Heavy Metal Contamination and Bioaccumulation in *Phragmites* Reedbeds

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Chapter 1: Heavy Metal Pollution in Wetlands

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Chapter 1: Heavy Metal Pollution in Wetlands

1. Introduction

Pollution is currently one of the major threats to the natural world. Understanding the impact different pollutants have on different environments is key in learning how to mitigate the impacts they have. Heavy metals have long been considered a threat to natural habitats and increasing focus has been placed upon them in recent decades. Heavy metals are some of the most persistent pollutants due to their inability to be degraded (Ali *et al.*, 2019) and can cause a wide range of harmful effects to both humans and other organisms (Mishra *et al.*, 2019; Wu et al., 2016).

Heavy metal is a term generally used to refer to metals with a higher atomic number than 20 and a density greater than 5 g cm^{-3}. Certain metalloids such as arsenic (As) and selenium (Se) are also often considered heavy metals due to their similar effects upon the environment (e.g. Lucia *et al.*, 2010; Bai *et al.*, 2012).

Almost all heavy metals are toxic, even those which are essential to life at low concentrations (Tchounwou *et al.*, 2012) and there are a number of human diseases which are caused as a result of interaction with heavy metal polluted water, such as Minamata disease which was the result of consuming fish from methylmercury contaminated waters (Harada, 1995), therefore, high levels of heavy metal pollution are a public health concern. Heavy metals have also been identified to cause lethal effects on birds (Fisher *et al.*, 2006), fish (Zhang *et al.*, 2017), invertebrates (Lanno *et al.*, 2019), plants (Pokorska-Niewiada *et al.*, 2018), and microorganisms (Madoni and Romeo, 2006). Heavy metals can bioaccumulate within organisms, potentially posing a greater threat to those higher on the food chain (Gall *et al.*, 2015). Therefore, heavy metal pollution can threaten whole ecosystems.

Wetlands are an interesting habitat to study in relation to pollution as they are simultaneously of high conservation importance and at risk of being damaged by heavy metals, while also sometimes being used to remediate heavy metal pollution. Wetlands are naturally able to filter heavy metals from water and this quality is being utilised as a cost effective, low energy method of wastewater treatment by creating constructed wetlands (Vymazal, 2010; Wojciechowska and Waara, 2011). Both freshwater and marine wetlands provide a wide range of ecosystem services in addition to removing pollutants from water;

they provide habitat for many species (Wahlroos *et al.*, 2015), protect surrounding land from flooding (Barbier *et al.*, 2013), and stabilise the soil (Ford *et al.*, 2016).

Due to the importance of wetlands, providing ecological and economic services it is important to promote their conservation. The Ramsar Convention in 1971 (The Convention on Wetlands of International Importance) was put in place to create a global agreement on wetland conservation and identify key sites of conservation importance. Since then, however, there has still been a decline in the area and quality of wetlands and many are still under threat (Dixon *et al.*, 2016), particularly in urban areas (Hettiarachchi *et al.*, 2015). The study of heavy metals in wetlands is therefore significant as heavy metals are an important group of pollutants which can have a major impact upon wetland systems.

2. Sources, and History of Heavy Metal Pollution into Wetlands

2.1. Sources of Heavy Metal Pollution

Heavy metal contamination can come from either natural or anthropogenic sources (Mishra *et al.*, 2019). The most common natural source of heavy metals is from the erosion of metal containing rocks (Xu *et al.*, 2014). Anthropogenic sources are however much more important in the introduction of heavy metals to wetland systems (Krishna and Govil, 2007). Heavy metal contaminated waste is produced by a wide range of industries including mining (Leon *et al.*, 2018), tanning (Yuan *et al.*, 2016), smelting (Blake *et al.*, 2007), and from sewage (Shrivastava *et al.*, 2003) amongst many other sources.

Mining is one of the biggest sources of heavy metal pollution in the world (Zhou *et al.*, 2020) and can release Cd, Cu, Pb, Zn, and As, among other metals into water bodies draining from mines (Hudson-Edwards *et al.*, 2008). This contamination can continue long after the mine has closed; the Environment Agency found that many of the river systems in England and Wales which had abandoned mines in their catchment areas contained high levels of heavy metal contaminants, many of which were above the levels expected to have a harmful ecological impact (Hudson-Edwards *et al*. 2008).

The relative importance of each source of heavy metal pollution varies in different places. In wetlands with catchments containing agricultural land a major pollution source would be the runoff of heavy metal contaminated fertilisers and pesticides (Otero *et al.*, 2005), in more urban areas the runoff from road surfaces can be an important source (Ignatavičius *et al.*, 2017). The area surrounding a wetland and its water sources can predict what pollutants may be present and how best to improve the water quality entering the wetland. For example, Nahlik *et al.*, (2019) found that there was positive correlation between the lead concentration in wetland soils in the USA and the road density and population density surrounding the wetland. Differing importance of pollution sources is also seen on a global scale; Zhou *et al.* (2020) analysed sources of heavy metal pollution in rivers and lakes and found that mining and manufacturing were the most important sources in Europe and Asia, whereas pesticide and fertiliser use, as well as rock weathering were greater heavy metal sources in Africa. Identifying the relative importance of different sources of pollution can be used to predict the types of metal pollution and the quantities which may enter a wetland, which can inform pollution mitigation efforts.

2.2. History of Heavy Metal Pollution

Heavy metals have been introduced to the environment by human activities for thousands of years, ever since humans started using metals; Lee *et al.,* (2008) examined the historical trends of metal usage using lake sediment cores in central China revealing that metals have been introduced to this lake for over 5000 years, since the start of the bronze age in China. Heavy metal pollution increased dramatically during the industrial revolution when metals were heavily relied upon for many industries (De Vleeschouwer *et al.,* 2007). In Lakes Geneva and Lucerne, Switzerland Thevenon *et al.*, (2011) found that prior to the industrial revolution the lead levels were indicative of natural weathering but after 1750 – 1850 the lead levels considerably exceeded the natural levels, coinciding with the European industrial revolution. This study also found a gradual decline in lead and mercury levels in the lakes' sediment since the 1970's, likely to be a response to more rigorous remediation efforts being implemented (Thevenon et al. 2011).

Despite modern regulations of heavy metal release in many places there is often a significant historical heavy metal load in natural environments that must be contended with when trying to remediate environments or to use a contaminated area (Hudson-Edwards *et al.,* 2008). An example of this was found by Enya *et al.* (2019) in the Mersey Estuary, UK, where heavy metal concentration increased with increasing depth, likely due to deindustrialisation over time leading to decreased deposition on the top layers of soil. The plants in the area still had elevated concentrations of heavy metals as the roots were able to reach the more polluted sediment, and this contamination of plants could put grazing animals at risk of exposure to heavy metals. Therefore, the historical load of heavy metal pollution remains a threat to the environment long after pollution sources have reduced output.

In recent years, some places have seen a decrease in heavy metal pollution in response to environmental regulations being enforced by governments; for instance, Hosono *et al.* (2011) identified a decrease in accumulation rates for zinc and lead from the late 1990s – 2006 in Jakarta Bay, Indonesia which coincided with the implementation of environmental protection laws in 1997. Some policies regarding the reduction of heavy metal usage have been successful; notably the decline of lead concentrations in the environment since the control of lead fuels (Von Storch *et al.,* 2003). Martín *et al.* (2015) found a decline of 126% of lead concentrations in herbarium leaf samples collected in 2012 compared to those collected in 1941 in Valencia, Spain. However, both Li *et al.* (2020) and Zhou *et al.* (2020) found that on a global scale the concentrations of heavy metals in water bodies has increased from the

1970s to the 2010s, indicating there is still a long way to go to bring heavy metal pollution under control.

3. Heavy Metal Removal from Water by Wetlands

Wetlands are an important part of remediating heavy metal pollution as their filtration ability is utilised to clean contaminated water. Both natural and constructed wetlands have been used in this capacity, with constructed wetlands often being created in such a way that the filtration ability is maximised (Yan and Xu, 2014). Therefore, it is of both economic and ecological importance to understand how heavy metals are removed from water by wetlands. Understanding where heavy metals end up in a natural wetland system can inform the best remediation strategies as well as how bioavailable the heavy metals are to organisms within the wetland.

It has been suggested that heavy metals are removed from water in wetlands via a range of pathways (Sheoran and Sheoran, 2006). These include: sedimentation which is facilitated by mechanisms such as precipitation, sorption, and hydrolysis; microbial uptake; and plant uptake. There have been varying opinions regarding the relative importance of each mechanism (Sheoran and Sheoran, 2006; Vymazal and Březinová, 2016a) with many factors, for example the presence of certain compounds (Almeida *et al.*, 2017) or the species of plant which are present (Kumari and Tripathi, 2015) having a major impact upon the fate of heavy metals.

3.1. Sedimentation

Sedimentation is the process of particles settling out of the water and becoming part of the sediment of the wetland. Sedimentation of heavy metals can only happen once the metals are in an insoluble form (Walker and Hurl, 2002). One method of sedimentation is through the process of adsorbtion when the metals undergo adhesion to soil particles either by chemisorption, where a chemical reaction occurs between the metal and the soil particle, or by cation exchange, where heavy metals in the form of cations are attracted to negatively charged particles of organic matter or to the media in the wetland (Sheoran and Sheoran, 2006). This process of adsorption is considered to be highly important in the removal of heavy metals in wetlands (Dan *et al.*, 2017). In general, lead and copper are more strongly adsorbed and so are less biologically available than zinc, nickel, and cadmium (Sheoran and Sheoran, 2006).

There are several other chemical processes which are involved in the removal process. Iron, manganese, and aluminium are able to undergo oxidation or hydrolysis under certain conditions to form a range of oxides and hydroxides, such as Fe oxyhydroxysulfates (Woulds and Ngwenya, 2004). The removal of these metals can depend upon the pH of the system as well as the redox potential which can change the ability of metals to precipitate. Fe^{3+} is insoluble, and so can settle, even at relatively low pH whereas Fe^{2+} is soluble in water up to pH 8. Therefore, in order for iron ions to become insoluble there must be alkaline conditions or Fe^{2+} must be oxidised to Fe^{3+}. In general, the mobility of metals is considered to be low when there are reduced conditions as metals are able to form insoluble compounds, such as metal sulphides (Tack *et al.*, 1996), meaning more metals remain part of the sediment. Certain macrophytes and microorganisms can promote these reductive conditions, including the wetland plant *Schoeneoplectus californicus* which promotes reduced substrate conditions (Dorman *et al.*, 2009). Another mechanism for removal is co-precipitation where metals can be precipitated alongside secondary minerals; for example, nickel can be co-precipitated alongside iron and manganese oxides (Rinklebe and Shaheen, 2017). This means that the proportion of different heavy metals found alongside each other can have an impact upon where they end up within the wetland.

