Measuring water pollution in the River Kent from a wastewater treatment plant (WWTP), with specific focus on faecal coliforms.

A dissertation thesis by

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Declaration:

I confirm that this piece of work that I have submitted is all my own work and that all references and quotations from both primary and secondary sources, including the internet, have been fully identified and properly acknowledged.

Signature: Jonathan Harte Date: 27/04/22

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Abstract:

This study was carried out to identify the inputs of faecal coliforms like *Escherichia coli* (*E. coli)* and other inputs which could influence water quality, using the River Kent as a case-study. With the increasing importance of conservation and the protection of the environment becoming a greater local, national and global priority, studying impacts on the environment is now more important than ever. As inputs from WWTPs can have a variety of impacts, an understanding of exactly what is entering our rivers from these sources is vital. With the support of a local environmental group called Sustainability and Energy Network in Staveley (SENS), I collected samples at three sites along the River Kent, two before the WWTP and one after it, with the aim of identifying what was being pumped in to the river. The results showed a significant increase in levels of *E. coli* after the WWTP with an increase of 1,114 CFU/100ml when compared to the site before. Other measures did not show any conclusive results of inputs from the WWTP. Further study is needed to form more conclusive results.

Introduction:

Why is wastewater management important?

Sanitation has been described by many as one of the key cornerstones of social and economic development, and as a result, has been an increasingly important focus for the global community over time (Cairncross, 2003; Mara *et al.,* 2010). In addition, alongside improvements in diet, medical practice and living conditions, developments and improvements in sanitation have been attributed to significantly reducing mortality rates, thus indicating clear health benefits (Harris & Helgertz, 2019). The history of sanitation practices can be dated back through the centuries, from cesspits and covered diches to the development of Roman sewers (Jansen, 2018). Initially, the aims of sanitation were not the same as they are today. However, they showed some understanding of the link between water and health (Jansen, 2018). It took many years for the practice to become widespread, and an understanding of the importance of wastewater treatment to be fully realised, and today, much more complex sanitation systems exist. Countries are now identifying the possibility of exploiting waste to achieve less wasteful circular economies. Using wastewater for biofuel is an example of this and this change in attitude is reflected in the European Union (EU) Bioeconomic strategy (Park *et al.,* 2011; European Commission, 2017; Kaszycki *et al.,* 2021).

Wastewater treatment is an important process which extracts and/or treats potentially hazardous organic materials that could create adverse water conditions. An example of this being organic enrichment, which is the input of excess nutrients such as nitrogen (Hellawell, 1986). Organic enrichment can lead to the depletion of dissolved oxygen, due to heterotrophic utilisation by microorganisms, and can result in some organisms which require high oxygen levels being misplaced (Hellawell, 1986). This is increasing concern and a decline in species that require high oxygen availability has already been noted within the United Kingdom (UK). For example, the white clawed crayfish (*Austropotamobius pallipes*), which has declined by about 70% since 1970 (John Cossee, 2021). Linked to this, the white claw crayfish is also being threatened by the invasive signal crayfish (*Pacifastacus leniusculus*). This breed of crayfish carries a deadly pathogen (*Aphanomyces astaci)* which is extremely harmful to the white clawed crayfish (Robinson *et al.,* 2018). As a result, it is incredibly important to protect the surviving population of white clawed crayfish and protecting their current habitat from pollution and its resultant consequences is a key aspect of this. This is just one example of the negative impact that wastewater effluent can have on the aquatic environment. A study carried out by Burdon *et al.,* (2020) identified that even on a microbial scale, wastewater can result in reduction in the rates of decomposition, and they went as far to say could even cause ecosystem ‘disservices’. Therefore, alongside its impact on public health, wastewater treatment can also have environmental consequences for the natural world.

As knowledge of potential impacts has increased, there has been an increase in the development of wastewater treatment technologies and legislation management. However, it is important to note that despite this, in the case of smaller villages and towns, wastewater treatment continues to sometimes lack the capacity and wastewater treatment processes to remove all of the possibly harmful substances in the effluent (Iwai *et al.,* 1990). Pathogen pollution is the primary example of this. This can be removed in the tertiary stage of wastewater treatment, but this aspect of treatment is generally optional, not compulsory.

Study site background:

Staveley is a small village located in the Northwest of England in Cumbria, and is situated between Kendal and Windemere. In 2020, it had a population of approximately 1,446 people and many local businesses (Citypopulations.de, 2020). Staveley is directly adjacent to the River Kent. The river is used by locals for swimming, angling, kayaking and other activities.

The River Kent itself has been designated as a Site of Special Scientific Interest (SSSI) as a result of being the home to both the white clawed crayfish and hosting important habitat, which helps to support local biodiversity (JNCC, n.d.). The focus site for this study is downriver from Staveley where the WWTP serving Staveley operates.

Rational behind the research:

The purpose of this investigation is to identify the degree to which wastewater effluent is being released into the River Kent and to analyse the type/content of any effluent noted. This was done to support the local SENS group, who are working close to the survey sites. The group is increasingly concerned about the River Kent and specifically, how the WWTP could be impacting their local environment and health. The importance of this work is also being highlighted by the former leader of the Liberal Democrats, Tim Farron, who has recently taken a bill to Parliament calling for tighter control and increased monitoring of discharges into our rivers and seas (Tim Farron, 2022).

The SENS group aim to achieve bathing water status, which would provide the river with certain protections that are currently not in place, despite it being a SSSI river with ecological importance. This is in part why this dissertation is so important, as it aims to help to clarify the inputs from the WWTP and can then potentially be used to identify possible risks to local people and the environment. Ideally, the findings of this study can be used as a starting point for future research, improvement and action.

Literature review:

Background of wastewater treatment:

Wastewater treatment plants (WWTP) play a key role in the removal of harmful substances, such as pharmaceuticals, pathogens and chemicals, preventing them from being released into waterbodies (e.g., rivers and lakes). Even though wastewater and sewage management has been around since the Roman era, modern wastewater treatment is a relatively new concept, evolving throughout the 20th century as shown in Figure 1. Prior to this, wastewater and sewerage management were very much neglected, resulting in events such as the infamous 'Great Stink' of 1858 (Halliday, 2001; Lofrano & Brown, 2010; Naden et al., 2016).

More recently, rising global trends in sanitation and wastewater management have triggered key legislation, including the European Union (E.U) Urban Waste-Water Directive of 1991 and the United Kingdom (U.K) Water Industries Act of the same year (Naden *et al.,* 2016). Such legislation is important as it helps to ensure that certain standards of wastewater treatment are met. In addition, it ensures that waste management practices are monitored, thereby seeking to limit adverse effects on the environment, i.e., eutrophication due to organic enrichment (Department for Environment, Food & Rural Affairs, 2012).

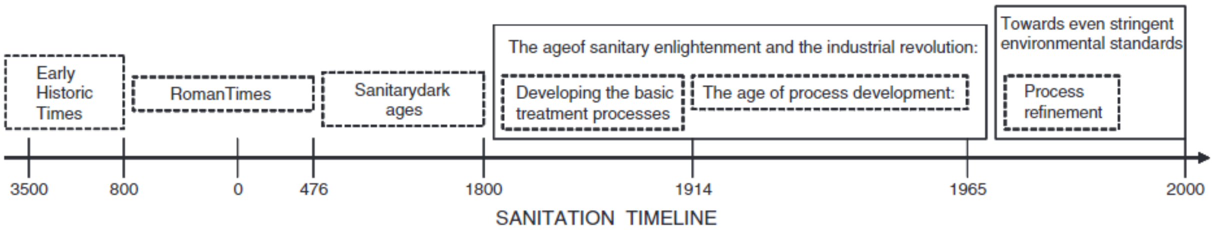


Figure 1- A timeline of the development of wastewater treatment plants and significant developments (Lofrano & Brown, 2010)

Types of wastewater treatment and their purposes:

There are many different types of wastewater treatment, with different stages being used to address the requirements of the area and its context. The basic design of primary and secondary wastewater treatment is shown in Figure 2. After initial screening to remove large objects, the wastewater is left to settle so that the heavier suspended solids can sink to the bottom to form ‘sludge’. The aqueous effluent then continues onto the secondary stage (Kesari *et al.,* 2021). The key purpose of the secondary stage is to reduce dissolved and particulate organic matter within the water. This activated sludge process is carried out by adding microbes and oxygen to the wastewater, which allows this matter to be broken down. The effluent is then left to settle again. This process removes nearly 85% of the biological oxygen demand (BOD), which is the oxygen demand for the breakdown of organic matter within the water (Kesari *et al.,* 2021). Some WWTP’s also use trickling filters, although this is now considered to be an outdated practice. This involves passing the effluent through a substrate, which can be made up of rocks or plastics, to reduce the organic matter content. Trickling filters are effective as they utilise biofilms. Biofilms are complex biological communities that grow on the substrate and help to remove organic matter as the waste flow percolates through the filter, in part due to the large surface area it creates for the activity of aerobic bacteria (McEldowney, 1993).

