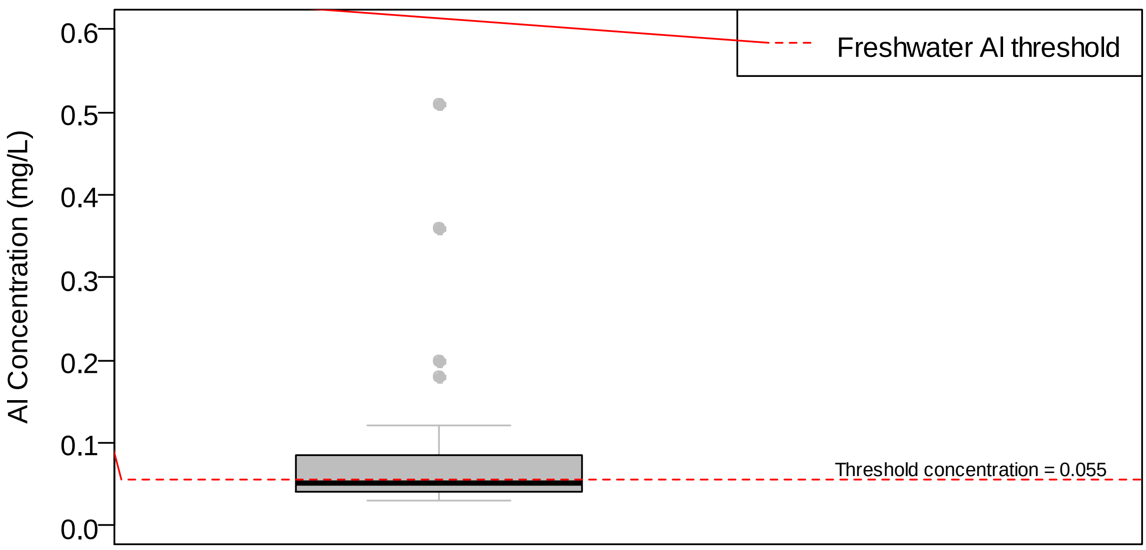

Figure 2: Boxplot of aluminium concentration observed across the site and its guideline thresholds

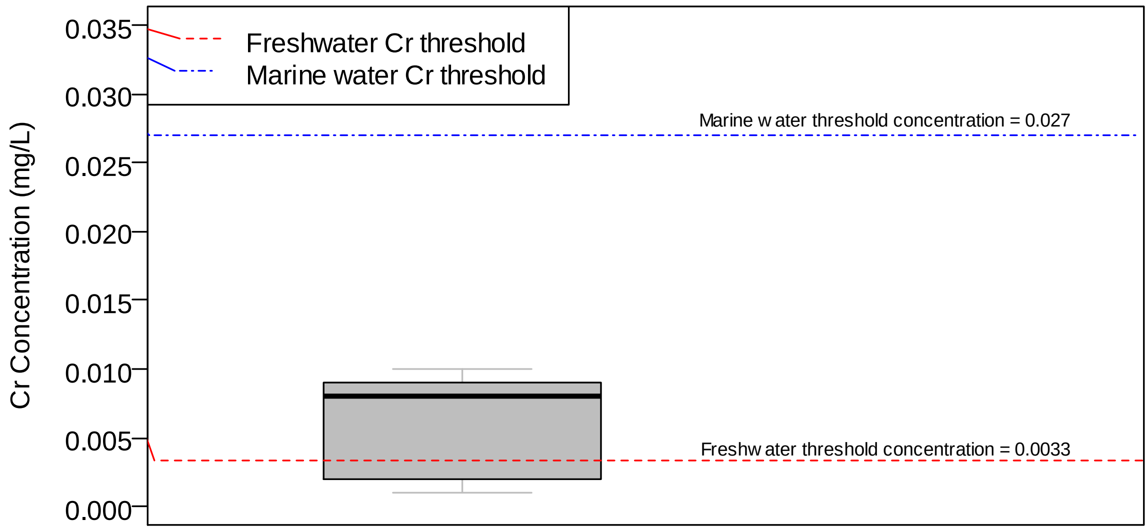

Figure 3: Boxplot of chromium concentration observed across the site and its guideline thresholds)

