Long term chemical and ecological recovery of Kinghorn Loch (Scotland, UK) following red mud pollution

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Declaration

The candidate confirms that the work submitted is her own, with the exception of the data on red mud leachate and natural waters within Kinghorn Loch used in Chapter 3, which were given by Edwards (1985) and Scottish Environment Protection Agency (SEPA). The thesis has been composed by the candidate and contains four chapters intended for publication in peer-reviewed journals. An outline of the candidate and co-authors contributions, are given below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others. No part of this work has been submitted for any other degree or professional qualification.

The candidate, as lead author, performed the sample collection, the experiment and was involved in the specialist laboratory analyses where Inductively Coupled Plasma Mass Spectrometry (ICP-MS), coupled ion chromatography and ICP-MS, and X-ray Fluorescence (XRF) methods were employed. The laboratory analysis of phosphorus concentrations in water, data analysis (except for generalised additive model analysis) and writing of the papers was carried out by the candidate. Co-authors provided support and guidance on the scope and design of the project, the specialist laboratory analyses and contributed to the editing of the manuscripts.

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Lay Summary

Inappropriate storage of red mud, a by-product of aluminium production, is of increasing environmental concern as illustrated by the October 2010 accident in Western Hungary that contaminated the Danube River. Estimated global production of red mud, which is caustic and contains elevated concentrations of metal and metalloids (e.g. arsenic, vanadium, aluminium) and phosphorus, is ~120 million t per year. Given the increasing industrial demand for aluminium worldwide and a risk associated with storage of red mud, it is crucial to understand the effects of red mud on the environment. Little is known about the long-term chemical and ecological responses in freshwaters following pollution with red mud. Kinghorn Loch, Fife, Scotland, is an important example of a red mud polluted aquatic ecosystem. This is due mainly to the long-term data documenting changes in the lake during the pollution and recovery following diversion of red mud leachate in 1983 to present.

Results showed a recovery of the surface water of Kinghorn Loch, represented by a significant decrease in pH and concentrations of all red mud constituents since the cessation of pollution into the lake. The chemical recovery period was long and took from 22 to 26 years for arsenic, vanadium and phosphorus. The 2012/2013 field survey showed that the contaminant concentrations were higher in deeper waters compared to surface waters and displayed seasonal peaks, with a range of physico-chemical factors controlling variations in water column contaminant concentrations. Sediment in Kinghorn Loch is still contaminated 30 years into the lake recovery period. Evidence from the laboratory experiment and the field observations suggests that pollutants are seasonally released from bed sediment into water. Aquatic plants in the lake contain relatively high concentrations of toxic arsenic. This indicated that recovery of the lake is not yet complete and that 30 years after the diversion of the pollution from Kinghorn Loch, the lake still processes red mud contaminants. The results of this research will help water resource managers to understand not only the environmental and human health effects of red mud pollution, but also the likely recovery time scales in relation to water quality targets.

Abstract

The estimated global production of red mud, a by-product of alumina production, is ~120 million t per year. Little is known about the long-term chemical, physical and ecological responses in aquatic ecosystems following pollution with red mud. To date, no whole-lake assessment of the impact of red mud and recovery trajectories has been conducted. Kinghorn Loch, Fife, Scotland, is an important example of a red mud polluted aquatic ecosystem. This is due mainly to the availability of long-term data documenting changes in the lake during the pollution and recovery following diversion of red mud leachate in 1983 to date. Long-term data were assessed to determine the time scale of recovery for a range of specific pollutants in the surface waters of Kinghorn Loch, and field surveys and a laboratory controlled experiment were conducted to investigate lake sediment-pollutant interactions and arsenic (As) species bioaccumulation in aquatic plants.

Results showed recovery of the lake water column, with a significant decrease of pH and the concentrations of all red mud constituents in surface water since the cessation of pollution. Using generalised additive models the chemical recovery period was 5 years for pH and from 22 to 26 years for As, vanadium (V) and phosphorus (P), with aluminium (Al) still not reaching the end-point recovery following this period. A 12-month field survey showed that concentrations of phosphate (PO_4-P), total phosphorus (TP), dissolved and total As and V in lake water varied significantly throughout the year, with depth of overlying water contributing to variation in P concentrations in water above sediment. A range of physico-chemical factors, including dissolved oxygen (DO), pH and concentrations of pollutant binding element controlled seasonal and spatial variations in water column contaminant concentrations. The behaviour of V differed from As and P concentrations in water, with maximum V concentrations occurring in spring and the highest concentrations of As and P in surface water and dissolved As and PO_4-P in water above sediment observed in summer. With the exception of V in deeper layers of the water column in spring and winter, observed total As and V concentrations met standards for Protection of Aquatic Life in the UK (50 and 20 $\mu g\ L^{-1}$, respectively).

Sediment in Kinghorn Loch is still contaminated 30 years into the lake recovery period. The highest seasonal mean concentrations in the upper (0-4 cm) sediment layer were 231 mg kg^{-1} for V and 185 mg kg^{-1} for As, the latter considerably exceeding Canadian Sediment Quality Guidelines for the Protection of Aquatic Life for As (17 mg kg^{-1}). Evidence from the laboratory experiment and the field observations suggests that the lake still processes red mud contaminants, with seasonal mobilisation of As and P from lake sediment under reducing conditions. In contrast, release of V from sediment into overlying water appeared not to be driven predominantly by redox conditions, but instead by interactions between pH, competitive ion concentrations and DO. Higher pollutant concentrations observed throughout the year in deeper layers of the water column compared to surface water indicated that the impact of sediment on the water column is generally confined to the bottom waters of the lake.

Macrophytes in Kinghorn Loch contained relatively high concentrations of As, predominantly in the inorganic form which is most toxic to organisms. The highest As content measured in roots of *Persicaria amphibia* (L.) Gray (40.4 – 218 mg kg^{-1}) greatly exceeded the 3 – 10 mg kg^{-1} range suggested as a potential phytotoxic level. Accumulation of toxic As species by plants suggested toxicological risk to higher organisms in the food web and indicated that ecological recovery of the lake is still in progress. The results of this research at Kinghorn Loch will help water resource managers to understand not only the environmental and human health effects of multiple pressures related to red mud pollution, but also the likely recovery time scales in relation to water quality targets.

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Table of Contents

List of Tables

List of Figures

Chapter 1 Introduction

1.1 Introduction

Little is known about chemical, physical and ecological responses in aquatic ecosystems following pollution with red mud, a by-product of the aluminium production process. Given the large scale of production of red mud worldwide (~ 120 million t a^{-1}; Power et al. 2011) it is important to understand the long-term fate of its constituents in the freshwater environment and likelihood of recovery following pollution release. To date, no whole-lake assessment has been conducted of the impact of red mud and the trajectories of recovery. This research aims to investigate the chemical and ecological responses and recovery trajectories following red mud pollution in Kinghorn Loch, Fife, Scotland.

Kinghorn Loch is an important example of a red mud polluted aquatic ecosystem. This is due mainly to the availability of long-term data (Edwards 1985; Tim Foster, Scottish Environment Protection Agency (SEPA) *pers. comm.* 2011-2015) documenting changes in the lake due to the pollution and during recovery following diversion of red mud leachate in 1983 up to the present day. Thus it provides a unique opportunity for research on the long-term effects of red mud pollution and processes of recovery. In this research the long-term data for Kinghorn Loch were assessed to determine the time scale of recovery for a range of specific pollutants. Field surveys and a laboratory controlled experiment were also conducted to assess lake sediment-pollutant interactions and pollutant bioaccumulation in aquatic plants. The results of this study will help water resource managers to understand not only the socioeconomic, environmental and human health effects of multiple pressures related to red mud pollution, but also the likely recovery time scales in relation to established water quality targets.

Chapter 1 reviews the scientific basis for the present study, beginning with information on the composition of red mud and the environmental risks associated with its production and storage. Next, an overview is presented of the behaviour of the constituents of red mud in the freshwater environment and their potential effects on the aquatic food web, followed by existing evidence of ecological degradation after the red mud pollution incident in Ajka, western Hungary in 2010. The potential effects of internal loading of contaminants deposited in sediment on recovery of freshwater ecosystems are also considered. Finally, the research aims and thesis overview are outlined.

1.2. Production and composition of red mud

Red mud is a by-product of alumina extraction from bauxite ore during the Bayer process. It is highly alkaline due to the addition of sodium hydroxide (NaOH) during the process and contains metal oxides and a variety of minor trace elements (Dubey and Dubey 2011; Edwards 1985; Gelencsér et al. 2011).

The solid phase of red mud typically comprises residual iron oxides (e.g. hematite), sodium aluminosilicates, quartz, titanium dioxide, calcium carbonate/aluminate, and sodium hydroxide and has a fine particle size (mostly <10 μm; Mayes et al. 2011; Ruyters et al. 2011). The aqueous phase contains elements dissolved from the bauxite during processing including aluminium (Al), arsenic (As), phosphorus (P), chromium (Cr), strontium (Sr), cobalt (Co), nickel (Ni), vanadium (V), molybdenum (Mo), lead (Pb), zinc (Zn) and selenium (Se) (Czop et al. 2011; Edwards 1985; Mayes et al. 2011; Ruyters et al. 2011). The elemental composition of red mud can vary, reflecting differences in the composition of the bauxite used in the Bayer process (Gelencsér et al. 2011; Mayes et al. 2011). Examples of composition differences in red mud from various bauxite sources are provided in Table 1.1.

Table 1.1. Examples of elemental composition of red mud (weight %) determined by X-ray fluorescence (XRF; Gräfe et al. 2011).

Bauxite origin	Refinery	Al_2O_3	Fe_2O_3	SiO_2	TiO_2	CaO	Na_2O	LOI[a]
Turkey	Seydisehir	20.2	39.8	15.2	4.15	1.8	9.43	na
Guinea	Aughinish	23.6	30.4	9.65	17.85	6.4	5.3	na
Ghana	Burntisland	23.4	36.3	18.3	5.97	4.38	12.4	na
Greece	Alumine de Greece	15.6	42.5	9.2	5.9	19.7	2.4	na
Southern Brazil	Laboratory	6.8	71.9	1.35	7.8	3.2	0.4	10.4
Jamaica	Laboratory	8.16	53.5	0.8	10.9	14.8	0.3	11.6
China	Pingguo	26.8	26.9	13.1	7.3	23.5		na
India	Renukoot	21.9	28.1	7.5	15.6	10.2	4.5	12.2
Hungary	Ajka	14.8	42.1	13.5	5.2	6.1	8.9	8.2

[a]LOI = loss on ignition measured between 900 and 1100°C

Table 1.2 shows an example of the composition of leachate from red mud, in this case for leachate entering Kinghorn Loch. It consisted of a caustic, highly alkaline solution of NaOH and carbonate, with mean pH of 12.1. The liquor contained very high concentrations of dissolved Al, V, As, as well as other trace elements (Zn, copper (Cu), Pb, Cr, cadmium (Cd)). It was also characterised by high concentrations of phosphate (PO_4-P), chloride (Cl), sulphate and total suspended solids (TSS). Most of the iron (Fe) input to the lake came from red mud solids that were sporadically released to the lake (Edwards 1985).

Table 1.2. Mean total concentrations (mg L^{-1}, apart from pH) of the constituents of red mud leachate entering Kinghorn Loch in 1981 – 1983; alkalinity reported as mg L^{-1} $CaCO_3$ (Edwards 1985).

Determinands	Leachate concentrations (mg L^{-1})
pH	12.1
$CaCO_3$	4125
TSS	87.2
PO_4-P	2.95
NO_3-N	1.80
NH_4-N	0.70
SO_4-S	154
Na	2006
Al	137
K	25.6
Ca	7.20
Mg	8.20
Si	7.06
Cl	67.8
Fe	0.51
Mn	0.06
V	5.30
As	3.56
F	3.16
Zn	0.01
Cu	0.03
Pb	0.03
Ni	0.06
Cr	0.11
Cd	0.01
B	0.82

1.3. Red mud pollution - the scale of the problem

The increasing industrial demand for aluminium has led to increasing production worldwide of alumina in the 21st century (from 55.0 in 2002 to 81.6 million t a^{-1} in 2008), with the main producing countries being China, Australia, Brazil and the USA (Li 2012). As a consequence, output of the production waste has increased (Li 2012). For every tonne of aluminium, approximately 3 t of red mud are produced (Li 2012). Estimated global production of red mud is about 120 million t a^{-1} (Power et al. 2011). Apart from the research following the Ajka spill in Hungary (e.g. Mayes et al. 2011; Burke et al. 2012), few scientific assessments of red mud pollution have been published in the peer reviewed literature (Czop 2011; Edwards 1985; Mora et al. 2015; Ouboter and de Dijn 1993). However, a review of available resources on the internet highlighted issues of red mud pollution in Jamaica (Encyclopaedia of the Nations 2008), India (Amnesty International 2011; Padel and Das 2011) and Brazil (Aluminium International Today 2012), indicating that the scale of the problem might be more widespread than can be concluded from peer reviewed literature.

Red mud disposal methods vary from utilising natural landscape depressions as storage sites through to storing the waste in well-engineered containment reservoirs or by discharge to sea (Power 2011). Failure of containment reservoirs can result in leachate entering aquatic ecosystems, which can have a severe negative impact on the receiving waterbodies and can lead to an increased risk to human health (e.g. Kinghorn Loch, Scotland; Edwards 1985; Gorka Pit Lake, Poland; Czop 2011). Dam failure of a red mud containment reservoir in October 2010 in Ajka, Hungary, resulted in the release of ~ 1 million m^3 of red mud waste, contaminating the Marcal River (catchment area 3078 km^2) and entering the Danube River (Klebercz et al. 2012). Spills from red mud sedimentation ponds in Venezuela have negatively impacted lagoons of the Orinoco River located in close proximity to the disposal sites (Mora et al. 2015). Most recently red mud disposal methods have favoured dry disposal (Power et al. 2011). This method reduces the land area required for storage and lowers the potential for leachate contamination of groundwater and surface waters (Power et al. 2011), but may cause air pollution by dispersal of dust from the waste deposit (Power et al. 2009).

Input of red mud in aqueous and solid phases can significantly alter aquatic ecosystems through increases in pH, salinity and contaminant concentrations, and interactions between the solid phase waste and natural sediments. The aqueous phase waste may also interact with natural sediments following incorporation into the food web and sedimentation to the bed (Edwards 1985; Mayes et al. 2011; Ruyters et al. 2011). Properties of the waste may also interact to increase the potential detrimental impacts of contaminants, e.g. elevated pH can increase the mobility of oxyanionic aqueous phase contaminants including As, Co, Mo, Cr and V (Czop et al 2011; Mayes et al 2011). The discharge of red mud pollution can severely reduce or even eliminate aquatic life in lakes and rivers (Edwards 1985; Mora et al. 2015), although the mechanisms of ecological deterioration are, largely, unknown, and evidence of recovery is sparse.

1.4. Transport and fate of red mud in the aquatic environment

Few studies have investigated the fate of red mud in aquatic ecosystems and little is known about the long-term behaviour of red mud constituents within lakes. However, the sediment-water interactions of some red mud constituents (e.g. P and As) have been investigated in lakes suffering from eutrophication or other forms of pollution. Such studies can indicate the potentially important impacts and processes of red mud pollution in aquatic systems.

Sediment contamination represents a potential threat to freshwater ecosystems and human health, due to the risk of the release of trace elements harmful to aquatic organisms and humans (Hirata et al. 2011; Rowe et al. 2002). Chemical mobility across the sediment-water interface and the risk of release to the water column of potentially harmful red mud constituents is likely to be constituent-specific. Results of a study of the sediments in Great Slave Lake in Canada, enriched with trace elements due to gold mine activities on the shore of the lake, indicated that Pb, Sb, Zn and Cu were not remobilised within the sediment and were isolated at depth by sedimentation (Andrade et al. 2010). In contrast, substantial solid-phase As enrichment in surface sediments and the presence of dissolved As in different layers of the sediment profile indicated post-depositional remobilisation of As (Andrade et al. 2010). To assess the impact of the release from sediments of red mud pollutants on the aquatic biota and on the recovery of freshwater ecosystems after red mud pollution, knowledge of the processes driving pollutant cycling within the sediment and factors affecting their release into the water column is required. The speciation of both As and P in sediments are driven by redox conditions, adsorption and desorption mechanisms and mineral phase solubility (Kleeberg and Kozerski 1997; Søndergaard et al. 2003; Toevs et al. 2008), all of which can interact with each other depending upon sediment elemental composition and physicochemical properties. The processes involved in the cycling of P and As between sediments and water are summarised in Figure 1.1.

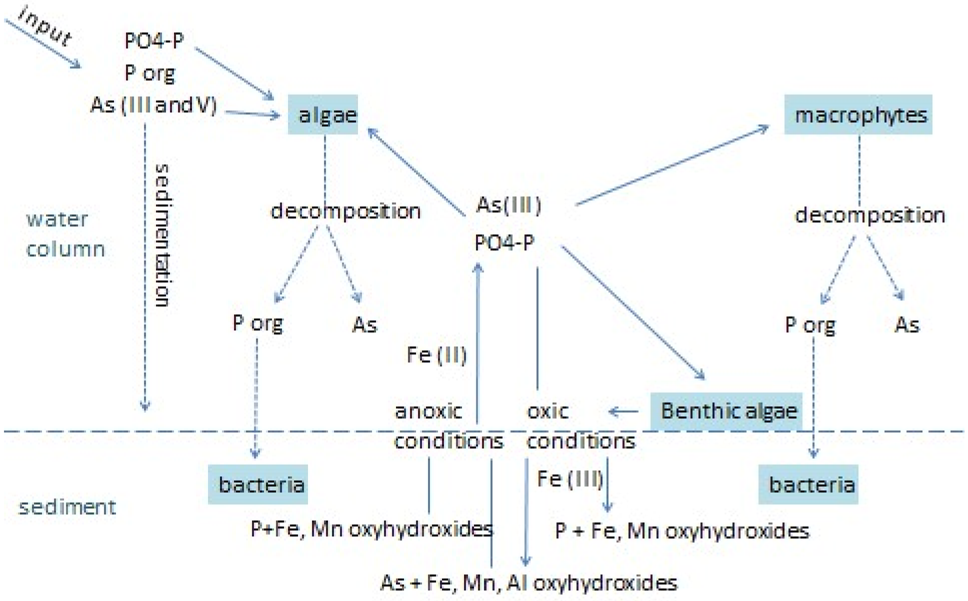

Fig. 1.1. Processes affecting cycling of P and As between water and sediment.

Arsenic can occur in waters in inorganic and organic forms, though the latter is uncommon (Partey et al. 2008). The inorganic forms of As are arsenate (As(V)), which is common under oxidising conditions and has low solubility, and arsenite (As(III)), which dominates under reducing conditions and has greater solubility (Partey et al. 2008; Whitmore 2008). In oxic conditions the diffusion of soluble As from sediments into overlying water is limited by sorption of arsenite and arsenate to Fe, Mn and Al oxyhydroxides (Toevs et al. 2008; Whitmore et al. 2008). Reducing conditions in sediments result in the reduction of Fe-As oxyhydroxides (Couture et al. 2010) which has been shown to positively correlate with increased dissolved As concentrations in the sediment pore-water (Toevs et al. 2008). During the reduction of Fe-As oxyhydroxides in sediments, As(V) is converted to As(III) and released to the porewaters. Under oxic conditions in the overlying water, dissolved As(III) is likely to be bound again by oxidised forms of Fe or Mn. If the overlying waters are anoxic, As(III) can persist in the water column (Whitmore et al. 2008). In the presence of sulfide, As(III) can also precipitate as an arsenic sulfide in anoxic zones, which decreases dissolved As (III) concentrations (Toevs et al. 2008).

Sediments play an important role in the regulation of P cycling in shallow lakes (Søndergaard et al. 2003). The retention of P in sediments is influenced by two processes: the downward flux that is the result of the sedimentation of particles continuously entering a lake or particles produced in a lake (e.g. algae or detritus), and the upward flux due to the release of P from sediment (Søndergaard et al. 2003). The internal loading of P from bed sediments to bottom waters is maintained through the high depositional rate of organic matter, especially in eutrophic lakes (Spears et al. 2007), creating the potential for high sediment mineralisation rates (Søndergaard et al. 2003). Microbial remineralisation of organic matter in the upper layers of bed sediments acts to elevate sediment P content adsorbed to Fe and Mn oxyhydroxides under aerobic conditions or to release dissolved P to the pore-water and bottom waters under reducing conditions (Boström et al. 1989; Spears et al. 2007). P can also be bound to other inorganic components, such as Al and Ca, which enhances its sedimentation from lake water or occurrence in sediment in organic P compounds. Organic P occurs in either labile forms or a refractory form which is not released during mineralisation processes and is permanently bound in sediment (Søndergaard et al. 2003).

A number of environmental drivers can regulate the mobilisation of red mud constituents in lake bed sediments including organic matter, temperature, light, redox potential, pH, and bacterial productivity (Czop et al. 2011). Haus et al. (2008) reported fluctuations in lake water level as a factor responsible for unstable redox conditions in bed sediments leading to an increase in As concentrations in pore-waters, with As much less mobile when water level is constant. Remineralisation of organic matter in sediments was identified as one of the major drivers of As cycling between bottom waters and bed sediments (Couture et al. 2010; Andrade et al. 2010). Eutrophication, and therefore, increased organic matter deposition and remineralisation, may lead to seasonally anoxic conditions in the hypolimnion, resulting in the release of As from redox sensitive oxyhydroxides in bed sediment. This negative influence of eutrophication on As precipitation was reported in a study of Little Lake Jackson in Florida (Whitmore et al. 2008).

Similar factors drive cycling and release of P from bed sediments. Sedimentation of organic matter and mineralisation processes affect the sorption capacity of sediments for P (Søndergaard et al. 1999) in a similar way as the sorption capacity for As. Transport and release of P from sediments are likely to be enhanced by increasing temperature and bacterial activity (Spears et al. 2006; Søndergaard et al. 1999), whilst increasing pH can increase the solubility of iron-phosphate compounds (Søndergaard et al. 1999). The mineral composition of sediment, in particular of elements with the capacity for binding and releasing P, such as Fe, Al, Mn and Ca, is another important factor affecting sediment P sorption capacity (Søndergaard et al. 1996). Biological factors that affect P behaviour in lakes include benthic primary production, which can enhance the oxidation of sediment and decreases P concentrations by direct P uptake, presence of submerged macrophytes and presence of benthivorous fish, e.g. common bream (*Abramis brama* L.) (Søndergaard et al. 2003). The foraging activities of the latter increase sediment resuspension and release of P. Enhanced nutrient release from sediment due to the feeding habits of another benthic fish, common carp (*Cyprinus carpio* L.), have also been reported (Skov et al. 2010).

1.5. Potential effects of red mud pollution on the aquatic environment

Although little is known about the combined effects of red mud on the aquatic environment following a pollution event, the toxic effects of specific red mud constituents have been assessed, in isolation, in many countries (Mayes et al. 2011). The composition of red mud leachate shown in Table 1.2 suggests four potential pressures that can affect the aquatic environment: (1) metal and metalloid pollution,

(2) eutrophication, (3) salinisation, and (4) alkalisation. The effects of these pressures are listed in Table 1.3, whilst the impact of metal and metalloids pollution and eutrophication are described in more detail in Sections 1.5.1 and 1.5.2. The summary in Table 1.3 indicates potential impacts of red mud on all trophic levels of aquatic ecosystems, ranging from changes in organism community structure and abundance to bioaccumulation and toxic effects.

Table 1.3. The effects on aquatic ecology of the pressures associated with red mud pollution.

Response	**Pressure**			
	eutrophication	**metals/ metalloids**	**alkalisation**	**salinisation**
phytoplankton	• decrease in diversity (Mason 2002) • shift in phytoplankton community structure, often dominated by cyanobacteria (Mason 2002) • changes in diatom communities (Mason 2002) • toxic cyanobacteria blooms (Mason 2002) • high phytoplankton biomass (Søndegaard et al. 2003) • decrease in top-down control of phytoplankton by zooplankton (Søndegaard et al. 2003)	• bioaccumulation (e.g. Chen et al. 2008; Mason 2002) • changes in phytoplankton community towards species resistance to metal/ metalloid stress, i.e. certain cyanobacteria species	• no studies found	• shift in phytoplankton community structure, likely dominance of cyanobacteria (Sonzogni et al. 1983) • changes in diatom community composition (Ziemann and Schulz 2011)
macrophytes	• decrease in diversity (Mason 2002), reduction or disappearance of submerged macrophytes (Søndegaard et al. 2003) • changes in aquatic plant abundance (Pedersen et al. 2006), increased growth of non-rooted macrophytes (Mason 2002)	• bioaccumulation (i.e. Mazej and Germ 2009; Mishra et al. 2008) • toxic effects (Mishra et al. 2008 ; Mišík et al. 2014)	• decrease in macrophyte abundance (Ouboter and de Dijn 1993)	• decreased growth with increasing salinities (Haller 1974) • influence on the community structure due to varying salt tolerance among species, e.g. large floating species (*Eichornia, Pistia*) and some submersed plants (e.g. *Hydrilla,Najas*) more susceptible to low salinity levels than *Myriophyllum* and small floating plants (e.g. *Lemna*) (Haller et al. 1974)

Table 1.3 continued.

Response	Pressure			
	eutrophication	**metal/metalloids**	**alkalisation**	**salinisation**
zooplankton	• changes in the community structure (Mason 2002)	• bioaccumulation (i.e. Chen et al. 2008; Mason 2002)	• decrease in diversity (Schell et al. 2001)	• toxic effects (Elphic et al. 2011)
macroinvertebrates	• decrease in diversity (Mason 2002)	• bioaccumulation (i.e. Arribére et al. 2011; Mason 2002) • toxic effect i.e. lethal effect (Edwards 1985)	• changes in invertebrate community (Ouboter and de Dijn 1993)	• decrease in diversity (Braukmann & Böhme 2011)
fish	• changes in fish community, coarse fish, e.g. perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), common bream (*Abramis brama*) often dominant (Mason 2002); • fish mortalities due to lack of oxygen (Mason 2002)	• bioaccumulation (i.e. Chen et al. 2008; Mason 2002) • toxic effects i.e. lethal effect, decrease in reproductive success (Hirata et al. 2011), decrease in growth, condition and population density (Lalonde et al. 2011) • changes in fish community (Lalonde et al. 2011)		• reduced adult growth and fitness with increase salinity (Prasad 2010) • negative effect on oocytes and larvae of fish (Prasad 2010)

1.5.1 Metal and metalloid pollution

The effects of metal and metalloid pollution on the aquatic environment have been the subject of many scientific publications (e.g. Hatcher et al. 1992; Lalonde et al. 2011; Mishra et al. 2008; Shah et al. 2009). For example, high concentrations of As have been reported to pose a threat to aquatic ecosystems and human health in Pakistan, Bangladesh, India, Taiwan (Shah et al., 2009), Japan (Hirata et al. 2011), China (Chen et al. 2008), Canada (Lalonde et al. 2011) and the USA (Rowe et al. 2002).

Bioaccumulation of metals and metalloids in aquatic biota varies at different trophic levels, depending on the feeding strategy, size and abundance of the organism. The contaminant uptake and transfer routes within the food web also vary by metal and metal concentration in the aquatic environment (Chen et al. 2008). Depending on the metal or metalloid, higher trophic levels can accumulate either higher (compared to lower trophic levels) concentrations of metals due to biomagnification or lower concentrations due to biodiminution (Chen et al. 2008). The capacity of aquatic plants to sequester and accumulate metals is species specific (Mishra et al. 2008). Concentrations of contaminants accumulated by plants can be higher than concentrations in water, but lower than in sediments (Mazej and Germ 2009). Different rates of plant growth, biomass accumulation and affinity for metal uptake may also influence element accumulation (Mishra et al. 2008). Uptake of As can reduce P, N, K, chlorophyll *a* and protein content of plant tissues (Mishra et al. 2008). Arsenic, due to its chemical similarity to P, can also interfere with some biochemical reactions in plants (Mishra et al. 2008).

Assessment of the ecotoxicity of river sediments contaminated with red mud from the Ajka spill indicated pronounced toxic effects of red mud contaminants on *Vibrio fischeri* bioluminescence and on the growth of *Lemna minor* L., as well as adverse toxic effects on roots and shoots of *Sinapis alba* Linn (Klebercz et al. 2012). In addition, genotoxicity experiments to investigate the effects of red mud released during the Ajka accident showed damage to genetic material in two higher terrestrial plants (*Tradescantia* and *Allium cepa* L.) which was attributed to the presence of soluble vanadium (vanadate; Mišík et al. 2014).

1.5.2 Eutrophication

Mason (2002) defined eutrophication as the enrichment of waters by inorganic plant nutrients, especially P and nitrogen (N), which results in an increase in primary production. Carpenter and Lathrop (1999) described eutrophication as a syndrome that develops when resilience mechanisms in a lake are broken down. Cultural eutrophication, which results from an increase in P and N due to human activities (Mason 2002), is a well-recognised, global scale problem and the subject of research and management efforts worldwide (e.g. Bennet et al. 2001; Carpenter et al. 1999; Hamilton and Landman 2011; Harper 1986; Jacquet et al. 2005; Mason 2002).

Eutrophication causes environmental and socioeconomic problems (Harper 1986). High concentrations of P can result in high phytoplankton biomass, turbid water and undesirable loss of biodiversity and a reduction in the functional capacity of the ecosystem. Impacts include a decrease in top-down control of phytoplankton by zooplankton, disappearance of submerged macrophytes and changes in fish community composition to one that favours cyprinids (Søndegaard et al. 2003). An increase in phytoplankton biomass commonly leads to an increased rate of organic deposition to the lake bed and an increased likelihood of anoxia. Bloom-forming cyanobacteria can dominate in eutrophic lakes and maintain feedback mechanisms to favour phytoplankton dominance over macrophyte dominance (Mason 2002).

During the last 10-30 years, major efforts have been made worldwide to address eutrophication by reducing external nutrient loading (Jeppesen et al. 2005). These have often led to a decrease in concentrations of total P and chlorophyll *a* in surface waters, reduction of phytoplankton biomass and increased water clarity (Jeppesen et al. 2007), followed by changes in phytoplankton, zooplankton and fish community structures (Jeppesen et al. 2005).

The release of P from sediments can be an important factor (after reduction of external nutrient loading) that delays recovery of lakes from eutrophication (Jeppesen et al. 2005, 2007; Søndegaard et al. 2003). To accelerate the recovery processes, external loading control may be combined with restoration methods such as biomanipulation (e.g. selective removal of planktivorous fish, stocking of piscivorous fish) and physico-chemical methods for the control of internal loading (e.g. sediment removal, application of P sediment capping agents) (Jeppesen et al. 2007). Sediment capping agents, such as lanthanum-modified bentonite clay (Phoslock$^{®}$) have been shown to increase the P-sorption capacity of sediment and therefore reduce the amount of P likely to be released from sediment into water under reducing conditions (Meis et al. 2013).

1.5.3 Potential multiple pressure interactions

Pollutants occurring together may act independently on aquatic organisms (in such situations the pressure with the strongest effect will be the most important; Table 1.3) or through interactions resulting in additive, antagonistic or synergistic effects (Mason 2002). Therefore, assessing the impacts of each pressure can be confounded by the independent or combined effects of multiple pressures. For example, eutrophication, salinisation and metal and metalloid stress may have combined effects on lake phytoplankton community. Eutrophication can lead to high phytoplankton biomass (Søndergaard et al. 2003) and dominance of cyanobacteria (Gibbs and Özkundakci 2011). Certain cyanobacteria species may also out-compete other phytoplankton in the presence of metals or metalloids (Dubey and Dubey 2011). A shift in phytoplankton community composition, with likely dominance of specific tolerant cyanobacteria species, has also been reported following salinisation (Sonzogni et al. 1983).

The influence of eutrophication on the effects of metals and metalloids on aquatic organisms is another example of interactions between pressures. Increased sedimentation of organic matter, as a consequence of increased algal production, can cause reducing conditions in lake bottom waters that may result in an increase of P and As release from bed sediments to the overlying water column (Czop 2011). Release of contaminants from bed sediments can not only lead to bioaccumulation of trace elements, but also creates a risk of sudden release of high concentrations of contaminants, which might result in mass mortality of organisms, e.g. as occurred in Lake Biwa in Japan (Hirata et al. 2011).

1.6. Evidence of ecological degradation following red mud pollution: Ajka, western Hungary

On 4 October 2010 a failure occurred in the dam of a red mud containment reservoir near Ajka, Hungary. This failure released about 700,000 m^3 of red mud from the reservoir (Gelencsér et al. 2011; Ruyters et al. 2011) and flooded parts of the city of Devecser, two villages (Ruyters et al. 2011) and around 40 km^2 of agricultural land along the Torna Creek and River Marcal (Gelencsér et al. 2011) in Veszprém County, western Hungary.

The dam failure resulted in the death of 10 people (due to drowning and severe burns from the alkaline solution) and more than 100 injuries, as well as irreversible damage to property. The receiving terrestrial and aquatic systems were contaminated with red mud (Ruyters et al. 2011). Research effort focused initially on assessing the public health risk associated with dust blowing from the red mud deposits (Gelencsér et al. 2011), phytotoxicity of red mud (to evaluate the soil contamination risk; Ruyters et al. 2011) and the impact of the pollution on the fluvial system (Mayes et al. 2011; Schöll & Szövényi 2011).

The industrial accident in Ajka was an unprecedented large scale release of red mud to a river system (Mayes et al. 2011). The highly alkaline pollution (pH 13.5) reached the Torna Creek and River Marcal, passed through the River Rába and entered the Danube, Europe's second longest river (Schöll and Szövényi 2011). To reduce the pH in contaminated rivers and streams, large amounts of calcium sulphate were applied (Enserink 2010). Due to the high toxicity, red mud not only had a catastrophic effect on the aquatic ecosystems close to the source of the pollution (Torna Creek, River Marcal), but also impacted aquatic organisms in river reaches further downstream. Data from a site in the River Danube, 120 km downstream from the pollution source, indicated a loss of the planktonic rotifer assemblages, despite the long hydrological distance and high level of contaminant dilution (Schöll & Szövényi 2011). The rotifer community biomass and diversity indices at this site decreased to zero a few hours after the passage of the main mass of contaminated water (Schöll & Szövényi 2011). However no significant changes in pH were recorded (Schöll and Szövényi 2011), indicating the sensitivity of aquatic biotic assemblages for determining the impacts of such contamination events.

Analysis of sediments and water in the Torna Creek and the River Marcal, conducted almost 2 months after the pollution event, indicated a recovery in pH which decreased with distance from the source of pollution from c. pH 13 (at the site closest to the pollution source area) to c. pH 8 downstream. The presence of elevated concentrations of As, Mo and V in water and As, V, Cr, Co and Ni in river sediment was limited to the Torna Creek and part of the upper Marcal River. Sediment analysis indicated the rapid dilution of red mud within the Torna Creek with the occasional red mud enrichment 'hot spot' in the upper Marcal River (Mayes et al. 2011). These results indicate the heterogeneous spatial distribution of red mud constituents in depositional systems. The comparison of As and V concentrations in water and sediment in affected watercourses (Kinghorn Loch and downstream of the Ajka accident) with existing water and sediment quality guidelines is presented in Tables 1.4, 1.5 and 1.6. In the absence of standards for As in sediments in the UK, Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment 1999a), which have been widely used for initial assessment of potential freshwater sediment toxicity, were employed. Such formal sediment quality guidelines have been not set for potentially toxic V (Burke et al. 2012). The water quality guidelines for V (EEC 1976) were repealed under WFD (EC 2000) in 2013. However in the absence of other informative guidance, these guidelines are used in the present study. The guideline values for V vary depending on the hardness of the water as specified in two class categories (0-200 mg L^{-1} $CaCO_3$ and > 200 mg L^{-1} $CaCO_3$; EEC 1976). With the exception of site K1 in the river system in Hungary, the total concentrations of As and V in Kinghorn Loch water before the diversion of the pollution were higher than those measured in the Torna Creek and the upper Marcal River. Sediment As concentrations in Kinghorn Loch in both 1983 and 2010 were considerable higher than As concentrations determined in the river system in Hungary and greatly exceeded the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (17 mg kg^{-1} dry weight; Canadian Council of Ministers of the Environment 1999a).

Table 1.4. Total As concentrations measured in water at Kinghorn Loch and at sampling sites along the river system downstream of the dam burst at Ajka, Hungary, compared against water quality guidelines.

Kinghorn Loch – lake outlet (µg L^{-1})		**Torna Creek and Marcal River 01.12.2010 (µg L^{-1})[a]**										**Water Quality Guidelines (µg L^{-1})**				
Mean value for 1981-1983	2010 (single measurement)	T2	K1	K2	K3	T1	T3	T4	T5	T6	M1	Drinking water		Protection of Aquatic Life		
												UK[b]	WHO[c]	UK[d]	UK[e]	Canadian[f]
321	7	<0.1	3926	224	181	108	124	43	37	33	<0.1	10	10	50	50	5

[a] Mayes et al. 2011

[b] UK Standards Ensuring the Quality of Water Intended for Human Consumption (EC 1998)

[c] World Health Organization (WHO) Guideline value for chemicals that are of health significance in drinking-water (WHO 2004)

[d] UK Standards for Protection of Surface Water Quality (EC 2000)

[e] UK Standards for Protection of Aquatic Life (EEC 1976)

[f] Canadian Water Quality Guidelines for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment 2001)

Table 1.5. Total V concentrations measured in water at Kinghorn Loch and at sampling sites along the river system downstream of the dam burst at Ajka, Hungary, compared against water quality guidelines.

Kinghorn Loch – lake outlet (µg L^{-1})		**Torna Creek and Marcal River 01.12.2010 (µg L^{-1})[a]**										**Water Quality Guidelines (µg L^{-1})**		
Mean value for 1981-1983	2010 (single measurement)	T2	K1	K2	K3	T1	T3	T4	T5	T6	M1	Drinking water	Protection of Aquatic Life (UK)[b]	
													0-200 mg L^{-1} CaCO$_3$	>200 mg L^{-1} CaCO$_3$
493	12.6	<0.1	6398	334	347	66	103	48	47	35	<0.1	none	20[c]	60[c]

[a] Mayes et al. 2011

[b] UK Statutory Guidelines for Protection of Aquatic Life (EEC 1976)

[c] These values are for total vanadium and represent the annual average.

Table 1.6. Arsenic concentrations measured in sediment at Kinghorn Loch and at sampling sites along the river system downstream of the dam burst at Ajka, Hungary, compared against sediment quality guidelines.

Kinghorn Loch – at the deepest site (mg kg^{-1})[a]		Torna Creek and Marcal River 01.12.2010 (mg kg^{-1})[b] (mean concentrations in acid-digested sediment)													Canadian Sediment Quality Guidelines (mg kg^{-1}) for the Protection of Aquatic Life[c]	
1985	2010	K1	FA	K2	K3	T1	T3	T4	S1	T5	T6	M2	M1	T2	ISQG[d]	PEL[e]
747	210	78.5	29.9	32.3	51.9	54.3	46.2	50.8	61.3	52.8	54.9	56.3	5.8	1.7	5.9	17.0

[a] measured in top 6 cm of sediment

[b] mean composition of acid-digested fluvial sediments (3 samples per site; Meyes et al. 2011)

[c] Canadian Council of Ministers of the Environment 1999a

[d] Interim Sediment Quality Guideline (ISQG) below this concentration adverse effects are expected to occur only rarely (predicting the absence of sediment toxicity)

[e] Probable Effect Level (PEL; predicting sediment toxicity)

1.7. Effects of internal loading from sediment on recovery

Recovery of freshwater ecosystems that have suffered from long-term pollution is likely to be affected by internal loading of pollutants deposited in sediments. A review of studies of aquatic ecosystems (i.e. lakes, rivers and reservoirs) in the USA affected by coal combustion ash residue which contains As, Cd, Cu, Hg, Pb, V, Se and Sr, indicated that recovery can be a slow process due to the cycling of pollutants between the bed sediments and the overlying water column (Rowe et al. 2002). Delayed recovery after external loading reduction of P, as a consequence of in lake cycling, has been reported in many lakes (Kleeberg and Kozerski 1997; Søndergaard et al. 2003; Spears et al. 2007). Shallow lakes are more resistant to recovery after the reduction of external nutrient loading (Kleeberg and Kozerski 1997) and the potential influence of sediment on lake water concentrations is greater than in deeper lakes (Søndergaard et al. 2003).

Understanding the processes regulating mobilisation of trace elements from sediments to the water column is essential when attempting to quantify recovery trajectories following pollution management. In the case of red mud pollution, the interactions between As and P within sediments can be an additional factor to consider. For example, in Lake Kasumigaura, Japan, the presence of P in the lake water led to a large decrease in As adsorption in sediments (Chen et al. 2011). A study of Lake Søbygaard, Denmark, revealed that, after the reduction of the external loading of P, sediment P was released from progressively greater sediment depths, which suggests that the deep sediment P-pool is not necessarily permanently buried (Søndergaard et al. 1999). From the similarity of cycling processes of P and As, it is likely that similar processes will regulate As mobilisation within sediments. Furthermore, both As and P are influenced by redox reactions in sediments which are in turn affected by many environmental factors, and by the sediment content of Fe.

1.8. Research aims and thesis overview

Based on the gaps in the knowledge on the effects of red mud on the aquatic environment identified in the literature review, this thesis aims to assess the long term chemical and biological recovery from red mud pollution of freshwater ecosystem with a higher retention time. To address this aim four main objectives were investigated in Chapters 3 to 6, respectively:

- the long-term recovery trajectories of red mud contaminants in the surface waters of a shallow lake;
- sediment contamination and pollutant cycling between the sediment and the water column of a waterbody affected by red mud 30 years after cessation of pollution;
- the impact of sediment on recovery in the water column and the physico-chemical factors that control the release of red mud constituents across the sediment-water interface;
- the As presence and risk to the aquatic food web 30 years after the beginning of recovery period, based on the bioaccumulation of As species within the macrophyte community.

Kinghorn Loch in Scotland is an ideal case study to investigate these research aims due to the existence of long-term data documenting approximately 30 years of changes in the lake since the diversion of pollution and a unique opportunity for research on the long-term effects of red mud pollution and the processes of recovery. This PhD project was based on field surveys conducted at Kinghorn Loch, a laboratory controlled experiment and analysis of existing long-term data documenting chemical changes in the lake.

The thesis is structured around four paper-style chapters (Chapters 3-6) preceded by the Methods chapter (Chapter 2) that provides an overview of the study site and describes in detail methods used for sample analyses and macrophyte community survey. Chapter 3 assesses the long-term chemical recovery in Kinghorn Loch following decades (until 1983) of red mud pollution. 28 years of data from the lake, documenting the last two years of the pollution and the subsequent recovery period, were used to address the following specific objectives to: (1) quantify the composition and catchment load of red mud pollutants to Kinghorn Loch during the peak pollution period, (2) quantify recovery trajectories of red mud pollutants over a 26 year period since pollution inputs ceased.

Chapter 4 describes variations in concentrations of pollutants in water and sediment in Kinghorn Loch measured monthly during a one year period 30 years after the beginning of the lake recovery. The contemporary data were used to test the following hypotheses: (1) concentrations of red mud constituents in Kinghorn Loch water vary spatially and across seasons, (2) concentrations of red mud constituents in lake sediment vary with the overlying water depth and across seasons, (3) pollutant concentrations in lake sediment have decreased over time since the diversion of the pollution.