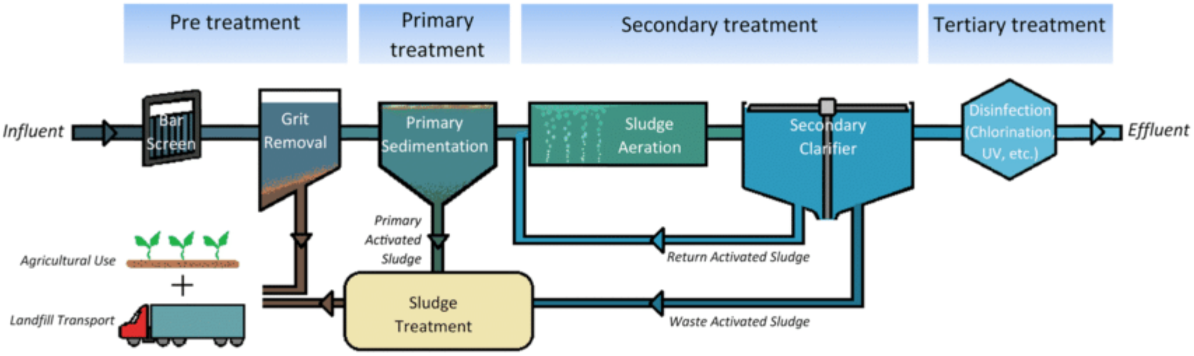


Figure 2 - Outline of WWTP processes and the different treatments involved in primary and secondary treatment (Birch *et al.,* 2020).

The tertiary stage of wastewater treatment is only used where there is a demand for a high-quality result (i.e., drinking water). This stage can be used to remove bacteria and to target other possible contaminants, as seen in Figure 2, through processes like chlorination and Ultraviolet (UV) treatment. In most cases, secondary treatment is not enough and recent studies identify the variability of secondary treatments, namely Campos *et al.,* (2016). Studies have shown that specific secondary and tertiary treatment was needed to lower some bacterial levels, for example *E. coli,* by any considerable degree (George *et al.,* 2002*;* Mason, 2002). The most effective treatments for the removal of bacteria like *E-coli* are ultraviolet treatment, sand filtration, activated sludge and chlorination. However, they are most effective when used in conjunction, in different combinations, depending on what you are trying to achieve (Perrot & Baron, 1995; George *et al.,* 2002). However, it is important to note that there is continued debate relating to the effectiveness of sand filtration and whether it has any significant impact on faecal bacteria when carried out in isolation (Zhang & Farahbakhsh, 2007). *E-coli* is extremely resilient to most primary and secondary treatments and certain strains have been seen to be more resistant to some tertiary wastewater treatments. In these cases, the only effective way to remove them from effluent is to use techniques such as chlorination, which is a highly chemical process or UV treatment (Shuia *et a.,* 2020).

The construction of wetlands is seen as a more natural form of wastewater treatment, and as such, have been the focus of much interest. Wastewater can be passed through these wetlands, and the macrophytes present help to remove some of the organic matter and chemical pollutants (e.g., Nitrates and Phosphates) as well as pacifying coliforms (Vymazal, 2005; Boutilier *et al.,* 2009). However, in some cases after a while, the carrying capacity of these wetlands can be exceeded and lead to leaching into the natural environment (Bastin *et al.,* 1999). The scale of this varies depending on the size and construction of the wetland (Bastin *et al.,* 1999). Using wetlands for wastewater management is cheaper than many alternatives. In addition, these wetlands can also benefit local biodiversity (Hsu *et al.,* 2011; Lu Su *et al.,* 2015).

Overall, managing wastewater treatment is a complex task. Many different pollutants can be present in effluent, including microplastics, heavy metals, nutrient ions and coliforms (Raouf *et al.,* 2019) and it can be incredibly problematic to remove all of the 'excess' contents of wastewater. These challenges are coupled with an increase in costs when higher levels of purity are required (Englande *et al.,* 2015).

UK sewage systems and issues related to them:

In the UK we have a combined sewer system. This means our domestic and industrial wastewater, together with storm drain water, goes into our sewage systems and treatments (Gardner *et al.,* 2013). Problems arise during exceptional rainfall events, meaning times of unusually high rainfall. At these times, the WWTP can release the wastewater before it has been fully treated to manage the load (Rathnayake & Anwar, 2019). This system has received much criticism as it can result in the overflow of different pollutants being released into aquatic environments. Unfortunately, despite this, the cost of improving the capacity of these waste management systems would in many cases be too great (Wiess *et al.,* 2002; Tibbets, 2005; Chen *et al.,* 2019).

As our water treatment is impacted by the weather and global warming has become an increasing concern. The frequency and intensity of extreme weather events has increased and is predicted to double between 2021 and 2030, as a result, flood events have also increased (Fowler & Hennessy, 1995; Chou *et al.,* 2012; Chinita *et al.,* 2021). The unfortunate and more frequent consequence of this is that the combined sewage systems will release more raw/untreated sewage into the natural environment when unable to manage the load.

Hunter (2003) identified the possibility of these increasing global temperatures also encouraging algal blooms and other bacterial communities which could, in turn, increase the risk of waterborne disease. In the UK, we have the Urban Wastewater Treatment Regulation 1994, which sets out requirements on discharges and permits relating to discharges (The Urban Wastewater Treatment, England and Wales, Regulations 1994). If needed and in exceptional circumstances, they set out that effluent must go through at least primary treatment (skipping other treatments) (The Urban Wastewater Treatment, England and Wales, Regulations 1994).

Why do we need wastewater treatment plants?

If stages of wastewater management can be skipped in certain situations, it begs the question as to whether we really need wastewater management in its current format at all, *and*, taking this point further, is pollution still really such a negative thing?

If we define pollution as a 'substances introduced into the environment by humans which have the possibility to induce harm to human health as well as damage to living recourses,' it is clear that pollution *is* a negative thing (Mason, 2002). Sewage can be harmful to human health. One example of this is the presence of coliforms, which can be present in wastewater, and are excreted from both humans and animals. These can make humans extremely unwell if ingested (Al- Bahlry *et al,.* 2009). Escherichia coli (*E. coli*), a coliform, is a very specific indicator of faecal pollution in wastewater. As a result, a number of countries prioritise the identification of faecal contamination over total coliforms, as they are viewed as less specific (Dufour, 1997; Edberg *et al.,* 2000; Haffhold, 2011; Sadowsky & Whitman, 2011).

However, worryingly, as previously mentioned, *E. coli* is highly resilient. There is evidence to suggest that *E. coli* can survive in extraintestinal environments and with their strong resilience, they can naturalise into pre-existing bacterial communities (Jang *et al.,* 2017; Li *et al.,* 2021). As a result, this could make the presence of *E. coli* less effective as an indicator of faecal pollution (Jang *et al.,* 2017; Li *et al.,* 2021). To tackle this, some treatments are aimed at removing *E. coli* and similar bacteria, for example chlorination. However, certain strains of E. *coli* are highly resilient to chlorine, more so than other bacteria, which means there is a higher possibility of identifying the bacteria in wastewater discharge even after treatment has occurred (Shuia *et a.,* 2020). However, it is important to note that results here depend on the concentration of chlorine used (Owoseni *et al.,* 2017). As a result of this, Hendricks & Pool (2012) concluded that more research into wastewater treatment and discharge is needed to identify how to prevent these pathogens from being released into the environment. They noted that this was due, in part, to the ineffective tertiary treatment at the WWTP used in their study, which still released coliforms after treatment (Hendricks & Pool, 2012).

