Impacts of artisanal and large scale gold mining on tropical rivers in West Africa: A case study from the Brong Ahafo Region of Ghana

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Impacts of artisanal and large scale gold mining on tropical rivers in West Africa: A case study from the Brong Ahafo Region of Ghana.

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MSc

This thesis is presented in fulfilment of the requirements for the degree of

Doctor of Philosophy

School of Science

Edith Cowan University

Perth, Western Australia

July 2016

USE OF THESIS

Disclaimer

I wish to disclose that Newmont Ghana Gold Limited (NGGL) paid for some of the water sample analysis used in this study. Historical data on the Subri River water quality (2004-2012) was also provided by NGGL, this data formed part of the company's regular monitoring program reported to Ghana EPA, which is publicly available. While I am aware of the potential perceived conflict of interests that may arise from the indirect relationship between NGGL and me, I confirm that the study and its results are independent of NGGL and of its business in the area.

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Abstract

Mining communities in more than 70 developing countries, mostly in the tropical regions, still practise artisanal and small scale gold mining (ASGM). ASGM commonly operates along rivers and streams for easy access to process water and as receptacles for mine water discharges. A largely unregulated industry, ASGM employs rudimentary mining and processing methods including the use of mercury amalgamation, and is often found near to larger scale and modern mining (LSM) operations.

The substantial use of mercury by ASGM has drawn the attention of agencies and researchers but so has its persistent economic role in providing much needed rural employment. Mercury toxicity to human and environmental health has attracted much of researches, however ASGM impacts on riverine ecology, particularly at biota community levels, remains understudied.

This study investigated the impacts of ASGM on the ecology of the Surow River and that of an LSM (the Ahafo mine) on the Subri River between February 2013 and April 2014. Both the Surow and Subri rivers are ephemeral tributaries of the Tano River, in Brong Ahafo, Ghana. The Ahafo mine, currently operated by Newmont Ghana Gold Limited (NGGL), has been operating on the Subri River catchment since December 2006, whilst ASGM started operations along the Surow River in 2007. Major ASGM operations ceased in May 2013 although smaller operators and processors remained.

Specifically, the study aimed to determine whether and how ASGM and LSM impacted the respective river's water and sediment quality, macroinvertebrate and microbial (Archaea and Bacteria) community structures and resulted in mercury biomagnification in fish. ASGM impact on river water and sediment quality was determined using a reversed BACI (Before/After and Control/Impact pairing) experimental design, whilst that of LSM used a conventional BACI design. Impacts on macroinvertebrate community structure were determined by comparing multiple control and impact sites sampled multiple times. The sequencing of 16S rRNA of Archaea and Bacteria was based on a one-off sampling and comparison between multiple control and impact sites on both rivers. The biomagnification of mercury in fish was tested via analysis of correlations between mercury concentrations in fish tissue and fish trophic level, fish length (proxy for age) and fish weight.

The study demonstrated that gold mining, regardless of size and methods, significantly impacts the river ecosystems studied. Sediment particulates and minerals naturally available in the rock formation but exposed to the environment by mining activities were the most significant pollutants in the affected riverine ecosystems. The study area is in the tropic and experiences intensive rainfall. This, results in excess water which may come into contact with exposed rocks and wastes in the mining areas that eventually runs into or is discharged into the Surow and Subri rivers. Changes in the sediment and water quality due to mining were reflected in the macroinvertebrate communities of both rivers, while the sediment microbial communities tended to respond to differences in water quality. The study, however, strongly indicated that the types, magnitudes and effects of the environmental impacts of ASGM were different from that of LSM. The use, or the lack of, environmental management systems to mitigate impacts appeared to be the most important differentiating factor. The study also witnessed significant improvements in both water and sediment quality in the Surow River with the cessation of major ASGM in the area.

Mercury, which was used in the ASGM (in relatively small quantities in Ghana compared to other countries) was detected in the Surow River sediment (despite naturally low concentrations of Hg in the local soils), but was largely undetectable in the waters. However, it posed health risks to humans and biota. This study found mercury biomagnified in fish from both the Surow and Subri rivers as well as the Tano River, indicating the presence of mercury in the rivers. The source of Hg, however, could not be clearly established but may have been from artisanal amalgamation processes and from smelting.

Although mercury remains a concern in ASGM impacted rivers, it is not the only contaminant of concern. Sedimentation and particulate bound elements such as Al, As, Cu, Fe, Hg, and Pb were the main river pollutants resulting from ASGM operations. Elevated concentrations of metals in the turbid water due to the lack of sediment controls exceeded the Ghanaian and US EPA standards for the protection of aquatic life as well as that of Ghanaian raw water to be processed as drinking water. ASGM also significantly elevated the concentrations of salt ions and sulfate in river water particularly due to discharges of water from mine dewatering. During the active ASGM period, concentrations of Cu, Cr, Hg and Ni in the Surow River's sediment exceeded the threshold effect level / TEL, lowest effect level / LEL, Australian effect low range /ERL and threshold effect level for *Hylella azteca* 28-day test or TEL HA28. Increased sediment load and decreased sediment quality in the Surow River were reflected in the macroinvertebrate community structure that was dominated by sediment-tolerant taxa but with only a few pollutant-sensitive taxa including Ephemeroptera and Trichoptera families.

In the Subri River affected by the Ahafo gold mine, the impacts of mining were ameliorated by sediment control measures applied by the mine. The sediment control measures on the Subri River included the use of environmental control dams (ECD), one on a major tributary stream to the river, the other on a minor tributary. The ECDs reduced not only turbidity and total suspended solids, but also electrical conductivity, concentrations of most salt ions, nitrates and sulfate, and most metals both as total and dissolved forms from in the mine water being discharged into the environment. The improved water quality in downstream Subri River compared to that of the mine site and upstream was also reflected in the sediment quality, which had lower concentrations of most pollutants than that of the Surow River. The mine affected area in downstream Subri River also had more sensitive taxa including Ephemeroptera families than the Surow River. Nevertheless, mine discharge in downstream Subri appeared to alter the ecosystem compared to upstream control sections. Cessation of mine discharges at closure could see downstream sections of the river return to conditions more consistent with upstream.

The exploratory microbial community study, a relatively novel study in the region, showed that the composition and diversity of the Archaea and Bacteria communities found in the Surow and Subri Rivers were comparable to those found in other studies including in the temperate regions. We also observed that microbial composition spatial variability within was greater than between rivers and that the variability was unrelated to riverine sediment chemistry but significantly related to water chemistry, particularly turbidity and concentrations of sulfate, Fe and FRP. The study also demonstrated a shift in sediment microbial community composition due to mine dewatering, particularly in the Surow River reaches affected by ASGM dewatering discharges. Given the one-off sampling nature of the sediment microbiology study, however, further study with repeated sampling regime is recommended.

Sedimentation at ASGM sites dramatically altered the river morphology and biota. Further, metals carried by the sediments were deposited along the river downstream during the dry season and remobilised during the rainy seasons. The use of simple small scale ECD equivalents would substantially sediment based pollution. Discharges from mine dewatering from ASGM activities increased conductivity of the river and under full scale operations would have been problematic for biota and water quality. Although discharges from the LSM were of higher quality, they were also in high quantity and substantially altered downstream water quality and biota. Although these changes resulted in increased sensitive taxa, the long-term sustainability of these discharges is unknown. This study demonstrated that impact assessment of ASGM or LSM on rivers should not be limited to the physical and chemical properties of water and sediment, but also include its riverine biota. This study supports the use of macroinvertebrate and potentially microbes as indicators of impact of ASGM and mining in tropical rivers. Moreover, an understanding of the ecological impacts of mining large and small can assist in the prioritising of impact mitigation efforts around ongoing operations and at closure.

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The miners' children watched us wading through the murky Surow River water near to an artisanal mining site, collecting macroinvertebrate samples on a hot September afternoon. They hardly can wait for us to leave so they can jump into the river to play. A village woman walked gracefully by and scolded the boys, "Why are you boys here? This Galamsey (artisanal small-scale gold mining in Ghanaian language) will end someday, it cannot provide for you forever. Go back to school!"

Clearly, it takes a village to educate a child. It also took a huge village spanning between Australia and Ghana, and all places in between to make a PhD out of me. I received support and assistance from many individuals in this long journey.

My deepest gratitude, first and foremost, goes to my principal supervisor Associate Professor Mark Lund. Mark has been my staunch mentor, teacher, discussion sparring partner and critic throughout the exciting, challenging, gruelling yet sometimes lonely journey. His three visits to Ghana during my research not only provided me with in-the-field lessons but also a strong feeling of support, that feeling of knowing that I was not alone in this journey. His expertise in hydro-ecology and creative-lateral thinking inspired me to undertake this study, and I am so glad he trusted me to be his student.

I sincerely thank Dr. Eddie van Etten, a member of my supervisory board, for taking me in and agreeing to supervise me, even when the going got tough. Eddie not only intelligently and diligently read my manuscript, he also taught me to think and write critically, with a patience that I almost envied. His expertise in ecology and an acute sixth sense for statistical correctness have tremendously guided me through the critical period of thesis completion.

I must acknowledge the inputs given by former members of my supervisory board Dr. Cherie McCullough and Dr. Melanie Blanchette. Cherie's significant input in the design of the project provided a strong framework to my field work. Dr Blanchette encouraged me to include the microbiology study in the project and provided her insights for the macroinvertebrate study.

I wish to express my gratitude to my alma mater, Edith Cowan University and the School of Science. ECU CPRS awarded me with the scholarship to conduct this study, without it the study wouldn't be possible. The School's faculty members including A/Prof. Mary Boyce, Prof. Pierre Horwitz, and A/Prof Andrea Hinwood afforded me with that very important behind the scene guidance and support. Yvonne and Lisa in the School's office and Mark Bannister in the lab provided me with that reliable and almost magical services that solved all administrative and logistical issues related to my study both in Ghana and Australia.

Although I lived in Ghana on and off for a couple years prior to my PhD study, working in an uncharted area in a foreign country was not particularly easy. I thank Yakubu Ibrahim and Mumin Zakaria, the field assistants extraordinaire, linguists and guards, for their hard work, support and assistance. They helped me putting things in a local context, seeing my study through the locals' eyes and carrying the heavy coolers of water samples and instruments. I will never forget our walk along the rivers to map out the area in the early days of the project. I wish them all the best for their continued training and education. Abudjei, Issifu and Charles "Rasta", the drivers extraordinaire, were also instrumental to my research in Ghana. I thank them not only for driving me to the labs or to the chemical supply shops in Kumasi, Accra and beyond, but also for their friendship.

They made the trips, often long and hard ones, easier. I also thank the artisanal and small scale miners in Kenyase and Hwidiem Villages of the Brong Ahafo Region in Ghana for giving me access to their mining areas and gold shops, for making me feel welcomed and safe, although we did not know each other. I also thank the Chiefs of Kenyase, Ntotroso and Hwidiem for their blessings and protections.

My sincere gratitude also goes to Mr. Paul Sowley and Mr. Tony Loh, Vice President and Manager of Environmental and Social Responsibility Department of Newmont Ghana Gold Limited (NGGL) respectively, who afforded me the analysis of my water samples at ACZ lab in the US and gave me access to the lab at the mine. At the Ahafo mine, I also owe my gratitude to Ms. Rita Lebene-Tibu, my first contact when I needed baseline data of the study area. The NGGL environmental lab team members Kwame, Lydia, Evans, Osei-Boakye, Sarfo, Paul, Moses and Mr. Dzah also played important roles in my lab and field works in Ghana. I thank them for making me feel so at home and welcomed in Ahafo. To Mr. Seth Ako, the senior geologist at Ahafo mine, I wish to express my sincere appreciation for his patience in teaching me the Geology 101 of the area. I also thank Seth's family for their friendship.

I owe my gratitude to various laboratories and experts in Ghana, USA and Australia. The Ghana Commission for Science and Industrial Research (CSIR) gave me access to their biomolecular lab in Kumasi and the macroinvertebrate lab in Accra. Mr Godwin Amugbe of the institution's macroinvertebrate lab validated my macroinvertebrate identifications, while Mr. Edem Kwami Amedorme, the ichthyologist of the institution identified the fish samples. The late Tony of ACZ lab in Colorado, USA and Peter Sarpong of SGS lab in Tema, Ghana were very helpful with my samples analysis too. Special appreciation goes to Associate Professor Richard Allcock at the Lotterywest State Biomedical Facility Genomics at the University of Western Australia for analysing my molecular biology samples and assisting me with the writing of the method in Chapter 7.

I thank PhD fellow students Meenu, Kate and Wilfred for being there for me, through the highs and lows. My office mates Flavia, Aldo, Federico and Rachel have been my family in the years of my candidature. Thankyou for being great mates, for keeping each other's sanity, the banters and espressos (Flavia, I still owe you that espresso machine).

My family in Indonesia, particularly Bapak and Ibu, are the main reason for my being here, in the pursuit of knowledge, integrity and happiness. I cannot thank them enough for what they have done for me, for being my inspirations including in this PhD journey.

Last but not least, I thank my husband and best friend Edward Currens Macdonald. With his unconditional love and dry-acute Gaelic sense of humour, he has been a very kind editing assistant and the research's 'co-funding agency'. More importantly, he is my north, south, east and west; the strongest supporter of my endeavours no matter how crazy they might sound (like doing a PhD research in a foreign country), and a true believer in what I can achieve.

All that is gold does not glitter,
Not all those who wander are lost;
The old that is strong does not wither,
Deep roots are not reached by the frost.

(J.R.R. Tolkien, The Fellowship of the Ring)

1 General Introduction

1.1 Background

Freshwater from streams, rivers and lakes is a vital resource because of its economic, cultural, social and environmental values. For most societies, freshwater is the main source of drinking water. Ecologically, freshwater also supports significant biodiversity, sustaining at least 100,000 species out of the approximately 1.8 million known species, or 6% of all described species (Dudgeon et al. (2006). Freshwater ecosystems, however, have received much less attention for research and protection than terrestrial or marine ecosystems, and rivers and streams have received the least attention of all (Allan et al., 1993).

Globally, freshwater resources are under threat with subsequent impacts on both humans and biodiversity (Dudgeon et al., 2006; Vorosmarty et al., 2010). Global decline in the quantity and quality of freshwater has been reported for decades (Alcamo et al., 1997; Assessment, 2005a; Zimmerman et al., 2008) with degradation and both loss of habitat and biodiversity in freshwater ecosystems occurring at greater rates than in other major ecosystems (Assessment, 2005b, 2005c). From human perspectives, freshwater insecurity is even more prominent in developing nations where access to clean water is often limited. In developed countries, for example, freshwater from natural sources is treated before being distributed, whilst in many developing countries millions of people depend on untreated freshwater collected directly from its source (Dudgeon et al., 2006; Macdonald et al., 2014; Zimmerman et al., 2008) for agriculture, industrial, cultural and domestic purposes, including drinking water. The United Nation Environmental Programme (UNEP) in its 2005 Millennium Ecosystem Assessment (MEA) reported that the ‘freshwater crisis’ is globally significant and accelerating, notably in sub-Saharan Africa, Latin America, North Africa and the Middle East. Growing demands for water, especially in rural areas as a consequence of population growth and development, as well as declining water quality due to pollution, are among the major environment threats in the aforementioned regions (Moyo, 2009). From a biodiversity perspective, the MEA also established, although incompletely, that fresh water ecosystems are in worse condition than terrestrial or marine ecosystems. The decline in riverine biodiversity, however, is not completely understood, partly due to the lack of knowledge and data on freshwater biodiversity. This is particularly true for invertebrates and microbes in the tropical ecosystems which support the majority of the world’s species (Dudgeon et al., 2006).

Although rivers in the tropical latitude regions make up to 40% of all rivers (Beckinsale, 1969) (Figure 1.1), research into river ecology has mostly concentrated on the permanent streams and rivers in temperate climate zones in the northern hemisphere, with little attention given to the tropics (Hamilton et al., 2005). Tropical rivers face similar stresses, perhaps in part due to fact that geopolitically most of them are situated in developing nations. Growing populations and development, pollution and waste, overexploitation, changing land use and cover, and climate change coupled with lack of institutional capacity of governments in many developing countries to mitigate the impacts contribute to the stress (Arthington et al., 2010; Dudgeon et al., 2006; Heino et al., 2009; Naiman et al., 2011; Vorosmarty et al., 2010).

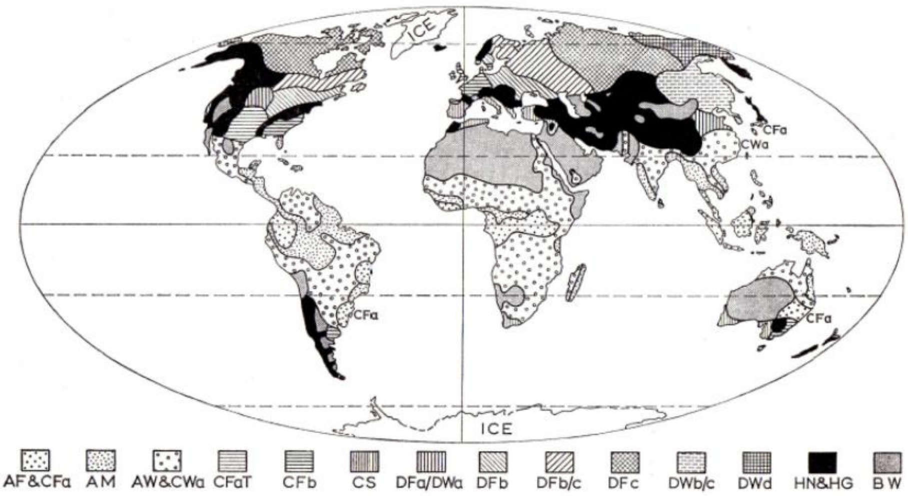


Figure 1.1 Classification of the global rivers (Beckinsale, 1969)
Rivers in the tropical regions are classified as AF&CFa, AM, AW&CWa, and BW. The rivers in Ghana fall either into AM or AF and CF regimes, characterised by warm rainy climate with appreciable runoff and some low water seasons.

Human activity is increasingly the major force implicated in the decline of freshwater ecosystems. Metal mining, including gold mining, is a significant anthropogenic source of river and stream pollutants. Past and contemporary gold mining at all scales of operation has also abstracted and discharged vast quantities of water to and from surrounding freshwater ecosystems (Younger et al., 2004). Although most commercial gold mining operations are now modernised to improve their economic and environmental performance, millions of people with limited access to capital and modern technology still practise artisanal and small scale gold mining (ASGM). A lack of both capital and access to appropriate technology separate ASGM from its modern and large-scale gold mining counterpart. The rudimentary technologies used in ASGM including the use of mercury amalgamation techniques, often result in environmental degradation. Of particular concern is the fact that ASGM is commonly situated near streams or rivers which provide process water and sites for mine water discharge (Macdonald et al., 2014). Ironically, ASGM is found mostly in tropical developing countries including Ghana, Tanzania, Brazil, Indonesia and the Philippines (Chibunda et al., 2009; Hilson, 2002; Lasut et al., 2010; Sousa et al., 2011a) where access to clean water is a key environmental and sustainability issue.

In Ghana, as in many other developing countries where ASGM is prevalent, it often operates alongside large-scale multinational gold operations. The close proximity of the two distinctively different scales and types of mining operations lead to additional environmental, social and legal conflicts (Aspinall et al., 2001; Hentschel et al., 2002; Hilson et al., 2007b). Encroachment of ASGM into the larger companies' legally-acquired concessional lands are often reported, endangering not only their environmental performance which was increasingly under state regulation, but also their long-term legacy post mining (Aspinall et al., 2001). On the other hand, authorities in developing nations where ASGM operates, such as Ghana, often lack the technical and institutional capacities to monitor, manage, mitigate and enforce environmental regulations, particularly in relation to ASGM (Hentschel et al., 2002). Driven by poverty, the potential for ASGM to provide a livelihood for millions of people globally has made it even more difficult for developing nations to eradicate the practice completely (UNEP, 2013).

Consequently, ASGM has persisted and its environmental impacts continue to be largely unreported, monitored and unmitigated.

The issues surrounding ASGM have been studied for the past 30 years (Hentschel et al., 2003), triggered by recognition as an important source of Hg emissions to the environment in the late 1980s (Bridge, 2004). The focus of the scientific literature, however, has been on mercury and its human health implications, including the amount of mercury emissions from ASGM operations (Appleton et al., 1999; de-Lacerda, 2003; Velásquez-López et al., 2010), mercury concentrations in fish used for human consumption (Barbosa et al., 2009; Basu et al., 2011; Bose-O'Reilly et al., 2010; Kambey et al., 2001; Lasut et al., 2010) and introduction of appropriate processing technologies to eliminate the use of mercury, including replacement with cyanide-based gold recovery technologies (Amankwah et al., 2010; Sousa et al., 2010; Veiga et al., 2009; Velásquez-López et al., 2011; Vieira, 2006). A review of 156 scientific articles on gold mining published between 1994 and 2000, for example, found that about 40% of the articles examined mercury pollution, with 70% of the mercury related articles were related to ASGM along the Amazon River and in nearby Brazilian areas (Müezzinogˇlu, 2003). Not only was the subject of this body of research limited almost exclusively to mercury, it also did not cover other regions in the tropics where ASGM is practised widely. Moreover, the ecological effects of ASGM pollutants and practices at the organism, population and ecosystem levels, as reflected in the community structure of aquatic biota, also remain understudied.

The environmental impacts of ASGM have also been of concern to governments, international development agencies and the wider public. The United Nations Environmental Protection (UNEP) and the United Nations Development Program (UNDP), for example, have been trying to improve the environmental, social and safety conditions of ASGM and have conducted a number of assessments and studies (Hinton, 2005; Sulaiman et al., 2007; Telmer et al., 2009). Their recommendations include, among others, the need for collaborative stakeholder efforts that include multinational and large-scale mining operators. Sharing of knowledge and technology in environmental management practices by the larger operators with ASGM operators and local authorities may contribute to improved ASGM health, social and environmental performances (Hentschel et al., 2002, 2003). The challenge with this proposition lies with their contrastingly different sizes, resources available, capacities, and access to capital and technology. It is particularly difficult to make a meaningful quantitative comparison because, unlike the large mining companies, the size, demography and impacts of ASGM operations are largely unknown due to their informal and transient nature (Hilson, 2005).

This PhD thesis presents and discusses results from my research conducted between February 2013 and April 2014 on the Surow and the Subri Rivers, two upper tributaries of the Tano River, in the Brong Ahafo District of Ghana. In the study area, ASGM and a large-scale gold mining company (the Ahafo mine) operate near to each other. ASGM operates on the Surow River catchment, while the large scale Ahafo mine operates on the catchment of the Subri River which runs alongside the Surow River. The research investigates possible changes in river ecology and differences in riverine impacts due to gold mining activities, specifically: 1) whether ASGM and large-scale mining alter river sediment and water quality, macroinvertebrate communities and microbial (Archaea and Bacteria) communities; 2) whether ASGM and large-scale mining resulted in different impacts on the sediment and water quality, macroinvertebrates and microbial communities of the two rivers; 3) which mining discharge components were most responsible for any alterations present; and 4) whether Hg used in ASGM was biomagnified in freshwater fish in the study area.

Although ASGM impacts on tropical rivers have been investigated in Ghana and other developing countries such as the Philippines, and Brazil, most of the studies have been conducted on large river systems such as the Amazon in Brazil (Santos et al., 2000; Telmer et al., 2006b) or the Pra (Donkor et al., 2005) and Ankobra Rivers in Ghana (Akabzaa et al., 2009) which have had extensive and long-established ASGM operations with chronic Hg inputs. The scale and age of these systems prevents identification of other possible impacts besides Hg contamination. In contrast, ASGM activities impacts on smaller rivers would be easier to trace due to acutely concentrated nature of measurable impacts (see Webster et al. 1992). Therefore, this study is different from previously published research because of the focus on a smaller river, with the intention of more clearly defining the suite of impacts from gold mining operations.

1.2 General approach to the study

A conceptual model linking factors arising from gold mining activities to riverine ecosystems has been developed to guide the research design (Macdonald et al., 2014). The model, depicted in Figure 1.2, has been developed based on the knowledge that land clearing, pitting and tailings disposal for mining often lead to erosion and in some cases acid mine drainage (AMD). These processes increase transport of sediments, nutrients and metals into the aquatic ecosystems. Sediments, nutrients and metals including Hg in ASGM are therefore key environmental stressors associated with gold mining in this model. The stressors may have impacts on aquatic ecosystems through changes in habitat quantity and quality. Increased suspended solid concentrations which lead to sedimentation, along with mine water discharge or river water abstraction, can change local hydrology including flow and wetted area which in turn may change habitat availability (Maddock, 1999). Metals, including Hg in the ASGM case, and in some places cyanide, used in processing can escape directly to the aquatic environment or indirectly to the soil and atmosphere which in turn are deposited into the aquatic ecosystems through rainfall and surface and subsurface runoff. Elevated concentrations of metals, nutrients and suspended solids in the water, may also result in poorer sediment and water quality which in turn can decrease (or increase) food for and reduce survival of aquatic biota resulting in changes in macroinvertebrate and microbial (Archaea and Bacteria) community structures and bio-toxicity. The focus of my project and parameters used in this study including river water and sediment qualities, community structures of macroinvertebrate and microbes (specifically Bacteria and Archaea) and potential for biomagnification and bioaccumulation of Hg in fish tissue are highlighted in the model (Figure 1.2). Potential impacts arising from ASGM and large-scale mining activities as depicted in the model, mining and processing methods employed by ASGM and large mining enterprises, and impact assessments methods commonly used for the industry are reviewed in section 1.3below.

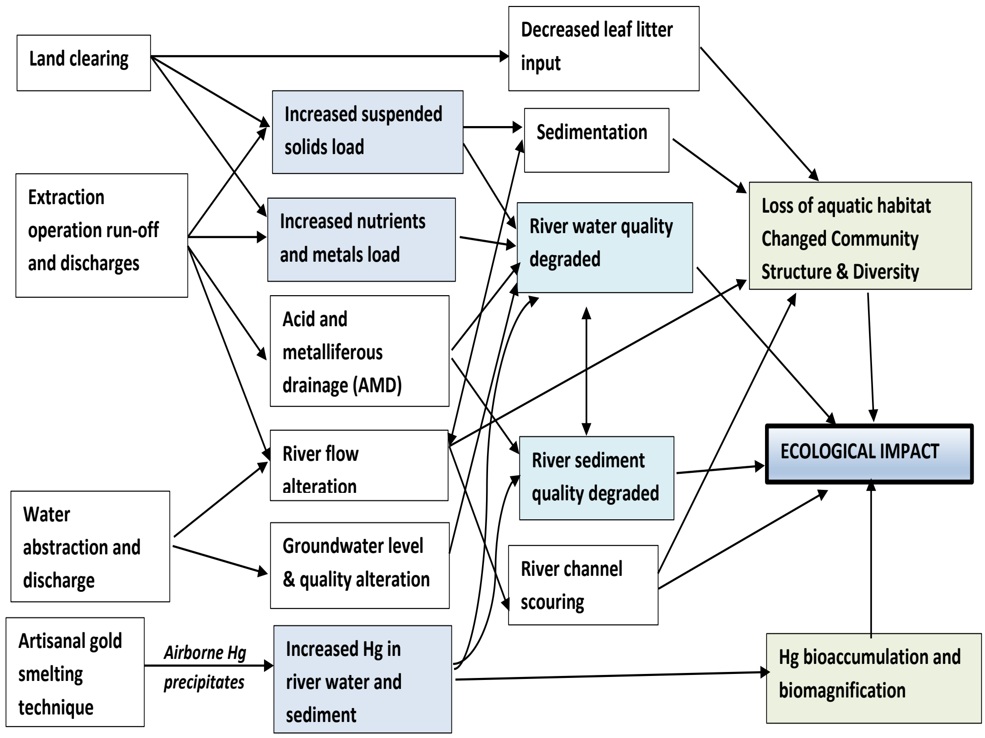


Figure 1.2 Gold mining impacts on riverine ecology.
Gold mining may impact riverine ecology through various factors and pathways. The highlighted boxes represented factors and pathways in the model which are the focus of this thesis.

1.3 Gold mining and its environmental impacts: global trends

Gold mining is an ancient industry, dated back to around 3500 BC when placers, oxidised residuals and quartz vein deposits were mined in Egypt, Nubia (Sudan) and Ethiopia (Mullen et al., 1998). Ancient metallurgy involved the recovery of gold by crushing and washing, followed capturing fine gold using sheepskin liners in sluices. Mercury amalgamation of gold, which was discovered sometime before 1000 BC by the Romans, significantly improved gold production at the time. In this method, mercury was used to adhere fine gold from crushed and washed material, which was then evaporated or burned off, leaving gold residue. The use of materials like the sheepskin to capture fine gold and the mercury amalgamation technique or combination of the two methods remains prevalent in contemporary ASGM operating in many developing countries today. Depleting grade of ore bodies in late 19th century had shifted the focus of development in mining techniques toward cost cutting and efficiency. The advancement in mining technology including the use of explosives and the development of heavy machinery have enabled mining of residual low grades of ores using the open cut mining technique combined with cyanidation (Mudd, 2007a; Mullen et al., 1998).

Gold mining, regardless of mining and recovery methods, always has some impact upon the environment, particularly on natural water resources. Such impacts may manifest at local, regional and global scales throughout the mine life cycle, and then even for millennia after the mine ceases (Thornton, 1996; Younger et al., 2004). The types and magnitude of impacts, however, varies between methods and equipment used. The old recovery method of panning visible gold nuggets, for example, potentially has less serious environmental impacts than modern underground or open cut mining followed by chemical metallurgical processes. The scale of operations also influences the degree and type of environmental impacts. The AGSM practised in many developing nations including Ghana, Indonesia and Brazil, for example, are known to still use mercury amalgamation methods, creating mercury pollution in soil, water and air and posing a risk to human health (Sulaiman et al., 2007; Telmer et al., 2009). Large gold mining operations, however, also have their own share of impacts. Large-scale mining techniques, including open-cut mining, remove waste rocks and topsoils and displace fresh water in quantities significantly larger that the small-scale operations.

World demand for gold continues to grow, by 2000, the global stock of gold reached around 146,000 tons, 70% of which was mined in this century alone, with 17% produced from 1985 to 1996. At the estimated production level of 31g of cumulative gold per person alive and with ongoing human thirst for gold, levels of gold production both by large operators and ASGM are predicted to continue to rise (Müezzinogˇlu, 2003; Mullen et al., 1998). As most high grade ores on earth have been exhausted, ore grades will continue to decline, and mining and extraction of gold will resort to more aggressive methods that potentially impact the environment to a greater extent (Korte et al., 1995; Mudd, 2007a, 2007b). If this trend is to be maintained, breakthroughs in exploration, metallurgy and environmental science are needed, or the financial and environmental costs of gold will continue to rise. Today, gold is mined all over the world, but, according to the World Gold Council, 75% of current gold is produced in 20 countries, many of them are developing countries in the tropics including Ghana, Brazil, Indonesia, Tanzania, Philippines and Papua New Guinea with direct investment from overseas mining corporations (Kumah, 2006). In these countries, large, modern and often foreign gold miners are often operating near the small-scale and artisanal operations, which are mostly operated by local residents.

1.3.1 Modern and Large Scale Gold Mining

Modern gold mining uses various gold extraction methods depending geological, mineralogical, metallurgical, geographical, environmental and economic factors (Marsden et al., 2006). However, about 90% of modern gold mining operations use a cyanidation process (Mudd, 2007b; Mudder et al., 2004). There are two types of cyanidation methods; the first type does not involve agitation and the second requires agitation. The non-agitated methods include vat leaching, which involves adding cyanide solution to ground ore in reactors; and heap leaching - system whereby crushed ore is piled onto a pit or surface lined with a heavy liner and cyanide solution is sprayed on top to leach out the gold. The leachate in both systems is collected for further processing and the cyanide solution is recycled. The agitated cyanidation method is used to treat ground slurries or reclaimed tailings. Gold is then recovered from the solutions that are separated from the treated slurries, or alternatively by activated carbon or resin to the gold cyanide pulp (otherwise known as the carbon in pulp/ CIP or resin in pulp / RIP techniques). The carbon in pulp process can also be incorporated into a leaching circuit (from heap leach or vat leach systems) known as the carbon in leach / CIL technique.

The environmental risks of working with cyanide is high not only because of its toxicity, but also because of cyanide's ability to react with metals other than gold that often present in the ores, such as zinc, nickel, cadmium, copper, iron, etc., forming different cyanide-related compounds (Mudder et al., 2004). Therefore, unless optimally managed, tailings from a cyanide based gold plant may contain a range of cyanide-metal complexes and their oxidised products such as ammonia, cyanate and thiocyanate. Seepage of the metal complexes may reach the surface and ground waters affecting freshwater ecosystems (Akcil et al., 2003; Müezzinogˇlu, 2003; Thornton, 1996). It is imperative that cyanide is removed from every step in the gold recovery processes before effluents are discharged into the environment. Although natural processes including microbial process can render cyanide non-toxic resulting in carbon dioxide and nitrogen compounds, the rate of natural degradation of cyanide depends on environmental variables such as pH, temperature, nutrient levels, oxygen and metal concentrations. Modern gold miners normally resort to the use of readily available robust and reliable chemical, physical and biological technologies to remove cyanide and cyanide complexes from their effluents (Akcil et al., 2003; Kuyucak et al., 2013). These technologies, alone or combined, are capable of achieving effluent levels protective of the environment (Mudder et al., 2004). Nevertheless, as environmental accidents due to cyanide usage in gold processing have occasionally happened, public scrutiny on gold mining cyanide practises remain high (Kumah, 2006) . This, among other reasons, has prompted the industry initiative to voluntary reporting on cyanide use. The "International Cyanide Management Code" (ICMI, 2013) and the Global Reporting Initiative Mining Supplement are examples of these initiatives (Akcil, 2010; Perez et al., 2009). Reporting on cyanide consumption is, however, not compulsory (Mudd, 2007a).

Cyanide is not the only potential source of environmental impacts from modern gold mining. With regards to freshwater ecosystems, environmental impacts of gold mining may come from the mining works, mineral processing and disposal of mine wastes, mine dewatering, post-mining flooding and uncontrolled discharge of polluted waters (Salomons, 1995; Younger et al., 2004). Pollutants from mines may result from not only controlled emissions subject to the regulatory process but also from the less well-defined fugitive emissions (Thornton, 1996).

Regardless of methods, mine works in modern mining, i.e. the sinking of mine shafts or open pits and the excavation of ores and overburden (waste rocks) inevitably disrupt the existing hydrological pathways that in turn effect the surface waters that are connected hydraulically with the disrupted groundwater (Booth 2002). According to Younger et al. (2004), such risks, however, tend to be relatively localised and limited compared to other impacts such as mine dewatering and acid mine drainage.

Mining activity also disturbs topsoils and produces vast quantity of waste rocks, with open cut mining producing more waste rocks than underground mining. Removal of topsoils and vegetation for mining can cause erosion and increases sediment loads in affected riverine ecosystems. Based on a historical review of reported data from the Australian gold mining industry, Mudd (2007b) reported that waste rock and tailing productions increased substantially since the introduction of open cut mining and the use of CIP / CIL methods in the late 20th century. He also reported that since 1985, annual waste rock production has exceeded the amount of milled. He also predicted it may be several times the amount of milled ore now and will continue to increase.

Waste rocks piled on the ground or in some cases in the exhausted pits as back fillers often contain oxidising minerals which may leach (Jeffery et al., 1988; Salomons, 1995). Seepage of contaminated leachate from waste rock during the active mining period or after the operations cease is a significant cause of contamination of surface and ground waters. Without proper management and remediation, metalliferous and / or acidic leachate from these piles can pollute the surrounding soil and water and have deleterious impacts on riverine ecosystems (Bridge, 2004; Ruiz et al., 2008).

Excavation of ores often borders productive aquifers, resulting in large pools of water in the mining voids underground or on the surface in the open pits. The issue of excessive water accumulations is more prevalent in the tropics where precipitation is high. To secure access to the reserves and ensure safety, mine dewatering is therefore often an integral part of mining activities. Mine dewatering impacts on natural freshwater systems may be summarised as two types: those related to discharge of the pumped water and those due to depression of the water table around the dewatered zone (Younger, 2004). Discharged mine water may pollute surface and ground water if the pumped water is of poor quality and may also increase flows in affected streams, particularly during the wetter seasons. The depression of the water table, may decrease flows in streams and other freshwater bodies that are in hydraulic continuity with the affected aquifers. Mine dewatering is therefore often subject to regulatory processes.

1.3.2 Artisanal Small-Scale Gold Mining

ASGM is gold mining by individuals, groups, families or cooperatives with rudimentary mining and processing methods (Hentschel et al., 2002). ASGM operations can be legal or illegal, but the sector is typically undercapitalized, unorganized, and transient in nature (Bridge, 2004). ASGM is recorded from more than 70 countries (Figure 1.3) but it mostly occurs in developing countries, where governments lack the technical and institutional capacity to provide adequate technical assistance or enforce compliance (Sousa et al., 2011b; Telmer et al., 2009). Ghana, Brazil and Indonesia are among the largest ASGM countries. Due to the informal nature of ASGM, little is known about ASGM demography, exact production levels, inputs and outputs of operations but it is predicted that more than 15 million people are employed in the sector globally, with 100 million more directly or indirectly depending on the sector (Hilson, 2005; ILO, 1999). The Artisanal Gold Mining Organisation claimed that, globally, as much as 30% of the world's gold is produced by ASGM.

ASGM commonly occurs in areas where ore deposits are relatively easy to mine with rudimentary technologies (de-Lacerda et al., 1998) such as the alluvial mining of material deposited in river beds. The mining methods used for these deposits include panning, dredging sand and gravel from the bottom of the rivers using a raft, and release of loose gravels from river and open pit banks using high pressure pumps (Aspinall et al., 2001). However, depending on location, geological conditions, level of technology and skills of miners, ASGM may also work primary ores such as altered upper quartz veins (Bridge, 2004; Spiegel et al., 2010). This process involves the direct removal of ore from the ground in quantities larger than dredging or panning. This method usually takes place at old mines, mining prospects and in or near larger operating mines. In this case, vertical shafts and tunnels are dug, often by hand, to depth of up to 30 m to reach gold bearing veins. The ore is then transported to a processing plant which can be located on site or in other places usually close to rivers for easy access to water.

When gold particles are large enough, panning is usually the end of the process. However, in most cases, ASGM involves further processing of ore and sluices to extract the gold. The historic mercury amalgamation process is the most common method used in ASGM because it is easy and relatively cheap (Sousa et al., 2011b; Telmer et al., 2009). Processing with cyanide has been introduced to ASGM in some countries but is not the preferred method among ASGM operators, particularly the smaller ones. Some ASGM operators, however, have now started to re-process mercury-laced tailings (from mercury-amalgamation gold process) with cyanide (Sousa et al., 2010; Veiga et al., 2009; Velásquez-López et al., 2011).

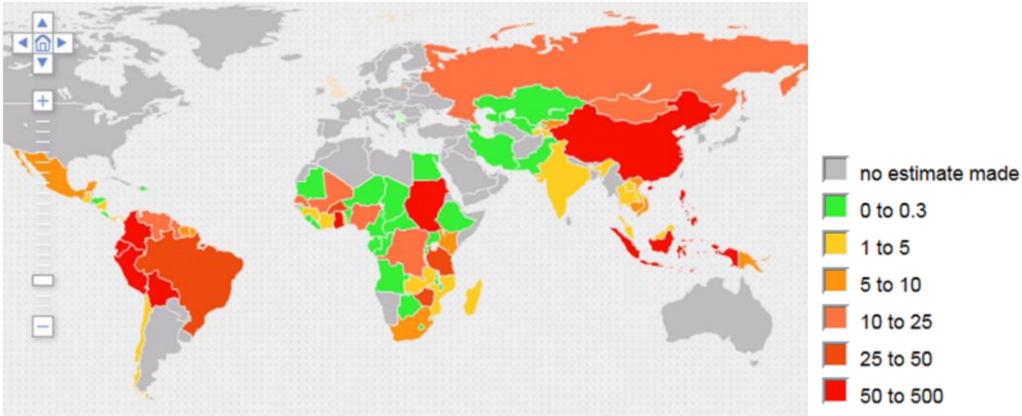


Figure 1.3 ASGM world distribution and estimate of mercury released from ASGM (t/y) (UN data on www.mercurywatch.org)

There are two types of mercury amalgamation methods used in ASGM. The first method is the whole ore amalgamation method whereby metallic Hg is added directly into ore in the grinding circuit, pump boxes or sluicing box (Figure 1.4). The trommel method used commonly in Indonesia is an example of this method (Sulaiman et al., 2007). The second method, commonly used in Ghana, is the gravity concentrates amalgamation (Hilson, 2002). In this method, a small amount of metallic Hg is added to gravity concentrates in barrels or mixing boxes and then separated by panning in water boxes, the river margin or pool (Figure 1.5). Mercury in the amalgam is then squeezed off through a piece of cloth by hand. The resulting amalgam is then burnt off, mostly in open air to vaporise the remaining Hg, leaving the gold. Figure 1.7 shows photographs of the actual process used by the ASGM operators in the study area. The residual material is then often dumped into the nearest river or creek, although in countries like Indonesia and Brazil operators re-process tailings with cyanide to extract any residual gold (Sulaiman et al., 2007).

The type of methods used in amalgamation significantly influence the amount of Hg used and emitted to the environment. The gravity concentrate method, commonly used in Ghana, releases smaller amounts of Hg than that of whole ore amalgamation. The Hg balance in gravity concentrates amalgamation is summarized by Veiga & Baker (2004) as shown in Figure 1.6. Mercury use in ASGM is particularly problematic due to its toxicity as well as sheer amount of emissions. In 2010, the UNEP estimated the annual Hg emission by ASGM to be 727 tonnes, or 35% of the total world anthropogenic emissions of Hg (UNEP, 2013).

The impacts of ASGM are however, not limited to Hg pollution. A range of potential environmental impacts of ASGM on rivers has been identified, including but not limited to changes in hydrology and water quality as a result of land clearing, erosion, mining and processing (Macdonald et al., 2014). Hydrological changes in rivers can alter available hydrological habitat for aquatic biota (Blanchette et al., 2013), and increased turbidity may lead to smothering of aquatic plants, habitats, and biota (Mol et al., 2004). Clearing of riparian vegetation, unregulated sewage from mining camps and rubbish disposal can impact on the rivers nutrient concentrations and habitats (Naiman et al., 1997). In tropical countries, these environmental impacts may be temporally variable. In the dry seasons, ASGM draws water from the nearest water bodies for processing. In the wetter seasons, run-off from unregulated ASGM elutriation boxes, slurry channels and sumps, tailing dumps and open pits elevates turbidity, total suspended solids, trace metals and nutrients in streams and rivers, resulting in sedimentation and changes to river morphology and water quality. In addition to reduced water quality, changes in water quantity of aquatic systems may also occur.

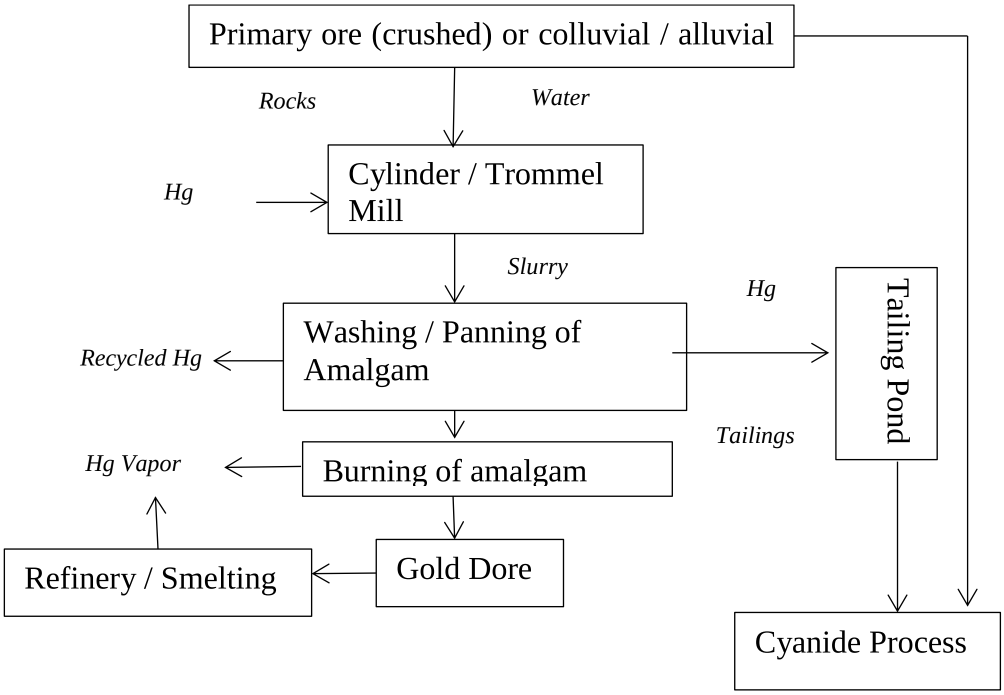


Figure 1.4 Whole ore amalgamation method in ASGM

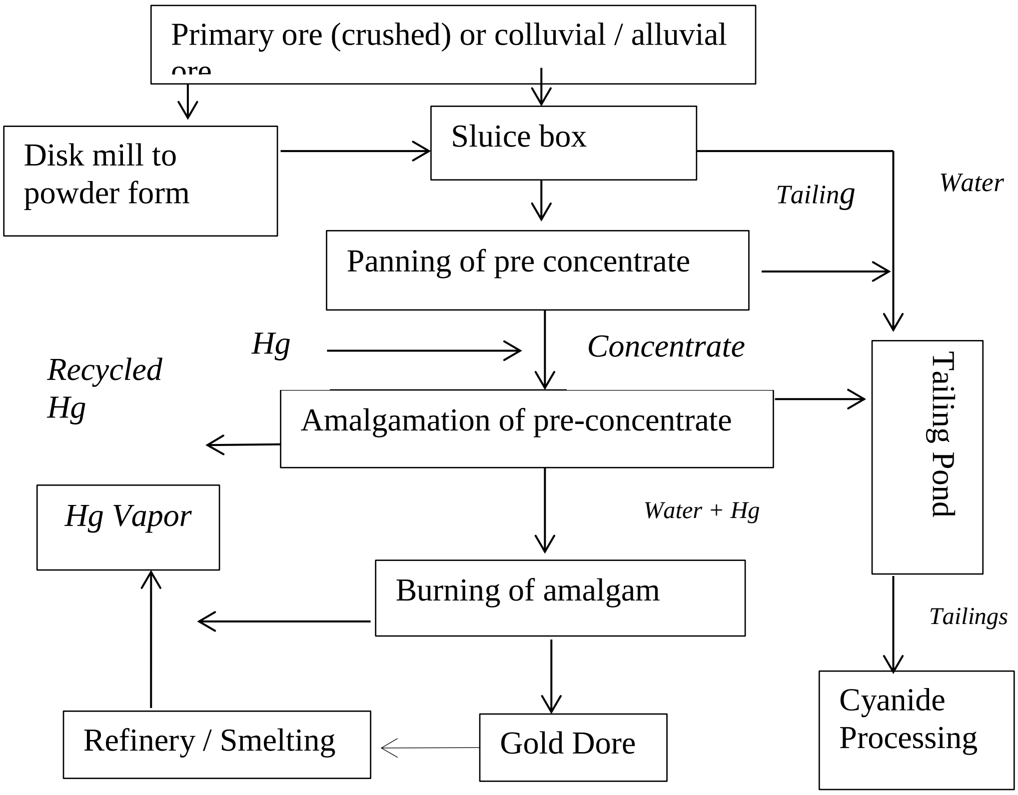


Figure 1.5 Gravity concentrate amalgamation method in ASGM

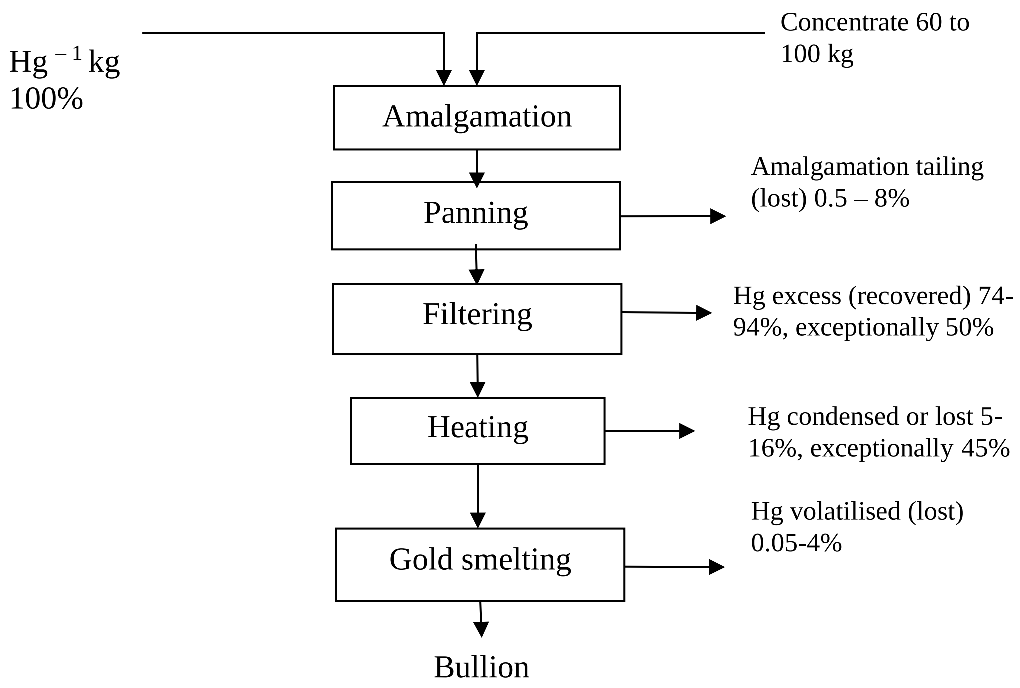


Figure 1.6 Mercury balance in the ASGM amalgamation (Veiga et al., 2004b)

1.4 Assessment of gold mining impacts on riverine ecosystems

The impacts of mining on the freshwater environment have been extensively studied, and have generally resulted in various standards and protocols applied to the water management in the modern mining industry across the globe. The standards and protocols, however, are largely based on the physical and chemical parameters that are known to impacted fresh and marine water resources, partly because these parameters are easily defined (Wilhm et al., 1968) and standardised methods are used in their analysis, and there are a wide range of current standards from North America, Europe and Australia (ANZECC, 2000; Apha, 2007). The use of physico-chemical parameters as water quality criteria in assessing mining impacts, particularly in flowing waters, however, has been viewed as insufficient by freshwater ecologists (Downes et al., 2002). Numerous substances in a wide range of concentrations can affect the quality of river water. In a flowing water environment, the types and concentrations of the substances vary continuously and erratically so that chemical survey can only represent stream conditions at the time and location of sampling (Chapman et al., 1996). Therefore, spills of highly concentrated substances that may occur occasionally, for example, may not be easily detected. Chemical testing can be particularly difficult when applied to wastewater that is highly treated as commonly seen in larger mining industry. Chemical testing on such samples may not reveal any evidence of pollution, while toxic substances at below detection limit concentrations may still seriously affect the aquatic organisms (Chapman et al., 1996; Wilhm et al., 1968). Moreover, the physical and chemical criteria of water quality are usually defined based on toxicological testing on aquatic biota. Such testing, however, is not always conducted in the countries / regions where the standards are applied (Hart, 1974). These shortcomings imply that the physico-chemical criteria should not be used exclusively when assessing stream water quality, but rather should be treated as a supplementary data to the more meaningful approach of the evaluating the biological conditions of the stream.

Figure 1.7 Artisanal and small-scale mining and processing methods commonly found on the Surow River catchment.

ASGM operations involve mining of deposit that is easy to access including alluvial material (A), grinding (B), gravity concentrating (C and D), mixing concentrates with a small amount of metallic mercury (E, F), recovering the amalgam and excess mercury (G,H) before burning the mercury off to get gold dorey (I) . Gold smelting and refinery (J, K, L) is mostly conducted in the refinery shops in nearby townships of Hwidiem and Kenyase I and II.

Biological studies can be used in monitoring of water quality for different purposes such as: i) toxicity studies and bioassays to link pollutants to observed impacts; ii) bio-concentration and bio-accumulation of pollutants in exposed organisms to predict likely effects along the food chain, (Fowle et al.); iii) the provision of baseline information to detect progressive changes; iv) indication of the pollutants present; and v) assessment of the impacts of pollutants on land use, water use and ecosystems (Norris et al., 1995). Due to the potentially extensive and intensive impacts of mining on riverine ecosystems, the mining industry often uses all types of the aforementioned biological studies to assess their impacts, often through the use of bioindicators (Humphrey et al., 1995).

The lack of study in the biological impacts of gold mining on rivers has likely been, in part, due to the lack of regulatory requirements on mining industry in many countries to monitor and assess its biological impacts (Humphrey et al., 1990; Humphrey et al., 1995). Much of the effort in incorporating biological parameters in the assessment and management of mining impacts on riverine ecosystems had been limited to Australia, New Zealand, North America, and Europe where biological parameters are part of their national water quality guidelines (Coysh et al., 2000; Friberg et al., 2006). The studies, however, have mainly used fish, algae and invertebrate communities as biomarkers (Faith et al., 1995; Pond et al., 2008).

1.4.1 Macroinvertebrates as indicators of mining impacts on riverine ecosystems

Aquatic macroinvertebrates perform various roles and functions in stream ecosystems. Due to their various feeding patterns, aquatic macroinvertebrates influence nutrient cycles, primary productivity, decomposition and translocation of materials in riverine ecosystems, and thus influence and reflect the river water quality. Macroinvertebrates are also an important source of food for fish so that the study of fish ecology should be linked to that of macroinvertebrates and their habitats (Wallace et al., 1996). Macroinvertebrates, consequently, are increasingly used as bio-indicators of river water quality and anthropological impacts on stream ecosystems because, unlike the physico-chemical characteristics of water, aquatic macroinvertebrates represent an integration of water conditions over a longer timescale (Connolly et al., 2004; Pearson et al., 1998). A concept originally developed in the United States, the use of macroinvertebrate in water quality assessment and management is now widely adopted in Australia (e.g. AusRivAs and the inclusion of biological monitoring in the Australian and New Zealand guidelines for fresh and marine water quality) and Europe (e.g. STAR-AQEM and the EU Water Framework Directive) (Coysh et al., 2000; Friberg et al., 2006).

The use of macroinvertebrate surveys for the assessment of mining impacts on river ecosystems have also been widely studied in North America, Europe and Australia (Bruns, 2005; Humphrey et al., 1995). Maret et al. (2003), for example, found lower taxa richness and densities of benthic invertebrates in sites downstream of an intensive metal mining site compared to that of upstream sites. Similarly, a study of macroinvertebrates in river ecosystems impacted by coal mining also showed a distinct difference between unmined and mined sites with regards to relative abundance, richness, composition, tolerance and diversity (Pond et al., 2008). Macroinvertebrate have also been used in the study of pollutant toxicity from the mining industry (Cain et al., 2004; Courtney et al., 2002; Harding, 2005; Humphrey et al., 1990). Nevertheless, the use of macroinvertebrates as monitors of mining impacts on riverine ecosystems is not sanctioned by mining regulations in most countries, nor is it widely practised in the industry, particularly in developing countries such as Ghana. In Africa, studies on macroinvertebrates have been mainly centred in the temperate climate of South Africa (Goetsch et al., 1997; Palmer et al., 1993; Palmer et al., 1997) with very few studies from other parts of Africa, with the exception of a few in Madagascar (Benstead et al., 2003), Algeria (Arab et al., 2004) and Lake Tanganyika (Donohue et al., 2004). A few studies involving macroinvertebrates were also conducted in Ghana and its neighbouring countries but limited to investigation of toxic substances or parasites in certain species such as the clams and crabs in the Volta river systems and mysidacea in the Ebrie lagoon in Cote d'Ivoire (Amisah et al., 2011; Kouassi et al., 2006; Obeng, 1966). It is sufficed to say that macroinvertebrates at community levels in tropical West African rivers are understudied.

The lack of study in macroinvertebrate and its role in water quality criteria in developing countries like Ghana is most likely due to the problems identified by Norris et al. (1995), which were previously also lacking in Australia, namely: i) the lack of directions from national or regional agencies; ii) lack of funding; iii) lack of biological guidelines; and iv) scarcity of freshwater ecologists and taxonomic experts. Studies in macroinvertebrate communities and how they react to anthropogenic forces including mining will contribute to the development of biological guidelines in the management and conservation of freshwater resources in tropical developing countries.

1.4.2 Studies of metals in fish for assessment of riverine ecological health

Metal pollution of riverine ecosystems is one of the possible impacts of metallic mining activities. The main ecological concern of toxic metals, including Hg, in the aquatic environment is its ability to build up in organisms along the food chain. In aquatic ecosystems, methyl mercury, for example, accumulates in fish to a level that may harm the fish and other animals that eat fish. Fish-eating birds and mammals have been identified as at risk, so are their predators. Studies have found methyl mercury in water and terrestrial birds (Aazami et al., 2012; Eagles-Smith et al., 2009; Jackson et al., 2011), eagles (Scheuhammer et al., 2008), seals and other endangered animals including polar bears (Atwell et al., 1998) and panthers (Barron et al., 2004). Concentration of metals in fish has therefore been used to establish levels of toxic metal pollution in aquatic ecosystems and their potential impacts on both environmental and human health.

Mercury concentrations in fish has been used widely in the study of gold mining impacts on the environment, particularly that of Hg from artisanal small-scale gold mining (Barbosa et al., 2003; Castilhos et al., 2006; Donkor et al., 2006; Tschakert, 2010). The study of biomagnification of metal in fish is based on evidence that the concentration of metal progressively increases along the trophic levels in the food chain. The difficulty in such study, among others, is to characterise the trophic structure accurately, particularly in a complex food web. Connecting pollutants detected in fish study with source of pollutants is another difficulty as mercury is also found in fishes from freshwater ecosystems without point source Hg pollution (Park et al., 1997). The measurement of the isotopes of N and C has become an alternative method to characterise the trophic structure quantitatively as well as tracing the possible sources of the pollutants (Atwell et al., 1998; Bowles et al., 2001; Bunn et al., 1999b).

1.4.3 Microbiology study in assessment of mining impacts on riverine ecosystems

Microbial organisms, particularly bacteria, are present in abundance in freshwater ecosystems. They perform critical roles in biogeochemical cycling and the control of water quality (Curtis et al., 2004; Küsel, 2003). The transformation of inorganic Hg into organic Hg (methylation) in streams, for example, is driven by microbial processes, by microbes including Sulfate Reducing Bacteria (SRB) in sediments (Cleckner et al., 1999; Lambertsson et al., 2006b; Regnell, 1994). In freshwater ecosystems, including flowing rivers, bacteria are sessile in the sediment and films, and are continually exposed to the water column (Wakelin et al., 2008). The ubiquitous presence of bacteria, their roles in controlling water quality and sessile habits in river ecosystems give rise to the potential for bacteria as indicators of water quality and anthropogenic impacts, including mining, on riverine ecosystems. The use of microbial organisms as bio-indicators however, has been limited partly by the limited information on microbial biodiversity. Despite its ubiquitous presence in the environment, only small fraction of microbial taxa has been identified (Riesenfeld et al., 2004; Torsvik et al., 2002), due to, until recently the difficulties in culturing microbes. Established methods of enumerating microbial organisms by culturing are expensive, time consuming, labour intensive and requiring taxonomic expertise (Wakelin et al., 2008). Furthermore, 99% of the microorganisms cannot be cultured by standard methods; and those uncultured fractions are not related or only distantly related to the cultured ones (Riesenfeld et al., 2004).

The difficulties in the identification of microorganisms in environmental science have been superseded in early 1990s by the newly found techniques using the microorganisms' DNA, enabling genomic analysis of microorganisms by identification of the their DNA without culturing, a technique which is commonly known as metagenomics (Taberlet et al., 2012; Tringe et al., 2005). Subsequently, microbiologists have been extracting microbial DNA directly from soil and analysing the genetic makeup of uncultivable microorganisms (Curtis et al., 2004; Taberlet et al., 2012). This, has facilitated the study of the ecology of environmental microorganisms, including that of marine and freshwater ecosystems (Chariton et al., 2010; Griebler et al., 2009; Newton et al., 2011; Wakelin et al., 2008).

Metagenomics has been gaining interest in the scientific communities and the mining industry. In the past decade, microbiologist and scientists in North America, Australia and Europe, have started using metagenomics to differentiate microbial assemblage in mine impacted area with elevated acid and metals differ from that of non-impacted areas (González-Toril et al., 2003; Gough et al., 2011; Rastogi et al., 2010; Rastogi et al., 2009). Despite the current trend in metagenomics technologies, microbial organisms in riverine ecology particularly in relation to mining impacts remain understudied and underexplored. This is especially prevalent in the tropics and developing countries such as Ghana. Microbial community studies by metagenomics in Africa has been limited to a few from the temperate regions of South Africa (MacLean et al., 2007; Tekere et al., 2013). Studies in microbial community in river sediment in the tropical West Africa would contribute to the knowledge in microbial biodiversity and their roles in freshwater ecosystems and open the discussion on the use of microorganisms as water quality criteria in the region, and more widely.

1.5 Approaches in study of river ecosystems

The most prevailing conceptual frameworks for the study of river ecosystems include the River Continuum Concept (RCC) (Vannote et al., 1980) and the Flood Pulse Concept (Junk et al., 1989). The RCC views a stream as a continuum of materials, energy and processes resulting in gradients in physical, chemical, morphological features which, in turn, are reflected in gradients in the biotic community along the river from head to mouth. The FPC, which more appropriately applies to tropical rivers (Johnson et al., 1995; Puckridge et al., 1998), stipulates that the principal driving force of the major biotic systems in a river is the flood pulse, which tends to occur in the wet seasons. While both concepts are widely accepted in the study of river ecosystems, particularly that of large rivers, other factors also influence river ecosystem structure and dynamics (Lake, 2000; Minshall, 1988; Minshall et al., 1985; Montgomery, 1999). Physical processes and features, including geology, topography, climate, tributaries, lithology, geomorphology and anthropogenic changes are among important factors affecting regional diversity in stream ecosystems (Gordon et al., 2006; Montgomery, 1999). Therefore, a study of river ecology must consider these factors at the local and regional levels.

Given the uniqueness of a river and the gradual changes that take place along a river, study designs that allow for the use of replicates with control sites are not easy to find and implement. To overcome the difficulties in finding valid control sites in the study of stream ecology, upstream (of the impact) sections are often used as controls for downstream ones. In this case, differences at downstream sites are assumed to be due to the impacts rather than some other intervening factor (Norris et al., 1995). Hurlbert (1984), however, warned of the confounding impacts arising from the continuum ecological properties of a river that sampling cannot be properly randomised with respect to the impact. In other words, there is much potential for pseudo replication, i.e. the differences between upstream and downstream can be due to other factors apart from impact. Consequently, this makes it difficult to employ ANOVA-type experimental or survey designs to come up with clear conclusion, known probabilities of error and relating cause and effect (Underwood, 2009). The before and after control intervention (BACI) design, on the contrary, is a stronger design than just comparing control and impact; it can also clearly separate cause and effect (Smith, 2002; Underwood, 1994). The BACI design, therefore, is used widely in river ecology studies, particularly in assessment of impacts of altered flow regimes and discharges of pollutants in rivers over a long period of time (Faith et al., 1995; Muotka et al., 2007; Ruiz et al., 2008).

The BACI design, however, requires baseline spatial and temporal data. Such data are rare because many development projects take place long before the interest or requirement to monitor its effects (Kilgour et al., 2004), a situation especially common to rivers in developing countries including Ghana (Appiah-Opoku, 2001; Biswas et al., 2013). To overcome these challenges, Norris (1995) suggests the use of repeated measures (Green 1993) and univariate statistical designs that emphasise sample variance (Underwood 1991, 1993) rather than means and spatial replication. The number of replicate samples depends on the magnitude and variability of effects; the smaller the effect size, the larger the required replication (McBride et al., 1993). Given the limited time and logistical resources available to the study, the limited replication can also result in the low significance in individual variables if it was analysed univariately. The use of composite variables such biological indices or similarity indexes in multivariate methods as new variables have proven useful in improving the significance in variables which otherwise is low when analysed by the univariate methods (Carlisle et al., 1999; Norris et al., 1995). Multivariate analysis is also widely used in the study of flowing water ecology because patterns of all variables together, be it physical and / or chemical variables or biota assemblages (community), can be more important than patterns of any individual variable (Downes et al., 2002). In studies with a priori groupings (Before/After, Control/Impact or Upstream/Downstream) such as in the study of river ecology, the multivariate analysis usually involves the creation of new variables or component out of the correlations (or covariates) between the sampling units (variables). To test the hypothesis about the new variables, a multivariate analysis of variance (MANOVA) is applied. This is then often followed by plotting the component scores against each sampling unit to show the relationships between the sampling units and the new variables. Downes et al. (2002) explained that that sampling units that are further apart on the plot are to be interpreted as also more different in their values for all variables together.

1.5.1 Research design

1.5.1.1 Hypotheses

It is hypothesised that ASGM and large-scale mining will impact the river sediment and water quality and the impacts of ASGM on the Surow River will be different from that of large mining on the Subri. It is also hypothesised that in each river there would be a separation between control and impact sites with regards to macroinvertebrate and microorganism communities. I also hypothesised that Hg is biomagnified in freshwater fish in the area.

1.5.1.2 Experimental design

I used a replicated ‘before/after’ (BA) and ‘control/impacted’ (CI) pairing (BACI) experimental design with multiple sites (Smith, 2002; Underwood, 1991, 1994) to determine impacts of mining on riverine water and sediment physico-chemistry characteristics. In this model, impacts of disturbance on the river’s characteristics are indicated by significant difference between control and impact, before and after, as well as in the interaction between BA and CI. The study was limited by the absence of pristine rivers as control sites, as well as the non-existence of baseline data for the Surow River because ASGM was already in place. Consequently, I used sites located upstream of mining operation on both rivers as control sites. For the study of impact of ASGM on the Surow River, I employed a reversed BACI experimental design (Michener, 1997; Smith, 2002; Underwood, 1994) whereby multiple sites were sampled before and after removal of the disturbance factor. This was made possible because the Ghanaian government effectively stopped AGSM during my study. To minimise the possible confounding effects that may arise from the continuum ecological properties of a river, I employed repeated and replicated sampling within both control and impacted zones of the river and used biological indices and multivariate ordinations as new variables to increase statistical power (See Norris et al., 1995; Underwood, 2009).

Baseline data on macroinvertebrates and microbiology communities, however, are not available for the before and after comparison. Replicated sampling for two way comparisons between control and impact and between rivers was therefore employed as an approach to assess differences between mining and non-mining sites with regards to macroinvertebrates and microbial community structures in the two rivers. Simple correlations between fish size, trophic level and concentration of Hg was employed to infer Hg bio-accumulation and biomagnification in fish in the study area. The designs, parameters and variables used in the study are summarised in Table 1.1.

Table 1.1 Research design, parameters and variables of study

Parameters	Research design and variables (factors)	
	Surow River	Subri River
Sediment Chemistry	• Control and Impact • Longitudinal variation • Before and After • BACI pairing (reversed)	• Control and Impact • Longitudinal variation • Temporal variation

Water Physicochemical parameters	• Control and Impact • Longitudinal variation • Seasonal • Before and After • BACI pairing (reversed	• Control and Impact • Longitudinal variation • Seasonal variation • Before and After • BACI pairing
Water – Total metal and nutrient concentration	• Control and Impact • Longitudinal variation • Seasonal • Before and After • BACI pairing (reversed)	• Control and Impact • Longitudinal variation • Seasonal variation • Before and After • BACI pairing
Water – Dissolved metal concentrations	• Control and Impact • Longitudinal variation • Seasonal • Before and After • BACI pairing (reversed)	• Control and Impact • Longitudinal variation • Seasonal variation
Macroinvertebrate Community	• Control and Impact • Longitudinal variation • Seasonal	• Control and Impact • Longitudinal variation • Seasonal
Microbiology Community	• Control and Impact • Longitudinal variation	• Control and Impact • Longitudinal variation
Fish mercury concentrations	• Correlations and modelling	

1.6 Thesis structure

The thesis consists of two non-data chapters (Chapters 1 and 2), five data chapters (Chapters 3 to 7) and one synthesis chapter (Chapter 8). The bio-physical environment of the study area in the North Asutifi District of Brong Ahafo Region in Ghana is explained in Chapter 2. The five data chapters discusses two main groups of riverine ecological parameters potentially impacted by mining, i.e. the physico-chemical parameters and biological parameters. The physico-chemical properties of sediment and water of the Surow River with possible impacts from ASGM are outlined in Chapter 3, whilst that of the Subri River potentially impacted by large and modern gold mining are outlined in Chapter 4. Chapter 5 covers Hg biomagnification and bioaccumulation potential in fish from both rivers. Macroinvertebrate and microbial (Archaea and Bacteria) communities in both rivers and how they might respond to gold mining impacts are discussed in Chapter 6 and 7 respectively. The last part of the thesis, Chapter 8, synthesises and integrates the research findings and discusses crosscutting concepts relevant to the study's subject matter with concluding remarks and recommendations. Chapters 3 to 7 are written as pre-cursors to standalone papers to facilitate future publication. Previous publication and conference papers related to the study are attached in Appendix 1 and

Appendix 2

2 Biophysical environment of the study area

2.1 Introduction

The Surow and Subri Rivers studied for this thesis run near each other and drain into the Tano River, a tropical trans-boundary river that flows from Techiman in the Brong Ahafo Region of Ghana to the Gulf of Guinea (Atlantic Ocean) at Aby Lagoon in Cote d'Ivoire (Fig. 2.1). The Tano River is a culturally, economically and ecologically important aquatic system in West Africa. Culturally, it is a sacred river for the Akan peoples of Ghana and Cote d'Ivoire. Economically, it is an important source of domestic and irrigation water for the Brong Ahafo region, the major food and cocoa producer of Ghana. The Tano River Basin belongs to the southwestern river system of Ghana that covers 22% of the country (52,478 km^2) and contributes 29.2 % to the total runoff of 8% of the total drainage in the country (Kankam-Yeboah et al., 2004; Yidana, 2009). The tropical forest reserves along the river are not only important for timber production, but also have areas set aside for conservation.

The Brong Ahafo region, where the Surow and Subri Rivers are located, shares borders with the Northern region of Ghana to the north, Ashanti and Western region to the south, Volta region to the east and to the west. Its climate is like that of the southern parts of neighbouring countries Le Cote d'Ivoire and Liberia. Although theoretically no two streams or rivers are the same (Hynes, 1975), the Surow and Subri Rivers may face challenges that are not only common to Ghana but also to the neighbouring countries and more broadly to tropical West Africa. Findings from this study may have similarity and applicability to a wider region within the Tano River Basin, Ghana, West African, or other tropical regions. On the other hand, the wider regions and local's biophysical environments may influence the findings of this study because regional and local climate, geology, topography, tributaries, lithology, geomorphology and anthropogenic impacts are among the most influential factors in river ecosystems (Minshall, 1988; Minshall et al., 1985; Montgomery, 1999).

The many factors influencing river ecosystems contribute to the complexity in the study of the river ecosystem, which is inherently challenging due to the nature of flowing waters (Fausch et al., 2002; Karr et al., 1986; Minshall, 1988; Minshall et al., 1985) and their link with their catchments (Downes et al., 2002). Because a stream or a river is a continuum of materials, energy and processes resulting in a gradient of physical, chemical, morphological features which are reflected in a gradient in biotic community composition along the river from head to mouth (Vannote et al., 1980), a study of river ecosystems must consider spatial changes of the features along the stream. At finer spatial scales, the conditions of the riparian environment of a river also influence the processes in a stream (Barker et al., 2006; Bunn et al., 1999a). The ecosystem of a stream, especially in the tropics, is also driven by the flood pulse (Johnson et al., 1995; Junk et al., 1989; Puckridge et al., 1998), so that temporal dynamics should also be taken into account. The complexity and heterogeneity of a stream ecosystem, consequently, calls for a multivariate approach in the study of stream and river ecosystems. It also necessitates an informed awareness of the spatial and temporal variability of the study area (Downes et al., 2002).

This chapter reviews the biophysical environment of the study area, focussing on the likely variables which influence the various components of this study. Being tributaries to the Tano River, biophysical characteristics of the Surow and Subri Rivers are similar to that of the Tano River which is generally influenced by biophysical characteristics of the tropical West African region. Consequently, it is important to review the general biophysical characteristics of the West Africa region, Ghana and the Tano River Basin prior to reviewing the local climate, hydrology, geology, ecology and anthropogenic influences on the Surow and Subri Rivers catchments. As this thesis aims to compare the impacts of two types gold mining on the Surow and Subri Rivers' ecology, it is important to establish the biophysical similarities or differences between the two rivers. Therefore, in this chapter we also discuss the riparian characteristics of the sampling sites based on a rapid riparian assessment conducted in April 2014 with an emphasis on the similarities and differences between the two rivers.

2.2 Study area description

The study area includes the Surow and Subri River catchments located west of the Tano River in the upper Tano Basin in the Asutifi district of the Brong Ahafo Region, Ghana, approximately 300 km northwest of the capital city of Accra and 40 km south of the regional capital city of Sunyani (Figure 2.1). Major land uses in the Surow and Subri Rivers catchment are gold mining and agriculture amongst tracts of natural forests. A large multinational gold mining company (the Ahafo mine) operates on the Subri River catchment and clusters of artisanal small-scale gold mining (ASGM) operators work on the Surow catchment. Farming activities in the area include cash crops (cocoa and palm), ranching and subsistence farming (vegetables and tubers). Subsistence fishing activities on the Surow and Subri Rivers are limited to the last two kilometres of the rivers upstream of their confluence with the Tano, particularly in the rainy seasons.

The Surow River is a seasonal stream of 16 km length set in a 3,500-ha catchment. It flows through Kenyase 1 and Kenyase 2 townships before it joins the Tano River about 2 km southeast of Hwidiem. The Surow main tributaries (head water) are called the Suntim and the Akantansu streams by local residents. These two streams confluence at Kenyase 1 and from then on it is called the Surow River by the locals. In this thesis, we refer to the Suntim/Akantansu/Surow system as the Surow River. Local residents, including the ASGM communities, used the River's water for agriculture and artisanal gold mining processing as well as for domestic purposes such as drinking and washing. Where public sanitary facilities were not available, nearby residents used the Surow as sewerage as well. During the dry season, farmers and miners abstract water from the river for agriculture and gold processing. The river also potentially receives discharges from ASGM operations including mining and processing activities at Kenyase 1 and Kenyase 2. ASGM operators occasionally pump mine water out of the mine shafts and discharge it into the river, particularly during the wetter months. The local communities also often use the Surow River as sewerage at places where infrastructure and sanitary facilities are limited.

The Subri River, which runs alongside the Surow River, has a length of 25 km and a catchment area of 12,900 ha. The Subri River receives water from several seasonal tributaries including the Subika/Samansu, Asundua and Apensu streams and passes through several hamlets before it joins the Tano River 2.5 km south of Subrisu town about 3 km upstream of the confluence between the Surow and Tano rivers (Figure 2.1). About half of the river catchment is within the concessional land of the Ahafo mine, therefore the river is potentially affected by the mine. Mine water that has been treated in environmental control dams (ECD) at the mine site to comply with the Ghanaian EPA guidelines is decanted into the river through one of its tributaries (the Asundua stream), and until recently treated mine water was also decanted into the Subri River through the lower reaches of the Subika stream. Agriculture activities in the Subri catchment include cocoa, palm, orange, teak as well as subsistence farming. The Subri River is a source of domestic water for some residents including for drinking, as well as for agriculture purposes.

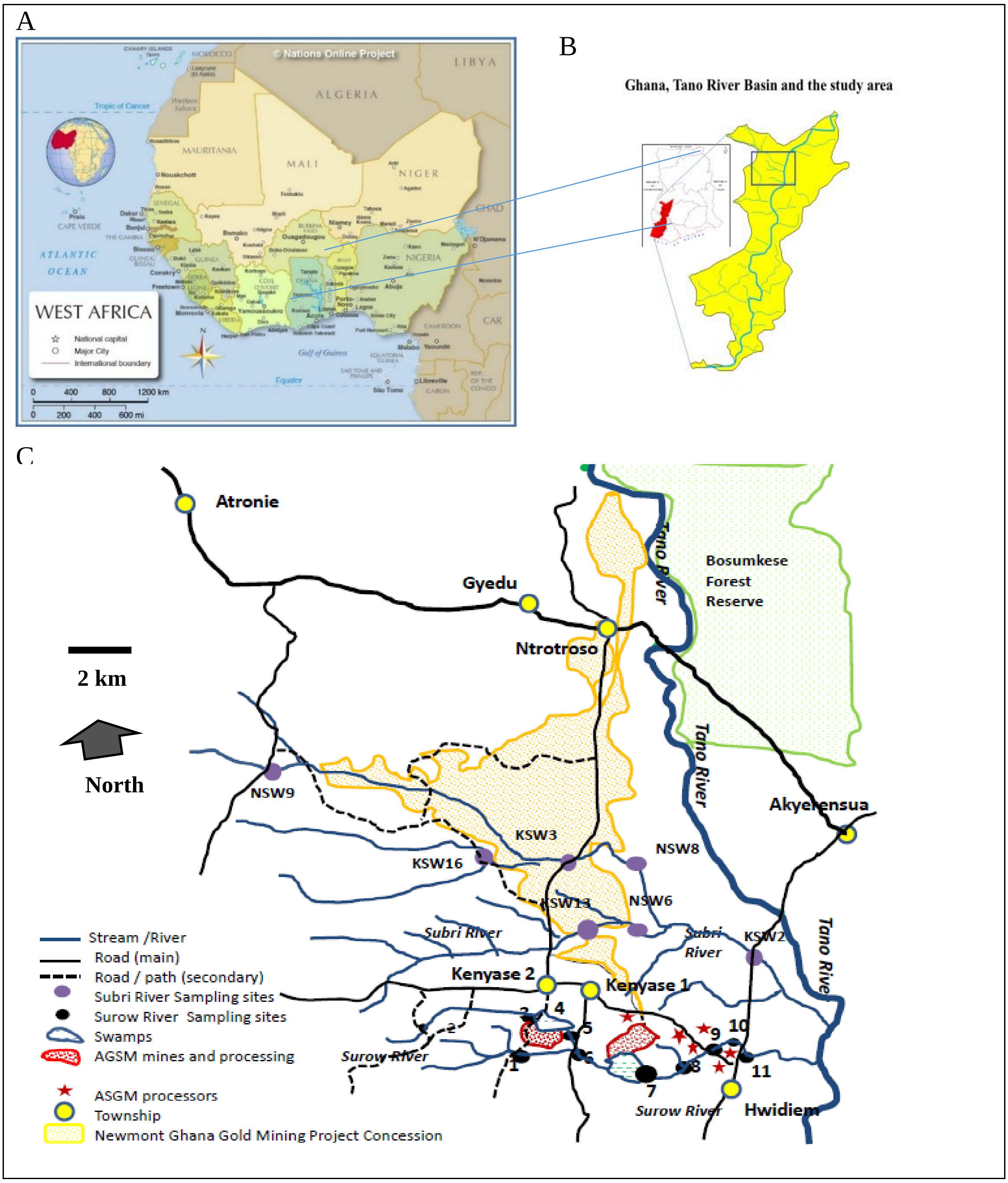


Figure 2.1 The study area is in the upper Tano River Basin (B) of Ghana, West Africa (A). Localities and sampling sites along the Surow and Subri Rivers are shown in C (the Tano River flows southwards).

Eleven sampling points on the Surow River and seven on the Subri River were selected based on access, safety, representativeness of catchment land uses and longitudinal position relative to mining activities (Figure 2.1, Table 2.1). Within the framework of BACI design, sites located upstream of known impacts (e.g. discharge from mining) were assigned as control sites, and those downstream as impact sites (Norris et al., 1995).

