THE IMPACT OF SEWAGE TREATMENT WORKS DISCHARGES UPON RIVERS

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THE IMPACT OF SEWAGE TREATMENT WORKS DISCHARGES UPON RIVERS

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Abstract

Sewage treatment works (STWs) are essential for urban sanitation but remain important sources of ecological pressure on river systems. Previous studies often lacked suitable controls, particularly for small datasets, or assessments of pollutant bioavailability and ecological risk. This study conducts a national-scale, multi-decadal assessment of STW impacts on river water quality across England, using long-term Environment Agency monitoring data and multiple statistical approaches.

Both metal (calcium (Ca), magnesium (Mg), cadmium (Cd), copper (Cu), nickel (Ni), iron (Fe), zinc (Zn), and manganese (Mn)) and non-metal (stream temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, phosphate, pH, suspended solids, and specific conductance) determinands were evaluated. Paired upstream–downstream and control site comparisons were used to assess local impacts and results were used to assess the impact of difference treatment technologies between sewage treatment works. The cumulative impact of sewage treatment discharges was assessed by examining the impact of discharge on chlorophyll-a concentration and the exceedance relative to water quality standards. The wider impact of the sewage treatment works discharges was assessed by comparison with river water quality data from all rivers across England using principal component analysis.

The results showed that:

- STW discharges had a significant impact for all determinands except COD and suspended solids, and while for BOD, pH, nitrate, specific conductance, and suspended solids the impact of STW discharge is significantly decreasing, the impact on phosphate concentrations was significantly increasing.
- STW discharges significantly increased the concentration of Ca, Mg, Cu, and Mn in the receiving river, but decreased the concentration of Fe and Zn. Equally, STW discharges significantly increased the bioavailable concentration of Zn, Mn, and Ni, but not the bioavailable concentration of Cu.

- The impact of STW discharges did significantly differ between types of secondary treatment, and tertiary treatment did significantly reduce the impact of STW discharges on the phosphate concentration.
- The combined impact of STW discharge did not change the probability of eutrophication incidents in the receiving rivers.

The principal component analysis has shown that for non-metal water quality determinands from STWs' the discharge was a dominant control on water quality across English rivers, for metals, STW discharges were not the dominant control and mining, and industrial discharges were more important.

Keywords:
sewage treatment works, river water quality, bioavailability, ANOVA, eutrophication, chlorophyll-a, principal component analysis, Water Framework Directive

Declaration and copyright

I confirm that no part of the material presented in this thesis has previously been submitted by me or any other person for a degree in this or any other university. Where relevant, material from the work of others has been acknowledged.

Signed:

Zihan Yang

Date:

23 June 2025

Acknowledgement

When I reached the UK border, it was nearly midnight. Late May in the UK can be surprisingly chilly – I was wrapped up in a down coat. So, for anyone planning to arrive around that time, do bring something warm – perhaps even a winter coat!

I arrived during the COVID period. For the first ten days, I was confined to a small room in Brook House, St Cuthbert's Society. I remember feeling overwhelmed – the chips were enormous, and the "COVID meals" were beyond generous: chips, ham, a Belgian pear, grapes… and yes, every meal came with a bag of crisps. Welcome to the UK! We really do love chips and crisps.

Although it was a frustrating time for everyone, Durham's stunning scenery brought a sense of peace. May is a lovely season here – the sky is so blue and clear, greenery is everywhere, and it stays light until nearly 9pm. A British friend of mine once told me that May is the best time of year – stepping into summer, when everyone begins to enjoy the weather again.

After lockdown, I finally met my first supervisor, Professor Fred Worrall – whom I'd like to thank most sincerely. I still remember him telling me that how far they could supervise me depended on how far I wanted to go. He answered all my silly questions without complaint. He wasn't one for small talk outside the office, but he always noticed when something had changed. If I looked lost in a meeting, he'd always double-check that I was still following. And most importantly – thank you, Fred, for helping carry my EGU poster all the way to Vienna.

My thanks also go to my second supervisor, Dr Julia Knapp. She and Fred always made sure I understood what I was doing. I remember, in the early days, she went through my R code line by line, making sure I understood each part. She was so careful with my writing, always spotting those tiny mistakes I tended to make.

At the same time, I want to thank my greatest supporters – my parents. A PhD is a long journey, and I wouldn't have made it this far without their help. And to my little sister – thank you for all the emotional support you've given me along the way.

Now, as this journey comes to an end, I find myself once again in the most beautiful season of the year, in the most beautiful corner of the country. I'm deeply grateful for these four years at the Department of Earth Sciences, Durham University. Special thanks to King Charles, who visited Durham during the final stretch of my studies – thank you for coming to see us!

Finally, thank you, Durham University.*Fundament eius super montibus sancta*.

Data Access Statement

The primary datasets used in this thesis were obtained from the Environment Agency (EA) under the UK Government's data access provisions. The water quality monitoring data covering river and sewage treatment works were derived from the EA's Water Information Management System (WIMS) database for the period 2000–2020. The M-BAT model used to calculate bioavailable metal concentrations is publicly available via https://www.wfduk.org. The R code used for statistical analyses can be made available upon reasonable request to the author. Due to licensing restrictions, direct redistribution of the raw EA monitoring data is not permitted.

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List of Abbreviation

STW	Sewage Treatment Works
SAS	Secondary Activated Sludge Treatment
SB	Secondary Biology Treatment
TAS	Tertiary Activated Treatment
TB	Tertiary Biology Treatment
PE	Population Equivalent
DWF	Dry Weather Flow
RCR	Risk Characterisation Ratio
EQS	Environmental Quality Standard
PCA	Principal Component Analysis
DOC	Dissolved Organic Carbon
M-BAT	Metal Bioavailability Assessment Tool
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
Chl-a	Chlorophyll-a
EA	Environment Agency
WFD	Water Framework Directive
WHO	World Health Organization
UKTAG	UK Technical Advisory Group (on the Water Framework Directive)
Calcium	Ca
Magnesium	Mg
Cadmium	Cd
Copper	Cu
Nickel	Ni

Iron Fe

Zinc Zn

Manganese Mn

Chapter 1: Introduction

1.1 Introduction

Sewage treatment works (STWs) are essential for managing wastewater and mitigating human impacts on aquatic ecosystems (Adam et al., 2021; Delgado et al., 2024; Pereao et al., 2021). Despite advances in treatment technologies, STWs continue to discharge effluents that alter the physical (Neal *et al.*, 2005; Wang *et al.*, 2022), chemical (Millier and Hooda, 2011; Roberts and Cooper, 2018), and biological properties (Lee *et al.*, 2023; Mills *et al.*, 2024) of receiving rivers. These changes impact critical water quality indicators, including temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH (Parmar and Bhardwaj, 2013; Liao *et al.*, 2015; Najafzadeh *et al.*, 2019); and the concentrations of metals like Cu (Cu), Zn (Zn), and Ni (Ni) (Udayakumar *et al.*, 2011; Zhang *et al.*, 2023). Understanding these impacts requires comprehensive statistical analysis and pattern recognition techniques. Despite extensive research, key knowledge gaps remain in understanding the impacts of STWs on rivers, these are:

i) Lack of appropriate comparison - few studies investigate whether water quality changes in STW-influenced rivers significantly differ from what would be expected for rivers, i.e. the studies lack controls. Without such comparisons, it is difficult to determine whether observed changes are due to STW discharges or broader environmental factors. Some studies assessing the impact of sewage treatment works (STWs) on water quality have focused solely on effluent discharge points without incorporating upstream-downstream comparisons or unaffected control sites. For example, Naji et al. (2021) investigated microplastic concentrations in effluent and sludge from wastewater outlets in Bandar Abbas, Iran, without comparing them to upstream concentrations. Martín et al. (2012) analysed pharmaceutical compounds in influent, effluent, and sludge from Spanish STWs, but not the concentration in the receiving waters prior to effluent discharge; and Menzies et al. (2019) measured dimethyl ammonium chloride (DEEDMAC) levels in effluent and sludge from 41 U.S. STWs and once again did not include upstream or non-impacted reference sites.

Consequently, although these works document pollutant concentrations within STWs and their immediate outflows, they do not clarify how those levels compare to baseline or unaffected conditions in the river ecosystem.

ii) Limited dataset - Few studies are based on large-scale dataset analysis, i.e. the number of replicates is small. Neal & Robson (2000) presented an analysis of river water quality in eastern UK rivers, based on data collected from 1993 to 1999, which highlighted seasonal trends and pollutant inputs to coastal environments, such as Chlorophyll event analysis. However, they only included 15 sampling sites. Neal et al. (2005) evaluated nutrient dynamics and sewage effluent impacts on phosphorus concentrations based on relatively limited datasets, comprising monthly to bi-monthly measurements from only six small STWs over approximately 16 months in the upper Thames Basin. Similarly, Jarvie et al. (2002) monitored nutrient chemistry and biological indices across fewer than 10 sites in the upper Thames Basin over approximately two and a half years at weekly intervals, also without explicitly defined pairs or control sites. Although a small number of national- or regional-scale studies exist (Rothwell *et al.*, 2010; Comber *et al.*, 2022), they generally did not evaluate correlations among different determinands or explicitly quantify the direct impact of STW discharges on downstream waters. Much of the literature still relies on single-river case studies—for instance, Bubb and Lester (1994) examined a 60-km reach of the River Stour to assess downstream metal concentrations influenced by STW discharges, and Cooper and Hiscock reported trends in the River Wensum under the EU WFD but provided only a broad overview without in-depth inter-determinand analysis. These examples underscore the need for more comprehensive and controlled datasets in water quality research, which the current study addresses through extensive, long-term monitoring at numerous paired sites and the rigorous use of control rivers.

iii) Concentration is not the same as impact; studies have focused on changes in concentration rather than impact. This difference takes two forms. Firstly, the complex mix of chemicals coming from STW discharges may interact, for example, limiting metal bioavailability. Second, an impact measured at the point of discharge may not persist in the wider catchment.

This thesis aims to address these gaps by assessing the impact of STW discharges on receiving rivers for a range of water quality determinands.

1.2 Aims and Objectives

This study aims to evaluate the influence of STWs on river water quality across England, with a particular focus on both metal and non-metal determinands, nutrient-driven eutrophication, and the bioavailability of trace metals. By integrating long-term monitoring data, statistical modelling, and regulatory frameworks, the research seeks to identify the ecological implications of STW discharges and inform future pollution management strategies.

The specific objectives are:

- To quantify the impact of STW discharges on non-metal water quality determinands, including temperature (T), biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate ($NO3^-$), phosphate ($PO4^{3-}$), suspended solids (SS), specific conductance (κ), and pH. Chapter 2 addresses this through comparative analysis of upstream and downstream sites, incorporating control river pairs and exploring the roles of STW characteristics and hydrological covariates.
- To assess the role of STW effluents in contributing to nutrient enrichment and eutrophication, with a focus on chlorophyll-a (Chl-a) and silicon dioxide (SiO_2) as indicators of algal growth. Chapter 4 expands the analysis to include incident-based evaluation of eutrophication risk, using both concentration and frequency metrics of Chl-a exceedance.
- To investigate the downstream changes in the concentrations of eight key metals -- calcium (Ca), magnesium (Mg), cadmium (Cd), copper (Cu), nickel (Ni), iron (Fe), zinc (Zn), and manganese (Mn) and bioavailability of Cu, Zn, Mn and Ni. Meanwhile, how STWs influence their transport and ecological risks. Chapter 3 conducts a spatially resolved analysis of metal distribution and evaluates treatment efficacy across different STW types.
- To explore pollution patterns and ecological risks of bioavailable metals across England, using principal component analysis (PCA) and the M-BAT model. Chapter 5 generalises the findings beyond paired sites to a national

scale, assessing Risk Characterisation Ratios (RCR) and highlighting spatial hotspots of concern.

1.2.1 Process-type classification used in this thesis

Activated sludge plants are analysed separately from other secondary biological treatments because they differ mechanistically (suspended biomass with controllable sludge age and aeration in activated sludge versus attached-growth systems with lower biomass inventories and shorter effective contact times) (Márquez *et al.*, 2022). These differences are expected to produce distinct effluent signatures (e.g., typically lower BOD and suspended solids but more oxidised nitrogen where nitrification is established) and, therefore, potentially different downstream impacts (Ramin *et al.*, 2022). Accordingly, secondary treatment is separated into secondary activated sludge (SAS) and secondary biological (SB) throughout the thesis. Tertiary configurations are also distinguished as tertiary activated sludge (TAS) and tertiary biological (TB) where information is available. Where differentiation between STW types was not possible due to lack of information, this constraint applies only to tertiary treatment processes; separation of SAS from SB was retained.

1.2.2 Explanatory inference is prioritised

To test whether, and by how much, STW discharges affect determinands, and decomposing effects by Type, Year, Month and Pair. Accordingly, analysis variance (ANOVA) was used to test the significance of main effects and interactions, to accommodate covariates (distance, altitude, percentile flow) in a paired design, and to report factor-level effect sizes—η^2 for ANOVA factors—together with coefficients for covariates. Principal component analysis (PCA) was employed to summarise multivariate structure with interpretable loadings and to reduce collinearity among determinands. Modern machine-learning approaches (e.g., random forests, gradient boosting, neural networks; for dimensionality reduction, t-SNE/UMAP) were not prioritised because the objective is explanation rather than prediction; factor-level effect sizes are required; and transparency for regulatory interpretation (WFD/EQS) is essential.

Together, these chapters contribute to a comprehensive understanding of how STWs affect riverine ecosystems, providing evidence to support targeted

interventions under regulatory frameworks such as the Water Framework Directive (WFD) and to help ensure compliance with Environmental Quality Standards (EQS).

1.3 Non-metal determinands

STWs influence water quality by altering nonmetal determinands such as temperature (T), BOD, COD, nitrate (NO_3^-), phosphate ($PO4^{3-}$), SS, specific conductance (κ), and pH. These parameters are critical indicators of aquatic ecosystem health (Whitehead et al., 1981; Kumar and Reddy, 2009; Lemessa et al., 2023). Nevertheless, effluent releases can alter physical and chemical characteristics—such as temperature or suspended solids—in receiving rivers, causing localised environmental stress (Dickenson *et al.*, 2011).

The ecological implications of these changes are substantial. Below, the impact of STW discharge on the key water quality determinands in the receiving rivers was reviewed.

1.3.1 Temperature (T)

Water temperature is a fundamental property of river water (Webb, 1996). However, given human-induced climate change, there is a concern that river water temperature will increase in a warming climate (Albini *et al.*, 2023). Warmer river waters will have multiple impacts, influencing the growth and performance of a range of aquatic organisms (Kedra, 2020). Warming of river water can lead to both acute and chronic impacts on fish, for example, Brown trout (Salmo trutta), which face a mortality risk at stream temperatures exceeding 24.7 °C (Krause *et al.*, 2005). Beyond ecological concerns, warmer river water also has economic (Dottori *et al.*, 2018) and social consequences (Lamborn and Smith, 2019). It can negatively affect industries such as tourism (Knoll *et al.*, 2019) and energy (Paltán *et al.*, 2021), as higher water temperatures reduce the efficiency of river water used for cooling in thermal power generation, including nuclear reactors (Fricko *et al.*, 2016). According to Heberling et al. (2015), once river temperature exceeds 23 °C, each additional 1 °C is associated with a 1.5% increase in water-treatment costs.

The main focus of recent studies on river temperature has been the impact of anthropogenically induced climate change (Gooseff *et al.*, 2005), and numerous

modelling studies have suggested rising stream temperatures as a result (Morrill *et al.*, 2005; Woltemade and Hawkins, 2016). Arnell (1998) estimate that river temperatures would rise by 1.6 °C in summer and 1.8 °C in winter; Hondzo and Stefan (1993) had predicted a rise in surface water temperature by 1.9 to 2.2 °C; and Van Vliet et al. (2013) found that the water temperature increase on average by 0.8 – 1.6 for 2071–2100 relative to 1971–2000. For the USA, Kaushal et al. (2010) analysed stream temperatures for 40 major rivers and showed significant, long-term warming trends for 20 rivers, which were significantly correlated with air temperature. In Japan, Ye and Kameyama (2017) found that between 1981 and 2016, 42% of 153 sites were warming faster than the air temperature. However, Worrall et al. (2022) found that over a 45-year period since 1974 for 263 catchments across the UK, the mean river temperature was well buffered against changes in air temperature – a 1°C rise in air temperature resulting in 0.37 °C in mean stream temperature.

The impact of STWs as a driver of anthropogenic warming of streams and rivers has received less study than the impact of climate change (Kinouchi *et al.*, 2007). Relatively warm water can come from several anthropogenic sources, including thermal power stations, land use and land-use change, urban paved areas, and urban wastewater. Wilson & Worrall (2021) demonstrate that sewage effluent can be utilised for heating, as it was, on average, 2.2°C warmer than the receiving water. Effluent normally occurs around the residential area, which might be relevant to the population. Thermal energy from effluent has been mentioned to be recovered under certain climate conditions, and this might be a method to reduce carbon emissions (Hawley and Fenner, 2012). Therefore, continued population growth in developed countries is likely to be a driver of rising stream temperatures.

In England, final sewage effluent temperatures must meet conditions agreed in site-specific discharge permits, which usually take the form of a maximum compliance limit, which no temperature sample should ever exceed, and a risk assessment to ensure water quality of the receiving water body does not deteriorate and that it meets its target quality standard. Temperature standards for rivers in England permit a 2 or 3 °C increase or decrease in relation to ambient river temperatures, as the 98th percentile of annual surface water discharges

(Environment Agency, 2014). Although final sewage effluent is regulated, it is typically warmer than the receiving waters.

1.3.2 Biochemical oxygen demand (BOD)

Biochemical Oxygen Demand (BOD) is a critical indicator of water quality. When BOD and levels exceed permissible standards, it leads to several issues, including challenges for drinking water treatment, harm to aquatic life, and plants (Kulkarni, 2016). The EU has set guidelines for BOD of 3.0 to 6.0 mg/L, aimed at protecting fisheries, aquatic life, and domestic water supplies, which would be impacted (Akan *et al.*, 2012). High BOD is typically associated with problems in aquatic ecosystems (Susilowati *et al.*, 2018), such as fish illness (Sharma *et al.*, 2022), a decline in macroinvertebrates, and a decrease in fish and crustaceans (Dyer *et al.*, 2003).

Human activity is the primary driver of increased BOD. A US study conducted in Virginia, USA (Zipper et al., 2002) reported that land use, geology, and landscape features were associated with elevated median BOD values between 1978 and 1995. While changes in land use driven by human activity can increase BOD levels, geological structure and landscape characteristics also contribute meaningfully to spatial variation in BOD concentrations (Zipper *et al.*, 2002). Research comparing groundwater and freshwater BOD levels has revealed the impact of agriculture on the organic matter in groundwater. Groundwater affected by livestock activities exhibits a BOD of approximately 3.7 to 11.6 mg/L, while groundwater impacted by cultivation shows a BOD of 1.3 mg/L. Elevated BOD levels observed in groundwater affected by agriculture stand in clear contrast to the lower concentrations typically found in freshwater environments, which range from 0.4 to 0.8 mg/L (Kim *et al.*, 2003). Population growth, leading to increased water depletion, is another contributing factor to high BOD levels (Kamarudin *et al.*, 2020). Wastewater discharge also plays a significant role (Ooi *et al.*, 2022; Fitriana *et al.*, 2023; Subramaniam *et al.*, 2023). In contrast, wet weather events can also drive BOD spikes due to the mobilisation of pollutants. For example, Mulliss et al. (1997) documented a five-fold increase in mean BOD concentrations during storm conditions, with peaks reaching 115 mg/L, largely attributed to combined sewer overflows and surface runoff at the onset of rainfall. These findings suggest that

BOD pollution can arise from both hydrological extremes – low flows that limit dilution and high flows that transport accumulated organic matter into receiving waters – depending on local infrastructure and land use. A model of organic matter breakdown in landfill leachate confirmed that higher rainfall and temperatures contribute to increased BOD (Bhatt *et al.*, 2016).

In the 17th century, industrial development and a population boom led to the discharge of large volumes of untreated wastewater, causing severe environmental problems (Whelan *et al.*, 2022). The widespread adoption of sewage treatment plants in the latter half of the 20th century, including the gradual replacement of trickling filters with the more effective activated sludge process in larger facilities, mitigated point source pollution (Johnstone and Horan, 1996; Whelan *et al.*, 2022). The European Urban Wastewater Treatment Directive (UWWTD, 1991), which established wastewater discharge standards based on population equivalence (PE) -- a parameter for characterising industrial wastewaters, also known as unit per capita loading or equivalent person-- played a crucial role in reducing BOD and promoting the recovery of aquatic habitats (Johnson *et al.*, 2019). Modern wastewater treatment, which incorporates primary and secondary treatment stages, can remove up to 85% of BOD from the effluent (Sonune and Ghate, 2004). More recently, Freeman et al. (2018) highlighted aerated wastewater treatment systems capable of removing up to 90% of BOD. Further advancements in BOD removal techniques continue to emerge. For instance, advanced oxidation processes applied to municipal wastewater have demonstrated a reduction in BOD from 8 mg/L to 2 mg/L within 60 minutes, and photocatalytic treatment using TiO_2-MMT has achieved a reduction from 9 mg/L to 4 mg/L in just 35 minutes (Pamuła *et al.*, 2022).

In England, sewage treatment plants must not exceed either a maximum concentration limit of 25 mg/L (Johnson and Mara, 2005) or a BOD limit, or achieve a minimum percentage reduction of 70-90% compared to the incoming wastewater (Del Solar *et al.*, 2005). BOD is considered compliant if either of these conditions is met. There's an additional, stricter limit of 50 mg/L O_2. Exceeding this limit is not necessarily a complete failure if the minimum percentage reduction is achieved, but such situations still have a negative environmental impact and must be reported to the Environment Agency (EA).

1.3.3 Chemical oxygen demand (COD)

The COD is a measure of the amount of oxygen required to chemically oxidize and break down organic in water (Akan *et al.*, 2012). It was developed to provide a more immediate measure of oxygen demand in contrast to BOD which takes 5 days to measure.

COD is a crucial indicator of water quality and its impact on aquatic life (Towrqseno *et al.*, 1992; Chaudhry *et al.*, 2022). High COD levels have been observed to lead to: a decline in fish species (Sarkar and Islam, 2020); aesthetically displeasing darker water coloration (Yang *et al.*, 2019); foul odours (Novita *et al.*, 2019); and increased greenhouse gas (GHG) emissions (Medeiros *et al.*, 2023; Hernaningsih *et al.*).

Elevated COD levels are often associated with areas of human activity (Kotti *et al.*, 2005; Chaudhry *et al.*, 2022). Anthropogenic activities, such as land use changes involving reclamation, agriculture, and urbanization, directly contribute to increased COD. A study of the Hun River in China (2009-2010) found higher COD levels at sampling points located near areas of intensive land use, classifying the water quality as among the worst in China (Wang *et al.*, 2013). Conversely, research has also demonstrated a negative correlation between forest and agricultural land cover and rising COD levels (Rather and Dar, 2020). Xu et al. (2020) identified sewage outfall as the primary driver of high COD during the dry season, while agricultural runoff was the key factor in the wet season.

The industrial revolution and population growth led to severe COD pollution, as effluents aggravated water quality issues: water quality reached its nadir in 1940 (Burton, 2003). However, with increasing awareness of environmental concerns and advancements in innovation and development, various techniques have been employed for COD removal.

Regulatory measures such as the Environmental Protection Act (https://www.legislation.gov.uk/), the WFD (https://environment.ec.europa.eu/), and the Environmental Permitting (England and Wales) Regulations (https://www.legislation.gov.uk/) have contributed to reducing COD. In addition to regulations, the activated sludge process, invented in the early 20th century, has

proven effective in removing 85% to 95% of organic matter, e.g., a 96% removal rate (The Environmental Protection (Duty of Care) Regulations 1991). Other technologies have also achieved greater than 80% removal of COD: membrane bioreactors (MBRs (Trouve *et al.*, 1994)) and anaerobic sludge blanket (UASB) Yetilmezsoy and Sakar, 2008).

There has been research into the impact of high COD levels from STW discharge on receiving waters. However, research in this area often focuses on a single river with a limited number of sampling points and short study durations. For instance, Maphanga et al. (2022) only analysed three sites of municipal COD discharge on the Crocodile River to assess pollution spatial distribution and areas requiring improved treatment. Similarly, Iloms et al. (2020) examined data from January to September 2017 on the Vaal River in South Africa, highlighting concerns about high COD levels in municipal effluent and their impact on the Vaal River's biodiversity.

In England, meeting the discharge standard is mandated, regardless of whether the minimum percentage reduction falls within the range of 70% to 90%, or if the concentration limit is set at 125 mg O_2/L. Even if the effluent concentration exceeds the maximum compliance threshold of 250 mg O_2/L, as long as the minimum reduction requirement is fulfilled, it does not constitute a maximum failure.

1.3.4 Nitrate (NO_3^-)

Nitrate serves as an indicator of water contamination (Chowdhury *et al.*, 2003), closely linked with the use of nitrogen-based fertilizers (Bijay-Singh and Craswell, 2021). Nitrate, a major end product of nitrification in the nitrogen cycle, can pose environmental risks when its concentrations become elevated. Excess nitrate can lead to eutrophication and hypoxia, adversely affecting fish, altering ionic balances, and disrupting algal communities (Sahay *et al.*, 2006; Chen *et al.*, 2011; Kocour Kroupová *et al.*, 2018). Nitrate from wastewater impacts human and animal health, being linked to methemoglobinemia, cancer, thyroid gland enlargement, diabetes mellitus, and obesity. Methemoglobinemia occurs when nitrate exposure oxidizes Fe^{2+} to Fe^{3+}, disrupting oxygen transport (Camargo and Alonso, 2006; Parvizishad *et al.*, 2017; McNulty *et al.*, 2022). Nitrogen can also contribute to aquatic ecosystem acidification, particularly through nitrification when pH exceeds 5.6 (Rudd *et al.*, 1988). Additionally, increased nitrogen fluxes due to human activities over the past four decades, leading to 6- to 20-fold increases in discharges to the North Sea, have exacerbated eutrophication, resulting in algae blooms in coastal waters (Smith, 2003). For inland water, algal blooms lead to foul odours and environmental damage, consequently raising environmental treatment costs (Maqbool *et al.*, 2016).

Anthropogenic activities often result in high nitrate emissions, posing risks to both the environment and human health. Agricultural practices, particularly nitrogen overuse, contribute to elevated nitrate levels, with nitrate's instability leading to its seepage into groundwater (Schaller, 1991). Wastewater discharge has been linked to eutrophication, reducing oxygen levels in water and soil, hindering nutrient absorption in mangroves, and posing risks to human health (Agraz-Hernández *et al.*, 2018; Xu *et al.*, 2018). To mitigate these risks, converting nitrate into non-toxic nitrogen gas (N_2) is considered the most effective approach for water treatment method (Meng *et al.*, 2023).

When investigating the various causal factors contributing to the rise in nitrate (NO_3^-) concentrations, it is crucial to consider the impact of effluent from STWs on the environment. Studies have highlighted the role of STWs in increasing nitrate concentrations in receiving waters. For instance, Spahr et al. (2024) found that STWs significantly contributed to elevated nitrate (NO_3^-) levels in streams within

Southwest Ohio, with the highest concentrations observed in watersheds receiving effluent discharge. Their findings suggest that STWs are a major driver of nitrate pollution, particularly during baseflow conditions when effluent can constitute a substantial portion of streamflow. Research has also shown that upgrading STWs can lead to improvements in water quality by reducing nitrogen levels in receiving water bodies. For example, a study focusing on two rivers dominated by wastewater demonstrated that the upgrade of STWs resulted in decreased nitrogen concentrations and overall improvements in water quality (Wang *et al.*, 2020). These findings underscore the significance of considering the impact of effluent on nitrite levels and the environment when assessing factors contributing to nitrite rise.

In the UK, there is a legal limit of 50 mg/L for nitrate concentration in drinking water, consistent with the guideline value set by the WHO. Water companies are tasked with monitoring and treating water sources to ensure compliance with this standard, as stipulated by the Environment Agency (https://www.gov.uk/). Additionally, Nitrate Vulnerable Zones (NVZs) designate areas at high risk of nitrate leaching, prompting the implementation of specific regulations for farmers operating within these zones (Nitrate vulnerable zones, 2018). It is essential for farmers in NVZs to adhere to these regulations to mitigate the risk of nitrate pollution and protect water quality in affected areas.

1.3.5 Phosphate ($PO4^{3-}$)

Phosphate is vital for the biological growth of algae and aquatic plants (Jalali and Jalali, 2016). However, excessive amounts can pose risks to water bodies, as they contribute to triggering algal blooms, oxygen depletion in the water, and aquatic life mortality (Schaum, 2018; Abdulwahid *et al.*, 2023). These algal blooms, caused by excessive phosphate, can also produce toxins harmful to both humans and animals.

Major sources of phosphate pollution include agricultural runoff from fertilisers, effluents from STWs, industrial discharge, and aquaculture waste (Rao and Prasad, 1997; Sileika *et al.*, 2002; Dumont *et al.*, 2005; Maqbool *et al.*, 2016; Ye *et al.*, 2017; Nathanailides *et al.*, 2023). Effective management of these anthropogenic

sources is essential to mitigate phosphate pollution and its ecological consequences (Lewis *et al.*, 2011).

Human activities are the primary contributors to phosphate pollution, and treated wastewater from STWs plays a crucial role in altering riverine phosphate levels, yet research on this aspect remains limited. For instance, a study on the Enoree River examined the effluent from four STWs, highlighting that phosphate concentrations remained elevated downstream of the Taylor STW, despite a decrease compared to upstream levels (Andersen *et al.*, 2014). Spahr et al. (2024) found that STWs contributed to phosphate concentrations being 30 times higher than background concentrations, though this study focused on a limited number of watersheds with only two upstream STWs.

In the UK, there is no specific drinking water regulation for phosphate, but its management falls under the EU WFD, which aims to achieve good ecological status for all water bodies. One relevant regulation is the Detergents (Amendment) Regulations 2016 (https://www.legislation.gov.uk/), which limits phosphate content in standard dishwasher detergent dosages to 0.3 g/L to help reduce phosphate pollution in surface waters.

1.3.6 pH

pH serves as a critical indicator of water quality (Dong *et al.*, 2015). For drinking water, maintaining a pH range of approximately 6.5 to 8.5 is considered safe, as extremes in either alkalinity or acidity can have adverse health effects (Arhin *et al.*, 2023). However, pH values can vary significantly across different industrial discharges (Odjadjare and Okoh, 2010). In aquatic environments, the abundance of species like Macrobrachium rosenbergii has been found to correlate with water pH levels. Acidic water conditions, with pH levels lowered to mildly acidic (6.4 ± 0.5) or acidic (5.4 ± 0.2), can adversely impact their feeding, survival, and growth, with implications for their larval development (Liew *et al.*, 2022). Similarly, in horticulture, plants are typically cultivated in mildly acidic environments, with a pH range of 5.5 to 6.5 considered optimal for leafy greens (Gillespie *et al.*, 2020; Alexopoulos *et al.*, 2021).

The discharge from anthropogenic activities often exhibits varying pH levels, which can pose challenges to environmental management and may be difficult to address. For instance, pH values outside the range of 6 to 9 can suppress the activity of microorganisms crucial for wastewater treatment, potentially leading to damage to treatment equipment and lower water quality at discharge (Gaya *et al.*, 2014). Additionally, pH plays a critical role in the removal of remazol red dye and associated COD; for example, at a pH of 4, reductions of red dye and COD are reported to be 91.24% and 71.13%, respectively (Yuniawati and Rahmayanti, 2023). Achieving the desired pH levels can contribute to resolving certain environmental issues by enhancing the physicochemical conditions for pollutant removal. Specifically, pH influences the ionisation state and solubility of organic pollutants such as PAHs, thereby improving their adsorption and degradation efficiency. It also affects the generation and stability of reactive species in advanced oxidation processes, contributing to the reduction of COD levels in industrial wastewater (Alhothali et al., 2021).

There is a paucity of studies addressing pH levels in effluent, despite its critical importance to the water environment. A study conducted in Algeria monitored daily inflow and outflow from a STW between 2016 and 2019 to assess whether treated wastewater quality met WHO and CCME permissible standards (Guasmi *et al.*, 2022).

Although the WFD does not establish a specific pH requirement for rivers and other water bodies in England, pH levels remain a crucial factor in assessing their overall ecological health. The EA ensures that these water bodies meet good ecological standards in accordance with the guidelines outlined by the WFD through the 2016 Environmental Permitting Regulations.

1.3.7 Suspended solids (SS)

Suspended solids (SS) are fine sediment particles in water and pose a long-term challenge in wastewater treatment due to their impact on aquatic ecosystems (Bilotta and Brazier, 2008; Quinteiro *et al.*, 2015). Their presence can degrade water quality and disrupt aquatic habitats, making effective removal essential for environmental protection (Abeyratne *et al.*, 2023).

Suspended solids originate from various anthropogenic activities, particularly in industries such as food processing and oil refining (Roccaro *et al.*, 2014). For instance, starch factories generate large amounts of suspended solids, but due to the high cost of wastewater treatment, untreated effluent is often discharged (Pidgeon and Ness, 2006). Palm oil mill effluent (POME) contains fine cellulosic materials, oil, and water, which can pose challenges for conventional biological treatment systems (Dominic and Baidurah, 2022). In addition to their environmental impact, suspended solids can lead to biofilter clogging, reducing treatment efficiency (Cui *et al.*, 2021). The potential for biofouling necessitates the implementation of effective treatment methods to ensure compliance with discharge standards.

Various techniques have been explored for SS removal, including biologically activated membrane bioreactors (BAMBi), which adjust retention times to eliminate non-biodegradable particles (Ravndal *et al.*, 2015), and sand filtration (Fernandes del Pozo *et al.*, 2022).

Although the WFD does not prescribe a specific standard for suspended solids, their concentrations are considered important indicators of hydromorphological and physico-chemical pressure. The Freshwater Fish Directive proposes a Guideline Standard of 25 mg/L (annual mean), which is not mandatory but is used as a reference for managing continuous discharges from activities such as mining and quarrying. While exceedance of this threshold does not in itself imply failure to meet "good" ecological status under the WFD, it may indicate environmental concern in naturally low-turbidity systems such as chalk streams.

1.3.8 Specific conductance (κ)

Specific conductance, known as Conductivity, is an important water quality determinand, which proves to have positive relationship with turbidity, BOD and COD (Pathan and Shinde, 2022) and it thought to have strong relationship with water contamination (Odjadjare and Okoh, 2010; Mainali and Chang, 2021). In wastewater monitoring, conductivity is widely used to indicate pollution levels by detecting significant changes in ion concentrations (Bersinger *et al.*, 2015; Bondarenko *et al.*, 2016; Mortadi *et al.*, 2020; Wu and Brant, 2020). Studies have observed substantial increases in Specific conductance at downstream sites and

final effluent points, reflecting elevated ion concentrations due to STW discharges (Andrianova *et al.*, 2014; Chusov *et al.*, 2014; Bondarenko *et al.*, 2016).

In this study, both upstream and downstream sampling points are used to directly evaluate the impact of STWs on water quality. WFD does not set specific standards for electrical conductivity, but it is used to support water body typology and indicate potential anthropogenic pressures. Low alkalinity waters typically show values below 70 µS/cm, moderate between 70–250 µS/cm, and high alkalinity waters exceed 250 µS/cm, reflecting underlying geological conditions. Significant deviations from these background ranges may indicate environmental concern.

1.4 Eutrophication – Chlorophyll (Chl -a) and Silica (SiO_2)

Eutrophication, characterized by excessive nutrient accumulation (e.g., phosphate and nitrate), is driven by agricultural runoff, urban stormwater, and STW discharges, introducing nitrogen and phosphorus into aquatic systems (Withers *et al.*, 2017; Roberts and Cooper, 2018; Smith *et al.*, 2020). These inputs can trigger harmful algal blooms, reducing water clarity, depleting oxygen, and disrupting aquatic food webs, leading to biodiversity loss and economic impacts (Heisler *et al.*, 2008; Graham *et al.*, 2015).

Chlorophyll concentration serves as a key indicator of algal biomass and eutrophication, providing insights into nutrient enrichment and ecosystem health (Kim *et al.*, 2020). A widely used threshold of 15 µg/L identifies chlorophyll incidents, signalling ecological disturbances such as algal blooms (Bowes *et al.*, 2019). This threshold is critical in regulatory frameworks for setting water quality targets and assessing eutrophication risks.

Diatoms, an essential phytoplankton group, require silica to form their frustules (Kröger *et al.*, 1999). Adequate silica availability supports diatom dominance (Yool and Tyrrell, 2003), which helps maintain water clarity (Marella *et al.*, 2020) and sustains productive food webs (Harvey *et al.*, 2019). However, eutrophic conditions often lead to silica depletion (Schelske and Stoermer, 1971), promoting harmful algal blooms and ecosystem imbalances (Wurtsbaugh *et al.*, 2019).

Monitoring chlorophyll concentrations over time helps evaluate nutrient impacts from STW effluents and assess the effectiveness of mitigation strategies. Integrated approaches, including advanced STW nutrient removal, improved land-use practices, and public awareness campaigns, are essential for managing eutrophication and maintaining ecological balance.

1.5 Metals

Metal concentrations in aquatic environments are often reported as dissolved concentrations, but only a fraction of this—known as the bioavailable concentration—is directly accessible for biological uptake and toxicity. Bioavailability is influenced by water chemistry parameters such as pH, dissolved organic carbon (DOC), and Calcium (Ca), which can alter metal speciation and interaction with biota (de Paiva Magalhães *et al.*, 2015). Therefore, relying solely on dissolved concentrations may still misrepresent ecological risk.

Metals such as Cadmium (Cd), Copper (Cu), Nickel (Ni), and Zinc (Zn) are recognized for their toxicity to aquatic ecosystems at elevated concentrations (Emenike *et al.*, 2022; Chen *et al.*, 2023). These metals can persist in the environment, bioaccumulate in organisms, and disrupt ecological balance. However, the bioavailability and toxicity of these metals are influenced by water quality parameters such as pH, DOC, and Ca, which modify their behaviour and ecological impact (Di Toro *et al.*, 2001; Heijerick *et al.*, 2003).

While Ca and Mg are essential nutrients, they regulate the bioavailability of toxic metals, competing for binding sites on aquatic organisms (de Paiva Magalhães *et al.*, 2015). STWs have been identified as sources of metals, particularly Cd and Ni, due to variations in sewage treatment efficiency and combined sewer systems (Chon *et al.*, 2012).

Previous studies have explored heavy metal impacts on rivers and estuaries but have often been limited in spatial or temporal scope. For example: Molloy and Hills (1996) focused on industrial pollution trends in the Firth of Clyde, assessing Zn, Manganese (Mn), Iron (Fe), Cu, Lead (Pb), and Ni, but did not evaluate STW influences. Similarly, Bubb and Lester (1995) demonstrated that sewage effluents

increased Cu, Cd, Fe, Pb, and Mn concentrations in the River Yare, UK, and enhanced metal complexation, but their analysis was limited to sediment-phase accumulation and did not assess bioavailability or downstream water column variation linked to STWs. Chon et al. (2012) examined metals in the Aire-Calder catchment and identified STWs as primary sources of Cd and Ni but found minimal contributions of STWs for Pb and Hg. Metals such as Cd, Cu, Ni, and Zn are highly toxic to aquatic organisms, even at trace concentrations (DeWitt *et al.*, 1996; Wang *et al.*, 2020). Once released into rivers, these metals bioaccumulate in aquatic organisms and bio magnify through the food web, posing risks to top predators, including humans (Izegaegbe *et al.*, 2020). Elevated metal concentrations can also alter ecosystem functions, disrupting nutrient cycling, microbial communities, and overall ecological balance (Dong *et al.*, 2021). Additionally, high metal levels contribute to degraded water quality, increasing turbidity and sedimentation, which can impact drinking water sources, recreational activities, and aquatic habitat sustainability (Birch and Olmos, 2008).

This study expands on prior research by focusing on eight metals—Ca, Mg, Cd, Cu, Ni, Fe, Zn, and Mn—to assess their concentration changes downstream of STWs and their bioavailability in receiving waters. Bioavailability, which determines the fraction of metals available for biological uptake, is a crucial factor in assessing ecological risk (Hering *et al.*, 2010). Unlike total metal concentrations, bioavailable fractions provide a more accurate indication of potential toxicity.

Excessive metal concentrations interfere with nutrient cycling and biological functions in aquatic organisms, leading to adverse effects such as reduced growth, reproduction, and survival rates. Bioaccumulation further amplifies these risks across trophic levels, ultimately impacting higher predators, including humans.

To mitigate ecological risks, regulatory frameworks such as the WFD and EQS establish permissible concentrations for metals in effluents. For instance, the UK EQS limits for Cd, Cu, Ni, and Zn are 0.05 mg/L, 0.5 mg/L, 0.5 mg/L, and 2 mg/L, respectively.

By integrating bioavailability models with long-term datasets, this study aims to provide a comprehensive assessment of metal impacts and inform management strategies for achieving ecological health standards in aquatic systems.

1.5.1 Calcium (Ca)

Calcium plays a pivotal role in regulating water hardness, buffering capacity, and pH levels (Harvey *et al.*, 2019; Weyhenmeyer *et al.*, 2019). It also plays a crucial role in modulating metal bioavailability and toxicity by competing for binding sites on aquatic organisms, thereby reducing the uptake of toxic metals such as Cu and Zn (Allen and Janssen, 2006). Additionally, calcium supports ecosystem stability and biodiversity, as many aquatic species rely on specific calcium-to-metal ratios for physiological functions (Wang *et al.*, 2014; de Paiva Magalhães *et al.*, 2015).

STWs influence calcium concentrations through their treatment processes, often using calcium-containing compounds (e.g., lime) for pH regulation and precipitation (Hering *et al.*, 2010; Liu *et al.*, 2011). As a result, effluent discharge can elevate calcium levels in receiving waters, altering water chemistry and buffering capacity (Bourg and Loch, 1995). While increased calcium levels can help mitigate acidity and metal toxicity, excessive inputs may also impact metal mobility and solubility, affecting their transport and bioavailability downstream (Miranda *et al.*, 2022).

Calcium also plays a role in metal sedimentation and nutrient cycling. Elevated calcium concentrations can reduce metal bioavailability by forming insoluble complexes or precipitates, limiting their uptake by aquatic organisms (Peters *et al.*, 2014). Conversely, fluctuations in calcium levels may disrupt ecological dynamics, particularly for species that require stable calcium concentrations for survival (Allen and Janssen, 2006). The interaction between calcium, pH, and DOC further influences metal solubility and toxicity, with higher calcium concentrations often leading to reduced metal solubility under neutral to slightly alkaline conditions (Romkens *et al.*, 1996; Tsui *et al.*, 2006).

Understanding calcium dynamics in aquatic systems is essential for developing effective pollution management strategies and ensuring compliance with regulatory frameworks such as the EU WFD. By linking calcium concentrations to water quality and ecosystem health, this study aims to provide insights into the broader implications of STW operations on river ecosystems.

There is currently no specific EQS standards or discharge limit set for Ca in the UK. However, calcium plays a key role in determining water hardness, which in

turn affects the behaviour and toxicity of various pollutants, particularly metals. WHO notes that water with hardness values of 200 mg/L or higher (measured as calcium carbonate) may lead to scale formation, whereas water with hardness below 100 mg/L can be more corrosive to pipework.

1.5.2 Magnesium (Mg)

As an essential element in aquatic ecosystems, magnesium plays an important role in regulating water chemistry and influencing ecological processes (Pleshchitser, 1958; Novak *et al.*, 2020). As a major contributor to water hardness, magnesium interacts with other metals and ions (Atouei *et al.*, 2016), regulating their solubility, mobility, and bioavailability (Berthon *et al.*, 1983; Blaquiere and Berthon, 1987). These interactions are crucial for understanding the dynamics of aquatic ecosystems and their susceptibility to pollution, especially in receiving waters affected by STWs (Ling *et al.*, 2017; Anggayasti *et al.*, 2023).

STWs influence magnesium levels through wastewater composition and treatment processes. Magnesium hydroxide, commonly used for pH neutralization and chemical precipitation, can increase Mg concentrations in effluent discharges (Hering *et al.*, 2010; Devlin *et al.*, 2019; Fouda *et al.*, 2021). Once released into rivers, these elevated Mg levels modify water chemistry, enhancing buffering capacity and mitigating the effects of acidic discharges (Bourg and Loch, 1995). However, excess magnesium may also alter metal speciation and transport, influencing the mobility of contaminants such as Cu and Cd (de Paiva Magalhães *et al.*, 2015).

Magnesium has a dual role in metal toxicity regulation. It can reduce metal bioavailability by competing with toxic metals such as Ni and Zn for binding sites on aquatic organisms, thereby lowering their toxicity (Allen and Janssen, 2006). Additionally, Mg interacts with DOC to form complexes, influencing metal transport downstream (Peters *et al.*, 2014). However, at excess concentrations, Mg can disrupt ionic balance, potentially affecting osmoregulation and physiological functions in aquatic organisms (Fagerbakke *et al.*, 1999; Mooney *et al.*, 2020).

The role of magnesium in pH stabilization and metal speciation has significant implications for pollution risk assessment and water quality management

(Semerjian and Ayoub, 2003; Cotruvo *et al.*, 2017). Higher magnesium concentrations can reduce metal solubility and toxicity under neutral to alkaline conditions, improving water quality (Brown and Markich, 2024). However, in ecosystems with disproportionately high magnesium inputs, ecological balance may be disrupted, requiring targeted management strategies (Mooney *et al.*, 2020).

Despite its importance, the role of magnesium in modifying metal toxicity remains underexplored compared to other water quality parameters. This study aims to assess the influence of STW discharges on Mg concentrations in receiving rivers and evaluate their ecological consequences. By linking Mg levels to key water chemistry parameters and ecosystem health, this research provides insights into STW-related aquatic pollution and contributes to sustainable water resource management.

In the United Kingdom, there are no specific EQS standards or discharge limits established for Mg in surface waters. However, magnesium, along with calcium, contributes to water hardness, which can influence the behaviour and toxicity of various pollutants.

1.5.3 Cadmium (Cd)

Cadmium is a highly toxic, bioaccumulative heavy metal and a priority pollutant due to its severe ecological and health risks (Emenike *et al.*, 2022). Even at trace levels, cadmium disrupts biological processes, reduces biodiversity, and accumulates in sediments and aquatic organisms, magnifying its toxicity through the food web (Taylor, 1983; Zhao and Marriott, 2013; Andresen *et al.*, 2016). Cadmium exposure disrupts enzyme activity, ion regulation, and reproduction in aquatic organisms (Paquin *et al.*, 2002). Chronic accumulation leads to toxic effects across trophic levels, including risks to human health via contaminated fish and water (Ali *et al.*, 2024).

Cadmium contamination in aquatic ecosystems is largely attributed to industrial discharges (Shrestha *et al.*, 2021), mining operations (Chan *et al.*, 2021), agricultural runoff (Jiraungkoorskul *et al.*, 2016), and sewage treatment works (STWs) (He *et al.*, 2025). The limited desorption efficiency of cadmium from sewage sludge suggests that STWs may not fully remove dissolved cadmium during conventional treatment processes (Choi and Yun, 2006). Additionally, cadmium can remobilise from sediments under changing redox conditions, further complicating its management (Phaenark *et al.*, 2024).

Cadmium bioavailability, which determines its ecological impact, is influenced by pH, water hardness, and DOC (Wang *et al.*, 2019). Under acidic conditions, cadmium solubility increases, leading to greater toxicity to aquatic organisms (Bourg and Loch, 1995). Conversely, high Ca and Mg concentrations in hard water can reduce cadmium's toxicity by competing for binding sites on organisms (de Paiva Magalhães *et al.*, 2015). DOC also plays a role by forming cadmium complexes, which reduce immediate bioavailability but facilitate long-distance transport (Peters *et al.*, 2014).

To mitigate cadmium pollution, regulatory frameworks such as the European Union's Urban Wastewater Treatment Directive (UWWTD) and EQS have established stringent discharge limits (0.05 mg/L in the UK). Compliance requires advanced treatment technologies such as chemical precipitation, ion exchange, and adsorption using activated carbon or biosorbents (Oliveira *et al.*, 2007). Recent innovations, including constructed wetlands and enhanced biological processes,

offer alternatives for cadmium removal, though efficiency varies depending on site-specific factors (Plaimart *et al.*, 2024).

1.5.4 Copper (Cu)

Copper is an essential trace element but becomes toxic at elevated concentrations, posing risks to aquatic ecosystems (Nor, 1987; Kiaune and Singhasemanon, 2011). Its persistence in water is enhanced by complexation with inorganic ions (e.g., sulfates, chlorides) and organic ligands (e.g., humic substances), making it difficult to remove via conventional wastewater treatment (Sarathy and Allen, 2005; Constantino *et al.*, 2015). This persistence is a major concern, particularly in STWs, urban runoff, industrial discharges, and diffuse sources like agriculture and from antifouling paints (Steiner and Boller, 2001; Sidondi *et al.*, 2024a).

Copper bioavailability is strongly influenced by pH, DOC, and water hardness (Di Toro *et al.*, 2001). Under acidic conditions, copper remains highly soluble and toxic (Bourg and Loch, 1995), whereas DOC complexation reduces bioavailability by binding copper into stable organic forms (Peters *et al.*, 2014). Additionally, higher water hardness (Ca and Mg levels) mitigates toxicity by competing for biological binding sites (de Paiva Magalhães *et al.*, 2015).

Elevated copper levels interfere with respiration and ion regulation in fish by damaging gill membranes, leading to reduced survival, growth, and reproductive success, particularly in sensitive species like salmonids (Heijerick *et al.*, 2003; Jonsson, 2023). Copper also bioaccumulates in lower trophic organisms (e.g., plankton, benthic invertebrates) and biomagnifies through the food web, impacting higher predators, including birds and mammals (Allen and Janssen, 2006).

Environmental policies, such as the WFD and UK EQS, set copper limits at 28 µg/L for freshwater and 3 µg/L for saltwater (Comber *et al.*, 2008). However, STWs remain a major contributor due to the ineffectiveness of standard filtration and precipitation methods in removing dissolved copper (Hutchinson, 2003; Chon *et al.*, 2012).

To address these challenges, recent advances in wastewater treatment focus on bioavailable copper removal rather than on the total concentration. Techniques such

as enhanced filtration, adsorption using activated carbon, and constructed wetlands show promise but require site-specific optimization (Rafiu *et al.*, 2007; Plaimart *et al.*, 2024). Understanding copper dynamics in river systems is crucial for improving management strategies and ensuring regulatory compliance to protect aquatic ecosystems.

1.5.5 Nickel (Ni)

Nickel is a transition metal that occurs naturally but is influenced by human activities (Heijerick *et al.*, 2003; Jonsson, 2023). Industrial discharges, mining activities, electroplating and alloy manufacturing, agricultural runoff, and STWs are major sources of nickel contamination in water bodies (Kumar and Dwivedi, 2021; Parades-Aguilar *et al.*, 2021). Despite STW treatment efforts, nickel often remains in dissolved or particulate forms, influencing its bioavailability and environmental persistence (Simpson *et al.*, 2014).

Nickel toxicity disrupts ecosystem functions by interfering with nutrient cycling and biodiversity. It impairs reproduction, survival, and enzyme activity in aquatic organisms, leading to oxidative stress and cellular damage (Naz *et al.*, 2023). Nickel also bioaccumulates in organisms, with long-term exposure posing risks through biomagnification up the food chain (Ali *et al.*, 2024).

The bioavailability of nickel is influenced by pH, redox conditions, and DOC (Peters *et al.*, 2014). Acidic conditions increase nickel solubility and toxicity, while higher pH and water hardness (Ca and Mg presence) reduce bioavailability by competing for biological binding sites (de Paiva Magalhães *et al.*, 2015). DOC forms complexes with nickel, which can decrease immediate toxicity but enhance downstream transport (Worrall *et al.*, 2019).

To mitigate nickel contamination, EQS limits nickel concentrations in freshwater to 20 μg/L in the UK. However, compliance remains challenging due to residual nickel in STW effluents (Hutchinson, 2003). Optimised treatment methods such as ion exchange, adsorption, and biological filtration have shown promise, yet site-specific variations affect their efficiency (Oliveira *et al.*, 2007; Plaimart *et al.*, 2024).

This study integrates bioavailability models with long-term datasets to refine nickel risk assessments and enhance pollution management strategies.

1.5.6 Iron (Fe)

Iron (Fe) is a naturally abundant element in freshwater ecosystems and plays a critical role in biogeochemical cycling. It primarily exists in two oxidation states: ferrous iron (Fe^{2+}), which is soluble and bioavailable, and ferric iron (Fe^{3+}), which is insoluble and forms precipitates under oxic conditions. This oxidation-reduction cycle, influenced by pH, redox potential, and dissolved oxygen levels, determines the mobility, bioavailability, and ecological effects of Iron (Bourg and Loch, 1995).

Iron enters aquatic systems through natural processes such as rock weathering, soil erosion, and groundwater discharge. However, anthropogenic activities, including STWs, mining, and industrial operations, elevate iron concentrations (He *et al.*, 2025). STWs contribute iron through the use of iron salts (e.g., ferric chloride, $FeCl_3$) in phosphorus removal, leading to iron-enriched effluents (Matthiessen *et al.*, 1999). Once released, iron can remain dissolved in anoxic conditions or precipitate as ferric hydroxide [$Fe(OH)_3$] in oxygen-rich environments, forming rust-coloured deposits in sediments.

Excessive iron in aquatic ecosystems can affect water quality and ecosystem health in several ways:

- Increased turbidity and sedimentation: Ferric precipitates reduce water clarity, alter sediment composition, and smother benthic habitats, affecting invertebrate and fish populations (Plaimart *et al.*, 2024).
- Photosynthesis inhibition: High turbidity limits light penetration, disrupting phytoplankton growth and reducing oxygen production (Hutchinson, 2003).
- Oxygen depletion: Microbial oxidation of Fe^{2+} to Fe^{3+} consumes dissolved oxygen, exacerbating hypoxic conditions, particularly in stratified water bodies (Worrall *et al.*, 2019).
- Formation of iron-organic complexes: Dissolved organic carbon (DOC) binds iron ions, reducing immediate toxicity but enhancing downstream transport, potentially affecting distant ecosystems (Peters *et al.*, 2014).

The toxicity of Iron to aquatic organisms depends on environmental conditions. Under anoxic or acidic conditions, Fe^{2+} remains soluble and highly bioavailable, leading to potential toxicity through osmoregulatory and respiratory interference (Price *et al.*, 2022). In contrast, neutral or alkaline conditions promote Fe^{3+} precipitation, reducing immediate toxicity but contributing to sediment pollution.

The WFD and EQS recommend dissolved iron concentrations below 1 mg/L (Comber *et al.*, 2008) to minimize iron-related ecological risks. Achieving compliance requires effective STW operations, advanced iron removal technologies, and continuous monitoring to track seasonal fluctuations in iron levels.

1.5.7 Zinc (Zn)

Zinc is an essential trace element for biological functions, but excessive concentrations in aquatic systems pose toxicity risks to organisms and disrupt ecological balance. Zinc contamination originates from industrial discharges, agricultural runoff, urban wastewater, and STWs (He *et al.*, 2025). Conventional STW processes struggle to remove zinc effectively, as dissolved Zn^{2+} ions persist through primary and secondary treatment (He *et al.*, 2025). While advanced treatment methods such as electrocoagulation and adsorption (e.g., chitosan, activated carbon) improve removal efficiency, cost and operational complexity limit widespread adoption (Plaimart *et al.*, 2024).

Excess zinc in freshwater systems can significantly alter water chemistry, microbial diversity, and aquatic fauna. It tends to adsorb onto sediments or form complexes with organic and inorganic ligands, reducing its mobility and altering its downstream bioavailability (Peters *et al.*, 2014). Elevated zinc concentrations have been shown to disrupt microbial communities, leading to decreased diversity and impaired nutrient cycling and other essential ecological processes (Matthiessen *et al.*, 1999). Furthermore, zinc can interfere with enzyme activity, osmoregulation, and metabolic functions in aquatic organisms, resulting in reduced growth and reproduction rates in fish and invertebrates (Naz *et al.*, 2023). Over time, zinc can also bioaccumulate in lower trophic levels and bio magnify through the food web, posing health risks to top predators, including humans who consume contaminated fish (Ali *et al.*, 2024).