Based on the Kinghorn Loch case-study, Chapter 5 investigates the long-term fate of red mud constituents in a shallow lake. This was achieved through a 12 month field campaign, examining concentrations of pollutants in surface water versus concentrations in water above lake bed sediment as evidence of internal loading of constituents, and a complementary laboratory experiment designed to trigger release of redox sensitive species from sediment to water. The following hypothesis was assessed: (1) that release of redox sensitive red mud constituents (P, As and V) from the bed sediment of Kinghorn Loch would persist 30 years after the beginning of the lake recovery period and be measurable in the field and the laboratory and (2) dissolved oxygen concentrations in the water column control the pollutant release from sediment to water and the process will be measurable in a laboratory.

Chapter 6 assesses the impact of As on macrophytes in Kinghorn Loch 30 years following the diversion of red mud leachate. Arsenic speciation analysis was applied to quantify inorganic As (arsenite, As(III) and arsenate, As(V)) and four organic As species: arsenobetaine, dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and tetramethylarsonium ions (Tetra)). Three hypotheses were tested: (1) macrophytes in Kinghorn Loch accumulate higher concentrations of inorganic As compared to organic As species, (2) accumulation of As species varies between above-sediment tissues (leaves and stems) of macrophytes and among macrophyte species, and (3) accumulation of As species varies between above- and below-sediment surface tissues of *Persicaria amphibia*, with the greatest concentrations accumulated in the roots of the plant.

The thesis concludes with an overall Discussion (Chapter 7) in which the key research results are summarised and their implications discussed, including the potential management options to minimise the chemical and ecological impacts of red mud on freshwater environments.

Chapter 2 Methods

This chapter is divided into two sections. The first describes the study site and the history of its pollution and monitoring. The second section lists the physico-chemical variables measured during the PhD research and the methods used. Detailed descriptions are provided of the laboratory analyses applied for determination of red mud constituents and of the macrophyte community survey.

2.1. Study site

Kinghorn Loch is a shallow lake that has suffered from long-term red mud pollution. It is a small lake of surface area 11.3 ha, mean depth 4.5 m and maximum depth 12.8 m (Edwards 1985), situated in Fife in Scotland (56°10′N, 3°11′W; Fig. 2.1).

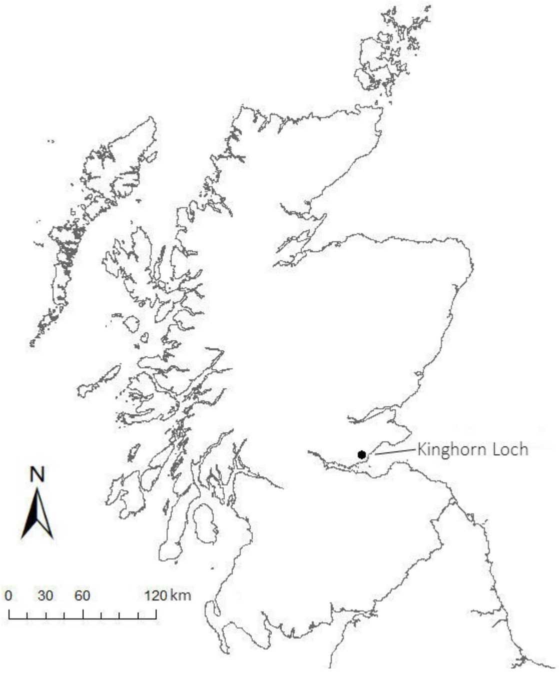

Fig. 2.1. Location of Kinghorn Loch in Scotland.

The catchment area is c.60 ha. The catchment geology consists of basalt and basaltic rocks that overlie calciferous sandstone and the dominant catchment land uses are livestock grazing and crop production. There are two inflows into the lake: one flowing through marshy ground in the northwest of the lake and another inlet in the North (Edwards 1985; Fig. 2.2).

Fig. 2.2. Locations of the former production plant and landfill site, and current location of the treatment plant in relation to Kinghorn Loch (image courtesy of Google Earth, taken in 2006).

The North inlet water was characterised by mean pH of 8.1 and a moderately high dissolved silicon concentration (mean value 4.3 mg L^{-1}) during the time period 1981 – 1983 (Edwards 1985). The measurements in 1981 – 1983 showed that the North inlet was responsible for only a minor contribution (approximately 5000 kg detrital solids each year) to the suspended load entering the lake (Edwards 1985). The only surface water outlet is in the southeast of the lake (Edwards 1985).

Kinghorn Loch received leachate from 1947 to 1983 from a nearby red mud landfill site (Whinnyhall Tip) operated by BA Chemicals Ltd., part of Rio Tinto Alcan (Fig. 2.2 and 2.3). The red mud was the by-product of bauxite processing to alumina at the BA Chemicals Ltd plant in Burntisland which operated from 1917 till 2002. The bauxite ore processed in the Burntisland plant was imported from Ghana. Initially the bauxite residue was stored in lagoons adjacent to the coast which were filled in during the 1940s. Subsequently red mud was stored in landfill, after pressing and filtering to reduce the water content. The original landfill area was unlined. After it was full in the late 1970s, landfilling continued in an adjacent area to the west which had a clay liner (Bob Cooper, Rio Tinto Alcan, *pers. comm.,* 2012). According to the anecdotal evidence, drainage from the landfill site was entering the northwest part of the lake with re-routed spring water which acted as a conduit for leachate into the lake and with groundwater.

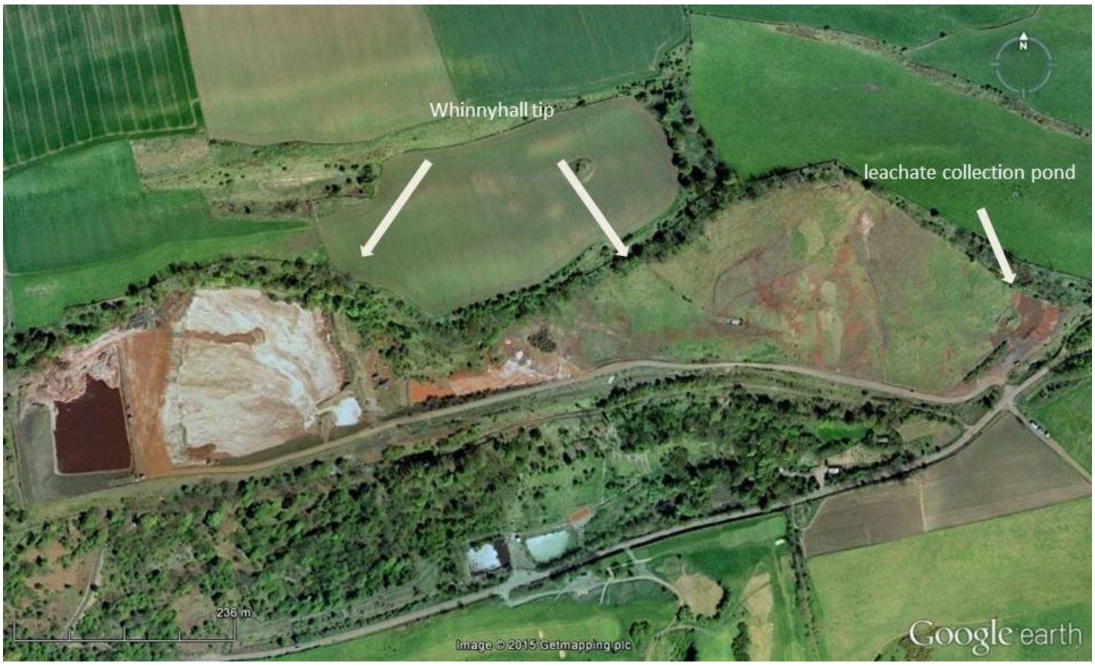

Fig. 2.3. The landfill site and leachate collection pond in 1999, 3 years before the site closure (image courtesy of Google Earth).

In May 1983 this discharge was diverted away from the lake as part of the scheme designed for suitable treatment and disposal of the leachate (Edwards 1985). A system of underground drains was installed to collect the polluted water leaching from the landfill site. However, as the landfill site is unlined, residual leaching beyond the collection drains may be apparent. This system of drains is still in operation following closure of the production site due to the contamination of the area used as a landfill site. The collected water is directed into a concrete-lined storage pond (Fig. 2.4 a) that feeds to the currently operating treatment plant and once treated, is released to the sea. Continuous pH monitoring is used in two areas of the treatment system: (1) automatically controlled pH dosing system to add correct amount of H_2SO_4 to the leachate to reduce the pH to 6-9; (2) monitoring of pH of discharge after treatment and before discharge to sea. In addition, a pond that used to collect and store leachate coming from the landfill site (Fig. 2.4 b) is now used for collection of surface runoff from the previous tip area (Fig, 2.2 and 2.3). pH monitoring of the pond water is applied as a quality check. Treatment of the leachate from the contaminated landfill area using the current system is estimated to be required for another 80 years (Bob Cooper, Rio Tinto Alcan, *pers. comm.*, 2012).

Fig. 2.4. a) Drains collecting water from the previous landfill site into the treatment plant pond, **b)** the collection pond.

Leachate reaching the lake, measured at the northwest inlet, consisted of a highly alkaline solution of NaOH and carbonate, with mean pH of 12.1. It contained high concentrations of dissolved Al, As, V, orthophosphate, sulphate and chloride (Table 1.2, Section 1.2). Spring water that diluted the leachate before entering the lake provided an input of Ca, Mg and Si, whilst red mud solids, entering the lake sporadically, were responsible for most of the input of Fe (Edwards 1985). Due to the composition of red mud, the discharge represented multiple pressures on the lake of alkalisation, metal pollution, salinisation and eutrophication.

As a result of the long-term red mud pollution, the lake became nutrient enriched (mainly with orthophosphate) and from the mid-1970s suffered from intense phytoplankton blooms (Edwards 1985). There was a significant increase in lake water pH, which in 1983 reached a value of 10.5. Concentrations in the lake surface water of dissolved As and V before the diversion of the leachate were the highest recorded in any water body in the UK, reaching 500 $\mu g\ L^{-1}$ (compared to <5 $\mu g\ L^{-1}$ in most waters in UK), and 494 $\mu g\ L^{-1}$, respectively. By 1983, 0.5 m depth of fine red mud had accumulated in the loch. Research conducted before the diversion of the discharge revealed localised zones in the lake that were characterised by low dissolved oxygen, elevated alkalinity, very high electrical conductivity and high concentrations of dissolved Mg, Ca, Al, As and Fe (Edwards, 1985).

Edwards (1985) assessed the impact of red mud leachate on the aquatic ecology of Kinghorn Loch up to 1985. Phytoplankton became almost entirely dominated by *Oscillatoria*, with only a small proportion of diatoms present. The pollution led to fish kills and severe reduction of zooplankton, macroinvertebrate and macrophyte species diversity and biomass (Edwards 1985).

During the first year after the diversion of the leachate, the loch pH decreased to 8.46 (annual mean). Arsenic, however, remained significantly elevated, with concentrations 30 – 50 μg L^{-1} in the water column and exceeding 1 mg L^{-1} in the sediment interstitial water. By 1985, two years after the diversion of the leachate from the loch, ecological improvements in the loch were apparent. There was a collapse in the phytoplankton activity, development of zooplankton and an increase in abundance of benthic macroinvertebrates (though no increase in species richness). Bioaccumulation studies of the plankton and macroinvertebrate populations in 1985 indicated no significant As bioaccumulation in Kinghorn Loch. The conclusions, based on the results of the first two years of recovery data, supported future management plans of stocking the loch with rainbow trout (*Oncorhynchus mykiss*) to establish a viable fishery. However, it was also concluded that there was a need for ongoing monitoring to assess the potential release of As and V from sediment over the longer-term (Edwards 1985).

The ongoing SEPA and the FRPB, its predecessor, monitoring programme that followed the diversion of the leachate from the lake has documented chemistry of the lake outlet water. No monitoring of lake sediment and chemistry of deeper layers of the lake water column has been conducted. Comparison of As and V concentrations in water from the lake outlet and lake sediment measured in 1983-1983 and in the lake outlet water in 2010 to existing water and sediment quality guidelines was given in Tables 1.4, 1.5 and 1.6 (Section 1.6).

2.2. Applied methods

A variety of methods were applied to measure concentrations of P and metals and metalloids in Kinghorn Loch water, and major and trace elements in lake sediment samples, alongside *in situ* measurements of water physico-chemical parameters (reported in Chapter 4 and 5). In addition, sediment cores were extracted from the lake for the laboratory controlled experiment (Chapter 5) and macrophyte samples were collected for the As accumulation study (Chapter 6). Concentrations of As species in macrophyte tissues were also determined.

The variables measured and the methods applied in Chapters 4-6 of the research are summarised in Table 2.1. In this chapter, detailed descriptions are given of the laboratory methods used to determine concentrations of elements in lake water, sediment and macrophyte samples, whilst most information relating to sample strategy, collection and preparation is given in the Methods sections of Chapters 4-6. In addition, Chapter 2 describes the survey method used for obtaining information about macrophyte species and colonisation depth in Kinghorn Loch. The data presented in Chapter 3 were provided by SEPA and therefore the methods for determining these variables are described in the Methods section of Chapter 3. The statistical analysis methods are described in Chapters 4-6.

Table 2.1. Variables measured in this PhD research and methods used.

Variable	Field method	Laboratory method	Chapter		
			4	**5**	**6**
Lake water					
pH[1]	pH probe	-----	x	x	
Temperature[1]	pH probe	-----	x	x	
Conductivity[1]	Conductivity probe	-----	x	x	
Dissolved oxygen (DO)[1]	Oxygen probe	-----	x	x	
Water depth	Handheld sonar system	-----	x	x	
PO_4-P and TP	Horizontal Van Dorn water sampler	Colourimetric analysis	x	x	
Dissolved and total metal(loid)s	Horizontal Van Dorn water sampler	Inductively Coupled Plasma Mass Spectrometry	x	x	
Lake sediment					
Major elements	HTH gravity corer	Fusion method (based on Norrish and Hutton 1969), X-ray fluorescence	x		
Trace elements	HTH gravity corer	Determined on pressed-powder samples, X-ray fluorescence	x		
Experiment					
Water pH[2]	-----	pH probe		x	
Water temperature[2]	-----	pH probe		x	
Water conductivity[2]	-----	Conductivity probe		x	
Dissolved oxygen (DO)[2]	-----	Oxygen probe		x	
Macrophytes					
Plant tissue content of As species	Collected by hand and a grapnel	Ion chromatography coupled to inductively coupled plasma mass spectrometry			x
Macrophyte community survey	Boat transects				x

[1] Measured *in situ* in the lake

[2] Measured *in situ* in sediment cores

2.2.1. Phosphorus concentration in water

Water samples were analysed for the determination of concentrations of soluble reactive phosphorus (SRP), which is the bioavailable portion of dissolved P occurring predominantly as orthophosphate, and also for total phosphorus (TP). The latter includes all inorganic and organic forms of P, both dissolved and in the solid particulate phase. Samples for PO_4-P analysis were filtered (Whatman GF/F; pore size 0.7 μm) on the day of sampling and stored frozen, together with unfiltered samples for TP analysis, at -18°C.

Prior to determination of TP, 5 ml of each unfiltered sample was digested using potassium persulfate ($K_2S_2O_8$) acid hydrolysis digestion as outlined by Eisenreich et al. (1975) to convert all forms of P into orthophosphate. For digestion, 0.1 mL of 30% sulphuric acid (H_2SO_4) and 0.5 mL potassium persulfate were added to each sample. The samples were then placed into an autoclave for 30 minutes at 120°C.

P concentrations in filtered and digested samples were determined according to the acid-molybdenum-blue colorimetric method of Murphy and Riley (1962). Colorimetric determination of orthophosphate in water relies on the reaction of orthophosphate with ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) and potassium antimonyl tartrate (PAT; $C_8H_4K_2O_{12}Sb_2$) in acid solution (H_2SO_4). The yellow phospho-molybdate complex formed during the reaction is reduced with ascorbic acid ($C_6H_8O_6$) to a more stable blue complex. The intensity of the blue colour is proportional to the concentration of orthophosphate present in solution and can be measured using colorimetry. The colorimetric analyses in the present study were conducted using a Varian Cary SoBio spectrophotometer (Varian, Inc. Palo Alto, CA. USA) connected to a SPS-B auto sampler. A calibration curve was produced based on the absorbance readings at 882 nm of five P standards in the range 0 - 400 μg L^{-1} and used for quantifying P concentrations in samples. Standard solutions were prepared before each analysis by diluting to appropriate volumes a 100 mg L^{-1} P (L^{-1} PO_4-P) stock solution, which was made by adding 500 ml of distilled water to 0.2195 g of potassium dihydrogen orthophosphate (KH_2PO_4). The detection limit for P was 3 μg L^{-1}. All glassware used during the analyses was acid washed (with 10%

hydrochloric acid) and then rinsed six times with distilled water before use to avoid contamination. The P analyses were conducted at the Centre for Ecology & Hydrology (CEH) laboratory using procedures that are cross-checked with the methods of the central CEH laboratory in Lancaster, covered by ISO accreditation.

2.2.2. Metal and metalloid concentrations in water

Water samples for the determination of dissolved metals and metalloid concentrations were filtered through a Millex syringe-driven 0.45 µm filter prior to preservation with 1% v/v nitric acid. Samples for total metal and metalloid determination were preserved with 1% v/v nitric acid, followed by 16 h digestion at 80^{o}C in polypropylene centrifuge tubes in an oven prior to analysis. Concentrations of metals and metalloids were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using an Agilent 7500ce (with octopole reaction system), employing an RF forward power of 1540 W and reflected power of 1 W, with argon gas flows of 0.82 L min^{-1} and 0.21 L min^{-1} for carrier and makeup flows, respectively. In collision gas mode, the gas flow was 6.5 L min^{-1} for He. Using a peristaltic pump, sample solutions were taken up into the Mira mist nebuliser at a rate of 0.2 mL min^{-1}. The instrument was operated in fully quant mode (three points per unit mass, spectrum acquisition) and each mass was analysed with an integration time of 0.1 s per point, giving a total integration time of 0.3 s per unit mass. Samples were analysed in triplicate. ^{51}V and ^{75}As are mono-isotopic and are subject to polyatomic interference by ClO (mass 51) and ArCl (mass 75), respectively. To remove this interference they were analysed using He as a collision gas. To correct for any instrument drift each sample was spiked with 20 µg L^{-1} ^{45}Sc as an internal standard. A range of standards from 0–100 µg L^{-1} were prepared as a multi-element solution by diluting 1000 mg L^{-1} stock solutions (ICP multielement standard solution IV 1000 mg L^{-1} from Merck KGaA, V solution 1000 mg L^{-1} and As solution 1000 mg L^{-1}, both from Fisher Scientific Ltd.) in 2 % v/v HNO_3 solution (VWR, Aristar grade). A Certified Reference Material (NIST SRM 1643e Trace Elements in Water) was employed as a quality control check. Information about the detection limits for metal and metalloids, and recovery of elements in CRM is presented in Table 2.2.

Table 2.2. Detection limits for metal and metalloids in water samples, and percentage recovery of the elements in the CRM used in ICP-MS analysis.

Element	LOD (µg L^{-1})	Recovery (%)
B	13.31	103
Mg	0.0005	134
Al	0.92	134
Ca	0.31	125
V	0.05	115
Cr	0.23	117
Mn	0.03	108
Fe	0.10	104
Co	0.002	111
Ni	0.07	113
Zn	0.32	119
Cu	0.09	124
As	0.45	106
Cd	0.002	107
Pb	0.002	124

2.2.3. Sediment major and minor elements

The content of major (N_2O, Al_2O_3, CaO, Fe_2O_3, K_2O, MgO, MnO, P_2O_5, SiO_2, TiO_2) and trace elements (V, Cr, Ni, Cu, Zn, As, Br, Rb, Sr, Zr, Mo, I, Ba, Y, Sc, Nb) in lake sediment samples was determined using X-ray Fluorescence (XRF).

Dry sediments were processed to form fused glass discs and pressed pellets for analyses of major and minor elements, respectively, following the methods outlined by Fitton at al. (1998). To prepare the glass discs, dry samples were first left overnight in an oven at 450°C to remove organic material. 0.9 - 1.0 g of each sample was then placed in a platinum crucible and ignited at 1100°C in a muffle furnace for 20 minutes to determine loss on ignition (LOI). The ignited sample was mixed with Johnson Matthey Spectroflux 105 in a sample:flux ratio of 1:5, based on the unignited sample mass, and fused at 1100°C. After the first fusion, the crucible was re-weighed and more flux was added to compensate for any flux weight loss. The sample was then fused for the second time over a Merker burner. The molten mixture was swirled several times to ensure homogeneity, cast on a graphite mould and flattened with an aluminium plunger into a thin glass disc. The mould and the plunger were kept at 220°C temperature using a hotplate.

To prepare the pressed pellets, 8 g of dry sample was mixed with four drops of a binding agent (2% aqueous solution of polyvinyl alcohol). The mixture was transferred into a 40 mm diameter aluminium disc placed in a stainless steel mould between two tungsten carbide discs and compressed in a press at 8 tonnes. The pressed and fused samples were analysed using a Philips PW2404 X-ray fluorescence spectrometer employing a Rh-anode primary X-ray tube. The spectrometer was calibrated for both major element and trace element concentrations (with the exception of As) using a suite of internationally recognised (by United States Geological Survey (USGS) and Centre de Researches Petrographiques et Geochemiques (CRPG)) standard materials (ACE – Granite Scotland, AGV Andesite Oregon USA, BHVO-1 basalt Hawaii USA, BIR basalt Iceland, DTS –Dunnite Washington USA, G2 - Granite Rhode Is. USA, GH Granite Algeria, GSP granodiorite Colorado USA, MAG1 Marine mud Gulf of Maine, PACS2 Marine sediment, SCO – Cody Shale Wyomming USA, SDO Ohio shale USA, SGR ShaleGreen River Formation SiO2 Quartz mineral, STM – Syenite Oregon USA), values for which are given in Govindaraju (1994) Geostandards Newsletter Sp. Iss. Vol. XVIII). Arsenic standard materials covering the range of concentrations in the lake sediment samples were not available. Thus, a suite of four arsenic internal standards covering the range of 100 to 1000 ppm was produced as follows. Suitable amounts of As_2S_3, weighed to a precision of 0.0001 g, were added to 10 g aliquots of dried, powdered granite the composition of which had been well established over 10 years of repeated analysis. The doped aliquots were mixed thoroughly by agitating in a ball mill over a period of several hours. Pressed powder pellets of the doped aliquots were prepared using the same method as for the sediment samples. Arsenic concentrations in these internal standards were established by simple mass balance calculation.

Theoretical alpha coefficients calculated using the Philips software, were used to correct for matrix effects on the intensities of major-element lines. The coefficients allowed for the effect of the extra flux, which replaced volatile components in the samples, so that analytical totals should be 100% minus the measured LOI. The major element results were derived as % of major element oxides in weight total (wt.%) and were corrected for the loss of the organic material in a sample during firing at 450°C. To calculate wt.% of major elements, wt.% element oxide was multiplied by the ratio of the molecular weight of the element to the molecular weight of the element oxide. Major and minor element concentrations are reported per unit of dry weight. To verify accuracy of the analytical procedure of minor and major element measurements, As standards were run as unknown samples, with the difference between measured and expected As concentrations being 4%. Detection limits for minor elements are listed in Table 2.3.

Table 2.3. Detection limits for minor elements in sediment samples.

Element	LOD (mg kg^{-1})
V	4.0
Cr	3.9
Ni	1.3
Cu	0.8
Zn	0.9
Sr	0.6
Ba	2.8
As	2.0

2.2.4. As speciation in macrophytes

Approximately 100 ± 5 mg of each powdered sample was weighed into 50 mL centrifuge tubes. Two blanks and two tubes containing 100 mg of standard reference material (NIST SRM 1568b rice flour) were included in the process to verify the accuracy of the analytical procedure. 10 mL of 1% nitric acid was added to each tube and the samples were placed in a CEM Mars 6 1800W microwave digester. The samples were gradually heated to 95°C for 50 minutes in the three stage digestion process (heat to 55°C in 5 minutes, hold for 10 minutes; then heat to 75°C in 5 minutes, hold for 10 minutes, then heat to 95°C in 5 minutes, hold for 30 minutes). After cooling to room temperature deionised water was added to each tube to make up to the final weight (10 g) of the digest and the tubes were centrifuged at 20°C, 3500 rpm for 50 minutes in a Sorvall Legend RT centrifuge. The separation method applied in the As speciation analysis in the present study was also used during the analysis of As speciation in rice-based products (Signes-Pastor et al. 2016) and was based on extensive trials on the effect of oxidation on the As species stability during the acid digestion conducted by the Institute for Global Food Security at Queen's University Belfast. The trials showed that the organic species remain unaffected by 1% acid extraction (Manus Carey, the Institute for Global Food Security, Queen's University Belfast *pers. comm.*, January 2016). 1 mL of each sample extract was transferred to a 2 mL polypropylene vial and 10 µL of hydrogen peroxide (30%) was added. The vials were placed into the ion chromatograph autosampler according to a predetermined random run order. Five calibration standards, including a blank, were made up in the range 0-5 µg L^{-1} using dimethylarsinic acid (Supelco, powder, matrix: 1% HNO_3), each in a 10 ml final volume. In addition, a mixed standard, containing c. 0.7 µg L^{-1} of each of the following components: sodium arsenite (Fisher Chemicals, powder), sodium arsenate dibasic heptahydrate (Sigma-Aldrich, powder), dimethylarsinic acid (Supelco, powder), arsenobetaine calibration solution (Reference material No 626, European Commission Bureau of reference BCR) and monosodium acid methane arsonate sesquihydrate (CHEM SERVICE, N-12495-100 MG, Chester) was prepared to identify the peaks of different As species during ion chromatography.

Arsenic speciation was determined by ion chromatography (Thermo Dionex IC5000 Ion Chromatograph) coupled to inductively coupled plasma mass spectrometry (Thermo Scientific iCap Q ICP-MS). The chromatograph system comprised a Dionex IonPac AS7 RFIC analytical column (2 x 250 mm) and a Dionex AG7 guard column. The mobile phase A and B consisted of 20 mM and 200 mM ammonium carbonate in deionised water, respectively. The flow rate was 0.3 mL min^{-1} using 100% mobile phase A when time = 0 mins, followed by a linear change to 100% mobile phase B when time = 15 mins, and finally followed by a linear change to 100% mobile phase A when time = 15.5 mins. The ICP-MS operating conditions were: forward RF power 1550W, nebuliser gas flow 1 L min^{-1} and nebuliser liquid sample flow rate ~0.35 mL min^{-1}. Helium was used as a collision gas at a flow rate of 4.5 mL min^{-1}. Data was acquired at mass 75 amu to monitor As, and the total analysis run time was 15.5 min per sample. As species in the solutions were identified by comparing their retention times with those of standards including inorganic As (As(III) and As(V)), arsenite, dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), tetramethylarsonium iodide (Tetra) and quantified by calibration curves with peak areas. Each individual result for samples, standards and blanks was an average of 3 replicates of 1 minute acquisition time. Detection limit for all As species was 0.001 mg kg^{-1}. The As species concentrations measured in CRM were within 25% of the certified values. The certified and measured values of As species in the CRM are listed in Table 2.4. The CRM did not contain arsenobetaine and tetra.

Table 2.4. Certified values and means (n = 2) of As species concentrations (mg kg^{-1} dry weight) measured in CRM**.**

Arsenic species	Certified value	Measured concentration	
		mean	s.d.
Arsenobetaine		< LOD	
DMA	0.180	0.216	0.003
Tetra		< LOD	
MMA	0.0116	0.014	0.000
Inorganic As	0.092	0.110	0.020

2.2.5. Macrophyte community survey

The macrophyte taxonomic data and maximum macrophyte growing (colonisation) depth for Kinghorn Loch were collected on 18.07.2013 following the method described in Gunn et al. (2010) and used previously to survey the macrophyte community in Loch Leven (Dudley et al. 2012). A wader transect technique often used in combination with the boat transect method (Gunn et al. 2010) was not applied due to the steep shore at Kinghorn Loch. The survey was conducted along three transects on the lake using a boat. Each transect was set from the mid-point of the lake to the northwest, west and southeast lake shore and divided into twenty approximately spaced intervals. At the end of each interval macrophyte samples were obtained using a double headed rake that was dragged along the lake bed within 4 m distance of each sampling point. The rake was made by combining back-to-back two garden rake heads with wire mesh tied to their surfaces (Dudley et al. 2012). A bathyscope was used to supplement the information obtained by using the rake. At each sampling point, all macrophyte species were recorded and the biomass of the vegetation was assessed applying a 0-3 point scale.

Chapter 3 Long term chemical recovery in a shallow freshwater lake (Kinghorn Loch, UK) following 40 years of red mud pollution

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The chapter has been prepared as a manuscript for submission to the journal "Environmental Science & Technology". The co-authors provided 1981-1983 data on red mud leachate and natural waters within Kinghorn Loch (Edwards 1985), the long-term data on 1954-1981 water pH (Edwards 1985) and 1981-2010 lake outlet water chemistry (given by SEPA), and conducted generalised additive model analysis (GAMs). The candidate, as lead author, carried out the remaining data analysis and writing of the paper. The co-authors provided support and guidance on the scope and design of the project and contributed to the editing of the manuscript.

3.1. Introduction

The red mud spill in October 2010 in Ajka, west Hungary, resulted in the release of c.1 million m^3 of red mud waste (Burke et al. 2012) which impacted on a catchment area of 3078 km^2 (Klebercz et al. 2012). Early research following the spill focused on short-term chemical and ecological impacts in aquatic ecosystems (e.g. Mayes et al. 2011; Schöll and Szövényi 2011; Klebercz et al. 2012) and on the potential human health effects associated with airborne red mud particles (Gelencsér et al. 2011). This research indicated that in aquatic ecosystems, with low residency times and well oxygenated surface sediments, red mud constituents including oxyanion forming elements (e.g. arsenic (As), vanadium (V) and chromium (Cr)) were contained within solid phase complexes and represented limited ecological threat. However, in depositional zones, where reducing conditions may be common, the liberation of such contaminants may be expected (Mayes et al. 2011). Little is known about the long-term chemical and ecological implications of red mud pollution with respect to recovery in depositional zones of rivers which have been impacted in Hungary (Mayes et al. 2011). The results of the surveys undertaken in 2013, three years after the Ajka spill, however, were encouraging, showing the rapid removal of much of the spill material from the Torna-Markal system, through either downstream transport and dilution, or remedial efforts (Anton et al. 2014).

Red mud pollution has been associated with multiple ecological pressures. pH values in the Torna Stream and Marcal river system (a tributary of the Danube River) reached 13.1 following pollution (Mayes et al. 2011). Mayes et al. (2011) reported elevated concentrations of aqueous phase V and As and particulate phase As, Cr, V and nickel (Ni) in excess of environmental quality standards within 2 km downstream of the spill site. In addition, As and V accumulation were reported in bed sediments within depositional zones more than 50 km downstream of the Ajka red mud disposal site (Mayes et al. 2011). These metalloids were present in weak acid extractable complexes (Mayes et al. 2011) and, as such, may be considered sensitive to release from the solid to aqueous phases under reducing conditions, more common in depositional zones (Whitmore et al. 2008). In addition, immobilisation of As by sorption to iron oxyhydroxides can be reduced by competition with phosphate for the same binding elements (Wällstedt et al. 2010). Monitoring of rotifera in the River Danube, 120 km downstream from the pollution source, indicated a loss of the planktonic rotifer assemblages, although the exact cause of this impact was not apparent (Schöll and Szövényi 2011). Red mud pollution can also impact on terrestrial ecosystems. For example, Ruyters et al. (2011) reported elevated concentrations of copper (Cu), iron (Fe), Cr and Ni in the shoots of barley grown in a soil medium containing 5% red mud and indicated that crop yield may be reduced by up to 25% as a result of elevated soil sodium (Na) content.

To date, no study has been published documenting the longer term chemical or biological recovery of freshwater ecosystems from red mud pollution. Information about recovery trajectories is required when considering the likely timescales, end point recovery conditions and legacy issues associated with red mud pollution in the aquatic environment. This is most important in ecosystems with higher retention times (i.e. lakes, wetlands and groundwater) where the residence time of pollutants can far exceed those in rivers and streams (Spears et al. 2012; Sharpley et al. 2013).

Here we use 28 years of data from a shallow freshwater lake with a well documented history of red mud pollution to address the following specific objectives: (1) quantify the composition and catchment load of red mud pollutants to Kinghorn Loch during the peak pollution period and (2) quantify recovery trajectories of red mud pollutants during 26 year period. This information was used in conjunction with environmental quality standards to assess the long-term environmental risk associated with these pollutants.

3.2 Methods

3.2.1 Study site

Kinghorn Loch is a small lake of area 11.3 ha, mean depth 4.5 m and maximum depth 12.8 m, situated in Fife in Scotland (56°10′N; 3°11′W). The lake received leachate from a nearby red mud landfill site (Fig. 2.1-2.2, Section 2.1) from 1947 to 1983, when the discharge was diverted away from the loch. Caustic liquor reaching the lake consisted of a solution of NaOH and carbonate, with average pH of 12.1. It contained high concentrations of dissolved Al, As, V, orthophosphate (PO_4-P), sulphate (SO_4-S) and chloride (Cl). Spring water that diluted the leachate before entering the loch provided an input of Ca, Mg and Si, whilst red mud solids, entering the lake sporadically, were responsible for most of the input of Fe (Edwards 1985).

3.2.2 Pollutant retention in Kinghorn Loch

Information about the frequency and nature of the sampling and analyses of the leachate entering the lake, lake inlet and outlet water is outlined in Table 3.1. Sampling and chemical analyses of the incoming leachate and lake water were conducted up to four times per year until 1981 by the Forth River Purification Board (FRPB). The decision to divert and treat the leachate was made by the FRPB in 1978 and was followed by a more detailed study conducted between 4 April 1981 and 24 May 1983, which consisted of weekly to fortnightly measurements of the flow rate of the leachate to the lake and analysis of the chemical composition of the leachate. The north inlet (the only unpolluted native water input; Fig. 2.2, Section 2.1) and the lake outlet were also sampled and analysed on a weekly to fortnightly basis from 1981 until 1983. The methods and results of analyses conducted until 1981 and during the 1981-1983 detailed study were originally cited in Edwards (1985) and are summarised below.

Table 3.1. Summary of leachate and Kinghorn Loch inlet and outlet sampling and analysis conducted until 2010.

Time period	Frequency	Location	Sampling and analyses
- 1981	up to 4 times per year	incoming leachate	pH, chemical composition
1954-1981	up to 4 times per year	lake water	pH
04.04.1981-24.05.1983	weekly to fortnightly	incoming leachate	physico-chemical, major ions, trace elements, TSS, flow rate
04.04.1981-24.05.1983	weekly to fortnightly	north inlet	physico-chemical, major ions, trace elements, TSS, flow rate
04.04.1981-24.05.1983	weekly to fortnightly	lake outlet	physico-chemical , major ions, trace elements, TSS, chlorophyll *a*, flow rate
1981-2010	weekly – 6 times per year	lake outlet	physico-chemical parameters, major ions, trace elements, TSS, chlorophyll *a*

The leachate and water samples were taken at mid-depth in running water in 1 and 10 L polythene or polypropylene bottles for metal and metalloid analysis and 1 L glass bottles for analysis of other determinands. The polythene and glass bottles were cleaned in 50% v/v nitric acid solution and 50% v/v chromic acid solutions, respectively, and rinsed with deionised water prior to sample collection.

Physico-chemical parameter measurements and sample filtration were conducted in a laboratory. pH values and fluoride concentrations were determined using a Radiometer PHM 63 meter and an ion selective electrode with a separate standard calomel reference electrode, respectively. Alkalinity was measured by titrating 100 mL samples against a standardised 0.05 M sulphuric acid solution to pH 4.5, with leachate samples being diluted 1/10 with deionised water prior the titration.

Samples for chlorophyll *a* determination were filtered through 0.45 μm membranes before extraction of pigment in the dark in 90% v/v acetone for 24 h at 4°C. Water samples for the determination of dissolved element concentration other than metals and metalloids were filtered through GF/C filters for Ca, Mg, F, SiO_2 and 0.1 μm filters for Al, Fe. Nitrate, ammonia, chloride, silica, orthophosphate, boron and sulphate were determined using air segmented continuous flow analysis with photometric detection performed on a Technicon AutoAnalyzer II system based on the assay reviewed by the Standing Committee of Analysts (HMSO 1980a). Detection limits for orthophosphate, nitrate, ammonia, chloride, silica, boron and sulphate were 0.01, 0.01, 0.04, 1.0, 0.1, 0.04, 2 mg L^{-1}, respectively. Fluoride concentrations were determined using an ion selective electrode with a separate standard calomel reference electrode, with a limit of detection of 0.01 mg L^{-1}.

Samples for dissolved metal and metalloid analysis were filtered through glass fibre discs and then through 0.1 μm polycarbonate filter membranes. 1 M spectrophotometric grade hydrochloric acid was used to adjust pH to 2-4 in leachate samples for metal determination and 2 cm^3 L^{-1} of hydrochloric acid was added to preserve samples prior to analysis. The samples were evaporated on a hotplate to approximately 80 cm^3 and made up to 100 cm^3 with deionised water, concentrating the samples of leachate and north inlet by 10 and 100 times, respectively. Metal and metalloid concentrations, with the exception of cadmium (Cd) and lead (Pb), were determined using flame atomic absorption spectroscopy performed on Instrumentation Laboratories IL357 equipped with an IL254 autosampler and based on the principles described in a document issued by HMSO (1980b). Limits of detection for As, Al, Ca, Cr, Cu, Fe, Mg, Mn, Ni, K, Na, V and Zn were 0.005, 0.5, 0.005, 0.03, 0.01, 0.01, <0.002, 0.005, 0.02, 0.004, 1.0, 0.3, 0.005 mg L^{-1}, respectively. Cd and Pb were measured by graphite furnace atomic absorption spectrophotometry, with detection limit 0.005 for Cd and 0.5 mg L^{-1} for Pb. Total suspended solids were determined by the removal of suspended matter from samples using vacuum filtration, followed by drying at 105°C and reweighing. Limit of detection was 2.5 mg L^{-1}.

The leachate load to Kinghorn Loch was estimated using mean discharge and determinand concentration data. The flow of the leachate to the loch was determined by measuring the water height above a 'V' notch weir at the dam of the leachate collection and settlement pond (Fig. 2.1-2.2, Section 2.1). The flow rate of the north inlet and below lake outlet were assessed by reference to gauge boards. Daily loads for the leachate and north inlet were extrapolated to provide an estimate of the annual load to the loch. The methods of the leachate and North inlet loads, and the outlet flow measurements are described in Edwards (1985).

The leachate and North inlet determinand concentration data from the period 1981 to 1983, combined with the flow rates, were used to determine the contaminant concentrations in mixed water in the loch (since the only source of As and V input was assumed to be the leachate). These values were compared with the concentrations measured in the lake outlet water to calculate the losses occurring within the lake body and subsequently the retention time of the contaminants.

3.2.3 Long-term chemical recovery of Kinghorn Loch

Loch pH data were available from monitoring conducted by FRPB and BA Chemicals Ltd. (pre-1981 period) and analysis of Kinghorn Loch outlet water samples 1981-2009 was conducted by the Scottish Environment Protection Agency (SEPA) and the FRPB, its predecessor. It was assumed that the results from the Kinghorn Loch outlet represented surface water conditions in the lake. A summary of the methods used for the analyses of the lake water outlet samples has been provided by SEPA (Tim Foster, *pers. comm.*, 2012-2015) and is given below.

Metal and metalloid concentrations were determined using graphite furnace or flame AAS, which was replaced by ICP-MS (As, V) in 1997 and ICP-OES (Ca, Mg, Fe, Na, K, Al) in 2001 based on HMSO (1996). Irrespective of the instrumentation or analysis methods the validation of measured concentrations would satisfy the same rigorous criteria, with the data being subjected to SEPA's internal quality procedures (*pers. comm.*). ICP-MS analysis was conducted on a Perkin Elmer Elan ICP-MS discrete analyser and, most recently, on a Nexion 300D instrument. Prior to analyses all samples were preserved by adding 1% v/v concentrated nitric acid. Samples for total metal and metalloid determination were then digested for 16 hours at 80°C (±5°C) in an oven prior to analysis. Blank and Independent Process Control samples were prepared along with the lake water samples. Limits of detection were 0.002 (for V and Al), 0.005 (As), 0.1 (Ca, Fe) and 0.5 mg L^{-1} (Mg, Na, K).

The remaining determinands in water outlet samples were analysed following standard procedures based on HMSO Methods for the Examination of Waters and Associated Materials publications listed in Table 3.2.

Table 3.2. Procedures used by SEPA for the analyses of Kinghorn Loch outlet water during the period 1981 to 2010.

Determinand	**Procedure based on:**
pH, alkalinity as $CaCO_3$, electrical conductivity	HMSO. 1981. The Determination of Alkalinity and Acidity in Water, 1981 - Methods for the Examination of Waters and Associated Materials. London.
Total Suspended Solids	HMSO. 1984. Suspended, Settleable and Total Dissolved Solids in Waters and Effluents, 1980 - Methods for the Examination of Waters and Associated Materials. London.
chlorophyll a	HMSO. 1983. The Determination of Chlorophyll a in Aquatic Environments, 1980. Methods for the Examination of Waters and Associated Materials. London; 13.2 ISO 10260: 1992(E). Water quality - Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration. 9pp. International Organization for Standardization, Genéve
NH_4	HMSO. 1982. Ammonia in Waters, 1981 - Methods for the Examination of Waters and Associated Materials. London.
NO_3-N	HMSO. 1982. London. Oxidised Nitrogen in Waters 1981 - Methods for the Examination of Waters and Associated Materials. London.
PO_4-P	HMSO. 1981. Based on Phosphorous in Waters, Effluents and Sewages, 1980 - Methods for the Examination of Waters and Associated Materials. London.
chloride	HMSO. 1982. Chloride in Waters, Sewage and Effluents 1981 - Methods for the Examination of Waters and Associated Materials. London.
SO_4-S	HMSO. 1989. Based on Sulphate in Waters, Effluents And Solids (2nd Edition) 1988 - Methods for the Examination of Waters and Associated Materials. London.
SiO_2	HMSO. 1981. Silicon in Waters and Effluents 1980 - Method For The Examination Of Waters And Associated Materials. London.

3.2.4 Statistical analysis

A non-parametric Mann-Whitney-Wilcoxon test was used to assess the significance ($P < 0.05$) of the difference between the determinand values in the lake outlet water in 1983 and 2009 using all determinand values recorded during both years. The frequency of sampling and analysis differed between the two years and was between 5 to 53 (depending on the determinand) in 1983 and 12 in 2009, with a similar spread of samples across seasons. Due to lack of measurements of Ca, Mg and Fe concentrations in 1983, data from year 1981 were used for these three determinands.