In addition, the release of E. *coli* and other pathogens, following wastewater treatment, can also come with several other potentially negative consequences for local people and the environment. The River Kent is designated as a Site of Special Scientific Interest (SSSI) and a Special Area of Conservation (SAC). This is primarily because it contains species of interest, such as the white-clawed crayfish (*Austropotamobius pallipes*), freshwater pearl mussels (*Margaritifera margaritifera*) and the bullhead (*Cottus gobio*) (JNCC, n.d.). The white-clawed crayfish has experienced population fluctuations over time, in part, due to its slow growth and low fecundity, coupled with issues like channelisation and pollution. This makes it easier for invasive, faster growing crayfish to compete (Holdich, 1991; Holdich & Reeve, 1991).

Pollution is a key threat and can exacerbate issues for these species of interest. This is because it can result in organic enrichment, which can lead to the deoxygenation of aquatic environments. This can make areas less habitable for species like the white clawed crayfish and the bullhead (Cottus Gobio), both of which tend to inhabit areas with little or no pollution and high oxygen availability (Reynolds & Reynolds, 1998; Seo *et al.,* 2019). Therefore, effective WWTP's can potentially reduce the occurrence of these negative impacts on the aquatic environment.

High levels of faecal coliforms can also become a threat to human health. A study by Abass *et al*., (2016) discussed the potential threat of coliforms within vegetation. Their study monitored a vegetable crop closest to the river and found that it had higher levels of coliform contamination. This was a specific threat to human health because the river was used to irrigate the arable land (Keraita *et al,.* 2008). Similarly, in Kumasi, Ghana, wastewater treatment is largely inadequate for the population. In addition, the effectiveness of their WWTPs varies. As a result, there is general concern relating to the health risks posed by polluted water adding further weight to the argument that effective wastewater treatment is necessary, beneficial and very much needed (Keraita *et al*., 2003; Nikiema *et al.,* 2010; Murray & Drechsel, 2011).

Arguably, moving forwards, water pollution should be seen as global, shared problem. It has implications for life in aquatic environments and for the people living near to them or using water from them. Water pollution can have a myriad of impacts, including the deaths of

14,000 people a day and significant threat to the survival of rare aquatic species (Chaudhry & Malik, 2017). This figure is due in part to inadequate effluent management (Chaudhry & Malik, 2017). There are many causes for this pollution, including domestic waste and farmland run off from pesticides and fertilisers (Chaudhry & Malik, 2017). Therefore, effective WWTPs may have a key role to play moving forwards in the work to tackle and resolve the issue of water pollution locally and globally.

Arguably, to do this, WWTPs must continue to evolve as their methods are not fully effective in their current form. Faecal coliforms continue to pose problems for WWTP's. Faecal coliforms are the main bacterial component of pollution from wastewater treatment plants, domestic waste, and farmland runoff, and can also come from on-site septic systems (Jamieson *et al.,* 2003). As previously discussed, their presence can have several negative consequences, but primarily, they can cause serious health implications for humans and can impact BOD, which can lead to a reduction in the oxygen availability for other organisms, such as freshwater invertebrates.

In addition, coliforms pose a particular threat to the River Kent. This is because part of the river is used for bathing and if ingested, coliforms in the water can lead to urinary tract infections (UTI's), hepatitis, diarrhoea and other such symptoms (Ajumobi & Olayinka, 2014). The degree and seriousness of illness will vary depending on the number of coliforms present and the individual's immune system (Ajumobi & Olayinka, 2014). This presents a strong case for the need for effective WWTP's.

Organic enrichment and nutrient pollution can also lead to many harmful impacts on an ecosystem. The importance of this is reflected by it being made a multinational priority for political bodies like the European Union (EU) (Woodward *et al.,* 2012; Grizzetti *et al.,* 2021). Eutrophication is a key issue caused by organic enrichment. It leads to excess plant growth, spurred by the increase in nutrients, and can result in harmful algal blooms. This can then lead to the decomposition of plants on the riverbed due to sunlight being blocked out by algae and surface plants (Smith & Schindler, 2009; Korpinen & Bonsdorff, 2015). This process can significantly impact the food and oxygen dynamics in aquatic areas, as decomposing material can lead to oxygen depletion (which can be harmful to some species as mentioned previously) and can also result in a shift in conditions. This shift may lead to species who are more resilient to these changes being more successful and could lead to trophic shifts in the ecosystem (Smith & Schindler, 2019; Van Der Lee *et al.,* 2021). This provides further evidence to support the need for effective and efficient WWTP's.

Ecosystem services and economic factors:

It is important to note the significance of ecosystem services, essentially, benefits that we derive from the environment. These services can be threatened by things like organic and faecal pollution. According to Constanza (1999), the ecosystem services provided by aquatic environments are highly valuable and can theoretically be valued up to $21 trillion per year. However, this argument is disputed, as ecosystems could continue to be harmed if there is seen to be a *more* beneficial financial 'trade-off.' Heal (2000) argued that it was hard to value ecosystems, as value in this sense may not include the importance of the ecosystem, just the exploitable amount (Farber *et al.,* 2002; Small *et al.,* 2017). Similarly, different people may value the same ecosystem differently from a cultural and economic perspective. This can be showed by the difference in opinions relating to the global value of ecosystem services. An example being Constanza *et al.,* (1997) which valued them somewhere between $16 and $54 trillion, but also noted that there is still a lot that we do not know about environmental processes which could be benefiting us, therefore, the value may still fluctuate (Constanza *et al.,* 2017)**.** With human population rising, the natural capital per individual diminishes and pollution further lowers the capacity of the environment to provide this service. Therefore, to enable the environment to continue to sustain the human population, we need to ensure that it is protected (Mason, 2002).

From an economic standpoint, WWTPs can be very expensive to build and maintain. The most expensive parts being used in the nutrient removal stage, costing €16,662,910 per annum and the Tertiary treatment costing €14,207,707 per annum, which includes the processes of UV treatment and chlorination. According to a study by Hernandez-Sancho et al., (2011) the total cost of all stages comes in at a staggering €128,101,780. It is important to note that these figures do not include the cost of initial construction, labour costs, the required infrastructure and the variation in types of wastewater treatment but is a good indicator of the sheer cost of waste management set up and maintenance. Arguably, these treatments safeguard humans against the cost of polluted water systems. In addition, studies on the potential impact of effluent on fish aquaculture, where many cases of infections within fish tissue and evidence of necroses and other health issues were noted, evidence that WWTP's also safeguard the health and survival of aquatic life (Mahmoud *et al.,* 2016). Discharges can also contain substances such as textile dyes. These can have a significant impact on reoxygenation and can increase the degree of anoxic conditions in water bodies. Chemicals in water could also result in bioaccumulation and negatively impact fisheries throughout the catchment (Gita *et al.,* 2017), therefore impacting aquatic and human health.

Concluding remarks:

To conclude, this literature review highlights the importance of measuring and monitoring domestic wastewater pollution. As evidenced, the impact of wastewater treatment can be seen at a local, regional, national and global level and affects aquatic, animal and human health. The River Kent provides an interesting case-study where the bioindicators and ecosystem services discussed can be examined and explored. This review and research support the purpose and design of this study and will provide evidence to support any conclusions made.

Method:

Aims:

To identify any significant inputs into the River Kent from the WWTP which could influence water quality, using statistically analysed data collected from the field.

To find out the number of coliforms entering the river from the WWTP and to compare this to current Environmental Agency (EA) water quality designations (Department for Environment Food & Rural Affairs, n.d.)

Hypothesis:

- There is unlikely to be to be evidence of large nitrate and phosphate inputs into the River Kent' due to the treatments carried out by the WWTP. However, there could be evidence of inputs of material influencing the dissolved solids, coliforms, and conductivity.
- There may be some coliforms seen upriver due to livestock and septic tank inputs. However, an increase in colony forming units within the water samples should still be seen after the WWTP.

Method summary:

The focus of this methodology was to identify wastewater effluent discharging into the river from the WWTP and the impact that this has on different water quality measures, in particular faecal coliforms, such as *E-coli*. The method was split into two parts, the initial surveys and water testing and then the coliform testing carried out towards the end of the survey period. The initial testing, where one test was carried out each survey day, tested for PH, conductivity, biological oxygen demand (BOD), total dissolved solids (TDS), temperature (℃), phosphate (PO $_4$) and nitrates (NO_3-). The second phase of testing involved testing for total coliforms and *E-coli*. The aims of these methods were to attempt to identify inputs from the WWTP and then to identify whether these inputs were significant.