There were 3 control sites and 8 impact sites on the Surow River, while on Subri River we had 3 control and 4 impact sites. These sampling sites were also selected to consider confluences between tributaries and the main river to allow for not only categorical analysis, but also gradient and multivariate analysis. Due to limited access and other safety considerations, most of the selected sampling sites were situated near to bridges or river crossings. However, to minimise the possible confounding effects of other disturbances, we moved a short distance, a minimum of 10 m, from tracks or bridges structures.

2.3 West African biophysical environment

West Africa covers Benin, Burkina Faso, the island of Cape Verde, Gambia, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Liberia, Mali, Mauritania, Niger, Nigeria, the island of Saint Helena, Senegal, Sierra Leone, Sao Tome and Principe and Togo (Figure 2.1). Geographically, the region is in the tropical African region, but the region has three sub climatic zones of (humid) Equatorial Forest, (semi humid) Guinea Savannah, and hot Semi-Arid Sudan Savannah on the Sahelian sub-region bordering with the Sahara.

The main drivers of West African climate are two masses of air: one originating and bringing moist air from the Atlantic Ocean moving north-easterly, and the other originating from the interior of the African continent moving south-westerly bringing dry air. The former is known as the South-West Monsoon and the latter is known as the Harmattan winds. The narrow gap between the two air masses is called the Inter Tropical Convergence Zone (ITCZ). The seasonality, characterized by rainfall, in West Africa is a result of the annual north to south shifts of the ITCZ (Hayward et al., 1987).

West Africa receives a total annual rainfall of up to 4000 mm (Hayward et al., 1987). The rainfall, however, varies significantly between subregions and over time (Hayward et al. (1987). Mean annual rainfall in southeast Ghana, Togo and Benin is relatively low at 1000 to 1200 mm compared to other places in the coastal zone (Figure 2.2 A and B). This is due to the low precipitation in August during which the whole coastal region between Abidjan in Cote d'Ivoire and Lagos in Nigeria experiences the short dry period from the end of July to early September.

In addition to the number of rainy days and total annual rainfall, an important characteristic of West African precipitation patterns is the intensity of the rain. Much of precipitation in West Africa falls in heavy storms and showers lasting for three to six hours with an intensity of 50 mm per hour or more, peaking at 200 mm per hour over short periods. During the storms, rain may fall in 100 drops of 2 mm in diameter or more per cm^2 (Hayward et al., 1987).

Average daily temperature in West Africa ranges between 14^o to 34^oC. Like other climatic features in the region, temperature differs temporally and spatially as summarised by Hayward et al. (1987) for the months of January, April, August and November (Figure 2.3).

Table 2.1 Sampling site description, hydrology and land use

Site Code	River / tributarie	Geographical position		Description	Max width (m)	Average Max Depth (m)	Dominant site hydrology	Dominant Substrate
		Latitude	Longitude					
1	Surow/Sur m	6.9701	2.4068	Control site, located at a river crossing on a dirt road at Kenyasi 2 Village. It is the head water of the Suntim Stream, main tributary of the Surow River. Surrounded by the remnants of secondary forest, this site is minimally impacted by human activities. The stream never dries up at this site, even in the dry seasons. No gold mine around and upstream this site. Being a head water of the stream, this site is considered sacred by the local citizen. A shrine near the sampling site is used to perform religious rites.	28	0.6	Run	Rocks and gravel, covered with periphyton and organic materials.
2	Surow/Aka ansu	6.9877	2.4109	Control site, located at a small bridge. Headwater of the Akantansu Stream, surrounded by village dwelling in between the remnants of secondary forest and cocoa farms in Kenyase 2 Village. The River at this site is a source of drinking / domestic water for some local residents. Although flows vary with between seasons, the River at this site never totally dries up. Traditional rites are often performed here.	2.8	1	Run	Gravel and sand with thin layers of algae in parts of the site.

3	Surow / Akantansu	6.9759	2.3942	Control site. A large body of water on Akantansu stream, about 400 m upstream of the Kenyase 2 ASGM mine. Surrounded by village dwelling amongst the remnants of secondary forest. Locals including ASGM miners use this body of water for bathing and other purposes including car washing. Local children swim here. Water is often abstracted to the ASGM site as process water, especially during the dry seasons. Wild life (water birds, snakes) as well as domestic animals (geese, ducks) are often spotted here	50	1	Slow/Pool	Fine sand and silt. Where the flow is very low, the bottom of the river at this site is covered with algae
4	Surow / Akantansu	6.9739	2.3918	Impact site. A large swamp adjacent to ASGM Kenyase 2 mine site. The mine is located on the hill on the right bank of the river. Locals reported to me of changes in the morphology of the river at this site. Prior to ASGM it was a channel, now it is a swamp. Personal observation within 12 months' period also witnessed the changes. In early 2013, the swamp was flooded with muddy water, in 2014 the swamp was covered with overgrown elephant grass and weeds. Water from the river is often abstracted for ASGM processing on its banks. The river at this site also receives sediments, some mine dewatering, and process water from the ASGM.	200	0.5	Swamp	Silt and fine sand. In many parts of the site, fresh silt sand from ASGM operations on the river banks are seen, particularly in the rainy season. In the drier season, the silt often covered by grass and other macrophyte. including elephant grass (*Pennisetum purpuretum*)
5	Surow / Akantansu	6.9716	2.3918	Impact site. A stream near to a Palm farm at Kenyase 2 Village. It is located about 300 m downstream of the Galamsey Kenyase 2 Swamp. The locals use the water to manually irrigate their farms. It is situated away from houses / dwellings.	28	0.5	Slow	Fine sand and silt in the copper and ash colour.
6	Surow	6.9632	2.3908	Impact site. A very fast flowing reach, resulted from the confluence between the Suntim and Akantansu Streams. From here onward, the River is called the Surow River by the locals. It is located downstream of Kenyase 2 mine and upstream of Kenyase 1 mine. Locals fetch water from this site for farming and other domestic purposes including drinking	3	.13 - .70	Run / Riffle	Mix of boulders, rocks, gravels and coarse sand

7	Surow	6.9438	2.3650	Impact site. A very slow flowing reach on a swamp at Kenyase 1 Village, about 500 m downstream of Kenyase 1 ASGM mine. River bottom is very muddy and mostly consisting of silty material. Sedimentation is visible. Locals reported to me that the swamp was previously a channel, but due to sedimentation the river water inundated the palm and cocoa farms along the banks. Palm trees still grow on the wetted area of the river at this site between elephant grass infestation. Locals / miners use the water as process water.	100	0.5	Swamp	Fine sand and silt with layers of algae and copper coloured film. Parts of the river at this site are also covered with organic litter
8	Surow	6.9440	2.3650	Impact site. The confluence between the main Surow River and a stream receiving discharges from K1 ASGM site. The site is surrounded by palm and subsistence vegetable farms. The channel at this site is well defined and dries up during the dry season. Travelling herds of cows occasionally were spotted around this site.	4	0.5 - 1.5	Run	Fine sand and silt
9	Surow	6.9427	2.3613	Impact site at a bridge on the Kenyase-Hwidiem road in Hwidiem township. A medium-fast flowing reach (depending on season), the site is surrounded by ASGM processors located upstream, downstream, on left and right banks. Sedimentation is visible at this site and it is overgrown by elephant grass and weeds. Local residents use water from this site as ASGM processing water as well other purposes including car washing. At this site, the river dries out at the peak of dry season and floods the road at the peak of rainy seasons	5	.50-2	Run	Silt and fine sand in the same colour of the ASGM tailings. In the drier season, parts of the site are covered with thin layer of algae and elephant grass (*Pennisetum purpuretum*)
10	Surow	6.9427	2.3594	Impact site located about 1 km downstream of the Hwidiem ASGM process plants. Sedimentation remains visible at this site although not as much as that of Site 9. In the dry season the river dries out at this site. Main land use here is vegetable and palm farming.	6.4	.74-1.5	Run	Boulders, silt and fine sands in the same colour of the ASGM tailings
11	Surow			Impact site located at a bridge on the main Hwidiem-Kumasi Road in Hwidiem about 1 km downstream of Site 10. Upstream of the bridge the river moves at medium to fast, but it slows down downstream of the bridge where the river channel turns into a swampy area overgrown with elephant grass and weeds. Land uses at this site include rural dwellings and vegetable farming. This site is also a river shrine for the traditional council of Hwidiem.	15	2	Pool/Slow	Mix of coarse and fine sands. Much of the river, downstream of this site, turns into a swamp covered with elephant grass (*Pennisetum purpuretum*)

NSW 9	Subri	7.0780	2.4223	Control site located at a bridge on the Ntotrososo – Mim Road. It is the headwater of a stream flowing into the water storage facility (freshwater dam) at the Ahafo mine site. Water moves fast upstream of the bridge, while in downstream of the bridge moves very slowly as it approaches a swampy area. Land uses include rural dwelling, animal ranching and minimal cocoa and palm farming among the remnants of natural forest.	3	.3 - 1	Slow / Pool	Mix of boulders, gravels and sand. These are often covered with layers of algae or periphyton. In the drier seasons, the edges of the river at this site is covered with grass.
KSW 16	Subri / Apensu	7.0186	2.3788	Control site located upstream of KSW3 on Subri River. Land uses: Teak plantation and farming			Slow /Pool	Mix of fine and coarse sand, often covered with organic materials and periphyton.
KSW 3	Subri/Apeı	7.0125	2.9283	Impact site on the Apensu stream. Although located at a bridge on the main road in Kenyase, the site is located within the Ahafo mine site. The stream at this site receives water from KSW16 and a stream flowing through the mine. Water from this site flows into an environmental control dam (ECD 4). Apart from mining, land uses at this site include public road and subsistence farming. In the peak of dry season, the stream at this site is reduced to a pool of water.	7.2	0.55	Pool	Silt and fine sand, often covered with thin layer of algae. Elephant grass (*Pennisetum purpuretum*) grows on the edges of the river at this site.
KSW 13	Subri/Subi	6.9846	2.3639	Control site for NSW6 on the Subika stream. A fast to medium flowing stream depending on season. In the dry season the stream flow is very low to dry. Land uses include teak plantation and subsistent farming.			Slow Run	Mix of boulders, rocks, gravel and sand. The boulders and rocks are covered in thin layers of algae
NSW 6	Subri/Subi Samansua	6.9828	2.3588	Impact site on the Subika or Samansua stream. It receives water from KSW13 and the Samansua stream that potentially carries runoff from the mine. An environmental control dam used to be operating upstream of this site during the mine construction period although it was already closed	6	0.6	Run	Coarse sand with organic litters

				prior to the study. Land uses at this site is mainly teak plantation and rural dwellings. The stream at this site dries out during the peak of dry season.				
NSW 8	Subri/ Asundua	6.9966	2.3483	Impact site on the Asundua stream joining the Subri. It receives mine water decanted from an environmental control dam at the Ahafo mine. As such, although flow at this sites varies, it never dries up during the dry season. The river channel at this site is well defined. Land uses include teak plantation and rural dwellings.	6.5	0.9	Run	Gravel and sand, often covered with organic litters from the remnant of secondary forest surrounding the site.
KSW 2	Subri	6.9623	2.3331	Impact site on the main Subri River at Subrisu village on the main road to Kumasi, about 2 km from its confluence with the Tano River. At this site is the confluence between the Subri Stream (NSW8) and Subika Stream (NSW6), cocoa and vegetable farming, rural dwellings.	6	.35 - 1.5	Run / Riffle	Mix of boulders, rocks, gravel and sand. Most of the boulders and rocks are covered in thin layers of algae

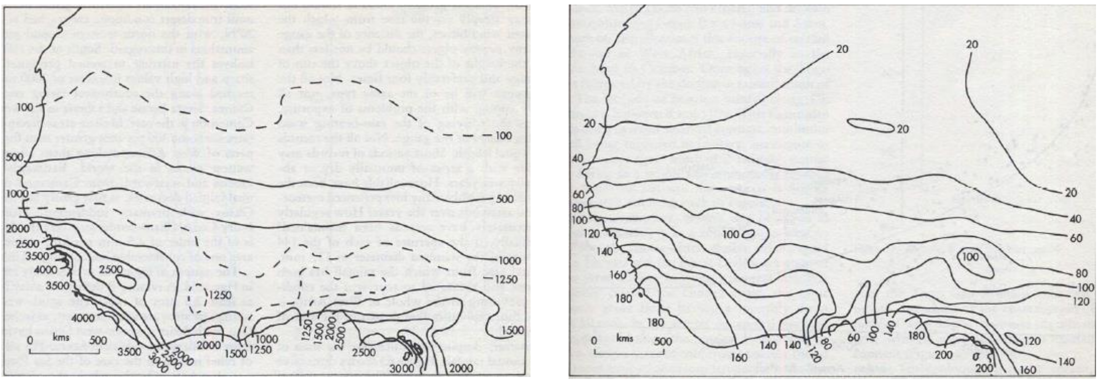


Figure 2.2 Mean annual rainfall (mm) (A) and number of rainy days (B) in West Africa (Hayward et al., 1987).

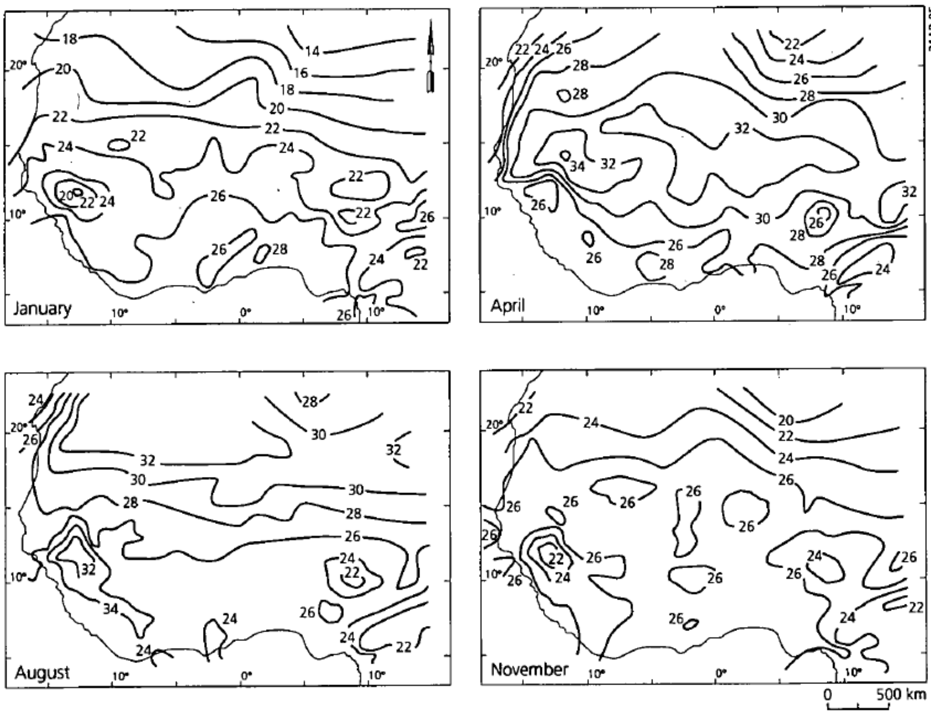


Figure 2.3 Mean daily temperature of West Africa in January, April, August and November. (Hayward et al., 1987)

2.4 Ghana and the Tano River Basin biophysical characteristics

Ghana's climate is mostly subequatorial (within the Equatorial Forest Zone of the West African zoning) and characterised by two rainy seasons, one from April to July, and a lesser one from September to November, resulting in an annual mean precipitation range of about 1,500 to 2,150 mm (Gyau-Boakye et al., 2002; Yidana, 2009). Dry seasons occur during the months of December to February and in the cool month of August. Rainfall, and hence surface water distribution in Ghana, is not only temporally variable, but also spatially variable. In the north, there is only one single rainfall regime in a year that runs between May to October with a peak in September. Half of the total annual rainfall in the northern part of Ghana, however, only falls from between June and September. The dry season in northern Ghana (November to March) has very little or no rainfall at all. On the contrary, the southern part of Ghana is characterised by two main rainfall seasons. The major rainy season occurs from March/April to the middle of July, peaking in June. The second or minor rainy season occurs in September to November with a peak in September/October (Gyau-Boakye et al., 2002). Southern parts of Ghana experience two very dry seasons that occur between December to February, and a short one in August. This rainfall pattern, is similar to the southern parts of neighbouring countries Ivory Coast and Liberia (Gyau-Boakye et al., 2002).

While temperature does not vary much across the country with a mean temperature between 26 to 29^{o}C, relative humidity varies between places and seasons, in the south it ranges from 85% in the wet season to 65% in the dry season (Shanahan et al., 2007), whilst in the northern region it averages 65% during the wet season and can be as low as 12% during the dry season (Ghana 2011).

The river basins of Ghana are grouped into three broad systems based on climate and other factors. The first group is the South-Western River System which covers 22% of the country and contributes 29.2 % of total national runoff. The system comprises of Pra, Ankobra, Tano, and Bia rivers. The second is the Coastal River System which covers 8% of the country's area and contributes 6.1% of total runoff. The Coastal System comprises of the Kakum, Ochi-Amisa, Ochi-Nakwa, Ayensu, Densu and Tordzie Rivers. The third is the Volta Rivers System which covers about 70% of the country and contributes about 65% of total runoff. The quantity and quality of these freshwater bodies face pressures from deforestation, declining rainfall, increasing temperature, pollution and sea water intrusion (Ansah-Asare et al., 2008; Gyau-Boakye et al., 2002; Kankam-Yeboah et al., 2004). The focus of my research are two rivers discharging into the Tano River, therefore the following discussion about Ghanaian biophysical characteristics and hydrology will be limited to that of Tano River and its basin. Information regarding the Tano River and its basin has been rather limited to that gathered and reported by the Ghanaian Water Resources Commission (WRC) for the state's Integrated Water Resources Management Plan (WRI, 2012). Most of the information regarding the Tano Basin presented in this section is originated from this Plan unless otherwise mentioned.

The Tano River basin lies between latitudes 5^{o} N and 7^{o}40' N, and longitudes 2^{o}00' W and 3^{o}15 W. Its 15,000 km^2 catchment area spans almost 35% of Brong Ahafo, 15% of Ashanti and 50% Western Regions of Ghana. The river flows for about 400 km from its source at Techiman in the Brong Ahafo Region at an altitude of 518 meters above sea level to the Aby Lagoon in Cote d'Ivoire. The Tano River basin is warm and moist with relative humidity between 75%-85% throughout the year. In the drier months of August and March, however, average temperatures can slightly drop to between 25^{o}C and 28^{o}C respectively. Like coastal Ghana and West Africa regions, the climate of the basin is sub-equatorial wet with two rainy seasons; one between April-July and the other October-November. Annual rainfall on the basin ranges from 1300 mm in the north to 2100 mm in the south and mean annual number of rainy days' ranges between 90 to 120 days. The Ghana Water Research Institute records that spatial and temporal distributions of rainfall are high and increasing southwards (Figure 2.4). A typical rainfall variation during the year at Sefwi-Bekwai meteorological station is illustrated in Figure 2.5 A and clearly shows the bimodal rainfall distribution of the area. The basin receives a total average of 1,500 mm of rainfall per year with a mean annual evapotranspiration ranging from 1,322 mm in the south to about 1,500 mm in the north. With an annual runoff of about 2,744 mm, the Tano River water originates mostly from rainfall. A typical seasonal flow as recorded at a gauging station in Tanoso (in upper Tano) is illustrated in Figure 2.5 B.

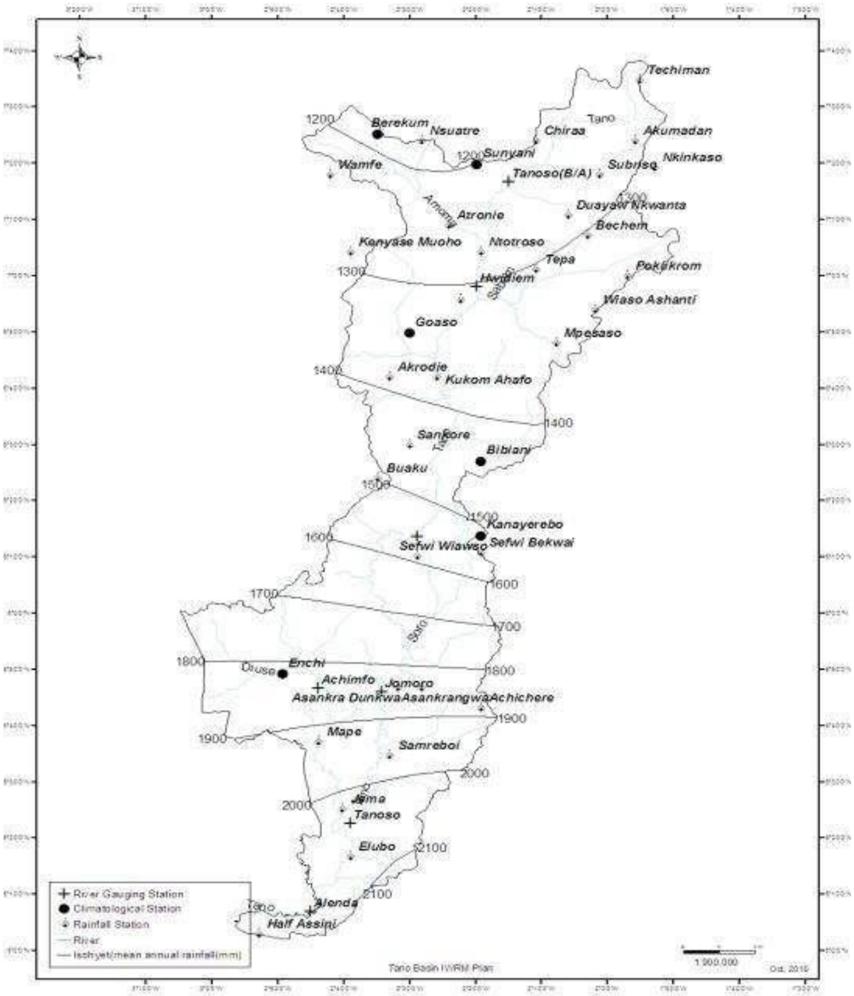


Figure 2.4 Annual rainfall distribution in the Tano basin (Source: WRI, 2012)

A B

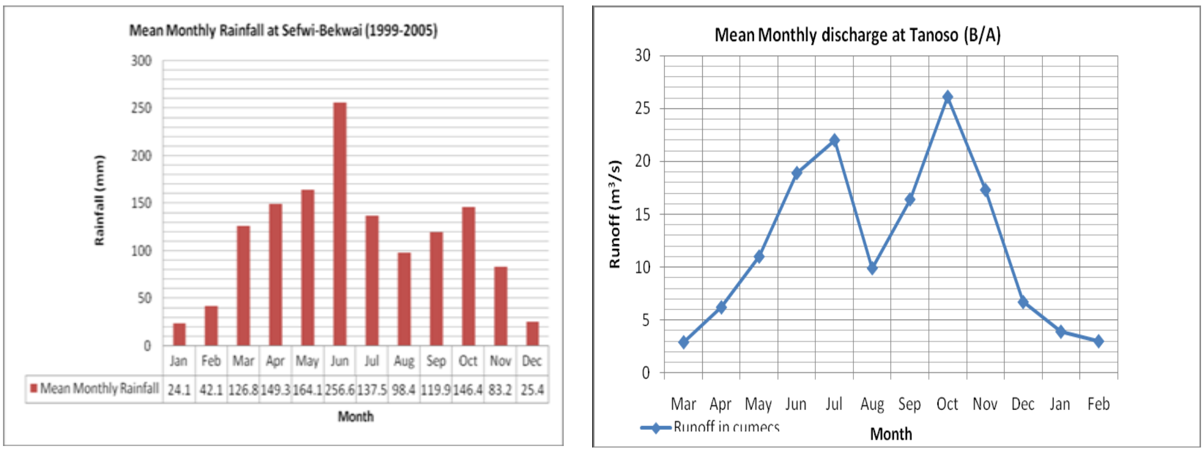


Figure 2.5 Mean monthly rainfall at Sefwi-Bekwai on the Tano basin (A) and mean monthly discharge of the Tano River at Tanoso (B) between 1999-2005 (WRI, 2012).

River ecological studies in West Africa, including Ghana, are mostly concerned with ichthyology and fish distribution (Abell et al., 2008; Hugueny et al., 1994; Tedesco et al., 2008; Thieme et al., 2005). In 1994, Hugueny and Leveque asserted that based on fish distribution, river basins in West Africa beared faunal similarities with each other although they recognised three main zoogeographic regions, namely the Eburneo-Ghanean biogeographical region covering rivers in Ghana and Cote d'Ivoire; Upper Guinea region covering Guinea and Liberia; and Lower Guinean region covering the rivers of Cameroon and Gabon (Hugueny et al., 1994). The latest freshwater bio-ecoregional classification (Abell et al., 2008) places the Tano River alongside the Pra and Ankobra rivers of Ghana in the Ashanti Freshwater Ecoregion, a sub-ecoregion of the Eburneo-Ghanean region.

A large part of the basin, particularly in the southern section, is within the wet-evergreen and the moist-semi-deciduous agro-ecological zones (AEZ) of Ghana, with about 40% of the total covered by forest, mostly protected area (WRI, 2012). The forest reserves along the river are designated for conservation of vulnerable and endangered species and some for timber production. Economic activities in the basin include cocoa farming, forestry, and mining. Towns along the river, including Kenyase and Hwidiem where the research was conducted, are known for their gold mining activities. Environmental challenges faced by the Tano River as perceived by the local stakeholders include: inadequate water supplies to meet the domestic, agricultural and industrial (including mining) demands; land degradation due to deforestation, agriculture and mining; water quality deterioration from household, commercial, industrial (including mining) and agricultural wastes (WRI, 2012). Similar to other freshwater resources in Ghana, the water resources of the Tano River and its coastal lagoon system are under the threat of pollution from nutrients from wastewater and farming and hazardous substances from mining which could have deleterious effects on the aquatic ecosystems(Kankam-Yeboah et al., 2004).

2.5 Local Climate

The climate of the study area is similar to that of southwestern Ghana and the Tano Basin. Major rains occur during April to July with an average precipitation of 294 mm/month and minor rains from September to November with an average precipitation of 234 mm/month. The major dry season coincide with the Harmattan season which runs between November and February with as little as zero precipitation and maximum evaporation, with an average precipitation of 16 mm/month and evaporation of 105 mm/month (Figure 2.6) (Unpublished meteorological report NGGL, 2013). The average temperature, however, is the lowest in the Harmattan due to the low temperatures experienced during the night. A short dry season occurs in August. Figure 2.7 illustrates rainfall patterns in the study area recorded at a weather station in Kenyase Village, Brong Ahafo, 2008 to 2012.

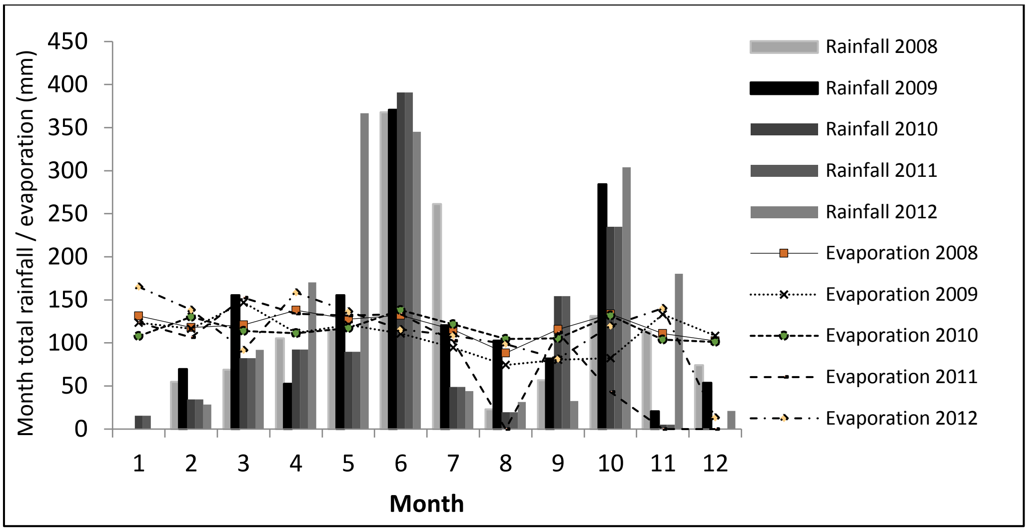


Figure 2.6 Historical total monthly rainfall and evaporation at Kenyase weather station 2008 – 2012.

2.6 Hydrology and morphology of the Surow and Subri Rivers

Baseline hydrological data of the Surow River (i.e. prior to the study) was not available, therefore, much of the hydrological features reported here are based on the hydrological measurement taken monthly between July 2013 and April 2014 (i.e. during the study period). July 2013 onward, on each water and biota sampling occasion (Chapter 3,4,6,7) and at each site, water depth and velocity were measured using a Marsh-McBirney Flowmeter. More frequent (twice a week) flow and depth measurements were taken from sites 3, 6 and 9 in August and September 2013.

The rivers and streams in the study area exhibit a classical wet-dry hydrological patterns that is driven by rainfall, typical of tropical rivers (Larned et al., 2010). The Surow River and most of the Subri River tributaries are seasonal streams that dry out from November to April over about half of the rivers' length (Figure 2.8 and Figure 2.10). On the contrary, heavy rains in the two rainy seasons often brings flood events

2.6.1 The Surow River

Between July 2013 and April 2014, the highest average daily discharge of the Surow River was recorded 1.3 m^3/s on the 7th of October 2013 at site 4, while the lowest was recorded at 0.1 m^3/s in January 2014 (Figure 2.8). In February and March 2014, sites 8, 9 and 10 dried up, effectively disconnecting the upstream sites from the downstream sites (Figure 2.9), whilst site 1 to 6 always have flowing water throughout the year albeit with very low flows.

Temporal patterns of river discharge and rainfall were observed but the Surow River's discharges did not correlate with one-week and day precipitation events (Pearson's r=0.04 and r=0.106 respectively). Site 6 and 8 were the exceptions where water discharges strongly correlated with total precipitation, both weekly and daily (Pearson's r=0.97; p=0.0001 and r=0.98; p=0.0001, respectively). Unlike other sites, the river at site 6 and 8 were in clearly defined channel forms.

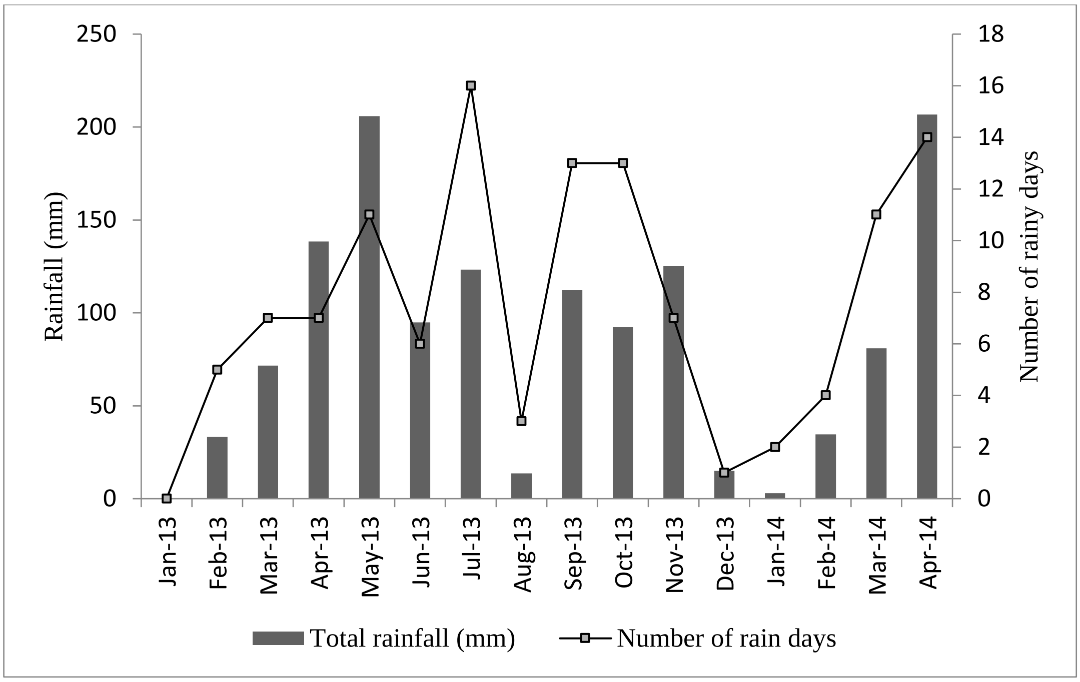


Figure 2.7 Total rainfall (mm) and number of rainy days per month measured at the weather station in Mensah Kumta Village, Kenyase, Brong Ahafo, Ghana from January 2013 –April 2014 (study period).

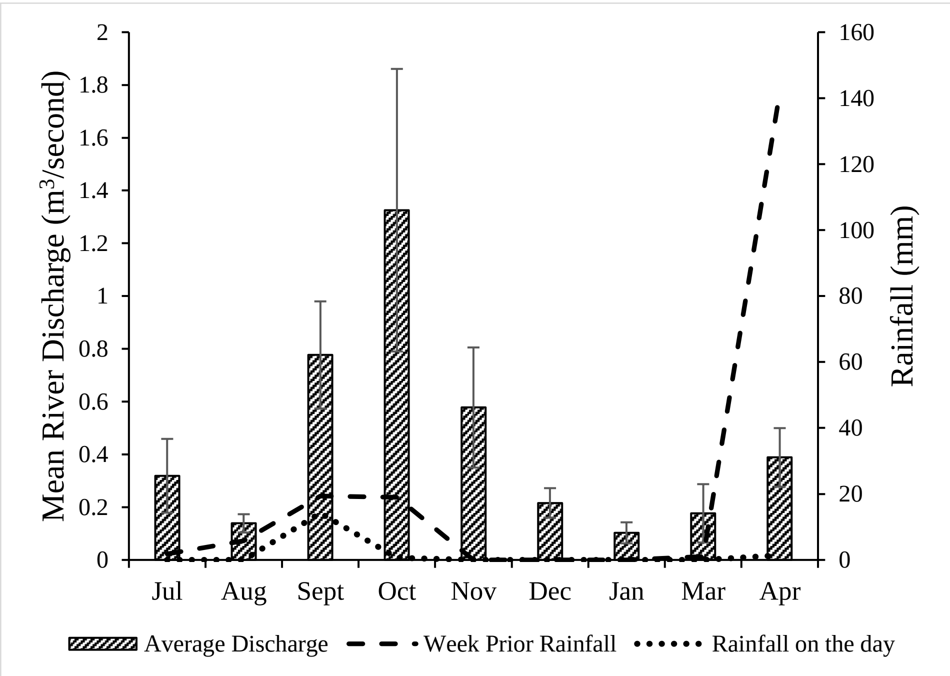


Figure 2.8 Mean water discharge along the Surow River with total rainfalls within one week prior to sampling and that of measured on the day of sampling. Data was collected between July 2013 and April 2014.

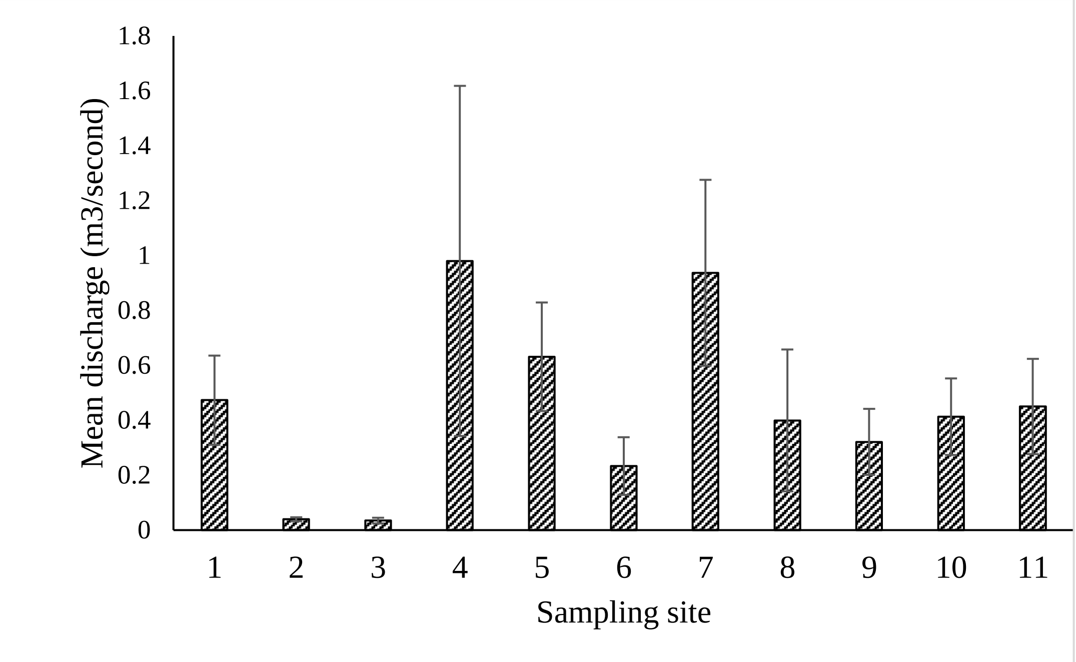


Figure 2.9 Mean river water discharge at each site along the Surow River. Data was collected between July 2013 and April 2014.

A

B

C

D

Figure 2.10 Contrasting river flows between the rainy season and dry season at the Surow (A and B) and Subri Rivers (C and D)

2.6.2 The Subri River

The Subri River has a more defined channel form that that of Surow River (as discussed in Chapter 3). At its source (site NSW9), the river is surrounded by secondary forest, urban dwelling and farms, and shows active widening process. Its river bottom and banks are overgrown with grass and other vegetation. As it flows closer toward the mine areas and the confluence with the Tano River, the river channel becomes more clearly defined.

Flow measurements taken at site KSW2 on the main Subri River channel from July 2013 to April 2014 indicated that river flow (discharge) varied with time and site. The highest river mean discharge of 1.9 $m^3 s^{-1}$ was recorded on the 7th of October 2013 while the lowest was recorded at 0.1 $m^3 s^{-1}$ in January 2014. The temporal variability of the river flows was measured in the field, particularly between the peak of dry and wet seasons. The most recent visit to the site at the peak of rainy season of October 2015, for example, coincided with the river flooding and recorded a flow of 25.9 $m^3 s^{-1}$. At the end of the dry season in February and March 2014, on the other hand, the river was dry at sites KSW13, NSW6 and KSW2, although pools of water could still be seen in the reaches downstream or upstream of the sampling sites. Sites NSW9, KSW16 and KSW3 can form large pools during the dry season. Site NSW8, on the other hand, always had flowing water throughout the study, although the flows varied with time (season). The flows at NSW8 were partially controlled by the amount of discharge of mine water from one of the environmental control dams (ECDs) at the mine.

2.7 Geology

The study area lies on the Bibiani-Sefwi Belt, a gold mineralised zone that extends southwest-northeast in the western part of Ghana (Figure 2.8). Gold in this area occurs with pyrite and quartz and is commonly associated with extensive disseminated sulphides and rarely with arsenopyrite. In the weathered zone, manganese oxides are common (NGGL, 2005). According to Newmont Ghana Environmental Impact Statement document (NGGL, 2005), the deposits at Kenyase extend over a distance of 13 km from southwest to northeast across the Subika and Apensu/Awonsu sub basins. The Apensu/Awonsu deposit which is mainly worked by the Ahafo mine has been tropically weathered, generally up to 30-40m in depth and consists of saprolite that is locally overlain by a zone of transported and in places cemented eluvial and alluvial fragments 1 to 7 m thick, with iron oxide pisoliths and cement known locally as duricrust. On the other hand, the Subika type of deposits which is also the typical deposits on the Surow River catchment where ASGM operates, is lacking the saprolite and duracrust which have been stripped due to recent erosion associated with the old Tano River. Oxidation in this type of deposit is limited to a thin (5-15 m) zone of complete oxidation of bedrock, followed by an irregular zone of partial oxidation extending up to 20 m into primary bedrock (Mr. Seth Ako, Principal Geologist with Newmont Ghana, pers. com).

A series of tests for potentially acid generating (PAG) rock on oxide and sulphide composite samples from the Kenyase deposits conducted by the Ahafo mine reported that the deposits ranged between neutral to highly basic. The sulphide composites were slightly basic to highly basic, whilst the oxide composites ranged from neutral to inert or slightly basic. These findings suggest that material taken from the study area had little tendency to produce acidity (Lottermoser, 2012; NGGL, 2005).

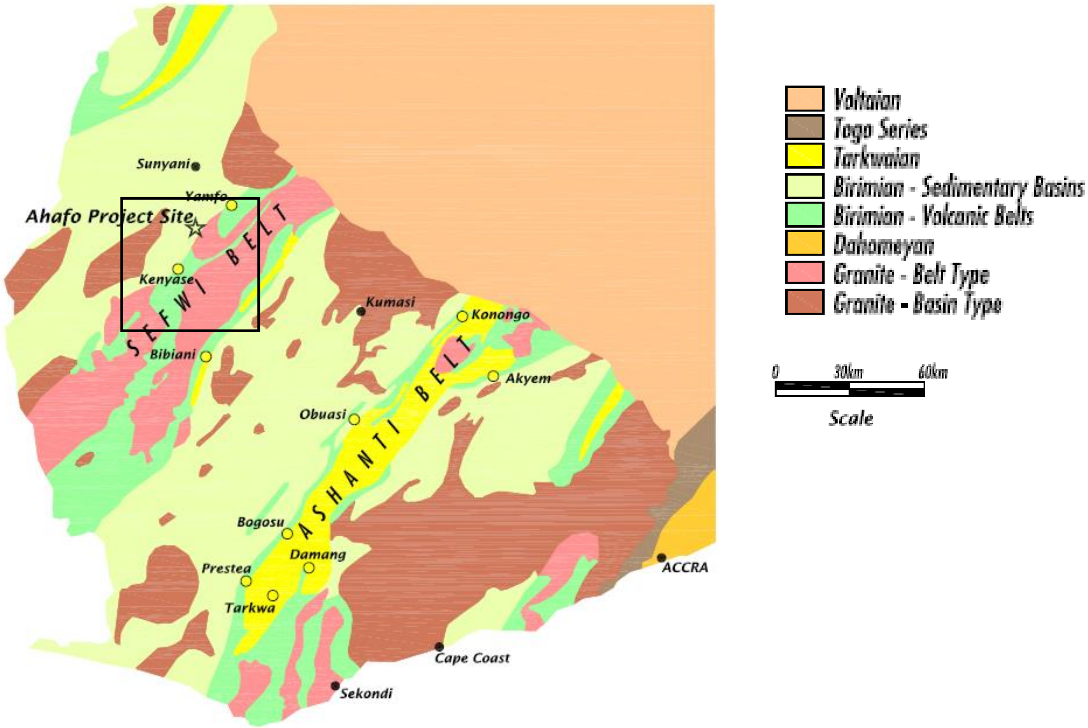


Figure 2.11 The geology of southwestern Ghana. The study area (marked with a rectangle) lies on the Sefwi Gold Belt of Ghana. The ASGM operators mostly work on the Birimian sedimentary and volcanic rocks whilst the Ahafo Gold Mine mostly works on the Granite as well as Birimian volcanic and sedimentary materials. (Source: Knight Piesold in NGGL (2005)).

2.8 Riparian conditions of study sites

2.8.1 Method

A rapid riparian assessment of the both sides of the two rivers at each sampling site was conducted once in April 2014. Variables of riparian characteristics used in the assessment included the canopy cover, understorey vegetation and grass cover, amount of organic litter on river banks, sediment size in river bottoms, slope, slumping or gullying of the banks, as well as presence of animals. Detailed data collection form containing variables and scoring system can be seen in

Appendix 3.

The riparian characteristics, which include canopy cover and health, banks slope, gullying, etc. (variables and scoring system is detailed in

Appendix 3), were then subjected to multivariate statistical analysis on the PRIMER v 6.1 and its PERMANOVA add-on (Anderson et al., 2008; Clarke, 1993). Data was log-transformed and normalised within the PRIMER suite of analyses prior to an analysis for Euclidian distance. Hierarchical agglomerative cluster analysis by means of the complete linkage was performed on the Euclidian distance matrix (Vega et al., 1998) . The correlation based Principal Component (PCA) ordination was then used to visualise the distance between sites, banks, rivers and level of impact (control or impact). The PCA was followed by Factor Analysis by plotting the PCs against original variables resulting in correlations (called loadings) which correspond with contributions of variables to the variance (Liu et al., 2003; Vega et al., 1998).

To test the hypotheses of no differences in riparian characteristics between rivers, banks and impact level (Control and Impact, see the factor labels for each sampling site in Table 2.1), Permutational Analysis of Variance (PERMANOVA) was performed. Pairs of factors that have significant differences ($p \leq 0.05$) were then analysed by SIMPER within the PRIMER package to identify variables contributing to the differences.

2.8.2 Results

Riparian characteristics of the Surow River were significantly different from the Subri (p PERMANOVA = 0.01; F = 2.425). Across rivers, riparian characteristics differed between sites (p PERMANOVA= 0.0001; F = 3.005) and between control and impact (p PERMANOVA =0.05; F=1.939), but did not differ with bank sides (left and right). On the Surow River, control differed significantly from impact, whilst on the Subri River the difference between control and impact was not significant. Detailed results from the PERMANOVA is presented in Table 2.2.

PCA of the riparian characteristics illustrated the distinct separation between the Subri and Surow rivers (Figure 2.12). Variables that significantly drove the separation as identified by the first PC appeared to correspond with vegetation conditions (understorey, grass cover, canopy cover, organic litter), whilst the second and third PCs were more driven by the morphology and physical conditions of the river channels and banks (gullying, undercut, slope, and sediment size). SIMPER analysis confirmed that the significant difference between riparian characteristics of the two rivers was contributed to by bank slopes and vegetation (canopy cover, grass cover) as well as organic litter (Table 2.3). The banks on the Subri River sites tended to have sharper slopes with less gullying than that of the Surow. The canopy cover and understorey vegetation on the Subri River were generally better than on the Surow River which had more open vegetation and higher grass and weed covers on its banks. This vegetation cover differences between the two banks probably explains the presence of organic litter which was more prominent on the Subri than on the Surow River.

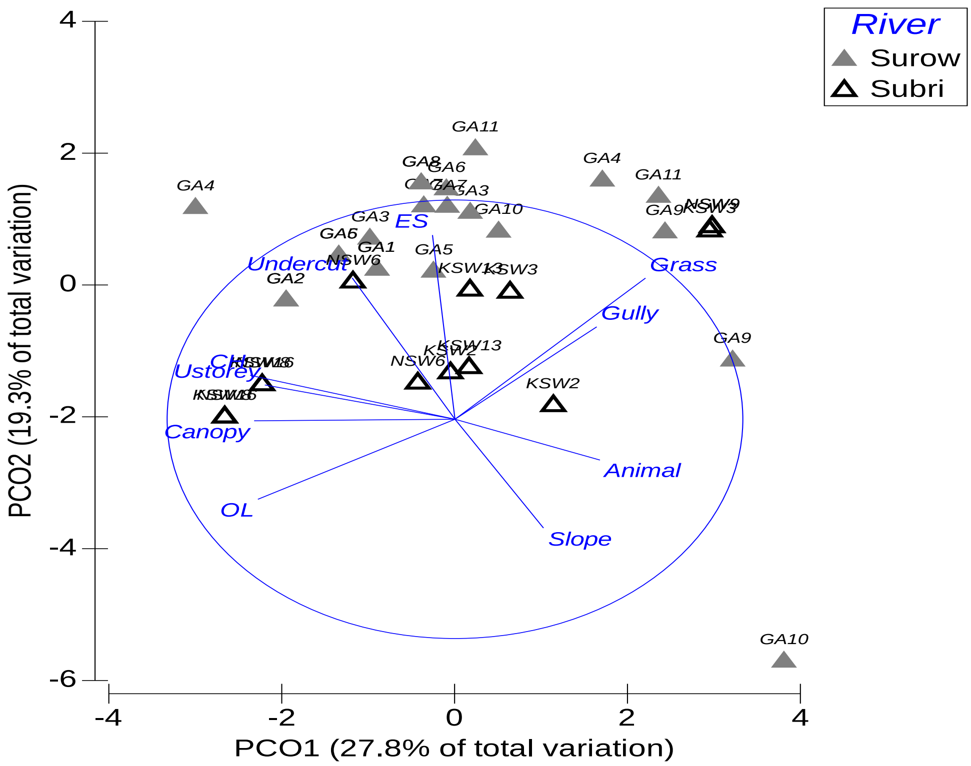
A separate PC analysis on each river indicated the distinct separation between control and impact on the Surow River (Figure 2.13A) but not on the Subri River (Figure 2.13B). As can be seen in Figure 2.13A, the control sites on the Surow River tended to have more canopy cover and healthier understorey vegetation than the impact sites. ASGM impacted sites (site 4, 9, 11) also tended to be overgrown with grass and weeds growing in the river channel and banks widen by sedimentation. Such separation between control and impact, however, was not clearly seen on the Subri (Figure 2.13B), although KSW2 and KSW3 tended to be separated from the rest due to gullying and sharper bank slopes.

SIMPER analysis also showed that the significant differences between riparian characteristics of control and impact sites across rivers were contributed by their differences in the amount of organic litter, gullying and the health of understorey vegetation on the banks (see Table 2.4). Control sites tended to have denser understorey vegetation and more organic litter cover on their banks than that of Impact sites. Gullying and bank slope also tended to be higher at Impact sites.

Table 2.2 PERMANOVA results of riparian characteristics on the Surow and Subri River sampling sites

Source of variance	df	SS	MS	Pseudo-F	P(perm)	Unique permutations
Across Rivers						
River	1	27.858	27.858	2.4154	0.0134	9923
Residual	34	392.14	11.534			
Total	35	420				
Bank Side	1	7.8432	7.8432	0.64701	0.7769	9924
Residual	34	412.16	12.122			
Total	35	420				
Site	17	310.58	18.269	3.0053	0.0001	9816
Residual	18	109.42	6.0791			
Total	35	420				
Control/Impact	1	22.665	22.665	1.9394	0.054	998
Residual	34	397.34	11.686			
Total	35	420				
Surow River						
River	10	165.68	16.568	2.1114	0.0013	9839
Residual	11	86.316	7.8469			
Total	21	252				
Bank Side	1	15.02	15.02	1.2676	0.2323	9211
Residual	20	236.98	11.849			
Total	21	252				
Control/Impact	1	39.522	39.522	3.7201	0.0013	7866
Residual	20	212.48	10.624			
Total	21	252				
Subri River						
River	6	132.24	22.041	6.2442	0.0001	7162
Residual	7	24.709	3.5298			
Total	13	156.95				
Bank Side	1	3.9932	3.9932	0.31327	0.9199	672
Residual	12	152.96	12.747			
Total	13	156.95				

Control/Impact	1	18.795	18.795	1.6325	0.1736	1192
Residual	12	138.16	11.513			
Total	13	156.95				



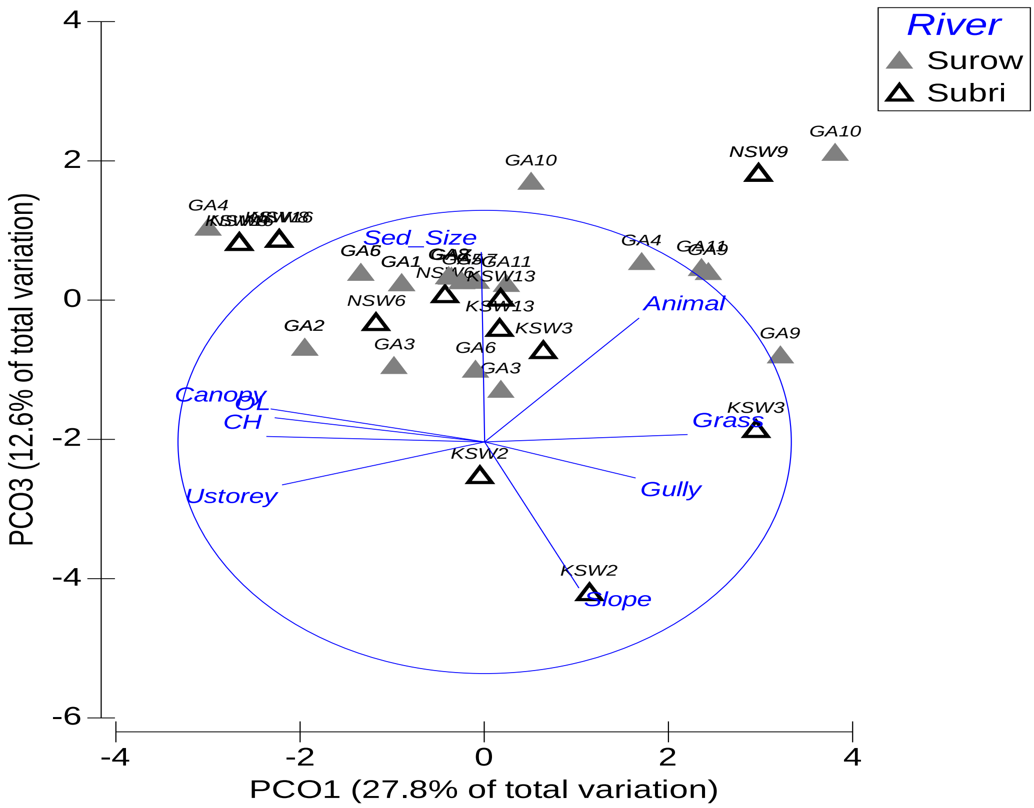
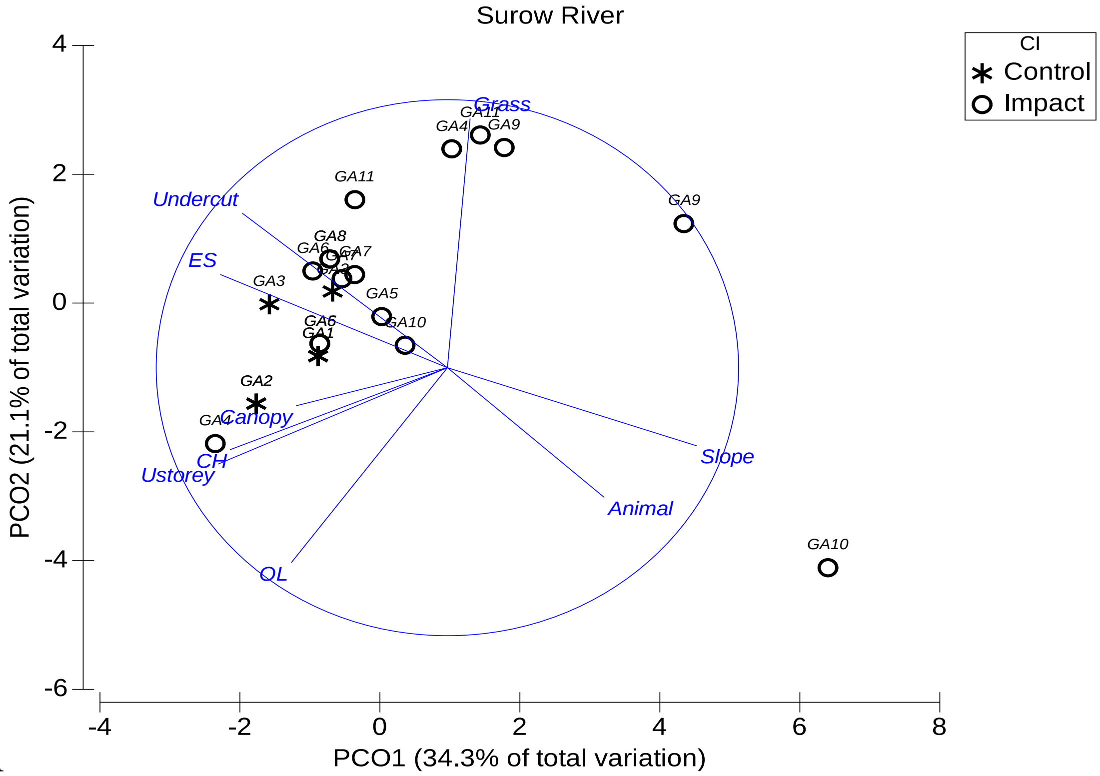


Figure 2.12 Principal component ordinations of riparian characteristics indicating the difference between the Subri and the Surow Rivers.
Variables significantly contributed (Pearson's r >0.5) to the PCs are indicated.



A

B

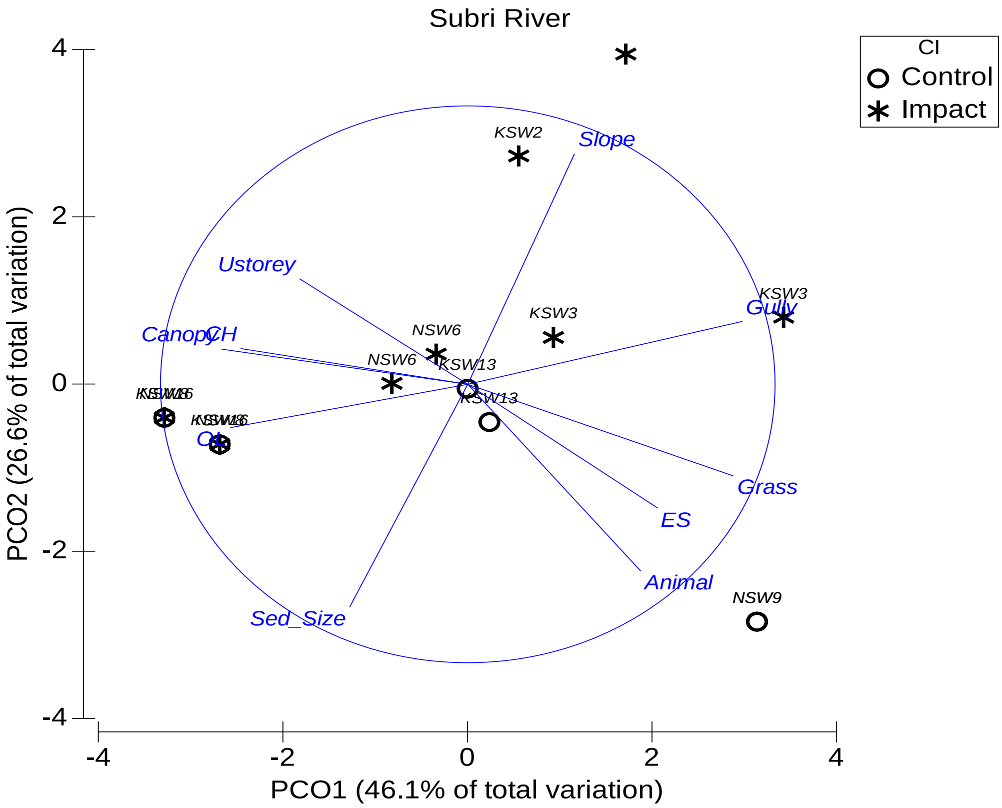


Figure 2.13 The principal component ordinations of the riparian characteristics indicating the difference between Control and Impact sites on the Surow (A) and the Subri (B) Rivers. Variables that contributed significantly (Pearson's r > 0.5) are indicated.

Table 2.3 Results of SIMPER analysis between the Surow and Subri Rivers riparian characteristics

Variable	Average Value		Av. Sq. Distance	Sq.Dist / SD	Contribution (%)	Cumulative contribution (%)
	Surow	Subri				
Slope	-0.375	0.589	2.68	0.84	10.65	10.65
Gully	0.337	-0.53	2.67	0.78	10.6	21.25
Grass	0.216	-0.34	2.23	0.88	8.87	30.12
OL	-0.26	0.408	2.2	0.85	8.76	38.88
Sed_Size	5.53E-02	-8.69E-02	2.14	0.51	8.51	47.4
CH	0.253	-0.397	2.11	0.93	8.38	55.78
Animal	-6.34E-02	9.96E-02	2.05	0.51	8.13	63.91
Canopy	1.72E-02	-2.71E-02	1.98	0.91	7.86	71.76
U_storey	0.108	-0.169	1.95	0.79	7.75	79.51
ES	0.137	-0.216	1.88	0.67	7.48	86.99
Slumping	-0.106	0.167	1.64	0.22	6.5	93.5

Table 2.4 Results of SIMPER analysis between riparian characteristics of Control and Impacts sites across rivers

Variable	Average Value		Av.Sq.Distance	Sq.Dist /SD	Contribution (%)	Cumulative contribution (%)
	Surow	Subri				
Gully	-0.487	0.244	2.32	0.75	9.82	9.82
OL	0.585	-0.293	2.28	0.9	9.66	19.49
U_storey	0.425	-0.212	2.25	0.81	9.52	29.01
Canopy	-0.4	0.2	2.23	0.98	9.43	38.44
Animal	0.174	-8.72E-02	2.19	0.53	9.26	47.71
CH	-0.133	6.63E-02	1.98	0.89	8.39	56.09
Grass	-0.195	9.73E-02	1.92	0.84	8.13	64.22
Sed_Size	-0.147	7.37E-02	1.9	0.58	8.03	72.25
Slope	-0.389	0.195	1.83	0.65	7.76	80.01
ES	0.153	-7.67E-02	1.72	0.55	7.28	87.29
Slumping	0.167	-8.33E-02	1.5	0.21	6.35	93.65

2.9 Discussion

The climate and hydrological features of the Surow and Subri rivers and their catchments exhibit the common features of tropical regions and rivers (Boyero et al., 2009). These features need to be taken into consideration when assessing and comparing anthropogenic impacts on the rivers' ecology. Tropical regions occur between the Tropics of Cancer and Capricorn at the latitude of ~23.5 North and South where the sun is directly overhead at solstice (McGregor et al., 1998; Seidel et al., 2008). Consequently, the tropical region is warm with temperatures that do not change much during the year or during the day (McGregor et al., 1998; Peel et al., 2007), except for major monsoonal areas, which includes the study area in Southern Ghana where, during the Harmattan, the temperatures drop significantly during the night. Another characteristic of tropical regions is the substantial precipitation in regions near to the equator while on the outer edges of the tropical belt it is notably drier (Peel et al., 2007; Seidel et al., 2008). Annual precipitation in the region may exceed 1000 mm (McGregor et al., 1998). The intensity of the rain in tropical West Africa during the rainy seasons typically produces high runoff that often leads to flooding, sheet and gully erosion, river scouring, surface crusting, soil compaction, and soil loss (Balek, 2011; Hayward et al., 1987), which were also evident in the study area. .

The Surow and Subri rivers, like other tropical rivers in Ghana and West Africa, are hydrographically dominated by rainfall (Moliere et al., 2006; Montgomery, 1999) (Beckinsale, 1969; Peel et al., 2007) and tend to be event driven (Smith et al., 2003). The strong seasonal precipitation that commonly occurs in the tropics produces seasonal patterns of river discharge and physicochemical properties. Water temperatures and conductivities tend to be lower during the long wet periods, while water depths, velocities, and dissolved oxygen concentrations tend to be greater.

The Surow and Subri are temporary rivers which, like many smaller tropical rivers, periodically cease to flow and are characterised by complex hydrological dynamics in the longitudinal dimension such as advancing and retreating wetted fronts, hydrological connection and disconnection and gradients in flow permanence. These dynamics influence biotic communities, nutrients and organic matter processing (Larned et al., 2010). The flood pulse forces, as proposed by Junk et al. (1989), play important roles in the cycling and fate of pollutants in tropical rivers such as the Surow and the Subri. During flood events, river-borne pollutants are often transported on to the floodplain with the deposition of contaminated sediments and infiltration of polluted water (Stewart et al., 1998). During the wet periods, organic nutrients in sediments are mineralized while inorganic materials are leached into flood waters which often extend the aquatic habitats onto the floodplains (Carignan et al., 1999; Sioli, 1984). The leached inorganic nutrients are in turn assimilated by macrophytes on the floodplain and returned into the water column and sediment during the falling water periods (Winemiller, 1990). The cycling of Hg, a pollutant commonly found in the Amazon riverine environment, for example, is thought to be associated with the flooding patterns. Guimaraes et al. (2000b) found that production of methyl mercury (MeHg), an organic and the most toxic form of Hg, on the floodplain of Tapajos River of the Amazon was at a higher rate during the inundation period. A study of fish Hg bioaccumulation in the Rio Madeira River of the Amazon, a river thought to be impacted by artisanal gold mining, showed that although accumulation of Hg by fish was species specific, the bioaccumulation trend in a few species was significantly different between the high and low water seasons (Bastos et al., 2007).

The gradual and continuous changes in water level in tropical rivers also leads to continuously assembled and disassembled habitats and community structures for riverine organisms (Arrington et al., 2005). A study on small tropical streams by Pringle et al. (1997) for example, showed how tropical storms and floods changed the assemblages of biotic communities through the scouring of substrates that reduce benthic food during these periods.

A

B

Figure 2.14 Sampling site NSW8 on the Subri (A) and the dense secondary forest on its banks (B). The river at this site receives discharges of mine water from the Ahafo mine

A

B

Figure 2.15 Sampling Site 4 on the Surow River at Kenyase 2 town. ASGM worked on the bank of the river at this site. Sedimentation on the river channel and die backs were visible (A). The river and banks were overgrown by tall grass (elephant grass) and weeds.

Although the Surow and the Subri rivers and their catchments share the same climatic characteristics, their riparian characteristics were different from each other as evident from the rapid riparian assessment. As riparian corridors are diverse mosaic of landforms, communities and environments of the larger landscape (Naiman et al., 1997; Naiman et al., 1993), the significant differences between riparian characteristics of the Surow and Subri rivers can be explained by the difference in river morphology and anthropological activities in their catchments, particularly at the river edge. The Subri River, with its much larger catchment area has more tributaries, sub-basins, and a more complex river and riparian system than the Surow River. Differences in type of land use and levels of management on the two river catchments may have also contributed to the differences in their riparian characteristics. Most of the Subri River catchment area is within the concessional area of the Ahafo mine whilst the Surow River catchment is not. Although the area cleared for mining on the Subri River is substantially larger than that of for ASGM mining on the Surow River, land clearing on the Subri catchment is managed by the Ahafo mining company and monitored by the Ghanaian environmental protection authorities, resulting in healthier and denser vegetated areas along the river compared to the Surow River. For example, the remnants of natural forest and teak forests (plantation) along the Subri River within the Ahafo mine concession (Site KSW16, KSW 13, NSW3, NSW8) are in a healthy condition with relatively dense canopy that provides ample supply of organic litter cover on the river banks (Figure 2.14). Most of the riparian area along the Surow, on the contrary, was either farmed, mined or overgrown with the elephant grass and other weeds (see Figure 2.15). Sedimentation that was visible along impacted sites on the Surow River was not visible on the Subri River except for Site KSW3 which was located within the mine operations site. The lack of sedimentation on the Subri River will be further explored and explained in Chapter 4. Discharges of mine water from the environmental control dams into the Subri, although likely to comply with regulations, may also have scoured the river's sediment and transported it downstream. On the contrary, sedimentation seen on the Surow River clearly indicated a lack of erosion control measures along the river. Water abstraction from the river by ASGM operators along the Surow River helped worsen sedimentation problems whilst occasional discharges of mine water seemed to have failed to scour and transport the sediment trapped between grass and weeds growing on the widening river.

2.10 Conclusions

The Surow and Subri rivers are typical tropical rivers hydrologically driven by rainfall. The rivers periodically cease to flow, notably during the peak of a dry season that runs between November and April, and flood during the two rainy seasons between April to July and September to November. Consequently, studies in river ecology and anthropogenic impacts on the two rivers must consider the natural temporal and spatial variations that takes place along the rivers due to the climatic and hydrographic changes.

The riparian characteristics on the Surow and Subri rivers are different to one to another. The differences are mainly due to the differences in vegetation types and cover and in the types of anthropogenic impacts on their catchments. The Surow River riparian features are characterised with sedimentation and grass growth along the channel and on the banks of the rivers particularly at sites downstream of ASGM sites. The Subri River riparian features, on the contrary, are characterised by healthier vegetation as indicated by higher canopy cover, understorey density and more prominent organic litter layers. The riparian characteristics on control sites are also different from impact sites on both rivers, although the difference was more significant on the Surow River. Generally, the control sites are characterised by denser understorey vegetation and more organic litter cover on their banks than that of impact sites. Gullying and bank slope also tended to be higher at impact sites

3 Impact of artisanal and small scale mining (ASGM) on water and sediment qualities in the Surow River, Brong Ahafo, Ghana.

3.1 Background

Artisanal and small-scale gold mining (ASGM) ventures, including those in the Surow River catchment, often operate near streams and rivers for easy access to alluvial ores, to supply water used in processing, and receptacle for mine water discharges. This chapter assesses ASGM impacts on sediment and water quality in the Surow River, a small tributary of the Tano River in Brong Ahafo, Ghana.

ASGM has been practised in many parts of Ghana for hundreds of years (Donkor et al., 2005); however, such operations are relatively new (<9 years) to the Surow River catchment (Figure 3.1). ASGM in this area started in 2005 following the commencement of a large multi-national gold mining project that discovered gold in the region. In Kenyase 1 and Kenyase 2 townships, small operators extracted secondary or tertiary alluvial gold ores easily found in the river banks, although increasing technical and financial capacities and depletion of alluvial resources have prompted larger operators to extract primary ores by mining underground using tunnels and shafts of up to 35 m deep.

Figure 3.1 Aerial photo of ASGM works on the Surow River taken on March 2014 shows active widening of the stream due to mining. (Photo courtesy of Newmont Ghana Gold Limited)

Ghanaian law stipulates that ASGM is exclusively reserved for Ghanaian citizens, and that foreigners and foreign investment in ASGM operation are forbidden (Ghana, 2006; "Small

Scale Gold Mining Act of 1989," 1989). The sector, nevertheless, received illegal foreign investments at least until April or May 2013 when the Ghanaian government deported as many as 4000 foreigners, mostly of Chinese nationality, involved in the industry across the country (Ibrahim, 2013).

At Kenyase 1 mine, more than 20 mine pits of up to 30 metres deep were created during the peak of ASGM activities in the area. These underground pits were reportedly interconnected with each other and would naturally fill with water (Figure 3.2 A). During the active mining period (up to April 2013), owners and operators pumped the water out to provide access for underground mining. The Ghanaian government eviction of illegal foreign ASGM financiers in end of April 2013 halted the operation of the power generator facility at the mine, the only source of power available to run the pumps and other mine utilities. Thus, most mining activities ceased in May 2013 and major ASGM operators left the mine but the underground mines appeared to be highly prized by the investors. Wishing to return to work on the mine someday, the pits were maintained, albeit minimally, by occasional dewatering. While most mine dewatering pumps ceased to operate since May 2013, I observed at least a couple of pumps were intermittently pumping water out of the mine shafts at Kenyase 1 throughout the study period, particularly in the rainy seasons. The untreated mine dewatering water discharged directly to the environment (Figure 3.2 B) flowing into the Surow River through a channel and swamp. Personal observation throughout the study period also showed that, although underground mining activity was substantially reduced, smaller mining operators and processors, comprised of local citizens, continued to operate after the deportation.

A B

Figure 3.2. An abandoned mine pit filled with water (A) and mine dewatering pump discharging mine water into a Surow River’s tributary channel at Kenyase I mine (B).

The cessation of major ASGM operations, or at least the downscale of ASGM operations in the study area in May 2013, was unforeseen when I commenced this project in January 2013. Originally I aimed to establish spatial and seasonal changes brought by ASGM to the physico-chemistry of the Surow River sediment and water by comparing multiple control and impact sites along the river over the 14 months’ period. Due to the cessation, therefore, I could only acquire three months’ worth of data of active ASGM impact from February to

April 2013. However, although the cessation disrupted the original experimental design, it had provided us with an opportunity to use the before/after and control/impact (BACI) experimental design to establish impact of ASGM on the riverine environments (Smith, 2002; Underwood, 1991, 1994). In this case, it is a reversed BACI because the impacted period preceded the time with no impact (Keough et al., 1997; Michener, 1997).

The main objective of this thesis chapter is to determine the impacts of ASGM on the Surow River sediment and water physico-chemistry by comparing multiple control and impact sites before and after the cessation of ASGM in the area. I also aim to identify the chemical elements contributing to any changes detected. The null hypotheses tested were therefore ones of no demonstrable changes in sediment and water quality before and after cessation of major ASGM operations in May 2013 as well as between upstream (control) and impacted sites across the river. The physico-chemical characteristics of water, nutrient and metal concentrations in water and sediment are the focus of this chapter, but some hydrological data influencing the river is also discussed.

3.2 Methods

3.2.1 Study site background

The Surow River is a tributary of the Tano River located in the upper Tano River Basin in the Brong Ahafo region of Ghana, approximately 300 km northwest of the capital city of Accra (**Error! Reference source not found.**). The Tano River is 400 km long with 15,000 km^2 of catchment whilst the Surow River is approximately 16 km long with 3,500 km^2 of catchment. Major land uses in the Surow catchment are ASGM and agriculture among tracts of natural forest. Farming activities in the area include cash crops (cocoa and palm), ranching and subsistence farming (vegetables and tubers). The biophysical environments of the study area are reviewed and discussed in details in Chapter 2.

3.2.2 Experimental and sampling design

The study employed a reversed BACI experimental design (Michener, 1997; Smith, 2002; Underwood, 1994) whereby multiple sites were sampled before and after removal of the disturbance factor and these were sampled within both control and impacted zones of the river. In all, eleven sites on the Surow River were selected based on access, safety, and representativeness of catchment land use (**Error! Reference source not found.** and Figure 3.3). Three out of the 11 sites were located upstream of Kenyase 2 ASGM mine work and designated as control sites (referred to as “control” hereafter). Eight sites located downstream of Kenyase 2 mine were designated as impact sites (referred to as “impact” hereafter). Table 3.1 summarises dominant hydrological feature and major land use and lists the spatial factors for each sampling site.

Temporal factors used in this study were the before and after cessation of major ASGM operation and “season”. Water samples were collected from February 2013 to April 2014 to capture potential effects of before and after the cessation and seasonality during the year as well as to minimise confounding impacts that may arise from gradient ecological properties of a river (Hurlbert, 1984; Norris et al., 1995; Stewart-Oaten et al., 1986; Underwood, 1994). Data acquired prior to the cessation of major ASGM operators in May 2013 was designated as “before” and that of May 2013 and beyond as “after”. Seasons were divided into “dry” and “rainy” based on rainfall data explained in Chapter 2. Table 3.2 lists the temporal factor for each sampling time

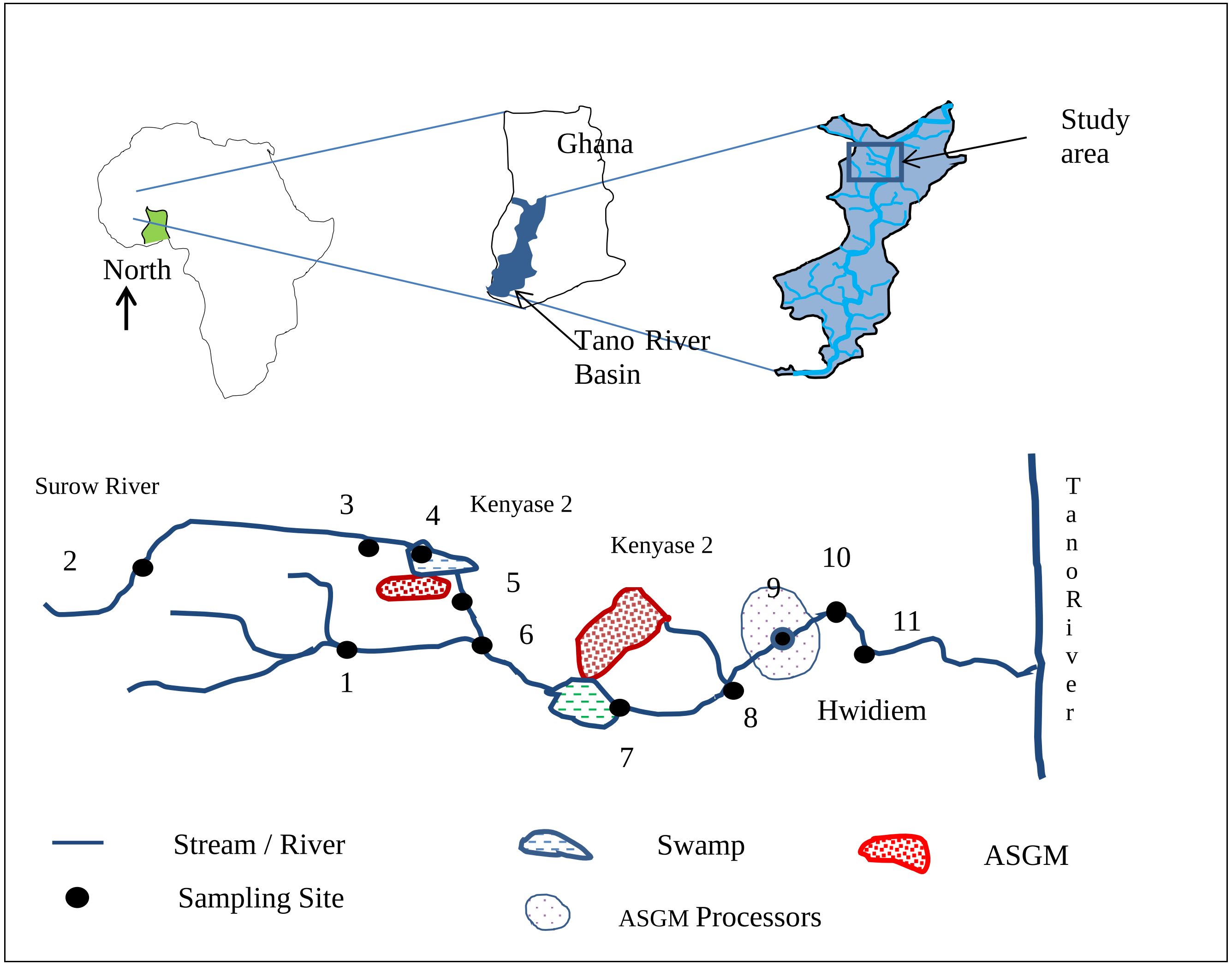


Figure 3.3 Study area on the Tano River Basin of Ghana and the sampling sites on the Surow River

Figure 3.3 Sampling sites 1 to 11 (A to K) and a mine dewatering pump at ASGM Kenyasi I mine site (L). Photos were taken at different times and seasons.

Table 3.1 Hydrology and land use (numbered upstream to downstream) of sampling sites with spatial factor designated to each site.

Sampling site code	Dominant site hydrology	Major land use	Spatial Factors	
			Location	Control/Impact
1	Run	Minimal use	Upstream	Control
2	Run	Minimal use, subsistence farming	Upstream	Control
3	Pool/slow run	Minimal use, rural dwelling	Upstream	Control
4	Swamp	ASGM (Mining, processing waste)	ASGM	Impact
5	Pool/slow run	Cocoa and Palm farming	Between	Impact
6	Riffle/Run. Confluence between Suntim and Akantansu	Minimal use, Subsistence farming	Between	Impact
7	Swamp	ASGM (Mining, processing waste, dewatering), cocoa farming	ASGM	Impact
8	Rifle/Run	Mine dewatering discharge, palm farming	ASGM	Impact
9	Run	ASGM (Processing)	ASGM	Impact
10	Riffle/Run	Cattle, vegetable farming	Downstream	Impact
11	Pool/slow run	Rural dwelling, farming	Downstream	Impact

Table 3.2 Temporal factors' labels assigned to each time of water sampling

Sampling time (Month)	Season	Before/After
February 2013	Dry	Before
March 2013	Dry	Before
April 2013	Rainy	Before
May 2013	Rainy	After
June 2013	Rainy	After
July 2013	Rainy	After
August 2013	Dry	After
September 2013	Rainy	After
October 2013	Rainy	After
November 2013	Rainy	After
December 2013	Dry	After
January 2014	Dry	After
February/March 2014	Dry	After
April 2014	Rainy	After

3.2.3 Sample collection and analysis

Water samples were collected monthly from February 2013 to April 2014 (14 months), except for February 2014 when parts of the river were dry. In addition, a sample of mine dewatering water at the Kenyase I ASGM site (Figure 3.3 L) was collected in April 2014. Water physical characteristics were measured *in situ*, while analysis for chemical components in the water were performed at the environmental laboratory at the mine site as well as at the ACZ commercial laboratory in Colorado, USA

At each time of sampling, we measured the physico-chemical parameters of pH, oxygen reduction potential (ORP), dissolved oxygen (DO), temperature, electrical conductivity (EC), and turbidity *in situ* using a Hydrolab Quanta (Hach, USA). Only during extreme weather conditions (dry or flooded) or when safety consideration did not permit, grab sampling was used. A preliminary analysis showed no difference between results from measurements of physico-chemical parameters of water using *in situ* and grab sampling (see

Appendix 4). July 2013 onward, on each sampling occasion and at each site, water depth and velocity were measured using a Marsh-McBirney Flowmeter. More frequent (twice a week) flow and depth measurements were taken from sites 3, 6 and 9 in August and September 2013.