Recovery in pH and red mud contaminants in lake surface water following the diversion of red mud leachate from the lake inlet in 1983 was defined as the point at which no further decrease in pH values and contaminant concentrations could be detected, as assessed using generalised additive models (GAMs; Wood, 2006), assuming normal errors. Since the aim of the modelling was to identify relationships between the concentrations with year after the diversion of the red mud leachate the following modelling strategy was employed. A Gamma error structure (log link function) was used in all models and an autocorrelation structure of AC1 (auto-regressive model of order 1) on year relative to the diversion of the leachate from loch inlet was applied. The relationship between the response (pH and pollutant concentrations) and the explanatory variable (year relative to the diversion of the pollution) was allowed to be a smooth function instead of restricting relationships to be linear. Due to the use of different smoothers for different seasons for V and the significance of the fixed effect of season in the pH analysis, separate models were fitted for different seasons for these two determinands. Data used in the models covered years 1981 – 2009 (the last two years of the pollution input and the recovery period that followed). Based on the recommendations of Zuur et al. (2009) the responses were not transformed prior to analysis. The changes in the red mud constituent concentrations were compared against relevant ecological quality standards. As there is no standard for PO_4-P or TP for Kinghorn Loch, the Trophic State Index for TP (Carlson et al. 1996) was used to provide ecological context for the lake water P concentrations.

The models were fitted using the GAM function in the mgcv package (1.7-6) (Wood 2011). The statistical analyses were conducted using R version 2.15.1. (R Core Team, 2012) available at http://www.r-project.org.

3.3 Results

3.3.1 Characteristics of red mud leachate entering Kinghorn Loch and loading estimates of red mud contaminants to the lake

The leachate entering the lake was characterised by pH of 12.1, comparable to the pH measured in Torna Creek in Hungary (13.1) a few months after the Ajka spill, and high alkalinity (4125 mg L^{-1}; Table 3.3). It contained very high concentrations of Al (138 mg L^{-1}), V (5.3 mg L^{-1}) and As (3.6 mg L^{-1}), with other trace elements present, i.e. Zn, Cu, Pb, Cr, Cd, at concentrations of 0.01 – 0.11 mg L^{-1}. The red mud leachate was also characterised by high concentrations of phosphate (PO_4-P; 3.0 mg L^{-1}) and TSS (87 mg L^{-1}). Compared to the unpolluted North inlet water, the leachate contained higher concentrations of Fe, K, Cl, F, SO_4-S and Na, with particularly high concentrations of the latter (2007 mg L^{-1}). The North inlet water, however, contained higher concentrations of Ca and Mg. The estimated mean leachate load of Al, As, V and PO_4-P to the lake during the period from January 1981 to May 1983 was 107, 2.3, 3.3 and 2.3 g m^{-2} yr^{-1}, respectively. The annual retention of Al, As, V and PO_4-P was 63.6, 0.6, 0.8 and 1.6 g m^{-2} yr^{-1}, respectively.

Table 3.3. Chemical composition of red mud leachate and natural waters within the Kinghorn Loch catchment taken from Edwards (1985). Values are mean values from monitoring (generally weekly to fortnightly) between 4 April 1981 and 24 May 1983 with the exception of bold values that are from single samples. Filtered samples are shown in brackets with < denoting a value that is lower than the detection limit of analysis. Data from Torna Creek (Hungary) taken from Mayes et al. (2012) for sample collection 1 December 2010. The leachate load to Kinghorn Loch was estimated using mean discharge and concentration data for the determinands during the period 4 January 1981 to 24 May 1983.

	Kinghorn Loch						***Torna Creek***
determinand	Caustic liquor before tipping (mg L^{-1})	Leachate susp. Solids (mg kg^{-1} d.w.)	Leachate (mg L^{-1})	North inlet (mg L^{-1})	Kinghorn Loch outlet (mg L^{-1})	Leachate load to loch (g m^{-2} yr^{-1})	Site K1 (mg L^{-1})
pH	**13.5**		12.1	8.1	9.9		13.1
Ca		**329750**	7.2 (4.0)	51 (43)	11 (3.4)	4.8 (3.1)	1513 (1.3)
Fe		**3300**	0.51 (0.12)	0.19 (0.08)	0.13 (0.06)	0.30 (0.09)	10 (<0.01)
Zn			**0.01**				0.45 (0.05)
Cu			**0.03**				0.39 (0.31)
Pb			**0.03**				0.27 (0.04)
V	**13.0**	**367**	5.3	0.05	0.49	3.3	6.4 (5.7)
Al	**3560**	**7700**	138 (138)	0.38 (0.17)	7.3 (6.6)	107 (1.5)	1228 (659)
TSS			87	6.2	31	66	
Mn			**0.06**				9.9 (<0.001)
Ni			**0.06**				0.27 (0.04)
Cr			**0.11**				0.36 (0.05)
Cd			**0.01**				0.06 (0.05)
As	**26.0**	**713**	3.6	0.01	0.32	2.3	3.9 (3.61)
Na	**(8990)**		(2007)	(21)	(252)	(1514)	(701)
K	**(14.5)**		(26)	(0.67)	(3.7)	(19)	115 (85)
Mg	**2.0**	**5150**	8.2 (0.10)	25 (22)	10 (4.3)	6.1 (0.72)	9.1 (0.001)
Cl	**130**		68	26	38	52	83
SiO_2-Si	**(7.9)**		(7.1)	(4.6)	(1.2)	(5.1)	499 (0.67)
PO_4-P	**(5.6)**		(3.0)	(0.04)	(0.13)	(2.3)	
NO_3-N	**(5.0)**		(1.8)	(7.3)	(1.8)	(1.4)	
F	**1.7**		3.2	0.15	1.5	2.7	
NH_3-N	**(1.0)**		(0.7)	(0.14)	(0.26)	(0.50)	
Alk			4125	133	427	3038	7160
SO_4-S			154	23	44	114	717
B			0.82	0.06	0.13	0.59	1.0 (0.90)

3.3.2 Responses in determinand values following 26 years of recovery

Fig. 3.1 shows variation in pH values in lake water in the period 1954 to 2010. It illustrates the increase of pH until the diversion of the leachate in 1983, with pH reaching 10.5, followed by a rapid decrease in the first few years of the recovery and pH fluctuations within the range around 7.5-9.5 during the remaining recovery period. The values of physico-chemical water parameters, and pollutant and chlorophyll *a* concentrations in lake outlet water were significantly lower in 2009 than in 1983, with the exception of total Ca and Mg, which had significantly higher concentrations in 2009 than in 1983 (Table 3.4, Figs. 3.2-3.3). Among determinands that decreased during the recovery period, concentrations of Cl, SO_4-S, SiO_2 and total K, Al and Na in loch water were > 1 mg L^{-1} in 1983, with the latter having the highest annual mean value (221 ± 36.4 mg L^{-1}) of the elements determined. Concentrations of total Fe, As and V were < 1 mg L^{-1} in 1983 and, together with Al, decreased to < 0.1 mg L^{-1} in 2009. Concentrations of PO_4-P decreased from 0.076 ± 0.045 mg L^{-1} to 0.012 ± 0.011 mg L^{-1}.

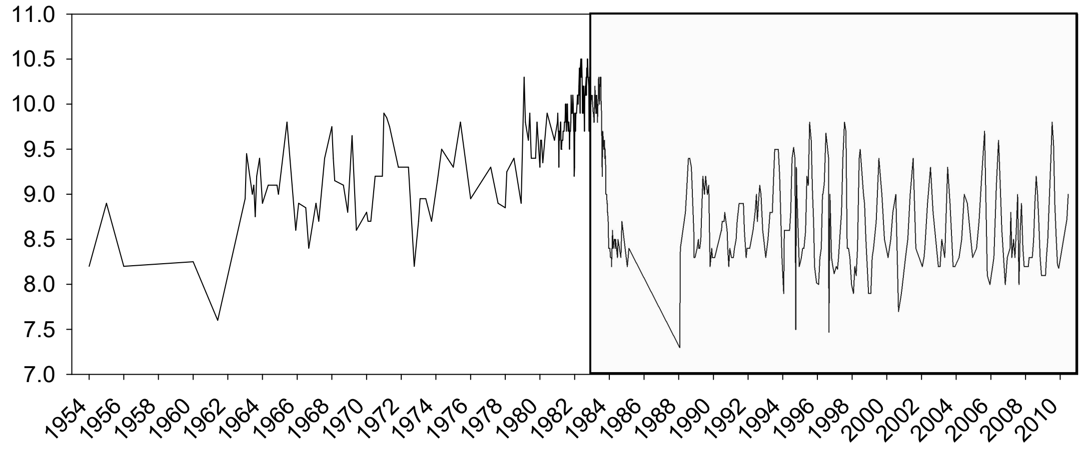

Fig. 3.1. Long-term trends in water pH in Kinghorn Loch. The shaded area represents the period of recovery following diversion of leachate from the lake inlet.

Table 3.4. Results of Mann-Whitney-Wilcoxon tests to assess the significance of the difference between determinand values in lake outlet water in 1983 (prior to diversion of the leachate) and in 2009 (after 26 years of recovery). Due to lack of measurements of Ca, Mg and Fe concentrations in 1983, data from year 1981 (^{a}) were used for these three determinands.

Response variable	1983 (^{a}1981)			2009			Test of difference	P-value
	mean value	S.D.	n_1	mean value	S.D.	n_2		
pH	9.70	0.526	53	8.69	0.625	11	512	< 0.001
Alkalinity as $CaCO_3$ (mg L^{-1})	417	69.1	5	112	22.9	11	55	0.002
Conductivity (µS cm^{-1})	936	148	53	325	49.3	12	636	< 0.001
Total Suspended Solids (mg L^{-1})	32.8	23.2	53	1.44	0.921	12	632	< 0.001
Chlorophyll a (µg L^{-1})	108	94.2	37	3.15	3.53	12	437	< 0.001
(mg L^{-1})								
NH_4-N	0.359	0.405	50	0.036	0.030	12	464.5	0.003
NO_3-N	0.259	0.192	47	0.015	0.011	12	517	< 0.001
PO_4-P	0.076	0.045	47	0.012	0.011	11	507.5	< 0.001
Cl	38.0	6.23	47	28.3	2.788	12	541	< 0.001
Ca^a	7.12	5.05	6	24.5	11.5	12	4	0.001
Mg^a	8.80	3.60	6	15.9	0.686	12	0	<0.001
Fe^a	0.220	0.325	5	0.017	0.010	12	60	< 0.05
SO_4-S	108	23.7	49	21.8	1.25	12	588	< 0.001
SiO_2	2.66	0.738	50	0.858	0.913	12	555.5	< 0.001
Na	221	36.4	49	24.5	0.522	12	588	< 0.001
K	3.12	0.539	49	0.961	0.439	12	579	< 0.001
Al	5.33	4.38	6	0.034	0.018	12	72	< 0.001
As	0.254	0.072	8	0.011	0.001	12	96	< 0.001
V	0.438	0.177	8	0.012	0.002	12	96	< 0.001

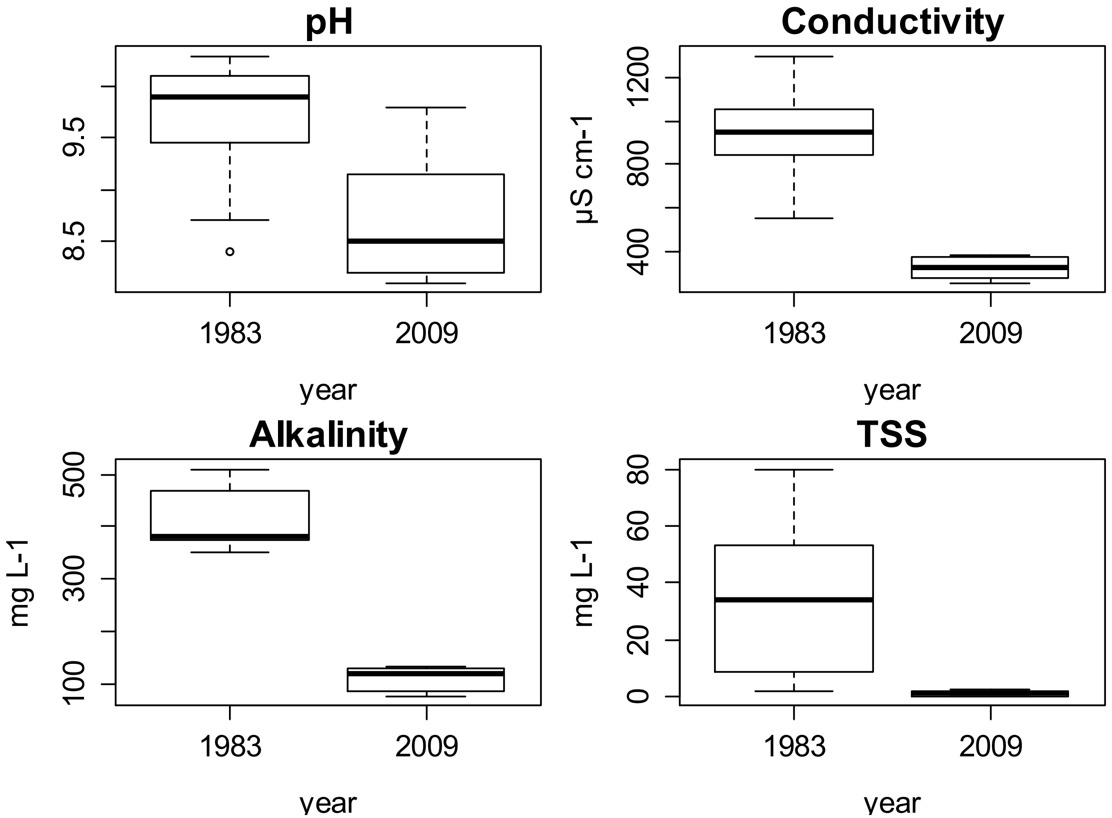

Fig. 3.2. pH, conductivity, alkalinity and TSS in Kinghorn Loch outlet in 1983 (prior to diversion of the leachate) and in 2009 (after 26 years of recovery). Data are summarised as boxplots showing the median and 25th and 75th quartiles, with whiskers representing the values outside the middle 50% and outliers shown as dots.

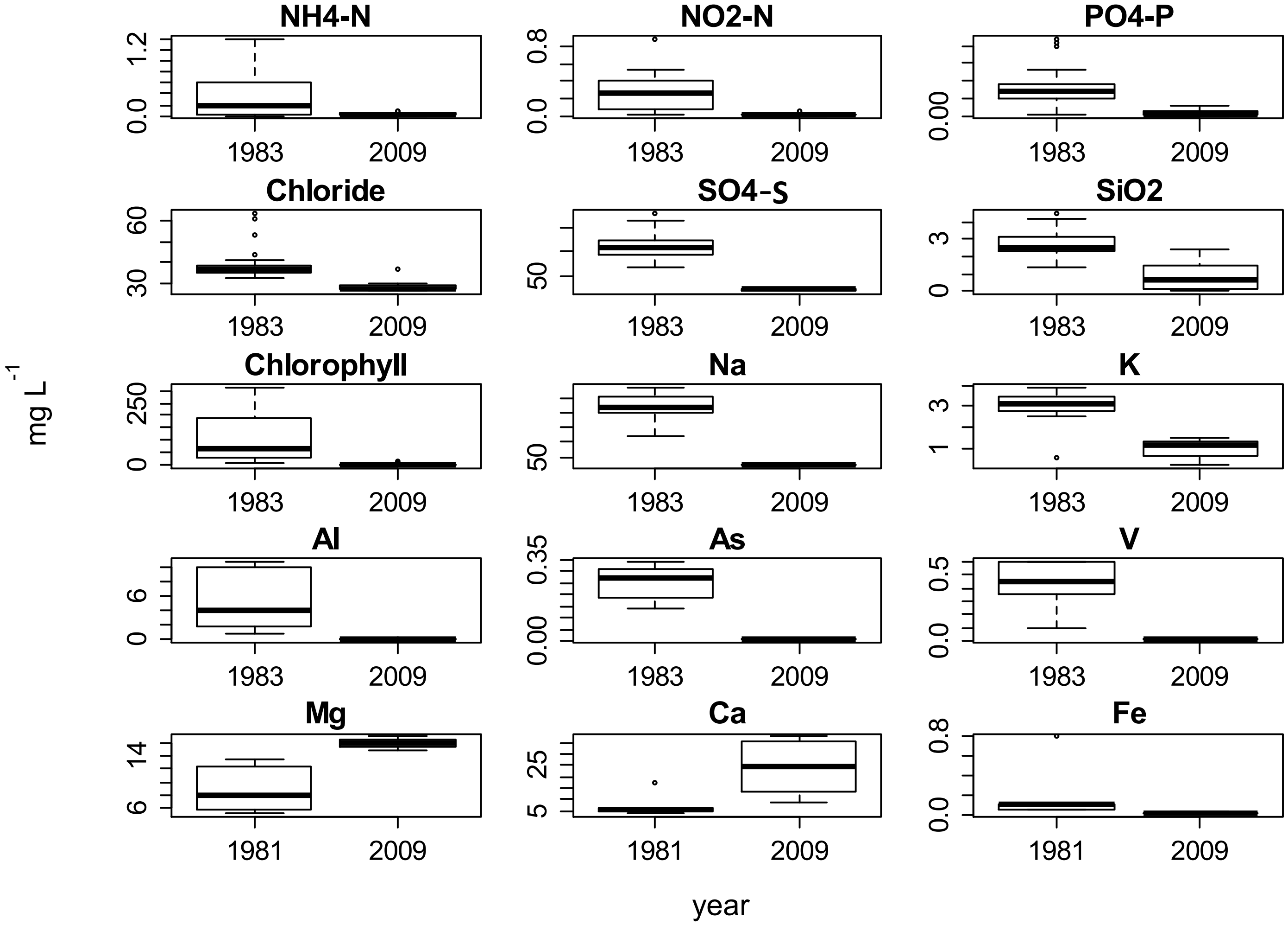

Fig. 3.3. Concentrations of pollutants in Kinghorn Loch outlet in 1983 (prior to diversion of the leachate) and in 2009 (after 26 years of recovery). Data are summarised as boxplots showing the median and 25th and 75th quartiles, with whiskers representing the values outside the middle 50% and outliers shown as dots.

3.3.3 Quantifying recovery trajectories for PO_4-P, total Al, As, V and pH in the lake outflow water

Fig. 3.4 shows changes in the concentrations of total Al, As, V and PO_4-P two years prior to and following the diversion of the leachate until 2010. The period of rapidly decreasing concentrations until 1985 was followed by fluctuating pollutant concentrations within considerably smaller value ranges compared to 1983, with occasional high concentrations. GAM models showed that the recovery times for pollution determinands in Kinghorn Loch outlet water varied depending on the determinand and, in some cases, on the season of measurement. The 2nd order derivatives of the GAM model indicated that there was no further significant decrease in PO_4-P concentration in Kinghorn Loch outlet water 24 years after leachate diversion (Table 3.5), i.e. after 2007. P concentrations were below the suggested Trophic State Index value of for TP 0.024 mg L^{-1} TP (Carlson et al. 1996) by 1986. For Al, the significant decrease in concentrations was maintained until the end of the observation period in 2009 when the Al concentration still exceeded the ecological quality standard of 0.015 mg L^{-1} (SEPA Supporting Guidance 2013). It took 22 years for As to stop significantly changing, with concentrations falling below the water quality guidelines of 0.050 mg L^{-1} (UK Standards for Protection of Aquatic Life; EEC 1976) in 2006. Depending on the season, it took from 18 to 26 years for the V concentrations to stop significantly decreasing, with the shortest recovery period observed for autumn and the longest for spring and winter concentrations. Concentrations measured in autumn were the first to decrease below the water quality guideline of 0.020 mg L^{-1} for total V in 2001 (UK Standards for Protection of Aquatic Life; EEC 1976) and spring concentrations the last, only falling below the guideline value in 2008. In contrast GAM models indicated that there was no further significant decrease in pH values in all seasons 5 years after the beginning of the observation period.

Table 3.5. Summary of generalised additive modelling (GAM) results for red mud contaminant recovery trajectories in Kinghorn Loch outlet water. Optimal models for V and pH predicted different recovery times for different seasons due to the use of separate smoothers for each season in the V model and the significance of the fixed effect of season in the pH model.

Response variable	r^2 adj	n	P-value	Deviance (%)	Recovery time (years)	End point value (mg L^{-1})	Season
PO_4-P	0.68	231	<0.001	60.8	24	0.004	-
Al	0.70	146	<0.001	87.5	26	0.020	-
As	0.79	192	<0.001	-	22	16.0	-
V	0.53	190	<0.001	83.2			-
					20	16.5	summer
					18	17.6	autumn
					26	10.3	winter
					26	19.2	spring
pH	0.58	237	<0.001	60.3			
					5	9.05	summer
					5	8.57	autumn
					5	8.28	winter
					5	8.63	spring

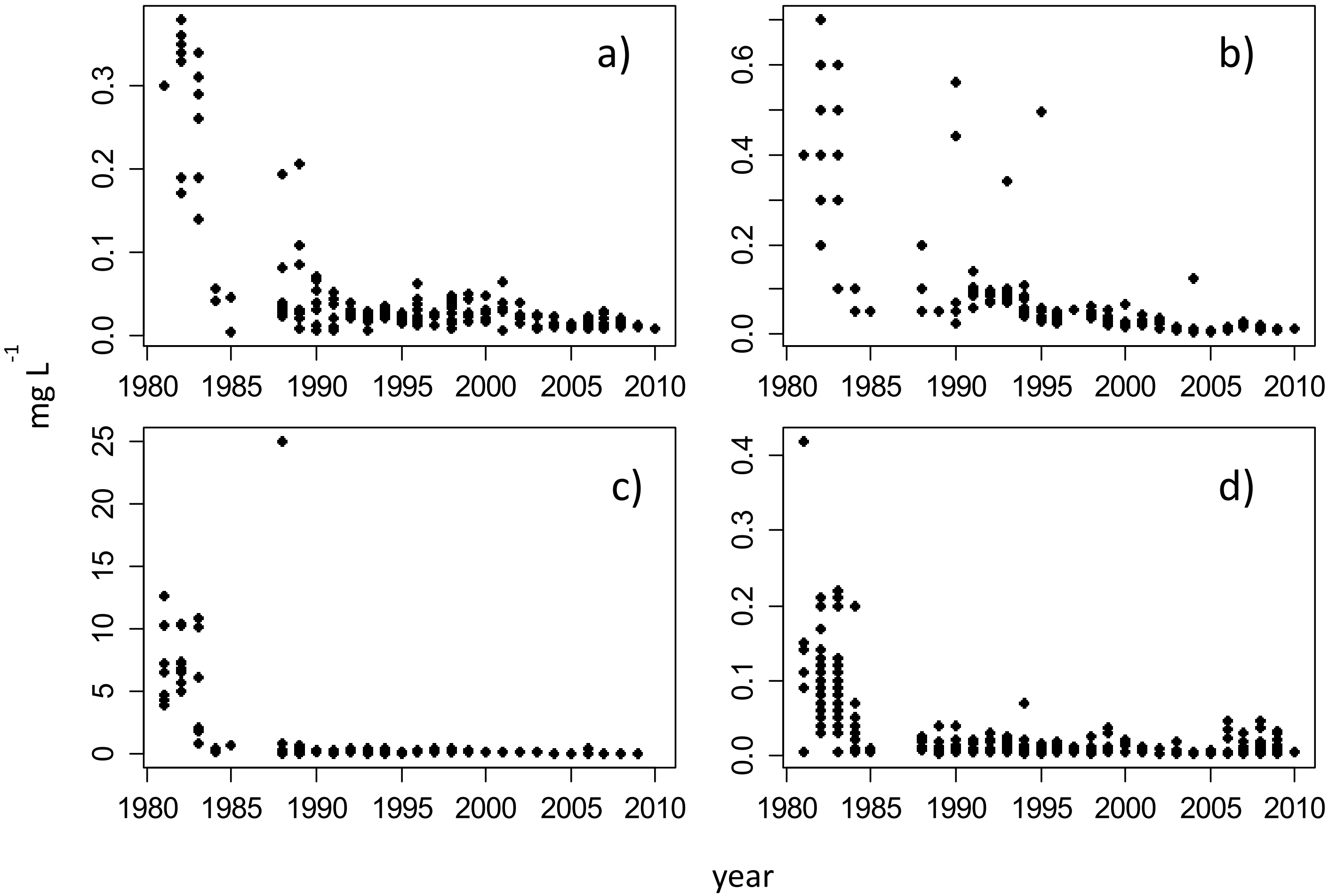

Fig. 3.4. Total As (a), V (b), Al (c) and PO_4-P (d) concentrations in Kinghorn Loch outlet prior to (1981-1983) and following (1984-2010) the diversion of the leachate from the loch inlet in 1983.

3.4 Discussion

3.4.1 Leachate impact on the lake chemistry prior to diversion of the red mud pollution

The composition of the red mud leachate, which entered Kinghorn Loch between 1947 and 1983, indicated four potential pressures that can affect the aquatic environment: high pH, metal and metalloid pollution, eutrophication and salinisation. Before the diversion of the leachate in 1981-1983, the mean pH of Kinghorn Loch was very high at 9.9. The high loadings of contaminants entering the lake (e.g. 107, 2.3, 3.3 and 2.3 g m^{-2} yr^{-1} of Al, As, V and PO_4-P, respectively), combined with the accumulated concentrations of several decades of leachate input, led to very high concentrations of a range of metals (e.g. Al - 7.30 mg L^{-1}, V – 0.49 mg L^{-1}), metalloids (e.g. As – 0.32 mg L^{-1}), PO_4-P (0.13 mg L^{-1}) and Cl (38.0 mg L^{-1}) in the loch water. The concentrations of some pollutants in the water column in 1983 greatly exceeded ecological quality standards for freshwaters. The concentrations of PO_4-P and As were approximately 3 and 5 times higher than the guidelines, whilst V and Al reached concentrations 22 and 355 times higher, respectively, than the recommended ecological quality values. The pressures in Kinghorn Loch, with the exception of eutrophication, were also observed in the river system downstream of the Ajka red mud spill in Hungary in 2010 (Mayes et al. 2011; Ruyters et al. 2011; Shöll and Szövényi 2011).

3.4.2 Processes controlling recovery times from red mud pollution of aquatic systems

26 years after the diversion of the red mud leachate pollution from Kinghorn Loch concentrations of all other determinands were significantly lower than in 1983, with the exception of Mg and Ca, suggesting overall chemical recovery of the lake water. Mg and Ca concentrations were significantly higher in 2009 than in 1983 (15.9 and 24.5 mg L^{-1} in 2009, respectively, compared to 8.80 and 7.12 mg L^{-1} in 1983). This result cannot be interpreted as evidence of a lack of recovery of the lake from red mud pollution, as the major input of these two elements did not come from the

leachate, but from the unpolluted north inlet. The significantly lower concentrations of Mg and Ca in lake outlet water in 1983 were likely to be a result of removal of any free Ca^{2+} and Mg^{2+} by reaction with the high carbonate alkalinity coming from the leachate and forming solid carbonates in the elevated pH conditions (Renforth et al. 2009). Such buffering mechanism occurred in the river system polluted after the Ajka accident when gypsum was added as a remediation measure to provide Ca^{2+} (Renforth et al. 2012). This prompted precipitation of calcium carbonate (Equation 3.1; Renforth et al. 2012), which consumes alkalinity and decreases pH, and decreased contaminant mobility due to metal(loid) scavenging by gypsum and ne-formed carbonate deposits (Renforth et al. 2012).

$$2OH^{-}_{(aq)} + CaSO_4\,2H_2O_{(s)} + 2CO_2 \leftrightarrow CaCO_{3(s)} + SO_4^{2-}{}_{(aq)} + 2H_2O_{(l)} \qquad (3.1)$$
$$+\, H_2CO_{3(aq)} \quad \Delta Gr = -692.5kJ/mol$$

The results of the GAM analyses indicated that the processes of chemical recovery in Kinghorn Loch were gradual and took up to three decades to complete. The time scale of the recovery differed among the determinands. The shortest recovery period (5 years) was observed for pH. In contrast, analysis of the river system affected by the Ajka red mud spill indicated rapid recovery in pH only 2 months after the pollution accident at sites further downstream from the pollution source (Mayes at al. 2011). The fast improvement in pH in this case was, however, a result of both acid dosing and dilution of the contaminated water in the river system (Mayes et al. 2011).

Recovery from high concentrations of contaminants in Kinghorn Loch water was much longer than from the elevated pH. The PO_4-P, As and V recovery times ranged from 18 to 26 years, with the concentrations of all three elements meeting the ecological quality standards only in 2009. Al continued to significantly decrease during the whole observation period, yet concentrations did not reach the ecological quality standard level even after 26 years. The latter results can be explained by much higher concentrations of Al entering the lake in the leachate compared to the other three pollutants. One of the reasons for the observed long chemical recovery period could be the accumulation of high pollutant loads from the leachate in Kinghorn Loch bed sediments (e.g. the mean annual retention of Al, As, V and PO_4-

P during 1981-1983 was 63.6, 0.6, 0.8 and 1.6 g m^{-2} yr^{-1}, respectively). After the cessation of external pollutant loading, sediment can act as an internal source of pollution, gradually releasing the accumulated contaminants under favourable physico-chemical conditions and delaying the processes of ecosystem recovery, as has been well documented in the case of P (Jeppesen et al. 2007; Søndergaard et al. 2003, Spears et al. 2012). Post-depositional remobilisation and internal cycling of pollutants have also been observed in a number of previous studies investigating the behaviour of As (Toevs et al. 2008; Whitmore et al. 2008) and V in water (Minelli et al. 2000). Decline in the release of P from lake bed sediments over decadal periods was positively correlated with decreasing P concentrations in the water column in another shallow lake - Loch Leven, Scotland (Spears et al. 2012). In addition, in Kinghorn Loch, immobilisation of P, As and V in sediment can be influenced by the competition between these elements for adsorption to iron oxyhydroxides (Wällstedt et al. 2010). High pH can also have negative effects on immobilisation of some elements, e.g. P (Søndergaard et al. 1999) and V (Naaem et al. 2007) and might therefore also account for longer recovery times. The likely impact of the elevated pH in Kinghorn Loch during the first years following the diversion of the leachate, as well as the potential competition for sorption sites between some red mud constituents, indicate that the interactions between the pressures might be another factor increasing the time of chemical recovery. The estimated residence time of Kinghorn Loch is eight months (Edwards 1985) and this is another likely reason for the long recovery time, as the higher hydraulic retention time in lakes than in fluvial systems, results in longer chemical and ecological recovery periods (Spears et al. 2012).

Mayes et al. (2011) reported the rapid dilution of sediment-bound pollutants, with the exception of depositional hotspots, in the river system affected by the Ajka accident. This result indicated the preferential transport out of the system of the contaminated material (Mayes et al. 2011), which may happen over much longer periods in lakes. Good ecological condition in the Torna Stream polluted in the Hungarian accident is expected to be achieved by 2021, some 11 years after the pollution event (Kovács et al. 2012). Our results indicate that this recovery time may be significantly longer in depositional zones with long hydraulic retention times.

Conclusions

This study showed a significant decrease of pH and the concentrations of all red mud constituents in Kinghorn Loch water after red mud leachate was diverted from the lake inflow waters. The chemical recovery period for four contaminants ranged from 22 to 26 years, depending on the determinand, and 5 years for pH. Al had not reached the end-point of recovery after 26 years. Fluctuating concentrations of the four contaminants (PO_4-P, V, As, Al) during, but also after the recovery period, indicate the persistence of internal cycling processes in maintaining water column concentrations of red mud constituents in Kinghorn Loch for more than 25 years following the control of red mud leachate inputs.

Chapter 4 A one year assessment of spatio-seasonal variation in distribution of red mud constituents in a shallow lake (Kinghorn Loch), following red mud pollution

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The chapter has been prepared as a manuscript for submission to the journal “Water Research”. The candidate, as lead author, performed the sample collection, and was involved in laboratory analysis of metal and metalloid content in water, and major and trace elements in sediment. The laboratory analysis of phosphorus content in water, data analysis and writing of the paper was carried out by the candidate. The co-authors provided support and guidance on the scope and design of the project, and specialist laboratory and data analysis related to the ICP-MS and XRF methods. The co-authors contributed to the editing of the manuscript.

4.1 Introduction

The failure of the red mud containment reservoir of an alumina plant in Ajka, western Hungary, in October 2010 led to the release of c.1 million m^3 of caustic, metal-rich waste (Burke et al. 2012), which contaminated the Marcal river catchment (area of 3078 km^2) and reached the Danube River (Klebercz et al. 2012). Research that followed the Ajka spill focused on the short-term effects of red mud pollution, in particular on the chemical and ecological impact on freshwaters, ecotoxicity of the contaminated fluvial sediment and soil, and the potential risk to human health associated with inhalation of red mud dust particles (e.g. Mayes et al. 2011; Ruyters et al. 2011; Schöll and Szövényi 2011; Klebercz et al. 2012; Mišík et al. 2014).

Red mud can introduce to the environment high concentrations of potentially toxic metals and metalloids, such as arsenic (As), cobalt (Co), chromium (Cr), nickel (Ni), and vanadium (V) (Ruyters et al. 2011; Klebercz et al. 2012) and phosphorus (P; Edwards 1985). Once the contaminants enter the freshwater environment they can persist, reaching concentrations that can be harmful to aquatic organisms and humans (Gupta et al. 2009). Elevated concentrations of aqueous phase As and V and particulate phase As, V, Cr and Ni were reported in river systems within 2 km of the Ajka spill site (Mayes et al. 2011). Considering the large volume of red mud waste that entered the fluvial system, the number of sites at which pollution was detected was relatively small. However toxic effects on aquatic biota were reported at red mud depositional hotspots with elevated concentrations of metals and metalloids downstream of the spill site (Klebercz et al. 2012).

Little is known about the long-term fate and behaviour of red mud constituents in freshwaters and the time scale of recovery of the affected water bodies. Once the input of pollution ceases, pollutants can remain in the system, particularly in stagnant areas of rivers, where water exchange is restricted (Ruhl et al. 2010) and in bed sediments of aquatic ecosystems with higher retention times than in fluvial systems, such as lakes (Spears et al. 2012, Sharpley et al. 2013). Previous studies showed that contaminated sediment can become a source of internal pollution, delaying the time of ecosystem recovery (Spears et al. 2012). Red mud pollutants, such as As, V and P that form oxyanions can cycle between bed sediment and the overlying water

(Minelli et al. 2000; Søndergaard et al. 2003; Whitmore et al. 2008). Mobility of oxyanion forming contaminants in the aquatic environment can be driven by a number of environmental factors, such as redox potential, pH and presence of pollutant-binding elements (Søndergaard et al. 1999; Bissen and Frimmel 2003; Wang and Wilhelmy 2009). Sorption of arsenite (As(III)), arsenate (As(V)) and P to absorbents such as oxyhydroxides of iron (Fe(III)), manganese (Mn(III/IV)) and aluminium (Al(III)) under oxic conditions limits upward diffusion of soluble As from sediments (Bissen and Frimmel 2003; Toevs et al. 2008). Dissolution of metal oxyhydroxides under reducing conditions can lead to the release of soluble As and phosphate (PO_4-P) into overlying water (Smolders et al. 2006; Whitmore et al. 2008). P can also be adsorbed to other inorganic components, such as Ca and clay (Søndergaard et al. 2003). Similar to P and As, V can also be bound to Fe oxides (Wällstedt et al. 2010). These processes of pollutant release from bed sediment and their distribution in water are sensitive to seasonal and spatial fluctuations of physico-chemical and ecological factors that drive their mobility in the aquatic environment (Kuhn and Sigg 1993; Sohrin et al. 1997; Spears et al. 2007; Spears et al. 2012).

The present study aims to investigate the seasonal and spatial variation of red mud constituents in the water column and bed sediment of a shallow lake 30 years following the control of red mud pollution. The focus of this and following chapters is on the three constituents (As, V and P), whose concentrations, with the exception of Al, were the highest among contaminants in the red mud leachate that entered Kinghorn Loch and which are mobile in aquatic environment. Aluminium is less mobile due to precipitation with silicon (Si), magnesium (Mg) (Edwards 1985) and Ca (Renforth et al. 2012), which are present in relatively high concentrations in the lake north inlet. Therefore, despite large Al input loads during the pollution period, the temporal and spatial changes in concentrations of this element were not investigated. Other contaminants, such as Cr, Cd and Pb, occurred in the leachate in lower concentrations than As, V and P (Section 3.3.1) and, due to the time limitation of this study, were not investigated further. The following hypotheses were tested: (1) concentrations of red mud constituents (As, V and P) in the lake water column vary spatially and across seasons, (2) concentrations of red mud constituents in lake sediment vary with the overlying water depth and across seasons, (3) pollutant concentrations in lake sediment have decreased over time since the diversion of the pollution.

4.2 Methods

4.2.1 Site description

Kinghorn Loch is a small (surface area 11.3 ha), shallow (mean depth 4.5 m, maximum depth 12.8 m) lake situated in Fife, Scotland (56°10′N; 3°11′W). The lake received leachate from a nearby red mud landfill site operated by an alumina production plant from 1947 to 1983 when the discharge was diverted. The liquor entering the lake consisted of a alkaline solution of NaOH and carbonate, with mean pH of 12.1. It also contained high concentrations of dissolved Al, As, V, orthophosphate, sulphate and chloride, whilst red mud solids, released to the loch sporadically, accounted for most of the input of Fe (Edwards 1985). A long-term monitoring programme of the lake water chemistry measured in the lake outlet (Fig. 4.1) was initiated in 1980 by the Forth River Purification Board and continued by the Scottish Environment Protection Agency (SEPA).

4.2.2 Water and sediment sample collection

Six sites were selected on a depth transect across Kinghorn Loch to allow the assessment of red mud constituent concentrations in lake water and bed sediment (Table 4.1, Fig. 4.1). Sampling of lake water and sediment was conducted from a boat at each site once a month from May 2012 to April 2013. Single sediment cores were collected at each site on each sampling date using a HTH gravity corer (core internal diameter 65 mm, length 500 mm; Pylonex, Umeå, Sweden). The cores were sectioned into 2 cm slices representing depths from 0-10 cm immediately after collection and stored frozen in polythene bags before being oven dried at 110°C to a constant weight and homogenised. To examine vertical changes of pollutant concentrations in water throughout a year, single 500 mL samples of lake surface water, bottom water 0.5 m above the sediment surface (sediment + 0.05 m water) and 50 mL samples of water 1 cm above the sediment surface (sediment + 0.01 m water;

collected from sediment cores) were taken on each sample occasion. Polypropylene acid washed bottles and sterile polypropylene centrifuge tubes were used for the collection of 500 and 50 mL samples, respectively. Each water sample was divided into four subsamples due to the different sample preparation and storage requirements of the analysis methods for various groups of elements. Dissolved oxygen (DO) concentration, pH, conductivity and water temperature were measured *in situ* in surface water and in water above the sediment surface (in a sediment core) at each of the six sample collection sites and on each occasion using a Hach HQ40d Portable Multi-Parameter Meter and probes (Hach Lange, Salford, UK). In addition, on each sampling date, depth profiles (from 0 to 11 m) of DO, pH, conductivity and temperature were recorded *in situ* at every 1 m water depth increment at the deepest site (12 m water depth). May and January pH depth profile measurements and February depth profile, surface water and water above sediment measurement of DO were not taken due to the failure of measuring probes.

Table 4.1. Site depth and National Grid Reference.

Site	Depth (m)	NGR
1	2	NT 25756 87422
2	6	NT 25844 87346
3	10	NT 25887 87310
4	12	NT 25921 87272
5	8	NT 25980 87229
6	4	NT 26012 87203

4.2.3 Water sample analyses

Water was analysed for redox sensitive red mud constituents (PO_4-P, total P (TP), dissolved and total As and V) and elements with pollutant binding capacity (dissolved and total Fe, Mn, Al, Ca). PO_4-P and TP concentrations in the water samples were determined according to the acid-molybdenum-blue colorimetric method of Murphy and Riley (1962). Samples for PO_4-P analysis were filtered (Whatman GF/F; pore size 0.7 µm) on the day of sampling and stored frozen, together with unfiltered samples for TP analysis, at -18°C. Prior to analysis, 5 mL of each unfiltered sample was digested using potassium persulfate ($K_2S_2O_8$) acid hydrolysis digestion following the procedure outlined by Eisenreich et al. (1975).

Samples for the determination of dissolved metals and metalloids were filtered through a Millex syringe-driven 0.45 µm filter prior to preservation with 1% v/v nitric acid. Samples for total metal and metalloid determination were preserved with 1% v/v nitric acid, followed by 16 h digestion at 80°C in an oven prior to analysis. Concentrations of metals and metalloids were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using an Agilent 7500ce. Detailed procedures of P and metal and metalloid determination are provided in Section 2.2.1 and 2.2.2, respectively.

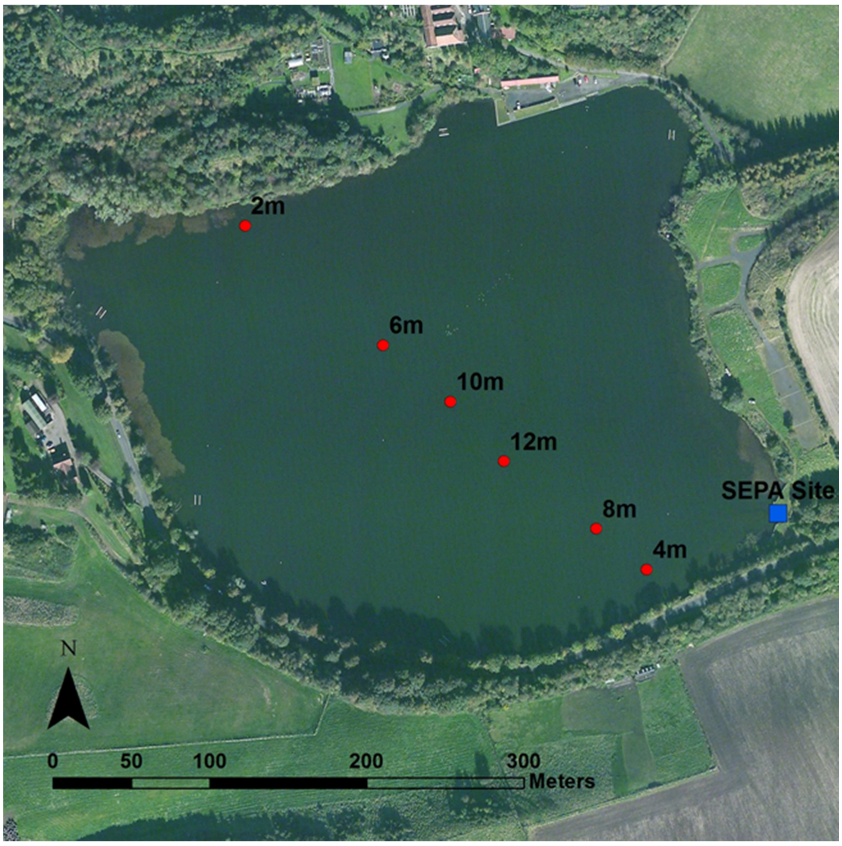

Fig. 4.1. Water and sediment sampling sites at Kinghorn Loch (ESRI ArcMap 10.1, image date 5.4.2011).