Phase one of data and sample collection:

After initial 'test runs' of the method, in November the first 'true' data collection was carried out starting on the 23rd of November. Subsequent collections were carried out on the 7th of December, 14th of January, 25th of January and 13th of February. Figure 3 shows the locations of all three data collection sites. These have been categorised into Site A, B and C, with A being the most upstream point of study and Site C being the most downstream point and after the WWTP. Site A and B were selected to act as a baseline to compare to Site C. Site A acted as control 'uninfluenced' by the presence of the WWTP or Staveley itself. Whereas Site B was used to identify the impacts of Staveley (the village itself) and to eliminate any possible variables in data created by it.

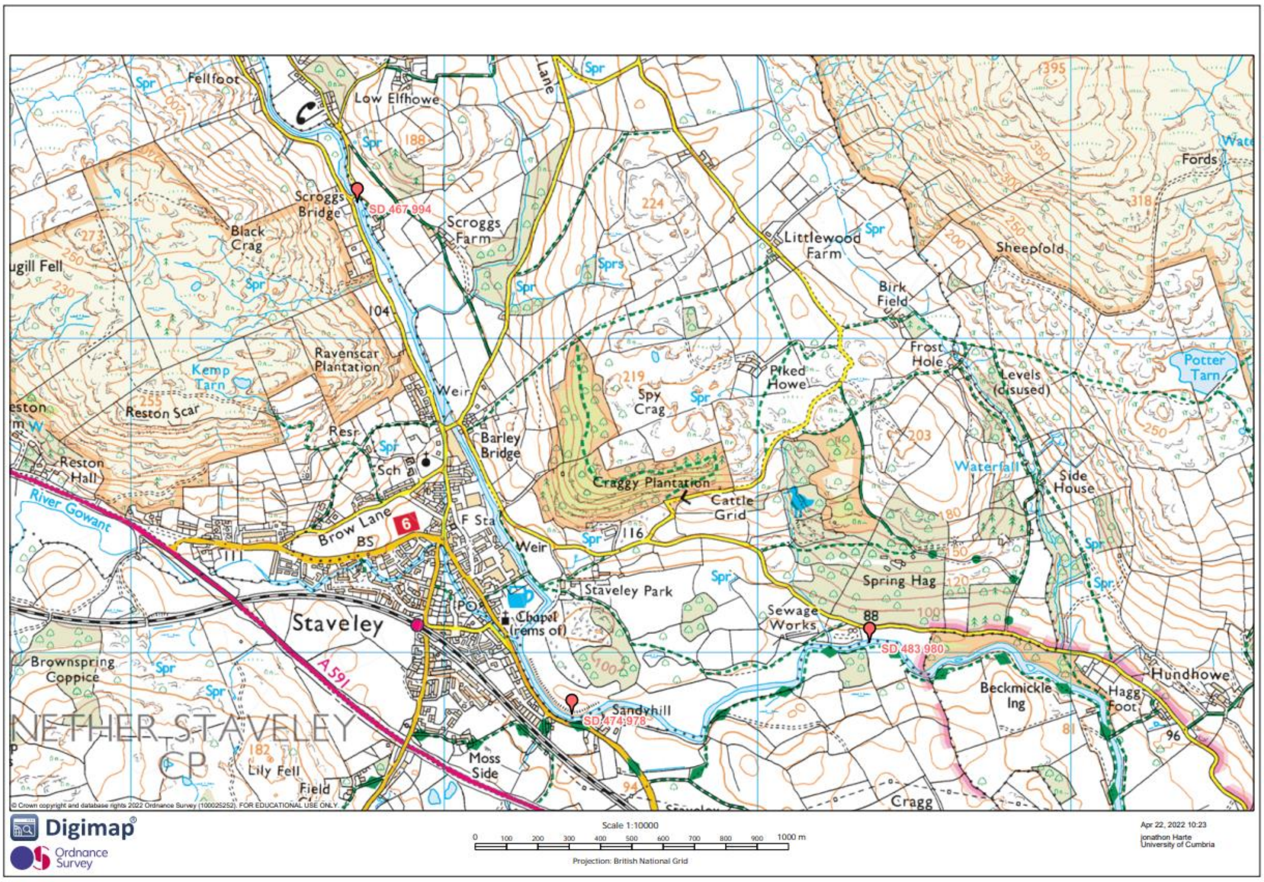


Figure 3- Map showing the site locations on the River Kent which goes through Staveley. Site A being SD 46 99, the most upstream point, Site B at SD 47 97 downriver of Staveley itself and Site C at SD 48 98, which is the site immediately after the WWTP (River Kent Staveley ordinance survey map, 2022)

A basic water quality assessment was carried out at each site using a HI1991300 Hanna Instruments probe which measured temperature, PH, conductivity, and TDS. By doing this, I was able to establish an understanding of the basic water quality measures. This data could support lab-based results and could also be used to form interesting comparisons. This way of monitoring physiochemical properties of water is an effective, cheap and easy way of taking these recordings in the field, this is the main reason I am using this method. Using probes like this has been used in a variety of studies across the literature and some do use Hanna Instruments probes in a variety of models (Arnold *et al.,* 2005; Khamis *et al.,* 2014; Ndione *et al.,* 2019)

Then, using waders and 1 litre plastic bottles, three water samples from each site were collected, each bottle containing a litre and each sample being taken from different points across the river. These samples were initially taken to the University of Cumbria laboratories in Ambleside, and some were frozen to later be taken to laboratories in the Fusehill Campus, Carlisle. All initial samples were collected on the same day and sampling was then repeated several times over a period of four months, from November through to February. The final collection, taken in February, were used for the coliform testing based in the laboratories in Carlisle.

Phase Two - Water sample testing and analysis:

In the Ambleside campus laboratories, measures of phosphate, nitrate and BOD were taken and recorded. This testing was completed within 48 hours of the initial data collection. Both nutrient tests were carried out using the palintest method and equipment. This method has been used in a number of studies, measuring rivers, lakes and even wetland and all involve some initial addition of reagents (Mutisya & Tole, 2010; Melaku *et al.,* 2020; Getnet *et al.,* 2021). This method was easy and a lot more specific than some of the other methods, an example being Nitrate strips.

To test for nitrates, 20ml of the water sample was mixed with a level scoop of Nitratest powder and 1 Nitratest Tablet. The solution was then shaken within the sample tube for one minute, then left to stand. After a further one-minute invert to aid flocculation, the sample was allowed to settle once again until all suspended reagents sank to the bottom. Following this, 10ml of the mixture was decanted into another sample tube. One Nitracol tablet was then crushed and added into the sample. The mixture was then left to stand for 10 minutes.

At this point, a blank was set up using an unaltered sample. This was placed in the photometer and a setting of Phot 63 was selected. This was covered with the lid and turned on so that a reading could be taken. The altered sample was then placed into the photometer, covered with the photometer lid, and a further reading was taken to provide the nitrate concentrations in mg/l.

For phosphate sampling, a phosphate test kit (which comes with the palintest photometer) was used. To complete the phosphate sampling, one Phosphate No 1 tablet and one Phosphate No 2 tablets were crushed and dissolved into 10ml sample. This was left to stand for ten minutes to allow time for the colour to develop.

Repeating the blank process outlined previously, this time Phot 28 was selected on the photometer to show the results for phosphates in mg/l.

Finally, samples were tested for BOD. This testing was completed using a dissolved oxygen probe (Oakton™ DO Six+ Portable Dissolved Oxygen Meters). Using glass BOD bottles, the dissolved oxygen in all the samples was measured. This testing was completed on samples taken from each survey day and forty-eight hours later, measured again using the same method. The data gathered helped to identify biological activity within the sample and could also be used as an indicator of pathogen pollution. However, during the last sampling day, the probe did not work. As a result, a BOD measure for the final survey day could not be taken. Arguably, the use of a probe was a lot easier than some of the alternative methods, an example being the Winkler method, which is a titration and would have required a lot more set up and reagents than the probe (Carpenter, 1965). Other studies have used the probe used in this study to measure dissolved oxygen in flood water effectively, which also supports its use (Clilverd *et al.,* 2009; Unger *et al.,* 2009)

For the coliform sampling two methods were used, the most probable number method (MPN) and the vacuum filtration method. The MPN method is a cheap and easy approach to identifying the presence of coliforms in a sample by creating conditions suitable for the coliforms to respire and produce gas. This can be seen in the inverted Durham tubes. This method uses a series of dilutions containing a lactose broth in various, specific dilutions. 10ml of sample are added to test tubes containing different dilutions of lactose broth and the inverted Durham tubes. If activity is observed, the test would be labelled as a positive test. This method has been criticised in the literature as having different results than other studies measuring colony forming units (CFU) however in this study it is being used as an indicator of faecal coliforms not necessarily to tests its quantity (Gronewold & Wolpert, 2008).