To mitigate zinc pollution, the WFD and EQS set dissolved zinc limits at 10.9 µg/L to minimize aquatic toxicity risks.

1.5.8 Manganese (Mn)

Manganese is an essential micronutrient for aquatic organisms and plays a crucial role in enzymatic processes and metabolic functions. However, manganese can become toxic at elevated concentrations, affecting aquatic ecosystems and posing risks to human health. Manganese contamination in rivers can arise from natural processes such as rock weathering, as well as anthropogenic sources, including STWs, industrial effluents, and mining activities (He *et al.*, 2025). Due to its complex geochemical behaviour, manganese presents considerable challenges for water quality management.

In STW effluent, manganese predominantly exists in the soluble form Mn^{2+}, which remains bioavailable under anoxic or low-oxygen conditions (Matthiessen *et al.*, 1999). Oxidation and Precipitation: In oxygen-rich environments, Mn^{2+} is oxidized to Mn^{3+} and Mn^{4+}, forming insoluble manganese oxides that precipitate as sediments (Plaimart *et al.*, 2024). This precipitation process increases turbidity and sedimentation, potentially smothering benthic habitats. Complexation with Organic Matter: Manganese can form complexes with dissolved organic carbon (DOC), enhancing its transport downstream while modulating its bioavailability (Peters *et al.*, 2014). Adsorption to Particles: Manganese ions can adsorb onto suspended sediments and clay particles, further influencing their distribution and ecological impact (Muliwa *et al.*, 2019).

The behaviour and bioavailability of Mn are dependent on environmental conditions. In STW effluents, Mn is predominantly present as soluble Mn^{2+}, which remains bioavailable under anoxic or low-oxygen conditions (Matthiessen *et al.*, 1999). Under toxic conditions, Mn^{2+} is oxidised to Mn^{3+} or Mn^{4+}, forming insoluble oxides that precipitate and increase turbidity (Plaimart *et al.*, 2024). Mn also forms complexes with DOC, which modulates toxicity and facilitates downstream transport (Peters *et al.*, 2014), and may adsorb onto suspended particles such as clay, further affecting its mobility (Muliwa *et al.*, 2019). Acidic pH enhances solubility and toxicity, while higher temperatures accelerate redox cycling (Bourg and Loch, 1995; Worrall *et al.*, 2019).

To safeguard aquatic ecosystems and human health, regulatory frameworks such as the WFD have established EQS for Mn. In the UK, concentrations in surface waters are generally required to remain below 0.3 mg/L to ensure compliance and minimise ecological risk.

1.6 Bioavailability and STW Influence on Metal Dynamics

While total metal concentrations provide a baseline for monitoring, bioavailability—defined as the fraction of metals available for uptake by organisms—determines actual ecological risk (Peijnenburg *et al.*, 1997; Vaananen *et al.*, 2018). Key factors influencing bioavailability include pH, water hardness, and DOC (de Paiva Magalhães *et al.*, 2015).

STWs discharge a complex mix of nutrients, organic matter, and residual metals, which may alter the water chemistry of receiving rivers (Srivastava *et al.*, 2022).These chemical changes can directly influence metal bioavailability through two main mechanisms. First, elevated levels of DOC downstream of STWs may bind with metals to form stable complexes, thereby reducing their immediate toxicity to aquatic organisms (Van Veen *et al.*, 2002). Second, STW effluents can lower pH, particularly in soft or poorly buffered waters, increasing the proportion of free ionic metal species, which are more bioavailable and toxic (Worrall *et al.*, 2019). These combined effects complicate metal speciation and transport and underscore the importance of bioavailability-based assessment in evaluating STW impacts.

Despite recognition of the importance of bioavailability, considerable knowledge gaps remain. Most existing studies focus on localised river sections, with limited assessments conducted at the catchment or national scale. Additionally, there is insufficient research on how STW discharges influence bioavailable metal concentrations under varying environmental conditions. Furthermore, the frequency with which metal concentrations downstream of STWs exceed bioavailability-based EQS thresholds remains unclear, raising important questions for regulatory policies.

1.7 Thesis Structure

This thesis is organised into five main chapters, each addressing specific aspects of how STWs affect river water quality in England:

- Chapter 2 evaluates the impact of STW discharges on non-metal water quality determinands, including temperature, BOD, COD, nitrate, phosphate, pH, suspended solids, and Conductivity, while considering geographical variation and STW characteristics.
- Chapter 3 investigates the influence of STW discharges on dissolved metal (Ca, Mg, Cd, Cu, Ni, Fe, Zn, Mn) and bioavailable metal (Cu, Zn, Mn Ni) concentrations, with attention to spatial patterns, STW characteristics, and bioavailability.
- Chapter 4 examines the role of STW discharges in nutrient enrichment and eutrophication, focusing on chl-a, SiO_2, and the frequency of eutrophication incidents.
- Chapter 5 expands the analysis to the national scale, assessing spatial and temporal patterns of bioavailable metal pollution and exceedances of EQS using the M-BAT model.
- Chapter 6 provides a synthesis of the findings, highlighting the overall significance of STW discharges for riverine water quality, outlining study limitations, and identifying directions for future research.

Collectively, these chapters provide a comprehensive assessment of the ecological and chemical implications of STW discharges, supporting the development of effective pollution management strategies under the WFD and EQS.

Chapter 2: Non-metal Determinands[1]

2.1 Introduction

This chapter analyses stream temperature (T), biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate (NO_3^-), phosphate ($PO4^{3-}$), pH, suspended solids (SS), and specific conductance (κ) in river waters to understand whether discharges from STWs have had an impact. These determinands have a direct or indirect impact on river water quality and their impacts have been reviewed in the previous chapter. The hypothesis proposed was that, if a STW discharge had a significant impact on river water quality (e.g., stream temperature), then the value of the determinand in the receiving river would be different downstream of the discharge. However, with every catchment having a different background river water quality, the comparison was performed as the difference between the upstream and downstream of the STW discharge. Furthermore, a water quality determinand, such as stream temperature, may be expected to change downstream anyway, and so the comparison upstream and downstream on a river reach with a STW's discharge must also be compared to changes across river reaches without a STW discharge.

Key questions of this chapter are then:

- Does the final sewage effluent discharge from STWs cause a significant change in the concentration, or value, of water quality determinands?
- If STWs discharges cause a difference to river water quality can these differences be explained by differences between the size and nature of the STWs?

[1] The majority of this chapter has been accepted (pending minor revisions) into *Ecohydrology*.

2.2 Approach and Methodology

The approach of this study was to consider the impact of discharges from STWs for a range of determinands while controlling for influence of the difference between years, months and control river reaches for a range of STWs. If significant impacts are demonstrated then the effect of each STW is compared to its properties of the sewage works to assess whether treatment approaches, technologies or the scale of the works influences its impact on receiving waters.

2.2.1 Study data

In this study, 21 years (2000 to 2020) of river water quality data from English rivers was used. All the water quality data used were collected by the Environment Agency (EA) – the UK government`s environmental protection agency in England. The monitoring of the EA includes chemical oxygen demand (COD); biochemical oxygen demand (BOD); river water temperature (T); suspended solids (SS); pH; specific conductance; nitrate concentration (NO_3^-); and orthophosphate concentration (PO_4^{3-}). Note that within this study BOD is henceforward referred to as a concentration where it is a rate or capacity, i.e. it is decline in concentration of dissolved O_2 over a period of 5 days. In addition to the water quality data from the river monitoring points above and below discharges and for control rivers, data measured in the final effluent discharge were also examined for comparative purposes although not included in the statistical analysis for this chapter. The analytical methods used by the EA are detailed by the UK government's Standing Committee of Analysts (http://standingcommitteeofanalysts.co.uk/).

Only routine water quality monitoring data were included in this study and data collected as part of unplanned reactive monitoring for the investigation of statutory failures were excluded as they would have biased analysis in favour of an impact of sewage discharges because they increased frequency of sampling at the time of suspected high sewage discharge. Further, only monitoring sites with at least 20 measurements over at least 20 years were included.

STW and Control Pairs

The study identified all monitored STWs discharges where the stream temperature of the final sewage effluent had been measured. Stream temperature was the most widely sampled of the determinands of interest and so this gave the largest initial dataset from which suitable sites could be selected. For each of these discharges, the nearest monitoring points on the receiving river were then examined. River water monitoring sites were chosen if a pair of river water monitoring sites could be found where one of the sites was upstream of the STW discharge and the other was downstream of the STW discharge. Further, pairs of sites were chosen if the two monitoring sites were on the same river and no identifiable stream, or other discharge, had joined the receiving river in between the two river water monitoring sites. These paired monitoring sites, one upstream and one downstream of the STW discharge are henceforward referred to as STW pairs (Figure 2.1).

It could be expected that river water quality would change downstream regardless of the presence or absence of a STW discharge and that any pair of sampling sites upon a river would be different. Therefore, all river water monitoring sites in England, where water quality had been monitored between 2000 and 2020, were examined. Pairs of sites were selected where they were up and downstream of each other without any known discharge between or any known stream joining the monitored river. Henceforth, these pairs of monitoring sites are known as Control pairs (Figure 2.1). In each case, the Control pair was chosen such that it did not have a common monitoring site with any STW pair. It would be hypothesized that, if STW discharges are changing a water quality determinand then the river water quality differences in STW pairs will be greater than that for Control pairs. For both the control and STW pairs river water quality determinand differences were calculated where an observation was made on the same day – it was not possible to pair data at a sub-daily level. If there was only one determinand value for a pair on a particular day, be that a STW or Control pair, then that pair was excluded from further analysis.

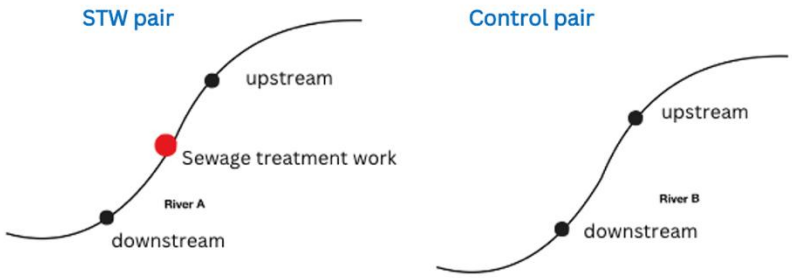


Figure 2.1 STW pair and Control pair.

Through this process, 442 STW and 419 Control pairs were initially identified from the stream temperature records. Visual inspection of the distribution of the STW and Control pairs shows no apparent bias in the location of the pairs (Figure 2.2). When completeness of data and determinands other than stream temperature was considered then the number of pairs that could be considered was smaller (Table 2.1). Once the number of pairs that could be matched for being sampled upon the same day then there were more pairs for pH than for stream temperature and the lowest number of pairs were for COD. Wherever available, the final effluent data for the STW within the each STW pair was also extracted from the EA WIMS database, but this final effluent concentration data was only ever used for comparison purposes and not used within the statistical analysis.

(a) STW pairs (b) Control pairs

Figure 2.2. Spatial distribution of STW and control site pairs.

Table 2.1. The number of pairs, both STW and Control, that could be included in this study.

Determinand	STW Pairs	Control Pairs
Temperature (°C)	256	168
BOD (mg O_2/L)	168	100
COD (mg O_2/L)	40	24
Phosphate (mg P/L)	317	232
Suspended solids (mg/L)	180	169
pH	202	150
Nitrate (mg N/L)	73	45
Specific conductance (µS/cm)	255	187

Covariates

Any difference between pairs of monitoring points, whether control or STW pairs, could be due to the physical arrangement of the pair, e.g., some water quality determinands can be expected to change downstream, with or without the presence of a sewage discharge. Therefore, if determinands could be expected to change downstream then the greater the distance between monitoring points the greater the expected difference in the water quality determinands. If the distance between monitoring points within a Control or STW pair could make a difference, then this study needs to show that difference due to the presence of a STW discharge is independent of the distance between monitoring sites in a pair. Therefore, the Euclidean distance between the monitoring points within each pair, both STW and Control pairs, was calculated and included as a covariate in the analysis.

Equally, it could be expected that for some determinants, e.g. stream temperature, the value of the determinant would be lower at higher altitudes to account for the physical relationship between monitoring sites within each pair, the altitude above sea level of both upstream and downstream sites for all pairs was included as a covariate in the analysis. Finally, river water quality determinands are known to vary across the hydrograph and so to control for differences in flow conditions at the time of each sampling flow the nearest river flow gauging station to each pair was located. River flow records for the UK were taken from the National River flow Archive (www.nrfa.ac.uk). If the nearest riverflow gauging station was on the same river as the Control or STW pair, then its flow record was downloaded. However, river flow would be expected to differ just because of the catchment area and location across the study region. So, to make pairs comparable with respect to their flow records at the time of water quality measurement each flow record was converted to its percentile flow record and then it was the percentile flow (percent rank) that was included as a covariate in the analysis. Therefore, three covariates were included in the subsequent analysis – Euclidean distance between monitoring points; altitude of the upstream and downstream monitoring points; and the percentile riverflow at the time of sampling. The visual inspection of the covariates between the pairs suggested no systematic bias in site selection (Table 2.2). However, sub-sampling necessary for each determinand may alter this selection.

Table 2.2. Mean properties of the covariates for the Control and STW pairs.

	Type	
	Control	STW
Euclidean distance (m)	1519	1823
Upstream altitude (m_asl)	107	89
Downstream altitude (m_asl)	81	81
Percentile flow (%)	51	52

2.2.2 Statistical analysis

Data from STW and Control pairs were evaluated using analysis of variance (ANOVA), incorporating four factors. The first factor, known as the Type factor, differentiated between STW and Control pairs. The Year factor accounted for the year of data collection, spanning from 2000 to 2020, creating 21 levels. The Month factor represented the month in which data were collected, adding another 12 levels to the model. Lastly, the Pair factor captured the variance between individual pairs, with the number of levels varying depending on the determinand under consideration and not uniformly distributed across the Type factor levels (refer to Table 2.1). The study design ensured that sample sizes were adequate for testing the significance of these factors and their interactions, with the Pair factor nested within the Type factor.

The primary objective of this investigation was to rigorously test all factors. ANOVA was initially performed without covariates, followed by a subsequent analysis including them.

Before conducting ANOVA, data distribution was tested for normality using the Anderson–Darling test, with log transformation applied as needed. Data distribution was then assessed with a QQ plot, and outliers were selectively removed, ensuring no more than 5% of the dataset was excluded. Unless stated otherwise, significance thresholds were set at a 5% probability, equating to a 95% confidence interval for the effects being non-zero. The impact of each statistically significant factor and its interactions was quantified using $\eta 2$, which measures the contribution of each factor to the observed variability in the variable. Additionally, if consecutive factors such as Year and Month were statistically significant, their trends were tested using a linear model.

The second aim of this study was to assess the reasons behind any significant difference caused to the receiving water caused by the individual STWs. Firstly, the main effects for each water quality determinand for each STW were correlated against each other with the aim of understanding whether an STW which shows a large impact for one determinand shows large effects for other determinands, eg, if an STW has a large, demonstrated impact for nitrate upon its receiving river then is it also likely to have a large impact for BOD, etc. The correlation analysis was

enhanced by the use of principal component analysis (PCA). Correlation analysis was used in conjunction with PCA because the PCA requires that the main effect for all determinands were available for an STW, whereas correlation analysis requires that only the two determinands to be correlated are available for a specific STW, i.e. correlation analysis has access to larger sample size than the PCA. The PCA was performed on z-transformed data and principal components with an eigenvalue ≥1 was considered for examination. Secondly, to discern the varying impacts of individual STWs, the results from STW pairs were classified into a binary outcome: the presence or absence of a significant effect for each determinand. The frequency of significant effects for each STW type was based on the characteristics of each STW using a Chi-squared test. Each STW was categorised by their treatment capabilities, including the presence or absence of secondary, tertiary, nitrogen removal, and phosphorous removal processes. Furthermore, the secondary treatment was subdivided as either secondary activated sludge (SAS) or secondary biological treatment (SB), and tertiary treatment was divided into tertiary activated sludge (TAS) and tertiary biological treatment (TB). The second approach was to use linear regression to compare the effect scale for each STW pair with the population equivalence (PE) and dry weather flow (DWF) of each STW. The PE and DWF of each STW being continuous variables, were excluded from the Chi-squared tests. The significance of these comparisons was determined based on a 5% probability threshold for the regression coefficients being greater than zero.

All statistical analyses were performed using RStudio v 4.3.1.

2.3 Results

An outline of the average final effluent concentration is given in Table 2.3 and in each case, except pH, the downstream mean value was greater than that observed upstream (Table 2.3). Therefore, a priori and independent of the relative magnitudes of discharge, it might be expected that STW final discharge will increase the concentration in the receiving river.

Arithmetic means were calculated for upstream, downstream and final effluent concentrations for each determinand using all available monitoring records. Mean values were used to characterise long-term central tendency and to enable comparison across site types and with effluent quality. They are not intended to characterise short-term variability or extreme discharge events.

2.3.1 Impact of STW discharges

The study has a large dataset for each determinand and with four factors the design is sensitive enough to find small significant differences. The smallest percentage difference found to be significant was for pH which showed a -0.4% decrease over the average upstream concentration, and the greatest difference was observed for phosphate (79%). The main effect, represented by the difference between the STW difference and the Control difference as shown in Figure 2.3, when divided by the average upstream value of the wastewater treatment plant, is used to assess the side effect of the sewage treatment (Table 2.3).

For all the determinands, except COD and suspended solids (Table 2.3), the Type factor was statistically significant, and the Type factor explained between 0.4% and 10% of the original variance in the datasets. Figure 2.3 illustrates that mean concentration differences in STW pairs consistently exceed those in Control pairs for most determinands, confirming the Type effect (see also Table 2.3). The strongest contrasts occur for phosphate and nitrate, while COD and suspended solids show no systematic separation. (Note that given that pH= $\mathbf{log_{10}}[\mathbf{H^+}]$then a statistically significant decrease in the value of pH is an increase in [H+] in the receiving river).

The Year factor accounted for a small amount of original variance in the datasets, with its most significant impact observed in the COD determinand, where it explained approximately 4% of the original variance in the dataset. Meanwhile, the interaction term Type*Year provided limited explanatory power regarding the original variance across all determinands (Table 2.4). However, from the perspective of this study the important term is not the Year factor rather the Type*Year interaction, i.e. has the significant impact observed for STW changed over time? The Type*Year interaction was significant for BOD, Phosphate, pH, Nitrate, and Specific Conductance (Table 2.4). For temperature, COD, and suspended solids there was no significant change in the impact over time. For BOD, Phosphate, pH, nitrate and specific conductance there was a significant Type*Year interaction, and these interactions were further analysed by significantly testing the trend over time by STW and Control. Figure 2.4 highlights that the STW–control gap changes over time for BOD, phosphate, pH, nitrate and specific conductance, but not for temperature, COD or suspended solids. For BOD, phosphate, pH and specific conductance the apparent improvement reflects deterioration in the controls while STW pairs remain approximately stable (Table 2.5), whereas for nitrate the impact declines because the STW effect reduces over time with no corresponding trend in controls. Table 2.5 shows that for BOD, Phosphate, pH and specific conductance there would be a decrease in the effect of STW on the receiving water over time, but in each case, it was because the water quality of the Control pairs was getting worse and there was no long-term change in the STW pairs. So, for BOD, Phosphate, pH and specific conductance, any long -term change in impact of STW discharge is due to the worsening of the context and not due to improvement in the treatment. For Nitrate the improvement in the impact was due to declining impact of STW in a context where there was no significant trend in the Control pairs.

The month factor explained less than 2% of the original variance in the data sets, and Type*Month explained little of the original variance - less than 0.2 % (Table 2.4). As above, and with respect to this study, the more important term was the Type*Month interaction, i.e. does any difference between STW and Control types persistent across the seasonal cycle? Temperature, suspended solids, and pH were found to be significant (Table 2.4). Temperature decreases during March, April,

May, and November for STW pairs. Suspended solids decrease in February, while pH levels drop from May through September for STW pairs. Temperature, suspended solids, and pH all showed significant seasonal trends in STW pairs, emphasizing the need for seasonal considerations in water quality management. Figure 2.5 indicates seasonality confined to temperature, suspended solids and pH: the STW–control difference reverses in March–May, June and November for temperature, in February for suspended solids, and from May to September for pH; in other months the direction of the STW effect is unchanged and effect sizes remain small (<0.3% variance explained).

The Pair factor was statistically significant for all determinands, explaining between 0.05% and 48% of the original variance in the datasets, with its influence being most important for Nitrate and least significant for Phosphate (Table 2.4). The Type*Pair interaction was significant for all determinands except COD and Nitrate, accounting for less than 12% of the variance. The highest impact was observed for Specific conductance, while Phosphate explained the least variance (Table 2.4). For all determinands studied in this Chapter, a summary of the mean difference of each Pair set in each Type greater than 0, equal to 0 and less than 0 has been shown in Table 2.7. The table presents the number of mean difference values for various water quality determinands categorised into greater than zero (>0), equal to zero (=0), and less than zero (<0), for Total, STW, and Control groups. Notably, the pair numbers for STW and Control do not have a one-to-one relationship, meaning the same pair number cannot be used to compare differences between STW and Control. Figure 2.6 shows substantial between-pair heterogeneity, with the widest spread for nitrate and specific conductance (Pair $\eta^2 \approx 0.48$ and 0.40, respectively) and minimal spread for phosphate ($\eta^2 \approx 0.0005$). Pairs are ordered from the lowest to highest overall mean difference, showing that some pairs have positive values while others are negative, consistent with the counts summarised in Table 2.7. The figure presents the main effect of Pair across both Types. Consistent with Table 2.4, the Type×Pair interaction was significant for all determinands except COD and nitrate, with the magnitude and, in some cases, the sign of the pair-level differences differing between STW and Control, as reflected in the Type-specific counts in Table 2.7.

In Table 2.7, Phosphate has the highest total number of pairs (549), while COD has the lowest (64). Phosphate has the highest number of pairs where mean difference was greater than zero (285), followed by Specific conductance (246) . The STW group generally has a higher number of total observations compared to the Control group for most determinands. Phosphate has the highest number of observations greater than zero in the STW group (193).

The number of differences equal to zero is relatively small across all determinands, indicating that most differences are either positive or negative. A priori, it would be expected that the proportion of STW pairs significantly different from zero would reflect the Type effect, which is true for all the determinands.

Table 2.3. Summary of the dataset and results for the analysis of impact of STW discharge. The significance of the impact of STW discharge for each determinand is stated as Yes if it was significant at a probability < 0.05 of being zero. The main effect (%) is main effect for each determinand for the Type factor. Values are compared to the mean for the upstream values for the STW pairs for that determinand (Main effect presented as the difference for STW minus the difference for the Control, which has been illustrated in Figure 2.3). N represents the total number of observation sampling points recorded at different times. N (without outliers) refers to the count of these pairs after removing outlier values.

Determinand (unit)	N	N without outliers	Mean upstream concentration	Mean downstream concentration	Mean Final effluent	Type Significant	Main effect	Main effect (%)
Temperature (°C)	23933	23033	10.8	10.91	13.3	Yes	0.12	1%
BOD (mg O_2/L)	13865	11351	2,26	2.37	8.88	Yes	0.07	3%
COD (mg O_2/L)	2135	1808	28.16	26.88	55.54	--	0.18	0.6%
Phosphate (mg P/L)	28760	22131	0.39	0.62	4.95	Yes	0.31	79%
Suspended solids (mg/L)	14595	12090	15.5	16.2	14.4	--	0.08	0.5%
pH	16919	15632	7.83	7.82	7.43	Yes	-0.03	-0.4%
Nitrate (mg N/L)	6067	4781	5.63	6.54	15.37	Yes	0.75	13.3%
Specific conductance (µS/cm)	6722	10431	731.55	781.73	891	Yes	5.00	0.6%

Table 2.4. The summary of the linear model for each determinand. The significance for each determinand is stated as Yes if it was significant at a probability < 0.05 of being zero. η2 indicates the extent to which a factor contributes to the observed variability in the variable.

Determinand	Type		Year		Type*Year		Month		Type*Month		Pair		Type*Pair	
	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2
Temperature (°C)	Yes	0.01	Yes	0.003	--	--	Yes	0.01	Yes	0.002	Yes	0.23	Yes	<0.01
BOD (mg O_2/L)	Yes	0.01	--	--	Yes	0.003	--	--	--	--	Yes	0.29	Yes	0.02
COD (mg O_2/L)	--	--	Yes	0.04	--	--	Yes	0.02	--	--	Yes	0.30	--	--
Phosphate (mg P/L)	Yes	0.04	Yes	0.0001	Yes	0.0007	Yes	0.0003	--	--	Yes	0.0005	Yes	0.0009
Suspended solids (mg/L)	--	--	Yes	0.003	--	--	--	--	Yes	0.002	Yes	0.11	Yes	0.02
pH	Yes	0.02	Yes	0.003	Yes	0.004	--	--	Yes	0.001	Yes	0.34	Yes	0.08
Nitrate (mg N/L)	Yes	0.10	Yes	0.03	Yes	0.01	Yes	0.01	--	--	Yes	0.48	--	--
Specific conductance (µS/cm)	Yes	0.004	Yes	0.01	Yes	0.003	--	--	--	--	Yes	0.40	Yes	0.12

*Table 2.5. The gradient of the trend over the entire study period for each determinand that showed a significant Type*Year interaction (Figure 2.4(a)-2.4(b)). Where not significantly different from zero, zero is given, otherwise the gradient is quoted with the standard error on that slope. Improvement is stated as Yes when the impact of STW significantly decreases with time. With respect to the control.*

	STW	Control	Improvement?
BOD (mg O_2/L)	0	0.004±0.002	Yes
Phosphate (mg P/L)	0	0.001±0.0002	Yes
pH	0	0.001±0.0005	Yes
Nitrate (mg/L)	-0.03±0.01	0	Yes
Specific conductance (μS/cm)	0	0.02±0.01	Yes

Table 2.6. The months of the year when the difference due to STW pairs was the opposite of the Type difference shown in Figure 2.5(a)-2.5(b).

Determinand	Months where difference is reverse
Temperature (°C)	March - May, June, November
Suspended solids (mg/L)	February
pH	May – September

Table 2.7. Number of mean difference values for each determinand categorised by type. 'N' represents the total number of mean difference values recorded. '>0' indicates the number of mean difference values that were greater than zero, '<0' represents the number of mean difference values that were less than zero, and '=0' denotes the number of mean difference values where the difference was not significantly different. (Figure 2.6(a)-2.6(b))

Determinand	Total				STW				Control			
	N	>0	=0	<0	N	>0	=0	<0	N	>0	=0	<0
Stream temperature (°C)	424	222	37	165	256	139	26	91	168	83	11	74
BOD (mg O_2/L)	268	109	63	96	168	75	37	56	100	34	26	40
COD (mg O_2/L)	64	31	13	20	40	19	10	11	24	12	3	9
Phosphate (mg P/L)	549	285	47	217	317	193	13	111	232	92	34	106
Suspended solids (mg/L)	349	160	52	137	180	76	22	82	169	84	30	55
pH	352	157	41	154	202	83	27	92	150	74	14	62
Nitrate (mg N/L)	118	73	3	42	73	49	1	23	45	24	2	19
Specific conductance (µS/cm)	442	246	21	175	255	143	9	103	187	103	12	72

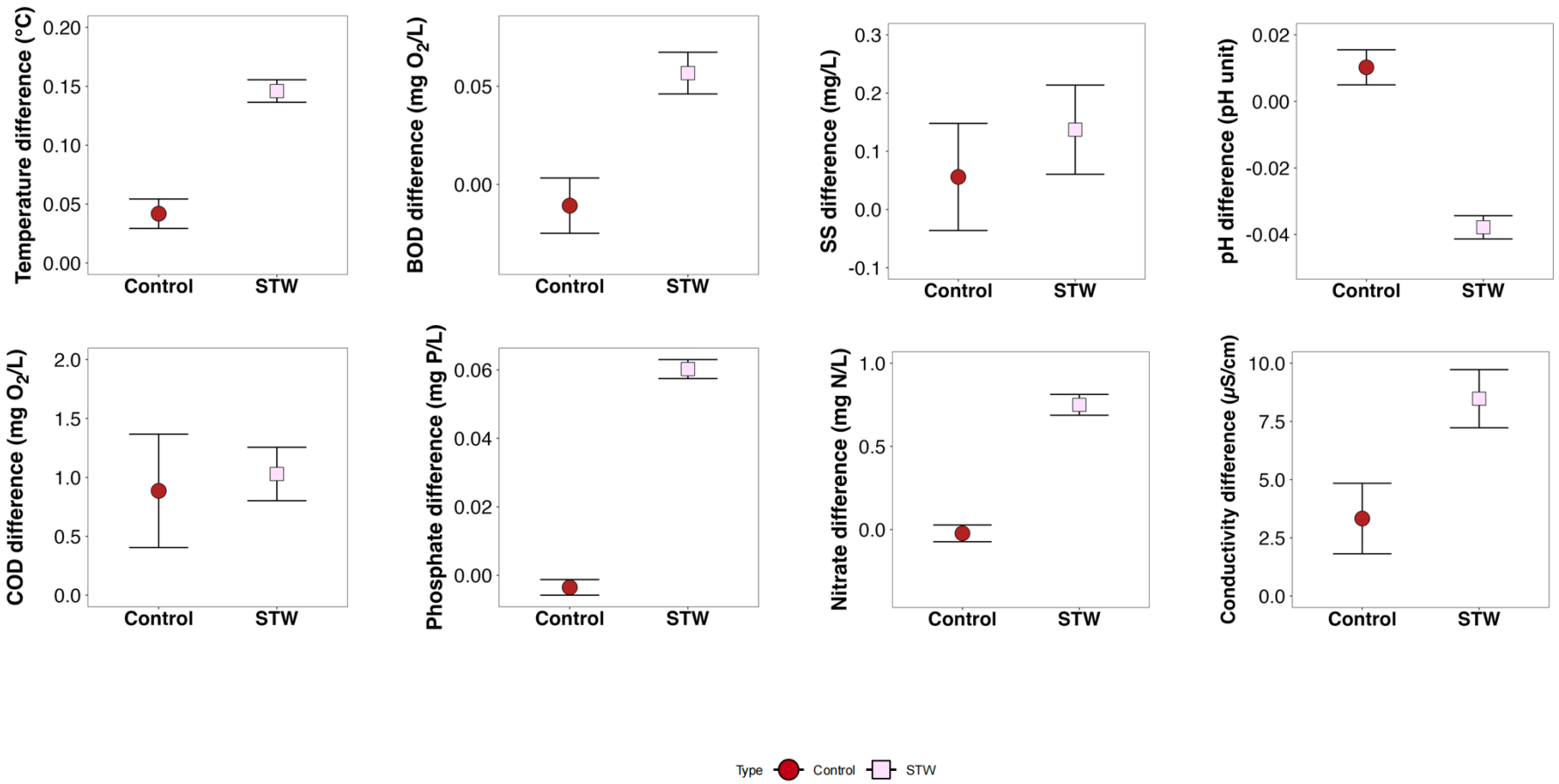


Figure 2.3. The main effects plot for the Type factor for the difference (diff). The values are presented as the marginal mean with the 95% confidence limits on that mean.

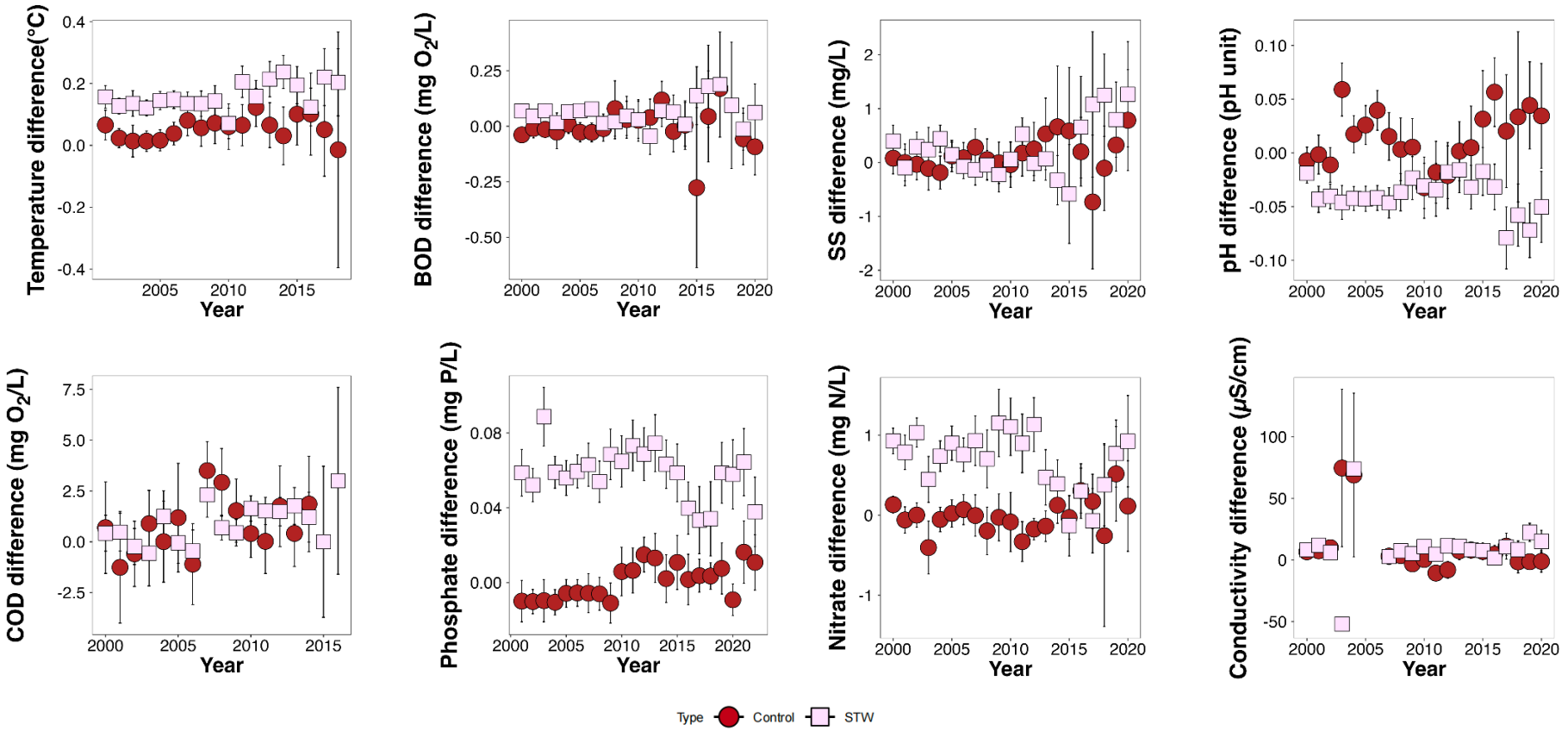


*Figure 2.4. The main effects plot of the Type*Year interaction. The values are presented as the marginal mean with the 95% confidence interval.*

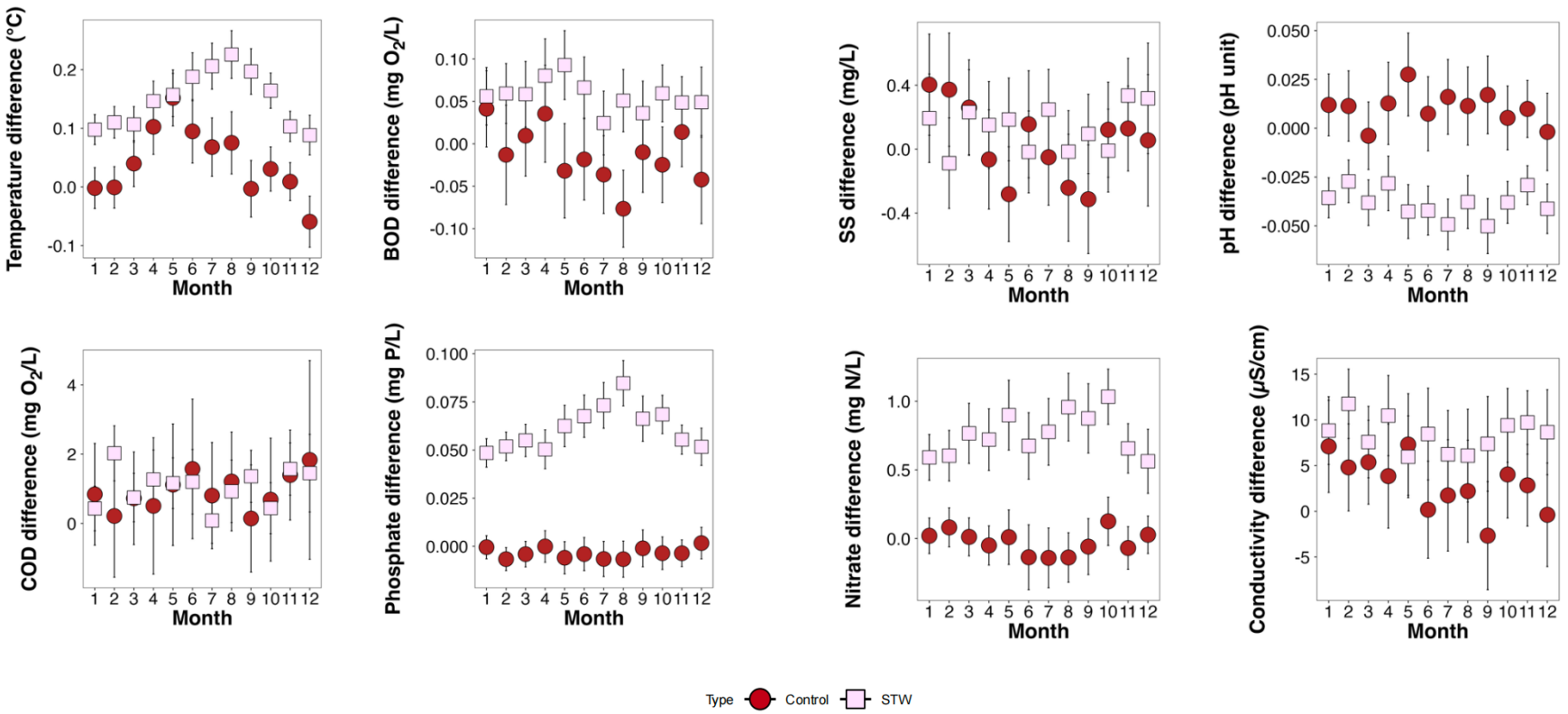


*Figure 2.5. The main effects plot of the Type*Month interaction. The values are presented as the marginal mean with the 95% confidence interval.*

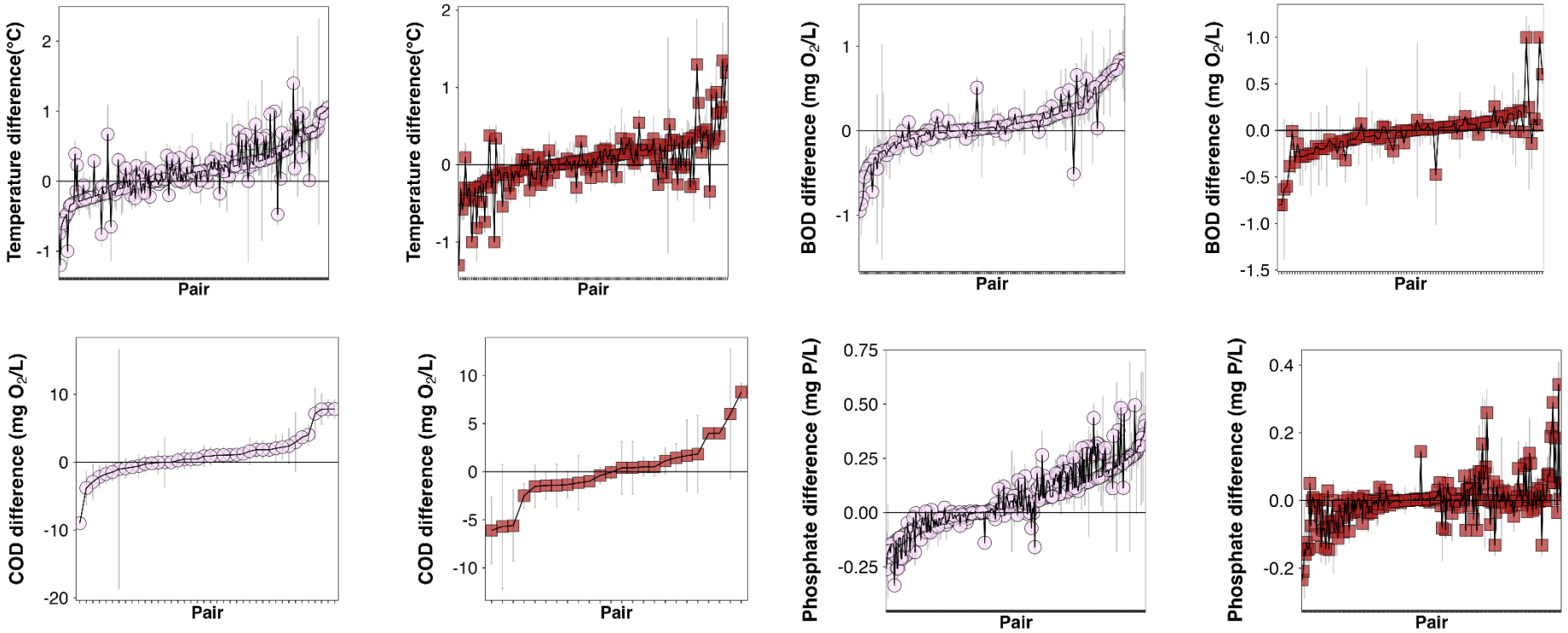


Figure 2.6(a). The main effects plot of the Pair factor. The Pairs are ordered from the lowest to greatest values of the difference. The points are the marginal mean with the 95% confidence interval.

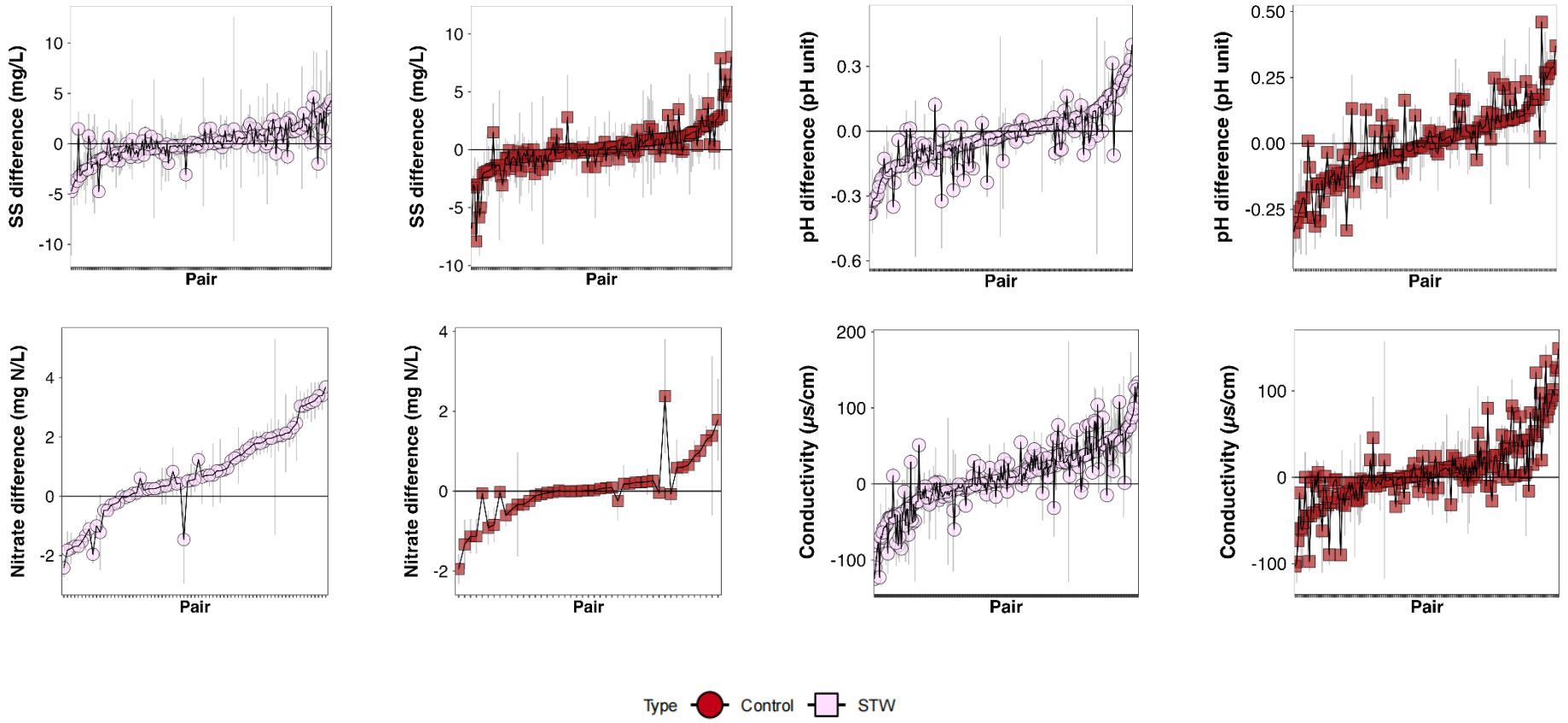


Figure 2.6(b). The main effects plot of the Pair factor. The Pairs are ordered from the lowest to greatest values of the difference. The points are the marginal mean with the 95% confidence interval.

2.3.2 Importance of covariates

In this study, the covariates considered included Euclidean distance, upstream altitude, downstream altitude, and percent rank flow. With respect to the Type factor statistical significance was unchanged for temperature, BOD, pH, Nitrate, and specific conductance, irrespective of the inclusion of covariates (Table 2.8). For COD, including covariates made the Type factor significant, with Euclidean distance being the important covariate. The COD dataset is the smallest in the study (Table 2.3), so results for some sites may have distorted the analysis, which is corrected by including the covariates. Conversely, for phosphate, the Type factor became insignificant when covariates were included, with both Euclidean distance and downstream altitude being important.

None of the covariates changed significance for BOD, suspended solids, or specific conductance. All covariates for temperature were significant, but their inclusion did affect the status or magnitude of the Type factor. Whenever Euclidean distance was significant, it positively correlated with the difference in the pairs, meaning the difference increased the further apart the pairs were. Similarly, when significant, upstream altitude was positively correlated with the difference across pairs. Conversely, percent rank flow was only significant for temperature, where differences declined with increasing percentile flow. When downstream altitude was significant, it had contrasting effects: for Nitrate, there was a positive correlation, while for Temperature, there was a negative correlation.

Table 2.8. The coefficient for each factor of each determinand and for each of the covariates. It will be marked as dark grey if it was significant at a probability < 0.05 of being zero. For the covariates, For the covariates, significant effects ($p < 0.05$) are highlighted in grey, and the coefficient values indicate the direction and magnitude of the effect.

	Type		Year		Month		Euclidean distance (m) Significant	up_altitude (m asl) Significant	down_altitude (m asl) Significant	Percent rank Significant
	Without covariate	With covariate	Without covariate	With covariate	Without covariate	With covariate				
Temperature (°C)	-0.21	3.60	0.01	0.03	--	0.002	0.001	0.11	-0.19	-0.09
BOD (mg O_2/L)	0.51	2.12	--	--	--	--	--	--	--	--
COD (mg O_2/L)	--	12.2	-0.22	--	0.31	--	0.000	--	--	--
Phosphate (mg P/L)	3.01	--	0.001	--	0.04	0.003	--	0.006	--	--
Suspended solids (mg/L)	--	--	0.0002	--	--	--	--	--	--	--
pH	-0.37	0.01	-0.007	-0.006	--	--	≈0	≈0	--	--
Nitrate (mg/L)	-0.35	2.72	0.47	1.17	0.35	--	0.001	0.08	0.003	--
Specific conductance (µS/cm)	34.29	47.95	0.67	--	--	--	--	--	--	--

2.3.3 Impact of the nature of the STWs

The efficacy of two distinct secondary treatment methods employed within STWs— Secondary Treatment (secondary activated sludge treatment (SAS) and secondary biological treatment (SB)); and Tertiary treatment (tertiary activated sludge (TAS)/ tertiary biological (TB)) in the STW– was tested by a series of Chi-squared tests (Table 2.9; Figure 2.7 – Figure 2.12). The results are defined as:

Main effect: The main effect is defined as the difference *observed for different* types of treatment. Specifically, for secondary treatment, the main effect is quantified as SAS−SB, and for tertiary treatment, it is calculated as TAS−TB.

Positive: Indicate that the difference between the treatments effectively reduced the value of the contaminant, meaning downstream values are lower than upstream. This is the desired outcome as it signifies that the treatment is working to remove or reduce the pollutant.

Negative: Indicate that the difference between the treatment was ineffective, or possibly that the condition worsened, as downstream values are higher than upstream. This could occur due to various reasons such as insufficient treatment capacity, suboptimal operation conditions, or external factors affecting the treatment process.

pH, and nitrate all showed statistical differences in both secondary and tertiary treatment (Table 2.9); Stream temperature, Specific conductance showed a statistical difference in Secondary treatment, while COD and phosphate showed a statistical difference in tertiary treatment. However, for BOD and suspended solids, neither secondary nor tertiary treatment showed a statistical difference. Significant results could be a positive or negative impact of that treatment on the water quality determinand. Of those showing a significant impact of either secondary or tertiary treatment the stream temperature impact of STWs was lower for SB than SAS (Table 2.9; Figures 2.7), i.e. a switch to biological treatment (SB) would decrease the impact of STW discharge.

While for BOD there was no significant impact of the differences between treatment types, tertiary treatment did make a significant difference for COD, TB made the impact of the STW worse than TAS (Figure 2.8). For the nutrient, Phosphate impact was significantly higher for TAS relative to TB, but type of

secondary treatment made no significant difference; similarly for Nitrate where TAS lowers impact relative to TB, and the SAS has lower difference compared to SB.

Table 2.9. Results of Chi-squared test for each determinand relative to technologies present at the STW. SAS = secondary activated sludge; SB = secondary biological; TAS = Tertiary activated sludge; TB = tertiary biological. It was significant when at a probability (P) < 0.05

Determinand (unit)		SAS	SB	P	Main effect (SAS-SB)	TAS	TB	P	Main effect (TAS -TB)
Stream temperature (°C)	Positive	415	1432	<0.05	0.26	95	122	0.15	--
	Negative	1071	1987			233	233		
BOD (mg O_2/L)	Positive	823	593	0.2	--	65	77	0.2	--
	Negative	325	267			29	50		
COD (mg O_2/L)	Positive	87	95	0.3	--	5	0	<0.05	-3.0
	Negative	105	91			1	53		
Phosphate (mg P/L)	Positive	3349	1801	0.34	--	199	205	<0.05	-0.06
	Negative	1232	700			200	356		
pH	Positive	1094	1587	<0.05	-0.07	62	95	<0.05	0.11
	Negative	191	517			125	54		
Suspended solids (mg/L)	Positive	1181	1163	0.72	--	63	84	0.40	--

	Negative	667	639			268	425		
Nitrate (mg N/L)	Positive	913	496	0.02	-0.35	13	94	<0.05	-0.1›
	Negative	150	113			1	129		
Specific conductance (µS/cm)	Positive	890	729	<0.05	20	49	68	0.90	--
	Negative	334	163			112	164		

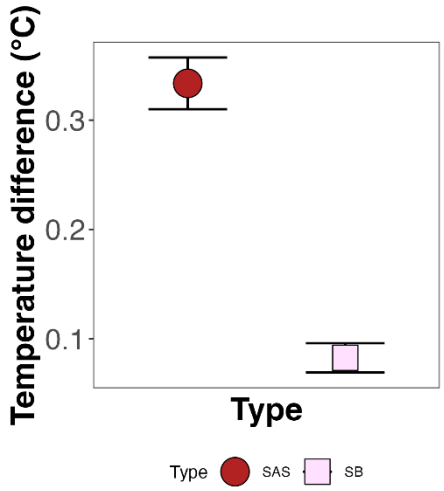


Figure 2.7. Effect of secondary treatment on mean stream temperature differences between upstream and downstream locations. Results are shown for STW pairs with activated sludge (SAS) and secondary biological treatment (SB), presented as marginal means with 95% confidence intervals.

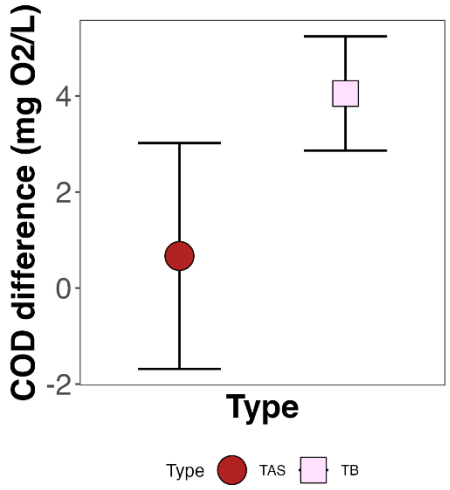


Figure 2.8. Effect of tertiary treatment on mean COD differences between upstream and downstream locations. Results are shown for STW pairs with tertiary activated sludge (TAS) and tertiary biological treatment (TB), presented as marginal means with 95% confidence intervals.

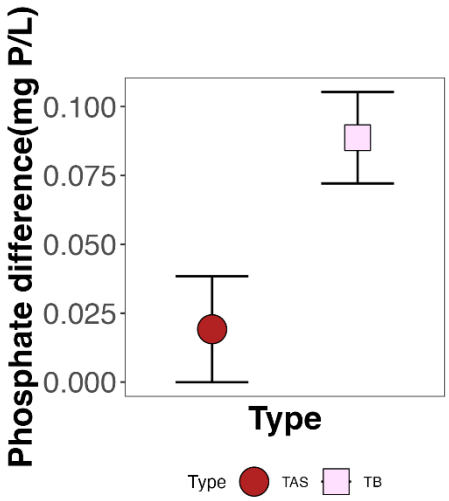


Figure 2.9. Effect of tertiary treatment on mean Phosphate differences between upstream and downstream locations. Results are shown for STW pairs with tertiary activated sludge (TAS) and tertiary biological treatment (TB), presented as marginal means with 95% confidence intervals.

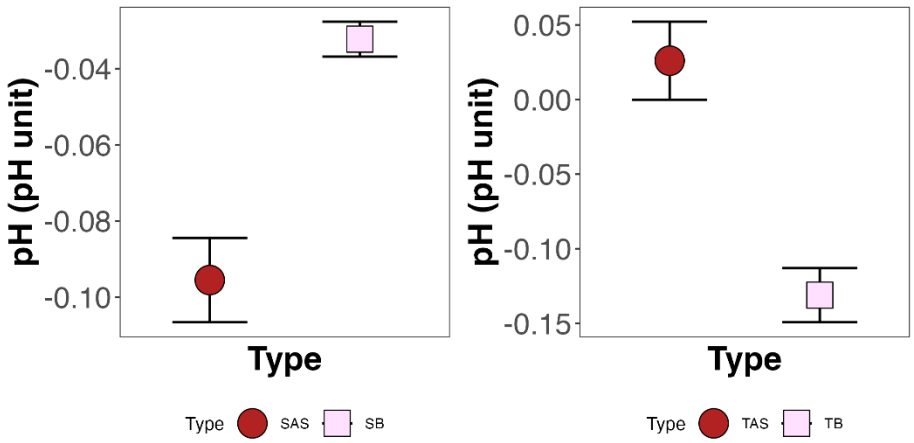


Figure 2.10. Effect of secondary and tertiary treatment on mean pH differences between upstream and downstream locations. Results are shown for STW pairs with secondary treatment: activated sludge (SAS) or secondary biological (SB); and tertiary treatment: tertiary activated sludge (TAS) or tertiary biological (TB), presented as marginal means with 95% confidence intervals.