Usually the majority of heavy metals within a wetland system will be found within the sediment (Ramachandra *et al.*, 2018; Keser *et al.*, 2020), indicating that sedimentation is one of the most important removal mechanisms for removing heavy metals from water. The retention of compounds is not guaranteed as many of the sedimentation processes are reliant upon certain conditions, one of the major of which is redox conditions. Sediment can act as a secondary source of heavy metals as changes to the environment can result in the metals becoming mobilised into the water and becoming more bioavailable (Saeedi *et al.*, 2013).

3.2. Microbial Uptake

Microorganisms can remove heavy metals from water by taking them up directly, either actively through bioaccumulation or passively though biosorption. Huang and Huang (1996) investigated the ability of *Aspergillus oryzae* and *Rhizopus oryzae* to take up Cu^{2+} and found that these filamentous fungi could remove copper from water. It has been suggested that biosorption is the more feasible mechanism for large scale wastewater treatment, given that active bioaccumulation would increase the nutrient and oxygen demand which could be difficult under the potentially anaerobic conditions of some constructed wetlands (Dixit *et al.*, 2015). Guiné *et al.* (2006) suggest that extracellular polymeric substances which are secreted by bacteria may be important in the retention of zinc. However, the exact metabolic pathways involved in the microbial uptake and storage or metabolism of heavy metals is still not completely understood and so it is unknown the exact mechanisms which are used and how important a part microbial uptake is in the treatment of heavy metals by wetlands.

3.3. Plant Uptake

Metals can be taken up by plants through the roots of emergent and floating rooted plants and through the leaves of submerged plants. The concentration of heavy metals that a plant can reach depends upon the species (Deng *et al.*, 2004). The bioavailability of metals can also vary, with metals which are in the form of inorganic precipitates or strongly bound to other solids being less available than those dissolved in water (Smith, 2009). Some plants can increase the bioavailability of metals by oxidising the metal species in the sediment; for example, Reboreda and Caçador (2007) found that the salt marsh species *Halimione portulacoies* oxidises sediment, releasing copper, cadmium, and lead into the ecosystem. Some plant species can act as hyper-accumulators of heavy metals and often have a high translocation factor which allows them to transport metals throughout the plant tissues and sequester them away from the cytoplasm (Rascio and Navari-Izzo, 2011).

Heavy metals can be taken up via passive diffusion or active transport which is facilitated by the presence of ion channels or carriers (Tangahu *et al.*, 2011). Jian *et al.* (2008) found that As^{3+} is able to enter the roots of rice (*Coryza sativa*) passively via a concentration gradient, mediated by the aquaglyceroporin Lsi1 which is an intrinsic protein in the cell membrane. Other proteins have been identified which are involved in the uptake of other metals; Nramp5 is a transporter protein capable of allowing manganese and cadmium uptake in rice, allowing rice plants to accumulate high concentrations of these metals (Sasaki *et al.*, 2012). The ability of plant species to accumulate heavy metals from wastewater is likely to depend upon the presence of metal specific transporter proteins in their root cell membranes.

Once within the plants heavy metals are able to be translocated to other tissues within the plant. Vymazal and Březinová (2016) found that the heavy metals that are sequestered within the stems and leaves of *Phragmites australis* is generally only a small fraction of the total heavy metals within the system. However, metals which are essential to plants are more likely to be mobilised to different tissues; Galletti *et al.* (2010) found that copper, which has an essential role in several enzyme systems, was able to be translocated until 34% of all copper in the wastewater was accumulated in the aboveground tissues. Bragato *et al.* (2006) also found that accumulation of heavy metals is greatest at the end of the growing season, suggesting that metal uptake is dependent upon the growth of plants and so may be limited by factors which limit growth such as nutrient deficiencies. Knowing how much of the heavy metals within the system are sequestered in the aboveground tissues of plants is also important in judging how efficient cutting of the plants would be to removing heavy metal pollutants from the wetland system.

The ability of plants to take up heavy metals has the potential to be used as a remediation mechanism in polluted wetlands to remove heavy metals from the wetland system (Leguizamo *et al.*, 2017). Plants chosen for this purpose should be tolerant of high levels of heavy metal exposure and hyperaccumulators of the specific heavy metal pollutants that are to be removed. In order to remove the heavy metals from the wetland completely the plants would have to be harvested; floating macrophytes have been suggested for this purpose (Rai, 2019). Rooted plant species which are able to translocate high concentrations of heavy metals into the stem and leaves are also useful as the aboveground biomass can be cut to remove the heavy metals from the wetland. This method of phytoremediation could be a lower cost and less disruptive alternative to other remediation methods such as dredging polluted sediment (Bert *et al.*, 2009).

4. Bioaccumulation of Heavy Metals

Bioaccumulation through trophic levels in a food web is a problem associated with heavy metals given their inability to be degraded, therefore potentially high levels of the metals can accumulate within the tissues of organisms (Alhashemi *et al.*, 2012; Sánchez-Chardi *et al.*, 2009). The ability of heavy metals to be passed through a food web means that metal pollution of a small area can lead to contamination through the wider community.

There are certain factors which can increase the risk a species has of bioaccumulation. may also be a difference in the ability of aquatic and terrestrial organisms to bioaccumulate pollutants from wastewater as aquatic animals may be able to take up pollutants via more mechanisms such as through gills or from the sediment (Streit, 1998), while terrestrial mammals and birds might only access the pollutants through feeding on contaminated food sources or drinking polluted water. Similarly, food source can have an influence on bioaccumulation; Heikens *et al.* (2001) found higher metal concentrations in detritovore Isopoda compared to herbivorous or carnivorous Coleoptera, suggested to be due to the greater contact with contaminated soil faced by detritovores. Another factor in bioaccumulation risk is feeding mechanism of a species; Cheruiyot *et al.* (2013) suggests that a chewing or swallowing predator will be more likely to receive a higher dose than those which feed via a sucking or piercing mechanism.

Aquatic species are in direct contact with the water and so can accumulate heavy metals to potentially toxic levels, in fish accumulation is often seen particularly in the gills and livers (Bawuro *et al.*, 2018). Ackerman and Eagles-Smith (2010) found that mosquitofish (*Gambusia affinis*) caged in rice wetlands accumulated 721 ng/fish of mercury within 60 days, indicating that mercury is easily accumulated within fish to sublethal toxic levels. Similarly, Alhashemi *et al.* (2012) found that multiple heavy metals were highly accumulated within several fish species in a wetland polluted with petrochemical wastewater, although each fish species showed differing levels of each metal and accumulation occurred within different regions. In one of the few studies done upon a constructed wetland Leung *et al.* (2017) recorded heavy metal bioaccumulation within fish, particularly of lead, reaching levels that were toxic if the fish were consumed by humans; this also suggests that any predator consuming fish from a constructed wetland may also be at risk of exposure to toxic levels of pollutants. Each of these studies show that at least some species of fish are clearly able to bioaccumulate fairly significant levels of heavy metals from polluted water, which could have lethal or sublethal effects upon either the fish themselves or any animals which predated upon them.

It should be noted that much of the work done on bioaccumulation within wetlands is done in developing countries which may not have stringent regulations regarding the water quality of wastewater which enters wetlands; it is possible that highly regulated water quality makes bioaccumulation a reduced problem. For example, in a constructed wetland in the USA, Nelson *et al.* (2000) found that invertebrates contained very low concentrations of aluminium, arsenic, silver, and mercury, at levels which were not considered to be biologically significant. The element which was of the greatest concern in terms of bioaccumulation was selenium which was recorded at levels which can have moderate toxic effects upon wildlife (Nelson *et al.*, 2000). However, even if pollutants are only at small concentrations in the lower trophic levels, if biomagnification up the food chain is able to occur then even small quantities of the pollutant within the wastewater have the potential to have negative impacts upon the ecosystem.

Some species may have the ability to limit metal accumulation by altering their behavioural responses. For example, Rathinasabapathi *et al.* (2007) found that grasshopper (*Schistocerca americana*) nymphs consumed significantly less of brake fern (*Pteris vittata*) which had hyperaccumulated arsenic within its leaf fronds. This feeding deterrent behaviour may be a method of avoiding heavy metal build up within an animal and species which without these behavioural responses could be more prone to the build up of heavy metal contaminants in the body.

The bioaccumulation rate is very species dependent and can also vary based on what sort of heavy metals are in an area. Certain metals are more likely to show high levels of bioaccumulation than others; specifically non-essential heavy metals including As, Cd, Pb, and Hg, as there are not excretion mechanism present unlike for essential heavy metals (Gall *et al.*, 2015). Bioaccumulation can have far reaching effects as more organisms are exposed to heavy metals than those directly in contact with contaminated sediment or water, as well as reaching concentrations that are orders of magnitude greater than the background concentrations, putting organisms at greater risk heavy metals building up to toxic levels (Hamed and Emara, 2006).

5. Ecological Consequences of Heavy Metal Pollution

Heavy metal toxicity has been extensively studied and the presence of metals can have a wide range of effects such as reduced plant growth (Chibuike and Obiora, 2014), reduced biodiversity in a habitat (Hernández and Pastor, 2008), and several can be lethal to animals (Pain *et al.*, 2019).

The levels of pollutants which are in a wetland may not be high enough to have a lethal effect upon the organisms which are living within the wetland system. However, many heavy metals can have sublethal effects which can alter their behaviour, reproductive output, or their inter- and intraspecific interactions. One example of this is seen in the alteration of foraging ability in Glass shrimp (*Paratya australiensis*) when exposed to levels of zinc found in untreated stormwater runoff; Oulton *et al.* (2014) observed a reduction in their ability to perceive a chemoattractant by up to 80% in shrimp exposed to zinc pollution. These sublethal effects should also be taken into consideration when determining if the levels of heavy metals in wastewater are at a safe level.

The toxic effects of pollutants have been examined at both the individual level, determining lethal doses and sub-lethal effects, and at the population level. For example, Goutte *et al.* (2014) found that breeding success was reduced in two skua species (*Catharacta* sp.) when exposed to mercury, although there was no reduction in individual survival. This reduction in reproductive rate when exposed to heavy metals is seen in a wide range of taxa; Xie *et al.* (2014) studied the effect of zinc pollution upon the aphid *Aphis medicaginis* and its predator the ladybird *Harmonia oxyridis*. Both species were found to have reduced reproduction; the aphid due to reduced expression of essential genes and the ladybird from reduced food availability due to avoidance of polluted prey (Xie *et al.*, 2014). Given that populations of animal species in polluted wetlands potentially have slower growth rate due to the presence of pollutants the populations may not be viable in the long term and they may not survive disturbances as well as populations from non-polluted habitats.

As the microbial community within a wetland is of importance in the removal of heavy metals from water it is important to understand how they will be affected by the presence of these pollutants, particularly over the long term. Deng *et al.* (2015) suggest that microbial community is both decreased in abundance and altered in structure when exposed to long term heavy metal pollution; this could have implications for the microbial communities within wetlands which are exposed to pollutants continuously for long periods of time.