Water samples were collected from 0.1 m below the water surface and immediately divided into unfiltered and filtered aliquots. Sample water filtration occurred in the field, through 0.5 µm GF/C (Pall Ltd Metrigard) for dissolved organic carbon, filterable reactive phosphorus (FRP) and dissolved metals analysis. Samples (250 ml) were preserved on site with 2 ml of 50% HNO_3 for dissolved metals, 2 ml of 25% H_2SO_4 for total phosphorus (TP), filterable reactive phosphorus (FRP), ammonia, nitrate as nitrogen (NOx), total Kjehdal nitrogen (TKN) and dissolved organic carbon (DOC) (APHA, 1998). . All samples were blind coded, stored at <4°C prior to analysis and airfreighted to ACZ Laboratory in Colorado, USA to be analysed within a week of sampling. At each time of shipment, a suite of blank and blindly coded samples of lab grade deionised water was sent to the laboratory along with the field collected samples to ensure quality of analysis.

At the ACZ Lab, most total and dissolved metals were analysed using inductively coupled plasma mass spectrometry (ICP-MS) following the USEPA Method 200.8 with lowest detection limit of; 0.0001 mg/L. Iron and Mg were analysed using ICP following the USEPA Method 200.7 with detection limits of 0.0005 mg/L and 0.2 mg/L respectively. Dissolved Hg was analysed using cold vapour atomic absorption (CVAA) following the USEPA Method 245.1 with a detection limit of 0.0002mg/L. Dissolved organic carbon was analysed following USEPA Method 5310B. On unfiltered samples, total Kjeldahl nitrogen (TKN) was analysed via block digester method (USEPA M351.2); and total phosphorus was analysed with an auto ascorbic acid method (USEPA M365.1).

At the environmental laboratory on the Ahafo mine site, I performed the analysis for ammonia/ammonium (NH_3-N), nitrate/nitrite (NOx-N) and sulfate (SO_4) on unfiltered water and FRP on filtered water using a Hach DR 2800 spectrophotometer following the APHA 21th 2005, 4500B&C, APHA 21th 2005 cadmium reduction (Apha, 2007), USEPA Method 375.4, and USEPA Method 365.2 ascorbic acid respectively.(USEPA, 2005).

At each water sampling site, sediment samples were collected from the bottom of the river using a 5-cm acrylic hand corer to a depth of 10 cm. Five replicate cores collected across different types of habitat were combined to give a more representative sample of the site, placed in new Ziploc bags, homogenised and stored in an ice box for transport to the laboratory. Sediment samples were stored frozen until analysed at the internationally accredited SGS laboratory in Accra, Ghana for total nutrient concentrations (N, P, C, S) following APHA (1998) and total metal concentrations (As, Mn, Pb, Cd, Hg) following the USEPA Standard Methods 200.8 (USEPA, 2005)

3.2.4 Data analysis

Water and sediment physico-chemistry data was subjected to univariate and multivariate statistical analyses with the reversed BACI model as the main statistical design (Michener, 1997; Smith, 2002). For the purpose of analyses, concentrations below the limit of detection (LOD) were replaced with LOD/$\sqrt{2}$ (Croghan et al., 2003; Verbosek, 2011). The univariate statistics of one-way and two-way ANOVA as well as correlation analysis were performed using the SPSS v23.

The multivariate analyses, including clustering and ordination, factor analysis and Permutational multivariate analysis of variance (PERMANOVA), were performed using the PRIMER v6 package and its PERMANOVA add on (Clarke et al., 2006).Prior to calculation of Euclidian distances between samples and variables, the data was log transformed ($\log_{10}$ (X+1)) and normalised, except for ORP due to the negative values often encountered in ORP data (Clarke et al., 2006; O'Hara et al., 2010). Within PRIMER, normalisation is carried out by subtracting the mean from each entry of single variable and dividing it by the standard deviation of the variable (Anderson et al., 2008; Clarke et al., 2006). Transformation and normalisation of data were required because water and sediment physico-chemical data suits contained a range of values with incomparable scales of measurements. For example, turbidity ranged between less than 50 to more than 2000 NTUs and water quality variables were measured in various incomparable units such as concentrations of dissolved metals (mg/L), turbidity (NTU), pH (unit), and temperature (^{o}C). Multivariate analysis is based on similarity or distance across the variables and factors (Anderson et al., 2008), therefore it is undesirable to assess similarity or distance between two factors (sites, control and impact, time of sampling, etc.) based only on the values of a handful of variables of high numerical values. Transforming data into log values will down-weight the importance of variables and measurements with high quantitative values so that similarity assessment can also consider the importance of variables or measurements with smaller quantitative values. Normalisation, in addition to transformation, can place the variables of incomparable units on a common scale (Anderson et al., 2008; Osbourne, 2002).

Hierarchical agglomerative cluster analysis by means of the complete linkage was performed on the Euclidian distance matrix (Vega et al., 1998) . Due to the complex mix of measurement scales in the environmental data, the correlation based Principal Component Analysis (PCA) ordination was used instead of the covariance based MDS ordination (Clarke et al., 2006). The PCA was followed by Factor Analysis by plotting the Principal Components (PCs) against the original variables resulting in correlations (called loadings) which corresponded with contributions of variables to the total variance (Liu et al., 2003; Vega et al., 1998).

Two-way PERMANOVA was used to test the hypotheses of no difference, across all or multiple variables, between before and after cessation of major ASGM (B/A), no difference between control and impact (C/I), and no significant interaction between the B/A and the C/I factors. Differences between sites, times of sampling and their interactions were also tested with PERMANOVA. Pairs of factors with significant differences ($p \leq 0.05$) were then analysed by SIMPER within the PRIMER package to identify variables mostly contributing to the differences. Two-way ANOVA and t-test were also used to determine the differences between B/A, C/I, and the interaction between B/A and C/I for individual; variables.

Sediment and water quality data were compared to applicable quality guidelines to assess their possible impacts on river biota and human health. The Ghana Water Company and Ghana EPA water quality guidelines were used for water quality. Sediment quality guidelines for preservation and conservation of river resources were unavailable in Ghana. We compared concentrations of selected metals in riverine sediment with the available guidelines including the threshold effect level / TEL (Smith et al., 1996), lowest effect level / LEL (Persaud et al., 1993), Australian effect low range /ERL (ANZECC, 2000) and threshold effect level for *Hylella azteca* 28 day test or TEL HA28 (MacDonald et al., 2000).

To determine levels of pollution in riverine sediment along the river, the Geo-Accumulation Index (I_{-geo}) and Enrichment Factor (EF) were used as indicators of contamination level and degree of anthropogenic modification respectively. I_{-geo} and EF were calculated following the formula given by Muller (1969) and Buat-Menard et al. (1979) explained below.

Equation 1 Geo-accumulation Index (I-geo) (Muller, 1969)

$$I - geo = \ln(\frac{Cn}{1.5} x\ Bn)$$

Cn = Concentration of examined element

Bn = Concentration of reference element at reference site

Equation 2 Enrichment Factor (Buat-Menard et al., 1979)

$$EF = \frac{\frac{Cn\ (sample)}{CRef\ (sample)}}{\frac{Bn\ (background)}{BRef(background)}}$$

Cn = Concentration of examined element

CRef= Concentration of examined element at reference site

Bn= Concentration of reference element at examined site

BRef = Concentration of reference element at reference site

An element that occurs in the environment in a small amount with little variance is usually used as a reference element although an element that occurs in high concentration may also be used provided it does not react with the examined element (Buat-Menard et al., 1979; Muller, 1969). Aluminium, iron and manganese are often used as reference elements in calculating I_{-geo} and EF (Abrahim et al., 2008 {Agyarko, 2014 #628; Agyarko et al., 2014; Buat-Menard et al., 1979; Loska et al., 1997). In this study, I used aluminium as the reference element because it consistently occurred along the river. I did not select

Mn or Fe as the reference element, although they also consistently occur in the environment, due to possible effects of Mn or Fe oxyhydroxides on concentrations and mobility of other trace metals in sediment including Hg and As (Chapman et al., 1998). Sampling site 1 was selected as the reference site due to the absence of mining activity near the site.

3.3 Results

3.3.1 Water

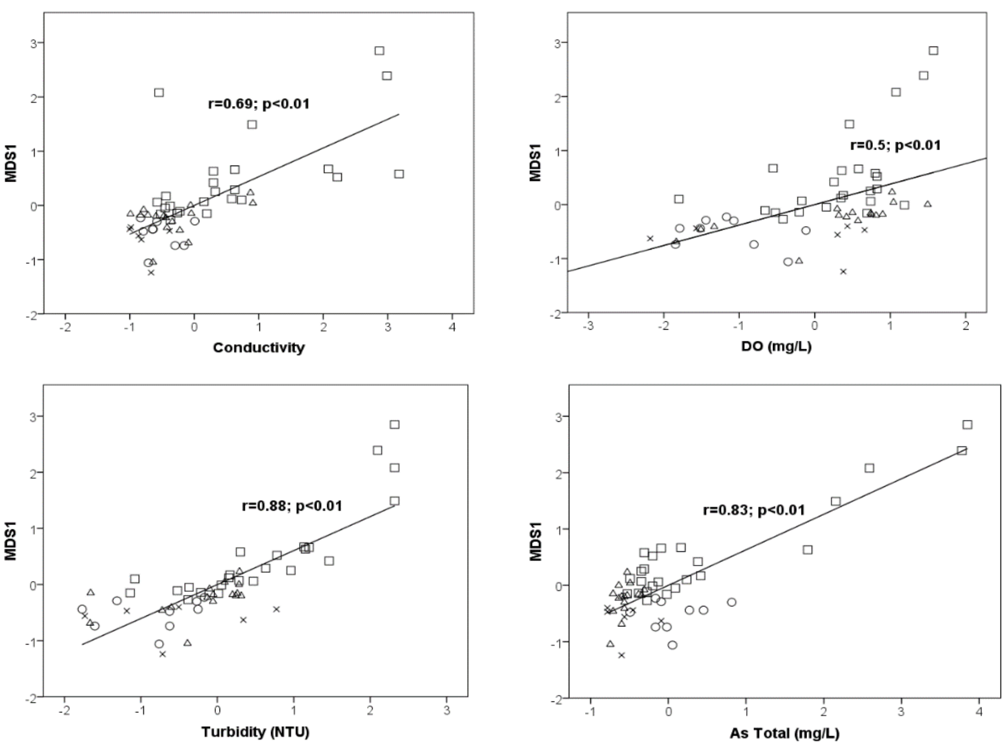
3.3.1.1 Impact of ASGM on river water quality (all variables)

Principal component ordination of all water quality variables between February and June 2013 indicated a separation of sites between before and after at control and impact (Figure 3.4;

Appendix 7). The first axis of the ordination (PC1) explained 39.7% of total variance and appeared to correspond with a spatial effect, separating control from impact, particularly before the cessation of ASGM (Figure 3.4A). The second axis explained 15.5% of variance and appeared to correspond with temporal effects, separating before from after. The third axis, which explained 9.9% of variance (Figure 3.4 B) also indicated some effect of temporal changes, although the separation of before from after ASGM cessation was not as clearly indicated as that of in PC2.

PERMANOVA confirmed that water quality in control was significantly different from impact ($p<0.01$;Table 3.3), before the cessation of ASGM was significantly different from after ($p<0.01$;Table 3.3); and their interaction was also significant ($p<0.05$,Table 3.3) demonstrating the impact of mining on the Surow water quality. The results were corroborated by pairwise tests that showed significant differences between control and impact ($p<0.01$;Table 3.4) before the cessation of major ASGM operations, but not significantly different after mining cessation. Water quality also differed with site ($p<0.01$,Table 3.3) and time (month) ($p<0.01$;Table 3.3).

SIMPER analysis (Appendix 8) identified the higher EC, turbidity, TDS, pH, DO, concentrations sulfate, total Al, Fe and Cu at impact sites compared to control and the lower concentrations of dissolved metals (Fe, Hg, As) and nitrate at impact compared to control, drove the significant difference between control and impact. The significant difference between before and after, as identified by SIMPER analysis (Appendix 9), were driven by EC, TDS, turbidity, concentrations of metals, sulfate, DO, pH and nitrates. After the cessation, EC, TDS, turbidity, concentrations of total metals and sulfate were lower than before, whilst DO, pH and nitrates were higher in after than before.



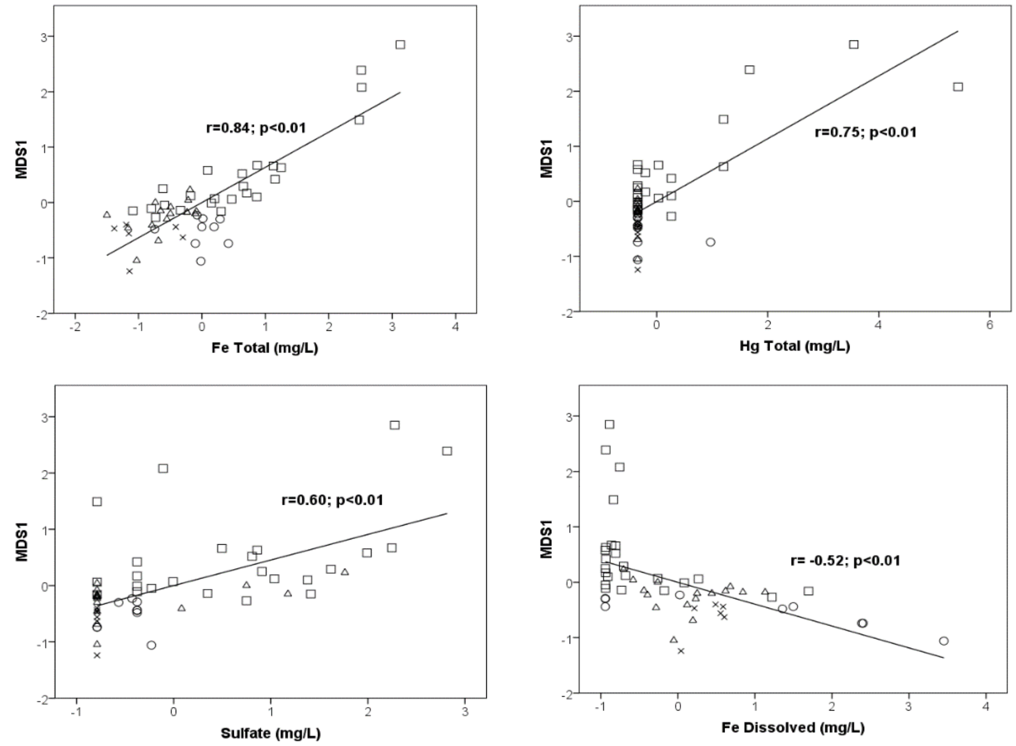


Figure 3.5 illustrates the strong and significant correlations between MDS coordinates of river water quality with log conductivity, DO, turbidity, concentrations of total As, Fe, Hg, sulfate and dissolved Fe. As can be seen in the figure, conductivity, turbidity, concentrations of total As, Fe, Hg, and sulfate at impact sites before the cessation of the major ASGM operations are higher than at control before and after the cessation, as well as than impact after the cessation (see Figure 3.6). The significance of the differences in these individual variables between before and after in control and impact sites are illustrated in the error bars in Appendix 11.

A closer examination of the box plots in Figure 3.6 also showed that extreme high values (indicated as outliers) of EC, turbidity, sulfate, total As, Cd, Cr, Cu, Fe, Mn, Pb and Hg were detected in February and March before the cessation of mining at sites 4, 7, 8 and 9 where the river received point and non-point discharges of process and mine waters. This suggested that sites 5, 6, 10 and 11 were not as highly impacted by the mines as sites 4, 7, 8 and 9. As explained in Chapter 2 and shown in **Error! Reference source not found.**, there was no ASGM activity adjacent to sites 5, 6, 10, and 11, although sites 5 and 6 were located downstream of Kenyase 2 mine (site 4) where ASGM was conducted and ore processing occurred, whilst site 10 and 11 were located downstream of site 9 adjacent to clusters of artisanal gold processors.

Dividing the impact sites into two levels of impact (i.e. sites 4,7, 8 & 9 as 'high impact' and site 5,6, 10 and 11 as 'medium impact') also demonstrated the gradient of impacts along the river. PERMANOVA (Appendix 10) showed that water quality significantly differed with level of impact ($p<0.01$, $F=9.03$), with before and after ($p<0.01$; $F=5.22$), and in the interaction between before/after and level of impact ($p<0.05$; $F=2.05$). Pairwise tests confirmed that before the cessation, control was significantly different from medium and high impact ($p<0.01$), and medium was also significantly different from high impact ($p<0.01$). After the cessation, control was different from high impact ($p=0.05$) but similar to medium impact ($p=0.65$), whilst medium was also similar to high impact ($p=0.5$). Concentrations of pollutants at impacted sites after the cessation were lower than before. In after, the concentrations in the medium impacted sites were also of similar levels to that of control sites.

Table 3.3 PERMANOVA analysis on all water quality variables from 11 sampling sites (February-June 2013) to distinguish differences between sites, times, and effect of ASGM cessation (before and after, B/A) at control and impact sites (C/I)
** denotes significant effect at the P<0.01 level and * at the P<0.05 level (2 tailed)

Factor	Source of variance	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	Site	10	323.63	32.363	2.3958	0.0002**	9852
	Residual	44	594.37	13.508			
	Total	54	918				
Time (monthly)	Month	4	237.37	59.342	4.3593	0.0001**	9897
	Residual	50	680.63	13.613			
	Total	54	918				
BACI	B/A	1	91.283	91.283	6.949	0.0002**	9934
	C/I	1	82.033	82.033	6.2448	0.0003**	9939
	B/A*C/I	1	30.772	30.772	2.3426	0.039*	9948
	Residual	51	669.95	13.136			
	Total	54	918				

Table 3.4 PERMANOVA– Pairwise analysis on all water quality variables (February-June 2013) to distinguish differences between control and impact before and after ASGM cessations and effect of cessations on water quality at control and impact.
** denotes significant effect at the P<0.01 level and * at the P<0.05 level (2 tailed)

Factors	Level	t	P(perm)	Unique perms
Control, Impact	Before	2.6478	0.0005**	9931
	After	1.2938	0.103	9293
Before/After	Control	1.8222	0.0004**	4328
	Impact	2.5817	0.0001**	9932

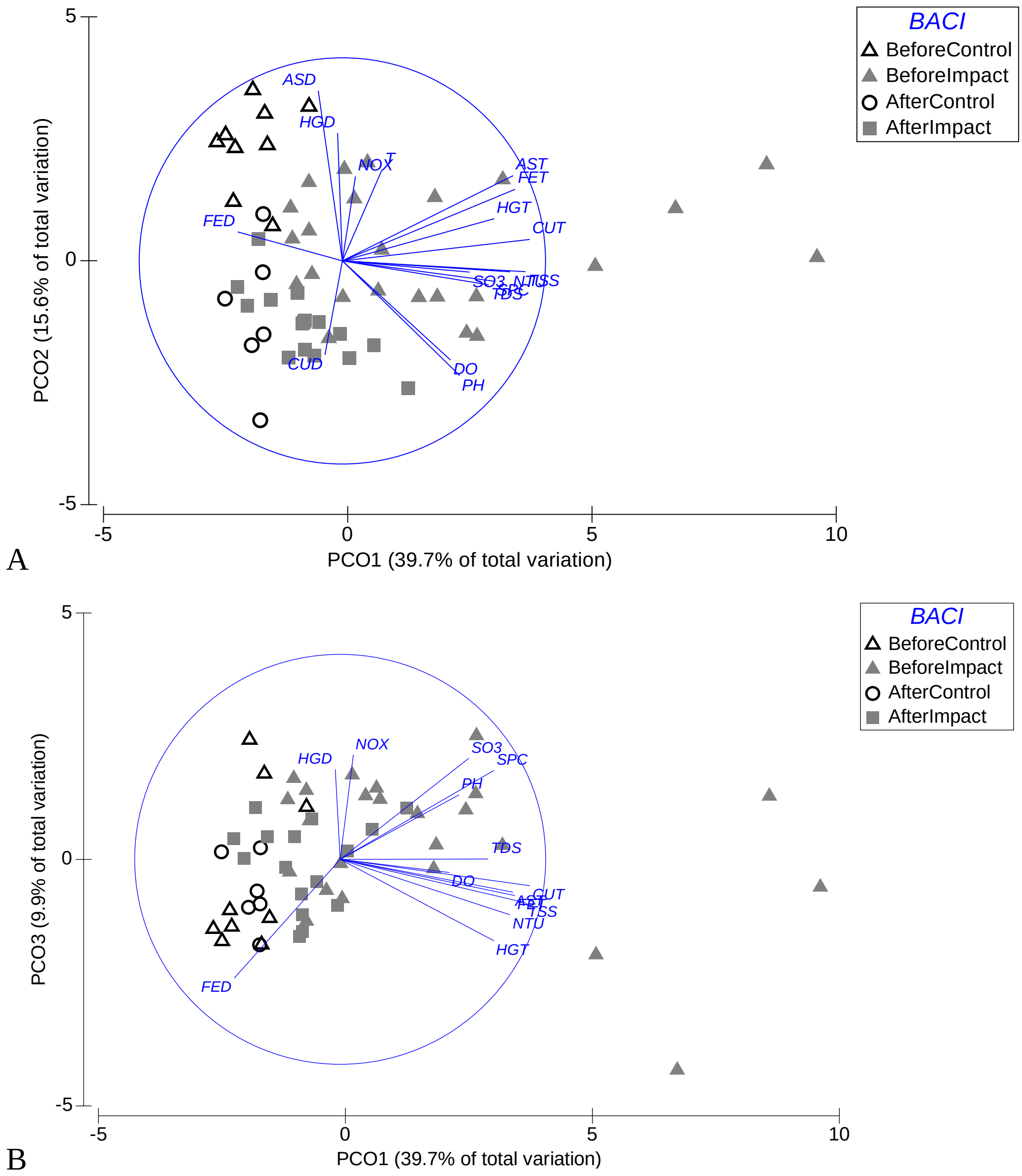


Figure 3.4 Principal component ordinations (PC1, PC2, and PC3) showing the effect of before and after cessation of ASGM on water quality at control and impact sites.

3.3.1.2 Dissolved metal concentrations

A separate multivariate analysis was run on dissolved metal data from 120 water samples acquired between April 2013 and April 2014. In this analysis, additional variables of dissolved Mg, Mn, Pb whose data became available from April 2013 onward were included.

BACI testing with PERMANOVA indicated that concentrations of dissolved metals in water before the cessation of ASGM were significantly different from after ($P<0.01$,Table 3.5), control were different from impact ($P<0.01$,Table 3.5) and the interaction between B/A and C/I was also significant ($P<0.05$,Table 3.5), demonstrating the impact of ASGM on the concentrations of dissolved metals in river water. This difference, was driven by by elevated concentration of dissolved Hg, Pb, As and Fe at control sites and elevated concentration of dissolved Cu at impact sites. Dividing the impacted sites into 'between' (site 5 and 6), 'ASGM' (site 4, 7, 8, and 9) and 'downstream' (site 10 and 11) locations provided an insight into the downgradient changes in dissolved metal concentrations along the river. Concentrations of dissolved metals at the upstream sites (site 1, 2 and 3) differed significantly from ASGM and downstream sites (

Table 3.6). The concentration of dissolved Fe, As and Pb at upstream sites were higher than ASGM sites whiltsconcentrations of dissolved Al, Mn and Cr at ASGM sites were higher than upstream sites. The elevated concentrations of dissolved Al, Mn and Cr detected at ASGM location persisted downstream. Dissolved metal and metalloids also differed significantly with site, time (month) and seasons (Table 3.5). SIMPER analysis showed that the concentrations of dissolved Fe, As, Al and Mn tended to be higher in the wetter month of April than the dry months of February and August.

Table 3.5 Permanova results for dissolved metal concentrations in Surow River
* denotes significant effect at the P<0.01 level and * at the P<0.05 level (2 tailed)

Source of variance	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	10	77.793	7.7793	1.3294	0.046*	998
Residual	120	702.21	5.8517			
Total	130	780				
Location	3	39.266	13.089	2.2502	0.0049**	9919
Residual	116	674.73	5.8167			
Total	119	714				
Month	11	159.1	14.464	2.772	0.001**	997
Residual	119	620.9	5.2177			
Total	130	780				
Season	1	38.449	38.449	6.6885	0.0001**	9931
Residual	129	741.55	5.7485			
Total	130	780				
C/I	1	39.039	39.039	7.1113	0.0002**	9955
B/A	1	37.646	37.646	6.8575	0.0001**	9929
C/IxB/A	1	14.593	14.593	2.6582	0.0332*	9942
Residual	116	636.81	5.4897			
Total	119	714				

Table 3.6 Pairwise comparison (PERMANOVA) between dissolved metal concentrations at Upstream, ASGM, Between and Downstream locations ** denotes significant effect at the P<0.01 level and * at the P<0.05 level (2 tailed)

Location	p values (PERMANOVA)		
	Upstream	ASGM	Between
ASGM	0.001**		
Between	0.11	0.879	
Downstream	0.001**	0.464	0.11

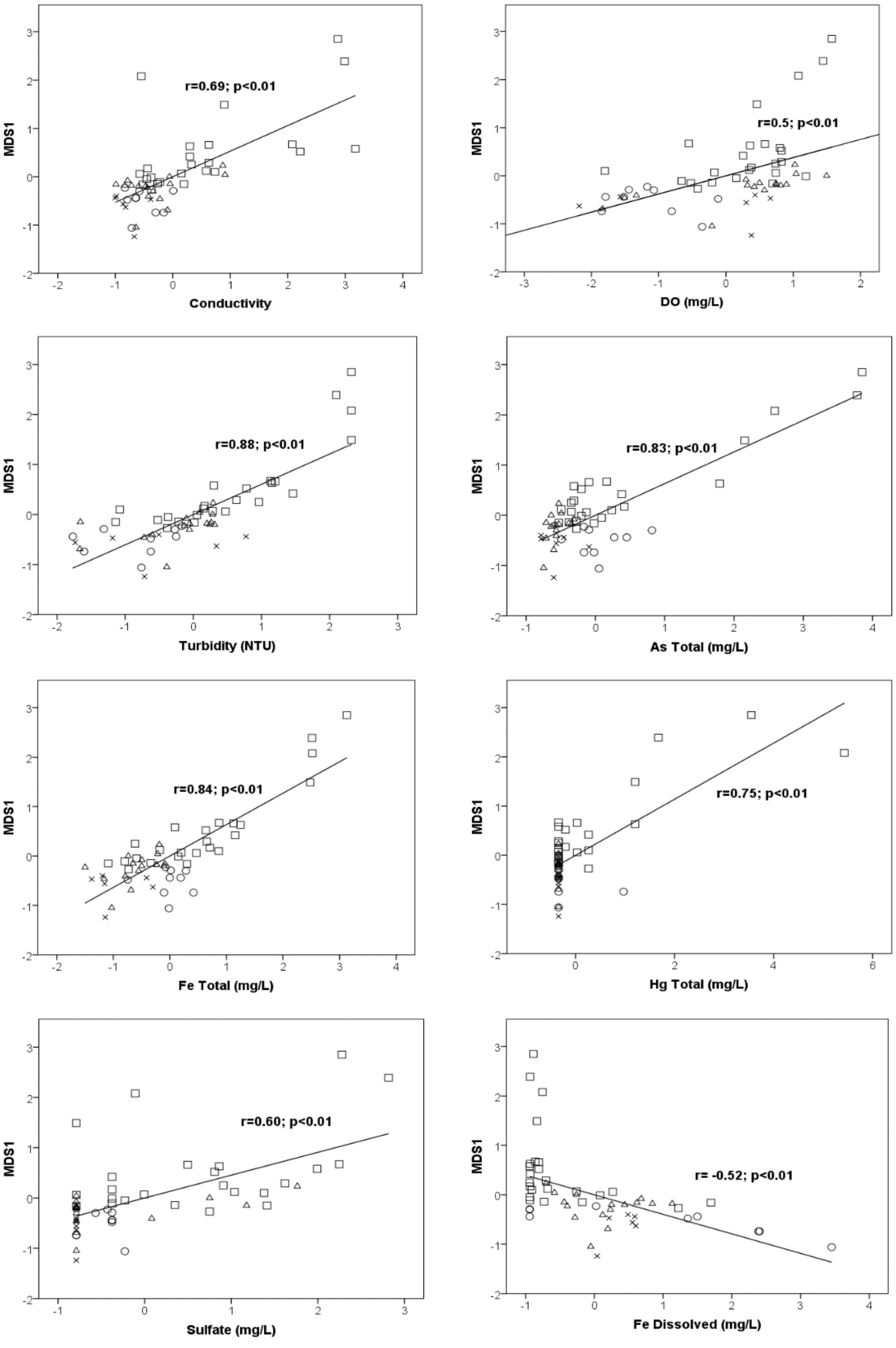


Figure 3.5 Correlations between MDS coordinate with log conductivity, turbidity, concentration of dissolved oxygen, total As, Fe, Hg, sulfate and dissolved Fe, with Control/Impact and Before/After overlay. ○ represents water quality in control before mine cessation, □ in impact before the cessation, X in control after the cessation and Δ in impact after the cessation. Pearson's r and the significance of correlations are stated in the graphs.

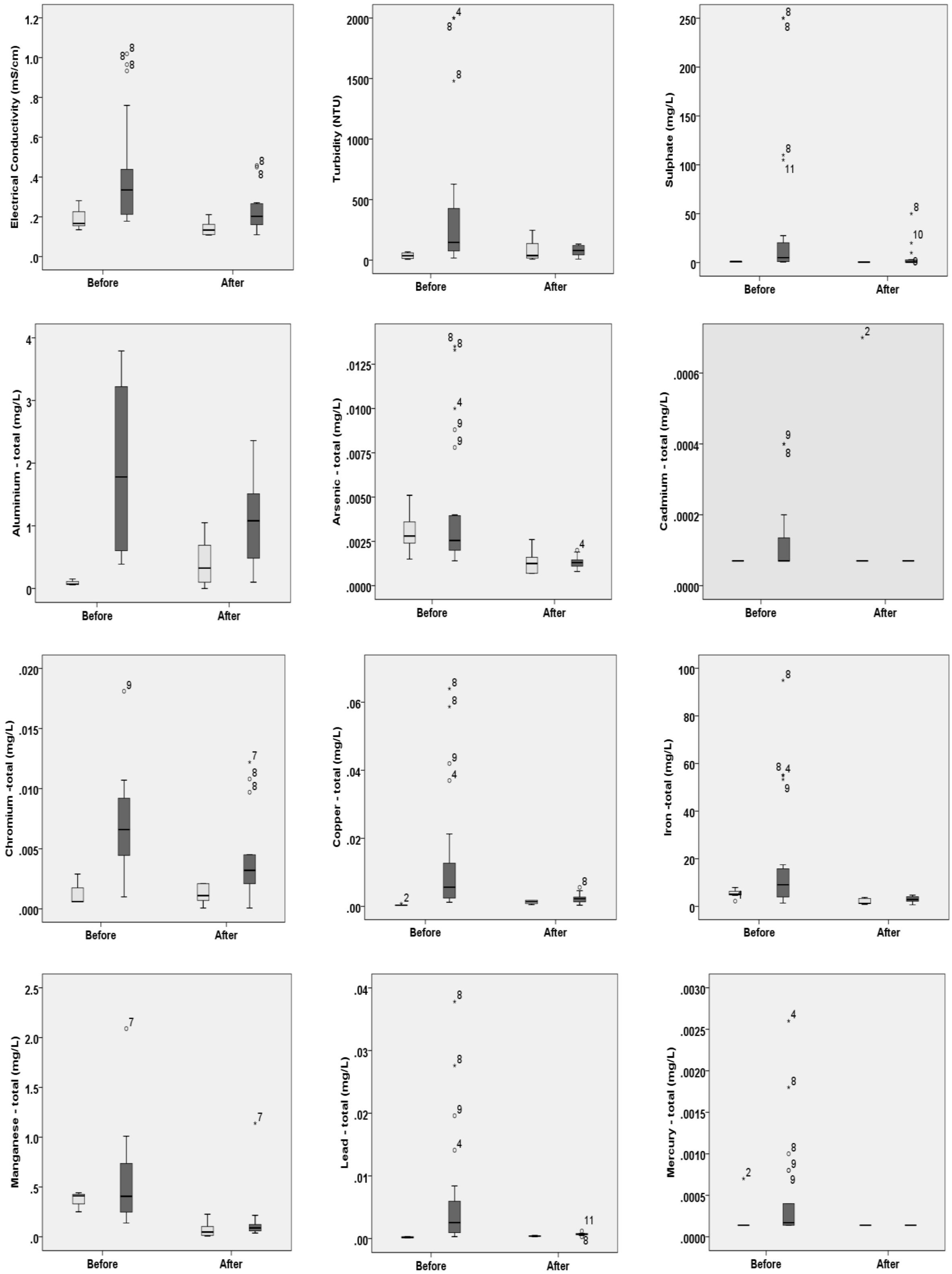


Figure 3.6 Water quality variables at control and impact sites (light and dark grey filled bars respectively) between February and June 2013 to distinguish effect of before and after cessation of ASGM. Outliers are identified with corresponding site numbers

3.3.1.3 Water quality

The Surow water quality characteristics are summarised in

Appendix 5. The elevated EC, turbidity, TDS, concentrations of sulfate and total metals at ASGM impacted sites 4, 7, 8 and 9 as identified by the univariate and multivariate analysis are clearly shown (

Appendix 6). At these sites the water quality variables often exceeded levels set by applicable guidelines for domestic use and environmental protection. At sites 4, 7, 8 and 9, turbidity often elevated to levels exceeding the instrument upper limit of detection (2000NTU) and the Ghana Water Company (GWC) standard of 500 NTU and Ghanaian EPA standard for environmental protection of 1000 NTU at site 4 and 7. Although average EC and TDS along the river over time remained below any water quality standard including the Ghanaian EPA's of 1500 µS/cm^2 and 1000 mg/L respectively, elevated EC and TDS were detected at site 4 and 8. Electrical conductivity exceeded the Ghanaian drinking water standard of 0.5 mS cm^{-1} five out of 13 times but not the EPA standard of 1.5 mS cm^{-1}. Sulfate concentration exceeded the drinking water standard of 250 mg L^{-1} on four occasions, particularly at site 8 where mine-dewatering water was discharged. Elevated concentrations of total Al, Fe, Hg and Mn particularly during the active mining period February to April 2013 were also seen at sites 4, 7, 8 and 9. Total aluminium concentrations at site 4 onward exceeded the GWC guidelines at all times. Iron concentration at the upstream sites (Site 1, 2, 3) exceeded the guidelines at all times, but the concentration was particularly high at site 7, 8, 9. Concentration of manganese was also elevated at the source (site 1 and 2) but was particularly high at sites 7, 8, and 9. Total mercury concentration was much lower than that of other ASGM impacted large rivers in Ghana and other countries, however, it exceeded the maximum concentration set by GWC of 0.01 mg/L in February 2013 at ASGM sites 4 and 8.

3.3.2 Sediment

3.3.2.1 Impact of mining on sediment quality

Sediment quality before the cessation of ASGM was significantly different from after ($p<0.01$;Table 3.7), control was different from impact sites ($p<0.05$;Table 3.7), but the interaction between B/A and C/I was not significant (Table 3.7). Pairwise PERMANOVA, however, showed that at control sites, sediment quality did not change with the cessation, whilst at impact sites it changed significantly with the cessation ($p<0.05$, Table 3.8). On the other hand, sediment quality at control differed significantly from impact before the cessation ($p<0.05$, Table 3.8) as well as after ($P>0.05$, Table 3.8), suggesting possible impact of mining operations on the river's sediment quality.

Principal component analysis (PCA) showed a clear separation of sites between before and after the cessation along the first axis (PC1), but the separation between control and impact sites both before and after cessation was not as clear (**Error! Reference source not found.**). Unsupervised clustering within the multivariate analysis procedure also showed that before the cessation, control (sites 1,2 and 3) was separated from impact which was divided into two clusters: the first consisted of sites 4, 5, 8, 10 and 11; and the second consisted of sites 9 and 7 (Figure 3.7). After the cessation, all sites but site 10 clustered closely together, although the impact sites (4, 7, 8, 9, 11) remained closer to each other than to the control sites (site 1, 2 and 3) as depicted in Figure 3.7. PCA also identified Co, K, Ba, S, Al, Mn, Ca, Fe and Hg as the variables strongly related (Spearman's $r>0.4$) to the first axis (63.5% of total variance) which corresponded with temporal variation, whilst the second component (13.8% of total variance) was related to Pb, V, Sr, Cu, and Zn and appeared to correspond with the spatial variations.

SIMPER indicated that the difference in sediment quality before and after the cessation of mining was due to the elevated concentrations of most elements, particularly S, K, Mg, Ba, Al, Co, Fe, Cr, and Mn (Appendix 13) whilst the difference between control and impact was due to the elevated concentrations of Co, Mn, Mg, Sr, Cu, and Ca at impact sites. Two-way

ANOVA on each variable corroborated the SIMPER results and indicated that sediment concentrations of Al, Ba, Ca, Co, Cr, Fe, Mg, Mn, P, K, S, Zn and Hg before the cessation were significantly different from after ($p<0.05$) Co, Mg, Mn, K, Sr and Hg at control were different from impact ($p<0.05$, Appendix 14) whilst significant interaction between B/A and C/I was evident in the concentrations of Ca, Co, Hg, Mg, Mn, K and S. The concentrations of elements significantly impacted by ASGM as indicated by the significant interactions beween B/A and C/I are depicted in the boxplots in **Error! Reference source not found.**

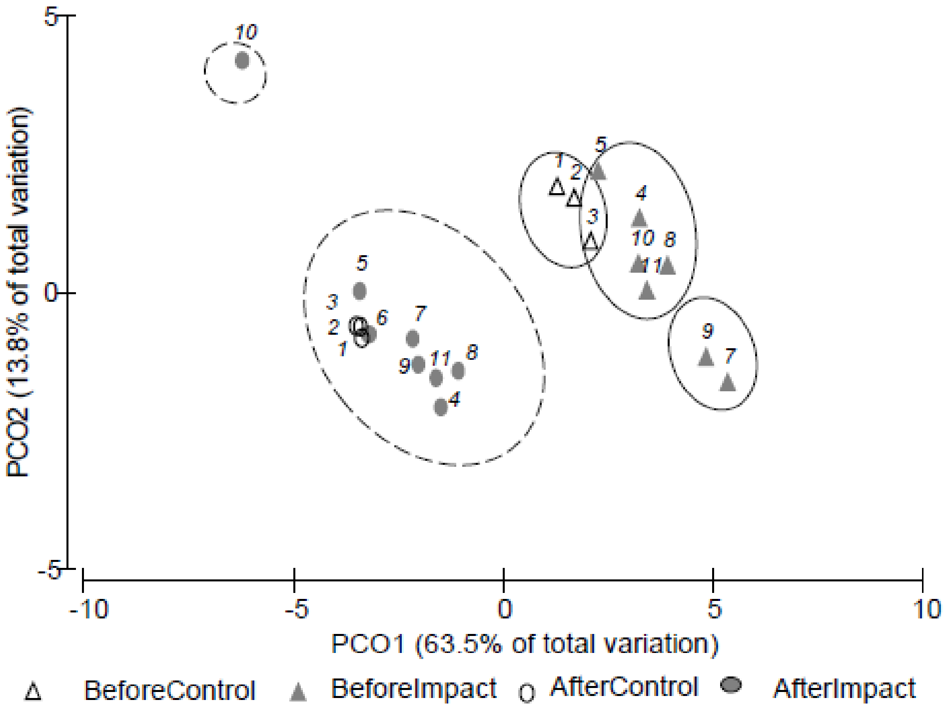


Figure 3.7 Principal component ordination of the Surow River sediment quality before and after cessation of mining and in controls and impact sitesment quality.
Clusters with solid line represent conditions before ASGM cessation and dashed line after. Contribution of each PC to the total variance are indicated in brackets.

Table 3.7 Results of Permanova distinguishing diferrences between control and impact and effect of ASGM cessation on the Surow riverine sediment quality

** denotes significant effect at the P<0.01 level and * at the P<0.05 level (2 tailed)

Test / Factors	Source of variance	df	SS	MS	Pseudo -F	P(perm)	unique permutations
BACI	B/A	1	152.64	152.64	22.211	0.0001**	9947
	C/I	1	30.241	30.241	4.4005	0.0149*	9935
	B/A x C/I	1	11.493	11.493	1.6724	0.1687	9947
	Residual	17	116.83	6.8721			
	Total	20	353.61				

Table 3.8 Permanova – Pairwise analysis of sediment quality within the Before/After and Control/Impact pairing (BACI) model.
** denotes significant effect at the P<0.01 level and * at the P<0.05 level (2 tailed)

Pairwise Test	Level of factor	t	P(perm)	unique permutations
Before x After	Control	3.7891	0.0931	10
	Impact	4.4615	0.0004*	5088
Control x Impact	Before	2.5229	0.0078*	120
	After	1.2034	0.1927	165

3.3.3 Sediment quality

Dominant elements in the river sediment at both times of sampling included Fe, Al, Ca, Mg, Mn, P, K, S, and V. Concentrations of As, Sn, and Se in riverine sediment along the Surow River were below detection limits of 2 mg/kg, 3 mg/kg and 3 mg/kg respectively in both February 2013 and April 2014 samples. Mercury in the sediment was detected at 9 sites in February 2013 (ranging from 0.1 to 0.9 mg/kg) while in April 2014 it was only detected at one site (site 4) at a concentration of 0.1 mg/kg. The concentrations of all metal and metalloids in the river's sediment in February 2013 and April 2014 are given in Appendix 12.

In February 2013, the sediment metal concentrations along the river exceeded the applicable sediment quality criteria due to elevated Cr, Cu, Hg and Ni, particularly at sites adjacent to ASGM operations (**Error! Reference source not found.**). Concentrations of Cr at all sites but site 5 were above the threshold levels set by all guidelines. Although concentrations of Cu at the upstream sites were lower than the criteria, mean concentration of Cu in the river sediment exceeded LEL value of 16 mg/kg. Copper was in high concentrations particularly at sites 4, 7, 8, 9, 10, and 11. Mean Ni concentration was below all guidelines but exceeded the LEL guidelines at site 8 and 10. Concentrations of Hg at all sites but site1, 2 and 11 also exceeded TEL, LEL, and ERL-Australia thresholds for aquatic life. The highest Hg concentration was found at site 7, followed by 4, and 9 (**Error! Reference source not found.** and **Error! Reference source not found.**). In contrast, in April 2014, after the cessation of major ASGM operations, the sediment quality met all criteria.

3.3.3.1 Geoaccumulation index and enrichment factor

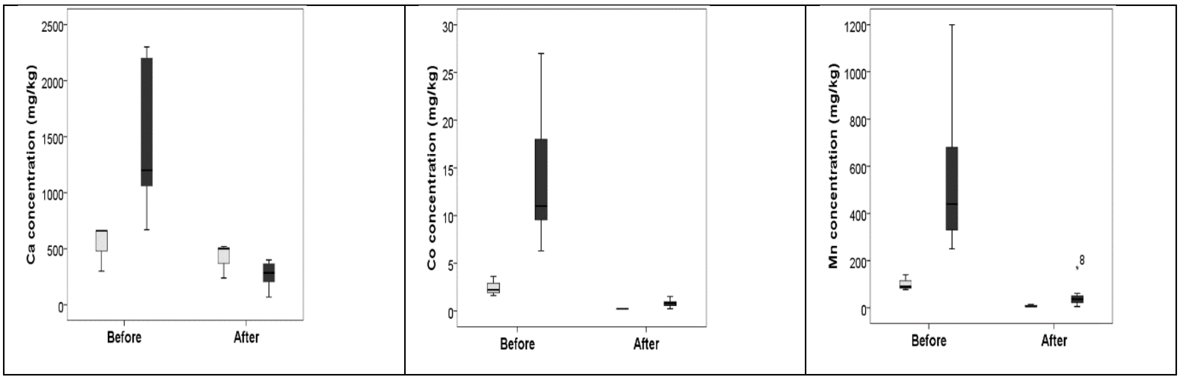
Aluminium was used as the reference metal in the calculations of Geoaccumulation Index (I_{-geo}) and Enrichment Factor (EF) because it did not vary significantly with sites as shown by one-way ANOVA (p=0.989, F=0.359). The average I_{-geo} value for all elements in February 2013 and April 2014 were 4.7 and 3.7 respectively. In February 2013 before the cessation, I_{-geo} for Al, Ba, Ca, Cr, Fe, Mg, Mn, P, K, Na, S, and V were > 5, indicating extreme contamination of the river sediment by these elements (Figure 3.10 A). Following the cessation on mining, $I\text{-}_{geo}$ values for Ba, Cr, K, part of Mg and Mn were below 5, although the values for Cu, Pb, Sr, Zn were higher than their values in February 2013 (Figure 3.10 B). $I\text{-}_{geo}$ values for As and Hg were consistently <1, suggesting that the river was not contaminated by As and Hg.

Enrichment Factor (EF) of elements in riverine sediment in February 2013 and April 2014 averaged 2.5 and 2.3 respectively. In February 2013, concentrations of Ba, Ca, Ni, K, Na, S and Zn were moderately modified (EF>3), Co, Mn, and Sr were severely modified (EF>5), and Mg and Hg were very severely modified (EF>10) by human activities (Figure 3.11 A). On the contrary, in April 2014 most metals, including Hg, had EF values of between <1.5 and

3, indicating no modification to minor modification by human activities with an exception for Cu and Mn whose EFs were > 10 at a few sites (Figure 3.11B).

Table 3.9 Concentrations of sediment metals in the Surow River in February 2013 and the sediment quality guidelines. Values exceeding the guidelines are printed in bold.

Site ID	Concentration of element in sediment (mg/kg dry weight)							
	As	Cd	Cr	Cu	Pb	Hg	Ni	Zn
1	1.41	0.21	**120**	9.6	7	0.03	3.2	8.8
2	1.41	0.21	**64**	8.2	7	0.03	5.2	15.0
3	1.41	0.21	**130**	13.0	9	**0.20**	4.2	17.0
4	1.41	0.21	**29**	**17.0**	6	**0.70**	11.0	13.0
5	1.41	0.21	15	9.3	6	**0.40**	6.0	16.0
7	1.41	0.21	**87**	31.0	29	**0.90**	13.0	18.0
8	1.41	0.21	**48**	**18.0**	7	**0.30**	**18.0**	16.0
9	1.41	0.21	**80**	**22.0**	18	**0.50**	14.0	36.0
10	1.41	0.21	**43**	**20.0**	8	**0.20**	**18.0**	16.0
11	1.41	0.21	**190**	**17.0**	18	**0.10**	9.9	15.0
Sediment Quality Guidelines								
TEL	5.9	0.596	37.3	35.7	35	0.174	18	123
LEL	6	0.6	26	16	31	0.2	16	120
MET	7	0.9	55	28	42	0.2	35	150
ERL	33	5	80	70	35	0.15	30	120
TEL_HA28	11	0.58	36	28	37	NG	20	98
ERL (Aust)	8.2	1.2	81	34	46.7	0.15	20.9	410



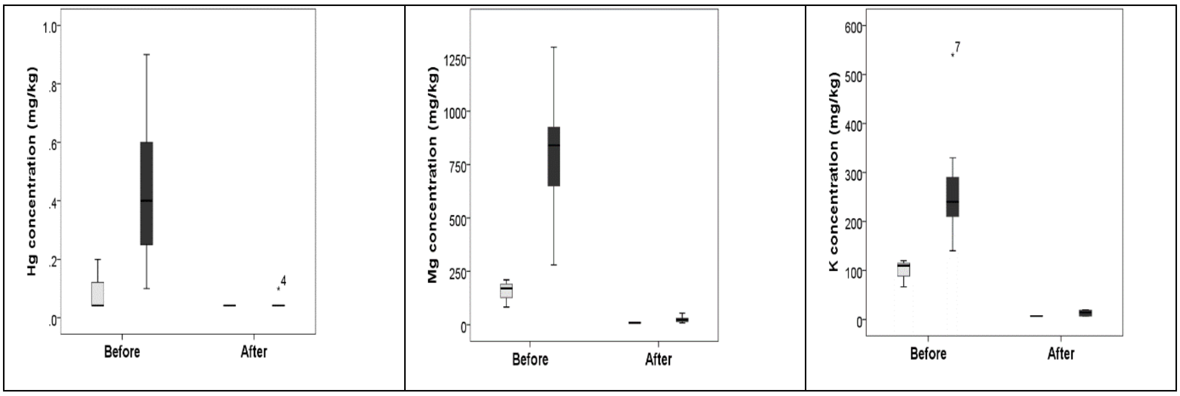


Figure 3.8 Concentrations of Ca, Co, Mn, Hg, Mg and S in the Surow River sediment at control (light grey box) and impact (dark grey box) before and after the cessation of ASGM distinguishing ASGM impacts on riverine quality. Outliers and their corresponding sources (site) are indicated

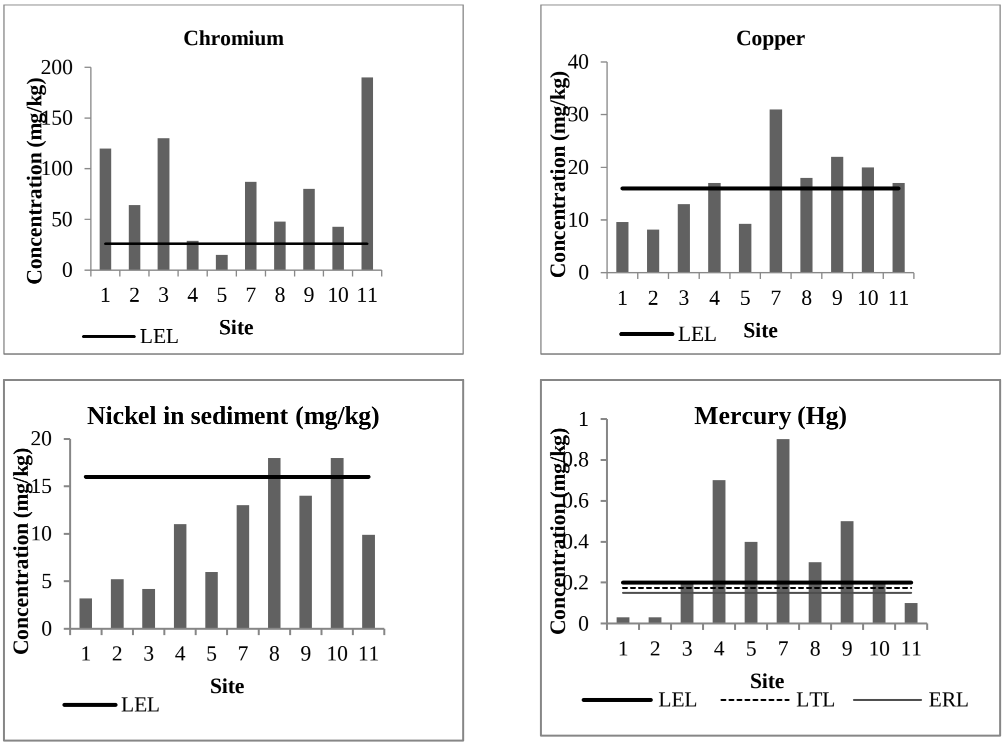


Figure 3.9. Concentrations of select metals (Chromium, Copper, Nickel and Mercury) in Surow river sediment in February 2013 and relevant sediment quality guidelines.

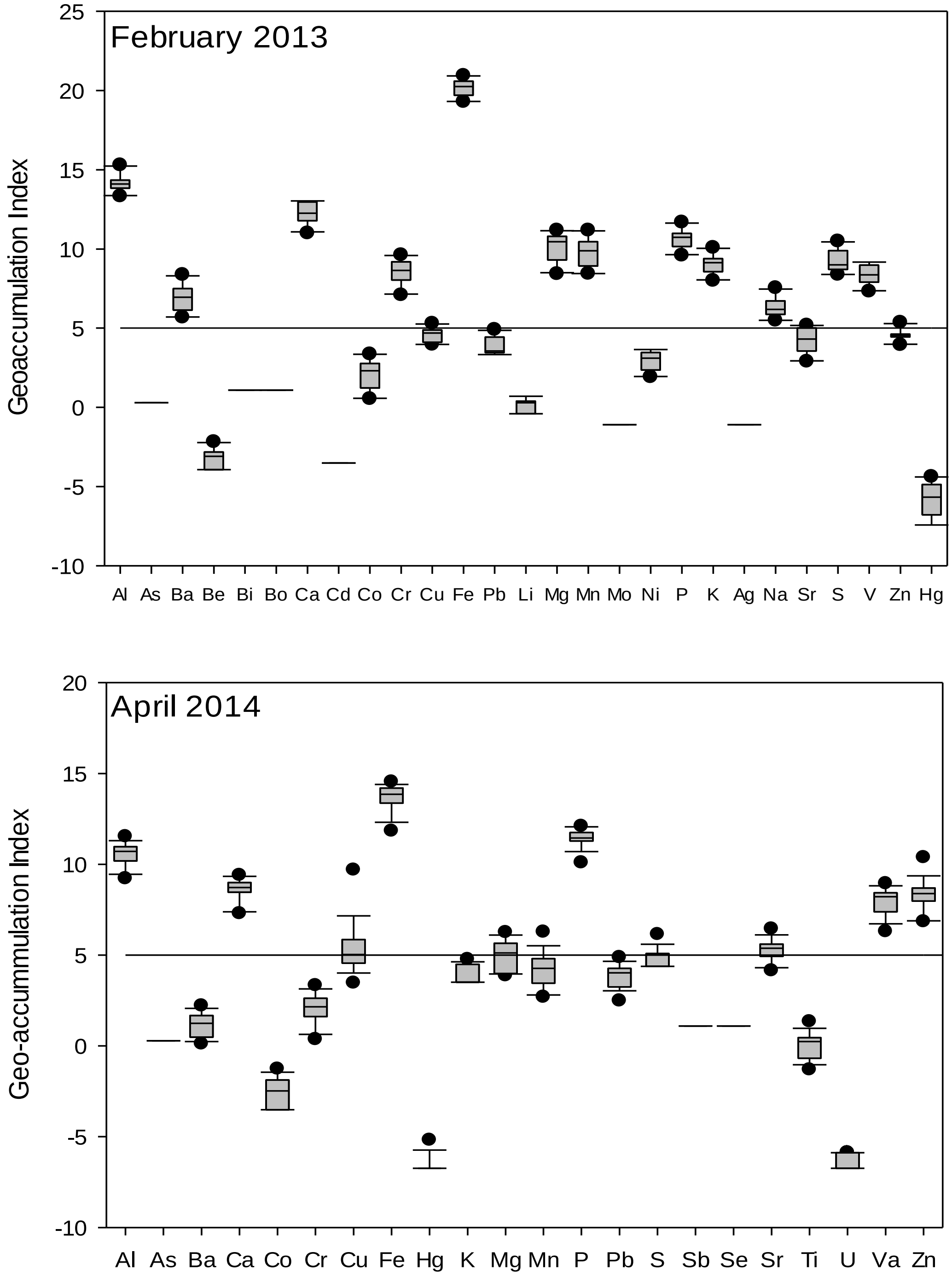
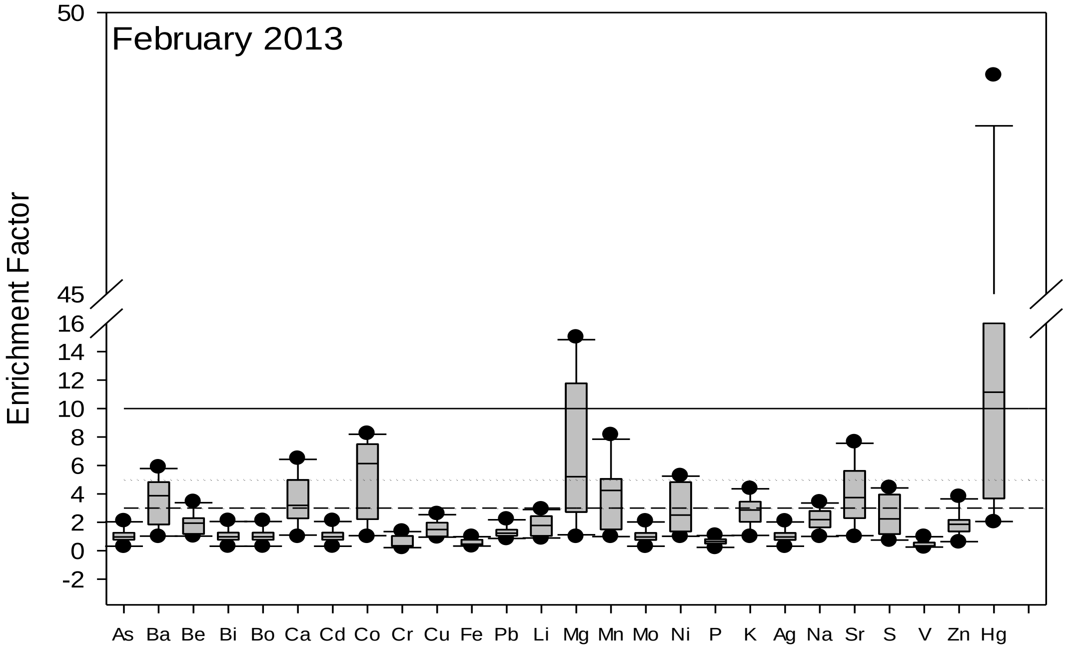


Figure 3.10 Geo-accumulation index of metal and metalloid element in riverine sediment Before (February 2013) and After (April2014) cessation of major ASGM operations along the Surow River. (The line indicates I_{-geo} value = 5, above which contamination is considered severe).



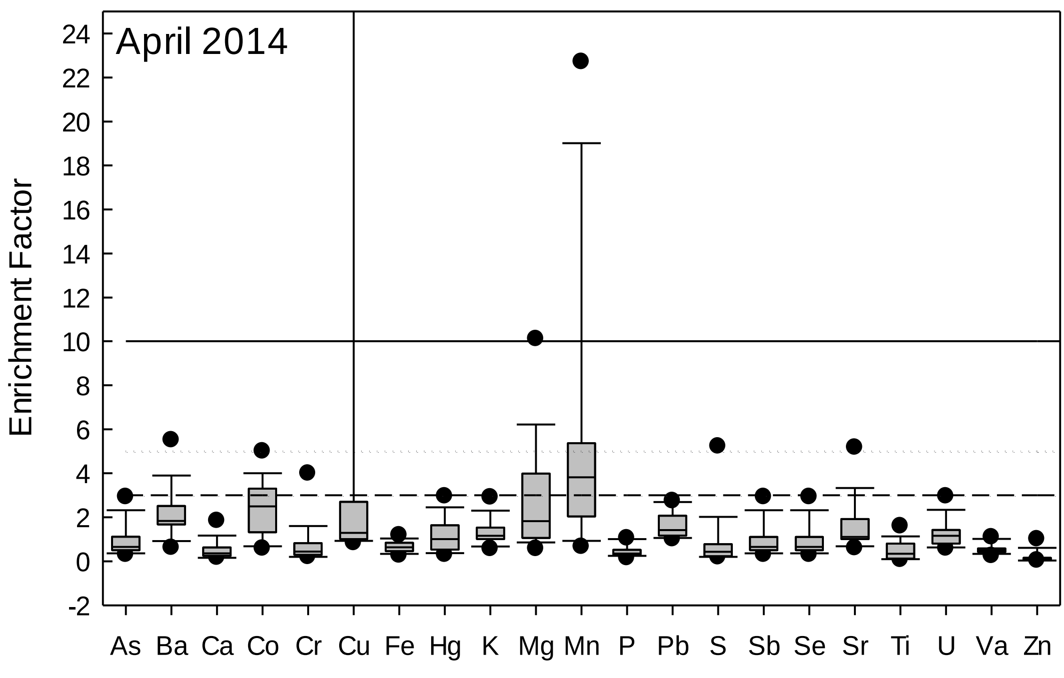


Figure 3.11 Enrichment Factors of each element in riverine sediment Before (February 2013) and After (April 2014) the cessation of major ASGM operations along the Surow River.Horizontal line and dashed lines indicate EF = 3, EF=5, and EF = 10, above which anthropogenic modification is considered moderate, severe and very severe.

Correlations between elements in riverine sediment

Concentrations of most elements in the sediment were correlated with each other. Aluminium, Fe, Mn, S, Ba, and Co were strongly and significantly correlated with each other, while Cr was only strongly correlated with Al, Fe, and S. Copper, however, was not correlated with any other element. Similarly, Zn and V were not correlated with any other elements except V was positively correlated with Pb. Mercury was correlated positively with all elements except Cr, Cu, Pb and Zn (Appendix 15).

Correlations between concentration of sediment metals and concentrations of metals in river water

Total Hg concentration in unfiltered water positively correlated with total concentrations of As, Cu, Fe, Pb and ammonia in corresponding water samples but uncorrelated with that of sediment samples. Concentrations of most dissolved metals were correlated with each other, but not Hg, Fe, Mg and Pb. Dissolved Fe correlated positively with other dissolved metals in the water and negatively with Sr in sediment. In this study we also found dissolved Hg to be negatively correlated with sediment concentrations of Al, Ba, Ca, Co, Fe, Mg, Mn, P and S in sediment, while dissolved Pb correlated positively with Fe and Cr (Appendix 16).

3.4 Discussion

The reversed BACI experimental design employed in this study has highlighted the significant impact of ASGM on the Surow River's water and sediment qualities. Elevated turbidity, EC, sulfate, metal and metalloid elements in water and sediment in the mining areas of the river were the main impacts of ASGM activities on the Surow River, as many of these impacts were reversed or nullified with the government's removal of major ASGM operations from the area at the end of April 2013. While the significant interaction between before/after and control and impact within the BACI model provide a very strong support for mining impact, the significant difference between control and impact highlighted the potential for the mining impact to persist for many years after mining, especially with respect to sediment.

3.4.1 Distribution of impacts

The study showed that although two-way PERMANOVA showed significant impacts of mining, the levels of impact on water and sediment quality variables were not widely distributed among impacted sites along the river (see Figure 3.6 and **Error! Reference source not found.** for illustrations). The study showed that site 5,6, 10 and 11 which were not adjacent to ASGM activities but categorised as impact sites in the study's BACI design due to their position down gradient of mining sites along the river, were not impacted as severely as site 4, 7, 8 and 9 where ASGM conducted mining and ore processing (Figure 3.4 and Figure 3.7). This suggested that although ASGM impacts on the Surow were significant, they were relatively localised to the river sections where ASGM operated on the adjacent upland, a condition that is common to low order and ephemeral streams in the tropics like the Surow River (Yuill et al., 2011).

The variability in water and sediment quality at impact sites along the river also highlights the difficulties inherent with the study of flowing water (Minshall et al., 1985; Vannote et al., 1980) as well as the different types of pollutants and impacts arising from ASGM (Meech et al., 1997; Telmer et al., 2006a; Veiga et al., 2004a) as identified in the impact model developed for this study presented in Chapter 1 (Macdonald et al., 2014). Mining activities, i.e. the extraction of ore from ground, emit pollutants that are different from ore processing or gold smelting and refinery (see Figure 1.3, 1.4 and 1.5), which effect the water and sediment quality in the adjacent water bodies. The water quality at site 8 which directly received mine dewatering discharges from the mine, for example, was characterised by high EC, sulfate, and metals commonly associated with mining and dilution of salt ions from the exposed rocks, whilst site 9 and 7 were characterised by elevated turbidity and metals including Hg associated with the by-products of gold processing. As such, to address and mitigate the environmental impacts of ASGM in countries where ASGM is prevalent, understanding the type of pollutants and their sources in each step of gold production in ASGM is important for local and regional environmental managers (Meech et al., 1998; Veiga et al., 2004b). Training and educational programs targeted to increase awareness among ASGM communities (Hilson et al., 2007a; Sousa et al., 2009) should also address this issue.

3.4.2 Impact of ASGM on water quality

3.4.2.1 Elevated turbidity

Results showed that the Surow River around ASGM area were associated with turbid water. The elevated concentrations of metals and metalloids in unfiltered water around ASGM sites, for example, was not detected in the filtered water (as dissolved metals), suggesting metals and metalloids affinity to the sediment particles in water. Because the river is of the first and second order where wash load sediment concentration is controlled only by sediment supply from adjacent upland areas and the runoff driven by major precipitation events (Yuill et al., 2011), it is reasonable to infer that the increased turbidity and sedimentation in the mining impacted areas along the Surow River was mainly from ASGM operations at Kenyasi 1 and 2.

Increased suspended solids, turbidity and sedimentation in surrounding water bodies are among the common impacts of mining (Younger et al., 2004). The impact is, however, more prevalent in ASGM impacted rivers (Akabzaa et al., 2009; Mol et al., 2004) due to the lack of erosion management and control measures at ASGM operations (Hinton et al., 2003; Sousa et al., 2010; Sousa et al., 2011b), as evident in this study. In Ghana, including in the study area, ASGM processors build makeshift sumps and ditches around their processing plants (Figure 3.12) to minimise tailings runoff into nearby streams and rivers (Babut et al., 2003). This effort is also financially driven by the current practice among ASGM operators to stockpile tailings from the mercury-amalgamation process to be reprocessed using the cyanide leaching method or sold to cyanide gold processors; a practice that has become more common in ASGM around the world (Sulaiman et al., 2007; Veiga et al., 2009; Velásquez-López et al., 2011). The sump and ditches, however, are obviously ineffective in preventing the sediment materials from entering nearby river systems. Fine sediment materials from tailings, ores, waste rocks and exposed soils due to clearing of riparian vegetation often remain uncontained and escape into the environment, particularly during the intense and heavy storms of 50 mm to 200 mm per hour (Hayward et al., 1987) which are common in the study area and which produces high runoff leading to flooding and soil loss (Windmeijer et al., 1993) (see Figure 3.13 for an example of a flood event on the Surow). Efforts to improve ASGM performance in Ghana and West Africa, therefore, should address this sediment control issue first and foremost, especially considering the climatic characteristics of the region.

Elevated turbidity in natural water, even when they are not necessarily toxic, can have deleterious effect on riverine biota (Henley et al., 2000). They can degrade habitat integrity that often results in reduced biodiversity (Akrasi, 2011; Arthington et al., 2010; Swaine et al., 2006). In assessing water quality parameters including turbidity of the Surow River which discharges into the Tano River, it is also paramount to consider the fact that in rural Ghana many local citizens often collect water directly from the rivers for domestic purposes including for drinking (Rossiter et al., 2010; Schäfer et al., 2009). When water is collected at the source, citizens rarely use proper water treatment including removal of sediment particles from the turbid water (Figure 3.14 A). For water treatment companies, turbid water and elevated suspended solids also increase water processing costs (Bilotta et al., 2008; Rossiter et al., 2010). The GWC who process raw water from the Tano River at Akyerensua village outside Hwidiem township (Figure 3.14 B), for example, had reported elevated concentrations of pollutants associated with the high suspended solids in the Tano River water which consequently elevated its costs of processing to meet the drinking water quality stipulated by the Ghanaian drinking water guidelines (Srem et al., 2013).

In a country, such as Ghana, where the capacity and funds to produce clean water are limited, an increase in clean water production costs may have downstream effects including, but not limited to, declining supply and quality of clean water as well as increasing price of clean water. As such, large industries discharging into Ghanaian rivers are normally required to comply with GWC guidelines. The guidelines, however, are not imposed on the ASGM operators so their discharges and incompliance with the guidelines with respect to elevated turbidity and total concentrations of Hg, Al, Fe, and Mn are unaddressed.

A B

Figure 3.12 Sump and ditches around an artisanal gold processor at Hwidiem, Brong Ahafo; the Ghanaian authorities could not prevent fine sediment material from entering nearby Surow River.

A B

Figure 3.13 The Surow River flooded onto the main road between Kenyasi and Hwidiem townships at site 9 (A) and the main road between Hwidiem and Kumasi at site 11 (B) after a storm event in September 2015. Site 9 (A) was surrounded by ASGM processors.

A

B

Figure 3.14 Streams and rivers in the study area are sources of drinking and domestic water for the local inhabitants (A) as well as the Ghana Water Company operating at nearby Akyerensua township (B).

3.4.2.2 Elevated concentrations of metal

Elevated total metal concentrations, including Al, Fe, Hg, and Mn in river water was among the ASGM impacts on the Surow River. While Hg in the system could have been introduced by ASGM through the mercury amalgamation process, Al, Fe and Mn are naturally available in abundance in the area (Donkor et al., 2005) as also evident in this study. Elevated concentrations of Al, Fe, and Mn in water around ASGM sites, however, indicated ASGM roles in accelerating the leaching of these metals in the system. Oxidation of exposed pyritic materials in uncontained waste rocks, ores and tailings at ASGM sites, assisted by high rainfall in the area are likely to have accelerated the release of the elements into the environment (Jeffery et al., 1988; Salomons, 1995).

The seasonal effect of rainfall on total and dissolved metal concentrations was evident in the system (Table 3.3 and) whereby concentrations of dissolved metal in the wet seasons were higher than in the dry seasons. The significant difference in dissolved metal concentrations between February and July, for example, reflected the difference in river discharge between the dry season and wet season of July. Effect of annual hydrological variations on dissolved metal concentrations over time was also evident in the significant difference between April 2013 (total precipitation 126 mm) and August 2013 (total precipitation of 13.6 mm). Changes in water physico-chemical properties with rainfall are typical of tropical and ephemeral rivers like the Surow (Larned et al., 2010), where the flood pulse forces (Junk et al., 1989) play important roles in the cycling and fate of pollutants. In tropical and ephemeral river systems, organic nutrients in sediments are mineralized while inorganic materials including metal and metalloids are leached into flood waters during the wet seasons (Carignan et al., 1999; Sioli, 1984). The leached inorganic materials deposited in the flood plains are in turn assimilated by the macrophytes on the floodplain and flushed back into the water column during the falling water periods (Stewart et al., 1998) (Winemiller, 1990). In the Surow River system, increased metal concentrations in the wet season were also associated with the discharge of mine dewatering water at site 8. Mine dewatering generally increases during the wet seasons as water inundated the mine shafts even more than in the dry seasons.

Elevated concentrations of Al, Fe, Hg, and Mn in riverine environment are of concern in riverine ecology. These metals can be bio-magnified/bioaccumulated by riverine biota (Pereira et al., 2010; Weber et al., 2013) following pathway explained by Chapman (1998) and summarised in Figure 3.15. Amisah et al (2011), for example, found elevated concentrations of iron and manganese in clams in the Volta Lake and river systems in Ghana, an indication of biomagnification of the elements in riverine biota. Blood dyscrasia or imbalances in blood components was also reported in fresh water fish exposed to elevated concentrations of manganese (Agrawal et al., 1980). Elevated metal concentrations in drinking water can also have negative effects on human health (Organization, 2004; WHO, 1989, 1990). Local citizen consuming untreated water from the Surow River whose total concentration of Fe ranges between 0.6 and 94.9 mg/L, for example, are potentially exposed to health problems associated with Fe. According to WHO, while Fe concentration of between 1 to 3 mg/L in water is considered safe, a long term consumption of water with Fe concentration >3 mg/L may have detrimental effects on human health.

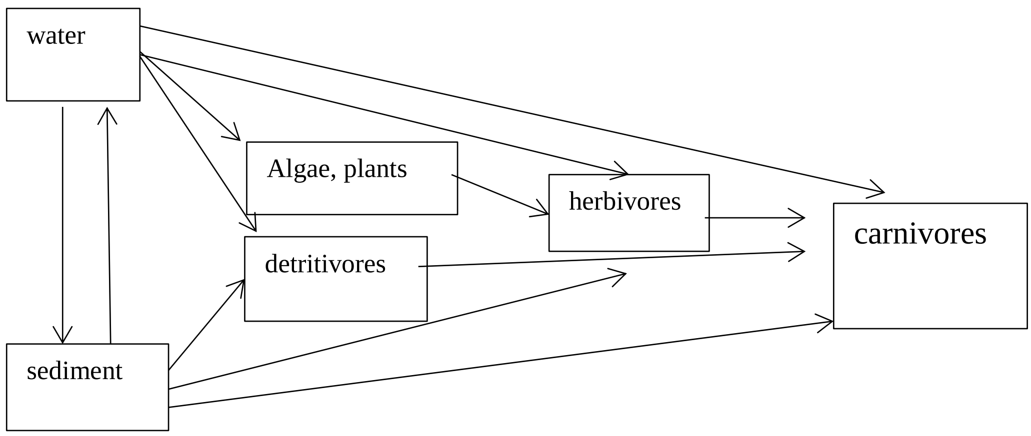


Figure 3.15 Metal pathway to benthic organisms (Chapman et al., 1998)

At a concentration ranging between 0.14 ppb and 2.6 ppb, the concentrations of total Hg in water in this study were not as high as that of reported from other ASGM studies. Total Hg in the Surow's water was also detected only during the active mining period. Despite the fact that ASGM operators use Hg in their gold extraction, dissolved Hg was undetected in most impacted sites but was found in higher concentrations at the upstream sites where ASGM was not present. The low concentrations of dissolved Hg in the river water around ASGM areas may reflect the relative effectiveness of the gravity amalgamation method employed by the ASGM operators in the study area. The amalgamation method practised in the study area requires only a very small amount of Hg compared to other methods including the whole-ore amalgamation method explained in Chapter 1 (Sousa et al., 2010; Sulaiman et al., 2007). Combined with the fact that Hg is highly prized among ASGM operators, the gravity amalgamation method appeared to have produced minimal Hg emission to the riverine environment in the study area.

The dissolved Hg detected in the upstream sites may be due to re-deposition of Hg vapour from the ASGM smelters in neighbouring villages (Schroeder et al., 1998). While metallic Hg is soluble in water; between 10 to 30% of dissolved Hg in waters is in the form of elemental Hg (Ullrich et al., 2001); it also vaporises at room temperature releasing Hg into the atmosphere (Leermakers et al., 2005). The airborne Hg eventually can be re-deposited in the aquatic systems (Pacyna et al., 2010; Telmer et al., 2009). At the peak of ASGM activities in the area, around 30 gold buying and refinery shops operated in Hwidiem and Kenyase. In these shops, shop owners smelted gold doré (unrefined gold-Hg amalgam) purchased from miners and processors to refine the gold and evaporate off the Hg from the amalgam. Veiga et al. (2004a) estimated Hg loss to the environment due to heating and smelting of gold amalgam commonly performed in such refinery shops to be between 5 and 20% of total Hg used in the mercury-gold amalgamation. Mercury vapour reportedly can travel a long range over a long period of time (Lim et al., 2001). Across the United States, for example, elevated Hg concentrations were found in freshwater fish, an indication of the presence of methyl Hg in the environment, even in places that lacked Hg point sources (Beaulieu et al.; Kamman et al., 2005; Riva-Murray et al., 2011). Therefore, deposition of atmospheric Hg originated from downstream Surow River into the river water at its source is plausible, although more detailed study on the extent of impacts of Hg vapour from artisanal gold refinery is needed.

The dissolved mercury at the upstream sites where there were no mining activities, however, can also be due to increased Hg methylation rates at these sites. Methylation of Hg can be stimulated by sulfate at a very low concentration (Benoit et al., 1999b; Gilmour et al., 1991; Krabbenhoft et al., 1995). Jeremiason (2006) asserted that an addition of a small amount of sulfate, which often associated with lower pH, increase methyl Hg (MHg) production in freshwater ecosystems where sulfate is limiting. The availability of fresh organic material in the bottom sediment of riverine ecosystem is important for the redox conditions necessary for the sulfate reduction which increase Hg methylation rate (Lambertsson et al., 2006b). On the Surow River, the canopy covered conditions of the upstream sites, particularly at site 1 and 2, the higher dissolved organic carbon (15.19 mg / l at site 1 and 14.72 mg/l at site 2) and the slightly lower pH at these sites, therefore, may have contributed to an accelerated methylation rate, resulting in the higher concentration of natural dissolved Hg in water.

3.4.2.3 Elevated Sulfate

Sulfate was elevated at ASGM impacted sites particularly at site 8 where mine-dewatering water was discharged and appeared to be the source of the sulfate. This sulfate comes from the mineralization of the ores in the area, which comprises mostly of sulphide composites containing pyrite (FeS_2) (NGGL, 2005) typical of the Sefwi belt of the Birimian host rocks, the main source of gold and diamonds that extends across Ghana (Akabzaa et al., 2009). The abundance of iron in dissolved or particulate forms in the Surow River systems corroborated the pyritic nature of the underlying geology. The spike in sulfate concentration at site 8 at the peak of the rainy season in April –to July 2014 is also typical of mining impacts in tropical river systems (Jeffery et al., 1988).

Elevated sulfate commonly occurs at mine sites rich in pyrites (FeS_2) where sulfate can be oxidised and leached from pyrite ores, waste rocks and tailings (Colmer et al., 1947; Salomons, 1995; Skousen et al., 1996). According to Salomons (1995), the oxidation of pyrite can take place both abiotically and / or biotically by microorganisms in a pH environment of above 4.5 via the following reaction:

$$7\ FeS_2 + 7O_2 + 2HO_2 \leftrightarrow 2\ Fe^{2} + 4SO_4^{2-} + 4H^{+} \qquad (a)$$

High concentrations of sulfate and iron in mine impacted aquatic environment is of a concern due to the potential for generating acid mine drainage (AMD), particularly in a very acidic environment. At pH between 2.5 – 4.5, the oxidation reaction (a) could continue, producing more iron and acid abiotically, as well as by bacteria following reaction (b) and (c).

$$4Fe^{2+} + 10H_2O + O_2 \leftrightarrow 4\ Fe(OH_3) + 8H^+ \quad \text{(b)}$$

$$2Fe^{2+} + O_2 + 2H \leftrightarrow 2Fe^3 + H2O \quad \text{(c)}$$

$$FeS_2 + 14Fe^3 + 8H_2O \leftrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16\ H^+ \quad \text{(d)}$$

With increased acidity to a pH of below 2.5, the oxidation can go further, releasing more iron and acid (reaction c and d) which take place completely by bacteria oxidation. Reaction b, c and d, are interrelated, and lead to acid rock drainage problems. The intensity of acid generating process depends on the availability of the primary and secondary factors. The primary factors are factors that promote production of acid, including availability of pyrite, oxygen and water, physicochemical factors such as temperature and pH, surface area of exposed metal sulphide materials, and population density of bacteria and nutrients availability. The secondary factors, which control the production of acid, include the availability of acid neutralising minerals such as calcites, dolomites, or carbonates of Fe, Sr or Mn (Salomons, 1995; Skousen et al., 1996). The presence of biocides such as mercury in the environment can also slow down the activity of acid producing bacteria (Bridge, 2004).