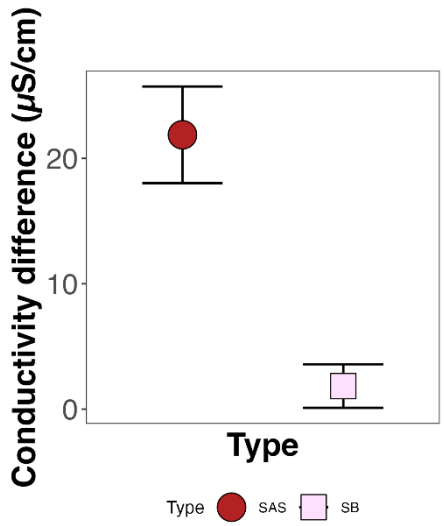


Figure 2.11. Effect of secondary treatment on mean Conductivity differences between upstream and downstream locations. Results are shown for STW pairs with activated sludge (SAS) and secondary biological treatment (SB), presented as marginal means with 95% confidence intervals.

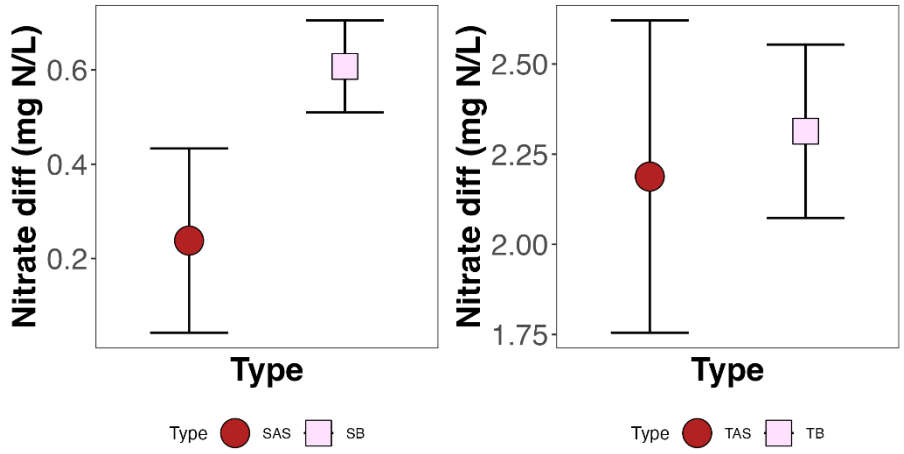


Figure 2.12. Effect of secondary treatment on mean Nitrate differences between upstream and downstream locations. Results are shown for STW pairs with activated sludge (SAS) and secondary biological treatment (SB), presented as marginal means with 95% confidence intervals.

Nitrate and Phosphate removal in Secondary Treatment and Tertiary treatment

In both secondary and tertiary treatment stages, there are differences in nitrate and phosphate removal at STWs. For secondary treatment, it was found that there was no significant difference in nitrate removal between SAS (Secondary Activated Sludge) and SB (Secondary Biological Treatment) systems (Table 2.10). However, phosphate removal showed significant differences depending on the type of secondary treatment, except at sites measuring pH and suspended solids, where no notable differences were observed (Table 2.10).

In terms of tertiary treatment, the presence or absence of tertiary treatment had a significant impact on nitrate and phosphate removal (Table 2.11). Chi-square tests indicated a statistically significant association between tertiary treatment and phosphate removal. Additionally, significant relationships were observed at sites measuring stream temperature, phosphate concentration, and specific conductance for nitrate removal. These findings suggest that tertiary treatment plays a crucial role in improving phosphate removal, while it does not affect nitrate removal in most cases.

Table 2.10 Results of Chi-squared test for each determinand relative Nitrate and Phosphate removal in Secondary treatment. It was significant when at a probability (P) < 0.05.

Determinand (unit)	Nitrate Removal	SAS	SB	P	Phosphate Removal	SAS	SB	P
Stream temperature (°C)	Yes	0	0	NA	Yes	905	1219	<0.05
	No	2725	4758		No	1820	3539	
BOD (mg O_2/L)	Yes	0	0	NA	Yes	637	842	<0.05
	No	1802	2943		No	1165	2101	
COD (mg O_2/L)	Yes	0	0	NA	Yes	201	166	<0.05
	No	411	471		No	205	305	
Phosphate (mg P/L)	Yes	0	0	NA	Yes	1217	1506	<0.05
	No	3377	5858		No	2160	4352	
Suspended solids (mg/L)	Yes	0	0	NA	Yes	909	908	0.50
	No	2183	2237		No	1274	1329	
pH	Yes	0	0	NA	Yes	599	1208	0.56
	No	1893	3915		No	1294	2707	
Nitrate (mg N/L)	Yes	0	0	NA	Yes	191	315	<0.05
	No	414	1790		No	223	1475	

Specific conductance (µS/cm)	Yes	0	0	NA	Yes	478	957	<0.05
	No	1201	3123		No	724	2166	

Table 2.11 Results of Chi-squared test for each determinand relative Nitrate and Phosphate removal in Tertiary treatment. It was significant when at a probability (P) < 0.05.

Determinand (unit)	Nitrate Removal	With tertiary	Without tertiary	P	Phosphate Removal	With tertiary	Without tertiary	P
Stream temperature (°C)	Yes	0	1738	<0.05	Yes	807	931	<0.05
	No	140	8066		No	2246	5960	
BOD (mg O_2/L)	Yes	0	1342	1	Yes	751	591	<0.05
	No	1	4890		No	1319	3572	
COD (mg O_2/L)	Yes	0	1	1	Yes	44	370	<0.05
	No	277	979		No	233	610	
Phosphate (mg P/L)	Yes	0	146	<0.05	Yes	1128	1411	<0.05
	No	2539	9719		No	2720	7145	
Suspended solids (mg/L)	Yes	0	1	1	Yes	685	1623	0.001
	No	1674	4457		No	989	2835	
pH	Yes	0	1	1	Yes	867	1567	<0.05
	No	1515	5959		No	648	4393	
Nitrate (mg N/L)	Yes	0	1	1	Yes	381	443	<0.05

	No	734	2437		No	353	1995	
Specific conductance (µS/cm)	Yes	0	61	<0.05	Yes	517	1349	<0.05
	No	1279	4466		No	762	3178	

2.3.4 Impact of population equivalence and dry weather flow

The difference across STW pairs for BOD, nitrate, pH, and specific conductance showed significant relationships with population equivalence (PE) (Table 2.12). For BOD and nitrate, positive correlations were found. Specifically, BOD and nitrate show an increase in impact of 0.04 mg O_2/L and 0.04 mg N/L per 1000 people, respectively. In contrast, pH and specific conductance exhibit negative relationships with PE.

All determinands, except suspended solids and pH, show positive correlations with dry weather flow (DWF, in thousands of cubic meters per day). Both BOD and nitrate have positive relationships with both population equivalence and dry weather flow. However, pH and suspended solids show negative correlations with DWF. Specific conductance, while showing a negative correlation with PE, exhibits a positive relationship with DWF.

DWF is a constant for a particular STW, so the observed relationships are not about changing flows but rather about the differences in the determinands due to the size of the varying flow levels among different STWs. Even though the models are statistically significant, the r^2 values for BOD and nitrate were small (0.03 and 0.04, respectively), so they explain only a small proportion of the variance in the data.

Table 2.12. Summary of the significance of STW discharge impacts on river water quality determinands. Significance is also assessed for Population Equivalence (per 1,000 population) and Dry Weather Flow (per 1,000 m³/day). Effects are stated as 'Yes' if significant at a probability of < 0.05 of being zero.

Determinand	Population equivalence		Dry weather Flow		r^2
	Sig	Coefficient	Sig	Coefficient	
Stream temperature (°C)	--	--	Yes	0.001	0.004
BOD (mg O_2/L)	Yes	0.04	Yes	0.02	0.03
COD (mg O_2/L)	--	--	Yes	0.0007	0.005
Phosphate (mg P/L)	--	--	Yes	0.002	0.004
Suspended solids (mg/L)	--	--	Yes	-0.003	0.02
pH	Yes	-0.002	Yes	-0.006	0.01
Nitrate (mg/L)	Yes	0.04	Yes	0.15	0.04
Specific conductance (µS/cm)	Yes	-0.006	Yes	0.03	0.004

Population equivalence and Dry weather flow correlation plot

Based on the overall analysis of PE and DWF, a strong positive correlation was found between the two variables, with a Pearson correlation coefficient of 0.88. This indicates that as both PE and DWF increase, the overall impact on the receiving water bodies also significantly increases (Figure 2.13). Figure 2.14 illustrates the scatter plots of Population Equivalence (PE) against Dry Weather Flow (DWF) for eight water quality determinands, including stream temperature, BOD, COD, phosphate, suspended solids, pH, nitrate, and specific conductance. These plots provide insights into the relationships between urbanisation (as measured by PE) and key water quality indicators.

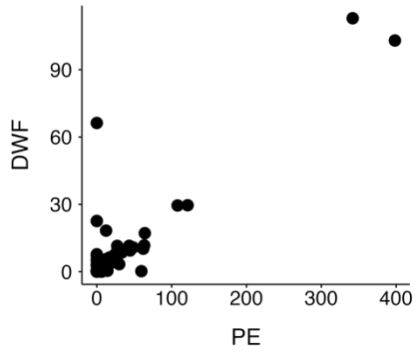


Figure 2.13 Scatter plot of Population Equivalence (PE) vs. Dry Weather Flow (DWF) for STWs with available PE and DWF data.

However, the relationships between PE and DWF vary across the determinands (Figure 2.14):

- **BOD (panel b)** shows the strongest positive correlation with PE.
- **Phosphate (panel d) and COD (panel c)** also show positive correlations with PE, although these are weaker than for BOD. COD levels follow a similar trend, but the correlation is less pronounced.

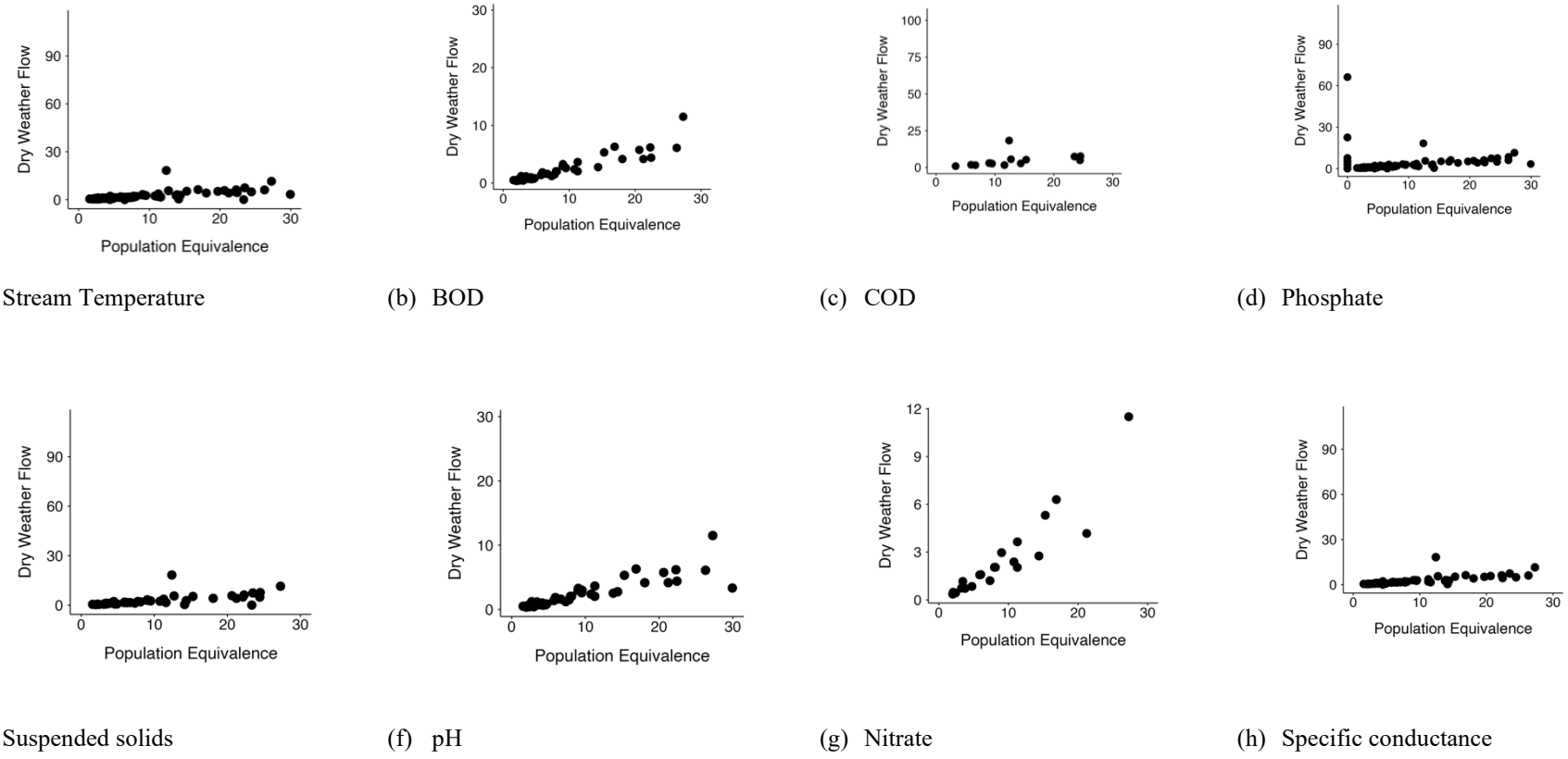
Conversely, several determinands exhibit little to no change in DWF despite rising PE, suggesting weak or absent correlations:

- **Stream Temperature (panel a)** remains relatively constant with increasing PE.
- **Suspended Solids (panel e)** show no clear relationship with PE.
- **Specific Conductance (panel h)** also shows minimal variation with PE.

A notable trend is observed in the relationship between PE and **nitrate (panel g)**, which displays two distinct patterns.

pH (panel f) displays several different trend lines with increasing PE and DWF.

Phosphate (panel d) also shows several values at PE = 0.



(a) Stream Temperature (b) BOD (c) COD (d) Phosphate

(e) Suspended solids (f) pH (g) Nitrate (h) Specific conductance

Figure 2.14. Scatter plot of Population Equivalence (×1000 people) vs Dry Weather Flow (×1000 m³/day)

In summary, while some determinands, such as BOD and phosphate, show strong positive correlations with PE and DWF, others like temperature, suspended solids, and specific conductance exhibit minimal change despite increasing population pressures. Nitrate concentrations display a more complex relationship, with distinct trends at different levels of PE.

2.3.5 Inter-determinands correlation

A pairwise evaluation was performed to understand the degree of association between the impact of STW discharge on the determinands. That is, if a STW shows a significant impact for one determinand does it show a significant impact for any others? The correlation matrix is shown in Table 2.13. It shows the correlation coefficients and probability values (p-values) between various determinands. Stream temperature has significant correlations with phosphate, nitrate, specific conductance and COD. BOD significantly correlates with COD and suspended solids. COD has significant correlations with phosphate, suspended solids, pH, nitrate, and specific conductance. Phosphate significantly correlates with nitrate and specific conductance. Suspended solids show significant correlation with pH. pH significantly correlates with Nitrate, and Specific conductance. Nitrate significantly correlates with specific conductance. These relationships help analyse the impact and interdependence of different water quality determinands, essential for understanding the overall effect of sewage treatment works (STW) discharges on water quality.

Table 2.13. Summary of inter-determinand correlation coefficients. Correlation coefficients range from −1 to 1 and are shown alongside their corresponding probability values. Coefficients with p-values < 0.05, indicating statistical significance, are highlighted in grey.

The correlation coefficient and p-values between two determinands								
Determinand (unit)	Stream temperature (°C)	BOD ($mg\ O_2/L$)	COD (mg O2/L)	Phosphate (mg P/L)	Suspended solids (mg/L)	pH	Nitrate (mg N/L)	Specific conductance (µS/cm)
Stream temperature (°C)		0.10	0.16	0.46	-0.15	0.15	0.40	0.37
BOD ($mg\ O_2/L$)			0.70	0.12	0.52	-0.07	0.08	0.03
COD ($mg\ O_2/L$)				0.57	0.61	-0.35	0.57	0.50
Phosphate (mg P/L)					-0.06	-0.10	0.91	0.84
Suspended solids (mg/L)						-0.20	-0.06	-0.12
pH							-0.22	-0.24
Nitrate (mg N/L)								0.90

2.3.6 Principal components analysis

By merging datasets for each determinand there were 168 data that could be considered across all determinands for all STW pairs. Three out of eight components had eigenvalues > 1 (Table 2.14) and these three components together explained 84% of the original variance. The contribution table on the components shows that the first component was correlated with Nitrate, Phosphate, Specific conductance and COD; the second component was correlated with Suspended solids, BOD and COD; and the third component was correlated with pH, Temperature and BOD.

Table 2.14. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1	PC2	PC3
Stream temperature (^{o}C)	0.25	-0.24	-0.53
BOD (mg O_2/L)	0.19	0.51	-0.37
COD (mg O_2/L)	0.42	0.39	-0.03
Phosphate (mg P/L)	0.48	-0.20	-0.04
Suspended solids (mg/L)	0.10	0.60	-0.06
pH	-0.16	-0.21	-0.74
Nitrate (mg N/L)	0.49	-0.19	0.10
Specific conductance (µS/cm)	0.47	-0.23	0.16
Eigenvalue	3.55	2.05	1.12
Percentage of variance explained	44%	26%	14%

The graph of the scores on the first two components shows a contrast with rivers plotting either on a trend along PC1 or trending along PC2, which is either dominated by a change in nutrients or by a change in COD/BOD (Figure 2.16). However, the constraint of merging the data so that all data were coming from the

same pair at the same time means that only 7 STW pairs within 4 rivers could be included in the analysis. Two of the STW pairs (River Alt D/S/Fazakerley Brook; and River Bela at Milnthorpe) plot along PC1; and the other STW pairs vary along PC2 (Figure 2.15).

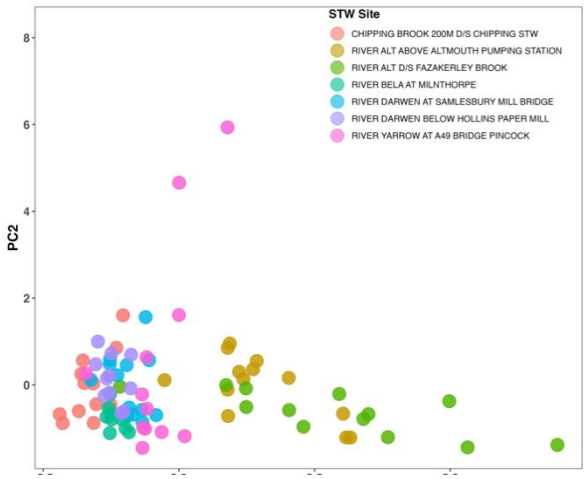


Figure 2.15. The scatter plot of the scores on PC1 and PC2.

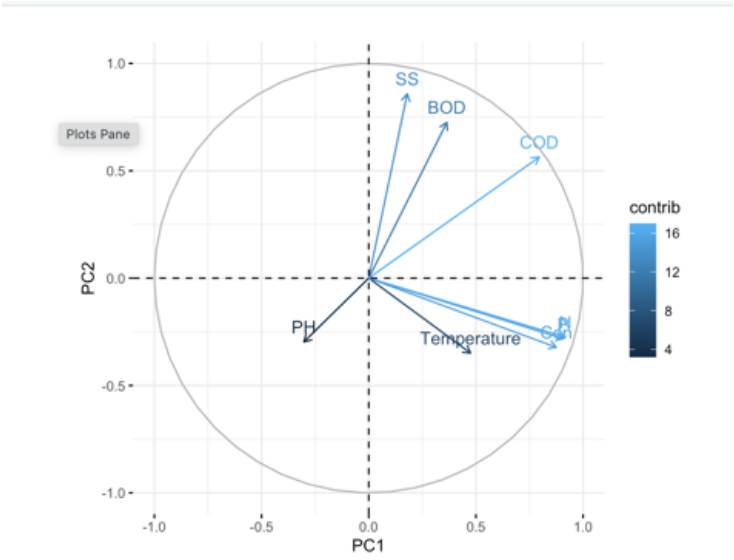


Figure 2.16. The loadings on PC1 and PC2.

2.4 Discussion

In this chapter, the impact of STW discharge on water quality has been demonstrated. A number of previous studies have shown the impact of STW discharge on receiving waters for the determinands considered in this study. For example, Kinouchi (2007)and Wilson & Worrall (2021) have highlighted the thermal effects of STWs, though the broader implications on water quality are less emphasized. This study, however, has extended the range of determinands, allowing for intercomparison (e.g., Table 2.13). The approach here utilized a clearer and more robust experimental design, employing control river reaches to better isolate the effects of STWs. This design allowed for a more comprehensive evaluation of spatial and temporal factors affecting river water quality, enhancing the reliability of our findings compared to earlier studies. Furthermore, the number of sites and rivers considered in this study was far larger than in previous studies, providing a more extensive dataset for analysis.

This study shows differences from the patterns observed by Mellal et al. (2024), suggesting a more complex interaction between STW discharges and river chemistry. Mellal et al.(2024) evaluated treated petroleum wastewater from a single site, focusing on seasonal variation in pH, temperature, phosphate, hydrocarbons, and COD. In contrast, this study also tests BOD, nitrate, and suspended solids, using a dataset spanning from 2000 to 2020, covering a larger time span and including more wastewater treatment plants. Additionally, control measures have been introduced, making the impact of the wastewater treatment plants on the river more straightforward; at the same time, this study includes 442 pairs of STW sites and 419 Control sites, which are more than in other studies.

Iloms et al. (2020) measured temperature, pH, BOD, COD, TDS, EC, and salinity concentrations at seven sampling sites, including wastewater treatment works (WWTW) inflows, effluents, and potable water sources from the Vaal River between January and September 2017. Barrenha et al. (2018) assessed the impact of one wastewater treatment plant (WWTP) on a river by analysing six sites between March 2011 and September 2013 and again from November 2013 to November 2014. Akinwole et al. (2021) studied five sites along Big Walnut Creek during September and October 2019 and February 2020, focusing on tertiary treated

effluents and their impact on physiochemical determinands and microbial biomass. These studies were limited in terms of temporal coverage, the number of sites, or the range of parameters analysed. The current research builds upon these works by encompassing a broader temporal range, analysing additional water quality parameters, and employing control measures to provide a more detailed assessment of the impacts of wastewater treatment plants on river systems.

Mellal et al. (2024) demonstrated that treated wastewater discharged from a petroleum refinery met regulatory standards and was of higher quality compared to raw influent water. Their study employed an artificial neural network (ANN) model, a machine learning-based approach, to predict water quality indices (WQI) with high accuracy using parameters such as pH, temperature, phosphate, and hydrocarbons. However, their analysis did not include upstream and effluent mean values. The current research addresses this limitation by providing a detailed comparison of these values (Table 2.3). While Iloms et al. (2020) analysed concentrations from various industries, they did not investigate temporal trends. This research extends the scope by examining yearly (Table 2.5) and monthly (Table 2.6) trends for each determinant, offering a more dynamic understanding of water quality changes. Pedro et al. (2018) identified no significant interaction between season and sampling sites but noted significant main effects. By incorporating characteristics ofSTWs) such as population equivalence, dry weather flow (Section 2.3.4), and treatment types (Section 2.3.3), this study provides a more detailed perspective on the factors influencing discharge quality. Akinwole et al. (2021) reported significantly higher microbial biomass at STW outfalls, whereas the current analysis examines a broader range of covariates (Table 2.8), offering a comprehensive assessment of STW impacts.

The inter-determinands correlations and relationships with STW characteristics provide deeper insights into the mechanisms affecting water quality. For instance, the positive relationship between temperature and nutrients (Table 2.13) suggests that higher water temperatures are associated with increased concentrations of phosphate and nitrate, likely due to accelerated microbial metabolism and enhanced enzymatic activity in warmer conditions, which promote nutrient cycling (Jarvie *et al.*, 2010). This finding aligns with Kinouchi (2007), who noted thermal impacts on river ecosystems, though our study extends this to nutrient dynamics across a

broader spatial scale. This pattern may also reflect thermal effects of treatment processes, as aeration in activated sludge systems can elevate effluent temperatures (Makinia *et al.*, 2005), while background temperature variation in control sites is largely driven by climatic and seasonal factors. In contrast, BOD's correlation with COD and suspended solids, but not with specific conductance or nutrients, implies significant organic pollution and particulate matter; however, the lack of significant relationship with nutrient levels or conductance indicates that organic pollution may not directly influence these parameters. This contrasts with Iloms et al. (2020), who observed stronger BOD-nutrient linkages in industrial effluents, possibly due to different pollution sources in their Vaal River study. Such differences may arise where primary sedimentation or secondary biological processes are insufficient to fully remove organic matter, while in control reaches elevated BOD can equally result from diffuse sources such as manure, leaf litter, or soil-derived organic carbon. Conversely, COD's associations with nutrients, specific conductance, and suspended solids suggest that areas with high COD often exhibit higher nutrient concentrations, suspended solids, and greater conductivity, pointing to overall poor water quality influenced by various pollutants. This is consistent with Akinwole et al. (2021), who linked high COD to organic loading in tertiary-treated effluents, though our larger dataset reveals a more widespread pattern across diverse STWs.

The scale of STWs, as measured by PE and DWF, further shapes these patterns. A strong positive correlation between PE and DWF (Table 2.12) indicates that as both increases, the overall impact on receiving waters rises, with higher PE closely associated with increased organic load, a key indicator of wastewater pollution. This supports findings by Barrenha et al. (2018), who noted increased organic loads with population size, though their single-site study lacked the spatial resolution of our 442 STW pairs. For BOD and nitrate, positive correlations with PE suggest that as PE increases, the differences in these determinands due to STWs also rise, reflecting greater organic and nitrogen loads. However, this influence diminishes for larger STWs as population size grows, suggesting a potential threshold effect where the impact of larger facilities becomes less notable. At lower PE values, nitrate concentrations are largely influenced by diffuse agricultural runoff (e.g., fertiliser and manure inputs), whereas at higher PE levels, effluent volumes and limited nitrogen-removal efficiency in many STWs result in stronger correlations

with DWF, reflecting the increasing contribution of sewage discharges to riverine nitrate loads. This shift likely reflects the increasing contribution of sewage treatment works to nitrate levels in more urbanised areas, where higher population densities lead to greater nitrogen loads in rivers, a pattern consistent with Jarvie et al. (2010), who observed significant STW impacts on nutrient levels in populated rural catchments. The stronger DWF-nitrate link at higher PE may result from increased effluent volumes from urban STWs, which overwhelm natural dilution and amplify nitrogen delivery during baseflow conditions.

DWF dynamics add complexity: pH and suspended solids show negative correlations with DWF, indicating reduced concentrations as flow increases, which may be driven more by environmental or operational factors within STWs (e.g., dilution or sedimentation) rather than flow alone. This aligns with Wilson & Worrall (2021), who attributed pH variability to buffering capacity rather than flow, though our study extends this to suspended solids dynamics. Specific conductance, despite a negative correlation with PE, exhibits a positive relationship with DWF, suggesting its concentration is influenced by factors beyond population equivalence, such as increased wastewater discharge volumes, industrial effluents, or groundwater contributions that vary with DWF. This finding diverges from Mellal et al. (2024), who linked conductance primarily to refinery effluents, highlighting the diverse industrial influences in our multi-site analysis.

Other determinands reveal additional environmental influences. Stream temperature likely reflects external factors such as seasonality or weather, consistent with seasonal models by Jarvie et al. (2010), who emphasised climatic drivers over sewage inputs. Suspended solids are more influenced by runoff, land use, or natural sediment transport than by population density or sewage flow, a pattern also noted by Akinwole et al. (2021) in catchment-scale studies. Suspended solids at control sites are strongly influenced by diffuse sources such as soil erosion and storm runoff, whereas elevated levels downstream of STWs can also reflect insufficient sedimentation in primary clarifiers. Specific conductance variations are likely tied to geological factors or industrial effluents rather than sewage discharge, supporting geological controls identified by Iloms et al. (2020). A notable shift in nitrate patterns, from agricultural runoff dominance at lower PE to STW contributions at higher PE, highlights the increasing role of urbanised areas with

greater nitrogen loads. The variability in pH may stem from changes in wastewater treatment, river buffering capacity, or other environmental conditions, a complexity also observed by Monea et al. (2020), who linked pH to treatment processes. Phosphate variations at low PE suggest influences from agricultural runoff or non-sewage sources, consistent with Jarvie et al. (2010), who emphasized diffuse pollution sources. These findings underscore the complex interplay of STW discharges and environmental factors, providing a foundation for targeted remediation strategies, particularly in managing nutrient enrichment and organic pollution. In addition, many STWs without chemical dosing or enhanced biological phosphorus removal show incomplete phosphate removal, explaining residual phosphorus in effluents.

These insights have practical implications for water quality management. The temperature-nutrient link suggests that thermal regulation at STW outfalls could mitigate eutrophication, while the PE-driven organic load highlights the need for upgraded treatment in urban areas. The DWF-dilution effect on pH and solids implies adaptive monitoring during flow variations, and the conductance-flow relationship underscores the importance of source tracking in industrial regions. Future research should explore treatment technology impacts and long-term ecological responses, building on this study's foundation. Although this study does not mention water discharge innovation, it provides a more detailed and useful analysis of the impact of wastewater treatment plant discharge. Despite the advances made with this study, there remain constraints. When the intercomparison was considered (e.g., PCA – section 2.3.6), the PCA requires all the data to be sampled together for all determinands on the same date for the same site. This constraint gave rise to PCA where only a limited number of STWs could be considered. In this context, all seven sites grouped into two directions, either impact varying for nutrients or varying with respect to BOD/COD. Consequently, this would mean that STWs are impacting the receiving waters for only one of two reasons, which could imply that remediation efforts need to be targeted accordingly.

Similarly, there was a lack of specific data on the techniques used at each STW site, which has limited the ability to fully assess the impacts of different tertiary treatments. However, this study has provided new insights into how secondary and tertiary treatment processes differ. For example, for temperature treatment, there is

a significant difference between different secondary treatment methods. Specifically, the temperature difference for SB (secondary biological treatment) is smaller compared to SAS (activated sludge secondary treatment), suggesting that SB is more effective in minimizing temperature changes (Figure 2.7). For phosphate treatment, the phosphate difference for TAS is smaller than for TB, showing TAS is more effective in treating phosphate. These contrasts highlight how specific treatment processes shape effluent quality: TAS plants with chemical precipitation achieve more effective phosphorus removal, while higher temperature shifts under activated sludge systems likely reflect the greater energy inputs from intensive aeration. Additionally, it is important to determine whether tertiary treatment is necessary to meet the discharge standards. Larger or more densely populated areas may challenge existing treatment capacities, leading to variations in treatment effectiveness. Dry weather conditions typically reduce the volume of water in sewage, potentially increasing the concentration of pollutants and presenting different treatment challenges. For instance, it is essential to understand how dry weather affects the biological treatment stages, as it may lead to reduced efficiency in nutrient removal due to higher concentrations of ammonia or organic matter.

This chapter has analysed key water quality determinants such as stream temperature, Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD), observing their significant impacts on river ecosystems. However, it has not addressed other water quality determinands. Chapter 3 will focus on metals.

The analyses presented in this chapter demonstrate how discharges from sewage treatment works (STW) influence a range of water quality properties that may contribute to secondary effects on eutrophication, potentially leading to enhanced nutrient enrichment. Overall, while STW processes clearly influence effluent composition, control sites demonstrate that diffuse sources such as agriculture, catchment geology, and urban runoff remain critical drivers of water quality, underlining the need for integrated management of both point- and non-point pollution sources. Chapter 4 will examine the impact of sewage discharges on eutrophication in more detail.

2.5 Conclusion

The objectives of this chapter were:

- Does the discharge from sewage treatment works (STWs) affect the concentration of water quality determinants in rivers?
- Can differences in river water quality due to STW discharges be attributed to the specific nature of the STW plants?

This chapter has shown that:

i) For all determinants, except Chemical Oxygen Demand (COD) and Suspended Solids, there was a significant change due to the presence of an STW discharge.

ii) Over the period of the study period there has been a notable yearly decrease in the impact due to Biochemical Oxygen Demand (BOD), Phosphate, pH, Nitrate and specific conductance from STWs.

iii) Different treatment types present at works did make a significant difference to the impact of the works on the receiving water, though this was not always positive.

iv) Tertiary treatment significantly improves Nitrate and Phosphate removal, while secondary treatment methods (SAS and SB) show no notable difference in nitrate removal but impact phosphate removal at most sites.

v) For all determinands the impact of an STW did increase with the size of the works, as measured by dry weather flow and population equivalence increase.

vi) Principal Component Analysis (PCA) showed there were two types of STW with respect to their impacts – impacting BOD/COD/SS or impacting nutrients.

This study highlights the potential for improvements in wastewater treatment technologies to mitigate pollution effectively without compromising water quality.

Chapter3: Metal determinands

3.1 Introduction

This chapter examines the impact of STW discharges on metal concentrations in rivers, specifically focusing on Ca, Mg, Cd, Cu, Ni, Fe, Zn, and Mn. These metals were selected due to their environmental importance and potential ecological impact. The concept of bioavailability is central to understanding the ecological risk of metals. Bioavailability refers to the fraction of total metal present in the environment that is in a form that can be absorbed by aquatic organisms, thus directly influencing metal toxicity. Several factors play crucial roles in determining metal bioavailability, including metal speciation, water hardness, pH, and DOC. Metal speciation, which refers to the different physical and chemical forms of a metal, is a key determinant of bioavailability, as different forms have varying levels of accessibility to organisms (Di Toro *et al.*, 2001; Heijerick *et al.*, 2003). Metals such as Cd, Cu, Ni, and Zn are known for their toxicity to aquatic life at elevated concentrations (Emenike *et al.*, 2022; Chen *et al.*, 2023; Naz *et al.*, 2023), while Ca and Mg, though essential nutrients, can influence the bioavailability and toxicity of other metals (Allen and Janssen, 2006; Wang *et al.*, 2014; Nys *et al.*, 2018). Monitoring these metals is essential for assessing pollution levels, understanding water chemistry interactions, and evaluating ecotoxicological risks (Nys *et al.*, 2018).

For this reason, understanding the factors that influence metal bioavailability and toxicity in receiving waters is crucial. Metal pollution presents a considerable threat to the global environment. Once metals enter an ecosystem, they can cause long-term toxic effects on organisms and the environment, a phenomenon known as chronic metal ecotoxicity (Paquin *et al.*, 2002). With the increasing severity of environmental pollution, understanding chronic metal ecotoxicity has become more critical than ever (Rainbow, 2002). The WFD requires European member states to ensure that all inland and coastal waters achieve "good" water quality status. EQS are used to meet this requirement, defining the concentration of chemicals in the water environment below which no adverse effects on aquatic life are expected (Hering *et al.*, 2010).

The toxicity of metals, as confirmed by EQS, is influenced by factors such as water hardness, pH, and DOC (Heijerick *et al.*, 2003). These parameters are crucial because they modify the bioavailability of metals, which in turn affects their toxicity to aquatic flora and fauna (Price *et al.*, 2022). By competing with harmful elements like Cu and Zn for binding sites on organismal surfaces (e.g., gills, cell membranes), water hardness—which is mainly regulated by Ca and Mg concentrations—reduces their bioavailability (de Paiva Magalhães *et al.*, 2015). Similarly, pH influences metal speciation, with acidic conditions increasing metal solubility and thus their bioavailability and toxicity (Bourg and Loch, 1995). DOC can form complexes with metal ions, potentially reducing their immediate bioavailability while facilitating their transport downstream (Peters *et al.*, 2014). Chapter 2 has shown that pH is significantly lowered in receiving rivers by the presence of STW discharges. Peters et al. (2014) have shown that DOC plays a critical role in binding metals, reducing their bioavailability, and thus lessening their toxic effects on aquatic life. Additionally, Worrall et al. (2019) demonstrated that DOC concentrations increase in receiving rivers due to STW discharges.

Given the complexity of factors such as water hardness, pH, and DOC in influencing metal bioavailability, it is crucial to assess how these parameters are altered by STW discharges and how these alterations subsequently affect metal toxicity. Assessing metal bioavailability, rather than solely relying on total metal concentrations, provides a more accurate and ecologically relevant measure of pollution risk in aquatic systems. This study investigates how STW discharges alter key water chemistry parameters, including pH, DOC, and hardness, and subsequently influence the bioavailability and toxicity of metals such as Cu, Zn, Ni, and Mn. Understanding these dynamics is crucial for assessing ecological risks and informing regulatory frameworks, such as the European Water Framework Directive, which aims to achieve good ecological status in all water bodies (Hering *et al.*, 2010). This study aims to explore these dynamics in detail, focusing on whether sewage effluent causes measurable changes — Cu, Mg, Ca, Cd, Zn, Fe, Mn, and Ni — in water quality and metal concentrations, and what role environmental and operational variables of the STW play in these changes. Studies have highlighted the presence of heavy metals in sewage discharge and their environmental implications. For instance, Rafiu et al. (2007) reported significantly

elevated concentrations of Cd, Pb, Mn, Zn, Cu, and Ni in water samples collected near STW, highlighting their contribution to downstream pollution. Similarly, Oliveira et al. (2007) investigated the removal efficiencies of metals such as Cd, Cu, Pb, Hg, Mn, Cr, and Zn in biological wastewater treatment, emphasising the variable performance of treatment processes in reducing metal loads. These studies underscore the importance of understanding how STW operations and environmental factors influence heavy metal concentrations, providing a foundation for the present investigation.

As identified in Chapter 2, previous studies of the impact of sewage on receiving waters have often been limited. For example, Bubb and Lester (1995) examined the impact of final effluents on the receiving waters of the River Yare in the UK, demonstrating that sewage effluents significantly increased metal concentrations -- Cu, Cd, Fe, Pb and Mn -- and facilitated metal complexation – Cu, Cd, Fe, Pb and Mn in the receiving waters, which had been mentioned in another study of Bubb and Lester (Buss and Lester, 1995), however, this was for only one river. Chon et al. (2012) analysed metals in the Aire-Calder catchment, identifying STWs as the primary sources of Cd and Ni, though their contribution to Pb and mercury (Hg) was minimal.

To study the bioavailability of metals in sewage effluent, several investigations have highlighted the environmental impacts of sewage discharge. Matthiessen et al. (1999) (2008) investigated the impact of STWs effluent on metal concentrations in UK rivers, focusing on Cu and Zn. The study utilised Environment Agency water quality data from 1995 to assess compliance with Predicted No-Effect Concentrations (PNECs) and evaluated the effectiveness of bioavailability-based approaches for regulatory compliance. Their findings indicated that Cu and Zn exceedances varied significantly depending on the assessment tier used. For Cu, a relatively small percentage of samples exceeded the PNEC, but most exceedances were also associated with background concentrations, suggesting that natural sources played a role. For Zn, exceedance rates were higher, particularly in areas with soft water, where existing EQS may be overly conservative. The study highlighted the limitations of traditional hardness-based EQS assessments and emphasised the importance of bioavailability corrections to improve regulatory accuracy. By demonstrating that metal exceedances were context-dependent, this

research contributed to the development of more refined water quality compliance strategies.

Building upon these studies, this research aims to address their limitations in temporal scope, range of metals considered, and ecological relevance. While Matthiessen et al. (1999) and Comber et al. (2008) provided valuable insights, they overlooked key metals like Mn and Ni, and the short monitoring periods hindered long-term trend analyses. By including Mn and Ni in addition to Cu and Zn, as highlighted in the UK River and Lake Assessment Method (https://www.wfduk.org), this study seeks to provide a more comprehensive understanding of metal bioavailability in sewage effluent. Mn plays a key role in redox-sensitive processes that influence metal mobility, while Ni is classified as a priority pollutant under international water quality standards due to its toxicity to aquatic life. By analysing Cu, Zn, Mn, and Ni over a 21-year period (2000-2020), this research addresses these gaps and investigates how variations in STW operations influence their downstream bioavailability. Unlike Comber et al. (2022), which primarily focused on compliance with EQS and demonstrated that catchment-scale contamination often drives metal pollution more than STW effluent, this study takes an ecologically relevant approach by directly assessing bioavailability and its implications for aquatic life. This focus improves upon the primary scope of earlier studies and provides insights into the long-term trends and risks associated with metal pollution in freshwater ecosystems.

This chapter poses the following questions:

- Does the final sewage effluent discharge from sewage treatment works cause a significant change in the concentration of metals in the receiving river?
- If sewage treatment work discharges result in differences in river water quality, can these differences be attributed to variations in the size and nature of the STW?
- How does the discharge from STWs affect the bioavailable concentrations of key metals (Cu, Zn, Mn and Ni) in downstream river sections compared to upstream levels?
- For which metals is the bioavailability significantly reduced by the STW?

- What factors significantly influence the variance in bioavailable metal concentrations?

3.2 Approach and Methodology

The approach of this study divides in two with the consideration of dissolved metal concentrations first and then their bioavailability. The work of this chapters builds on the same systematic approach to assess the impact of STWs discharges on various water quality determinands as developed in Chapter 2.

3.2.1 Study data

This chapter utilizes 21 years (2000 to 2020) of data for the elements Ca, DOC, pH, Mg, Cd, Cu, Ni, Fe, Zn, and Mn. For the M-BAT model also requires pH, DOC and Ca concentration data. All sourced from the Environment Agency (EA) in England. The analytical methods and data quality constraints employed are consistent with those detailed in Chapter 2.

3.2.2 STW and Control Pairs

Chapter 3 advances the investigative framework established in Chapter 2, which identified and analysed monitored discharges from sewage treatment works and their impact on rivers. The methodology remains consistent: for each STW discharge, the proximate river monitoring points were scrutinized, and pairs of sites were carefully chosen based on their positions relative to the STW discharge, ensuring no additional streams or discharges influenced the data in between. As with the previous chapter, a control group of river monitoring sites—known as 'Control pairs'-was selected to account for natural variations in water quality and to provide a baseline for comparison. These control sites were deliberately chosen to ensure no overlap with the STW sites, providing a clear demarcation for assessing changes attributed solely to STW discharges. The hypothesis remains that if STW discharges significantly alter water quality determinants, this effect will be reflected in a greater difference in the measurements between upstream and downstream sites in STW pairs compared to Control pairs.

Continuing with the established protocol from Chapter 2, observations made on the same day were compared, excluding any pairs lacking concurrent data. By

applying this consistent methodology to the analysis of the new determinant in Chapter 3, the same rigorous approach from Chapter 2 was maintained. Chapter 3 advances the investigative framework established in Chapter 2, which identified and analysed monitored discharges from sewage treatment works and their impact on rivers. The methodology remains consistent: for each STW discharge, the proximate river monitoring points were scrutinised, and pairs of sites were carefully chosen based on their positions relative to the STW discharge, ensuring no additional streams or discharges influenced the data in between. As with the previous chapter, a control group of river monitoring sites—known as 'Control pairs'—was selected to account for natural variations in water quality and to provide a baseline for comparison. These control sites were deliberately chosen to ensure no overlap with the STW sites, providing a clear demarcation for assessing changes attributed solely to STW discharges. The hypothesis remains that if STW discharges significantly alter water quality determinants, this effect will be reflected in a greater difference in the measurements between upstream and downstream sites in STW pairs compared to Control pairs.

In Chapter 2, 442 STW pairs and 419 Control pairs were initially identified based on-stream temperature records. Visual inspection confirmed that these pairs were evenly distributed across locations, indicating no apparent spatial bias. However, when considering data completeness and additional determinands beyond stream temperature, the number of pairs that could be analysed for dissolved metal concentration was smaller (Table 3.1). Their locations are represented in Figure 3.1.

Table 3.1. The number of pairs, both STW and Control, that could be included in this study.

Determinand	STW Pairs	Control Pairs
Ca (mg/L)	11	9
Mg (mg/L)	3	12
Cd (µg/L)	22	18
Cu (µg /L)	91	54
Ni (µg/L)	28	21
Fe (µg/L)	23	17
Zn (µg/L)	22	19
Mn (µg/L)	16	14

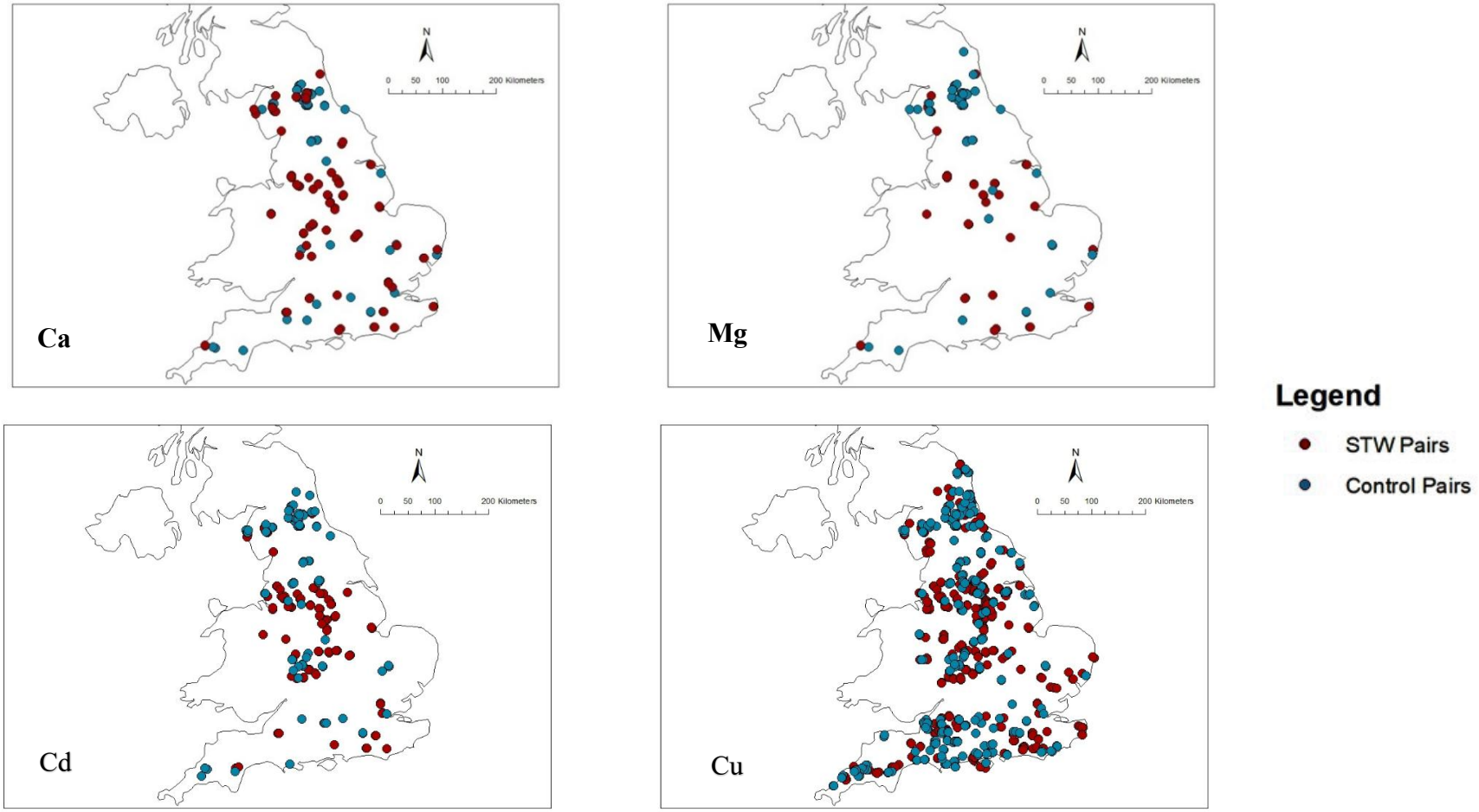


Figure 3.1(a). Location of STW and Control pairs for each metal determinand.

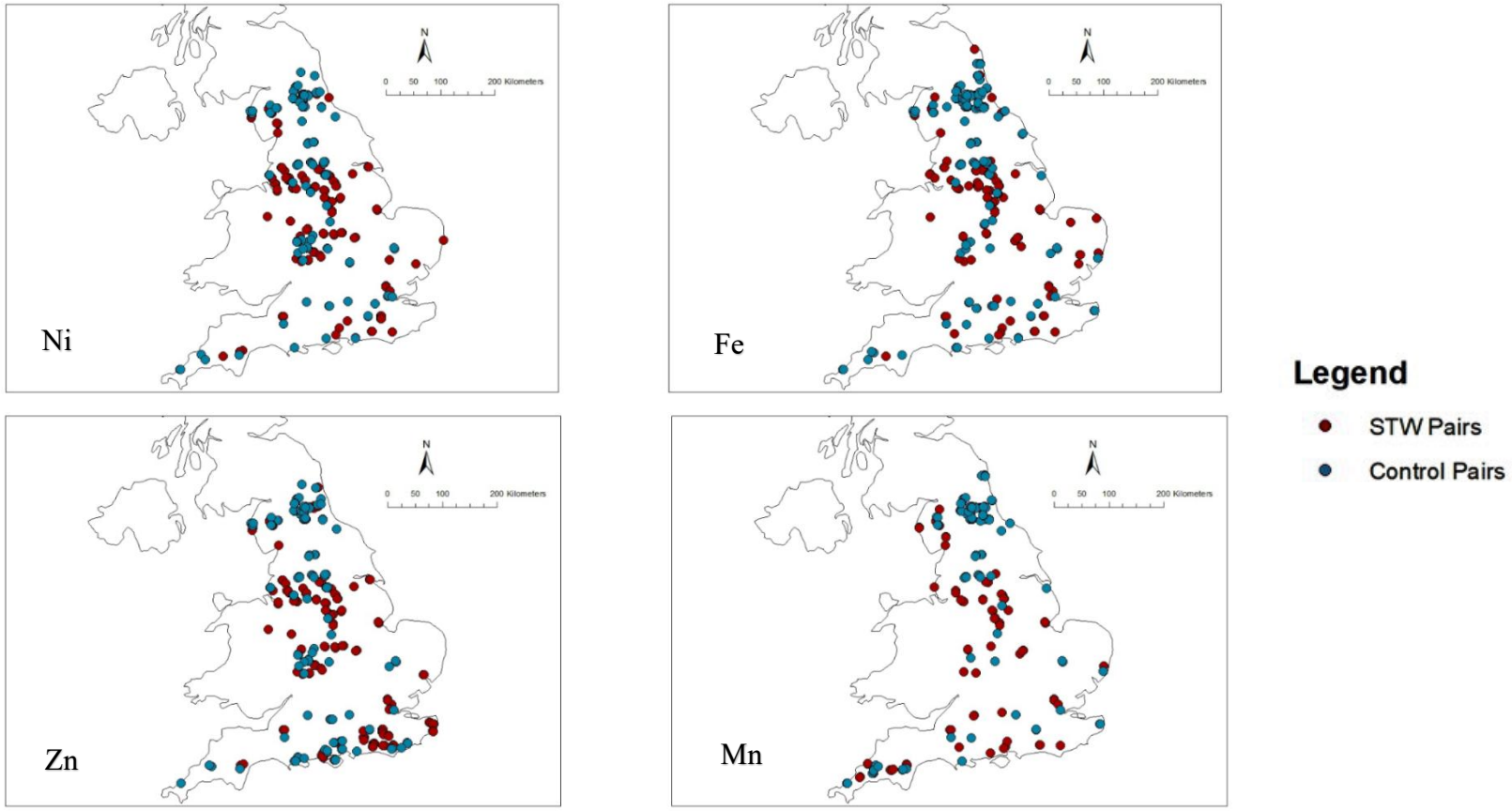


Figure 3.1(b). Location of STW and Control pairs for each metal determinand.

Covariates

As discussed in Chapter 2 (Section 2.2.2), certain water quality determinands can naturally change downstream, regardless of the presence of sewage discharges. To account for this and ensure that any differences between control and STW pairs are attributed to STW discharges rather than the physical arrangement of monitoring sites, four covariates were incorporated into the analysis:

Euclidean distance between monitoring points within each pair (both STW and control) was included to control for the expected changes in water quality determinands as a function of distance in the ANOVA. Altitude of the upstream and downstream monitoring points were considered, as certain determinands, such as stream temperature, are known to vary with elevation. Percentile riverflow at the time of sampling, derived from river flow records from the National River Flow Archive (NRFA), was used to account for variability in flow conditions. The flow records were converted to percentile flow to ensure comparability across different catchments and locations. These covariates were included to isolate the effect of STW discharges on water quality from geographical and hydrological factors. Visual inspection of the covariates indicated no systematic bias in site selection (Table 2.2), though sub-sampling for individual determinands may affect the final analysis.

Bioavailable metal concentration

Due to the complexity of directly measuring bioavailable metal concentrations, a predictive model was used to estimate the bioavailable metal concentration from measured dissolved metal levels (https://www.wfduk.org/). For this, the UK's Metal Bioavailability Assessment Tool (M-BAT), a simplified model based on each metal's Biotic Ligand Model (BLM) (https://www.wfduk.org/), was employed. The M-BAT uses the measured concentration of the metal and calculates the bioavailable concentration of that metal based upon the pH; and the DOC and Ca concentrations measured in the same sample. The M-BAT was used to predict the bioavailable metal concentration for each of Cu, Zn, Mn and Ni, and then calculate the risk characterization ratio (RCR). The RCR was calculated by comparing the predicted bioavailable concentration of each metal under site-specific conditions with the Environmental Quality Standard for Bioavailable Metals ($EQS_{bioavailable}$). An RCR greater than 1 indicates a potential risk due to exceedance of the EQS.

Given the prerequisite of simultaneous measurement of DOC, pH, Ca, and the metal of interest within a single sample for bioavailability and RCR calculations, the number of viable sample pairs was substantially diminished in comparison to those available for dissolved metal concentration analysis. Table 3.2 gives the number of samples and STW and Control pairs for which bioavailable metal concentration and RCR could be calculated.

Table 3.2. The number pairs and samples for each metal that could be considered within the M-BAT.

M-BAT	Samples	Samples (without outliers)	STW Pairs	Control Pairs
Cu (µg/L)	3202	3042	8	9
Zn (µg/L)	3016	2530	8	8
Mn (µg/L)	1206	1076	4	8
Ni (µg/L)	2945	2645	7	8

3.2.3 Statistical analysis

In this chapter, ANOVA was employed for the analysis of the concentration data for the dissolved metal concentration and the bioavailable metal analysis just as outlined in Chapter 2, Section 2.2.2. For the dissolved and bioavailable metal concentration data the impact of sewage effluent on the receiving the ANOVA was performed with four factors—Type, Year, Month, and Pair—where the factors are defined as in Chapter 2. Further this ANOVA of the dissolved and bioavailable metal concentrations was analysed first without and then with covariates (Euclidean distance between pairs, upstream altitude, downstream altitude, and percentile flow).

To meet the assumptions of ANOVA, data distribution was assessed using the Anderson-Darling test (Anderson and Darling, 1952). Where necessary, log transformations were applied to normalise the data. Post-transformation, the data distribution was checked using a QQ' plot, and outliers were selectively removed, ensuring no more than 5% of the dataset was excluded.

Because the dataset for bioavailable metals is considerably smaller than that for the dissolved metals it would not necessarily be a fair comparison to compare between the results for the bioavailable metal concentration to the dissolved metal concentration. So, for those samples where there was a sufficient information to calculate a bioavailable metal concentration the ANOVA was repeated using the dissolved metal concentration data only for those samples where there was a sufficient information to allow the calculation of the bioavailable concentrations. The same factors and interactions were considered as for previous analysis. Therefore, there is an additional comparison, i.e. not only do we consider whether there is a difference between Control and STW pairs for those sites where there was sufficient information to calculate bioavailable metal concentration, but also that the same difference be observed for the dissolved metals at those sites.

To understand the impact of the STW treatment processes on dissolved metal concentration, and as in Chapter 2, a Chi-squared test was conducted to assess the effectiveness of STW processes, determining whether treatment outcomes varied significantly across categorical variables. Differentiation between tertiary types was not possible in this chapter owing to incomplete metadata; this limitation

applies only to secondary processes. Correlation was used to test the relationship between PE, DWF, and the concentration differences in metals.