The impacts heavy metal pollution can have on microorganisms can also be a public health risk; for example, Christopher *et al.* (2014) found that in bacteria isolated from wetlands in the Lake Victoria basin which displayed chromium tolerance also displayed resistance to the antibiotic cefuroxime, suggesting metal tolerance and antibiotic resistance is linked. This relationship has been found many times in different species of bacteria, including clinically significant species such as *Escherichia coli* (Mandal *et al.*, 2020; Samanta *et al.*, 2012).

Wetlands are often not polluted by a single metal, rather there are frequently mixtures of metals present and there can also be other pollutants. This can alter the biological effects of the heavy metal as they can act together to be additive (Renella *et al.*, 2003), synergistic (Chu and Chow, 2002) or antagonistic (Yang *et al.*, 2018). There can even be both synergistic and antagonistic effects at different doses of the same mixture of pollutants such as the combination of the herbicide siduron and cadmium which was found to show synergistic toxic effects on *Eisenia fetida* earthworms at lower effect levels and antagonism at higher effect levels (Uwizeyimana *et al.*, 2017). The conditions of a wetland can also alter the toxicity of heavy metals; for instance, an increase in pH or dissolved carbon concentration have been found to increase the dose of copper required to cause a 50% reduction in reproduction of *Daphnia magna* (De Schamphelaere and Janssen, 2004).

There are multiple different ways in which heavy metals can impact organisms living in polluted areas, both lethal and sublethal toxicity as well as having impacts on an individual, population, and community level, and when determining the ecologically safe levels of heavy metals in wetlands it is important to take all of them into account. Wetlands are also chemically and biologically complex systems where there are often multiple heavy metals present in different forms alongside other pollutants, as well as factors such as pH and water hardness which can influence metal toxicity. This can make creating environmental quality standards a complex process (Bass et al. 2008).

6. Conclusion

Heavy metals are a major group of pollutants that threaten the environment. There are still high levels of metals entering wetlands from current industrial sources, and there can also be historical pollution loads that must be considered. It is clear that wetlands as an environment hold a special place in the study of heavy metal pollution, as ecological systems they are at threat from pollution entering them while simultaneously being used to remediate pollution. Much of the literature currently focusses on wetlands as a ‘green’ method of removing heavy metals from wastewater, rather than recognising wetlands as a habitat, one which is of conservation concern in many places, which can be negatively impacted by the presence of heavy metals.

It is important to understand the dynamics of heavy metals within a wetland system in order to predict the ecological dangers associated with them and identify potential methods of remediation. An understanding of the fate of heavy metals within wetlands can also identify how constructed wetlands can be created which can be used as a treatment for heavy metal contaminated wastewater. These processes can be difficult to understand in a real-world situation as each wetland is unique and has its own complex interactions within it.

Finally, when studying the ecological impacts that heavy metals can have on wetlands, particularly when determining environmental quality values, both lethal and sub-lethal effects of heavy metals must be taken into consideration at both the individual species and community levels in order to promote the conservation of wetland communities in the long term.

7. References

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Chapter Two: Trace Element Distribution in Water, Sediment, and Plant Tissues of *Phragmites australis* in a Reedbed at King's Mill Reservoir Nottinghamshire

1. Introduction

1.1. Pollution in Reedbeds

Wetlands are a massively important ecosystem, which provide a wide array of ecosystem services. They have been said to act as 'nature's kidneys' as they are capable of filtering waters that pass through them, improving water quality (Shutes, 2001). They are also valuable for the reduction of flood risk and the sequestration of carbon, with estimates of up to 30% of the global soil carbon being contained within wetlands (Nahlik and Fennessy, 2016). Wetlands also sustain biodiverse communities, providing a unique habitat that transitions between aquatic and terrestrial (Gibbs, 2000). Due to wetlands' ability to produce high levels of biomass they can support high biodiversity. It has been estimated that up to 31% of all plant species in the continental USA are found within wetlands, although they only cover 5% of the land area (Office of Water, Wetlands, Oceans, and Watersheds, 2001).

Reedbeds are a type of wetland which are dominated by the common reed; *Phragmites australis*. Within the UK, reedbeds are considered a priority habitat according to the Biodiversity Action Plan (BRIG, 2008), which promotes the creation, management, and restoration of reedbeds. The protection of reedbeds in the UK is fuelled by the dependence of priority species upon the habitat; bitterns *Botaurus stellaris*), harvest mice (*Micromus minutus*), and water voles (*Arvicola amphibius*) are all to some degree reedbed specialists and are charismatic species which conservation concern is focussed on in the UK (BRIG, 2007). In addition to being one of the most important habitat for birds in the UK, reedbeds are also of great importance for invertebrates, with 40 species being solely dependent upon *P. australis* (Hawke and José, 1996).

Despite the ecological importance of wetlands, they are one of the most threatened ecosystems on Earth; over half of the world's wetlands have been lost during the 20th Century (Davidson, 2014) even with efforts to protect them such as the Ramsar Convention. Threats to wetlands include: drainage to provide land for agriculture or urban development (Davis and Froend, 1999); invasive species competing with natives (Houlahan and Findlay, 2004); and pollution causing a deterioration in wetland condition (Lamers *et al.*, 2002). Despite being threatened by pollution, wetlands also have a role in countering it. Both natural and constructed wetlands are being utilised as nature-based solutions, taking advantage of their function as natural filters to treat water contamination. Wastewaters being treated this way include water polluted by heavy metals (Cheng *et al.*, 2002), agricultural waste (Vymazal and Březinová, 2015), and sewage (Brix and Arias, 2005). There is a wealth of knowledge regarding the ability of wetlands to remove traditional pollutants, improving the carbon oxygen demand, total suspended solid, and nutrient levels in the water passing through them. There has also recently been a lot of interest in the use of constructed wetlands to treat emerging contaminants, such as pharmaceuticals and pesticides (Du *et al.*, 2014). There has, however, been limited study into the effects of pollutants upon the wetland ecosystem itself. As wetlands are promoted as a 'green' solution to pollution remediation or wastewater treatment, more needs to be known about the potential harm pollutants could do to the wetland species. If there are no or very limited negative impacts upon the ecological community within the reedbed then habitat provision can be viewed as an added benefit of using wetlands for pollutant remediation, whereas if there are severe impacts to wildlife then this cost needs to be taken into consideration when judging the usefulness of wetlands as a treatment method.

1.2. Trace Element Pollution

The introduction of trace elements, most prominently heavy metals, to the environment has been a problem for centuries (Nriagu, 1996) and has led to concerns about food safety (Tóth *et al.*, 2016), human health impacts (Duruibe et al., 2007), and detrimental effects they cause to natural ecosystems (Maanan *et al.*, 2015). Even in countries, such as the UK, where the release of trace elements has decreased in recent years (Vincent and Passant, 2006), trace element pollution remains a major concern for environmental safety. For example, it has been recorded that 9% of rivers in England and Wales are at risk of failing to meet the environmental targets due to the pollution from abandoned metal mines (Environment Agency, 2008).

Within the UK the levels of several, potentially harmful, trace elements in aquatic systems are regulated as part of the Water Framework Directive (WFD). Water quality standards are given in Table 1, taken from the proposed standards set by the Department for Environment, Food, and Rural Affairs to meet WFD aims (DEFRA, 2014), taking into account the ecological risk of each pollutant. There are many difficulties in setting environmental quality standards (EQS) given the variability of aquatic systems as well as the fact that many toxicity tests are done within a laboratory setting. The Environment Agency described some of the issues with setting EQSs including: the impact of chemical variables such as pH, water hardness, and organic content on toxicity; the varying bioavailability of metals, such as free metal cations being more readily available to organisms; and the interaction of multiple metals influencing toxicity (Bass *et al*. 2008).

While there are currently standards for water quality given in the UK, there is a lack of specific UK standards for concentrations of trace elements in sediment. In 2008 an Environment Agency report provided draft sediment quality criteria for England and Wales but this is not mandatory (Hudson-Edwards *et al*. 2008). These values are given in Table 1.

Guidance as to what concentrations are potentially harmful can be found in the Dutch quality standards (Dutch Target and Intervention Values, 2000) which considers the concentrations of pollutants at which environmental remediation is required, and the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment, 2001) which set thresholds based upon the toxicological effects upon aquatic organisms of certain sediment concentrations. The Canadian Sediment Quality Guidelines for the Protection of Aquatic Life gives values for the threshold effect level, below which adverse effects due to the presence of these trace elements rarely occur, and the probable effect level, which shows the concentrations above which negative impacts due to the presence of the pollutant are likely to occur. These guidelines are given in Table 1.

Table 1: Water and sediment quality guidelines given by DEFRA, Environment Agency draft guidelines (Hudson-Edwards et al. 2008) Threshold Effect Level (TEL) and Probable Effect Level (PEL), CCME (TEL and PEL), and the Dutch Target and Intervention Values regarding the concentrations of trace elements which are harmful to the environment.

	UK DEFRA Water Quality Standards / µg L^{-1}	Environment Agency Draft Sediment Quality Guidelines / mg kg^{-1}		Canadian Sediment Quality Guidelines for the Protection of Aquatic Life / mg kg^{-1}		Dutch Intervention Values / mg kg^{-1}
		TEL	PEL	TEL	PEL	
Beryllium	-			-	-	30
Vanadium	-			-	-	250
Chromium	4.7 (Cr III)/ 3.4 (Cr VI)	37.3	90	52.3	160	380
Cobalt	-			-	-	240
Nickel	-	18	35.9	-	-	210
Copper	1 (bioavailable)	36.7	197	18.7	108	190
Zinc	10.9 (bioavailable) + background level	123	315	124	271	720
Arsenic	50	5.9	17	7.24	41.6	55
Selenium	-			-	-	100
Molybdenum	-			-	-	200
Silver	-			-	-	15
Cadmium	-	0.596	3.53	0.7	4.2	12
Barium	-			-	-	625
Thallium	-			-	-	15
Lead	-	35	91.3	30.2	112	530
Iron	1			-	-	-
Manganese	123 (bioavailable)			-	-	-

1.2.1. Toxicity of Trace Elements

The concentrations at which trace elements become toxic depend upon the element, hence why the quality guidelines differ between each element. Trace elements in the water can have an array of severe effects upon aquatic organisms such as; decreased growth of fish in the presence of high chromium, lead, and copper concentrations in the water (Canli and Atli, 2003), the alteration of aquatic macroinvertebrate community composition (De Jonge *et al.*, 2008), and the increase of embryonic mortality in aquatic birds (Ohlendorf *et al.*, 1986). Trace elements in soil and sediment have been recorded having adverse effects upon plants (Song and Lee, 2016), sediment-living animals (Wijayawardena *et al.*, 2018), and microorganisms (Sobolev and Begonia, 2008). This study is the first into trace element pollution at King's Mill Reservoir and as such will be focussing mainly on the toxicity of trace elements to plants.