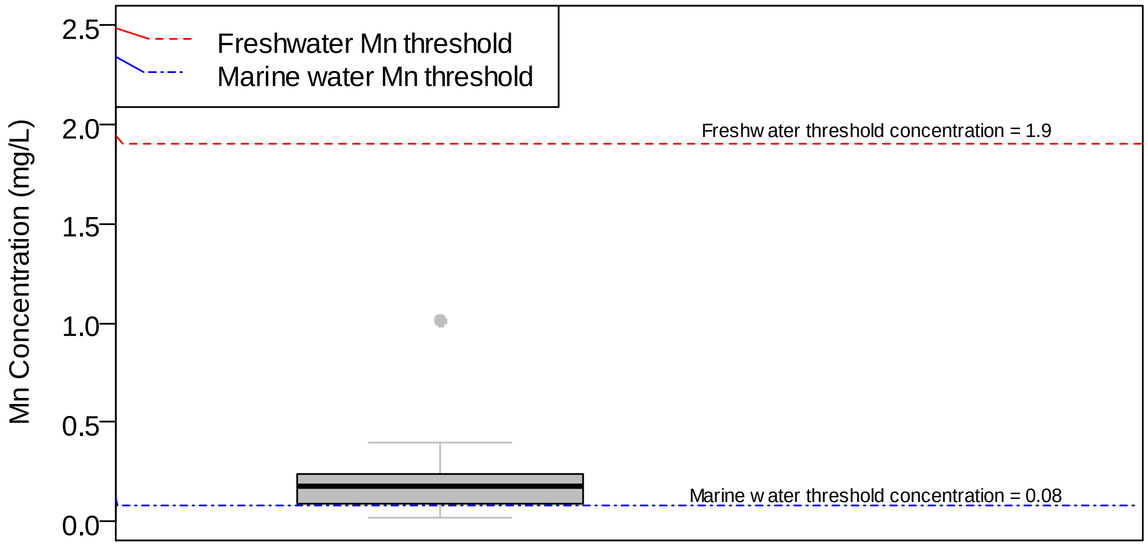

Figure 4: Boxplot of manganese concentration observed across the site and its guideline thresholds

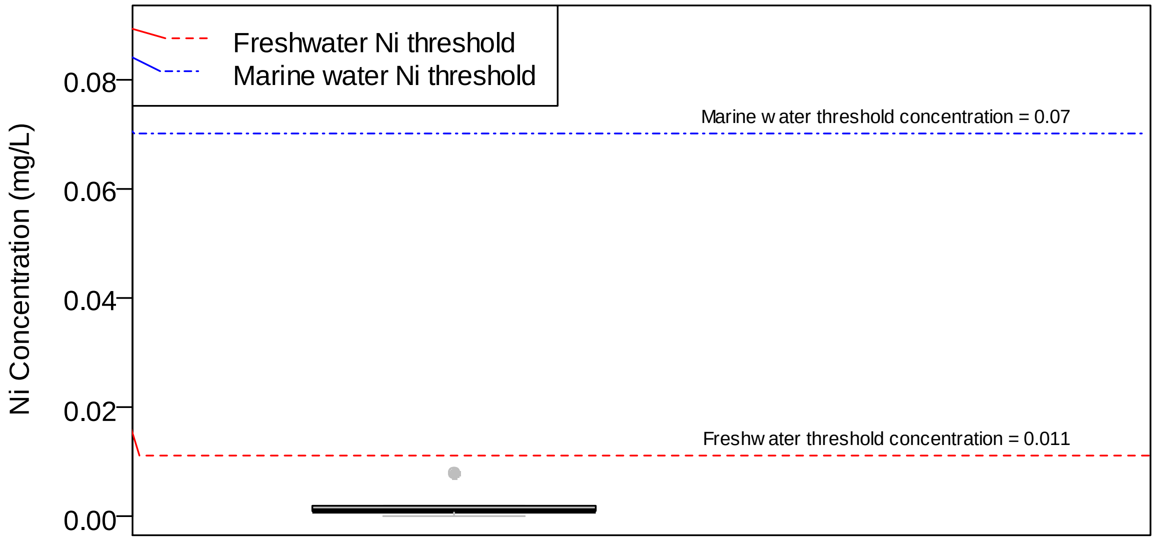

Figure 5: Boxplot of nickel concentration observed across the site and its guideline thresholds

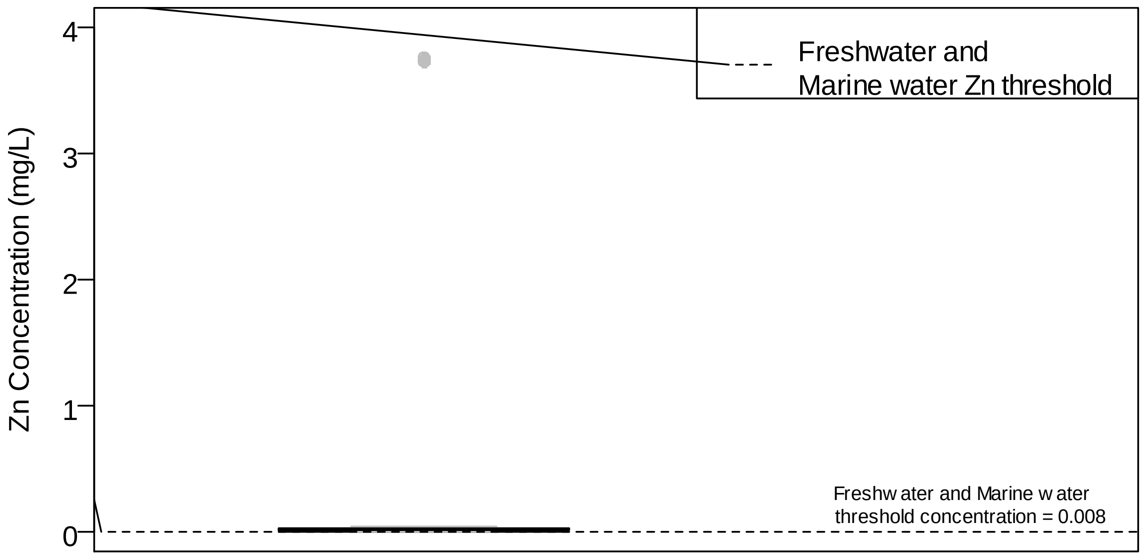

Figure 6: Boxplot of zinc concentration observed across the site and its guideline thresholds

The Shapiro-Wilk test for all toxicants returned p-values of less than 0.05; therefore, we rejected the null hypothesis and concluded that the datasets significantly deviated from a normal distribution (*Table 2*). This led us to use the one-sample Wilcoxon signed-rank non-parametric test where the null hypothesis is that the population's median is equal to the specified value and the alternative hypothesis is that the median is greater than the specified value.