For bioavailable metals, correlation was performed to explore the relationship between bioavailable metal concentrations and the concentrations of Ca, DOC, dissolved metals, and pH. The significance and coefficients for each factor were derived using a General Linear Model (GLM). The coefficient for each factor represents the proportional change in bioavailable metal concentration per unit change in the corresponding factor. Specifically, these coefficients quantify how much the concentration of bioavailable metals (e.g., Cu, Zn, Mn) changes when the concentration of Ca, DOC, or pH varies by one unit. For instance, a negative coefficient for pH indicates that a decrease in pH leads to a decrease in bioavailable metal concentration, while a positive coefficient for Ca suggests that higher Ca concentrations are associated with higher bioavailable metal concentrations.

Finally, PCA was used to examine patterns in both the dissolved metal concentrations, and separately, the bioavailable metal concentrations and prior to PCA a correlation analysis was performed.

3.3 Results

3.3.1 Dissolved metal concentrations

A summary of the average dissolved metal concentration at the upstream sites and the average final effluent concentration is shown in Table 3.3.

Table 3.3. Summary of the dataset and results for STW discharge impact analysis. The significance of the impact ($p < 0.05$) is marked as "Yes" for each determinand. The main effect (%) represents the difference in values for STW pairs compared to upstream means, excluding control differences (illustrated in Figure 2.3). N is the total number of recorded pairs, and N (without outliers) excludes outliers. Upstream, downstream, and final effluent refer to their respective mean concentrations.

Determinand	N	N without outliers	upstream	downstream	final effluent	Type Significant	Main effect	Main effect (%)
Ca (mg/L)	192	140	76.27	77.62	73.24	Yes	1.1	1.4
Mg (mg/L)	210	160	8.00	12.67	17.56	Yes	0.03	0.38
Cd (µg/L)	509	509	0.20	0.18	0.21	--	--	--
Cu (µg/L)	6522	5263	3.19	4.21	7.88	Yes	0.18	5.6
Ni (µg/L)	884	884	4.63	6.13	65.29	--	--	--
Fe (µg/L)	937	721	96.20	66.19	249.30	Yes	-4.30	-4.5
Zn (µg/L)	972	766	49.60	33.64	58.46	Yes	0.65	1.3
Mn (µg/L)	511	379	112.61	160.82	137.09	Yes	-5.0	-4.4

Correlation analysis of dissolved metal concentrations

Significant correlations (Table 3.4) were found between: Ca and Mg (0.57), Ca and Cu (0.41), Cd and Ni (0.57), Cd and Zn (0.92), and Ni and Zn (0.59) – Table 3.11. Mn has positive correlation with all other determinands. These relationships suggest that these determinands may share common pollution sources or exhibit similar geochemical behaviours, indicating that changes in one could potentially predict changes in another.

Table 3.4. Summary of inter-determinand correlation coefficients. Correlation coefficients range from −1 to 1 and are shown alongside their corresponding probability values. Coefficients with p-values < 0.05, indicating statistical significance, are highlighted in grey.

Determinand (unit)	Ca (mg/L)	Mg (mg/L)	Cd (µg/L)	Cu (µg/L)	Ni (µg/L)	Fe (µg/L)	Zn (µg/L)	Mn (µg/L)
Ca (mg/L)		0.57	-0.06	0.41	0.06	-0.37	-0.08	0.20
Mg (mg/L)			0.004	0.33	0.09	-0.25	-0.007	0.18
Cd (µg/L)				-0.12	0.57	-0.03	0.92	0.15
Cu (µg/L)					0.34	0.009	-0.15	0.27
Ni (µg/L)						0.19	0.59	0.48
Fe (µg/L)							0.01	0.23
Zn (µg/L)								0.19

Impact of STW discharges on dissolved metal concentration

The mean final effluent concentrations for most determinands, except for Ca, are higher than their respective mean upstream concentrations (Table 3.3). Furthermore, the mean downstream concentrations of Ca, Mg, Cu, Ni, and Mn are higher than their corresponding mean upstream concentrations.

The Type factor was statistically significant for all metals except Cd and Ni (Table 3.3 & Table 3.5, Figure 3.2)), explaining less than 28% of the original variance in the datasets (Table 3.5). The Type factor was most important for Ca (28% of original variance explained) and least important for Zn (1% of original variance explained). The largest impact was observed for Cu, which showed a 5.6% increase, followed by Fe (-4.5%) and Mn (-4.4%). Mg had the smallest main effect.

This study focuses on the interaction of Type*Year rather than the individual effects of the Year factor alone. The year factor explained a portion of the variance for all metals except Mg and Cd; among them, Ca explained 63% of the original variance, and other metals explained less than this value (Table 3.5). The key interest lies in whether the observed impacts for the Sewage Treatment Works (STW) have changed over time.

For Mg, Cu and Ni there was a significant Type*Year interaction but there was no significant interaction for Zn. For Cu, the STW pairs showed a decrease when compared to Control pairs, which is an improvement in the impact of STW discharge (Table 3.6, Figure 3.3 – Cu difference). For Ni, the STW pairs showed an increase over time, but showed no improvement in the STW discharge (Table 3.6, Figure 3.3 – Ni difference). Due to the instability and absence of data in certain years (such as the missing data for Ca) (Figure 3.3), the overall coherence of the dataset is compromised. This inconsistency makes it more challenging to analyse annual trends and reduces the reliability of the study's conclusions.

Figure 3.3 illustrates the predicted marginal means for each metal, based on the ANOVA model, with a particular focus on the Type*Year interaction. This plot highlights how the effects of STW vary over time, presenting the predicted values for both Control and STW sites. The error bars represent the 95% confidence intervals, providing a measure of uncertainty around the predicted means. Figure

3.3 emphasizes the yearly variations in metal concentrations and shows how STW influences these concentrations compared to Control sites over the study period.

Appendix 1 provides a detailed breakdown of the Type*Year interaction, presenting the marginal means and 95% confidence intervals for Metals differences across years. The Ni difference for STW sites shows a steady increase over the study period. The Cu difference for STW sites indicates a slight decline with minor variations, and the Mg difference for STW sites demonstrates a consistent decrease across the years.

Month factor explained less than 23% of the original variance in the datasets and Type*Month explained an even smaller proportion of the original variance (less than 6% - Table 3.5). As above, and with respect to this study, the more important term was the Type*Month interaction, i.e. does any difference between STW and Control types persist across the seasonal cycle?

In this study, Cu, Ni and Mn showed a significant Type*Month interaction (Table 3.5). For Cu, the predicted values for STW pairs from January to December were higher than Control pairs (Table 3.7; Figure 3.4 – Cu difference); for Ni STW pairs showed higher values from January to May, and Control pairs showed higher values than STW pairs from June through December (Table 3.7; Figure 3.4 – Ni difference); Mn showed that from November to December the values for STW pairs were higher than those for Control pairs (Table 3.7; Figure 3.4 – Mn difference).

Figure 3.4 illustrates the predicted marginal means for each metal, based on the ANOVA model, with a particular focus on the Type*Month interaction. This plot highlights how the effects of Sewage Treatment Works (STW) vary across months, presenting the predicted values for both Control and STW sites. The error bars represent the 95% confidence intervals, providing a measure of uncertainty around the predicted means.

To further explore the monthly trends, Appendix 2 offers a more detailed breakdown of the Type*Month interaction. The values shown in Appendix 2 provide additional insights into how the differences in metal concentrations evolve across months, contributing to a deeper understanding of the seasonal dynamics at play.

The Pair factor was statistically significant for all determinands except Cd, explaining between 28% and 89% of the original variance in the datasets, with its influence being most important for Ni and least important for Cu.

The Type*Pair was only significant for Cu, which accounted for 0.01% of the original variance (Table 3.5).

A summary of the mean difference of each Pair greater than 0, equal to 0 and less than 0 is given in Table 3.8. A priori, it would be expected that the proportion of STW pairs significantly different from zero would reflect the Type effect. However, for Mg and Zn, the mean value at STW sites is lower than that at Control sites when considering only positive differences.

Figure 3.5 illustrates substantial between-pair heterogeneity in the concentration differences for the selected metals and metalloids, with a widespread observed for Ni (Pair $\eta^2 = 0.89$) and Mn ($\eta^2 = 0.51$), and a narrower spread for Cu ($\eta^2 = 0.28$) and Cd (non-significant). The points are ordered along the x-axis by the overall mean difference across both Type groups, from lowest to highest, and show both positive (downstream increase) and negative (downstream decrease) values. Notably, the position of points on the x-axis does not necessarily correspond to the magnitude of their mean differences along the y-axis. For example, some points on the left side of the x-axis have higher y-values than those on the right side, reflecting the mixed effects observed across different pairs. This highlights the complex nature of STW impacts on metal concentrations across various sites, as quantified in Table 3.8. Note: This is not a cumulative distribution function (CDF), but a plot showing the ordered mean differences across pairs.

Table 3.5. The summary of the ANOVA for each determinand. The significance for each determinand is stated as Yes if it was significant at a probability < 0.05 of being zero. η2 indicates the extent to which a factor contributes to the observed variability in the variable.

Determinand	Type		Year		Type*Year		Month		Type*Month		Pair		Type*Pair	
	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2
Ca (mg/L)	Yes	0.28	Yes	0.63	--	--	Yes	0.23	--	--	Yes	0.54	--	--
Mg (mg/L)	Yes	0.04	--	--	Yes	0.07	--	--	--	--	Yes	0.35	--	--
Cd (µg/L)	--	--	--	--	--	--	Yes	0.01	--	--	--	--	--	--
Cu (µg/L)	Yes	0.04	Yes	0.009	Yes	0.01	--	--	Yes	0.005	Yes	0.28	Yes	0.01
Ni (µg/L)	--	--	Yes	0.27	Yes	0.03	Yes	0.06	Yes	0.06	Yes	0.89	--	--
Fe (µg/L)	Yes	0.02	Yes	0.11	--	--	Yes	0.05	--	--	Yes	0.38	--	--
Zn (µg/L)	Yes	0.01	Yes	0.11	--	--	--	--	--	--	Yes	0.46	--	--
Mn (µg/L)	Yes	0.05	Yes	0.27	--	--	Yes	0.08	Yes	0.06	Yes	0.51	--	--

*Table 3.6. The gradient of the trend over the entire study period for each determinand that showed a significant Type*Year interaction. Where not significantly different from zero, zero is given, otherwise gradient is quoted the stand error on that slope. Improvement is stated as Yes when the impact of STW would be significantly decreasing with time with respect to the control.*

	STW	Control	Improvement?
Mg (µg/L)	-0.26	0	Yes
Cu (µg/L)	-0.22	0	Yes
Ni (µg/L)	0.32	0	No

Table 3.7. Months where STW-predicted concentrations were greater than Control-predicted concentrations for each determinand.

Determinand	STW predicted > Control predicted
Cu (µg/L)	January – December
Ni (µg/L)	January – May
Mn (µg/L)	November – December

Table 3.8. Number of mean difference values for each determinand for each pair categorized by type. 'N' represents the total number of mean difference values recorded. '>0' indicates the number of mean difference values that were greater than zero, '<0' represents the number of mean difference values that were less than zero, and '=0' denotes the number of mean difference values where the difference was not significantly different.

Determinand	Total				STW				Control			
	N	>0	=0	<0	N	>0	=0	<0	N	>0	=0	<0
Ca (mg/L)	20	12	4	4	11	7	1	3	9	5	3	1
Mg (mg/L)	15	6	4	5	3	1	1	1	12	5	3	4
Cd (µg/L)	40	12	19	9	22	8	9	5	18	4	10	4
Cu (µg/L)	145	59	42	44	91	42	22	27	54	17	20	17
Ni (µg/L)	49	14	22	13	28	8	15	5	21	6	7	8
Fe (µg/L)	40	27	5	8	23	16	3	4	17	11	2	4
Zn (µg/L)	41	14	13	14	22	6	6	10	19	8	7	4
Mn (µg/L)	30	13	7	10	16	8	3	5	14	5	4	5

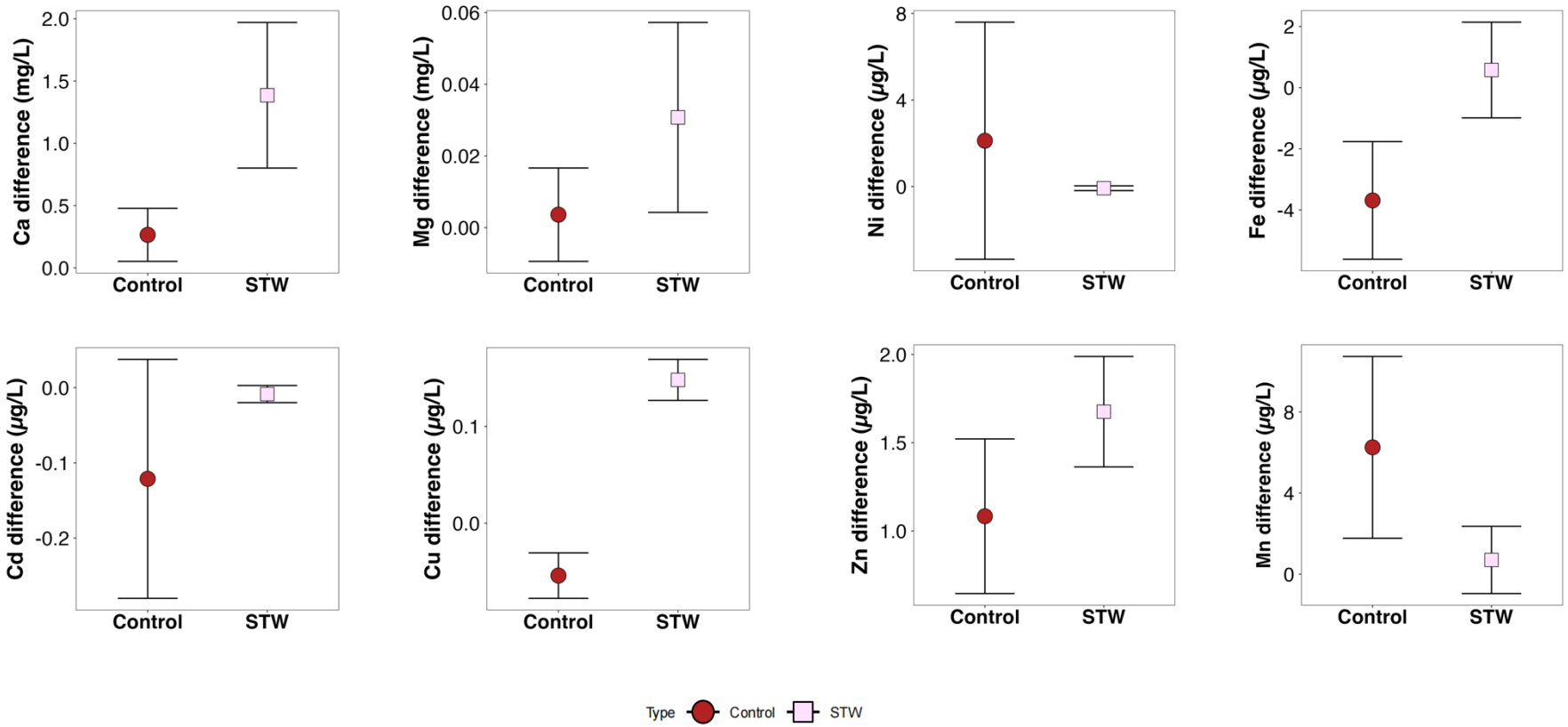


Figure 3.2. The main effects plot for the Type factor for the difference. The values are presented as the marginal mean with the 95% confidence limits on that mean. NB. The y axis for each metal is different.

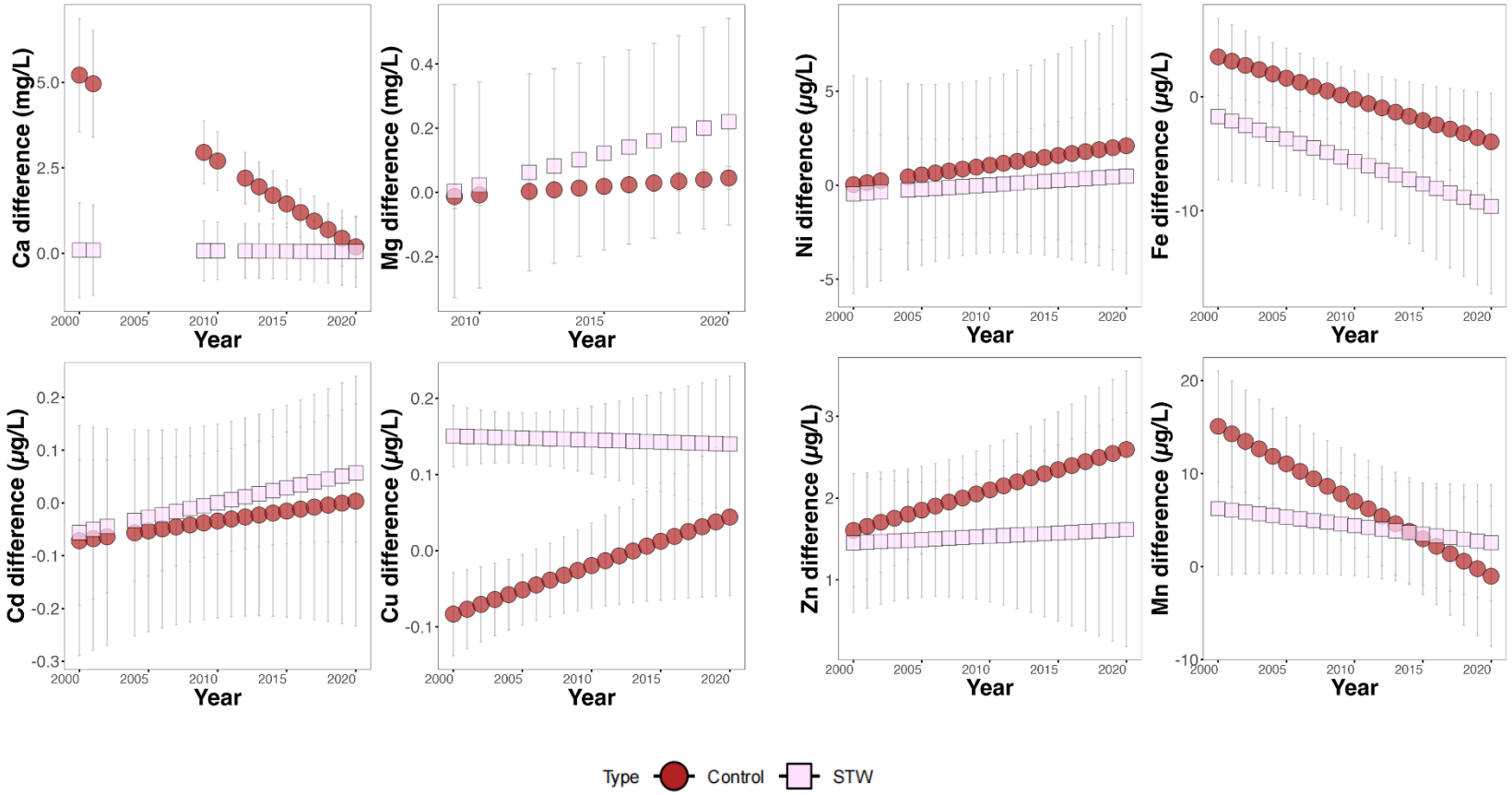


*Figure 3.3. Main effects plot based on ANOVA-predicted marginal means for metal differences across months. These values represent the estimated marginal means for each Type*Year combination, averaged across the levels of other design factors (Month, Pair), and are not raw observed data*

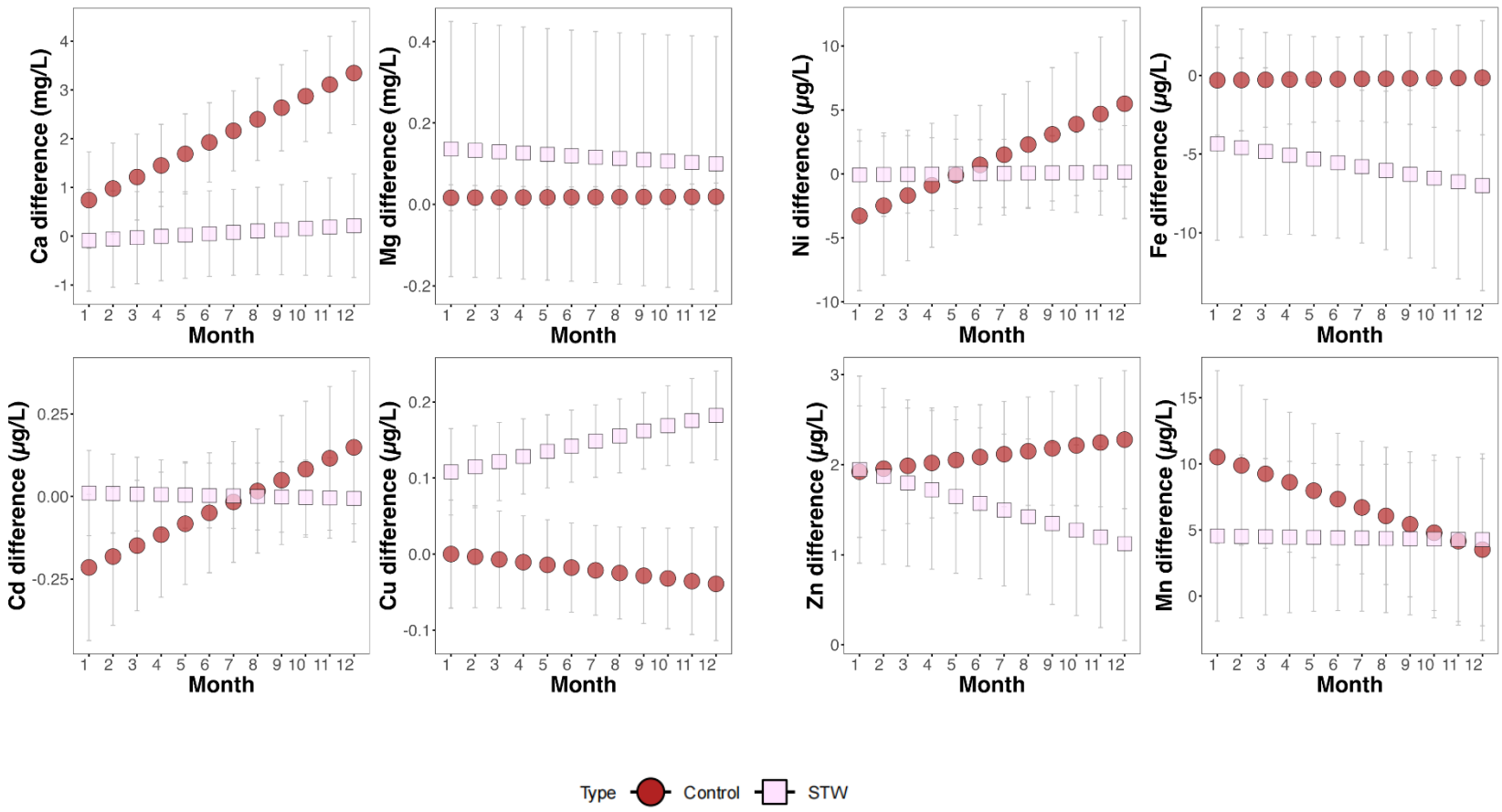


*Figure 3.4. Main effects plot based on ANOVA-predicted marginal means for metal differences across months. These values represent the estimated marginal means for each Type*Month combination, averaged across the levels of other design factors (Year, Pair), and are not raw observed data.*

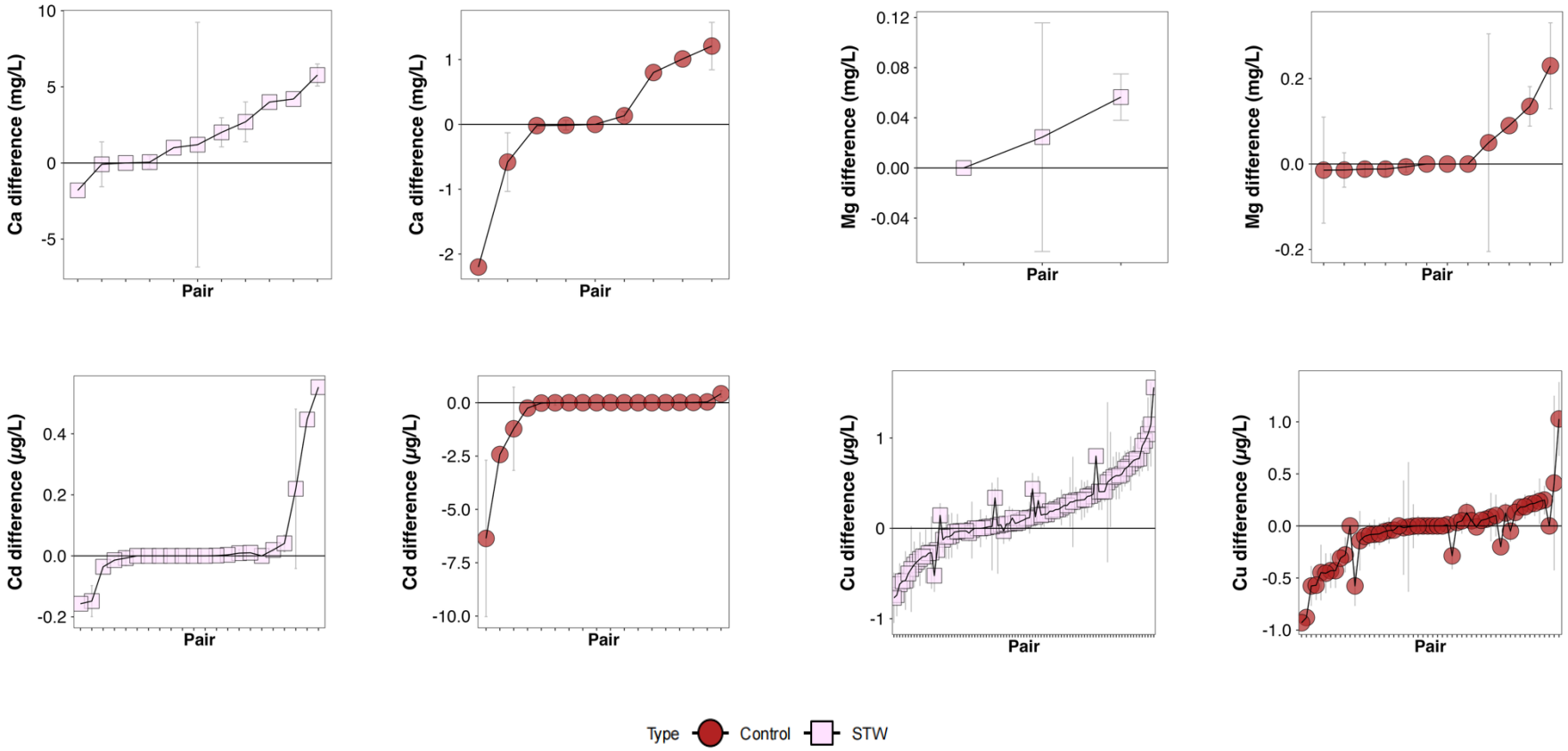


Figure 3.5(a). The main effects plot of the Pair factor. The Pairs are ordered from the lowest to greatest values of the difference. The points are the marginal mean with the 95% confidence interval.

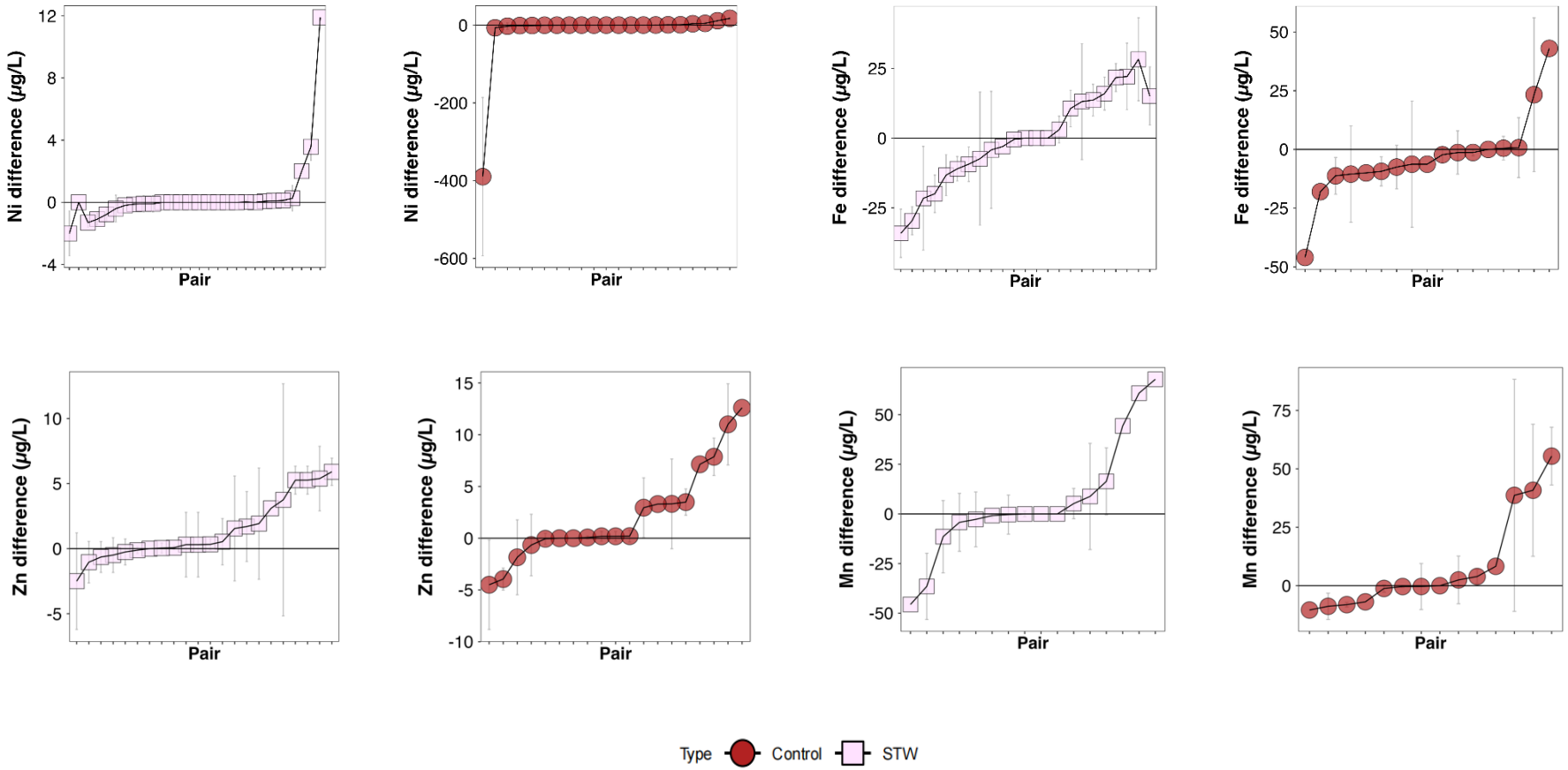


Figure 3.5(b). The main effects plot of the Pair factor. The Pairs are ordered from the lowest to greatest values of the difference. The points are the marginal mean with the 95% confidence interval.

Importance of covariates for dissolved metal concentrations

The inclusion of covariates (length, upstream altitude, downstream altitude, and Percentile flow) resulted in changes to the significance and coefficients of the original factors (Type, Year, Month, Pair) (Table 3.9). Specifically, after including the covariates, the significance of Type, Year, Month, and Pair changed, with the coefficients of these factors also being altered. For instance, Percentile flow had a significant effect on Cd, Cu, and Fe, with coefficients of -—0.0002, -0.02, and -0.23, respectively, while Euclidean distance, upstream altitude, and downstream altitude showed no significant effect on the dissolved metal concentrations.

Table 3.9. The significance and η^2 for each factor of each determinand. The significance and coefficient for each of the covariates was assessed at a probability < 0.05 of being zero.

Determinand	Type		Year		Month		Pair		Euclidean distance (m)		up_altitude (m asl)		down_altitude (m asl)		Percentrank Significant	
	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	coefficient	Sig	coefficient	Sig	coefficient	Sig	coefficient
Ca (mg/L)	Yes	0.001	--	--	Yes	0.33	--	--	--	--	--	--	--	--	--	--
Mg (mg/L)	Yes	0.69	Yes	0.62	Yes	0.37	--	--	--	--	--	--	--	--	--	--
Cd (µg/L)	Yes	0.01	Yes	1	Yes	0.05	Yes	0.05	--	--	--	--	--	--	Yes	-0.0002
Cu (µg/L)	Yes	0.008	Yes	0.06	Yes	0.01	Yes	0.19	--	--	--	--	--	--	Yes	-0.02
Ni (µg/L)	Yes	1	Yes	1	--	--	--	--	--	--	--	--	--	--	--	--
Fe (µg/L)	Yes	0.02	Yes	0.08	Yes	0.04	Yes	0.18	--	--	--	--	--	--	Yes	-0.23
Zn (µg/L)	Yes	0.04	Yes	0.09	Yes	0.03	Yes	0.33	--	--	--	--	--	--	--	--
Mn (µg/L)	--	--	Yes	0.02	Yes	0.04	Yes	0.25	--	--	--	--	--	--		

Impact of the nature of the STWs on the dissolved metal concentration

The Chi-squared test results showed that there was only a significant difference between activated sludge secondary treatment (SAS) and secondary biological treatment (SB) for Fe and Cu (Table 3.10). In both cases the presence of SB treatment is more effective for Fe and Cu removal (Figure 3.6).

Table 3.10 Results of Chi-squared test for each determinand relative to technologies present at the STW. SAS = secondary activated sludge; SB = secondary biological. It was significant when at a probability (P) < 0.05.

Determinand		SAS	SB	P	Main effect (SAS-SB)
Ca (mg/L)	Positive	0	7	--	--
	Negative	0	23		
Mg (mg/L)	Positive	0	0	--	--
	Negative	0	11		
Cd (µg/L)	Positive	0	4	--	--
	Negative	0	16		
Cu (µg/L)	Positive	134	803	0.0001	0.5
	Negative	407	1008		
Ni (µg/L)	Positive	1	12	--	--
	Negative	1	16		
Fe (µg/L)	Positive	32	116	0.004	28
	Negative	67	115		

Zn (µg/L)	Positive	0	8	--	--
	Negative	3	18		
Mn (µg/L)	Positive	0	24	--	--
	Negative	0	24		

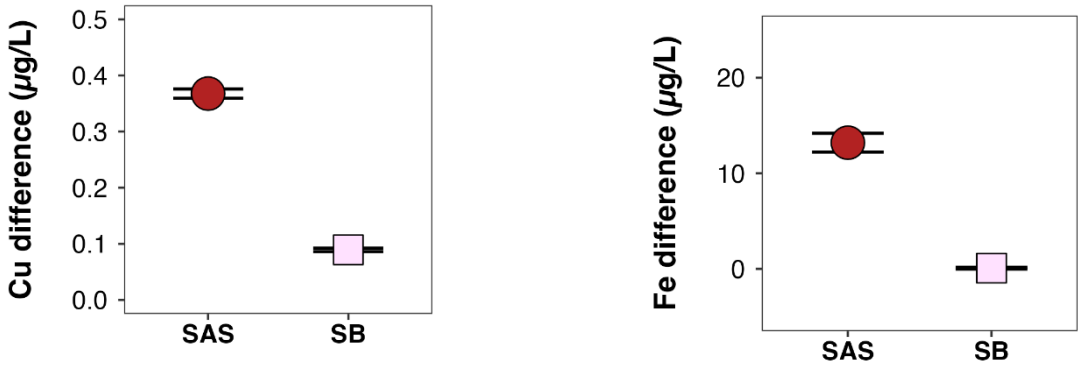


Figure 3.6. Secondary treatment difference for Cu and Fe. The main effects and the 95% confidence limits are plotted – they are quite small relative to the plot size.

Impact of population equivalence and dry weather flow on dissolved metal concentrations

As discussed in Section 2.3.4, a positive correlation exists between population equivalence (PE) and dry weather flow (DWF) (Pearson correlation coefficient of 0.88), indicating that increases in both variables significantly impact receiving water bodies (See Figure 2.13).

While previous analyses focused on the overall relationship between PE and DWF, this chapter examines the relationship between individual determinand concentrations and PE and DWF (Table 3.11). For Ca and Ni, increase PE led to a decrease in metal concentration, while Zn was positively correlated with PE. With respect to DWF then Fe was negatively correlated while both Mg and Cu showed positive correlation.

Table 3.11. Significance of the impact of STW discharge on each determinand, Population Equivalence (rPE), and Dry Weather Flow (DWF). A result is marked as "Yes" if the impact was statistically significant at $p < 0.05$ (i.e. the probability of the effect being zero is less than 5%).

Determinand	Population equivalence (PE/1000)		Dry weather Flow (DWF/1000)		r^2
	Sig	Coefficient	Sig	Coefficient	
Ca (mg/L)	Yes	-0.39	--	--	0.47
Mg (mg/L)	--	--	Yes	19.11	0.69
Cd (µg/L)	--	--	--	--	0.008
Cu (µg/L)	--	--	Yes	0.28	0.10
Ni (µg/L)	Yes	-0.002	--	--	0.08
Fe (µg/L)	--	--	Yes	-4.83	0.04
Zn (µg/L)	Yes	0.42	--	--	0.30
Mn (µg/L)	--	--	--	--	0.003

Principal components analysis of dissolved metal concentrations

By merging datasets for each determinand there were 387 data that could be considered across all determinands for all STW pairs. Three out of eight components had eigenvalues > 1 (Table 3.12) and these three components together explained 76% of the original variance. The contribution table on the components shows that the first component (PC1) was correlated with Zn, Cd and Ni; the second component (PC2) was correlated to Ca, Mg and Cu; and the third component was highly correlated with Fe and Mn. The graph of the scores on the first two components shows a quasi-orthogonality with STWs plotting on trends along PC1 or trending along PC2. After data processing there was data for 16 STWs. One STW appears distinct - Nent North of Crookbank – with variation dominantly along PC1 and nearly orthogonal to all other STWs (Figure 3.7).

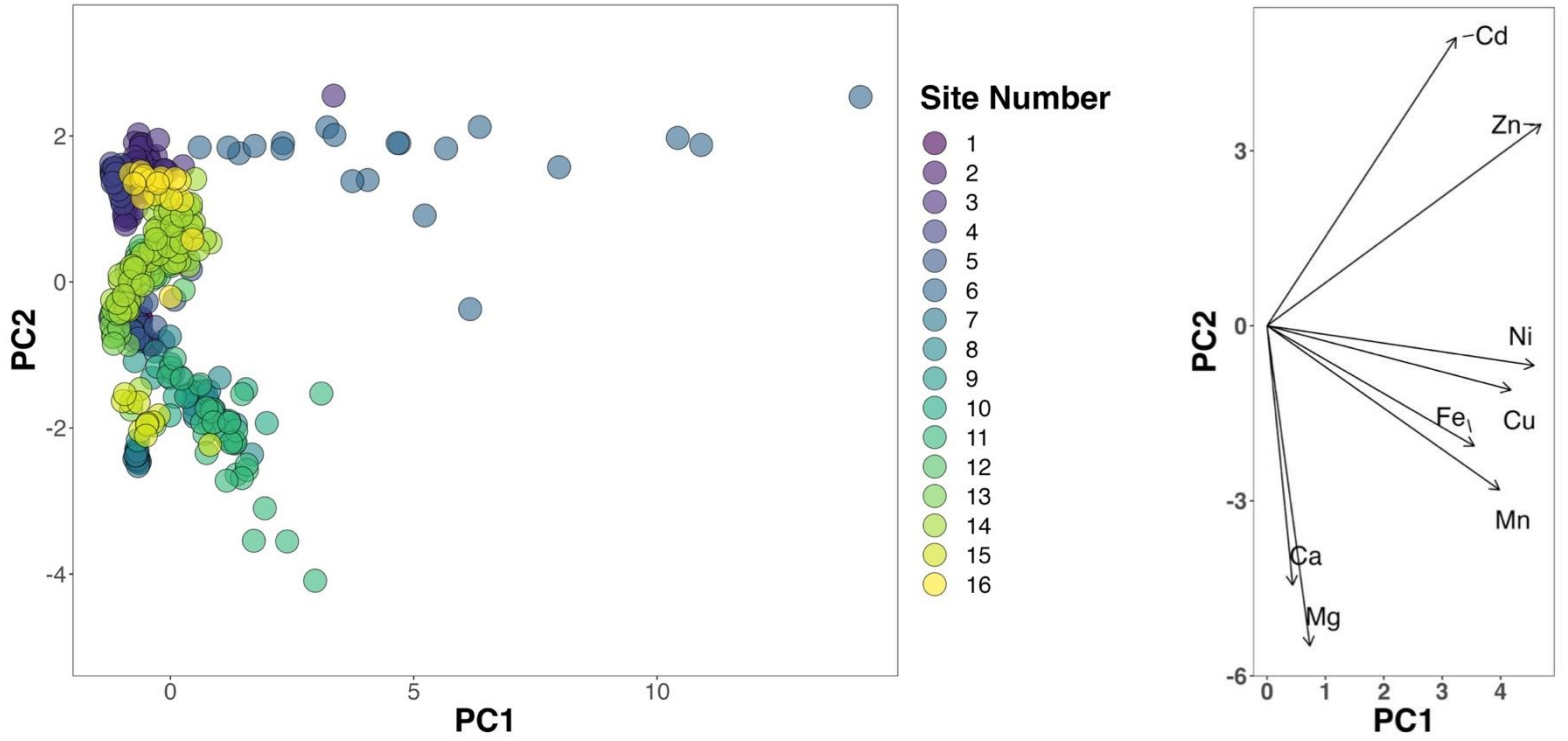


Figure 3.7. PCA score and loading for the first two principal components (PC1 and PC2). The list of sites with their corresponding numbers is as follow: 1 — Chess Stream, Park Farm; 2 — Cressbrook Pool Outfall; 3 — River Derwent at Carricks Picnic Site (Ruffside); 4 — Glenridding Beck at A592; 5 — Lydden at Lydlinch; 6 — River Nent north of Crookbank Cottage; 7 — Owlands Wood Dyke at Cornmill Farm; 8 — Pennington Brook at Black Harry Bridge; 9 — River Gipping upstream of Horseshoe Weir; 10 — Rainworth Water at Robin Dam Bridge; 11 —

River Alt above Altmouth Pumping Station; 12 — River Amber at Dalebank; 13 — River Roe at Gaitsgill; 14 — Rookhope Burn at Eastgate; 15 — Strine Brook at Longford Footbridge; 16 — Threlkeld EA Flow Station.

Table 3.12. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1	PC2	PC3
Ca (mg/L)	0.06	-0.57	-0.19
Mg (mg/L)	0.11	-0.52	-0.18
Cd (µg/L)	0.53	0.20	-0.30
Cu (µg/L)	0.12	-0.47	0.33
Ni (µg/L)	0.54	-0.04	0.19
Fe (µg/L)	0.08	0.24	0.66
Zn (µg/L)	0.54	0.21	-0.27
Mn (µg/L)	0.34	-0.20	0.42
Eigenvalue	2.58	2.13	1.39
Percentage of variance explained	32%	27%	17%

3.3.2 Bioavailable metal concentration

A summary of the bioavailable concentrations for each metal is shown in Table 3.13. For metal bioavailability, the presence of STW discharge had varied impacts across metals. Zn exhibited a significant decrease in main effect (-258% compared to Control), indicating that the increase in Zn concentrations downstream of STW sites was smaller than the natural variation observed in control sites. For Mn, the bioavailable concentrations increased (main effect of 21%), suggesting a notable contribution of STW discharge to downstream Mn levels. Similarly, Ni showed a modest increase in bioavailable concentrations (main effect of 13%). For all metals for which bioavailable concentrations could be calculated, except for Cu, the Type factor was significant but explained no more than 2% of the variance in the original dataset (Figure 3.8 and Table 3.14). Except for Zn, all bioavailable metals had higher mean concentrations in STW pairs than in the Control pairs (Figure 3.8).

The Year factor was significant for all metals, explaining no more than 10% of the original variance (Table 3.14). Additionally, the Type*Year interaction was only significant for Cu, although it explained only 0.2%. The yearly trends for the bioavailable concentration of Cu with STW pairs showing a significant decrease at the rate of -0.004 µg /L per year, while the Control pairs exhibited an increase at the rate of 0.003 µg /L per year, i.e. the impact of STW discharge for Cu was diminishing with time through the study.

The Month factor was significant for Cu, Zn, and Ni, contributing to less than 4% of the variance in the original dataset. However, the Type*Month interaction was insignificant for all bioavailable metals, so the seasonal differences were not analysed further.

The Pair factor accounted for no more than 35% of the original variance, with the highest contribution observed for Zn, explaining 35% of the original variance. In contrast, Ni and Cu contributed minimally to the variance, and the effect was not significant for Mn. Additionally, the Type*Pair interaction was found to be insignificant for all bioavailable metals.

Table 3.13. Summary of the dataset and results for the analysis of the impact of STW discharge for each Bioavailable metal. The main effect (%) is the main effect for each determinand for the Type factor. Values are compared to the mean for the upstream values for the STW pairs for that determinand. N represents the total number of pairs recorded at different times. N (without outliers) refers to the count of these pairs after removing outlier values. Main effect = Mean STW bioavailable concentration difference – Mean Control Bioavailable concentration difference.

Bioavailable metal(unit)	N	N (without outliers)	Mean upstream	Mean Downstream	Type Significance	Main effect	Main effect (%)
Cu (µg/L)	3202	3042	0.16	0.162	--	--	--
Zn (µg/L)	3016	2530	5.92	8.97	Yes	-15.3	-258
Mn (µg/L)	1206	1076	27.62	28.52	Yes	5.83	21
Ni (µg/L)	2945	2654	0.97	0.95	Yes	0.13	13

Table 3.14. The summary of the General Linear Model for each determinand Bioavailable metal. The significance for each determinand is stated as Yes if it was significant at a probability < 0.05 of being zero. η^2 indicates the extent to which a factor contributes to the observed variability in the variable.

Bioavailable metal (unit)	Type		Year		Type*Year		Month		Type*Month		Pair		Type*Pair		Dissolved concentration		Type*Dissolved concentration	
	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2
Cu (µg/L)	--	--	Yes	0.02	Yes	0.002	Yes	0.006	--	--	Yes	0.008	--	--	Yes	0.61	Yes	0.06
Zn (µg/L)	Yes	0.02	Yes	0.10	--	--	Yes	0.04	--	--	Yes	0.35	--	--	Yes	0.93	Yes	0.01
Mn (µg/L)	Yes	0.02	Yes	0.004	--	--	--	--	--	--	--	--	--	--	Yes	0.66	Yes	0.02
Ni (µg/L)	Yes	0.005	Yes	0.02	--	--	Yes	0.003	--	--	Yes	0.03	--	--	Yes	0.92	Yes	0.008

Comparison of Type effect between bioavailable and dissolved metal concentrations

Due to differences in the size of the dataset size used for analysing the dissolved and bioavailable metal concentrations, the dissolved metal concentration data was re-analysed only for those samples where the bioavailable metal concentration could be calculated. The effect of Type on bioavailable metal concentrations was compared to that for dissolved metal concentrations in the same set of samples (Figure 3.8). In this analysis, the Type factor was significant for all bioavailable metals, except for Cu. Among the metals, Zn showed the largest difference between the Control and STW samples.

When comparing the bioavailable metal concentrations to the dissolved metal concentrations, similar patterns were observed between the two sets of data, indicating that the Type effect (STW vs. Control) generally followed a similar pattern for both bioavailable and dissolved metals.

- For Mn and Ni, the bioavailable concentrations increased downstream of the STW discharge, consistent with the observed increases in their dissolved concentrations.
- For Zn, the bioavailable concentration decreased in the STW impacted waters, mirroring the reduction observed for dissolved Zn.
- For Cu, while the dissolved concentration increased downstream of the STW, there was no significant difference in bioavailable Cu concentrations between STW and Control.

These results suggest that, for most metals, the bioavailable concentration closely align with dissolved metal concentration patterns, except Cu, where the increase in dissolved levels was not accompanied by a corresponding increase in bioavailability.

Bioavailable concentration and its relationship with pH, Ca, DOC and Relevant dissolved metal

In the statistical analysis, correlation was performed to explore the relationships between bioavailable metal concentrations and variables such as Ca, DOC, dissolved metal concentrations, and pH. In Table 3.15, the bioavailability of each metal (Cu, Zn, Mn, and Ni) was measured on the same day, along with pH, Ca, and DOC. A negative correlation was observed between all bioavailable metals and DOC, except Mn. Zn, Mn and Ni exhibited a negative correlation with Ca, while all bioavailable metals showed positive relationship with pH.

When comparing the effects of Ca, pH, and DOC on bioavailable metal concentrations, it appears that pH and DOC have a more substantial impact on bioavailable metal concentrations than Ca. For instance, the pH coefficient for Mn is 9.42, meaning that an increase in pH by one-unit results in a increase in bioavailable Mn concentration by approximately 9.42 units. This positive relationship reflects the increased solubility of Zn at higher pH levels. Similarly, the DOC coefficient for Zn is -0.73, showing a similar inverse relationship, while the Ca coefficient for Zn is only 0.43, indicating a relatively weaker effect of Ca on Zn concentration.

Similarly, although the pH coefficient for Cu is 0.06 and the DOC coefficient for Cu is -0.01, the Ca coefficient for Cu is just 0.0002, which is considerably lower in comparison to the effects of DOC and pH.

Table 3.15. The summary of the General Linear Model for each determinand Bioavailable metal with Ca, DOC, pH and relevant dissolved metal. The significance for each determinand is stated as Yes if it was significant at a probability < 0.05 of being zero. η^2 indicates the extent to which a factor contributes to the observed variability in the variable. The coefficient quantifies how much the concentration of the bioavailable metal changes.

Bioavailable metal (unit)	Ca			DOC			pH			Dissolved concentration		
	Sig	η^2	Coefficient	Sig	η^2	Coefficient	Sig	η^2	Coefficient	Sig	η^2	Coefficient
Cu (µg/L)	Yes	0.12	0.0002	Yes	0.12	-0.01	Yes	0.23	0.06	Yes	0.38	0.03
Zn (µg/L)	Yes	0.43	-0.02	Yes	0.01	-0.73	Yes	0.04	0.64	Yes	0.75	0.27
Mn (µg/L)	Yes	0.001	-0.11	--	0.003	0.08	Yes	0.03	9.42	Yes	0.61	0.35
Ni (ug/L)	Yes	0.01	-0.002	Yes	0.001	-0.05	Yes	0.09	0.39	Yes	0.75	0.24

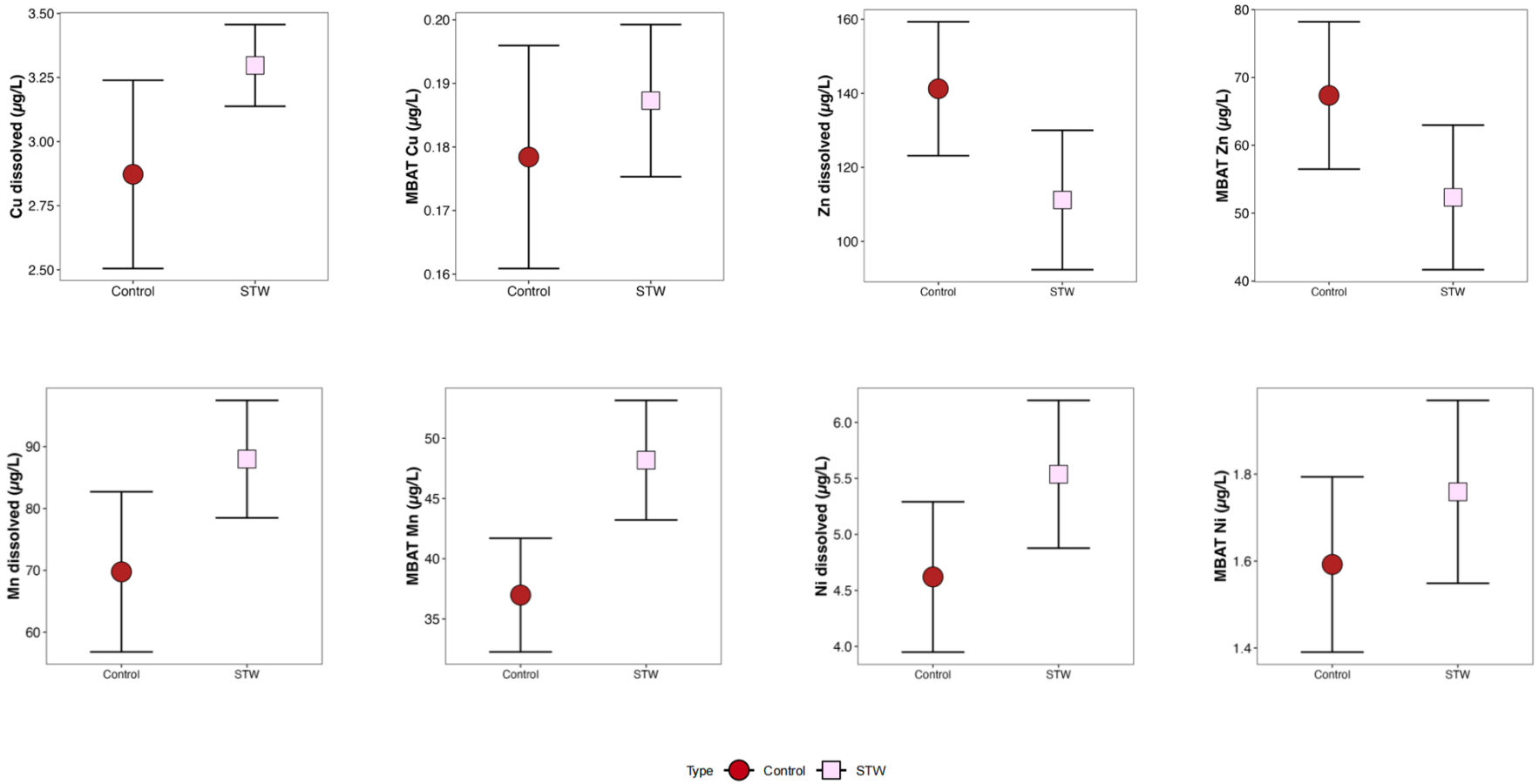


Figure 3.8 Type difference of dissolved metal and bioavailable concentration. The values are presented as the marginal mean with the 95% confidence limits on that mean. NB. The y axis for each metal is different.

RCR proportion

Table 3.16 displays the proportions of the Risk Characterisation Ratio (RCR) calculations that exceeded 1 for each bioavailable metal under site-specific conditions. An RCR greater than 1 indicates that the metal's bioavailable concentration surpasses the Environmental Quality Standard ($EQS_{bioavailable}$), suggesting potential environmental risks.

For Mn and Ni, the RCR never exceeded 1, indicating that these metals do not pose an environmental risk at the sites analysed based on bioavailable concentrations. Cu and Zn were observed to have RCR greater than 1 in this study, a proportion of 0.22 and 0.004, indicating a potential risk due to exceedance of the $EQS_{bioavailable}$ at some sites.

This summary allows for a comparative evaluation of the environmental risk across different metals, with Zn showing the highest likelihood of exceeding environmental safety thresholds in the locations studied.

Table 3.16. Summary of RCR. N represents the total number of sampling points recorded at different times.

Bioavailable metal(unit)	N of RCR	N of RCR >1	Proportion of RCR >1	Median RCR
Cu (µg/L)	3042	11	0.004	0.13
Zn (µg/L)	2530	559	0.22	0.37
Mn (µg/L)	1076	0	0	0.12
Ni (µg/L)	2654	0	0	0.18

Inter-determinand correlation for bioavailable metal concentrations

Before conducting the PCA analysis, correlations between bioavailable metal concentrations on the same day were analyzed (Table 3.17). Cu showed a significant negative correlation with both Zn and Mn, with correlation coefficients of -0.23 and -0.11, respectively. Conversely, Cu exhibited a significant positive correlation with Ni, with a value of 0.37. Zn had a significant positive correlation with Mn and Ni, with values of 0.10 and 0.09, respectively. Additionally, Mn displayed a significant positive correlation with Ni (0.32). Overall, the bioavailable metals were significantly interrelated, with particularly strong relationships observed between Cu and Ni, as well as Mn and Ni.