In plants some trace elements are essential micronutrients, required for the normal biological functioning of organisms, and only become toxic at higher concentrations (Nagajyoti et al., 2010). Copper, for instance, is a necessary micronutrient in plants for ATP synthesis among other functions, but at excessive levels, can cause oxidative stress by allowing the generation of reactive oxygen species, reducing plant growth (Lombardi and Sebastiani, 2005). Other trace elements have no known biological function, including arsenic, chromium, and lead, all of which can be highly toxic at very low concentrations (Pourrut *et al.*, 2011; Finnegan and Chen, 2012; Shanker *et al.*, 2005). For example, as chromium is non-essential there is no specific uptake mechanism. However, non-specific carriers in the roots can allow chromium uptake into the roots (Shanker *et al.*, 2005). Concentrations of chromium as low as 500 µg kg^{-1} have been demonstrated to cause reduced root growth and produce hard, brittle seedlings in *Triticum aestivum* (Hasnain and Sabri, 1997). Lead has been recorded causing a number of harmful effects on plants including inhibition of seed germination and reduced stem elongation and leaf expansion (Nagajyoti et al., 2010), likely due to the inhibition of enzymatic activity. The presence of any of these non-essential trace elements within an environment may be a cause for concern if they can be accessed by plants.

1.2.2. Bioaccumulation of Trace Elements

The bioaccumulation of trace elements in plants is of importance for both the bioremediation of pollutants, as plant uptake is an important mechanism of pollutant removal in treatment wetlands (Bonanno, 2013), and when determining the ecological risk pollutants pose to an ecosystem, due to their transfer through trophic levels (Gall et al., 2015). The trace elements which are accumulated within plant tissues can be passed to herbivores, where the pollutants can bioaccumulate (Notten *et al.*, 2005) and in turn be passed to predators.

The most common route for the uptake of trace elements by plants is via the roots. Usually uptake is from the elements within the soil solution where they are more readily available (Gall et al., 2015), while most of the trace elements in the soil are within the solid phase, adsorbed or complexed with clay, organic compounds, or iron and manganese oxides (Hooda, 2010), and less available for uptake. There are multiple chemical, physical, and biological factors which can alter the uptake of trace elements; one of the most extensively studied is pH (Wang et al., 2000; Bravo *et al.*, 2017). Zeng *et al.* (2011) reported that when pH was above 7.0 there was a significantly lower uptake of Cd, Cr, Fe, Mn, and Pb by rice (*Oryza sativa)* than when the soil pH was more acidic. Soil bacteria can also alter the bioavailability of certain trace elements to plants; the removal of rhizospheric bacteria reduced the concentration of Zn, Cd, Cu, and Pb in the roots and leaves of *Sedum alfredii* (Xiong *et al.*, 2008).

Different species of plant have differing abilities to take up trace elements (Eid and Shaltout, 2016). Some plants are capable of acting as hyper-accumulators, able to take up concentrations of trace elements to levels 100-1000 times greater than other plant species without experiencing harmful effects (Pollard et al., 2014). Cicero-Fernández *et al.* (2016) suggested that under certain conditions, when some metals were highly bioavailable, *P. australis* could act as a hyper-accumulator. This, along with its known tolerance for metals (Srivastava et al., 2014), makes *P. australis* an ideal species for phytoremediation of polluted areas. However, if *P. australis* is capable of containing high trace element concentrations then any animal feeding on it may be exposed to harmful levels.

1.2.3. Sources of Trace Element Pollution

Trace elements occur naturally in the soil, but concentrations can become elevated when they are deposited by either natural or anthropogenic means. Natural sources include the erosion of rocks (Schütz and Rahn, 1982), the deposition of volcanic emissions (Nriagu, 1989), and release by forest fires (Burton *et al.*, 2016). However, anthropogenic sources are responsible for much higher levels of trace element deposition in the environment (Nriagu, 1989). Yang and Rose (2005) found that even relatively remote lakes in the UK had elevated trace element levels compared to background concentrations, attributed to over 100 years of atmospheric deposition from anthropogenic emissions. They also found that pollution was greater in those lakes closer to urbanised areas (Yang and Rose, 2005). Historically one of the largest anthropogenic sources has been atmospheric release (Pan and Wang, 2015), but in the past few decades there has been a massive reduction in atmospheric release due to a decrease in the use of leaded petrol (Von Storch *et al.*, 2003), and the implementation of equipment to control emissions (Pacyna et al., 2009). For example, Pacyna *et al.* (2009) found that cadmium emissions in Europe decreased by approximately 60% between 1987 and 2005. Despite reductions, Nicholson *et al.*, (2003), still found that atmospheric emissions accounted for between 25% and 75% of heavy metal deposition onto agricultural areas in the UK. Other than atmospheric release, important sources of trace element pollution include sewage sludge (Fang *et al.*, 2017), agricultural waste (He et al., 2005), and road runoff (Zhao *et al.*, 2010).

1.2.4. Trace Elements in Reedbeds

Reedbeds are an ideal environment in which to study the bioaccumulation of trace elements as they are fairly self-contained and have relatively simple linear food chains through which the passage of trace elements can be followed. It is also important to understand the levels of trace elements these valuable habitats are being exposed to in order to understand the risks they are facing. One of the most dramatic examples of how trace elements can cause serious toxic effects in a wetland is seen in the case study of the Kesterton National Wildlife Refuge, California, USA. This wetland was used to treat agricultural drainage water which contained elevated levels of selenium and resulted in multiple species showing reduced survival and physical abnormalities (Ohlendorf *et al.*, 1986). This was one of the first investigations into the impacts trace elements can have, not upon human health, but upon the whole ecosystem. Cases such as these can act as a warning as to what can occur if the risks of trace element pollution in wetlands are not recognised and addressed.

The majority of studies into elemental contaminants in reedbeds focus on a small number of heavy metals with the most commonly studied pollutants being highly hazardous heavy metals such as Zn, Pb, Cd, Cu, Fe, Hg, and As (e.g. Janadeleh *et al*. 2017; Allinson *et al.* 2015). However, there are several other trace elements which can act as pollutants and have a harmful effect upon the environment such as selenium, which can cause spinal defects and induce mortality in fish (Lemly, 2018), and aluminium, which can induce oxidative stress in algae (Ameri *et al*. 2020). Trace elements can also have an impact upon the toxicity of each other; for instance, selenium has been shown to reduce cadmium toxicity in chickens (*Gallus gallus domesticus*) (Hao *et al.* 2017). It may therefore be useful to identify the presence of these elements to understand the biological threat. It is useful to study a broad range of potential elemental pollutants in a wetland as it provides additional context for hazardous pollutants and may identify lesser studied pollutants which could be a threat to the environment.

1.3. Study Site

The site used in this study was a reedbed in King's Mill Reservoir, Sutton-in-Ashfield, Nottinghamshire. King's Mill Reservoir was historically a mill pond which was flooded in 1839 to create the current reservoir. The reservoir currently covers approximately 31.8 ha, of which the reedbed used in this study covers about 0.30ha. The reedbed is located at the south-west end of the reservoir, at the inlet where the River Maun enters the reservoir via three large culverts passing below the A617 (see Figure 1).

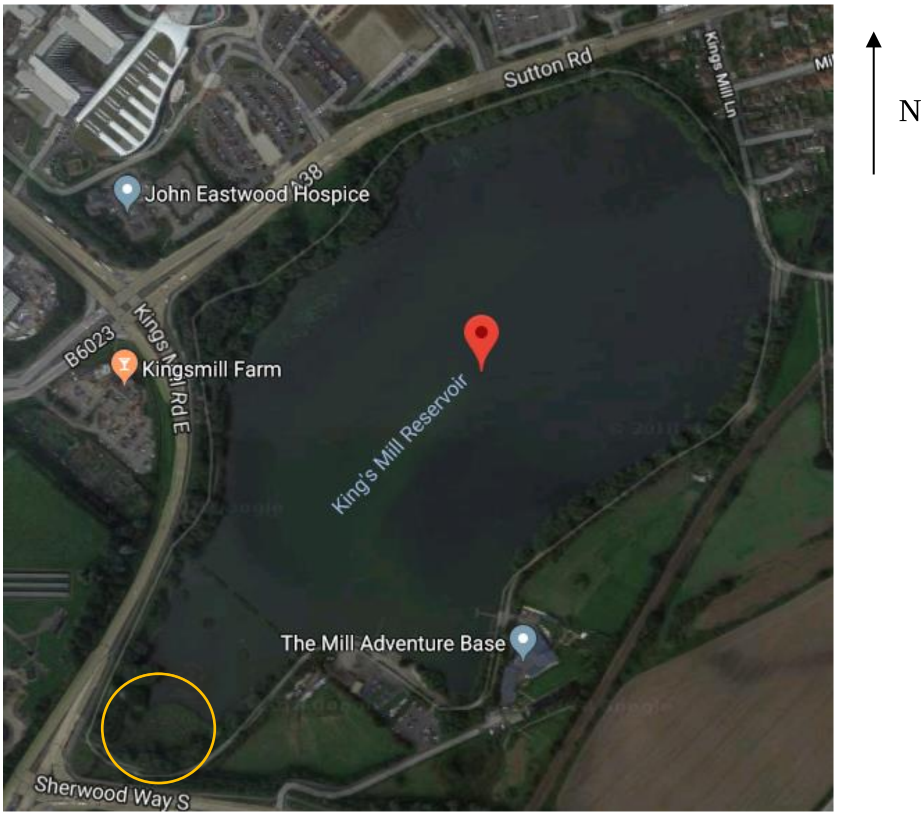

Figure 1: Map of King's Mill Reservoir, Sutton-in-Ashfield, Nottinghamshire, with the reedbed circled (yellow).

King's Mill Reservoir is positioned early in the flow of the River Maun, with the source of the river occurring only 2 km upstream. At the inlet to King's Mill Reservoir the catchment of the River Maun covers an area of 9.7km^2, made up of the sub-catchment areas of four separate tributaries. Most land use in this area is residential and industrial, with the Maun

Valley Industrial Park close to the reservoir. There are also two main roads directly adjacent to the reservoir: the A617 along the southern boundary of the site, and the A38 to the north and west. The reservoir receives surface water runoff from a number of sources including road drainage and from hardstanding around Kingsmill Hospital. There are also consented discharges of trade effluent from the hospital and both treated sewage effluent and storm sewer overflow from the Severn Trent Water (STW) treatment works adjacent to the reservoir.

The industrialised, built-up catchment area of the River Maun and the runoff directly into the reservoir could cause significant concentrations of heavy metals to occur within the reservoir. In the past there have also been algal-blooms at King's Mill Reservoir, likely as the result of nutrient enrichment of the reservoir causing an increase in algal biomass production. Therefore, the concentration of ions such as nitrate and phosphate coming into this site are of interest to determine whether eutrophication is still a continuing threat to the ecological condition of the reservoir.

1.4. Aims and Objectives

This study aimed to look into the presence of trace element pollution within the a reedbed at King's Mill Reservoir and the distribution of them between the water, sediment, and *P. australis* compartments within the reedbed, in order to examine potential risks to the reedbed ecosystem and the wider reservoir. This study provides an exploration into the presence of a wider range of potential trace element contaminants than are usually studied in environmental quality studies, which typically focus on a small number of heavy metals.