The second method for measuring coliforms involved filtering 100ml of sample through a filter paper using the vacuum filtration method. This method creates a vacuum that pulls the water through the filter paper, trapping the desired coliforms. This method used 45um filter paper. Machines are sometimes used, but in this case, a water aspirator was used to create the vacuum and was connected to the flask with rubber tubing. This created a vacuum in the flask which pulled the water through the Büchner funnel and the filter paper, and then into the flask. Following this process, tweezers were sterilised and then used to move the filter paper onto the Chromocult agar, face down. The filter paper was then quickly covered using the agar plate lid and incubated on top of the Chromocult agar at 35℃, then left for twenty-four hours. Following this, the number of pink and blue colonies could be counted, blue being *E-coli* and pink being other coliforms. This sampling was carried out on all of the fresh samples and repeated three times to ensure that the results were accurate. However, only three of the frozen samples could be tested due to the lack of available Chromocult plates (due to their cost). The membrane filtration technique used in this study is a commonly used method. Some argue that the Colilert method is more effective, however the membrane filtration method was well referenced and easier to acquire (Geldreich *et al.,* 1965; Dufour, 1977; Buckalew *et al.,* 2006).

Statistical analysis:

The data was recorded and then collated in a Microsoft Excel spreadsheet. This data was then transferred into SPSS v.25 (IBM, Chicago, USA), the software used for data analysis in this study. The Shapiro-Wilks normality test was then used to ascertain whether the data was parametric or non-parametric for the different measures (i.e. PH, TDS, conductivity and BOD). These tests were carried out over the three sites and on different days in order to identify any factors that might influence the data, such as time and weather conditions. For post hoc testing depending on the results of the Shapiro-Wilks normality results we would:

Where the results were parametric, an ANOVA test was used to test the variance in the results.

Where the results were non-parametric a Kruskal-Wallis one way ANOVA was used.

Results:

PH, TDS, conductivity and temperature:

This data indicates that there was no significant impact on the temperatures recorded before and after the WWTP. The levels of PHunits also remained within a similar range before and after the WWTP and between the sites in general. These findings were consistent between the different test dates and test sites, with no significant change being noted. A steady increase in the measure of TDS and conductivity was noted as the water travelled between each site.

		Measurements taken			
Site	Date	Temperature (℃)	Total Dissolved Solids (ppm)	Conductivity (um)	PH
A	23-Nov	5.6	40	79	7.6
B	23-Nov	5.4	45	89	7.67
C	23-Nov	5.1	53	107	7.82
A	07-Dec	5.2	30	63	7.88
B	07-Dec	5.6	41	81	7.86
C	07-Dec	5.1	45	90	7.76
A	14-Jan	5.7	36	71	8.22
B	14-Jan	5.8	41	82	7.97
C	14-Jan	6	45	91	7.82
A	25-Jan	5.1	43	86	8.11
B	25-Jan	5	51	102	8.22
C	25-Jan	5.4	60	120	7.97

Figure 4- Table showing the measurements for Temperature (℃), Total dissolved solids (ppm), Conductivity (um) and PH (PHunits), Refer to figure 2 for site locations.

Nitrates and phosphates:

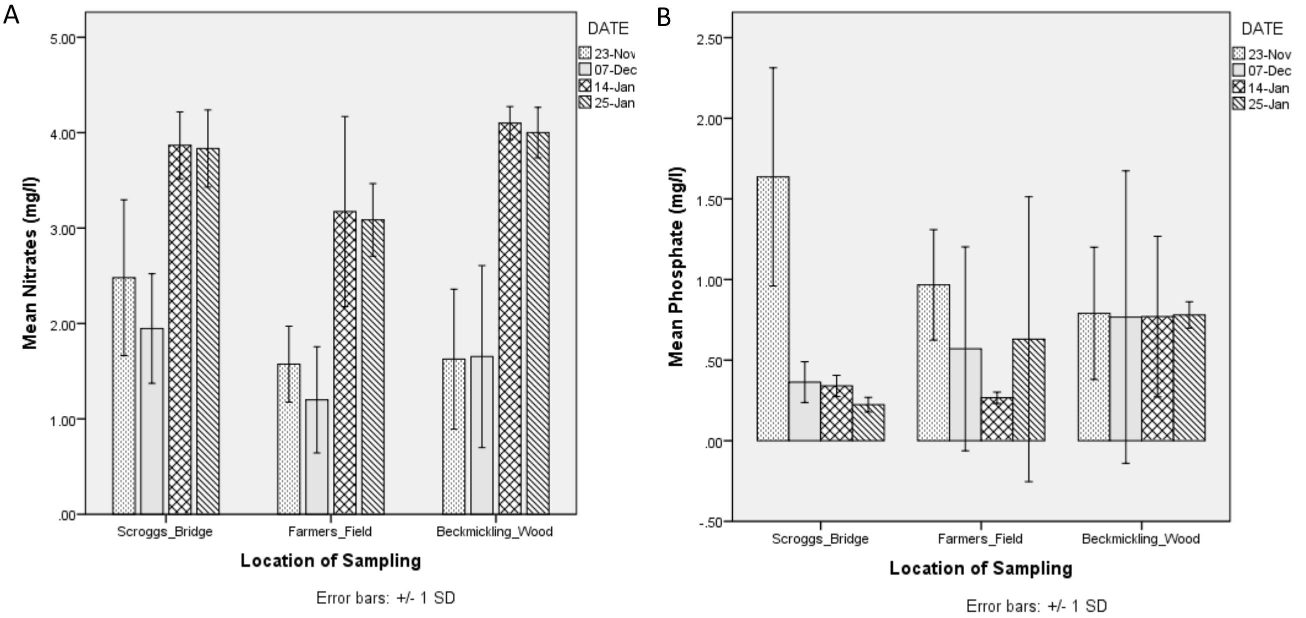


Figure 5- Chart A shows the data for the nitrate testing in mg/l showing the sites in the X axis and levels in the Y axis. Sites are also identified in the Legend. Chart B shows the data for phosphates in mg/l in the same way as Chart A. The raw data for both can be seen in Appendix A.

The data recorded in Figure 5, Chart A evidence that levels of nitrate varied significantly between the dates of sampling. In January, the levels were at their highest (compared to the levels recorded in November and December). In January, a slight increase in levels of nitrate was noted at site C (after the WWTP). Levels did not increase in the same way in November and December at this site. Levels appeared to peak at approximately 4.3 mg/l. The lowest level of nitrates recorded during the test period was 0.7 mg/l. The standard deviations overlap for some of the results recorded.

Figure 5, Chart B shows the phosphate levels recorded, evidencing a significant degree of variation across the dates and the three sites. There are no notable trends in the data. In January and December, a marginal increase in phosphate levels was identified between Scroggs Bridge, Farmers Field and Beckmickling Wood. However, in November, phosphate levels decreased in these areas.

Coliform testing:

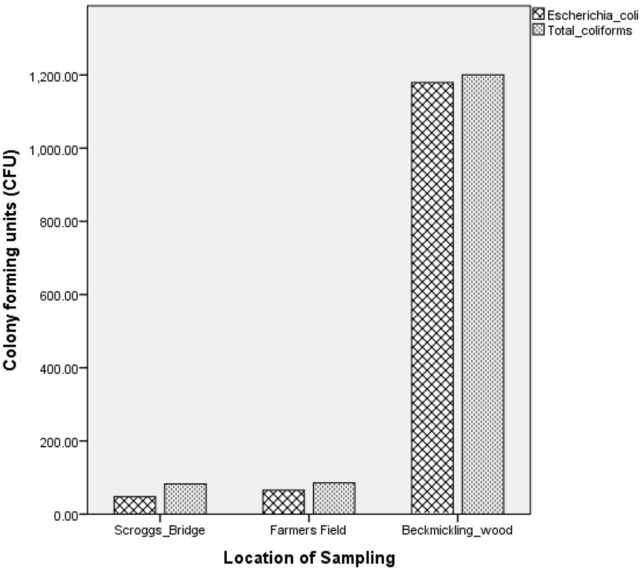


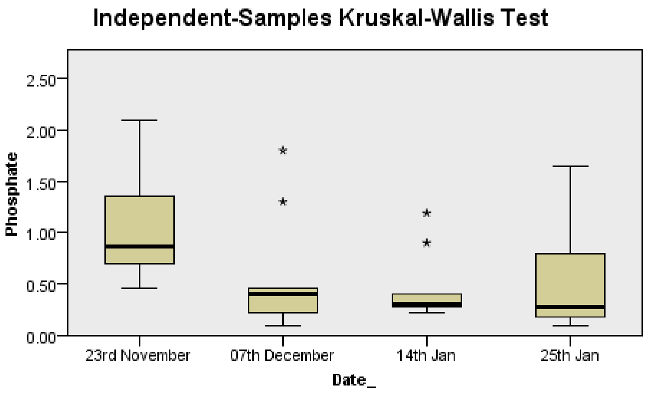
Figure 6: This graph shows the difference in the colony forming units (CFU) for all 3 of the study sites, with different bars representing the *E-coli* and total coliforms which can be distinguished in the legend.