Table 3.17. Inter-determinand correlation coefficients (range: -1 to 1) and their corresponding p-values.Coefficients with p-values < 0.05, indicating statistical significance, are highlighted in grey.

Determinand (unit)	Cu (µg/L)	Zn (µg/L)	Mn (µg/L)	Ni (µg/L)
Cu (µg/L)		-0.23	-0.11	0.37
Zn (µg/L)			0.10	0.09
Mn (µg/L)				0.32

Principal components analysis of the bioavailable metal concentrations

A total of 497 data points were used in the PCA analysis for bioavailable metal concentrations, consisting of 249 STW samples and 248 Control samples. Among the four principal components, two had eigenvalues greater than 1 (Table 3.18), explaining 68%, 70.5%, and 68% of the variance for the entire dataset, STW sites, and Control sites, respectively. The contribution of each component reveals that Cu and Mn concentrations primarily influence the first component, while the second component is strongly associated with Mn.

As shown in Figure 3.9 the Control samples exhibit a tighter clustering, reflecting a more consistent and lower bioavailability of metals under natural conditions, with less variability in metal behaviour in the absence of significant STW discharge. Despite this, the distribution of STW and Control samples overlaps considerably, which can be better seen in the separate PCA scatter plot of STW (Figure 3.10) and Control (Figure 3.11), with Control samples occasionally extending beyond the boundary defined by the STW data points.

The loadings of the bioavailable metals on PC1 and PC2 (Figure 3.9) indicate that as one moves to the right along PC1, certain metals, such as Ni, are more associated with higher bioavailability in STW samples. In contrast, metals like Cu appear to contribute more to explaining bioavailability in the Control group, as reflected in their positioning along PC2.

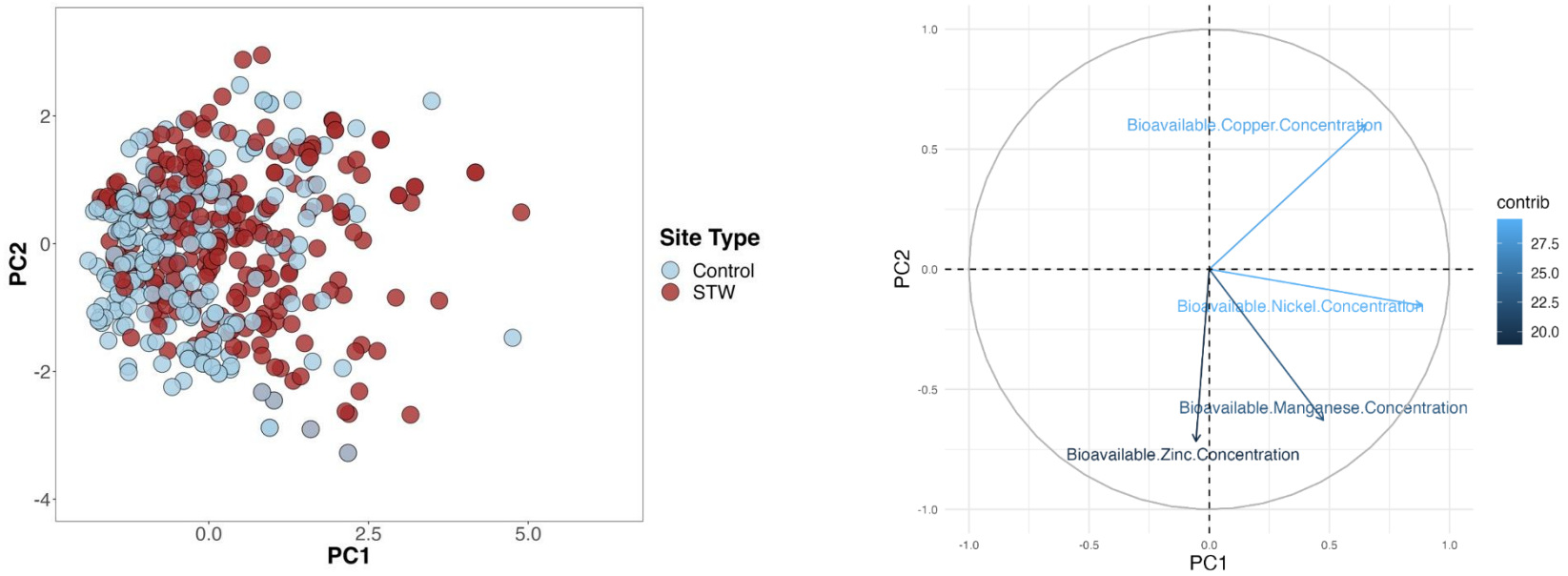


Figure 3.9. The scatter plot of the scores and loadings on PC1 and PC2 for all sampling sites.

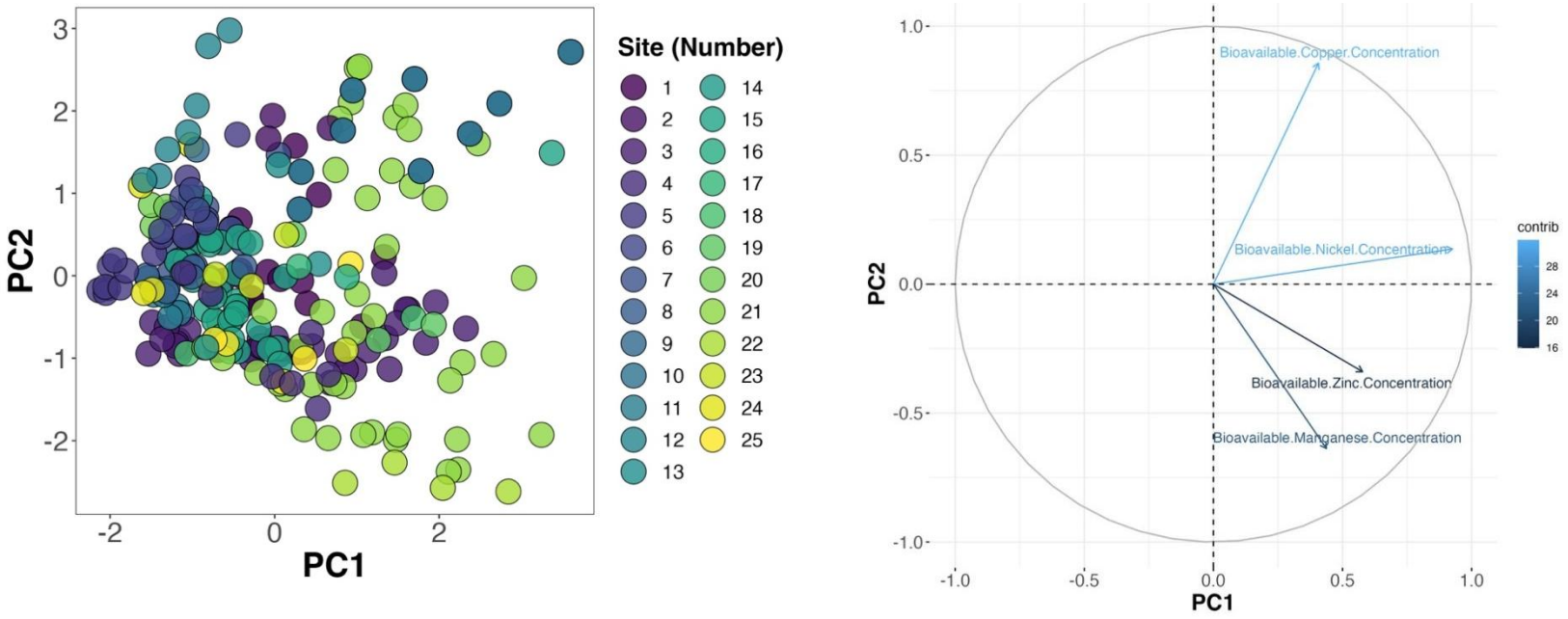


Figure 3.10. The scatter plot of the scores on PC1 and PC2 for STW sampling sites. The list of sites with their corresponding numbers is as follow: 1 — Bentley Brook at A636 Road Bridge; 2 — Hole Brook, PTC River Darwen; 3 — River Derwent at Carricks Picnic Site (Ruffside); 4 — River Derwent upstream of Blanchland STW; 5 — River Alt above Altmouth Pumping Station; 6 — Pearl Brook below Horwich ETW; 7 — Rookhope Burn at Eastgate; 8 — Rookhope Burn upstream of Rookhope STW; 9 — North Killingholme Main Drain downstream of Killingholme SW; 10 — River Glenderamackin upstream of Gategill Beck; 11 — Threlkeld EA Flow Station; 12 — River Dove above confluence with River Dearne; 13 — River Erewash downstream of Kirkby in Ashfield STW; 14 — River Erewash at Pyebridge; 15 — Seymoor Drain at Cottam; 16 — River Amber at Dalebank; 17 — River Amber at Hockley Quarry Footbridge; 18 — River Wye at Ashwood Quarry; 19 — Strine Brook at

Longford Footbridge; 20 — Bobs Brook downstream of Lower Gornal STW; 21 — River Gipping upstream of Horseshoe Weir; 22 — Old Park Watercourse at Whistley Bridge; 23 — North Stream at Eastry; 24 — River Dever at Bransbury; 25 — Hog Dyke at Raunds Farm Bridge upstream of A45 Road Bridge.

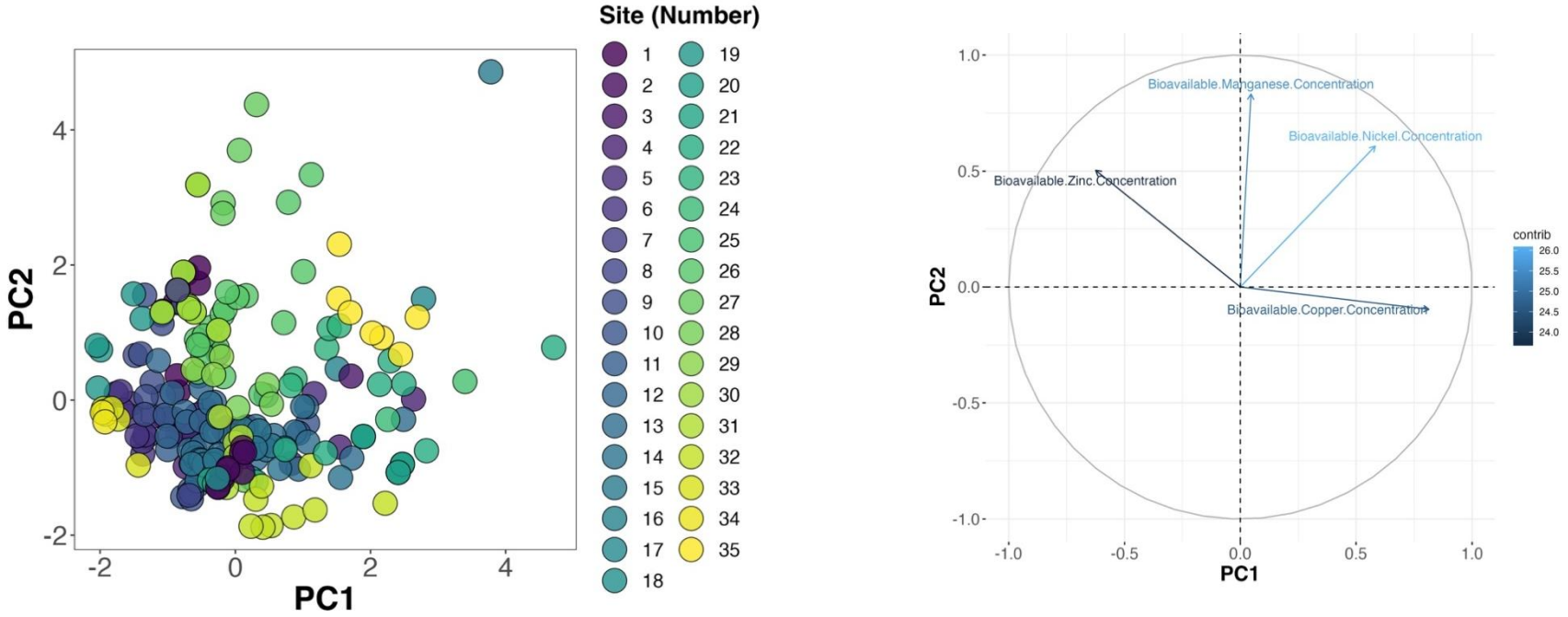


Figure 3.11. The scatter plot of the scores on PC1 and PC2 for Control sampling sites. The list of sites with their corresponding numbers is as follow: 1 — Barney Beck upstream of Old Rake; 2 — Beverley Brook at Motspur Park, downstream of Road; 3 — Bowling Beck at Lingwell Gate Lane; 4 — Daddry Shield Burn upstream of Quarry Level; 5 — Daddry Shield Burn downstream of Middle Level; 6 — Daddry Shield Burn upstream of Middle Level Portals; 7 — Downstream of Large Spoil Heap; 8 — Fosse Gill at Turner Bridge; 9 — Great Eggleston Beck at Middle End Bridge; 10 — Gunnerside Gill upstream of Bunton Lead Level; 11 — Honeycrook Burn at Ford, Tony's Patch; 12 — Honeycrook Burn at Whinnetley Farm Road Bridge; 13 — Hudeshope Beck downstream of Marl Beck; 14 — Hudeshope Beck at Road Bridge upstream of Marl Beck; 15 — Lee Moor Beck at A61 Bridge; 16 — River Leven at Bense Bridge; 17 — River Leven at East Angrove; 18 — Middle Tongue Dike at

Paradise Farm; 19 — River Nent at Caplecleugh; 20 — River Salwarpe at Porters Mill; 21 — River Caldew at Mosedale Bridge; 22 — River Cary downstream of North Barrow Brook; 23 — River Esk at Ruswarp; 24 — River Lemon downstream of River Sig confluence; 25 — River Sig upstream of River Lemon confluence; 26 — Rookhope Burn just upstream of Ripsey Breakout; 27 — Rookhope Burn upstream of Rookhope STW; 28 — Rushyford Beck 200 m downstream of tributary Windles STW; 29 — Settlingstones Burn upstream of Newburn Burn at Footbridge; 30 — Shildon Burn at Road Bridge to Pennypie; 31 — Stony Burn downstream of Stonyburn Bridge; 32 — Tathal Brook at Lodge Farm Footbridge; 33 — River Allen at Hindley Wrae Ford; 34 — River West Allen at Blueback Bridge; 35 — West Beck at Stone Bridge.

Table 3.18. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1			PC2		
	Total	STW	Control	Total	STW	Control
Cu (µg/L)	0.69	-0.34	0.69	0.07	0.75	0.07
Zn (µg/L)	-0.54	-0.46	-0.54	-0.43	-0.30	-0.43
Mn (µg/L)	0.02	-0.35	0.02	-0.72	-0.57	-0.72
Ni (µg/L)	0.48	-0.75	0.48	-0.54	0.11	-0.54
Eigenvalue	1.40	1.55	1.40	1.32	1.28	1.32
Percentage of variance explained	35%	39%	35%	33%	32%	33%

3.4 Discussion

Sewage treatment works have been extensively studied for their role in influencing downstream water quality, particularly in relation to compliance with Environmental Quality Standards (EQS) for trace metals. Comber et al. analysed data from over 600 sites across the UK using the BioMet bioavailability model. Their findings indicated that, after accounting for bioavailability, compliance rates for metals such as Cu and Zn exceeded 95%. The local impacts of STW discharges on downstream metal concentrations were quantitatively minor; for instance, median increases in Cu and Zn concentrations were 19% and 33%, respectively, both remaining below EQS thresholds ($p < 0.05$). These findings suggest that, while localised impacts were limited in magnitude, catchment-scale contamination, with widespread EQS exceedances in upstream areas, remains a critical issue that requires broader management interventions. However, while Comber et al. (2022) primarily focused on trace metals and applied a bioavailability-driven compliance framework, this study expands the scope to include a wider range of determinands—Cu, Ni, Ca, Mg, Zn, Cd, Fe, and Mn. Each determinand was analysed for yearly and monthly trends relative to control sites, along with covariates such as river length, altitude, and percentile flow. The nature of STWs was also examined for its influence on metal concentrations, highlighting its potential to mitigate or exacerbate downstream impacts.

Recent large-scale analyses, such as Comber et al.(2022), examining over 600 STWs sites across England, found minimal local impacts for many contaminants but confirmed STW-driven elevations in metals like Ni and Mn, reinforcing the need for site-specific assessments beyond catchment-wide trends. This chapter's inclusion of a 21-year dataset (2000–2020) and multiple rivers addresses limitations in earlier studies, such as Matthiessen et al. (1999) and Comber et al. (2008), which focused on shorter monitoring periods for Cu and Zn compliance, overlooking key metals like Mn and Ni. The observed temporal trends—e.g., decreasing annual differences for Cu and increasing for Ni—provide novel insights into long-term dynamics. These trends align with the WFD's emphasis on sustained monitoring of metal concentrations to assess ecological status, highlighting the need for targeted WFD interventions to mitigate ecological risks.

In line with these studies, Buss and Lester (1995) investigated STW discharges on the River Ivel and reported increased Cd, Cu, Fe, and Mg concentrations downstream. Building on this, Bubb and Lester (1995) highlighted the role of DOC from effluents in influencing metal bioavailability through significant metal complexation in receiving waters. These findings underscore the importance of considering both concentration changes and bioavailability in assessing STW impacts, providing a foundation for the current study.

These findings on the role of DOC in influencing metal bioavailability observations are supported by Peters et al. (2014), who demonstrated that DOC forms complexes with metal ions, reducing immediate bioavailability while facilitating downstream transport, a mechanism likely relevant to the current findings. Meanwhile, the relationship between dissolved and bioavailable Cu concentrations, where increased dissolved Cu levels do not consistently increase bioavailability, may be influenced by competition from Ca and Mg for biotic ligand sites, as supported by Väänänen et al. (2018) using the UKTAG M-BAT to evaluate metal uptake. For Mn and Ni, the rise in both forms suggests heightened ecological risk, particularly in soft-water regions, while Zn's lower bioavailability despite higher dissolved levels aligns with Hargreaves et al. (2018)'s findings on ligand binding in sediments. This framework integrates bioavailability corrections to improve the accuracy of EQS and highlights the need for site-specific risk assessments.

While bioavailability provides a more ecologically relevant measure of metal toxicity, its relationship with dissolved metal concentrations is not always linear. In this study, Cu showed a significant increase in dissolved concentrations downstream of STW discharges, yet its bioavailable concentration remained largely unchanged. This suggests that complexation with DOC or other competing ions, such as Ca^{2+} and Mg^{2+}, may have reduced its bioavailability. In addition, treatment processes that do not effectively remove DOC may enhance metal–ligand complexation, reducing apparent bioavailability despite elevated dissolved loads (Wood *et al.*, 2011). Conversely, for Mn and Ni, both dissolved and bioavailable concentrations increased, indicating a higher risk of ecological impact. Such

patterns may reflect limited removal efficiencies for Mn and Ni in conventional biological processes, while diffuse sources such as urban runoff and catchment geology can further elevate background concentrations in control sites.

Zn presented a contrasting trend, where bioavailable concentrations were significantly lower in STW-impacted waters compared to control sites, despite higher dissolved concentrations. This suggests that Zn may have been strongly bound to organic ligands or particulates, reducing its bioavailability. Control sites with higher suspended solids from diffuse erosion may also promote Zn adsorption onto particulates (Marttila *et al.*, 2013), indicating that both effluent chemistry and catchment processes influence its availability. The results indicate that SB is more effective for Fe and Cu removal, whereas SAS shows better performance for Mg. These findings highlight the importance of considering both dissolved and bioavailable fractions when assessing the environmental impact of STW discharges. Regulatory frameworks based solely on dissolved metal concentrations may overestimate or underestimate actual ecological risks depending on site-specific conditions. This pattern is consistent with Hargreaves et al. (2018)'s sediment studies, which found Zn binding to organic matter reduces its ecological availability. The discrepancy between dissolved and bioavailable Zn underscores the need for bioavailability-based EQS, as advocated by the Environment Agency's tiered framework, to accurately reflect site-specific risks.

Further advancing the understanding of bioavailability, Vaananen et al. (Vaananen *et al.*, 2018) emphasized the importance of bioavailability in ecological risk assessment and highlighted the use of tools like the UKTAG M-BAT in England to adapt EQS for metals, such as Cu, Mn, and Zn, based on local water chemistry. This approach is particularly relevant this study, as M-BAT's hardness and DOC adjustments could explain the observed Cu and Zn bioavailability patterns, enhancing the precision of EQS compliance assessments across diverse UK river conditions.

The study also draws on the work of Crane et al. (2010), who evaluated Cu and Zn removal efficiencies across three biological wastewater treatment processes: Activated Sludge (AS), Trickling Filters (TF), and Membrane Bioreactors (MBR). Their findings demonstrated that AS achieved the highest Cu removal (99%),

followed by TF (84%) and MBR (47%). For Zn, TF outperformed AS and MBR, with removal efficiencies of 84%, 68%, and 50%, respectively. MBR's poor performance was attributed to long sludge retention times (SRT > 40 days), which produced organic molecules that chelated metals, preventing adsorption. These findings provide a baseline for this study's comparison of SAS and SB processes, where SB's superior Fe and Cu removal may reflect enhanced biological reduction under anaerobic or low-oxygen conditions. The variability in removal efficiencies aligns with Oliveira et al. (2007)'s observations on biological treatment performance, suggesting that process optimization could further reduce downstream metal loads.

In comparison, this study evaluates the performance of SAS and SB treatment processes for a broader range of metals, including Cu, Ni, Zn, Cd, Fe, Mg, and Mn. The results indicate that SB is more effective for Fe and Cu removal, whereas SAS shows better performance for Mg. These contrasts reflect underlying process mechanisms: SB may enhance reductive removal of Fe (Lies *et al.*, 2005) and Cu (Huang *et al.*, 2015) under low-oxygen biofilm conditions , whereas SAS maintains higher Mg removal through greater aeration and cation exchange (Uludag-Demirer and Othman, 2009; Jiang *et al.*, 2013). Furthermore, this study incorporates bioavailability assessments, highlighting the relationships between metals and environmental factors such as pH, DOC, and Ca. By analysing temporal (monthly and yearly trends) and spatial variations (upstream vs. downstream), and calculating RCR values for environmental risk assessment, this study provides a more comprehensive evaluation of wastewater treatment impacts, extending the scope of the work carried out by Crane et al. (2010).

The inclusion of bioavailability assessments builds on Crane et al. (2010)'s focus on removal efficiency, revealing that SB's effectiveness may also mitigate bioavailable metal risks, while SAS's Mg focus could stabilise hardness-related effects. Temporal and spatial analyses further align with Worrall et al. (2019)'s findings on DOC-driven metal dynamics, suggesting that STW design influences both concentration and ecological impact over time.

The 21-year dataset enhances trend detection compared to shorter studies like Matthiessen et al. (1999), while the 16-site limitation, driven by data availability, is

mitigated by Chapter 5's broader scope, potentially validating localised findings across England. This chapter considered more determinands over a longer time period (2000 to 2020) and across more rivers than any previous study. Further, there was always a control, and the STW design allowed for factors such as year and month to be accounted for. Although this study examined water quality data from across England, the constraints of the rigorous experimental design and the requirement that all determinands be measured coincidentally meant that only 39 STW sites could be included (figure 3.10). While this chapter was limited to studying impacts where data allowed, Chapter 5 will adopt a different approach, considering all English river data and comparing them to the final effluent from STW discharges to assess whether broader impacts can be discerned. Overall, the findings emphasise that STW design and operational processes are key determinants of effluent metal profiles, but control sites highlight the parallel importance of diffuse inputs from agriculture, geology, and urban runoff, necessitating integrated management approaches.

3.5 Conclusion

i) All determinands, except Cd and Ni, showed significant changes in the receiving river due to STW discharges. For Ca, Mg, Cu, and Mn, concentrations increased in the receiving river, while Fe and Zn concentrations decreased.

ii) The annual trend for the difference made by STW discharges showed a decrease for Mg and Cu, but an increase for Ni.

iii) Different secondary treatments impact the removal of Fe and Cu; SB is more effective than SAS at removing metals.

iv) Larger STWs have a greater impact on receiving waters. PE affects changes in Ca, Ni, and Zn concentrations, while DWF influences Fe, Mg, and Cu.

v) Principal Component Analysis (PCA) identified two types of STWs based on their impacts: one type affecting Zn, Cd, and Ni, and the other type impacting Ca, Mg and Cu.

vi) STW discharges significantly impacted bioavailable Zn, Mn, and Ni, while bioavailable Cu remained unchanged despite increased dissolved concentrations.

vii) Zn showed the highest proportion of samples that had RCR > 1 and so exceeded their environmental quality standard.

Chapter 4: Non-metal Determinands -- National scale impact of STW discharge on English rivers

4.1 Introduction

The purpose of Chapter 4 is to study the broader impact of sewage treatment works (STW) discharges on nutrient pollution, specifically focusing on their potential leading to eutrophication (Preisner *et al.*, 2021). The process of eutrophication is defined by an excessive accumulation of nutrients, such as phosphate and nitrate, in aquatic ecosystems, which can lead to the overgrowth of algae and aquatic plants (Bhat and Qayoom, 2021). The over-enrichment of water can lead to detrimental algal blooms, lower oxygen levels, and deteriorate water quality, all of which will pose serious threats to aquatic biodiversity, ecosystem health, and the water-based economy (Withers *et al.*, 2014). Chlorophyll concentration is regarded as the test of algal biomass in aquatic systems (Spaulding *et al.*, 2024). Elevated concentrations of chlorophyll typically indicate nutrient enrichment (Howell and Benoit, 2021), often linked to sewage effluent discharges (McLaughlin *et al.*, 2021). Monitoring chlorophyll concentrations provides critical insight into the extent to which nutrients from STWs stimulate algal growth (Bennett *et al.*, 2021). Diatoms are one of the main aquatic plants and their growth requires silica to construct their frustules – a silica-based cell wall (Kröger *et al.*, 1999). So, along nutrient, silica concentrations should be considered when assessing eutrophication potential (Lim and Lee, 2017).

As this study showed in Chapter 2, the discharge from STWs normally caused an increase in downstream stream temperature, Nitrate, and Phosphate concentrations, with the increases being 0.01%, 79%, and 13.3%, respectively (Table 2.3). Although there has been considerable research on nutrient pollution and eutrophication, the precise role of sewage treatment works (STW) discharges in contributing to increased nutrient levels (Albini *et al.*, 2023) and subsequent increases in chlorophyll concentration remains unclear. This chapter addresses that knowledge gap by providing a data-driven analysis to clarify the impact of STW effluents on nutrient pollution and consequent changes to aquatic ecosystems. Specifically, it investigates how elevated concentrations of nitrogen and

phosphorus, resulting from STW discharges, influence key ecological parameters such as chlorophyll-a levels, primary productivity, and the overall health of aquatic communities. By linking nutrient concentrations to observable ecological changes, this study aims to refine our understanding of the role of STWs in driving eutrophication processes and their broader implications for water quality management and conservation strategies.

Beyond chlorophyll concentration, this chapter examines the broader effects of STW effluent by integrating discharge data with river water quality measurements, aiming to uncover potential correlations between effluent discharges and changes in key water quality indicators (Wallace *et al.*, 2016). Combining these datasets is crucial for understanding the full scope of STW impacts on water bodies, as their interactions with natural processes can be complex. This analysis builds on Chapter 2's findings, focusing on several water quality parameters—stream temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, phosphate, pH, suspended solids, and specific conductance—which are key indicators of water quality and provide a foundation for evaluating nutrient-related effects on river systems.

The chapter investigates whether STW discharges contribute to eutrophication by examining questions related to three key questions:

1. Chlorophyll Concentration: Does the discharge from STWs lead to an increase in chlorophyll concentrations, indicating higher algal growth?
2. Threshold Exceedance: Do these increases in chlorophyll concentration surpass specific ecological thresholds that signal a risk of harmful eutrophication?
3. Does the effluent from STW impact the river from a wider version? If so, what river is polluted the worse by what determinand?

4.2 Approach and Methodology

This chapter builds upon the previously discussed methods in Chapter 2. The study focuses on evaluating the impact of nutrient discharges from sewage treatment works (STWs) on water eutrophication. So, the primary objective is to determine any significant impacts of these discharges on river water quality. This evaluation

is conducted across multiple years and months, considering different control sections of rivers associated with a variety of STWs. If significant impacts are identified, the effect of each STW is further analysed in relation to its characteristics, such as treatment approaches, technologies, and the scale of operations, to assess their influence on the receiving waters. This analysis follows exactly that for Chapter 2 but is now focused on two new determinands – Chlorophyll (Chl-a) and SiO_2, key for understanding the impact on eutrophication. These determinands are essential for understanding how nutrient pollution contributes to algal blooms and changes in aquatic ecosystems. The analysis also explores the relationship between these determinands and other environmental variables, such as nitrate, phosphate, and temperature, which are crucial for understanding eutrophication dynamics and which been analysed in Chapter 2.

Unlike for Chapter 2, impact of STW discharges on eutrophication should also consider events and not just concentration. Chlorophyll incidents were judged to have occurred when chlorophyll levels exceeded 15 µg/L (Bowes *et al.*, 2019), a common threshold used to assess algal biomass. In this Chapter, Chl-a events were analysed as binary outcomes where an event "incident" (Chl-a > 15 µg/L) and "non-incident" cases (Chl-a < 15 µg/L), enabling a comparison of the frequency of eutrophication events between STW-impacted and control sites, as well as between upstream and downstream of each type.

To further understand the wider impact of STW discharges, river and final sewage effluent data were analysed by PCA to identify patterns among non-metal determinands and understand how STW discharges impact the broader river system.

4.2.1 Study data

In this chapter, the study utilizes 21 years (2000 to 2020) of river water quality data from English rivers, collected by the Environment Agency (EA)—the UK government's environmental protection agency in England. The water quality data includes measurements for chlorophyll-a and silicon dioxide (SiO_2), which are key additional indicators for assessing eutrophication. This data was collected from river monitoring points located above and below sewage treatment works (STWs) discharges, as well as from control rivers. Additionally, data measured in the final

effluent discharge were examined for comparative purposes, although they are not included in the statistical analysis for this chapter.

For the Principal Component Analysis (PCA), the same determinands as in Chapter 2 were considered. However, unlike Chapter 2, which analysed specific pairs of determinands, this chapter jointly utilizes the full range of available SiO_2 and Chl-a data from 2000 to 2021 to ensure a comprehensive analysis.

The data regulation and selection criteria applied in this chapter are consistent with those in Chapters 2 and 3. Only routine water quality monitoring data are included, while data from unplanned reactive monitoring—conducted to investigate statutory failures—are excluded to prevent bias. Additionally, monitoring sites are only included if they have at least 20 measurements over a span of 20 years or more. In analysing the impact of STW discharges on SiO_2 and Chl-a concentrations and events, this chapter utilises the same data source as in Chapters 2 and 3. The study utilizes 21 years (2000 to 2020) of river water quality data from English rivers, collected by the Environment Agency (EA)—the UK government's environmental protection agency in England. The water quality data includes measurements for chlorophyll-a and silicon dioxide (SiO_2), which are key additional indicators for assessing eutrophication. This data was collected from river monitoring points located above and below sewage treatment works (STWs) discharges, as well as from control rivers. Additionally, data measured in the final effluent discharge were examined for comparative purposes, although they are not included in the ANOVA within this chapter.

For the Principal Component Analysis (PCA), the same determinands as in Chapter 2 were considered. However, unlike Chapter 2, the PCA was performed on all river water data and effluent data.

STW and Control Pairs

The selection of STW and Control pairs for the analysis of eutrophication determinands in this chapter follows the same methodology as outlined in Chapters 2 and 3. Specifically, pairs were chosen where one monitoring site is upstream and the other is downstream of a sewage treatment works (STW) discharge, ensuring that no other discharges or streams join the river between these sites. These pairs of monitoring sites are referred to as STW pairs. Similarly, Control pairs were selected

where the monitoring sites are also upstream and downstream of each other but without any known discharge or stream joining the river between them, and without sharing a common monitoring site with any STW pair.

In keeping with the established methodology, observations made on the same day are compared to maintain consistency in the analysis. Any pairs lacking concurrent data on the same day are excluded from further analysis. This chapter focuses on analysing eutrophication determinands while preserving the comparative analysis framework set out in previous chapters.

The initial identification of 442 STW pairs and 419 Control pairs from the stream temperature records, as described in Chapter 2, remains consistent and was used in this chapter for the study of eutrophication (Table 4.1; Figure 4.1). As before, any available final effluent data for the STWs within each STW pair are extracted from the EA WIMS database, although this data is used for comparative purposes only and not included in the statistical analysis.

Table 4.1. The number of pairs, both STW and Control, that could be included in this study.

Determinand	STW Pairs	Control Pairs
Chl-a (μg/L)	22	9
SiO_2 (mg/L)	26	19

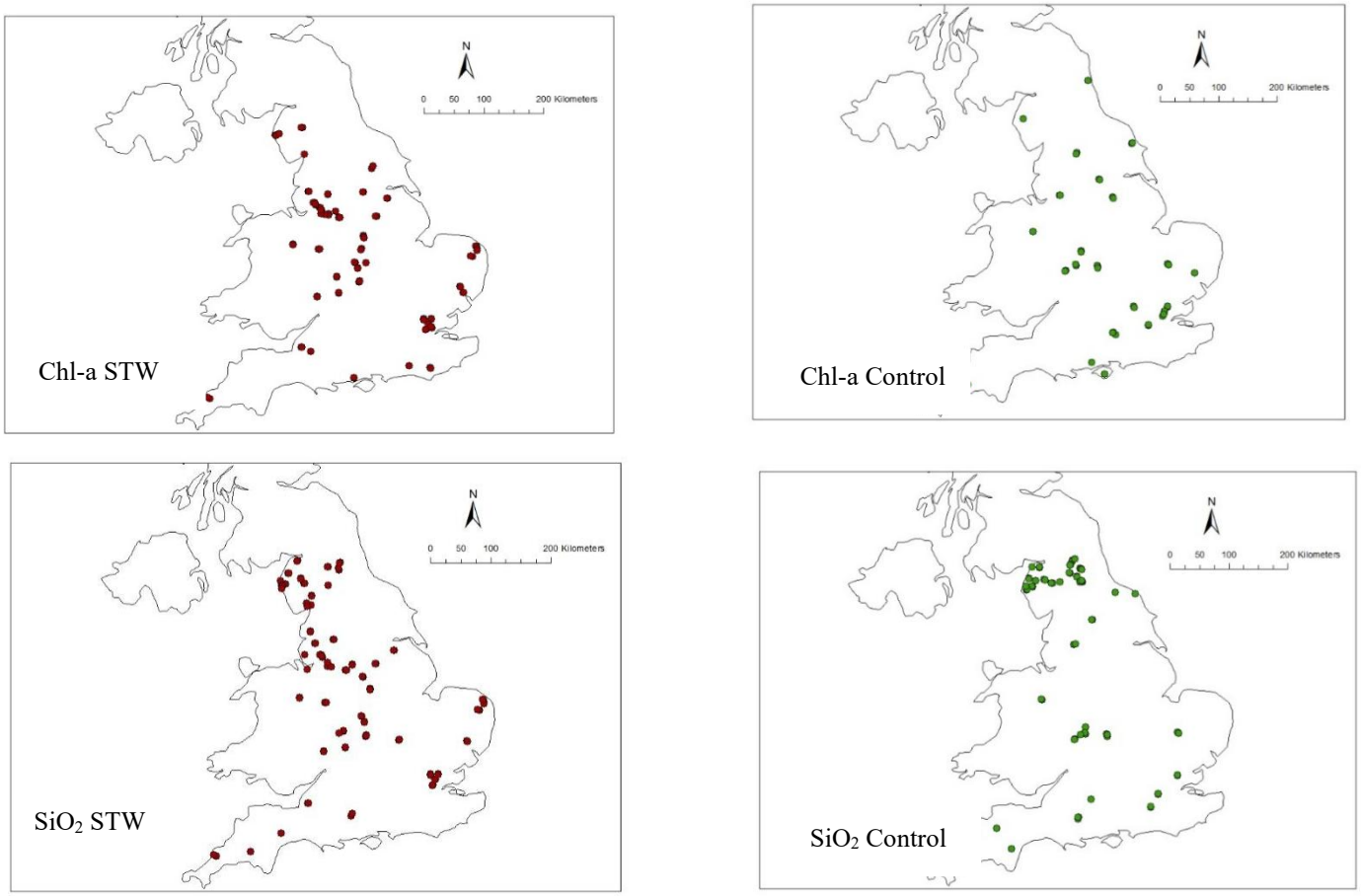


Figure 4.1. Location of the STW and Control pairs for each determinand considered in this chapter.

Covariates

In this eutrophication study, covariates such as the Euclidean distance between site pairs, upstream and downstream altitudes, and percentile flow will continue to be utilized to ensure a more robust analysis. By accounting for these factors, the study aims to isolate the effects of sewage discharge more effectively, thus enhancing the reliability and precision of the findings.

Binomial analysis

Chlorophyll (Chl-a) events were analysed using a binomial approach, where Chl-a concentration data were categorized into two distinct groups:

- Incident: Cases where Chl-a concentrations exceeded the eutrophication threshold of 15 µg/L, indicating a potential algal bloom or high eutrophication risk.
- Non-Incident: Cases where Chl-a concentrations were at or below 15 µg/L, representing lower eutrophication risk.

The binomial analysis enabled the comparison of the frequency of these events in different conditions:

- STW-Impacted vs. Control Sites

To assess whether STW discharges were associated with a higher frequency of eutrophication events compared to control sites.

- Upstream vs. Downstream Comparisons

To evaluate how Chl-a concentrations changed from upstream to downstream in relation to STW discharges and whether STWs contributed to significant downstream eutrophication effects.

The statistical significance of differences in event frequencies between groups was determined using a Chi-square test. Additionally, estimated marginal means were calculated to compare Chl-a levels between STW and control groups, controlling for potential confounding variables. Differences were deemed significant if the p-value was less than 0.05 and if the confidence intervals of the marginal means did not overlap.

4.2.2 Statistical analysis

The primary approach was to assess the impact of STW discharge on receiving rivers using the same ANOVA as described in Chapter 2. The difference in concentration of SiO_2 and Chl-a for the paired data were tested to the factors: Type, Year, Month, and Pair. Before conducting the ANOVA, outliers were removed, and data distribution was examined using the Anderson-Darling test. The ANOVA was performed without and then with covariates. Significance thresholds were set at a 5% probability level, corresponding to a 95% confidence interval for non-zero effects. The magnitude of each statistically significant factor, or interaction, was quantified using η^2, which measures the contribution of each factor to the observed variability in the variable.

The second objective was to assess the reasons for significant differences in receiving waters caused by the processes of the individual STWs. As in Chapter 2, the main effects of each determinand were considered using Chi-square analysis to assess the frequency of significant effects across different types of sewage treatment works (STWs). In addition to the Chi-squared analysis, linear regression was used to evaluate the impact of Population Equivalent (PE) and Dry Weather Flow (DWF) on the impact of STWs, i.e. the calculated main effect for each STW pair was compared to PE and DWF. The significance of these comparisons was determined based on a 5% probability threshold for the regression coefficients being greater than zero.

Analysis of chlorophyll (Chl-a) events

For Chl-a concentrations in UK rivers, thresholds for algal blooms typically range from 15 µg/L to 100 µg/L (Bowes *et al.*, 2019). This analysis specifically focused on chlorophyll-a concentrations exceeding the threshold value of 15 µg/L, which indicates potential eutrophication events.

Firstly, Chi-square test was applied to compare the proportion of measurements above the 15 µg/L threshold compared to the STW and Control pairs both up- and downstream. Secondly, binomial regression was used to test the impact of the Type factor on the probability of an event over 15 µg/L.

Principal Component Analysis

A PCA was performed for all non-metal determinands as listed in Chapter 2 without pair control. This PCA was not restricted to just the data from the STW and Control pairs but was performed on all English river and final effluent data within the WIMS database. This data consisted of all samples where determinands were measured on the same sample for any river or stream in England and for any STW final effluent as long as they were sampled as part of routine monitoring. The PCA was conducted on z-transformed data, and principal components with an eigenvalue ≥1 were considered for examination. To assess the influence of sewage treatment works (STW) discharges on the receiving river, principal component analysis (PCA) was conducted separately on the river water data, the final sewage effluent data, and the combined dataset.

All statistical analyses were conducted using RStudio v4.3.1.

4.3 Results

As discussed in previous chapters, the average concentrations of Chlorophyll-a and SiO_2 at upstream, downstream, and final effluent points highlight important trends in water quality. The comparison between upstream and downstream concentrations at STW sites shows that downstream levels for both Chlorophyll-a and SiO_2 are higher than upstream, suggesting an increase potentially linked to effluent discharge from sewage treatment works (STW).

The main effect, however, represents the difference between the changes observed at STW sites and those at Control sites (i.e., STW - Control), rather than simply comparing upstream and downstream concentrations. The main effect (%) expresses this difference as a percentage of the upstream STW concentration. For Chl-a, the main effect percentages range from -6.15% to -1.38%, indicating that the increase in downstream Chl-a concentrations at STW sites is small relative to the upstream levels. In contrast, the main effect percentages for SiO_2 range from -11.08% to -4.34%, reflecting a more substantial proportional increase relative to the upstream SiO_2 concentrations at STW sites.

This distinction emphasizes that the observed differences in downstream concentrations are not only influenced by the STW effluent itself but also by how these changes differ compared to Control sites, helping to contextualize the impact of STW discharges on downstream water chemistry.

4.3.1 Impact of STW discharges

As in the previous study, a four-factor design was employed to analyse Chlorophyll and silica (SiO_2), allowing for the detection of even small significant differences. The main effect (%) is calculated as described in Chapter 2, representing the relative impact of the STW, by dividing the main effect by the mean STW upstream concentration.

In this chapter, SiO_2 shows a significant relationship with the Type factor (Table 4.2, Table 4.3), whereas Chl-a does not. This is reflected in Figure 4.2, where overlapping error bars suggest that the Type factor for Chl-a does not show a significant difference between STW and Control sites. Despite the lack of significance for Chl-a, the main effect ranges from -6.15% to -1.38%, and for SiO_2, from -11.08% to -4.34%. The Type factor for SiO_2 indicates a significant decrease in silica concentrations at STW sites relative to Control sites.

For the Year factor, SiO_2 accounts for 2% of the variance, with a significant Type*Year interaction explaining 4% of the variance (Table 4.3). Chl-a, on the other hand, does not show statistical significance for either the Year factor or the Type*Year interaction. Figure 4.3 illustrates the year-to-year changes for both Chl-a and SiO_2. For SiO_2, significant separation between STW and Control occurs in 2000 and 2001, but the differences in most years are minimal. Chl-a shows similar trends with less fluctuation. Note that Control sites showed relatively high SiO_2 differences prior to 2003 but exhibited a marked decline thereafter, whereas STW sites remained comparatively stable across the study period (Figure 4.3).

Regarding the Month factor, Chl-a shows a 0.4% variance, while SiO_2 shows no significant variance (Table 4.3). However, the Type*Month interaction is not significant for either of the two determinands. In Figure 4.4, although September and November for SiO_2 show significant differences, most months show overlapping error bars, indicating no distinct separation between STW and Control for both determinands.

For the Pair factor, SiO_2 explains 4% of the variance, with a significant Type*Pair interaction (which explains 5% of the variance0 (Table 4.3). In contrast, Chl-a shows no significant variance for the Pair factor. Figure 4.5(a) shows that the differences in Chl-a between STW and Control sites vary across pairs but remain

within overlapping confidence intervals. This indicates that the TypePair interaction for Chl-a is not significant, suggesting that the influence of STW discharges on chlorophyll concentrations is relatively consistent across sites and not strongly dependent on pair-specific conditions. For SiO_2, Figure 4.5(b) illustrates the variability in differences across individual STW and Control pairs, with some pairs showing pronounced positive or negative deviations. This heterogeneity in pair-specific responses underpins the significance of the Type*Pair interaction, indicating that the effect of STW discharges on SiO_2 concentrations is not uniform across sites but depends strongly on local conditions. Table 4.4 confirms this asymmetry, with 27 of 45 SiO_2 comparisons being positive compared with 15 negative and 3 non-significant. This heterogeneity in pair-specific responses underpins the significance of the TypePair interaction, indicating that the effect of STW discharges on SiO_2 concentrations is not uniform across sites but depends strongly on local conditions.

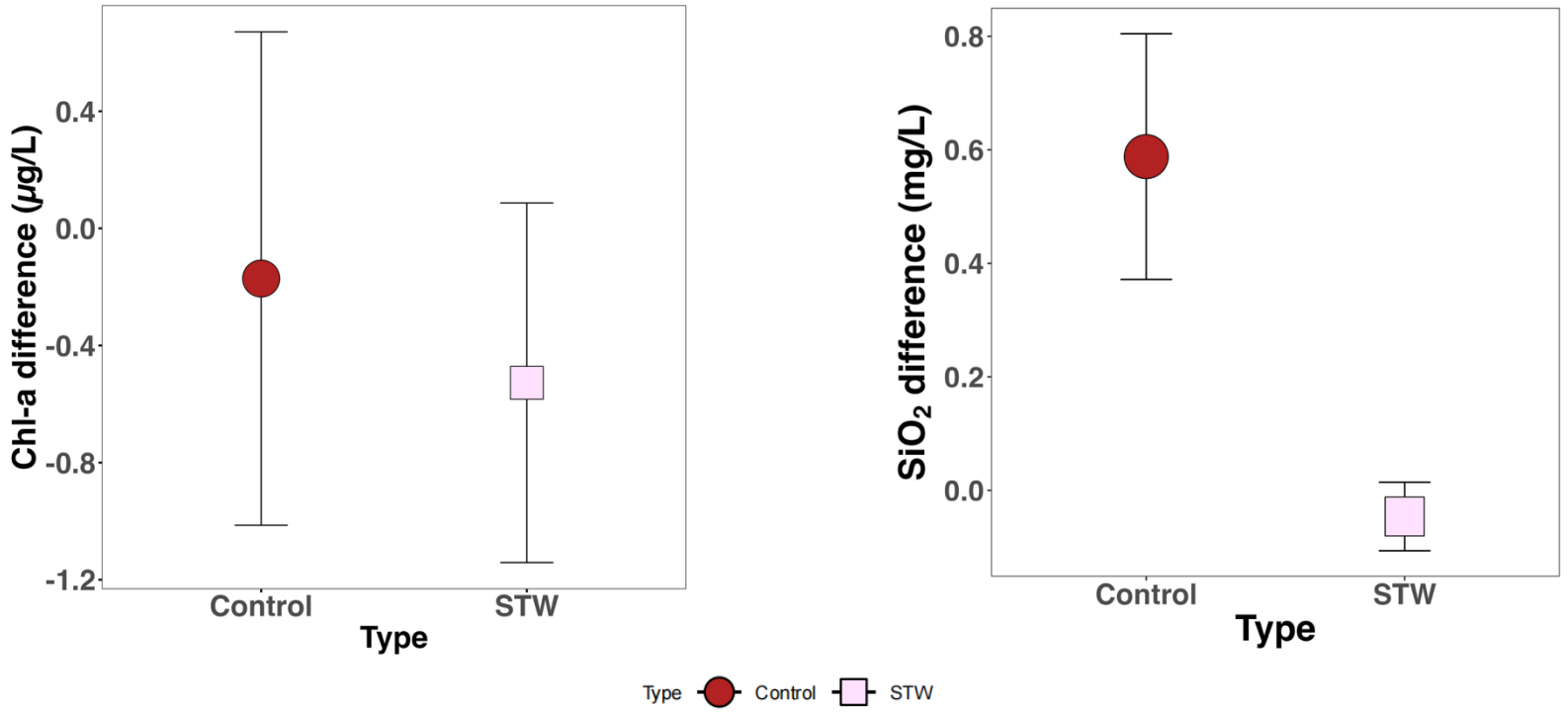


Figure 4.2. The main effects plot for the Type factor for the difference. The values are presented as the marginal mean with the 95% confidence limits.

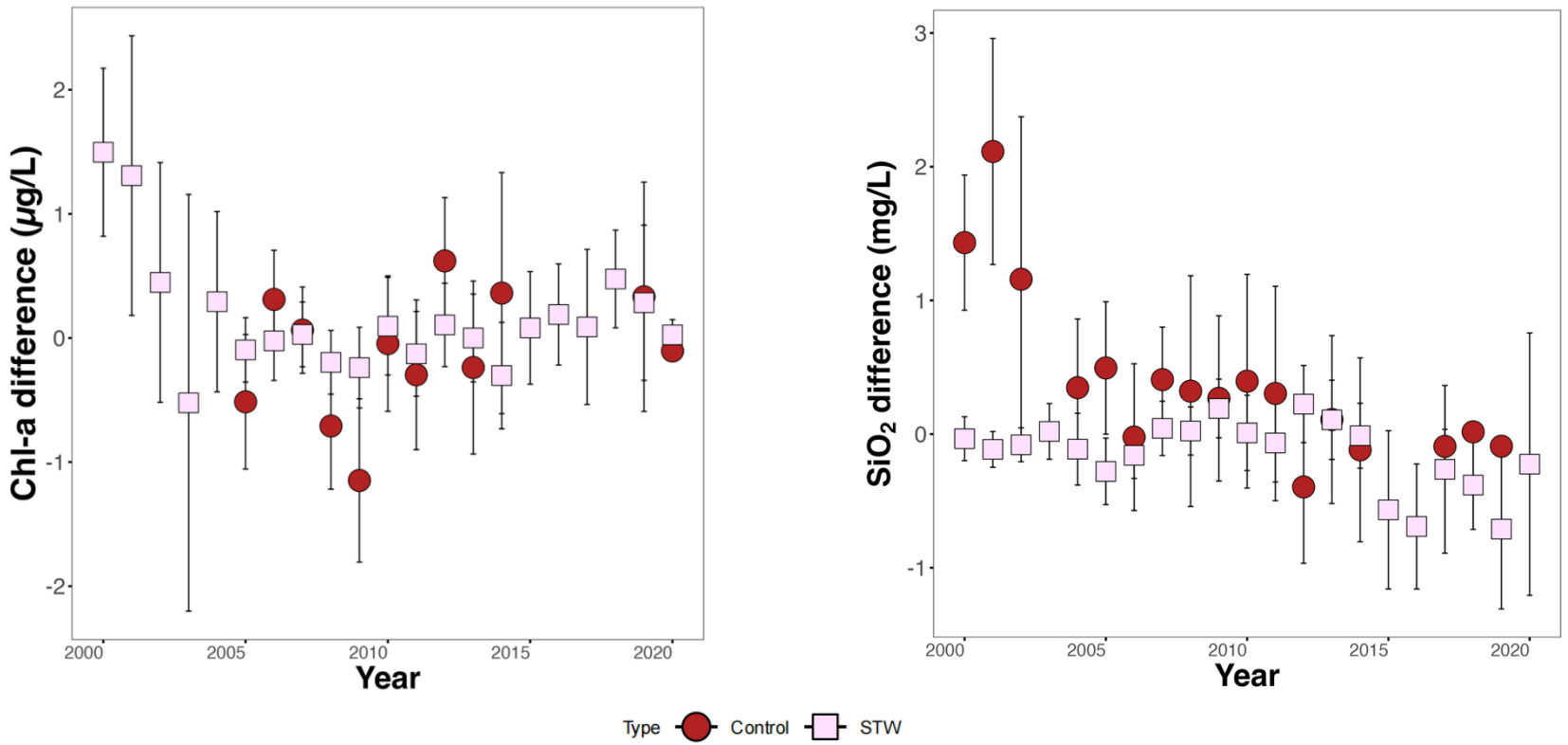


*Figure 4.3. The main effects plot of the Type*Year interaction. The values are presented as the marginal mean with the 95% confidence limits.*

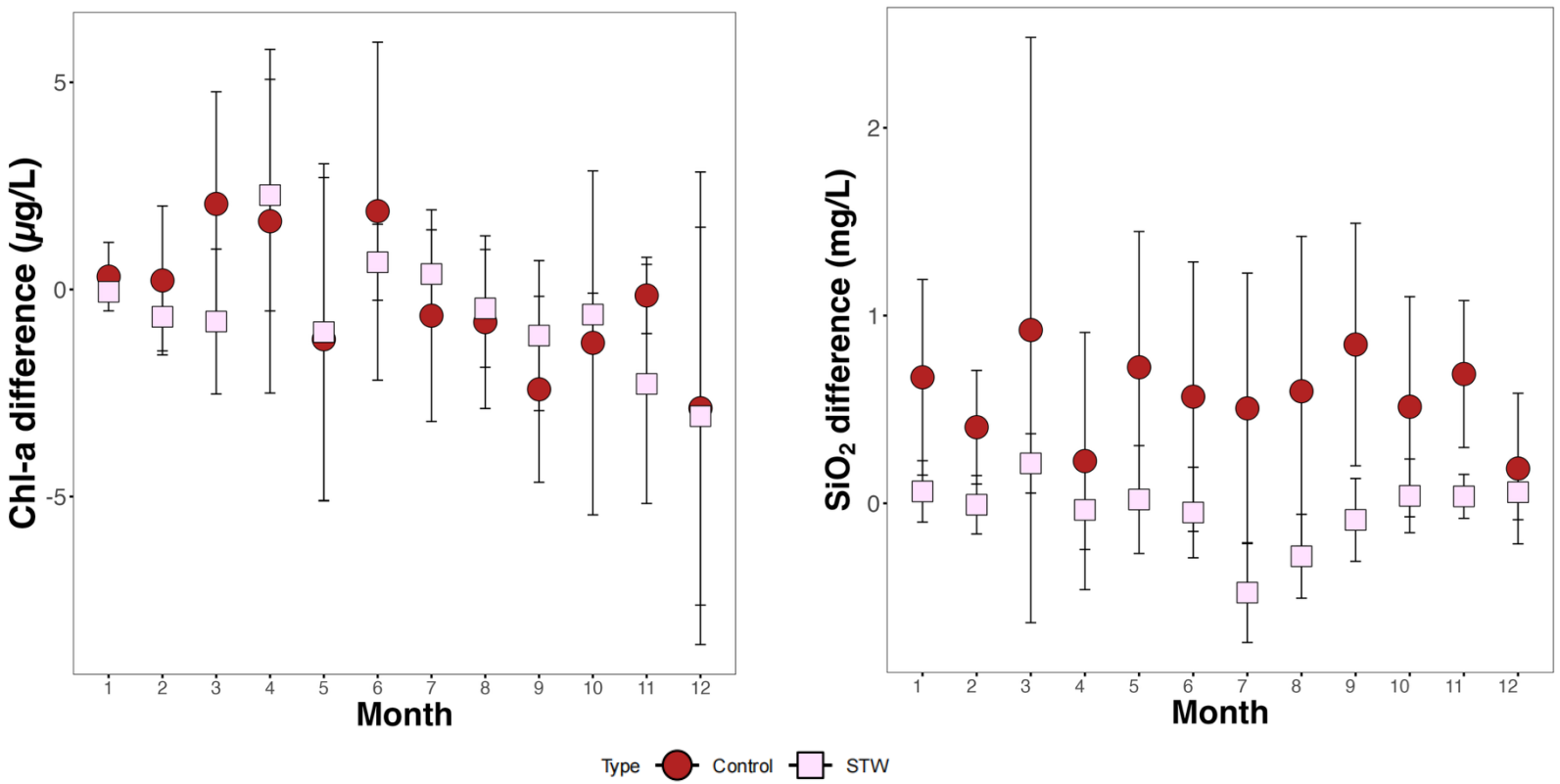


*Figure 4.4. The main effects plot of the Type*Month interaction. The values are presented as the marginal mean with the 95% confidence limits.*

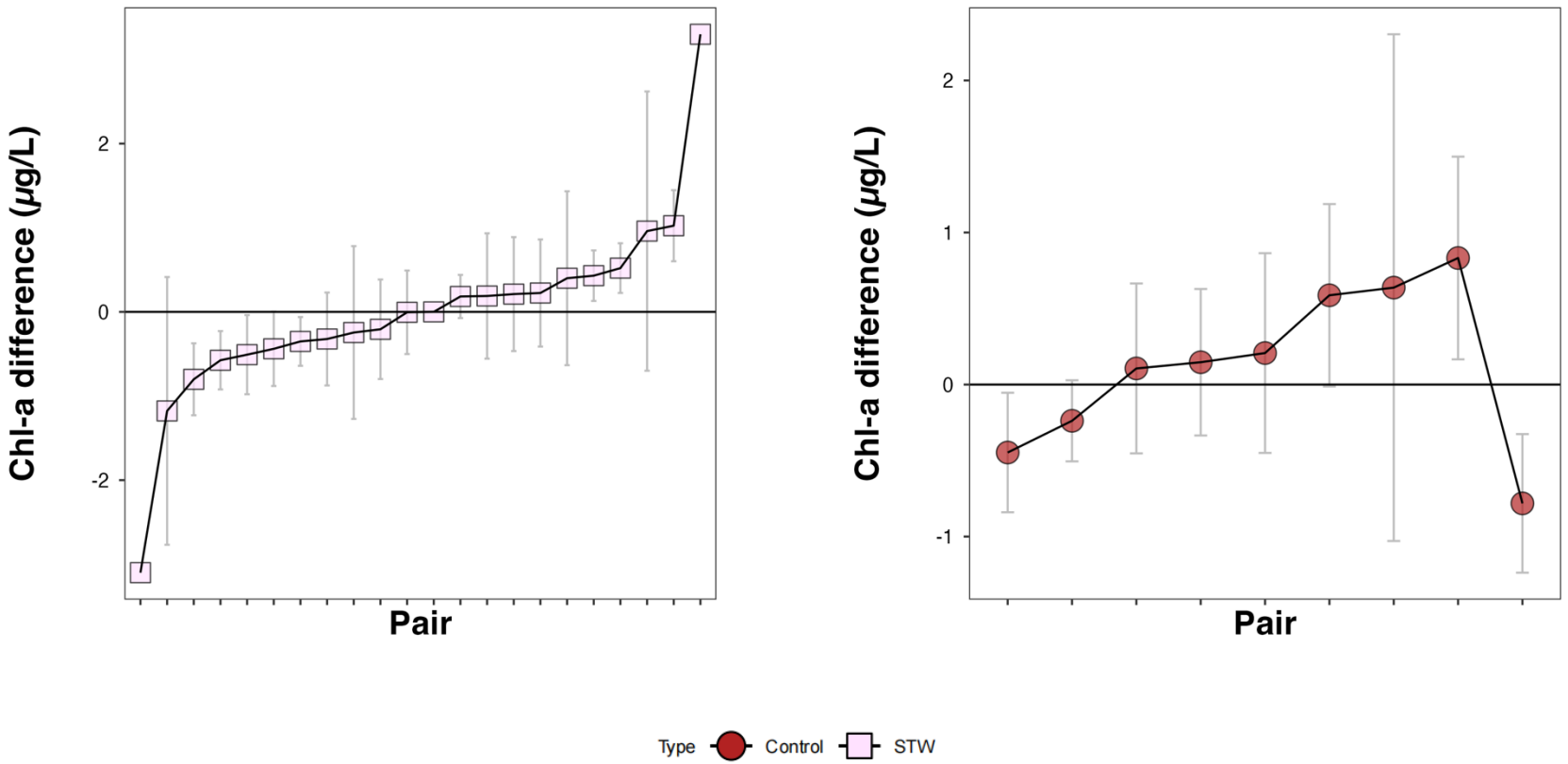


Figure 4.5(a). The main effects plot of the Pair factor. The Pairs are ordered from the lowest to greatest values of the difference. The points are the marginal mean with the 95% confidence interval.