Table *3* shows that the p-values from aluminium, manganese and nickel were greater than 0.05 for freshwater thresholds, whereas the p-values for chromium and zinc were smaller than 0.05. For marine water threshold, the p-values for chromium and nickel were greater than 0.05, while the p-values for manganese and zinc were smaller than 0.05.

Table 2: P-value results from Shapiro-Wilk test of each toxicant

Toxicant	Aluminium (Al)	Chromium (Cr)	Manganese (Mn)	Nickel (Ni)	Zinc (Zn)
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Alpha level is set at 0.05

Table 3: ANZECC & ARMCANZ Guideline thresholds with 95% level of species protection for each metal toxicant, p-value results from one-sided t-tests and the number of samples that exceeded the guideline thresholds

Toxicant	Guideline threshold value (mg/L)		P-value from comparison against the threshold		Number of samples that exceeded the threshold	
	Freshwater threshold	Marine water threshold	Freshwater threshold	Marine water threshold	Freshwater threshold	Marine water threshold
Aluminium (Al)	0.055	-	0.254	-	10	-
Chromium (Cr)	0.0033	0.027	<0.001	1	23	0
Manganese (Mn)	1.9	0.08	1	<0.001	0	27
Nickel (Ni)	0.011	0.07	1	1	0	0
Zinc (Zn)	0.008	0.008	0.0137	0.0137	14	14

Note: Alpha level is set at 0.05

Spatial patterns of toxicants

Aluminium concentrations were above the threshold for all drains, with extremely high concentration along Woolcock drain (*Figure 7*). Chromium concentrations were above the freshwater threshold along the Chapman and Kitchener drains and in NW, SW, N, and NE wetlands (*Figure 8*). Several extreme chromium concentrations were observed between S Wetland 2 and SE Wetland, in NW wetland and around where the Chapman drain meets the Swan River (*Figure 8*). Concentrations of manganese were above the marine water threshold along all three drains, with exceptionally high concentration detected below S Wetland 2 along Chapman drain (*Figure 9*). *Figure 10* illustrates that nickel concentrations were low throughout the site, with one relatively higher concentration along Woolcock drain but not higher than any threshold. Similar observations were made for zinc, but the higher concentration in Woolcock drain exceeded the guideline thresholds (*Figure 11*).

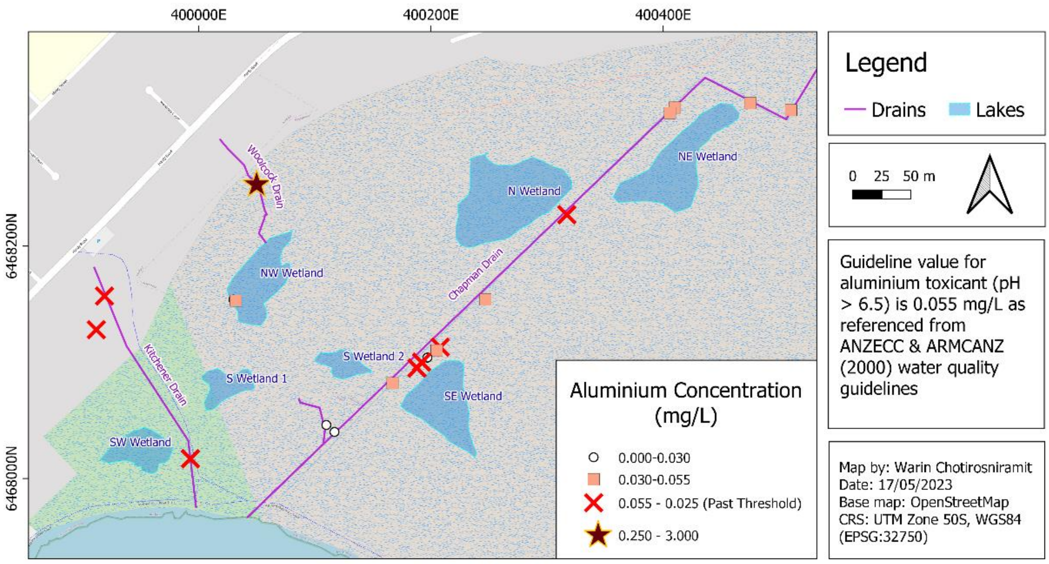

Figure 7: Spatial patterns of aluminium concentration in water samples collected at Ashfield Flats