With its high pH between 6.7 and 8.5 (the highest recorded pH at any site during the study), the high sulfate and iron concentrations at site 8, equation (b), (c) and (d) are not happening in the area, at least for the time being. Although we did not do analyses for carbonates of Fe, Sr and Mn, the underlying hydrogeology of the area were known to be saturated in calcites, dolomites or carbonates (Banoeng-Yakubo et al., 2009), which are well known as neutralising materials. The abundance of Fe, Sr and Mn in the Surow River sediment can be an indication of abundant availability of the neutralising materials. The presence of mercury in sediment particularly during the active mining period may also act as biocides to oxidising bacteria and contribute in preventing the generation of acid mine drainage in the river.

The apparent current lack of acid rock drainage within the Surow River system shouldn't, however, be taken for granted because acid rock drainage can take place long after a mine ceases (Salomons, 1995), even at mine sites previously thought to have high pH environment and non-AMD generating properties (Skousen et al., 1998). In the presence of enough soluble iron II and III (see reaction b, c and d), sulfate and an appropriate environment conducive for growth of oxidising bacteria and availability of nutrients, a mine can still generate AMD in the future, particularly in the absence of proper AMD potential prediction, prevention measures and regular monitoring as seen in the ASGM operations. Prevention of AMD commonly applied in the modern mining industry includes the use of alkaline materials (e.g. lime) to increase acid neutralising capacity of the environment, encapsulation of AMD generating rocks in materials to prevent contact with O_2 and water, and regular monitoring (Lottermoser, 2012; Salomons, 1995; Skousen et al., 1998). Such AMD prevention and mitigation measures are not currently practised by ASGM operators. Not only do ASGM operators expose their tailings, waste rocks, mine pits and discharge mine water indiscriminately during their active operations, but also they often leave the mine site un-rehabilitated when they cease mining (Hinton et al., 2003), as can be seen at Kenyase 1 and Kenyase 2 ASGM sites. The fact that the mine pits at Kenyase 1 are still maintained and discharging mine dewatering water after being shut down by the government in April 2013, also shows that the discharge of pollutants including sulfate, iron and salts will continue. It is also an indication of willingness and plans of ASGM operators to return to these mining sites, when the time is right, particularly when other income generating opportunities are not available (Banchirigah, 2008; Hilson, 2010).

3.4.3 Impact of ASGM on riverine sediment quality

The analysis of I_{-geo} and EF values showed that while small amount of trace metals occur naturally in riverine ecosystems, elevated concentrations of trace metals in sediment at the bottom of water column can be a good indicator of anthropogenic-induced pollution rather than that of natural enrichment due to natural geological weathering (Muller, 1969). In this study, for example, the level of contamination by Hg in the sediment was low even during the active ASGM operations prior to April 2013; however, the presence of Hg in the system indicated a pollution by anthropogenic activities, which most likely was the ASGM. On the other hand, while anthropogenic activities did not modify the sediment concentrations of Fe, P, V, and Zn; the river sediment was contaminated by these elements, probably due to reasons other than anthropogenic activities.

Elevated metals, including Cr, Cu, Hg and Ni in sediment, as evident in the Surow ASGM areas, are of ecological concern because of their toxicity at various biological levels (tissue, organ, organism, system) and their potentials for biomagnification (Hakanson, 1980; Pereira et al., 2010) following the metal pathway explained by Chapman et al. (1998) (Figure 3.21). In the study of ASGM impacts on the environment, however, Hg has always been the subject of interest due to its association with the Hg amalgamation process commonly used in ASGM and its toxicity. Although Hg is poisonous in all forms, methylmercury, which forms up to 30% of mercury in water, is the form of most concern in aquatic ecology due to its high toxicity, bioavailability and potential to biomagnify in fish to a level that may harm the fish and other animals that eat fish (Leermakers et al., 2005). Fish-eating birds and mammals have been identified as at risk, so are their predators. Mercury has been found, for example, in water and terrestrial birds (Aazami et al., 2012; Eagles-Smith et al., 2009; Jackson et al., 2011), eagles (Scheuhammer et al., 2008), seals and other endangered animals including polar bears (Atwell et al., 1998) and panthers (Barron et al., 2004). Mercury is a neurotoxicant, mutagen, teratogen and carcinogen (Eisler, 2004; Wolfe et al., 1998). Lethal concentrations of mercury to sensitive aquatic organisms range from 0.1 to 0.2 µg/L medium. Sub-lethal effects of Hg on fish and other aquatic biota include inhibition of reproduction, reduced growth rates and reduced ability to capture prey (Eisler, 2004). In birds and mammals, Hg at a very low concentration can adversely affect metabolism, histology, reproduction, growth development, and motor coordination (Eisler, 2004; WHO, 1990). The reproductive effects of Hg in mammals range from behavioural deficit after birth to foetal death (Wolfe et al., 1998), while in birds reduced egg production and poor hatching success have been reported (Seewagen, 2010).

Mercury, which was elevated in the sediment around the ASGM sites before the cessation, may naturally occur in sediment and water (Ullrich et al., 2001) especially in areas with ferruginous and fossilized materials (Brabo et al., 2003). In the case of the Surow River sediment in February 2013, however, Hg was below detection limit of 0.03 mg/kg at the source of the river but was particularly high at ASGM sites exceeding the recommended maximum concentrations of 0.1 to 0.2 mg/kg for protection of freshwater ecosystems. The longitudinal trend of Hg concentrations and the fact that there was no other industry but ASGM along the river corroborated the inference that ASGM was the main possible source of Hg enrichment in the Surow riverine sediment. ASGM operators released Hg into the environment during the pre-concentration process of slurry and open air amalgamation, which eventually reach waterways directly by downgradient movement of water and sediment or indirectly from the air and rainfall (Barbosa et al., 2003; Brabo et al., 2003; Telmer et al., 2006b; Telmer et al., 2009). The positive correlations between mercury and Al, Ba, Ca, Co, Fe, Mg, Mn, P, K, Sr and S concentrations in sediment suggest that the elevated concentrations of Al, Ba, Ca, Co, Fe, Mg, Mn, P, K, Sr and S may also be ASGM induced. In this case, ASGM activities appeared to have accelerated the leaching of metals which are already available in abundance in the river system such as Al, Fe, and Mn. Metals originally found in smaller concentrations at the sources of the river, such as Cr, Ni, and Sr, also appeared to be weathered severely downstream, particularly during the active mining period.

Elevated Hg concentrations in sediment were also reported in various ASGM studies in other countries such as the Brazilian Amazon (; Brabo et al., 2003; Dominique et al., 2007; Telmer et al., 2006a), Indonesia (Limbong et al., 2003), the Philippines (Appleton et al., 1999) as well as other ASGM areas in Ghana (Donkor et al., 2006). Although lower than values from contaminated historic mine sites such as the Carson River of Nevada (reported range of 2.0 to 156 mg/kg), the February 2013 average concentration of Hg in the Surow River sediment (0.34 mg/kg) was comparable to and even slightly higher than reported values from the Tapajos River in the Brazilian Amazon (average 0.29 mg/kg;(Malm, 1998), the Pra River in Ghana (0.27 mg/kg;(Kehrig et al., 2003) and the Talawaan and Katingan Rivers of Indonesia (0.154 to 0.48 mg/kg;(Bose-O'Reilly et al., 2010). The latter rivers are much larger river systems affected by chronic and present ASGM operations in comparison to the Surow River. Unlike most ASGM operators within the Surow catchment, ASGM in the Indonesian and Brazilian studies are known to use the whole Hg amalgamation method explained in Chapter 1, which reportedly emits more Hg into the environment than the gravity concentration amalgamation method (Veiga et al., 2004b). In higher ordered rivers such as the aforementioned rivers in Brazil and Indonesia and the Pra in Ghana, sources of sediment and pollutants cover wider catchment areas, including a network of streams and its tributaries (Yuill et al., 2011). On the other hand, in first and second order streams and rivers like the Surow, the source of pollutants and sediment supplies most likely is the adjacent upland areas (Yuill et al., 2011). The similarity in riverine sediment Hg concentrations between the Surow River and the Amazonian and Indonesian rivers is therefore alarming. It may indicate the acute effects of Hg in the Surow River environment. The low discharge and often discontinuous nature of the Surow River particularly in the drier seasons, may also have allowed for longer residence time of the water and its contaminants in the river, resulting in higher concentrations of pollutants in the sediment. Bose-O'Reilly et al. (2010) asserted that a site with an average sediment Hg concentration of 0.317 should be considered 'mercury hotspot' in need for intervention. The Surow River in early 2013 certainly qualified as such using the criteria. Fortunately, sediment showed a general decrease in metal and metalloid concentrations in April 2014 following the cessation of mining, including Hg particularly at impact site, which strongly support our hypothesis that mining is responsible for the elevated metal in the river's sediment.

The lack of statistical correlations between sediment metals and concentrations of most metals in water (as total and dissolved) in this study was also found in similar studies (Bose-O'Reilly et al., 2010; Brabo et al., 2003; Donkor et al., 2006). However, in this study we found significant negative correlations between dissolved mercury in water and sediment concentrations of iron, manganese and sulphur. This is consistent with the roles of Fe, Mn, sulfate and availability of fresh organic matter in sediment in determining the bioavailability of trace metals in anaerobic sediments, including methylation of Hg. In the presence of sulfate and the sulfate reducing bacteria (SRB), Fe and Mn form iron monosulfides (FeS) and manganese monosulfides (Ankley, 1996; Chapman et al., 1998). Due to the higher solubility of FeS and MnS than other trace metals sulphides (MeS), metals including Hg will displace Fe and Mn to form more insoluble MeS. The results may be beneficial for the riverine ecology because the binding of other potentially toxic metals with sulphide make them less bioavailable, which appears to substantiate in the case of the Surow River.

3.4.4 Effect of cessation of major ASGM operators and potential for pollution mitigation and remediation

The study has shown reductions in pollution levels in the Surow River sediment and water since the Ghanaian Government's decision to deport foreign investors and illegal ASGM operators from the area in 2013. The changes in riverine metal and metalloid concentrations, however, may have coincided with at least two events: (1) the cessation of major ASGM operators from the Surow catchment in May 2013, and (2) the floods associated with heavy precipitation in the two rainy seasons between February 2013 and April 2014, or a combination of the two. The first was supported by the significant interaction between before/after and control and/impact within the BACI model. The effect of the second event (floods), however, was also plausible as indicated by the declined concentrations in both control and impact sites particularly for sediment, although control remained different from impact and the decline at impact was more substantial than at control (see Figure 3.8). Extreme scouring of river bottom and sheet and gully erosion are typical of tropical West African riverine environments due to the high intensity of the rain in the region during the rainy seasons which typically produces high runoff that often lead to flooding, soil compaction and soil loss (Balek, 2011; Hayward et al., 1987).

The consequences of the findings from this study with regards to the decline in sediment pollutants, therefore, are two-fold. First, it suggests the possibility for natural remediation of contaminated riverine environment, albeit locally, if the discharge of pollutants is reduced or stopped altogether. Partial recovery in a mercury polluted river was also observed by Tarras-Wahlberg et al. (2001) in the Puyango River in Ecuador where ASGM operators halted their operations after an El-Nino heavy rainfall and flooding event in 1997-1998. The reduced discharges to the river had resulted in improved sediment quality and the return of benthic macroinvertebrate taxa previously absent in the area. Second, the reduced concentrations of metals and metalloids in the Surow River sediment and water however, remain an ecological concern. The sediment and pollutants most likely have been scoured off the river's bottom and transported downstream including into the Tano River and beyond (Walling et al., 2003), deposited on the floodplains particularly during the flood events (Stewart et al., 1998), or buried in the river bottom as a sink. Either way, the pollutants will remain a risk to the environment. Studies in the impact of mining on riverine sediment and water at historical mine sites in Europe and north America have shown that metal and metalloid elements including mercury, remain an ecological issue long time after the mine ceases (Bonta, 2000; Thorslund et al., 2012; Whyte et al., 2000). Fluxes of heavy metals in sediment downstream of such contaminated sites and changes in the water quality particularly with regards to increasing concentration of dissolved metals were observed (Whyte et al., 2000), necessitating continuous monitoring.

3.5 Conclusion

ASGM significantly impacted riverine sediment and water quality in the Surow River through elevated turbidity, EC, TDS and total metal concentrations. At mine impacted areas, turbidity often exceeded the Ghanaian EPA Guidelines for freshwater ecology, whilst total concentrations of metals including Fe, Hg, and Mn exceeded the water quality guidelines applicable in the region, particularly during the active mining period. Elevated concentrations of metals including Cr, Fe, Hg, Mn in riverine sediment around ASGM sites also exceeded the threshold levels of sediment quality for the protection of aquatic life.

ASGM impacts on water quality were seasonal. Increased dissolved metal and metalloid concentrations along the river were detected during the heavy rainy season of the year. High precipitations also intensified the differences between mining and non-mining related sites, indicating that mineral and pollutants deposited in solid materials on the river catchment may still be diluted and washed off during the rainy season long after ASGM cease to operate in the area.

Most metal and metalloid concentrations in riverine sediment did not correlate with their corresponding total and dissolved concentrations in river water, suggesting the presence of a more complex bio-geomorphological process in the water. The negative correlations between dissolved mercury in water and concentrations of Fe, Mn, Hg and S in the sediment, however, were evident and indicated the roles of Fe, Mn and S, which were available in abundance, in limiting the bioavailability of Hg in the system.

Most of the key features of impact reversed with the cessation of major ASGM operations along the river. While flood events may have contributed to the decline in pollutant levels particularly in sediment, the reversal of impacts indicates the river's ability to recover longitudinally, at least at local scales.

The government's act to deport illegal foreign financiers of ASGM to the effect of closing down major ASGM operations has been effective in improving the sediment and most water quality characteristics of the Surow River. The exception was concerning the mine-dewatering discharge site, where elevated EC, TDS, sulfate and metals in water were detected even after the closure of major ASGM operators at the end of April 2013. This suggests persistent impacts of mine dewatering and leaching of minerals from piles of waste rocks and tailings left un-rehabilitated by the ASGM operators.

The study's results emphasise the importance of implementing sediment control measures in ASGM areas and the need to increase awareness among ASGM communities and local and regional environmental managers of ASGM impacts from each step of ASGM process on the riverine environments. Given the lack of baseline data prior to mining or the study, I also strongly recommend monitoring of water and sediment characteristics at ASGM impacted areas along the river and beyond for any physico-chemistry changes in the water and sediment future environmental impacts. The full extent of ecological impacts of elevated concentrations of pollutants in riverine sediment and water needs to be further investigated by assessing their potentials for biomagnification and their impacts on biota at community levels, which will be addressed in Chapter 5, 6, and 7.

4 Impacts of large commercial gold mining on riverine sediment and water

4.1 Introduction

Large-scale commercial mining activities such as those at gold mines in Ghana impact ground and surface waters due to the potential release of pollutants, and water usage including abstraction, and discharge (Mudd, 2008). In the gold mining region of Ghana precipitation is very high and the rainfall during the two rainy seasons is intensive (see Chapter 2), although the area also experiences some dry months, typical of the West African tropics (Hayward et al., 1987). Consequently, unlike most Australian or some North American mines whose environmental water issues are often related to the limited water supply for mining (Mudd, 2007b, 2008) resulting in negative water balances (i.e. consumptive water balance), the water issues at tropical mines are characterised by a positive water balance, i.e. excessive availability of water which necessitates discharge off-site. The discharge can be polluting due to acid mine drainage (AMD), metals, cyanide, and fine particulates (Ashton et al., 2001; Monjezi et al., 2009; Salomons, 1995; Younger et al., 2002). These pollutants may arise from open pits (Akcil et al., 2006; Bowell et al., 2005; Monjezi et al., 2009), exposed waste rocks, processing and processed waste including tailings, and run off water from exposed surfaces (Lapakko, 2002; Salomons, 1995; Skousen et al., 1996).

In an adjacent catchment to the ASGM area discussed in Chapter 3, a multinational mining company operates the South Ahafo gold mine (hereafter called the Ahafo mine) in the catchment of the Subri River. The Subri River discharges into the Tano River. The Subri River is longer, has more tributaries, and drains a larger catchment area than the Surow River (see Chapter 3). Unlike the ASGM operations on the Surow River catchment, the Ahafo mine employs modern gold mining technology supported by an environmental management system and regulated by Ghanaian Governmental environmental regulations and applicable international mining industry standards and guidelines. Further, in its environmental impact statements, the owners of the Ahafo mine acknowledge the possible impacts of water discharge, and are bound by applicable laws and agreed their own obligations to mitigate them. The Subri River receives discharges of treated mine water and potentially also seepage from the mining area.

The Ahafo mine is an open-cut mine currently owned and operated by Newmont Ghana Gold Limited (NGGL), a subsidiary of Newmont Mining Corporation (NMC) after an acquisition of the South Ahafo reserve in 2002 from Normandy Mining Ltd. After obtaining mining permits and licences including approval of its Environmental Impact Assessment (EIA) in 2005, construction of the mine facilities commenced in July 2006 whilst mine operations started in December 2006 and gold was poured for the first time in January 2007. In 2005, the mine reserve was predicted to have 105 million tonnes (MT) of ore, yielding 6.8 million ounces of gold. To mine this resource, a total of 2,174 hectares (ha) of surface area was disturbed for the mine’s facilities which include four open-cuts, a waste rock disposal area, mill and the Carbon in Pulp (CIP) based processing plant (explained in Chapter 1 and Figure 4.1), water storage facility, tailings storage, environmental control dams (ECD) including stormwater and sediment control dams, and other ancillary facilities such as road and resettlement areas for communities impacted by the project (NGGL, 2005). Two of the mine pits, the water storage facility, tailings storage, the CIP processing plant and ancillary facilities, and two ECDs are located in the Subri River catchment.

Mitigation of possible impacts at the Ahafo mine include engineering works to address each identified impact at its source, continuous monitoring programs and audits by external parties to ensure compliance (NGGL, 2005). The environmental engineering works include facilities to address potential impacts arising from dewatering of the open pits which are inundated with groundwater inflow and surface runoff water. At Ahafo mine, groundwater inflow is routed and pumped into a tailings pit or water storage facility to be used for process water. Although the company's geological data indicated that the area did not have significant potentially acid generating (PAG) rocks, waste rock disposal and management was designed to encapsulate any PAG with acid neutralising rocks in such a way that prohibited contact between water and the PAG over an extended period of time, a technique commonly practised in contemporary mines to prevent AMD (see Jeffery et al., 1988; Salomons, 1995). The waste rock is also surface compacted to further prevent water infiltration that could promote water runoff. A network of surface water ditches is built around the perimeter of the rock piles and where necessary to intercept and divert potential water runoff, preventing it from flowing back into the pits, the waste rock dumps or ores. Water from these ditches flows into a series of clay-lined environmental control dams (ECD). Two of the ECDs, ECD 4 and ECD 6 are on the Subri River catchment. Located down gradient from waste rock disposal facilities and pits with an average capacity of about 100,000 m^3 each and average maximum height of 4 meter, ECD 4 and 6 were designed to intercept, collect and settle runoff water from these disturbed areas. ECD 4 is built on the Asundua stream, intercepting the stream before it joins the main Subri River (Figure 4.2). KSW3 drains into ECD4. ECD 6 is an impoundment on the bank of a tributary stream of the Samansua Stream draining into the Subri. Unlike ECD 4, ECD 6 does not intercept the stream, but occasionally discharges into NSW6 on the Subri. Both Samansua and Aundua streams are the main tributaries to the Subri River (Figure 4.2). The ECDs hold water until the water quality meets the Ghanaian EPA standards for mining discharges (EPA, 2005) and the Ghana Water Company guidelines (see Appendix 20)Occasionally, compliant water from the ECDs is decanted into the Subri River above NSW6 and NSW8 (NGGL, 2005). The company is obligated to conduct monthly sampling of the ECDs water and quarterly sampling of adjacent streams potentially impacted by the mine. Sampling of water physico-chemical properties, total metal and nutrient concentrations have been conducted since two years before the actual mining activities begun in December 2006 until present. Sediment quality, however, is not a criterion in the environmental monitoring for compliance.

This chapter aims to determine the effects of Ahafo mine's discharge on the Subri River sediment and water. Specifically, the objectives of this work are: (1) to determine whether water and sediment quality of the Subri River are altered by mining discharge; and (2) to determine whether the ECDs are an effective environmental and mine water management strategy. The key hypotheses are tested in this work are: (1) discharge has no impact on water quality and sediment in the Subri River (no difference between control and impact, before and after, with a non-significant interaction) and (2) no difference between sites located downstream of ECDs and that of inside the mine.

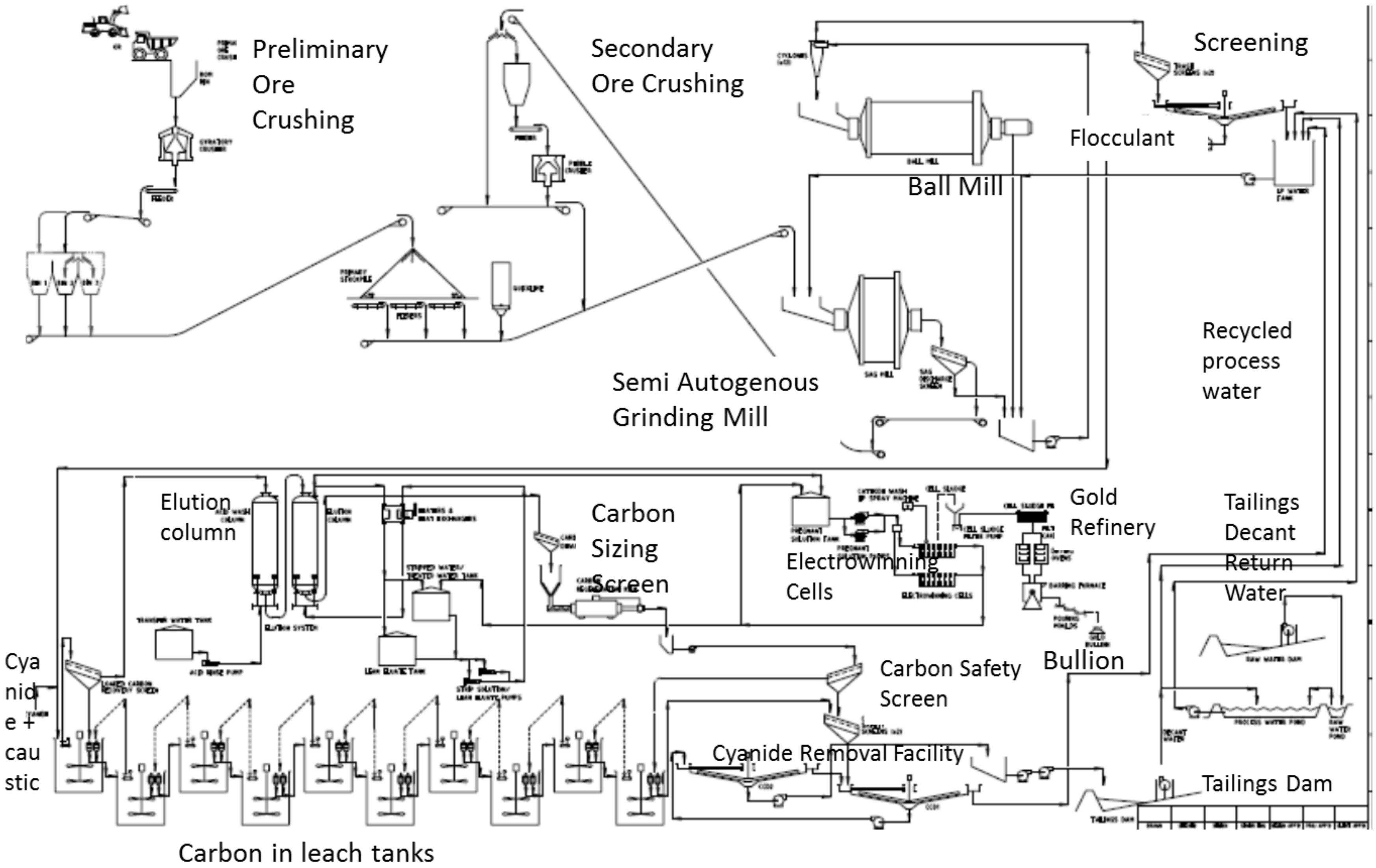


Figure 4.1 Flow chart of mining and processing methods at the Ahafo gold mine (NGGL, 2005)

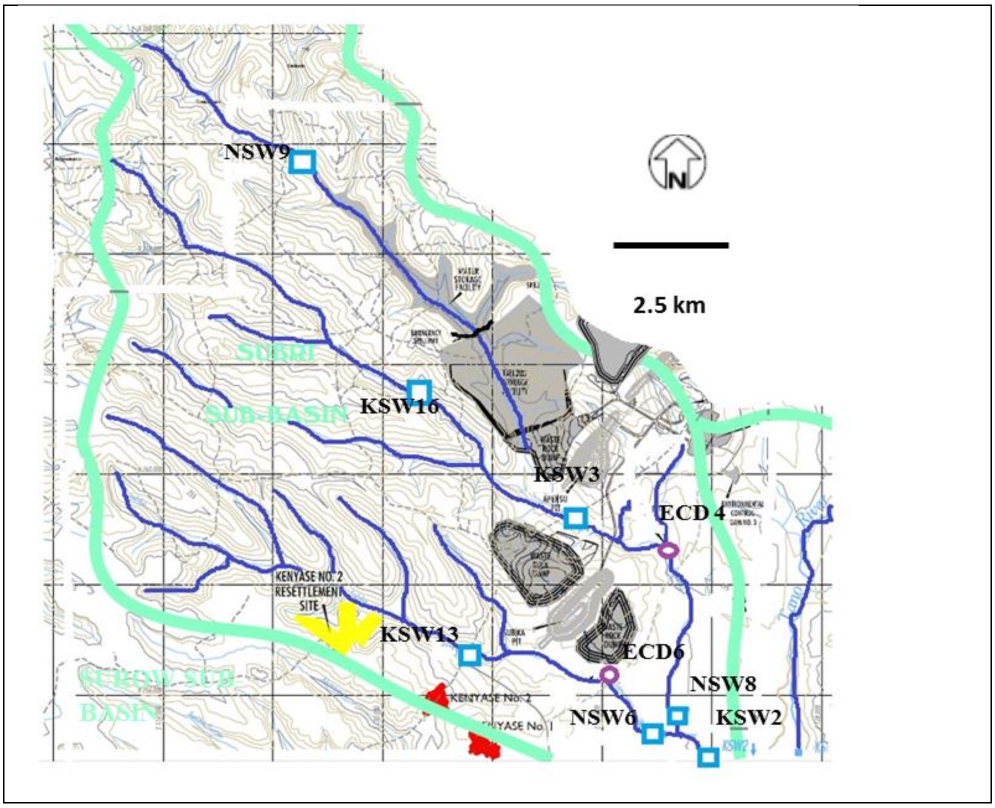


Figure 4.2 The Ahafo mine operations along the Subri River and water and sediment sampling sites (blue squares).
Mine areas and facilities are highlighted in grey and environmental control dams (ECDs) are indicated. The map is adapted from the Ahafo mine's Environmental Impact Assessment (EIA) document (NGGL, 2005)

4.2 Methods

4.2.1 Study and sampling design

The sampling program is based around a BACI design (Smith, 2002; Underwood, 1994) which compares multiple un-impacted sites ('control', C) to multiple impacted sites ('impact', I), across multiple 'before' (B) to multiple 'after' (A) time periods. Since 2004, the Ahafo mine has conducted regular monitoring of physico-chemical parameters and concentrations of total metals in the water at all sampling sites and this data has been used in this study. Data prior to December 2006 (commencement of the mine) was used for B data and the remainder was used as A data. As dissolved metal concentrations were only monitored after December 2006 and sediment samples were only taken in my study (2013-2014), the BACI design cannot be applied to the sediment and dissolved metal data - comparison between multiple control and impact sites are used instead.

Seven sampling sites were selected to capture longitudinal changes along the Subri River, incorporating control sites and those potentially impacted by the mine. The sampling sites were parts of NGGL sampling sites for their regular reporting. Three of the seven sites (site NSW9, KSW16, KSW13) were considered control sites, three sites located downstream of the mine (NSW6, NSW8, KSW2) were assigned as impact, whilst a site inside the mine (KSW3) was designated as the representative of the condition at the mine sites ('mine', M). To test the changes along the river due to mining and facilities put in place by the mining company to mitigate the impact, comparison between control, impact, and mine sites was made. In particular, two way comparisons by pairing mine and impact (IM) factor with before and after (BA) factor was also made to test the effectiveness of the mine water management system.

Sampling was conducted over a year to encompass seasonal (dry and rainy) differences on river water quality. Designation of seasons corresponds to that given in Chapter 2. The characteristics and typical land use around each sampling site are described in Chapter 2.

4.2.2 Sampling and analysis

Ahafo mine conducts water sampling every quarter from most of its sampling sites although some sites that needed more detailed attention were sampled monthly. Between February 2013 and April 2014, I accompanied the mine's environmental monitoring team to sample and process the water samples from sites relevant to my study using their standard methodology. The environmental monitoring team consists of experienced and qualified people, using reliable instruments calibrated regularly by the Ghana Standards Agency. Sampling and analysis protocols and data management are audited regularly by internal and external auditors including for ISO 1400 accreditation. As sediment quality is not a part of regular and reportable monitoring program at the mine, I conducted sediment sampling independently in February 2013 and April 2014.

Water and sediment sampling protocols, sample preparations and methods of analysis are as per Chapter 3. Water samples from the Subri River were analysed by ACZ laboratory in Colorado, USA; and sediment samples by SGS laboratory in Accra, Ghana and its associated laboratory in Canada.

4.2.3 Data

Regular monitoring data of the sampling sites collected by Ahafo mine from 2004 to March 2013 were combined with data I collected between February 2013 – 2014. A total of 400 water data points with 45 water quality parameters from 7 sites were analysed. Sediment quality data was measured from all sites in April 2014 and 3 sites in February 2013. Two additional sediment samples were also collected from the Tano River downstream of Hwidiem township in January 2014.

4.2.4 Data analysis

Univariate and multivariate statistical analysis were used in this study. The multivariate statistical analysis procedures are detailed in Chapter 3. Due to the large number of water quality variables analysed, a factor analysis was conducted to distinguish variables with high loadings from those with lower loadings prior to PERMANOVA (Appendix 18)

The Geo-accumulation Index ($I\text{-}_{geo}$) and Enrichment Factor (EF) were also calculated to indicate contamination level and degree of anthropogenic modification following the formula given by Muller (1969) and Buat-Menard et al. (1979) as outlined in Chapter 3. In this case, sampling site NSW9 was used as the reference site due to its position at the source of the Subri River and the absence of mining activity near to the site.

4.3 Results

4.3.1 Water

4.3.1.1 Impacts of mining on water quality

Principal component ordination of water quality variables selected through factor analysis (Appendix 18) indicated a separation between control and impact, and between before and after, as illustrated in Figure 4.3. PCA of dissolved metal concentrations in water also showed a distinct separation between control site and impacted sites along PC1 axis, whilst separation between impact and the mine site was not as distinct (Figure 4.4).

Two-way PERMANOVA confirmed that the Subri River water quality at impact sites was significantly different ($p<0.01$) from control and that before was significantly different from after mining. The impact of mining on the changes of water quality seen was confirmed by the significant interaction between B/A and C/I (Table 4.1). Pairwise SIMPER analysis indicated that the significant difference between control and impact was driven by the alkalinity, pH, TDS, concentrations of ions of Na, Ca, Mg, sulfate and nitrates, and As, Fe, Cl, Pb, Mn and TSS (Appendix 21). Independent t-test on these variables confirms that alkalinity, pH, TDS, concentrations of ions of Na, Ca, Mg, sulfate and nitrates in impact were higher than control, whilst the concentrations of As, Fe, Cl, Pb, Mn and TSS in impact were lower than control (Appendix 22)

Water quality at impact sites (I) located downstream of the ECDs was also significantly different from that of the mine site (M), as were the interactions between BA and MI factors, all at $p<0.01$ (Table 4.1 B), distinguishing the impact of ECDs on the river water quality downstream of the mine from that upstream of ECDs. PERMANOVA of dissolved metals data from December 2006 to April 2014, however, showed that the controls were significantly different ($p<0.01$) from impact and mine sites (all $p<0.01$, Table 4.2), but mine and impact sites were not significantly different ($p>0.05$) (Table 4.2). This, therefore suggested that the ECDs were unable to reduce the concentration of dissolved metals in the river water.

After the conception of the mine, the significant difference between the mine site and impact sites was attributed to TSS, TDS, concentration of total Mn, Fe, Al, Ni, nitrates, sulfate, Ca, Na, K, pH, fluoride and concentration of total Co, as identified by SIMPER. Independent t-Test analysis on the individual variables confirms that turbidity, conductivity, TDS, concentrations of Ca, Mg, Na, K, Cl, nitrates, sulfate, total Fe, Mn, Cu, Al and Se in downstream Subri after the mine were significantly lower than in the mine sites, whilst they were similar before the mine (Appendix 23).

The gradient changes in the river's water turbidity, pH, EC, TDS, concentrations of Ca, Mg, NOx, sulfate, Fe, Mn, Al and As are depicted by the box plots (Figure 4.6) which illustrate impact of mining and effects of ECDs on the river's water quality. Plotting MDS coordinates against individual variables identified by SIMPER as contributors to the differences between B/A and C/I (**Error! Reference source not found.**) provides an insight into the correlations between these individual variables and the water quality in general. As can be seen in Figure 4.5, pH, conductivity, concentrations of Ca and Mg at impact sites after mining tended to be higher than that at control sites before and after mining. On the other hand, TSS, concentrations of metals including Fe, Cu, and As at impact sites after mining are lower than at control sites before and after mining.

Water quality also differed with site, time (month) and season at $p<0.01$ (Table 4.1 C and D). Pairwise comparison between individual sites showed that almost all sites were significantly different from one another at $p<0.01$, except NSW8 and NSW6 which were similar ($p>0.05$) to each other. A closer look at the outliers identified in the box plots in Figure 4.6 suggested that spikes in the concentrations of salt ions including Ca, Na, K, Cl and Mg were mostly detected in the beginning of rainy seasons (March, April, September, November), whilst spikes in nitrates and sulfate were detected in the drier months.

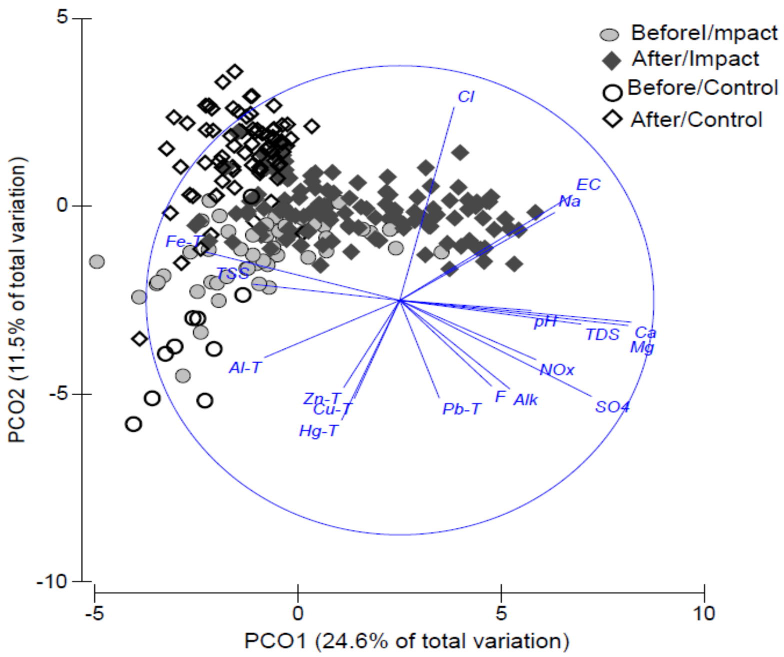
Table 4.1 Results of PERMANOVA on water quality data between 2004 and 2014 distinguishing impacts of mining on the Subri river water quality, impact of mine water treatment on the downstream water quality, and temporal effects

	Source	df	SS	MS	Pseudo-F	P(perm)
A	Before/After	1	421.71	421.71	27.409	0.0001
	Control/Impact	1	320.81	320.81	20.851	0.0001
	BAxCI	1	224.21	224.21	14.573	0.0001
	Residual	258	3969.6	15.386		
	Total	261	5120.8			
B	Before/After	1	444.68	444.68	28.337	0.0001
	Impact/MineSite (IM)	1	131.85	131.85	8.4021	0.0001
	BAxIM	1	126.61	126.61	8.0683	0.0003
	Residual	245	3844.7	15.692		
	Total	248	4768.3			
C	Season	1	89.387	89.387	5.0254	0.0007
	Residual	335	5958.6	17.787		
	Total	336	6048			
D	Month	11	608.71	55.338	3.3064	0.0001
	Residual	325	5439.3	16.736		
	Total	336	6048			

Table 4.2 Results of Pairwise PERMANOVA between dissolved metal concentrations in Subri River water at control, mine site, and impact

Groups	t	P(perm)	unique perms
Impact, Control	3.0547	0.0001	9921
Impact, Mine	1.2754	0.0942	9945
Control, Mine	3.0417	0.0001	9915

A



B

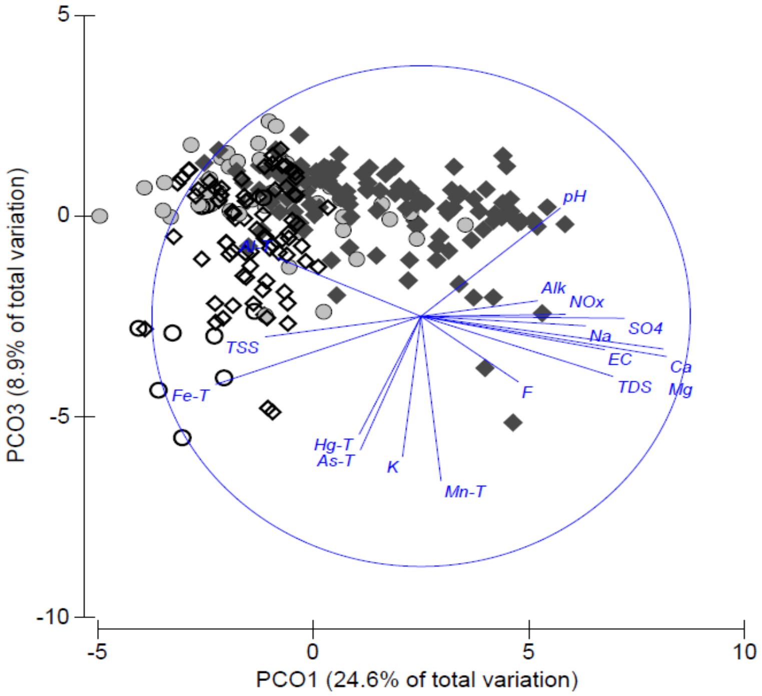


Figure 4.3 Principal component ordinations showing control and impact sites before and after mining a) PC axes 1 and 2 and b) PC axes 1 and 3 in the Subri River water quality. Variables significantly (Pearson's r >0.4) correlated to the ordination space are shown.

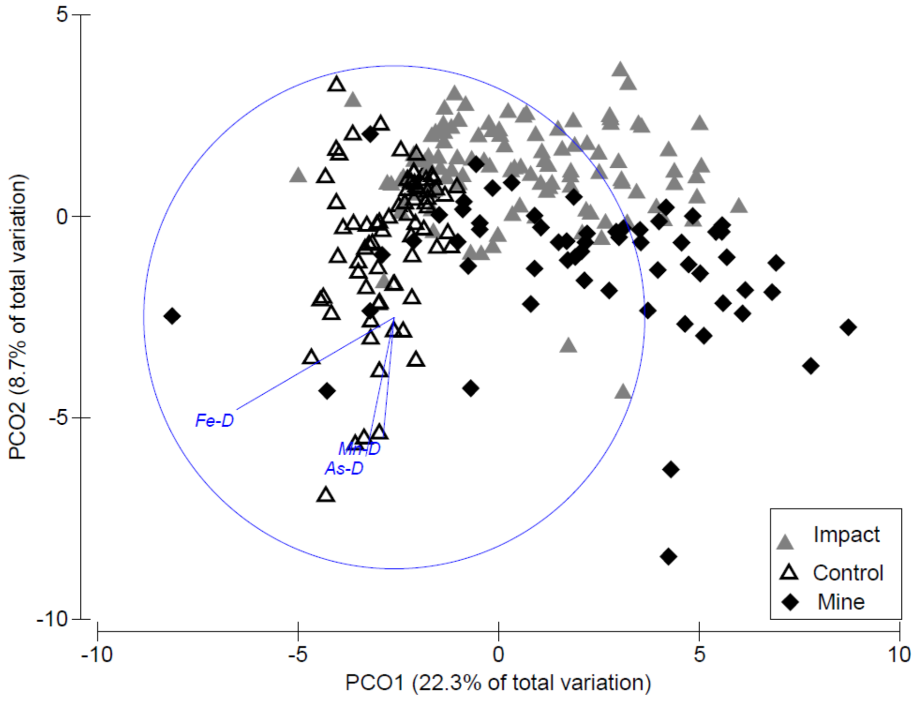


Figure 4.4 Principal component analysis of concentration of dissolved metals in water between December 2004 and April 2014 across control, impact and mine sites in the Subri River. Variables significantly (Pearson's r >0.4) correlated with the ordination space are shown.

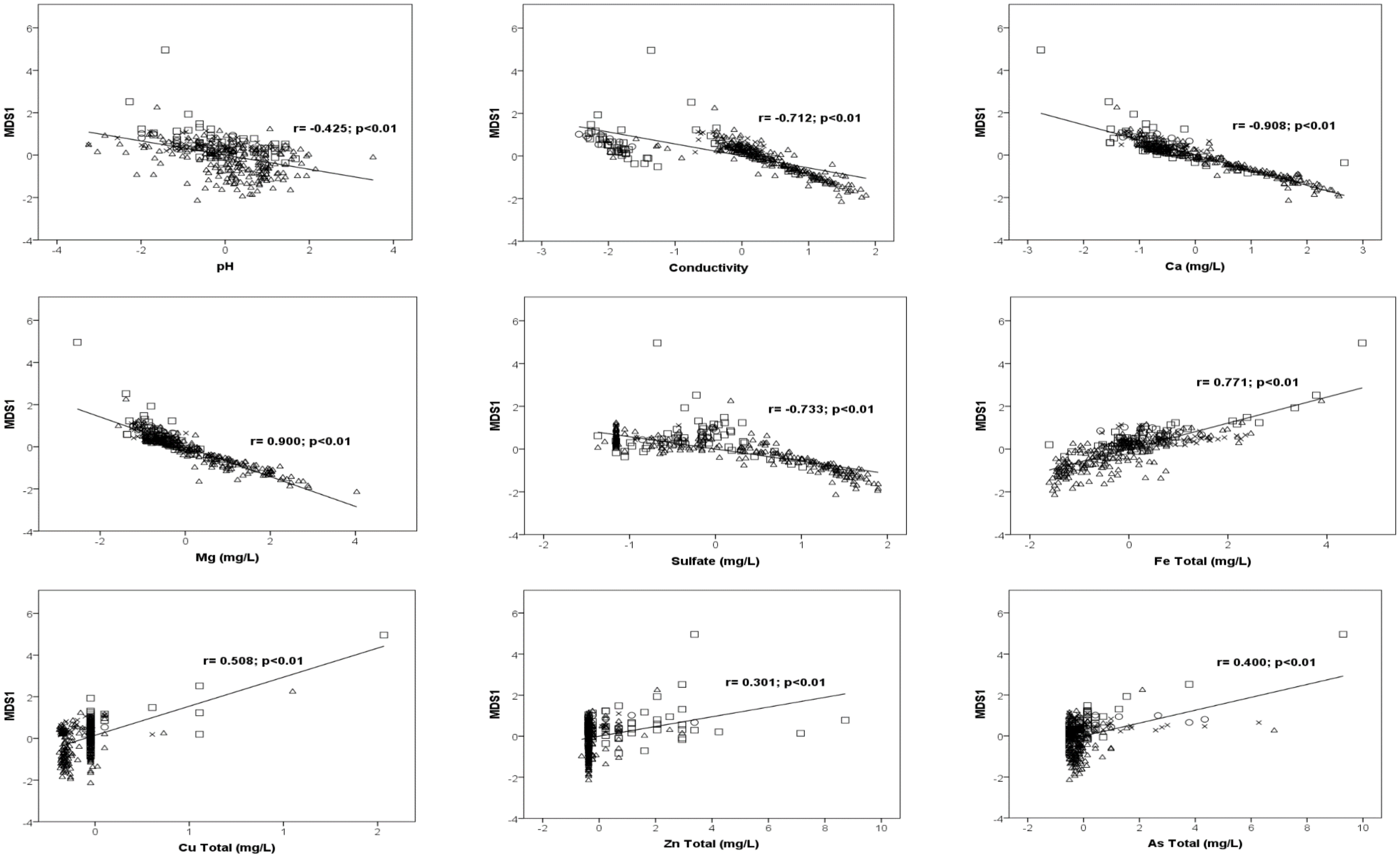

MDS1
pH
r= -0.425; p<0.01
Conductivity
r= -0.712; p<0.01
Ca (mg/L)
r= -0.908; p<0.01
Mg (mg/L)
r= 0.900; p<0.01
Sulfate (mg/L)
r= -0.733; p<0.01
Fe Total (mg/L)
r= 0.771; p<0.01
Cu Total (mg/L)
r= 0.508; p<0.01
Zn Total (mg/L)
r= 0.301; p<0.01
As Total (mg/L)
r= 0.400; p<0.01

Figure 4.5 Correlations between MDS coordinate with log pH, conductivity, concentration of dissolved Ca, Mg, sulfate, total Fe, Cu, Zn and As, with Control/Impact and Before/After overlay. ○ represents water quality at control before mining, □ impact before mining, x control after mining and Δ impact after mining. Pearson's r and the significance of correlations are stated in the graphs

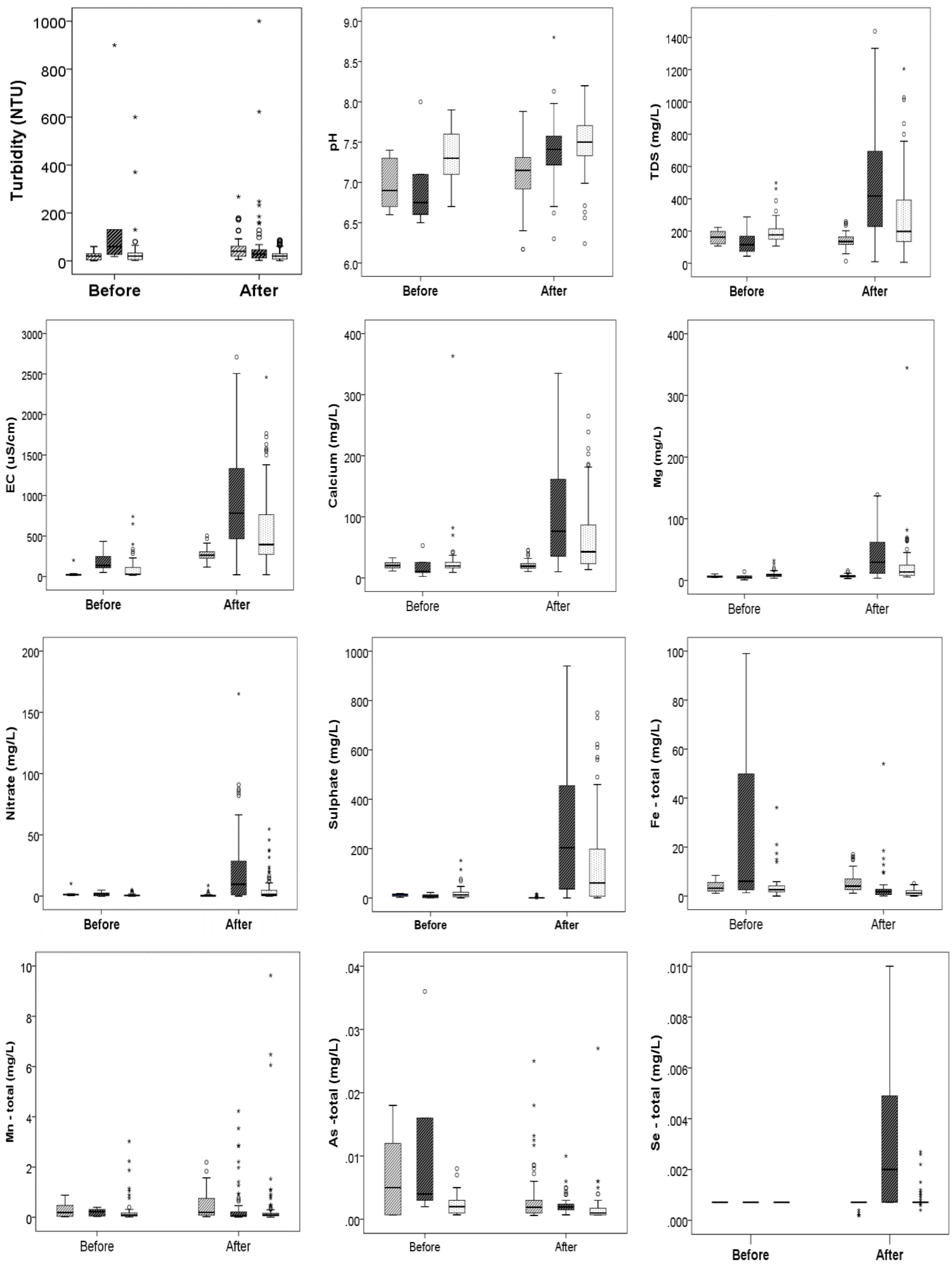


Figure 4.6 Box and whisker plots of key Subri River water quality variables (turbidity, pH, TDS, EC, Ca, Mg, nitrates or NOx, sulfate, total Fe, Mn, As, Se) showing differences between control ( ), mine ( ), and impact ( ) sites before and after mining. Selenium was not measured before the mine started.

4.3.1.2 Water quality

The Subri River water was generally neutral to mildly basic (mean pH 7.37 ± 0.025), with a minimum pH of 5.9 which was recorded once from a pool of water at KSW13 at the end of a dry season in March 2014, and a maximum of 8.8 at KSW3 also in March 2014. Water pH significantly correlated with concentration of salts. The waters were fresh with a mean EC of 612 ± 32.6 µS cm^{-1} and a mean TDS of 317.4 ± 17.7 mg L^{-1}. The river water was often turbid particularly at control sites in the dry season, but less turbid in the wet season and downstream of mining areas, averaging at 43 ± 5 NTU with a mean TSS of 43.2 ± 7.7 mg L^{-1}. The Subri River water, similarly to the Surow River water, was also characterised by a low oxygen concentration (mean 4.2 ± 0.1 mg L^{-1}) with a minimum of 0.3 mg L^{-1} and a maximum 8.5 mg L^{-1}. Increased pH, EC, TDS and alkalinity at the mine and impact sites was noted, whilst turbidity and TSS tended to decrease (Figure 4.6). Iron, Al, Mn and Zn were the most abundant metals (as total and dissolved) in the water. Arsenic, Cr, Se, Ni, Pb and Cd were rarely above detection limits. Mercury in the river was only above detection limits of 0.0002 mg L^{-1} three times between 2004 and 2014, once at the source of the river (NSW9) before the mine started and on two occasions at the bottom of the Subri River near to Subrisu village (site KSW2). Elevated concentrations of total metals tended to occur at the control more than at the impact sites. In spite of the use of cyanide at the mine, no concentration of cyanide ever exceeded the detection limit of 0.003 mg L^{-1} in the river water. Water temperature did not vary significantly, with an average of 26.0 ± 0.3 C.

Overall, the physico-chemical characteristics of the river water were within the Ghanaian and US EPA standards for mine effluent (EPA, 2005; USEPA, 2015) and the Ghana Water Company Guidelines for raw drinking water supply most of the time. The exceptions include some exceedances for EC, TDS, nitrate and sulfate at the mine site (KSW3). The river water characteristics and applicable standards are summarised in Appendix 20.

4.3.2 Sediment quality

4.3.2.1 Impact of mining on sediment quality

Sediment quality did not differ with site, location (control, mine and impact), nor in the interaction between time and location, although it significantly differed with time (PERMANOVA $p < 0.01$,

Table 4.3).

Principal component ordination of the riverine sediment quality Figure 4.7shows a distinct separation between February 2013 and April 2014 data along the first axis (PC1) (Figure 4.7). Some spatial variation was also evident along PC1 with elevated metal and metalloid concentrations aligned (Pearson's r>0.4) with mine site (KSW3).

The significant difference between sediment quality in February 2013 and April 2014 according to SIMPER analysis (Appendix 25) was mostly due to the decreased concentrations of metal and metalloid concentrations in 2014 compared to that of 2013. The concentrations of select metals and metalloids in sediment at mine and impact sites along the river in February 2013 and April 2014 are illustrated in Figure 4.8.

Table 4.3 Results of PERMANOVA on sediment quality (February 2013 and April 2014) from the Subri River between location and time

Source	df	SS	MS	Pseudo-F	P(perm)	unique perms
Site	7	124.170	17.739	0.908	0.584	5345.000
Residual	3	58.613	19.538			
Total	10	182.780				
Location	2	30.816	15.408	1.610	0.226	9763.000
Time	1	59.687	59.687	6.238	0.0149*	9590.000
Location x Time	2	6.397	3.199	0.334	0.886	9777.000
Residual	5	47.839	9.568			
Total	10	182.780				
Control/Impact	1	3.021	3.021	0.265	0.863	560.000
Time	1	32.680	32.680	2.871	0.119	280.000
CIxTi	1	3.017	3.017	0.265	0.867	560.000
Residual	4	45.529	11.382			
Total	7	84.704				

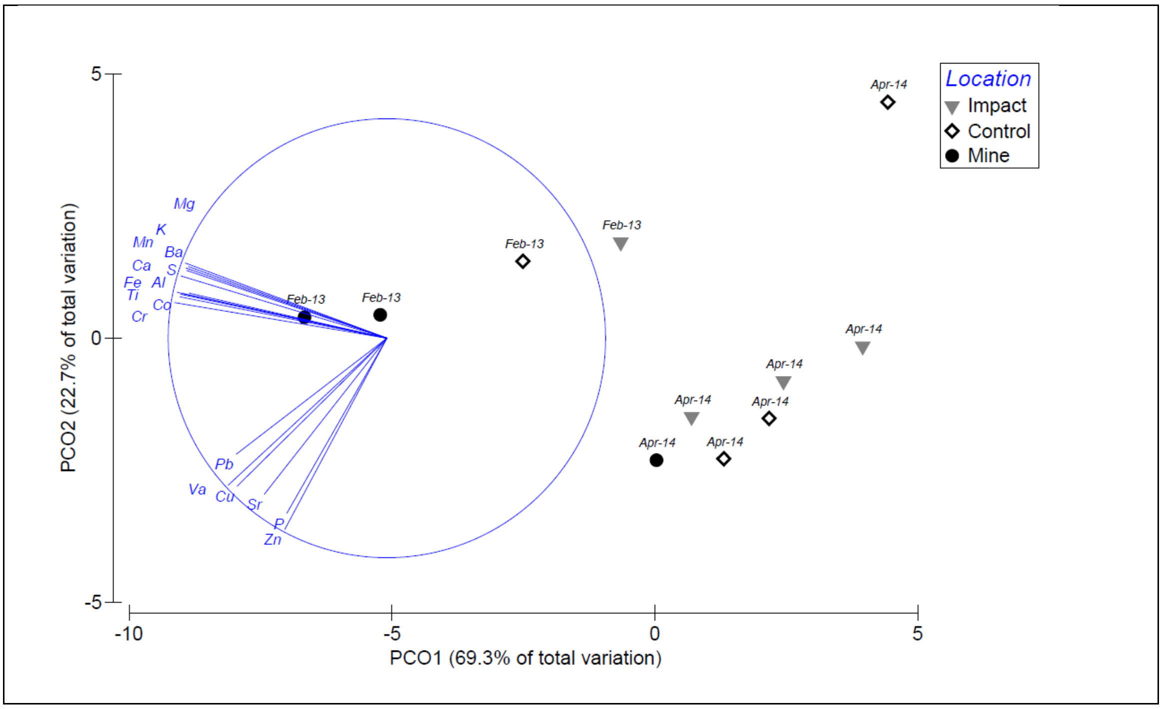


Figure 4.7 Principal component ordination of sediment metal and metalloid concentrations in February 2013 and April 2014 across control, impact and mine sites in the Subri River. Variables significantly (Pearson's r >0.4) correlated with the ordination space are shown.

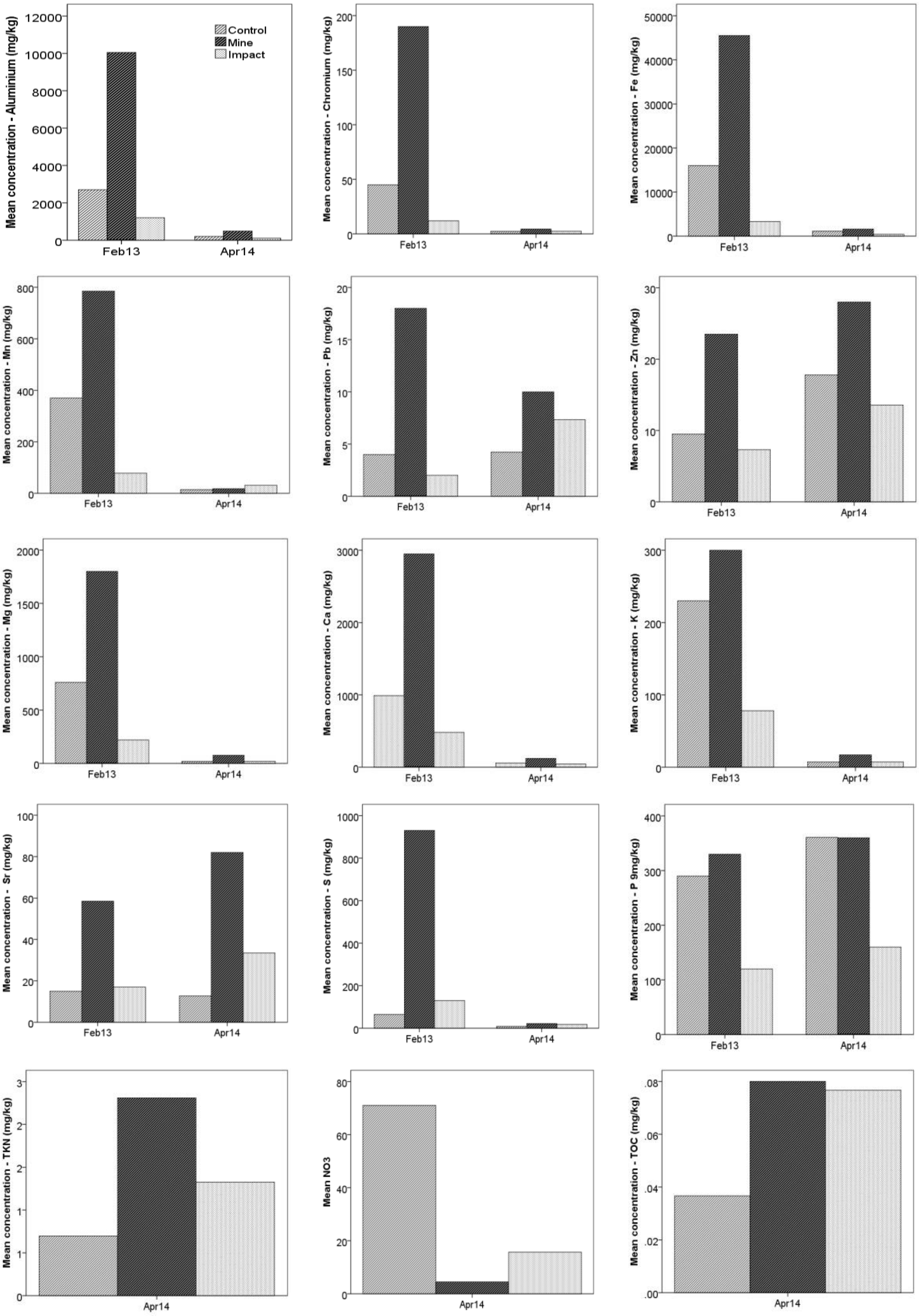


Figure 4.8 Mean concentrations of select metals and metalloids in Subri River sediments at control (medium grey), mine (dark grey) and impact (light grey) sites in 2013 and 2014. Nitrate, total Kjeldahl nitrogen (TKN) and total organic carbon were measured only in 2014.

4.3.2.2 Sediment characteristics: February 2013 – April 2014

Sediment complied with sediment quality guidelines for aquatic protection across all sites except for KSW3. In February 2013, concentrations of Cr at site KSW3 exceeded all guidelines and in 2014 elevated concentrations of Cu exceeded the lowest level effect or LEL (Persaud et al., 1993), minimal effect thresholds or MET (MENVIQ, 1992) and the threshold effect level on *Hyllalea azteca* on 28 days test or TEL_HA28 (MacDonald et al., 2000)(Appendix 26).

Concentrations of As, Hg, and Sb in the river sediment was below the detection limits of 2, 3 and 0.06 mg/kg respectively. Iron was the most abundant element in the sediment (6450 ± 3920 mg kg^{-1}), followed by Al (1340 ± 794 mg kg^{-1}), Ca (489 ± 295 mg kg^{-1}), Mg (288 ± 173 mg kg^{-1}), P (268 ± 57.8 mg kg^{-1}), Mn (97.3 ± 46.2 mg kg^{-1}), and S (95.7 ± 63.9 mg kg^{-1}). Potassium, V, Sr, Cr, Ba, Zn, Pb and Co were also detected in the sediment (See Appendix 24 for concentrations). Sediment concentrations of Fe, Al, Ca, Mg, Mn, S, K, Cr, and Ba were not evenly distributed across the sites along the river, with p (ANOVA) <0.01 and <0.05. Concentrations of P, Va, Sr, Zn, Pb and Co, on the other hand, do not significantly vary between sites along the river as tested with ANOVA.

The average index of geo-accumulation for Fe, Al, Mn, P, Ca, Mg, Va, Mn, Zn, Sr and S were > 5, indicating extreme contamination of the river by these elements, while the average index for As, Co, Hg and Ti were < 1 (Figure 4.9 A and Appendix 27) suggesting that the river generally was not contaminated with As, Co, Hg and Ti.

Although the I_{geo} values for many metals in the river were above 5, average enrichment factor (EF) for all elements across all sites was 1.6 (

Appendix 28 and Figure 4.9), indicating the absence of or a minor presence of anthropogenic modification to the concentrations of metals and metalloids in the sediment. In other words, the abundant presence of metals in the Subri River sediment mostly was not a result of anthropogenic sources. Site KSW2 on the main Subri River channel near to the confluence with the Tano River at Subrisu village, however, was an exception, with EF values for Ba, Co and Mn of 9, 13.5, and 11.8 respectively, indicating a severe to very severe enrichment. Based on the EF calculation, site KSW2 was also moderately polluted with Pb (EF= 3.5), Sr (EF= 3.2) and Mg (EF= 3.8).

Significant positive correlations between all elements measured, except for P, Sr, and S, in the Subri River sediment samples were evident. However, sediment concentrations of metals and metalloids were not correlated with the river water, either as total (unfiltered water) or dissolved (filtered water).

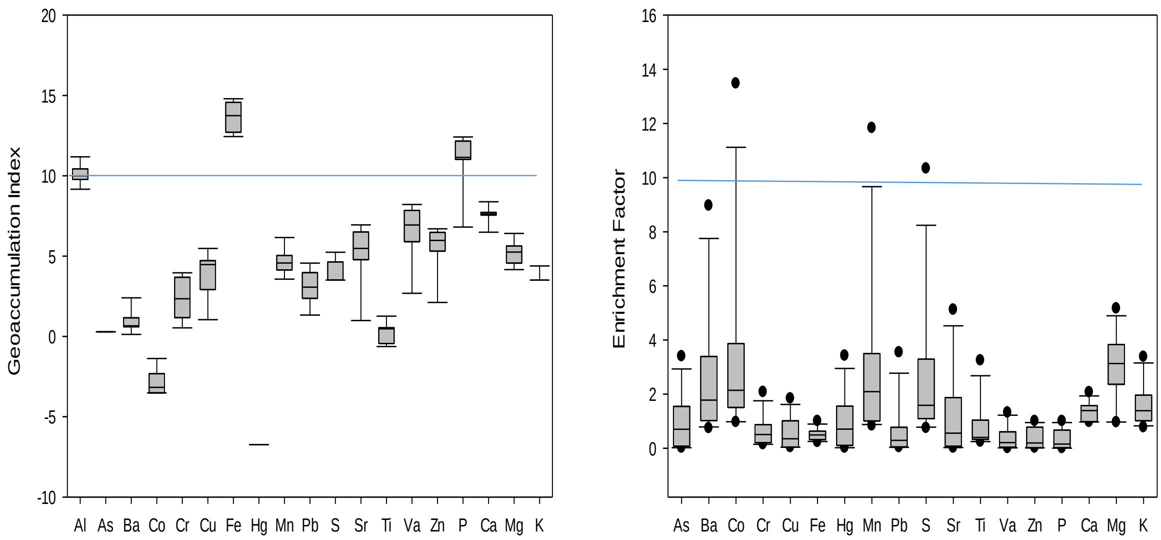


Figure 4.9 Geo-accumulation index (I-geo) and Enrichment Factor (EF) of the Subri River sediment

4.4 Discussion

The Ahafo mine changed the Subri River’s physico-chemistry parameters, increased concentrations of suspended and dissolved solids, salt ions, nitrate and sulfate in its mining impacted site. Elevated concentrations of pollutants in the water, however, were reversed or reduced by ECD 4 on the Asundua stream before entering the Subri River’s main channel downstream of the mine. This resulted in reduced concentration of most metals, TSS, turbidity in the water and improved sediment quality in downstream Subri compared to upstream and the mine site. Concentrations of salt ions, nitrate and sulfate at downstream Subri are also lower than the mine site. The impact of the mine varied by month and season, reflecting variations in precipitation. Concentrations of salts and dissolved metals increased in the rainy season, while concentrations of metals and metalloids in sediment decreased after a rainy season. The following sections discuss the identified impacts, its mitigation and implications.

4.4.1 Elevated salt ions and turbidity

The most obvious impact of mining on the Subri River water physico-chemistry were increased TDS, EC and the concentrations of Na, Ca, K, Mg, Cl, F, nitrate and sulfate. These major ions are typically high in aquatic environments impacted by mining activities (Kunz et al., 2013; Younger et al., 2002; Younger et al., 2004). At mining sites, salts are usually among the first elements to leach from exposed rocks, stockpiles of ores or tailings (Salomons, 1995) because of their hygroscopic natures, particularly in the presence of carbonic acid (Gorham, 1961). Pit walls are normally the main source of soluble salt released as pit water rises or through leaching by the rainfall (Bowell et al., 2005; Kwong et al., 1997; Pellicori et al., 2005; Price et al., 1998). At Ahafo mine, however, the pit water is contained or diverted into a water storage facility and not discharged into the ECDs or surrounding natural streams. Therefore, the sources of salt ions and elevated EC, alkalinity and TDS at the mine site (KSW3) are most likely from the burden, waste rocks and stockpiles that leach into run off water which then enters streams (Akcil et al., 2006; de Lacerda et al., 1998; Jeffery et al., 1988; Salomons, 1995), particularly in the rainy seasons.

The elevated EC and concentrations of major salts including Ca, Mg, and Na was positively correlated with pH which tended to be elevated around the mine site. Neutral to high pH is very desirable in gold metal mining areas, not only in preventing the creation of hydrogen cyanide (HCN) where cyanide is used as a reagent, but also to prevent the formation of acid mine drainage (Jeffery et al., 1988; Skousen et al., 1998). At Ahafo mine, the elevated pH at the mine site is probably also due to the addition of lime ($CaCO_3$) in the CIP gold processing to prevent the formation of toxic HCN (NGGL, 2005). Conductivity often exceeded 1500 µS/cm around the mine site, which can have negative effects on the freshwater ecosystems, particularly on biota with low tolerance to salts (Zalizniak et al., 2006). Nielsen et al. (2003) suggested that salinity of more than 1000 mg l^{-1} (1500 EC), which is also the maximum Ghanaian EPA standard value for mine discharges, will adversely affect the viability of eggs belonging to macroinvertebrates and seeds of aquatic plants. Management efforts to reduce elevated salinity in mine water before it enters the natural aquatic environments are therefore crucial to ensure long-term ecological integrity.

Mining activities increased turbidity in surrounding aquatic environments. At Ahafo, this is particularly evident in the first months of its site preparation in the end of 2006. Land clearing for mining and mining services facilities exposes land surfaces including the top soil to the rain, resulted in erosion, increased turbidity and sedimentation. This may also lead to increased concentrations of metal and metalloids in affected rivers surrounding the mine, which was also evident in the end of 2006 on the Subri River. This, however, discontinued when construction completed, probably also due to the sediment control systems put in place.

4.4.2 Elevated sulfate

The current mean (across all sites) sulfate concentration of 134 ± 13.0 mg l^{-1}, sulfate in the Subri River water was below the maximum level of 300 mg l^{-1} recommended by applicable water quality guidelines. However, exceedances in sulfate concentrations were also recorded 15% of the time, all of which took place at the mine site (KSW3). Similarly to Na, Mg and Ca, elevated concentrations of sulfate in water are common in mine sites. Although sulfate is naturally available in ground water (Skousen et al., 1998; Spalding et al., 1993) in areas with sulfate rocks such as at Ahafo, it is most likely leached from exposed soil and rocks including waste rocks and pyrite (FeS_2) ore stockpiles, following the formula given by Salomons (1995) explained in section 3.4 of Chapter 3. Leaching of sulfate from sulphite rocks is normally accelerated in acidic environments; nevertheless, the leaching can take place at any pH both abiotically and / or biotically by microorganisms (Salomons, 1995), including in environments like the Ahafo mine.

Sulfate pollution in mining areas can also be an indication of acid mine drainage, particularly in acidic environments. The presence of favourable microbes and Fe, which is naturally abundant in the study area, can accelerate the creation of acid mine drainage which can have deleterious effects on freshwater ecosystems (Jeffery et al., 1988; Salomons, 1995). As previously discussed in Chapter 3, the intensity of the acid generating process depends on the availability of factors that promote and control production of acids. Promoters of the production of acid include availability of pyrite, oxygen and water, physicochemical factors such as temperature and pH, surface area of exposed metal sulphide materials, and population density of bacteria and nutrients availability. On the contrary, the controllers of the production of acid include the availability of acid neutralising minerals such as calcites, dolomites, or carbonates of Fe, Sr or Mn (Salomons, 1995; Skousen et al., 1996). Given the availability of pyrite, high rainfall and warm temperatures typical of tropical West Africa, the high sulfate concentrations at Ahafo have probably resulted from the formation of AMD in the area. However, a high pH of 7.37 ± 0.025 and availability of the neutralising minerals including carbonates of Fe, Sr and Mn, calcites and dolomites which are the underlying geology of the area (Banoeng-Yakubo et al., 2009) prevent substantive acidification. The abundance of Fe, Sr and Mn in the Subri River sediment, therefore, can be a suggestive of abundant availability of the neutralising materials of carbonates of Fe, Sr and Mn. Further, the lack of acid mine drainage within the Subri River system may also be due to the prevention measures applied by Ahafo mine. At the mine, alkaline materials (e.g. lime) are used to increase acid neutralising capacity of the environment along with the encapsulation of AMD generating rocks in materials to prevent contact with O_2 and water, and regular monitoring (See Lottermoser, 2012; Salomons, 1995; Skousen et al., 1998).

The present lack of AMD at Ahafo, however, should not be taken for granted because acid mine drainage can take place long after a mine ceases (Salomons, 1995), even at mine sites previously thought to have high pH environment and non-AMD generating properties (Skousen et al., 1998). Given the high rainfall at the Ahafo mine, leaching of sulfate and iron from the exposed rocks and pit walls may intensify with time, particularly as the pits gets deeper and groundwater rises to form pit lakes (Bowell et al., 2005; Pellicori et al., 2005; Price et al., 1998). Given the net-positive water balance at the mine, excess water from the water bodies at the mine most likely would also have to be discharged or spilled over into the natural water bodies surrounding the mine, potentially polluting the environment. Consequently, management of mine water, particularly with regards to sulfate, as well as continued monitoring are crucial in the prevention of AMD at Ahafo mine.

4.4.3 Elevated nitrates

The average nitrate concentration along the river of 8.13 ± 1.24 mg l^{-1} suggests that the nitrates concentrations in the river's water were generally high, although remained under the standard (Ghana EPA) value for protection of aquatic life of 16 mg L^{-1}. Elevated nitrate in the river water may be due to natural occurrences (Fahrner, 2002; Spalding et al., 1993) or other anthropogenic impacts such as farming (Chang et al., 2002). However, unlike in the Surow River where nitrate did not significantly vary with site and was not impacted by mining, downgradient increase in the concentrations of nitrates in the Subri River strongly support impact from mining. This is corroborated by the finding that exceedances above the Ghanaian guidelines were recorded 10% of the time, particularly at the mine site (KSW3), suggesting the mine as the potential source of nitrate pollution.

In mining areas, including Ahafo, the main source of elevated nitrate is the detonating agents used in mining that commonly contain ammonium nitrate, calcium nitrate or sodium nitrate (Huisman et al., 2006; Koren et al., 2000; Zaitsev et al., 2008). According to Forsyth et al. (1995), nitrates can be introduced into the mine water in the mine works (pits or tunnels) or at waste rock disposal areas, originating from spillage during transport or charging, leaching of explosive in wet blast holes or residual undetonated explosive agents in blasted broken rocks. While improvements in mine blasting efficiency have been the subject of study among mining engineers and specialists, nitrates in mine waters continue to pose challenges because elevated nitrates are toxic to biota and can create eutrophication which poses a serious threat to freshwater ecosystems (Chang et al., 2002; Jarvis et al., 1997; Kunz et al., 2013; Lamers et al., 2002). The adverse effects of chronically elevated nitrate on freshwater macroinvertebrates and macrophytes have been widely studied (Camargo et al., 2006; Camargo et al., 2005; Chang et al., 2002; Jarvis et al., 1997; Soucek et al., 2005). The concerns with eutrophication and elevated nitrates in aquatic environments are highlighted by their ability to be transported down the river into the receiving marine ecosystems as well as terrestrial environments (Burgin et al., 2007; Smith et al., 1999). Therefore, although the current water management systems at Ahafo have been reducing the concentration of nitrates in effluent water downstream of the mine, a conservative approach to reduce nitrates at the source to prevent further increase in nitrates in mine waters should be taken. At Ahafo, this is especially important considering the long projected life of the mine and the likelihood of a net positive water balance at the site. The excess water can fill up mine pits and other water storage facilities on the mine which heightens the potential for leaching of more nitrates from the pit walls and exposed rocks, and subsequent decant to the surrounding aquatic ecosystems. Removal of nitrates from mine water before it leaves the mine therefore is crucial, especially whilst blasting efficiency is yet to be improved.

Various technology to remove nitrates from water are available, which include the use of zeolite (Bhatnagar et al., 2011; Guan et al., 2010), ion exchange and reverse osmosis (Häyrynen et al., 2009; Malaiyandi et al., 1981), filtration using membrane technology (Awadalla et al., 1994) and biological processes using denitrification bacteria including *Nitrosomonas* and *Nitrospira* (Koren et al., 2000; Zaitsev et al., 2008). Although application of biological removal of nitrates has been practised in municipal and industrial waste water treatments, it has not been widely used in mining. In temperate regions, the application of biological process to remove nutrients in water is often limited by the lack of organic carbon supply and the low ambient temperature. On the other hand, in the warm, tropical and fertile regions like Ghana, the biological process using bacteria alone or combined with phytoremediation to remove nitrates from water in mine impacted areas should have a much better chance for success (Mattila et al., 2007; Zaitsev et al., 2008).

4.4.4 Metals and metalloids

Concentrations of metal and metalloids, including Fe, As, Cu, Al and Se, in the Subri River water were increased by the mine. At mine site, the main source of metal and metalloids in surrounding water is mineralisation / leaching of metal and metalloids from ore stockpiles, rocks in open pits and the waste rock facility (Bowell et al., 2005; Bright et al., 1994; Luo et al., 2008; Smedley et al., 2002). At the Ahafo mine, this was seen in the increased concentrations of Fe, Al, As, Cu, Se, Zn in water at the mine site (KSW3) although they decreased downstream. An increase in the concentrations of Fe and As, however, was particularly obvious even before the mine operations commenced (Figure 4.6). Although the groundwater in the study area were typically characterised by elevated concentrations of Fe and As (Agyarko et al., 2014; NGGL, 2005), the elevated concentrations of Fe and As could have resulted from land clearing and construction works in the last quarter of 2006, prior to the commencement of the mine's operations in December 2006.

Beyond the mine site, the overall results indicated a decline in the concentrations of metals and metalloids in the river water compared to the mine site to levels and similar to or lower than upstream levels, suggested a positive impact of the water management systems and sediment control measures applied at the mine. For example, the concentrations of Fe and Mn at sites downstream from the mine are lower than that of upstream sites (see Figure 4.5 and Figure 4.6). The general decline in total metal concentrations downgradient of the mine site most likely was the result of the precipitation of metal and metalloid particulates in the ECDs which may have been supported by the increased salinity and alkalinity in the water around the mine site. Flocculation of metal and metalloid particulates with organic materials in river water is known to accelerate in the presence of salt ions (see Sholkovitz, 1976; Sholkovitz et al., 1981). Metal and metalloids also tend to precipitate with increasing pH to > 7 (Al-Abed et al., 2006; Smedley et al., 2002), as evident in the negative correlations between concentrations of select metal and metalloids including Fe, Al and As with pH in this study. The sediment control measures at the mine, the collection and settlement of mine runoff water in the ECDs, the increased salinity and pH of water around the mine, therefore, have assisted in the improvement of mine water quality with regards to concentrations of metal and metalloid.

4.4.5 Sediment quality

The relatively low concentrations of contaminants in the Subri River water was also reflected in the quality of its riverine sediments except for that of in the mine area. The Subri riverine sediment quality complied with sediment quality guidelines for aquatic ecology at all sites but the mine site (KSW3) due to elevated chromium in February 2013 and elevated copper in April 2014. The study demonstrated that, unlike the ASGM discussed in Chapter 3, the Ahafo mine did not negatively impact the Subri River's sediment quality as evident in the non-significant difference between control and impact sites. This, most likely, is attributable to the sediment control measures employed by the mining company. The sediment control measures at Ahafo, beside the ECDs, include the application of best management practices (BMP) which stipulate, amongst others, that land clearing is only done when necessary during the dry season, revegetation of the disturbed area is to commence immediately, and placement of silt fences and straw bales down-slope of disturbed area (NGGL, 2005).

Sediment quality, however, differed with time, probably reflecting the difference between dry and rainy seasons, particularly at the mine impacted sites. Concentrations of almost all metals and metalloids in the river sediment were significantly lower in the rainy season, suggested the scouring effects of the intense rain which leads to transport of materials downstream, typical of tropical West African conditions (Hayward et al., 1987; Windmeijer et al., 1993). The effect of the rain was more prominent at sites downstream from the mine site than it was at control sites, possibly due to the addition of the larger amount of water being discharged from the ECDs in the rainy seasons.