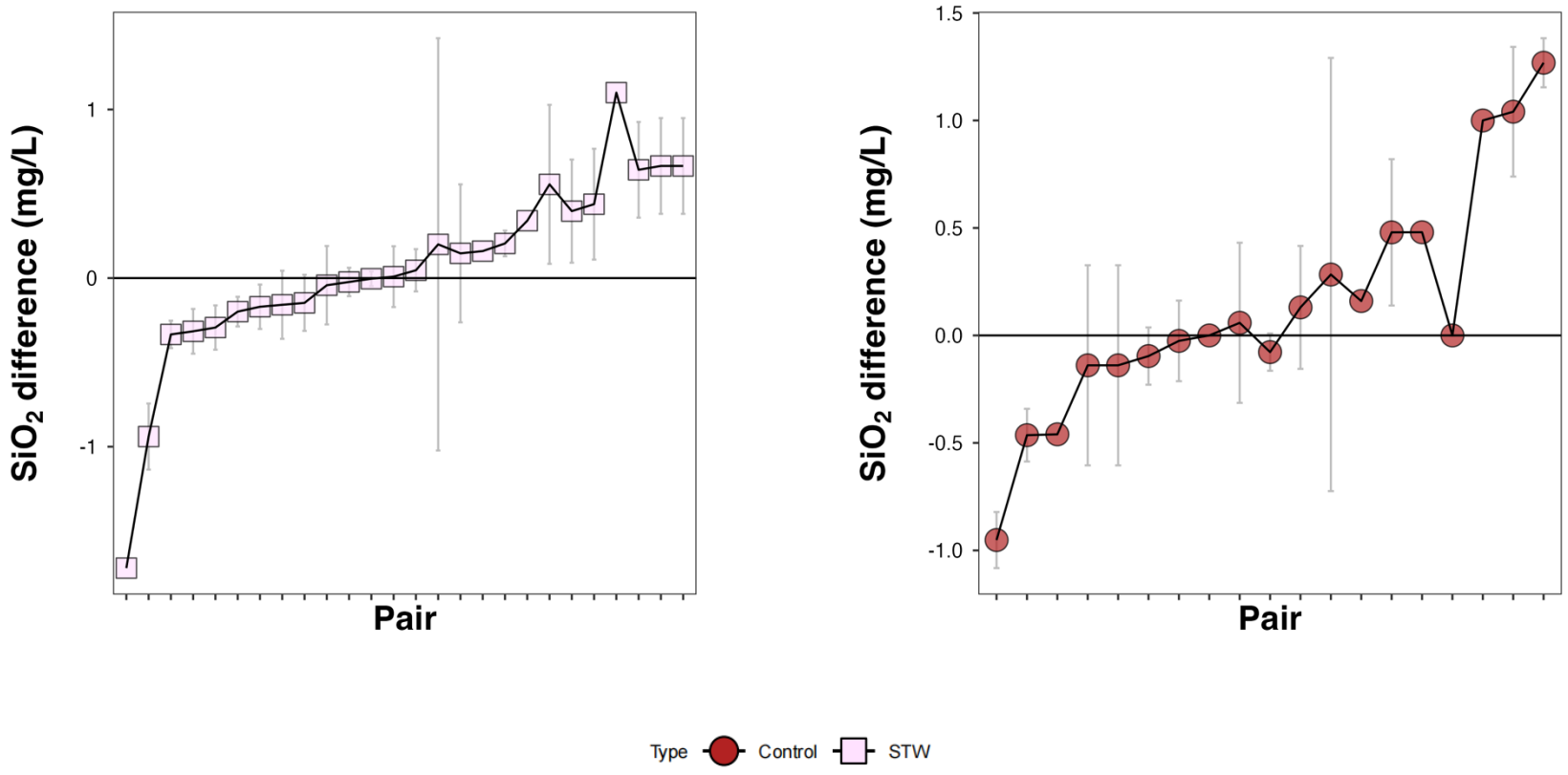


Figure 4.5(b). The main effects plot of the Pair factor. The Pairs are ordered from the lowest to greatest values of the difference. The points are the marginal mean with the 95% confidence interval.

Table 4.2. Summary of the dataset and results for the analysis of impact of STW discharge. The significance of the impact of STW discharge for each determinand is stated as Yes if it was significant at a probability < 0.05 of being zero. The main effect (%) is main effect for each determinand for the Type factor. Values are compared to the mean for the upstream values for the STW pairs for that determinand (Main effect presented as the difference for STW minus the difference for the Control, which has been illustrated in Figure 4.1). N represents the total number of pairs recorded at different times. N (without outliers) refers to the count of these pairs after removing outlier values.

Determinand (unit)	N	N (Without outliers)	Mean upstream concentration	Mean downstream concentration	Mean Final effluent	Type Significant	Main effect	Main effect (%)
Chl-a (µg/L)	1546	1257	9.43 ± 0.74	10.73 ± 0.99	8.63 ± 7.12	--	[−0.58, −0.13]	[−6.15%, −1.38%]
SiO_2 (mg/L)	1333	1189	8.30 ± 0.07	8.58 ± 0.06	11.31 ± 0.17	Yes	[−0.92, −0.36]	[−11.08%, −4.34%]

Table 4.3. The summary of the linear model for each determinand. The significance for each determinand is stated as Yes if it was significant at a probability < 0.05 of being zero. η2 indicates the extent to which a factor contributes to the observed variability in the variable.

Determinand	Type		Year		Type*Year		Month		Type*Month		Pair		Type*Pair	
	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2
Chl-a (µg/L)	--	--	--	--	--	--	Yes	0.004	--	--	--	--	--	--
SiO_2 (mg/L)	Yes	0.04	Yes	0.02	Yes	0.04	--	--	--	--	Yes	0.04	Yes	0.05

Table 4.4. Number of mean difference values for each determinand categorised by type. 'N' represents the total number of mean difference values recorded. '>0' indicates the number of mean difference values that were greater than zero, '<0' represents the number of mean difference values that were less than zero, and '=0' denotes the number of mean difference values where the difference was not significantly different. (Figure 4.4)

Determinand (unit)	Total				STW				Control			
	N	>0	=0	<0	N	>0	=0	<0	N	>0	=0	<0
Chl-a (µg/L)	31	15	2	14	22	10	2	10	9	5	0	4
SiO_2 (mg/L)	45	27	3	15	26	15	1	10	19	12	2	5

4.3.2 Importance of covariates

When all covariates were included in the analysis, Type became a significant factor for both determinands. Similarly, Year continued to show significance for both determinands. Month became significant specifically for Chl-a, but after accounting for the covariates, Pair was significant only for SiO_2. Importantly, none of the covariates were significant for SiO_2. In contrast, for Chl-a, Euclidean distance, upstream altitude, and downstream altitude were significant. Among these, upstream altitude had a notable effect: for each unit increase in upstream altitude, Chl-a increased by 0.018 μg /L (Table 4.5).

Table 4.5. The η^2 values for each factor and the coefficients for each covariate. Significance was indicated for values with a probability < 0.05 of being zero.

Determinand	Type		Year		Month		Pair		Euclidean distance (m)		up_altitude (m asl)		down_altitude (m asl)		Percentrank Significant	
	Sig	η^2	Sig	η^2	Sig	η^2	Sig	η^2	Sig	coefficient	Sig	coefficient	Sig	coefficient	Sig	coefficient
Chl-a (µg/L)	Yes	0.02	Yes	0.06	Yes	0.03	--	--	Yes	0.000	Yes	0.02	Yes	0.000	--	--
SiO_2 (mg/L)	Yes	0.008	Yes	0.03	Yes	0.05	Yes	0.40	--	--	--	--	--	--	--	--

4.3.3 Impact of the nature of the STWs

As previously discussed in Section 2.3.3, the efficacy of two distinct secondary treatment methods (SAS vs. SB) was assessed using Chi-squared tests (Table 4.6; Figure 4.6). The main effects, representing the differences between treatments, followed the same pattern as outlined earlier: positive values indicate successful pollutant reduction, while negative values suggest ineffective treatment or worsening conditions.

Table 4.6. Results of Chi-squared test for each determinand relative to technologies present at the STW. SAS = secondary activated sludge; SB = secondary biological. It was significant when at a probability (P) < 0.05.

Determinand (unit)		SAS	SB	P	Main effect (SAS-SB)
Chl-a (µg/L)	Positive	111	147	0.0005	-0.375
	Negative	62	163		
SiO_2 (mg/L)	Positive	12	35	0.92	--
	Negative	27	89		

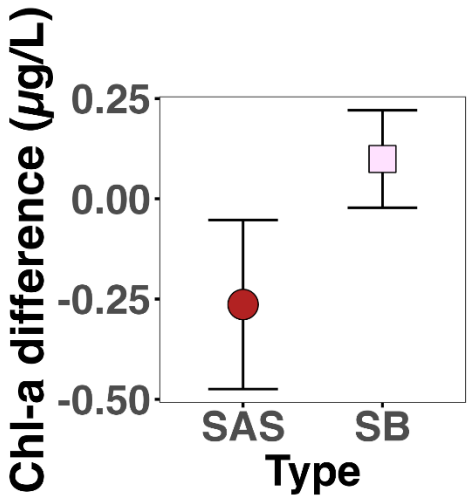


Figure 4.6. Effect of secondary treatment on mean chlorophyll-a (Chl-a, µg/L) differences between upstream and downstream locations. Results are shown for STW pairs with activated sludge secondary treatment (SAS) and secondary biological treatment (SB), presented as marginal means with 95% confidence intervals.

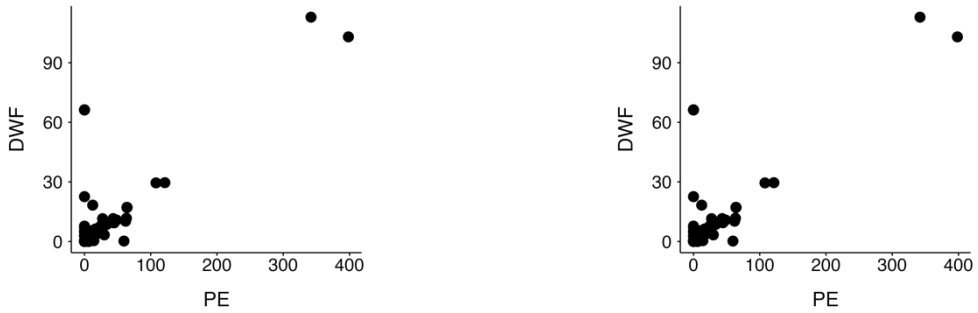
4.3.4 Impact of population equivalence and dry weather flow

As previously discussed in Section 2.3.4.2, a strong positive correlation was identified between PE and DWF (Pearson correlation coefficient of 0.88), highlighting their significant combined impact on receiving water bodies (Figure 2.13). While the earlier analysis emphasized the overall relationship between PE and DWF, this chapter delves deeper into the specific relationships between individual determinand concentrations and these variables (Table 4.7). PE shows a negative relationship with Chl-a, and DWF shows no statistical significance to Chl-a. When both PE and DWF were considered in explaining changes in Chl-a concentrations, the model only accounted for 1% of the variance. PE shows no significant relationship with SiO_2, while DWF shows a positive relationship with SiO_2. When both determinands were included in the model, it explained 2% of the variance in SiO_2 concentrations (Table 4.7).

Figure 4.7 presents scatter plots showing the relationships between PE and DWF with Chl-a (a) and SiO_2 (b). The patterns in both plots are similar, with dots clustering around lower values of PE (0 to 100) and DWF (0 to 30).

Table 4.7. Significance of STW discharge effects on determinands, Population Equivalence (per 1000 population), and Dry Weather Flow (per 1000 m³/day). A result is marked as "Yes" if the effect was statistically significant at $p < 0.05$.

Determinand	Population equivalence		Dry weather Flow		r^2
	Sig	Coefficient	Sig	Coefficient	
Chl-a (µg/L)	Yes	-0.01	--	--	0.01
SiO_2 (mg/L)	--	--	Yes	0.01	0.02



(a) Chl-a (b) SiO_2

Figure 4.7. Scatter plot of Population Equivalence (x - axis) VS Dry Weather Flow (y – axis)

4.3.5 Chlorophyll events

In this section, chlorophyll data has been analysed based on Type, Pair, Year, and Upstream or Downstream locations. The probability of chlorophyll concentrations exceeding 15 µg/L in the Upstream and Downstream of the sewage treatment works (STW) and Control sites is provided below (Table 4.8).

Table 4.8. Proportion of chlorophyll events (Chl-a concentration > 15 µg/L) relative to location on the stream reach.

Pair	Upstream	Downstream
Control	0.112	0.120
STW	0.113	0.119

Chi-Square of the incident data

Two Chi-squared tests were conducted for the analysis: one comparing the STW and Control, and the other comparing the upstream and downstream sites between the STW and Control.

The first comparison evaluates the Upstream and Downstream sites of the Sewage Treatment Works (STW) against the Upstream and Downstream sites of the Control. This analysis examines whether there is a significant difference in the proportion of chlorophyll concentrations exceeding 15 µg/L between the two locations. A Chi-squared test was applied to determine if the combined values from the STW (Upstream and Downstream) differ significantly from the combined values of the Control. The P-value was 0.98, indicating no significant difference between the STW and Control sites (Table 4.9).

Table 4.9. Proportion of chlorophyll incident (value >15) based on STW and Control.

	Incident number	Non-incident number	Whole number	Proportion
Control	839	6378	7217	0.98
STW	1599	12181	13780	

The second comparison was separate tests for the Upstream and Downstream sites. The Upstream site of the STW was directly compared to the Upstream site of the Control, and the Downstream site of the STW was directly compared to the Downstream site of the Control. This method assessed whether there was a significant difference in the proportion of chlorophyll exceeding 15 μg/L at each specific location (Upstream vs. Upstream, and Downstream vs. Downstream). The P-value for the Upstream comparison is 0.87, and for the Downstream comparison is 0.93, indicating no significant difference between the STW and Control for both the Upstream and Downstream sites (Table 4.10).

Table 4.10. Proportion (Pr) of Chlorophyll Incident (Value > 15 µg/L.). Based on Upstream and Downstream Comparisons of STW and Control.

	Upstream				Downstream			
	Incident number	Non-incident number	Whole number	Proportion	Incident number	Non-incident number	Whole number	Proportion
Control	375	2977	3352	0.87	464	3401	3865	0.93
STW	827	6478	7305		772	5703	6475	

Binomial regression of Chl-a incidents

When considering only the effect of Type there was no significant effect (Table 4.11), indicating that there is no strong evidence of a difference between STW and Control sites in the overall chlorophyll incidents (Table 4.11).

Table 4.11. Proportion of chlorophyll incident (value >15) for Chi square.

	Predicted probability	Confidence interval
Control	0.03	0.01~ 0.04
STW	0.04	0.03 ~ 0.06

4.3.6 Principal components analysis

In the previous sections, PCA was conducted on paired sampling points located upstream and downstream of sewage treatment works (STWs). In this section, PCA is applied to all river data, final effluent data, and their combined dataset from the 2002 dataset for England.

By merging datasets for each determinand on the same day for PCA analysis, 6,348 (5512 with catchment information) river data points, 236 effluent data points, and 6,584 combined data points are identified. This section will analyze river sampling points, effluent sampling points, and their combined data using PCA.

River data from all years

Three out of eight principal components had eigenvalues > 1 (Table 4.12), and these three components together explained 62% of the original variance. PC1 was mainly influenced by COD and BOD, reflecting the impact of organic wastes in freshwaters; PC2 is mainly influenced by Phosphate and Nitrate, reflecting the impact of nutrients in the water; and PC3 is mainly influenced by pH (Table 4.12).

Table 4.12. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1	PC2	PC3
Stream temperature (°C)	0.08	-0.28	-0.04
BOD (mg O_2/L)	0.60	0.31	-0.15
COD (mg O_2/L)	0.65	0.26	-0.06
Phosphate (mg P/L)	0.29	-0.58	0.18
Suspended solids (mg/L)	0.18	0.05	0.21
pH	-0.11	-0.10	-0.65
Nitrate (mg N/L)	0.25	-0.60	0.22
Specific conductance (µS/cm)	0.15	-0.22	-0.27
Eigenvalue	2.06	1.68	1.23
Percentage of variance explained	26%	21%	15%

The PCA plot (Figure 4.8) illustrates the projection of different river sampling sites onto the principal components, with each point representing a site. Clusters of points indicate sites with similar profiles based on the variables contributing to PC1 and PC2, while dispersed points indicate differences. PC1 is primarily influenced by COD and BOD, meaning that sites with high PC1 scores have elevated concentrations of these organic pollutants. PC2 is mainly influenced by phosphate and nitrate, indicating that sites with high PC2 scores have higher nutrient concentrations. Rivers such as the Alt and Ribble, with high PC1 scores (right side), are heavily affected by organic pollution, while rivers such as the Ribble and Lune, with high PC2 scores (upper side), are characterized by high nutrient levels. In contrast, rivers clustered in the lower left corner have low scores on both PC1 and PC2, indicating relatively low levels of organic pollutants and nutrients.

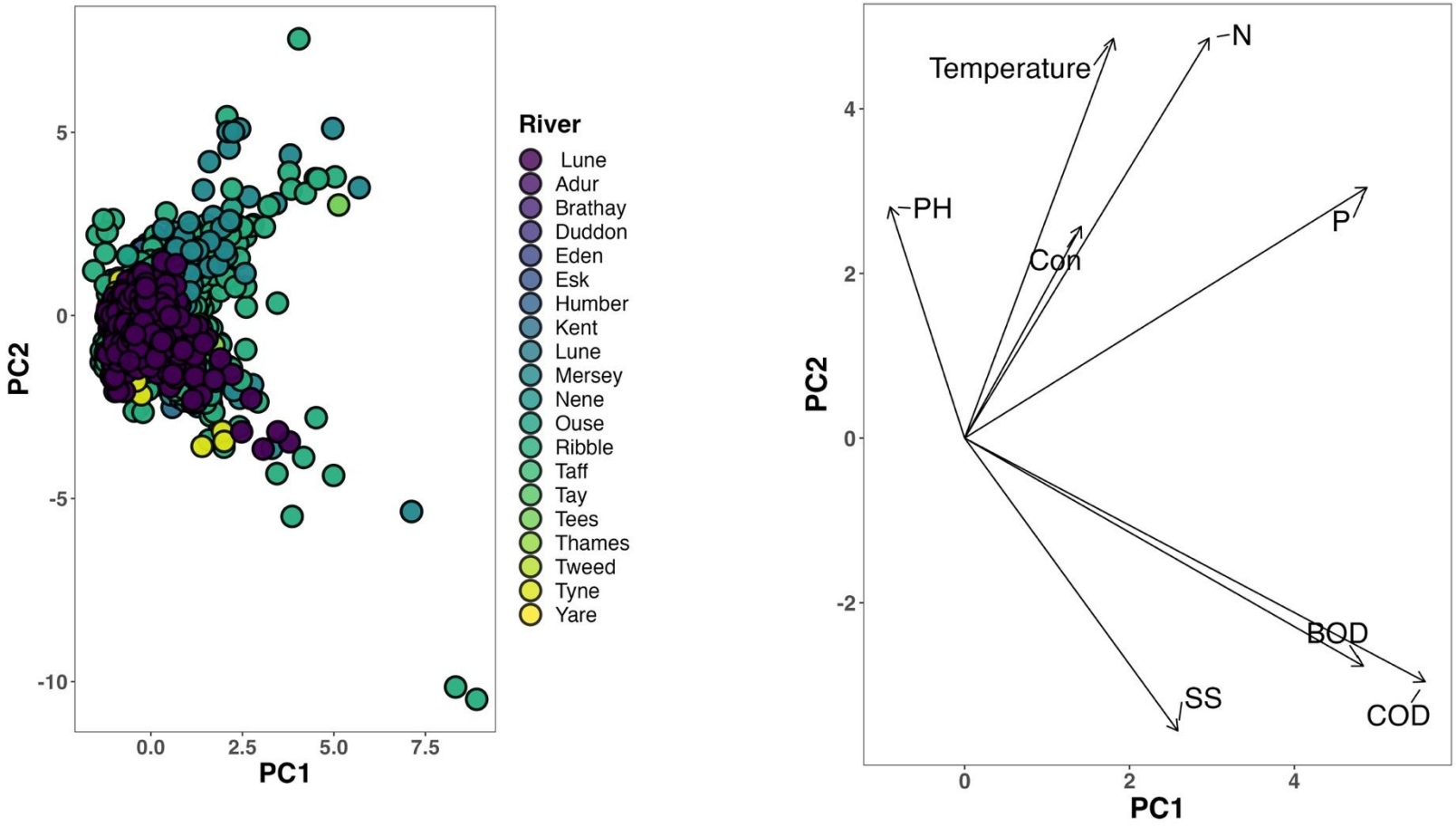


Figure 4.8. PCA and loading plot for River Sampling Points.

Final effluent data from all years

In the previous chapters, final effluent data was only used to perform an illustrative comparison with Upstream concentration values. In this chapter, Final effluent data are directly used and analysed as they represent the end member for demonstrating the wider impact. Determinands in Chapter2 for whole England has been collected and merged without Pair control. Same day data has been merged. 236 data have finally been found.

For these 236 data PCA analysis, three out of eight components had eigenvalues >1 (Table 4.13) and they explained 71% of the original variance. PC1 is medium highly positive to BOD and COD, showing, as with the above PCA, that PC1 is controlled by presence of organic wastes; PC2 mediumly relates to Phosphate, Temperature and Nitrate, presenting the nutrients impact; and PC3 highly positively relates to pH, and mediumly negatively relates to Nitrate.

Table 4.13. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1	PC2	PC3
Stream temperature (°C)	0.03	0.52	0.27
BOD (mg O_2/L)	0.54	-0.12	-0.16
COD (mg O_2/L)	0.56	-0.09	-0.15
Phosphate (mg P/L)	0.26	0.56	-0.17
Suspended solids (mg/L)	0.35	-0.23	-0.10
pH	0.14	0.09	0.79
Nitrate (mg N/L)	-0.21	0.46	-0.46
Specific conductance (µS/cm)	0.36	0.34	0.10
Eigenvalue	2.78	1.64	1.17
Percentage of variance explained	35%	21%	15%

Plotting the data by sampling site (Figure 4.9), PC1 has higher BOD and COD loading, so rivers having higher score on PC1 have more organic pollutants in the final effluent. PC2 mediumly correlated with Phosphate, Stream temperature and Nitrate, which means the rivers possessing higher score on PC2 have higher nutrient determinands in the final effluent. Figures 4.8 and 4.9 appear to show three end-member mixing system where all the final effluent and river samples can be described by three end-members, and those three end-members would be:

- High PC1, low PC2 – this would represent the high suspended solids and high organic wastes end member.
- Low PC1 – the low concentration end-member for all determinads.
- High PC2 – this end-member would represent the nutrient end-member.

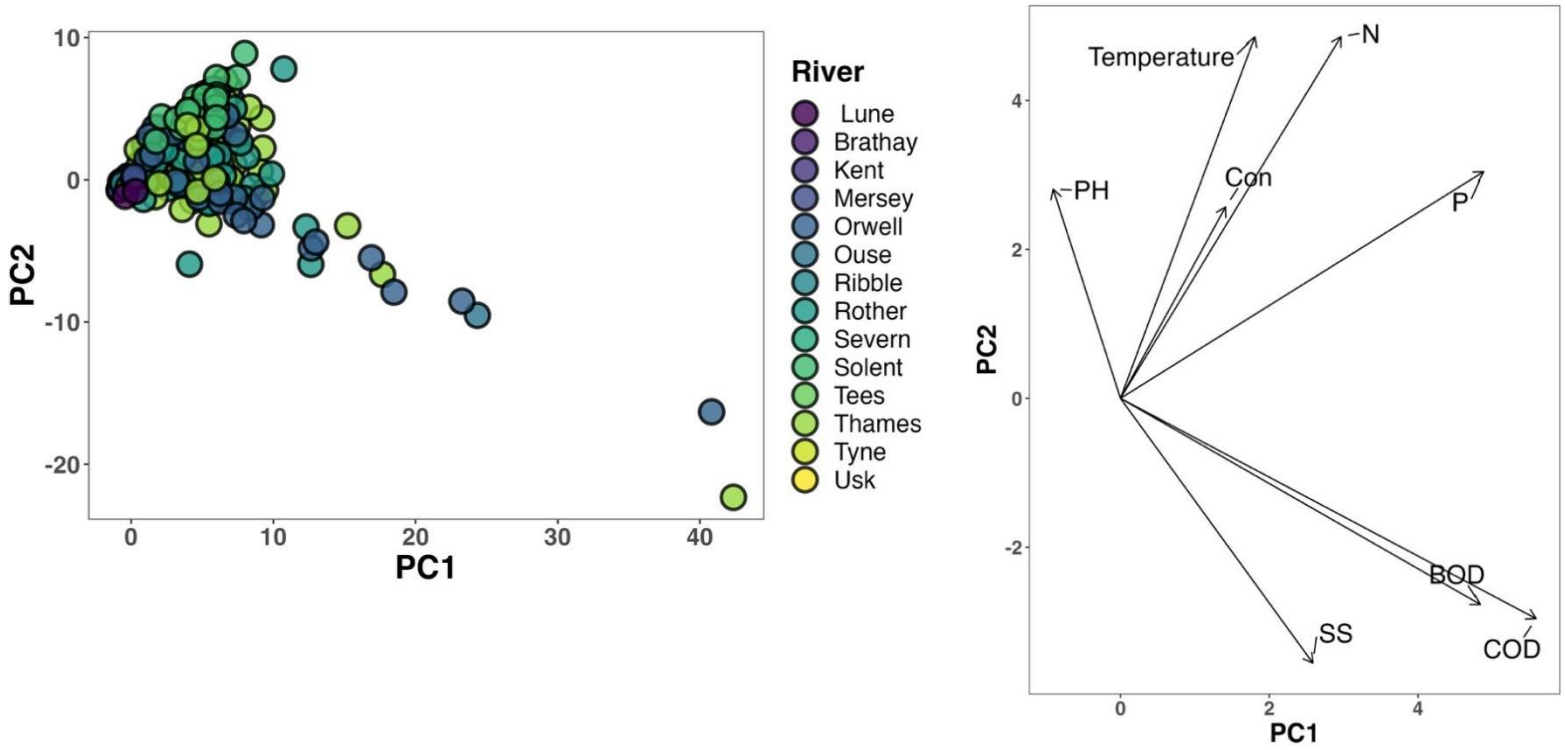


Figure 4.9. PCA for Final Effluent Sampling Points.

Combination of River data and Final effluent

For this section, three out of eight components had eigenvalues >1 (Table 4.14), which explained 63% of the original variance. PC1 is correlated with BOD, COD, and Phosphate; PC2 is related to Phosphate and Nitrate; and PC3 is highly related to pH and mediumly correlated with Stream temperature.

Table 4.14. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1	PC2	PC3
Stream temperature (°C)	0.18	0.39	0.47
BOD (mg O_2/L)	0.54	-0.39	0.18
COD (mg O_2/L)	0.59	-0.35	-0.09
Phosphate (mg P/L)	0.42	0.45	-0.19
Suspended solids (mg/L)	0.20	-0.12	-0.18
pH	-0.10	0.13	0.71
Nitrate (mg N/L)	0.29	0.55	-0.31
Specific conductance (µS/cm)	0.16	0.20	0.27
Eigenvalue	2.26	1.57	1.18
Percentage of variance explained	28%	20%	15%

The overall PCA plot on the top panel shows the projection of different water samples (river water and sewage) on the principal components (Figure 4.10). Most river water samples cluster in the lower-left corner of the plot, indicating low scores on both PC1 and PC2, thus reflecting lower levels of organic indicators (BOD and COD) and nutrients. In contrast, data from final sewage effluent are more widely distributed, with many scores high on both PC1 and PC2, indicating higher levels of organic waste indicators (COD, BOD) and nutrients (Nitrate, Phosphate). The PCA plot for sewage data is shown in Figure 4.9, and the PCA plot for river data is shown in Figure 4.8. Both of them illustrate the variation in samples, with arrows indicating the contribution of each determinand.

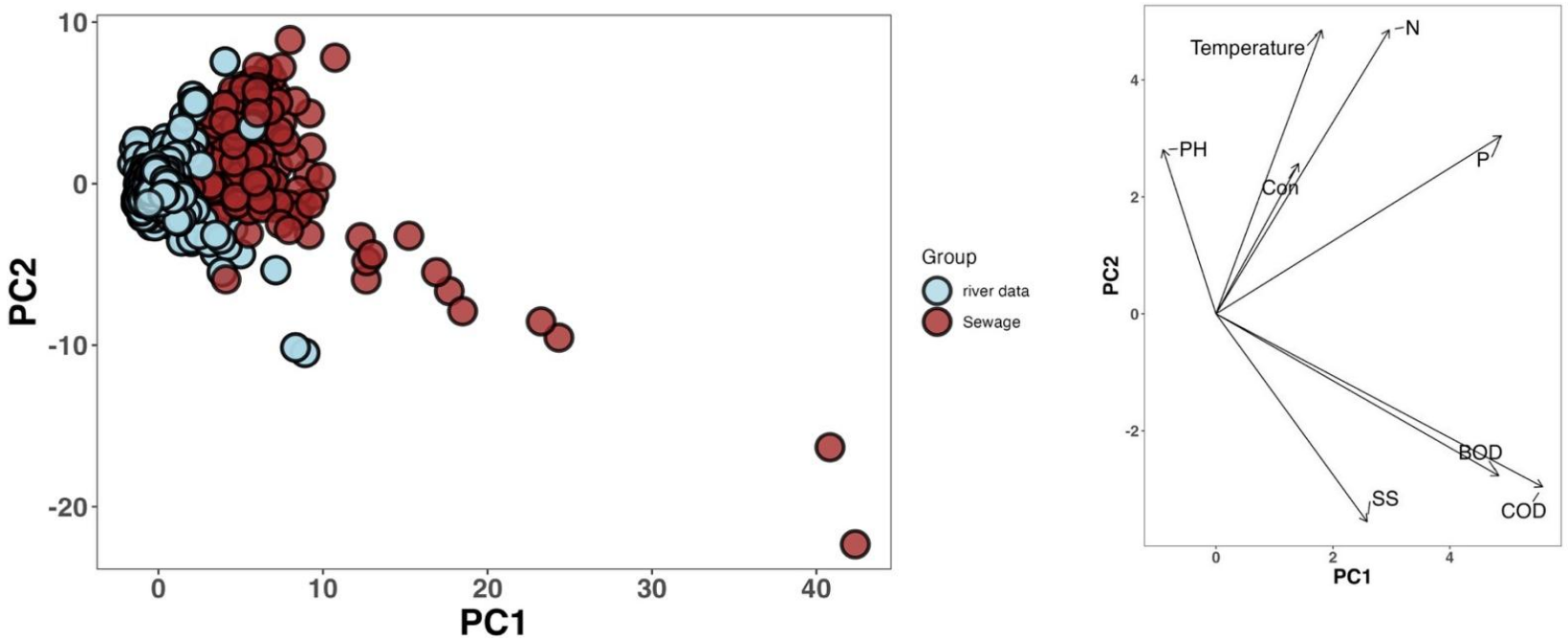


Figure 4.10. PCA for both River data and Final Effluent Sampling Points.

4.4 Discussion

This chapter's purpose was to understand the wider impact of sewage effluent upon receiving rivers and specifically whether the increased flow of warm, nutrient-rich water from sewage treatment works would lead to increased eutrophication. The study built upon results in previous chapters but had to add a consideration of chlorophyll and silica.

This study analysed 22 STW pairs and nine control pairs for chlorophyll, and 26 STW pairs and 19 control pairs for SiO_2 (Table 4.1). The Type factor for SiO_2 was statistically significant, indicating that the difference between STW and Control for silica concentrations is meaningful (Figure 4.2 and Table 4.2). And the main effect for SiO_2 indicates that STWs contribute to a measurable decrease in silica concentrations, with a proportional change ranging from -11.08% to -4.34% relative to upstream concentrations.

In contrast, the Type factor for Chl-a did not result in significant differences between the STW and Control pairs for chlorophyll. The overlapping confidence intervals in Figure 4.1 suggest that there is no statistically significant distinction between the STW and Control.

Even though statistical significance was not detected, certain time periods or locations may exhibit more pronounced effects. Like in this study, for Chl-a, the main effect ranges from -6.15% to -1.38%, suggesting minimal and non-significant changes when comparing STW and Control sites. The downstream increase in Chl-a concentration at STW sites may be more influenced by local nutrient conditions rather than a direct effect of STW discharges. The observed pattern may reflect variation in nutrient-removal efficiency across STWs, where plants lacking advanced phosphorus or nitrogen removal processes contribute more strongly to local enrichment, while those with tertiary treatment exert weaker effects. In contrast, control reaches may be more affected by diffuse inputs from agriculture and urban runoff, which can sustain elevated chlorophyll even in the absence of direct effluent discharges. Additionally, silica availability may indirectly impact Chlorophyll-a levels, particularly where diatoms—a major contributor to chlorophyll biomass—depend on silica for growth.

So, long-term monitoring to reveal trends that clarify the influence of STW discharges on chlorophyll and SiO_2 levels is important. Based on the analysis, the Type*Year variance suggests that the trend in SiO_2 concentrations varies between STW and Control sites over time, and the significant interaction indicates that the yearly changes in SiO_2 differ between the two groups, possibly reflecting an increasing impact of STW discharges on SiO_2 concentrations in receiving waters. Since SiO_2 is typically not removed during wastewater treatment, effluent inputs may alter riverine SiO_2 concentrations by dilution or by shifting nutrient stoichiometry. Conversely, the decline in control sites may reflect enhanced diffuse inputs of nitrogen and phosphorus from agriculture, which lower the Si:N ratio and create conditions of potential silica limitation. Note that the significant decline in control SiO_2 differences after 2003 (Figure 4.3) may not be directly attributable to STW regulation but could reflect broader catchment processes. Agricultural inputs of N and P have been reported to increase during this period, which may lower the Si:N ratio and enhance the potential for silica limitation, particularly for diatom growth. While there is no clear evidence of a widespread post-2000 decline in silica concentrations in UK rivers, nutrient enrichment and changing nutrient stoichiometry have been noted as important drivers of ecological change(Makareviciute-Fichtner *et al.*, 2024). This suggests that the observed control SiO_2 behaviour may be linked to diffuse nutrient pressures in addition to regulatory changes under the WFD.

For Chl-a, the Month factor was significant, indicating that chlorophyll concentrations fluctuate seasonally. However, the Type*Month interaction was not significant, suggesting that the trends in chlorophyll concentration are consistent across both STW and Control Sites (Table 4.3).The near balance between positive and negative Chl-a differences (Table 4.4) suggests that while some STWs may stimulate downstream algal growth through nutrient enrichment, others may reduce concentrations via effective nutrient removal, dilution, or site-specific factors. This bidirectional influence explains the lack of a significant overall effect. By contrast, for SiO_2, both Figure 4.5(b) and Table 4.4 show a consistent skew towards positive differences, with 27 of 45 pairs exhibiting downstream increases compared with only 15 negative and 3 non-significant cases. This alignment between the graphical and tabular results reinforces the significance of the Type*Pair interaction,

indicating that STW discharges more frequently elevate SiO_2 concentrations, though the magnitude of change varies across sites.

Several studies have addressed eutrophication by monitoring changes in chlorophyll concentrations in water bodies. For instance, Slijkerman et al. (2014) studied 11 locations (834 data points) from November 2011 to May 2013 along Bonaire Harbour, analysing dissolved inorganic nitrogen, phosphate, and chlorophyll levels. While this study is similar in scope, it did not specifically examine the impact of STW effluent on nutrient levels or chlorophyll. In contrast, the key focus of this study was to assess STW discharge as one of the primary anthropogenic influences. Does STW discharge impact chlorophyll concentrations? Is there a relationship between chlorophyll and other determinants such as nitrate, phosphate, and temperature? This study analysed chlorophyll data from 2000 to 2020, with 1546 samples collected from paired sites identified in Chapter 2 within the UK.

In another study, Bowes et al. (2012) noted a peak in chlorophyll levels that coincided with a decrease in silica concentrations. Their research examined changes in chlorophyll-a, nitrate, phosphate, and temperature in the Thames basin. Bowes et al. (2012) reported that peaks in chlorophyll and reductions in silica ceased at temperatures between 15°C and 21°C. However, their study did not focus on the impact of STW discharges on receiving rivers, nor did it investigate the interrelationships between chlorophyll, silica, nitrate, phosphate, and temperature. Moreover, they did not report chlorophyll incidents related to eutrophication. In contrast, this study has explored chlorophyll incidents and their influence on different water types, as well as their upstream and downstream effects relative to STW discharges.

Oliveira & Goulder (2006) examined STW impacts in their study of three streams and six sites in North Yorkshire, conducted from May to June 2000. They found that high volumes of STW effluent had a mild impact on micro-algal epilithic communities, coupled with an increase in inorganic nutrients and oxygen demand. Their study focused on sites upstream and downstream of STW outfalls and analysed epilithic community similarities between different bacterial groups. However, their study did not include control data or multivariate analysis, which

limits the robustness of their conclusions. In contrast, this study included control data and employed multivariate analysis, making the results more reliable.

Alternatively, this study considered the composition of the final sewage effluent for these key determinads in the context of all English rivers (Figure 4.9). 6584 data and their composition of the year 2002 have been considered. There is not enough time within this thesis to explore all the implications of these results, but several implications are worth raising. This result would mean that river water composition, only with respect to the determinands considered, was actually controlled by sewage effluent; in effect, river water is dilute sewage.

Above all that, in this study, PE and DWF were considered to see the STWs' nature on eutrophication determinands. Chlorophyll shows a significant relationship with population equivalence, exhibiting a negative correlation implying that as the population equivalence of the sewage treatment works (STW) decreases, the concentration of chlorophyll increases. The negative correlation between PE and chlorophyll may indicate that smaller STWs, often lacking advanced nutrient-removal processes, exert proportionally greater influence on local eutrophication, whereas larger facilities with tertiary treatment achieve more consistent nutrient control. At the same time, control sites in agricultural catchments may maintain high nutrient loads independent of PE, explaining the weaker associations observed for SiO_2. In contrast, population equivalence is insignificant for SiO_2. However, dry weather flow (in thousands of cubic meters per day) has a significant impact on SiO_2 concentrations, consistent with previous findings regarding dry weather flow (Grout *et al.*, 1999). The scatter plots (Figure 4.7) illustrate the relationship between Population Equivalence (PE) on the x-axis and Dry Weather Flow (DWF) on the y-axis for both Chlorophyll and SiO_2. In both plots, there is a cluster of points with relatively low PE and DWF values, with a few points extending to higher PE values, indicating that as the population equivalence increases, the DWF also tends to rise, though the relationship appears non-linear and somewhat scattered. However, this interpretation should be treated with caution, as diffuse agricultural runoff, livestock wastes, and urban stormwater remain important nutrient and organic matter sources in control sites, and their contributions may overlap with or mask the role of STW discharges. Furthermore,

it would imply that there is no role for other sources of nutrients or organic wastes, e.g., agricultural runoff.

4.5 Conclusion

This chapter has shown that:

i) There was no significant impact of sewage discharge on the chlorophyll concentration of receiving rivers.
ii) The inclusion of covariates suggested that there was a possible significant increase in Chl-a concentration due to sewage discharge.
iii) The trend in the impact of sewage discharge on SiO2 suggests that STW discharges will significantly impact receiving rivers in the future.
iv) SAS (secondary activated sludge) is more effective than SB (secondary biological treatment) in reducing chlorophyll in receiving waters.
v) The comparison between final effluent and receiving river composition shows that English rivers could be considered as dilute sewage discharge.

Chapter 5: Metal Bioavailability and Pollution Risk -- National scale impact of STW discharge on English rivers

5.1 Introduction

Due to their environmental persistence, heavy metals can have a considerable impact on river ecosystems (Zhao and Marriott, 2013; Emenike *et al.*, 2022), disrupt aquatic life (Grove and Sedgwick, 1998), and pose long-term environmental and health risks (Ali *et al.*, 2019). These metals (such as Cd, Cu were deposited into the UK environment after the peak mining in the 19th century (Grove and Sedgwick, 1998), often accumulating in riverbeds and bioaccumulating in organisms, thereby affecting biodiversity (Bryan and Langston, 1992). Other sources of heavy metal pollution include industrial discharges, ongoing mining activities, agricultural runoff, and sewage treatment works. For example, industrial activities are an important source of heavy metal contamination in water bodies (Hutchinson, 2003).Rapid industrialisation, large-scale agricultural and urban development have increaseed the heavy metal burden on aquatic ecosystems (Phaenark *et al.*, 2024). Sartorius et al. (2024) found that the mine discharge increaseed the downstream heavy metal concentration, and it had a detrimental impact on the ecosystem (Sartorius et al., 2024). Over time, exposure to high concentrations of heavy metals has toxic impacts on aquatic species, affecting reproduction, growth, and even the economy. For instance, higher concentrations of heavy metals can enter the food chain, impacting human populations that rely on rivers for drinking water or fishing (Ali *et al.*, 2024). Similarly, heavy metal pollutionaffects the economybased on water recreational activities, such as sailing and fishing, etc (Sidondi *et al.*, 2024b).

The purpose of this Chapter is to explore the broader impact of heavy metals and bioavailable metals in the river ecosystems across England. This chapter builds on the work presented in Chapter 3, which focused on demonstrating the direct impact of STW discharges on the receiving river immediately downstream of the discharge. This chapter expands the analysis to encompass all river sampling data from 2000 to 2020, providing a more comprehensive view of heavy metal contamination nationwide. For this purpose, principal component analysis (PCA) is used to identify patterns and relationships within river water quality data from across

England. The dataset is divided into two categories: river data and sewage discharge data and then analysed in combination.

In Chapter 3, bioavailable metals were studied within STW pairs and Control pairs. For this chapter, all river sampling data from 2000 to 2020 are analysed using the M-BAT model to estimate bioavailability across England, showing STW discharges in the context of overall river water quality.

This chapter focuses on identifying broader patterns of pollution and the factors influencing the concentrations of bioavailable metals across various regions. The specific research questions were:

- What are the dominant sources and compositional characteristics of bioavailable metals at a national scale, and how do they compare to the patterns identified in more localised STW-impacted sites (Chapter 3)?
- To what extent do STWs contribute to bioavailable metal exceedances at the national scale, and how does their influence compare to other regional factors after demographic and spatial normalisation?

5.2 Approach and Methodology

The aim of this chapter was to explore the broader impact of STW on metal pollution in rivers. Two approaches were used:

- A principal component analysis was performed using all river data for metals and the same determinand data for metals in the final effluent. This analysis was conducted to determine whether final effluent from STWS was a source to rivers at all scales.
- The bioavailable concentration was calculated for all metals that are compatible with the M-BAT model. This was done for all English river sites where sufficient data were available to apply the model.

5.2.1 Study data

For the analysis of total metal concentrations, data were downloaded from the Environment Agency, covering the period from 2000 to 2020. This dataset includes measurements of Ca, Mg, Cd, Cu, Ni, Fe, Zn, and Mn, and was divided into two categories: samples of river water and samples of STW final effluent.

Data for Principal Component Analysis (PCA)

PCA was performed to examine pollution patterns and assess whether STW discharges contribute to river metal concentrations at a national scale. The dataset for PCA included: total metal concentrations in river water, and metal concentrations in STW final effluent. The approach used all available river sampling points across England.

Data for Bioavailable Metal Analysis (M-BAT Model)

The bioavailable metal analysis focused on Cu, Zn, Mn, and Ni. These metals use the same river dataset as the total metal concentrations but were further refined by merging them with site-specific data on pH, dissolved organic carbon (DOC), and Ca recorded on the same day and at the same location.

RCR greater than 1

To assess potential heavy metal contamination, a threshold of Risk Characterisation Ratio (RCR) ≥ 1 was applied, following established regulatory guidance (WFD-UKTAG, 2014) . An RCR greater than 1 indicates that the bioavailable

concentration of a metal exceeds its Environmental Quality Standard (EQS_bioavailable), implying a potential risk to the aquatic environment. Only samples meeting this criterion were included in subsequent analyses. Within this subset, linear models were constructed to evaluate the relationships between bioavailable metal concentrations and key water quality parameters, including pH, DOC, Ca, and the corresponding dissolved metal concentrations.

Spatial attribution and data Normalisation

Spatial Attribution of Sampling Points

To enable the aggregation of monitoring data at the administrative county level, spatial matching was performed in R using the sf package. STWs sampling points, recorded by their easting and northing coordinates, were converted into spatial objects using the OSGB36 coordinate reference system (EPSG:27700). County boundary shapefiles were similarly transformed to the same projection to ensure consistency.

Each STW sampling point was assigned to the corresponding county using a spatial join operation (st_join with st_intersects). Where necessary, nearest-neighbour matching (st_nearest_feature) was applied to associate sampling sites located near county boundaries with the nearest county centroid. This approach ensured that every sampling point was consistently attributed to a single administrative unit. This spatial allocation to counties facilitates regional analysis of pollution patterns and enables subsequent integration with county-level socio-environmental indicators, such as population density and the spatial distribution of STWs.

Acquisition of County-Level Attributes

County-level demographic and geographic attributes were obtained from the Office for National Statistics via the UK Government's Geoportal. The datasets included total population and total area (in km^2) for each county. Spatial joins were used to associate each county polygon with these attributes.

Aggregation and Normalisation of Monitoring Data

Following spatial attribution, the metal monitoring dataset was aggregated at the county level. For each county, the number of monitoring records exceeding a Risk Characterisation Ratio (RCR) threshold of 1 was calculated for each metal of

interest (e.g., Cu, Zn, Ni, Mn). These exceedances were summarised into county-level totals.

To enable meaningful comparisons across counties of differing sizes and populations, the exceedance counts were normalised:

- **Per Capita**: the number of RCR exceedances per 1,000 population.
- **Per Area**: the number of RCR exceedances per square kilometre (km^2).

Normalised exceedance metrics were calculated by dividing the total number of exceedances by the respective county population or area and scaling as appropriate. It is acknowledged, however, that the population data represent entire administrative regions and may not spatially coincide with the precise locations of RCR exceedances. This potential spatial mismatch should be considered when interpreting per capita results.

5.2.2 Statistical analysis

The purpose of this chapter was to investigate the relationships between water quality determinands and to identify key pollution patterns across study sites in England, focusing on total and bioavailable metal concentrations.

To achieve this, two main statistical approaches were applied:

Principal Component Analysis (PCA)

PCA was employed to explore overall pollution patterns and identify groups of correlated determinands. The analysis aimed to identify the primary pollution components affecting various river sites. The steps involved standardising metal concentrations to account for differences in units and magnitudes, extracting principal components (PCs) to identify dominant pollution factors, interpreting high-loading determinands to assess their potential sources (e.g., STW discharge, natural inputs), and mapping high-PC locations to visualise spatial pollution trends in English rivers.

Bioavailable Metal Assessment (M-BAT Analysis)

To evaluate the ecological risk of bioavailable metals, the following statistical analyses were conducted:

Pearson correlation analysis examined the relationships between bioavailable metal concentrations and key water quality parameters, including pH, DOC, and Ca. Used a significance threshold of $p < 0.05$.

Risk Characterisation Ratio (RCR) calculation - computed the proportion of sites where RCR >1, indicating potential ecological risk. Comparison of dissolved vs. bioavailable metal concentrations: Visualised differences and trends to understand the variability in metal bioavailability.

Prior to analysis, all predictor and response variables were standardised by z-transformation (centred to a mean of zero and scaled to a standard deviation of one). This step ensured comparability of effect sizes across variables and stabilised interpretation of interaction terms.

Linear regression models were subsequently applied to investigate the influence of water quality parameters on the bioavailable concentrations of metals (Cu, Zn, Mn, Ni). The independent variables included pH, dissolved organic carbon (DOC),

Ca, and corresponding dissolved metal concentrations (Cu, Zn, Mn, Ni). To account for potential interaction effects, two-way interaction terms (pH*DOC, pH*Ca, DOC*Ca) were incorporated into the models.

Separate models were developed for each metal, and analyses were conducted across two datasets, firstly, the full national dataset; and, secondly, the subset comprising observations where the Risk Characterisation Ratio (RCR) was ≥ 1.

Model evaluation was based on regression coefficients, p-values for significance testing, and partial R^2 values, which indicated the proportion of variance uniquely explained by each predictor or interaction term.

This modelling framework allowed the identification of both primary predictors and context-dependent interactions influencing metal bioavailability across spatial and contamination gradients.

All statistical analyses were performed using RStudio v 4.3.1.

5.3 Results

A summary of river and sewage data from 2000 to 2020 is provided in Table 5.1 (Figure 5.1(a) – Figure 5.1(d)). For river data, Cu has the most records, followed by Fe, Ni, Zn, Cd, Mn, Ca, and Mg. In contrast, for sewage data, Fe had the most records, while Mn, Ca, and Mg have limited data availability. This limitation will impact the subsequent principal component analysis (PCA) analysis.

In addition to total sample counts, the table also presents the range of sample numbers per site, highlighting the variation in monitoring intensity across locations. For instance, Cu was measured at river sites with sample counts ranging from 1 to 372, and at sewage sites from 1 to 380.

Although data availability is uneven, particularly in the sewage dataset, all eight determinands were retained for PCA analysis to ensure consistency with the river data and allow comparative interpretation. However, the limited sample size for Mn, Ca, and Mg in the sewage data may reduce the robustness of the multivariate patterns derived from these variables.

Table 5.1. Summary of each determinand for the whole data number.

Determinand (unit)	N of river data	Per Site samples range (river data)	N of sewage data	Per Site samples range (sewage data)
Ca (mg/L)	66365	1-248	188	1-20
Mg (mg/L)	42265	1-248	189	1-20
Cd (µg/L)	109431	1-301	3421	1-198
Cu (µg/L)	611355	1-372	5311	1-380
Ni (µg/L)	150119	1-301	3739	1-198
Fe (µg/L)	166926	1-343	7833	1-382
Zn (µg/L)	139112	1-309	2828	1-198
Mn (ug/L)	67632	1-329	665	1-99
N of PCA data	19393	1-204	20	1-10

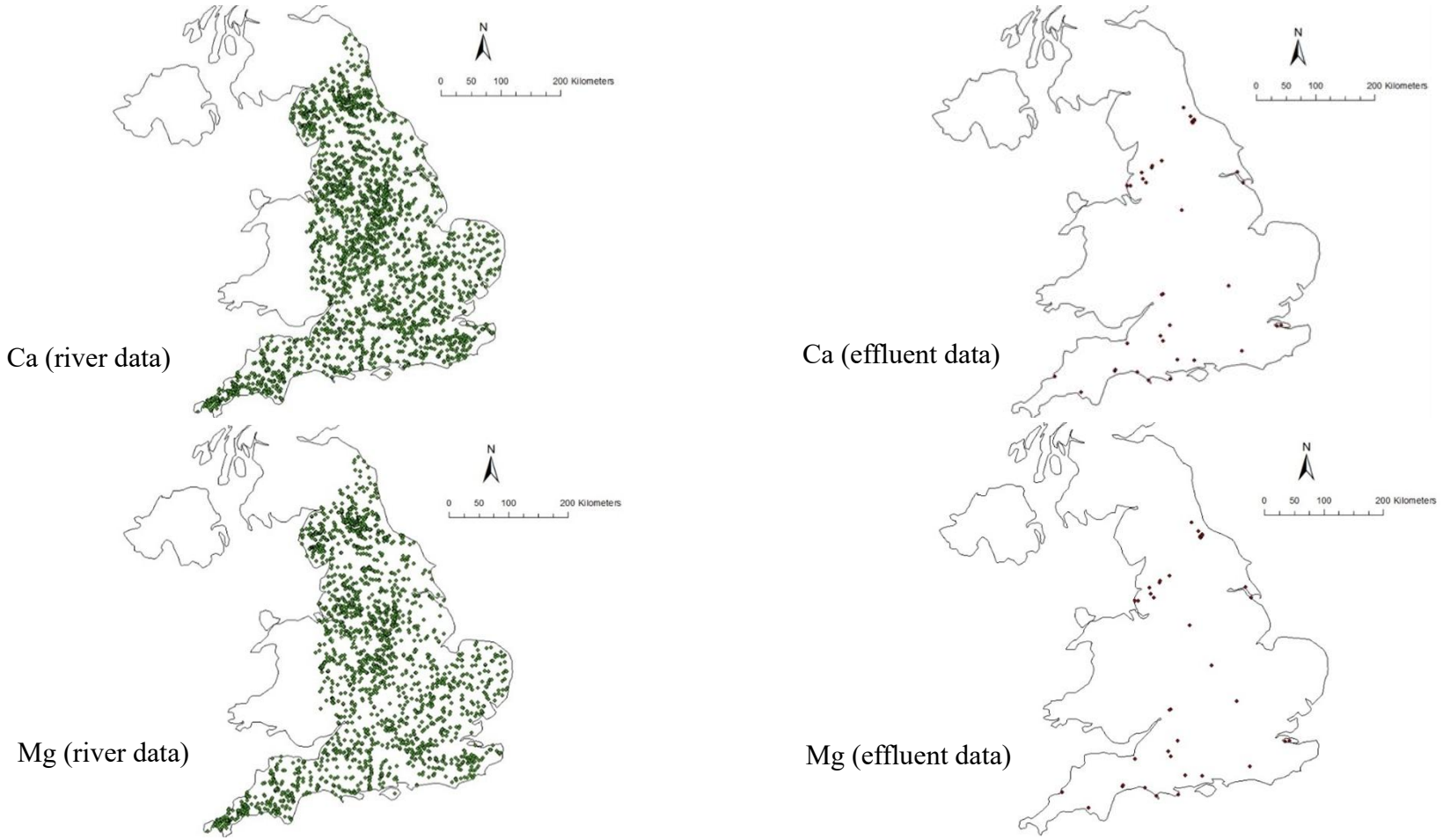


Figure 5.1(a). Map each determinant for river sampling data and final effluent data.