The results shown in Figure 6 indicate a significant increase in the number of *E. coli* present in the samples after the WWTP, compared to those taken before the WWTP. At site A, there was approximately 48 CFU /100ml. At site B, 65 CFU/100ml was measured adjacent to the farmer’s field. At Beckmickling Wood, a higher count was recorded of approximately 1179 CFU/100ml. This is represented in Figure 6, where the increase in coliforms and *E. coli* is notably higher at Beckmickling Wood.

The data provided by the SENS group showed a peak of approximately 2500 CFU/100ml in Beckmickling Wood, with much lower quantities upstream. This data can be seen in Appendix C. The results from SENS varied depending on the dates of sampling, with the same site showing a measurement of approximately 590 CFU/100ml within the same month. The data from James Cropper Paper Mill in Burneside showed similar variability, again depending on the dates that the samples were taken. For example, on one day in November, the sample showed 690 CFU/100ml. A subsequent sample was taken in December at the same location, showing 8700 CFU/100ml. This data can be found in Appendix B.

Statistics:

The Shapiro-Wilks test for normality showed the results for the *E. coli* sampling were non-parametric and so rejected the null hypothesis (**H_0**= The is no difference between the number of coliforms in the river between all 3 sites). When testing whether any of the other results varied between sites, the results were parametric and there was no significant difference for PH, nitrate, phosphate, temperature, TDS, conductivity and BOD. However, when testing the data to see if there was a significant difference between the dates the surveys were taken phosphates were the only non-parametric sample, other than *E. coli,* with p values of p=.000 for site A and p=0.17 for site B. Post hoc testing for nitrates and phosphates, with a one-way ANOVA, showed a p value greater than 0.05, so we accept the **H_0**=. There was no difference between the nitrate and phosphate levels in the river between all three sites. The same can be said for temperature and PH. However, post hoc testing for TDS and conductivity had p values of p=0.032 and p= 0.028 respectively (Kruskal-Wallis). Therefore, for these measures, we reject the **H_0**=. There was no difference between the TDS and conductivity between the sites. Post hoc testing of the phosphate data showed that the data was mainly biased towards the 23rd November, which means we reject the **H_0**=. There is no significant difference between the levels of phosphates and the dates recorded.



Total N	36
Test Statistic	9.176
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.027

1. The test statistic is adjusted for ties.

Figure 7- Results from a Kruskal-Wallis test looking at where the significance is in relation to dates. The main significance comes from the 23rd November sampling.

Discussion:

Summary of the key findings

This overall study shows somewhat mixed results. The measurement for coliforms indicates a dramatic increase compared to areas upriver from the WWTP. Whereas, the other measures, for example, PH levels, conductivity and TDS, show little evidence of being impacted by the WWTP. Primarily, this study aimed to identify whether there were any significant inputs from the WWTP which could be impacting the water quality or the river in general. The test data did evidence significant inputs of coliforms. It also identified that other inputs were minor. However, it is important to note that this study represents only a snapshot of the coliform presence in the River Kent. A comprehensive, longer-term scheme is being carried out by the SENS group which will help to provide a more representative picture of coliform presence over time.

Discussion of the findings and interpretations

E. coli was the most significant measure taken during this study. A dramatic increase of 2400% greater than the baseline levels before the WWTP was noted. Furthermore, the data provided by James Cropper Paper Mill and SENS supports these findings. Interestingly, higher levels than those recorded in this study were evidenced by SENS at a similar location in Staveley, and Burneside further downstream. However, it is important to note that all data is also influenced by background input, such as the surrounding farmland and septic tanks. These and other inputs could contribute to the data relating to levels of *E.coli*. However, the intense peak of *E.coli* next to the WWTP strongly suggests that levels must be influenced by a factor/s in addition to the background inputs noted.

Between sites A and B, which covers a 2 km distance, an increase of only 17CFU/100ml in faecal coliforms was noted. However, between sites B and C, which covers a smaller distance of 1.1km, 1112CFU/100ml was recorded, thus representing a significant increase in the presence of coliforms. The WWTP is situated between sites B and C. It is unlikely that any other additional inputs could have contributed within this small area. Leading to the conclusion that these levels of *E. coli* must be generated from the WWTP. However, it is important to concede that unforeseen variables and/or additional inputs that have not been noted could have contributed to this increase.

This data supports the hypothesis that there is a significant input of coliforms going into the River Kent based on the *E.coli* primary dependent variable. The most likely causal factor is the WWTP, primarily because of its location in the study and the significance of the increase of coliforms noted, representing a 2400% increase. Other inputs could also contribute to this increase, but the extreme nature of the increase and lack of other notable variables, would suggest that the WWTP was responsible.

One of the main aims of this study was to compare the results taken in the River Kent to current Environmental Agency UK (EA UK) designations. The EA has identified poor water quality as anything ≤900 CFU/100ml (Department for Environment Food & Rural Affairs, n.d.). The results from this study, and those collated by SENS on certain dates, were higher than this figure, refer to appendix B and C. Therefore, at certain times, the water quality in the River Kent could be designated as poor. In these cases, the EA advises against swimming to protect health. Pollution risk forecasting could be used to identify the days where water quality would be too poor for swimming. However, this measurement only focuses on the risk to human health and does not consider the potentially harmful impact that this may have on local biodiversity and aquatic life.

One of the other aims of this study was to identify if there were any other significant inputs going into the river from the WWTP, which included the other parameters measured in this study. When testing whether the data was parametric or non-parametric, I separated the data to test for normality between the different sites and between the different dates. All of the samples were tested for PH, temperature, conductivity, BOD and TDS. These tests indicated that phosphates and nitrates were parametric, showing little change based on the proximity of the WWTP. There was a slight increase between the sites in terms of conductivity and TDS, which increased between sites A and C and could be seen in the one-way ANOVA. However, this increase was steady and was not significant enough to be a result of the WWTP. It was most probably caused by inputs from the surrounding land. The parametric data were generally normally distributed. However, phosphate monitoring on the 23$^{rd\ of}$ November at site A indicated a very high measure (see figure 6B). This could represent an anomaly in the results or could be caused by an unexpected cause upriver. In this case, this could represents an anomaly as the standard deviation does overlap in many of these situations. It could also be the result of incorrect treatment of the sample before using the Palintest photometer. This would require more research to explore fully.

Comparing this data to the data provided by James Cropper Paper Mill and SENS was extremely useful as it supported the data collected in this study relating to *E. coli.* The data tests for *E.coli* had to be taken on one survey day and therefore provided only a snapshot of data. This could be influenced by individual events impacting the water source. The data collected by SENS was collected over time and supports the conclusions drawn from this study data, therefore allows us to be more certain about the results. In addition, the results from the James Cropper Paper Mill's results were also useful. This data allows us to identify that the issue could be significant, reaching as far as Burneside, therefore could be having impacts downriver. This distinction is important as this study does not address the scale, or range of the impacts that the discharges could be having on the local environment.