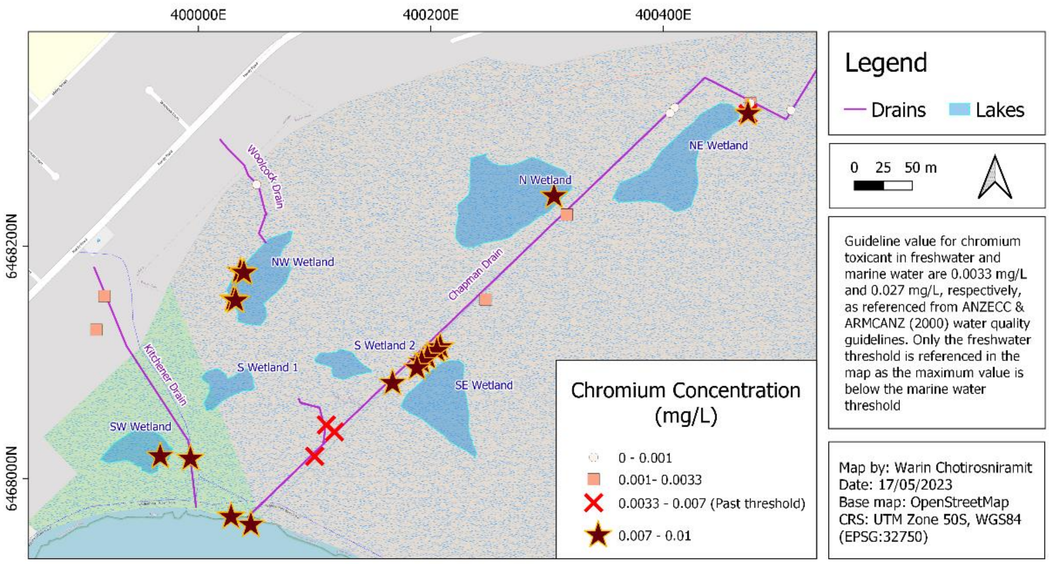

Figure 8: Spatial patterns of chromium concentration in water samples collected at Ashfield Flats

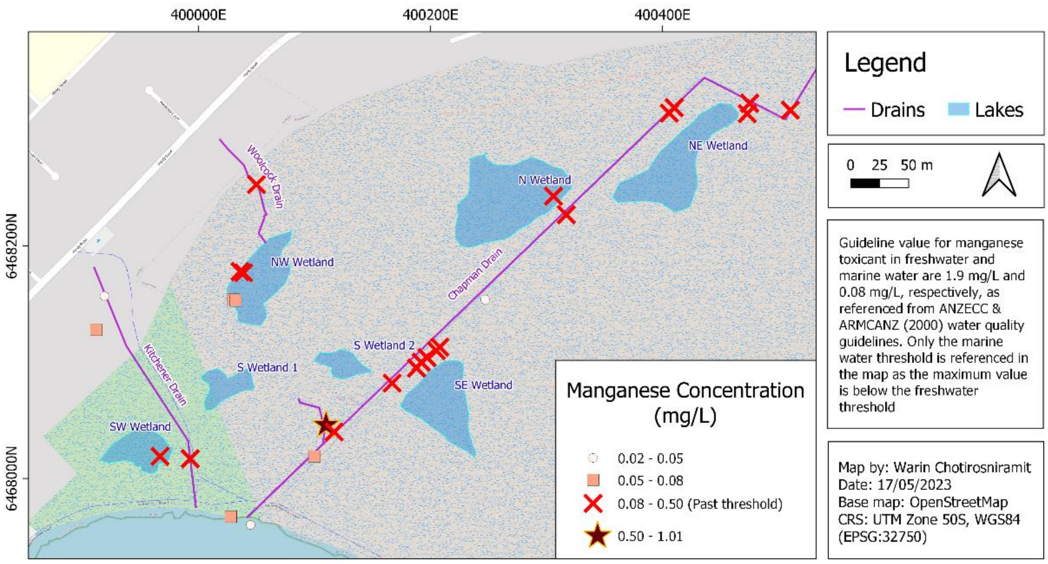

Figure 9: Spatial patterns of manganese concentration in water samples collected at Ashfield Flats

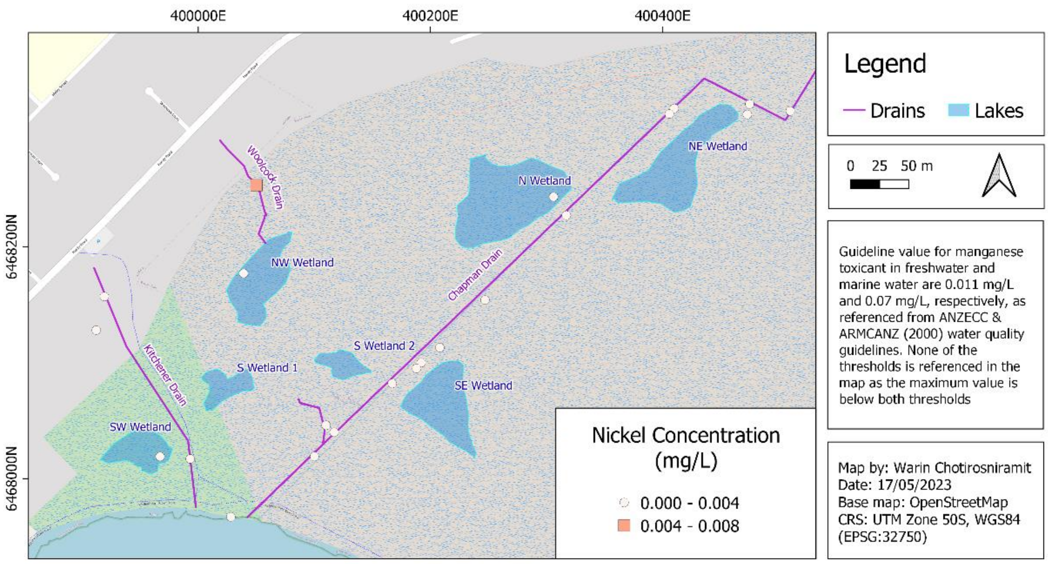

Figure 10: Spatial patterns of nickel concentration in water samples collected at Ashfield Flats