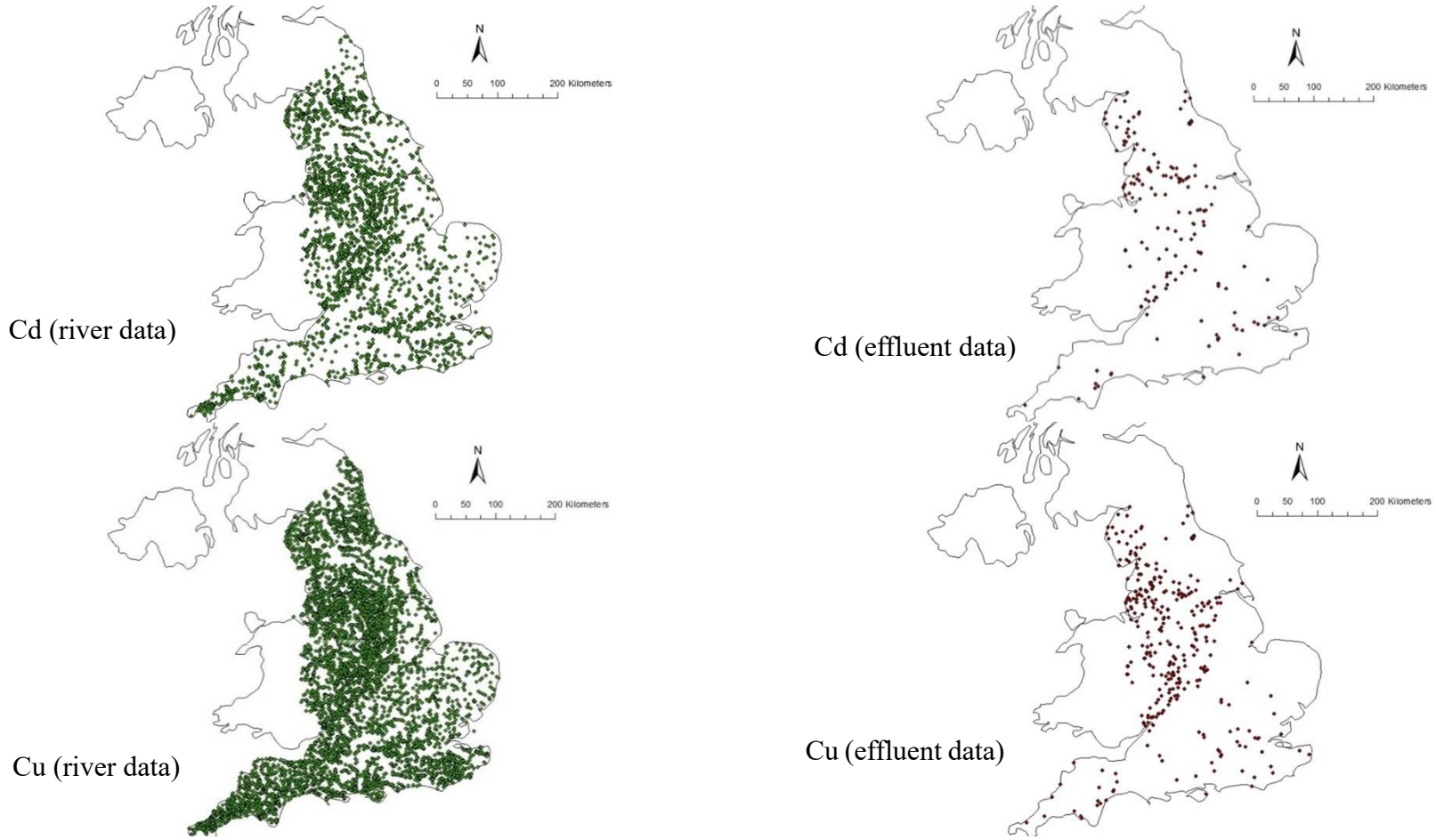


Figure5.1(b). Map each determinant for river sampling data and final effluent data.

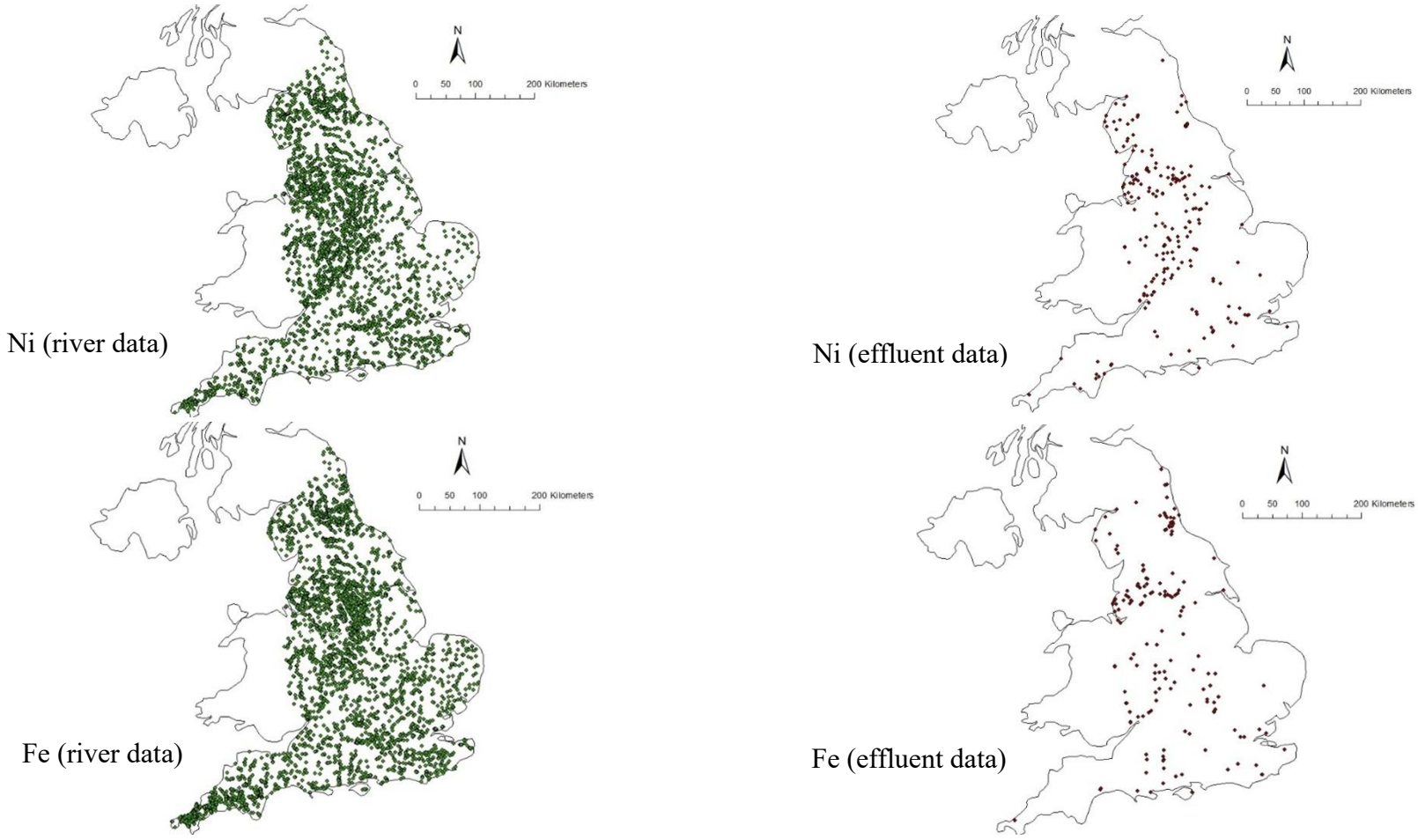


Figure5.1(c). Map each determinant for river sampling data and final effluent data.

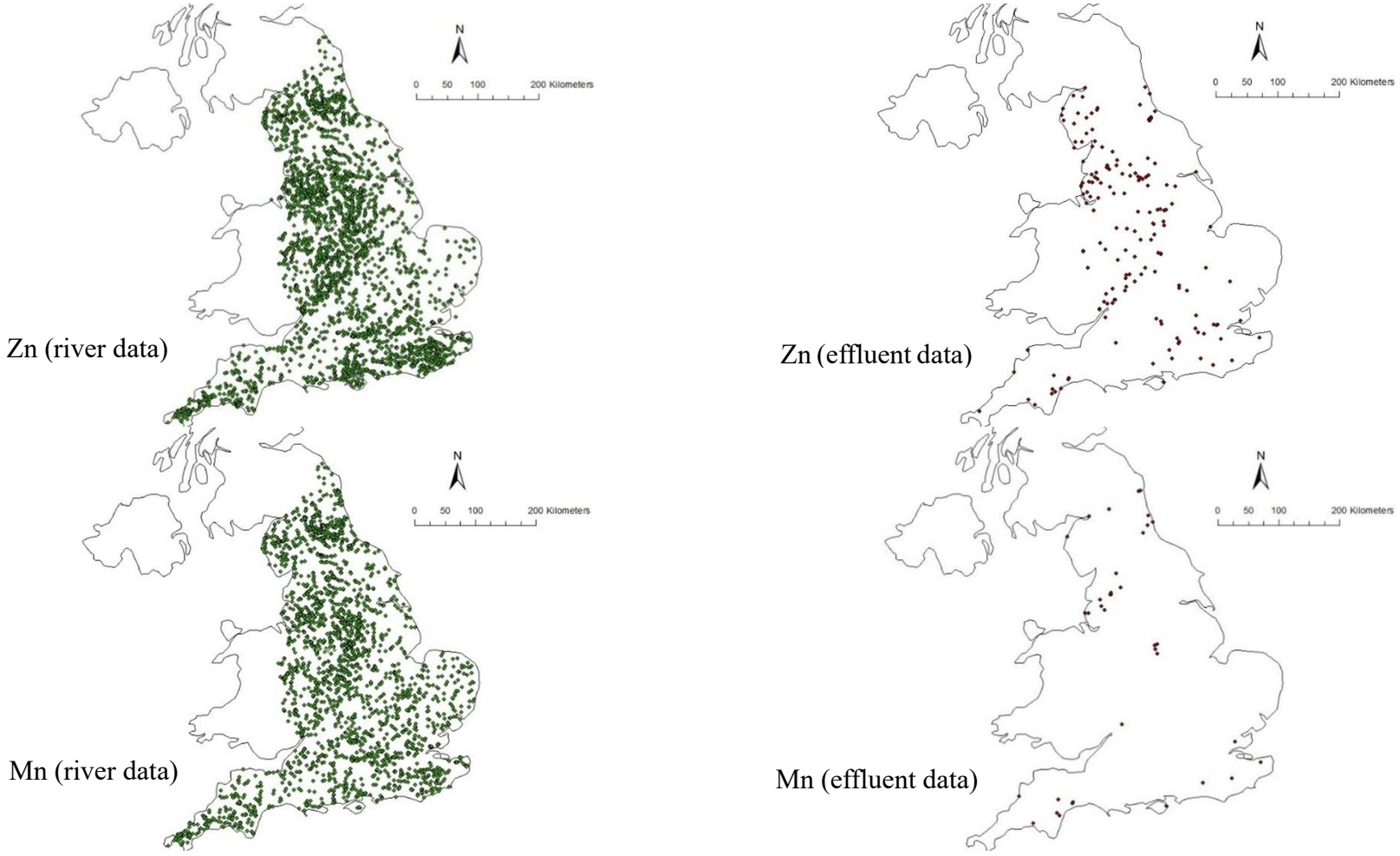


Figure5.1(d). Map each determinant for river sampling data and final effluent data.

5.3.1 Inter-determinand Correlation

The correlation matrix (Table 5.2) demonstrates that significant positive correlations ($p < 0.05$) exist between most pairs of metals, with the exceptions of Mg vs. Cd, Ca vs. Cu, and Mg vs. Zn. Among the strongest correlations, Cd and Zn show the highest association ($r = 0.84$; p-values < 0.000), followed by Ni and Mn ($r=0.68$; p-values < 0.05), Ni and Zn ($r=0.65$; p-values < 0.05), and Cu and Fe ($r=0.61$, P-values < 0.05). Overall, significant correlations ranged from $r = 0.02$ to $r = 0.84$. In contrast, no statistically significant correlation was detected between Mg and Cd, Ca and Cu, or Mg and Zn.

Table 5.2. Correlation coefficients between determinands (range: -1 to 1) and their corresponding p-values.Statistically significant coefficients ($p < 0.05$) are highlighted in grey.

Determinand (unit)	Ca (mg/L)	Mg (mg/L)	Cd (ug/L)	Cu (ug/L)	Ni (ug/L)	Fe (ug/L)	Zn (ug/L)	Mn (ug/L)
Ca (mg/L)		0.22	0.04	-0.01	0.02	0.06	0.02	0.09
Mg (mg/L)			0.01	0.02	0.12	0.06	0.01	0.17
Cd (µg/L)				0.19	0.33	0.15	0.84	0.17
Cu (µg/L)					0.56	0.61	0.52	0.48
Ni (µg/L)						0.39	0.65	0.68
Fe (µg/L)							0.37	0.40
Zn (µg/L)								0.43

5.3.2 Principal Components Analysis

In this chapter, all river data including the river and sewage data of Ca, Mg, Cd, Cu, Ni, iron, Zn, and Mn from 2000 to 2020 England were collated. All the data was merged on the basis of being sampled on the same day at the same site: A total of 19393 river data points were found, which include 1151 sites on 95 rivers. In contrast, for the final effluent data, there were only 20 data points, which include 8 sites on 4 rivers. Note that the PCA dataset was constructed per site and included only those dates where all selected determinands were available for that site. That is, the date-determinand matching was done separately for each site, rather than requiring a common date across all sites. For example, at Site A, only dates with complete values for all 8 determinands (Ca, Mg, Cd, Cu, Ni, Fe, Zn, Mn) were retained; this filtering was repeated per site. The final PCA dataset of 19393 river data points thus represents the combined total across all sites after this filtering.

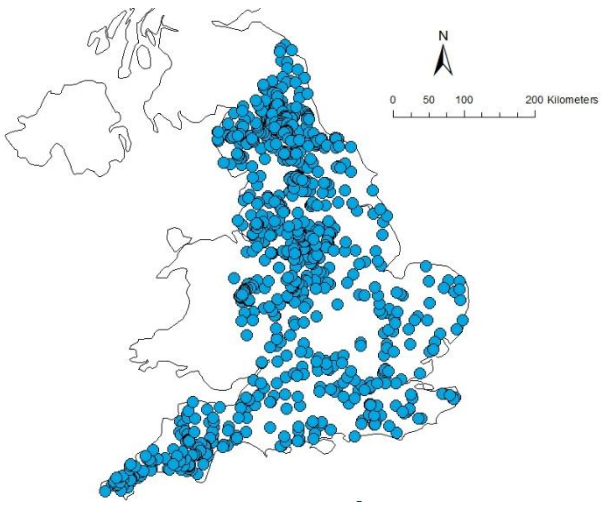


Figure 5.2. The location of the sampling sites that could be included in the PCA.

The map (Figure 5.2) shows the spatial distribution of the sites across England that could be used in the PCA. The distribution highlights the coverage of the dataset, ensuring a comprehensive assessment of metal contamination patterns in relation to STWs.

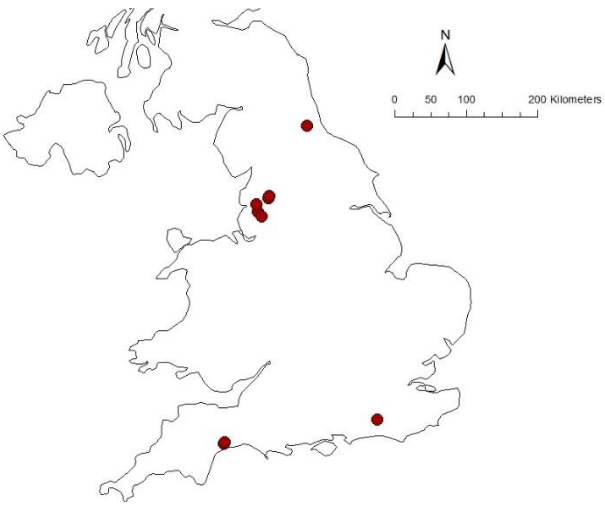


Figure 5.3. Location of the sewage sites that could be used in this PCA.

The map (Figure 5.3) displays the geographical distribution of the sewage treatment works (STW) included in the PCA analysis, i.e. where all the metals were analysed together on the same day. As can be seen from Figure 5.3, it is rare that all metals of interest are sampled simultaneously at the same site, which limits the spatial coverage and comparability of the dataset. The spatial distribution suggests a concentration of studied STWs in northern and southwestern England, highlighting regional differences in effluent characteristics and potential influences on receiving water bodies.

River data from all years

Three out of the eight components had eigenvalues greater than 1, collectively explaining 73% of the original variance. PC1 explains 42% of the variance and has the highest loadings from Ni, Cu, and Zn. PC2 accounts for 16% of the total variance and has the highest loading for Mg, followed by Ca and Mn. PC3 accounts for 15 % of the original variance, and Ca has the highest loading, followed by Fe (Table 5.3).

Table 5.3. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1	PC2	PC3
Ca (mg/L)	0.04	-0.44	-0.53
Mg (mg/L)	0.07	-0.55	-0.42
Cd (µg/L)	0.32	0.49	-0.49
Cu (µg/L)	0.42	-0.11	0.34
Ni (µg/L)	0.46	-0.07	0.06
Fe (µg/L)	0.35	-0.21	0.32
Zn (µg/L)	0.47	0.35	-0.25
Mn (µg/L)	0.4	-0.28	0.11
Eigenvalue	3.32	1.29	1.17
Percentage of variance explained	42%	16%	15%

The PCA biplot of PC2 vs PC1 (Figure 5.4) shows the relationships between metal determinands and their contributions to the principal components. PC1 is predominantly influenced by metals such as Mn, Fe, Cu, and Ni.

PC2 appears to reflect a gradient between natural water chemistry and anthropogenic metal enrichment. It is defined by a contrast between positive loadings for Cd and Zn—often associated with anthropogenic sources such as urban runoff or industrial discharge—and negative loadings for Ca and Mg, which are typical of natural background water chemistry. Thus, PC2 differentiates between sites with elevated anthropogenic contamination (high Cd and Zn) and those with more geochemically buffered water (high Ca and Mg).

Figure 5.4 suggests that there are four to five distinct sources of metals in rivers and one common source. The common source plots at the origin and then a series of apparent mixing lines with other sources being presented by high positive values of PC2; high negative values for PC2; and then several potential high PC1 sources. Figure 5.4 then appears as a series of mixing lines spreading out from the origin.

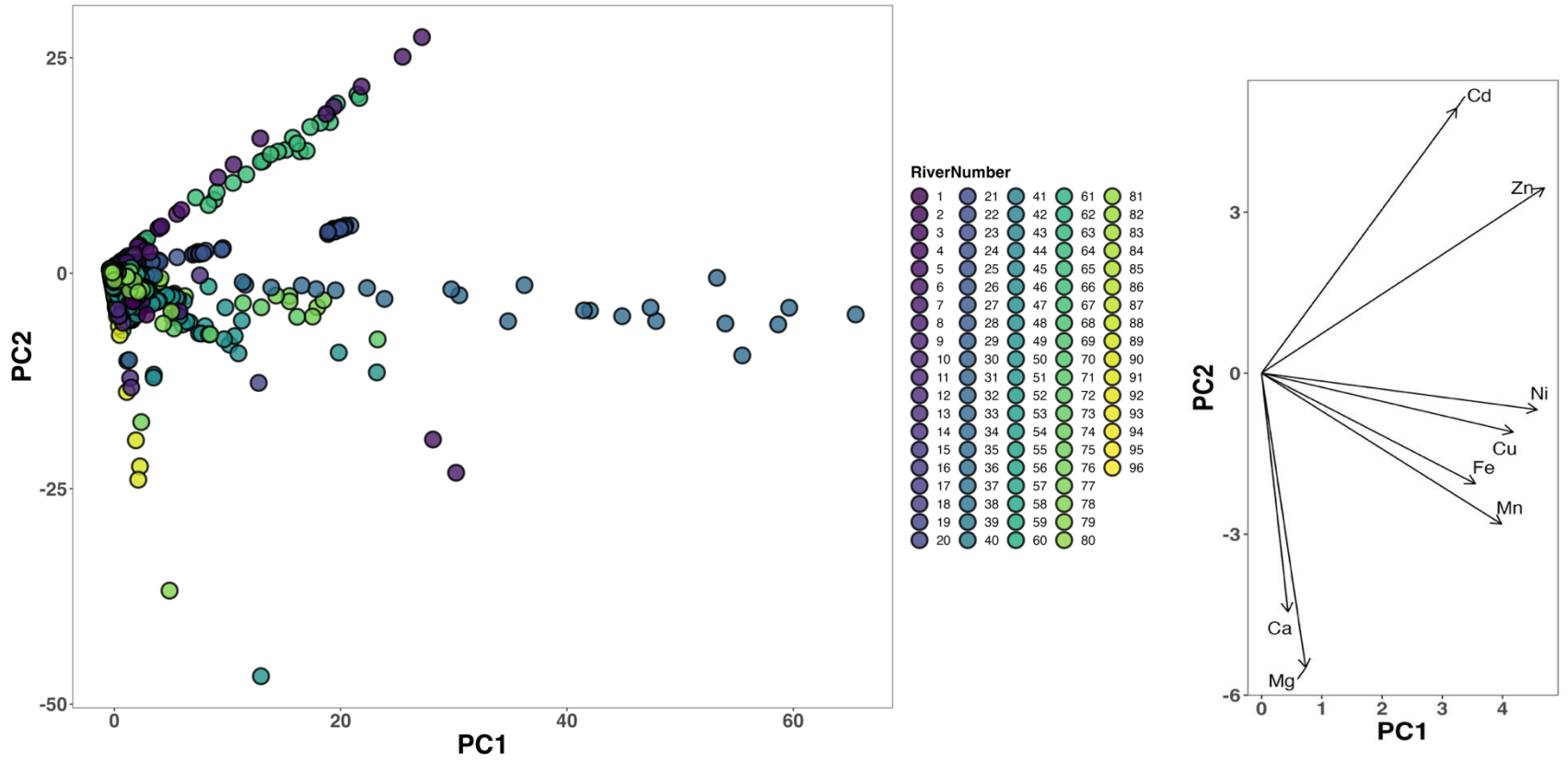


Figure 5.4. PCA plot and loading for PC1 and PC2. The list of rivers with their corresponding numbers is as follows:1--Avon, 2--Duddon, 3--Eden, 4-- Ouse, 5--Solent, 6--Adur, 7--Aire, 8--Aln, 9--Alt, 10--Annas, 11--Arun, 12--Avon, 13--Axe, 14--Blyth, 15--Brue, 16--Bure, 17-Camel, 18--Cober, 19--Colne, 20--Coquet, 21--Crake, 22--Crouch, 23--Cuckmere, 24--Dart, 25--Derwent, 26--Derwent, 27--Don, 28--Eden, 29--Ehen, 30--Ellen, 31--Esk, 32--Exe, 33--Fal, 34--Fowey, 35--Frome, 36--Gannel, 37--Hayle, 38--Heddon, 39--Helford, 40--Holywell Stream, 41--

Hornsea Mere, 42--Humber estuary, 43--Itchen, 44--kent estuary, 45--Leven, 46--Lune, 47--Medway, 48--Mersey, 49--Mount's Bay, 50--Nene, 51--Orwell, 52--Otter, 53--Ouse, 54--Par, 55--Parrett, 56--Plym, 57--Portreath Harbour, 58--Red, 59--Ribble, 60--Roach, 61--Rother, 62--Rother, 63--Seaton, 64--Severn, 65--Sid, 66--Skelton Beck, 67--Solent, 68--St Austell Bay, 69—Stiffkey, 70--Stour, 71--Tamar, 72--Tamar, 73--Taw, 74--Tees, 75--Teign, 76--Test, 77--Thames, 78--Torridge, 79--Trent, 80--Trent, 81--Tweed, 82--Tyne, 83--Ure, 84--Ure, 85--Wallington,86--Wampool, 87--Wansbeck, 88--Waveney, 89--Weaver, 90--Welland, 91--Witham, 92--Wye, 93--Wyre, 94--Yare, 95--Yealm.)

Final effluent data from all years

Among the eight principal components analysed, three had eigenvalues greater than 1, collectively explaining 82% of the total variance (Table 5.4). The first principal component (PC1) accounts for 39% of the variance, with the highest positive loading for Mn, but with high negative loadings for Ca and Mg. The second principal component (PC2) explains 25% of the variance, with the highest loadings on Zn, followed by Mn. The third principal component (PC3) accounts for 18% of the variance, with the highest loadings on Cu, followed by Mg, as shown in Table 5.4.

Table 5.4. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1	PC2	PC3
Ca (mg/L)	-0.56	-0.11	-0.23
Mg (mg/L)	-0.56	0.19	0.18
Cd (µg/L)	0	0	0
Cu (µg/L)	-0.48	0.23	0.42
Ni (µg/L)	0	0	0
Fe (µg/L)	-0.26	-0.13	-0.81
Zn (µg/L)	-0.06	0.73	-0.19
Mn (µg/L)	0.27	0.59	-0.2
Eigenvalue	2.49	1.57	1.16
Percentage of variance explained	39%	25%	18%

The PCA biplot of PC2 vs. PC1 (Figure 5.5) illustrates the relationships between metal determinands and their contributions to the principal components. PC1 is primarily driven by metals such as Mn, Fe, Cu, and Ni. PC2 reflects a compositional gradient between anthropogenic enrichment and geochemical background conditions, with positive loadings for Cd and Zn—indicative of potentially untreated or industrial waste inputs—and negative loadings for Ca and Mg, which are commonly associated with natural water hardness. Sites with high PC1 scores exhibit generally elevated metal concentrations, while those with high PC2 scores are characterised by enhanced levels of Cd and Zn and reduced Ca and Mg. Figure 5.5 represents a single mixing line, with the dominant variation being in the concentrations of Fe, Mn, Cu, and Ni, rather than in Ca, Mg, Cd, or Zn.

Sites such as Horwich STW generally exhibit higher PC2 scores, indicating elevated levels of Cd and Zn, while Browney STW is associated with higher concentrations of Cu and Ni. In contrast, Walton Le Dale STW is characterised by elevated levels of Fe and Mn. These patterns highlight site-specific variations in effluent composition, reflecting differences in wastewater treatment processes and potential sources of metal contamination in the influent water (Figure 5.6).

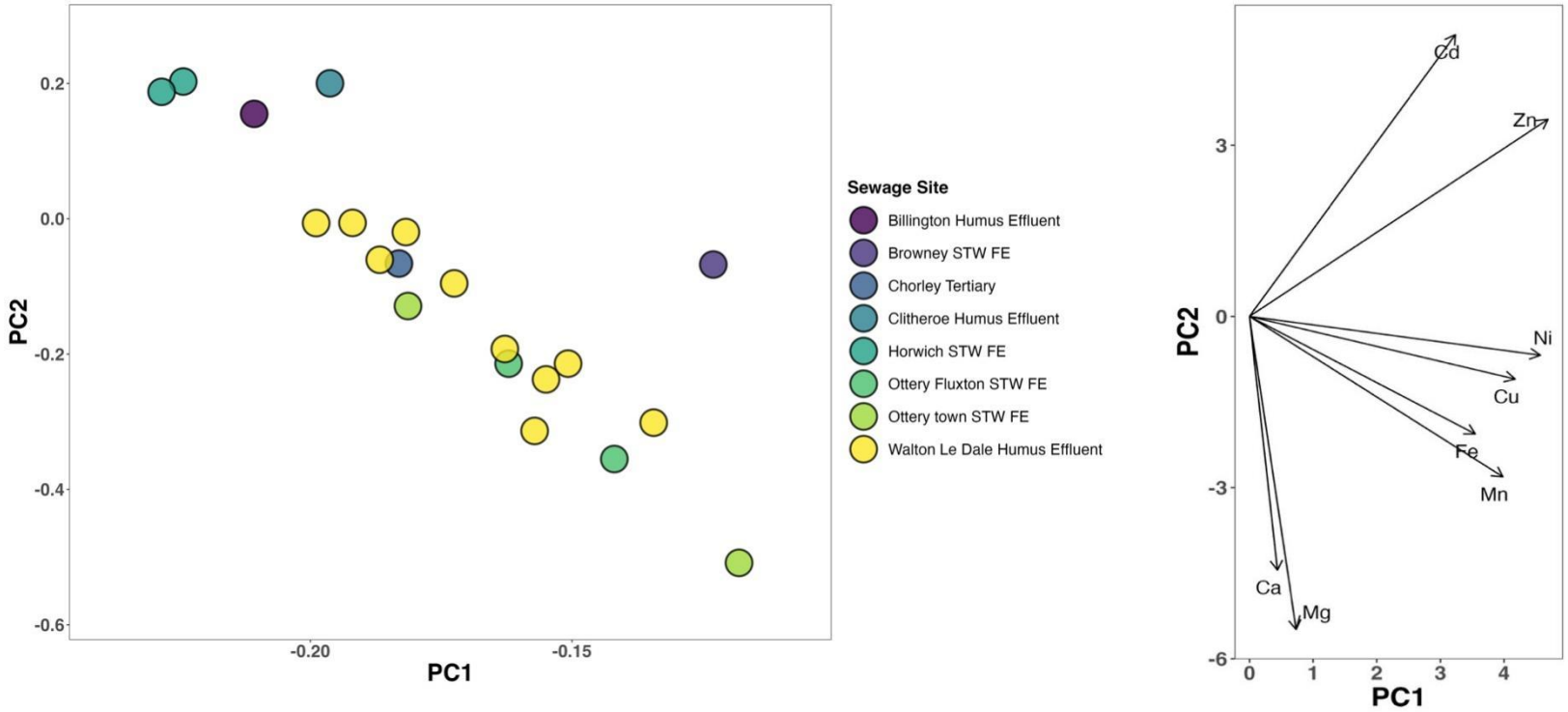


Figure 5.5. The scatter plot of the final effluent scores on PC1 and PC2.

Combined data from all years (rivers + final effluent)

Among the eight determinands analysed, three principal components had eigenvalues greater than 1, collectively explaining 73% of the total variance (Table 5.5). The first principal component (PC1) accounts for 42% of the variance, with the highest loadings on Zn (0.47), followed by Ni (0.46), Cu (0.42), and Mn (0.40), indicating strong associations with metals linked to industrial discharges. The second principal component (PC2) explains 16% of the variance, with the highest loadings on Mg (0.55) and Ca (0.44), reflecting geogenic contributions likely associated with natural background waters. The third principal component (PC3) accounts for 15% of the variance, with the highest loadings on Ca (0.53), followed by Cd (0.49) and Mg (0.42).

Table 5.5. Loading of each determinand on each principal component with eigenvalue > 1.

Determinand	PC1	PC2	PC3
Ca (mg/L)	0.04	0.44	0.53
Mg (mg/L)	0.07	0.55	0.42
Cd (µg/L)	0.32	-0.49	0.49
Cu (µg/L)	0.42	0.11	-0.34
Ni (µg/L)	0.46	0.07	-0.06
Fe (µg/L)	0.35	0.21	-0.32
Zn (µg/L)	0.47	-0.35	0.25
Mn (µg/L)	0.4	0.28	-0.11
Eigenvalue	3.32	1.29	1.17
Percentage of variance explained	42%	16%	15%

It is perhaps not surprising that the PCA biplot of PC2 vs PC1 for the PCA performed on the combined river and STW dataset (Figure 5.6) appears very similar to that of the river data only PCA (Figure 5.4), as the combined dataset dominantly consists of the river data. However, what is clear is that the STW data plots at the

apex of the trends in the river data implying that STW final effluent is a common endmember for river metal concentrations.

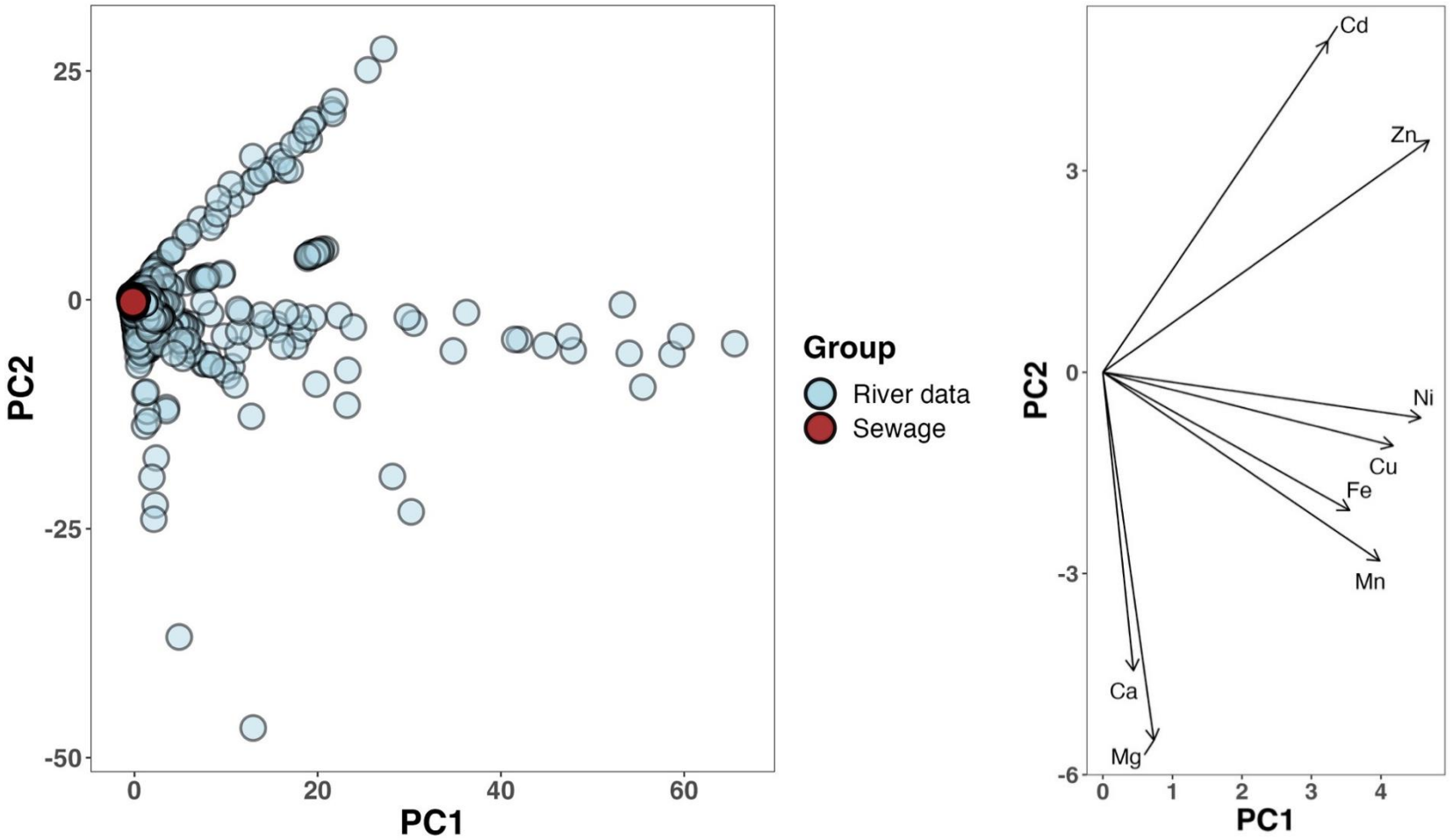


Figure 5.6. PCA and loading plot for both River data and Sewage.

5.3.3 Metal bioavailability

This section aims to assess the bioavailability of four metals (Cu, Zn, Mn, Ni) (Figure5.7) in aquatic environments across England and so assess the importance of sewage effluent in comparison to other sources of pollution.

Summary data analysis of bioavailable metals

The number of samples and sites for which the bioavailable metal concentration could be calculated are detailed in Table 5.6, and Table 5.7 shows the data without the STW upstream and downstream data used in Chapter 3.

Table 5.6. Summary of the dataset N represents the total number of sampling points recorded at different times.

Bioavailable metal(unit)	N	N of Sites	Median RCR	Proportion of RCR >1	Dissolved concentration
Cu (µg/L)	33756	1546	0.1	0.001	0.175 ~ 430
Zn (µg/L)	20148	1477	4.28	0.67	0.5 ~54600
Mn (µg/L)	10542	1708	0.13	0.16	10 ~ 12200
Ni (µg/L)	33673	1443	0.15	0.03	0.5 ~ 181

Table 5.7. Summary of the dataset without the sampling sites that were previously included in Chapter 3.

Bioavailable metal(unit)	N without sampling points in Chapter 3	N of Sites	Median RCR	Proportion of RCR >1	Dissolved concentration
Cu (µg/L)	33328	1455	0.1	0.01	0.175 ~ 430
Zn (µg/L)	19723	1389	4.34	0.67	0.5 ~54600
Mn (µg/L)	10262	1642	0.13	0.17	10 ~ 12200
Ni (µg/L)	33201	1359	0.15	0.03	0.5 ~ 181

Among the metals for which the bioavailable concentration could be calculated, Zn had a median RCR of 4.28, which is the only bioavailable metal with a median RCR above 1, while Zn showed the highest proportion of samples with RCR >= 1 (67%). Cu showed the lowest proportion of samples with RCR >= 1 at 0.1% (Table 5.6). When the STW upstream and downstream data used in Chapter 3 were excluded from the analysis (Table 5.7), the proportion of Cu samples exceeding RCR >1 increased from 0.001 to 0.01 compared to the full river dataset analysis (Table 5.6), representing a tenfold increase. The increase in Cu exceedances after removing STW upstream and downstream sites indicates that these locations likely influenced metal concentrations, possibly through dilution or localised retention effects. This finding suggests that STW effluent primarily affects water quality at a local scale rather than driving widespread contamination across the entire river network.

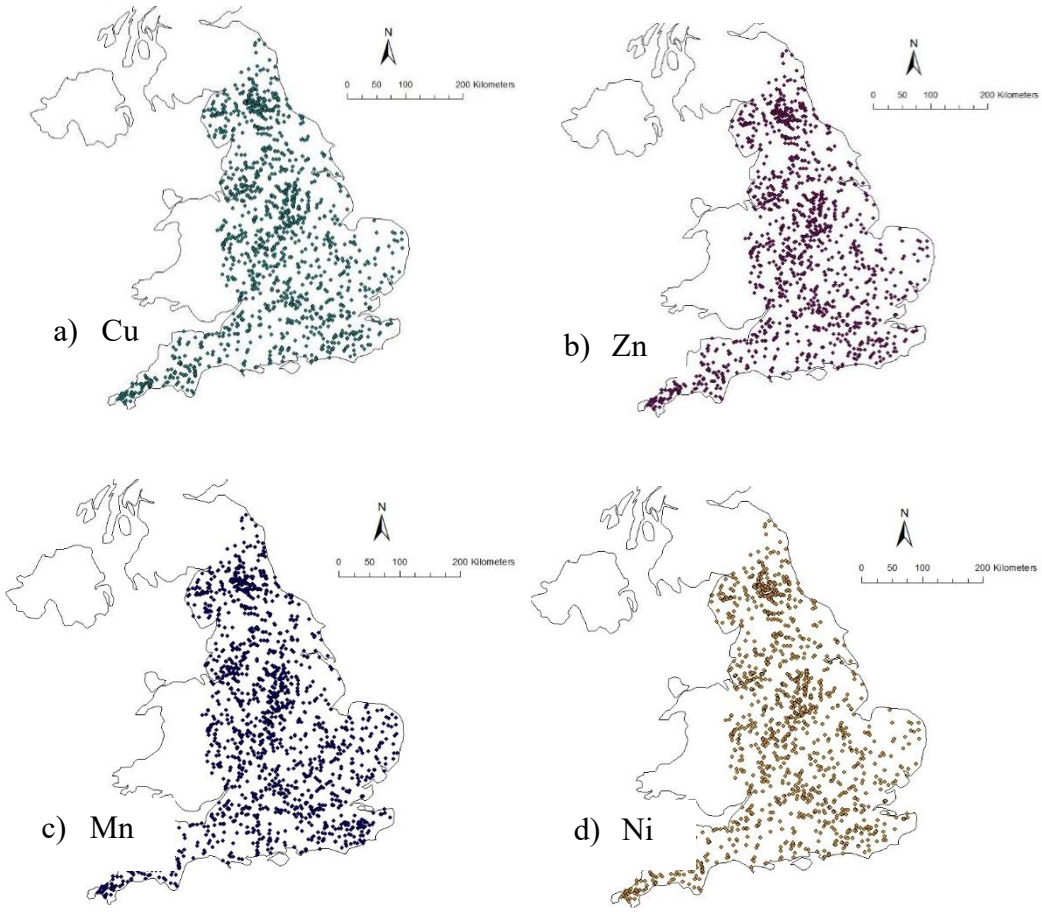


Figure 5.7. Locations of monitoring sites for each determinand at which M-BAT was used to calculate bioavailable concentrations.

Analysis of bioavailable metal concentrations using the M-BAT model framework (Table 5.8) incorporated z-transformation of all predictors and response variables to enable cross-parameter comparability of effect sizes. Statistically significant relationships were identified between bioavailable Cu, Zn, Mn, and Ni and key water chemistry variables, including pH, dissolved organic carbon (DOC), Ca, and the corresponding dissolved metal concentrations.

Comparison with the more localised STW-impacted dataset in Chapter 3 (Table 3.15) reveals both consistencies and divergences in predictor strength. Dissolved metal concentrations remained the dominant explanatory variable across all metals, with consistently high partial R^2 values (e.g., Zn remained stable at 0.92–0.98, and Cu increased from 0.62 to 0.76), highlighting a robust and scale-independent relationship between dissolved and bioavailable metal fractions.

In contrast, the explanatory power of other water chemistry variables declined at the national scale. For example:

- The partial R^2 for Zn and DOC decreased from 0.0054 in Chapter 3 to 0.0002 in Chapter 5.
- For Ni, the partial R^2 associated with DOC increased modestly from 0.0053 to 0.0220, while the contribution of Ca remained relatively stable (0.0018 to 0.0022).
- The influence of pH became less pronounced, with partial R^2 values for Cu and Zn declining (Cu: 0.012 → 0.0001; Zn: 0.0007 → 0.0001).

At broader spatial scales, DOC, Ca, and pH appear to exert weaker predictive effects compared to those observed under more localised STW influences. By contrast, dissolved metal concentrations consistently explained the majority of variance in bioavailable metals, reinforcing their importance in both local and national assessments.

Overall, the findings from Chapter 5 confirm that dissolved metal concentrations are primary predictors of bioavailable metal risk across spatial scales, whereas the predictive roles of pH, DOC, and Ca exhibit greater context dependency. These correlations were undertaken to clarify the relative influence of water chemistry determinands on bioavailability and to evaluate whether the effects of pH, DOC, and Ca on bioavailable metal concentrations identified in Chapter 3 at site-specific STW and control locations are also evident at the national scale, as examined in Chapter 5. The observed context dependency suggests that while some predictors exert strong universal influence, others are modulated by catchment-specific factors, supporting the chapter's aim to identify both generalisable trends and regionally contingent mechanisms in bioavailable metal distribution.

Table 5.8. The summary of the regression analysis for each determinand Bioavailable metal. The significance for each determinand is stated as Yes if it was significant at a probability < 0.05 of being zero. Partial R^2 indicates the extent to which a factor contributes to the observed variability in the variable.

Bioavailable metal(unit)	pH		DOC		Ca		Dissolved concentration		pH*DOC		pH*Ca		DOC*Ca	
	Sig	partial R^2	Sig	partial R^2	Sig	partial R^2	Sig	partial R^2	Sig	partial R^2	Sig	partial R^2	Sig	partial R^2
Cu (µg/L)	Yes	0.0001	Yes	0.0011	Yes	0.0014	Yes	0.7637	Yes	0.0000	Yes	0.0000	Yes	0.0006
Zn (µg/L)	Yes	0.0001	Yes	0.0002	Yes	0.0001	Yes	0.9803	Yes	0.0001	Yes	0.0000	Yes	0.0001
Mn (µg/L)	Yes	0.0464	Yes	0.0010	--	0.0015	Yes	0.5458	Yes	0.0192	Yes	0.0088	Yes	0.0001
Ni (µg/L)	Yes	0.0102	Yes	0.0220	Yes	0.0022	Yes	0.7103	Yes	0.0052	Yes	0.0164	Yes	0.0014

Comparison between the mean dissolved concentration and the mean bioavailable metal concentration

Figure 5.8 shows that bioavailable metal concentrations (BioCu, BioZn, BioMn, BioNi) are consistently lower than their corresponding dissolved metal concentrations (Cu, Zn, Mn, Ni). Specifically, the bioavailable concentrations are approximately 15% for Cu, 50% for Zn, 20% for Mn, and 30% for Ni of the dissolved concentrations. Note that the narrow 95% confidence intervals suggest that the variation within each group was low, and that the large sample size and consistent data quality contributed to high precision in the estimated means.

One key finding is that Zn has the highest bioavailable fraction (50%) compared to the other metals, suggesting that Zn remains more available in river systems despite variations in water chemistry. In contrast, Cu has the lowest bioavailable fraction (15%), likely due to strong complexation with dissolved organic matter, which limits its free ion availability. Similarly, the relatively low bioavailability of Mn and Ni (20% and 30%, respectively) suggests that their speciation is influenced by sorption to particulates or precipitation of carbonates. These differences highlight that bioavailability is not solely dictated by total dissolved metal concentrations but also by chemical interactions that govern metal partitioning.

The distinction between total dissolved and bioavailable concentrations is crucial when assessing the risks of metal pollution. Metals with a higher bioavailable fraction, such as Zn, pose a greater potential for biological uptake and toxicity, even at lower total concentrations, whereas metals like Cu may be less bioavailable despite higher dissolved concentrations. These findings emphasise the importance of assessing both dissolved and bioavailable fractions when evaluating the ecological risks of metal pollution, particularly in regulatory frameworks that consider bioavailability in water quality standards.

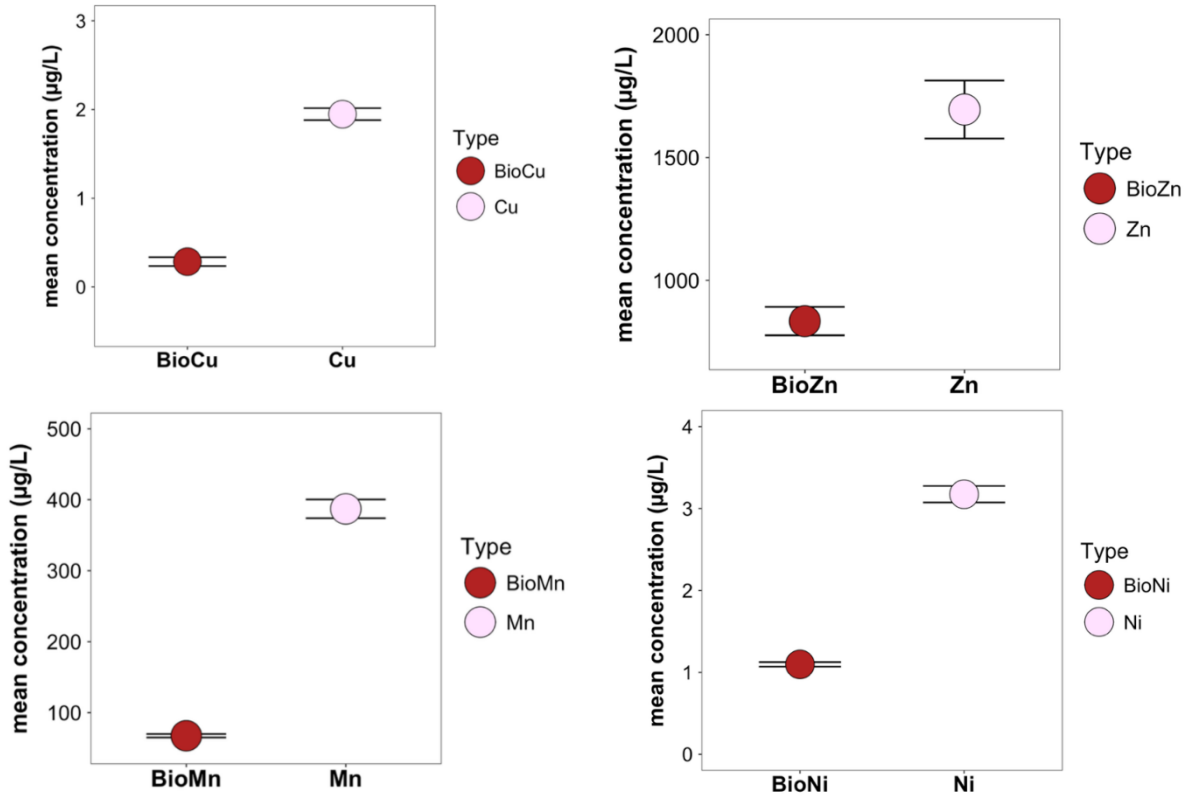


Figure 5.8. Comparison of dissolved metal concentration mean & bioavailable metal concentration mean. The values are presented as the marginal mean with the 95% confidence limits on that mean.

RCR greater than 1 subset

Regression analysis was conducted on the high-risk subset (RCR ≥ 1) to evaluate the influence of pH, dissolved organic carbon (DOC), Ca, and dissolved metal concentrations on the bioavailability of Cu, Zn, Mn, and Ni (Table 5.9(a); Table 5.9 (b)). Number of the sampling points and number of sites are presented in Table 5.9 (a). All metals showed statistically significant relationships with at least some predictors, although not all covariates or interaction terms reached significance.

pH emerged as a stronger driver of bioavailable metal concentrations in the RCR ≥ 1 subset compared to the full dataset. It was particularly important for Mn (partial $R^2 = 0.36$) and, to a lesser extent, for Ni (partial $R^2 = 0.04$). These values were substantially higher than those observed at the national scale, highlighting the amplified role of acid–base conditions under high-risk contamination scenarios.

DOC remained a statistically significant predictor for all metals, although its explanatory power varied across different metals. It contributed moderately to Cu (partial $R^2 = 0.02$) and Ni (0.07) but had minimal influence on Zn (0.0007) and Mn (0.003). The increased partial R^2 for Cu and Ni relative to the full dataset suggests that DOC-mediated complexation may be more relevant at highly contaminated sites.

Ca showed metal-specific effects. It remained a significant predictor for Zn, Mn, and Ni, but not for Cu. For Mn (partial $R^2 = 0.05$) and Ni (0.02), Ca contributed more strongly to the high-risk subset than in the whole dataset, implying a possible role of ionic competition or precipitation processes under elevated metal burdens.

Dissolved metal concentration continued to explain the greatest variance for Zn (partial $R^2 = 0.98$) and Cu (0.80), affirming its dominant role in predicting bioavailable fractions. However, its explanatory power was reduced for Mn (0.27) and Ni (0.50) compared to the full dataset, suggesting that additional environmental factors gain influence at high-risk sites.

Interaction terms (pH*DOC, pH*Ca, DOC*Ca) generally exhibited higher partial R^2 values in the RCR ≥ 1 subset than in the full dataset. For Mn and Ni, interactions between pH and DOC or Ca explained an additional 2–5% of the variance, indicating that interactive chemical processes are more pronounced in

high-contamination settings. However, not all interactions were statistically significant, particularly for Cu (pH*DOC, pH*Ca) and Zn (pH*Ca).

Table 5.9(a). The number of RCR >= 1 metal and its linear model relationship with pH, DOC, Ca and Dissolved metal. N represents the whole sampling number, and N of sites represents the number of sites.

Bioavailable metal(unit)	N	N of sites	pH		DOC		Ca		Dissolved concentration	
			Sig	partial R^2	Sig	partial R^2	Sig	partial R^2	Sig	partial R^2
Cu (µg/L)	336	79	Yes	0.0064	Yes	0.0195	--	0.0044	Yes	0.7953
Zn (µg/L)	13457	415	Yes	0.0002	Yes	0.0007	Yes	0.0006	Yes	0.9773
Mn (µg/L)	1721	103	Yes	0.3564	Yes	0.0034	Yes	0.0453	Yes	0.2722
Ni (µg/L)	940	89	Yes	0.0358	Yes	0.0745	Yes	0.0169	Yes	0.4986

Table 5.9(b). The number of RCR >= 1 metal and its linear model relationship with pH, DOC, Ca and Dissolved metal. N represents the whole sampling number, and N of sites represents the number of sites.

Bioavailable metal(unit)	pH*DOC		pH*Ca		DOC*Ca	
	Sig	partial R^2	Sig	partial R^2	Sig	partial R^2
Cu (µg/L)	--	0.0013	--	0.0004	Yes	0.0091
Zn (µg/L)	Yes	0.0001	--	0.0000	Yes	0.0001
Mn (µg/L)	Yes	0.0279	Yes	0.0545	Yes	0.0027
Ni (µg/L)	Yes	0.0064	Yes	0.1219	--	0.0036

Spatial distribution of sites with RCR > 1

The spatial distribution of those sites where RCR >= 1 is shown for each metal in Figure 5.9, while Figure 5.10 displays the locations of all sites where RCR ≥ 1 for any metal. These maps should be compared to Figure 5.7, which shows all the sites where sufficient data were available to calculate the bioavailable concentration. This comparison illustrates that the risk from bioavailable metal concentration is geographically restricted.