4.2.4 Sediment analyses

The content of major and trace elements in lake sediment samples was determined using X-ray Fluorescence (XRF). The upper two sediment samples (0-4 cm), which contained a higher proportion of water compared to deeper layers of sediment, were combined together for each core to provide sufficient dry material for analysis. 0-4 cm slices from all twelve sampling dates and 4-6, 6-8 and 8-10 cm samples from four mid-season months (Jul 2012, Oct 2012, Jan 2013 and Apr 2013) were analysed. Oven-dried sediment samples were processed to form fused glass discs and pressed pellets for analyses of major and minor elements, respectively, following the methods outlined by Fitton at al. (1998). The element concentrations were determined using a Philips PW2404 X-ray Fluorescence spectrometer employing a Rh-anode primary X-ray tube. A full description of the procedure is outlined in Section 2.2.3.

4.2.5 Statistical analyses

Monthly observations (n = 12) from each water depth and surface layer of sediment (0-4 cm) were allocated to seasons (winter: Dec 2012 – Feb 2013, spring: Mar – May 2013, summer: Jun – Aug 2012, autumn: Sep – Nov 2012), whilst quarterly observations from the sediment depth profiles were presented as annual means for each sampling site. Two-way analysis of variance (ANOVA, total degrees of freedom = 71, $\alpha = 0.05$) was used to assess the variation in pollutant concentrations (P, As and V) in water and surface sediment between the four seasons and six sampling sites (six overlying water depths), with the interaction between season and overlying water depth included in the models. Plots of residuals versus fitted values and theoretical quantiles versus standardized residuals (Q-Q plots) were used to examine homogeneity of variance and the normality assumption, respectively. Several response variables (dissolved and total As in surface water, sediment + 0.5m water and sediment + 0.01 m water, PO_4-P and TP in sediment + 0.5m water and sediment + 0.01 m water, and dissolved and total V in sediment + 0.01 m water) were $\log_{10}$ transformed to meet homogeneity and normality assumptions, which in the case of PO_4-P in bottom water and water above the sediment required adding a constant to remove negative values.

Multiple regression following backward regression selection of variables was applied to assess the relationship between pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V) and potential explanatory variables in both surface water and water above sediment. Explanatory variables included in the analyses were pH, DO, conductivity and temperature, concentrations of dissolved or total forms of pollutant-binding elements in water (depending on the form of response variable), pollutant-binding elements in sediment (in sediment + 0.01 m water models) and sediment P, As and V (for models with P, As and V as response variables, respectively). The explanatory variables were tested for collinearity using pairwise scatter-plots, and pairwise correlation and variance inflation factor (VIF) calculations, with collinear covariates being omitted from the analyses (following the procedure described in Zuur et al. 2010). Homogeneity of variance and the normality assumption were examined by plotting residuals versus fitted values and theoretical quantiles versus standardized residuals (Q-Q plots), respectively, and $\log_{10}$ transformation was applied to PO_4-P, TP and dissolved and total V in water above sediment to meet the assumptions. Only significant relationships ($P < 0.05$) were included in the final multiple regression models.

Spearman's correlation coefficients were calculated to evaluate the relationships between concentration of dissolved As, dissolved V, PO_4-P, total As, total V and TP in surface water and water overlying sediment, with $P < 0.05$ being taken as statistically significant. Statistical software R version 3.1.3 (http://www.r-project.org, R Core Team, 2013) was used for conducting the statistical analyses.

4.3 Results

4.3.1 Water column physico-chemical parameters

The water column in Kinghorn Loch was generally well mixed throughout the year, with the exception of the three summer months, when a decrease in temperature, DO and pH, and an increase in conductivity with increasing water depth were observed (Fig 4.2). Stratification was observed during two summer months, with anoxic conditions occurring from 5 m and 4 m below the water surface at the deepest sampled site in July and August, respectively.

The lowest mean DO concentrations occurred at all sites in autumn and the highest in spring, with small differences in DO among the sites in both seasons (Table 4.2). The highest pH and temperature values were measured at each site in summer. Surface water mean conductivity was very similar at all sites in each season, with the lowest seasonal mean value recorded in summer and the highest seasonal conductivity in spring. Seasonal mean DO concentrations in sediment + 0.01 m water ranged considerably from 1.19 ± 0.63 mg L^{-1} (summer, 10 m water depth) to 13.1 ± 2.04 mg L^{-1} (spring, 2 m water depth; Table 4.3). Summer was the season characterised by the greatest difference in DO among sites, with mean DO concentrations at the shallowest site being at least twice as high as mean DO concentrations at the other five deeper sites. Similarly to DO, the lowest seasonal mean pH in sediment water was recorded at all sites (apart from site 1, 2 m) in summer and the highest in spring. At all sites the lowest seasonal mean temperature in sediment + 0.01 m water occurred in winter and the highest in summer.

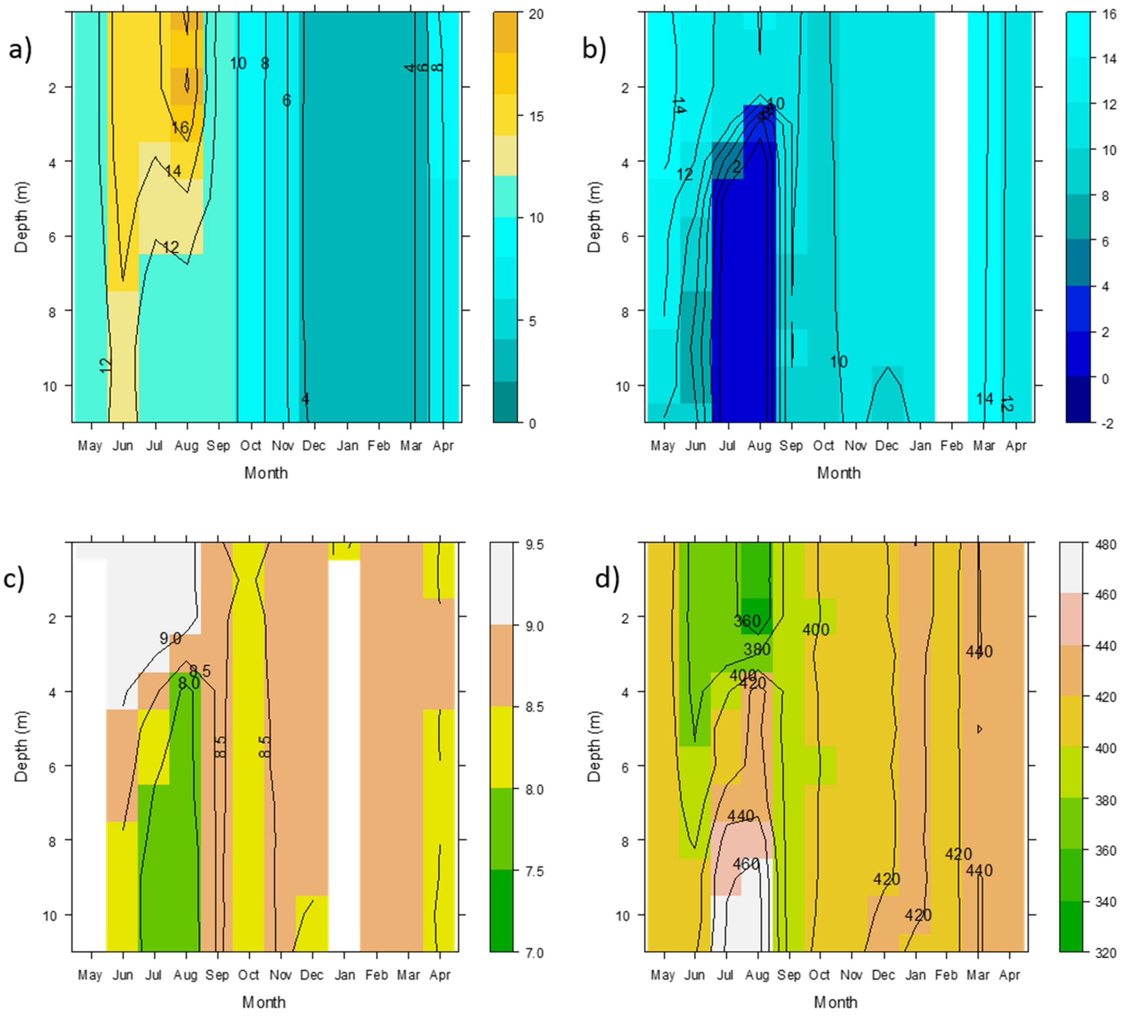

Fig. 4.2. Depth profiles (0-11 m) at the deepest site (site 4) at Kinghorn Loch from May 2012 to April 2013 of (a) temperature (^{o}C), (b) DO (mg L^{-1}), (c) pH and (d) conductivity (μS cm^{-1}).

Table 4.2. Means and standard deviations of the means of pH, conductivity (µS cm^{-1}), temperature (^{o}C) and DO (mg L^{-1}) across seasons and at 6 sites in surface water in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

		Site											
	Season	**1 (2 m)**		**2 (6 m)**		**3 (10 m)**		**4 (12 m)**		**5 (8 m)**		**6 (4 m)**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
pH	Winter	8.06	0.19	8.58	0.05	8.49	0.16	8.57	0.12	8.66	0.17	8.56	0.11
	Spring	8.67	0.64	8.76	0.34	8.88	0.53	8.84	0.35	8.97	0.40	8.90	0.46
	Summer	9.03	0.17	9.07	0.10	9.11	0.15	9.14	0.03	9.02	0.15	9.11	0.10
	Autumn	8.36	0.23	8.36	0.16	8.50	0.14	8.47	0.36	8.55	0.15	8.50	0.17
conductivity	Winter	418	9.02	418	11.5	417	9.07	421	17.7	417	9.02	418	10.2
	Spring	430	15.0	430	16.8	430	16.5	431	13.7	429	17.2	429	17.7
	Summer	361	18.0	361	18.0	361	18.2	360	16.5	359	18.3	359	19.4
	Autumn	403	7.02	401	6.51	401	5.51	399	7.21	399	5.57	400	5.00
temperature	Winter	2.87	0.81	3.00	0.78	3.03	0.84	2.87	0.55	3.00	0.87	2.77	0.47
	Spring	7.47	3.73	7.43	3.74	7.50	3.76	7.77	3.90	7.67	3.83	7.70	3.84
	Summer	16.2	1.72	16.2	1.57	16.3	1.65	16.6	1.42	16.5	1.66	16.3	1.80
	Autumn	8.87	2.26	8.97	2.30	8.97	2.26	9.03	2.35	9.17	2.25	9.10	2.31
DO	Winter	12.1	0.78	11.4	0.52	11.4	0.30	11.4	0.18	11.3	0.43	11.5	0.10
	Spring	13.4	2.17	13.4	2.23	13.4	2.21	13.2	2.07	13.3	2.18	13.4	2.12
	Summer	11.0	1.00	11.5	1.43	11.5	1.63	12.1	0.96	12.1	2.19	11.7	1.61
	Autumn	10.3	1.10	10.2	0.85	10.3	0.98	10.5	1.03	10.7	1.17	10.7	1.13

Table 4.3. Means and standard deviations of the means of pH, conductivity (µS cm^{-1}), temperature (^{o}C) and DO (mg L^{-1}) across seasons and at 6 sites in sediment + 0.01 m water samples in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

		Site											
	Season	**1 (2 m)**		**2 (6 m)**		**3 (10 m)**		**4 (12 m)**		**5 (8 m)**		**6 (4 m)**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
pH	Winter	8.41	0.09	8.33	0.25	8.58	0.12	8.50	0.09	8.61	0.09	8.57	0.06
	Spring	8.97	0.46	8.82	0.32	8.98	0.36	8.82	0.28	8.89	0.28	8.79	0.13
	Summer	8.84	0.14	8.16	0.24	7.78	0.09	7.69	0.11	7.94	0.07	8.10	0.15
	Autumn	8.47	0.17	8.47	0.13	8.53	0.12	8.53	0.11	8.52	0.06	8.24	0.45
conductivity	Winter	422	11.9	421	13.1	420	10.6	432	22.0	422	10.1	420	11.0
	Spring	437	5.51	464	38.4	461	36.4	441	1.41	468	52.6	447	8.96
	Summer	371	14.2	411	11.2	443	14.5	457	10.4	428	1.53	413	25.9
	Autumn	406	4.73	404	5.00	404	5.51	403	5.51	402	4.58	402	7.00
temperature	Winter	2.57	1.15	2.77	0.71	2.73	1.06	3.10	0.82	3.03	0.60	2.93	0.55
	Spring	7.20	3.32	7.27	3.56	7.27	3.54	6.77	3.14	7.20	3.49	7.10	3.70
	Summer	16.4	1.89	14.8	0.44	13.3	0.70	14.0	0.71	14.2	0.36	15.9	1.27
	Autumn	8.90	2.16	9.33	2.41	9.33	2.34	9.43	2.35	9.47	2.32	9.17	1.96
DO	Winter	10.9	0.69	11.3	0.50	11.4	0.32	8.78	3.51	10.4	0.30	11.2	0.26
	Spring	13.1	2.04	11.8	2.10	12.5	1.79	11.4	2.43	11.9	2.06	11.2	2.78
	Summer	8.33	0.70	2.64	2.28	1.19	0.63	1.55	0.78	1.49	0.66	3.26	2.59
	Autumn	9.72	0.93	10.1	0.84	9.86	1.10	10.1	0.91	10.0	0.74	10.0	0.76

4.3.2 Water column pollutant concentration – seasonal and spatial variation

Figure 4.2 shows variations of surface water pollutant concentrations across seasons and at six sites during a year of field observations. Detailed information is provided in Appendix A.1 and A.2. Seasonal mean concentrations of PO_4-P varied from 1.83 ± 6.05 (spring, 2 m water depth) to 16.3 ± 8.11 µg L^{-1} (summer, 8 m water depth). Summer and autumn mean PO_4-P concentrations were at least twice as high as spring PO_4-P concentrations recorded at each site. Seasonal mean concentrations of TP ranged from 16.4 ± 0.95 (winter, 8 m water depth) to 34.4 ± 6.91 µg L^{-1} (summer, 6 m water depth), with the highest mean concentration observed at all sites in summer. Seasonal mean dissolved and total As concentrations in surface water ranged from 6.39 ± 0.34 µg L^{-1} (winter, 2 m water depth) to 14.4 ± 2.85 µg L^{-1} (summer, 8 m water depth) and from 8.38 ± 1.06 µg L^{-1} (winter, 2 m water depth) to 15.2 ± 4.05 µg L^{-1} (summer, 4 m water depth), respectively. At all sites, the highest mean concentrations of As, both dissolved and total forms, occurred in summer, whilst the lowest were observed in winter. Seasonal mean dissolved V concentrations in surface water varied from 7.52 ± 0.65 µg L^{-1} (autumn, 2 m water depth) to 12.7 ± 0.58 µg L^{-1} (spring, 8 m water depth), whilst concentrations of total V ranged between 8.23 ± 1.81 µg L^{-1} (autumn, 2 water depth) and 14.5 ± 0.74 µg L^{-1} (spring, 6 m water depth). The lowest mean concentrations of dissolved and total V were recorded in autumn and the highest occurred in spring for all sites.

Seasonal mean PO_4-P concentrations in sediment + 0.01 m water ranged from 4.17 ± 5.71 µg L^{-1} (spring, 6 m water depth) to 75.9 ± 30.2 µg L^{-1} (summer, 12 m water depth; Fig 4.3, Appendix A.3), whilst concentrations of TP ranged from 29.1 ± 4.39 µg L^{-1} (autumn, 6 water depth) to 208 ± 225 µg L^{-1} (spring, 10 m water depth; Appendix A.4). Seasonal mean concentrations for dissolved and total As in sediment + 0.01 m water varied from 5.96 ± 1.79 µg L^{-1} (winter, 2 water depth) to 23.6 ± 1.87 µg L^{-1} (summer, 10 m water depth) and from 10.1 ± 4.67 µg L^{-1} (winter, 2 m water depth) to 29.8 ± 20.8 µg L^{-1} (spring, 10 m water depth), respectively.

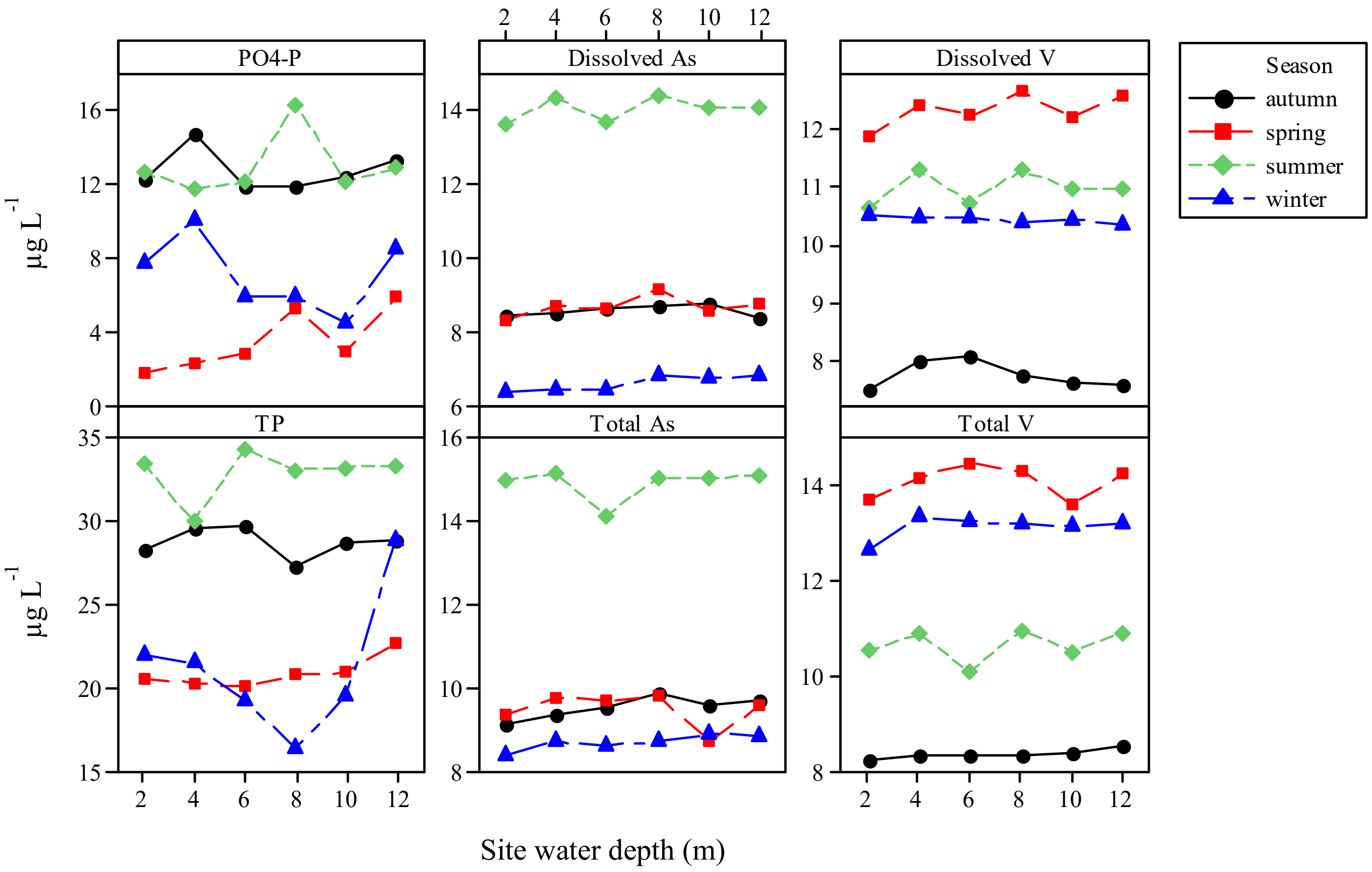

Fig. 4.3. Variation of pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V) across seasons and at sites of different water depth in surface water in Kinghorn Loch from May 2012 to April 2013.

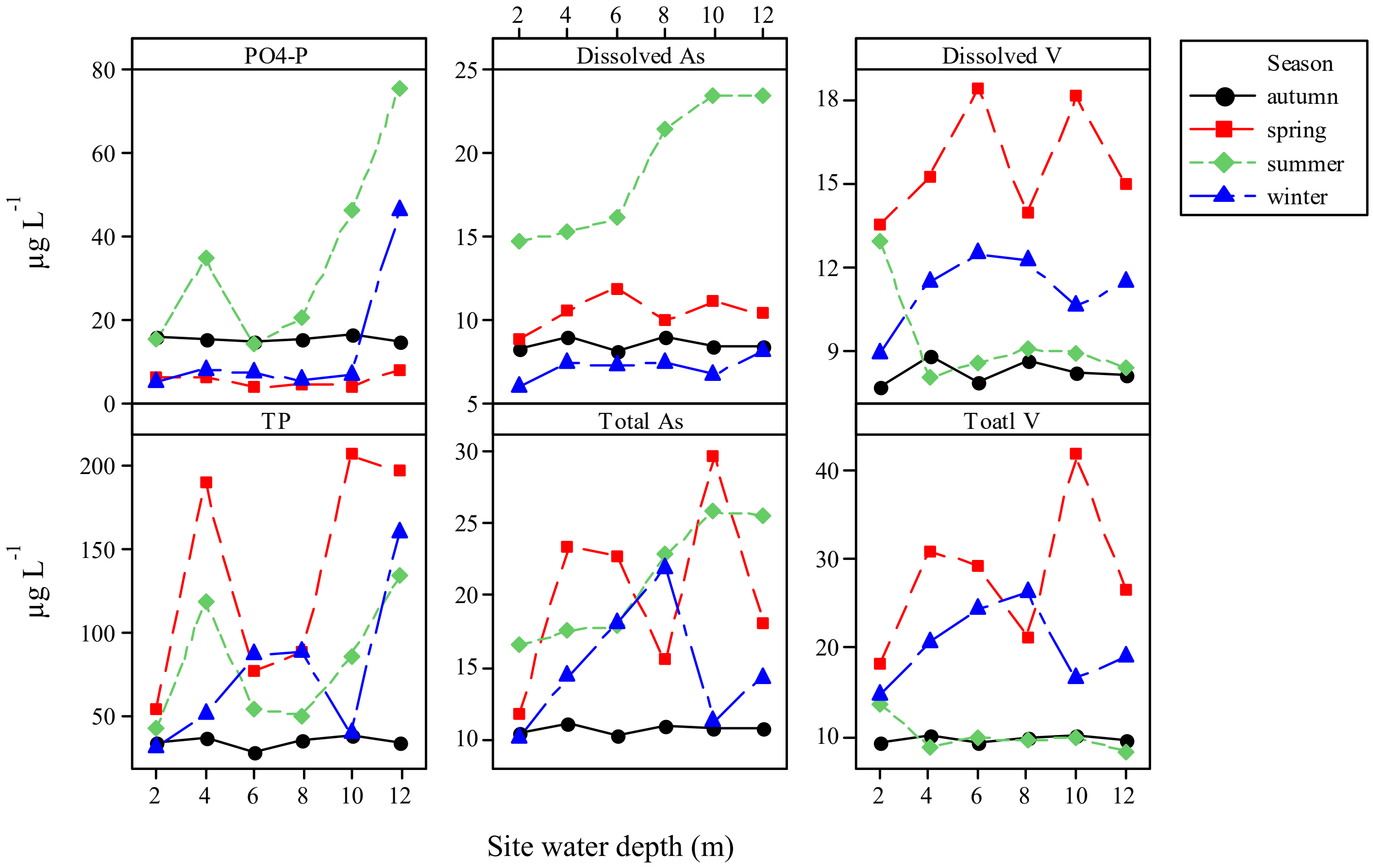

Fig. 4.4. Variation of pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V) across seasons and at sites with different water depths in sediment + 0.01 m water in Kinghorn Loch from May 2012 to April 2013.

The lowest mean dissolved As concentration occurred at all sites in winter, whilst the highest was in summer. Seasonal mean concentrations of dissolved V in sediment + 0.01 m water ranged from 7.76 ± 0.71 µg L^{-1} (autumn, 2 m water depth) to 18.4 ± 11.3 µg L^{-1} (spring, 6 m water depth), with the highest mean concentrations at each site occurring in spring and the lowest (with the exception of site 6) in autumn. Seasonal mean total V concentrations varied between 8.46 ± 1.37 µg L^{-1} (summer, 12 m water depth) to 42.0 ± 21.2 µg L^{-1} (spring, 10 m water depth). Statistical analysis indicated a significant influence of season on concentrations of PO_4-P, TP, dissolved and total As, and dissolved and total V at both surface and sediment + 0.01 m water sample depths (Table 4.4). Overlying water depth, however, had a significant effect only on PO_4-P and TP in sediment + 0.01 m water. Interaction between season and depth was not found to have significant effect on any of the three pollutants.

The highest seasonal means of As and P in sediment + 0.5 m water occurred, as in surface water, in summer (Appendix A.5 and A.6) and were higher than in surface water, but lower compared to the highest means measured in sediment + 0.01 m water. The range of seasonal mean concentrations of both forms of V in sediment + 0.5 m water was similar to V means observed in surface water. Similarly to surface water the ANOVA analysis indicated only significant effect of season on concentrations of PO_4-P, and dissolved and total forms of As and V in sediment + 0.5 m water (Table 4.4). However, TP, as in sediment + 0.01 m water, was significantly influenced by both season and overlying water depth, with the highest concentration measured at the deepest site (85.5 ± 25.8 µg $L^{-}1$).

4.3.3 Relationship between water column pollutant concentrations and physico-chemical factors

Variations in dissolved and total concentrations of each of the pollutants were explained by a combination of physico-chemical water parameters and elements with pollutant binding abilities (seasonal means of pollutant-binding elements are provided in Appendix A.7–A10). The proportion of variation in pollutant concentration explained by

Table 4.4. Results of two-way ANOVA to assess the significance of the difference between pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V (µg L^{-1})) across seasons and at 6 sites in surface water, sediment + 0.5 m water and sediment + 0.01 m water in Kinghorn Loch from May 2012 to April 2013.

Depth	Pollutant	Season		Site		Interaction	
		F-value	P-value	F-value	P-value	F-value	P-value
Surface water	Dissolved As	34.160	< 0.001	0.115	0.989	0.022	1.00
	Dissolved V	106.172	< 0.001	0.415	0.836	1.444	0.166
	PO_4-P	13.727	< 0.001	0.371	0.866	0.270	0.996
	Total As	18.396	< 0.001	0.038	0.999	0.043	1.00
	Total V	37.513	< 0.001	0.128	0.985	0.054	1.00
	TP	17.306	< 0.001	0.612	0.691	0.400	0.972
Sediment + 0.5 m water	Dissolved As	45.845	< 0.001	0.894	0.493	0.419	0.966
	Dissolved V	79.922	< 0.001	0.584	0.712	0.852	0.619
	PO_4-P	20.393	< 0.001	1.296	0.281	0.295	0.994
	Total As	28.702	< 0.001	1.102	0.372	0.454	0.952
	Total V	47.073	< 0.001	0.190	0.965	0.179	1.00
	TP	19.041	< 0.001	3.353	0.011	1.611	0.106
Sediment + 0.01 m water	Dissolved As	50.858	< 0.001	1.312	0.275	0.644	0.823
	Dissolved V	33.476	< 0.001	0.082	0.995	1.444	0.166
	PO_4-P	21.068	< 0.001	2.616	0.036	0.711	0.761
	Total As	9.286	< 0.001	1.350	0.260	0.745	0.728
	Total V	29.886	< 0.001	0.197	0.962	0.913	0.556
	TP	6.847	< 0.001	2.787	0.027	0.768	0.705

multiple regression models ranged from 38% (for surface and sediment + 0.01 m water PO_4-P, and sediment + 0.01 m water dissolved V) to 75% (for sediment + 0.01 m water total As; Table 4.5).

To avoid problems with collinearity, only DO, conductivity, pH, dissolved Fe and Mn, total Fe and Ca were used in the surface water models. The VIF for all explanatory variables that were included in the regression analysis for each pollutant was < 3, ensuring that there was no strong collinearity between variables (Zuur et al. 2010). The final models for surface water dissolved and total As concentrations showed significant positive relationships with pH, but negative relationship with conductivity. Surface water dissolved and total V were significantly positively related with oxygen and conductivity. There was also a significant positive correlation between dissolved V and dissolved Al and pH. Surface water TP was significantly positively related to pH and both forms of P were negatively correlated with conductivity. Pollutant-binding elements were present in final models of all pollutants.

To avoid collinearity among explanatory variables, only DO, conductivity, dissolved Al and Ca, total Fe, sediment Fe and Mn, and sediment As, V or P (depending on the response variable) were included in regression models for sediment + 0.01 m water. The VIF for all explanatory variables entered into each model was < 3. Final models indicated a significant negative correlation of dissolved and total sediment water As with oxygen and a significant positive correlation with conductivity. Sediment water dissolved and total V were significantly positively correlated with DO and conductivity. There was a significant negative correlation of sediment water PO_4-P and TP with DO, and a significant positive correlation of TP with conductivity. Pollutant-binding elements were present in final models of all contaminants with the exception of PO_4-P.

Table 4.5. Results of multiple regression following backward regression to examine the relationship between pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V) and potential explanatory variables (dissolved Fe, Mn, Al, Ca, total Fe, Mn, Al, Ca, pH, DO, conductivity, temperature and sediment As, V, P, Fe, Mn, Al, Ca) in both, surface water and sediment + 0.01 m water. The multiple regression was conducted at alpha = 0.05. Only significant results are reported.

	Pollutant concentration		P-value	Equation of line y = ….	R^2 (%)
Surface water	Dissolved	As	< 0.001	- 9.004 + 0.289 Fe + 3.93 pH[a] - 0.043 conductivity[a]	65
		V	< 0.001	- 24.622 – 0.154 Fe + 0.095 Al + 2.559 pH[a] + 0.353 DO + 0.021 conductivity[a]	46
		PO_4-P	< 0.001	31.196 + 1.021 Fe - 0.215 Al – 0.063 conductivity[a]	38
	Total	As	< 0.001	- 5.244 + 0.123 Ca[b] + 4.298 pH[d] – 0.066 conductivity[c]	48
		V	< 0.001	- 14.544 – 0.02 Fe[e] + 0.615 DO + 0.049 conductivity[c]	55
		P	< 0.001	55.957 + 0.324 Ca[b] + 4.977 pH[d] – 0.216 conductivity[c]	58
Sediment + 0.01 m water	Dissolved	As	< 0.001	11.80 – 1.13 DO[f] + 0.05 conductivity – 0.23 Ca	73
		V	< 0.001	0.80 + 0.04 DO[f] + 0.004 conductivity – 0.02 Ca	38
		PO_4-P	< 0.001	3.89 – 0.14 DO[f]	38
	Total	As	< 0.001	1.35 – 1.08 DO[f] + 0.008 Fe[g] + 0.05 conductivity	75
		V	< 0.001	0.27 + 0.05 DO[f] + 0.0003 Fe[g] + 0.004 conductivity	70
		P	< 0.001	2.26 – 0.05 DO[f] + 0.0005 Fe[g] + 0.007 conductivity – 10.3 $Mn_{sediment}$	55

[a] collinear with dissolved Mn, Ca and temperature

[b] collinear with temperature and total Al

[c] collinear with temperature and total Mn

[d] collinear with temperature

[e] collinear with Al

[f] collinear with pH, temperature, dissolved and total Mn, and dissolved Fe

[g] collinear with total Al and Ca

4.3.4 Relationship between pollutant concentrations in surface water and water above sediment

With the exception of TP, there was a significant positive correlation between concentrations of each pollutant in surface water and sediment + 0.01 m water (Table 4.6). The strongest correlation was observed between dissolved As concentrations (Spearman's rho = 0.91) and the lowest between concentrations of total As (Spearman's rho = 0.56).

Table 4.6. Spearman's rank coefficients of correlation (rho) between concentrations of each pollutant (dissolved As, dissolved V, PO_4-P, total As, total V and TP) measured at 6 sites in surface water and sediment + 0.01 m water in Kinghorn Loch from May 2012 to April 2013, shown together with their significance.

Pollutant	Spearman's rank coefficients of correlation (rho)
Dissolved As	0.91^{***}
Dissolved V	0.66^{***}
PO_4-P	0.70^{***}
Total As	0.56^{***}
Total V	0.74^{***}
TP	0.11^{NS}

^{NS}not significant ($P > 0.05$),
***significant ($P < 0.001$)

4.3.5 Sediment pollutant concentrations – seasonal and spatial variation

Figure 4.5 shows concentrations of As, V and P in the top 4 cm of bed sediment across seasons and at six sites. Seasonal mean sediment P concentration (Appendix A.11) varied from 690 ± 1.0 mg kg^{-1} (2 m water depth, autumn) to 1210 ± 100 mg kg^{-1} (10 m water depth, winter). Mean P content at the three shallowest sites (2 – 6 m) was ≤ 1000 mg kg^{-1} during all seasons, whilst mean P at the three deepest sites (8 – 12 m) was > 1000 mg kg^{-1}. Seasonal mean concentrations of As ranged from 65.4 ± 9.32 (2 m water depth, winter) to 197 ± 19.4 mg kg^{-1} (10 m depth, spring). Seasonal mean concentrations of V varied from 163 ± 7.17 (2 m water depth, summer) to 234 ± 13.5 mg kg^{-1} (12 m water depth, winter).

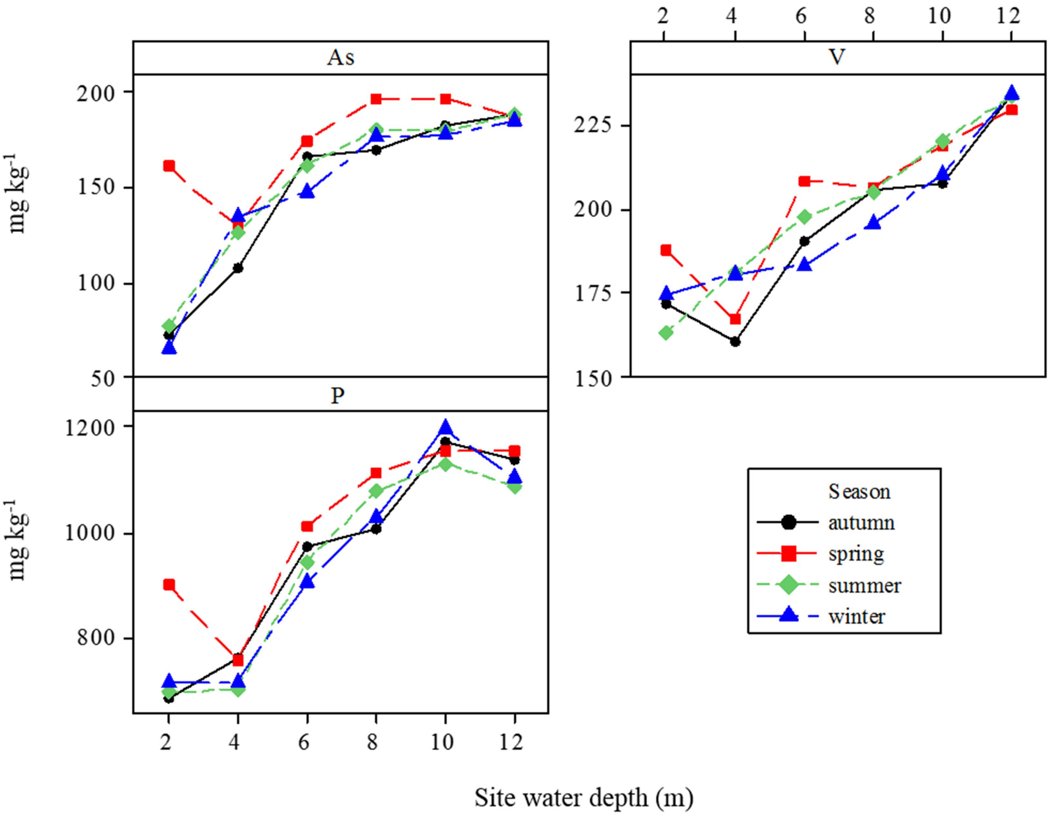

Fig. 4.5. Variation of pollutant concentrations (As, V and P) across seasons and at sites with different water depths in the top layer of sediment (0-4 cm) in Kinghorn Loch from May 2012 to April 2013.

Overlying water depth was found to have a significant influence on all three examined red mud constituents in the top layer of sediment (Table 4.7), with pollutant concentrations generally increasing with water depth (Fig. 4.5). Season had a significant effect on As and P concentrations, however interaction between season and overlying water depth was significant only for As.

Table 4.7. Results of two-way ANOVA analyses to assess the significance of the difference between pollutant concentrations (As, V and P (mg kg^{-1})) across seasons and at 6 sites (water depths) in the sediment surface (0-4 cm) in Kinghorn Loch from May 2012 to April 2013.

Pollutant	Season		Site		Interaction	
	F-value	P-value	F-value	P-value	F-value	P-value
As	6.36	0.00106	37.054	< 0.001	1.960	0.040
V	0.707	0.552	19.566	< 0.001	0.568	0.885
P	4.171	0.0105	84.383	< 0.001	1.352	0.210

4.3.6 Sediment pollutant concentrations - variation across sediment depth

Annual mean P content in the top 10 cm of sediment varied from 640 ± 30 mg kg^{-1} (60; 6-8 and 8-10 cm sediment layer, 2 m water depth) to 1230 ± 30 mg kg^{-1} (4-6 cm, 12 m water depth; Appendix A.12-A.13). The lowest range of P content was observed at the two shallowest sites, however no clear trend was evident in P across sediment depth. Annual mean concentrations of As continuously increased with sediment depth at all six sites, with the mean concentrations in the top sediment layer ranging from 86.6 ± 36.9 mg kg^{-1} (2 m water depth) to 185 ± 19.1 mg kg^{-1} (12 m water depth) and mean concentrations in the deepest sediment layer varying from 136 ± 38.4 mg kg^{-1} (2 m water depth) to 397 ± 150 mg kg^{-1} (12 m water depth). A continuous increase with sediment depth was also observed for annual mean V concentrations, with the exception of site 2 where mean V in the 6-8 cm sediment layer (239 ± 8.02 mg kg^{-1}) was slightly higher than in the 8-10 cm layer (241 ± 29.6 mg kg^{-1}). Annual mean concentrations of V in the 0-4 cm layer ranged from 164 ± 6.45 mg kg^{-1} (2 m water depth) to 231 ± 23.0 mg kg^{-1} (12 m water depth), whilst concentrations in the deepest sediment layer varied from 238 ± 6.90 mg kg^{-1} (8 m water depth) to 311 ± 87.2 mg kg^{-1} (12 m water depth).

4.4 Discussion

4.4.1 Evidence of translocation of As, P and V in the lake water column and underlying processes

The present study showed that concentrations of As, V and P (both dissolved and total forms) in the water column of Kinghorn Loch varied significantly across seasons at all three water column sampling depths, and in the case of P in deeper layers of the water column, also across sites. The spatial variation was, however, weaker than the seasonal one. Multiple regression analyses identified several physico-chemical factors driving variance in concentrations of red mud pollutants. DO content appeared to be driving variation in concentration of all pollutants in water above sediment, but in surface water DO was only significantly related to V.

4.4.1.1 Arsenic and phosphorus

The highest concentrations of both dissolved and total P and As in surface water were measured in summer (although P concentrations were also high in autumn), with particularly pronounced differences between summer and the other three seasons observed for both forms of As. A peak in concentrations in the warmest season was also documented for PO_4-P and dissolved As in sediment + 0.01 m water. Increased summer P concentrations in shallow eutrophic lakes have been attributed in a number of previous studies to internal loading from sediment driven by seasonal changes in redox conditions (Jensen et al. 1992; Søndergaard et al. 2003; Søndergaard et al. 2013). The occurrence of an internal loading related peak in TP concentrations in the water column may also extend into early autumn, which was observed in Loch Leven, a shallow, eutrophic lake in Scotland (Spears et al. 2012), and corresponds with high autumn P concentrations in surface water in the present study. Internal loading might occur when higher temperature enhances decomposition of organic matter in bed sediments by microorganisms leading to depletion of DO in bottom water (Søndergaard et al. 2003), which in turn causes reduction of pollutant-binding metal oxyhydroxides in sediment and release of redox sensitive, soluble forms of elements into the overlying water (Smolders et al. 2006; Partey et al. 2008; Couture et al. 2010). A significant negative relationship between As and P in sediment + 0.5 m water with DO, as well as a negative correlation of DO with temperature (correlation coefficient = - 0.729, $P < 0.001$) and a decline in DO concentrations below 4 and 5 m in the water column in summer suggests that this process occurs in Kinghorn Loch. The occurrence of the greatest difference in dissolved As concentrations between summer and other seasons at the deepest sites, where the DO was lowest, also supports this interpretation.

The multiple regression results showed no influence of DO on As and P variations in the lake surface water. Significant positive correlations between PO_4-P and dissolved and total As in sediment + 0.01 m water and surface water, however, suggest that the concentration of these pollutants in the upper water column were influenced to some extent by the As and P content in deeper waters. The absence of the correlation between TP concentrations at the two sampling depths indicates that other factors than TP concentration in deeper water drive TP variation in surface water. TP and both forms of As in surface water were significantly positively correlated with pH, and the highest seasonal pH in surface water was measured in summer, corresponding with the highest seasonal concentrations of these two pollutants. The collinearity between DO and pH in sediment + 0.01 m water (correlation coefficient = 0.817, $P < 0.001$) suggests that an increase of As and P concentrations in deeper parts of the water column might also be explained by increased pH. The highest concentrations of TP and total As in sediment + 0.01 m water were recorded either in spring or in summer, which indicates that pH (characterised by the highest values in sediment + 0.01 m water in spring) and DO (with lowest values occurring in summer) together play a role in the behaviour of these two contaminants in deeper waters. High pH leads to elevated concentrations of pollutants due to its negative effect on the binding capacity of Fe, resulting in the release of iron-bound elements (Søndergaard et al. 2003; Scheffer 2004). Water pH in productive lakes can fluctuate significantly across seasons with variation in algal biomass (Maberly 1996) and its elevated values are related to enhanced photosynthetic activity (Kneebone and Hering 2000; Scheffer 2004).

The similarity in seasonal patterns of As and P observed in the present study in the lake water column is in agreement with seasonal variation of inorganic As and nutrients, especially between As (V) and PO_4-P, reported in surface water of Lake Greifen, Switzerland (Kuhn and Sigg 1993). Due to the chemical similarity of inorganic As to P (Mishra et al. 2008) and its relationship to PO_4-P transporters (Favas et al. 2012), As(V) can be accumulated by photosynthesising aquatic organisms using the same uptake pathways as the nutrient (Rahman et al. 2007; Foster et al. 2008; Xue et al. 2012). This can lead to variation of both elements in the water column being driven in a corresponding way by biological factors. Kuhn and Sigg (1993) related the depletion in spring of dissolved As together with PO_4-P in the epilimnion to uptake of both elements by algae. The lowest PO_4-P and low As concentrations in surface water in spring in the present study indicate that uptake by algae might be seasonally controlling to some extent the concentrations of these two pollutants in Kinghorn Loch as well.