The main objectives of this study were:

a. to determine the concentrations of a wide variety of trace elements, including several which have the potential to have harmful effects upon the environment in high enough quantities, within the water, sediment, and different plant tissues of *Phragmites australis* of King's Mill Reservoir, Nottinghamshire.

b. to examine the mobility of these trace elements between different tissues of *P. australis* to determine the bioaccumulation of trace elements within *P. australis* and the potential bioavailablity of these elements to herbivores.

c. to compare the concentrations of trace elements found within the reedbed with environmental standards to predict any possible ecological risks of trace element pollution within this reedbed system.

d. to examine the spatial variability of trace elements within the reedbed, and between the reedbed and the inflow of the reservoir.

e. to measure the concentrations of chloride, nitrate, and sulphate in order to determine whether there is a risk of nutrient enrichment in the reedbed.

2. Methods

2.1. Sample Collection

Water, sediment, and plant samples were collected from King's Mill Reservoir on 4th April 2018. Within the reedbed, four transects were created going from dry land out into the reservoir and samples were taken approximately every 5m (see Figure 2). At each sampling point 75 ml water was filtered through a 0.22µm filter. At each sampling point, approximately 150 ml sediment was dug from the surface with a trowel and stored within a sealed plastic bag. Plant samples were collected from the roots and from the top third of the reed stem, at each sampling point approximately six plant roots and six plant stems were collected. The plant samples were stored within sealed plastic bags. All samples were kept cool (approx. 4°C) until they were returned to the lab, within 24 hours.

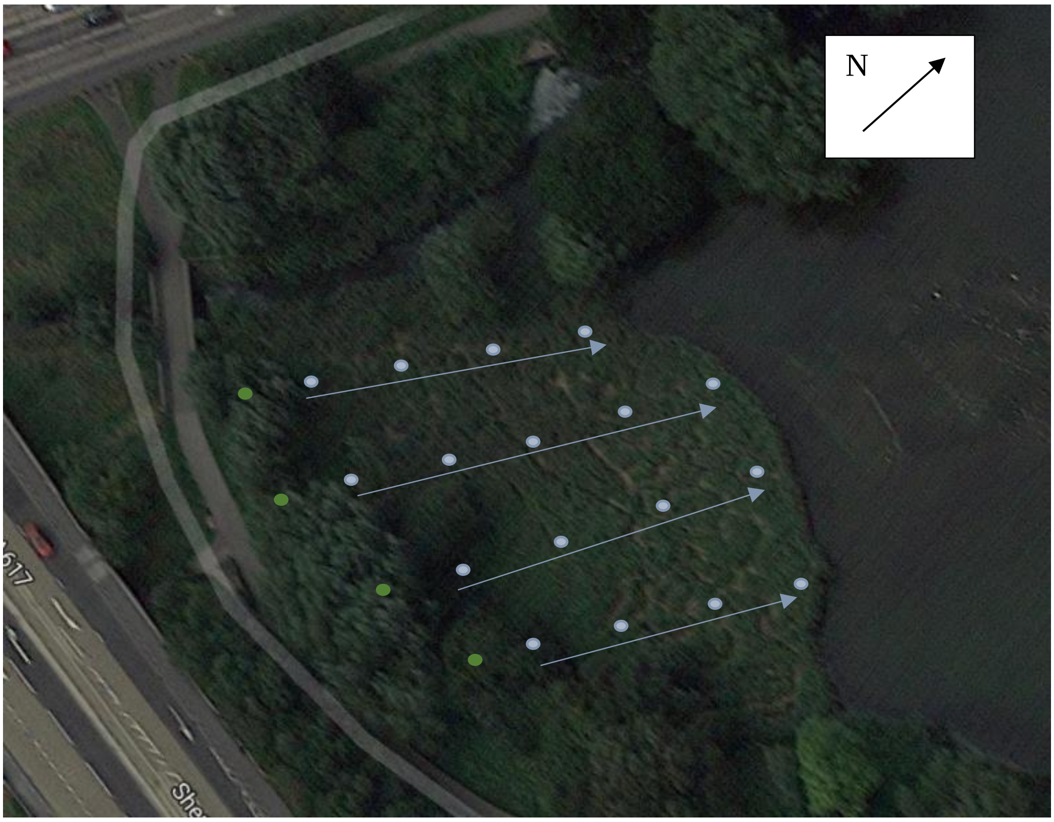

Figure 2: Map of the reedbed at King's Mill Reservoir with the approximate positions of the sampling points within the reedbed where water, sediment, and plant samples were taken (blue), transects into reedbed shown by the arrows and the points where soil was taken on the land adjacent to the reedbed (orange).

Water and sediment samples were also taken at three points along the River Maun between an outflow from STW treatment plant and the reedbed at the inlet to the reservoir (Figure 3). Soil samples were taken from four points along the dry land corresponding with the start points of the transects into the reedbeds. These were used to determine trace element concentrations in areas which are only rarely flooded by the water from the reservoir (see Figure 2 for positions).

Figure 3: Map of the inflow of the River Maun into King's Mill Reservoir, with the sampling points (blue) marked (0m, 100m, and 150m from the storm outflow), and the position of the outflow (yellow) of storm water overflow from the adjacent ST water treatment works.

2.2. Water Sample Analysis

The trace element concentrations in the water samples were analysed by inductively coupled plasma – mass spectrometry (ICP-MS), for which samples were acidified using 0.4 ml 50% nitric acid in 9.6 ml sample. The concentration of chloride, sulphate, nitrate, fluoride, and phosphate ions in the water samples was measured by ion chromatography (Dionex ICS-1100 Ion Chromatography).

2.3. Sediment Sample Analysis

Sediment samples were air dried (19 ± 1°C) over three days and then oven dried at 90°C overnight. The dried sediment was broken up and ground using a mortar and pestle to a fine dust. Each sample was then split into three separate samples to be analysed simultaneously. The samples (approximately 0.2g) were fully digested using 2.0 ml nitric acid and 1 ml perchloric acid, then 2.5 ml hydrofluoric acid, and then another 2.5 ml nitric acid and 2.5 ml MilliQ water. The mixture was made up to 50 ml with MilliQ water. Trace element concentrations were determined by inductively coupled plasma mass spectrometry (Thermo-Fisher iCAP-Q ICP-MS). Montana II Soil (NIST Standard Reference Material 2711a) was used as the reference material in this analysis.

2.4. Plant Sample Analysis

Plant samples were oven dried in an electric oven at 65°C over four days until completely dry. They were then acid digested; 8 ml nitric acid and 2 ml hydrogen peroxide were added to approximately 0.2 g sample and left overnight. The samples were then heated to 95°C for two hours, left to cool and then made up to 50 ml using MilliQ water and filtered (0.22 µm). The samples were then analysed using ICP-MS. Tomato leaves (NIST Standard Reference Material 1573a) were used as the reference material.

2.5. Statistical Analysis

The differences between concentrations of elements in samples from the reedbed, reservoir inlet, and soil, were analysed using analysis of variance (ANOVA) or Kruskal-Wallis tests depending upon whether the residuals were normally distributed, determined by the Shapiro-Wilk test. The differences between concentrations of elements at different distances from the shore within the reedbed were determined by the same statistical tests. A Bonferroni correction was applied to overcome inflated Type 1 errors caused by multiple testing.

Correlations between concentrations of trace elements within the roots and within the sediment and water were determined using Pearson correlations. All statistical tests were calculated using R (Version 3.5.0).

The bioaccumulation factor (BAF), which indicates the ability of a plant to take up trace elements from the sediment into the roots, was calculated by:

$$BAF = C_{root}/C_{sediment}$$

where C_{root} is the mean concentration of the element in the roots of *P. australis* and $C_{sediment}$ is the mean concentration of the element in the sediment.

The translocation factor (TF), which indicates the mobility of an element between the roots and aboveground tissues of *P. australis*, was calculated by:

$$TF = C_{shoot}/C_{root}$$

where C_{shoot} is the mean concentration of the element in the stem and leaves of *P. australis* and C_{root} is the mean concentration of the element in the roots.

3. Results

3.1. Ion Concentrations

The mean concentrations of ions detected in the water of the reedbed and the reservoir inlet are reported in Table 2. The ions tested for fluoride and phosphate are not shown as they were below the level of detection. There was a significantly higher concentration of chloride and nitrate in the reservoir inlet compared to the reedbed. There was no significant difference in any of the ion concentrations at different distances from the shore within the reedbed.

Table 2: Mean concentrations of ions in the water at Kings Mill Reservoir, found in the reedbed and in the reservoir inlet. A significant difference between the concentrations in the reedbed and the reservoir inlet are given to thresholds of 0.5 (*) and 0.01 (**).

	Mean Concentration in Reedbed / mg L^{-1} (±SE)	Mean Concentration in Reservoir Inlet / mg L^{-1} (±SE)
Chloride *	59.384 (4.66)	99.813 (10.54)
Nitrate **	1.110 (0.29)	7.197 (0.38)
Sulphate	7.051 (1.28)	29.176.86)

3.2. Trace Elements in Sediment and Water

Trace element concentrations in sediment and water are given in Table 3. The concentrations of trace elements were consistently higher in sediment than in the water, often over 1000 times greater in concentration.

Table 3: Mean concentrations of trace elements in the water and sediment at Kings Mill Reservoir. Water concentrations are given in μg L^{-1} and sediment concentrations are given in mg kg^{-1}.

	Mean Concentration in Water (±SE) / μg L^{-1}		Mean Concentration in Sediment (±SE) / mg kg^{-1} (dry weight)		
	Reedbed	Reservoir Inflow	Reedbed	Reservoir Inflow	Soil
Boron	57.128 (6.12)	76.455 (2.91)	NA	NA	NA
Sodium	28858.872 (1789.10)	55677.279 (4774.10)	1277.819 (64.20)	1597.370 (144.71)	909.523 (54.92)
Magnesium	20473.161 (1096.08)	28031.171 (1658.40)	6950.943 (445.64)	11975.910 (886.93)	6552.804 (883.32)
Phosphorus	161.735 (35.33)	18.405 (2.65)	4242.464 (585.20)	1002.118 (88.70)	1009.894 (96.91)
Sulphur	7.730 (1.15)	28.139 (2.81)	NA	NA	NA
Potassium	4399.214 (242.11)	4952.147 (382.00)	7586.306 (523.46)	10438.173 (170.75)	11245.315 (1085.70)
Calcium	54184.171 (1927.44)	68667.164 (3980.14)	25008.357 (2932.65)	37469.691 (3330.96)	13392.740 (1120.62)
Titanium	11.930 (0.68)	16.642 (0.98)	343.796 (23.85)	355.089 (48.75)	394.392 (33.37)
Lithium	5.140 (0.50)	22.738 (3.58)	29.586 (2.08)	26.600 (2.21)	40.809 (1.89)
Beryllium	0.013 (0.002)	0.008 (0.004)	1.459 (0.12)	1.193 (0.08)	2.020 (0.10)
Aluminium	2.863 (0.35)	13.490 (0.78)	22053.632 (1031.88)	21863.941 (1365.34)	20620.338 (762.65)
Vanadium	0.395 (0.05)	1.139 (0.16)	47.324 (2.80)	42.467 (4.44)	50.632 (3.05)
Chromium	3.178 (2.94)	0.620 (0.02)	67.091 (4.60)	66.140 (7.76)	55.497 (3.53)
Manganese	693.886 (259.51)	1.335 (0.22)	1657.722 (287.65)	743.347 (70.85)	373.562 (26.72)
Iron	145.447 (99.20)	6.982 (0.36)	28549.796 (2630.83)	18436.660 (1884.27)	17699.984 (1586.22)

Table 3 Cont.