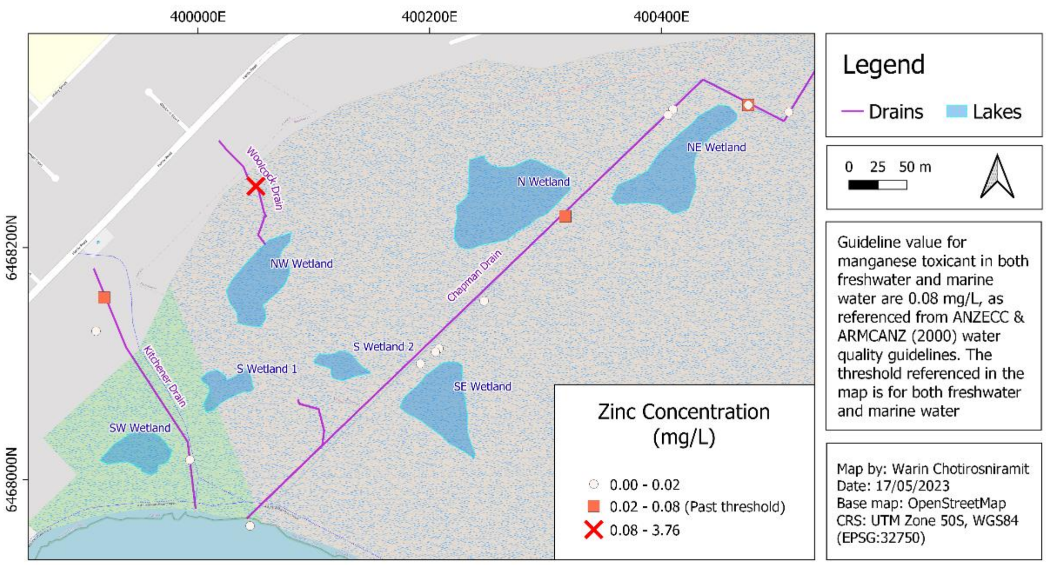

Figure 11: Spatial patterns of zinc concentration in water samples collected at Ashfield Flats

Relationship between heavy metal concentrations and species richness

The eDNA analysis was preprocessed to include only fish and frog species because they are less mobile, making them more susceptible to heavy metal contamination. The mean number of these species at each site is displayed in *Table 4* along with the mean concentration of heavy metals. The Shapiro-wilk test returned p-values smaller than 0.05 for all variables; therefore, we rejected the null hypothesis and concluded that the datasets did not follow a normal distribution. Hence, we used Spearman's correlation where the null hypothesis is that there is no relationship between the two variables.

Table 4: Mean number of species and mean concentration of different toxicants at each sampling site in Ashfield Flats

Group ID	Site	Species	Al	Cr	Mn	Ni	Zn
1	Upper Chapman outside fence	2.5	0.035	0.001	0.205	0.001	0.01
2	Chapman Bend	2	0.04	0.0015	0.24	0.002	0.02
3	Mid-Chapman drain	1.5	0.05	0.002	0.05	0.001	0.01
4	Lower Chapman	7.25	NA	0.007	0.07	0.002	NA
5	Swan River	7.25	NA	0.008	0.06	0.001	0.01
6	SW Wetland	6.25	NA	0.008	0.09	0.001	NA
7	Upper Kitchener	2.5	0.19	0.002	0.04	0.0015	0.02
8	Lower Kitchener	2.75	0.085	0.0085	0.175	0.001	0.01
9	NW Wetland	1	0.035	0.0085	0.085	0.001	NA
10	Chapman side drain	5.25	0.03	0.0065	0.58	0.001	NA
11	NE wetland pond	1.75	NA	0.0075	0.155	0.002	NA
12	Upper Chapman below bend	1.75	0.05	0.001	0.31	0.002	0.015
13	Mid-Chapman between N & NE wetlands	1.5	0.12	0.002	0.18	0.001	0.04
14	Mid-Chapman drain	3.5	0.056	0.01	0.34	0	0
15	Mid-Chapman between S2 & SE wetlands	7	0.05	0.01	0.2	0	NA
16	Woolcock Drain	1.25	0.435	0.001	0.185	0.008	3.745
17	N wetland pond	1.5	NA	0.008	0.13	0.002	NA

Table 5: P-value results from Shapiro-Wilk test of the distribution of mean number of species and mean concentrations of each toxicant

Variable	Species	Al	Cr	Mn	Ni	Zn
P-value	0.00248	0.000143	0.00429	0.00955	<0.001	<0.001

Figure 12 shows that aluminium, nickel and zinc had a negative correlation with the number of species, whereas chromium had a positive correlation and manganese had no correlation. *Table 6* shows that only Species-Zinc, Nickel-Chromium and Zinc-Nickel had p-values of less than 0.05. *Figure 13* illustrates that the actual observations of aluminium, chromium, manganese and nickel deviate largely from the trendlines in their relationship with the number of species, which could explain why they were insignificant. In contrast, it is visible that the residuals are smaller for zinc, suggesting that zinc is more accurate in explaining the variation in the number of species (*Figure 13*).