4.4.1.2 Vanadium

Similarly to As and P, the aqueous geochemistry of V has been shown to be controlled by redox processes (Peacock and Sherman 2004). The present study confirmed the influence of DO on seasonal variations of V, both in surface water and sediment + 0.01 m water. In contrast to the other two pollutants, however, V concentrations were significantly positively related to DO, with the highest V concentrations observed in spring, when DO was the highest, and the lowest concentrations in autumn (in surface water) and summer and autumn (in sediment + 0.01 m water). The results from Kinghorn Loch are also in contrast with V measurements in coastal waters of Long Island Sound in the USA, where the dissolved V concentrations were significantly higher in summer compared to spring (Wang and Wilhelmy 2009). The multiple regression analysis indicated pH as another factor controlling V concentrations in Kinghorn Loch. pH was significantly positively related with dissolved V concentrations in surface water, and the significant positive correlation between DO and pH in sediment + 0.01 m water, suggested possible impact of pH also in deeper parts of the water column. The highest V concentrations in sediment + 0.01 m water corresponding with the highest seasonal pH appears to confirm this driver and is supported by the study of Naeem et al. (2007) who reported a linear decrease in V adsorption onto metal oxides with an increase of pH from 4 to 11.6.

pH however, did not control total V concentrations in Kinghorn Loch surface water and there was a positive correlation between this pollutant and DO. A study of V seasonality in Kinghorn Loch surface water between 2000 – 2009 showed a strong positive correlation between total V and chlorophyll *a*, with their highest peaks observed in spring (Watt 2015). Watt (2015) hypothesised that accumulation by phytoplankton might be one of the drivers of V seasonality, with an increase in total V concentrations due to a net accumulation during spring blooms, followed by a decrease in autumn related to a decline in phytoplankton productivity and a removal of V from surface water with phytoplankton biomass. This, together with likelihood of V transport from deeper parts of the water column due to enhanced mixing in spring (indicated by the significant positive correlation between V in surface and sediment + 0.01 m water) may be potential explanations of the surface water results in the present study. Furthermore the peak of chlorophyll *a* in spring indicates potential indirect influence of algae on the V cycling through rise of pH, and subsequently a decreased adsorption of V in bed sediment, which has been previously documented in Lake Biwa, Japan (Harita et al. 2005). Given that highest DO in Kinghorn Loch occurred in spring and DO is also positively related with V, phytoplankton may also indirectly control the V cycling by increasing oxygen concentrations during a period of enhanced photosynthetic activity. Further research is required to better understand processes driving behaviour of V in Kinghorn Loch and other aquatic systems.

4.4.1.3 Evidence of relationship between pollutant binding elements and As, P and V

The redox reactions driving seasonal variation in pollutant concentrations in lakes can be controlled by biological (e.g. phytoplankton abundance) and chemical factors (e.g. Mn and Fe oxides; Kuhn and Sigg 1993). Pollutant binding elements appeared in final regression models for all the contaminants, both in surface water and sediment + 0.01 m water, with the exception of the model for PO_4-P in sediment + 0.01 m water that contained DO as a sole significant explanatory factor. The highly significant negative correlation between DO in sediment + 0.01 m water and dissolved Mn (correlation coefficient = - 0.863, $P < 0.001$) and Fe (correlation coefficient = - 0.751, $P < 0.001$), however, indicated a likely positive relationship also between the two elements and concentrations of PO_4-P in sediment + 0.01 m water. The importance of Mn and Fe oxyhydroxides in cycling of As and P in aquatic ecosystems has been recognised in previous studies (e.g. Muller et al. 2002; Søndergaard et al. 2003; Whitmore et al. 2008), as has the importance of Fe oxyhydroxides in V cycling (Wällstedt et al. 2010). Similarly the important role of Fe and Al in regulating P cycling in lakes has been previously identified (Olila and Reddy 1997). Strong positive relationships of sediment As concentrations with Fe, and between Fe and Al were reported in Lake Coeur d'Alene, Idaho (Toevs et al. 2008), and in Little Lake Jackson, USA (Whitmore et al. 2008), respectively. Also Ca capability to bind P has been previously identified (Søndergaard et al. 2003). The highest seasonal concentrations of dissolved Fe and Mn were, with two exceptions, recorded in surface water and water above sediment in summer. This, and the variation in the occurrence of the highest concentrations of the Al and Ca throughout the year, suggests a stronger influence of Fe and Mn on the concentrations of P and As in Kinghorn Loch water. The results of the multiple regression analysis did not indicate a significant influence of the sediment content on the variations of pollutant concentrations in lake water. Pollutant binding elements in the sediment were present only in the model for TP in sediment + 0.01 m water and there was no indication of significant impact of pollutant concentrations in sediment on water column pollutant concentrations. No correlation between concentrations of TP in water and P content in sediment was previously reported by Jensen et al. (1992), who studied P cycling in 15 Danish lakes. The authors indicated, however, that the water P concentrations were instead related to the ratio of surface sediment TP and sediment total Fe concentrations. The results from the latter study imply that the influence of concentrations of pollutant binding elements in the sediment on the variations in pollutant concentrations in Kinghorn Loch water should not be excluded.

Total As concentrations in water measured in Kinghorn Loch were within UK Standards for the Protection of Aquatic Life (50 $\mu g\ L^{-1}$; EEC 1976). The Standard for Protection of Aquatic Life for total V (20 $\mu g\ L^{-1}$; EEC 1976) was met in surface and sediment + 0.5 m water. However, with the exception of the shallowest site, it was exceeded in spring and winter in the deepest layer of the water column (sediment + 0.01 m samples), indicating a potential risk to aquatic organisms. Observed concentrations of TP exceeded the Trophic State Index value of 24 $\mu g\ L^{-1}$ for TP, above which a lake is classified as eutrophic (Carlson et al. 1996), at all three water sample depths at all sites.

4.4.2 Red mud pollutant content in lake sediment and relevance to environmental health

The present study showed a recovery of Kinghorn Loch sediment from contamination since the control of the red mud leachate in 1983, with a decrease in As and V concentrations in the upper sediment layer in the deepest area of the lake from 749 and 2554 mg kg^{-1} in 1985 (Edwards 1985), to 207 and 281 mg kg^{-1} in 2010 (unpublished data) and 185 and 231 mg kg^{-1} (present study), respectively. Although the concentration of As has decreased about four times and V about ten times since the pollution input ceased, they still remain very high. In comparison, mean As and V concentrations in sediment of Lake Baikal (Siberia), reported as natural background levels of these trace elements and controlled by weathering of rocks in the lake drainage basin, were 8.24 mg kg^{-1} and 81.1 mg kg^{-1}, respectively (Muller et al. 2002). Sediment surface As content in the present study, varying across sites from 85.6 to 185 mg kg^{-1}, was comparable to the As concentrations observed in surface sediment of contaminated freshwater sites, such as a section of Lake Dianchi, China, polluted by industrial drainage (mean As 97 mg kg^{-1}, Zhang et al. 2013) and Lake Mohawk, USA (90.8 – 462 mg kg^{-1}; Barringer et al. 2011). Arsenic concentration in Kinghorn Loch sediment was also higher than in Little Lake Jackson, USA, contaminated by organic arsenical herbicides applied to nearby golf courses (mean As concentration 47.3 mg kg^{-1}; Whitmore et al. 2008). In the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life the probable effect level (PEL) for As in freshwater sediment is 17 mg kg^{-1} dry weight, above which adverse biological effects are expected to occur more frequently, such as decreased benthic invertebrate abundance and increased mortality (Canadian Council of Ministers of the Environment 1999a). Annual mean concentrations of As in Kinghorn Loch sediment at all six sampled sites still greatly exceed this guideline 30 years into the recovery period.

Limited research has been conducted on the behaviour of V in the environment (Cappuyns and Swennen 2014), in particular, on the presence of V in freshwater ecosystems, and no environmental standards exist for this element in freshwater sediment. The Canadian Soil Quality Guideline for the Protection of Environmental Health is 130 mg kg^{-1} dry weight (Canadian Council of Ministers of the Environment 1999b) and previous research indicated that V concentrations in soil lower than this value can have toxic effects on plants (Smith et al. 2013). Genotoxic effects on higher plants attributed to high V concentrations were also observed in a study on the impact of red mud from the contaminated sites in Hungary (Mišík et al. 2014), where the V content in the uppermost soil sample (899 mg kg^{-1}) is much higher than the V concentrations in Kinghorn Loch sediment. The present study indicates that V content in sediment in Kinghorn Loch remains sufficiently elevated to have a toxic effect on aquatic organisms. In contrast to As and V, P content in surface sediment showed an increasing trend from 430 mg kg^{-1} in 1985 (Edwards 1985) to 1090 mg kg^{-1} in 2010 (unpublished data) and 1150 mg kg^{-1} in the present study, respectively. This may be due to a continued increase in P loading from catchment agricultural activities during a period when inputs of other constituents had decreased. However, there is no data on P loading from the agricultural sources available to confirm this. An alternative, or additional, explanation may be that P is more sensitive to redox mediated liberation and accumulation in surface sediment than other red mud contaminants which are more readily confined to deeper sediment layers with time.

As and V concentrations in Kinghorn Loch increased with sediment depth in the top 10 cm of sediment. Andrade et al. (2010) reported a similar trend for As in the upper layers of sediment from the Great Slave Lake, Canada. The authors of the study reported an increase in As concentrations at the sediment water interface, a result of the post-depositional mobility exhibited by this element (Andrade et al. 2010). Surface sediment interstitial water was not sampled in the present study, however, the higher As concentrations in the sediment + 0.01 m water compared to upper layers of the water column appear to indicate post-depositional mobility of As in Kinghorn Loch. The decrease of As concentrations towards the top of the sediment cores was also observed in Little Lake Jackson, USA (Whitmore et al. 2008). P content remained relatively constant with sediment depth at Kinghorn Loch corresponding with other studies which demonstrated that the upper 10 cm layer of sediment generally contains mobile P and interacts with the lake water (Søndergaard et al. 2003).

The distribution of the three red mud constituents in the top layer of lake sediment was strongly related to the overlying water depth, with higher pollutant concentrations accumulated in deeper areas of the lake. Significant spatial variation of TP in the top layer of sediment was also documented in Loch Leven (Spears et al. 2007). The highest content of the most abundant sediment P fraction (reductant-soluble P) was, however, observed at intermediate depths of Loch Leven. In addition to spatial variation, As and P concentrations in Kinghorn Loch sediment changed seasonally, with higher content recorded at most sites in spring. This variation might be explained by seasonal changes in pollutant retention in sediment.

Conclusions

The present study shows that concentrations of As, V and P in the water column of Kinghorn Loch varied seasonally, with P also displaying spatial variation. Pollutant distribution in the surface layer of bed sediment was strongly influenced by the overlying water depth, and to a lesser extent by season. The variation in pollutant concentrations in the water column appeared to be driven by the combined effects of a number of physico-chemical factors including DO, pH and concentrations of pollutant-binding elements. The elevated content of red mud constituents in the lake sediment indicates that, although As and V concentrations in water generally meet environmental quality standards, the lake has not fully recovered 30 years after the diversion of the pollution. Furthermore, due to the likelihood of internal loading from bed sediment, the results raise questions about the potential influence of the contaminated sediment on persistent water column pollution many years following the discharge control, as well as about an impact on aquatic organisms in Kinghorn Loch.

Chapter 5 Assessing the legacy of red mud pollution in a shallow lake (Kinghorn Loch, UK): bed sediment as a residual pollution source to the water column

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This chapter has been prepared as a manuscript for submission to the journal "Inland Waters". The candidate, as lead author, performed the sample collection, the experiment and was involved in laboratory analysis of metal and metalloid content in water. The laboratory analysis of phosphorus content in water, data analysis and writing of the paper was carried out by the candidate. Co-authors provided support and guidance on the scope and design of the research and expertise in ICP-MS. Co-authors contributed to the editing of the manuscript.

5.1 Introduction

In Chapter 3 it was demonstrated that the recovery of Kinghorn Loch surface water from red mud pollution exceeded 25 years for some red mud contaminants. One explanation for the long recovery time is the continued mobilisation of accumulated pollutants from lake sediment after the diversion of the leachate. The seasonal increases in red mud contaminant concentrations reported in Chapter 4, with higher concentrations in the deeper parts of the water column compared to lake surface water, support this hypothesis. Hence to confirm this explanation and explore the main drivers the process of mobilisation of redox sensitive red mud constituents from sediment will be investigated in this chapter.

Inappropriate storage of red mud, a by-product of alumina production, is of increasing concern as illustrated by the October 2010 Ajka accident in Hungary, which resulted in the release of ~1 million m^3 of red mud waste (Burke et al. 2012), contaminating the Marcal river (catchment area 3078 km^2) and entering the Danube River (Klebercz et al. 2012). Nevertheless the chemical and ecological impact-response trajectories following red mud pollution in freshwaters are largely unknown. This is confounded by a lack of knowledge of the changes in composition of red mud that occur as a result of interactions with sediments and freshwater organisms during transport through aquatic ecosystems (Klebercz et al. 2012). Research after the Ajka accident focused on the short-term impact of red mud waste on freshwaters. In aquatic ecosystems with a short residence time and well oxygenated surface sediment layer, oxyanion forming contaminants (e.g., arsenic (As), vanadium (V), and chromium (Cr)) were contained within solid phase complexes, representing little ecological threat (Mayes et al. 2011). In depositional zones, where reducing conditions are likely to occur, the release of these elements may be expected (Mayes et al. 2011). Little is known, however, about the long-term fate of red mud constituents in freshwaters or of their impact on recovery processes. This knowledge is expected to be particularly important for achieving the effective recovery of longer retention time aquatic ecosystems, such as lakes, where the residence time of pollutants can far exceed those in rivers and streams (Spears et al. 2012, Sharpley et al. 2013).

Sediments play an important role in regulating seasonal phosphorus (P) dynamics in shallow lakes (Søndergaard et al. 2003). Cycling of P, as well as As, between sediment and overlying waters is driven by redox conditions, adsorption and desorption mechanisms, and mineral phase solubility (Kleeberg and Kozerski 1997, Søndergaard et al. 2003, Toevs et al. 2008). P can be either permanently buried in the sediment or released in dissolved form into the water column. Loosely adsorbed inorganic and organic fractions and redox sensitive forms of P adsorbed to iron (Fe) are considered potentially mobile (Søndergaard et al. 2003). Phosphate (PO_4-P) release can occur due to the dissolution of iron oxyhydroxides during anoxic conditions (Smolders et al. 2006), when Fe(III) is reduced to Fe(II) and both Fe and P are released into porewaters (Søndergaard et al. 2003). P can also be bound to other inorganic components, such as aluminium (Al), manganese (Mn), calcium (Ca), or clay (Søndergaard et al. 2003). Similar to P, the diffusion of soluble As from sediment into overlying water is limited by sorption of arsenite (As(III)) and arsenate (As(V)) to Fe, Mn and Al oxyhydroxides (Toevs et al. 2008, Whitmore et al. 2008). During the reduction of Fe oxyhydroxides in sediments under anoxic conditions, As(V) can be converted to As(III) and released to the porewaters. When waters overlying the sediment are well aerated, dissolved As(III) is likely to be co-precipitated with oxidised forms of Fe or Mn (Whitmore et al. 2008). Vanadium (V), like P and As, can be adsorbed to Fe oxides (Wällstedt et al. 2010). It occurs in many oxidation states; V(V) is expected to dominate in oxidising water environments, and V(IV) in reducing conditions (Minelli et al. 2000). The conversion between V species depends on redox potential, pH, and V concentration (Minelli et al. 2000), but there is limited evidence in the literature from which the behaviour of V in lakes can be predicted. In addition to redox potential, a number of environmental conditions are likely to regulate the mobilisation of red mud constituents in lake bed sediments, including water temperature, light, pH, organic matter, and bacterial productivity (Czop et al. 2011).

The aim of this research was to investigate the long-term fate of red mud constituents in a shallow lake. This was achieved through a 12-month field campaign, examining concentrations of pollutants in surface water versus concentrations in water above lake sediment as evidence of internal loading of constituents, and a complementary laboratory experiment designed to trigger release of redox sensitive species from sediment to water. We assessed the following specific hypotheses: (1) release of redox sensitive red mud constituents (P, As and V) from the bed sediments of Kinghorn Loch would persist 30 years after the beginning of the lake recovery period and be measurable in the field and (2) dissolved oxygen (DO) concentrations in the water column control the pollutant release from sediment to water and the process will be measurable in a laboratory.

5.2 Methods

5.2.1 Study site

Kinghorn Loch is a small lake of area 11.3 ha, mean depth 4.5 m and maximum depth 12.8 m, situated in Fife, Scotland (56°10′N; 3°11′W). The lake received highly alkaline leachate from a nearby red mud landfill site (Fig. 2.1-2.2, Section 2.1) from 1947 to 1983 when the discharge was diverted. Caustic liquor reaching the loch consisted of a highly alkaline solution of NaOH and carbonate, with mean pH of 12.1. It contained high concentrations of dissolved Al, As, V, orthophosphate, sulphate and Cl, whilst red mud solids, released to the loch sporadically, accounted for most of the input of Fe (Edwards 1985). A long-term monitoring programme of the lake water chemistry measured in the outlet of the lake was initiated in 1980 by the Forth River Purification Board and continued by the Scottish Environment Protection Agency (SEPA).

5.2.2 Water sample collection - field campaign

A one-year field campaign was conducted to assess cycling of red mud pollutants in the lake. Lake water sampling was carried out from a boat once a month from May 2012 to April 2013 at a site chosen within the deepest part of Kinghorn Loch (12 m water depth). To test the influence of sediment on vertical changes of pollutant concentrations in the water column throughout a year, single 500 mL samples of lake surface water and 50 mL samples of bottom water (i.e. sediment + 0.01 m water, from a sediment core) were taken using polypropylene acid washed bottles and sterile polypropylene centrifuge tubes, respectively. The water samples were analysed for concentrations of three redox sensitive red mud constituents (PO_4-P, total P (TP), dissolved and total As and V) and four elements with pollutant binding ability (Fe, Mn, Al, Ca). Due to the different sample preparation and storage requirements for the various groups of elements, each water sample was divided into four subsamples.

5.2.3 Sample collection – laboratory experiment

Sediment cores for the laboratory experiment were collected by boat, close together (within an area of ~10 m^2 to minimise variability between them) from the deepest point of the loch using a HTH gravity corer on 31 July 2012. The cores had internal diameter 65 mm and consisted of c.25–33 cm depth sediment and 14–21 cm overlying water and were returned to the laboratory within 2 h of collection. DO concentration, conductivity, pH and water temperature were measured *in situ* at the site of water sample and sediment core collection (Table 5.1) using a Hach HQ40d Portable Multi-Parameter Meter and probes (Hach Lange, Salford, UK).

Table 5.1. Field measurements of Kinghorn Loch water at the site of sediment core collection on 31 July 2012.

Depth	Secchi depth (m)	Water temp. (^{o}C)	Conductivity (μS cm^{-1})	pH	DO (mg L^{-1})
surface	1.22	16.6	316	7.99	11.6
1 m above sediment		10.3	336	7.09	0.2

5.2.4 Oxygen manipulation experiment

The experiment was conducted in a controlled temperature room in which the temperature was set at the lake water temperature measured 1 m above the sediment (10.3^{o}C) at the time of the core collection. Collected sediment cores were randomly assigned to the oxic and anoxic treatments. To investigate the influence of oxygen conditions on the release of red mud contaminants from the lake sediments into the water column air was bubbled through the overlying water in five of the sediment cores (Tetratec Whisper AP 200 pump, TetraWerke, Melle, Germany) to create oxic conditions and N_2 through the overlying water in five other sediment cores to create an anoxic environment. Cores were continually bubbled with air or N_2 for 8 days. The 7-day experimental period started after a 24 h acclimatisation period. DO, conductivity, pH and temperature were measured in the water overlying each core, first after the acclimatisation period and then between 14:30-15:30 h every day of the experiment. 50 mL water samples were collected daily (after measurement of the water physico-chemical parameters) from 1 cm above the sediment surface using a syringe. The sampled water was replaced with 50 mL filtered (through Whatman GF/F filter) lake surface water collected at the same time as the sediment cores and stored at the same temperature. This experimental set-up has been previously used in similar experiments (Spears et al. 2008).

5.2.5 Analyses of water samples from the field campaign and the experiment

PO_4-P and TP concentrations in the water samples were determined using the acid-molybdenum-blue colorimetric method (Murphy and Riley 1962), preceded by a persulphate digestion for the latter (as described in Eisenreich et al. 1975). Samples for determination of dissolved metals and metalloids were filtered through a Millex syringe-driven 0.45 µm filter prior to preservation with 1% v/v nitric acid. Samples for total metal and metalloid determination were preserved with 1% v/v nitric acid, followed by 16 h digestion at 80°C in an oven prior to analysis. Concentrations of metals and metalloids were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using an Agilent 7500ce. A detailed description of both methods is provided in Sections 2.2.1-2.2.2.

5.2.6 Statistical analyses

Monthly field observations (n = 12) were allocated to seasons (winter: Dec 2012 – Feb 2013, spring: Mar – May 2013, summer: Jun – Aug 2012, autumn: Sep – Nov 2012) and 2-way analysis of variance (ANOVA, total degrees of freedom = 23, $\alpha = 0.05$) was applied to examine the variation in concentrations of pollutants (P, As and V) and conductivity, pH, temperature and DO in lake water between the four seasons and between the two sample collection depths. Due to conditions in the lake water column varying from stratification to mixing throughout year, the pollutant concentration-sample collection depth relationship may change across seasons.

Therefore the sample collection depth-season interaction term was added to the models. Assessing residuals showed violation of homogeneity (an important assumption in analysis of variance; Zuur et al. 2010) for the PO_4-P, TP, dissolved V, pH and temperature observations. Log_{10} transformations were applied to these datasets, but the residuals still indicated homogeneity violation. Hence, a statistical technique that allows for heterogeneity - generalised least squares (GLS; Zuur et al 2009) - was applied to analyse both forms of P, dissolved V, pH and temperature data. For each of the five response variables, four GLS models - a model with no variance covariate and 3 models with heterogeneity incorporated as VarIdent variance structure (allowing a different variance per stratum either for season, water depth, or combined season-water depth) - were produced and the best model was chosen by comparing Akaike's Information Criterion (AIC) values. If a model term was non-significant in the model output, the model was re-run without the term and the AIC values were used to compare the simplified and full version of the models. Models with the lowest AIC values were selected for further analysis. Planned multiple regression following stepwise multiple regression was conducted to examine the relationships between pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V) and potential explanatory variables (pH, DO, conductivity and temperature) in both surface water and sediment + 0.01 m water. Explanatory variables used in the models were selected by running first a stepwise regression model to choose variables that did not correlate ($P > 0.15$) with each other. Only significant relationships ($P < 0.05$) were included in the final regression models. The normality assumption was examined with a normal probability plot of the residuals and Anderson-Darling tests. All data were log_{10} transformed to meet the normality.

A repeated measures one-way analysis of variance (ANOVA; total degrees of freedom = 69, $\alpha = 0.05$) was conducted on the results of the experiment to test the effects of oxic and anoxic conditions on the release of elements from sediment into overlying water and also to assess the effects of oxygen on water column conductivity, pH and temperature. Statistical analyses were conducted using R version 2.15.2. (http://www.r-project.org), with the exception of the planned multiple regression, which was carried out using Minitab statistical software version 16 (Minitab Ltd., Coventry, UK).

5.3 Results

5.3.1 Field observations: pH, conductivity, temperature and oxygen profile

Measurements of DO, pH and conductivity during a year at two water depths are shown in Fig. 5.1c, f and i, respectively. Seasonal mean concentrations of DO ranged from 1.55 ± 0.78 mg L^{-1} (sediment + 0.01 m water, summer) to 13.2 ± 2.07 mg L^{-1} (surface water, spring; Table 5.2). Mean DO values were similar in the surface and sediment + 0.01 m water in autumn and higher in the surface compared to sediment + 0.01 m water in winter and summer. The greatest difference in DO oxygen between the two water depths occurred in summer indicating the presence of lake water stratification in this season. The effects of season, depth and interaction between the two variables on DO concentrations were found to be significant (Table 5.3). Mean seasonal values of pH were very similar in surface and sediment + 0.01 m water throughout most of the year with the exception of summer, in which the highest seasonal mean pH (9.14 ± 0.03) was recorded in surface water and the lowest seasonal mean pH (7.69 ± 0.11) in sediment + 0.01 m water. Statistical analysis indicated significant effects of season, depth and interaction on water pH. The lowest (360 ±16.5 µS cm^{-1}) and highest (457 ± 10.39 µS cm^{-1}) mean seasonal conductivity were also recorded in summer. The lowest mean conductivity value was observed at the top of water column and the highest in the sediment + 0.01 m water, respectively. During the other three seasons conductivity values were slightly lower in the surface than sediment + 0.01 m water. All three explanatory variables were found to have a significant effect on conductivity. Seasonal mean temperature varied throughout the year from 2.87 ± 0.55 ^{o}C (surface water winter) to 16.6 ± 1.42 ^{o}C (surface water, summer). The difference in temperature between the two water depths in autumn - spring was small, with the greatest difference observed in summer. Higher surface water temperatures compared to sediment + 0.01 m water were recorded in spring and summer. Only season was found to have a significant effect on temperature.

Table 5.2. Means and standard deviations of the means of pH, conductivity (μS cm^{-1}), temperature (^{o}C) and DO (mg L^{-1}) across seasons and at 2 water column depths in Kinghorn Loch at the deepest site (12 m) from May 2012 to April 2013.

	Winter				**Spring**				**Summer**				**Autumn**			
	Surface		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**	
	mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
pH	8.57	0.12	8.50	0.09	8.84	0.35	8.82	0.28	9.14	0.03	7.69	0.11	8.47	0.36	8.53	0.11
conductivity	421	17.7	432	22.0	431	13.7	441	1.41	360	16.5	457	10.4	399	7.21	403	5.51
temperature	2.87	0.55	3.1	0.82	7.77	3.90	6.77	3.14	16.6	1.42	14.0	0.71	9.03	2.35	9.43	2.35
DO	11.4	0.18	8.78	3.51	13.2	2.07	11.4	2.43	12.1	0.96	1.55	0.78	10.5	1.03	10.1	0.91

Table 5.3. Results of two-way ANOVA and GLS analyses to assess the significance of the difference between pH, conductivity (μS cm^{-1}), temperature (^{o}C) and DO (mg L^{-1}) across seasons and at 2 water column depths in Kinghorn Loch at the deepest site (12 m) from May 2012 to April 2013.

	Season		**Depth**		**Interaction**	
	F-value	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
pH	4.44	<0.0189	292	<0.0001	65.8	<0.0001
conductivity	7.06	0.0035	29.9	<0.0001	15.0	<0.0001
temperature	175	<0.0001	1.22	0.2867	2.38	0.1078
DO	10.9	0.0006	30.4	0.0001	11.0	0.0006

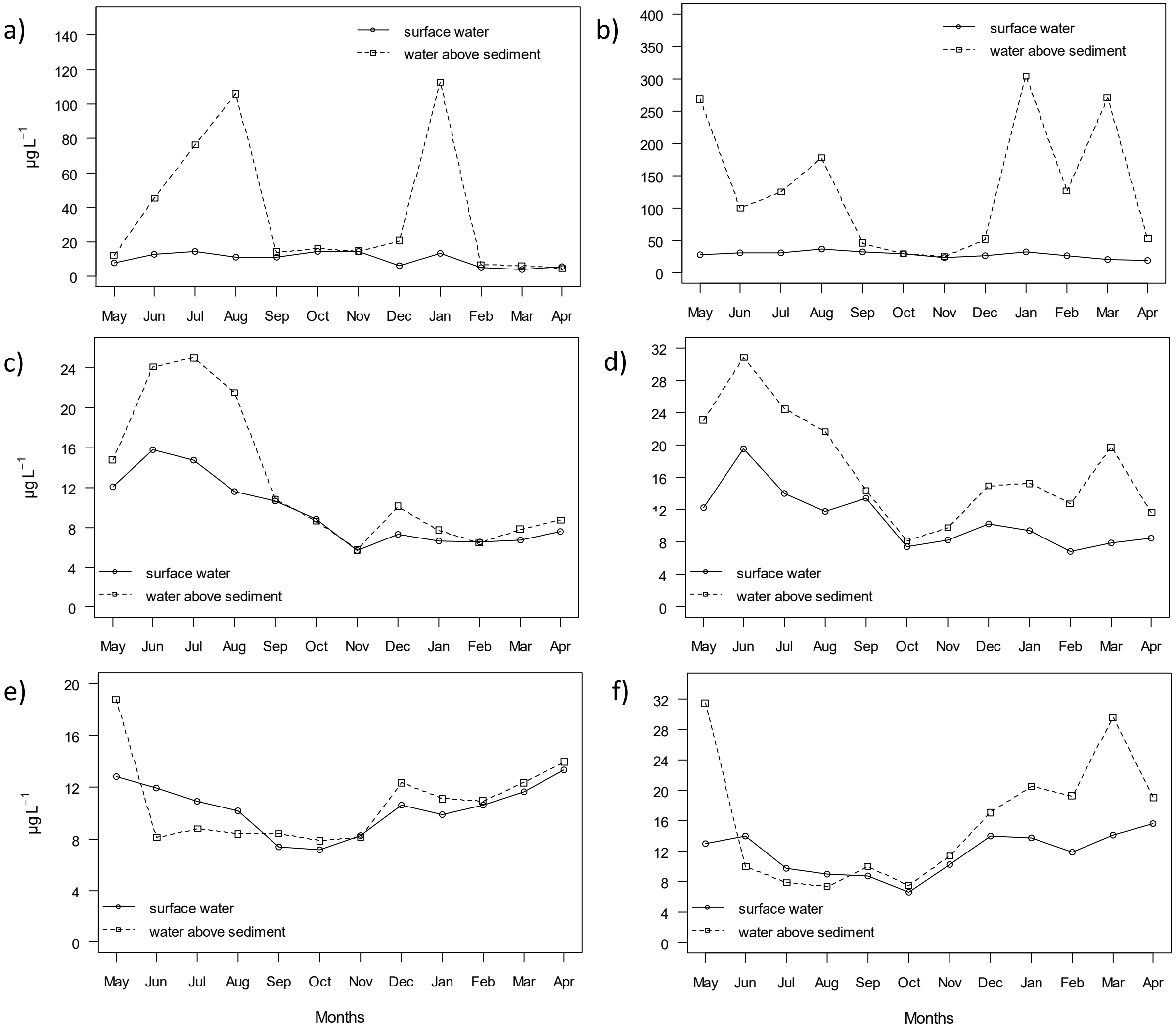

Fig. 5.1. Monthly pollutant concentrations (PO$_4$-P (a), TP (b), dissolved (c) and total As (d), dissolved (e) and total V (f)) in surface water and water 1 cm above the sediment in Kinghorn Loch at the deepest site (12 m) from May 2012 to April 2013.

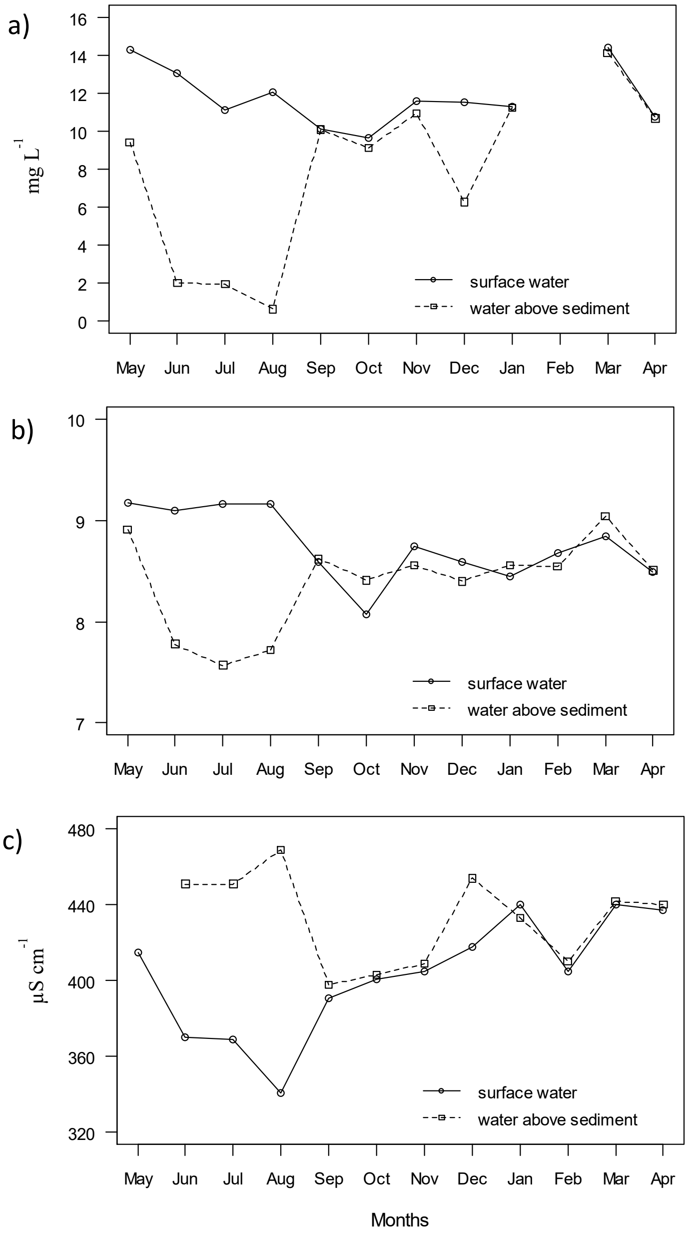

Fig. 5.2. Monthly water physico-chemical parameters (DO (a), pH (b), conductivity (c)) in surface water and sediment + 0.01 m water 1 cm in Kinghorn Loch at the deepest site (12 m) from May 2012 to April 2013. February DO measurements were not taken due to the failure of a measuring probe.

5.3.2 Field observations: water column pollutant concentrations

Fig. 5.1 shows monthly pollutant concentrations throughout the monitoring period in both surface and sediment + 0.01 m water. Seasonal mean concentrations of PO_4-P and TP (Table 5.4) ranged from 5.90 ± 1.90 (surface water, spring) to 75.9 ± 30.2 µg L^{-1} (sediment + 0.01 m water, summer) and from 22.7 ± 4.41 (surface water, spring) to 198 ± 125 µg L^{-1} (sediment + 0.01 m water, summer), respectively. Season and interaction between season and depth had a significant effect on PO_4-P, whilst season and depth were found to have significant influence on TP (Table 5.5). Seasonal mean dissolved and total As concentrations varied from 6.82 ± 0.43 µg L^{-1} (surface water, winter) to 23.5 ± 1.83 µg L^{-1} (sediment + 0.01 m water, summer) and from 8.88 ± 1.77 µg L^{-1} (surface water, winter) to 25.7 ± 4.72 µg L^{-1} (sediment + 0.01 m water, summer), respectively. Results indicated significant effects of season and depth on both dissolved and total concentrations of As, although interaction between the two explanatory variables was significant only in the case of the former. Seasonal mean concentrations of dissolved V ranged between 7.60 ± 0.58 µg L^{-1} (surface water, autumn) to 15.0 ± 3.35 µg L^{-1} (sediment + 0.01 m water, spring), whilst total V concentrations varied from 8.46 ± 1.37 µg L^{-1} (sediment + 0.01 m water, summer) to 26.7 ± 6.65 µg L^{-1} (sediment + 0.01 m water, spring). Season and interaction between season and depth were found to have a significant influence on the variation in V (dissolved and total) with depth having a significant effect only on the total V concentrations.

Whilst the highest P and As concentrations occurred in sediment + 0.01 m water in summer, dissolved and total V concentrations were highest in sediment + 0.01 m water in spring and V was the only element among the three pollutants for which the dissolved and total concentrations in summer were higher in surface water than in water close to sediment. For all three contaminants the smallest difference between the mean seasonal concentrations (both dissolved and total) in surface and sediment + 0.01 m water occurred in autumn. Within the three red mud constituents, the greatest observed difference between surface and sediment + 0.01 m water mean concentrations was for P, with PO_4-P in summer and TP concentrations in spring being six and eight times higher in sediment + 0.01 m water compared to surface water, respectively (Table 5.4). Of the elements that are likely to bind the three pollutants in sediment, mean dissolved Ca was the only element with similar (with the exception of surface water in summer) concentrations across the year and at the two water depths (Table 5.6). Concentrations of dissolved and total Al, Mn, Fe, and total Ca were higher in sediment + 0.01 m water in all seasons. The highest dissolved and total Mn and dissolved Fe concentrations were observed in summer, whilst the highest total Al, Ca, Mn and Fe occurred in spring and winter. Means and standard deviations of the means of other metals and metalloids that were measured are shown in Appendix B.1.

Table 5.4. Means and standard deviations of the means of PO_4-P, TP, dissolved and total As, dissolved and total V (μg L^{-1}) across seasons and at 2 water column depths in Kinghorn Loch at the deepest site (12 m) from May 2012 to April 2013.

Pollutant		Winter				Spring				Summer				Autumn			
		Surface		Sediment + 0.01 m		Surface		Sediment + 0.01 m		Surface		Sediment + 0.01 m		Surface		Sediment + 0.01 m	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Dissolved	As	6.82	0.43	8.09	1.86	8.80	2.90	10.5	3.79	14.1	2.20	23.5	1.83	8.37	2.55	8.40	2.55
	V	10.4	0.42	11.5	0.77	12.6	0.85	15.0	3.35	11.0	0.88	8.42	0.32	7.60	0.58	8.14	0.28
	PO_4-P	8.53	4.51	46.7	57.5	5.90	1.90	7.80	4.14	12.9	1.50	75.9	30.2	13.4	1.79	15.1	1.08
Total	As	8.88	1.77	14.3	1.41	9.59	2.38	18.2	5.90	15.2	4.01	25.7	4.72	9.73	3.26	10.8	3.25
	V	13.2	1.11	19.0	1.75	14.3	1.29	26.7	6.65	10.9	2.68	8.46	1.37	8.57	1.82	9.67	1.94
	P	28.9	3.04	162	130	22.7	4.41	198	125	33.4	3.25	135	39.6	29.0	4.11	34.1	11.3

Table 5.5. Results of two-way ANOVA and GLS analyses to assess the significance of the difference between pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V (μg L^{-1})) across seasons and at 2 water column depths in Kinghorn Loch at the deepest site (12 m) from May 2012 to April 2013.

Pollutant		Season		Depth		Interaction	
		F-value	P-value	F-value	P-value	F-value	P-value
Dissolved	As	27.6	<0.0001	9.74	0.0066	4.67	0.0158
	V	40.5	<0.0001	0.00	0.9952	10.3	0.0005
	PO_4-P	19.2	<0.0001	3.48	0.0805	4.49	0.0181
Total	As	9.27	0.0009	18.7	0.0005	1.95	0.1619
	V	21.5	<0.0001	12.8	0.0025	7.49	0.0024
	TP	3.99	0.0268	15.0	0.0013	1.83	0.1818

Table 5.6. Means and standard deviations of the means of metals and metalloids (µg L^{-1}) across seasons and at 2 water column depths in Kinghorn Loch at the deepest site (12 m) from May 2012 to April 2013.

Pollutant		**Winter**				**Spring**				**Summer**				**Autumn**			
		Surface		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Dissolved	Al	9.48	4.30	13.0	3.36	5.28	5.25	13.0	2.34	9.47	1.23	10.5	1.67	7.09	7.36	13.21	7.46
	Ca[a]	43.6	1.02	43.3	2.83	42.9	4.82	41.9	5.11	31.1	3.89	45.5	3.28	43.1	2.89	42.6	3.61
	Mn	0.42	0.28	23.0	27.9	0.38	0.30	9.89	7.23	2.02	0.52	407	17.3	0.37	0.22	6.2	5.94
	Fe	3.89	1.01	9.30	0.44	2.09	1.26	11.4	13.2	7.53	1.91	60.8	4.08	5.10	4.40	18.9	13.6
Total	Al	91.1	12.6	793	560	53.9	19.5	1541	1038	39.7	25.7	87.8	63.6	63.7	37.7	134	47.6
	Ca[a]	51.2	7.01	63.4	10.8	40.3	2.51	62.1	16.1	33.3	6.05	47.8	4.97	43.4	1.73	46.0	1.48
	Mn	5.05	1.43	48.9	17.8	4.20	3.76	75.1	40.0	13.8	1.90	368	40.0	8.64	4.52	16.4	7.38
	Fe	61.5	9.41	524	385	26.1	5.44	1005	776	28.1	1.41	121	29.4	61.4	39.7	111	41.8

[a] concentrations given in mg L^{-1}

5.3.3 Field observations: relationship between water column pollutant concentrations and physico-chemical parameters

DO was significantly positively correlated with dissolved V in surface water and negatively correlated with dissolved As and PO_4-P in sediment + 0.01 m water, accounting for 45% of the variation in V, 72% in As and 49% in PO_4-P concentrations (Table 5.7). There was a significant positive correlation between pH and dissolved and total concentrations of As in surface water and between pH and total V in sediment + 0.01 m water (34, 39 and 59% of variation explained, respectively). No significant effect of temperature on any of the pollutants was identified.

Table 5.7. Results of planned multiple regression following stepwise multiple regression to examine the relationship between pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V) and potential explanatory variables (pH, DO, conductivity and temperature) in both, surface water and sediment + 0.01 m water. Planned multiple regression was conducted at alpha = 0.05. Only significant results are reported.

	Pollutant concentration		P-value	Equation of line y = ….	R^2 (%)
Surface	Dissolved	As	0.047	- 3.80 + 5.05 pH	34
		V	0.024	- 0.171 + 1.10 DO	45
		PO_4-P	NS		
	Total	As	0.03	- 3.55 + 4.85 pH	39
		V	NS		
		P	NS		
Sediment + 0.01 m	Dissolved	As	0.001	1.40 - 0.433 DO	72
		V	NS	NS	NS
		PO_4-P	0.019	1.95 – 0.758 DO	49
	Total	As	NS		
		V	0.004	- 5.42 + 7.12 pH	59
		P	NS		

5.3.4 Oxygen manipulation experiment: pH, conductivity and temperature profile

A significant difference between DO concentrations measured under the two treatments (Table 5.8) demonstrated that they were effective in maintaining distinct oxic and anoxic conditions. Mean DO concentrations under the air treatment (11.0 ± 0.35 mg L^{-1}; Table 5.8) were similar to surface water conditions in Kinghorn Loch at the time of sampling (11.6 mg L^{-1}). Likewise DO concentrations under the N_2 treatment (0.66 ± 0.19 mg L^{-1}) were similar to anoxic conditions in the lake sediment + 0.01 m water (0.20 mg L^{-1}). Conductivity, pH and temperature values were significantly different under oxic and anoxic treatments, although the temperature difference was small. Conductivity and temperature were significantly higher in cores supplied with air, whilst pH was significantly higher in cores treated with N_2 (Table 5.8). The conductivity and pH in the experimental cores contrasted with the field measurements, where higher pH and lower conductivity were recorded in oxygenated surface water compared to anoxic bottom water in Kinghorn Loch. Conductivity under the air treatment and pH under both treatments were higher than Kinghorn Loch field measurements.

Table 5.8. Results of repeated measures one-way ANOVA analysis to assess the significance of the difference between pH, conductivity (μS cm^{-1}), temperature (°C), DO (mg L^{-1}) and pollutant concentrations (PO_4-P, TP, dissolved and total As, dissolved and total V (μg L^{-1})) under oxic and anoxic treatments during the 7-day incubation experiment.