Cobalt	0.299 (0.08)	0.119 (0.01)	9.256 (0.41)	8.029 (0.82)	11.607 (1.32)
Nickel	1.092 (0.14)	0.925 (0.04)	36.519 (2.73)	36.687 (4.95)	35.115 (1.97)
Copper	2.464 (0.24)	3.582 (0.41)	126.155 (10.22)	109.598 (19.07)	66.438 (10.10)
Zinc	8.655 (1.10)	23.385 (1.03)	640.469 (35.01)	563.409 (69.81)	238.721 (26.29)
Arsenic	0.736 (0.12)	0.521 (0.01)	11.071 (1.07)	7.831 (0.68)	11.099 (0.42)
Selenium	0.213 (0.03)	1.197 (0.05)	2.313 (0.17)	0.950 (0.07)	1.222 (0.26)
Rubidium	5.027 (0.70)	2.970 (0.20)	40.630 (1.89)	42.183 (3.01)	44.745 (3.61)
Strontium	123.083 (6.09)	143.705 (11.03)	87.720 (3.52)	82.444 (6.87)	73.636 (7.37)
Molybdenum	1.803 (0.26)	1.221 (0.06)	2.530 (0.13)	3.294 (0.59)	1.618 (0.11)
Silver	0.005 (0.001)	0.001 (0.000)	5.025 (1.09)	3.310 (0.90)	3.656 (1.00)
Cadmium	0.021 (0.004)	0.061 (0.002)	2.147 (0.15)	1.732 (0.23)	0.806 (0.12)
Caesium	0.003 (0.000)	0.010 (0.000)	2.549 (0.12)	2.237 (0.22)	2.937 (0.36)
Barium	64.481 (5.97)	56.977 (2.32)	518.012 (35.16)	470.282 (49.72)	618.576 (260.40)
Thallium	0.006 (0.001)	0.010 (0.000)	0.434 (0.03)	0.343 (0.03)	0.513 (0.02)
Lead	0.090 (0.01)	0.157 (0.01)	218.756 (24.15)	184.129 (27.78)	166.082 (68.52)
Uranium	0.209 (0.05)	0.794 (0.05)	1.333 (0.08)	1.346 (0.15)	1.475 (0.02)

The distance from the shore did not have a significant impact on the concentration of any elements in either the sediment or water once a Bonferroni correction was applied to give a p value of 0.0017.

Multiple elements showed significant differences between the reedbed and the reservoir inlet, for water samples, and between the reedbed, reservoir inlet, and soil, for sediment samples (see Figures 4 and 5 respectively). In the water the concentrations of Al, Cd, Cs, Li, Se, Na, U, V, and Zn were all significantly different between the reservoir inlet and the reedbed. For all of these elements the reservoir inlet had a greater concentration than the water in the reedbed. In the sediment samples Cd, Mg, and Zn had significantly different concentrations between the reservoir inlet, the reedbed, and the soil on the shore adjacent to the reedbed. Cadmium was at higher concentrations within the reedbed. Magnesium was at higher concentrations in the sediment at the reservoir inlet. Zinc had higher concentrations in the reservoir inlet and the reedbed than in the soil.

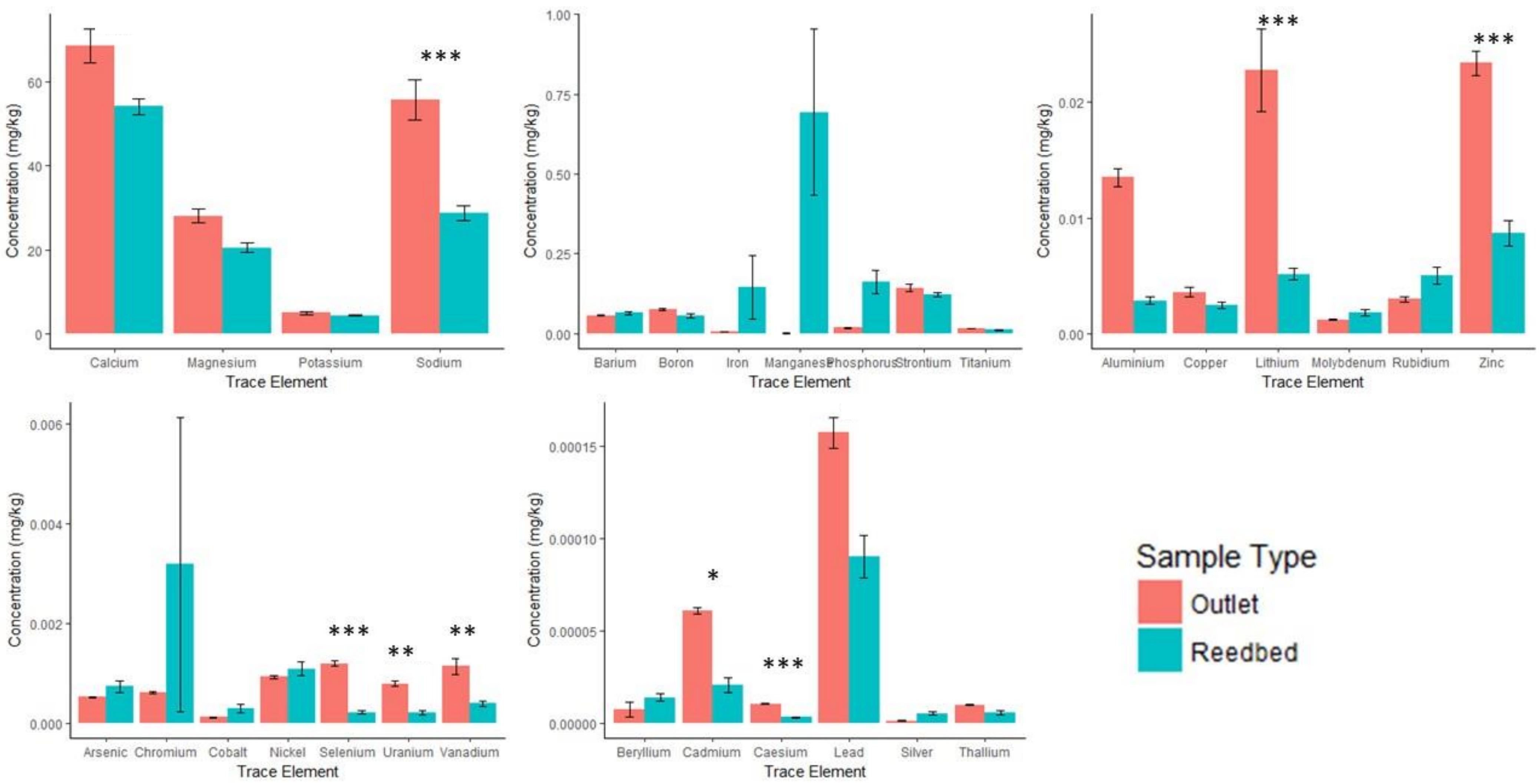

Figure 4: The mean concentration of trace elements (± standard error) in water samples taken from the reservoir inlet and within the reedbed at King's Mill Reservoir, Nottinghamshire. Significant differences between sample types are given to Bonferroni corrected threshold values of 0.0017 (*), 0.00034(**), and 0.000034 (***).

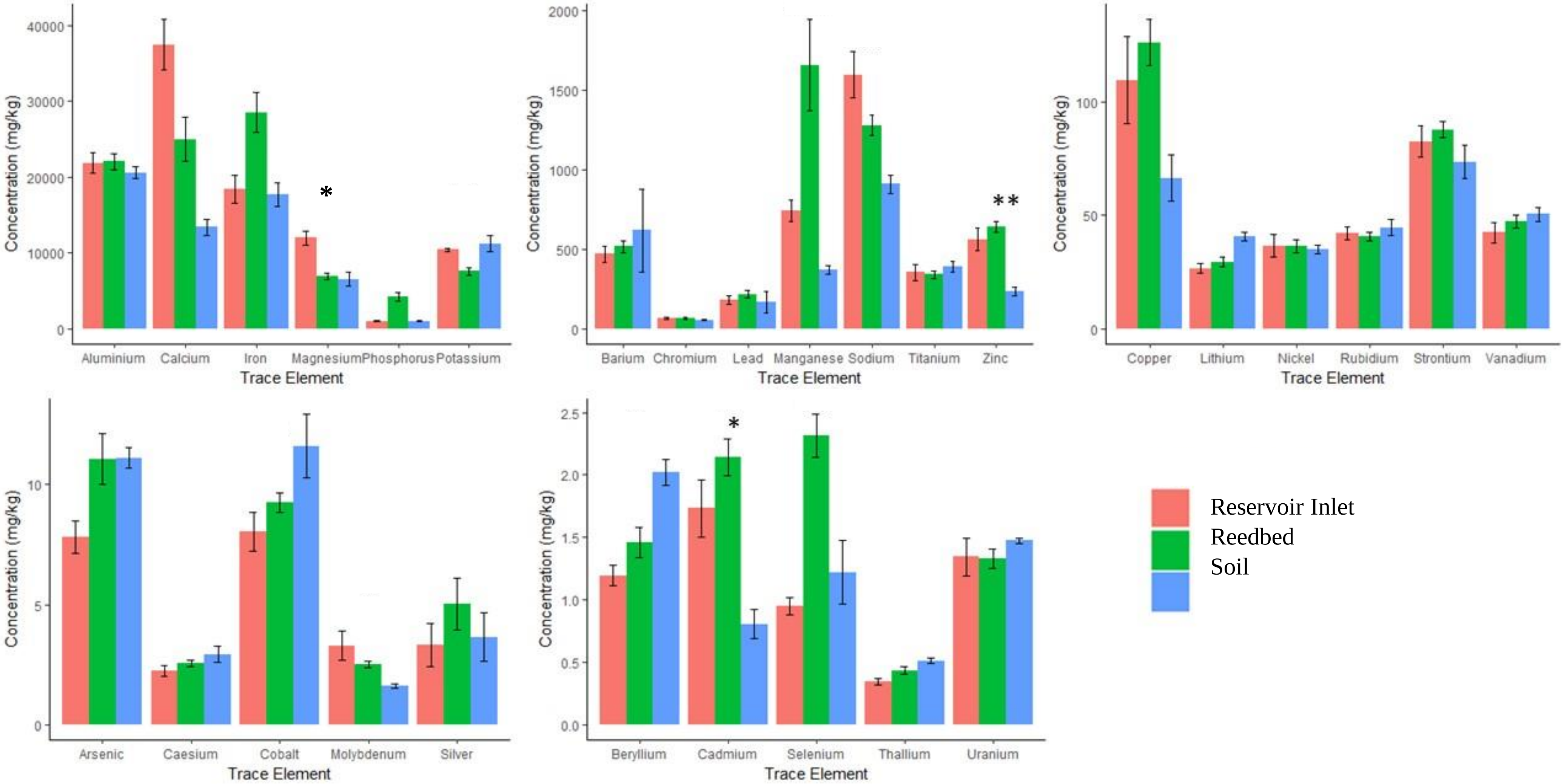

Figure 5: The mean concentration of trace elements (± standard error) in sediment samples taken from the reservoir inlet, within the reedbed, and from the soil on the shore adjacent to the reedbed at King's Mill Reservoir, Nottinghamshire. Significant differences between sample types are given to Bonferroni corrected threshold values of 0.0017 (*) and 0.00034(**).