Limitations of the Study

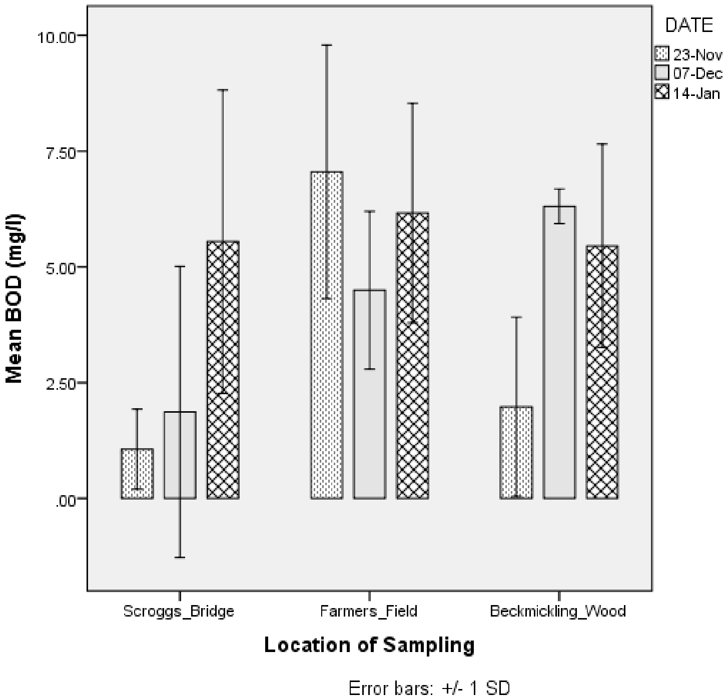


Figure 8- A Graph showing the mean BOD in mg/l at each site and each date measurements were repeated with error bars representing the standard deviation, created using SPSS.

The standard deviations for a lot of the water quality measures did overlap with the samples taken. This made it difficult to fully differentiate the data and thus identify clear trends that could be caused by the WWTP. This can be seen in the measures for BOD (see figure 8). These results show that the standard deviation overlaps for a large number of the samples. This is particularly clear when looking at the data recorded on the 14th of January at all sites, which clearly overlap. This means that some of the measures were the same at the different sites, which makes it difficult to draw clear conclusions from the data.

One of the limitations of this study is that the coliform sampling was only carried out on one day. As a result, the reliability of this data is reduced, as one-off events could influence the data sets. This limitation was mitigated by using the data from studies carried out by both the SENS group and the James Cropper Paper Mill to support any conclusions drawn. In their studies, they noted a large variability in *E. coli* CFU at different dates. This could be a result of the weather conditions. In certain weather conditions, WWTPs carry out an emergency release. This would potentially significantly impact data and could account for the fluctuations noted. This would need further exploration and cross referencing with weather records. As a result, the results of this study and those carried out by SENS and James Cropper Paper Mill suggest a clear concern in terms of *E. coli*, with levels being high enough to be considered a risk but cannot be seen as conclusive. They could however, help to inform future research on this topic, an example being the pilot testing being done within Staveley.

A further potential limitation is the influence of human error and/or technological reliance. An example of this was technological problems encountered during the study. The dissolved oxygen metre broke, and as a result, BOD could not be recorded for the final sampling date. This does pose questions relating to the functionality of the machine in question and therefore the reliability of the other readings. In terms of human error, test data could be impacted. For example, when using the phosphate and nitrate reagents, the palintest could be carried out incorrectly. In addition, due to microbiological inexperience, cross contamination of samples, or insufficient antiseptic techniques could influence results.

Whilst the data is supported by that provided by both SENS and James Cropper Paper Mill, there are very few studies on the impact of the WWTP in Staveley, other than smaller studies previously mentioned in this discussion. These studies and their data are also potentially subject to limitations, and their methodology would need to be fully explored in order to identify the validity of data included therein.

Finally, it is important to note that there may be more effective methods to collect and analyse this data. An informal study carried out by the Rivers Trust took samples at similar sites to the one used in this study (The Rivers Trust, 2020). They used the colilert method, which involved a pre-prepared reagent which can be added to the sample. This method is favourably reviewed by several papers (Yakub *et al.,* 2002; Macy *et al*., 2005). However, it is expensive. Therefore, although it may produce better results than other methods, such as the membrane filtration method, it was not viable for this project (Wohlsen *et al.,* 2008).

Recommendations for further investigation

The monitoring period and frequency of testing used for this study limited the solidity of conclusions drawn. For future research, creating a longer survey period with more frequent monitoring, each day if possible, would be advisable. It would also be interesting to investigate and assess the impact of weather on coliform discharge to see whether higher levels can be identified. By doing this, you would be able to better predict the coliform presence in the river under certain weather conditions.

It would also be interesting to expand the investigation to identify the impact/s of the WWTP on biodiversity, in particular benthic invertebrates like the white clawed crayfish. This could be done using, kick samples similar to those used by the Riverfly Project, but with a particular focus before and after the WWTP (Brooks *et al.,* 2019). Studies could be carried out to record the aquatic plant species present, testing migratory fish health as an example. This would develop and expand this research, helping to draw conclusions evidencing the impact of the WWTP on aquatic life.

Finally, as previously mentioned there is currently no evidence relating to the scale of the problem and how far-reaching it could be. Therefore, an important aspect of future research could be to investigate how far this pollution could and/or has reached and importantly, how long it lasts. There are studies on the naturalisation of *E. coli* in water bodies and reproducing in soil and sediment which could suggest that these strains could survive long periods in aquatic environments (Jang *et al.,* 2017). This could suggest that the impacts of faecal coliform inputs could be long lasting and resilient. This also could bring into question the use of *E. coli* as a bioindicator of pollution, as it will be hard to distinguish between naturalised strains and strains from the WWTP when monitoring without using genetic analysis.

Conclusions:

To conclude, this study, based on evidence gathered from the River Kent, has achieved its primary aim of identifying the main inputs from the WWTP. The main input was found to be *E. coli*, with approximately 1,114 CFU/100ml being measured (background input has been considered to produce this figure). The levels of coliforms measured varied depending on the date that the samples were taken. This could be a result of the changes in weather, farmland use and/or other variables.

The data gathered for the purposes of this study is supported by the data collected by SENS and the James Cropper Paper Mill. It is important to note that both of these groups place high value on the water quality of the river in terms of socioeconomic and environmental factors.

The results for this study were taken over a three-month period. Moving forward, further monitoring, over an extended period of time, would be needed in order to draw solid conclusions relating to the level of coliforms entering the River Kent over time. This study has identified the WWTP as the potential problem/cause of increased levels of coliforms in Staveley. However, this hypothesis would require further investigation and exploration in order to be confirmed. This is important, as there are gaps in this study that need to be addressed before we can be certain of the impacts and implications of the releases from the WWTP. However, this study has been successful in identifying areas for further study in terms of the River Kent and the importance of completing this work.

The results included in this study are concerning and raise clear questions relating to the quality of the water in terms of human use, the health of the water in terms of aquatic life and the impact of the WWTP in terms of the conservation of biodiversity within this area.

Appendices:

Appendix A:

Table showing the raw data for Nitrate, Phosphate and BOD in the River Kent across the 3 sites including repeats. Site A being Scroggs Bridge, B being the farmers field and Site C being Beckmickling wood, refer to figure 2 for a map of the sites.

		N (mg/l)			**P (mg/l)**			**BOD (mg/l)**		
Site	**Date**	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3	Repeat 1	Repeat 2	Repeat 3
A	23-Nov	1.84	2.2	3.4	0.86	1.95	2.1	2.01	0.85	0.32
B	23-Nov	2.02	1.26	1.44	1.35	0.86	0.69	4.2	9.66	7.29
C	23-Nov	0.78	2.06	2.04	1.25	0.46	0.66	4.11	1.48	0.33
A	07-Dec	2.6	1.52	1.72	0.41	0.46	0.22	5.48	0.38	-0.26
B	07-Dec	1.1	0.7	1.8	1.3	0.22	0.19	2.55	5.73	5.2
C	07-Dec	2.5	0.62	1.84	1.8	0.1	0.4	6.17	6.02	6.73
A	14-Jan	3.9	4.2	3.5	0.4	0.27	0.35	4.21	9.28	3.15
B	14-Jan	4.1	3.3	2.12	0.23	0.3	0.27	6.19	3.78	8.52
C	14-Jan	4.2	3.9	4.2	0.9	0.22	1.19	4.81	7.9	3.65
A	25-Jan	3.6	3.6	4.3	0.27	0.18	0.22			
B	25-Jan	3.1	2.7	3.46	1.65	0.14	0.1			
C	25-Jan	4.3	3.9	3.8	0.8	0.69	0.85			

Note: Site are labelled as Scroggs Bridge= Site 1, Farmers Field= Site 2 and Beckmickling wood= Site 3