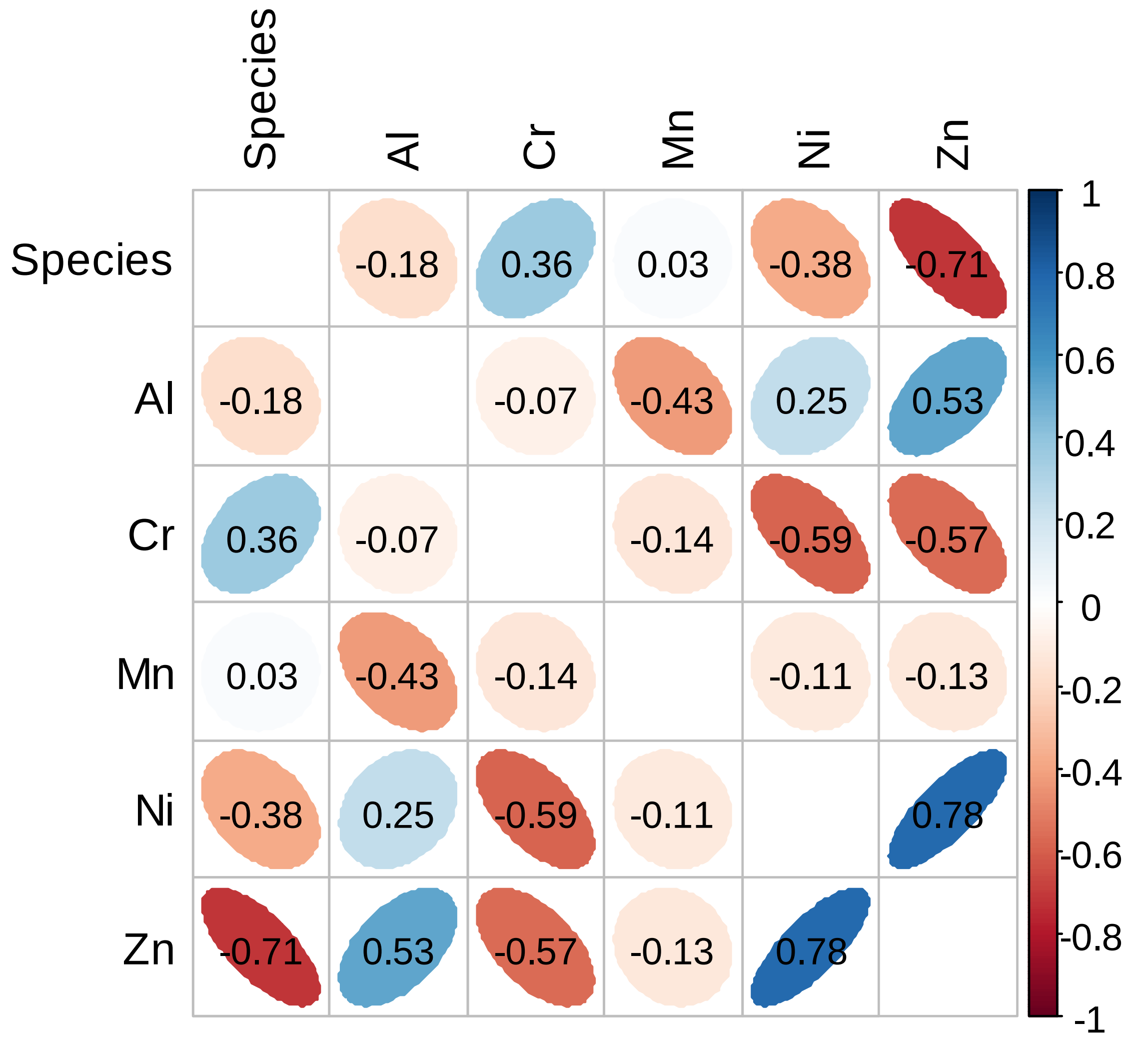

Figure 12: Correlation heatmap from Spearman's correlation matrix for mean heavy metal concentrations and mean number of species of each site in Ashfield Flats. The ellipsoids' shape and orientation represent the strength and direction of the relationship.

Table 6: Pairwise two-sided p-values of the Spearman's correlation matrix for mean heavy metal concentrations and mean number of species of each site in Ashfield Flats

Variables	Species	Al	Cr	Mn	Ni
Al	0.578				
Cr	0.150	0.829			
Mn	0.910	0.166	0.596		
Ni	0.137	0.441	0.013*	0.670	
Zn	0.021*	0.142	0.088	0.730	0.008*