When compared with water quality standards (see Table 1) the trace elements which had a water concentration greater than the recommended environmental standards given by the UK Water Framework Directive (WFD, 2015) were copper and iron, in both the reedbed and the reservoir inlet; manganese, in the reedbed; and zinc, in the reservoir inlet. This however does not take into account how much of the zinc and manganese in the water that was measured here is bioavailable, as this is what the standards measure, as opposed to total concentration as measured here.

The sediment standards are given in Table 1. Chromium, cadmium, and arsenic levels in the sediment are all between the threshold effect level and the probable effect level given by the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (CCME, 2001). Copper, zinc, and lead levels were above the probable effect level from the Canadian Sediment Quality Guidelines. The levels of trace elements measured here did not exceed the Dutch Intervention Values.

3.3. Trace Elements within Plants

The concentrations of trace elements found in the roots and stems/leaves of *P. australis* are shown in Table 4, along with the phytotoxic levels of certain trace elements. Most trace element concentrations were well below the phytotoxic levels. The only elements which show a concentration above the phytotoxic levels were lead, chromium, and manganese.

Table 4: The concentrations of trace elements in the roots and stems/leaves of *Phragmites australis* from King's Mill Reservoir, Nottinghamshire, and phytotoxic levels of certain elements. Significant correlation between: a - sediment conc. and root conc. And b – water conc. and root conc. 1 - Kabata-Pendias (2000), 2 - Djingova *et al.* (2003), 3 – Chaney (1989).

	Mean Concentration in Roots (±SE) / mg kg^{-1}	Mean Concentration in Stem/Leaves (±SE) / mg kg^{-1}	Phytotoxic Levels / mg kg^{-1}
Boron	2.893 (0.21)	3.830 (0.21)	50-200[1]
Sodium	441.149 (58.49)	167.193 (30.63)	-
Magnesium	490.323 (30.74)	473.220 (26.26)	-
Phosphorus	540.731 (51.27) [a]	329.797 (40.75)	-
Potassium	2475.713 (324.36)	699.399 (89.25)	-
Calcium	1676.604 (212.78)	877.927 (49.16)	-
Titanium	17.807 (1.72)	17.896 (1.47)	-
Lithium	0.146 (0.03)	0.043 (0.01)	-
Beryllium	0.055 (0.01)	0.048 (0.01)	-
Aluminium	78.666 (18.93) [a]	20.208 (2.35)	1000-3000[1]
Vanadium	0.412 (0.09)	0.114 (0.01)	5-10[1]
Chromium	0.921 (0.17)	0.778 (0.24)	0.5[3]
Manganese	113.354 (18.64)	45.294 (3.64)	5-500[3]
Iron	410.572 (97.78) [a]	93.584 (7.59)	1000-3000[1]
Cobalt	0.145 (0.03)	0.062 (0.01)	15-50[1]
Nickel	0.726 (0.14)	0.598 (0.16)	-
Copper	10.791 (1.08)	4.236 (0.33)	25-40[1]
Zinc	45.884 (3.96)	57.035 (4.37)	500-1500[3]
Arsenic	0.243 (0.05)	0.059 (0.00)	-
Selenium	0.073 (0.01)	0.063 (0.01)	-
Rubidium	3.255 (0.43)	0.880 (0.16)	-
Strontium	4.308 (0.49)	5.117 (0.52)	1000[1]
Molybdenum	0.449 (0.07)	0.979 (0.15)	10-50[1]
Silver	0.093 (0.02)	0.024 (0.00)	-
Cadmium	0.064 (0.01)	0.026 (0.00)	5-700[3]
Caesium	0.020 (0.00)	0.006 (0.00)	-
Barium	12.576 (1.67) [b]	13.207 (0.95)	500[1]
Thallium	0.011 (0.00)	0.004 (0.00)	-
Lead	3.201 (0.75)	1.010 (0.12)	0.01[2]
Uranium	0.016 (0.00)	0.003 (0.00)	-

Position within the reedbed had no significant effect upon the trace elements which were found within the *P. australis*. Only three elements showed a significant correlation between the concentration in the sediment and the concentration in the roots; phosphorus which showed a negative correlation, and iron and aluminium which showed a positive correlation. Only barium showed a significant correlation between its concentration in the root and concentration in the water.

The bioaccumulation factors and translocation factors are given in Table 5. The uptake of most elements was very low when compared with the concentrations found in the sediment. The elements with the greatest bioaccumulation factor were sodium, potassium, and phosphorus. The majority of trace elements were found at a higher concentration within the roots than the stems, the exceptions, which had a translocation factor greater than 1, were B, Ti, Zn, St, Mo, and Ba.

Table 5: Bioaccumaltion factor and translocation factors of trace elements in the organs of Phragmites australis from the reedbed at King's Mill Reservoir, Nottinghamshire.

	Bioaccumulation Factor (root/sediment)	Translocation Factor (stem/root)
Boron	NA	1.32
Sodium	0.345	0.37
Magnesium	0.071	0.96
Phosphorus	0.128	0.60
Potassium	0.326	0.28
Calcium	0.067	0.52
Titanium	0.052	1.00
Lithium	0.005	0.29
Beryllium	0.038	0.87
Aluminium	0.004	0.25
Vanadium	0.009	0.27
Chromium	0.014	0.84
Manganese	0.068	0.39
Iron	0.014	0.22
Cobalt	0.016	0.43
Nickel	0.020	0.82
Copper	0.086	0.39
Zinc	0.072	1.24
Arsenic	0.022	0.24
Selenium	0.032	0.85
Rubidium	0.080	0.27
Strontium	0.049	1.18
Molybdenum	0.178	2.18
Silver	0.019	0.25
Cadmium	0.030	0.40
Caesium	0.008	0.31
Barium	0.024	1.05
Thallium	0.024	33.33
Lead	0.014	31.55
Uranium	0.012	22.06

4. Discussion

Trace element pollution seems to be of some concern at King's Mill Reservoir; certain elements within the reedbed at King's Mill Reservoir were measured at levels which concentration guidelines would suggest could have adverse ecological impacts. The distribution of these elements was primarily in the sediment, with concentrations in the water being considerably lower. The bioaccumulation factor of all elements *by P. australis* were lower than one, indicating that none were accumulated to concentrations greater than those in the sediment.

The elements which show the greatest levels in water are sodium, calcium, magnesium, and potassium which corresponds with the main cations typically found in natural waters (Nikanorov and Brazhnikova, 2009). The main sources of these elements are from deposits of rocks eroded or dissolved. As expected, potassium concentrations occurred here at lower levels than the other major elements, probably due to its uptake from water by aquatic plants and microorganisms. The chloride and sulphate measured in the water are also major components of natural waters (Nikanorov and Brazhnikova, 2009). When the sulphate concentration is compared to the sulphur results from the ICP-MS it can be seen that most of the sulphur in the water was in the form of sulphate ions.

The concentration of nitrate in the water at the reservoir inlet is considerably greater than the 1.5 mg L^{-1} total nitrogen level which Barker *et al.* (2008) suggested would act as an indication of unfavourable conditions within a water body. Here only the nitrate concentration is measured, and it is already above the 1.5 mg L^{-1} threshold, without addressing other species of nitrogen which may also be entering the reservoir. High concentrations of nitrate are associated with the propagation of phytoplankton and a decline in species richness (Barker *et al.*, 2008), and the levels recorded at the inlet to King's Mill Reservoir indicate that potentially harmful levels of nitrate are entering the site, suggesting the site remains at high risk for algal-blooms, as have occurred previously. However, the nitrate concentrations are much lower within the reedbed, which could be a result of the natural plant and microbial nitrogen removal mechanisms exhibited within wetlands. This could mean than an increased area of reedbeds being introduced to the reservoir could be used as a remediation technique to reduce the concentrations of nitrogen, as has been done on many other sites (e.g. Zhao *et al.*, 2012; Rodrigo *et al.*, 2018).

Within the sediment the elements of highest concentration were calcium, iron, aluminium, magnesium, potassium, phosphorus, and sodium. These major elements are typically found in any soil, although the exact composition may vary (Strawn *et al.*, 2015). Of more interest regarding pollution are the potentially environmentally harmful trace elements which are present.

The heavy metal concentrations in the water are consistent with levels found in water polluted with urban runoff recorded in Melbourne, Australia (Allinson *et al.*, 2015) and Stockholm, Sweden (Karlsson *et al.*, 2010), but lower than levels found in urban runoff and industrial runoff in Singapore (Joshi and Balasubramanian, 2010). It is therefore possible that a considerable amount of the pollution seen in King's Mill Reservoir could be due to runoff from the surrounding urban area, including the neighbouring busy roads, but may not include severe runoff from heavily industrialised areas. Karlsson *et al.* (2010) compared the concentrations of trace elements in both the dissolved and particulate phases in water and found considerably higher levels in the particulates in the water, especially that of lead, which had particulate concentrations of up to 16 $\mu g\ L^{-1}$ compared to dissolved concentrations of <1 $\mu g\ L^{-1}$, and chromium which had particulate and dissolved concentrations of over 8 $\mu g\ L^{-1}$and <0.5 $\mu g\ L^{-1}$respectively. This could mean that the water contains higher concentrations of these elements than are measured here as the samples tested were filtered before analysis. In future works the particulates in the water could also be analysed for trace element concentration to get a more comprehensive view of the composition of the water.

The sediment was the compartment of the reedbed which contained the highest concentrations of all trace elements. This is typical of aquatic systems where higher concentrations of metals are generally associated with the particulate matter (e.g. Alhashemi *et al.*, 2011). Elemental pollutants can be sequestered into the sediment and held there for long periods of time. However, as the pollutants are elemental they cannot be degraded and so the concentrations in the sediment are only going to increase over time as more polluted water enters the system and, if conditions, such as pH or oxygen availability, change, then sediments can act as a source of trace elements and become accessible by living organisms (Peng *et al.*, 2009). As chemical conditions within a wetland are important in determining the risks associated with heavy metal pollutants the lack of information here on factors such as pH and water hardness limit the ability of this study to make definitive conclusions regarding the ecological risks, however, these results do provide a snapshot of the concentrations of many pollutants which can be reached at King's Mill Reservoir which can be used to inform further study.