The transport of materials down the river was also apparent in the analysis of index of geo-accumulation and enrichment factors in sediment down the river. This study showed that the riverine sediment across all sites was contaminated ($I_{-geo} > 5$) with Fe, Al, Mn, P, Ca, Mg, Va, Mn, Zn, Sr and S. The abundant presence of metals in the Subri River sediment, however, was not a result of anthropogenic modifications as indicated by average enrichment factor (EF) < 2. This finding suggested that the enrichment of riverine sediment by the elements was of natural causes, such as the natural weathering of underlying rock, scouring and transport effects of flooding commonly seen in the tropics (Junk et al., 1989). While the concentrations of metals in sediment at almost all sites were not resulted from anthropogenic modifications, site KSW2 in downstream Subri River channel was an exception. Enrichment factor values at KSW2 suggested that the site was the most polluted of all sites along the river with Ba, Co, and Mn, and moderately polluted with Pb (EF= 3.5), Sr (EF= 3.2) and Mg (EF= 3.8). The elevated concentrations metals at KSW2 indicated sediment transport down the river and accumulation of material near the bottom of the Subri River, although the site's close proximity with the river's confluence with the Tano River may have also contributed to presence of the pollutants. The Tano River is a much larger river than the Subri River and drains a larger catchment area, and therefore is potentially more exposed to pollutants including Ba, Co, Mn, Pb, Sr and Mg, which could be deposited onto its floodplains and tributaries including the Subri River during flood events (Förstner et al., 2004; Nakamura, 2003; Stewart et al., 1998). The contamination at KSW2 needs to be addressed by the local and regional environmental managers in the area, due to possible accumulative impacts in the Subri River, but also in the Tano River basin and beyond.

4.4.6 Environmental control dams (ECD)

A feature of the Ahafo mine that separates its impact mitigation measure on the Subri River from that of ASGM on the Surow River is the ECDs on the Subri. Turbidity, TSS, EC and the concentrations of nitrates, sulfate, metal and metalloid elements in the Subri River downstream of the mine site and ECDs were similar to or lower than upstream, and lower than the mine site, signifying the roles of the ECDs in preventing the elements released by mining from entering the Subri River. This, suggests that the ECD is effective in reducing the pollutants concentrations in downstream Subri compared to that of the mine site, by allowing particulates and associated metals and other ions to settle, whilst allowing dissolved ions, except for NOx and sulfate, to pass through untreated. The concentrations of Ca, Mg and dissolved Fe, Al, and Sb persisted beyond the ECDs, although their concentrations remained below the maximum levels for protecting aquatic environments as required by the Ghanaian EPA guidelines. The major salt ions contributing to river water's conductivity, in high concentrations, can also be toxic to biota as well, with ionic stress shown to be toxic to mussel, amphipod, cladoceran and mayfly (Kunz et al., 2013). Dissolved metals are more bioavailable than in their particulate form (Salomons et al., 1980), and aquatic biota takes up and accumulates metal, whether they are essential or not, which can have detrimental effect on survival or reproduction (Harding, 2005; Hare, 1992; Maret et al., 2003; Rainbow, 2007). Consequently, monitoring of water quality in the ECDs for dissolved ions prior to discharge is important due to potential toxicity downstream.

Nitrate and sulfate, elevated at the mine, are also reduced by the ECDs, possibly due to the presence of bio-geochemical processes involving sulfate, iron and nitrate reducing bacteria in the systems (Lyew et al., 2001; Mattila et al., 2007; Zaitsev et al., 2008). Much of the microbial cycling of sulfate, iron and nitrate takes place in the upper layers of sediment in aquatic environments (Küsel, 2003; Lambertsson et al., 2006a) so that surveying of microbial community and sediment characteristics of the water storage facilities at the mine including the ECDs would be beneficial to better understand and potentially optimise the processes.

The improved water and sediment quality downstream from the ECDs compared to of the mine site and upstream, is expected to alter the river ecology, including macroinvertebrate and microbial communities in the river, which are discussed in Chapter 6 and 7. The potential adverse ecological effects of dams (Anderson et al., 2006; Baxter, 1977) of both ECD 6 (upstream of NSW6) and ECD 4 (upstream of NSW8) should also remain a consideration. The effects of stream damming and flow regulation on the river ecology have been studied extensively (Anderson et al., 2006; Benstead et al., 1999; Concepcion et al., 1999; Greathouse et al., 2006). The direct ecological effects of dams include fragmentation of habitat, blockage of migration routes, mortality of larvae and juveniles at water intakes, alteration of natural hydro-geomorphic regimes, declines in biodiversity, alteration of natural food webs, and the shift in the water physico-chemistry of the coastal zones (Baxter, 1977; Benstead et al., 1999; Poff et al., 2002). Given their small sizes (average capacity of less than 15 ha each), the ECDs may have effects on the local hydrology, productivity and diversity of riverine biota including microorganism, macroinvertebrate and fish (Ligon et al., 1995; Poff et al., 2002; Power et al., 1996), although there is a potential for full recovery after removal of small dams (Doyle et al., 2005). Therefore, although the ECDs have proven to be beneficial in reducing mine-associated pollutants from entering the natural aquatic environments, the biological effect of the ECD on the riverine ecosystem components including macroinvertebrate and microbial communities should also be monitored. This is because the biological disturbance can have different effect on selectiveness (Poff et al., 2002) and more interactive effects of productivity on biotic assemblages and species diversity (Svensson et al., 2010) than that of physico-chemical disturbance. The biological effects also usually take longer to rehabilitate. Biological monitoring of the ECDs affected streams is important not only to ensure minimal disturbance to the current ecological integrity of the Subri River and beyond (See Ligon et al., 1995), but also at closure or removal of the dams at mine closure (Doyle et al., 2005).

4.5 Conclusion

The Subri River water quality was impacted by the Ahafo mine's operations in its catchment. Significant increases in conductivity, TDS, (as EC and TDS), alkalinity, concentrations of major ions of Na, Ca, Mg, sulfate, nitrates, chloride and fluoride were evident in the mine sites and impacted sites downstream from the mine. In spite of the elevated concentrations of salt ions and the temporarily increased concentrations of metals including Fe due to land clearing and mine site preparation; the mine's environmental management and impact mitigation measures including the ECDs had proven effective in mitigating the impacts of mining on the river water's quality in downstream Subri. Turbidity, TSS, and concentrations of total As, Fe, Cl, Pb, and Mn in downstream Subri were reduced to levels similar to or lower than upstream and before mining.

Despite the spatial and temporal changes due to gold mining operations, water quality of the Subri River complied with the Ghanaian EPA water standards for mine related discharges and Ghana Water Company Guidelines for water to be processed as drinking water most of the time. However, exceedances in the concentrations of sulfate, nitrate, EC and TDS did take place particularly in the mine site, indicating the potential for the leaching and release of elevated sulfate, nitrate and salt ions from the mine that could have detrimental effect in the long run. Control and management of sulfate, nitrate and salt ions in mine water, therefore, should be the target of Ahafo mine's water management systems.

The sediment control measures applied by the mine have also proven effective in preventing and if any, mitigating impacts of the mine on sediment quality. Similarly to the water quality, riverine sediment quality complied with sediment quality guidelines for aquatic ecology at all sites. The mine did not appear to impact riverine sediment quality in the area, although down gradient and lateral transport of sediment materials by flood events was possible, resulted in possible accumulation at the river mouth.

5 Mercury biomagnification and bioaccumulation in fish from the Surow and Subri rivers

5.1 Introduction

At places with limited analytical laboratory capacity, such as ASGM impacted areas in developing countries, detection and speciation of mercury in natural water samples is often too difficult and expensive to be practical. As a consequence, environmental Hg is often unmonitored. The main concern with Hg pollution is its capability to biomagnify and bioaccumulate in aquatic biota (Amisah et al., 2011; Bryan et al., 1992; Pereira et al., 2010; Rainbow et al., 2011).. This chapter focuses on bioaccumulation and potential biomagnification of metals potentially discharged by AGSM activities in the local area, particularly Hg, in fish caught in the Surow and Subri rivers. While Hg is not used in the modern gold mining operations on the Subri River's catchment, it is used in ASGM operations on the Surow River catchment to extract gold and may be released into the aquatic environment during the pre-concentration process of slurry and the open air amalgamation. Additionally, smelting of the amalgam in the towns within the catchment releases Hg to the atmosphere creating an aerial source, which can eventually reach waterways (Barbosa et al., 2003; Brabo et al., 2003; Telmer et al., 2006b; Telmer et al., 2009).

Bioaccumulation of metals in aquatic biota is a complex process (Luoma et al., 2005) controlled by a large number of factors, including but not limited to species, trophic position, and specific regulatory mechanisms within the body (Rainbow, 2007) as well as other environmental factors governing the bioavailability of the metals (Bryan et al., 1992). Bryan et al. (1992) asserted that metal bioavailability was influenced by (1) mobilisation of metals to water and their speciation, (2) transformation (e.g. methylation), (3) control played by other elements in sediment such as Fe and Mn to which metals can be bound, (4) competition between metals for uptake sites in organism, (5) bioturbation, and (6) physico-chemical parameters such as salinity, pH, and redox. Mercury can concentrate in fish to a level that may harm the fish and other animals that eat fish. Fish eating birds and mammals have been identified as at risk, so are their predators. Mercury has been found, for example, in water and terrestrial birds (Aazami et al., 2012; Eagles-Smith et al., 2009; Jackson et al., 2011), eagles (Scheuhammer et al., 2008), seals and other endangered animals including polar bears (Atwell et al., 1998) and panthers (Barron et al., 2004). Concentration of metals in fish has therefore been used to establish levels of toxic metal pollution in aquatic ecosystems and their potential impacts on both environmental and human health.

In the environment, Hg occurs in three valence states; i.e. pure or elemental (metallic) (Hg^0), monovalent (Hg(I)) and divalent (Hg(II)). Hg(I) and Hg(II) may be present in various physical and chemical forms as inorganic and organic mercury compounds or salts which include mercuric sulphide, mercuric chloride, phenylmercury, and methylmercury (Leermakers et al., 2005; Ullrich et al., 2001; UNEP, 2002a). The solubility, mobility, and toxicity of Hg in aquatic ecosystems depends on the nature and forms of Hg. Elemental Hg and organic methyl and dimethyl Hg (MMHg, DMHg respectively) form dissolved Hg found in aquatic systems. While metallic Hg is not soluble in water, it readily forms ions that are soluble in water. It also vaporises at room temperature releasing mercury into the atmosphere which can eventually be re-deposited in aquatic systems. Between 10 to 30% of the dissolved Hg in waters is in the form of elemental Hg (Ullrich et al., 2001). MMHg and DMHg naturally occur in aquatic systems, formed after being transformed from inorganic Hg through various bio-geochemical processes (Figure 5.1). While DMHg is the dominant methylated Hg species in deep ocean waters, MMHg is more common in freshwaters and estuarine systems (see (Leermakers et al., 2005).

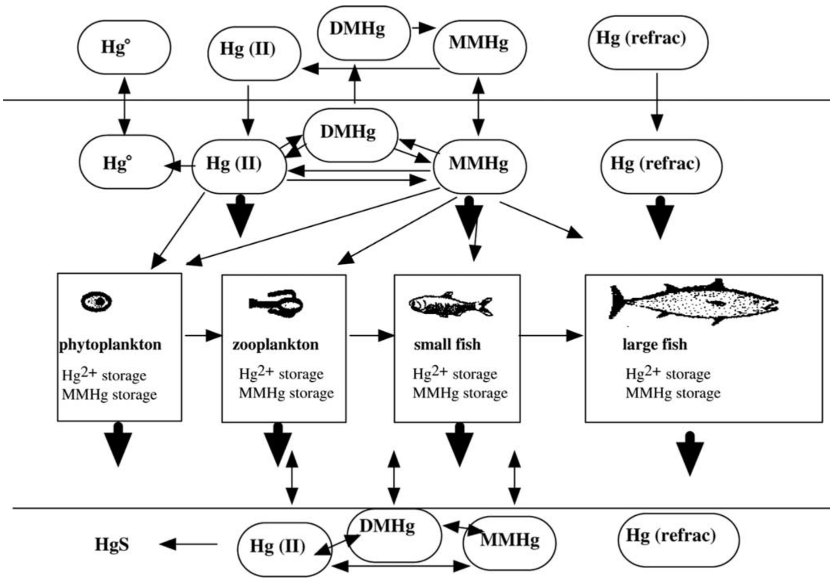


Figure 5.1 Hg transformation and biomagnification in the aquatic systems (Leermakers et al., 2005)

Methylation of inorganic mercury to MMHg is a complex process, generally mediated by the sulfate reducing bacteria (SRB) in the sediment and to a lesser degree in the water column. Although the presence of Hg is a prerequisite for MMHg production, factors such as redox potential, sulfate concentrations, pH, dissolved organic carbon (Lambertsson et al., 2006b; Regnell, 1994), and nutrient concentrations (Cleckner et al., 1999) which affect SRB are important in Hg methylation activities in freshwater and estuarine ecosystems (Lambertsson et al., 2006b). At low concentrations sulfate can stimulate methylation (Benoit et al., 1999b; Gilmour et al., 1992; Jeremiason et al., 2006). At high sulfate concentrations, however, MMHg production is inhibited by the accumulation of sulphide (Gilmour et al., 1991; Lambertsson et al., 2006b). Gilmour et al. (1992) proposed a sulfate concentration range of 0.2 to 0.5 mM SO_4^{2-} as optimal for methylation. Fresh organic material availability in the bottom sediment of aquatic ecosystem is important for creating the redox conditions necessary for sulfate reduction which influences the Hg methylation rate (Lambertsson et al., 2006b). Therefore, flood or high flow events which alter the sediment morphology of a river also strongly influence Hg methylation (Barbosa et al., 2003; Bastos et al., 2007; Brabo et al., 2003; WHO, 1989, 1990). MMHg concentrations in the water column of a stream was seasonal (Schuster et al., 2008) and strongly negatively correlated with eutrophication level (Hurley et al., 1998). Local-scale environmental factors including levels of Hg deposition were also important to Hg bioaccumulation particularly in a topographically heterogeneous landscape (Riva-Murray et al., 2011). Studies showed that Hg concentrations in fish responded rapidly to an increase in Hg input in freshwater ecosystems. For example, the addition of enriched stable Hg isotopes to a lake in Northern Ontario, Canada, and its watershed resulted in a significant increase in MMHg concentrations in algae and benthic macroinvertebrates, and in Total Hg in fish within three years (Harris et al., 2007; Paterson et al., 2006).

Although Hg is poisonous in all forms, MMHg is the form of most concern in aquatic ecology due to its high toxicity, bioavailability, and potential to biomagnify (Leermakers et al., 2005). MMHg is a neurotoxicant, mutagen, teratogen and carcinogen (Eisler, 2004; Wolfe et al., 1998). Lethal concentrations of Hg to sensitive aquatic organisms range from 0.1 to 0.2 µg/L. Sub-lethal effects of Hg on fish and other aquatic biota include inhibition of reproduction, reduced growth rates and reduced ability to capture prey (Eisler, 2004). In birds and mammals, MMHg at very low concentrations can adversely affect metabolism, histology, reproduction, growth development, and motor coordination (Eisler, 2004; WHO, 1990). The reproductive effects of MMHg in mammals range from behavioural deficit after birth to foetal death (Wolfe et al., 1998), while in birds reduced egg production and poor hatching success have been reported (Seewagen, 2010).

The study of Hg and MMHg concentrations in fish has mostly concerned marine species used for human consumption. Nevertheless, MMHg in freshwater ecosystems has also been increasingly studied, particularly in places where Hg emissions are known to be an environmental issue. In countries such as Brazil and Indonesia, for example, elevated Hg concentrations were reported in freshwater ecosystems affected by ASGM practices (Bastos et al., 2007; Castilhos et al., 2006; Telmer et al., 2006b; Tschakert, 2010). Other fish studies in ASGM impacted rivers such as the Pra River in Ghana, however, reported fish Hg concentrations below the WHO recommended limit of 0.5 µg g^{-1} for consumption (Donkor et al., 2006; Oppong et al., 2010). In West African communities including those surrounding the ASGM communities in Ghana, freshwater fish caught from the wild are an important source of protein (Brashares et al., 2004; Kadye et al., 2012; Oppong et al., 2010).

This chapter aims to determine the potential for Hg biomagnification and bioaccumulation in fishes caught in the Surow and Subri rivers. In addition, concentrations of arsenic (As), cadmium (Cd) and lead (Pb) in fish tissues were also investigated. Specific objectives of this study are: (a) to measure the effects of fish weight, length, and trophic levels on Hg, As, Cd and Pb concentrations in fish muscular tissue; and then (b) to compare the effects between rivers, fish foraging habits and trophic levels. My hypothesis is that fish in the Surow River show evidence of Hg bioaccumulation and biomagnification due to the presence of AGSM, but that will not be the case for the Subri River where Hg is not used in mining.

5.2 Methods

5.2.1 Study and sampling design

Concentrations of metals (As, Cd, Pb, Hg) in muscle tissue of fish samples from the Surow and Subri rivers were analysed to determine their potential to bioaccumulate with weight and total length (surrogate measure of fish age) and biomagnify through the trophic levels. Correlations between metal concentrations and fish weight, total length and trophic levels were used to predict the effects of fish weight and age on fish metal concentrations (bioaccumulation) and effects of trophic levels on fish metal concentrations (biomagnification). Results from each river and fish grouping (by foraging habit and diet) were then compared to each other to detect effects variability.

Local belief in the Brong Ahafo region forbids fishing on and consumption of fish from the upper Tano River, although fishing from its tributaries is allowed. Bibiani (Western Region), is located about 50 km south of Hwidiem, where fishing on the Tano River is not restricted. Fishermen on the Subri River normally work near to the confluence between the Subri and the Tano rivers where water levels support fishing almost all year round. Fishermen on the Surow River also work on the reaches approaching its confluence with the Tano River as well as on the swampy part of the Surow near to sampling site 11 (see **Error! Reference source not found.** and Figure 3.3 in Chapter 3). Fishing from sampling site 11 on the Surow River, was seasonal (only during the rainy seasons). At the Surow, Subri, and Tano rivers, catches of the day are usually sold to customers waiting on the river shores when fishermen pulled in their catches in the morning (Figure 5.2 A and B) or smoked to preserve them (Figure 5.2 D).

A B

C

D

Figure 5.2 Fisherman at the Tano (A and B) River with bamboo fish traps commonly used in the region (C). A fisher wife at Subrisu fishing village on the Subri River smokes fish to preserve catch of the day (D).

Fish samples were purchased from fishermen working on the Surow and Subri rivers at Hwidiem Township on three days over a two week period in July 2013 and once in January 2014 from a Tano River fisherman near to Bibiani. Fresh fish were purchased in the mornings not targeting specific species, sizes or number of samples. At the time of purchase, each fish was labelled with sample number, source (river) and date of sampling, identified, photographed for possible further identification, weighed, placed individually in a clean Ziploc bag and packed in an ice box for transport to the laboratory for further processing and analysis. Fish were measured for total length at the time of purchase or within hours of purchase. Methods of handling, packaging, scaling/skinning, filleting, and storage of samples followed USEPA (2000, 2001).

5.2.2 Analysis

Further identification and validation using photographs of samples was performed by Mr. Edem Kwami Amedorme, the ichthyologist at the Fishery Division of the Water Research Institute (WRI) within the Ghana Council for Scientific and Industrial Research (CSIR) in Accra using keys provided by Paugy et al. (2003). Trophic levels and foraging habit information (habitat hereafter) were acquired from Fishbase.org (Froese et al., 2008), whose database is widely accepted and used in fish research (Reynolds et al., 2005; Romanuk et al., 2011). The trophic levels in Fishbase database were calculated by adding 1 to the mean trophic position of all food items consumed by a fish species, weighted by relative abundance (Pauly et al., 2002) and cross-validated with nitrogen stable isotopes by Kline et al. (1998) and Mancinelli et al. (2013).

At the SGS laboratory in Tema, Accra, fish tissue samples were digested for As, Cd, and Pb analysis following USEPA Method 3050 and for Hg using the USEPA 7471 Method. The total concentration of Hg was analysed by Cold Vapour Atomic Absorption (CVAA) spectrometry with limits of detection (LOD) of 0.05 $mgkg^{1}$, while As, Cd and Pb were determined on ICP-OES with a LOD of 0.05, 0.05 mg kg^{-1} and 1 mg kg^{-1} respectively. Concentrations of Hg in fish refers to MMHg, either directly measured as methyl mercury (MMHg) or as Total Hg (THg). As MMHg is the form of Hg that is accumulated in biota samples and MMHg comprises >95% of total Hg in fish tissue (Bloom, 1992; Grieb et al., 1990), fish tissue samples were analysed for THg.

5.2.2.1 Data analysis

Descriptive statistics and hypothesis testing were undertaken with SPSS Statistics (v 22, IBM). Metal/metalloid concentrations below limits of detection (LOD) were substituted by LOD/√2 (Croghan et al., 2003; Verbosek, 2011). Prior to analysis, data was tested for homogeneity using the Levene's test and Non-Parametric Levene tests (Nordstokke et al., 2010) and normality via the Shapiro-Wik test. Non homogeneous and non-normal data were log10 transformed. After transformation, the variances in fish length and weight were homogeneous and normally distributed by rivers and trophic levels. Mercury concentrations, however, remained non-homogeneous and not normally distributed after transformation. Given this, non-parametric statistics were applied to mercury concentrations following Luengo et al. (2009) and Pereira et al. (2010)

Analysis of variance (ANOVA) with Bonferroni *post hoc* tests were performed to compare the fish weight, length, concentrations of metal/metalloids, trophic levels, taxa, and habitat among rivers. For variables that were not homogeneous or normal after transformation, non-parametric statistics including Mann-Whitney and Kruskall-Wallis tests and Spearman's correlations were used instead of ANOVA and Pearson's correlations (Luengo et al., 2009). Simple linear regression analysis was employed to analyse correlations between metal/metalloid concentrations, trophic levels, fish weight and length with a level of significance at $p<0.05$.

5.3 Results

5.3.1 Fish taxa

A total of 60 fish were collected across the three rivers, 15 species from 10 families and 5 classes were identified, with Cichlidae (n = 24), Clariidae (n =11) and Claroeitidae (n = 8;Table 5.1) being the most common families. Fish samples from the Surow River (n=19) consist of 8 species from 3 families and 3 classes and were dominated by Cichlidae or the Tilapia family (12 specimen). Almost all of the species obtained for the Surow River samples were also found in the Subri River samples; the exception was *Heterotis niloticus* (Figure 5.4 E) which was not found in both Subri and Tano River samples. Twelve out of the 19 Surow River samples were benthopelagic fishes and the rest were demersal with a mean trophic levels of 2.45 ± 0.13. From the 32 Subri fish samples, 14 species from 10 families and 4 classes were identified with fishes from Chichlidae family as the most common (12 out 32 samples), followed by Clariidae and Claroteidae. Five species, i.e. *Parachanna obscura, Ctenopoma petherici, Hepsetus odoe, Brycinus imberi* and *Barbus roseopunctatus* (Figure 5.4 A to D), found in the Subri River samples were not found in either Surow or Tano river samples. Unlike the Surow River samples, the Subri River samples contained more demersal (18 out of 32) than benthopelagic species, and more carnivorous than herbivorous species with a mean trophic level of 3.08 ± 0.11.

Fish weight, length and trophic levels were significantly different between the two rivers (Mann-Whitney $p<0.01$; $p<0.05$; and $p<0.01$ respectively); fish samples from the Subri River were also of significantly higher trophic levels, as well as smaller and shorter than that of from the Surow River. Three species were identified from the 9 Tano River fish samples, all but one was also seen in the Subri River samples. *Synodontis bastiani* (Mochokidae) was the only benthopelagic in the Tano River samples and was absent from both Surow and Subri river samples. All of the Tano River fish species were carnivorous (mean trophic level 3.2 ± 0.04) and two were demersal.

Table 5.1 Taxonomy, trophic levels, habitat type, weight, length, total mercury concentration in muscle tissue and number of fish samples collected from the Surow, Subri and Tano Rivers

Fish taxonomy, trophic level and habitat type	Parameter	N	Minimum	Maximum	Mean ± SE
Chromidotilapia guntheri	Weight (g)	9	17.0	50.0	33 ± 4.77
(F:Cichlidae, C: Perciformes); Trophic = 2.6,	Length (cm)	9	11.5	16.0	13.28 ± 0.53
benthopelagic	Hg (mg/kg)	9	0.10	0.30	0.19 ± 0.02
Heterotis niloticus	Weight (g)	2	1300.0	3900.0	2600 ± 1300
(F: Arapaimidae, C: Osteoglossiformes); Trophic =	Length (cm)	2	46.0	78.0	62 ± 16
2.6, benthopelagic	Hg (mg/kg)	2	0.20	0.40	0.3 ± 0.1
Clarias anguillaris	Weight (g)	6	150.0	1790.0	956.67 ± 272.28
(F:Clariidae, C: Siluriformes); Trophic = 3.3,	Length (cm)	6	22.5	63.0	48.08 ± 6.29
demersal	Hg (mg/kg)	6	0.04	0.60	0.37 ± 0.09
Oreochromis niloticus	Weight (g)	7	200.0	2420.0	700.29 ± 291.88
(F: Cichlidae, C: Siluriformes); Trophic = 2.0,	Length (cm)	7	23.0	66.0	33.29 ± 5.57
benthopelagic	Hg (mg/kg)	7	0.04	0.80	0.21 ± 0.1
Heterobranchus bidorsalis	Weight (g)	1	420.0	420.0	420
(F: Clariidae, C: Osteoglossiformes); Trophic = 3.7,	Length (cm)	1	36.0	36.0	3
demersal	Hg (mg/kg)	1	0.30	0.30	0.30
Tilapia zillii	Weight (g)	1	230.0	230.0	230
(F: Cichlidae, C: Siluriformes), Trophic = 2.5,	Length (cm)	1	24.0	24.0	2
demersal	Hg (mg/kg)	1	0.30	0.30	0.30
Clarias gariepinus	Weight (g)	4	110.0	560.0	395±98.25
(F: Clariidae, C: Osteoglossiformes)	Length (cm)	4	22.0	43.5	36.88±5.00
Trophic = 3.2, benthopelagic	Hg (mg/kg)	4	0.04	0.40	0.29±0.08
Sarotherodon galilaeus	Weight (g)	7	25.0	280.0	139.29±29.65
(F: Cichlidae, C: Perciformes, Trophic = 2.0,	Length (cm)	7	13.0	25.0	19.21±1.37
demersal	Hg (mg/kg)	7	0.04	0.10	0.09±0.008

Parachanna obscura (F: Channidae, C: Perciformes), Trophic = 3.4, demersal	Weight (g)	2	375.0	375.0	375 ± 25.28
	Length (cm)	2	34.0	36.0	35 ± 1.38
	Hg (mg/kg)	2	0.30	0.40	0.35 ± 0.14
Hepsetus odoe (F: Hepsetidae, C: Characiformes), Trophic = 4.1, demersal	Weight (g)	6	55.0	225.0	109.17 ± 25.28
	Length (cm)	6	22.0	32.0	25.67 ± 1.38
	Hg (mg/kg)	6	0.30	1.10	0.72 ± 0.14
Chrysichthys nigrodigitatus (F: Claroteidae, C: Osteoglossiformes), Trophic = 3.2, demersal	Weight (g)	8	40.0	500.0	238.13 ± 53.30
	Length (cm)	8	20.0	39.0	28.06 ± 2.36
	Hg (mg/kg)	8	0.30	0.70	0.49 ± 0.05
Ctenopoma petherici (F: Abanantidae, C: Perciformes), Trophic = 3.2, benthopelagic	Weight (g)	2	50.0	100.0	75 ± 25
	Length (cm)	2	14.0	20.0	17 ± 3
	Hg (mg/kg)	2	0.20	0.37	0.29 ± 0.09
Labeo roseopunctatus or *Barbus* (F: Alestidae, C: Cypriniformes), Trophic = 2.3, benthopelagic	Weight (g)	1	1750.0	1750.0	1750
	Length (cm)	1	51.0	51.0	51
	Hg (mg/kg)	1	0.10	0.10	0.10
Brycinus imberi (F: Cyprinidae, C: Cypriniformes), Trophic = 3.3, demersal	Weight (g)	3	50.0	80.0	63.33 ± 8.82
	Length (cm)	3	17.5	17.5	17.5 ± 0
	Hg (mg/kg)	3	0.10	0.60	0.40 ± 0.15
Synodontus bastiani (F: Mochokidae, C: Osteoglossiformes), Trophic = 2.9, benthopelagic	Weight (g)	1	45.0	45.0	45
	Length (cm)	1	22.0	22.0	22
	Hg (mg/kg)	1	0.70	0.70	0.70

A

B

C

D

E

F

Figure 5.3 Fish species commonly seen in the Surow, Subri and Tano Rivers fish samples
(A) *Sarotherodeon galilaeus* (B) *Oreochromis niloticus* (C) *Tilapia zilii* (D) *Chromidotilapia guntheri* (E) *Clarias anguillaris* (F) *Chrysichthys nigrodigitatus*

A

B

C

D

E

F

Figure 5.4 Fish species only seen in the Subri (A to D), the Surow (E) and the Tano (F) Rivers
(A) *Parachanna obscura*, (B) *Hepsetus odoe*, (C) *Brycinus imberi*, (D) *Barbus roseopunctatus*, (E) *Heterotis niloticus* and (F) *Synodontis bastilus*

5.3.2 Trace element concentrations

Concentrations of As, Cd, and Pb in all fish muscle tissue samples were below the LOD of 0.05 mg kg^{-1}, 0.05 mg kg^{-1} and 1 mg kg^{-1} respectively. Mean concentrations of THg across all samples was 0.33 ± 0.03 mg kg^{-1}, which is below the WHO recommended maximum concentration of 0.5 mg kg^{-1} for human consumption (WHO, 1991). Only 6 species had THg concentrations over the maximum levels for human consumption, they were *O. niloticus* (Surow River), *H. odoe* (Subri River; had the highest concentration at 1.1 mg kg^{-1}), *C. anguillaris* (Tano River), *S. bastiani* (Tano River), *B. imberi* (Subri River) and *C. nigrodigitatus* (Subri and Tano Rivers) concentrations.

Concentrations of THg varied significantly with species (Kruskal-Wallis $p<0.01$), family (Kruskal-Wallis $p<0.01$), trophic level (Kruskal-Wallis $P<0.01$), habitat (demersal and benthopelagic) (Mann-Whitney $p<0.05$) and rivers (Kruskal-Wallis $p<0.01$). Mean Hg concentration was 0.22 ± 0.04 mg kg^{-1} for the Surow River samples, 0.33 ± 0.05 mg kg^{-1} for the Subri River samples, and 0.55 ± 0.04 mg kg^{-1} for the Tano River samples. Mean Hg concentrations in fish muscle, did not differ significantly between the two rivers (Mann-Whitney, $p>0.05$).

Table 5.2 summarises the correlations between fish muscle Hg concentrations and fish weight, length and trophic levels in various data sets, whilst Figure 5.5 to Figure 5.6 show significant correlations. Analysis on all fish sample from the Surow, Subri, and Tano Rivers (N=60) demonstrated significant correlations between concentrations of Hg with total length (Spearman's $r = 0.36$; $p <0.01$) and trophic level ($r = 0.62$; $p<0.01$) as depicted in Figure 5.7 A and B. Concentration of Hg, however, was poorly correlated with fish weight. Among the carnivorous (trophic level ≥ 3) fishes across rivers, Hg concentrations (mean Hg concentration of 0.45 ± 0.05 mg kg^{-1}) were not correlated with fish weight, lengths and trophic levels. Similarly, Hg concentration in herbivorous (trophic level <3) fishes across rivers (with a mean Hg concentration of 0.19 ± 0.04 mg kg^{-1}) was not correlated with weight and length, but correlated positively with trophic levels ($r=0.54$, $p<0.01$). Strong correlations between Hg concentration with length and trophic level were seen in demersal fishes (Figure 5.7 C and D), whilst in benthopelagic fishes the correlation was only seen between Hg concentration and fish length.
In the Surow River (N=19) where most fishes were herbivorous, Hg concentration did not correlate with weight but correlated strongly and positively with length and trophic level with Spearman's $r= 0.57$, $p<0.5$ and $r=0.61$, $p<0.01$ respectively (Figure 5.5). Concentration of Hg in the Subri River fish samples (N=32) was significantly correlated with trophic level (Spearman's $r=0.70$; $p<0.01$) (Figure 5.2 C) but did not correlate with fish weight and length. In the Tano River fish samples, Hg concentration did not correlate with trophic level, weight nor length.

Variability in Hg concentrations in the carnivores (trophic levels 3.2 to 3.4 trophic levels) was high. A Kruskal-Wallis test on these carnivorous fish samples from the Surow and Subri Rivers (N=17) indicated a significant difference in weight and length of fish between the two rivers ($p<0.01$). Mercury concentrations between these trophic levels were not significantly different in the two rivers and did not correlate with fish weight, length or trophic levels (Table 5.2).

Table 5.2 Spearman's Correlations between Hg concentration in fish muscle tissue and fish weight, length and trophic levels in various data sets
* denotes significance at p<0.05; ** denotes significance at p<0.01.

Data set	Spearman's correlations with Hg			
		Weight	Length	Trophic Levels
All rivers, N=60	Correlation Coefficient	.131	.355**	.619**
Surow River, N=19	Correlation Coefficient	.436	.569*	.614**
Subri River, N=32	Correlation Coefficient	.155	.330	.698**
Carnivorous fishes, N=33	Correlation Coefficient	-.048	.088	.163
Herbivorous fishes, N=27	Correlation Coefficient	-.043	-.053	.544**
Benthopelagic, N=26	Correlation Coefficient	.109	.203	.401*
Demersal, N=34	Correlation Coefficient	.076	.375*	.552**

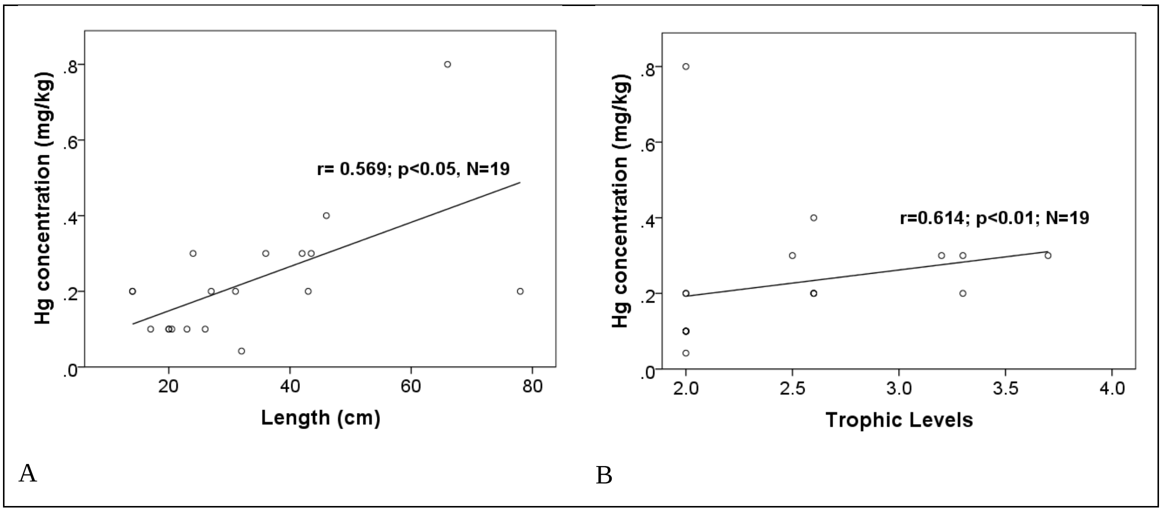


Figure 5.5 Concentration of Hg in the Surow River fish samples (N=19) positively correlated with fish length (A) and trophic level (B)

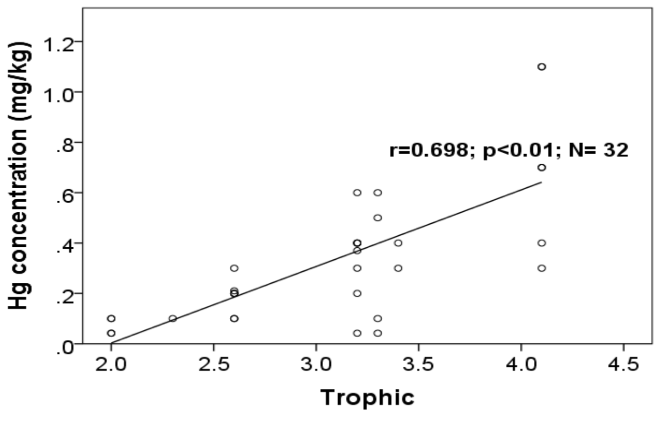


Figure 5.6 Concentration of Hg in the Subri River fish samples (N=32) positively correlated with fish trophic level

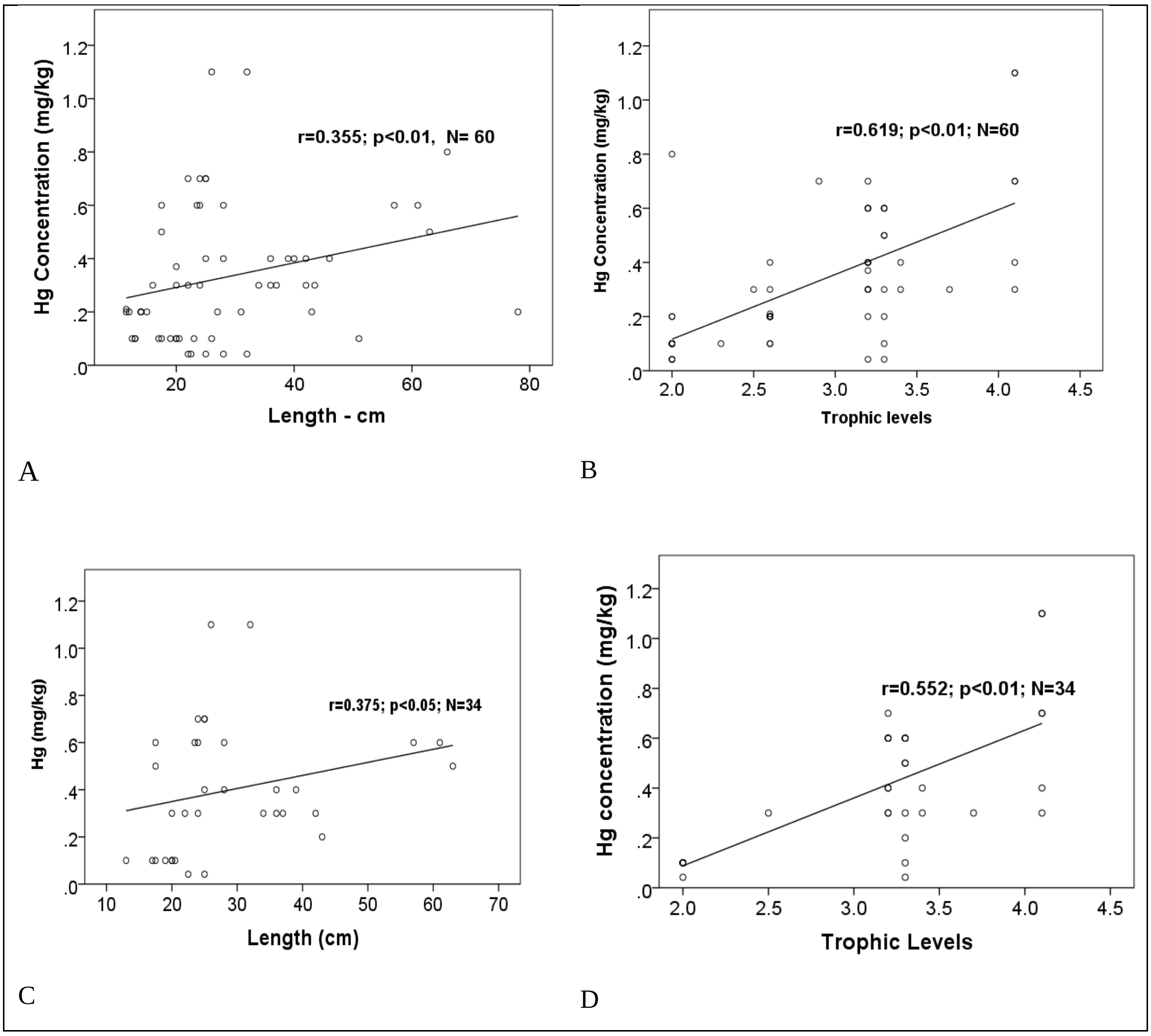


Figure 5.7 Concentration of Hg in all fish samples (N=60) from the Surow, Subri and Tano Rivers positively correlated with fish length (A) and trophic level (B), particularly in the demersal fishes (N=34) (C and D)

5.4 Discussion

5.4.1 Fish samples diversity

This study did not aim to examine fish diversity across the study area. However, it is important to observe the differences in fish samples collected and trophic levels between rivers in order to analyse their differences in metal and metalloids bioaccumulation and biomagnification potentials. Based on the fish samples collected, the Surow and Subri rivers differed in fish species diversity. Subri River's samples were more diverse than the Surow's although most of the fish samples from both rivers belong to the same classes of Siluriformes (mud fish) and Perciformes (including the tilapia fishes) commonly found in West Africa (McConnell et al., 1987; Winemiller et al., 2008). The difference in sample diversity between the two rivers may reflect differences in catchment size and longitudinal zonation from upstream to downstream, hence habitat diversity, as also observed by Araújo et al. (2009) in their work. The Subri River has a larger catchment (12,900 ha) than the Surow‘s (3,500 ha). Hugueny (1989) found that species richness in West African rivers positively correlated with catchment surface area; the larger the catchment area the more diverse the fish community. The Subri River is also wider and longer (25 km of main channel length) than the Surow River (16 km main channel length), which create opportunities for more trophic levels. The difference in species diversity between the Surow and Subri Rivers fishes was also seen in fish weight, length and trophic levels. The higher diversity of the Subri River may also be a result of the seasonal flooding of the Tano River in the rainy season which may displace fishes to its floodplains and tributaries, a phenomenon commonly seen in tropical rivers (Winemiller, 1990; Winemiller et al., 2008). The Subri River's morphology of a defined channel, compared to the Surow's often swampy channels, may also make it easier for fish migration from the Tano River. The one-off fish sampling from the Tano River in January (a dry season) identified species that were seen mostly in the Subri River samples but were not found in the Surow River (e.g. *C. nigrodigitatus*), further suggesting that some fishes in the Subri River may have originated from the Tano River.

5.4.2 Mercury biomagnification and bioaccumulation potentials in the Surow and Subri Rivers

Concentrations of As, Cd and Pb in all fish in this study were below the LOD of 0.05, 0.05 mg kg^{-1} and 1 mg kg^{-1} respectively. Studies have shown that some metal/metalloids, including As, Cd, and Pb, may biodilute, biomagnify, or not change along food chains, depending on various factors including the bioavailability of the elements and their biological roles (Hare, 1992; Rainbow, 2007). In aquatic invertebrates, As, Cd and Pb had been found to be accumulated on the surface of their exoskeleton. Further, concentrations in their body tissues are the net balance between metal influx rate from the environment and metal efflux from the organism (Giguère et al., 2004; Hare, 1992) and can consequently, be below the LOD. In fish, concentrations of Cd and Pb, for example, were also found to decrease with fish age and changed with seasons and quality of the water (Canli et al., 2003; Giguère et al., 2004). Canli (2000) also reported that concentrations of Cd and Pb in highly contaminated *Tilapia zillii*, a fish commonly found in African rivers and lakes, can be significantly reduced after immersing them in clean uncontaminated water for 30 days. The low concentrations of As, Cd, and Pb in fish samples from the Surow, Subri, and Tano rivers in this study, therefore, are possibly due to the low concentrations of As, Cd and Pb in river water and sediment as discussed in Chapter 3 and 4.

Mercury, however, was detected in most fish samples. The higher Hg concentrations were in carnivorous species. Nevertheless, some herbivorous fishes particularly the largest (and longest) *O. niloticus,* also contained high concentrations of Hg. This indicated a potential for bioaccumulation and biomagnification of Hg in both rivers. The positive significant correlations between Hg concentrations with fish length across all rivers suggested that the Hg concentrations were affected by potential differences in fish age. In other words, Hg was bioaccumulated with age. The relatively high concentration of Hg observed in some large specimens of *H. niloticus* (0.4 mg kg^{-1}) and *O. niloticus* (0.8 mg kg^{-1}), is an example of how Hg is accumulated by age even in herbivorous fishes.

Mercury accumulation by age indicated the long-term bioavailability of mercury in the system. However, although the high Hg concentrations in *H. niloticus* and *O. niloticus* may be due to accumulation of and exposure to Hg with time, it may also be due to their opportunistic feeding strategies, as benthopelagic fishes may shift from benthic to pelagic, and from planktivory or omnivory to piscivory, depending on food availability - which in the tropics is often governed by the seasons (Bastos et al., 2007). Bastos et al. (2007) in their study on Hg in Amazonian fish in Brazil, for example, found that while Hg bioaccumulation was species specific, it was also influenced by changes in feeding strategies brought by flooding seasons without systematically affecting the accrual of Hg in higher trophic fish. Consequently, in an environment where Hg is bioavailable, omnivores may accumulate Hg more than the carnivores (Yousafzai et al., 2010). The significant positive correlation between trophic levels and Hg concentrations in the herbivorous group of fish samples (trophic levels 2.0 to 2.9) seen in this study, therefore, may also be explained by the omnivorous nature of some fishes studied (see Table 5.1). On the other hand, Hg concentrations in a smaller *C. angulliaris* and *C. gariepinus* (catfish) samples in this study were below LOD despite them being carnivorous fishes, while some larger samples of these species had Hg concentrations of above 0.4 mg kg^1. Studies of metallic concentrations in *C. gariepinus* in Nigeria (Kadye et al., 2012) and South Africa (Avenant-Oldewage et al., 2000) showed that the catfish was a complex predator; the smaller fish appeared to feed lower in the food chain, whilst the larger fish were top predators, influencing mercury uptake by this species at different stages of their lives. The limited numbers and sizes of individual species collected in this study may be responsible for no significant correlations between Hg and weight.

The positive and highly significant correlations between Hg concentration and trophic level in both Surow and Subri rivers (seeTable 5.2) confirmed that Hg was not only accumulated in fish tissue but was also biomagnified along the food web in the systems. The exceptions were in the carnivorous and the Tano River data, where correlations between Hg concentrations and trophic levels were not significant, most likely due to lack of variability in the trophic levels of the samples collected. A comparison between carnivorous fish (trophic levels 3.2 to 3.4) from the Surow and Subri rivers confirmed that concentrations of Hg did not differ significantly between rivers. The finding highlights the potential for Hg biomagnification in both rivers, and that the effect of trophic levels on accumulation of Hg was more significant than age or spatial variability (McIntyre et al., 2007; Watras et al., 1998; Weber et al., 2013).

A source of Hg in the Surow River's fishes is likely to be from the ASGM operating in its catchment, especially during the active ASGM period of February to April 2013 as discussed in Chapter 3. The presence of Hg and bioaccumulation of Hg in the Subri River fishes, however, cannot be positively correlated to THg concentrations in the river's sediment and water, which were generally below detection limits of 0.06 mg kg^{-1} and 0.002 mg l^{-1} respectively (See Chapter 4). ASGM were not known to be operating along the Subri or Tano (above its confluence with the Subri) rivers. Concentrations of Hg in the Tano River's sediment and water acquired from a one off sampling (N=3) near to the fishermen landing site at the time of fish sampling were also below detection limits. The lack of correlations between Hg concentrations in different environmental compartments, however, is not unusual and has been reported in other studies (Barbosa et al., 2003; Brabo et al., 2003). This is possibly due to the lack of information regarding concentrations of bioavailable Hg (as MMHg) in sediment and water, as most of Hg concentrations in sediment and water are measured as THg. Mercury has also been detected in freshwater fishes at places that lacked Hg point sources particularly in forested streams where Hg body burden was at an increasing probability to exceed human and wildlife health guidelines (Beaulieu et al.; Brabo et al., 2003; Kamman et al., 2005; Riva-Murray et al., 2011). Bioaccumulation of Hg in lotic ecosystems without Hg point sources were reported to be strongly correlated to land cover characteristics particularly forest cover (Riva-Murray et al., 2011), wetland extent and connectivity, hydrologic alteration (Hurley et al., 1998), aqueous MMHg, dissolved organic carbon (Stewart-Oaten et al.), suspended sediment concentrations (Brigham et al., 2009) and pH in the water column (Krabbenhoft et al., 1995; Riva-Murray et al., 2011; Schuster et al., 2008).

The source of Hg in the aquatic environments includes that of from the atmosphere. Atmospheric Hg is gaseous in form and can redeposited to the ground and water. The gaseous Hg can also be evaporated back into the air after it reaches the ground (Schroeder et al., 1998). When atmospheric Hg is deposited in water, however, it becomes soluble and bioavailable. Globally, ASGM is recorded the second largest source of anthropogenic atmospheric Hg after fossil fuel combustion (Pacyna et al., 2010; Telmer et al., 2009; WHO, 2013). For example, atmospheric Hg in Colombian ASGM sites was reported to be 10 times higher than WHO recommended level of 1000 ng m^{-3} (Cordy et al., 2011). Once emitted into the air, atmospheric Hg may reside in the air for months up to two years. Gaseous Hg also disperses regionally and globally, although about 10% may be redeposited locally within 50 km from its point source by precipitation or dryly (Lindqvist et al., 1985). Therefore, the source of bioavailable Hg in fishes from all three rivers may have been atmospheric, including that of emitted by ASGM smelting operations in the study area, although we could not positively confirm this.

Another possible source of bioavailable Hg in the Subri and Tano rivers, despite a lack of point sources, is adjacent waterways and lands contaminated by Hg. Rivers and streams in the tropics, including the study area, are highly connected by seasonal flooding (Arrington et al., 2005; Junk et al., 1989; Winemiller, 1990; Winemiller et al., 2008). Floods directly and indirectly transport materials, including Hg, between rivers. During a flood, Hg may be deposited on to flood plains where it undergo a complex speciation process and redeposited into surrounding streams and rivers in another flood (Bastos et al., 2007). Mercury also naturally occurs in some sediments and waters (Ullrich et al., 2001) especially in areas with ferruginous and fossilized materials (Brabo et al., 2003). This also provides a plausible explanation to the concentrations of Hg in fishes from the Surow, Subri and Tano rivers, in spite of the lack of obvious Hg point sources in the Subri and Tano rivers.

5.4.3 Level of Hg in fish tissue

Concentrations of THg in fishes across rivers in this study (mean = 0.33 ± 0.03 mg kg^{-1}), interestingly were comparable to, and even higher in certain carnivorous species, than other rivers chronically impacted by ASGM in Ghana, such as the Pra River (Donkor et al., 2006; Oppong et al., 2010) . The mean concentration, however, is lower than that of Amazonian fishes reported by Brabo et al. (2003) (mean concentration 1.274 ppm) or that of chronically ASGM impacted rivers in North Sulawesi, Indonesia (mean 0.58 ± 0.45 ppm, maximum value 2.60 ppm) as reported by Bose-O'Reilly et al. (2010).

Although the mean concentrations of Hg in fishes across all rivers sampled were below the WHO recommended maximum concentration of 0.5 mg kg^{-1} for human consumption (WHO, 1991), 25% of the samples were equal to or exceeding the standard. High concentrations of Hg were particularly seen in carnivorous species including various catfishes popular for eating among the local inhabitants (*H. odoe*, the Clariases and *C. nigrodigitatus*) and larger herbivorous such as *H. niloticus*.

Human exposure to Hg from fish consumption depends on Hg concentrations in fish, amount of fish consumed and type of fish (Barbosa et al., 2003). While fish consumption rate among the inhabitants in the study area is beyond the study’s scope, for fish consumption advisories purposes, it is important to highlight the potential for Hg accumulation in larger and older fishes as well as Hg biomagnification in the carnivorous fishes in the area. All of the fish species purchased for this study, including the carnivorous ones, are consumed by the local citizens, potentially putting them at risk of Hg contamination, particularly important for pregnant woman and children (Anderson et al., 2004; Oken et al., 2005). Most of the local inhabitants also consume fish as a whole, not only as fillets (fish muscle), which may increase their risks related to Hg uptake, as concentrations of Hg in skin and organs were often reportedly higher than in the muscle tissues (Mason et al., 2000; Pereira et al., 2010).

5.5 Conclusion

This study has shown that although the concentration of Hg in fish muscle from the Surow and Subri rivers vary with taxa, weight and length, and between rivers, it has the potential to bioaccumulate in both rivers with increasing size and age, even in herbivorous fishes. It also has a potential to biomagnify with increasing trophic level. Accumulation of Hg due to vertical transfer along the food web is, however, more significant than that of due to fish size and age.

The bioaccumulation potential of mercury in the Surow and Subri rivers was similar to each other regardless of the significant difference in fish weight, length and trophic levels between fish samples from the two rivers and the assumed lack of point source Hg emission from the Subri River catchment. The biomagnification potential was also similar across trophic levels as well as foraging characteristics of fish.

Mean concentrations of Hg in fish tissue across rivers in this study remained below the WHO advisory limit of 0.5 ppm. However, individual concentrations of Hg in some larger herbivorous fish and carnivorous fish in both Subri and Surow rivers equalled or exceeded the health guidelines. All fish samples from the Tano River, which were carnivorous, also contained Hg in concentrations exceeding the WHO guidelines. As fish caught from the wild remained an important source of protein in the study area, fish consumption advisory for the inhabitants in the study area should take into account the Hg bioaccumulation and biomagnification potentials in fishes of the Surow, Subri and Tano Rivers. Indiscriminate consumption of fish from these rivers may pose health risks to the inhabitants.

While uncertainties regarding the potential source of the Hg accumulated and biomagnified in the rivers fish, particularly those of the Subri, and Tano Rivers, remained, results from this study can be used as a starting point for future biomonitoring impact of gold mining in the area, or studies to evaluate the potential risks of Hg to the freshwater aquatic environments and to the health of the population who depend on fish as source of protein in their diet.

6 Impacts of gold mining on macroinvertebrates community

6.1 Introduction

This chapter addresses the potential impacts of gold mining on macroinvertebrate communities in the Surow and Subri Rivers. Monitoring of stream water quality, such as that discussed in Chapter 3 and 4, normally involves collection of 'grab' samples, that represent the conditions at the time of sampling (Pearson et al., 1998). Consequently, spills of highly concentrated or toxic substances into streams and rivers that may occur occasionally are not easily detected using this approach. Chemical testing also does not evaluate potential synergistic effects of chemicals, particularly those below limit of detection, that might occur and have chronic or acute toxicity to riverine organisms (Wagenhoff et al., 2012). Continuous and frequent monitoring of water quality, particularly over an extended period, is also expensive (Pearson et al., 1998), especially for local and regional environmental managers in developing countries where access to adequate laboratory facilities and resources are limited. These shortcomings of water quality monitoring have encouraged the monitoring of aquatic biota to address these limitations.

Aquatic macroinvertebrates influence nutrient cycles, primary productivity, decomposition and translocation of materials in riverine ecosystems due to their various feeding patterns, that influence river water quality (Wallace et al., 1996). Stream invertebrates are also known to be sensitive to the condition of habitats, water quality and disturbances. As such, macroinvertebrates have been used as bio-indicators of river water conditions and anthropological impacts, including mining, in stream ecosystem, where they effectively integrate water quality conditions over a prolonged time period (Pearson et al., 1998), particularly in temperate Australia, New Zealand, North America and Europe (Coysh et al., 2000; Friberg et al., 2006).

Macroinvertebrate also respond to concentrations of toxic concentrations of metal and metalloids (Bruns, 2005; Harding, 2005; Smolders et al., 2003), nutrient enrichment (Smith et al., 2003; Sponseller et al., 2001), water acidification (Guerold et al., 2000), as well as changes in the geo-morphology and hydrology of the stream (Boulton, 2003; Dewson et al., 2007; Elosegi et al., 2010). Macroinvertebrate species can be sensitive to elevated metal concentrations and stream acidification, especially where associated as acid mine drainage. For example, high reductions in the diversity of macroinvertebrates and a total eradication of Ephemeroptera from some streams in Europe were reported to be an impact of stream acidification (Barber-James et al., 2008) and metal pollution (Clements et al., 2000). Effect of elevated N and P on macroinvertebrate community may be direct and indirect through various mechanisms including toxicity and eutrophication. Effects of changes in river flows in relation to macroinvertebrate communities have been reviewed by, amongst others, Dewson et al. (2007) and Poff et al. (2010). Macroinvertebrate taxa react differently to both increased and decreased flows. A decrease in water discharge is commonly associated with lower velocity, reduced volume of water, less wetted perimeter, increased sedimentation and changed water chemistry which can result in fewer habitats. Therefore decreased water discharge, consequently often results in decreased macroinvertebrate diversity due to the reduced habitat diversity, whilst abundance may increase or decrease (Dewson et al., 2007). Cushman (1985) studied the effects of short but recurring elevated flows resulted from daily discharge of water from a reservoir found that macroinvertebrate abundance, diversity and productivity were significantly reduced. According to Cushman (1985) the reduced biotic productivity of macroinvertebrates was directly the result of the flow changes, and indirectly by changes in water depth and scouring of sediment, which many taxa had limited capacity to adapt to.

Metal mining can significantly impact stream and river ecosystems via increased sediment load, concentrations of metal, metalloids and nutrients, acidification in water and changes in the river's geomorphology and hydrology (Jarvis et al., 1997; Younger et al., 2004). These impacts on water and sediment quality, consequently, will have an impact on the macroinvertebrate community. Macroinvertebrate abundance, richness, composition, tolerance and diversity are known to be impacted by increased sedimentation and associated turbidity (Gray et al., 1982; Henley et al., 2000; Ryan, 1991; Wood et al., 1997; Yule et al., 2010). An increase of 20-80 mg l^{-1} above background levels for suspended solids, for example, can cause a 45-70% decrease in total abundance of macroinvertebrates (Gammon, 1968; White et al., 1976). Gray et al. (1982) also found that midge larval (Chironomidae) density decreased during release of suspended solids, but then recovered and grew quickly at the expense of other species. Increase in densities of some mayflies (Ephemeroptera) and worms (Oligochaeta) were also reported to be highly correlated with increases in suspended solids.

The use of macroinvertebrates communities for the assessment of mining impacts on river ecosystems has been widely studied in North America, Europe and Australia (Bruns, 2005; Humphrey et al., 1995; Maret et al., 2003). Applications of macroinvertebrate community assessment for stream and river monitoring in the tropics, however, has not been widely practised (Morse et al., 2007) (Dudgeon, 2000; Jacobsen et al., 2008). Studies in macroinvertebrate community in tropical rivers have been limited to a few, including in Malaysia (Wells et al., 2008; Yule et al., 2009), Indonesia (Yule et al., 2010), and Papua New Guinea (Yule, 1996). Aquatic macroinvertebrates in tropical Africa been particularly understudied (see (see Jacobsen et al., 2008). In West Africa, it has been largely limited to studies conducted in Ivory Coast (Camara et al., 2012; Dejoux et al., 1986; Statzner, 1982). In Ghana, macroinvertebrate studies have been limited to macroinvertebrate surveys of urban and agriculture polluted streams (Baa-Poku et al., 2013; Thorne et al., 2000) (Ansah et al., 2012), studies concerning macroinvertebrate vectors for diseases posing health risk to humans (Benbow et al., 2014), effects of insecticides on macroinvertebrates (Kurtak et al., 1987; Walsh, 1985) or concentrations of metals in molluscs (Amisah et al., 2011). Less attention has been given to macroinvertebrate community responses to mining impacts, and much less attention has been given to that of related to Artisanal Small Scale Gold Mining (ASGM) impacts, in spite of the intensity of ASGM activities in the region and macroinvertebrate's potential as bio-monitor of anthropogenic impacts on stream ecology.

This chapter aims to assess macroinvertebrate community responses to the possible impacts of ASGM and large-scale gold mining in the Surow and Subri Rivers respectively. The specific aims of this study are:

(1) To assess whether macroinvertebrate assemblage and diversity differs between

(a) the Surow and Subri Rivers;

(b) mine impacted and un-impacted sites on each river; and

(c) seasons

(2) To assess relationships between macroinvertebrate community assemblage and water physico-chemical and river hydrological variables

I hypothesise that macroinvertebrate community abundance, taxa richness and diversity would respond to the different sediment and water qualities between the two rivers, and between control and impact sites, and seasonally. The influence of mining is hypothesised to be increased abundance and reduced diversity of taxa due to the nature of the impacts seen in Chapters 3 and 4.

6.2 Methods

6.2.1 Study design and macroinvertebrate sample collection

The study design involved sampling of macroinvertebrates across multiple mining impacted sites (impact) and non-impacted sites (control) along both the Surow and Subri Rivers on four occasions, July 2013 (beginning of a dry season), October 2013 (end of a rainy season), February 2014 (end of dry season) and April 2014 (mid rainy season). Macroinvertebrate sampling was completed in conjunction with water sampling for these occasions as described in Chapters 3 and 4.

Macroinvertebrate samples were collected from the 11 water sampling sites on the Surow and 7 sites on the Subri Rivers; the locations are shown in Chapter 2, 3 and 4. On each sampling occasion, three 3 m transects were sampled at each site using a dip net measuring 25 x 25 cm with a mesh size of 250 µm. Each sample was collected across all available habitats (edge, riffle, sandy pool, etc), ensuring no overlap between transects. Large macroinvertebrates were live-picked from each sample at each site immediately after collection for 30 minutes. Sorted specimens and the remaining unsorted sample were then preserved with 100% ethanol to give an approximate 70% ethanol in the sample. In the laboratory, smaller specimens were picked from remaining samples under a dissecting microscope. Blind numbering and labelling was assigned to each sample to ensure objectivity.

In February 2014, at the end of the long dry season when sections of the rivers dried up into pools or became completely dry, samples were collected from pools. In contrast in April and October water levels were often very high and unsafe to sample at some sites. Consequently, some sites were sampled less than three times or not sampled at all when the river dried up or flooded. Table 6.1 summarises the number of samples collected from each site on each sampling period.

6.2.2 Specimen Identification

All specimens were identified to the lowest practicable levels with available keys including those commonly used for West African macroinvertebrates (Brown et al., 1993; Dejoux et al., 1986; Gooderham et al., 2002) and temperate and tropical Australian regions (Gooderham et al., 2002) and tallied for abundance. Identification was predominantly to family level, except for some taxa such as Hirudinea, Oligochaeta, and Collembola which were identified only to higher levels of taxonomy.

Identified samples from the first sampling program (July 2013) were then sent to the Macroinvertebrate Laboratory of the Ghana Council for Scientific and Industrial Research (CSIR) in Accra to be confirmed by Dr Godwin Amungbe, the Council's macroinvertebrate expert. Dr Amungbe also identified eight samples from the first sampling round down to species level, where possible. Hydrology and water quality characterisation

River depth, width and flow (velocity) were measured at the time of macroinvertebrate sampling. Rainfall was measured daily at the Newmont Mining Corporation weather station in Kenyase village (see Chapter 3). Rainfall data used in this chapter is the accumulated rainfall for 7 days prior to the day of macroinvertebrate sampling. River water was sampled and analysed for physico-chemical parameters monthly for 14 months, including in the months of macroinvertebrate sampling occasions described in Chapter 3 and 4.

Table 6.1 Number of samples analysed from each site across all sampling times

Site	River	Number of samples analysed			
		July 2013	October 2013	February 2014	April 2014
1	Surow	3	3	3	3
2	Surow	3	3	3	3
3	Surow	3	3	3	3
4	Surow	3	3	3	3
5	Surow	2	3	3	3
6	Surow		3	3	3
7	Surow	3	3	3	3
8	Surow	2	3	2	3
9	Surow	3	3	2	3
10	Surow	3	3	2	2
11	Surow	3	3	3	3
NSW9	Subri	3	3	2 pools	3
KSW16	Subri	3	3	2 pools	Not sampled
KSW3	Subri	3	3	3	3
KSW13	Subri	3	3	Not sampled	3
NSW6	Subri	3	3	Not sampled	3
NSW8	Subri	3	3	3	3
KSW2	Subri	3	3	2 pools	3

6.2.3 Data analysis

Descriptive analysis and hypothesis testing regarding variance between replicates, rivers, sites, control and impacted sites, and times (season or month) were made using univariate and multivariate statistical methods. The following metrics were calculated using a facility within the PRIMER package for biotic community data: macroinvertebrate abundance or number of individuals per sample (N), taxa richness or number of taxa per sample (S), Margalef's diversity index (d'), and Shannon-Wiener diversity index (H' log-e).

Two way ANOVA with two levels of rivers (Surow and Subri) and two levels of mine impacts (Control and Impact) and their interactions were used to investigate the possible differences in N, S, d, and H between impacts of ASGM on the Surow and impacts of the

Ahafo mine on the Subri. Similarly, analysis of temporal variability in the indices in the two rivers was also performed by ANOVA. These univariate analyses were performed using the SPSS v 22 package.

Multivariate analysis within the PRIMER package (Clarke et al., 2001; Clarke, 1993) was used to analyse variance in macroinvertebrate community compositions (assemblage) (Johnston et al., 2009; Wilsey et al., 2005). For the multivariate analysis, square-root transformation was applied to the abundance of each taxon (O'Hara et al., 2010) prior to calculating resemblance between sites using the Bray-Curtis distance. Ordination with non-metric multidimensional scaling (MDS) was used to explore and illustrate the site resemblance data. Permutational multivariate analysis of variance (PERMANOVA) were used to test for differences in species composition between factors (rivers, C/I, time), whilst SIMPER was used to identify variables (taxa) contributing to significant differences.

The relationships between macroinvertebrate assemblage and environmental variables (hydrology and water physico-chemistry variables) were investigated using with the distance-based redundancy analysis (dbRDA), also within the PRIMER / PERMANOVA package. DbRDA performs multivariate multiple regressions of macroinvertebrate community data against predictor variables (Anderson et al., 2008; McArdle et al., 2001). Variables with strong significant relationships with Pearson's $R>0.4$ were chosen.

River hydrology and water physico-chemistry data used was from July 2013, October 2013, February 2014, and April 2014 sampling accordingly. Below LOD (limit of detection) data were substituted with $LOD/\sqrt{2}$, whilst environmental parameters that constantly gave below LOD, including the concentration of dissolved Hg, were omitted. Explanatory variables used in the analysis and modelling are listed in Table 6.2. Environmental data was $\log_{10}$ transformed and normalised prior to analysis of resemblance via Euclidian distance.

Table 6.2 List of variables for DistLM analysis across rivers

No.	Variable	Symbol
1	Velocity	V
2	Depth	D
3	Width	W
4	Discharge	Disc
5	Week rain	Rain_1week
6	Temperature	T
7	EC	EC
8	DO	DO
9	pH	pH
10	TDS	TDS
11	Turbidity	Turb
12	Aluminium	Al
13	Arsenic	As
14	Copper	Cu
15	Iron	Fe
16	Manganese	Mn
17	Lead	Pb
18	Magnesium	Mg
19	Calcium	Ca

20	Nitrate	NOx
21	Filterable Reactive Phosphorus	FRP
22	Sulfate	SO

6.3 Results

6.3.1 Macroinvertebrate taxa and diversity

In total, 198 samples consisting of 39,965 individuals were collected; 126 samples containing 24,906 individuals were from the Surow River; and 72 samples containing 15,059 individuals were from the Subri River. Eighty eight taxa, distributed among 22 orders, were identified mostly to family levels. Seventy eight taxa were found in the Surow River and 80 taxa were found in the Subri River. The taxa, their number of occurrences and total abundance in each river are listed in

Appendix 30. Although identification of organisms in this study was up to the family level, specimens from eight samples from the first sampling period were identified to lowest practical taxa by Dr Godwin Amungbe, for exploratory purposes, with 39 families yielding 77 taxa, also presented in

Appendix 30. These were samples corresponding to sites 6, 4 and 9 on the Surow and site KSW 13 on the Subri. The number of macroinvertebrates (N), species richness (S), Margaleff's diversity (d) and Shannon-Wiener's diversity indices of each site are presented in boxplots in Figure 6.1.

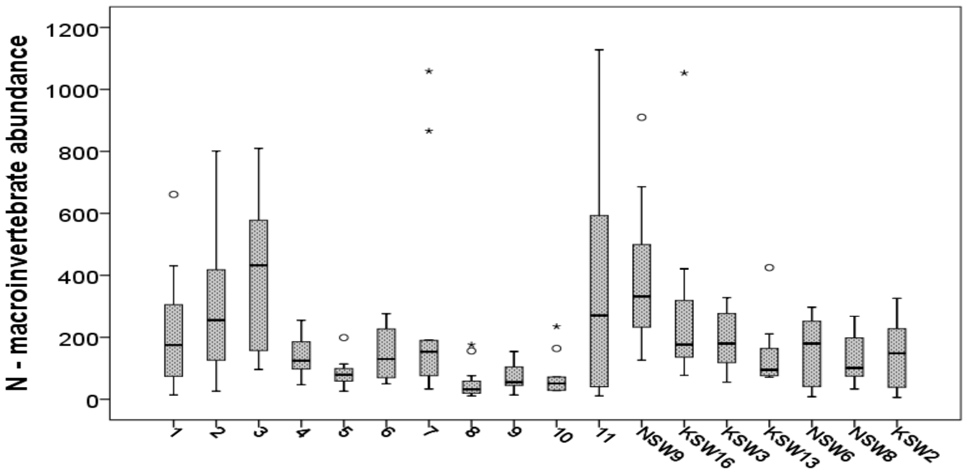
The composition of macroinvertebrate groups (orders) in the Surow and Subri rivers is summarised in Figure 6.2. Diptera, Gastropoda, Ephemeroptera, Coleoptera, and Decapoda are the most abundant macroinvertebrates in both rivers. Diptera, Ephemeroptera and Trichoptera are analysed in the following section due to their importance as indicators of pollution in aquatic ecosystems. Most dipteran families are less sensitive to pollutants, whilst Ephemeroptera and Trichoptera belongs to the sensitive to pollutant group known as EPT (Ephemeroptera, Trichoptera and Plecoptera). Trichoptera, however, was not found in this study.

6.3.2 Diptera, Ephemeroptera and Trichoptera

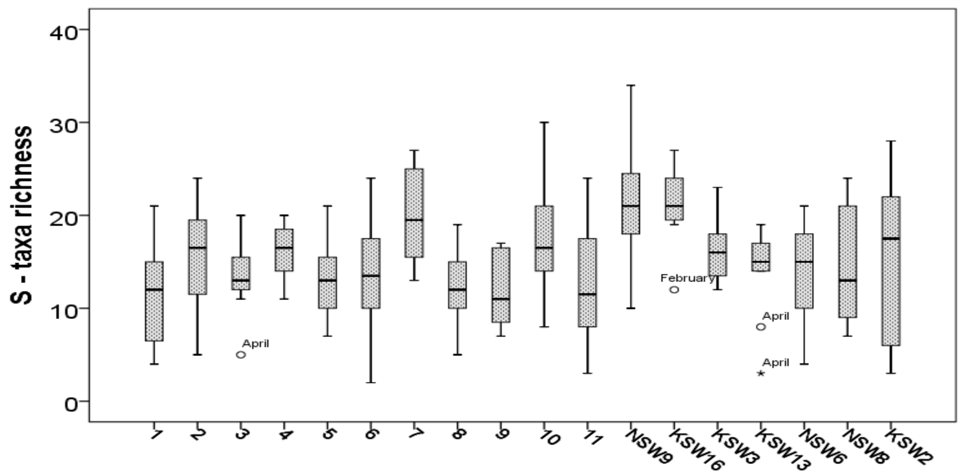
6.3.2.1 Diptera

Dipteran families contributed 37% to the total number of specimens (combined Surow and Subri Rivers) as shown in **Error! Reference source not found.**. Within Diptera, 16 families were identified, with Chironomidae being the most abundant and common (63.5%), followed by Ceratopogonidae (32.6%) and Culicidae (2.5%). Two way PERMANOVA confirmed that dipteran community assemblage differed with river ($p<0.01$, $F=7.3$, Table 6.3) between control and impact ($p<0.01$, $F=5.5$, Table 6.3) and in the interaction between river and CI factors ($p<0.01$, F3.1, Table 6.3), suggesting that mining impacted on Diptera community assemblage in the two rivers. Pairwise SIMPER (

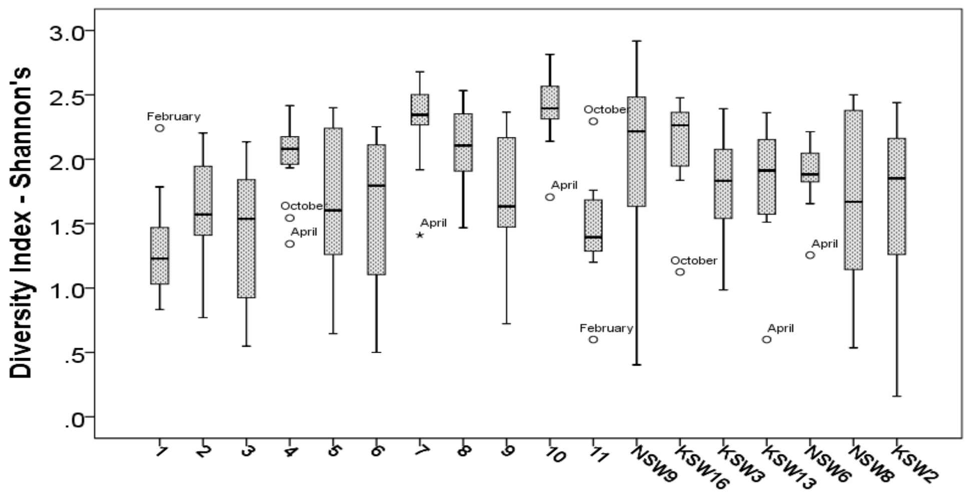
Table 6.4) identified Chironomidae, Ceratopogonidae, Culicidae, Tipulidae and Simulidae drove the difference between Diptera community composition in the impact sites of the Surow and impact sites of the Subri. The impact sites of the Surow had more Chironomidae, Ceratopogonidae and Culicidae than impact sites of the Subri. Tipulidae, on the contrary, was found more abundant at impact sites of the Subri compared to that of the Surow.



A



B



C

Figure 6.1 Spatial variability in boxplot of macroinvertebrate abundance (N), taxa richness (S) and Shannon-Wiener's diversity index across all sampling sites on the Surow River (site 1 to 11) and the Subri River (site NSW9 – KSW2) between July 2013 and April 2014.

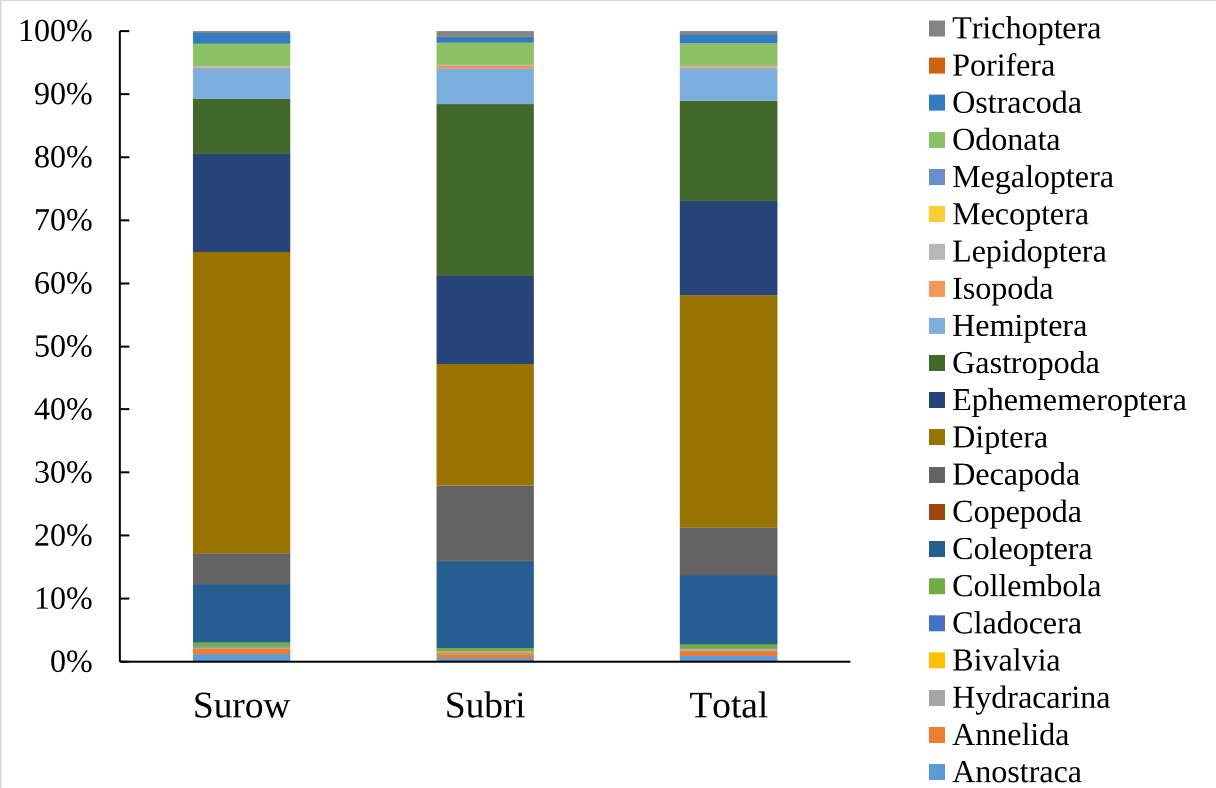


Figure 6.2 Relative abundance of macroinvertebrate orders in samples collected from the Surow and Subri Rivers between July 2013 and April 2014.

6.3.2.2 Ephemeroptera

Ephemeroptera, one of the pollutant sensitive taxa (EPT) commonly focused on in macroinvertebrate monitoring programs, contributed 15% of the total macroinvertebrate abundance in this study. A total of 5531 ephemeropterans were collected and six families identified, ie. Baetidae, Caenidae, Heptagenidae, Leptophlebidae, Trichorythidae and Policentrophlebidae. Policentrophlebidae was not found in the Surow River whilst Trichorythidae was not found in the Subri River. Baetidae were the most abundant, accounting for more than 75% of the total abundance of Ephemeroptera, followed by Caenidae (19.4%) and Heptagenidae (4%). The community composition of Ephemeroptera families varied with river and between control and impact ($p<0.01$, F 4.2 and $F=7.9$ respectively), but the interaction between river and C/I was not significant (Table 6.3). Further pairwise test showed that at control, the composition of Ephemeroptera families did not differ, but they differed at impact ((each with PERMANOVA $p<0.01$). The difference between the two rivers at impact, according to SIMPER (

Table 6.5), was due to the abundances of Baetidae, Caenidae, and Heptagenidae. Baetidae was found in higher abundance at impact sites on the Surow compared to that of the Subri. On the other hand, Caenidae and Heptagenidae were found in higher abundances at impact sites on the Subri compared to that of the Surow. Overall, across rivers, Baetidae and Caenidae were found in abundance at control sites, whilst Heptagenidae was found in higher density at impact.