Measured variables		**Oxic conditions**		**Anoxic conditions**		**Test statistic (F)**	**P-value**
		mean value	**s.d.**	**mean value**	**s.d.**		
Treatment variables	pH	8.36	0.16	9.18	0.21	335.1	<0.001
	Conductivity	401	163	305	5.50	12.03	0.001
	Temperature	9.76	0.42	9.39	0.14	23.65	<0.001
	DO	11.0	0.35	0.66	0.19	23310	<0.001
Pollutants (dissolved)	As	16.1	3.06	18.1	2.21	10.11	0.002
	V	9.36	3.35	4.04	0.89	82.39	<0.001
	PO_4-P	45.1	38.2	136	35.0	108.4	<0.001
Pollutants (total)	As	20.3	3.23	24.8	4.35	23.96	<0.001
	V	10.4	3.43	4.84	0.91	84.73	<0.001
	TP	69.1	39.7	201	45.3	169.2	<0.001

5.3.5 Oxygen manipulation experiment: water column pollutant concentrations

There were differences in pollutant concentrations between the treatments observed on every day of the experiment (Fig. 5.2). PO_4-P and TP (Fig. 5.2a and b) concentrations throughout the 7-day incubation period were significantly higher under anoxic conditions than oxic (Table 5.8). Similar differences were observed for As, with dissolved and total concentrations (Fig. 5.2c and d) significantly higher under anoxic conditions. Dissolved and total V (Fig. 5.2e and f) also differed significantly under different redox conditions. However, in contrast to P and As, V concentrations were higher under oxic conditions compared with anoxic. Both dissolved and total V concentrations decreased during the experiment under oxic conditions. Among the three contaminants, the difference between the mean concentrations under oxic and anoxic conditions was smallest for As (dissolved and total) and greatest for P, with mean PO_4-P and TP concentrations three times higher under anoxic conditions compared to the oxic treatment (Table 5.8, Fig. 5.2).

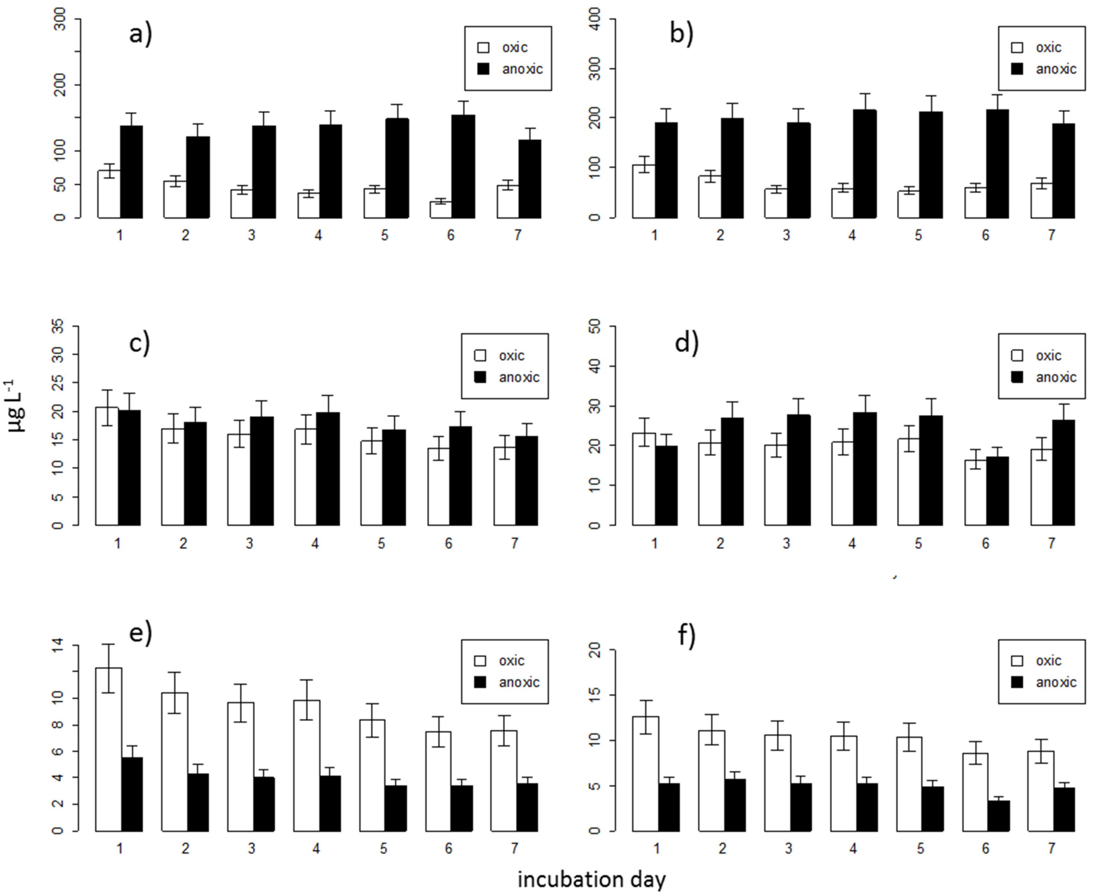

Fig. 5.3. Water column pollutant concentrations (PO_4-P (a), TP (b), dissolved (c) and total As (d), dissolved (e) and total V (f)) under oxic and anoxic treatments during the 7-day incubation experiment. Bars represent the mean of 5 cores from each treatment and error bars standard deviation of the mean.

Mean concentrations of the four pollutant-binding elements did not differ significantly between the treatments (Table 5.9). However, Al, Mn and Fe (with the exception of dissolved Al) were higher under anoxic conditions. Concentrations of dissolved and total Ca remained similar under both treatments. The high standard deviations for Al, Mn and Fe are likely to be a result of analytical uncertainty due to the sensitivity of the instrument used for the analysis, rather than inherent variability in lake water. The results for other measured metals and metalloids are included in Appendix B.2.

Table 5.9. Results of repeated measures one-way ANOVA analysis to assess the significance of the difference between water column concentrations of dissolved and total pollutant-binding elements (μg L^{-1}) under oxic and anoxic treatments during the 7-day incubation experiment.

Measured variables		Oxic conditions		Anoxic conditions		Test statistic (F)	P-value
		mean value	s.d.	mean value	s.d.		
Pollutants (dissolved)	Al	44.1	181	20.1	26.08	0.60	0.440
	Ca[a]	42.1	2.62	41.3	2.72	1.56	0.216
	Mn	173	305	248	77.4	1.95	0.167
	Fe	28.3	24.3	35.9	18.0	2.18	0.144
Pollutants (total)	Al	33.3	15.4	36.0	5.39	0.923	0.340
	Ca[a]	47.7	5.14	50.0	9.04	1.71	0.195
	Mn	243	336	289	85.9	0.60	0.443
	Fe	90.7	53.3	117	65.2	3.47	0.067

[a] concentrations given in mg L^{-1}

5.4 Discussion

This study, combining both field observations and a laboratory experiment, indicates that the internal cycling of three red mud constituents (As, V and P) in Kinghorn Loch is persistent, 30 years following the control of the leachate, with redox conditions being one of the factors controlling the process. Arsenic, V, and P showed significant seasonal variations in lake water, with higher concentrations in deeper water, and there were significant differences in pollutant concentrations between the oxic and anoxic treatments during the experiment. This is generally consistent with the investigations of the behaviour of P (Søndergaard et al. 2003, Smolders et al. 2006), As (Toevs et al. 2008, Whitmore et al. 2008) and V (Minelli et al. 2000) in aquatic environments. Low oxygen levels can affect the sediment sorption capability by reduction of pollutant-binding Fe and other metal oxyhydroxides, resulting in the release of soluble forms of elements into the overlying water (Smolders et al. 2006, Partey et al. 2008, Whitmore 2008, Couture et al. 2010). The operation of this mechanism was indicated in bottom waters in Kinghorn Loch in summer, when DO concentrations, which changed significantly across seasons, were the lowest during the year and corresponded with the stratification of the lake. The concentrations of dissolved Fe and Mn were much higher in summer in surface and sediment + 0.01 m water compared to other seasons. The highest seasonal dissolved and total As at both water depths, and PO_4-P concentrations in sediment + 0.01 m water, also occurred in summer. As and P concentrations varied significantly during the year and the results of the multiple regression indicated that dissolved As and PO_4-P in bottom water were significantly and negatively related to DO across the 12-month period. This pattern was also evident in the sediment core experiment, where significantly higher P and As concentrations were measured in the anoxic treatment. The role of the pollutant-binding elements, however, was not as evident in the laboratory controlled environment as Fe, Mn, Al and Ca concentrations were not significantly different between the oxic and anoxic treatments. These results, together with the field observations of variation in As and P concentrations, indicate the importance of DO levels for regulating P and As release from sediments in Kinghorn Loch, which can result in seasonal increases in concentrations in the lake water column (Fig. 5.1), especially under stratified conditions.

A number of previous studies have also observed seasonal variations in release of P from bed sediments related to changing DO concentrations, with an increase in sediment sorption capacity for P in winter and decrease in summer (e.g., Kleeberg and Kozerski 1997, Søndergaard et al. 1999, Spears et al. 2012). The release of As from sediment under anoxic conditions during the present experiment and the observation of the highest concentrations of As in bottom water in Kinghorn Loch during summer stratification are in contrast with the results from Crowley Lake, USA. The latter study found no evidence for significant sediment-water exchange of total As under stratified conditions (Kneebone and Hering 2000). However, high concentrations of As have been measured in surface sediment from both lakes, ranging from 86 to 185 mg kg^{-1} in Kinghorn Loch (Section 4.3.5.2) and from 140 to 260 mg kg^{-1} in Crowley Lake (Kneebone and Hering 2000). The difference in As behaviour can be explained by immobilisation of As in sediment at Crowley Lake due to its occurrence as the sulphide phase, which is stable under reducing conditions (Kneebone and Hering 2000). Similarity between the variation of dissolved and total As, and also between PO_4-P and total P in the current study (Fig. 5.1) indicates strong dependence of total concentrations of these two elements on their soluble forms. This is confirmed by the similarity between the proportions of total As, which comprised dissolved As (79% in oxic and 73% in anoxic conditions), and of TP, which comprised PO_4-P (65% in oxic and 68% in anoxic conditions) under both treatments.

Significant differences in dissolved and total As and TP concentrations between water depths in Kinghorn Loch indicate that the As and TP seasonally mobilised from sediment is mostly contained within bottom waters (Fig. 5.1). This is also apparent for PO_4-P, with ANOVA results showing a significant influence of season on the concentration-water column depth relationship. Similar PO_4-P concentrations between depths were only observed in spring and autumn, when the lowest annual sediment + 0.01 m water concentrations of PO_4-P were close to low PO_4-P observed in surface water. The summer and winter release of PO_4-P from sediment, reflected by much higher concentrations in sediment + 0.01 m water during these two seasons, did not affect the surface water concentrations, which remained low throughout the year, showing little variation. The multiple regression analysis did not show any significant correlation between surface water concentrations of P and measured physico-chemical parameters. The analysis indicated, however, that increased concentrations of dissolved and total As in surface water were significantly positively related to pH. The highly significant positive correlation between pH and DO in sediment + 0.01 m water (correlation coefficient = 0.899, $P < 0.001$) suggested that pH might also be one of the factors responsible for driving release of As and P from loch sediment. Increased presence of the dissolved As fraction, due to the enhanced mobility of this element at high pH, was previously reported in streams in Sweden (Wällstedt et al. 2010).

V adsorption to iron oxides, together with redox potential, have been shown to strongly control the aqueous geochemistry of this element (Peacock and Sherman 2004), and thus it was hypothesised that V behaviour would be similar to As and P in this study. However, significant differences in V concentrations occurred in the two experimental treatments, with higher dissolved and total V concentrations in the overlying waters observed under oxic compared to anoxic conditions. The experimental finding is consistent with the fieldwork measurements in Kinghorn Loch bottom waters in summer, when mean dissolved As and PO_4-P concentrations were highest compared to other seasons and dissolved V concentrations (together with the autumn observations) were lowest. Furthermore, multiple regression analysis showed a significant positive relationship between dissolved V concentrations and DO concentrations in surface waters. Dissolved and total V varied significantly throughout the year with the highest seasonal concentrations recorded for both sampling depths in spring. These results are in contrast with the cycling pattern of V, characterised by significantly higher concentrations of dissolved V in summer compared to spring, reported in coastal waters of Long Island Sound, USA (Wang and Wilhelmy 2009). Together, the evidence from the experiment and the Kinghorn Loch field observations suggest that the internal cycling of V in Kinghorn Loch differs from cycling of As and P, and is not controlled predominantly by redox processes.

Although interest in V behaviour in the environment is growing (Cappuyns and Swennen 2014), processes controlling seasonal changes in concentrations of dissolved V in aquatic ecosystems are still not well understood (Wang and Wilhelmy 2009). In addition, the majority of studies have investigated the V behaviour in controlled laboratory conditions, oceans, estuaries and groundwater (e.g. Blackmore et al. 1996; Audry et al. 2006; Pourret et al. 2012; Wright et al. 2014; Cinti et al. 2015), rather than in lakes. pH, together with redox conditions, are the main drivers of V speciation. V exists in three oxidation states in the aqueous environment - V^{3+}, V^{4+} and V^{5+} (Wehrli and Stumm 1989) - which are characterised by different solubility and adsorption behaviour (Minelli et al. 2000; Wright and Belitz 2010; Fig. 5.4). The thermodynamic data suggest that in oxic and alkaline water formation of soluble V(V) oxyanions occur, in more reducing and acidic conditions occurrence of less soluble V(IV) oxyanions is favoured, whereas under reducing conditions over a wide pH range V(III) dominates (Wright and Belitz 2010). V(V) behaves in a similar way to PO_4-P and forms surface complexes with hydrous oxides by ligand exchange (Pourret et al. 2012).

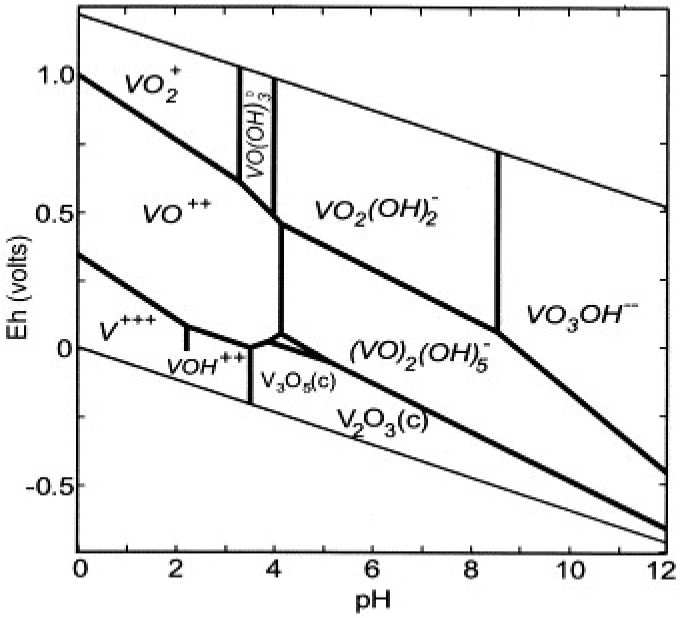

Fig. 5.4. Eh-pH diagram for V aqueous species illustrating the occurrence of V species as a function of pH and redox potential (from Peacock and Sherman 2004).

Harita et al. (2005), in a laboratory experiment using water and sediment from Lake Biwa, Japan demonstrated that the release of V from sediment observed under oxic conditions in the lake was a response to increase in pH, which was the predominant driver of V mobilisation. Naeem at al. (2007) reported a strong effect of pH on V adsorption onto metal oxides in laboratory controlled conditions, with an increase in adsorption of V as pH increased from 2 to 4 and a linear decrease as pH rose from 4 to 11.6. The results of these studies seems to be in agreement with the Kinghorn Loch field observations, in which the highest seasonal V concentration in sediment + 0.01 m water corresponded with the season of highest pH and oxic conditions, and V and pH trends were similar throughout the year (Fig. 5.1). pH varied significantly across the seasons and depths and regression analysis indicated a significant positive relationship between pH and total V concentrations in sediment + 0.01 m water. It is, however, inconsistent with the experimental results in which adsorption of V was higher under the N_2 treatment characterised by higher mean pH compared to the oxygen treatment.

However, Wright and Belitz (2010) indicated that, although the effect of pH on V concentrations was evident in oxic groundwater, it was a less significant driver under anoxic conditions, perhaps due to V precipitation as insoluble oxyhydroxides and/or strong adsorption of reduced cationic forms of V(IV and III) to negatively charged mineral surfaces. This may explain the lower V concentrations under the anoxic treatment compared to oxic conditions observed in this study, despite pH being higher in the former. Moreover, Wright and Belitz (2010) reported significantly higher V concentrations in oxic compared to anoxic groundwater, similar to other studies of groundwater (Wright et al. 2014; Cinti et al. 2015). In addition, Wright et al. (2014) measured higher concentrations of V than As in oxic groundwater and the opposite in anoxic groundwater samples, indicating a different behaviour of As and V in response to varying redox conditions in aqueous systems. Cinti et al. (2015) suggested that mobilisation of V was favoured under oxidising conditions at near neutral pH due to the formation of highly soluble V(V) oxyanion, whereas under reducing conditions relatively insoluble species of V(III) were prevalent. The experimental results for V, as well as the summer field observations at Kinghorn Loch, appear to be in agreement with these studies. They are also consistent with the study of a river-wetland-lake system in the Balaton Region, Hungary, which indicated V removal under anoxic conditions in one of the lakes (Elbaz-Poulichet at al. 1997). Similar behaviour of V in response to redox conditions has also been observed in seawater. Dissolved V concentrations were found to be lower in anoxic ocean basins compared to oxic sea water, due to the removal of the element into anoxic sediment (Emerson and Huested 1991). Audry et al. (2006) indicated removal of V in the sulphate reduction zones, following initial adsorption to Fe oxides, in the freshwater reaches of the Gironde Estuary, France. Reduction of V(IV) to V(III) under the presence of sulphide with subsequent precipitation of V(III) as an oxide solid or co-precipitation of V(III) with Fe oxides were indicated as two possible explanations for the removal of V under anoxic conditions in groundwater (Wright et al. 2014).

Competition for binding sites can be another factor controlling the cycling of V, as well as P and As. The extent of anion sorption depends on its relative affinity for the sorption material. Wällstedt et al. (2010) indicated that vanadate (VO_4^{3-}) should be more strongly adsorbed to ferrihydrite than PO_4^{3-}, which in turn shows higher adsorption strength than arsenate (AsO_4^{3-}). The results for dissolved As and PO_4-P in the present study appear to reflect this relationship, with a greater difference between PO_4-P concentrations in oxic and anoxic conditions compared to As, suggesting that PO_4-P was more strongly absorbed by ferrihydrite. The V results, however, contradict a stronger adsorption of V compared to PO_4-P.

Blackmore et al. (1996), in a study assessing the removal of V from corrosion inhibitor effluent, showed that with decreasing concentrations of iron oxyhydroxides, the effect of pH and presence of PO_4-P on the extent of adsorption of VO_4^{3-} increased. Moreover, the study indicated that the presence of PO_4-P in aqueous solution at higher concentration than V will severely interfere with the adsorption of the latter, not only by competing for adsorption sites with V, but also by interfering with the co-precipitation process. The higher PO_4-P compared to dissolved V concentrations in the Kinghorn Loch water column measured in sediment + 0.01 m water in summer and under reducing conditions in the laboratory experiment, suggest that competitive adsorption of PO_4-P could result in the persistence of V under oxidising conditions. This also appears to be in agreement with the increased concentrations of dissolved forms of V and As attributed to high PO_4-P concentrations (and also the effect of high pH in some streams) previously reported in streams in Sweden (Wällstedt et al. 2010). To better understand the mechanisms driving the internal cycling of V in Kinghorn Loch the interactions between pH, competitive ion concentration, and redox conditions need to be assessed further and speciation of V should be examined. An investigation on the influence of other determinands, such as sulphate, nitrate and total alkalinity would also help to understand the results recorded in the present study and is recommended for any future research to better understand the processes that drive cycling of V, as well as As and P.

Even though this study has demonstrated a seasonal increase in the water column in red mud contaminants released from sediment, the higher concentrations appear to be confined to the bottom waters of Kinghorn Loch. The total As concentrations measured during the experiment and field campaign, as well as total V in the experiment did not exceed Standards for Protection of Aquatic Life in the UK (50 µg L^{-1} (EEC 1976) and 20 µg L^{-1} (EEC 1976), respectively). The standards were not, however, met for total V in Kinghorn Loch sediment + 0.01 m water in spring 2013. TP concentrations measured during the experiment and field campaign (with the exception of surface water in spring) exceeded the Trophic State Index value of 24 µg L^{-1} for TP, above which a lake is classified as eutrophic (Carlson et al. 1996).

Conclusions

The results of the laboratory experiment indicated that the bed sediment of Kinghorn Loch still acts as a source of As, P and V to the water column, as was confirmed by the higher concentrations of red mud constituents in sediment + 0.01 m water compared to surface water. The laboratory experiment suggested seasonal mobilisation of As and P from lake sediment under reducing conditions and this was also indicated by the field observations. The V release from sediment was significantly higher under oxic conditions and appeared to be controlled by the interaction of multiple factors which may include competitive ion concentration, pH and redox conditions.

Chapter 6 Accumulation of As species in macrophytes from a shallow lake recovering from red mud pollution (Kinghorn Loch, Scotland, UK)

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The chapter has been prepared as a manuscript intended for submission to the journal "Environmental Science & Technology". The candidate, as lead author, performed the sample collection and preparation and was involved in laboratory determination of arsenic species in plant tissues. The data analysis and writing of the paper was carried out by the candidate. Co-authors provided support and guidance on the scope and design of the project and expertise in coupled ion chromatography and ICP-MS methods. Co-authors contributed to the editing of the manuscript.

6.1 Introduction

The estimated global production of red mud, a by-product of alumina production, is ~120 million t a^{-1} (Power et al. 2011). In October 2010, failure of a containment reservoir in Ajka, Western Hungary, resulted in the release of ~1 million m^3 of red mud waste, contaminating the Marcal River (catchment area 3078 km^2) and entering the Danube River (Klebercz et al. 2012). The Hungarian red mud spill raised concerns about inappropriate storage of red mud and the impact of the waste on the receiving environment.

Little is known about the geochemical behaviour of red mud in freshwaters (Klebercz et al. 2012) or of the short- and long-term ecological impacts and likelihood of recovery following its release into the environment. Red mud is highly alkaline due to the addition of sodium hydroxide (NaOH) during the production process and contains metal oxides and elevated concentrations of a range of minor trace elements (Dubey and Dubey, 2011; Edwards 1985; Gelencsér et al. 2011), which can be toxic to species throughout the aquatic food web. Initial research after the Ajka accident focused on the short-term impact of the pollution on freshwaters. Mayes et al. (2011) showed the abundance of some contaminants, e.g. arsenic (As), vanadium (V), chromium (Cr) and nickel (Ni), in fluvial sediment downstream of the spill. These elements when present in well oxygenated surface sediment layers were associated mainly with 'hard-to-leach' residual phases, unlikely to be mobile in the environment. However, in reducing conditions, which can occur in depositional zones, release of these elements may be expected (Mayes et al. 2011). Furthermore river sediments contaminated with constituents of red mud have been shown to have pronounced toxic effects, causing reduced bioluminescence of *Vibrio fischeri* bioluminescence and growth of *Lemna minor* and roots and shoots of *Sinapis alba* (Klebercz et al. 2012). In aquatic ecosystems with short residence times the long-term effects of pollution might be spatially limited to depositional zones, allowing system recovery (Klebercz et al. 2012). However the residence time of pollutants in higher retention time aquatic ecosystems can exceed those in fluvial systems (Spears et al. 2012; Sharpley et al. 2013). Therefore, long-term monitoring of the red mud impact on aquatic life is particularly important in lakes, which can have potentially longer time-scales of recovery.

Among red mud contaminants, As appears to be a pollutant that poses a very high risk to the environment. It is a toxic element (Rahman et al. 2008a), with high concentrations posing a threat to aquatic ecosystems and human health reported worldwide, e.g. in Pakistan, Bangladesh, India, Taiwan (Shah et al. 2009), Japan (Hirata et al. 2011), China (Chen et al. 2008) and the USA (Rowe at al. 2002). In macrophytes, uptake of As can reduce phosphorus (P), nitrogen (N), potassium (K), chlorophyll *a* and protein content in tissues and, due to the chemical similarity of As to P, can interfere with some biochemical reactions (Mishra et al. 2008).

Concentrations of contaminants accumulated by plants can be higher than in water, but lower than in sediment (Mazej and Germ 2009). The bioavailability of trace elements to plants depends on the concentration of contaminants in the environment, trace element speciation, exposure time, sampling period, abiotic factors and type of absorption mechanism (Mazej and Germ 2009). In addition, the capacity of aquatic plants to sequester and accumulate metals is affected by many factors, such as plant growth rate, biomass accumulation and affinity for metal uptake (Mishra et al. 2008). Previous studies have shown that As bioaccumulation varies among macrophyte species and among aquatic plant tissues (Taggart et al. 2005; Mishra et al. 2008; Zhang et al. 2008; Mazej and Germ 2009).

Arsenic can occur in inorganic and organic chemical forms, or species, which are associated with different levels of toxicity (Koch et al. 2000). For example, the inorganic species arsenite (As(III)) is more toxic than organic arsenobetaine to plants and animals (Koch et al. 2000). The uptake mechanism (Rahman et al. 2008b) and level of accumulation in plants (Koch et al. 2000; Rahman et al. 2011) also vary between As species. Significant differences between concentrations of different As species have been reported in rice (*Oryza sativa* L.), with the predominant form being inorganic As (Abedin et al. 2002; Rahman et al. 2011). Determination of As speciation in plants is important for assessing the plant As toxicity to consumers at higher levels of the food chain (Zhao et al. 2010). Most previous research on As species in plants has focused on crops and As speciation in plants in the aquatic environment has not been extensively studied, with most of the existing knowledge based on controlled laboratory experiments (e.g. Rahman et al. 2008a, Rahman et al. 2008b, Zhang et al. 2008). Consequently, little is known about variation of individual As species between tissues of aquatic plants. Koch et al. (2000), Rahman et al. (2007) and Rahman et al. (2008a) determined inorganic and organic As species in whole macrophytes, but did not examine concentrations of As species in different plant tissues. Determination of the species of As accumulated in macrophytes provides important information about the toxicity of the contaminant that can be transferred to higher organisms in aquatic food web (Alam et al. 2002; Gómez et al. 2004). This allows for more accurate assessment of the environmental risk, which increases with the occurrence of inorganic As and is particularly important at sites where fish and waterfowl are used for human consumption.

The present study aims to assess the impact of As on five macrophyte taxa - three submerged species (*Potamogeton pectinatus* L., *Elodea nuttallii* (Planch) H. St. John, *Myriophyllum spicatum* L.), one submerged species with floating leaves (*Persicaria amphibia* (L.) Gray) and one multicellular algae (*Chara* spp.) in Kinghorn Loch 30 years after the diversion of red mud leachate. Arsenic speciation analysis was applied to quantify inorganic As (arsenite, As(III) and arsenate, As(V)) and four organic As species: arsenobetaine, dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and tetramethylarsonium ions (Tetra). The following hypotheses were tested: (1) macrophytes in Kinghorn Loch accumulate higher concentrations of inorganic compared to organic As species, (2) accumulation of As species varies between above-sediment tissues (leaves and stems) of macrophytes and among macrophyte species, and (3) accumulation of As species varies between above- and below-sediment surface tissues of *Persicaria amphibia*, with the greatest concentrations accumulated in the roots of the plant.

6.2 Methods

6.2.1 Study site

Kinghorn Loch is a small lake of surface area 11.3 ha, mean depth 4.5 m and maximum depth 12.8 m, situated in Fife, Scotland (56^{o}10′N; 3^{o}11′W). The lake received highly alkaline leachate from a nearby red mud landfill site from 1947 to 1983 when the discharge was diverted. Caustic liquor reaching the loch consisted of a highly alkaline solution of NaOH and carbonate, with mean pH of 12.1. It contained high concentrations of dissolved Al, As, V, orthophosphate, sulphate and chloride. Red mud solids, released to the loch sporadically, accounted for most of the elevated input of Fe (Edwards, 1985). Long-term monitoring of water chemistry of the loch was initiated in 1980 by the Forth River Purification Board and continued by the Scottish Environment Protection Agency (SEPA). 30 years after the diversion of red mud pollution from Kinghorn Loch, concentrations of As in lake surface sediment ranged from 191 to 277 mg kg^{-1} (in 2010; unpublished data) and exceeded the Canadian Sediment Quality Guidelines for Protection of Aquatic Life, which predicts toxicity from sediment concentrations ≥ 17 mg kg^{-1} of As (Canadian Council of Ministers of the Environment 1999a). These concentrations of As indicate a potential risk to lake macrophytes.

6.2.2 Plant sampling

Plant sampling was conducted on 18 July 2013 when whole plants were collected by hand and using a double-headed rake from a boat at three sites in Kinghorn Loch (Table 6.1, Fig. 6.1).

Table 6.1. National Grid References of plant sample sites in Kinghorn Loch and approximate distance site distance from the NW part of the lake.

Site	NGR	Distance (m)
1	NT 25756 87422	100
2	NT 25771 87179	250
3	NT 26071 87285	430

The location of the sites was chosen to represent areas at different distances and directions from the NW part of the lake, where the pollution entered the waterbody. Between 3 and 10 plants per taxa (depending on the species specific volume of fresh plant material) were collected at each site. Samples represented the five most abundant macrophyte taxa in Kinghorn Loch as determined by a full lake survey - *Persicaria amphibia, Potamogeton pectinatus*, *Elodea nuttallii*, *Myriophyllum* *spicatum and Chara* spp. The macrophyte community survey method followed the procedure outlined in Gunn et al. (2010) and is described in detail in Section 2.2.5.

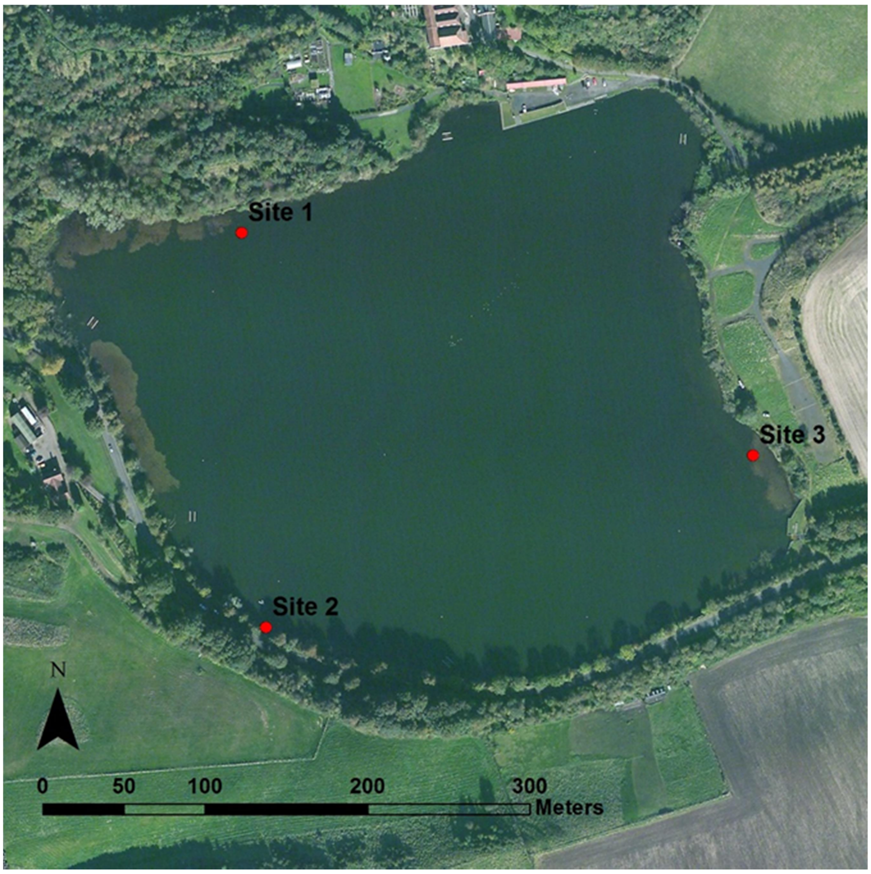

Fig. 6.1. Macrophyte sampling sites at Kinghorn Loch (ESRI ArcMap 10.1, image date 5.4.2011).

6.2.3 Sample preparation

The macrophytes were stored in polyethylene bags and stored at 4°C until processing in the laboratory the next day. Any sediment and macroinvertebrates on the plant material were removed by rinsing in distilled water. To test for any variation in As accumulation in different parts of macrophytes, *P. pectinatus*, *E. nuttallii* and *M. spicatum* samples were divided into leaves and stems, and samples of *P. amphibia*, the only collected species with well-developed roots, into leaves, stems and roots. Axes and branches of multicellular algae *Chara* spp. were also separated and are referred to as stems and leaves, respectively, for the purpose of statistical analysis. Tissue samples of each macrophyte tissue type and species were combined by site from a number of individual plants to obtain enough dry material for As analyses.

Samples were then oven dried at 80°C and ground using a Retsch mixer mill MM200.

6.2.3 Chemical analysis

Three replicate samples of each tissue type from each macrophyte species were analysed. Approximately 100 mg ± 5 of each powdered sample was digested with 1% nitric acid using a CEM Mars 6 1800W microwave digester. Arsenic speciation was determined by ion chromatography (Thermo Dionex IC5000 Ion Chromatograph) coupled to inductively coupled plasma mass spectrometry (Thermo Scientific iCap Q ICP-MS). Arsenic species in the solutions were identified by comparing their retention times with those of standards containing inorganic As (As(III) and As(V)), arsenite, dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), tetramethylarsonium iodide (Tetra) and quantified from calibration curves using peak areas. Species with retention times not matching any of the standards were classified as unknown species. Total identified As species were calculated as the sum of identified inorganic and organic As species (Equation 6.1). The detection limit for each As species was 0.001 mg kg^{-1}. The method for determination of As speciation is described in detail in Section 2.2.4.

$$\text{Total identified As species} = \text{inorganic As} + \text{arsenite} + \text{DMA} + \text{MMA} + \text{Tetra} \quad (6.1)$$

6.2.4 Statistical analyses

The means of three replicate samples were used for all statistical analyses. Concentrations of different As species in *P. amphibia* tissues (leaves and stems) were analysed together with data for other macrophytes species to examine across species variation in the above-sediment parts of macrophytes, and separately (concentrations in leaves, stems and roots) to test for differences between As concentrations in below- and above-sediment parts of *P. amphibia.*

A one-sided two sample t-test was used to test whether the mean concentration of inorganic As was greater than the mean concentration of total organic As in the above sediment tissues of macrophytes and also whether below-sediment surface tissues of *P. amphibia* contained higher concentrations of As species than above-sediment tissues.

Mixed effect modelling was conducted to examine variations in concentrations of each As species across macrophyte species and tissue type (within shoots). Root samples were not included in these models, as they were only available for one species. Model validation was conducted on the final models. Homogeneity of variance was assessed by plotting residuals versus fitted values and the normality assumption was evaluated by plotting theoretical quantiles versus standardized residuals (Q-Q plots). In models with all macrophyte species, several response variables (i.e. inorganic As, arsenobetaine, total organic and the sum of all identified As species) were $\log_{10}$ transformed to meet homogeneity and normality assumptions. The random effect part of the model allowed for variations among sites and incorporated a split plot design of the data. The analysis started with the model containing both nominal fixed explanatory variables (i.e. macrophyte species and macrophyte tissue) and the two-way interaction between these two terms. The model selection process followed the procedure described in Zuur et al. (2009). Maximum likelihood ratio (ML) tests were performed, using the ANOVA function, to select the best-fit model by comparing AIC values (as described in Section 5.2.6) and to determine the significance ($P < 0.05$) of dropped terms. The final model was re-run using restricted maximum likelihood (REML). Statistical analyses were conducted using R version 3.0.1 (http://www.R-project.org, R Core Team, 2013), using the 'nlme' package (Pinheiro et al. 2014) for the mixed modelling.

6.3 Results

6.3.1 Difference between inorganic and organic As concentrations across macrophyte species

Fig. 6.2 shows variations in As species concentrations across five macrophyte species and different plant tissues of each of the species. The above-sediment parts of the five macrophyte species in samples combined across sampling sites contained significantly higher concentrations of inorganic As species than total organic As, with mean values of 8.80 mg kg^{-1} d.w. (s.d. 13.8) and 0.56 mg kg^{-1} d.w. (s.d. 0.44), respectively (Table 6.2). Higher concentrations of inorganic As were measured compared to total organic As concentrations in both tissues of all macrophytes species (Table 6.3).

P. amphibia (combined above and below-sediment surface tissues) also contained significantly higher concentrations of inorganic As than total organic As, with mean values of 40.0 mg kg^{-1} d.w. (s.d. 65.7) for inorganic and 0.25 mg kg^{-1} d.w. (s.d. 0.11) for organic forms (Table 6.2).

Table 6.2. Results of one-sided t-test analysis to assess if inorganic As concentration is greater than total organic As concentration (mg kg^{-1}) measured in tissues of five macrophyte species and in *P. amphibia* in Kinghorn Loch in July 2013.

	Total inorganic As		**Total organic As**		**t-value**	**df**	**p-values**
	mean	**s.d.**	**mean**	**s.d.**			
All macrophyte species (above sediment tissues)	8.80	13.8	0.56	0.44	3.2607	29.06	0.001
P. amphibia (leaves, stems, roots)	40.0	65.7	0.25	0.11	2.0072	10.00	0.036

6.3.2 Differences in As species accumulation in above-sediment macrophyte tissues

The lowest mean concentrations of inorganic As in the above sediment-surface tissues were measured in the leaves and stems of *P. amphibia* (0.76 mg kg^{-1} ± 0.14 and 1.07 mg kg^{-1} ± 0.29, respectively) and the highest in leaves of *E. nuttallii* (45.1 mg kg^{-1} ± 20.7; Table 6.3). Two organic As species (MMA and Tetra) were below limit of detection in all tissue types of *Chara* spp., with MMA also not detectable in leaves of *M. spicatum.* Mean concentrations of the four organic As species ranged from 0.01 mg kg^{-1} d.w. (DMA in *Chara sp*. branches, MMA in both tissues of *P. amphibia*, Tetra in *P.amphibia* leaves) to 1.16 mg kg^{-1} (arsenobetaine in *Chara* spp. branches). Unknown As species were present in the leaves of *P. pectinatus* and *M. spicatum* and in both tissues of *Chara* spp., with the highest mean concentration (1.04 mg kg^{-1} ± 0.47) measured in the latter species.

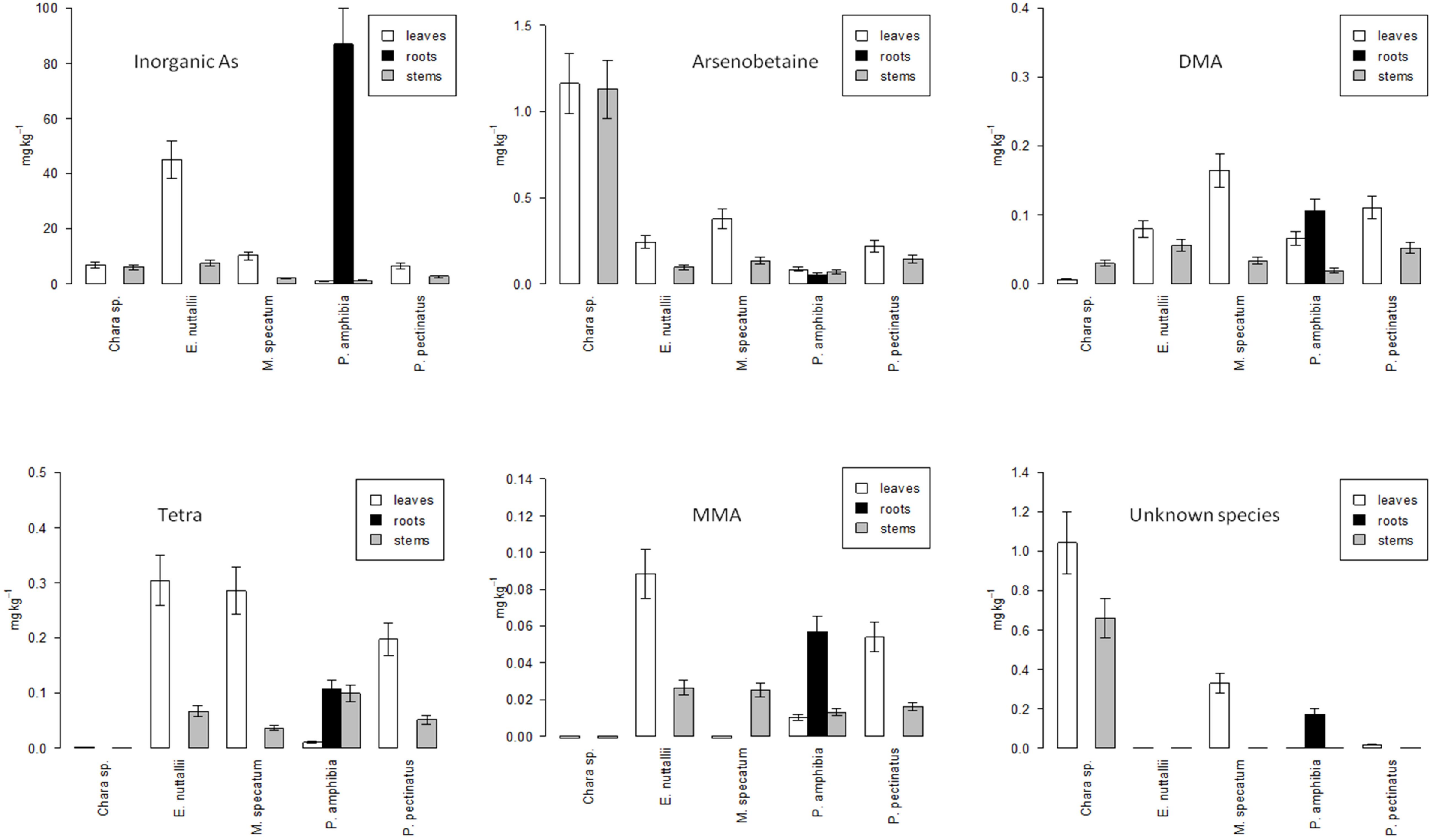

Fig. 6.2 Concentrations of As species in tissues of five macrophyte species in Kinghorn Loch in July 2013. Root tisues were only analysed for *P. amphibia*. Bars represent the mean of 3 samples from each plant tissue and error bars standard deviation of the mean. Note the different y-axis scales.

Table 6.3. Means and standard deviations of As species concentrations (mg kg^{-1}) measured in tissues of five macrophyte species in Kinghorn Loch in July 2013. Each value represents the mean ± s.d. of n = 3 plant samples.