The concentrations of zinc, copper, and lead in the roots and leaves of *P. australis* are comparable to those which have been recorded in the literature from both natural and constructed wetlands (Vymazal *et al.*, 2007). The cadmium and nickel concentrations found at this site were at a lower concentration than the ranges recorded in Vymazal *et al.* (2007); 0.9 – 9.7 mg Cd kg^{-1} and 6 – 86 mg Ni kg^{-1} in the roots. Although the cadmium concentration in the stem/leaves was similar to that found by Vymazal *et al.* (2009) in a constructed wetland treating municipal wastewater. The concentrations of heavy metals in plant tissue show a similar pattern to that found by Vymazal *et al.* (2009) and Bonanno and Giudice (2010) with the most abundant trace elements in plant tissues being iron, aluminium, zinc, and manganese. This can be explained by these elements having the greatest concentrations in the sediment. Similarly, the elements with the lowest concentrations in *P. australis*; Cd, U, Tl, Ag, Se, Cs, and Be, correspond to the elements with the lowest concentration in the sediment.

4.1. Spatial Distribution of Trace Elements

Certain trace elements within the water were found at higher concentrations at the reservoir inlet next to the outflow of the storm sewage overflow, where there was a significant difference. This suggests that these elements, including cadmium, zinc, and selenium, are entering King's Mill Reservoir from either the storm overflow or from road runoff into the River Maun prior to its entry to the reservoir. This is also informative if remediation strategies may be implemented in the future as it indicates a major point where several pollutants are entering the reservoir. Other elements show no difference in concentration in samples from different points. This could indicate a more diffuse source of these elements entering the reservoir, so the water concentrations are similar across the reedbed and the reservoir inlet. However, it should be noted that these concentrations were only measured in samples taken on a single day, and across other days and at different times of the year the sources of pollutants may vary, such as following high rainfall (Banerjee *et al.* 2016).

Within the reedbed there was no difference in trace element concentration depending upon distance from the shore. In many wetland systems pollutants tend to be on higher concentration closer to the inlet zone (e.g. Kongroy et al. 2011; Murray-Gulde *et al*. 2005), due to the exposure to the highest concentrations of pollutants. This pattern can also be seen in toxicity rates; for example, Murray-Gulde *et al.* (2005) noted that in a wetland fed copper contaminated wastewater there was a much lower (2%) survival rate of *Ceriodaphnia dubia* at the inlet than at the outlet (96%). However, this sort of pattern is often documented in constructed wetlands which have a very specific source, within the King's Mill reedbed pollutants could be entering from the reservoir inlet which runs through the reedbed or there could be other sources around the reservoir.

4.2. Bioaccumulation and Translocation of Trace Elements at King's Mill Reservoir

The bioaccumulation factors calculated here are considerably lower than some others recorded in other studies; for example, Bonanno (2013) found the BAF of zinc (0.29), arsenic (0.32), and particularly nickel (2.44) was much higher than those seen at this site; 0.072, 0.022, and 0.020 respectively. There are a number of reasons why the BAF is lower in this study. For instance, the season can have a large effect upon the concentrations of trace elements within plant tissues; the samples examined here were taken in the spring and so were one-year old stems, not in their growing season, but in *P. australis* the greatest concentration of metals is generally seen at the end of the growing season, in the autumn (Kastratović *et al.*, 2013). Another reason could be that these pollutants at King's Mill Reservoir are in forms which are less bioavailable, as they are more associated with the solid phase of the sediment rather than the solution (Rieuwerts, *et al.*, 1998).

Most of the trace elements had a translocation factor of less than 1, indicating that the majority of trace elements are accumulated to a higher concentration in the roots. This general trend of most trace elements being found in higher concentrations in roots than stems is consistent with other studies into trace elements in *P. australis* (Bonanno, 2011; Vymazal *et al.*, 2009). This pattern is also seen in many other wetland species, including *Typha latifola* and *Juncus effusus* (Deng *et al.*, 2004). It has been suggested that this could be a mechanism utilised by plants to prevent reaching toxic levels of harmful metals in the aboveground organs (Bonanno, 2013). Mays and Edwards (2001) found that the majority of each metal within the roots was found in the root coating, with as much as 93% of iron found within the root coat, which could allow greater tolerance to toxic elements.

The fact that low concentrations of heavy metal pollutants are being stored in the aboveground organs can have implications for phytoremediation; unless the whole plant is removed from the system then the pollutants sequestered within them will return to the sediment when the plant dies and degrades, and so aboveground cutting will not remove the majority of the trace elements which have been taken up by the plants. The phytoremediation of pollutants at King's Mill Reservoir could be increased by the presence of other plant species, particularly hyperaccumulators which are able to translocate large concentrations to the leaves and stems.

Of the elements which had a translocation factor greater than one, boron, zinc, and molybdenum are considered to be essential elements while titanium and strontium have been recorded as beneficial to plant growth at certain concentrations (Lyu *et al.*, 2017), and so plants have established mechanisms to allow their movement (e.g. Takano *et al.*, 2008). However, barium has no known biological function and has been recorded to cause toxic effects including the inhibition of carbon fixation and stomatal shutdown (Suwa *et al.*, 2008), therefore, it would be expected that plants should limit barium mobility. Although knowledge of barium uptake and mobility in plants is scarce, Myrvang *et al.*, (2016) suggested that barium uptake could be enhanced by calcium uptake. The element with the greatest translocation factor was strontium, which corresponds with Li *et al.*, (2011) who found the concentrations of strontium in the leaves of *P. australis* were almost double that in the roots, although the ratio of strontium in the roots and leaves varies considerably between species and appears to be highly dependent upon external conditions such as pH (Moyen and Roblin, 2010).

The mobility of trace elements within *P. australis* are important when considering the potential for bioaccumulation further up the food chain as many animals feed on the leaves and stems of *P. australis,* and so a greater concentration in these organs will lead to a greater risk of exposure.

4.3. Potential Ecological Risks of Trace Elements at King's Mill Reservoir

In the water iron, manganese, and zinc all reached concentrations which could be considered potentially harmful under water quality guidelines. Iron concentrations between 0.1 and 1.0 mg L^{-1} in freshwater lakes have been associated with blooms of blue-green algae (Morton and Lee, 1974). This is of concern at King's Mill Reservoir as there have been problems with algal blooms in the past and, when combined with the elevated nitrate levels entering the reservoir, it seems the risk remains high.

Regarding manganese, the lethal dose for aquatic invertebrates has been recorded as low as 0.8 mg L^{-1} for *Daphnia magna* (Reimer, 1999), and within the reedbed in this study manganese levels reached as high as 3.2 mg L^{-1}, indicating that aquatic invertebrates may be at risk from the manganese levels in the water. This does assume, however, that enough manganese is bioavailable, further work needs to be done to investigate the true bioavailable levels of trace elements rather than relying on the total concentrations.

Chromium, cadmium, and zinc concentrations in the sediment were all between the threshold effect level and the probable effect level of the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment, 2001) which means that they are in the range in which adverse effects of these toxic elements can occasionally occur. More likely to cause harmful effects are the elements which were above the probable effect level; copper, zinc, and lead, which predicts that these levels will have adverse effects in 56%, 65%, and 58% of cases respectively.

For the Environment Agency draft sediment quality guidelines lead, nickel, and zinc were all above the probable effect level meaning it is likely to have a negative impact upon the environment (Hudson-Edwards *et al.* 2008). Arsenic, cadmium, chromium, and copper were all between the TEL and the PEL. This indicates that King's Mill Reservoir is at risk from the levels of heavy metal pollution which are present in the reedbed and remediation measures should be taken to alleviate the negative consequences which are very real possibilites.

The highest concentration of lead measured in the sediment (477 mg kg^{-1}) is greater than the predicted lethal dose for the earthworm *Eisenia fetida*, and concentrations as low as 250 mg kg^{-1} are associated with reduced body weight and cocoon production (Žaltauskaitė and Sodienė, 2010). This suggests that the levels of lead could have a negative impact upon sediment living organisms. Lead levels in sediment of only 68 mg kg^{-1} have also been linked with a 40%-70% reduction in the biomass of *P. australis* (Windhamt *et al.*, 2001). Even at levels lower than the lethal dose, toxic trace elements have also been shown to have sub-lethal effects; for example, amphibians exposed to chromium concentrations as low as 20 µg L^{-1} are linked to morphological abnormalities and developmental delays (Fernando *et al.*, 2016). Given the elevated levels of certain toxic metals within the reedbed, it may be important that remediation methods for this site are considered to treat the current pollution and prevent it from getting any worse and further impacting upon the wildlife.

Potentially phytotoxic levels of chromium, lead, and manganese were recorded in the tissues of *P. australis*. Both chromium and lead are non-essential in plants and can be highly toxic (Shanker *et al.*, 2005; Fahr *et al.*, 2013). The relatively high levels of manganese in plants may be explained by the high concentration in the water, where it could be more bioavailable (Magalhaes et al. 2015). It must be recognised that these elements are not acting alone, and the presence of other elements and compounds can alleviate or increase the toxicity of certain trace elements; for example, selenium can reduce chromium toxicity by decreasing the production of superoxide free radicals and so reducing oxidative stress (Qing *et al.*, 2015).

5. Conclusion

This study has shown that King's Mill Reservoir has a moderate load of heavy metals. These include heavy metals such as lead, copper, nickel and zinc, which are at levels of biological concern within the sediment. Lead, chromium and manganese are being accumulated by *P. australis* to potentially phytotoxic concentrations. This suggests that the pollution entering the reedbed at King's Mill Reservoir could be a threat to any organisms living in it and so should be addressed as soon as possible.

Future work at this site could include the examination of trace element concentrations during different seasons, to determine the seasonal dynamics of elemental pollutants within the reedbed. Also, as this study only measured the total concentrations of elements then the bioavailability needs to be looked into in order to more clearly identify the risks which trace element pollution poses to wildlife at this site. The species of certain pollutants could also be studied to give a greater understanding of both the source of the pollution, which could help inform how to regulate it, as well as the dynamics of the pollutant within the reedbed itself. Looking into the concentrations of trace elements across multiple days in different seasons will provide a more accurate snapshot of where trace element concentrations are the greatest and how bioaccumulation patterns change over the life cycle of *P. australis.*

While there has been considerable work done into the bioaccumulation of trace elements by plants and, particularly by species such as *P. australis* due to their importance as phytoremediators in constructed wetland systems, there has been very little done on bioaccumulation further up the wetland trophic levels. The work done at this site should be built upon to investigate the trace element accumulation within species which face different pollutant exposure; stem-dwelling invertebrates, aquatic invertebrates, and terrestrial invertebrates, as well as animals in higher trophic levels such as the small mammals which inhabit the reedbed.

6. References

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