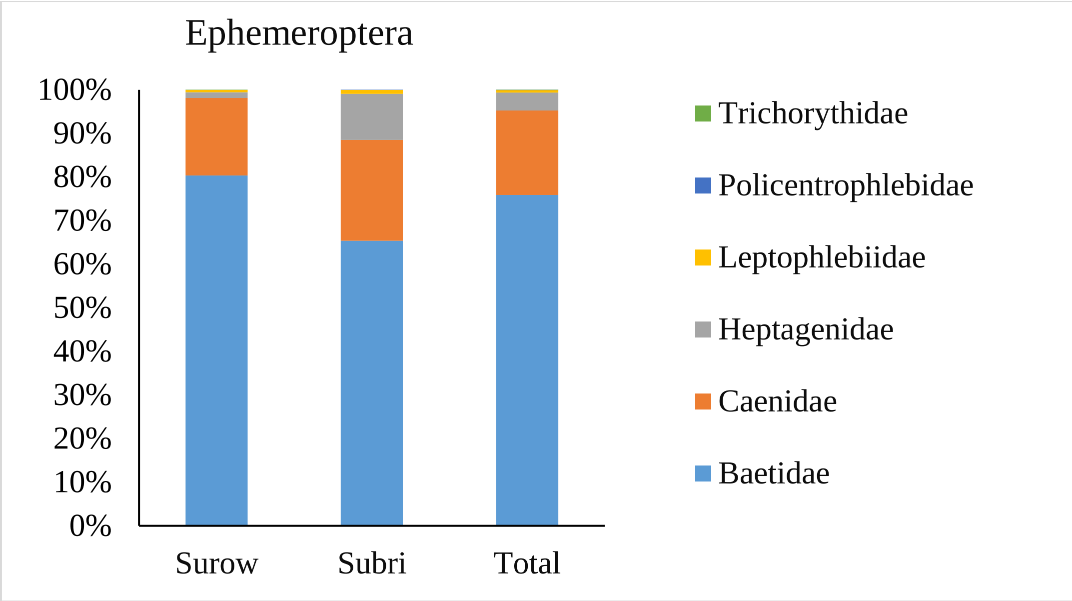


Figure 6.3 Relative abundance of the Ephemeroptera families in the Subri and Surow Rivers between July 2013 and April 2014

6.3.2.3 Trichoptera

Across rivers, Trichoptera contributed only <1% to the total macroinvertebrate abundance. However, similarly to Ephemeroptera, the abundance and diversity of Trichoptera families is given an attention here due to its attribute as one of the most sensitive to pollutant taxa.

Out of the 208 Trichoptera specimens, 135 were found in the Subri River, and only 73 were found in the Surow River. A total of eight families were identified (see **Error! Reference source not found.**); the Surow River only had four of them (Ecnomidae, Hydropsychidae, Hydroptilidae and Leptoceridae), whilst the Subri River had all of them. Leptoceridae were the most common trichopteran family across rivers (51% of total Trichoptera abundance), followed by Hydropsychidae and Hydroptilidae, whilst Barbarochthonidae, Calamoceridae, Psychomyidae and Policentropodidae were scarce and only found in the Subri River. With only seven sampling sites (compared to 11 sampling sites on the Surow), the Subri River not only had higher abundance but more Ephemeroptera morphospecies than the Surow River. **Error! Reference source not found.** Figure 6.4 depicts the relative abundance of each family within the Trichoptera collected in this study.

Two way PERMANOVA, however, showed that the community assemblages of Trichoptera families did not differ with river, between control and impact, and the interaction between river and C/I was not significant (p=0.07, F=2.5; Table 6.3).

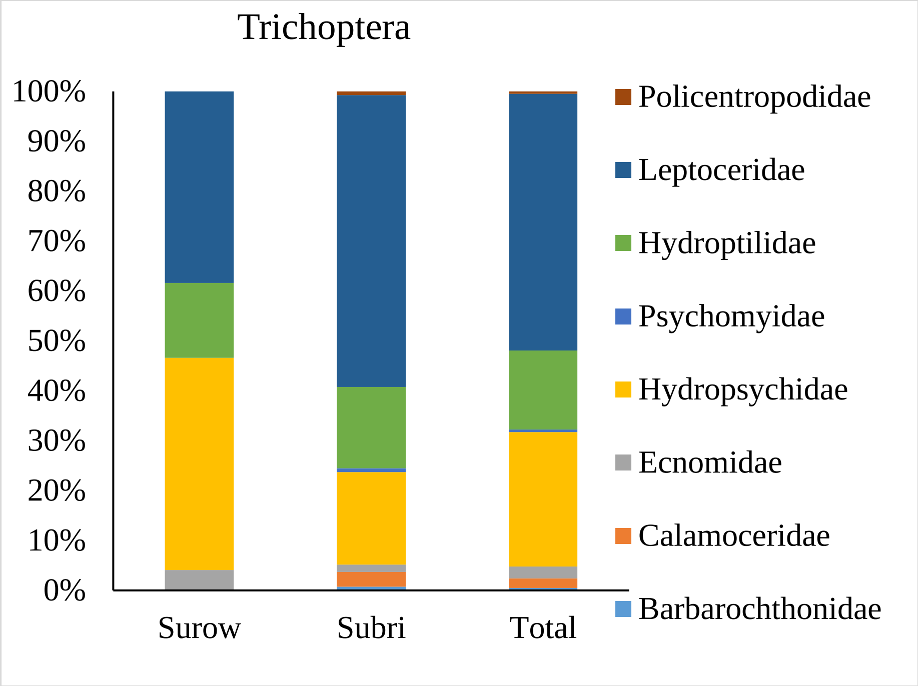


Figure 6.4 Relative abundance (%) of Trichoptera taxa in the Surow and Subri rivers between July 2013 and April 2014.

Table 6.3 Results of two way PERMANOVA on community compositions of Diptera, Ephemeroptera and Trichoptera families with river (Ri), control and (CI) as factors.

	Source	df	SS	MS	Pseudo-F	P(perm)
Diptera	Ri	1	13,274	13,274	7.24	0.00
	CI	1	9,988	9,988	5.45	0.00
	RixCI	1	5,766	5,766	3.14	0.01
	Res	171	313,650	1,834		
	Total	174	343,670			
Ephemeroptera	Ri	1	7,268	7,268	4.19	0.01
	CI	1	13,771	13,771	7.93	0.00
	RixCI	1	1,642	1,642	0.95	0.43
	Res	171	296,800	1,736		
	Total	174	320,360			
Trichoptera	Ri	1	2,130	2,130	1.14	0.30
	CI	1	2,149	2,149	1.15	0.31
	RixCI	1	4,641	4,641	2.48	0.07
	Res	51	95,543	1,873		
	Total	54	105,980			

Table 6.4 SIMPER analysis identifying Diptera families contributing to the significant difference between Diptera community composition in the impact sites of the Surow River and the impact sites of the Subri River

Family	Av.Abundance at Surow Impact	Av.Abundance at Subri Impact	Av.Dissimilarity	Diss/SD	Contrib %	Cum. %
Chironomidae	4.84	3.99	25.21	1.3	44.08	44.08
Ceratopogonidae	2.66	0.74	15.13	0.94	26.46	70.54
Culicidae	0.75	0.3	6.15	0.73	10.75	81.29
Tipulidae	0.18	0.34	3.26	0.6	5.71	87
Simuliidae	0.22	0.15	2.71	0.39	4.74	91.74

Table 6.5 SIMPER analysis identifying Ephemeroptera families contributing to the significant difference between Ephemeroptera community composition in the impact sites of the Surow River and the impact sites of the Subri River

Family	Av.Abundance at Surow Impact	Av.Abundance at Subri Impact	Av.Dissimilarity	Diss/SD	Contrib %	Cum. %
Baetidae	2.61	1.88	32.6	1.17	48.93	48.93
Caenidae	0.94	1.57	21.74	1.08	32.64	81.57
Heptagenidae	0.27	0.83	9.99	0.68	15	96.56

6.3.3 Temporal variability

In both the Surow and Subri rivers, temporal variations in taxa richness, Margalef's (d) and Shannon-Wiener's (H) diversity indices were significant ($p<0.01$). Macroinvertebrate abundances (N), however, did not vary with time in both rivers. Boxplots in Figure 6.5 depict the temporal variability in macroinvertebrate abundance, taxa richness and diversity (Margalef's and Shannon-Wiener's). Similarly, macroinvertebrate community composition (assemblage) in the Surow River significantly differed with time (PERMANOVA $p<0.01$, $F=3.55$**Error! Reference source not found.**), as it did in the Subri (PERMANOVA $p<0.01$, $F=3.42$). The presence and absence of macroinvertebrate species also varied with time (PERMANOVA $p<0.01$).The month of July had the highest mean taxa richness in the Subri and the second highest in the Surow rivers. The highest mean abundance and Shannon-Wiener's diversity index in both rivers occurred during the second rainy season in the year (October). Macroinvertebrate community in the first rainy season of the year (April), on the contrary, were generally characterised by low mean abundance, low taxa richness, and hence lower diversity, whilst February was characterised by high mean density but relatively low taxa richness and lower diversity (Figure 6.5).

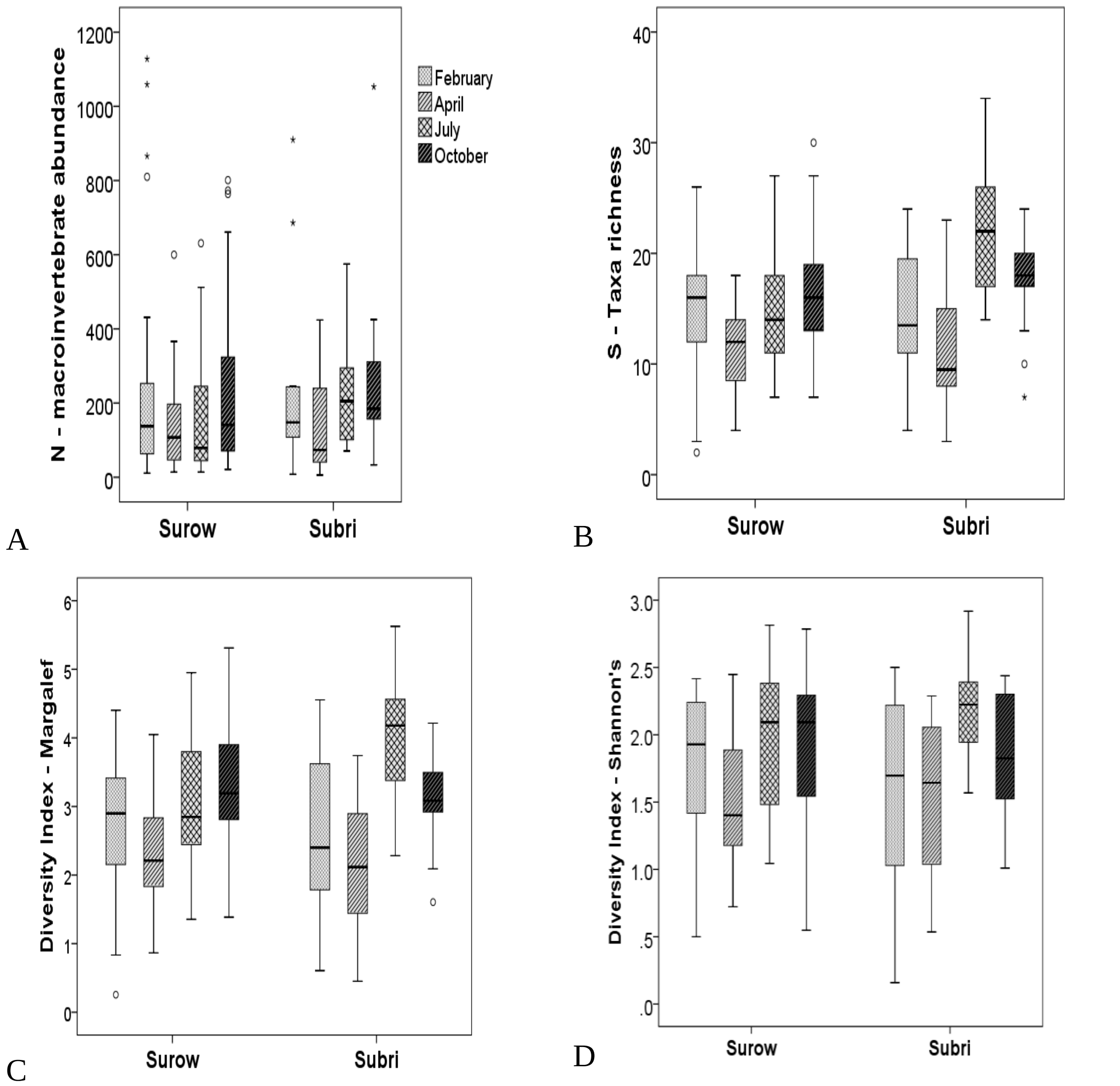


Figure 6.5 Seasonal variability in boxplots of macroinvertebrate abundance (A), taxa richness (B), Margalef's and Shannon-Wiener's diversity indices (C and D) in the Surow and Subri rivers between July 2013 and April 2014

6.3.4 Impact of mining on macroinvertebrate community: comparison between control and impact in the two rivers.

Macroinvertebrate abundance (N), taxa richness (S), Margaleff diversity (d) and Shannon diversity (H) indices did not differ with river nor between control and impact across rivers, although the interaction between river and C/I factors for S, d and H were significant ($p<0.01$) (

Appendix 31). Box plots (Figure 6.6) depict the indices variability between control and impact in each river.

Results of PERMANOVA on macroinvertebrate community compositions, however, showed that macroinvertebrate community compositions in the Surow were significantly different from that of the Subri, ($p<0.001$, $F=10.35$), control was different from impact ($p<0.001$, F 7.8) and the interaction between River and CI factors was also significant ($p<0.001$, $F = 3.5$). This suggests that the macroinvertebrate community compositions at impact sites in the Surow were different from that of impact sites in the Subri. Further SIMPER analysis (Appendix 32) identified the abundances of Chironomidae, Baetidae, Ceratopogonidae and Dysticidae at impact sites in the Surow were higher than in the impact sites of the Subri. Contrastingly, the abundances of Thiaridae, Atydae, Elmidae, Planorbiidae, Coenagrionidae, and Caenidae in the impact sites of the Subri were higher than the impact sites in the Surow. The sensitive to pollutant Ephemeroptera and Tricoptera families of Heptagenidae, Leptoceridae were also more abundant in the impact sites of the Subri than in the impact sites of the Surow.

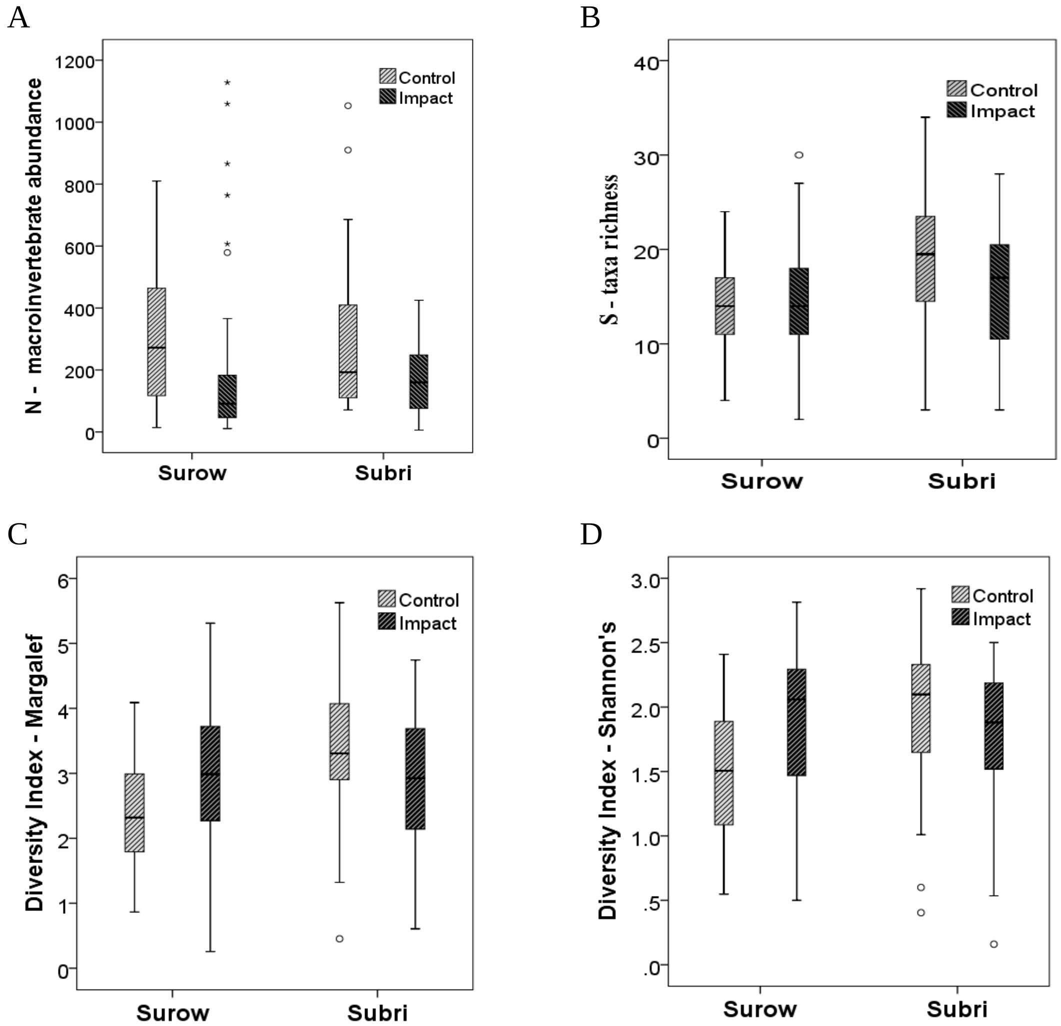


Figure 6.6 Spatial variability in boxplot of macroinvertebrate abundance (A), taxa richness (B), and diversity index (C and D) at Control and Impact sites in the Surow and Subri rivers between July 2013 and April 2014.

Table 6.6 Results of two-way PERMANOVA on macroinvertebrate community compositions between rivers (Ri), control and impact (CI) and the interaction between Ri and CI

Source	df	SS	MS	Pseudo-F	P(perm)
Ri	1	24336	24336	10.35	0.0001
CI	1	18276	18276	7.77	0.0001
RixCI	1	8224	8224.3	3.50	0.0004
Res	193	453810	2351.4		
Total	196	507590			

As shown in the MDS ordination (Figure 6.7), macroinvertebrate assemblages at control sites on the Surow River are clustered closely together, indicating relative similarity to each other, whilst in the Subri River the assemblages at control sites are widely scattered along the axis, indicating differences between the control sites. The wide separation between samples from the Subri River control sites was most likely due to them being located on three different tributaries to the main Subri, draining different sub-catchments of different sizes and morphology. This was not the case of the Surow River's control, where the sites, except for site 1, were on the main channel of the river, resulting in less variability between control sites macroinvertebrate assemblages. Consequently, macroinvertebrate assemblage at control in the Surow was different from that of the Subri. The significant difference between the two control sites, based on the SIMPER analysis, was due to the differences in the density of Chironomidae, Baetidae, Coenagrionidae, Belastomatidae, Atydae, Thiaridae, Dytiscidae, Hydroptilidae, and Libellulidae. The first three taxa were more abundant in the Surow River's control sites, whilst the rest were more abundant in the Subri River control sites. Macroinvertebrate assemblage at impact sites also differed between rivers (Appendix 34).

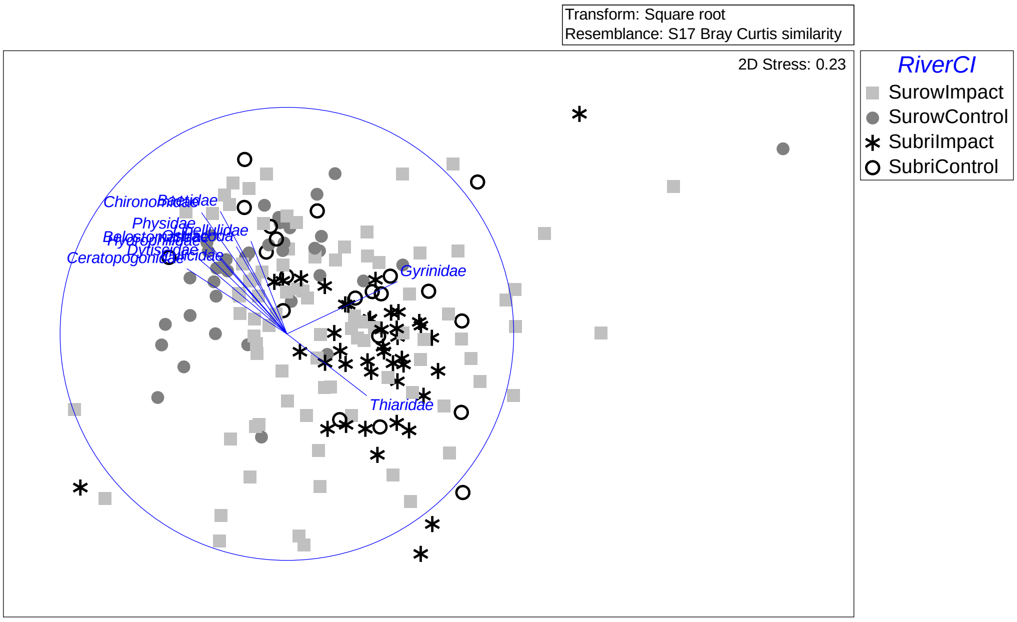
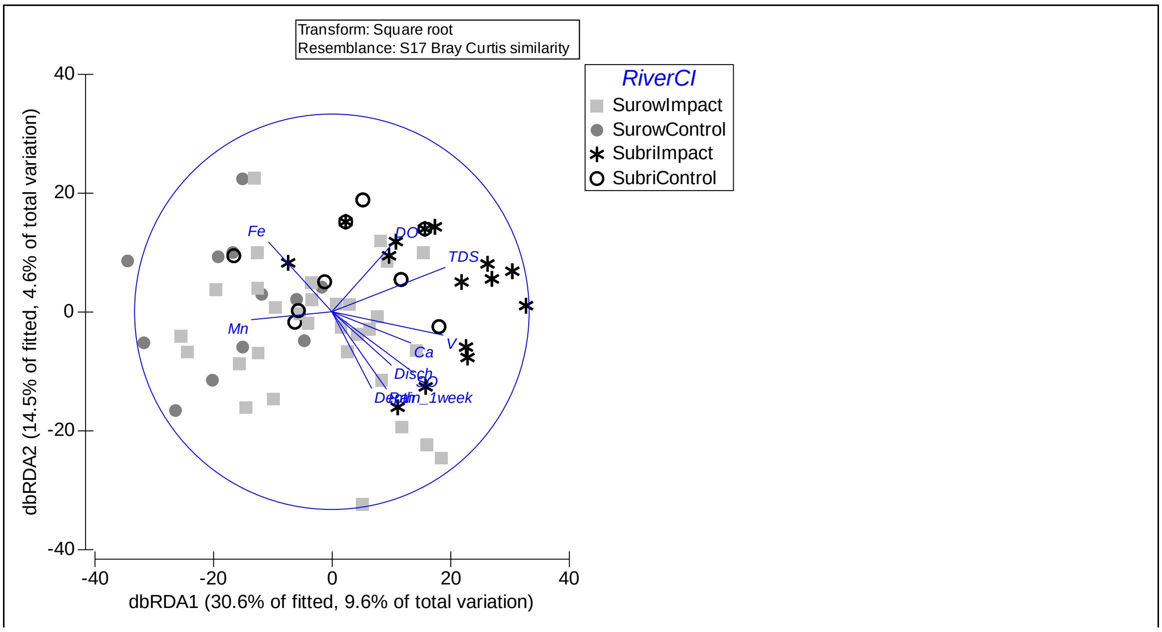


Figure 6.7 MDS ordination of macroinvertebrate assemblages in all samples (N=219 samples) illustrates differences between the Surow and Subri rivers and between Control and Impact sites within each river.

6.3.5 Macroinvertebrate assemblage correlations with river hydrology and physico-chemistry

Distance-based redundancy analysis (dbRDA) was employed to identify water quality and river hydrology variables that significantly correlate with the macroinvertebrate assemblage. Across rivers, 45% of total variance can be explained by the first two axes of dbRDA of the relationship between macroinvertebrate assemblage and the environmental factors, and another 19.6% of total variance are explained by the third and fourth axes of dbRDA (Figure 6.8A). The dbRA identified concentrations of Fe, Mn, Ca, sulfate, nitrates, TDS as well as river water velocity, depth, discharge and rainfall as significantly correlated (Pearson's r>0.4) with dbRDAs which are surrogate variables for macroinvertebrate assemblage. The correlations between environmental variables and dbRDA are presented in Table 6.7. Plotting the environmental variables with strong dbRDA Pearson's r against MDS ordinates as surrogate for the macroinvertebrate assemblage provide an insight into the correlations between these variables and macroinvertebrate assemblages (Figure 6.9). As can be seen in Figure 6.9, concentrations of Fe, Mn, and TDS strongly correlated with MDS1, whilst MDS2 tend to strongly correlate with the hydrological variables (river velocity, depth, discharge, rainfall) and MDS3 tend to strongly correlate with nutrients, sulfate and Ca.



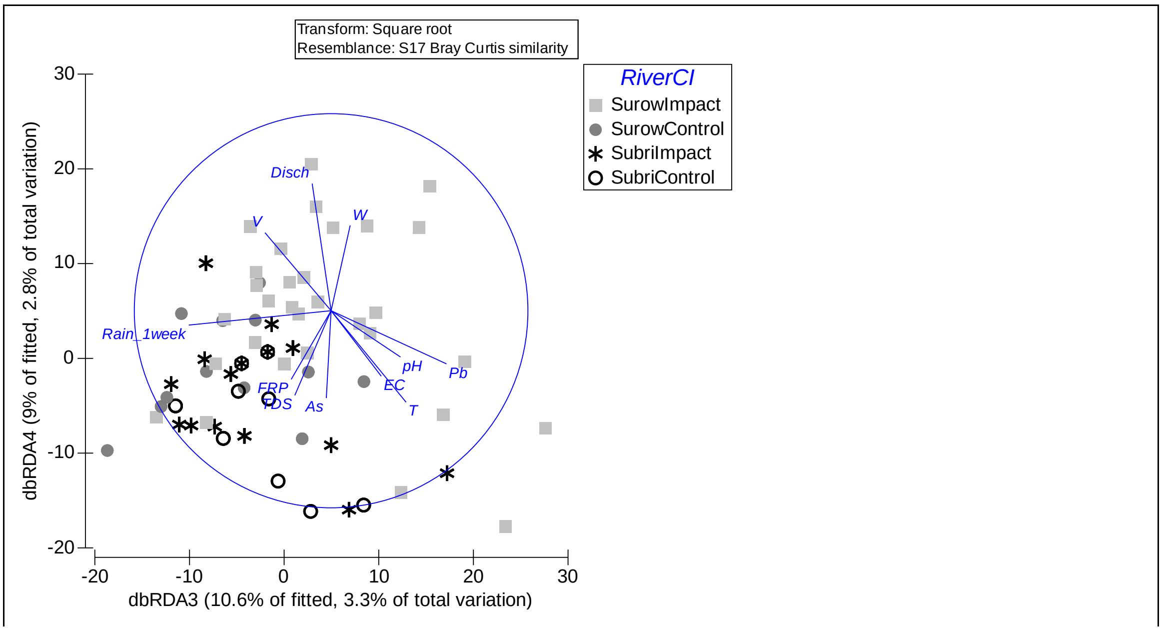
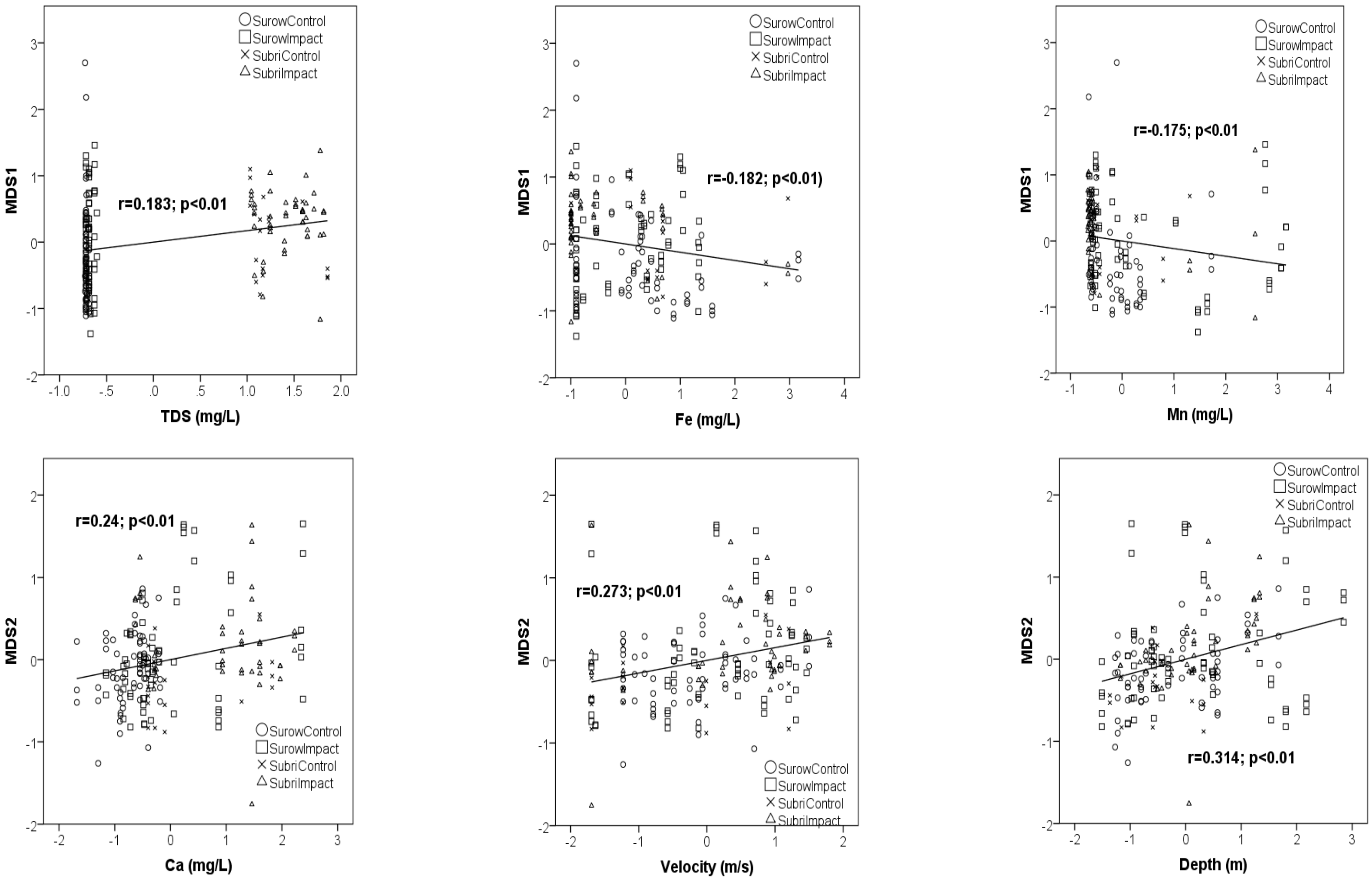


Figure 6.8 Distance-based redundancy analysis (dbRDA) (A = axis 1 and 2; B= axis3 and 4) on the relationships between macroinvertebrate community composition and environmental variables, explaining nearly 65 % of variance in overall macroinvertebrate assemblage. The vectors represent environmental variables with strong correlations (Pearson's r >0.4) with the macroinvertebrate community compositions.

Table 6.7 Relationships between dbRDA coordinate axes and environmental variables (multiple partial correlations). Strong and significant correlations (Pearson's r) are in bold

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA5	dbRDA6	dbRDA7	dbRDA8	dbRDA9	dbRDA10
V	**0.467**	0.044	-0.251	0.23	-0.05	-0.069	0.292	**-0.382**	-0.017	-0.211
Depth	0.042	-0.213	0.118	0.227	-0.195	-0.153	-0.009	**0.401**	0.106	-0.07
W	-0.151	0.121	0.03	**0.344**	**0.384**	**0.385**	0.089	-0.058	0.119	**0.401**
Disch	0.095	-0.227	-0.027	**0.352**	-0.168	-0.166	**-0.341**	0.078	-0.139	0.173
Rain_1week	0.063	**-0.392**	**-0.599**	-0.155	0.21	0.098	-0.092	-0.04	0.273	-0.065
T	-0.034	**-0.336**	0.243	**-0.331**	-0.172	0.015	-0.33	**-0.359**	0.3	-0.096
EC	0.236	-0.216	0.126	-0.223	-0.037	0.2	0.1	0.025	-0.285	0.024
DO	**0.348**	**0.306**	0.153	0.124	-0.114	0.028	0.037	0.06	0.091	0.011
pH	0.077	0.098	**0.304**	-0.214	0.041	0.053	0	-0.113	0.181	0.042
TDS	**0.432**	**0.347**	-0.104	**-0.332**	0.146	-0.096	-0.28	0.247	**0.304**	0.23
Turb	-0.093	-0.028	0.16	0.02	0.132	-0.186	0.115	**0.367**	0.034	-0.212
Al	0.095	-0.228	0.163	0.198	-0.007	0.172	-0.258	-0.051	0.081	0.092
As	-0.288	-0.065	-0.15	**-0.311**	-0.004	-0.063	0.049	0.191	-0.299	0.202
Cu	0.089	0.068	-0.023	0.077	-0.008	-0.151	-0.005	-0.32	-0.237	-0.15
Fe	-0.243	**0.318**	-0.131	0.248	-0.194	0.246	**-0.518**	-0.053	0.084	-0.16
Mn	-0.199	-0.003	0.164	-0.008	**0.384**	0.011	-0.065	**-0.304**	-0.169	-0.049
Pb	-0.067	0.144	**0.336**	-0.117	0.16	-0.093	0.081	-0.124	0.164	-0.052
Mg	0.122	-0.023	0.222	-0.09	**-0.341**	0.253	-0.063	0.002	-0.266	-0.021
Ca	0.154	-0.018	-0.034	-0.057	-0.041	-0.17	-0.128	-0.172	-0.281	0.64
Nox	0.225	0.077	-0.051	-0.144	0.271	**0.445**	-0.241	0.208	**-0.398**	**-0.317**
FRP	-0.215	0.231	-0.224	-0.191	**-0.502**	**0.321**	0.265	-0.062	0.105	0.109
SO	0.163	**-0.325**	0.161	0.109	-0.004	**0.423**	0.271	0.108	0.204	0.146



SurowControl
SurowImpact
SubriControl
SubriImpact
r=0.183; p<0.01
MDS1
TDS (mg/L)
r=-0.182; p<0.01)
MDS1
Fe (mg/L)
r=-0.175; p<0.01
MDS1
Mn (mg/L)
r=0.24; p<0.01
MDS2
Ca (mg/L)
r=0.273; p<0.01
MDS2
Velocity (m/s)
r=0.314; p<0.01
MDS2
Depth (m)

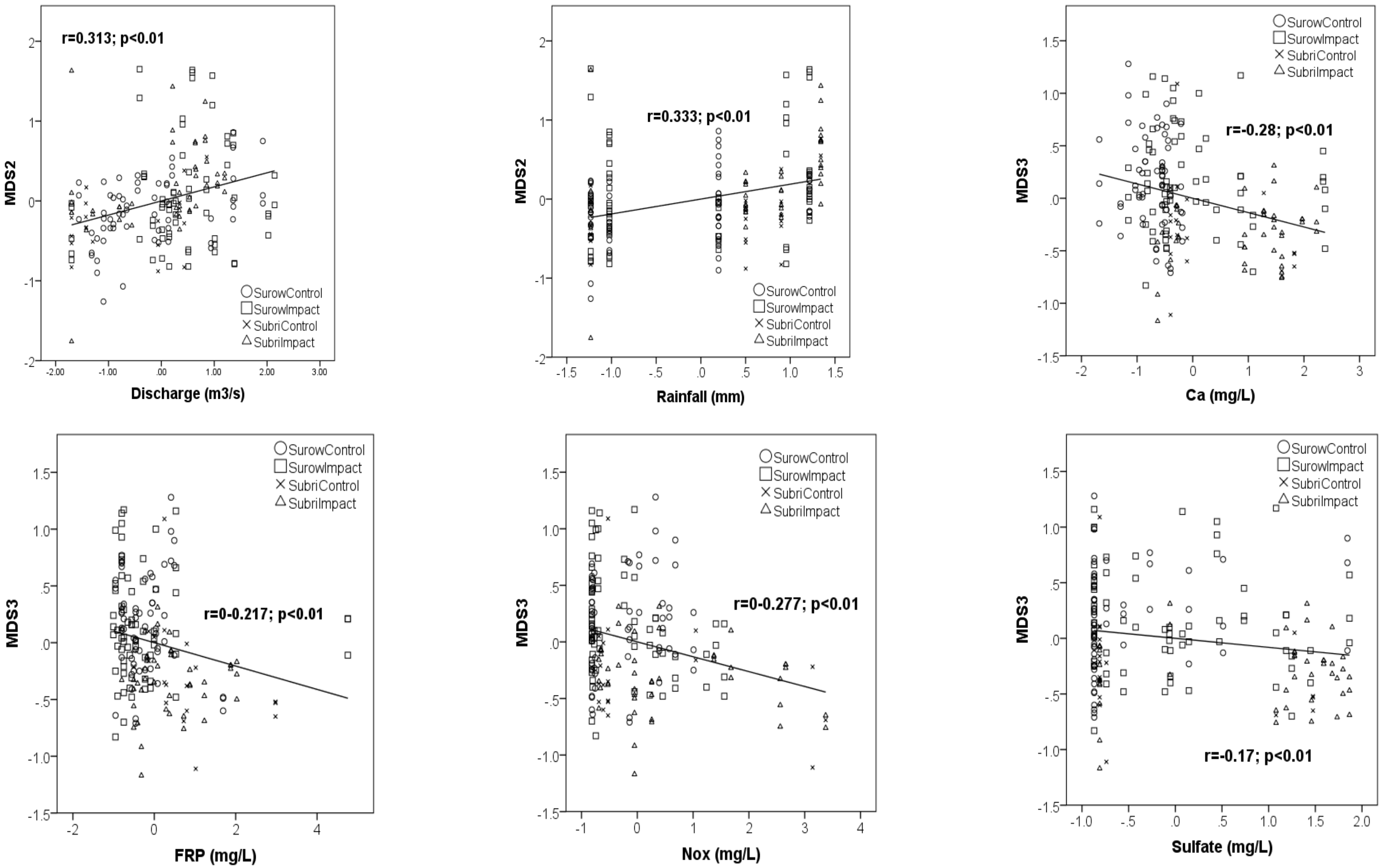

r=0.313; p<0.01
MDS2
Discharge (m3/s)
SurowControl
SurowImpact
SubriControl
SubriImpact
r=0.333; p<0.01
MDS2
Rainfall (mm)
SurowControl
SurowImpact
SubriControl
SubriImpact
r=-0.28; p<0.01
MDS3
Ca (mg/L)
SurowControl
SurowImpact
SubriControl
SubriImpact
r=0-0.217; p<0.01
MDS3
FRP (mg/L)
SurowControl
SurowImpact
SubriControl
SubriImpact
r=0-0.277; p<0.01
MDS3
Nox (mg/L)
SurowControl
SurowImpact
SubriControl
SubriImpact
r=-0.17; p<0.01
MDS3
Sulfate (mg/L)
SurowControl
SurowImpact
SubriControl
SubriImpact

Figure 6.9 Correlations (Pearson's) between MDS coordinates of macroinvertebrate assemblages and the concentrations of Fe, Mn, TDS, Ca, FRP, nitrates, sulfate, river flow, depth, discharge and rainfall with Rivers X Control/Impact overlay. The Pearson's r and significance levels of the correlations are indicated.

6.4 Discussion

The study has resulted in at least four important findings. First, macroinvertebrate taxa richness and diversity (family) in this study were comparable to that of previous studies in the region and in the temperate region, and potentially higher should they be identified to the species or genus levels. Second, macroinvertebrate community compositions in both rivers varied with seasons. Third, macroinvertebrate community indices (diversity, richness, abundance) are similar between rivers, and between control and impact. However, macroinvertebrate community assemblage (compositions) are different between rivers, between control and impact, and the interaction between the two factors. Fourth, hydrological variables (velocity, river depth, discharge, rainfall) and concentrations of metals (Fe, Mn), salt ions (Mg, Ca) as well as sulfate and nutrients strongly correlated with macroinvertebrate assemblage. The following sub-sections will discuss the findings.

6.4.1 Macroinvertebrate abundance and diversity

With 88 macroinvertebrate taxa at family or higher taxonomic levels, this study has found more taxa than previous studies in Ghana and neighbouring countries. The taxa richness in this study would have been much higher if identification was completed to species level as demonstrated by the identification of 77 taxa by CSIR within 8 macroinvertebrate samples (Appendix 6-1). The potentially much higher taxa richness was also evident during the identification process where variation within a family were visible. For example, three different types of Ostracoda, three sub-families of Veliidae, and several sub-families of Planorbidae, Thiaridae, Dytiscidae and Chironomidae were visible in the samples although these could not be practically named.

The families seen in this study are also commonly found in temperate regions, with a few exceptions. Plecoptera, an important indicator group in the temperate region and a part of the EPT taxa richness index often used to measure pollution, was not collected in this study. Plecoptera species are rare in West Africa (Cloudsley-Thompson, 1982; Thorne et al., 2000) although Camara et al. (2012) reportedly found 3 species of Plecoptera in a study in the Ivory Coast. Amphipod and isopod crustaceans, which are abundant in temperate zones and often act as indicator of medium pollution, were almost negligible in this study (less than 1% of total abundance) and concentrated only at site 7 on the Surow River in July, possibly indicating pollution at the site. However, instead of amphipods and isopods, we found the freshwater prawn (Atyidae) and crabs (Potamidae), consistent with the study of Thorne et al. (2000) in this region as well as Dudgeon (2000).

Many of the taxa found in this study were also found in other studies in Ghana, although taxa richness and density were almost twice as high as that of clean and polluted streams in Accra as reported by Thorne et al. (2000) and Baa-Poku et al. (2013), and that of an aquaculture impacted stream reported by Ansah et al. (2012). The higher macroinvertebrate taxa richness and density may be due to this study's intensive sampling program, which covered not only more sampling sites but also four different seasons, capturing the effect of seasonality in taxa richness. Thorne's study for example, only sampled 8 sites using multiple samplers that were immersed for six weeks, yielding 31 taxa of 27 families. The difference in taxa richness between Thorne's and this study is also evident in the difference in the number of Ephemeroptera and Trichoptera families. Thorne's study only found 2 Ephemeroptera (Baetidae and Caenidae) and two Trichoptera (Hydropsychidae and Policentropodinae) families which mostly were found in the cleaner part of the river; whilst our study found a total of 6 ephemeropteran and 8 trichopteran families including those also found in Accra. However, we cannot make a distinct comparison between the Surow or Subri river water quality and that of the Accra stream. Water quality in the Accra stream in Thorne's study was sampled less extensively than ours (5 times over a 6 week period) and metal concentration in water was not sampled. The polluted part of the stream was reportedly characterised by high BOD, TSS, pH and low dissolved oxygen, which was comparable to Surow and Subri rivers. Unlike in our study area, however, ammonia and nitrate in the Accra stream were quite low. The riparian conditions of this study site / sampling sites, as explained in Section 2.8 in Chapter 2 may also contribute to the taxa richness. Although patchy, most of the sites in this study had some canopy with plenty of organic input (see Chapter 2), which was in contrast with the urban environment of Accra where Thorne, Baa-Poku and Ansah conducted their studies.

The number of family taxa recorded in this study was slightly higher than a study in Ivory Coast (Camara et al., 2012), a country sharing borders and climatic conditions with Ghana. It is also significantly higher than that of reported by Arab et al. (2004) from a study in Algeria, North Africa. Camara et al (2012) reported 74 macroinvertebrate families and 132 taxa from a year study on the Banco stream (9 km long) comparable to the Surow and the Subri rivers in size, located within the Banco National Park in Ivory Coast. Unlike the Banco stream study, no Plecoptera were encountered in the Surow or Subri rivers. The Banco stream lies within a national park, and is less likely to suffer more anthropogenic impacts than the Surow or Subri Rivers. Differences in water quality between Banco stream and the Subri and Surow Rivers might account for the difference in presence of Plecoptera, however Camara et al (2012) did not provide any metal or nutrient water quality data for comparison.

6.4.2 Temporal variance in macroinvertebrate community assemblage

Temporal variability in macroinvertebrate community abundance and diversity in the Surow and Subri Rivers is typical of tropical rivers, driven by seasonality in rainfall (Blanchette et al., 2013; Pringle et al., 2000). In the tropics, including in the study area in Ghana, floods and low water flows are two dominating hydrological characteristics of rivers that control habitat availability and heterogeneity (Puckridge et al., 1998). Macroinvertebrate abundance and richness in rivers normally correspond with habitat heterogeneity and availability. In the study area, the rainy seasons often bring floods to the rivers in April and late September, while the long dry season between December and February often temporarily disconnect the rivers into a series of pools, particularly in the lower reaches. Across rivers, macroinvertebrate assemblages in July and October were similar, and abundance and richness were the highest, despite the separation by a very dry season that occurs in August. This suggests that the August dry period has limited impact on the river hydrology and therefore macroinvertebrate community. The high richness and abundance recorded in July and October, may also be explained by the high flows during the months, which provide maximum habitat availability in the river, away from the disturbance associated with extreme flows in April and September or the low/no flows in February (Puckridge et al., 1998; Welcomme, 1979).

Despite regular discharges of mine dewatering water from the ASGM on the Surow River and the Ahafo mine on the Subri River, these do not prevent the rivers from becoming disconnected into shallow pools during February. According to Williams (1996), such drying process poses significant biophysical challenges to the riverine biota, including macroinvertebrates. Depending on whether the drying process is predictable (e.g. due to annual climatic changes seen in this study) or unpredictable and length of the dry season, biota adapt to the shrinking of habitat size and availability. The adaptation include ‘terrestrialisation’ of their habitats, migration, and modification of life cycle (Williams, 1996). During drought, many macroinvertebrates including ephemeropterans and odonates may survive as eggs (Lehmkuhl, 1973), chironomids may survive as eggs or larvae (Williams et al., 1977) while coleopterans and hemipterans can survive and migrate as adults (Larson, 1997; Macan, 1939). The puddles formed in downstream of the rivers during the long dry season, often provide refuges (Robinson et al., 2011) for drought intolerant taxa and opportunistic taxa including some families from the order Diptera (Chironomidae and Ceratopogonidae), Coleoptera and Hemiptera (Williams, 1996) and Thiaridae (Gooderham et al., 2002). In this study in April, when the first rainy season began, these refugia were incorporated back into the river, with loss of some species and recolonisation of new habitat. This resulted in the similar assemblage of April samples to that of February, although April had the lowest abundance and diversity per sample across the rivers. The drop in community abundance and taxa richness in the first flush of rainy season was also observed by Larson (1997) and Camara et al. (2012).

In the Surow River, the natural annual drought was often worsened by water abstraction from the drying river, mostly by miners, for ASGM related activities (Figure 6.10). If water abstraction continues and intensifies, the loss of refugia in pools might significantly impact survival of some macroinvertebrate taxa. On the other hand, water discharge from mine dewatering on both rivers especially on the Subri, may disturb the natural cycle of drought in the river.

A

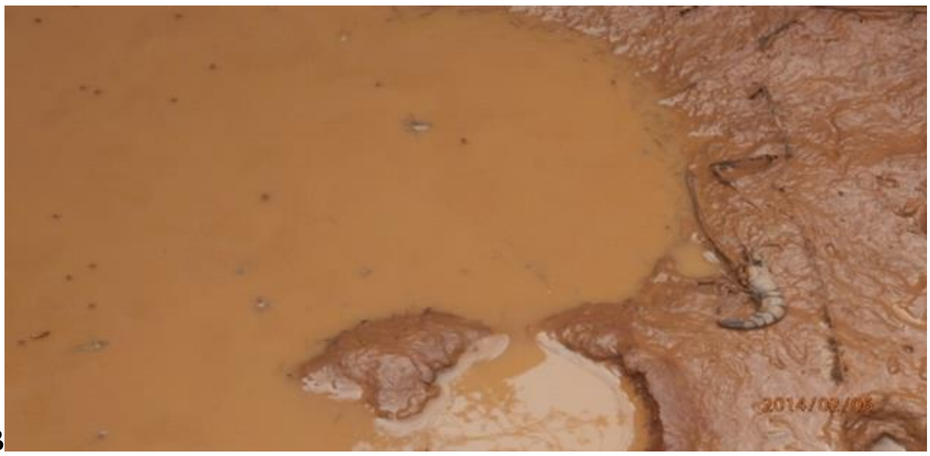

B


Figure 6.10 Abstraction of river water by miners on the Surow in dry seasons (A) often worsen the naturally reduced habitats for macroinvertebrates (B).

6.4.3 Impact of mining on macroinvertebrate communities

Macroinvertebrate abundance, taxa richness and diversity in each of the Surow and Subri River declined at impact compared to control. Two-way ANOVA, however, showed that the indices did not differ with river nor between control and impact, suggesting the indifference between ASGM and large mining impacts on macroinvertebrate abundance, taxa richness, and diversity. Analysis of variance in macroinvertebrate communities however, cannot be based solely on their indices. Two communities may have exactly the same number of macroinvertebrates, the same number of taxa and diversity indices; but they are not necessarily the same, because the community assemblage in each community may be different from one another, and their responses to pollutants can vary as well. Consequently, the univariate analysis of macroinvertebrate community indices should be accompanied by the multivariate analysis of the community structure (Anderson et al., 2008) as we discuss in this section

In this study, taxonomic richness decreased at impact sites compared to control in each of the Surow and the Subri rivers, but the decline in the Surow River mean taxa richness was greater at 20% compared to the 7% decline in the Subri. The decrease in taxa richness in both Surow river maybe explained by the decrease in habitat heterogeneity and availability (Eggleston et al., 1998) although it was not so obvious for the Subri. In the Surow River, increased sediment loads and concentrations of metals and metalloids in ASGM impacted sites (Chapter 3) most likely contributed to the decrease. A decline in macroinvertebrate abundance and taxa richness in rivers impacted by mining has been seen in similar studies in temperate and tropical regions (Bruns, 2005; Faith et al., 1995; Pond et al., 2008; Smolders et al., 2003).

Impact sites on the Subri River, in contrast to that of the Surow River, were characterised by low suspended solids (sediment particle) and metal concentrations in water, but the water in downstream Subri was high in salinity (TDS), nutrients and associated salt ions (Ca, Mg, K). The less turbid water at impact site resulted from the sediment control measures and environmental control dam (ECD) put in place by the Ahafo mine (see Chapter 4) is discharged into the Subri, upstream of NSW8. Despite the sediment control measures, however, the decrease in taxa richness at impact sites in the Subri River remains significant. The discharge of mine water into the Subri River can contribute to the decrease in taxa richness and macroinvertebrate abundance at impact sites due to increased flows in downstream Subri River flows. This can also have a scouring effect on macroinvertebrate habitats at the impacted sites, reducing habitat availability and heterogeneity, which can result in reduced taxonomic richness as well as macroinvertebrate density. ECD may also have captured much of the suspended food in the water and reflected in the taxa lost in the impact areas.

Abundant tolerant to pollutant taxa also inhabited impact sites in both rivers, which is often an indication lack of competition from the more sensitive to pollutant taxa; hence an indication of pollution (Wagenhoff et al., 2012). However, macroinvertebrate assemblages at impact sites of the two rivers were not similar, most likely due to the variability in types of impacts seen. The Subri impact sites had more highly sensitive taxa including Elmidae, Ephemeroptera and Trichoptera families than the Surow. Ephemeroptera (including Heptageniidae) and Trichoptera are considered the most sensitive to pollution. For example, Clements (2004) found that a moderate level of metal pollution can reduce heptageniid mayflies (Ephemeroptera; Heptageniidae) by more than 75%. Barber-James et al. (2008) also reported a decline and eradication of Ephemeroptera population in some European streams affected by acid mine drainage associated with high concentrations of metals. Consequently, an increase in taxa richness within ephemeropteran group can be considered a reflection of improved water quality (Barbour et al., 1992; Plafkin et al., 1989). The higher taxa richness within Ephemeroptera in the downstream Subri river (see Figure 6.3), therefore, suggested a more favourable water and sediment quality than in downstream Surow river affected by ASGM. Similarly, the more abundant dipteran families of Chrironomidae and Culicidae, which are known to be very tolerant to pollutant (Chessman, 2003; Gooderham et al., 2002), in the impact sites of the Surow River compared to that of the Subri can also be considered a reflection of the dominance of the tolerant taxa due to the lack of competition from the more sensitive to pollutant taxa. Hence, it can also be considered an indication of a worse water and sediment quality in downstream Surow River than that of downstream Subri River.

This assertion is consistent with the water quality of the rivers discussed in Chapter 3 and 4. As discussed in Chapter 3 and 4, riverine sediment and water quality in the Subri was generally less polluted than that of the Surow River. The lower sediment load and metal concentrations at Subri River impact sites seemed to have resulted in a more favourable environment for all taxa, tolerant or intolerant to pollution. The clear water with lower metal concentrations also added to the depth and velocity of the river's flow, resulted in a steadier and more rapid flow favourable to the sensitive and rapid flow inhabitant taxa such as Elmidae (Gooderham et al., 2002), which was found in most impact sites on the Subri River. This, also resulted in more competition for the sediment and pollutant tolerant taxa such as Hydroptillidae, Dytiscidae, Syrphidae and Psychodiidae, as reflected in the decrease in their abundance at impact sites on the Subri River. In other words, macroinvertebrate community at impact sites on the Subri River had more pollutant-sensitive taxa, whilst at control sites, macroinvertebrate community consisted of pollution-tolerant taxa, reflecting the altered water quality at impact sites.

The Surow River's impact sites were characterised by high turbidity and concentrations of metals. Elevated turbidity and sedimentation often resulted from mining, particularly alluvial mining activities (Kelly, 1988) (Wagener et al., 1985a) and were detected and visible in the reaches of the Surow River, including sites 4, 5, 7, 9 and 11. At these sites, turbidity levels often reached >2000 NTU. Turbidity levels as low as 5 NTU was reported to decrease aquatic primary productivity by 3 -13 % (Ryan, 1991). Elevated turbidity and sedimentation are reported to decrease macroinvertebrate abundance and biomass as well as increase drift rates (Henley et al., 2000; Ryan, 1991; Wagener et al., 1985b; Yule et al., 2010) which eventually changes the macroinvertebrate community composition. This phenomenon was also seen in my study whereby macroinvertebrate abundance significantly decreased at impact sites on the Surow River whilst diversity increased.

Increased sediment load and sedimentation, which are common in rivers impacted by mining, have been known to reduce macroinvertebrate abundance and taxa richness (Yule et al., 2010) through reduced habitat availability and heterogeneity in riverine ecology (Gray et al., 1982; Wagenhoff et al., 2012; White et al., 1976). Initially, increased fine sediment provides subsidy to pollution-tolerant taxa and macroinvertebrate abundance, but it is consistently followed by negative effects on other taxa, taxa richness, and abundance of pollution sensitive taxa such as the EPT (Ephemeroptera, Plecoptera, Trichoptera).

The effects of increased sediment load were apparent in the Surow River, where the abundance and taxa richness of sensitive Ephemeroptera and Trichoptera decreased at impact sites. Chironomids abundance also decreased at impact sites of the Surow, although the decline was less than that of in the Subri. However, although fine sediment is a significant single stressor, Wagenhoff et al. (2012) warned that macroinvertebrate communities commonly react to multiple stressors and rarely respond to a single stressor. In his study on chironomids, for example, Wagenhoff showed that the decline in chironomids abundance was a result of a complex synergistic pattern of various stressors, including sediment. Decreases in chironomid abundance as seen in the impact sites in this study, therefore, should not be explained merely by increased sediment load, but other factors should also be considered, as discussed in Section 6.4.4.

6.4.4 Relationships between macroinvertebrate community assemblage and river environmental parameters

The variance in macroinvertebrate assemblages between sites, rivers, and seasons and control and impact sites in the study area appeared to reflect the differences in water chemistry and temporal changes. The study, however, could not arrive at one statistically superior model to predict macroinvertebrate assemblage using the river's environmental attributes as predictor variables. The difficulties in modelling macroinvertebrate assemblage is not uncommon, particularly when dynamic environmental variables such as pH, concentrations of DO, pollutants and river depth and flows are used as predictors (Ambelu et al., 2010; Hoang et al., 2001). These variables, in lotic systems, may change in a very short time as the river water flows while macroinvertebrate assemblage respond to longer terms stressor exposure. In many cases, the difficulties in modelling are also caused by the limited number of macroinvertebrate taxa in a community that can be modelled (Ambelu et al., 2010). Unlike this study, most macroinvertebrate modelling have used metrics or presence / absence data as the response variables rather than macroinvertebrate assemblage and a few selected static variables such as altitude and river length (Ambelu et al., 2010; D'heygere et al., 2003; Dedecker et al., 2004; Hoang et al., 2001).

The application of principal component analysis in the dbRDA method, however, has enabled us to predict the relationships between macroinvertebrate assemblage and various environmental variables. In the Surow and Subri rivers, hydrological variables such as water velocity, river depth, discharge and rainfall strongly and significantly correlated with macroinvertebrate assemblage (see Figure 6.9). The finding is in agreement with Wills et al. (2006) who modelled the relationships between water flows and the insects density. Wills' model showed that a 90% decrease from the baseflow drastically reduced the EPT insects' density. In Ahafo, while much of hydrological variability was seasonal (e.g. river flows, depth, one-week rainfall), flows in the Surow and Subri rivers were both positively and negatively affected by mining activities. In the Surow River, discharge of mine dewatering water usually took place during the wet season, and water abstraction by ASGM operators in the dry season. Mine dewatering discharges by the Ahafo mine into the Subri River, however, were more regular, with lack of discharge in the dry season of Harmattan as an exception, resulting in more predictable flows downstream of the river for the rest of the year.

The dbRDA also shows that concentrations of metals (Fe, Mn, As), salt ions (Mg, Ca) as well as sulfate, nitrates and FRP strongly correlated with macroinvertebrate assemblage. The differences in concentrations of these elements separated the rivers, control from impact sites, as well as wet from dry seasons in regards to macroinvertebrate assemblage

(Figure 6.8). Concentrations of these elements were positively associated with the drier months (February and late October), corroborating Williams (1996) assertion that in the drier seasons macroinvertebrate must cope with the increasing water salinity due to more concentrated metals and ions. Salt ion concentrations of Ca, Mg and sulfate, as well as TDS, however, were more greatly associated with the seasonal effect of rainstorms in April across rivers than spatial factors, representing the 'first flush' effect (Beasley et al., 2002; Faulkner et al., 2000) on both physico-chemistry of river water and macroinvertebrate community structure. These floods affect macroinvertebrate abundance as discussed in section 6.4.2. The April floods also transport pollutants from rivers catchments and dilute salts ions (Ca, Mg, sulfate) from waste rocks, tailings, and ores in the open cut and underground mines associated with both small-scale and large-scale gold mining (Jeffery et al., 1988; Salomons, 1995).

Turbidity, which was the main characteristics of the Surow River water, however, did not strongly correlate with macroinvertebrate assemblage, but strongly correlated in the Subri. This, could be explained by the variance of turbidity in each river. In the Surow River, turbidity was continuously high and at some sites downstream of ASGM, turbidity levels were above the probe's highest limit of detection of 2000 NTU (and recorded as 2000 NTU instead). This might result in insignificant differences between samples, which is the basis of Euclidian distance used in PCA/dbRDA, lead to the insignificant correlations between macroinvertebrate assemblages and turbidity in the Surow River. On the contrary, turbidity in the Subri River, which was a lot lower than in the Surow, changed significantly down the river, particularly at impacted sites as a result of the sediment control measures (ECDs), resulting in the significant correlation as per PCA/dbRDA.

Filterable reactive phosphorus (FRP) also correlated significantly with macroinvertebrate assemblage across rivers. As can be seen in Figure 6.8, differences in FRP concentrations contributed to the separation of macroinvertebrate assemblages in control from that of impact. Higher concentrations of FRP were associated with the upstream sites on both rivers, where canopy cover was higher and agriculture activities were more intensive (personal observation). The correlation between NOx and macroinvertebrate assemblage, however, was only significant in the third axis of MDS (Figure 6.9), which mostly associated with the elevated concentrations of NOx (alongside TDS and Mg) at site KSW3 and NSW8. These sites were situated within the Ahafo mine tenement, directly receiving water from the mine that most likely containing salts including Mg and nitrate commonly associated with mine impacted areas (Jeffery et al., 1988). Whilst high concentrations of NOx can be toxic to freshwater macroinvertebrates (Camargo et al., 2005; Camargo et al., 1992), the effects of nutrients in macroinvertebrate community, however remained unclear. Pearson et al. (1998) reported that elevated nutrient concentrations often did not significantly change macroinvertebrate abundance, diversity and evenness, unless the disturbance was extreme and for a very long time. Matthaei et al. (2010) also reported that the effects of nutrients were less common than that of sediment addition and flow reduction. Nutrients, however, can impact macroinvertebrate community structure through eutrophication. The presence of algal grazers such as Thiaridae and Physidae in abundance was an indication of eutrophic environments. Although the elevated concentrations of NOx in KSW3 and NSW8 on the Subri River remained in compliance with the Ghanaian and USEPA guidelines for the protection of aquatic life (see Chapter 4), the elevated density of Thiaridae and other Gastropod families at these sites and further downstream suggested the effects of elevated nutrients on the abundance of these taxa. However, the proportion of variance explained by dbRDA on all 88 families, were relatively low, ranging between 40 and 45 % within two axes and between 51 and 55% within 3 axes. This, suggests that there remains much variation which are unexplained by the large number of environmental variables assessed in this study to predict the community assemblage.

6.5 Conclusions

This study shows that the diversity, taxa richness and composition of the macroinvertebrate communities found in the Surow and Subri Rivers are comparable to previous studies in the region as well as those from the temperate regions. The Subri River generally supports more taxa with higher diversity and abundance than the Surow. The higher taxa richness in the Subri River may reflect its larger catchment area as well as the different types and levels of stressors than that of the Surow. Macroinvertebrate abundance, taxonomic richness, diversity indices and community compositions in the rivers are also seasonal.

The abundance, taxonomic richness and diversity indices of the macroinvertebrate communities, however, did not differ with rivers nor between control and impact, and the interaction between rivers and control and impact was not significant. However, macroinvertebrate community compositions were different between rivers, control and impact, and the interaction between the two factors was also significant. Further pairwise analysis showed that the macroinvertebrate community compositions at impact sites on both rivers were also significantly different from each other. More sensitive to pollutant taxa, including Ephemeroptera, were found in downstream Subri than in downstream Surow, whilst more tolerant to pollutant taxa, including Chironomidae, were found in downstream Surow than downstream Subri.

While the study does not have the before and after information to positively demonstrate whether gold mining had impacted macroinvertebrate community in the Surow and Subri rivers, this study shows strong significant relationships between macroinvertebrate community assemblage and some physico-chemical and hydrological features associated with the effects of ASGM and the Ahafo gold mine activities on receiving rivers. Differences in concentrations of metals/metalloids (Fe, Mn), salt ions (Ca, Mg, sulfate), TDS, nutrients (FRP and NOx) as well as hydrological features (velocity, depth, discharge, rainfall) significantly correlate with differences in macroinvertebrate assemblages between rivers, between control and impact sites, and between the wet (flood) season and drier ones.

This study shows that macroinvertebrate community assemblage can be used in the study of impacts of both artisanal and large scale mining in tropical regions. Monitoring of riverine macroinvertebrate not only will provide the mining communities, local government and other stakeholders with an alternative way to monitor mining impacts in the area, but also an important step in understanding the ecology of freshwater ecosystems necessary for conservation.

7 Changes in riverine sediment microbial communities due to gold mining activities

7.1 Introduction

This chapter explores possible changes in the riverine sediment microbial community due to artisanal gold mining operations on the Surow and modern gold mining on the Subri River. As discussed in the preceding chapters, gold mining can affect riverine ecology due to potential releases of solutes used, released or modified by mining and metallurgical processes. These potential pollutants include sediment, nutrients, metals and metalloid elements, including Fe, Mn, Ca, Mg, and NOx (Chapter 3 and 4), as well as elements used in gold processing such as Hg for ASGM and cyanide for modern gold mining. In a riverine ecosystem, these elements may affect or be affected by the biogeochemical cycling of carbon, nitrogen and sulphur performed by microbes (see Küsel, 2003; Offre et al., 2013).

In the carbon cycle, microbes dominate carbon assimilation, mineralisation, and methanogenesis pathways (Offre et al., 2013). Crenarchareota and Euryachaetoa, for example, can assimilate carbon from inorganic compounds (Berg et al., 2010) including carbonate materials commonly unearthed by mining activities. Archaea and Bacteria are also central to the global nitrogen cycling through oxidation of ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-) (Camargo et al., 2006; Fierer et al., 2012; Ramirez et al., 2012), whose concentrations can be elevated by mining discharge into surrounding waters (Häyrynen et al., 2009; Koren et al., 2000; Malaiyandi et al., 1981). Other important roles of microbes are in the cycling of Fe and S by performing sulphide oxidation of sulphide minerals such as pyrite, marcasite and chalcopyrite, which are commonly found in mining areas (Offre et al., 2013; Salomons, 1995). In the oxidation process, some bacteria leach metals into the aquatic environment which may lead to poor water quality (Küsel, 2003). Sulfate-reducing bacteria (SRB) can also convert heavy metals, which are often released by mining, into their methylated forms, which can be even more toxic and bioavailable (Macalady et al., 2000). On the other hand, sulphite oxidation by SRB can also be inhibited by elevated heavy metals (Kandeler et al., 1996; Sobolev et al., 2008). Certain bacteria including *Thiobacillus* are also capable of breaking down cyanide commonly used in gold mining (Akcil et al., 2003; Koren et al., 2000).

Microorganisms play important roles in the biogeochemical processes in mine affected environments. They are essential in the formation of AMD (González-Toril et al., 2003; Lear et al., 2009; Rowe et al., 2007), methylation of heavy metals (Macalady et al., 2000; Sánchez-Andrea et al., 2011) and leaching of ammonia and nitrates at mine affected environments (Leininger et al., 2006), but they can also remediate the impacts (Akcil et al., 2003). For these reasons, microbial communities in areas affected by mining are increasingly studied and used as indicators of environmental health, particularly that of riverine ecosystem.

Microbes including Archaea and Bacteria can provide useful information on aquatic ecosystems (Singh et al., 2009) because of their ubiquitous presence in river water and sediment and their roles in the biogeochemical transformations. Microbes in riverine sediment and biofilms are especially important because they are more abundant than in the water column (Torsvik et al., 2008; Torsvik et al., 2002). The sessile habits of microbes in surface biofilm expose them to the water column making the community a potentially longer-term indicator of stress and disturbances in the river water than water column microbes (Lear et al., 2009). However, most of the studies on the use of microbial communities as bio-indicators, at least until recently, were based on microbes in the water column (Griebler et al., 2009), partly due to difficulties in culturing most sediment microbes (Torsvik et al., 2002) and the limited number of bacteria that have been formally identified (Lombard et al., 2011). Established methods of enumerating microbial organisms by culturing can only identify a small fraction of soil / sediment microorganism community. About 99% of the microorganisms cannot be cultured by standard methods; and those uncultured fraction are not related or only distantly related to the cultured ones (Riesenfeld et al., 2004). Furthermore, the culturing based methods are expensive, time consuming, labour intensive and requiring of much taxonomic expertise (Wakelin et al., 2008).

The challenges and pitfalls of the culturing based methods, however, have been superseded by advances in high-throughput DNA sequencing technology, which has enabled the identification of microbial communities without having to culture them (Hall, 2007; Tringe et al., 2005). The technique has enabled genomic analysis of microorganisms by identification of their DNA which are directly extracted (without culturing) and cloned from an assemblage of microorganisms, a technique which is commonly known as metagenomics (Taberlet et al., 2012; Tringe et al., 2005). Further developments in metagenomic techniques, such as the use of 454 pyrosequencing technologies in the 2000s, makes it possible to skip the cloning steps. Subsequently, microbiologists have been extracting microbial DNA from soil and analysing the genetic makeup of uncultivable microorganisms (Curtis et al., 2004; Taberlet et al., 2012), facilitating the study of the ecology of environmental microorganisms, including that of marine and freshwater ecosystems (Chariton et al., 2010; Griebler et al., 2009; Newton et al., 2011; Wakelin et al., 2008). This, has lead to the potential use of sediment microorganisms as bio-indicators for disturbances to riverine ecology, including those impacted by mining (Gough et al., 2011; Singh et al., 2009; Torsvik et al., 2002). The studies related to mining impacts, however, have been largely conducted in the temperate regions. In Africa, environmental microbiology studies remain limited to a few in the temperate region of South Africa (MacLean et al., 2007; Tekere et al., 2013) or in relation to agriculture in Kenya (Bossio et al., 2005). Studies in the use of environmental DNA and microbiology with regards to river ecology and mining impacts in the tropics, especially in West Africa, remain very limited

For this chapter, I set out to investigate the potential changes in the riverine sediment microbial community due to artisanal and modern gold mining activities on the Surow and Subri rivers respectively. Specifically, my objectives were to determine: (i) whether the microbial community in the Surow River differed from that of the Subri; (ii) whether the microbial community at impact sites differed from that at the control sites on each river; and (iii) the mining effluent components most responsible for any change in microbiota.

In Chapters 3 and 4, I established that sediment and water qualities in the Surow and Subri rivers differed between control and impact sites. The Surow River water was characterised by elevated turbidity and concentrations of metal and metalloids elements particularly at impact sites, whereas the Subri River water was characterised with low turbidity and metal concentrations but elevated ions including sulfate and NOx particularly in sites downstream of mine water discharge outflow. Therefore, I hypothesise that the microbial community in the Surow River sediment will be different from that of the Subri. I expect to see differences in microbial communities between control and impact sites of both rivers. I also expect microbial communities in the riverine sediment samples to be correlated with sediment and water quality variables linked to mining impacts. Unlike the studies in Chapter 3, 4 and 6, this microbial community study involved a one-off sampling of multiple sites impacted and un-impacted by gold mining and, consequently, temporal variability cannot be analysed.

7.2 Methods

7.2.1 Sediment and water sampling

Riverine sediment and water samples were collected in April 2014 from the water sampling sites outlined in Chapter 2. Additional sediment and water samples were collected at Kenyase 1 artisanal mine site (near site 8) from direct discharge of the mine dewatering pump, a site on the mine dewatering channel located about 500 m upstream of its confluence with the Surow River at Site 8, and a site on the outskirt of Kenyase 1 swamp (near site 7). Additional samples from Kenyase 2 mine site were taken from the swamp near to the mine: a sample each was taken from below the ASGM mine dewatering and waste outfall in the middle of the swamp, upstream of the swamp, and outskirt of the swamp at the confluence between a community sewer and the swamp on the Surow River.

At each sampling site, sediment samples were taken from 50 mm depth using a clean plastic coring tube (20 ml plastic syringe with top removed), placed in new ziplock plastic bags, homogenised and transported in an ice box to the laboratory for further processing. At the laboratory, each sample was divided into three parts, one part was sent to SGS analytical laboratory for chemical analysis, another used for DNA extraction. Extraction of DNA was performed within 72 hours of sampling on samples held at 4^{o}C. The remaining sample was frozen and stored in individual double sealed bags in the Molecular Biology Laboratory of the Ghana CSIR in Kumasi.

Water physical characteristics were measured *in situ* at the time of sampling using a handheld Quanta Hydrolab. Water samples were sent to the SGS analytical laboratory in Accra within 48 hours of sampling to be analysed for dissolved metals, nutrients and elements as explained in Chapter 2, 3, and 4.

7.2.2 DNA extraction and sequencing

7.2.2.1 DNA extraction

DNA was extracted in duplicate from 750 µg of wet sediment sample from each site using the FavorPrep^{TM} Soil DNA Isolation Mini Kit according to the manufacturer's direction. The concentration of DNA in the samples was quantified using QubitTM 3.0 Fluorometer (Invitrogen) from Thermo Fisher Scientific Inc. Samples were then held at -80^{o}C prior to analysis.

7.2.2.2 Miocrobiome analysis 16S rRNA sequencing

DNA samples were supplied to Associate Professor Richard Allcock at the Lotterywest State Biomedical Facility Genomics at the University of Western Australia for miocrobiome analysis 16S rRNA sequencing using primer sequences and protocol following Caporaso et al. (2012), with local modifications. At the facility, 1 ng samples were amplified using the 16S V4/5 primers (515F :GTGCCAGCMGCCGCGGTAA and 806R : GGACTACHVGGGTWTCTAAT). Specifically, the facility used a mixture of gene-specific primers and gene-specific primers tagged with ion torrent-specific sequencing adaptors and barcodes. The tagged and untagged primers were mixed at a ratio of 90:10. Using this method, the approximately 10 cycle inhibition observed by using long tagged primers could be reversed and hence we achieved amplification of all samples using 18-20 cycles, thus minimising primer-dimer formation and allowing streamlined downstream purification. Amplification was performed using a 5Prime-Hostart MasterMix Kit (5Prime, USA) and was confirmed by agarose gel electrophoresis. PCR products formation was quantified using a Qubit fluorometer (Thermofisher Scientific). Up to 100 amplicons were diluted to equal concentrations and adjusted to a final concentration of 15 pM. Templated Ion Sphere Particles (ISP) were generated on a One Touch 2 (ThermoFisher Scientific) using a 400 bp templating kit and sequenced on a PGM (ThermoFisher Scientific) for 850 cycles using a 400 bp sequencing kit yielding a modal read length of 309 bp. Reads were trimmed for quality purposes using TorrentSuite 5.0.

7.2.2.3 Analysis of 16S RNA sequences

Metagenomic analysis using culture-independent high throughput 16SSU rRNA quantitative gene sequencing and microarrays was performed on the PCR derived sequences. The data was analysed using the Quantitative Insights into Microbial Ecology (QIIME, version 1.8). The following commands were applied to the derived 16S rRNA sequence: (i) the rRNA sequence FASTq reads were separated into two separate libraries, one containing “sequences (FASTA files)” and the other “quality of DNA information (QUAL)” scores; (ii) each file in the sequence library was assigned a unique subject identity barcode, creating a “mapping” library; (iii) PCR “mixed sequence” chimeras were removed using a reference file and identification of “de novo” chimeric sequences; and (iv) operational taxonomic units based on 97% specific16S rRNA gene sequence identities were used to distinguish different species of microbe and these were grouped into their most likely phylum/class/order/family/genus using GreenGenes database, Version 12_10). The reads were repeated to refine the dataset to a consistent number of reads and taxonomic identification. Genomic analysis was obtained from taxonomic Level 1-6, but not including Level 7 species subtype identification.

7.2.3 Data and statistical analysis

To establish possible impacts of the two different types of gold mining on microorganism communities in the Surow and Subri rivers, two-way univariate and multivariate statistical analysis were performed. Factors used in the two-way analysis are two levels of river (the Surow and the Subri), control and impact, proximity to a mine and direct connectivity to mine-dewatering water discharges. Samples located within 200 m radius from a mine working, mine processing, or mine wastes were assigned to the ‘mine’ group, otherwise to the ‘no-mine’ group. Discharge sites were those located up to 500 m downstream of mine dewatering outflow or other water discharges. Site 7 on the Surow, for example, was assigned to the groups ‘impact’, ‘mine’ and ‘no discharge’ because it was located close to Kenyase 1 mine works but was not connected with mine dewatering discharges.Table 7.1 lists the factors assigned to each sample and site. Due to the one-off sampling scheme, unlike chapter 3, 4, and 6, temporal factor was not used in this chapter. The factors assigned to each sample (site) are listed in Table 7.1.

Table 7.1 Spatial factors assigned to samples from each site for the multivariate anlaysis

Site	River	Control/Impact	MineDewatering	Proximity to mine
1	Surow	Control	No discharge	No
2	Surow	Control	No discharge	No
3	Surow	Control	No discharge	Yes
4	Surow	Impact	Discharge	Yes
4 Upstream	Surow	Impact	No discharge	Yes
4 Midstream	Surow	Impact	Discharge	Yes
4 Sewer	Surow	Impact	No discharge	Yes
5	Surow	Impact	No discharge	No
6	Surow	Impact	No discharge	No
7	Surow	Impact	No discharge	Yes
8	Surow	Impact	Discharge	Yes
9	Surow	Impact	Discharge	Yes
11	Surow	Impact	No discharge	No
8C (mine dewatering channel)	Surow	Impact	Discharge	Yes
K1_P (mine dewatering pump)	Surow	Impact	Discharge	Yes
K1_M (swamp)	Surow	Impact	No discharge	Yes
NSW9	Subri	Control	No discharge	No
KSW16	Subri	Control	No discharge	No
KSW3	Subri	Impact	Discharge	Yes
KSW13	Subri	Control	No discharge	Yes
NSW6	Subri	Impact	Discharge	Yes
NSW8	Subri	Impact	Discharge	Yes
KSW2	Subri	Impact	No discharge	No

The operational taxonomic units (OTU) generated by Qime are reported as relative abundances of all the OTU assigned to particular taxa. The univariate analysis was performed on microbial community indices including taxa richness (N), diversity (Shannon's diversity index; H'=Sum (Pi x Log(Pi)) and evenness (Pielou's J'=H'/Log(S)) of each sample using SPSS v22 (SPSS, 2013). These indices were calculated using the transformation function within the PRIMER package. Multivariate analysis was performed on the OTUs at the Phylum, Family and Genera taxonomic levels on the PRIMER v6 (Clarke et al., 2006) with its PERMANOVA add on (Anderson et al., 2008). The OTU data was standardised (by total) prior to a fourth-root transformation (O'Hara et al., 2010). A resemblance matrix was generated via Bray-Curtis similarity. Clustering and non-metric multidimensional scaling (MDS) were undertaken to investigate the resemblance between samples. PERMANOVA within the PRIMER package was used to test for differences between factors. Factors with significant PERMANOVA results were then subjected to SIMPER analysis to investigate variables responsible for the differences.

Sediment and water physico-chemistry data used for this chapter was from April 2014, which was sampled concurrently with the sediment microbial community sampling. Values below the limit of detection (LOD) were substituted with LOD/√2 (Verbosek, 2011). Environmental parameters that were frequently below LOD, were omitted (e.g. Hg). All sediment and water physico-chemistry data, with the exception of ORP, was $\log_{10}$ transformed and normalised prior to the generation of resemblance matrix using Euclidian distance. Principal component analysis (PCA) was used to plot the distance between samples.

To test correlations between microbial community structure and environmental variables (sediment and water physico-chemistry) the RELATE procedure was used with a total of 999 random permutations. The models that would best explain variance in the microbial assemblage and diversity using the BEST / Biota and Environmental Matching (BIOENV) procedure with a total of 999 permutations were tested (Anderson et al., 2008). Variables or group of variables with the highest significant correlations were selected. This was followed with a distance-based redundancy analysis (dbRDA), also within the PRIMER / PERMANOVA package, to perform multivariate multiple regressions of microbial community data against the environmental data as predictor variables (Anderson et al., 2008; McArdle et al., 2001). The dbRDA was also utilised to plot the variances and correlations between microbial community and the predictor variables. Only variables with strong significant relationships with Pearson's $R>0.4$ were chosen.