Plant tissue	**Arsenic concentration (mg kg^{-1} dry weight)**						
	Arsenobetaine	**DMA**	**Tetra**	**MMA**	**Total organic As**	**Inorganic As**	**Unknown species**
P. amphibia stems	0.07 ± 0.02	0.02 ± 0.01	0.12 ± 0.07	0.01 ± 0.01	0.23 ± 0.08	1.07 ± 0.29	< LOD
P. amphibia leaves	0.07 ± 0.03	0.06 ± 0.02	0.01 ± 0.00	0.01 ± 0.00	0.15 ± 0.04	0.76 ± 0.14	< LOD
P. amphibia roots	0.05 ± 0.04	0.11 ± 0.04	0.11 ± 0.07	0.06 ± 0.03	0.32 ± 0.12	86.9 ± 75.8	0.17 ± 0.22
P. pectinatus stems	0.14 ± 0.08	0.05 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.26 ± 0.09	2.56 ± 1.19	< LOD
P. pectinatus leaves	0.22 ± 0.07	0.11 ± 0.02	0.20 ± 0.01	0.05 ± 0.05	0.58 ± 0.11	6.32 ± 1.82	0.02 ± 0.03
E. nuttallii stems	0.10 ± 0.03	0.06 ± 0.01	0.07 ± 0.04	0.03 ± 0.01	0.25 ± 0.08	7.35 ± 4.68	< LOD
E. nuttallii leaves	0.24 ± 0.06	0.08 ± 0.01	0.30 ± 0.08	0.09 ± 0.03	0.71 ± 0.06	45.1 ± 20.7	< LOD
Chara spp. *axes*	1.13 ± 0.50	0.03 ±0.03	< LOD	< LOD	1.16 ± 0.48	5.92 ± 0.15	0.66 ± 0.24
Chara spp. *branches*	1.16 ± 0.69	0.01 ± 0.01	< LOD	< LOD	1.17 ± 0.70	6.89 ± 0.53	1.04 ± 0.47
M. spicatum stems	0.13 ± 0.03	0.03 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	0.23 ± 0.03	1.96 ± 0.39	< LOD
M. spicatum leaves	0.37 ± 0.25	0.16 ± 0.22	0.29 ± 0.06	< LOD	0.82 ± 0.19	10.1 ± 2.07	0.33 ± 0.44

The model selection process indicated a model with plant tissue, plant species and the interaction term included as an optimal one for inorganic As, arsenobetaine, MMA, Tetra, total organic As, and all identified As species (Table 6.4). Due to lack of a significant effect, the interaction term was omitted from the model for unidentified As. None of the explanatory variables was found to have a significant association with plant DMA concentration.

E. nuttallii had significantly higher and *P. amphibia* significantly lower concentrations of inorganic As than *Chara* spp., while there was no significant difference in concentration between *P. pectinatus* and *M. spicatum* and the latter species (Table 6.5). The results also indicated that *E. nuttallii, M. spicatum* and *P. pectinatus* accumulated lower concentrations of inorganic As in stems than in leaves, whilst in *P. amphibia* higher inorganic As was measured in stems compared to leaves. There was no significant difference between concentrations of this As species in leaves and stems of *Chara* spp.

Compared to *Chara* spp., the other four macrophyte species accumulated significantly lower concentrations of arsenobetaine (Table 6.5). The arsenobetaine concentrations measured in stems of *E. nuttallii* and *M. spicatum* were lower than in the leaves, while there were no significant differences in the other three species between the concentrations in the leaves and stems.

MMA concentrations in *P. pectinatus* and *E. nuttallii* were significantly higher than in *Chara* spp. and MMA in stems of *E. nuttallii* was significantly lower compared to MMA in leaves of this species (Table 6.5). There was no significant difference between MMA in stems and leaves of *Chara* spp.

The model for Tetra indicated no significant difference in concentration between *P. amphibia* and *Chara* spp. (Table 6.5). The Tetra concentrations in the other three species were higher than in *Chara* spp., with concentrations in the stems of these species significantly lower than in the leaves. In contrast, Tetra in *P. amphibia* was significantly lower in leaves compared to stems.

Table 6.4. Ranking of mixed models explaining variation in As species concentrations in macrophytes in Kinghorn Loch in July 2013. For each response variable, models are presented in descending order of fit, with the best fit model selected as the one with the lowest AIC value if the difference in AIC was significant (P < 0.05). Explanatory variables in the models are species, tissue and interaction between species and tissue (Species*Tissue). *k* is the number of parameters in the model, ΔAIC refers to the difference in AIC between each model and the best-fit model. Only response variables with significant difference between a more complex and reduced models are shown.

Response variable	Model	*K*	AIC	ΔAIC
Arsenobetaine	Species + Tissue + Species*Tissue	10	48.76	
	Species + Tissue	6	52.14	3.38
	Species	5	58.04	9.28
	Tissue	2	76.04	27.28
MMA	Species + Tissue + Species*Tissue	10	-139.43	
	Species + Tissue	6	-125.65	13.78
	Species	5	-124.83	14.60
	Tissue	2	-115.28	24.15
Tetra	Species + Tissue + Species*Tissue	10	-93.25	
	Species + Tissue	6	-50.19	43.06
	Species	5	-41.06	52.19
	Tissue	2	-41.70	51.55
Inorganic As	Species + Tissue + Species*Tissue	10	25.44	
	Species + Tissue	6	64.52	39.08
	Species	5	77.99	52.55
	Tissue	2	90.61	65.17
Unknown As species	Species + Tissue + Species*Tissue	10	3.76	1.51
	Species + Tissue	6	5.27	
	Species	5	7.57	2.30
	Tissue	2	23.31	18.04
Total organic As	Species + Tissue + Species*Tissue	10	24.05	
	Species + Tissue	6	51.08	27.03
	Species	5	60.17	36.12
	Tissue	2	69.09	45.04
Total identified As species	Species + Tissue + Species*Tissue	10	24.25	
	Species + Tissue	6	63.86	39.61
	Species	5	77.02	52.77
	Tissue	2	88.97	64.72

Table 6.5. Coefficient estimates of the optimal mixed models explaining variation in arsenic species concentrations in macrophytes in Kinghorn Loch in July 2013. The differences between levels (macrophyte species and tissues) of explanatory variables are related to the baseline category which is *Chara* spp. The means of the levels are presented in Table 6.3. DMA is not included in the table as none of the explanatory variables was found significant.

Response variable	**Explanatory variable**	**Estimate**	**Std. error**	**t-value**	**p-value**
Arsenobetaine	Intercept	0.0445	0.2691	0.1655	0.872
	Species (*E. nuttallii*)	-1.4871	0.3806	-3.9078	0.005
	Species (*M. spicatum*)	-1.2297	0.3806	-3.2315	0.012
	Species (*P. amphibia*)	-2.7348	0.3806	-7.1865	< 0.001
	Species (*P. pectinatus*)	-1.5994	0.3806	-4.2028	0.003
	Tissue (stems)	0.0036	0.2678	0.0133	0.990
	Species (*E. nuttallii*):Tissue (stems)	-0.9246	0.3788	-2.4410	0.035
	Species (*M. spicatum*):Tissue(stems)	-0.8671	0.3788	-2.2894	0.045
	Species (*P. amphibia*) :Tissue(stems)	0.0067	0.3788	0.0178	0.986
	Species (*P. pectinatus*) :Tissue(stems)	-0.5179	0.3788	-1.3673	0.201
	s.d. of the random intercept: 1.2060e^{-05}				
MMA	Intercept	-0.0013	0.0109	-0.1226	0.905
	Species (*E. nuttallii*)	0.0897	0.0154	5.8308	< 0.001
	Species (*M. spicatum*)	-0.0000	0.0154	-0.0004	0.999
	Species (*P. amphibia*)	0.0110	0.0154	0.7128	0.496
	Species (*P. pectinatus*)	0.0552	0.0154	3.5884	0.007
	Tissue (stems)	0.0000	0.0147	0.0010	0.999
	Species (*E. nuttallii*):Tissue (stems)	-0.0619	0.0208	-2.9842	0.014
	Species (*M. spicatum*):Tissue(stems)	0.0263	0.0208	1.2677	0.234
	Species (*P. amphibia*) :Tissue(stems)	0.0040	0.0208	0.1936	0.850
	Species (*P. pectinatus*) :Tissue(stems)	-0.0379	0.0208	-1.8241	0.098
	s.d. of the random intercept: 8.6490e^{-07}				

Table 6.5. continued.

Response variable	**Explanatory variable**	**Estimate**	**Std. error**	**t-value**	**p-value**
Tetra	Intercept	0.0019	0.0235	0.0826	0.936
	Species (*E. nuttallii*)	0.3020	0.0333	9.0727	< 0.001
	Species (*M. spicatum*)	0.2834	0.0333	8.5129	< 0.001
	Species (*P. amphibia*)	0.0084	0.0333	0.2523	0.807
	Species (*P. pectinatus*)	0.1947	0.0333	5.8492	< 0.001
	Tissue (stems)	-0.0018	0.0312	-0.0563	0.956
	Species (*E. nuttallii*):Tissue (stems)	-0.2353	0.0441	-5.3403	< 0.001
	Species *(M. spicatum*):Tissue(stems)	-0.2477	0.0441	-5.6203	< 0.001
	Species (*P. amphibia*) :Tissue(stems)	0.1155	0.0441	2.6203	0.026
	Species (*P. pectinatus*) :Tissue(stems)	-0.1441	0.0441	-3.2690	0.008
	s.d. of the random intercept: $9.6756e^{-07}$				
Inorganic As	Intercept	1.9275	0.2196	8.7793	< 0.001
	Species *(E. nuttallii*)	1.7889	0.2684	6.6656	< 0.001
	Species (*M. spicatum*)	0.3729	0.2684	1.3895	0.202
	Species (*P. amphibia*)	-2.2187	0.2684	-8.2670	< 0.001
	Species (*P. pectinatus*)	-0.1153	0.2684	-0.4294	0.679
	Tissue (stems)	-0.1497	0.1472	-1.0170	0.333
	Species (*E. nuttallii*):Tissue (stems)	-1.7470	0.2082	-8.3920	< 0.001
	Species (*M. spicatum*):Tissue(stems)	-1.4903	0.2082	-7.1589	< 0.001
	Species (*P. amphibia*) :Tissue(stems)	0.4828	0.2082	2.3194	0.043
	Species (*P. pectinatus*) :Tissue(stems)	-0.7975	0.2082	-3.8311	0.003
	s.d. of the random intercept: 0.1912				
Unknown As species	Intercept	0.9235	0.1128	8.1902	< 0.001
	Species (*E. nuttallii*)	-0.8510	0.1522	-5.5907	< 0.001
	Species (*M. specitum*)	-0.6874	0.1522	-4.5162	0.002
	Species (*P. amphibia*)	-0.8510	0.1522	-5.5907	< 0.001
	Species (*P. pectinatus*)	-0.8429	0.1522	-5.5378	< 0.001
	Tissue (stems)	-0.1451	0.0672	-2.1576	0.049
	s.d. of the random intercept: $2.2881e^{-06}$				

Table 6.5. continued.

Response variable	Explanatory variable	Estimate	Std. error	t-value	p-value
Total organic As	Intercept	0.0462	0.1892	0.2444	0.812
	Species (*E. nuttallii*)	-0.3876	0.2676	-1.4487	0.186
	Species (*M. spicatum*)	-0.2605	0.2676	-0.9736	0.359
	Species (*P. amphibia*)	-1.9326	0.2676	-7.2233	< 0.001
	Species (*P. pectinatus*)	-0.6065	0.2676	-2.2667	0.053
	Tissue (stems)	0.0358	0.1600	0.2238	0.827
	Species (*E. nuttallii*):Tissue (stems)	-1.1303	0.2263	-4.9956	< 0.001
	Species (*M. spicatum*):Tissue(stems)	-1.3169	0.2263	-5.8206	< 0.001
	Species (*P. amphibia*) :Tissue(stems)	0.3309	0.2263	1.4627	0.174
	Species (*P. pectinatus*) :Tissue(stems)	-0.8719	0.2263	-3.8535	0.003
	s.d. of the random intercept: 4.3937e^{-06}				
Total identified As species	Intercept	2.0862	0.2145	9.7272	< 0.001
	Species (*E. nuttallii*)	1.6496	0.2753	5.9916	< 0.001
	Species (*M. spicatum*)	0.2939	0.2753	1.0676	0.317
	Species (*P. amphibia*)	-2.1919	0.2753	-7.9613	< 0.001
	Species (*P. pectinatus*)	-0.1841	0.2753	-0.6687	0.523
	Tissue (stems)	-0.1322	0.1404	-0.9416	0.369
	Species (*E. nuttallii*):Tissue (stems)	-1.7418	0.1985	-8.7730	< 0.001
	Species (*M. spicatum*):Tissue(stems)	-1.4775	0.1985	-7.4416	< 0.001
	Species (*P. amphibia*) :Tissue(stems)	0.4714	0.1985	2.3742	0.039
	Species (*P. pectinatus*) :Tissue(stems)	-0.8042	0.1985	-4.0504	0.002
	s.d. of the random intercept: 0.1559				

Chara spp. contained significantly higher concentrations of unidentified As species than the other four macrophyte species, with significantly higher concentrations in the leaves than stems (Table 6.5).

The model showed that the four macrophyte species had lower total organic As concentrations than *Chara* spp., but the difference was only significant in *P. amphibia*. *E. nuttallii, M. spicatum* and *P. pectinatus* had significantly lower concentrations of total organic As in stems compared to leaves (Table 6.5).

Two species had significantly different total identified As concentrations in comparison with *Chara* spp. - *E. nuttallii* and *P. amphibia* - which accumulated higher and lower concentrations, respectively (Table 6.5). Total identified As was significantly lower in stems of *E. nuttallii*, *M. spicatum* and *P. pectinatus* compared to leaves and significantly higher in stems than leaves of *P. amphibia*.

6.3.3 Differences in As species accumulation within *Persicaria amphibia*

Inorganic As in roots was substantially higher (86.9 ± 75.8 mg kg^{-1} d.w.) than mean concentrations of any other identified As species measured in *P. amphibia,* which were $\leq$ 0.12 mg kg^{-1} d.w. (s.d. 0.07; Table 6.3). All analysed organic species of As were measured in each tissue of *P. amphibia*, whilst unknown As species were only detected in plant roots (mean 0.17 mg kg^{-1} d.w. ± 0.22).

Below-sediment surface tissue of *P. amphibia* contained significantly higher concentrations of inorganic As, DMA, MMA and total organic As and identified As species than above-sediment tissues (Table 6.6). There was no significant difference in concentrations of arsenobetaine and Tetra between tissues above and below the sediment surface.

Table 6.6. Results of one-sided t-test to assess if mean concentrations of As species in below-sediment surface tissue are greater than means of As species (mg kg^{-1}) measured in above-sediment tissues of *P. amphibia* in Kinghorn Loch in July 2013.

	Below-sediment surface tissue		**Above-sediment tissues**		**t-value**	**df**	**P-value**
	mean	**s.d.**	**mean**	**s.d.**			
Arsenobetaine	0.05	0.04	0.07	0.02	-0.8661	6.291	0.791
DMA	0.11	0.04	0.04	0.03	3.2193	7.007	0.007
Tetra	0.11	0.07	0.07	0.08	0.8955	8.923	0.197
MMA	0.06	0.03	0.01	0.00	3.5631	4.156	0.011
Total organic As	0.32	0.12	0.19	0.07	2.1963	6.213	0.035
Inorganic As	86.9	75.8	0.91	0.27	2.5372	4.000	0.032
Unknown As species	0.17	0.22	0.00	0.00	-1.7466	4.000	0.922
Total identified As	87.2	75.9	1.11	0.33	2.5377	4.000	0.032

6.4 Discussion

6.4.1 Macrophytes in Kinghorn Loch accumulated potentially toxic concentrations of As

The present study showed that lake macrophytes contain relatively high concentrations of As, 30 years following the diversion of red mud leachate from Kinghorn Loch, with the mean concentrations in shoots varying from 0.91 to 45.8 mg kg^{-1} and mean As in roots of *P. amphibia* 87.4 mg kg^{-1}. The As concentrations in shoots reported here were higher than those reported in two other lakes (i.e. 1.4 to 5.3 mg kg^{-1} in Lake Dianchi in China; Zhang et al. 2013; and < 1 mg kg^{-1} in Lake Velenjsko, Slovenia; Jackson et al. 1991) characterised by elevated concentrations of As in surface sediment. In addition, the As concentration in *E. nuttallii* leaves in Kinghorn Loch was in excess of the maximum plant shoot content of As reported for wetlands affected by the Aznalcóllar mine spill in Spain (0.01-28.5 mg As kg^{-1}; Taggart et al. 2009). However, the concentration range in plant roots documented by Taggart et al. (2009; 1.39-1089 mg kg^{-1}) was greater than that measured in root samples in Kinghorn Loch (40.5-218 mg kg^{-1}).

There are no widely accepted toxicity threshold concentrations for As in plant tissues (Taggart et al. 2005). Most of the mean concentrations measured in macrophyte tissues in Kinghorn Loch, however, were higher than the ranges 0.01-1.00 mg kg^{-1} and 3.00-10.0 mg kg^{-1}, which were suggested by Chaney (1989) as 'normal' and potentially phytotoxic ranges of inorganic As concentrations in dry foliage, respectively. Furthermore, the mean As concentration observed in macrophyte roots in the present study exceeded 50 mg kg^{-1}, which Chaney (1989) indicated as a maximum dry diet level tolerated by livestock.

The five most dominant macrophyte taxa in Kinghorn Loch are perennial (Moore 1986; Preston and Croft 2001), and although some, such as *Chara* spp. do not die down in winter (Moore 1986), others, such as *M. spicatum* and *P. pectinatus* die back to roots and tubers developed on rhizomes (Preston and Croft 2001). Decomposition of plant shoots that contain relatively high As concentrations may lead to an increase of As concentrations in water. However, such impact was not traceable in the seasonal changes in the metalloid concentrations in the lake water column during the one year monitoring period.

6.4.2 Concentrations of inorganic As in macrophytes were significantly higher compared to organic As

Inorganic As dominated As speciation in the studied macrophytes in Kinghorn Loch. Although As speciation in plants in the aquatic environment has not been extensively studied, these field observations are in agreement with the results from controlled laboratory experiments that have shown much higher uptake of arsenate and arsenite, compared to DMA and MMA, by *Spirodela polyrhiza* L. (Rahman et al. 2008a), and by *Azolla caroliniana* Willd. and *Azolla filiculoides* Lam. (Zhang et al. 2008), and higher arsenate uptake in comparison to DMA by *Salvinia natans* L. (Rahman et al. 2008b). The present study also confirms the results of the survey investigating As species accumulation in a range of aquatic plants sampled in a wetland affected by As from mining activities in Yellowknife, Canada (Koch et al. 2000). These observations made on aquatic plants are generally consistent with the results of wider research, which have investigated As speciation in several plants used for human consumption, e.g. rice *Oryza sativa* (Rahman et al. 2008c; Rahman et al. 2011), as well as other terrestrial plants (Meharg and Hartley-Whitaker 2002).

It is not known if the presence of various organic As compounds in tissues is a result of uptake or transformation of As by plants (Meharg and Hartley-Whitaker 2002). The present study identified four organic As species within macrophyte tissues. Each organic As species was recorded in all four plant species. However, in *Chara* spp. only two, arsenobetaine and DMA, were detected. Apart from arsenobetaine in *Chara* spp., concentrations of organic As species were < 1 mg kg^{-1} in all plant tissues. DMA, MMA and Tetra, but not arsenobetaine, were previously identified in aquatic plants from Yellowknife, Canada (Koch et al. 2000). A review of As speciation in terrestrial plants showed that methylated As forms were also reported (as a minor fraction of the total accumulated As) in a wide number of plant species (Meharg and Hartley-Whitaker 2002). The presence of arsenobetaine has been previously reported in terrestrial plants, however, in a smaller number of species compared to methylated As compounds (Meharg and Hartley-Whitaker 2002). In addition to the As compounds discussed above, the results of the present study indicate that *Chara* spp., *P. pectinatus* and *M. spicatum* all accumulated a small fraction of As species that were not identified by the analysis procedure employed. A few aquatic plant species from the Yellowknife study, such as *Myriophyllum sp.*, contained arseno-sugars (Koch et al. 2000). Koch et al. (2000) suggested that the presence of arseno-sugars in submerged plant tissues could be explained by contamination of samples with organisms containing this form of As, e.g. epiphytic algae growing in physical contact with some macrophytes. Thus it is possible that arseno-sugars accounted for some of the unidentified As species present in macrophytes at Kinghorn Loch.

6.4.3 Accumulation of As species varied between above-sediment tissues of macrophytes and among macrophyte species

Most studies have examined variation in As accumulation only for total As. The extent of metal(loid) accumulation investigated at the total concentration level has been shown to differ between macrophyte species and tissues (Cardwell et al. 2002; Grudnik and Germ 2010; Favas et al. 2012) and thus, it was hypothesised that the concentrations of different As chemical forms within plants in Kinghorn Loch will also vary depending on plant tissue and species. Variation in total identified As, and consequently variation of inorganic As, which had the highest contribution to the total As concentrations in this study, confirmed that hypothesis. With the exception of DMA, organic As species concentrations were also reported to vary significantly with plant species and tissue type. Across-species As variation observed in the present study is in agreement with the differences in the uptake of inorganic and organic As forms by different macrophyte species previously reported by Koch et al. (2000) and Zhang et al. (2008). Variation in metal(loid) uptake may be related to differences in plant growth rate and absorption efficiency for metals (Mishra et al. 2008). In addition, some macrophytes can accumulate greater concentrations of As than others, but might show less signs of damage due to more effective mitigation mechanisms (Leão et al. 2014). In the present study, the highest shoot concentrations of inorganic As were observed in *E. nuttallii*. Previous estimates of metal(loid) (Hg and Cd) uptake by *E. nutallii* shoots indicated high and rapid bioaccumulation of metals, with shoots showing very small stress level, due to protection of cellular machinery by metal storage in cell walls and highly effective intracellular tolerance mechanisms (Larras et al. 2013). This might explain the capability of this species to accumulate high concentrations of As in Kinghorn Loch. Lower content of inorganic As in above-sediment tissues of other taxa in the present study might indicate different plant defence mechanisms in comparison to *E. nuttallii*, such as limiting As absorption. In order to decrease As uptake and enhance their survival in the presence of arsenate, plants can suppress phosphate uptake pathways used for arsenate absorption (Meharg and Hartley-Whitaker 2000), which may be the defence process occurring in some of the macrophyte species in Kinghorn Loch.

This study showed a generally consistent trend in variation of As content in shoots. Concentrations of all detected As species in macrophytes in Kinghorn Loch were higher (apart from DMA in *Chara* spp.) in leaves compared to stems of the four macrophyte taxa sampled. The only exception was *P. amphibia*, in which total As concentrations were significantly higher in stems than leaves. The present study result for *P. amphibia* is not consistent with the higher metal concentrations (cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb)) reported in leaves compared to stems for *Persicaria* sp. by Cardwell et al. (2002). This may be due to a difference in uptake and transportation pathways of different metal(loids) by the *Persicaria* sp. However, the significantly higher concentrations of inorganic and organic As in leaves compared to stems in *M. spicatum* in the present study were in agreement with total As distribution in shoots of this species in Kadin Creek, Turkey (Yabanli et al. 2014).

6.4.4 Roots of *P. amphibia* accumulated significantly higher concentrations of most identified As species compared to the above-sediment tissues

Most previous studies have shown that a major fraction of total As is accumulated in the roots of aquatic plants (Taggart et al. 2005; Mishra et al. 2008; Mazej and Germ 2009; Rahman and Hasegawa 2011; Favas et al. 2012). To date, few plants have been identified with the ability to translocate high amounts of As from roots to above-sediment tissues (Rahman and Hasegawa 2011). This was also demonstrated for *P. amphibia*, in the present study. Chou et al. (2014), suggested that minor translocation of As from roots to shoots in rice plants was indicative of adaptive survival behaviour in As contaminated sediments. The results for inorganic As from Kinghorn Loch indicate that this might also be the strategy of *P. amphibia.* Favas et al. (2012) suggested that As speciation appears to play an important role in both the uptake mechanism and further translocation of As between plant tissues. Arsenate is an analogue of phosphate (Meharg and Hartley-Whitaker 2002) and uptake, accumulation and translocation of inorganic As is thought to be affected by its relationship with phosphate transporters (Favas et al. 2012) and the presence of iron (Fe) and sulphur (S; Zhao et al. 2010). Fe plaque on plant roots can act either as a sink or a source of As to plants and S can help detoxify As through complexation and restricting its translocation to shoots (Zhao et al. 2010). In contrast, Rahman et al. (2011) reported that uptake of the organic As species, DMA, through roots and shoots of rice plants was not affected by Fe. Moreover DMA and arsenate are suggested to be taken up by different pathways (Rahman et al. 2008a). Despite these differences, concentrations of DMA in the present study were, similarly to inorganic As species, significantly higher in the plant roots than in shoots. Knowledge of the mechanisms of translocation and accumulation of organic As species in plants is, however, limited.

6.4.5 Toxicological implications of measured As species concentrations in macrophytes in Kinghorn Loch

Information about the relative concentrations of As species present in macrophytes can provide important toxicological context. Organic As species, recorded in the present study at concentrations comparable to background levels of total As, are generally less toxic to organisms, including aquatic plants, than inorganic As compounds (Tamaki and Frankenberger 1992). Moreover, arsenobetaine, the organic As species present in the highest concentration of those analysed in the present study is reported to have non-toxic properties (Caumette et al. 2012).

The dominance of inorganic As forms, which are present in elevated concentrations in all macrophyte species in Kinghorn Loch indicates that, even following 30 years of recovery, red mud pollution still poses a risk to the macrophytes of this lake, and potentially to higher organisms in the food web. Inorganic As species are highly toxic to plants (Meharg and Hartley-Whitaker 2002). It is possible that the macrophyte community that has developed in Kinghorn Loch is tolerant to elevated As concentrations. For example, two of the macrophytes species included in the study, *M. spicatum* and *P. pectinatus*, have been reported to accumulate high metal concentrations, persisting under conditions of concentrations in tissues higher than those regarded as toxic for plants (Samecka-Cymerman and Kempers 2004). In addition, *P. pectinatus* can quickly colonise polluted waters and environments unsuitable for other species (Samecka-Cymerman and Kempers 2004) and *Elodea sp*. is known to be able to adapt to a wide range of environmental conditions (Regier et al. 2013). These traits have led researchers to consider the use of *M. spicatum*, *P. pectinatus* and *E. nuttallii* for phytoremediation (Lesage et al. 2008; Peng et al. 2008; Larras et al. 2013). The presence of species that have been shown to be persistent in heavily polluted waters suggests that the elevated As concentrations in the sediment may still influence the macrophyte community structure in Kinghorn Loch.

Conclusions

Arsenic was accumulated in macrophytes in Kinghorn Loch, 30 years following the diversion of red mud pollution from the lake. Arsenic species concentrations varied with macrophyte species and tissue type. The dominance of toxic inorganic As species suggested that there was an increased toxicological risk to these macrophyte species and that this risk is also present for higher organisms in the food web utilising macrophytes as a food source.

Chapter 7 Overall Discussion

The discussion brings together the findings from Chapters 3-6 to address the objectives listed in Chapter 1. Firstly, an assessment of the time frame of the chemical recovery of water in a shallow lake from red mud pollution is made. Secondly, legacy issues of red mud pollution are described in terms of sediment contamination and its impact on water recovery, focusing on factors driving cycling of pollutants between bed sediment and water column. This is followed by an assessment of ecological recovery of the lake based on the presence of arsenic (As) species in macrophytes and implications for higher trophic food levels. Finally, the consequences of the findings of the present study for the management of red mud polluted aquatic ecosystems are outlined, along with recommendations for future research and the wider implications of the results.

7.1 Long-term chemical recovery of water in a shallow lake from red mud pollution

Knowledge of the effects of red mud on the aquatic environment is limited. The majority of studies examining the effects of, and recovery from, red mud pollution are based on recent research after the Ajka spill, Hungary, in October 2010. As a consequence, the existing body of research describes only the short-term impact of red mud and its behaviour in flowing waters following the pollution event. Red mud impacts on standing waters are reported in only three publications (Gorka Lake, a pit lake in southern Poland (Czop et al. 2010); Topibo Lake and Coropina swamp, Suriname (Ouboter and de Dijn 1993) and lagoons on the lower Orinoco River, Venezuela (Mora et al. 2015)) and the aquatic environments examined were still receiving red mud leachate. The study of Kinghorn Loch has allowed investigation of the time frame of recovery and the long-term effects of red mud in a shallow aquatic ecosystem over both short (i.e. months to years) and long (i.e. decades) time scales.

The analysis of long-term monitoring data documented a recovery of the surface water of Kinghorn Loch, represented by a significant decrease in pH and concentrations of all red mud constituents in surface water since the cessation of pollution into the lake in 1983. The chemical recovery period estimated for contaminants using general additive models (GAMs) was long, comprising 5 years for pH and from 22 to 26 years for As, vanadium (V), aluminium (Al) and phosphorus (P). Indeed end-point recovery for Al had not been reached following this period (Chapter 3). In contrast, pH in the river system affected by the Ajka red mud spill showed a rapid recovery of only 2 months (Mayes et al. 2011). Recovery in lakes can take longer compared to rivers and streams due to the higher retention time of standing waters, which prolongs the release of contaminants by the ecosystem (Spears at al. 2012; Sharpley et al. 2013). Moreover, the decrease in pH in Hungary was due to the combined effect of dilution and acid dosing, whilst at Kinghorn Loch similar restoration measures were not applied.

Although the long-term changes in Kinghorn Loch surface water indicated recovery of the lake, the 2012/2013 survey showed that the As, V and P concentrations were higher in deeper water compared to surface water and displayed seasonal peaks. Furthermore, V concentrations exceeded standards for the Protection of Aquatic Life in the UK (20 $\mu g\ L^{-1}$; EEC 1976) in water sampled 0.01 and 0.5 m above the bed sediment in spring (Chapter 4). This indicated that recovery of the lake is not yet complete and that 30 years after the diversion of the pollution from Kinghorn Loch, the lake still processes red mud contaminants.

7.2 Legacy issues of red mud pollution – sediment contamination and its impact on lake recovery

Despite a considerable decrease in sediment pollutant concentrations since the cessation of the pollution in 1983 (with the exception of P), sediment in the lake remains contaminated (Chapter 4). In contrast, after the Hungarian red mud spill rapid dilution of sediment-bound contaminants was reported, with the exception of depositional hotspots in the river system affected by the pollution (Mayes et al. 2011). This indicates considerably shorter recovery times in fluvial systems in comparison to lakes. However, it also suggests that the residence time of contaminants in lakes might be relevant to predicting the recovery time of depositional zones in rivers. Persistence of pollutants in sediments years after the cessation of pollution has been documented in previous studies investigating the recovery of high retention water bodies from eutrophication (Jeppesen et al. 2007;

Søndergaard et al. 2003; Spears et al. 2012) or metal and metalloid contamination (Lemley 1997; Harrington et al. 1998). Many of the studies of aquatic systems affected by eutrophication reported prolonged recovery due to the occurrence of internal loading of pollutants from bed sediment into the water column (e.g. Jensen et al. 1992; Søndergaard et al. 2003; Søndergaard et al. 2013). Internal cycling has also been observed in a number of previous studies investigating the behaviour of As in lakes (Toevs et al. 2008; Whitmore et al. 2008), whilst release from aquifer rocks into groundwater related to physico-chemical conditions have been reported for V (Minelli et al. 2000). Although pollutant concentrations in the surface water of Kinghorn Loch decreased significantly during the 30 year period after cessation of red mud pollution inputs, with As, V and P reaching end-points in surface water (Chapter 3), higher concentrations of red mud constituents were observed in water 0.01 m above sediment compared to surface water. This indicates that the bed sediment of Kinghorn Loch still acts as a source of these constituents to the water column (Chapter 4 and 5), as was confirmed by the laboratory experiment (Chapter 5).

The highest concentrations of PO_4-P and total P (TP) in surface water, and PO_4-P in water above the sediment surface occurred in summer, corresponding with a summer P concentration peak commonly observed in shallow lakes affected by internal P loading related to seasonal changes in redox conditions (Jensen et al. 1992; Søndergaard et al. 2003; Søndergaard et al. 2013). Peaks in concentrations of dissolved and total As in Kinghorn Loch surface water and dissolved As in water 0.01 m above sediment in summer also indicate that the long term recovery of As in the water column might be related to the sediment contribution. The release of contaminants from bed sediment driven by redox conditions observed during the laboratory experiment (Chapter 5) appears to confirm the field observations. With the exception of Al and Ni, elevated concentrations of dissolved trace elements were not observed in the water of Orinoco River lagoons receiving red mud leachate (Mora et al. 2015). This is likely due to immobilisation of contaminants by sorption or coprecipitation in the pH conditions measured in the lagoon water (between 5.52 and 9.91; Mora et al. 2015). Although pH in Kinghorn Loch water reached a value of 10.5 before the diversion of the leachate, pH measured in the present study (7.69-

8.98) was within the range measured in the lagoon waters. The absence of elevated concentrations of red mud contaminants in lagoon water might be attributed to differences in the trace element content of red mud entering the lagoons (e.g. no As and V) compared to the leachate input to Kinghorn Loch and, therefore, different mobility of the contaminants in the aquatic environment.

The environmental conditions triggering internal loading in Kinghorn Loch were shown to be similar for As and P, but different for V. Although aqueous V geochemistry has been shown previously to be strongly controlled by redox through adsorption to iron oxides (Peacock and Sherman 2004), mechanisms controlling seasonal changes in dissolved V concentrations in aquatic ecosystems are still not well understood (Wang and Wilhelmy 2009). The experiment (Chapter 5) showed that anoxic conditions led to the release of As and P from sediment, which is consistent with previous studies (Smolders et al. 2006; Couture et al. 2010). In contrast, the behaviour of V was characterised by increased concentrations in the water during oxic conditions (Chapter 5). Similar differences between the behaviour of As and V under oxic conditions in groundwater have been previously reported by Wright et al. (2014). The experimental results confirmed the field observations which indicated that the bed sediments can act as a sink or source of pollutants during different seasons, and that the release conditions are pollutant specific. For As and P, release occurred during depletion of dissolved oxygen (DO) at the sediment-water interface, whereas V concentrations were highest in spring, coinciding with high DO levels (Chapter 4). Enhanced surface water As concentrations in summer coincided with high P concentrations, as reported previously in eutrophic Lake Biwa, Japan (Sohrin et al. 1997).

The 2012/2013 survey of Kinghorn Loch showed that, in addition to the redox conditions, contaminant mobility in the water column was controlled by a combination of physico-chemical factors, including pH, conductivity and concentrations of pollutant binding elements, with likely influence of phytoplankton growth that was not measured in the present study (Chapter 4). These results are consistent with the existing literature on the geochemical behaviour of the pollutants (Søndergaard et al. 1999; Bissen and Frimmel 2003; Wang and Wilhelmy 2009).

pH is one of the most important factors influencing the adsorption of metal ions on solids (Vega et al. 2003). The linear decrease in adsorption of V with increasing pH from 4 to 11.6 reported by Naeem et al. (2007) is consistent with the field observations during the present study. The highest V concentrations in water 0.05 m above sediment in Kinghorn Loch corresponded with the season of highest pH (Chapter 4 and 5), and total V and pH were significantly positively correlated throughout the year in these samples at the deepest site (Chapter 5). The experiment conducted in the present study did not test for an effect of pH, however laboratory experiments using Lake Biwa water and sediment demonstrated that the release of V from sediment observed under oxic conditions in the lake was a response to increased pH, which was the predominant driver of V mobilisation (Harita et al. 2005). Furthermore the field survey results from Kinghorn Loch for TP and total As suggested that pH together with DO play a role in the behaviour of these two contaminants in deeper waters (Chapter 4). Maberly (1996) showed that lake water pH can significantly fluctuate across seasons with variation in photosynthetic activity. The indirect impact of algae on element cycling through pH was indicated by the results from Lake Biwa (Harita et al. 2005) that showed influence of pH variation, driven by biological activity, on the transportation of V between the solid phase and water. It was also suggested by the positive relationship between total V concentrations and both chlorophyll *a* and pH in Kinghorn Loch surface water in years 2000 – 2009 (Watt 2015). Watt (2015) hypothesised that phytoplankton might also be driving seasonality of V through accumulation of this element in spring blooms, leading to a seasonal increase in total V concentrations at this time of year.

Another factor that should be considered in understanding As, V and P cycling in Kinghorn Loch is the interaction between the three contaminants. Due to analogous chemical structures phosphate, arsenate and vanadate have similar adsorption properties (Rietra et al. 1999). Hence competition between these elements for pollutant-binding elements, shown in previous studies (Blackmore et al. 1996; Dixit and Hering 2003; Wällstedt et al. 2010), can affect pollutant mobility (Bissen and Frimmel 2003). Enhanced arsenate mobilisation with addition of phosphate has been previously indicated in studies of soil-water systems affected by red mud (Lockwood et al. 2014) and of red mud leachate from Ajka, Hungary (Burke et al. 2013). In addition, due to the chemical similarity of inorganic As to P (Mishra et al. 2008) and its relationship to PO_4-P transporters (Favas et al. 2012), As(V) can be accumulated by photosynthesising aquatic organisms using the same uptake pathways as the nutrient (Sohrin et al. 1997; Rahman et al. 2007; Foster et al. 2008; Xue et al. 2012). The lowest PO_4-P and low As concentrations in surface water measured in the present study were in spring (Chapter 4), when abundance of phytoplankton is high (Watt 2015), indicating that algal uptake might to some extent also be a control on the seasonal patterns of concentrations of these two pollutants in Kinghorn Loch. Sohrin et al. (1997) indicated that uptake of As by phytoplankton in Lake Biwa, Japan, may have been decreased by the presence of high concentrations of P, with eutrophication influencing not just the concentration, but also the speciation of As in the lake. Further assessment of the interactions between redox conditions, pH and competitive ion concentrations, as well as determination of As and V speciation in water (due to the differing biogeochemical behaviour of As and V species in the aquatic environment; Sohrin et al. 1997; Harita et al. 2005) is required to better understand the processes that drive the behaviour of these three elements in Kinghorn Loch.

The results of the present study highlight the importance of monitoring vertical and seasonal changes in pollutants in the water column to improve understanding of in-lake processes and thus provide information on the risk assessment and management of polluted sites. In addition to impacting the recovery time of the water column, sediment contamination in Kinghorn Loch indicates the presence of toxic risk to the aquatic food web, in particular from As and V, given the high concentration of these two contaminants in the surface sediment. The current study investigated the impact on macrophytes of As, present in the lake sediment at some sites at concentrations more than ten times the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life (17 mg kg^{-1} dry weight; Canadian Council of Ministers of the Environment 1999a).

7.3 Ecological recovery – the presence of As species in macrophytes and implications for higher trophic levels

The majority of studies of As speciation in plants have investigated accumulation in terrestrial plants (e.g. Meharg and Hartley-Whitaker 2002), with the main focus on plants that are used as a food source for humans (Rahman et al. 2008c; Rahman et al. 2011). Few studies, however, have examined accumulation of As species in macrophytes (Rahman et al. 2008a; Rahman et al. 2008b; Rahman et al. 2008d; Zhang et al. 2008), and among them only one (Koch et al. 2000) has been based on field observations (from a contaminated wetland environment) rather than controlled laboratory experiments. Pollution with red mud can introduce high loads of As to the environment as demonstrated for Kinghorn Loch in Chapter 3. In addition to Al production, anthropogenic activities, such as gold (Koch et al. 2000), copper, lead, zinc (Taggart et al. 2009) and coal mining (Mishra et al. 2008) and the use of arsenic-based herbicides (Whitmore et al. 2008) have been documented as sources of As to the aquatic environment. As reported in this study (Chapter 4), As can persist in freshwater ecosystems, being mobilised under reducing conditions (Chapter 5), which suggests potential long-term impact of this element on the food web.

Arsenic toxicity depends on its chemical form (Bissen and Frimmel 2003), therefore, determination of As species is important for evaluating risk to aquatic organisms and human health. The present study showed that macrophytes in Kinghorn Loch contained relatively high, in comparison to other polluted sites, concentrations of As, predominantly in the inorganic form which is most toxic to organisms (Chapter 6), indicating that the ecological recovery of the lake is still in progress. The highest As content was measured in the roots of *Persicaria amphibia* (L.) Gray (40.4 – 218 mg kg^{-1}) greatly exceeding the 3.00 – 10.0 mg kg^{-1} range indicated by Chaney (1989) as a potential phytotoxic level. The dominance of toxic inorganic As species suggested that there was an increased toxicological risk to the sampled macrophyte species in Kinghorn Loch and that this risk may propagate through the food web to higher organisms.

Three of the five most abundant macrophyte species in Kinghorn Loch have been considered for use in phytoremediation due to their persistence under conditions of elevated As tissue concentrations, and their ability to colonise polluted waters (Samecka-Cymerman and Kempers 2004; Regier et al. 2013). This suggests that although macrophyte abundance in the lake has recovered greatly following periods of absence (Edwards 1985), it is possible that the community structure that developed in Kinghorn Loch after 1984 is driven by plant tolerance to elevated As concentrations. Ruyters et al. (2011) showed that, immediately after a red mud pollution event, bioavailability of contaminants to plants is a secondary issue compared to the presence of elevated Na in terms of toxic effects on plant growth and plant composition. The Kinghorn Loch results indicate that in a long-term perspective the contaminant bioavailability might be an important factor affecting the ecological recovery of contaminated systems, due to the persistence of red mud constituents in the aquatic environment.

The presence of elevated As concentrations in macrophytes (Chapter 6) and sediments (Chapter 4) indicates a potential risk of transfer to higher organisms at Kinghorn Loch. Fish can not only assimilate As by ingestion of food and fine particles suspended in water, but also by ion exchange of soluble metal species across lipophilic membranes (e.g. gills) and adsorption on membrane surfaces and tissue (Alam et al. 2002). Kinghorn Loch was stocked with common carp (*Cyprinus carpio* L.; Saul Gardiner, Scottish Carp Group, *pers. comm.* 2012) for recreational fishery purposes. As a sediment-dwelling, bottom feeder this large-bodied cyprinid is likely to have higher exposure to contaminants in sediments compared to pelagic fish species (Alam et al. 2002). In the current study common carp could not be examined for As accumulation due to difficulties with obtaining fish for analysis. However, given the high content of As in lake surface sediment (Chapter 4) and accumulation of As in a variety of fish species (planktivores, piscivores, omnivores and bottom feeders) documented in a number of studies (Chen et al. 2008; Shah et al. 2009; Hirata et al. 2011), there is a likelihood of As being transferred to fish in Kinghorn Loch. Although it has been demonstrated in some studies that, despite sediment contamination with As, the concentrations in fish did not exceed Canadian Guidelines for Chemical Contaminants and Toxins in Fish and Fish Products (3.5 mg kg^{-1} edible weight; Alam et al. 2002; Lalonde et al. 2011), other studies have measured As concentrations high enough to pose a risk to human health (Chen et al. 2008; Shah et al. 2009).