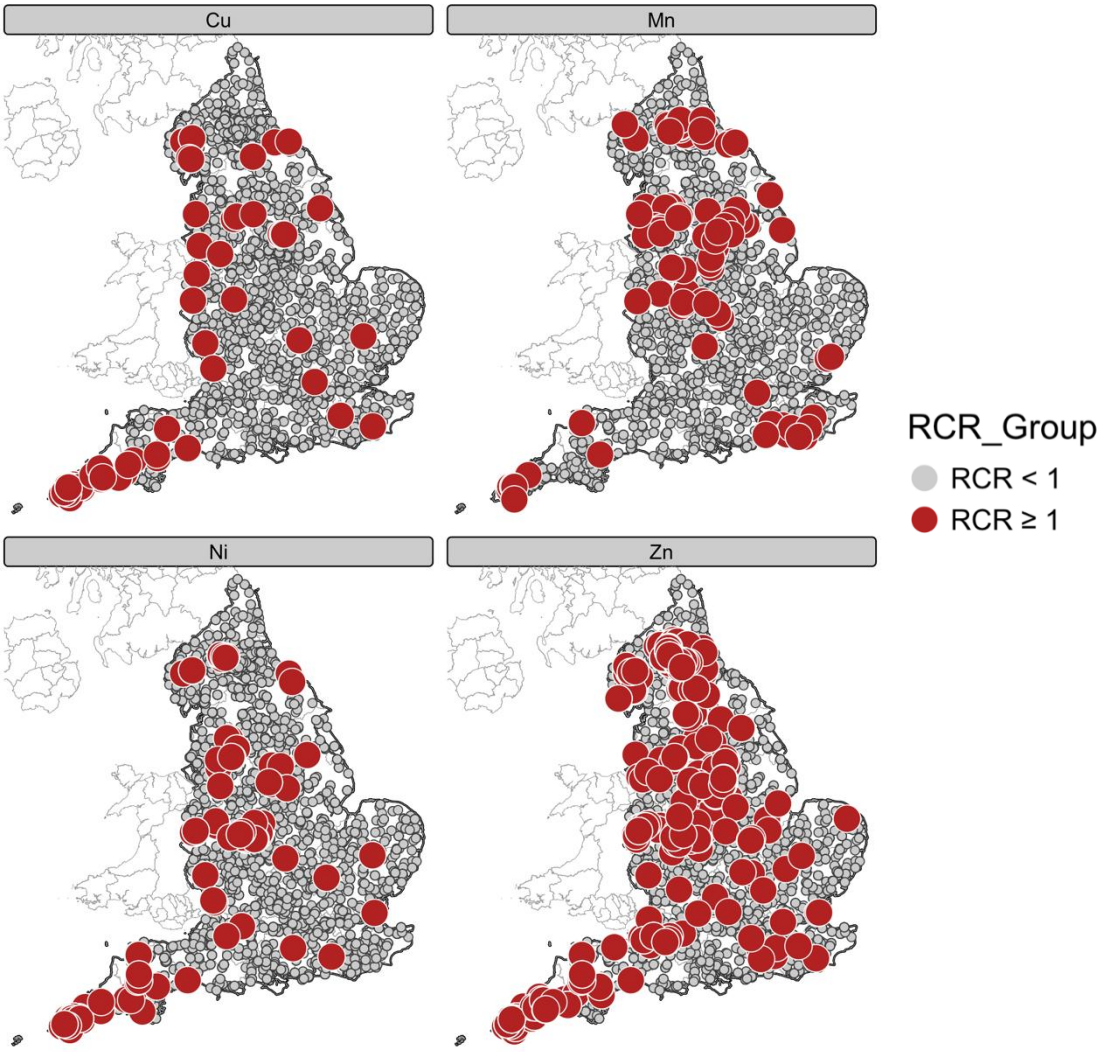


Figure 5.9. The red points are sites where RCR > 1, and the grey points are sites where RCR <1.

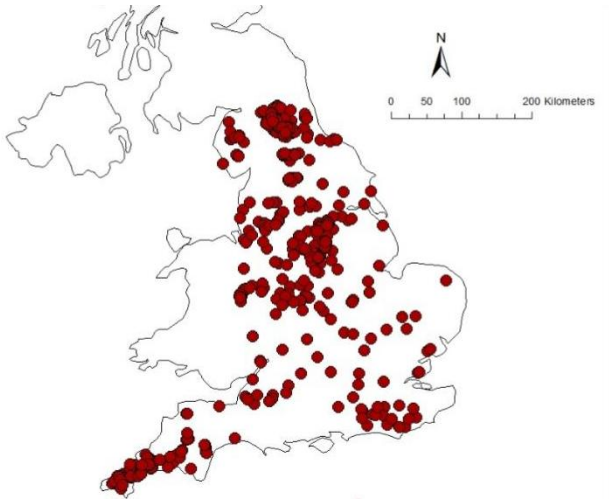


Figure 5.10. The location of all sites where RCR > 1 site for all metals.

Assessment of RCR Exceedance Across Study Areas for Bioavailable Metals

The spatial distribution of sites shown in Figure 5.10 may represent population density, with a greater number of sites having RCR > 1 in areas of higher density, and therefore more STWs. To make a fairer comparison the sites were matched to English council districts based up on their coordinates. The table below (Table 5.10) summarises the distribution of areas where RCR > 1 for four different metals:

- Zn: Poses the least concern among the four metals, with 79.6% of council districts showing RCR < 1, indicating a relatively low pollution risk across most regions.
- Ni: Stands out as the most prevalent contaminant, with only 48.1% of council districts having an RCR < of 1, making it the most significant concern in terms of pollution.
- Mn and Cu Exhibit similar patterns, with approximately 70% of council districts (73.3% for Mn and 71.7% for Cu) having an RCR < of 1, indicating moderate pollution levels.

These results highlight that Ni contamination is the most widespread issue, while Zn poses the least risk.

Table 5.10. Proportion of council districts with RCR < 1 for bioavailable metals.

Bioavailable metal	Total	RCR < 1	Percentage RCR < 1
Cu (µg/L)	138	99	71.7
Zn (µg/L)	137	109	79.6
Mn (µg/L)	135	99	73.3
Ni (µg/L)	135	65	48.1

Risk Characterisation of Bioavailable Metals

Spatially Normalised Exposure

To enhance comparability between council districts, site-level RCR > 1 exceedances were aggregated per region and normalised by both the population of each county and the area of each county using a single composite metric: the per capita per km² ratio. This indicator reflects the demographic exposure burden relative to the spatial density of pollution, providing a more equitable basis for inter-regional comparison. If sewage effluent were the only source of RCR > 1, then we would expect an even distribution of RCR occurrences per population density.

Summary statistics for all metals for all council districts are given in Tables A1 to A4

To evaluate whether the spatial distribution of RCR > 1 exceedance events deviated significantly from an even demographic-based expectation, a chi-square test was conducted for each bioavailable metal. The total number of observed exceedances was compared against the expected counts, which were calculated by proportionally distributing the national total of RCR > 1 events based on the population of each administrative area.

Results, summarised in Table 5.11, indicate that for all four metals—Cu, Zn, Mn, and Ni—the observed distributions differ significantly from their expected values (χ^2 ranging from 1,868 to 404,823, all $p < 0.000$). This statistical significance suggests that RCR > 1 occurrences are not evenly distributed across regions in proportion to their population sizes.

Table 5.11. Summary of Chi-square Test Results Comparing Observed and Expected RCR > 1 Exceedance Counts for Bioavailable Metals.

Bioavailable metal	Total Population	Total RCR > 1 (observed)	Total RCR > 1 (expected)	χ^2	P value
Cu (µg/L)	53718496	336	336	1868	< 0.000
Zn (µg/L)	52650096	13457	13457	404823	< 0.000
Mn (µg/L)	53788296	1721	1721	12926	< 0.000
Ni (µg/L)	51688200	940	940	53815	< 0.000

Spatial Allocation of Sewage Treatment Works (STWs) by County

While raw RCR > 1 exceedance maps (Figure 5.11(a) – Figure 5.11(b), left column) visually align with areas of higher STW counts, particularly in the Southwest and Northwest, this apparent association diminishes when demographic normalisation is applied (right column). Normalised exposure burdens (RCR per 1,000 capita per km^2) display a much more even spatial distribution, and no clear correlation is observed between STW density and population-adjusted risk levels. This suggests that STWs, while necessary for explaining point-source discharges, are not the sole drivers of spatial exceedance patterns. Instead, regional variation may reflect differences in population vulnerability, dilution capacity, local hydrochemistry, or diffuse sources beyond STW discharges. However, it is acknowledged that the population figures used in the normalisation process refer to entire administrative regions, which may not spatially coincide with the exact locations of RCR exceedance. In some cases, high RCR values may occur in upstream or rural areas with limited local population, while the overall population of the region is dominated by urban centres unrelated to the contamination site. This potential spatial mismatch should be considered when interpreting population-normalised exposure burdens.

To visualise spatial patterns, choropleth maps were generated showing the distribution of normalised RCR exceedances across counties. Additionally, bubble maps were created, where the bubble size represented the number of STW facilities within each county. Maps were produced using the tmap package in R, with consistent symbology and log-transformed classification intervals to accommodate skewed data distributions

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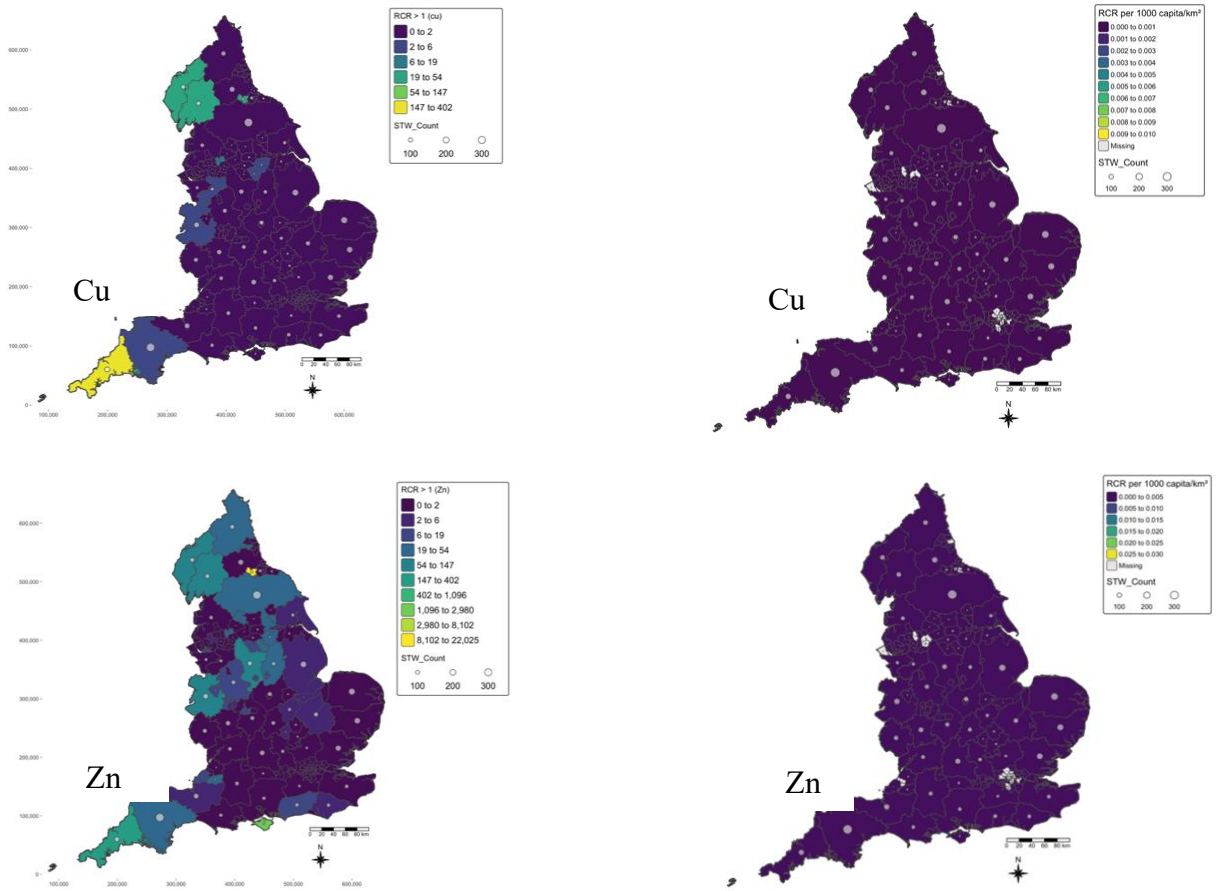

Figure 5.11(a). Comparison of Raw RCR >1 (left column) and Population-Normalised Exposure Burdens（right column）

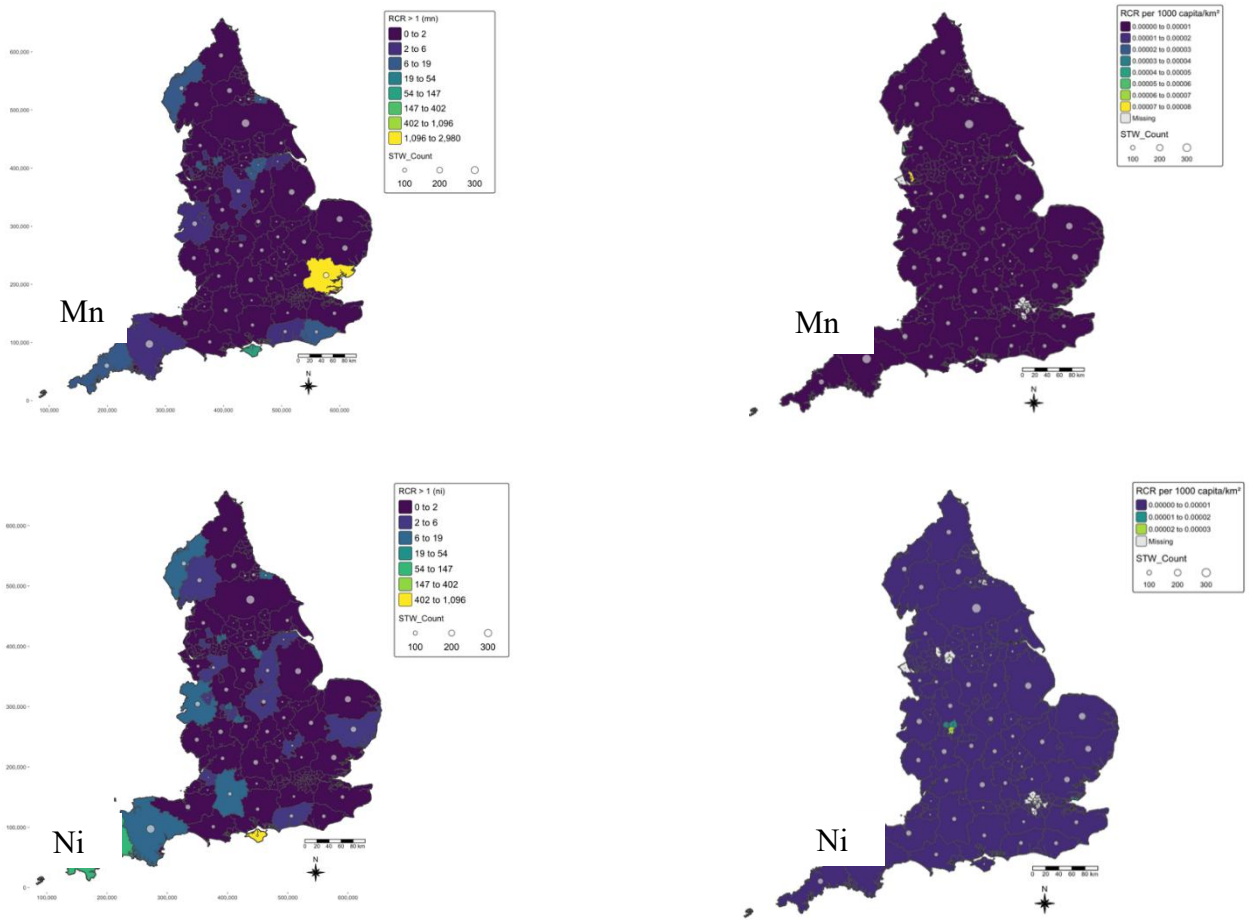

Figure 5.11(b). Comparison of Raw RCR >1 (left column) and Population-Normalised Exposure Burdens（right column）

While the per capita per km² ratio provides a normalised view of population-level exposure intensity, it is also valuable to examine where different bioavailable metals co-occur in exceedance across the same regions. Among the 96 counties where at least one RCR > 1 observation was recorded for any metal, only six counties—Cornwall, Cumberland, Shropshire, Rochdale, Rotherham, and Devon—exhibited exceedances for all four bioavailable metals (Cu, Zn, Mn, and Ni), as indicated by non-zero RCR > 1 ratios for each metal (Table 5.12).

This geographic overlap was identified using proportion-based exceedance metrics, which normalise the number of RCR > 1 observations by the total number of observations for each metal in each county. This approach ensures fairer inter-regional comparisons, accounting for potential variation in data availability or monitoring effort.

Table 5.12. Proportion of sampling records with RCR > 1 for each bioavailable metal in counties exhibiting multi-metal exceedance. Ratios are calculated as the number of exceedance records divided by the total number of observations permetal and county.

Area	Cu_RCR_Ratio	Zn_RCR_Ratio	Mn_RCR_Ratio	Ni_RCR_Ratio
Cornwall	0.14	0.26	0.01	0.08
Cumberland	0.13	0.52	0.04	0.04
Devon	0.01	0.19	0.02	0.09
Rochdale	0.18	0.30	0.13	0.33
Rotherham	0.05	0.35	0.10	0.06
Shropshire	0.03	0.70	0.01	0.07

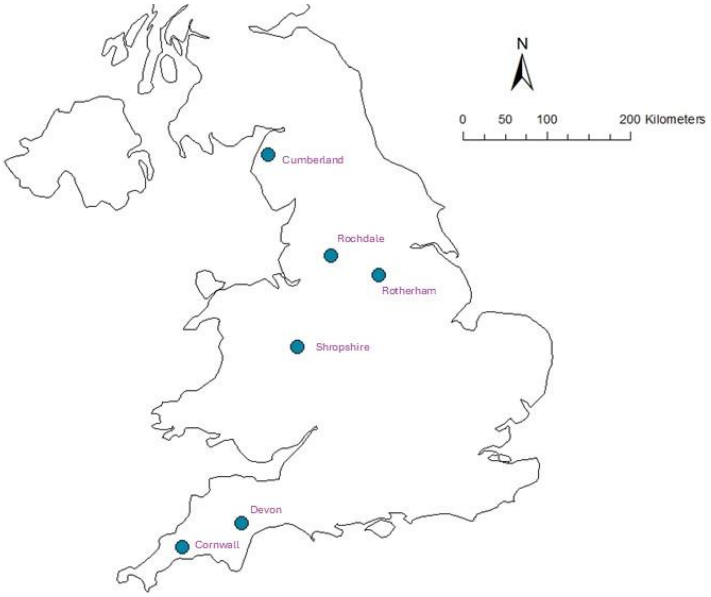

Figure 5.12. The areas which show RCR > 1 for all bioavailable metals.

5.4 Discussion

While previous studies, such as those by Comber et al. (2022), have undertaken large-scale assessments of bioavailable metals at national levels, particularly focusing on the upstream–downstream dynamics of STW effluent, there has been limited research that integrates population-based exposure with spatial pollution density across multiple regions and decades. Most studies have focused on the types of heavy metals and their spatial and temporal distributions. For example, Barak and Mason (1989) examined the concentration changes of Hg, Cd, and Pb in Eastern England, finding that small towns contributed metal contaminants downstream. They also observed that high flows could dilute heavy metal accumulation in aquatic systems. Their study, conducted between September and November 1987, included 15 water samples and 40 sediment samples per site across four study sites. Similarly, Dawson and Macklin (1998) highlighted the importance of understanding the chemical speciation and mobility of heavy metals—Pb, Zn, Cu, and Cd—in river sediments, influenced by the physico-chemical environment. Their research involved 16 samples collected in October 1994, with two sites resampled in February 1995. Zhao and Marriott (2013) found that heavy metal concentrations in floodplain sediments decreased with increasing distance from former mining sites, primarily due to dilution and the breakdown of meta-coarse particles into finer sediments. Near mining sites, heavy metals were associated with coarser sediment grains, whereas further downstream, they were bound to finer particles. Additionally, flooding could alter pH conditions, facilitating the remobilisation and translocation of heavy metals. Rowland et al. (2011) conducted a study in north-west England, collecting data every four weeks from 18 February 2008 to 31 March 2010. They observed low concentrations of Ni, Cd, and Pb in the rivers, with seasonal and flow-dependent variability primarily driven by diffuse sources. Their findings also highlighted compliance with environmental quality standards. However, unlike these studies, this research encompasses data from across England for multiple decades (2000 to 2020) and includes multiple metals. Thus, this study provides a broader and more comprehensive perspective, offering insights that extend beyond the spatial and temporal limitations of previous studies.

For bioavailability studies, most analyses of heavy metals typically focus on a single river, or a specific area affected by anthropogenic activities. Beane et al.

(2016) investigated historic mining at Wheal Betsy in southwest England, collecting 12 sediment samples from Cholwell Brook. Their study found that metal concentrations, particularly for Pb and Cd, significantly exceeded the EQS set under the WFD. Lead concentrations in stream sediments were up to 76 times higher than the Canadian sediment guidelines' "Probable Effect Level." Additionally, they observed a decline in benthic invertebrate species richness near the mine site, with Pb and Cd identified as the primary causes of ecological impact. Their analysis also revealed that the main mine drainage admitted was the most significant source of metal contamination, contributing approximately 50% of the total metal load in the area, and up to 88% for Ni. Mano and Shinohara (2020) utilised a bioavailability model to investigate the acute toxicity of Ni on Daphnia magna. Their study, conducted in five non-polluted rivers in Japan, involved exposing Daphnia magna to varying concentrations of dissolved Ni while observing changes in mobility and mortality. They determined that the Effect Concentration (EC50) ranged from 0.52 to 4.0 mg/L, while the Lethal Concentration (LC50) ranged from 0.62 to 5.3 mg/L.

While the studies above did not consider the influence of sewage effluent on metal bioavailability in rivers, Comber et al. (2022) directly addressed this issue. Their study analysed water quality upstream and downstream of over 600 STWs across the UK using the BioMet bioavailability model (https://bio-met.net/), which incorporates site-specific parameters such as pH, dissolved organic carbon, and Ca to assess compliance with Environmental Quality Standards (EQS) for trace metals. They found that accounting for bioavailability significantly improved compliance rates with EQS values and concluded that while the local impact of STWs was generally limited, catchment-scale contamination remained a concern.

However, the primary focus of Comber et al. (2022) was on regulatory compliance rather than a detailed investigation into the broader hydrochemical and ecological implications of STWs' discharges. In contrast, the current study extends this analysis by incorporating additional water chemistry parameters and employing statistical techniques, such as Principal Component Analysis (PCA) and Linear Modelling (LM), to evaluate the broader environmental consequences of wastewater inputs on riverine systems.

Väänänen et al. (2018) conducted a comprehensive review on the role of metal bioavailability in ecological risk assessment (ERA) for freshwater systems,

highlighting advancements in science and regulation across different countries. They emphasised that traditional risk assessments based on total metal concentrations may lead to either underestimation or overestimation of ecological risk, as the bioavailable fraction primarily drives metal toxicity. Their review explored the integration of bioavailability-based methods into environmental regulation, noting that while countries such as the USA and Canada have adopted site-specific bioavailability models (e.g., BLMs) for water and sediment assessment, European implementation has been more gradual, with tools like the UKTAG Metal Bioavailability Assessment Tool (M-BAT) becoming more widespread.

Väänänen et al. (2018), however, identified significant gaps in the assessment of metal mixture toxicity and sediment contamination within Europe, as well as the absence of bioavailability-based regulations in countries such as China. A similar situation applies to the UK, where water quality standards are still based on total dissolved metal concentrations. They concluded that future efforts should focus on developing streamlined bioavailability assessment tools, establishing regulatory guidelines, and enhancing training for environmental practitioners to ensure consistent application of bioavailability in risk assessment frameworks.

Despite the valuable contributions of these studies, none have included as many determinands or spanned such a long temporal period as the current research. Unlike Chapter 3, in which sewage treatment works were directly linked to individual monitoring sites, the scale of the dataset in this chapter precluded a paired control analysis. Nevertheless, this broader-scale approach enables the identification of spatial patterns and regional trends in bioavailable metal exposure, offering important insights into the cumulative impact of wastewater discharges on freshwater ecosystems across England.

From the inter-determinand correlation (Section 5.3.1), Cd and Zn's high association indicate a likely shared source or similar geochemical behaviour. This correlation is consistent with industrial processes, as Cd is frequently found in Zn ores and is released during metal smelting and mining (Shiel *et al.*, 2010). Industrial sectors such as electroplating, battery manufacturing (Wang *et al.*, 2021), and smelting operations (Danczak *et al.*, 2020) contribute to this pattern, where both metals are simultaneously discharged into the environment and exhibit similar mobility (Lei *et al.*, 2020). Mg and Cd, Ca and Cu, or Mg and Zn suggests differing

geochemical behaviours and sources. While anthropogenic activities often introduce Cd and Cu, Mg is predominantly derived from natural sources such as the weathering of dolomite and serpentine (de Obeso *et al.*, 2021). Similarly, the weak correlation between Mg and Zn may be attributed to differences in their solubility and transport mechanisms in aquatic environments (Santos *et al.*, 2023). Overall, the results highlight the interplay between natural and anthropogenic influences on metal distributions. The strong associations between certain metals, such as Cd-Zn and Ni-Cu, suggest common pollution sources, whereas the lack of correlation among others indicates the presence of multiple contributing factors. These findings emphasise the importance of source identification for effective pollution management, warranting further investigation into industrial emissions and natural geochemical processes.

The PCA results based on combined datasets of river monitoring and sewage effluent proved the relationships from the inter-determinand correlation, which indicate that Ni, Cu, and Zn are the dominant contributors to PC1, suggesting shared compositional characteristics between sewage discharges and metal-enriched river sites. However, the observed clustering also reveals that sewage effluent samples occupy a relatively narrow range in PCA space compared to the broader variation seen in river data. This suggests that while STW discharges may contribute to the overall metal signature in rivers, additional sources or in-river processes likely influence spatial variability (Figure 5.4 & 5.5); And itreflects the limited capacity of most STWs to remove dissolved trace metals during conventional treatment, meaning that downstream signatures largely depend on effluent volume rather than specific removal processes, while control variability arises from diffuse geological and land-use drivers.

Furthermore, the M-BAT-derived RCR analysis highlights regions where bioavailable metal concentrations exceed regulatory thresholds, offering a spatial perspective on potential contamination risks. While some alignment exists between high RCR regions and areas of greater STW density, this relationship weakens after normalising by population and area. This spatial pattern suggests that wastewater discharges are one of several contributing factors, alongside other localised sources such as historic mining, agricultural runoff, and background geochemistry. In particular, elevated Ni and Cd risks in certain upland regions are more consistent

with legacy mining and mineralised geology than with STW density, whereas Zn hotspots often coincide with intensive agricultural areas where manure and fertiliser inputs act as diffuse sources.

This study expands upon previous findings by providing a spatially normalised assessment of bioavailable metal risks across England. The M-BAT-derived RCR analysis identifies several regions where concentrations exceed regulatory thresholds. While some alignment exists between these regions and areas with high STW density, the relationship becomes less apparent after normalisation by population and area. This indicates that although STW discharges contribute to local metal burdens, they are unlikely to be the sole driver of exceedances. Instead, a combination of factors—including historic mining, land use, agricultural runoff, and regional geochemistry—appears to shape spatial metal risk patterns. Moreover, it should be noted that the population figures used for normalisation refer to entire administrative regions, which may not spatially correspond to the specific locations of RCR exceedance. This spatial mismatch may affect the interpretation of population-normalised exposure burdens, particularly where high RCR values occur in sparsely populated upstream areas.

One limitation of this study is that while M-BAT provides a useful framework for bioavailability assessment, it is only valid for a subset of metals (Mn, Cu, Ni, Zn); therefore, it cannot be applied to other metals. Additionally, a regression analysis was initially used to identify key individual predictors of bioavailable metal concentrations. Subsequent models incorporating interaction terms between covariates (e.g., pH*DOC, pH*Ca, DOC*Ca) revealed that these interactions, although often secondary to main effects, contributed additional explanatory power, particularly under high-risk conditions (RCR $\geq$ 1). This highlights the importance of considering both direct and interactive effects in understanding the drivers of metal bioavailability (Table 5.8 & Table 5.9). Future studies incorporating more comprehensive geochemical models, such as the Biotic Ligand Model (BLM), could improve our understanding of bioavailability in complex riverine environments.

The findings presented in this chapter have important implications for environmental policy and river basin management. By expanding the analysis beyond STW–Control site pairs to a national dataset, this study offers a broader

perspective on the drivers of bioavailable metal exposure. Although STWs contribute to localised metal inputs, the spatial patterns of population-normalised RCR values indicate that STWs are not the primary determinant of regional risk distributions. Instead, catchment-scale factors such as background geology, legacy contamination, diffuse pollution, and hydrochemical variability play substantial roles. For example, in Table 5.12, the co-occurrence of multiple metals exceeding risk thresholds in these counties highlights potential zones of cumulative contamination pressure, possibly influenced by a combination of legacy mining, industrial activity, and contemporary discharges from STWs. These spatial patterns underscore the importance of integrated, catchment-scale approaches that address both historical and contemporary pollution sources in assessing and managing ecological risk. From a treatment perspective, the findings also highlight the need to evaluate how advanced processes (e.g., chemical precipitation, filtration, or adsorption) might reduce specific bioavailable metal fractions that conventional biological treatment does not address.These results underscore the importance of integrated management strategies that address both point-source and diffuse inputs, and reinforce the value of bioavailability models, such as M-BAT, in informing risk-based water quality standards within frameworks like the Water Framework Directive.

5.5 Conclusion

i) Final effluent from STWs is a plausible source of trace metals in rivers, with evidence of compositional similarity for key metals.

ii) Dissolved metal concentrations consistently emerged as the strongest predictors of bioavailable metal levels, regardless of spatial scale or contamination severity.

iii) Demographic normalisation indicates that the influence of STW discharges on RCR exceedance patterns is minimal despite their localised contributions.

iv) Bioavailable metal contamination shows pronounced regional disparities, shaped by a combination of historical, geochemical, and anthropogenic factors beyond STW discharges.

Chapter 6: Conclusions

Discharges from sewage treatment works (STWs) are an essential part of urban infrastructure but can pose considerable risks to river ecosystems. While many studies have explored the effects of STW discharges on receiving water bodies, this thesis has conducted a national-scale and multi-annual analysis of the impact of STW discharges across a wide range of determinands—both non-metal and metal—on rivers. This study considered more determinands over more sites and in more statistically rigorous manner than previous studies.

This research was guided by aims introduced in Chapter 1: to evaluate the influence of STW discharges on river water quality across England, with particular emphasis on both metal and non-metal determinands, eutrophication indicators, and bioavailable trace metals. Through the integration of long-term monitoring data, and statistical models, the study sought to assess the ecological implications of these discharges and to inform pollution management strategies aligned with frameworks such as the European Water Framework Directive (WFD) and the UK Environmental Quality Standards (EQS). These aims, first introduced in Chapter 1, are systematically addressed and evaluated in the subsequent chapters.

- Chapter 2: Impact on non-metal Water Quality Determinands
  Aim**:** To assess the effect of STW discharges on common water quality determinands such as stream temperature, BOD, COD, nitrate, phosphate, pH, suspended solids, and specific conductance.

- Chapter 3: Impact on Metal Concentrations
  Aim: To evaluate how STW discharges affect dissolved and bioavailable concentrations of metals (e.g., Cu, Zn, Mn, Ni) in river systems.

- Chapter 4: Contribution to Eutrophication
  Aim: To determine if STW discharges promote eutrophication through increased chlorophyll-a and nutrient concentrations.

- Chapter 5: National-scale Metal Pollution Patterns
  Aim: To assess whether STW discharges contribute significantly to bioavailable metal exceedances at the national scale compared to regional sources.

- Chapter 6: Synthesis and Conclusions

Aim: To synthesise the findings from the preceding chapters, highlight the overall significance of STW discharges for river water quality, discuss study limitations, and identify directions for future research.Collectively, the findings demonstrate that STW discharges significantly impact river water quality and the wider river network.

6.1 Impact of non-metal determinands

- The impact of STW discharges on receiving rivers was significant for all determinants except COD and Suspended Solids.
- Over the study period (2000 – 2022), a decrease in STW-related impacts was observed for BOD, pH, nitrate, specific conductance, and suspended solids, while for phosphate concentrations there was a significant rise in STW-related impact over the study period. For, Temperature and COD there was no change in the STW-related impact over time.
- The type of treatment used in STWs played a significant role in controlling the impacts of STW discharge on receiving waters, with some types of treatment proving less favourable than others.
- While tertiary treatment is effective in reducing phosphate levels, secondary methods such as secondary activated sludge treatment (SAS) and secondary biological treatment (SB) show minimal differences in nitrate removal but significantly improve phosphate removal.
- STW size, as indicated by dry weather flow (DWF), was associated with greater impacts on all determinands, whereas population equivalence (PE) did not show a consistent relationship with the determinands being considered.
- Principal component analysis (PCA) showed that STWs could be categorized into two impact types: one focused on BOD, COD, and SS, and the other on nutrient-related determinands.

6.2 Impact of metals and bioavailability shown in study pairs

- Except for Cd and Ni, all analysed determinands were significantly affected by STW discharges. Ca, Mg, Cu, and Mn showed elevated concentrations downstream, whereas Fe and Zn demonstrated reductions.
- Over time, the effect of STW discharges on Mg and Cu concentrations significantly declined, whereas their impact on Ni concentrations significantly increased.
- The efficiency of metal removal varied between secondary treatment types; SB was more effective than SAS in reducing Fe and Cu concentrations.
- The magnitude of STW impact on river water quality increases with plant size. Specifically, PE was linked to changes in Ca, Ni, and Zn, while DWF influenced levels of Fe, Mg, and Cu.
- From PCA analysis, STWs were differentiated into two impact types—those contributing mainly to Zn, Cd, and Ni pollution, and those affecting Ca, Mg, and Cu.
- STW discharges significantly impacted bioavailable Zn, Mn, and Ni, while bioavailable Cu remained unchanged despite increased dissolved concentrations.
- Zn showed the highest proportion of samples that had risk concentration ratio (RCR) > 1 and so exceeded their environmental quality standard.

6.3 Eutrophication

- STW discharges have no significant effect on the chlorophyll concentrations of the receiving waters.
- The inclusion of covariates indicated a possible significant effect of sewage discharge on increasing Chl-a concentrations.
- Trends in the influence of sewage discharge on SiO_2 indicate that future impacts on receiving rivers may become more important.
- Compared to SB treatment, SAS demonstrated greater effectiveness in lowering chlorophyll levels in downstream waters.
- Analysis of 2002 data indicates that the chemical composition of receiving rivers in England closely resembled that of final effluent, implying that many rivers could be considered as dilute sewage effluent.

6.4 Impact of STW metal discharges on the wider English river network and national-scale bioavailability situation

- Final effluent from STWs is a plausible source of trace metals in rivers, with evidence of compositional similarity for key metals.
- Dissolved metal concentrations consistently emerged as the best predictors of bioavailable metal levels, regardless of spatial scale or contamination severity.
- Demographic normalisation indicates that the influence of STW discharges on RCR exceedance patterns is minimal despite their localised contributions.
- Bioavailable metal contamination shows pronounced regional disparities, shaped by a combination of historical, geochemical, and anthropogenic factors beyond STW discharges.

6.5 Study limitations

This study was designed to address three key knowledge gaps outlined in Chapter 1: the lack of appropriate comparisons in study design, the limited scale of existing datasets, and the tendency to focus on pollutant concentrations rather than ecological impact. While significant progress was made, several limitations remain.

First, although upstream-downstream site pairings were used to provide appropriate control conditions—an improvement over many prior studies—paired data availability varied across determinands. For some parameters, limited paired samples may have reduced statistical power and restricted the detection of smaller or less consistent effects.

Second, the study utilized one of the most extensive datasets currently available, addressing the issue of small-scale sampling noted in previous work. However, access to critical metadata such as STW treatment type and population equivalence (PE) was constrained due to the inaccessibility of key data sources following Brexit. As a result, some analyses—particularly those evaluating differences among treatment processes or load-based assessments—could not be conducted or were excluded beyond Chapter 3.

Third, while this research went beyond simple concentration reporting by assessing bioavailable metals and EQS exceedances, it could not fully evaluate the ecological consequences of chemical mixtures or downstream pollutant dynamics. These limitations stem from data availability and the complexity of capturing in-situ chemical interactions and transport processes at a national scale.

In summary, although this study advances the field by addressing previously overlooked aspects of STW impacts, it remains constrained by data pairing limitations, treatment process metadata gaps, and unresolved challenges in assessing long-range and interactive ecological effects. These findings underscore the need for improved infrastructure data transparency and further research into catchment-scale pollutant behaviour.

6.6 Future work

Building on the findings and methodological framework of this study, several directions for future research are proposed. These fall into three main categories: (1) enhanced data analysis for source attribution, (2) integration with experimental data and emerging contaminant monitoring, and (3) scenario-based modelling to inform management and policy development.

6.6.1. Advanced Data Analytics and Source Attribution

Future work could apply more sophisticated multivariate techniques—such as Principal Component Analysis (PCA)—to further unravel the complex structure of pollution sources. PCA offers the potential to differentiate between overlapping geogenic, anthropogenic, and legacy industrial signals, particularly in regions where multiple inputs obscure attribution. Building upon PCA outputs, targeted end-member analysis could be employed to define representative pollution signatures, allowing clearer identification of dominant contributors to metal and nutrient concentrations. In addition, incorporating chemical speciation and isotopic data would enhance source discrimination and improve understanding of pollutant behaviour and bioavailability under different environmental conditions.

6.6.2. Integration with Experimental Monitoring and Emerging Contaminants

The current framework could be extended through integration with field work data. For instance, coupling field observations with laboratory-based chemical speciation

experiments would offer a more detailed view of bioavailability and ecological risk. Furthermore, as monitoring programs expand, the analysis could be broadened to include emerging contaminants such as pharmaceuticals, microplastics, and endocrine-disrupting compounds—key pollutants that pose growing threats to aquatic ecosystems but are often omitted from routine surveillance.

6.6.3. Scenario-Based Modelling and Policy Applications

Finally, future studies could incorporate scenario-based modelling to assess the potential outcomes of different management interventions. Using the spatial models developed in this study as a foundation, simulations could explore the effectiveness of upgraded treatment technologies, tighter effluent standards, or alternative nutrient removal strategies. These models would provide valuable tools for regulators and policymakers seeking evidence-based strategies to improve river water quality under changing environmental and socio-economic conditions.

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Appendix Table A1. Cu: RCR > 1 Site-Level Exposure Metrics by Region.This table presents site-level exceedance statistics for bioavailable Cu across English council districts. Included metrics are the number of sites exceeding the RCR > 1 threshold, total monitored sites, population, area (km²). The final column reports the per capita per km² ratio, reflecting spatial-demographic normalised exposure intensity.

Area	Exceed_Site_Count	Total_Site_Count	Population	Area_km2	PerCapita_per_km2_Ratio
Blackpool	1	3	141000	33.560	0.000
Cheshire East	1	4	400500	1168.795	0.003
Cornwall	27	71	572000	3573.835	0.006
Croydon	1	4	390500	85.995	0.000
Cumberland	9	46	274000	1560.000	0.000
Darlington	1	18	618800	198.812	0.000
Devon	2	50	814400	6578.755	0.008
Doncaster	2	16	308700	567.277	0.002
East Sussex	1	14	546900	1721.902	0.003
Essex	1	15	1506300	3459.960	0.002
Halton	1	12	128600	80.375	0.001
Herefordshire	1	13	187600	2180.535	0.012
Hillingdon	1	2	304800	115.522	0.000

Isles of Scilly	1	1	2100	13.575	0.006
Kingston upon Hull	1	6	266500	70.984	0.000
Kirklees	1	4	433400	405.307	0.001
Milton Keynes	1	7	288200	309.327	0.001
Plymouth	5	21	264700	81.991	0.000
Redcar and Cleveland	1	12	136600	248.289	0.002
Rochdale	3	13	224100	156.240	0.001
Rotherham	1	29	266200	289.186	0.001
Shropshire	1	36	324700	3188.263	0.010
Somerset	1	26	573100	3462.370	0.006
South Gloucestershire	1	8	290700	500.484	0.002
Stockton-on-Tees	1	4	197000	205.217	0.001
Westmorland and Furness	10	55	227600	3667.000	0.000
Wolverhampton	1	4	264000	69.466	0.000
Wrexham	1	8	135100	506.884	0.004

Appendix Table A2. Zn: RCR > 1 Site-Level Exposure Metrics by Region. This table provides exceedance data for Zn, a metal generally showing lower regional risk. Metrics follow the same format to allow consistent comparison.

Area	Exceed_Site_Count	Total_Site_Count	Population	Area_km2	PerCapita_per_km2_Ratio
Bath and North East Somerset	6	17	192400	348.612	0.002
Birmingham	1	7	1142500	269.027	0.000
Bracknell Forest	1	6	125200	108.234	0.001
Bradford	5	17	547000	368.387	0.001
Bristol	1	4	470,000	111.321	0.000
Cambridgeshire	2	21	680400	3056.066	0.004
Cornwall	50	71	572000	3573.835	0.006
Cumberland	21	41	274000	1560.000	0.000
Darlington	12	18	618800	198.812	0.000
Derby	5	18	261100	78.270	0.000
Derbyshire	16	26	796800	2549.661	0.003
Devon	12	50	814400	6578.755	0.008
Doncaster	6	16	308700	567.277	0.002

Dudley	1	8	323600	97.265	0.000
Durham	105	145	521300	2231.233	0.004
East Riding of Yorkshire	1	8	343100	2409.308	0.007
East Sussex	2	14	546900	1721.902	0.003
Gateshead	4	15	196200	147.489	0.001
Gloucestershire	1	20	646600	2683.579	0.004
Halton	3	11	128600	80.375	0.001
Herefordshire	1	12	187600	2180.535	0.012
Isle of Wight	3	6	140900	379.786	0.003
Isles of Scilly	1	1	2100	13.575	0.006
Kirklees	1	4	433400	405.307	0.001
Leeds	1	5	809000	550.578	0.001
Leicester	1	7	366000	73.589	0.000
Leicestershire	1	13	712600	2087.261	0.003
Lewisham	1	3	299800	34.352	0.000
Lincolnshire	1	14	769500	5950.271	0.008

Liverpool	1	1	357700	112.981	0.000
Luton	1	4	224800	44.068	0.000
Manchester	1	5	549900	111.896	0.000
Milton Keynes	2	7	288200	309.327	0.001
Norfolk	1	14	918400	5374.494	0.006
North Northamptonshire	3	6	360400	993.518	0.003
North Somerset	3	8	573100	373.572	0.001
North Yorkshire	10	27	618800	8054.935	0.013
Northumberland	13	39	321600	5032.156	0.016
Nottingham	5	25	319600	75.870	0.000
Nottinghamshire	6	19	826300	2087.469	0.003
Oxfordshire	1	28	726500	2608.130	0.004
Peterborough	2	18	769500	341.527	0.000
Plymouth	5	19	264700	81.991	0.000
Reading	1	6	173200	41.326	0.000
Rochdale	6	14	224100	156.240	0.001

Rotherham	10	28	266200	289.186	0.001
Salford	1	5	270800	97.967	0.000
Sandwell	1	3	341700	85.221	0.000
Sefton	1	7	279700	153.865	0.001
Sheffield	4	11	554400	366.749	0.001
Shropshire	20	33	324700	3188.263	0.010
Solihull	3	10	216700	177.542	0.001
Somerset	2	24	573100	3462.370	0.006
Southend-on-Sea	2	4	321000	44.408	0.000
Staffordshire	2	7	877900	2628.223	0.003
Stockport	1	6	295200	125.536	0.000
Sunderland	1	7	274200	136.155	0.000
Surrey	1	9	1205600	1667.758	0.001
Swindon	1	13	233700	227.716	0.001
Telford and Wrekin	2	11	185800	291.803	0.002
Torbay	3	12	139400	65.488	0.000

Wakefield	1	5	353800	337.270	0.001
Walsall	6	13	284300	104.027	0.000
West Sussex	4	17	885100	1996.349	0.002
Westmorland and Furness	16	47	227600	3667.000	0.000
Wigan	1	6	329800	188.329	0.001
Wiltshire	1	7	513400	3258.226	0.006
Wolverhampton	2	3	264000	69.466	0.000
Worcestershire	1	17	604900	1743.230	0.003
York	1	10	201700	275.330	0.001

Appendix Table A3. Mn: RCR > 1 Site-Level Exposure Metrics by Region As above, this table summarises regional exceedance metrics for Mn . It enables comparison of exposure burden across districts accounting for both population size and land area.

Area	Exceed_Site_Count	Total_Site_Count	Population	Area_km2	PerCapita_per_km2_Ratio
Blackburn with Darwen	3	6	154900	139.386	0.001
Blackpool	2	3	141000	33.560	0.000
Brighton and Hove	2	7	276300	85.542	0.000
Cornwall	5	60	572000	3573.835	0.006
Coventry	3	19	343300	99.799	0.000
Cumberland	3	51	274000	1560.000	0.000
Darlington	1	18	618800	198.812	0.000
Derby	1	20	261100	78.270	0.000
Derbyshire	4	28	796800	2549.661	0.003
Devon	3	50	814400	6578.755	0.008
Doncaster	5	12	308700	567.277	0.002
Durham	21	126	521300	2231.233	0.004
East Riding of Yorkshire	1	7	343100	2409.308	0.007

East Sussex	4	24	546900	1721.902	0.003
Essex	2	23	1506300	3459.960	0.002
Gateshead	1	13	196200	147.489	0.001
Isle of Wight	1	5	140900	379.786	0.003
Kent	1	33	1578500	3543.664	0.002
Kingston upon Hull	1	6	266500	71.000	0.000
Knowsley	1	1	155000	86.258	0.001
Leicestershire	1	25	712600	2087.261	0.003
North East Lincolnshire	1	4	157200	190.562	0.001
North Lincolnshire	2	6	169900	849.080	0.005
Redcar and Cleveland	4	17	136600	248.289	0.002
Rochdale	4	14	224100	156.240	0.001
Rotherham	5	28	266200	289.186	0.001
Salford	1	9	270800	97.967	0.000
Sandwell	1	3	341700	85.221	0.000
Sheffield	1	14	554400	366.749	0.001

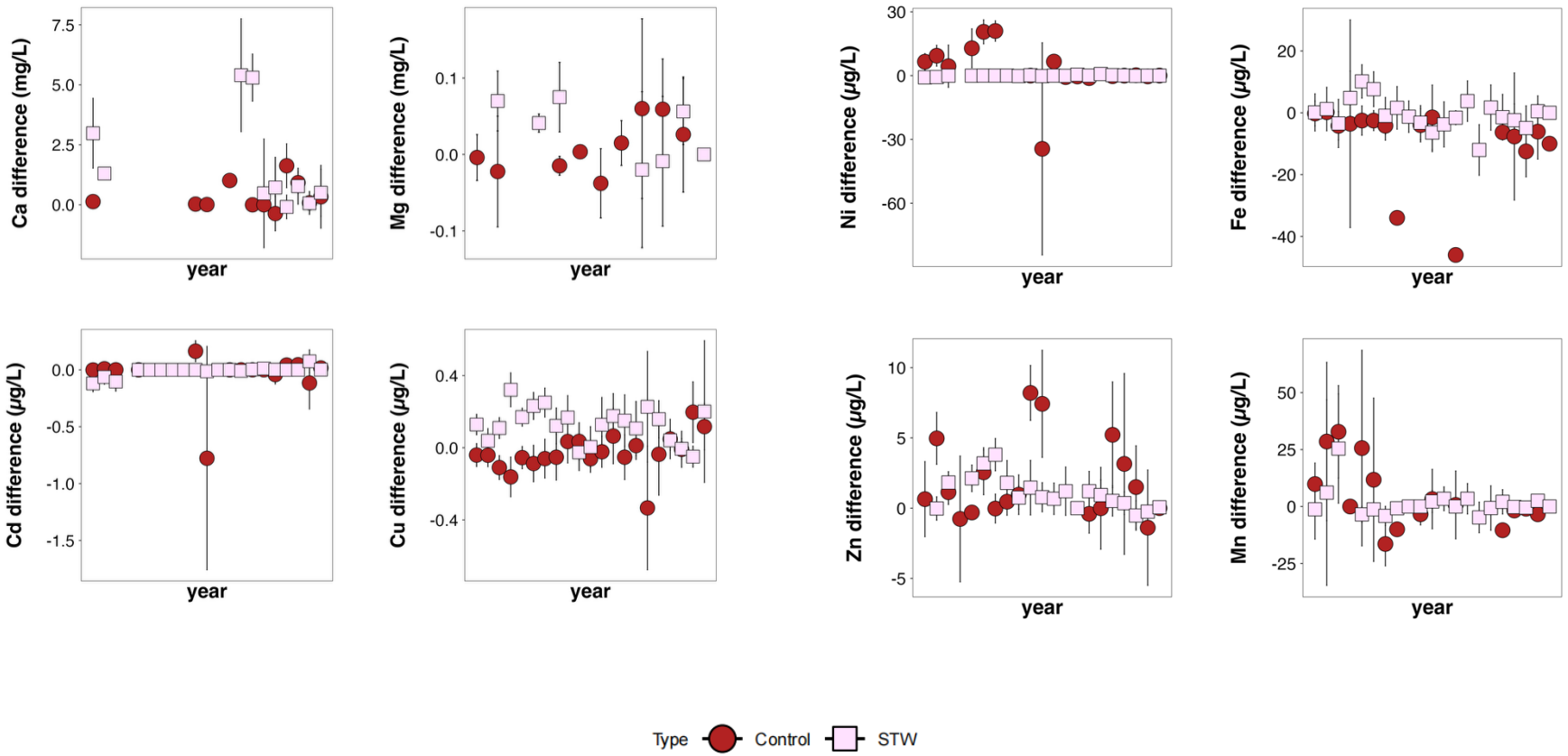
Shropshire	1	35	324700	3188.263	0.010
Slough	1	4	158300	32.202	0.000
Staffordshire	1	5	877900	2628.223	0.003
Stoke-on-Trent	3	14	258000	92.989	0.000
Telford and Wrekin	1	10	185800	291.803	0.002
Wakefield	1	8	353800	337.270	0.001
Walsall	3	10	284300	104.027	0.000
Warwickshire	1	21	599200	1974.139	0.003
West Sussex	2	15	885100	1996.349	0.002
Wigan	4	7	329800	188.329	0.001

Appendix Table A4. Ni: RCR > 1 Site-Level Exposure Metrics by Region. This table details the distribution of RCR > 1 exceedances for Ni, including region-specific exceedance probabilities, exposure per capita, and spatial intensity per km².

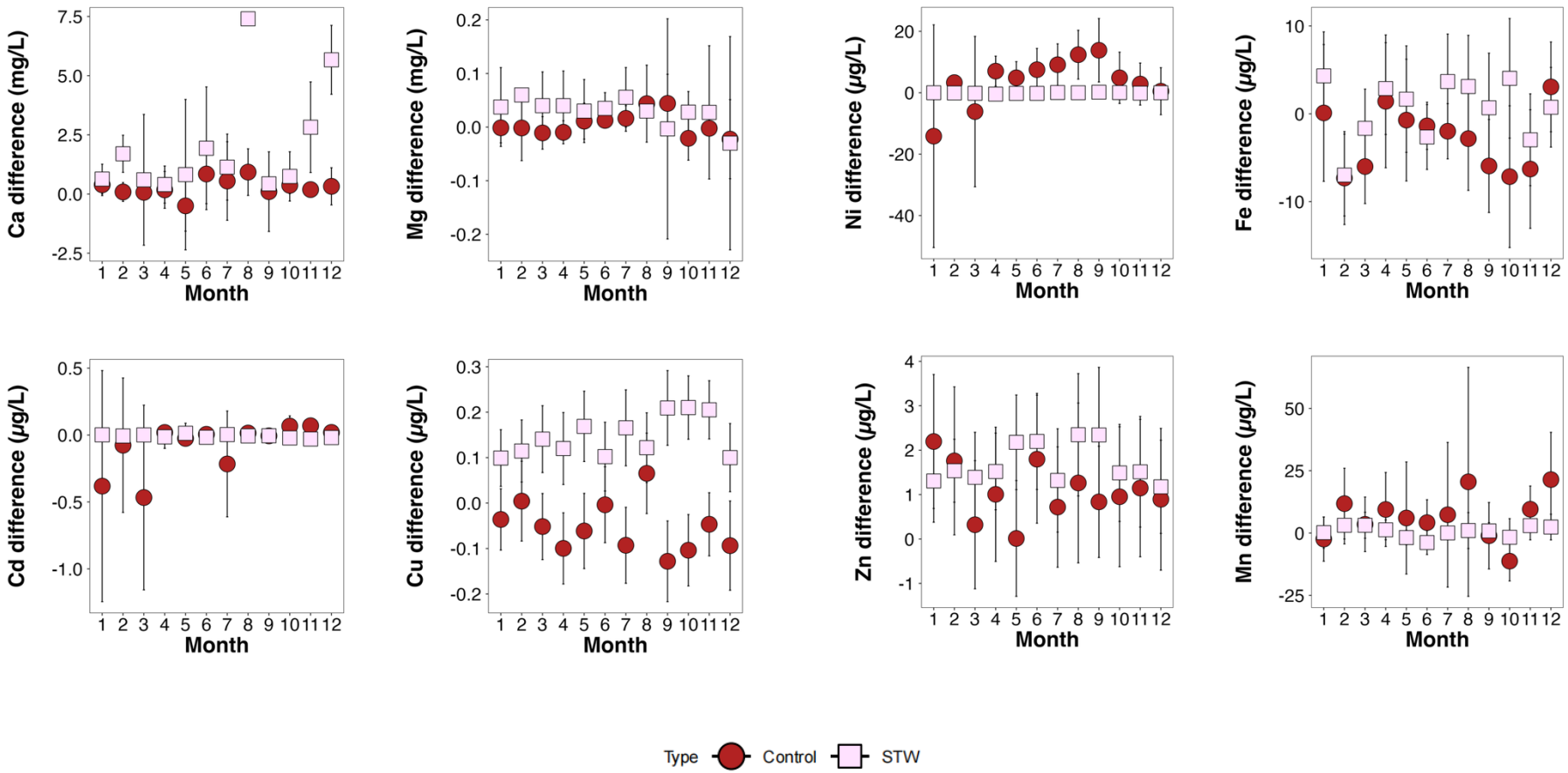
Area	Exceed_Site_Count	Total_Site_Count	Population	Area_km2	PerCapita_per_km2_Ratio
Bath and North East Somerset	1	16	192400	348.612	0.002
Birmingham	1	6	1142500	269.027	0.000
Blackburn with Darwen	2	6	154900	139.386	0.001
Central Bedfordshire	1	4	295500	709.158	0.002
Cheshire East	1	4	400500	1168.795	0.003
Cornwall	19	56	572000	3573.835	0.006
Cumberland	3	40	274000	1560.000	0.000
Derbyshire	1	26	796800	2549.661	0.003
Devon	7	49	814400	6578.755	0.008
Durham	5	149	521300	2231.233	0.004
Hampshire	1	5	1406200	3675.415	0.003
Herefordshire	1	11	187600	2180.535	0.012
Isle of Wight	1	6	140900	379.786	0.003

Leicestershire	2	13	712600	2087.261	0.003
North Lincolnshire	1	5	169900	849.080	0.005
Nottinghamshire	1	21	826300	2087.469	0.003
Plymouth	4	18	264700	81.991	0.000
Redcar and Cleveland	1	12	136600	248.289	0.002
Rochdale	5	9	224100	156.240	0.001
Rotherham	3	30	266200	289.186	0.001
Sandwell	2	3	341700	85.221	0.000
Shropshire	2	33	324700	3188.263	0.010
Solihull	3	11	216700	177.542	0.001
Somerset	1	22	573100	3462.370	0.006
South Gloucestershire	2	8	290700	500.484	0.002
Southend-on-Sea	1	4	180600	44.408	0.000
Suffolk	1	14	763400	3823.292	0.005
Telford and Wrekin	2	11	185800	291.803	0.002
Trafford	1	7	235500	108.001	0.000

Walsall	6	14	284300	104.027	0.000
West Northamptonshire	1	8	426500	1374.391	0.003
West Sussex	1	16	885100	1996.349	0.002
Westmorland and Furness	2	47	227600	0.000	0.000
Wigan	1	6	329800	188.329	0.001
Wiltshire	1	7	513400	3258.226	0.006
Wolverhampton	1	3	264000	69.466	0.000



*Appendix Figure 1. The main effects plot of the Type*Year interaction. The values are presented as the marginal mean with the 95% confidence interval.*



*Appendix Figure 2. The main effects plot of the Type*Month interaction. The values are presented as the marginal mean with the 95% confidence interval.*