7.3 Results

7.3.1 Adequacy of sampling

The adequacy of sampling program is tested using the rarefaction curves which estimate the taxa richness (here identified to genus level) in larger samples based on various diversity estimators including S_{obs} (total number of genera observed), Chao1, Chao2 (for replicated incidence data), Jacknife 1 (for abundance data), and Jacknife 2 (for incidence data) (Figure 7.1). The diversity curves for Chao 1 is flat due to the absence of doubleton (Gotelli et al., 2011), whilst S_{obs}, Chao 2, Jacknife 1 and 2 are asymptotic, suggesting that the estimates for richness are complete. This, suggests that, although this study involved a one-off sampling of 23 sediment samples, additional sampling will not yield any additional species (genera). In other words, the sampling methods and number of samples in this study are adequate (Gotelli et al., 2011)

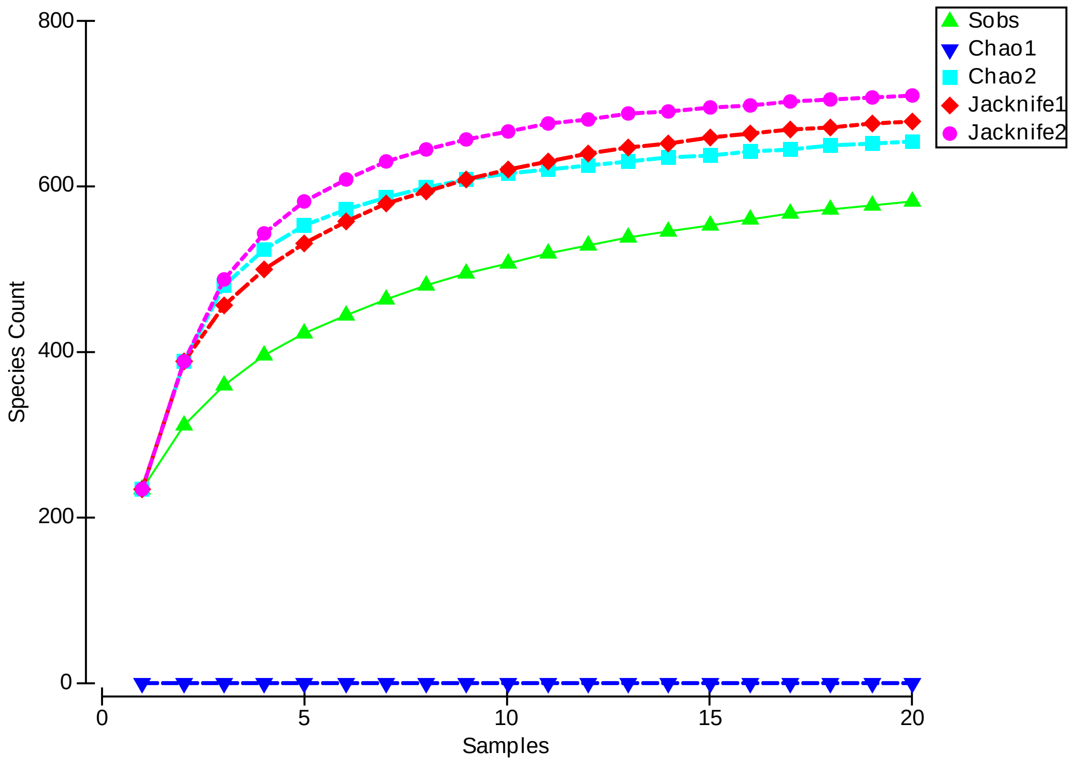


Figure 7.1 Rare curves showing the estimates for species (genera) counts in larger samples with S_{obs} (total number of genera observed), Chao1, Chao2 (for replicated incidence data), Jacknife 1 (for abundance data), and Jacknife 2 (for incidence data) as estuimators.

7.3.2 Microbial community identities and relative abundance across rivers and in each river

Out of the 23 sediment samples, only 20 DNA samples were processed for sequencing and taxonomy classification. Insufficient DNA for downstream applications could be extracted from samples from site KSW3 (located within the Ahafo Gold mine) on the Subri River and site 4M (midstream of Site 4 on the Surow River at the ASGM overflow). The DNA sample from site 10 on the Surow River was also unavailable for analysis due to the rarefaction calculations in assigning OTUs, consequently, a sample has to be omitted from the final results.

The OTUs were assigned to a total of 31 phyla, 88 classes, 148 orders, 283 families and 583 genera across both rivers. The total number of OTUs assigned to each taxonomic level in the Surow River was higher than in the Subri River (Figure 7.2), but there were twice as many sediment samples from the Surow River compared to Subri River. Contribution of each family to the microbial community across rivers is presented in **Error! Reference source not found.** with emphasis on the dominant taxa (relative abundance $\geq$ 1%). At the kingdom level, three Archaea, 27 Bacteria and an unknown OTU were identified. Proteobacteria was the most dominant phyla (47%), followed by unknown bacteria (22%), Acidobacter (8%), Bacteroidetes (5%), Firmicutes (5%) and Actinobacter (4%). Figure 7.3 depicts the mean relative abundance of dominant phyla in the Surow and Subri rivers. At the family level, an unknown Bacteria and an unknown Betaproeibacteria were the most abundant taxa, followed by an unknown Proteibacteria, Rhizobiales, unknown Gammaproteibacteria and unknown Deltaproteibacteria (Figure 7.4). Unknown OTUs were prevalent in this study. At the phyla level, one Bacteria OTU was unknown, at the Family level 22 OTUs were unknown whilst at the genera level 154 OTUs were unknown; this contributed 72% to the total relative abundance of all genera.

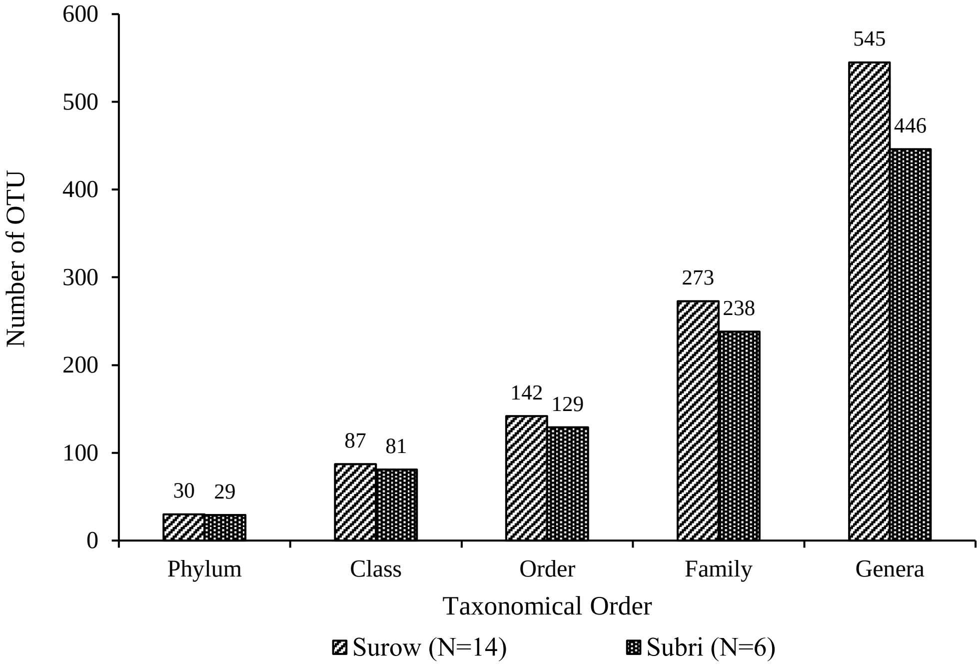


Figure 7.2 Comparison between total numbers of OTUs assigned at five taxonomic levels in the Surow and Subri Rivers. The Surow River samples were taken from 14 sites whilst the Subri River samples were taken from only 6 sites.

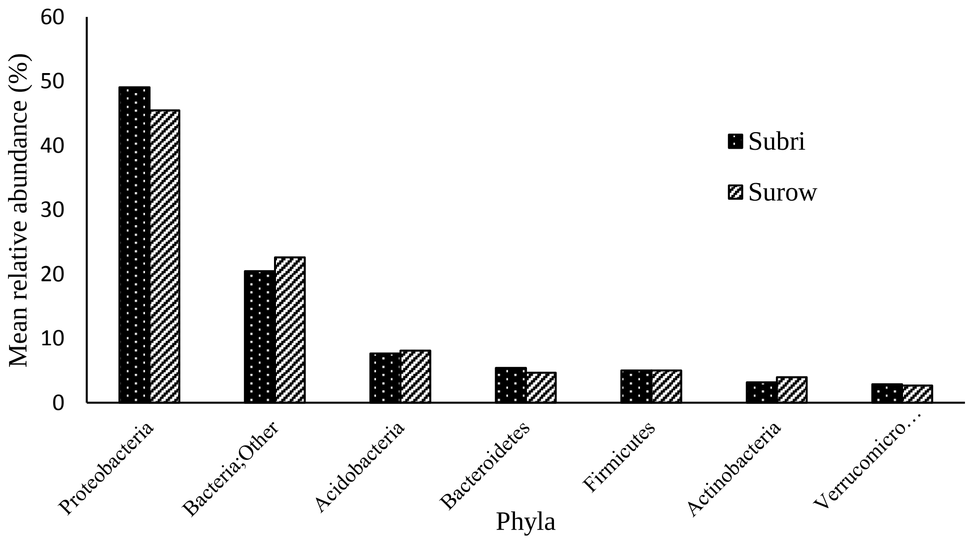


Figure 7.3 Dominant Archaea and Bacteria phyla (relative abundance ≥ 3%) in the Subri and Surow Rivers' sediment. 'Other' denotes unknown taxa.

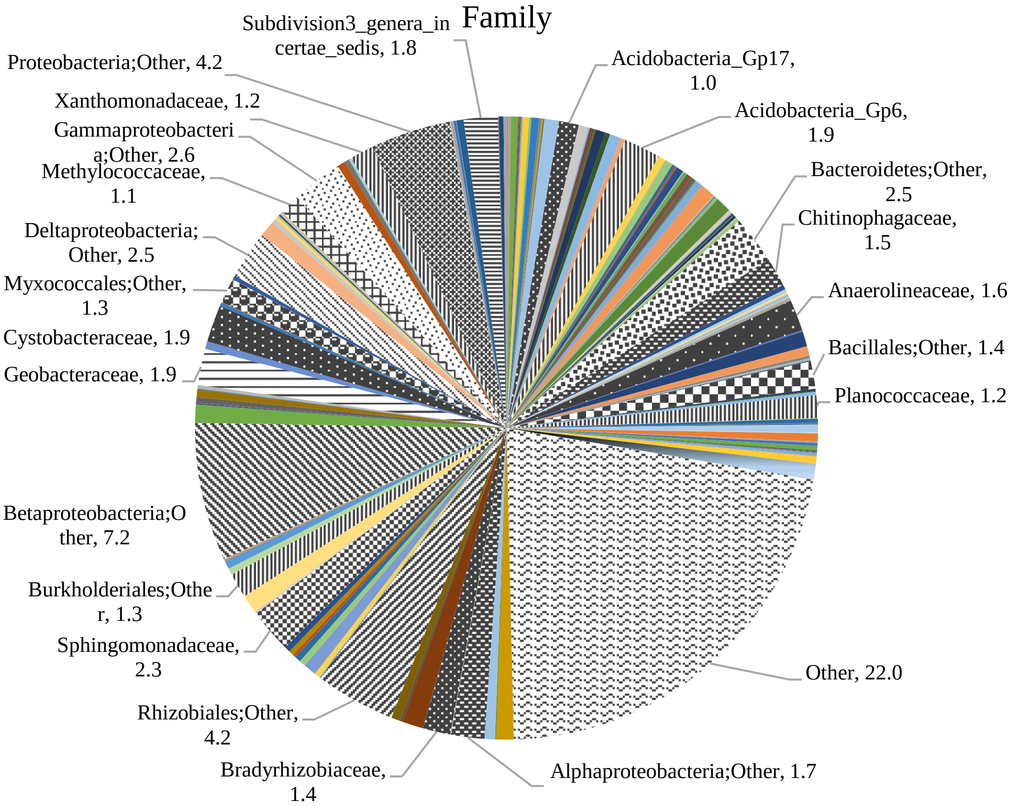


Figure 7.4 Contribution of dominant families (relative abundance ≥ 1%, in grey patterned wedges) to the microbial communities across Surow and Subri Rivers. Relative abundance in % is given following each family name. 'Other' denotes unknown taxa.

7.3.3 Dominant Phyla

7.3.3.1 Proteobacteria

Proteobacteria contributed 49% and 45% to the total microbial abundance in the Subri and the Surow rivers respectively. Alpha, Beta, Delta, Epsilon, and Gamma Proteobacteria classes were identified as was an unknown proteobacteria. Across both rivers, Alpha proteobacteria were the most dominant Proteobacteria, followed by the Beta, Delta, Gamma and the unknown Proteobacteria (Figure 7.5). The mean relative abundances of Delta and Gamma Proteobacteria in the Surow River were slightly higher than in the Subri, whilst relative abundances of Alpha, Beta, Epsilon and unclassified Proteobacteria in the Surow River were lower than in the Subri River. Epsilon Proteobacteria was rare, it was absent from site KSW13 on the Subri River and sites 5, 6, 8C and 8 on the Surow River.

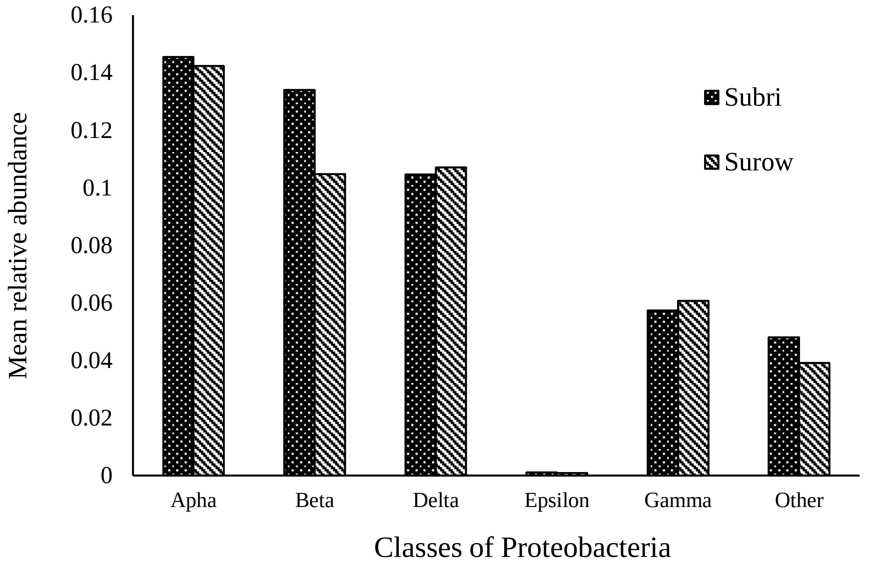


Figure 7.5 Comparison between mean relative abundance of classes of Proteobacteria in the Subri and Surow rivers. 'Other' denotes unknown taxa.

7.3.3.2 Acidobacteria

Acidobacteria contributed 8% to the total microbial abundance across both rivers. Twenty two classes of Acidobacteria were identified with one of them unknown (Figure 7.6). Acidobacteria groups 6, 17, 3, 16, 4, 18 and 7 were the prominent classes in both rivers while groups 9, 15, 19, 20 and 21 were uncommon. Group 19 and 20 Acidobacteria were only found in the Surow River; group 19 was found at site 3, 5, 6, 7, and 8C in the vicinity of Kenyase 1 and 2 ASGM sites; while Group 20 was found at site 6 only.

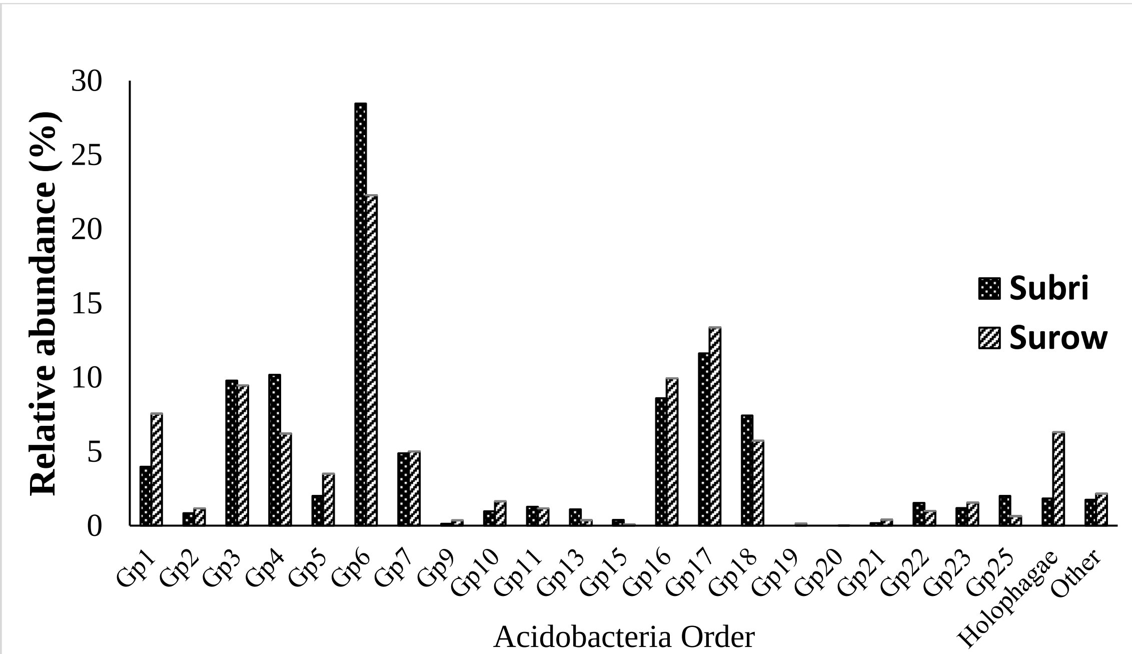


Figure 7.6 Relative abundance of the classes of Acidobacteria in the Subri and the Surow rivers. 'Other' denotes unknown taxa.

7.3.3.3 Bacteriodetes

The phyla of Bacteriodetes contributed 5% of OTUs to the total microbial community across both rivers. Four Bacteriodetes classes were identified and another was unknown. The unknown Bacteriodetes dominated all other classes in abundance, followed by Sphingobacteria and Flavobacteria (Figure 7.7). Contribution of each Bacteriodetes class to the phyla was not significantly different between rivers.

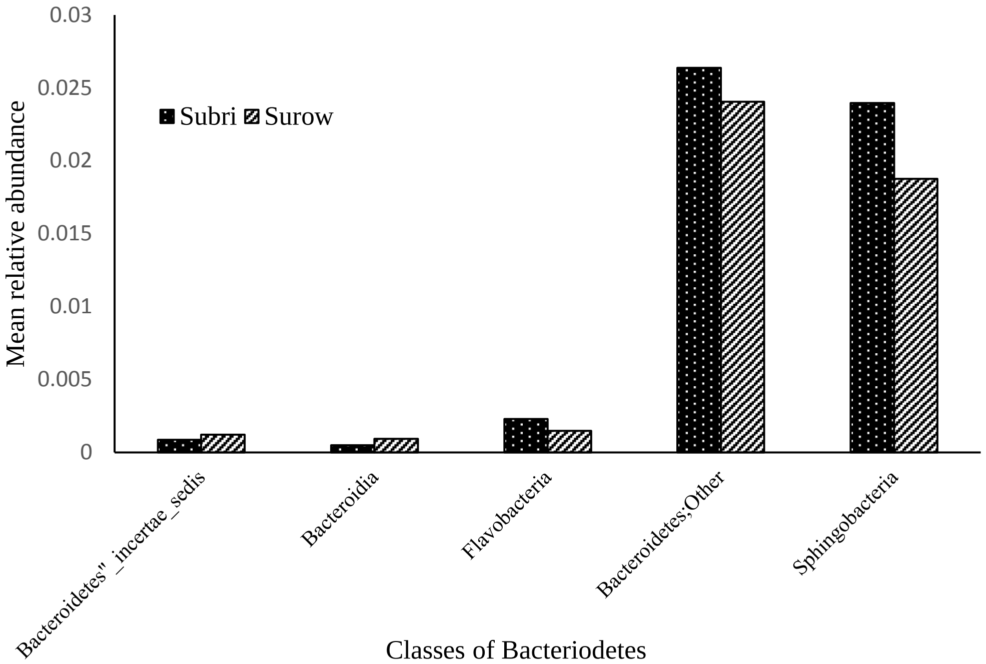


Figure 7.7 Comparison between mean relative abundance of Bacteriodetes classes in the Subri and Surow rivers. ‘Other’ denotes unknown taxa.

7.3.3.4 Firmicutes

The Bacteria phyla of Firmicutes contributed 5% to the total abundance of the microbial community across both rivers. Four classes of Firmicutes were identified with one unknown. Bacilli and Clostridia classes were the most abundant Firmicutes, whilst Ersypelotrichia, Negativicutes and unknown were uncommon, contributing < 0.05% to the total microbial community each (Figure 7.8). On the Subri River, Ersypelotrichia was found only at site NSW8 whilst on the Surow River it was found at site 5, K1_M and the Sewer at site 4.

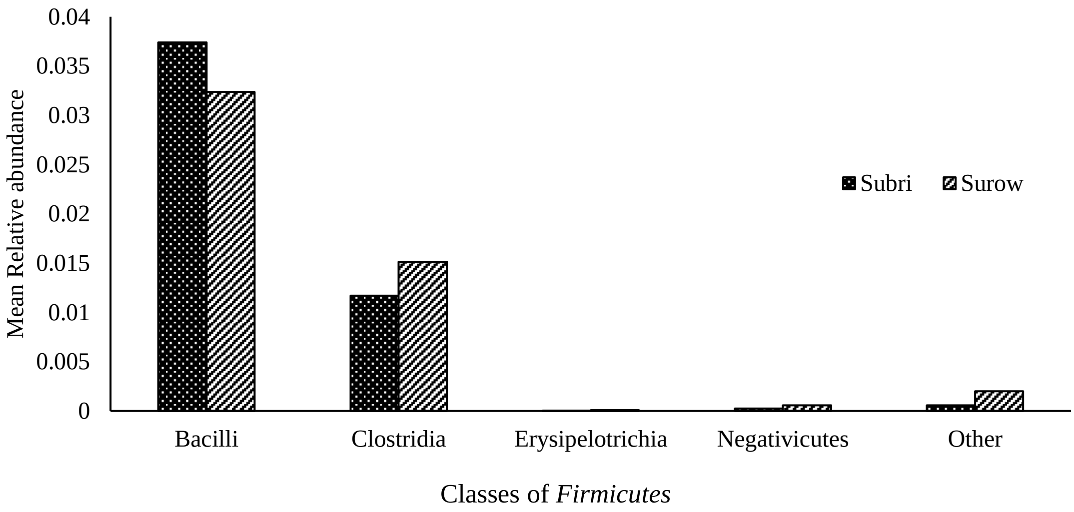


Figure 7.8 Comparison between mean relative abundance of classes of Firmicutes in the sediment of the Subri and Surow rivers. 'Other' denotes unknown taxa.

7.3.3.5 Actinobacteria

Phylum Actinobacteria contributed 3% to the microbial community in the Subri and Surow rivers. Eight classes of Actinobacteria were identified, only 7 of the 8 classes were known. The classes of Actinomycetales, Acidomicrobiales and the unknown were the most abundant Actinobacteria and were found in all samples from both rivers. Coriobacteriales, Euzebyales, Nitriliruptorales and Rubrobacterales were uncommon, contributing < 0.4% each to the total microbial abundance (Figure 7.9). The classes of Coriobacteriales, Euzebyales, Nitriliruptorales were identified only in the Surow River samples.

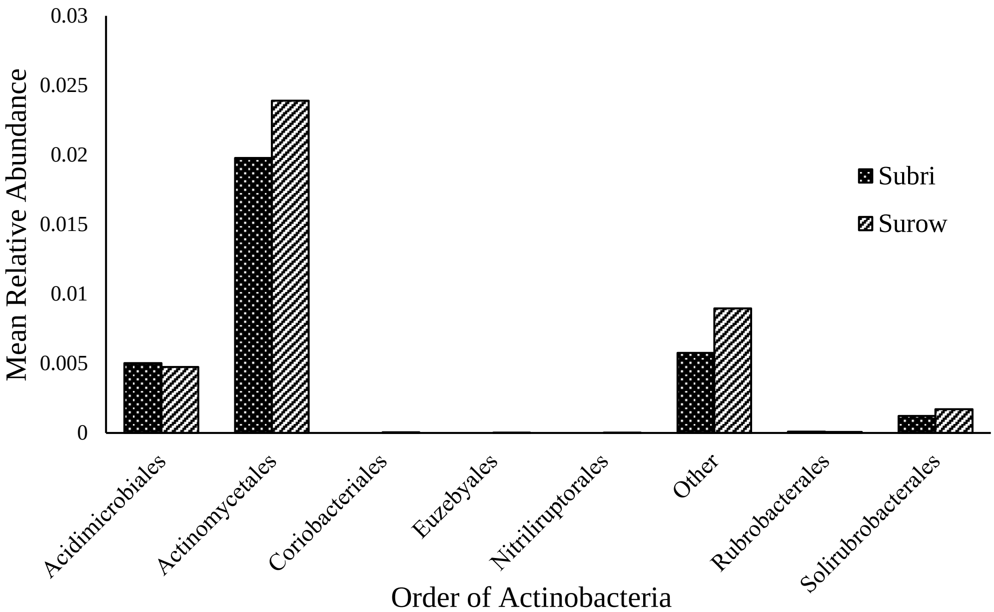


Figure 7.9 Comparison between mean relative abundance of each Actinobacteria order to the all Actinobacteria phyla in the Subri and Surow Rivers sediment. 'Other' denotes unknown taxa.

7.3.4 Rare Phyla

Nineteen out of the 31 OTUs identified to the phyla levels contributed < 1% each to the overall abundance and were therefore considered rare (See Hua et al., 2015). Collectively, the 19 rare phyla contributed <1.6% to the total abundance of the microbial community across both rivers. Amongst the rare phyla, an unknown Archaea, Bacterial phyla of Gemmatimonadetes, Chlamydiae, Armatimonadetes, Chloroby and Chrenarcaeota as well as an unknown phylum were the most abundant across rivers. Bacterial phyla of Elusimicrobia, Synergitetes, OD1 and Fibrobacteres were very rare (Figure 7.10). Elusimicrobia and Synergitetes were only found in the Surow River at sites 8 and 3 respectively; OD1 was only found at site KSW13 on the Subri River, whilst Fibrobacteres, was found only at site NSW8 and KSW13 on the Subri River and site 8 on the Surow River.

Overall, the abundance of the rare phyla did not differ between rivers. The abundances of the rare phyla BRC1 and SR1 across rivers, however, differed between discharge and no-discharge (ANOVA p=0.006, F=9.633 and p=0.005, F=4.431 respectively). Sites received mine water discharges had less BRC1 and SR1 than those with no-discharge (Figure 7.11).

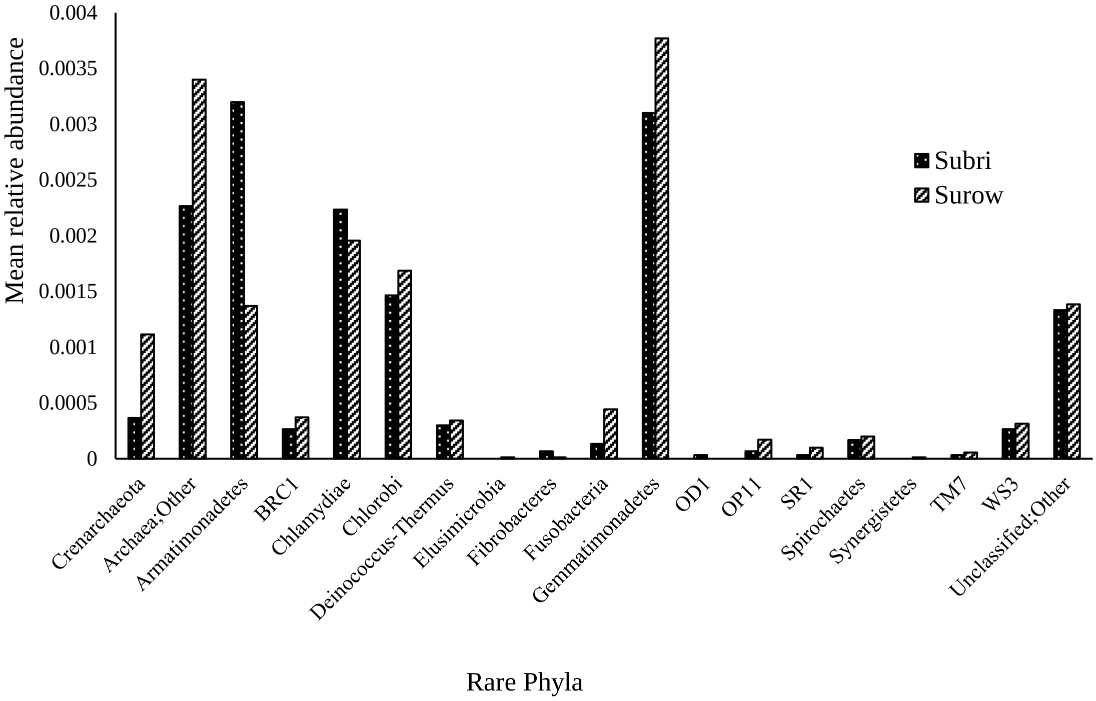


Figure 7.10 Comparison between mean relative abundance of rare phyla in the Subri and Surow Rivers sediment. Rare phyla are those contributed < 1% to the total microbial community abundance across both rivers. 'Other' denotes unknown taxa.

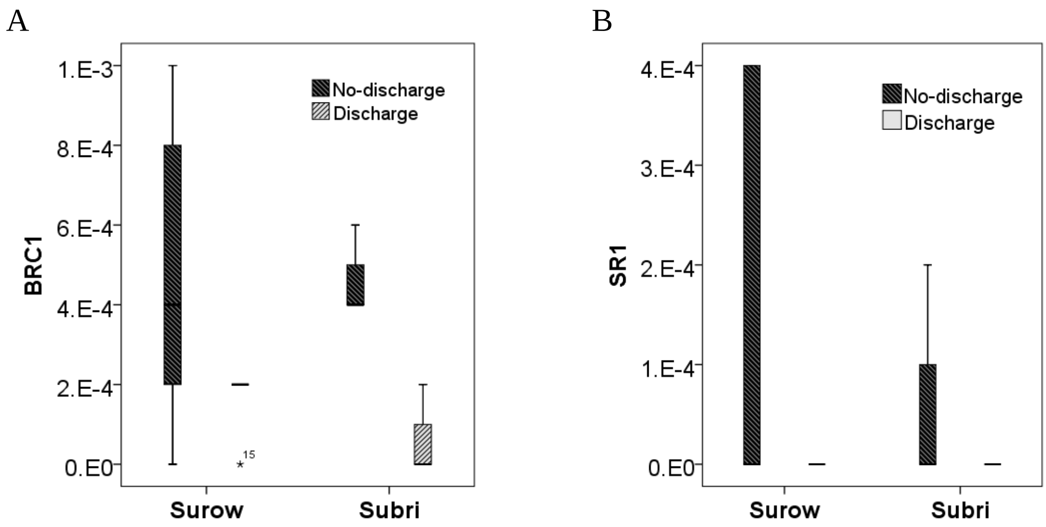


Figure 7.11 The abundance of BRC1 (graph A) and SR1 (graph B) on the Surow and Subri Rivers in sites with mine water discharges are different from those without discharge.

7.3.5 Microbial community diversity indices

Across the Surow and Subri rivers, the number of identified OTUs at family level (S) per site ranged between 132 and 183, diversity (Shannon's diversity index; H'=Sum (Pi x Log(Pi)) ranged between 3.19 and 3.91; and evenness (Pielou's J'=H'/Log(S)) ranged between 0.65 and 0.78. The highest richness was recorded at site 4_US which was located upstream of an ASGM outflow of mine water and waste discharge on the Surow River, whilst the lowest richness was recorded at site 2 also on the Surow River. The highest diversity was recorded at site NSW6 located downstream of an Ahafo environmental control dam on the Subri River and the lowest was that of site 5 which was located downstream of the Kenyase 2 ASGM swamp site on the Surow River. Site K1_P located at the mine dewatering pump at the Kenyase 1 ASGM site had the highest microbial community evenness, whilst the lowest evenness (0.67) was recorded at site 7 and K1_M, both located on the swamp downstream of Kenyase 1 mine.

At the family level, mean number of taxa, evenness and Shannon diversity index for the Surow River were slightly lower than that of the Subri River, impact sites slightly higher than control and discharge lower than no-discharge in both rivers (Figure 7.12 and Table 7.2). The differences in the diversity indices between the Surow and Subri rivers, however, were nearly significant with one-way ANOVA p=0.52 and F=0.435.

At the genera levels, the number of identified taxa per site ranged between 184 and 310; Shannon's diversity index ranged between 3.32 and 4.28; and evenness between 0.63 and 0.77. Similar to that of family taxonomic level, the diversity indices at genus levels in the Subri River were also slightly higher than that of the Surow River, impact sites were higher than control, mine higher than no-mine, and no-discharge higher than discharge in both rivers (see **Error! Reference source not found.**).

Table 7.2 Diversity indices of microbial community identified to the family level in the sediment of the Surow and Subri rivers.

Index		N	Mean	Std.Error	Minimum	Maximum
Number of family (S)	Subri	6	151.33	7.71	132	180
	Surow	13	156.08	3.43	136	183
	Total	19	154.58	3.29	132	183
Evenness (J)	Subri	6	0.71	0.01	0.68	0.75
	Surow	13	0.71	0.01	0.65	0.78
	Total	19	0.71	0.01	0.65	0.78
Shannon (H’)	Subri	6	3.55	0.09	3.36	3.84
	Surow	13	3.59	0.06	3.19	3.91
	Total	19	3.58	0.05	3.19	3.91

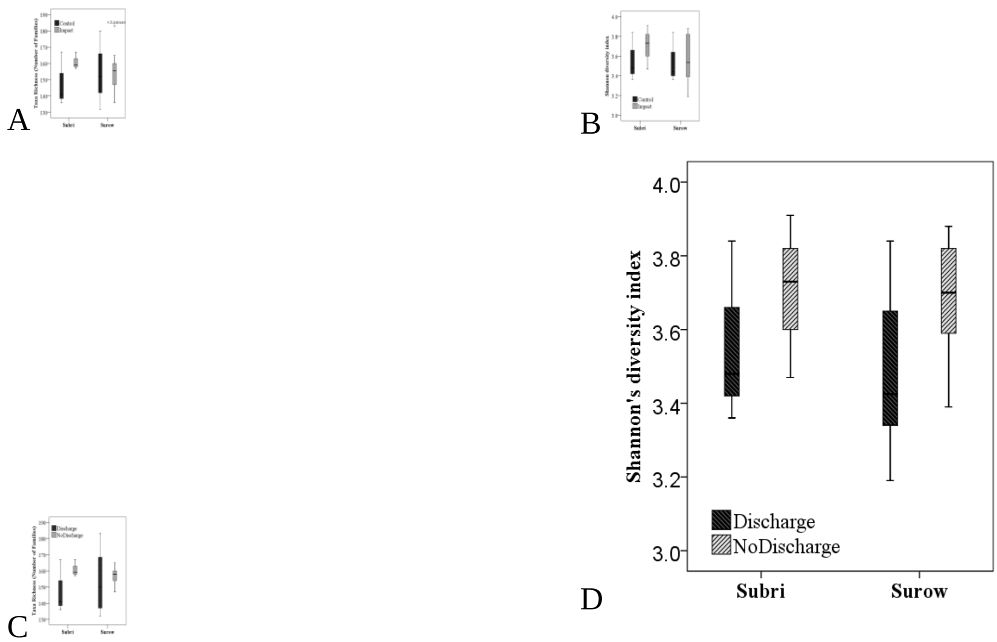


Figure 7.12 Mean taxa richness (number of family) and Shannon’s diversity index of microbial communities at control and impact sites (A and B) and at sites without mine water discharge and with discharge sites (C and D) on the Subri and Surow Rivers.

Table 7.3 Diversity indices of microbial community identified to the genus level in the sediments of Surow and Subri Rivers.

Index		N	Mean	Std. Error	Minimum	Maximum
Number of genus (S)	Surow	14	234.36	10.08	184	310
	Subri	6	240.33	12.10	198	271
	Total	20	236.15	7.79	184	310
Evenness (J)	Surow	14	0.69	0.01	.63	.77
	Subri	6	0.70	0.02	.66	.74
	Total	20	0.69	0.01	.63	.77
Shannon's diversity (H')	Surow	14	3.74	0.08	3.32	4.28
	Subri	6	3.83	0.12	3.50	4.17
	Total	20	3.77	0.07	3.32	4.28

7.3.6 Microbial community structure

Microbial community composition was analysed based on the abundance of each OTU in each factor (river, site, control/impact, mine/no-mine, discharge /no-discharge). Preliminary multivariate analysis of all OTUs from the 20 sampling sites suggested that site 11 was an outlier as depicted in a non-metric multidimensional (MDS) plot (Figure 7.13). The strong separation of site 11 on the Surow River and the remaining sites made it difficult to clearly delineate differences between microbial communities in the rest of the samples, therefore site 11 was excluded from further analysis.

At genera levels, automated clustering of samples indicated a distinctive separation between groups of OTUs from sites with mine works and no-mine works (Figure 7.14), which can also be seen in the MDS plot (Figure 7.15). The pattern of grouping and separation by the factors, however, were less clear in the OTUs at the higher taxonomic levels (family and phyla), and in the rare species and presence / absence data sets.

One way PERMANOVA indicated that in the Subri River, microbial community compositions identified to phyla, family and genera levels at impact, mine and discharge sites were not different from that of control, no-mine and no-discharge (Table 7.4). In contrast, in the Surow River, microbial community's compositions were different between control and impact, no mine and mine; and no-mine water discharge and mine water discharge (PERMANOVA $p \leq 0.01$ and $p \leq 0.05$; Table 7.4). The rare phyla community structure, which was analysed separately, did not vary spatially along the Surow River.

Two-way PERMANOVA, however, showed that microbial community compositions identified to both family and genera levels were not different between the Surow and Subri, between control and impact; between no mine and mine, and between no-mine water discharge and mine water discharge. The interactions between factors were also insignificant. Further, pairwise test on the family OTUs showed that whilst mine water discharge and no-discharge sites in the Subri River were similar, in the Surow they were different (p=0.01, Table 7.5). The significant difference between discharge and no-discharge sites was due to, among others, the more abundant OP11 Bacteria, Gemmatimonadetes, Nitrospira and Actinobacteria in the sites affected by mine dewatering; and the less abundant Chloroflexi, Fusobacteria, an unknown Archaea and an unknown Bacteria in the area affected by mine dewatering (Appendix 35).The significant difference between discharge and no-discharge sites on the Surow River was also driven by the absence of some families from certain sites. Methanospirilliacea (Euryacheota), Marinilabaceae (Bacteroidales), Chlorobiaceae (Chlorobi) and Desulfomicrobiaceae (Deltaproteobacteria) were present in the no-discharge sites but absent from the discharge. Correlations between MDS spatial coordinates of microbial community assemblage and some families of Archaea and Bacteria Phyla identified as contributors to the significant difference between microbial community in areas affected by mine dewatering discharge and that of not affected by mine dewatering discharge are illustrated in Figure 7.16.

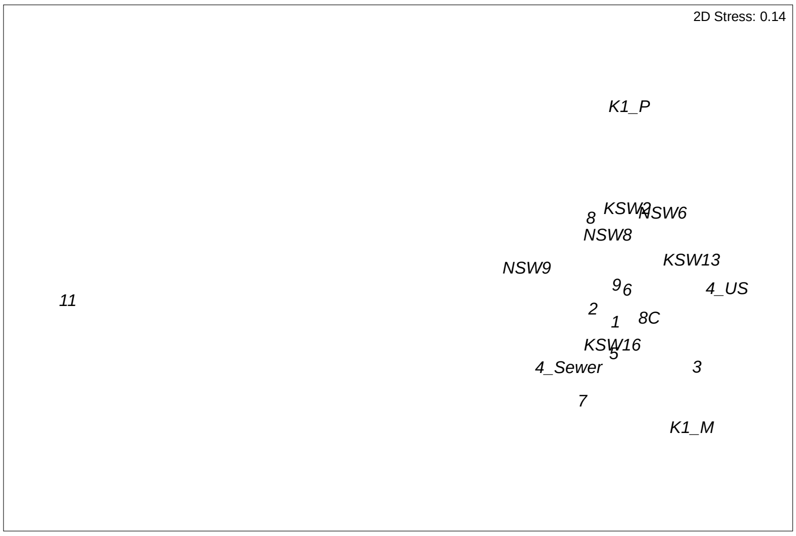


Figure 7.13 Non-metric multidimensional plot of OTUs from all sampling sites showing the wide separation between Site 11 (on the Surow River) and the rest. Site 11 is considered an outlier and was removed from further multivariate analysis.

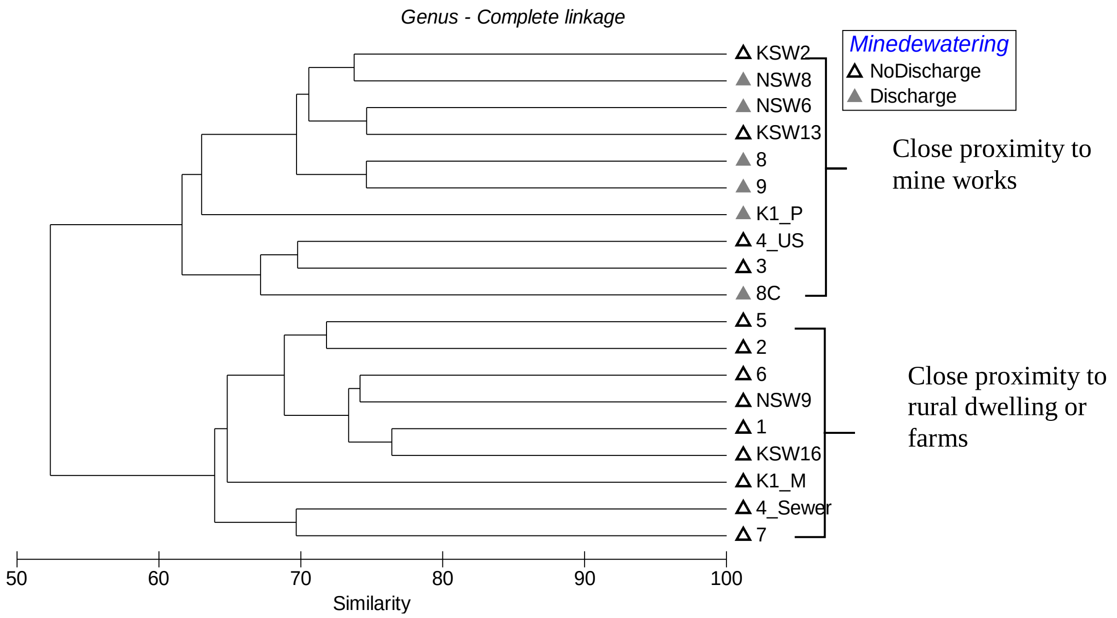


Figure 7.14 Dendogram of similarity in microbial community abundance and diversity at genera level between samples across rivers.

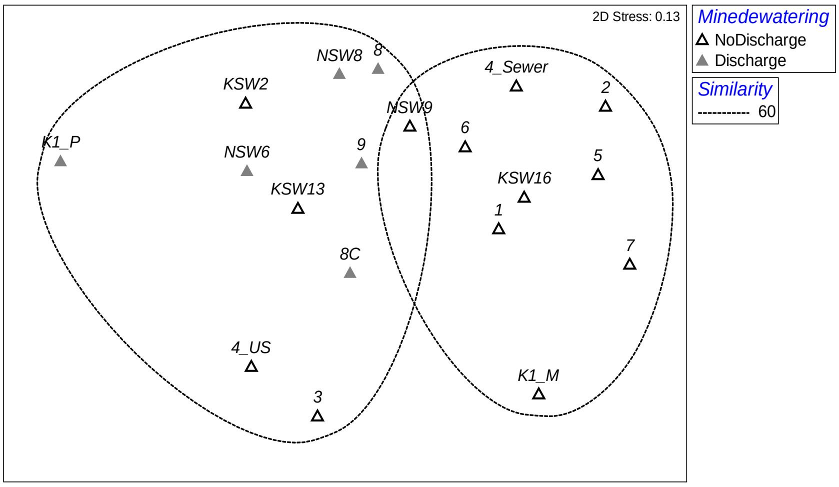


Figure 7.15 Non-metric multidimensional scaling (MDS) plot of microbial community relative abundance and diversity at genera level across rivers showing a separation of sites that were not closely affected by mine water discharges from those unaffected

Table 7.4 Results of one way PERMANOVA on riverine sediment microbial community identified at the phyla, family, genus level, rare phyla (relative abundance ≤ 1%), and presence/absence of microbial families across rivers, in the Subri River and the Surow River.
* significant at p≤0.01. ** significant at p ≤ 0.05

Source of variance	Phyla		Family		Genus		Rare Phyla		Presence (Family)	
	F / t	p	F / t	p	F / t	p	F / t	p	F / t	p
Subri River										
Control and Impact	1.22	0.30	1.23	0.20	1.22	0.20	1.20	0.30	1.16	0.19
Proximity to mine	1.37	0.21	1.07	0.30	0.99	0.50	1.40	0.11	1.04	0.50
Proximity to mine water discharge outflow	1.09	0.27	1.04	0.48	0.93	0.61	1.28	0.13	1.14	0.20
Surow River										
Control and Impact	0.89	0.61	0.88	0.77	0.94	0.58	0.91	0.59	0.99	0.49
Proximity to mine	0.90	0.63	1.20	0.10	1.27	0.05*	0.91	0.59	1.09	0.26
Proximity to mine water discharge	1.49	0.02*	1.42	0.01**	1.47	0.01**	1.40	0.07	1.23	0.04*

Table 7.5 Results of pairwise test between river and mine-water discharge factors

Level	Factor	t	p (PERM)
No-Discharge	Subri X Surow	1.1338	0.1717
Discharge	Subri X Surow	0.94358	0.7355
Subri	No-Discharge x Discharge	1.0398	0.4785
Surow	No-Discharge x Discharge	1.4151	0.0128*

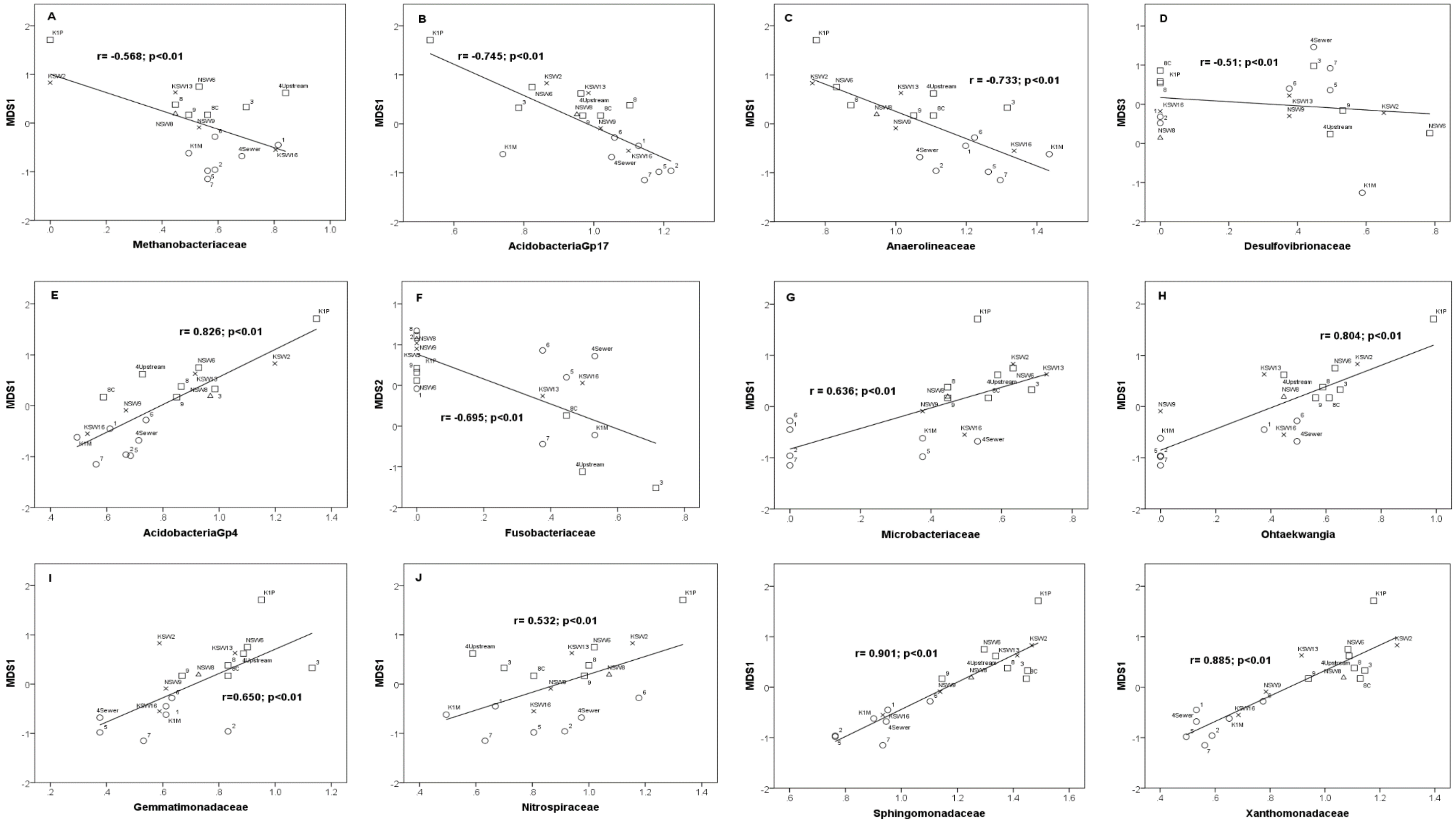


Figure 7.16 Correlations between MDS coordinates of microbial community assemblages and the relative abundance of some Archaea and Bacteria families with Rivers X Control/Impact overlay. The Pearson's r and significance levels of the correlations are indicated ○ represents microbial community assemblage in the Surow without direct connection to mine dewatering, □ in the Surow with mine dewatering, x in the Subri without mine dewatering and Δ in the Subri with mine dewatering.

7.3.7 Sediment and water quality

The sediment and water quality of the Surow and Subri Rivers analysed over time are presented and discussed in Chapters 3 and 4. The April 2014 sediment and water quality, however, is also presented here in relation to the microbial community study.

In April 2014, sediment quality in the Surow River differed significantly from the Subri River ($p<0.05$; $F=2.552$). The Surow River's sediment was characterised by elevated metal concentrations including Al, Fe, Pb, especially at mine impacted sites (site 4, 7, 8, and 9), whilst the Subri River sediment was characterised by elevated concentrations of sulphur, organic carbon, NOx, P, Zn, Cu especially at mine impacted sites and Mn, Mg, Ba, and Co near to its confluence with the Tano River. The MDS of sediment quality is plotted in Figure 7.17.

In the Surow River, water quality at mine dewatering affected sites was significantly different ($p<0.05$) from the non-affected sites. In the Subri River, water quality at mine impacted areas was not different between sites ($p>0.05$) although EC, TDS, concentrations of sulfate, Al and Mn were elevated at sites NSW8 and KSW2. The MDS of the rivers' water quality is plotted in Figure 7.18.

7.3.8 Correlations between microbial community assemblage and sediment and water physicochemical variables

Microbial community structure at phyla, family and genera levels was poorly correlated with sediment quality. Water quality was only weakly correlated with microbial community at genus level (Spearman's $r = 0.25$; $p <0.05$), whilst at the phyla and family levels the correlations were not significant. Rare phyla and microbial family presence also did not correlate with sediment and water qualities.

The Biota and Environmental Matching analysis (BIOENV), however, showed that more than 45% of variance in microbial community abundance and diversity at the phyla, family and genera levels was explained by combinations of a few water quality variables. At the phyla level, 47% of variance was explained ($p< 0.05$) by turbidity, dissolved oxygen (DO) and sulfate in the water. Similarly, at the genera level, 47% of variance was explained by turbidity and concentrations of DO, sulfate, As and Pb. Possible models to explain the variance in microbial community abundance and diversity of the rare species and at the family level, however, were not significant ($p > 0.05$). BIOENV analysis also revealed insignificant correlations between microbial communities at phyla, family and genera levels and sediment quality variables. Consequently, sediment quality was not used in the dbRDA analysis below.

OTUs identified to the family levels and water quality were used in a distance based redundancy analysis (dbRDA). Although it does not create models, dbRDA can identify environmental variables that significantly correlate with microbial assemblage. Across rivers, 80% of total variance in microbial abundance and diversity can be explained by the first 10 dbRDAs According to the dbRDAs, environmental variables strongly correlated with microbial assemblage and contributed to the 80% variance were temperature, turbidity, DO, EC, FRP, sulfate, ammonia, NOx, Al, As, Fe, Mn and Pb (Figure 7.19 and

Appendix 36). Plotting the values of these water quality variables against the MDS of microbial community assemblages, however, demonstrated that only concentrations of sulfate, Fe, FRP and turbidity correlated strongly and significantly with microbial community assemblage (Figure 7.20 and Appendix 37).
The dbRDAs also indicated that between rivers there were two broad types of microbial communities differentiated by mine water discharge and distance from mine works. The first type is associated with discharge outflows and mine works. This type was dominated or characterised by the presence of Actinobacteria, Synergistetes, SR1 Bacteria, Cyanobacteria, Gemmatiomanadetes, Deinoccus-Thermus, Bacteroidetes, Fibrobacteres, Nitrospira and Proteobacteria groups. Dominant genera in this group included *Terrimonas* (Chitinophagaceae, Sphingobacteraceae, Proteobacteria), an unknown *Chitinophagaceae* (Sphingobacteraceae) and unknown *Xanthomonadaceae* (Gammaproteobacteria). The second type is associated with no-discharge sites and is dominated by Fusobacteria, Euryachaeota, Chloroflexi, Chlorobi, Acidobacteria, an unknown Bacteria and an unknown Archaea groups. These groups include unknown genera of Fusobacteria, unknown *Anaerolinidaceae* (Chloroflexi), *Gp17* (Acidobacteria) and an unknown Bacteria.

In the Surow River where microbial community at sites receiving mine water discharges was different from those without discharge, dbRDA identified concentrations of DO, As, Mn, Fe, Cu, sulfate, ammonia, NOx, turbidity and FRP to be strongly correlated with dbRDA orthogonal axes (Figure 7.21), although only turbidity, concentrations of DO, Fe, sulfate and FRP had strong and significant correlations with the MDS coordinates as surrogate for microbial community assemblages (Figure 7.22). The first axis of dbRDAs of the Surow also indicated that microbial community associated with mine water pump site (K1_P site) was separated from the rest. microbial communities in the Surow River (**Error! Reference source not found.** A). The mine water pump site was dominated by the phyla of Fibrobacteres, Bacteroidetes, Gemmatimonadetes, Actinobacteria, Nitrospira and Proteobacteria, whilst the rests were dominated by the phyla of Acidobacteria, Cyanobacteria, Fusobacteria, Euryachaeotha, Chloroflexi, Chlorobi, and unknown Bacteria. Acidobacteria was dominant at sites 8, 8C and 9 which were located directly downstream of the mine water pump site K1_P.

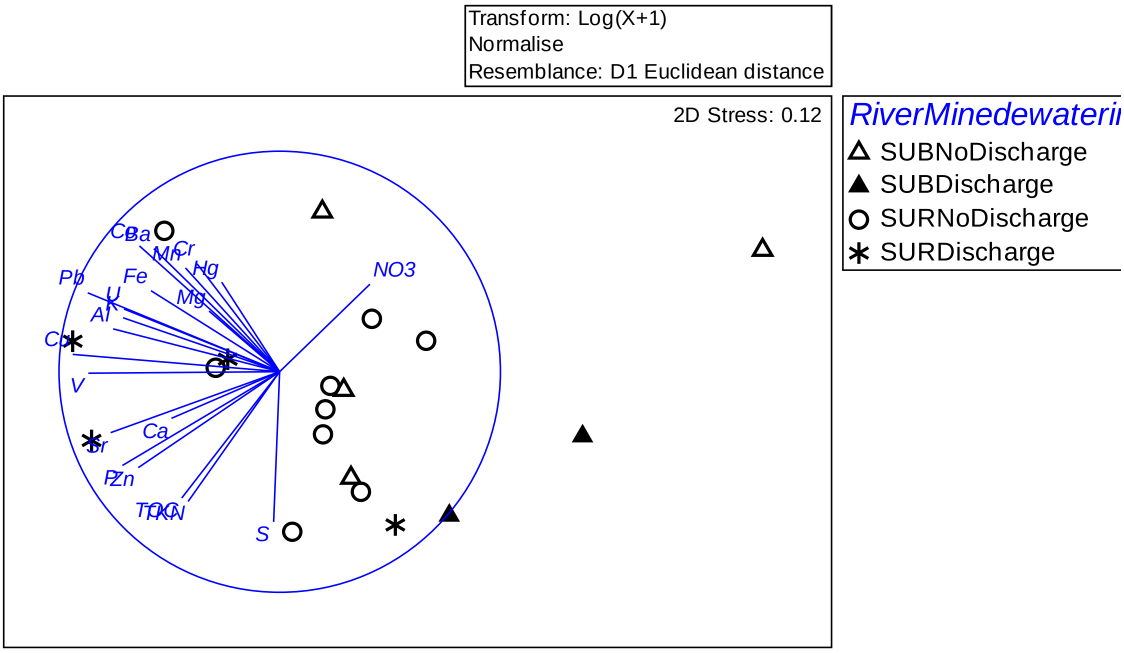


Figure 7.17 MDS plots of sediment quality in the Surow and Subri rivers in April 2014 with rivers and mine water discharge as overlay. The vectors represent river sediment quality variables that are strongly correlated (Pearson's r >0.4) with the MDS coordinate axes.

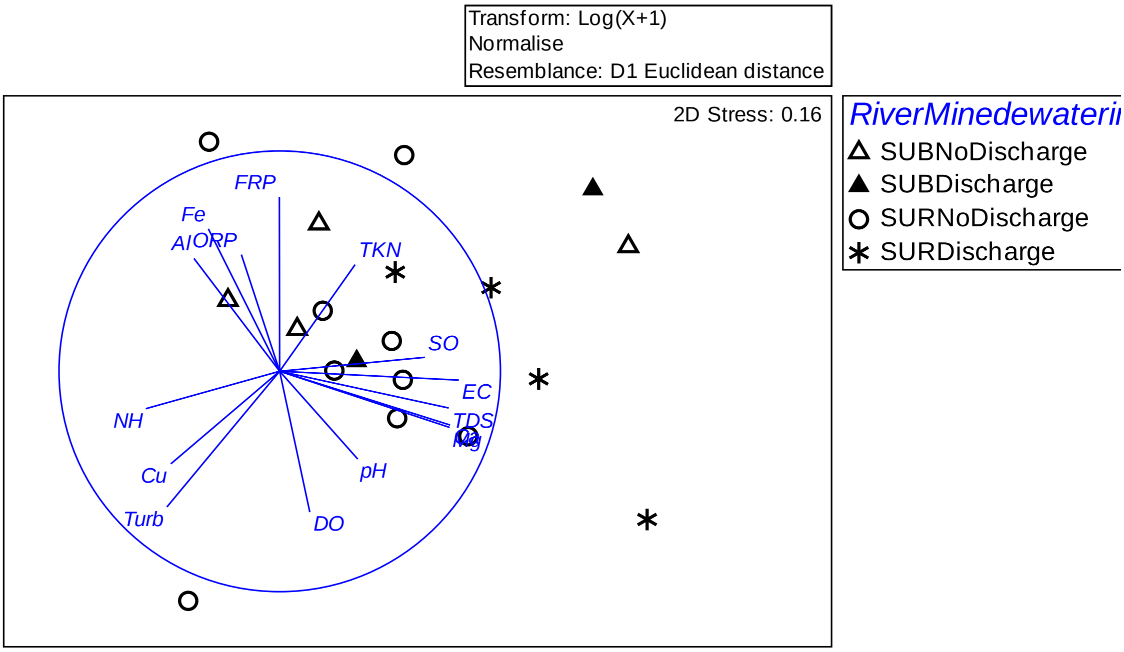


Figure 7.18 MDS plots of water quality in the Surow and Subri rivers in April 2014 with rivers and mine water discharge as overlay. The vectors represent river water quality variables that are strongly correlated (Pearson's r >0.4) with the MDS coordinate axes.

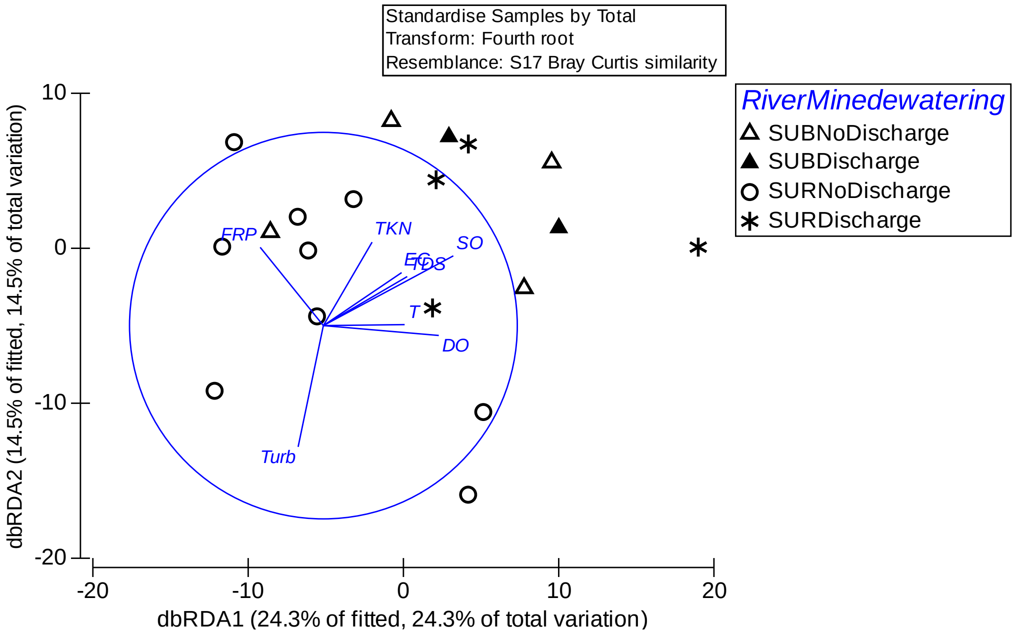
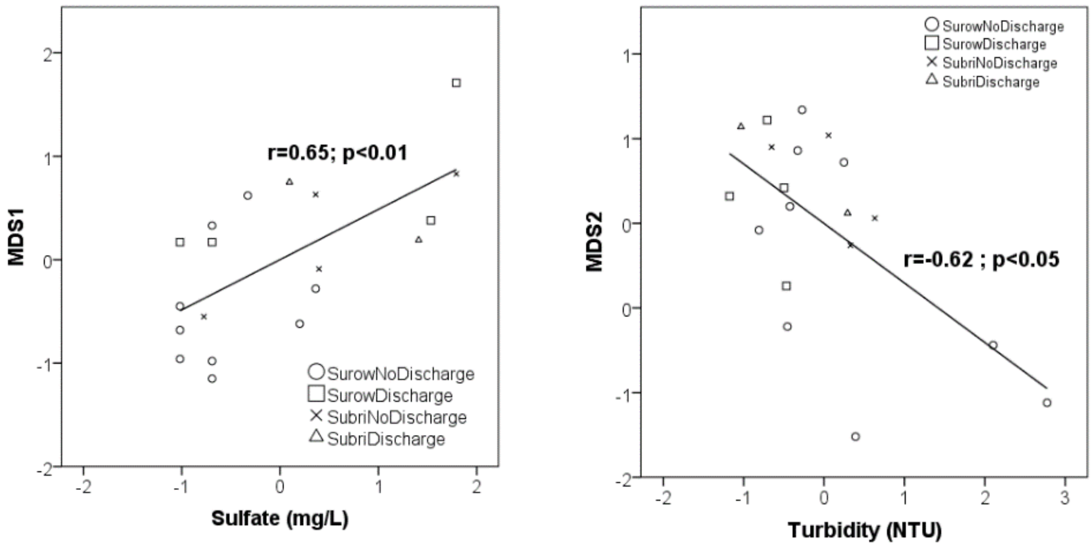


Figure 7.19 Distance based redundancy analysis showing the difference between microbial community at sites receiving mine water discharge and non-discharge. The vectors represent water quality variables with significant correlations (Pearson's r >0.4) with the first and second dbRDA axes



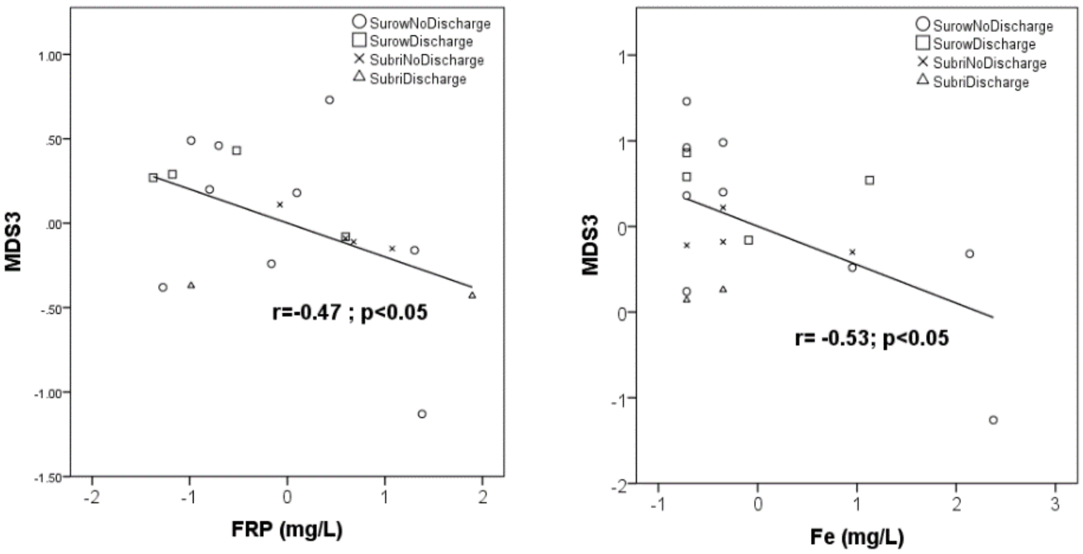
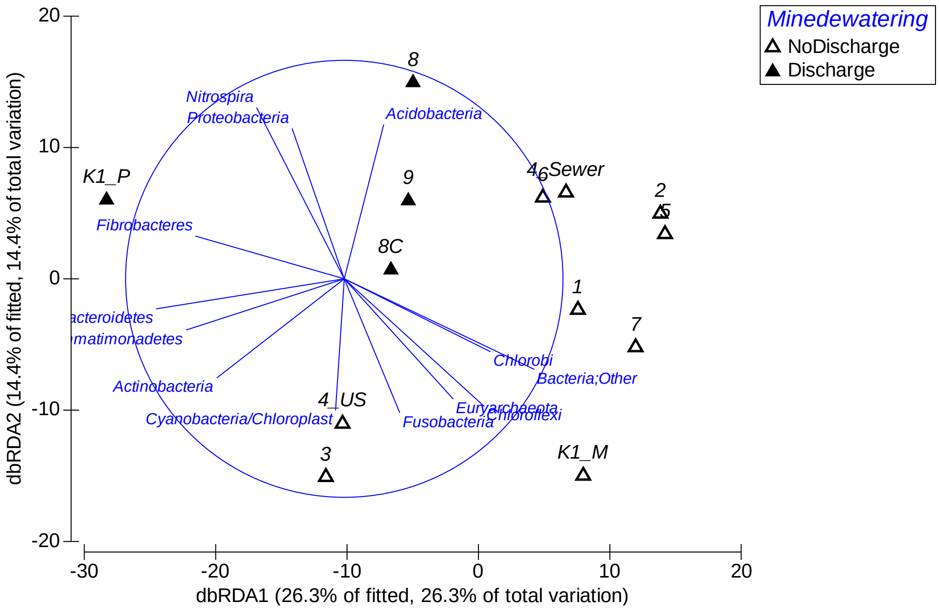
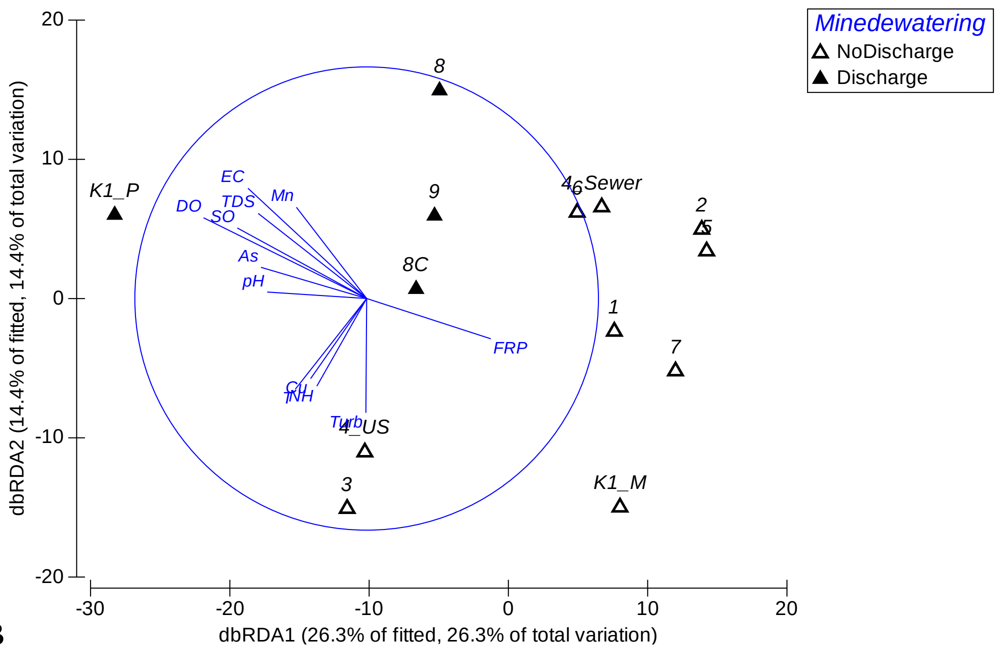


Figure 7.20 Relationships between MDS coordinate axes of microbial community assemblage and log concentrations of sulfate, filterable reactive phosphorus (FRP), Fe and turbidity of the rivers water with river and mine water discharge as overlay.



A



B

Figure 7.21 Distance based redundancy analysis of microbial community and water quality in the Surow River showing that microbial community at the dewatering pump site (K1_P) was different from that of other sites (A). Water quality at K1_P was characterised by increased DO, EC, sulfate and TDS (B).

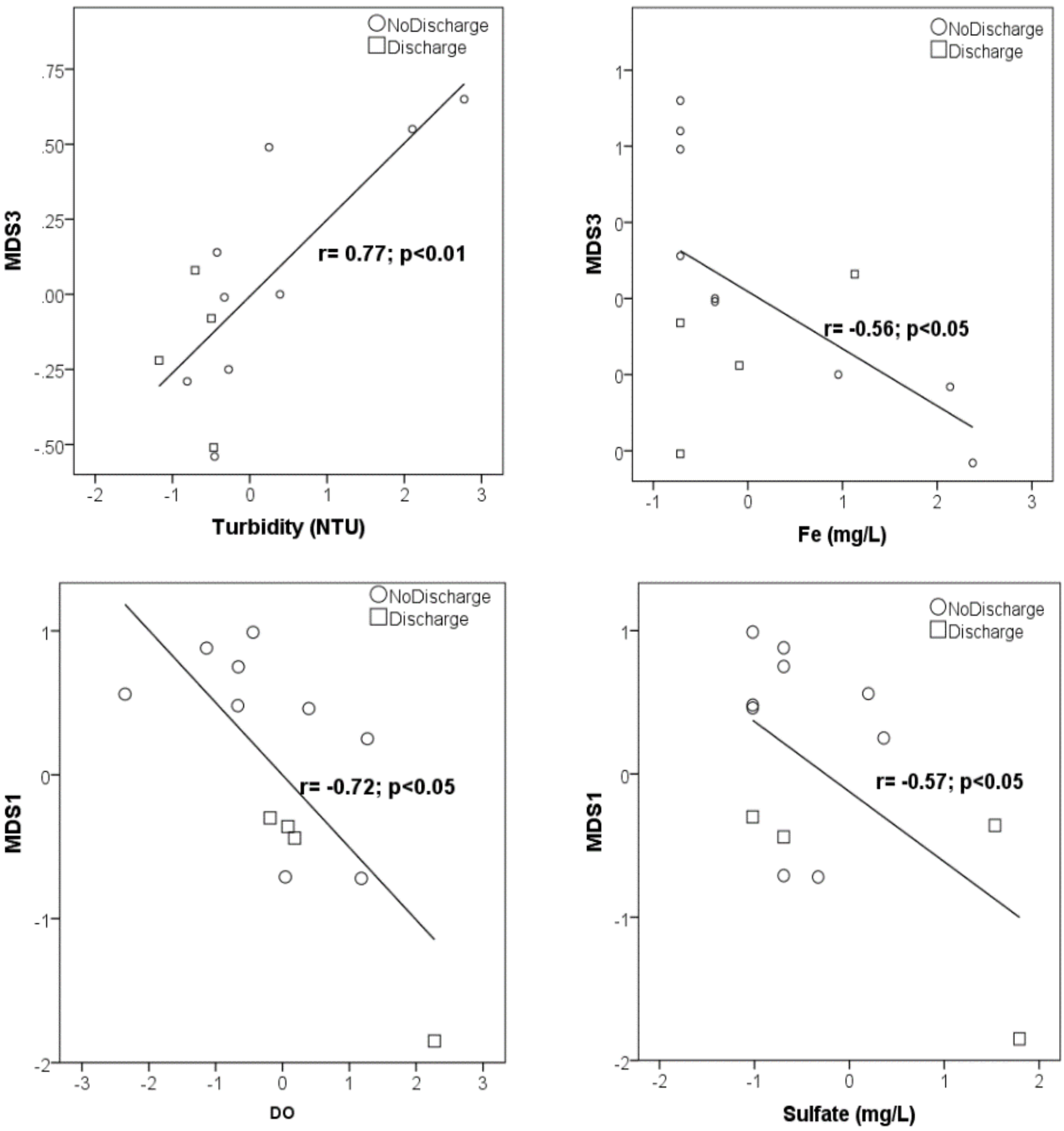


Figure 7.22 Correlations between MDS coordinates of microbial community assemblage and log turbidity, concentration of Fe, DO and sulfate in the Surow River, with mine water discharge and no-discharge as overlay.

7.4 Discussion

There are four important findings from the one-off microbiological study of the Surow and Subri sediments. Firstly, the microbial community composition and diversity in the study area is comparable to other studies. Second, spatial variability within is greater than between rivers and that the variability was unrelated to riverine sediment chemistry but significantly related to water chemistry. Third, mining activities, particularly mine dewatering, shifted riverine sediment microbial community composition. Fourth, the shift in microbial community composition in sites affected by mine dewatering from artisanal and small-scale gold mining (ASGM) operations is more significant than in sites affected by the large and modern mining operations. In this section, the four significant findings are presented separately and discussed.

7.4.1 Microbial community diversity and identity

This study, most likely the first into riverine sediment microbiology in Ghana, has revealed the microbial community diversity of riverine sediment in the region. Comparing this work to previous studies, however, can be problematic because the previous studies have used different methods with very different levels of resolution and samples from different sites, as also previously observed by Fierer et al. (2006) as well as Roesch et al. (2007). However, based on the total number of identified genera as well as by the Shannon diversity index, the sediment microbial community diversity in this study was comparable or even higher than related studies. For example, 70 genera of Actinobacter were identified in this study, compared to 48 Actinobacter genera from a study of soil microbiology in Brazil (Suela Silva et al., 2013). The composition of dominant phyla in this study, which include Proteobacteria (particularly Alpha and Beta Proteobacteria), Acidobacteria, Firmicutes, Bacteroidetes and Actinobacteria, are also comparable to findings from soil microbial studies in other places including France and North America (Constancias et al., 2015; Fierer et al., 2007a) as well as Canada and Brazil (Roesch et al., 2007).

Although biases are likely to be present in this type of work (Rajendhran et al., 2008; Wintzingerode et al., 1997), the ubiquitous presence of an unknown Bacteria and Archaea phyla comprising > 22% of the total abundance in this study is too substantial to be regarded as an error. The prevalence of unknown OTUs in soil microbiology studies is not uncommon (Chan et al., 2014), potentially due to error in computation of sequences or simply because they were novel organisms yet to be identified. A study of soil microbiology from 5 states across America, for example, found that between 6 to 12% DNA sequences were unknown even when the largest library of the time was used (Roesch et al., 2007). A study on coastal water microorganism in tropical Malaysia also reported unknown bacteria that contributed more than 40% to the total identified microbial community (Chan et al., 2014). Given the taxonomic classification procedure of the rest of the taxa, contamination is also highly unlikely. The unknown OTUs also tend to be more prevalent at sites near to farms and rural dwellings reflecting the influence of anthropogenic impacts that alter the biodiversity in the rivers (Chan et al., 2014). Considering the relative novelty of river sediment microbial study in Ghana and West Africa, the unknown OTUs are probably novel phyla, orders, families or genera requiring further investigation.

The sediment samples from the sites KSW3 and 4M that resulted in an almost zero count of DNA may have contained microbial taxa that are not already identified and classified in the other samples. Although these sites are situated within a mine site, it is almost implausible to assume that these sediment samples did not contain any microbial DNA, because previous studies showed that Archaea and Bacteria are present in most habitats, even in extreme environments (Rowe et al., 2007; Torsvik et al., 2008). The most plausible explanation for failure in extracting DNA from the two mine affected sediment samples may lie in the DNA extraction process. Some substances in sediment and soil are known to interfere with DNA extracting process (Wintzingerode et al., 1997). Humic acids and other humic compounds are among major contaminants in sediment samples that can inhibit DNA modifying enzymes (Porteous et al., 1991; Rajendhran et al., 2008) and hybridization specificity (Steffan et al., 1988). These substances, although untested in this study, may have been present in abundance in sediment samples at site KSW3 on the Subri and 4M on the Surow. Further investigation on these samples or similar samples may provide better results and explanation.

7.4.2 Variability in microbial community structure between rivers

Despite the difference in river length, catchment area, types of mining on their catchment and riverine sediment quality, sediment microbial community assemblages and diversity in the Subri and Surow rivers were not different from each other. This finding is interesting given similar studies often found otherwise (Lear et al., 2008; Lear et al., 2009) and theoretically, soil microbial community composition vary spatially (Constancias et al., 2015; Delmont et al., 2011), even at fine spatial scales (Roesch et al., 2007; Torsvik et al., 2008).

The similarity between sediment microbial communities in the Surow and Subri rivers most likely is due to the edaphic factors that include local geological characteristics and soil pH (Fierer et al., 2006) as a result of geographical proximity and similarity (elevation, climate) between the two rivers (Córdova-Kreylos et al., 2006) and land use and management (Bossio et al., 2005; Chung et al., 2007; Constancias et al., 2015). Although we did not conduct plant density, land use and crops surveys, personal observation suggested that there was a general similarity in land use, crops and agricultural methods used by inhabitants within the rivers' catchments which may also have contributed to the similarity between microbial communities in the two rivers. The similarity, therefore, suggested that despite the stark contrast between ASGM and the Ahafo gold mine in terms of scale of operations, the impacts of the two types of gold mining on river sediment microbiology were generally comparable at river scales.

7.4.3 Changes in microbial community and diversity due to gold mining

Despite the similarity at broader scales, spatial changes in microbial community composition due to environmental factors can be seen at smaller spatial scales, driven by mine water discharges and proximity to mine works. Although conductivity and concentrations of Al, Mn, As, NOx and ammonia as well as temperature contributed to the differences between microbial communities and water qualities; across rivers, turbidity, Fe, sulfate and FRP were the most significant water quality variables responsible for the changes in microbial community. In the Surow River affected by ASGM, turbidity, Fe, DO, sulfate and FRP are the drivers of the microbial community shift, particularly at sites receiving mine dewatering discharge. Mine dewatering water generally contained higher concentrations sulfate and phosphate which are leached into the water from rocks exposed by mining, as evident in the water quality data and discussed in Chapters 3 and 4. The pumping and discharging of this water also aerates it, resulting in the elevated dissolved oxygen concentrations and reduced turbidity.