Note: Alpha is set at 0.05, * indicates p-value smaller than 0.05

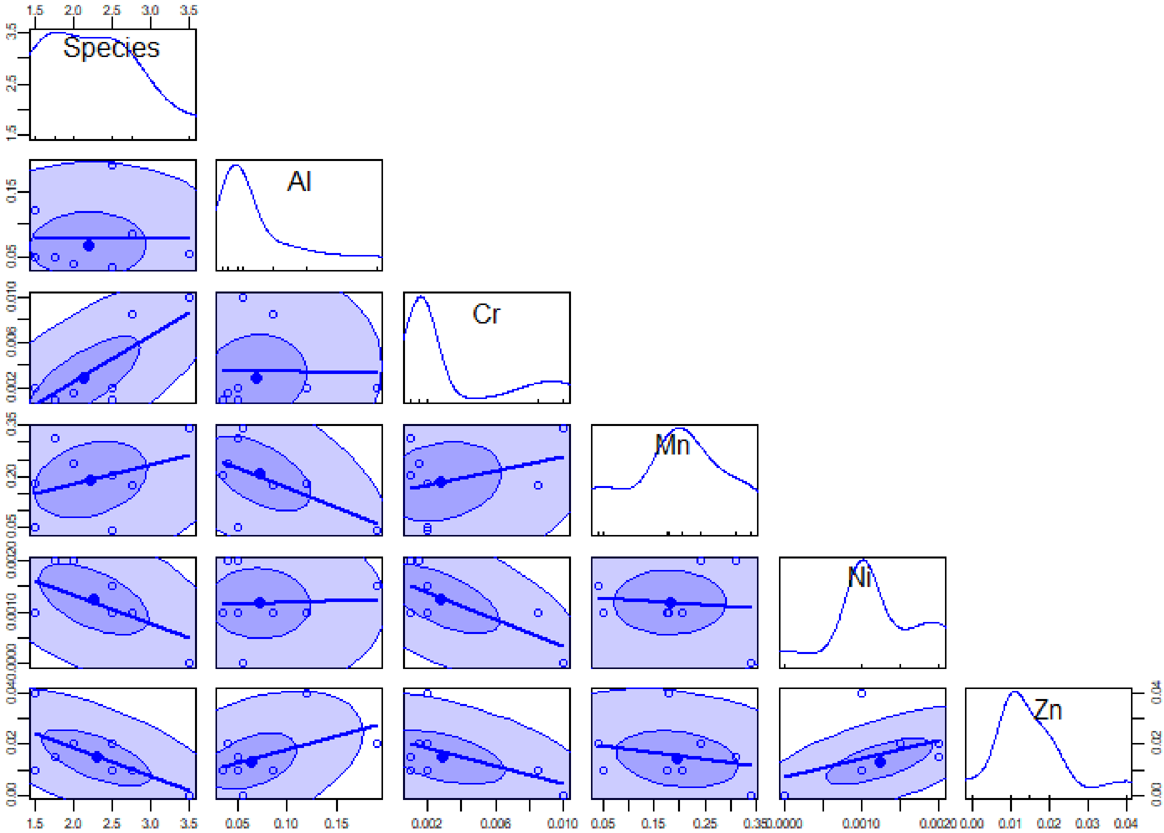

Figure 13: Scatterplot matrix showing the relationship between variables where observations are displayed by hollow points and the strength and direction of relationship are indicated by the trendlines and the ellipsoids. Note: The outlier of zinc at site 16 was removed to improve the visualization of trend

Discussion

Implications of toxicant concentration levels according to guideline thresholds

For freshwater threshold, the Wilcoxon test returned p-values greater than 0.05 for aluminium, manganese and nickel (*Table 3*), indicating insufficient evidence to state that their median concentrations surpassed the thresholds. Conversely, we obtained p-values smaller than 0.05 for chromium and zinc for freshwater threshold (*table 3*); leading to the rejection of the null hypothesis and the conclusion that their median concentrations exceeded the thresholds (*Table 3*). These results imply that over 50% of chromium and zinc concentrations exceeded the freshwater threshold, posing potential harm to the ecosystem. For instance, elevated chromium levels can cause blood-related issues like anemia and thrombocytopenia in fish, and high zinc concentrations disrupt gill functionality and increase mortality (Asghar et al., 2015; Aslam & Yousafzai, 2017).

For marine water threshold, the test returned p-values greater than 0.05 for chromium and nickel; therefore, we did not reject the null hypothesis and concluded that their median concentrations were lower than the thresholds. As for manganese and zinc, the p-values were smaller than 0.05, so we rejected the null hypothesis and concluded that their median concentrations exceeded the marine water thresholds (*Table 3*). These findings suggest that more than half of the samples collected had manganese and zinc concentrations surpassing the marine water thresholds, potentially causing adverse effects such as immune system impairment in fish and symptoms similar to oxygen deficiency in aquatic environments (Niemiec & Wisniowska-Kielian, 2015).

Although these heavy metals play an important role as micronutrients in organisms, excessive amounts can have adverse effects on ecosystems' health (Lin et al., 2008; Narain et al., 2011; Quigg, 2016). Guideline values, such as thresholds from World Health Organization or Environmental Protection Authority, are used worldwide to keep heavy metal concentration at safe levels (Mollo et al., 2022). At a global or national level, management strategies include formulating and enforcing heavy metal guidelines and conducting Environmental Impact Assessments on current and planned projects that may lead to heavy metal contamination (Naser, 2013). A limitation of guideline thresholds is the specified values likely do not apply to all species; for example, the ANZECC & ARMCANZ values were derived from very few native Australian and New Zealand species; therefore, using these values may not produce the best outcome for them (Hickey & Pyle, 2002).