Appendix B

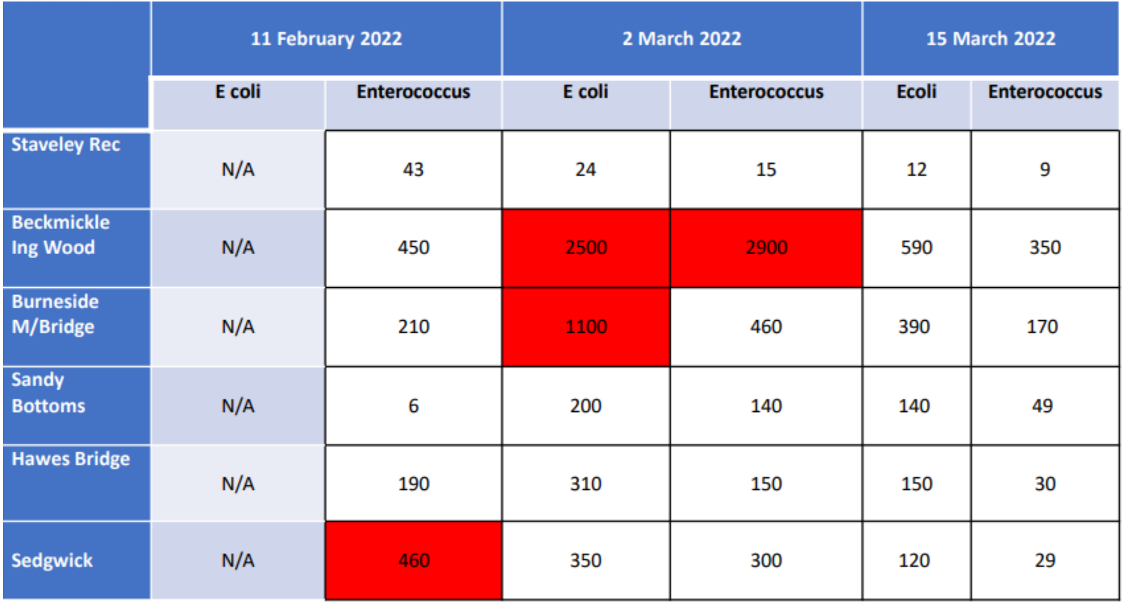
Table showing data provided by James Cropper Paper, a paper mill downstream of Staveley measuring the water in the River Kent over similar starting period to my data collection.

	Dates of recordings:		
Measurement taken:	11/10/2021	15/11/2021	06/12/2021
Nitrate as N (mg/l)	0.806	0.956	0.886
Temperature of Water (℃)	11.3	9.9	5.9
Oxygen, Dissolved as O2 (mg/l)	11.3	10.9	12.4
Escherichia coli : Confirmed : MF (cfu/100ml)	1400	690	8700
pH	7.68	7.56	7.65

Note: Data collected from a 'Sonde' monitoring devise in Burneside downriver from Staveley

Appendix C:

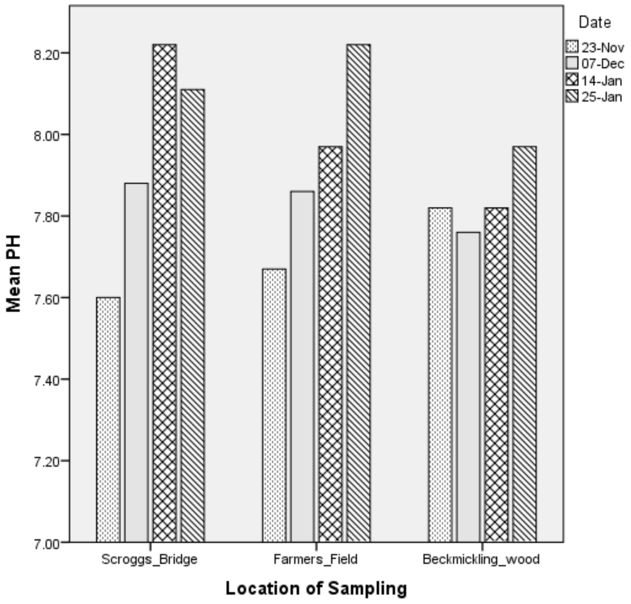
A Table showing the SENS data for *E. coli* and enterococcus across 3 days of sampling at a number of locations.



	11 February 2022		**2 March 2022**		**15 March 2022**	
	E coli	**Enterococcus**	**E coli**	**Enterococcus**	**Ecoli**	**Enterococcus**
Staveley Rec	N/A	43	24	15	12	9
Beckmickle Ing Wood	N/A	450	2500	2900	590	350
Burneside M/Bridge	N/A	210	1100	460	390	170
Sandy Bottoms	N/A	6	200	140	140	49
Hawes Bridge	N/A	190	310	150	150	30
Sedgwick	N/A	460	350	300	120	29

Appendix D

A Graph showing the mean PH in Phunits at each site and each date measurements were repeated with error bars representing the standard deviation, created using SPSS.



Appendix E

A chart showing the results for normality testing for nitrates data across the 3 sites

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
Nitrate	1	.227	12	.089	.898	12	.150
	2	.135	12	.200*	.962	12	.815
	3	.258	12	.027	.863	12	.054

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix F

A chart showing the results for normality testing for phosphate data across the 3 sites

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
Phosphate	1	.356	12	.000	.669	12	.000
	2	.295	12	.005	.821	12	.017
	3	.148	12	.200*	.962	12	.816

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix G

A chart showing the results for normality testing for PH data across the 3 sites

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
PH	1	.217	4	.	.955	4	.745
	2	.181	4	.	.993	4	.971
	3	.349	4	.	.865	4	.279

a. Lilliefors Significance Correction

Appendix H

A chart showing the results for normality testing for Temperature data across the 3 site

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
Temperature	1	.252	4	.	.882	4	.348
	2	.192	4	.	.971	4	.850
	3	.276	4	.	.870	4	.298

a. Lilliefors Significance Correction

Appendix I

A chart showing the results for normality testing for TDS data across the 3 sites

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
PPM	1	.188	4	.	.973	4	.858
	2	.271	4	.	.848	4	.220
	3	.287	4	.	.864	4	.276

a. Lilliefors Significance Correction

Appendix J

A chart showing the results for normality testing for conductivity data across the 3 sites

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
Conductivity	1	.165	4	.	.990	4	.956
	2	.249	4	.	.866	4	.281
	3	.279	4	.	.883	4	.350

a. Lilliefors Significance Correction

Appendix K

A chart showing the results for normality testing for BOD data across the 3 sites

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Site	Statistic	df	Sig.	Statistic	df	Sig.
BOD	1	.182	9	.200[*]	.887	9	.184
	2	.117	9	.200[*]	.981	9	.968
	3	.164	9	.200[*]	.954	9	.730

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Appendix L

A chart showing the results for normality testing for *E. coli* data across the 3 sites

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Ecoli	.380	3	.	.762	3	.026

a. Lilliefors Significance Correction

Appendix M

A chart showing the results for normality testing for total coliforms data across the 3 sites

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
coliforms	.384	3	.	.752	3	.004

a. Lilliefors Significance Correction

Appendix N

A chart showing the results for normality testing for Nitrate, PH, Temperature, TDS and conductivity in relation to the dates recorded.

Tests of Normality

		Kolmogorov-Smirnov[b]			Shapiro-Wilk		
	DATE	Statistic	df	Sig.	Statistic	df	Sig.
Nitrate	07-Dec	.364	3	.	.800	3	.114
	14-Jan	.253	3	.	.964	3	.637
	23-Nov	.336	3	.	.856	3	.257
	25-Jan	.211	3	.	.991	3	.817
Phosphate	07-Dec	.240	3	.	.974	3	.693
	14-Jan	.291	3	.	.925	3	.471
	23-Nov	.312	3	.	.895	3	.371
	25-Jan	.228	3	.	.982	3	.746
PH	07-Dec	.328	3	.	.871	3	.298
	14-Jan	.232	3	.	.980	3	.726
	23-Nov	.260	3	.	.958	3	.605
	25-Jan	.198	3	.	.995	3	.868
Temperature	07-Dec	.314	3	.	.893	3	.363
	14-Jan	.253	3	.	.964	3	.637
	23-Nov	.359	3	.	.812	3	.142
	25-Jan	.292	3	.	.923	3	.463
PPM	07-Dec	.285	3	.	.932	3	.497
	14-Jan	.196	3	.	.996	3	.878
	23-Nov	.227	3	.	.983	3	.747
	25-Jan	.182	3	.	.999	3	.935
Conductivity	07-Dec	.253	3	.	.964	3	.637
	14-Jan	.193	3	.	.997	3	.890
	23-Nov	.241	3	.	.974	3	.688
	25-Jan	.182	3	.	.999	3	.935

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