Birds feeding on macrophytes can also be at risk from the long-term transfer of contaminants through the aquatic food chain (Taggart et al. 2006). A study of five waterfowl species from Doñana, SW Spain, 2-3 months after pollution of the wetland with metal rich sludge indicated that As had entered the bird food chain, although concentrations were below levels that would cause ecotoxicological concern (Taggart et al. 2006). Elevated As was also measured in bird species at the same site two years after the spill, with greylag goose (*Anser anser* L.) being one of the four species accumulating higher concentrations than other bird species (Gómez et al. 2004). Greylag geese, which are present at Kinghorn Loch (Wallace, *pers. comm.,* 2012-2014), feed extensively on below sediment surface macrophyte material, such as bulbs and rhizomes (Taggart et al. 2009). In the present study the plant tissues of *P. amphibia* below the sediment surface contained the highest As concentrations observed in plants at Kinghorn Loch (Chapter 6). Herbivorous bird species can also be exposed to As through consuming sediment particles attached to macrophyte roots (Taggart et al. 2009) which, given the high concentrations of As remaining in Kinghorn Loch sediment (Chapter 4), indicates further the potential risk of As transfer to bird species.

Given the high content of As still present in the lake sediment (Chapter 4), the sediment-associated As is likely to remain a toxic hazard to the aquatic food web in Kinghorn Loch for a long period of time, despite 30 years of recovery since the cessation of pollution. In comparison, Torna Stream polluted in the Hungarian accident is expected to achieve good ecological condition by 2021, some 11 years after the pollution event (Kovács et al. 2012), showing much quicker recovery in a fluvial system. Overall, the results from Kinghorn Loch suggest that the presence of As in higher levels of the aquatic food web be considered fully in sites recovering from red mud pollution, especially for higher retention time freshwater ecosystems. This is particularly important at sites where fish and waterfowl are used for human consumption (Taggart et al. 2009).

7.4 Implications of the results for the management of red mud polluted aquatic ecosystems

Considering the long recovery period of Kinghorn Loch following red mud pollution (Chapter 3), the presence of legacy issues, such as contaminant cycling between sediment and water (Chapter 4 and 5) and pollutant presence in the aquatic food web (Chapter 6), management actions might be required to enhance natural recovery processes in bauxite residue polluted water bodies. Mora et al. (2015) indicated removal by pumping of red mud contaminated sediment from Ontario River lagoons as the most effective way of reducing pH and Al in water to background values. A number of restoration methods are available for treatment of lakes containing high concentrations of P, such as flushing, sediment dredging, aeration of bottom water and sediment capping (Beutel and Horne 1999; Scheffer 2004; Spears et al. 2013). Previous studies have also indicated using materials with binding properties for the removal of As from drinking water (Kundu and Gupta 2006; Partey et al. 2008; Zaspalis et al. 2007). Therefore, the application of capping materials used to prevent P release from sediment, such as lanthanum modified bentonite (Spears et al. 2013), might have potential for the dual control of As, P, and, potentially, also V, due to its chemical similarity. The effectiveness of such measures in aquatic ecosystems impacted by red mud or other pollution associated with multiple pressures, however, would have to be carefully examined due to the competition for sorption sites between P and other oxyanion forming pollutants, such as As and V, and also the difference in behaviour of the contaminants. This is particularly important in the case of V, which is not substantially removed from red mud leachate by a range of neutralisation methods (Burke et al. 2013). Aeration of bottom water has been used as a method of reducing sediment P release by maintaining oxidising redox conditions at the sediment-water interface (Beutel and Horne 1999). This method may also be effective in decreasing As release from sediments in lakes due to the similarity of As and P response to changes in redox conditions, as shown in the present study (Chapter 4 and 5) and literature. However, such treatment might result in an undesirable response in V, leading to a release of this metal from the bed sediments to the water under oxidising conditions, illustrated in spring in Kinghorn Loch and in oxic treatment of sediment cores (Chapter 4 and 5). A thorough assessment of the polluted aquatic ecosystem would therefore be necessary for obtaining crucial data on contaminant behaviours and the processes that drive them before considering such a management option.

7.5 Recommendations for future studies

Due to the chemical similarity and interactions between the three studied contaminants documented in previous studies, it can be expected that co-occurrence of elevated concentrations of As, P and V can add complexity to inner-annual variation in cycling of these pollutants in lakes, as well as affecting the impact of the contaminants on lake ecology compared to freshwater environments affected by only one of these pollutants. Therefore, increasing knowledge about the interactions between these contaminants is needed for better understanding of the effects of these processes on recovery mechanisms and for providing information for restoration of water bodies affected by multiple pressures, such as eutrophication and metal(loid) pollution.

The findings of the present study lead to questions for future research such as, do elevated P concentrations observed, for example, in summer in Kinghorn Loch (Chapter 3) decrease the impact of As and V present in the lake on phytoplankton and plants due to P outcompeting metals in uptake by organisms? Can recovery of P in aquatic ecosystems lead to higher impact of metal(loid)s on plants? Arsenate uptake by macrophytes *Spirodela polyrhiza* L. and *Ceratophyllum demersum* L. was previously shown to be considerably inhibited by increased phosphate concentrations in laboratory controlled conditions, due to competition between arsenate and phosphate in the same uptake pathways in aquatic plants (Rahman et al. 2007; Xue et al. 2012). To obtain a better understanding of the potential impact of interactions among red mud contaminants on the ecological recovery of aquatic ecosystems an assessment of As and V intake by macrophytes under varying concentrations of phosphate, As and V would need to be conducted. Furthermore, determination of species of As and V in fish and waterfowl at red mud polluted sites would help assess the toxic risk of the contaminant presence in the aquatic food web to human consumers.

Determination of As and V species in water would allow for more complex understanding of the factors that drive the mobilisation of these contaminants from bed sediment into the water. Examining in laboratory controlled conditions effects of changing P concentrations in water on the release of As and V species from sediment, as well as assessment of a combined impact of variation in pH and oxygen conditions would further improve knowledge of the recovery processes. Analysis of the effectiveness of application of sediment capping materials on red mud contaminated sediment in terms of reducing release not only of P, but also of metal(loid)s, would help identify management actions for enhancing processes of recovery in red mud contaminated sites, given the risks associated with sediment removal. A summary of the recommended future research and associated outcomes is provided in Table 7.1.

7.6 Wider implications of the results

The worldwide production of alumina, the raw material for aluminium production, has been rising (from 55.0 in 2002 to 81.6 million t a^{-1} in 2008; Li 2012), reflecting the increasing industrial demand for aluminium (Li 2012) in construction, packaging and transportation (Power et al. 2011). As a consequence, the global inventory of the by-product of alumina extraction, red mud, estimated at 2.7 billion tonnes in 2007, has been increasing by approximately 120 million t a^{-1} in recent years (Power et al. 2007). Li (2012) identified 51 red mud pollution incidents worldwide in the time period from the 1940s to 2012 from news reports and published literature, with an online survey identifying further under-recorded incidents. Given the large and increasing scale of alumina production worldwide and risk associated with storage of the by-product of alumina extraction, it is crucial to understand the long-term effects of multiple pressures associated with red mud pollution and the long-term fate of red mud constituents in the environment. This thesis provides novel insight into the processes driving the recovery of a shallow lake and indicates potential management options, as well as areas of further research required to better understand and manage red mud polluted aquatic ecosystems.

Table 7.1. Summary of the suggested future research questions and topics, and expected associated outcome.

Future research questions and topics	Outcome
Determination of As and V species in Kinghorn Loch water together with other physico-chemical determinands	Providing greater understanding of As and V cycling, and factors that drive mobilisation of these pollutants from bed sediment into water.
As and V speciation under changing redox conditions	A laboratory controlled experiment examining speciation of As and V under changing redox conditions would complement the field study and allow better insight to be gained into factors and processes controlling cycling of these two elements.
Impact of varying P concentrations in water on the As and V remobilisation from sediment	Examining the effects of changing P concentrations, together with the assessment of combined effects of variation in pH and oxygen conditions on As and V release from sediment in the laboratory controlled environment would help obtain more comprehensive understanding of interactions between the three contaminants and their impact on the recovery processes.
Assessment of V bioaccumulation and speciation in macrophytes	Understanding of V potential bioavailability, providing important knowledge for assessment of risk related to presence of V in water and sediment.
Determination of As and V speciation in fish and waterfowl at red mud polluted aquatic environments	Obtaining knowledge for the assessment of toxicity risks of As and V presence in aquatic food webs to human consumers.
Impact of elevated P concentrations on bioaccumulation of As and V by macrophytes	An experimental study, investigating uptake of As and V by macrophytes under controlled, varying P concentrations in water would help understand interactions between the three pollutants, and the implication of these interactions for the ecological recovery of freshwaters.
Effectiveness of application of sediment capping materials on red mud contaminated bed sediment	Providing knowledge on potential use of such products in reducing release not only of P, but also metal(loid)s, would help identify management actions for red mud contaminated waterbodies.

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Appendix A

Table A.1. Means and standard deviations of the means of PO_4-P, dissolved As and dissolved V ($\mu g\ L^{-1}$) across seasons and at 6 sites in surface water in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

Pollutant	**Season**	**Site**											
		1 (2 m)		**2 (6 m)**		**3 (10 m)**		**4 (12 m)**		**5 (8 m)**		**6 (4 m)**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Dissolved As	Winter	6.39	0.34	6.43	0.27	6.76	0.26	6.82	0.43	6.83	0.33	6.43	0.83
	Spring	8.32	2.24	8.63	2.63	8.58	2.50	8.80	2.90	9.20	2.57	8.72	2.82
	Summer	13.7	2.14	13.7	1.87	14.06	1.89	14.06	2.20	14.4	2.85	14.3	2.85
	Autumn	8.45	2.61	8.66	2.77	8.79	2.30	8.37	2.55	8.70	2.11	8.54	2.57
Dissolved V	Winter	10.54	0.34	10.5	0.50	10.5	0.66	10.4	0.42	10.4	0.66	10.5	0.54
	Spring	11.9	1.45	12.3	0.86	12.2	0.57	12.6	0.85	12.7	0.58	12.4	0.85
	Summer	10.7	0.61	10.8	0.40	11.0	0.84	11.0	0.88	11.3	1.55	11.3	1.39
	Autumn	7.52	0.65	8.07	0.76	7.61	0.55	7.60	0.58	7.75	0.44	8.02	0.47
PO_4-P	Winter	7.80	10.9	6.00	6.91	4.53	6.57	8.53	4.51	5.93	7.05	10.03	7.16
	Spring	1.83	6.05	2.90	6.58	2.93	6.49	5.90	1.90	5.37	8.95	2.33	5.13
	Summer	12.7	1.04	12.1	1.15	12.2	0.79	12.9	1.50	16.3	8.11	11.8	1.17
	Autumn	12.3	1.37	11.9	0.86	12.4	1.43	13.4	1.79	12.0	1.10	14.8	4.78

Table A.2. Means and standard deviations of the means of TP, total As and total V (μg L^{-1}) across seasons and at 6 sites in surface water in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

Pollutant	Season	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Total As	Winter	8.38	1.06	8.65	1.31	8.92	1.85	8.88	1.77	8.73	1.60	8.73	1.31
	Spring	9.35	2.57	9.73	2.38	8.75	1.85	9.59	2.38	9.83	2.80	9.77	2.16
	Summer	15.03	3.74	14.2	3.98	15.06	3.38	15.2	4.01	15.09	4.64	15.2	4.05
	Autumn	9.17	1.42	9.57	2.84	9.60	2.17	9.73	3.26	9.89	3.39	9.40	2.24
Total V	Winter	12.7	0.86	13.3	0.78	13.2	0.87	13.2	1.11	13.2	0.70	13.4	0.93
	Spring	13.7	1.77	14.5	0.74	13.6	1.87	14.3	1.29	14.3	1.14	14.2	1.82
	Summer	10.6	2.31	10.1	2.39	10.5	2.52	10.9	2.68	11.0	3.23	10.9	2.44
	Autumn	8.23	1.81	8.35	1.73	8.40	1.52	8.57	1.82	8.36	1.62	8.35	1.82
TP	Winter	22.07	4.24	19.4	1.95	19.6	1.39	28.9	3.04	16.4	0.95	21.6	2.93
	Spring	20.6	10.4	20.1	10.6	21.00	11.0	22.7	4.41	20.8	9.57	20.4	8.55
	Summer	33.6	3.87	34.4	6.91	33.3	4.03	33.4	3.25	33.2	3.62	30.07	4.75
	Autumn	28.4	4.95	29.8	7.72	28.8	4.14	29.0	4.11	27.3	5.03	29.6	5.66

Table A.3. Means and standard deviations of the means of PO_4-P, dissolved As and dissolved V (µg L^{-1}) across seasons and at 6 sites in sediment + 0.01 m water in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

Pollutant	**Season**	**Site**											
		1 (2 m)		**2 (6 m)**		**3 (10 m)**		**4 (12 m)**		**5 (8 m)**		**6 (4 m)**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Dissolved As	Winter	5.96	1.79	7.28	0.43	6.71	0.71	8.09	1.86	7.51	0.78	7.42	0.82
	Spring	8.89	1.53	11.9	5.64	11.1	5.09	10.5	3.79	10.01	2.84	10.7	4.34
	Summer	14.9	2.59	16.2	0.91	23.6	1.87	23.5	1.83	21.5	3.47	15.3	2.03
	Autumn	8.26	2.01	8.16	1.92	8.51	3.11	8.40	2.55	8.97	2.58	8.99	1.97
Dissolved V	Winter	8.93	1.86	12.5	1.38	10.6	0.78	11.5	0.77	12.2	1.33	11.5	0.32
	Spring	13.6	0.11	18.4	11.3	18.1	8.44	15.02	3.35	13.9	0.31	15.3	2.07
	Summer	12.9	2.06	8.58	2.42	8.96	0.99	8.42	0.32	9.08	1.32	8.10	2.44
	Autumn	7.76	0.71	7.90	1.18	8.22	0.59	8.14	0.28	8.67	0.55	8.82	1.09
PO_4-P	Winter	5.40	2.38	7.27	2.34	7.03	3.97	46.7	57.5	5.57	4.54	8.27	3.47
	Spring	6.10	6.20	4.17	5.71	4.30	7.88	7.80	4.14	4.57	7.39	6.37	8.50
	Summer	15.3	2.52	14.1	0.70	46.3	22.8	75.9	30.2	20.7	5.89	35.00	24.5
	Autumn	15.9	2.18	15.0	1.86	16.8	3.07	15.10	1.08	15.7	1.20	15.6	2.41

Table A.4. Means and standard deviations of the means of TP, total As and total V (μg L^{-1}) across seasons and at 6 sites in sediment + 0.01 m water in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

Pollutant	Season	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Total As	Winter	10.09	4.67	18.08	6.10	11.4	3.24	14.3	1.41	21.9	9.94	14.4	4.72
	Spring	11.8	3.02	22.8	18.2	29.8	20.8	18.2	5.90	15.6	7.42	23.5	16.5
	Summer	16.6	5.51	17.9	2.67	25.9	5.70	25.7	4.72	22.9	3.29	17.6	2.68
	Autumn	10.5	1.43	10.3	2.15	10.8	2.83	10.8	3.25	11.00	3.33	11.2	2.54
Total V	Winter	14.8	5.24	24.5	8.18	16.8	2.75	19.00	1.75	26.4	9.34	20.7	5.14
	Spring	18.4	2.63	29.4	22.03	42.0	27.2	26.7	6.65	21.3	12.2	31.07	22.2
	Summer	13.8	3.73	9.98	5.33	10.05	3.61	8.46	1.37	9.56	1.85	8.97	2.78
	Autumn	9.47	0.93	9.38	1.70	10.1	1.32	9.67	1.94	10.04	2.15	10.3	3.47
TP	Winter	30.8	4.85	86.8	65.7	39.4	7.31	162	130	88.4	47.3	51.1	13.7
	Spring	54.9	33.8	77.6	54.8	207.9	224.8	198	125	89.3	93.4	190.7	159
	Summer	42.2	6.20	54.6	24.06	86.5	35.6	135	39.6	50.7	7.33	119	42.4
	Autumn	33.9	5.75	29.1	4.39	38.5	5.69	34.07	11.3	36.3	10.7	36.8	7.99

Table A.5. Means and standard deviations of the means of PO_4-P, dissolved As and dissolved V (µg L^{-1}) across seasons and at 6 sites in sediment + 0.5 m water water in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

Pollutant	**Season**	**Site**											
		1 (2 m)		**2 (6 m)**		**3 (10 m)**		**4 (12 m)**		**5 (8 m)**		**6 (4 m)**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Dissolved As	Winter	6.82	0.57	6.78	0.55	6.73	0.59	7.27	1.10	6.61	0.69	6.97	1.06
	Spring	8.35	2.18	8.38	1.98	9.18	2.84	9.65	3.77	9.34	3.29	9.30	3.27
	Summer	13.7	2.04	14.04	0.99	16.6	0.43	22.01	2.68	17.2	2.65	13.9	2.75
	Autumn	8.24	2.11	8.32	2.43	8.52	2.15	8.39	1.95	8.34	2.67	8.35	2.83
Dissolved V	Winter	10.5	0.63	10.5	0.71	10.5	0.75	10.7	0.90	10.4	0.73	10.8	0.72
	Spring	12.3	0.70	12.5	0.63	12.8	0.62	12.5	0.48	12.7	0.80	12.7	0.83
	Summer	10.9	0.89	9.99	0.93	8.92	1.46	8.30	1.40	9.49	1.78	10.1	2.35
	Autumn	7.26	0.40	7.54	0.91	7.42	0.41	7.72	0.59	7.79	0.67	8.02	0.66
PO_4-P	Winter	11.5	7.67	9.97	6.57	11.3	10.2	14.7	10.04	7.40	3.10	9.27	4.67
	Spring	2.97	4.80	2.90	7.65	3.57	7.50	4.73	3.53	2.50	6.27	3.97	6.73
	Summer	12.5	0.55	12.00	0.72	14.9	5.25	36.8	13.7	12.9	2.05	11.7	0.85
	Autumn	12.5	0.95	12.9	1.04	12.07	1.00	12.9	1.67	12.5	1.47	12.2	1.12

Table A.6. Means and standard deviations of the means of TP, total As and total V (µg L^{-1}) across seasons and at 6 sites in sediment + 0.5 m water in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

Pollutant	Season	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Total As	Winter	8.95	1.73	8.76	2.09	8.93	1.46	9.47	2.02	9.04	1.53	8.67	2.10
	Spring	9.78	2.28	9.96	3.31	9.98	1.75	10.7	4.05	9.60	2.00	10.2	2.37
	Summer	14.8	3.99	14.06	3.57	19.06	2.43	23.7	3.94	18.3	3.41	15.2	4.04
	Autumn	9.47	2.74	9.59	1.96	10.2	3.14	10.4	2.64	10.3	2.94	9.93	2.22
Total V	Winter	13.5	0.93	13.4	1.15	13.6	1.17	13.8	1.62	13.6	1.03	13.2	1.71
	Spring	14.6	0.46	14.6	0.60	14.07	1.08	14.3	0.87	13.7	1.77	14.6	1.26
	Summer	10.6	2.68	9.48	2.95	9.21	2.83	8.40	1.21	9.28	2.39	10.1	3.04
	Autumn	8.54	1.64	8.38	1.80	8.54	1.29	8.61	1.72	8.44	1.97	8.65	1.92
TP	Winter	22.3	1.65	23.6	2.69	24.6	3.20	35.2	4.28	20.07	0.35	17.03	4.11
	Spring	24.5	13.6	23.07	12.05	19.4	9.07	27.2	9.61	18.7	10.1	26.9	5.12
	Summer	32.2	6.08	33.1	1.61	47.1	18.4	85.5	25.8	40.6	4.67	32.7	8.52
	Autumn	32.3	6.08	32.2	6.71	28.9	6.14	28.6	5.46	31.8	3.44	30.5	5.76

Table A.7. Means and standard deviations of the means of dissolved Al, Mn, Fe (µg L^{-1}) and Ca (mg L^{-1}) across seasons and at 6 sites in surface water in Kinghorn Loch from May 2012 to April 2013.

Pollutant	**Season**	**Site**											
		1 (2 m)		**2 (6 m)**		**3 (10 m)**		**4 (12 m)**		**5 (8 m)**		**6 (4 m)**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Al	Winter	7.58	1.83	9.76	5.03	13.4	2.00	9.48	4.30	9.51	3.98	10.58	5.63
	Spring	12.7	13.0	5.67	4.08	4.84	6.33	5.28	5.25	7.25	2.48	5.84	4.67
	Summer	20.08	12.0	8.41	0.70	11.05	2.33	9.47	1.23	9.35	1.66	7.18	4.58
	Autumn	9.90	5.29	6.53	5.77	4.62	3.65	7.09	7.36	7.91	6.68	14.7	17.6
Ca	Winter	43.6	2.49	43.6	0.89	44.0	0.99	43.6	1.02	43.8	0.75	43.7	1.01
	Spring	42.9	5.80	41.4	6.32	42.4	6.16	42.9	4.82	44.2	5.14	42.1	4.81
	Summer	30.7	2.09	30.3	3.78	31.0	2.41	31.1	3.89	31.2	3.39	30.8	3.19
	Autumn	41.9	1.57	43.8	1.31	43.3	2.27	43.1	2.89	42.9	2.99	44.5	4.74
Mn	Winter	0.35	0.16	0.33	0.21	0.41	0.31	0.42	0.28	0.49	0.27	0.54	0.42
	Spring	0.60	0.35	0.60	0.76	0.54	0.45	0.38	0.30	0.81	0.70	0.47	0.38
	Summer	1.08	0.23	1.17	0.44	1.29	0.52	2.02	0.52	1.90	0.70	1.32	0.35
	Autumn	0.35	0.12	0.32	0.29	0.15	0.16	0.37	0.22	0.31	0.23	0.43	0.19
Fe	Winter	3.09	0.28	3.51	0.82	4.23	1.73	3.89	1.01	4.92	3.14	4.65	0.43
	Spring	3.53	1.07	4.21	3.98	2.94	1.66	2.09	1.26	2.42	1.32	2.66	2.17
	Summer	7.65	1.47	4.64	0.85	6.29	1.55	7.53	1.91	8.18	1.26	5.78	2.87
	Autumn	7.14	3.60	4.78	1.95	3.82	1.84	5.10	4.40	4.63	3.86	10.7	7.29

Table A.8. Means and standard deviations of the means of total Al, Mn, Fe (µg L^{-1}) and Ca (mg L^{-1}) across seasons and at 6 sites in surface water in Kinghorn Loch from May 2012 to April 2013.

		Site											
Pollutant	**Season**	**1 (2 m)**		**2 (6 m)**		**3 (10 m)**		**4 (12 m)**		**5 (8 m)**		**6 (4 m)**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Al	Winter	80.5	21.2	94.2	17.5	89.9	12.2	91.05	12.6	116	50.6	114	23.6
	Spring	59.3	9.18	37.1	11.7	45.04	14.9	53.9	19.5	41.0	17.6	48.7	5.89
	Summer	69.0	49.9	39.09	19.04	40.7	13.5	39.7	25.7	36.3	13.2	32.3	14.7
	Autumn	47.0	27.3	68.1	33.8	74.01	51.4	63.7	37.7	73.0	32.6	59.9	39.8
Ca	Winter	49.4	6.25	50.9	5.60	49.9	5.80	51.2	7.01	51.7	5.96	50.7	6.75
	Spring	41.1	1.45	41.3	2.55	38.08	3.75	40.3	2.51	40.0	3.03	41.06	2.80
	Summer	31.3	7.43	30.0	5.52	31.8	5.53	33.3	6.05	32.1	7.23	32.4	5.22
	Autumn	42.1	1.34	43.7	2.10	44.7	2.79	43.4	1.73	45.0	3.51	43.9	0.87
Mn	Winter	4.80	1.23	5.45	1.95	4.88	1.48	5.05	1.43	5.28	1.07	5.60	1.70
	Spring	4.97	4.63	5.05	5.54	4.10	3.28	4.20	3.76	4.67	3.96	4.30	3.52
	Summer	14.4	1.94	11.7	1.87	13.6	2.36	13.8	1.90	13.1	3.03	12.03	1.24
	Autumn	6.87	2.75	9.55	5.68	8.96	3.57	8.64	4.52	9.66	5.67	8.69	4.01
Fe	Winter	56.7	13.7	82.9	24.8	63.7	10.5	61.5	9.41	73.2	21.1	74.3	4.26
	Spring	36.2	6.66	20.6	5.15	22.1	0.79	26.06	5.44	21.6	4.11	22.5	5.78
	Summer	66.5	51.0	25.3	1.90	27.7	0.15	28.08	1.41	27.0	2.10	23.7	4.65
	Autumn	46.7	27.2	72.08	35.3	72.6	51.2	61.4	39.7	62.7	34.5	56.3	38.7

Table A.9. Means and standard deviations of the means of dissolved Al, Mn, Fe (μg L^{-1}) and Ca (mg L^{-1}) across seasons and at 6 sites in sediment + 0.01 m in Kinghorn Loch from May 2012 to April 2013.

Pollutant	Season	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Al	Winter	11.9	6.79	13.3	2.30	15.2	2.62	13.0	3.36	14.6	5.40	13.9	2.02
	Spring	85.7	140	20.2	19.4	9.35	0.82	13.0	2.34	13.2	2.25	7.09	9.49
	Summer	19.4	6.15	11.6	4.27	10.2	6.16	10.5	1.67	8.5	2.47	8.59	2.71
	Autumn	10.5	6.16	14.1	4.38	19.5	6.87	13.2	7.46	18.5	8.36	15.2	9.47
Ca	Winter	34.4	13.9	45.8	3.66	41.7	2.64	43.3	2.83	43.06	2.28	42.9	2.09
	Spring	42.9	9.47	41.4	7.78	42.8	6.90	41.9	5.11	42.3	3.23	43.4	2.39
	Summer	31.0	2.32	38.5	1.82	44.5	2.32	45.5	3.28	41.2	1.22	40.3	3.46
	Autumn	42.5	1.82	41.8	2.70	42.02	3.24	42.6	3.61	43.1	4.37	44.3	3.77
Mn	Winter	10.1	9.50	5.25	4.98	4.59	2.23	23.0	27.9	5.64	4.63	3.70	2.75
	Spring	8.29	13.0	6.97	7.12	11.8	19.09	9.48	7.23	2.31	1.58	34.6	58.8
	Summer	14.1	15.6	111	83.01	387	9.35	407	17.3	309	71.1	181	103
	Autumn	3.09	3.63	1.48	0.36	5.04	3.93	6.20	5.94	3.37	2.77	3.27	1.90
Fe	Winter	8.63	4.11	9.19	5.45	10.9	5.25	9.30	0.44	6.81	2.71	6.73	3.58
	Spring	80.00	133	12.3	14.9	2.69	1.24	11.4	13.2	7.30	6.86	4.32	3.04
	Summer	18.06	7.91	25.8	11.7	45.9	11.1	60.8	4.08	25.4	10.4	26.8	7.66
	Autumn	8.99	5.65	9.51	3.87	14.29	8.60	18.9	13.6	20.3	3.41	16.04	10.9

Table A.10. Means and standard deviations of the means of total Al, Mn, Ca and Fe (µg L^{-1}) across seasons and at 6 sites in sediment + 0.01 m water in Kinghorn Loch from May 2012 to April 2013.

Pollutant	Season	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Al	Winter	268	113	1734	1750	564	213	793	560	1903	917	951	515
	Spring	615	475	1917	2533	3954	5184	1541	1038	1188	1836	2415	3821
	Summer	297	186	297	377	224	195	87.9	63.6	191	93.03	184	86.9
	Autumn	177	142	102	2.19	190	81.0	134	47.6	184	103	204	79.2
Ca	Winter	57.4	30.8	75.2	20.8	59.8	14.3	63.4	10.8	79.5	24.5	68.9	17.08
	Spring	51.6	12.2	57.7	14.2	85.8	68.8	62.09	16.1	53.7	24.7	75.0	54.7
	Summer	39.2	10.8	43.3	10.7	48.5	11.7	47.8	4.97	43.5	3.59	45.8	6.58
	Autumn	48.08	2.28	46.2	0.39	46.2	1.83	46.01	1.48	47.0	2.44	47.6	2.51
Mn	Winter	27.3	17.3	48.8	31.8	22.6	8.64	48.9	17.8	56.7	19.6	33.4	6.87
	Spring	34.9	28.6	65.1	47.7	162.8	196	75.1	40.0	43.03	44.1	132	111
	Summer	43.8	23.2	114	47.4	349	28	368	40.0	272	67.9	174	95.4
	Autumn	24.3	12.4	11.6	4.46	19.6	8.18	16.4	7.38	18.0	12.2	17.6	5.81
Fe	Winter	227	103	893	753	403	130	524	385	1029	402	582	237
	Spring	479	406	1003	1046	2485	3620	1005	776	735	1149	1516	2400
	Summer	213	112	182	183	187	104	121	29.4	124	48.7	144	48.2
	Autumn	167	162	82.04	14.6	150	95.1	111	41.8	162	80.7	178	40.7

Table A.11. Means and standard deviations of the means of As, V and P (mg kg^{-1}) across seasons and at 6 sites (water depths) in sediment surface (0-4 cm) in Kinghorn Loch from May 2012 to April 2013. Site water depths are given in brackets next to site numbers.

		Site											
Pollutant	**Season**	**1 (2 m)**		**2 (6 m)**		**3 (10 m)**		**4 (12 m)**		**5 (8 m)**		**6 (4 m)**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
As	Winter	65.4	9.32	147	17.4	178	11.4	184	11.24	176	15.3	135	26.3
	Spring	162	50.7	175	38.7	197	19.4	188	18.39	196	3.88	129	14.3
	Summer	77.4	11.3	162	18.0	180	2.60	188	9.57	180	28.3	126	20.4
	Autumn	72.0	16.6	166	19.04	183	29.3	188	17.60	170	32.3	108	5.40
V	Winter	174	4.23	183	12.2	210	21.4	234	13.5	196	6.08	181	20.8
	Spring	188	41.9	208	23.7	219	11.5	229	20.7	206	6.51	167	16.7
	Summer	163	7.17	198	2.27	220	3.09	233	18.3	205	11.3	181	10.6
	Autumn	172	19.2	190	20.6	207	25.1	234	24.0	206	25.00	161	15.1
P	Winter	720	10	920	30	1210	100	1120	80	1030	40	720	50
	Spring	910	220	1000	60	1160	40	1160	10	1110	20	760	80
	Summer	690	30	940	30	1130	20	1090	60	1080	80	710	60
	Autumn	690	10	980	30	1180	30	1140	50	1010	70	760	50

Table A.12. Means and standard deviations of the means of trace elements (mg kg^{-1}) at 6 sites (water depths) in top 4 layers of sediment surface (0-4, 4-6, 6-8 and 8-10 cm) in Kinghorn Loch sampled in July and October 2012, and January and April 2013.

Pollutant	Sediment depth	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
As	0-4	86.6	36.9	167	26.3	182	4.31	185	19.1	173.7	19.8	127	21.03
	4-6	88.1	18.1	197	33.4	200	24.5	221	17.09	220	26.7	173	34.6
	6-8	113	29.7	232	30.8	249	20.7	281	50.9	297	43.3	226	58.4
	8-10	136	38.4	249	53.3	318	47.4	397	150	373	12.01	278	73.5
V	0-4	164	6.45	196	21.9	218	12.4	231	23.0	198	6.52	165	14.06
	4-6	188	8.40	215	30.4	251	16.2	282	10.8	235	2.57	198	24.4
	6-8	241	56.2	241	29.6	275	26.8	282	9.86	238	11.1	226	28.4
	8-10	287	45.08	239	8.02	264	23.3	311	87.2	238	6.90	245	26.2
Cr	0-4	103	10.8	105	19.4	109	4.93	104	3.84	97.2	7.14	73.4	6.35
	4-6	115	19.6	115	14.2	129	6.67	128	6.05	118	4.16	88.6	14.6
	6-8	130	27.4	128	7.17	140	10.2	135	5.91	124	2.29	103	16.4
	8-10	151	32.3	123	20.0	136	3.18	142	4.36	126	3.15	113	20.2
Ni	0-4	57.0	6.31	59.0	14.07	62.4	1.63	57.5	3.44	55.5	5.31	40.5	3.73
	4-6	67.7	14.1	64.4	1.59	76.4	3.84	60.0	5.95	60.6	0.66	53.5	10.5
	6-8	69.9	16.7	66.7	3.51	70.05	3.96	55.7	7.28	61.2	3.22	57.9	8.11
	8-10	68.0	13.03	56.6	10.4	70.4	3.96	64.6	2.56	47.3	7.03	58.7	13.4

Table A.12. continued.

Pollutant	Sediment depth	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Zn	0-4	66.3	2.34	102	15.8	134	42.0	110	6.45	102	8.02	74.3	5.18
	4-6	66.3	4.14	110	13.2	133	3.36	160	36.9	117	0.59	85.5	10.4
	6-8	73.4	9.39	116	6.65	132	5.69	113	19.4	111	7.84	93.6	10.6
	8-10	78.5	13.6	106	15.0	120	9.84	89.9	12.6	98.2	1.04	97.0	9.81
Cu	0-4	25.2	1.73	37.9	5.32	41.03	1.00	40.9	2.69	37.5	2.22	28.03	0.89
	4-6	25.6	1.06	39.9	4.47	48.6	2.42	49.3	1.63	43.08	2.28	31.4	2.49
	6-8	27.5	3.21	43.4	2.32	52.2	6.27	44.3	6.96	42.8	1.45	33.8	3.36
	8-10	29.8	4.11	40.2	5.52	46.7	2.38	38.4	1.95	40.8	0.68	36.0	3.13
Ba	0-4	215	7.44	204	21.5	199	8.94	200	5.85	193	7.00	177	6.44
	4-6	215	14.5	209	6.72	215	3.75	206	6.81	201	3.15	186	5.64
	6-8	222	21.2	208	7.81	214	9.67	203	5.78	197	6.70	194	2.00
	8-10	227	20.3	199	7.20	203	4.18	196	21.07	188	0.33	198	9.21
Sr	0-4	474	19.2	462	28.9	382	4.84	398	10.6	412	27.1	517	7.46
	4-6	462	27.3	459	37.2	360	11.0	390	24.2	400	16.5	494	53.4
	6-8	445	41.9	450	35.4	387	27.6	482	118	472	66.03	507	12.8
	8-10	453	43.8	484	45.1	457	54.02	599	49.6	551	10.8	520	32.2

Table A.13. Means and standard deviations of the means of major elements (mg kg^{-1}) at 6 sites (water depths) in top 4 layers of sediment surface (0-4, 4-6, 6-8 and 8-10 cm) in Kinghorn Loch sampled in July and October 2012, and January and April 2013.

Pollutant	Sediment depth	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
P	0-4	770	10	970	30	1150	30	1150	30	1060	70	730	50
	4-6	660	40	1040	60	1200	30	1230	30	1100	30	720	50
	6-8	640	30	1050	30	1170	30	1160	10	1070	70	710	60
	8-10	640	60	1020	70	1160	40	1030	10	1050	20	750	50
Mg	0-4	16400	1860	18220	1990	18260	520	18190	900	17250	1290	13860	1040
	4-6	18080	2370	19450	1540	21070	1110	21240	1060	20130	1450	16160	2100
	6-8	20720	2300	21920	1220	23060	1690	24440	2710	23490	1850	18450	3670
	8-10	23160	4740	26460	3870	25210	1920	28880	5270	26450	360	22160	4100
Al	0-4	11300	900	17280	2490	18210	540	17770	950	16960	1780	11570	1420
	4-6	12310	1540	19240	2560	21620	1680	20520	910	20670	1450	14500	2860
	6-8	14320	2360	22460	1750	23710	1590	21760	1220	23870	1170	17320	4530
	8-10	16240	3770	25200	1010	24910	1480	23260	3590	25770	420	20870	4730
Ca	0-4	210000	4900	190050	15260	169300	1810	175840	5220	182060	6490	224390	6520
	4-6	212840	13670	187070	12480	155940	2850	170050	3510	176970	4670	226100	12010
	6-8	203820	17700	178490	7430	161150	8340	181300	22720	180140	10150	208680	22820
	8-10	196950	23990	169990	19000	171150	7470	199990	7850	189080	2840	205600	21100
Mn	0-4	810	30	720	80	660	30	610	20	690	80	530	30
	4-6	660	40	740	30	640	30	580	40	650	30	560	70
	6-8	690	70	770	20	640	30	530	30	690	30	590	90
	8-10	730	80	820	20	650	20	520	20	710	10	680	140

Table A.13. continued.

Pollutant	Sediment depth	Site											
		1 (2 m)		2 (6 m)		3 (10 m)		4 (12 m)		5 (8 m)		6 (4 m)	
		mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Fe	0-4	9520	1270	10400	2060	11080	220	10670	430	10000	850	7020	670
	4-6	10670	2000	10950	1140	13010	250	11830	410	11340	130	8350	1050
	6-8	11790	2880	11840	610	13500	850	11320	2330	11060	600	8880	1340
	8-10	13480	2840	13420	2690	12360	520	9840	1190	10360	240	9800	1220
Si	0-4	90470	5200	95970	7700	106360	2670	105470	1620	97240	4970	82460	1790
	4-6	93760	8120	96990	4370	112860	2000	107420	5400	97960	2460	82910	4140
	6-8	97560	16460	97170	5360	109110	8760	96580	17750	89680	9240	81390	6190
	8-10	103910	16410	97970	12310	97310	6020	80050	11670	79300	880	83920	7780
Na	0-4	690	120	640	150	710	50	650	60	630	50	380	20
	4-6	790	170	760	200	900	190	830	130	760	60	480	70
	6-8	1040	380	770	60	900	180	840	310	790	60	590	130
	8-10	1100	360	1100	510	770	60	690	140	690	40	650	140
Ti	0-4	2550	460	2360	530	2490	60	2330	120	2200	220	1540	140
	4-6	2990	660	2520	300	3080	140	2710	100	2620	40	1880	380
	6-8	3305	960	2760	180	3200	300	2650	600	2550	170	2090	390
	8-10	3820	970	3640	1440	2890	110	2280	330	2380	40	2280	350
K	0-4	940	60	1170	250	1260	10	1180	70	1140	110	800	10
	4-6	990	120	1200	150	1510	40	1340	50	1260	40	930	170
	6-8	1100	250	1310	80	1480	130	1210	230	1220	130	990	170
	8-10	1200	290	1630	590	1310	90	990	140	1020	70	1090	180

Appendix B

Table B.1. Means and standard deviations of the means of metals and metalloids (µg L^{-1}) across seasons and at 2 water column depths in Kinghorn Loch at the deepest site (12 m) from May 2012 to April 2013.

Pollutant		**Winter**				**Spring**				**Summer**				**Autumn**			
		Surface		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**		**Surface**		**Sediment + 0.01 m**	
		mean	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Dissolved	B	29.3	5.54	32.4	20.8	52.5	2.90	49.9	2.97	66.5	2.95	69.8	2.00	57.0	8.79	58.4	10.6
	Mg[a]	18.1	0.27	17.9	1.15	18.6	0.36	18.4	0.75	19.5	0.46	20.2	0.81	18.4	0.96	18.2	1.40
	Cr	0.28	0.04	0.37	0.22	0.21	0.20	0.27	0.25	0.50	0.12	0.91	0.45	0.27	0.15	0.57	0.23
	Co	0.08	0.01	0.12	0.03	0.08	0.01	0.11	0.01	0.12	0.01	0.21	0.03	0.15	0.05	0.17	0.04
	Ni	0.21	0.02	1.11	0.79	0.21	0.20	0.53	0.21	0.71	0.37	1.67	1.04	0.39	0.20	0.58	0.24
	Zn	2.89	1.81	10.3	2.99	2.24	0.82	3.72	1.14	2.03	0.80	6.21	1.54	3.09	0.74	7.83	3.48
	Cu	<LOD	0.33	<LOD	0.36	0.94	1.22	0.27	0.66	0.59	0.05	0.46	0.21	0.41	0.19	0.58	0.08
	Cd	0.00	0.00	0.02	0.01	0.00	0.00	0.01	0.00	0.11	0.00	0.11	0.00	0.00	0.00	0.00	0.00
	Pb	0.14	0.01	0.33	0.18	0.20	0.09	0.45	0.36	0.71	0.65	2.04	2.84	0.09	0.13	0.13	0.19
Total	B	74.3	29.0	73.4	25.4	83.8	28.5	87.1	26.1	71.3	6.92	71.3	2.74	57.9	3.49	61.1	1.66
	Mg[a]	19.3	2.42	20.2	2.31	17.9	0.90	17.7	1.25	19.7	2.71	20.1	1.25	18.9	1.45	19.1	1.19
	Cr	0.44	0.23	2.29	1.04	0.27	0.06	2.35	1.73	0.59	0.21	1.07	0.82	0.48	0.30	1.06	0.42
	Co	0.11	0.01	0.38	0.17	0.09	0.01	0.57	0.34	0.18	0.01	0.29	0.05	0.14	0.07	0.19	0.07
	Ni	0.33	0.06	2.72	0.84	0.25	0.35	2.59	1.72	0.61	0.30	0.98	0.92	0.49	0.25	0.98	0.13
	Zn	5.45	2.89	24.6	9.98	6.21	4.85	15.4	11.3	10.3	13.5	6.17	2.87	6.23	4.49	7.78	3.12
	Cu	<LOD	0.33	4.31	2.75	0.36	0.20	2.86	1.85	4.06	5.52	1.00	0.58	0.48	0.54	0.68	0.72
	Cd	0.00	0.01	0.04	0.03	<LOD	0.01	0.03	0.05	0.01	0.00	0.02	0.03	0.00	0.00	0.01	0.01
	Pb	0.05	0.28	3.02	3.42	<LOD	0.30	3.97	4.13	1.90	2.68	6.89	11.2	0.22	0.06	0.42	0.01

[a] concentrations given in mg L^{-1}

Table B.2. Results of repeated measures one-way ANOVA analysis to assess the significance of the difference between water column concentrations of dissolved and total metals and metalloids (μg L^{-1}) under oxic and anoxic treatments during the 7-day incubation experiment.

Measured variables		Oxic conditions		Anoxic conditions		Test statistic (F)	P-value
		mean value	s.d.	mean value	s.d.		
Pollutants (dissolved)	B	57.7	5.58	59.8	5.52	2.49	0.120
	Mg[a]	17.7	0.75	17.6	0.73	0.83	0.367
	Cr	1.00	0.90	1.04	0.65	0.06	0.813
	Co	0.26	0.10	0.24	0.07	1.52	0.222
	Ni	4.32	2.43	4.06	4.27	0.10	0.748
	Zn	12.9	7.22	10.8	9.35	1.12	0.293
	Cu	18.0	60.0	1.45	3.33	2.65	0.108
	Cd	0.03	0.02	0.02	0.01	5.33	0.024
	Pb	1.52	0.65	1.46	0.58	0.16	0.688
Pollutants (total)	B	65.8	6.44	68.2	15.2	0.75	0.391
	Mg[a]	20.2	1.57	21.3	3.07	3.76	0.057
	Cr	2.06	4.08	1.75	1.55	0.18	0.675
	Co	0.23	0.21	0.18	0.08	2.07	0.155
	Ni	5.93	6.50	5.22	6.98	0.19	0.662
	Zn	25.8	55.9	21.5	8.19	0.21	0.65
	Cu	28.6	104	1.45	1.40	2.39	0.127
	Cd	0.10	0.09	0.05	0.02	7.75	0.007
	Pb	3.22	2.64	2.25	0.80	4.30	0.042

[a] concentrations given in mg L^{-1}