Spatial analysis of pathways of heavy metal contamination

Spatially monitoring heavy metal pathways is a local management strategy to mitigate contamination (Naser, 2013). Our spatial analysis revealed elevated concentrations of aluminium, manganese and zinc in Chapman drain; high concentrations of aluminium, chromium and manganese in Kitchener drain; and substantial concentrations of aluminium, manganese and zinc in Woolcock drain, all exceeding the guideline values. Much of these data shared similarities with observations in the wetlands, for example, manganese contaminations in the drains were consistent with concentrations in the wetlands nearby. However, an exceptionally high concentration of chromium was observed in NW wetland, but its concentration in Woolcock drain that leads to the wetland was well below the guideline value. This suggests that chromium persists in the environment for a long time due to its non-biodegradable nature (Sharma et al., 2022). From *figure 12* and *table 6*, we found that nickel and zinc were positively correlated as they had a p-value of less than 0.05; this data is supported by *figures 10* and *11*, which show similar distribution of concentrations of nickel and zinc. These findings suggest that the two metals could share the same source or pathway.

A hydrological study of Ashfield Flats by McGrath (2021) corroborated our results, for instance, they also found that aluminium concentrations in the Chapman drain and zinc concentrations in the Woolcock drain greatly exceeded the ANZECC & ARMCANZ values. However, their findings of high zinc concentrations in Kitchener drain differ from our study, but this could be due to temporal variation as their samples were collected in 2019 (McGrath, 2021). Chapman and Kitchener drains discharge stormwater directly into the Swan River; therefore, the effect of polluted runoff is minimal compared to Woolcock drain, which discharges stormwater into the NW wetland (McGrath, 2021). However, wetlands along Chapman and Kitchener drains are especially prone to contamination during flooding periods as water from the drains and the wetlands will likely mix; this effect is exacerbated by increasing sea levels caused by climate change, which is predicted to bring major flooding to the Swan and Canning Rivers (Kuhn et al., 2011; McGrath, 2021). Extreme concentrations of aluminium and zinc in Woolcock drain likely emanated from contaminated groundwater, which is intercepted by Woolcock drain. The sources of this contamination are associated with landfill storage of pyritic waste and former fertilizer and sulphuric acid manufacturing facilities (DWER, 2019; Kellenberger, 1998).

The impact of heavy metal concentrations on aquatic species richness

Among the toxicants analyzed, only zinc showed a significant relationship with the number of species as it was the only element with p-value smaller than 0.05 from the correlation analysis. The negative Spearman's coefficient indicates an inverse correlation between the concentration of zinc and the number of species. Interestingly, a study by Sun et al. (2019) found no evidence that concentrations of zinc, aluminium and nickel in stormwater affected macroinvertebrate biodiversity. This study may suggest that macroinvertebrates are not affected by heavy metals contamination, but it does not exclude larger organisms like fish and amphibians from the impacts. Another study in Norway by Johansen (2013) found that over 400 tadpoles in a sedimentation pond were killed as a result of a tunnel wash containing high concentrations of zinc and copper, implying that high concentrations of heavy metals can have detrimental effects on the amphibian communities.

Several studies found that species richness increased as the distance from the sources of heavy metal contamination increased (Alsherif et al., 2022; Blanár et al., 2019; Boutin & Carpenter, 2017). This trend is inconsistent with our findings for most of the metals since we found that aluminium, chromium, manganese and nickel had no significant relationship with the number of species. There are a few limitations to our study that could lead to these inconsistent results. Firstly, our sample size was very limited, which decreases the statistical power to detect meaningful correlations, and ultimately decreases the reliability of the results. A limitation of using eDNA in this study is that DNA from both living and dead organisms contributed to the eDNA pool (Beng & Corlett, 2020). This is a problem because we are only interested in the former, and the counts of dead organisms can lead to false interpretations as it falsely increases the species richness. Moreover, eDNA may be transported rapidly from where it was released, which means that eDNA detected may come from organisms in the river that were never affected by heavy metals. This could cause any correlation between heavy metals and biodiversity to be false, especially in Chapman and Kitchener drains which are connected to the river.

Conclusion

This study aimed to investigate the complex impacts of heavy metals on the aquatic systems in Ashfield Flats Reserve. The findings provide compelling evidence that the concentrations of aluminium, chromium, manganese and zinc significantly exceeded ANZECC & ARMCANZ guideline values. The high concentrations of these heavy metals were not confined to the drains but also affected surrounding wetlands, with the NW wetland being particularly affected. Among the heavy metals analyzed, only zinc was found to have a significant negative impact on the aquatic species richness. However, it is important to acknowledge the limitations of small sample size and technical challenges of eDNA, which may have hindered our ability to detect significant correlations with other toxicants.

Our findings led to important implications for the management of this site. Firstly, our spatial analysis identified drains as the pathways of the toxicants; however, the specific sources of these contaminants still need to be properly identified, so that remediation efforts can be targeted. Secondly, establishing a regular monitoring program will enable early detection of contamination trends and facilitate timely interventions. Lastly, restoration and habitat preservation should be prioritized in areas that are affected the most by heavy metals to allow recovery of species and increase resilience of ecosystems.

Future research should enhance the impact assessment of heavy metals by increasing the sample size and incorporating complementary techniques in addition to eDNA to quantify biodiversity. This will provide a more comprehensive understanding of the effects of heavy metals and may identify other toxic elements that need to be prioritized in management strategies.

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