The study revealed that in the Surow River, the characteristics of mine dewatering water appeared to be reflected in the microbial communities at sites closely affected by mine water and mine works. The cooler and more aerated mine dewatering water discharged into the rivers, for example, allowed for aerobic microbial community including Gemmatiomonadetes group to flourish (see Figure 7.16 I), while supressing anaerobic Archaea and Bacteria communities including the methanogenic Methanobacteriaceae and Anaerolineaceae which were more abundant in sites with low river flows due to sedimentation including site 1, 5 and 7 on the Surow River and site NSW 9 and KSW16 on the Subri River (see Figure 7.16 A and C) (DeBruyn et al., 2011; Offre et al., 2013). The abundance of Proteobacteria groups including Sphingomonadaceae and Xanthomonadaceae (Figure 7.16 K and L respectively) in sites affected by mine dewatering including site 8 and mine dewatering pump on the Surow and NSW6, NSW8 and KSW3 on the Subri, may be explained by the elevated concentrations of sulfate.

Sulfate should be favourable to the sulfate-reducing bacteria (SRB), including the Proteobacteria families. Sulfate reducing bacteria, often found in pore water, are important in the cycling of Fe and S by performing sulphide oxidation of sulphide minerals such as pyrite, marcasite and chalcopyrite, which are commonly found in mining areas (Offre et al., 2013; Salomons, 1995), including on the Surow and Subri rivers catchment. In the oxidation process, some bacteria leach metals into the aquatic environment which may lead to poor water quality, particularly in acidic environment (Küsel, 2003). Sulfate-reducing bacteria (SRB) can also convert heavy metals including Hg, which are often released by mining, into their methylated forms, which can be even more toxic and bioavailable (Benoit et al., 1999b; Macalady et al., 2000). Given the potential for the release of Hg by artisanal mining activities, the abundance of sulfate reducing bacteria in ASGM affected rivers, therefore, should be of concern and monitored.

Sulfate in mining areas is likely to be leached from carbon-rich sulphide minerals (Offre et al., 2013; Salomons, 1995), therefore bacteria which are known to correlate positively with carbon mineralisation including Bacteroidetes and Alphaproteobacteria families such as Sphingobacteraceae as well as Gammaproteobacteria (Fierer et al., 2007a) also flourished in the sites affected by mine dewatering on the Surow and the Subri rivers.

Mine dewatering water was also characterised by elevated concentrations of ammonia which explained the increased relative abundance of ammonia-reducing bacteria including Nitrospira, Proteobacteria, Bacteriodetes and Actinobacteria (Campbell et al., 2010; Fierer et al., 2007b) in sites impacted by mine dewatering, particularly on the Subri River. On the contrary, Acidobacteria and Firmicutes, which were less abundant at sites affected by mine dewatering on both rivers, were previously known to correlate negatively with ammonia-enriched soil and sediment (Campbell et al., 2010)

The presence of rare bacterial phyla of Deinoccus-Thermus, Elusimicrobia, Synergitetes, OD1, and Fibrobacteres in a few sites close to the mines or affected by mine water discharges, namely 8 and 3 on the Surow River and NSW8 and KSW13 on the Subri River, may also indicate their biogeochemical roles. For example, Deinoccocus-Thermus families including Geodermatophilaceae, which corresponded with mine water discharge and mine impacted sites including the ASGM mine dewatering pump site on the Surow and NSW6 and on the Subri, are stone-inhabiting bacteria, known to be highly resistant to low oxygen, heavy metals and light, often found in rock samples, even those buried deep underground and in arid environment (Gtari et al., 2012; Urzì et al., 2001). Their resistance to pollutants associated with mining, therefore, needs to be further investigated.

Similarly, unknown taxa may have important roles in the biogeochemical processes in the rivers. Unknown Bacteria and Archaea, for example, were also among dominant microorganisms at sites unaffected by mine water but closer to rural dwellings and farms (sites 3 and 7) on the Surow River and site NSW9 on the Subri River. Although roles of most microorganisms are not well understood (Philippot et al., 2010; Torsvik et al., 2002), it remains interesting to investigate the roles of the unknown prokaryotes in the biogeochemical processes of gold mining impacted aquatic ecosystems, highlighting the importance of identification of the unknown taxa.

The findings from this study suggest that water quality is a more significant factor in determining riverine sediment microbial community composition than the edaphic factors. This is consistent with the fact that sediment Archaea and Bacteria live inside water-filled pores and in the water films surrounding sediment particles (Torsvik et al., 2008), supporting the potential for use of metagenomics as monitor water quality, particularly in mining affected rivers.

7.4.4 The difference between impacts of ASGM and modern-large scale mining and its implications

This study found that the shift in microbial community composition in sites affected by mine dewatering discharge by ASGM was more significant than in sites affected by mine dewatering discharge by the large and modern mining operations. This, however, may have also been contributed by the sampling size of the microbiology study. We only had 6 sediment samples from the Subri River compared to 14 samples from the Surow which may partially explain the low spatial variability in the Subri River.

Given the exploratory nature of the river sediment microbiology study and its limitation, further study with more detail sampling design and replicates is needed to arrive to more decisive assertion regarding the difference between impacts of the two types of mining on microbial community assemblages. However, the significant shift in microbial community assemblage in sites affected by mine dewatering discharge in the Surow River reflected the nature of mine dewatering by ASGM. Mine dewatering water and waste from the ASGM works are discharged or allowed to run off directly into surrounding natural aquatic environments without treatment. This practise, unfortunately, continued even after most of ASGM operations was shut down by the government.

Unlike the ASGM, the Ahafo mine operated ECD was designed to contain mine water and 'treat' it to meet the environmental standards prior to decanting it into the Subri River. This may explain the reduced variability in the sediment microbial community assemblage in downstream Subri River, compared to variability in the Surow. The network of ECDs at the Ahafo mine through site NSW8. The ECDs work as settlement pond / lake for the sediment materials, suspended solids and pollutants that may come with mine waters (NGGL, 2005). In addition, the microbial communities in the ECDs, like in freshwater lakes, may perform biogeochemical processes that may reduce concentrations of nutrients and some metals (Ledin et al., 1996; Newton et al., 2011).

The effectiveness of the ECD in improving mine water and wastes as well as mitigating significant changes in the environment due to mining could be replicated at ASGM sites. A settling dam like the ECD can be relatively simple and easy to implement. This is particularly important considering the efforts in eliminating ASGM from Ghanaian society have proven to be ineffective, mostly due to the social and economic issues often related to ASGM communities, including lack of alternative employment opportunities (Banchirigah et al., 2010; Hilson, 2007, 2010) .

7.5 Conclusion

Gold mining activities can change river water and sediment quality, which in turn may change riverine sediment microbial community assemblages. River water quality, however, is a more significant factor than riverine sediment quality in determining microbial community assemblage.

Discharge of mine dewatering water into the rivers has the most significant effects, particularly on the Surow River affected by ASGM discharge of untreated mine dewatering water. Turbidity, concentrations of sulfate, Fe, and FRP are correlated with the shift present. The shift in microbial community due to mine dewatering discharge in the Subri River, however, is not significant, possibly due to the mitigating effects of the ECDs in the Ahafo mine. However, this may also be due to the limitation associated with the one-off sampling and exploratory nature of this study, as well as the fact that the sample size in the Subri River is far less than the Surow. Further study with replicates and more proportionate sample size is recommended to arrive to a more conclusive result.

This study demonstrated that the microbial community assemblage and diversity in the sediment of the Surow and Subri rivers are comparable to other similar studies, although more than 22% of the total abundance remain unknown. This study also shows that riverine sediment microbial communities have potential to be used as biological monitors for gold mining impacts in tropical environments. Further study needs to consider the use of finer spatial scales in sediment sampling as well as investigating the temporal variations of microbial community.

8 Impact of gold mining on riverine ecology in tropical West African region: comparison between artisanal small-scale gold mining and contemporary large scale gold mining

8.1 Synthesis

Thorough investigation of the Surow and Subri rivers, two tributaries of the Tano River in the Brong Ahafo Region in Ghana, has demonstrated that gold mining significantly impacts adjacent river ecosystems. This study has established that sediment particulates and minerals naturally available in the rock formations, but exposed and introduced to the aquatic environment by mining activities, were the most significant pollutants in the affected riverine ecosystems. Situated in the tropics with intense rainfall, the impacts were exacerbated by excess surface water flows from the mining areas that discharged into the Surow and Subri Rivers. Changes in the sediment and water quality due to mining were quantified and reflected in the macroinvertebrate communities of both rivers, while the water microbial communities tended to respond to the differences in water quality only. Mercury, which was used in ASGM, was detected in the Surow River sediment but barely detected in the river water, except for the upstream sites during the peak of ASGM operations. Mercury, however, was biomagnified in fish from both the Surow and Subri Rivers as well as the Tano River.

The study also strongly indicated that the types, magnitudes and effects of the environmental impacts of ASGM were different from that of the contemporary large-scale gold mining (the Ahafo mine), and that many of the impacts of ASGM on the physical environment of the Surow River were naturally remediated with the cessation of most ASGM in the area. While differences in the scale and method of operations most likely contributed to the differences between the two modes of mining, the use, or the lack of, environmental management systems to mitigate impacts appeared to be the most important differentiating factor. In the Subri River affected by the Ahafo gold mine, the impacts were mostly ameliorated by the intensive sediment control measures applied on the mine, whilst on the Surow River such control measures barely existed for ASGM. The sediment control measures on the Subri River included the use of environmental control dams (ECDs). It was demonstrated that the ECDs significantly reduced not only the river water turbidity and TSS, but also TDS, EC, concentrations of most salt ions (including NOx and sulfate), and most metals (both as total and dissolved in water). The improved water quality downstream of mining in the Subri River was also reflected in its less contaminated sediment compared to the Surow River.

The impacts of mining on the Surow and Subri Rivers are depicted in the linkage diagrams created for each river system (Figure 8.1 and Figure 8.2respectively), highlighting sediment and mine water as the main sources of impacts. These linkage diagrams are the extended versions of the original conceptual diagram with hypothetical links constructed as an underlying framework to the study (Figure 1.2, Chapter 1), only this time the diagram incorporated actual findings as well as postulations arising from the study. The following sections discuss the impacts and main components of gold mining effluents impacting the riverine ecology, i.e. mercury, sediment and mine water. Potential mitigating actions for the impacts are also addressed.

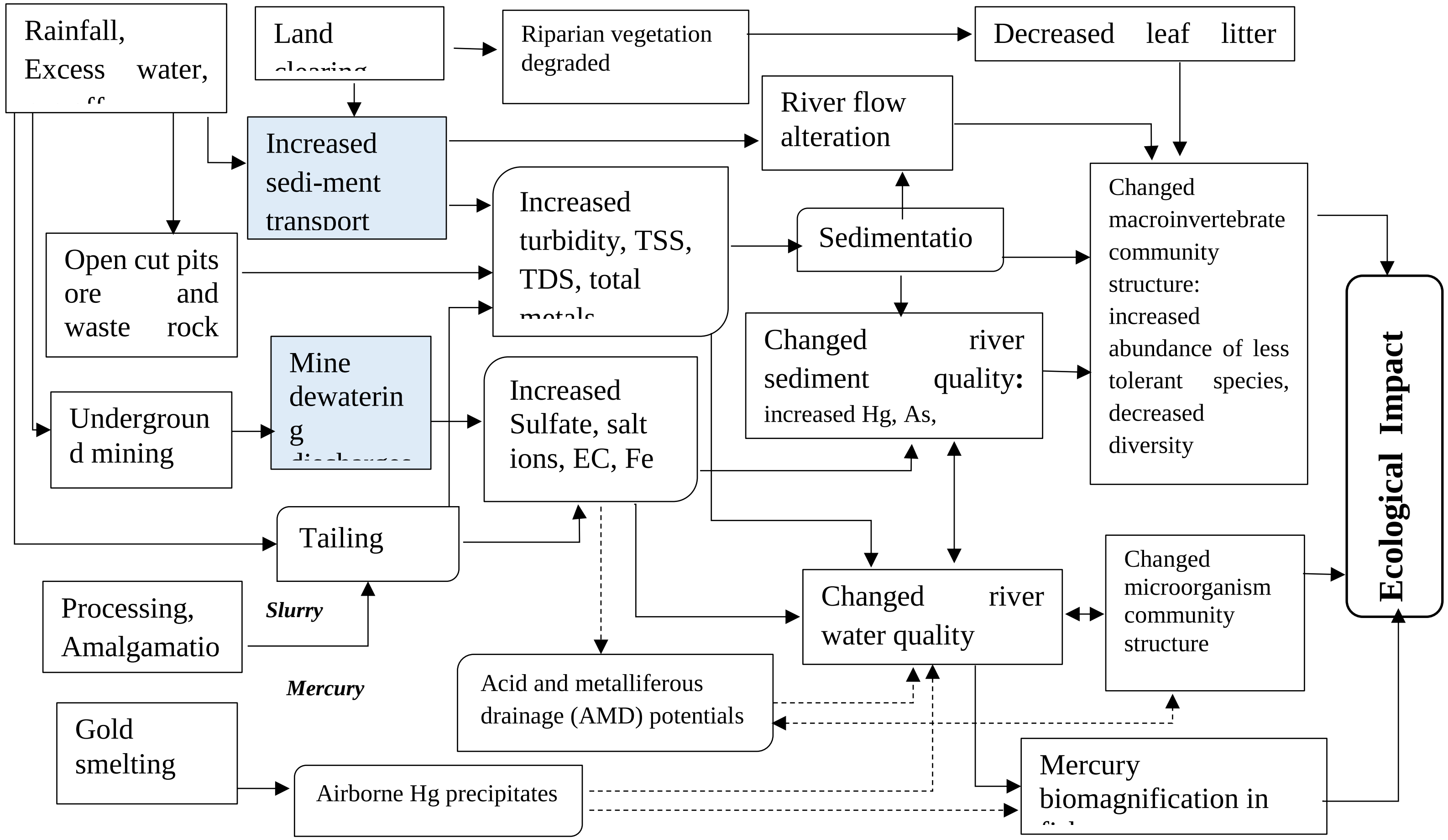


Figure 8.1 Model of the ecological impacts of artisanal and small scale gold mining on the Surow River.
Arrows depict links that were directly or indirectly demonstrated in the study.

Broken arrows represent tentative links or links between cases that were not evident in this study, e.g. AMD was not detected in this study but remains a possibility. The blue boxes represent sources of impacts which are demonstrated significant in the study.

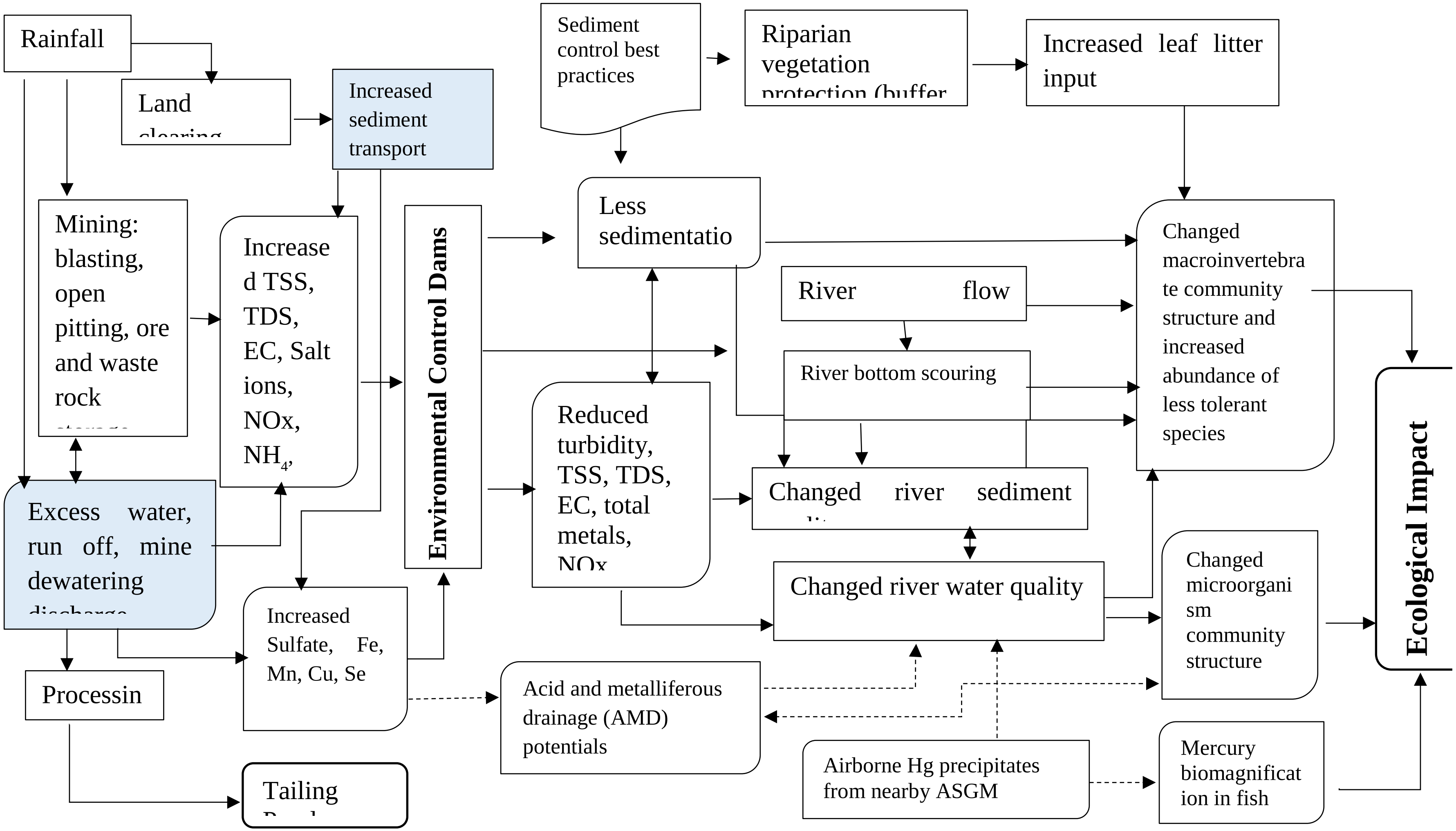


Figure 8.2 Model of ecological impacts of Ahafo gold mine on the Subri River environment
Arrows depict links that were directly or indirectly demonstrated in the study. Broken arrows represent tentative links or links between cases that were not evident in this study, e.g. AMD was not detected in this study but remains a possibility. The blue boxes represent sources of impacts which are demonstrated significant in the study.

8.2 Mercury pollution by ASGM

Studies of the impacts of artisanal and small-scale gold mining have mostly concentrated on Hg because of its use in the amalgamation process and its toxicity and potential to have deleterious effects on the environment (Appleton et al., 2006; Cordy et al., 2011; Dominique et al., 2007). In this study, however, Hg was not detected in high concentrations in both unfiltered and filtered (also called dissolved Hg; see Lasorsa et al. (1995)) water in the ASGM affected areas of the Surow River most of the time. The only exception was seen during the active ASGM period when Hg was detected in the river water upstream of the mining activity. Elevated concentrations of Hg above the safe standard for aquatic life were also detected in the Surow river sediment in February 2013 when ASGM was actively working in the area. The Hg enrichment of the Surow River sediment was also evident from the I-geo and EF analysis (Chapter 3). The sediment Hg, however, dissipated after the cessation of major ASGM operation. This is an indication that the Hg-contaminated sediment was scoured of the bottom of the river and transported downstream or onto the flood plains by the flooding events in the following rainy seasons during September or in April after the cessation of ASGM (Guimaraes et al., 2000a; Guimaraes et al., 2000b).

In the Subri River water and sediment, Hg was also undetected most of the time, except for three occasions between 2005-2014 when Hg concentrations in water were above the detection limit of 0.0002 mg/L in the control part of the Subri River and downstream area nearing to its confluence with the Tano River. Spot sampling of water and sediment samples from the Tano River also resulted in concentrations of Hg below detection levels. However, the fish Hg study strongly supports our hypothesis that Hg was biomagnified in fish in both the Surow and Subri rivers, as well as the Tano River which receives water and sediment from both rivers. The fish study strongly suggests that despite its low concentration in water, mercury existed in the riverine ecosystems and was taken up by riverine biota in the area, even in places lacking of point source mercury emission like the Subri River. The Tano, on the contrary, has other tributaries that may be polluted with Hg released by ASGM operating within their catchments. This study could not establish the exact sources of the Hg being magnified in the fish, particularly those in the Subri River where anthropogenic point sources of Hg were not known to exist.

Undetected Hg in river water, as experienced in this study, can also be due to the complications inherent with Hg analysis of natural water samples or the generally low concentrations of Hg (Bank, 2012; Swartzendruber et al., 2012). We chose the CVAA methods (EPA 245.1 method) for the analysis of total mercury because it has the lowest limit of detection (0.0002 mg/L) amongst other methods that were available to us economically. The achievable low detection limits of CVAA methods available for the analysis, however, are not low enough for ambient water samples (Lasorsa et al., 2012), this most likely contributed to the very low to undetected concentrations of dissolved Hg in filtered water.

The difficulties in detecting Hg in water is due to its volatility and instability. Mercury in the environment is available in various forms, each may need specific method of analysis (Bank, 2012). As explained in section 5.1, Hg changes forms in water between gaseous elemental Hg (Hg^0 or GEM), dimethyl Hg (DMHg), monomethyl Hg (MMHg), Hg ion (HgII), dissolved gaseous Hg (DGM), reactive gaseous Hg (RGM), particulate-bound Hg (PHg), colloidal Hg (Co-Hg) and reactive Hg (HgR) via complex bio-geochemical processes involving sulfate reducing bacteria (Benoit et al., 1999a; Swartzendruber et al., 2012).

In rivers, anthropogenic Hg occurs mostly as PHg and HgII. Particulate-bound Hg and HgII predominantly are of atmospheric origin and precipitated both dry and wet into rivers and catchments (Selin, 2014; Swartzendruber et al., 2012), settle in riverine sediment and be transported downstream. Particulate-bound Hg, therefore, is most likely the Hg detected in the unfiltered water and riverine sediment during the active ASGM period in the dry season of February 2013. Unlike PHg, DGM, HgII, and Hg^0 deposited in water are often quickly methylated into MMHg and DMHg (Swartzendruber et al., 2012) in both the river column and sediment. Mercury methylation is particularly prevalent in wetland environments where long period of water inundation support anaerobic reactions (Engstrom, 2007; Jeremiason et al., 2006; Lambertsson et al., 2006a). A study using a stable isotope of Hg introduced to a wetland, for example, showed that the Hg transformed into methylated forms within days and moved downward to below water table and horizontally into a nearby lake through the groundwater within months (Branfireun et al., 2005). The Surow River is also characterised by some wetland-like pools of slow moving water along its course, particularly around ASGM mines and downstream of the river nearing to its confluence with the Tano River. Inorganic Hg potentially emitted through tailings and run-off water in the river, therefore, can be trapped and methylated in the wetlands. In other words, much of Hg in the Surow River ecosystems may be in the forms of monomethyl or dimethyl mercury. Unfortunately, the specialised analysis of methyl mercury was not available in Ghana nor in the ACZ lab where we sent the water samples for analysis.

of Hg used in gold processing by ASGM in the area. Unlike the ASGM in Indonesia, China or Peru who applied the direct ore amalgamation method (Figure 1.4, Chapter 1) using the trammel (James, 1994; Sulaiman et al., 2007; Veiga et al., 2006), ASGM operators on the Surow catchment employed the amalgamation of gravity concentrates method. In the direct ore amalgamation method commonly used in Indonesia, for example, 1 kg Hg is added to a steel grinding mill containing 40 kg of ore resulting in the loss of Hg to the environment, as expressed by the ratio of Hg lost to gold produced, of up to 100:1 (Veiga et al., 2006). The amalgamation of concentrates only method used in Ghana, on the other hand, processes very small amounts of gold-containing material (concentrates) thereby requiring a small amount of Hg (less than 30 grams of Hg, my personal observation) to recover the gold. Consequently, this results in significantly reduced Hg losses to the tailings or the environment (Veiga et al., 2006).

Tailings, however, are not the only source of Hg emission to the environment in ASGM. The heating and smelting of gold amalgam in ASGM potentially releases Hg into the environment more than that of from tailings. According to de Lacerda (2003), about 30% of Hg emission from ASGM is due to amalgamation process through tailings, whilst 70% of is due to heating and smelting. Veiga et al. (2004a) estimated Hg loss to the environment due to heating and smelting of gold amalgam commonly performed in ASGM refinery shops ranging between 5 and 20% of the total Hg used in the Hg-gold amalgamation. The use of retorts (Jønsson et al., 2009), can be significantly reduce the amount of Hg vapour emitted to the atmosphere, reportedly by up to 95% (Hinton, 2005). Unfortunately, the ASGM smelters in the Surow River catchment, like many others in the world, do not use the retort due to the costs associated with retorts or lack of awareness of and trust in the benefits of retorts (Hilson et al., 2007a; Jønsson et al., 2009; Spiegel et al., 2006; Sulaiman et al., 2007). Consequently, Hg is vaporised off the gold-mercury amalgam into the air during the extreme heating process. Mercury vapour, primarily in the HgII and Hg^0 forms, can reportedly travel long distances as they remain airborne for a long period of time (Lim et al., 2001). This Hg will eventually be redeposited into rivers and receiving aquatic environments, methylated and readily taken up by aquatic biota including fish (Engstrom, 2007) as depicted by a model in Figure 8.3, even in places that lacked Hg point sources (Beaulieu et al.; Kamman et al., 2005; Riva-Murray et al., 2011).

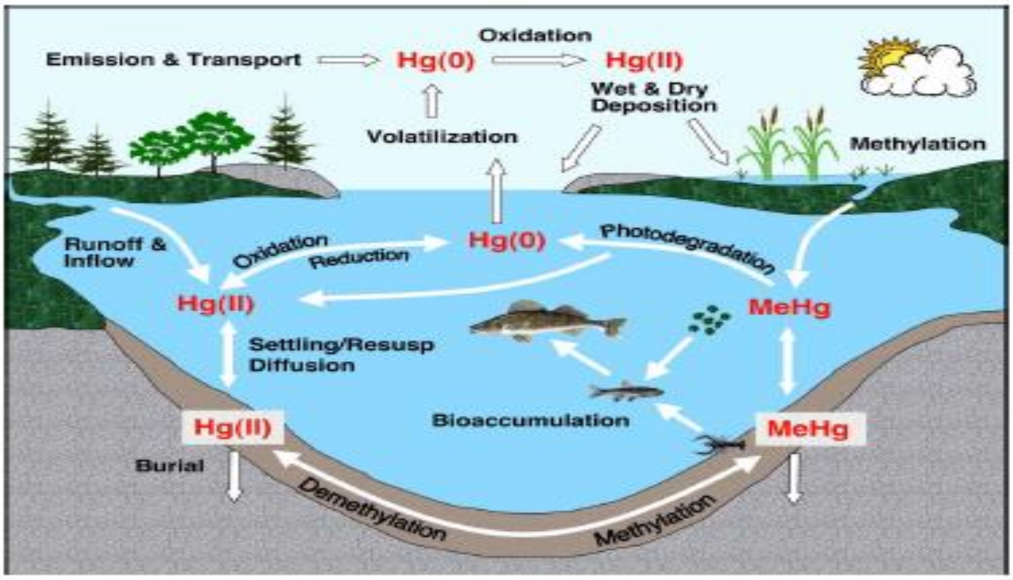


Figure 8.3 Biogeocycling of mercury in a lake ecosystems (Engstrom, 2007).

The biomagnification of Hg in fish in rivers that are not directly affected by ASGM such as the Subri and the Tano Rivers (Chapter 6) strongly indicates the presence of methyl mercury in the rivers. It also indicates transport of mercury into these rivers. In this study, we did not investigate the exact source of Hg that was biomagnified in fish nor the extent of mercury vapour transport in the air. However, considering the absence of other industrial and anthropogenic sources of Hg in the area, the Hg vapour from ASGM smelters has become one of the most plausible sources of the Hg that was biomagnified in the fish. This study, therefore, suggests that ASGM contributes to the broad-scale Hg enrichment of riverine environments and biota. This study also demonstrates that the ecological risk arising from Hg is present not only in the Surow River which is directly affected by ASGM, but also in the nearby riverine ecosystems not directly affected by ASGM.

The potential for ASGM to emit Hg vapours into the air highlights the global problem of atmospheric Hg (Lindqvist et al., 1985; Pacyna et al., 2010; Schroeder et al., 1998). The Global Mercury Project (UNEP, 2013; UNEP, 2002b) and the Minamata Convention and Global Mercury Treaty (Bank et al., 2014; Selin, 2014) initiated by the United Nations as parts of the global efforts to curtail the problem, found that ASGM is the largest global source of mercury emission (UNEP, 2013). With the chronic global atmospheric Hg being predicted to continue to rise in spite of the global initiatives (Pacyna et al., 2010), more serious measures need to be taken to cut the anthropogenic sources of Hg emission, including from ASGM (Selin, 2014). Such Hg reduction efforts can be undertaken at local and regional levels among ASGM operators around the world by promoting the exclusive use of the gravity concentrates amalgamation methods over the whole-ore amalgamation methods (Veiga et al., 2006; Velásquez-López et al., 2010) and the use of retorts in the gold-amalgam heating and smelting (Amankwah et al., 2010; Jønsson et al., 2009; Spiegel et al., 2006) to capture the Hg vapour preventing it from emitting into the atmosphere. This should also be supported by research into the transport and distribution of Hg vapour from ASGM smelting shops. Considering the complications of Hg speciation in water and sediment and its analysis, assessment and monitoring of Hg in riverine environment should employ a multi-tier investigation involving biological assessment of the river's biota (Donkor et al., 2006; Gammons et al., 2006; Lasorsa et al., 1995). This will provide not only information about the extent of Hg pollution in the area, but also the potential for biomagnification of Hg in biota to better understand its ecological impacts. Although studies in environmental Hg have been of significant interest over the past decades, the current and previous body of knowledge on Hg biogeochemical cycling remains insufficient to accurately predict the magnitude of increase in atmospheric Hg (Bank et al., 2014; Selin, 2014). Sharing and integration of research in mercury, including biomagnification in biota (Bank et al., 2014; Gustin et al., 2016) at the local, regional and global scales are therefore very important.

8.3 Sediment as significant source of pollutants in riverine ecosystem

This study demonstrated that regardless of scales and methods of mining and extraction of gold, sediment is a significant pollutant in riverine ecosystems originating from mining activities. Consequently, sediment control at its source and beyond is crucial in mitigation of mining impacts on riverine ecology.

The lack of sediment control in ASGM areas was evident in this study, resulting in the degradation of the Surow River water and sediment quality, particularly due to elevated concentrations of particulate-bound elements, including Hg, Fe and various nutrients. Sedimentation that changed river morphology and degraded riparian vegetation was also evident in the Surow River ecosystems. The contaminants carrying sediment is particularly an issue in tropical rivers where intense rainfalls often result in regular flood events (Hayward et al., 1987; Junk et al., 1989) that transport and deposit the sediment and pollutants downstream and on to floodplains. The pollutants, once deposited, can be mineralised within floodplains and wetlands before being transported back into rivers by subsequent flood events, hence creating an ongoing cycle of pollution (Bastos et al., 2007; Guimaraes et al., 2000b).

Contrary to the ASGM practices on the Surow River, the Ahafo gold mine practises sediment control measures that include engineering works and the application of best management practices (BMP) in sediment and erosion control (Macdonald et al., 2003) to prevent sediment materials from escaping the mine site and entering the Subri River. The sediment control measures have proven effective in controlling sediment particulates transport in the Subri River as evident by the decrease in turbidity and TSS downstream of the dams compared to that of upstream. Elevated turbidity and TSS, however, was recorded at impacted sites on the river during the first months of the mine development before it improved to background levels. This can be explained as a lag time between the commencement of disturbance due to mining project (land clearing, development, etc.) and the water quality improvement due to the implementation of BMP (Meals et al., 2010).

The study also strongly indicated that water and sediment quality at downstream Subri River was also less contaminated than that of the Surow River. The settlement and control of sediment materials at Ahafo mine removed most of particulates bound elements from mine waters entering the river, lowering total metal and metalloid concentrations in water downstream. Macroinvertebrate communities respond acutely to suspended sediment and sediment pollution (Smolders et al., 2003; Wagenhoff et al., 2012; Wood et al., 1997; Yule et al., 2010), therefore the difference in water and sediment quality between the two rivers was also reflected in the respective macroinvertebrate communities. The macroinvertebrate community assembly in the lower Subri River had higher taxa richness and abundance than that of unaffected areas on upstream Subri River and the ASGM affected Surow River. The mine-affected area in the lower Subri River also had more taxa that were sensitive to pollution (such as the ephemeropteran and trichopteran families) than that found in the Surow River, an indication of more suitable water quality in the lower Subri River downstream of mining. On the contrary, the Surow River's macroinvertebrate community was dominated by taxa tolerant of sediment pollution with reduced ephemeropteran and trichopteran taxa, an indication of the negative effects of sediment addition and flow reduction on the Surow River due to ASGM (Matthaei et al., 2010). Clearly, sediment addition via mining has significantly impacted the aquatic ecosystems of the receiving rivers and its control is crucial in the mitigation of mining impacts on riverine ecosystems.

8.4 Management of mine water

This study shows that impacts of gold mining on riverine ecology are not only due to contaminants, but also to the quantity of water being abstracted from, discharged to, or in contact with waste that escaped to surrounding riverine environments. The difference between impacts of ASGM and that of the contemporary large-scale gold mining on the surrounding aquatic ecosystems can also be explained by the water management systems implemented, or the lack of them, at each mine. In the dry seasons, ASGM operators abstracted water from the Surow and its ephemeral tributary streams, while also discharging mine dewatering water from mining shafts indiscriminately into river. Unlike in some drier places where water abstraction for mining is an environmental issue, such as in Australia (Cote et al., 2012; Mudd, 2007b, 2008), North America and Europe (Pusch et al., 2000), at the Ahafo mine water is readily available in abundance so the mine does not need to abstract water from the Subri River or nearby bores for its operational needs. The abundant water is built up by the intense rainfall in the area and groundwater flowing into the four Ahafo mining pits, and is available in excess of what the mine needs or can retain. The water surplus has to be managed to ensure that the quantity and quality of water being stored or discharged does not interfere with the integrity of the aquatic ecosystems around the mines, highlighting the importance of water balance calculations for management (Rapantova et al., 2007).

Mine water management systems can be explained by a model adapted from that of the Australian Department of Resources, Environment and Tourism (DRET, 2008) and shown in Figure 8.4.

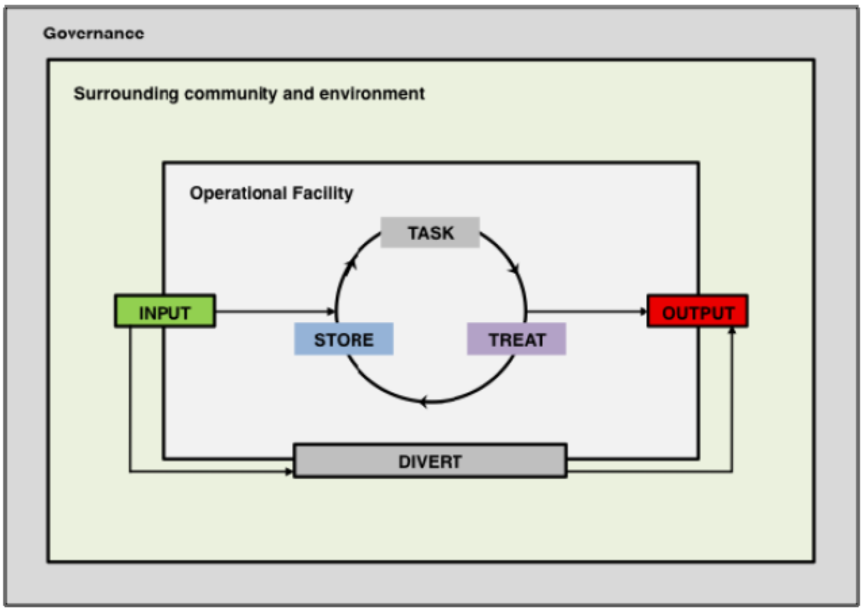


Figure 8.4 Water system map (DRET, 2008) adapted for mining

In this model, a mine water system consists of:

1. Input, representing water received from the environment which includes rainfall, ground water, or other sources such as water abstracted from nearby rivers or lakes for operational purposes.

2. Store, tasks and treat cycle, representing water use in mine operations which include processing, mining, dust supressing, tailing management and other mine supporting facilities. In this cycle, water often has to be stored for future use and treated to meet processing standards or other regulatory standards.

3. Divert, representing water that is not a part of mining or processing and needs to be moved around or through the operations. This includes the runoff water that potentially touches mining materials, rocks or wastes at the mine.

4. Output, representing the removal of water from the mine site to the environment. This includes back flow and seepage into the ground water, evaporation from water storages or discharges and outflows onto surface water environments such as nearby water bodies.

The accounting of water, the comparison between input and output, describes whether a mine is a net positive consumer (Output-Input > 0) or a net negative water consumer (Output-Input < 0). It is important to note that in this model, the calculation of the flows involve not only the quantity of water but also its quality (i.e. the elements that comes with the water). Water passing through a mine also never disappears, but continues to exists in one form or another (Kemp et al., 2010; Moran, 2006). Therefore, in an environment where water is scarce, the magnitude of water input into a mine is comparable to the local water resources availability. In an environment where water is abundant, such as in the study area, there is an increased likelihood for water to be in contact with the rock surface on the pit walls or waste materials. In the case of excess mining pit water, this enriched water may overflow or spill over into the environment. While it is important to remove the water from flooded pits to make way for mining and avoiding spillage, it is also important to remove the pollutants before the water enters the environment. Consequently, in mines with net positive water balance, surplus mine water storage and treatment facilities are often necessary.

At Ahafo mine, water input comes from rainfall and mining pits containing surface water and groundwater. Mining pit water, which is available in abundance, is used for processing. At the plant site, process water is recycled so that the used process water does not leave the mine to contaminate surrounding water bodies (NGGL, 2005). Any excess used process water is directed to the tailing storage facility to manage the tailings. At the time of study, fresh excess mine pit water was stored in the pits and other water storage facilities. Meanwhile, surface water runoff, which potentially had been in contact with waste rocks and materials was diverted to and contained in the ECDs, which in turn discharged into the Subri River when water quality complied with the Ghanaian EPA standard for aquatic ecology. The ECD system and its impacts on the Subri River ecosystem are discussed in Section 8.5 below. Although at the time of my study water storage was not an issue, given the net positive balance of water in the system, the current water storage capacity is predicted to be exceeded. In the future, regular discharge of this mine dewatering water, therefore, is predicted to be necessary to prevent flooding of mine pits and overflows (pers. communication with Mr. Kwame Yeboah MSc, Newmont Ghana Environmental Water Monitoring Coordinator). Consequently, the current water management system at Ahafo mine includes a plan for a water treatment system for the future discharge of mine pit water.

Surplus mine water often contains pollutants, including metals, metalloids, salts and nutrients that can have deleterious effects on riverine ecology (Kemp et al., 2010; Younger et al., 2004). Elevated metal, metalloids and salt ions in mine water can arise from seepage of groundwater into mining pits, mineralisation / leaching of elements from pit walls, burden, waste rocks, tailings, etc (Akcil et al., 2006; de Lacerda et al., 1998; Jeffery et al., 1988; Salomons, 1995) and fugitive sediment particles. While sulfate is available naturally in groundwater in areas with sulfate rock formations (Spalding et al., 1993), in a mine site sulfate also leaches from the rocks upon contact with water following the formula given in section 3.4.2 (Chapter 3) by Salomons (1995) at any pH, but especially in acidic environments. Similarly, nutrients, particularly ammonia and nitrates, are normally elevated in mining areas due to residues of explosives used in mining (Huisman et al., 2006; Koren et al., 2000; Zaitsev et al., 2008).

Although we did not investigate mine pit water or mine dewatering water quality at Ahafo mine, the quality of runoff water at the mine site which was diverted to the ECDs provided an approximation to the quality of mine water at the mine, including that of the mine pits and mine dewatering. At Ahafo mine site, as evident in the study, the mine water often contained elevated salt ions (hence the elevated TDS and EC), particularly nitrates and sulfate, exceeding the Ghanaian standard criteria for protection of aquatic life. The elevated nitrates and sulfate in mine water reflects the effects of mining activities which include blasting, mine pitting and rock waste storage which expose the rock materials to water. Unlike the solid particles and other particulate-bound elements including metals, dissolved nitrates and sulfates cannot simply settle out alongside sediment but may need specific technology such as nano-flitration or reverse osmosis to remove it (Malaiyandi et al., 1981). Discharge or overspill of nutrient and sulfate rich water, especially in large quantities and high frequencies, could disrupt the integrity of the surrounding natural aquatic ecosystems (Camargo et al., 2005; Camargo et al., 1992; Matthaei et al., 2010) through eutrophication (Lamers et al., 2002; Lamers et al., 1998; Smith et al., 1999), macroinvertebrate community structure (Sponseller et al., 2001; Wagenhoff et al., 2012) and changes in the microbial function and community structure (Wakelin et al., 2008). Therefore, effective treatment to remove nutrients and sulfate from mine dewatering water must be performed before the surplus water is discharged or overflown into the natural environments due to the intense precipitation in the area.

ASGM operations, unlike the Ahafo mine, lacked a mine water management system. ASGM operators commonly abstract water from the Surow River and its tributary streams for processing, including washing of ore in sluicing boxes and other uses at their mining camps (Figure 8.5 C) particularly in the dry seasons. Shallow sumps are often built under the elutriation boxes to capture some tailings that escaped with the elutriation water in the ASGM processes for sale to cyanide processors, effluent water from this process typically flows back to the river, particularly during the rainy seasons (Figure 8.6). In other words, proper storage and treatment of process water is absent from the ASGM operation, whilst run-off surface water that has been in contact with tailings and rock materials is not diverted nor treated. Miners' lack of awareness of environmental conservation and limited capital most likely have hindered development of water management facilities in ASGM areas (Hilson et al., 2007a). The absence of monitoring program and policy regarding ASGM impacts does not assist with the situation either.

Similar to the Ahafo mine, ASGM operators in the area also faced issues related to groundwater inundating their mining shafts, an indication that their tunnel shafts have intercepted the water table. Miners had to dewater the shafts regularly to make way for mining, and discharged it indiscriminately into the Surow River. The mine dewatering activities particularly increases during the rainy seasons when surface water also flows into the shafts. Mining shaft dewatering continued to happen even after the main cessation of ASGM in the area, although it was less frequent and possibly designed to maintain the shafts with a hope to return to the mine sometime in the future when conditions permit. Unlike at Ahafo mine, however, the quantity and quality of untreated mine water discharges from ASGM are not monitored nor regulated (pers. observation). The study showed that the mine dewatering water from ASGM was contaminated with salts and minerals along with sediment particulates. Elevated nitrates and ammonia, however, was not a significant issue with the ASGM mine water discharged into the Surow River. This reflects the manual and rudimentary method of mining applied by ASGM operators in the area which does not require the use of explosives. As such, contamination by nitrates and ammonia from explosives in ASGM operations in the area was negligible. The low nitrate and ammonia concentrations in the river may also be caused by the biological processes in the wetland-like sections along the Surow River which can promote the removal of nutrients (Chan et al., 1982; Whitmire et al., 2005). River water at sites receiving mine dewatering discharges on the Surow, however, typically had elevated EC, concentrations of metal particularly Fe and Mn, sulfate, and salt ions.

A

B

C

D

Figure 8.5 Lower Surow and Subri rivers dry up in the dry seasons (A and B) while the flows are very low upstream (C and D).

Figure 8.6 Sedimentation and tailing sump at an ASGM processing station.

Although the elevated concentrations of sulfate and other salt ions were comparable to or lower than that of Ahafo mine site, the concentrations of metal and metalloids in ASGM mine dewatering water were higher than that of originating from the Ahafo mine. This, most likely was due to the metals' affinity for sediment particles available in abundance in the Surow River. This, suggests that the difference between water quality of the ASGM and Ahafo discharges mostly is due to the difference in sediment control measures and mining methods (with or without explosives) applied in the two modes of gold mining. Nevertheless, the elevated sulfate and metals, particularly Fe and Mn, in the Surow River environment is of environmental concern, considering their potential to create of AMD if other environmental conditions support (Akcil et al., 2006; Jeffery et al., 1988; Lottermoser, 2012). This concern is heightened by the fact that ASGM operation lacked measures to control or prevent AMD. Elevated sulfate is even alarming in an area where Hg is known to be emitted into the environment such as the Surow River catchment. This is because sulfate can enhance the methylation of Hg (Jeremiason et al., 2006; King et al., 2002), making it more bioavailable to the riverine food chain.

Based on the water quality categories and standards in
Table 8.1, for example, compliant mine water from ASGM and the Ahafo mine could potentially be used to supply the local water company to be treated to the drinking water quality. At the very least, excess water could be used for agriculture or aquaculture (Annandale et al., 2002; McCullough et al., 2006; Otchere et al., 2004) that can support the human and economic development of the area. Water trading and transporting with drier mines could also be considered (Barrett et al., 2010) to minimise discharge of excess water while at the same time conserving water resources in the region.

Table 8.1 Mine water quality category and possible uses (Kemp et al., 2010)

Water Category	Summary description	Characteristics
Category 1	Close to the standards of drinking water, only requires minimum treatment (disinfection) to be safe for human consumption. Can be used for all purposes	Characterised by a total dissolved solids (TDS) concentration of 100 mg/L and concentration of other physical and chemical constituent below agreed threshold (for example the Ghana Water Company or WHO 2008 standards)
Category 2	Water that requires treatment to remove TDS and other constituents to be safe for human consumption, but it can be used without treatment for many agricultural and recreational purposes	Characterised by a TDS ranging between 1000 mg/L and 5000 mg/L and concentrations of other elements not meeting the criteria listed above
Category 3	Hypersaline water that cannot be used for any agricultural purposes without removal of TDS	Characterised by a TDS higher than 5000 mg/L

8.5 Roles of ECD in mitigating mining impact on riverine water and sediment quality

The study strongly supports the positive effects of the environmental control dams (ECD) in mitigating gold mining impacts on the Subri River ecosystem. The ECDs have shown to be effective primary treatment for runoff and storm water diverted from the mine by ‘settling out’ solids, floating particles and particulate-bound pollutants (including metals such as Fe, Cu, Zn and Se) from the liquid part (Al-Abed et al., 2006). This reduces the discharge of pollutants into receiving environment (river) which otherwise can further disrupt the integrity of the river ecosystems.

The positive effects of the ECDs on mine water runoff, however, was made possible by the regular monitoring of water quality, regular discharge of in-compliance water and removal of silt and sediment built up in the bottom of the ECDs. Thus, although the earthen dam construction may be relatively simple, operation of an ECD requires some levels of planning, especially in terms of hydrology, and ongoing management for it to function effectively. This needs to be considered if similar systems are to be adopted in the ASGM sectors, or when planning for mine closure at the end of the mine. Decommissioning of the ECDs and reclamation of the area at mine closure can be considered to prevent the risks arising from overflows and spillage. This, however, should include rehabilitation of the sediment built up at the bottom of the dam which is potentially contaminated with trace metals and nutrients (Hinton, 2002), calling for regular monitoring of sediment quality in the ECDs during the active mining periods to provide baseline data for future closure plans. On the other hand, having been a part of the Subri River ecosystems for more than 10 years now and proven beneficial for the river’s ecosystems, the removal or closure of the ECDs may also have impact on the Subri River ecosystems, which also needs to be assessed. If the water and sediment quality in the ECDs meets the standards (see **Error! Reference source not found.** for example) and the ecological impacts of damming the river are minimal, retaining the ECDs for other purposes including for agricultural irrigation or fish farming (Otchere et al., 2004) can be considered, provided the local authorities and surrounding communities have the capacities to manage it. In the long terms, if ECDs are to be retained post-mine closure, a monitoring program must be established. Such a monitoring program should look for any changes in water and sediment chemistry, if the water pH changes over time, and whether macroinvertebrate and microorganism community structures, change.

8.6 Recommendations within a global context

Ghana is only one of more than 70 countries where ASGM is practised (UNEP, 2013), with most of these being developing countries located in the tropics. As witnessed in this study, ASGM are commonly found operating near to water bodies including streams and rivers (Ribeiro, 2006; Telmer et al., 2009). The ASGM impacts identified in this study, therefore, very likely represent a fraction of the global impacts of ASGM on freshwater ecosystems. This is particularly alarming considering many of ASGM practising countries are located in the tropics, whose rivers are known for their high biodiversity (Ramirez et al., 2008). Due to its important role in providing rural employment in many developing countries and its ubiquitous impacts on the environment, ASGM has been of considerable interest to national governments, as well as the United Nations. The United Nation Development Program (UNEP), for example, carried out the Global Mercury Project (GMP) aimed to improve the environmental performance of ASGM in the developing countries. The objective of GMP, among others, was the gradual eradication of the use of Hg in ASGM through various integrated programs. However, while the main goal is yet to be achieved, the GMP also recommends formalisation (e.g. through some form of licensing) and organisation of ASGM to better control and mitigate its impacts (Hinton, 2005; Ribeiro, 2006). Many governments of countries with ASGM problems, such as Ghana and Indonesia (Spiegel et al., 2005; Sulaiman et al., 2007), have implemented the UN recommendations by incorporating provisions for the small-scale mining sector in their mining laws (2006; 2009). The laws encourage the formalisation of ASGM, for miners to work in cooperatives licensed to work in mining areas designated for ASGM, and for the improvement of control and monitoring by authorities (Macdonald et al., 2014). Within this framework, with an assumption that the impacts of ASGM on adjacent riverine ecosystems in the study area in Ghana would be similar to that of other tropical parts of the world where ASGM is practised, I recommend the following based on my studies and experiences:

1. Concerted efforts to prevent sediment transport from ASGM to adjacent rivers and streams at the source are crucial in mitigating ASGM impacts on the environment. Sediment particulates and particulate-bound elements are the main sources of pollutants from ASGM operations. Identification and control of sediment release at each step of mining and processing in ASGM operations should be institutionalised and a part of requirements if ASGM is to be formalised.

2. Development of ECDs in the areas designated for small-scale mining. The research findings with regards to the ECDs suggests that most of the sediment-related impacts of ASGM on the Surow River can also be mitigated by environmental control dams. While total eradication of ASGM from the area remains challenging due to the associated socio-economic issues and the lack of viable employment alternatives to overcome poverty, measures that can mitigate its environmental impacts should be encouraged. Development and the use of ECDs within ASGM communities to control the release of sediment and particulate-bound pollutants into surrounding water bodies is one of them. ECDs in ASGM communities can serve as primary mine effluent treatment facility to settle solids and floating particulates in run off and mine pit waters before it reaches the natural aquatic environment. This will mitigate many of the ASGM impacts on riverine biota, including those on microorganisms and macroinvertebrates. Water stored in the ECD can also be used for ASGM processing so abstraction of river water by ASGM can also be prevented or reduced.

3. Mercury is not the only contaminant in ASGM affected rivers, but remains a concern. This implies that studies in the environmental impacts and management of ASGM should not solely concentrate on Hg. Other pollutants including sediment, nutrients, other trace metals and sulfate should also be carefully assessed, because their impacts on riverine ecosystems can also be significant. On the other hand, the presence of Hg in different compartments of the environment has to be carefully monitored as well given its toxicity at low concentrations. Regular monitoring of Hg in water, sediment and riverine biota must be carried out in ASGM areas and beyond. In the study area, for example, the presence of mercury is indicated by the elevated Hg concentrations in sediments during active ASGM mining as well as the mercury bioaccumulation in fish in the both ASGM affected and unaffected rivers.

4. Ideally, the use of Hg in ASGM should be banned. However, where the use of Hg in ASGM remains prevalent, the exclusive gravity concentrate amalgamation method should be promoted over the whole ore amalgamation method. Although in this study we only investigated impacts of ASGM that used the gravity concentrate amalgamation method,

other studies showed that the whole ore amalgamation method required and emitted significantly higher quantity of Hg. Therefore, within the context of formalisation of ASGM, the shift to using gravity concentrate amalgamation should be a part of the enforcement and development process of the formalisation.

6. Improve smelting techniques to reduce release of Hg in to the atmosphere (Amankwah et al., 2010). The use of retort to capture Hg vapour in the gold smelting and refinery shops should be introduced to miners and facilitated. Then, at a later stage, it can be made compulsory to smelters and gold shop owners as a part of the formalisation and organisation of ASGM.

7. Rehabilitation of ASGM-impacted riparian areas should be improved. Awareness of the need for concurrent rehabilitation of mine worked areas should be built among miners and be made part of the permitting and licensing processes. Rehabilitation can also be enforced by, among others, temporarily and progressively closing down certain areas impacted by ASGM. This study has demonstrated that local recovery of ASGM impacted river is possible (Allan et al., 1993), particularly in the tropics where rainfalls are high. In the tropics, the high flow associated with the rainy seasons has the capacity to dilute and flush out contaminants in river water and sediment, while the riverine biota can have a chance to recover which typically happen quite swiftly (Boulton et al., 2008; Yule et al., 2010).

8. Future study on the impacts of ASGM should not be limited to the physical and chemical properties of riverine water and sediment, but also include impacts on riverine biota. The implications of ASGM impacts on the physical and geochemical properties of streams and rivers may span across the food web. Understanding the ecological impacts can assist in the prioritising of impact mitigation efforts. This study has shown that macroinvertebrates can be used as indicators of impact of ASGM and mining in tropical rivers. This study also demonstrates the potential for the use of the microorganism community as an indicator of mining impacts on riverine ecosystems. The use of riverine biota, including macroinvertebrate and microorganism composition as indicators of the river health not only will complement the conventional water and sediment quality surveys, but also provide opportunities for environmental education for the mining communities (Ramirez et al., 2008). Future studies in sources of Hg that is biomagnified in fish, the magnitude of Hg vapour released by ASGM into the atmosphere and the distance travelled by Hg vapour emitted ASGM will be very useful and can fill the knowledge gaps we encountered in this study. Speciation of Hg and the use of alternative methods to assess Hg in environmental samples could also provide more detailed insight into the extent of ASGM Hg pollution. Socio-economic features of ASGM (e.g. size of operations, number of people involved, etc) were another significant knowledge gap in this study.

9. Large mining companies operating adjacent to ASGM communities need to be aware of the risks posed by AGSM operations to their environmental management performance. The environmental regulations applied to large miners are generally much stricter standards than AGSM operators. Further, many of the local inhabitants are unable to distinguish between AGSM impacts and those of the larger companies, particularly with regards to the water resources surrounding them. One solution is for larger miners to support the capacity development of local authorities to monitor and mitigate AGSM impacts, particularly on water, using existing local laws. Such efforts are particularly relevant in the developing countries like Ghana where a lack of clean water supplies is part of a greater sustainability and human health issues.

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Appendices

Appendix 1 Paper presented at and published in the proceedings of the 12th International Mine Water Association (IMWA) Conference, Xu Zhou, China, 2014. http://www.mwen.info/docs/imwa_2014/IMWA2014_Macdonald_401.pdf Awarded the 2nd best Student Paper at the Conference.

Regulation of artisanal small scale gold mining (ASGM) in Ghana and Indonesia as currently implemented fails to adequately protect aquatic ecosystems.

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Abstract:

Artisanal small scale gold mining (ASGM) operations are largely unregulated, informal and transient. Rudimentary mining and processing techniques used in ASGM often result in degraded environmental, safety, health and social conditions. ASGM requires permanent sources of water, placing most operations close to natural water bodies. Until recently, the impact on these environments has been largely overlooked, with most studies focussing primarily on mercury contamination and health concerns. Based on Ghanaian and Indonesian experiences, regulation of ASGM is a good step toward improvement, but here we argue that regulation alone is insufficient to improve environmental performance, particularly when the impacts of ASGM on aquatic ecosystems are largely unknown.

Keywords: Mercury, mine water, Mine Closure, Sustainability, ASGM

Background

The international mining industry is increasingly regulated and required to implement stringent procedures to prevent or mitigate its environmental impacts (Jones et al., 2012). In contrast, poverty, high commodity prices and poor governance have attracted many people in developing countries to the largely unregulated artisanal gold mining (ASGM). Currently ASGM activity is documented in more than 70 countries and provides direct employment to at least 15 million people and indirect employment to more than 100 million people around the world (WHO, 2013). ASGM generally refers to gold mining by individuals, groups, families or cooperatives with rudimentary mining and processing methods (Hentschel et al., 2002). An ASGM operation can be formal (registered business) or informal, but the sector is typically unorganized and without external investment. The combination of these factors explains much of the low productivity in ASGM (ILO 1999), while the lack of long term planning highlights its unsustainable nature (Hinton, Veiga, & Veiga 2003). Due to an intrinsically poor geological exploration capacity, ASGM typically operates in areas where mineable reserves are known, usually discovered by larger commercial mining companies with legal concessional titles of the land. The presence of ASGM in such areas often creates additional legal, social and environmental conflicts to existing regulated mining operations (Aspinall et al., 2001;

Hinton et al., 2003). AGSM activities near to regulated mines may also complicate regulation of these mines and their operational and closure environmental and social performances (Aspinall et al., 2001; Mauric et al., 2012). Mercury use in ASGM is particularly problematic due to its toxicity as well as sheer amount of emission. In 2010 UNEP estimated the annual mercury emission by ASGM to be 727 tonnes, or 35% of the total world anthropogenic emission of mercury (UNEP, 2013).

ASGM mining and processing methods

ASGM operators usually work on secondary or tertiary alluvial ores easily found in river sediment by panning, dredging, or hosting sediments down river banks or open pits using high pressure pumps. More recently, ASGM operators have been working on primary ore mined underground, typically by manual digging of vertical shafts or tunnels up to 30 to 35 meters deep. Loose gravel, sands and milled ores in ASGM are usually processed via semi-mechanical crushing, elutriation and, in most cases, mercury amalgamation followed by gold smelting and refining. During the processes, most ASGM emits mercury to the environment by (1) disposal of mercury-laced tailings and process water to the ground and water bodies, and (2) atmospheric emission of mercury vapours from the smelting of the gold-mercury amalgam. The amount of mercury used and emitted into the environment by ASGM is often determined by the type of processing method rather than regulatory requirements. For example, the total mercury used in and emitted by the whole ore amalgamation method widely practised in Indonesia, is substantially more than that of the partial gravity-amalgamation method commonly used in Ghana.

Processing with cyanide has been introduced to ASGM operators as an alternative to mercury amalgamation because unlike mercury, cyanide breaks down rapidly and does not bio-accumulate as readily. The cyanide processing, however, still not preferred amongst smaller operators because it requires larger capital investment and production scales (Sousa et al., 2010; Sulaiman et al., 2007). It is, however, common practice for ASGM processors in Indonesia to sell their mercury-laced tailings to larger ASGM operators or processing centres to be reprocessed with cyanide. In North Sulawesi, Indonesia, two thirds of gold produced in the area is obtained by cyanidation of these tailings (Sulaiman et al., 2007). In Ghana, tailings from ASGM are also illegally sent to larger processors elsewhere in the country as well as neighbouring countries such as Burkina Faso and Cote d'Ivoire for cyanidation. The mercury-cyanide complexes resulting from these larger processors potentially create more pollutions which are yet to be established (Veiga et al., 2014).

Legal framework of ASGM

The legality of ASGM varies among countries, with some providing a legal framework for small scale mining activities, and others simply banning such activities. In an effort to manage and promote an efficient ASGM sector, the Ghanaian and Indonesian Mining Laws, for example, have provisions for small scale mining. The *Ghanaian Mining Act (2006)* and *Indonesian Mineral and Coal Law (2009)* stipulate that Ghanaian and Indonesian citizens, as individuals or cooperatives of up to ten people, can apply for a licence to mine on a maximum of10 Ha land in areas designated for small scale mining. In Ghana, extension offices of the Minerals Commission comprising representatives of several governing agencies (e.g., the Environmental Protection Agency and Precious Metals Marketing Company) have been formed in the nine main ASGM regions to process AGSM mining applications as well as monitor activities (such as mercury trade control) and purchase gold (Mr. Kofi Tetteh, Minerals Commission 2014. *pers. com.*). In Indonesia, while a mechanism of licensing, permitting, management and control of small scale mining is not clearly stipulated, the management and control of ASGM is fully decentralized to regional governments. The use of mercury in mining is illegal in Indonesia (signatory to the 2013 UNEP International Treaty on Mercury), while limited use of mercury in ASGM is legal in Ghana.

Despite the regulatory attempts to legalise ASGM operations in Ghana and Indonesia, ASGM continue to grow, mostly illegal due to operators' lack of permits and/or mine concessions / rights, or the use/misuse of controlled substances such as mercury or cyanide. Miners have found permits and licences hard and expensive to acquire while law enforcement is poor and often unevenly applied. In Ghana, according to a 2008 report by the Ghana Chambers of Mines, there were only 300 registered ASGM operators in Ghana, and between 300,000 to 500,000 miners currently in operation – a clear sign of ASGM persistence, despite government regulatory efforts (Bush, 2008). The latest (2013) enforcement effort in Ghana included the arrest and deportation of almost 4000 foreigners working directly or indirectly in ASGM which resulted in a temporary cessation of many operations around the country (Mensah, 2013). However, personal observations by the author (FM) of ASGM communities in Ghana showed that operations were rapidly reinstated, this time being run mostly by local citizens. In Indonesia, illegal ASGM grew from 50,000 miners operated in 576 areas in 2006 to 250,000 miners operated in about 800 areas in 22 provinces in 2010 (Ismawati, 2014). The decentralization of authority to the local governments in Indonesia was seen as a significant contributing factor to the unintended growth in illegal ASGM.(Gita et al., 2012).

While the regulations pertinent to ASGM in Indonesia and Ghana require monitoring and control of mining operations, the impacts of ASGM on aquatic ecosystems are often under monitored or not monitored at all. The illegality of most ASGM operators makes it even harder for these impacts to be monitored. The regulatory requirements of ASGM operators to perform environmental impact assessments often do not fit within the reality of ASGM, as operators lack capacity to produce impact assessments. Essentially, like many governments in developing countries where ASGM commonly is found, Ghana and Indonesia lack the institutional and technical capacity to provide adequate assistance to assess impacts or enforce compliance, especially at the local and regional levels (Sousa et al., 2011b). A lack of information and data about AGSM practises adds to the challenges in implementing environmental management and due diligence principles (Hilson, 2005). The sheer numbers of ASGM miners and locations, combined with the poorly understood temporal and spatial variability of impacts on aquatic ecosystems complicate efforts of local and regional environmental managers to regulate activities.

The impacts of ASGM on aquatic ecosystems

The toxicity of mercury to people involved with ASGM and, to a lesser extent, to the environment, has been well studied (Bose-O'Reilly et al., 2010; Castilhos et al., 2006; Donkor et al., 2006). As most ASGM operations occur near to lakes or along streams and rivers for easy access to water needed for operations, its impacts on aquatic ecosystems can be significant (fig.1). The impacts of ASGM on aquatic ecosystems vary both spatially and temporally due to the volume and concentration of contaminants being released. During the dry season, ASGM operators draw water from the nearest water bodies for processing. In the wetter seasons, run-off from unregulated ASGM elutriation boxes, slurry channels and sumps, tailing dumps and open pits elevates turbidity, total suspended solids, trace metals and nutrients in streams and rivers, resulting in sedimentation and changes to river morphology and water quality. In addition to reduced water quality, changes in water quantity of aquatic systems may occur, due to the large volume of untreated mine water pumped directly out of mine pits and shafts into rivers or other water bodies. The Ghana Water Company who supplies water for domestic and industrial purposes has complained of increased costs of treatment due to elevated contaminants in raw water drawn from rivers impacted by ASGM (Srem et al., 2013).

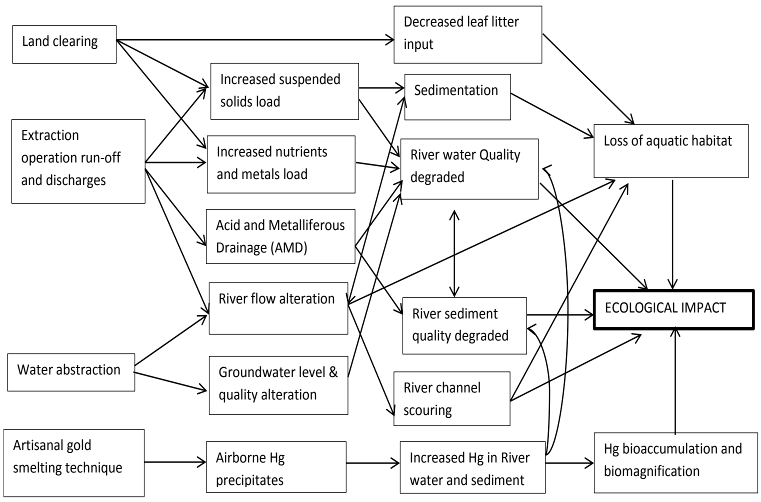

Figure 1. The impacts of artisanal small-scale gold mining (ASGM) on riverine systems.

Conclusion

Regulations on ASGM alone have proven ineffective in curbing impacts to aquatic ecosystems. To be effective, the regulations should be accompanied by a comprehensive approach that includes training and educational programs, targeted at miners, processors and local/regional authorities, toin recognise, control and monitor impacts. While studies in impacts of ASGM mercury on the environment and human health remain important, studies and efforts to find effective methods to prevent, identify, monitor and control other ASGM pollutants and processes affecting waterways (e.g., sedimentation, alterations in flow regime) are needed. Practical, economical and appropriate aquatic environmental monitoring measures should be introduced to environmental managers at local and regional levels while use of cleaner methods can be gradually introduced to miners. Such efforts are particularly relevant in the developing countries like Ghana and Indonesia where lack of clean water supplies is part of a greater sustainability issue.

Acknowledgements

This paper forms a part of FM's PhD research project on effects of ASGM on aquatic ecology conducted in the Brong Ahafo Region of Ghana 2012 – 2014, she was supported with an Australian Postgraduate Award. The authors wish to acknowledge support and assistance received from various institutions and individuals, including but not limited to: (1) the Mine Water and Environment Research Center (MiWER) ECU, Perth, Australia, (2) Newmont Ghana Gold Limited at Ahafo, Ghana, (3) Mr. Mumin Zakaria and Mr. Yakubu Ibrahim of Hwidiem, Ghana, (4) Ms. Yuyun Ismawati, the IPEN Lead for Mercury Reduction in ASGM/Mining, (5) Nana Berima Twireku Ampem III, Chief of Ntrotroso Town and Nana Osuodumgya

Berima Apiedwaa Boafo II, Chief of Hwidiem Town in the Brong Ahafo Region, Ghana, and (8) Kenyasi I Galamsey Committee, Brong Ahafo, Ghana.

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Impacts of artisanal small-scale gold mining on water quality of a tropical river (Surow River, Ghana).

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ABSTRACT

Rivers in Ghana provide environmental and economic services such as fishery and farming, and are also the main sources of clean drinking water. Artisanal small-scale gold mining (ASGM), a significant industry in Ghana, typically occurs near streams and rivers in order to obtain a source of water for processing and waste discharge. ASGM is subsistence mining carried out by individuals or small collectives using rudimentary technologies for both extraction and processing of ore. Using small quantities of mercury for gold extraction, ASGM also releases high quantities of sediment, (along with metals and other contaminants) into local water bodies, posing environmental and downstream human health risks. In Ahafo, Ghana, we undertook a detailed assessment of the effect of ASGM on the water quality of the Surow River over one year (January 2013 to April 2014). Physico-chemical properties of the water at 11 sites along the river (above and below ASGM sites) were measured monthly. Our research indicates that the impacts of ASGM extend beyond Hg contamination, with the main effects of ASGM on river systems being changes in water conductivity, sediment loads, and metals, as well as alteration of river morphology. Dewatering water was responsible for significant increases in conductivity. We did not detect mercury above drinking water standards, with the exception being at the headwaters, presumably from natural sources. In general, we found that sites with associated ASGM activities had water qualities that did not meet Ghanaian national standards for drinking water, with manganese at particularly high concentrations. We also saw temporal variability in water quality parameters, likely due to the combination of fluctuating ASGM activities and the natural seasonal hydrology of tropical river systems.

Keywords: ASGM, sedimentation, mine dewatering, river ecosystems

INTRODUCTION

Rivers in Ghana provide not only environmental and economic services such as fishery and farming, but are also the main source of clean drinking water. Rural communities, particularly in areas where access to clean water is limited, often use untreated river water for domestic purposes including drinking. Where water is treated before consumption, declines in water quality within the rivers from pollutants and sediment loads from agriculture, industry, mining and forestry increase treatment costs (Fianko et al., 2010; Gyau-Boakye et al., 2002). Coupled with declines in water quality, increasing demand (Gyau-Boakye et al., 2002), and declining rainfall (Gyau-Boakye et al., 2000; Owusu et al., 2009), rivers in Ghana are under pressure.

Artisanal small-scale gold mining (ASGM) is a globally-significant industry, providing rural employment directly to at least 15 million people and indirectly to over 100 million in more than

70 countries (WHO, 2013). Many ASGM operations occur near streams and rivers for easy access to alluvial ores, but also to supply water used in processing and as a receiving environment for mine waters. Although ASGM contributes to rural economies, it often results in degraded environmental, safety and social conditions due to the rudimentary mining and processing techniques used (Hilson, 2002; Telmer et al., 2009). ASGM traditionally relied upon secondary and tertiary materials easily found near to the surface or river banks. However, due to depletion of alluvial resources and increased technical and financial capacities, contemporary operators are increasingly mining primary ore found underground, by manually digging vertical shafts or tunnels up to 30 to 35 m deep. These shafts and tunnels often require dewatering, with large volumes of untreated dewatering water often pumped out of these underground operations into nearby rivers and streams. Metal released from processing, dewatering or acid rock drainage can further degrade river water quality. Particularly concerning in ASGM is the widespread use of mercury amalgamation techniques in processing, although cyanide processing is increasingly being used in reprocessing of tailings (de Andrade Lima et al., 2008; Velásquez-López et al., 2011). Mercury processing emits toxic vapours, with predicted global mercury emission by ASGM to be 727 tonnes: 35% of the total world anthropogenic emission of mercury (UNEP, 2013). The toxicity of mercury derived from ASGM operations to people and, to a lesser extent, the environment, has been well studied (Bose-O'Reilly et al., 2010; Castilhos et al., 2006; Donkor et al., 2006). However, the impact of AGSM operations on the broader water quality of these river and streams has been largely overlooked.

Previously, we identified a range of potential environmental impacts of ASGM on rivers, such as changes in hydrology and water quality (particularly increased turbidity), as a result of land clearing, erosion, mining and processing (Macdonald et al., 2014). Hydrological changes in rivers can alter available hydrological habitat for aquatic biota (Blanchette et al., 2013), and increased turbidity may lead to smothering of aquatic plants, habitats, and biota. Clearing of riparian vegetation, unregulated sewage from mining camps and rubbish disposal can impact on the rivers nutrient concentrations and habitats. In Ghana, these environmental impacts are temporally variable, with ASGM demands for water during dry seasons and excess water in wet seasons altering the flow of the river/stream (pers. obs.). Further, degradation of the river water quality and ecology can have flow-on impacts to cultural values associated with the river, as well as fishing and suitability for drinking.

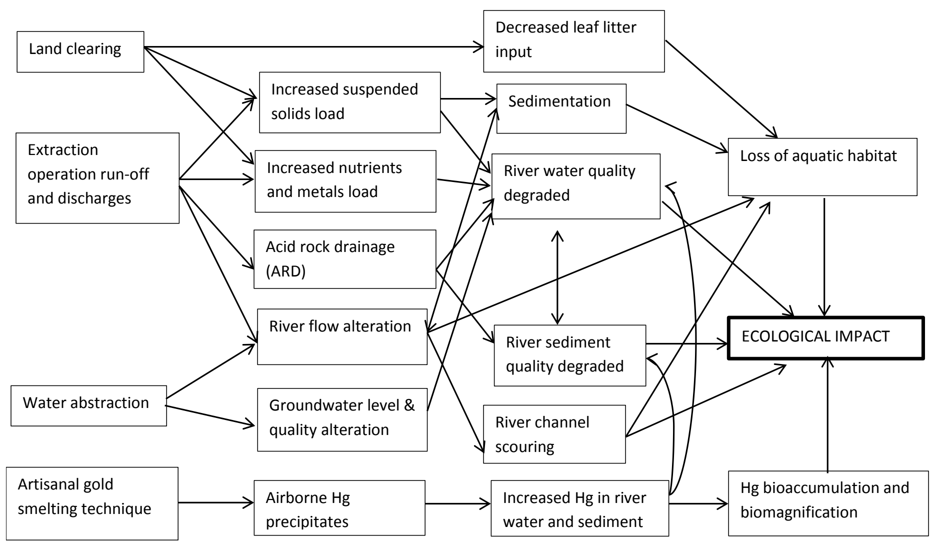


Figure 1 The impacts of artisanal small-scale gold mining (ASGM) on riverine systems (from Macdonald et al., 2014).

The impact of ASGM on tropical rivers has been investigated in Ghana, the Philippines, and Brazil, but the focus of these studies has been on elevated mercury concentrations and mercury cycling as a result of rudimentary processing techniques (Appleton et al., 2006; Bastos et al., 2007; Brabo et al., 2003). These studies were conducted on large river systems such as the Amazon in Brazil (Santos et al., 2000; Telmer et al., 2006b) or the Pra (Donkor et al., 2005)and Ankobra Rivers in Ghana (Akabzaa et al., 2009) which have extensive and long-established ASGM operations with chronic mercury inputs. However, the scale and age of these systems prevents identification of other possible impacts besides mercury contamination. In contrast, ASGM activities in smaller rivers are easier to trace due to acutely concentrated nature of measurable impacts (see Webster et al. 1992). Therefore, this study is different from previously published research because of the focus on a smaller river, with the intention of more clearly defining the suite of impacts from ASGM operations.

The aim of this study was to identify the possible impacts of ASGM operations on water quality in the Surow River, a small tributary of the Tano River in Brong Ahafo, Ghana.

METHODOLOGY

Study site background

The Surow River catchment is in the upper Tano River Basin in the Brong Ahafo region, Ghana, approximately 300 km northwest of the capital city of Accra (Figure 2). Major land uses in the Surow catchment are ASGM and agriculture among tracts of natural forest. Farming activities in the area include cash crop (cocoa), ranching and subsistence farming (vegetables and tubers). The Tano River (400 km long and 15,000 km^2 of catchment) is a major source of potable and domestic water for south west Ghana, and the Surow River is approximately 16 km long with a 3,500 ha. catchment. Located in a wet tropical region, the major rains occur during April to June (average precipitation 294 mm/month) with minor rains from September to November (average precipitation 234 mm/month) and the driest months are from December to February (average precipitation 16 mm/month and evaporation 105 mm/month) (unpublished meteorological report NGGL, 2013). Therefore, rivers in the Tano Basin exhibit classical wet-dry hydrological patterns, driven by rainfall.

ASGM has been practiced in many parts of Ghana for hundreds of years (Donkor et al., 2005). However, operations are relatively new (9 years) to the Surow River catchment. ASGM in this area was started in 2005, following the commencement of a large multi-national gold mining project that discovered gold in the region. During the study period (February 2013 to April 2014), ASGM communities operated along the river at Kenyase I, Kenyase II and Hwidiem townships (Figure 2). At Kenyase I and II, small operators extracted secondary or tertiary alluvial ores easily found in the river banks, while larger operators extracted primary ore mined underground. Ores from these two sites are processed on site as well as sold to other processors mainly scattered near to the river at Hwidiem township. Loose gravel, sands and milled ores are processed via mechanical crushing, elutriation and, in most cases, mercury amalgamation followed by gold smelting and refining (see Macdonald et al. 2014).

Ghanaian legal provisions on mining exclude foreigners and foreign investments in ASGM operations. The sector, nevertheless, received foreign investments at least until March/April 2013 when the Ghanaian government deported as many as 4000 foreigners involved in the industry. As a result, many ASGM operators across the country (including those in Kenyase I and II) ceased most of their operations in May 2013, mostly due to lack of financial support previously provided by foreign investors. Although underground mining activity was substantially reduced, dewatering of existing mine pits continued, especially during the wetter months. Smaller mining operators and processors, comprised of local citizens, continued to operate after the deportation.

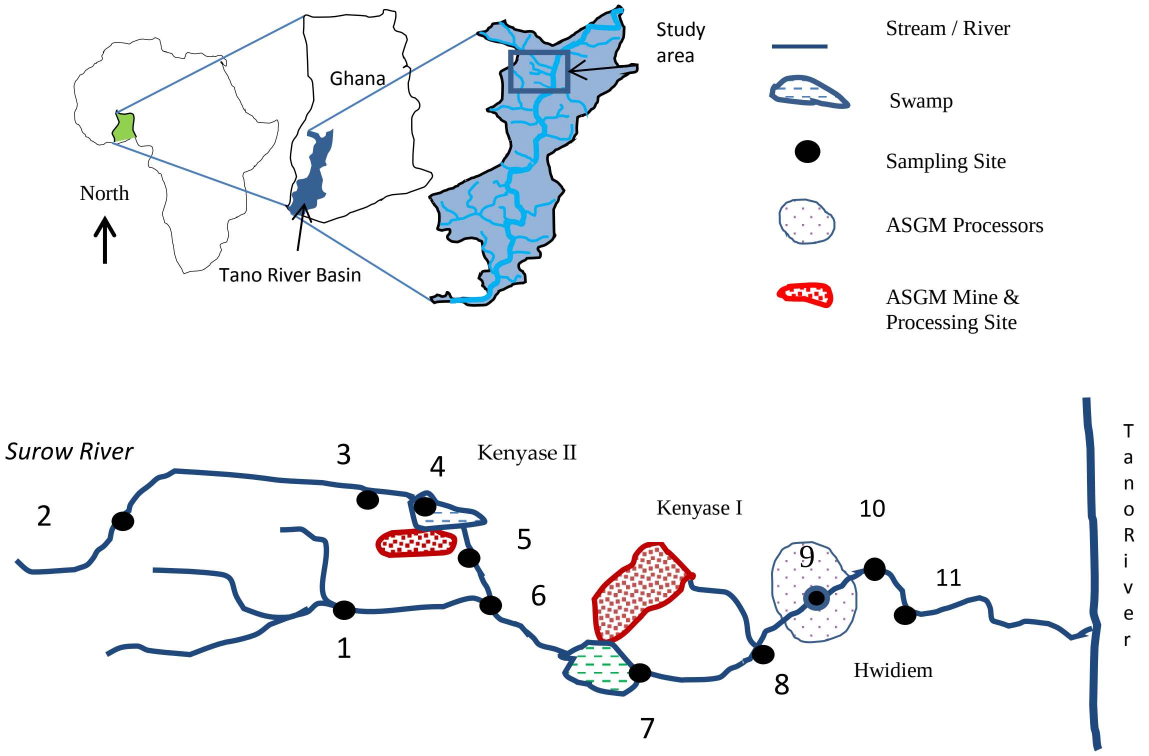


Figure 2. Location of sampling sites (1-11) along the Surow River, Ghana (not to scale).

Sampling program

Eleven sites on the Surow River were sampled monthly for 14 months from February 2013 to April 2014, typically within a 12 h period. Sites were chosen based on access, safety, and representativeness of catchment land use (Table 1). In addition, direct sampling of dewatering water at the Kenyase I ASGM site (sample site 8) was conducted once in April 2014.

Table 1. Hydrology and land use of sites (numbered upstream to downstream) on the Surow River, Ghana (February 2013-April 2014).

Sample site	Dominant site hydrology	Major land use
1	Riffle/run	Minimal use
2	Riffle/run	Minimal use
3	Pool/slow run	Minimal use, rural dwelling
4	Swamp	Mining, processing waste
5	Riffle/run	Minimal use
6	Run	Minimal use
7	Swamp	Mining, processing waste
8	Rifle/Run	Dewatering water

9	Riffle/Run	Processing
10	Riffle/Run	Cattle, cocoa farming
11	Pool/slow run	Rural dwelling

On each sampling occasion, and at each site, water depth and velocity (Marsh-McBirney Flowmeter, USA) were measured. Physico-chemical parameters of pH, oxygen reduction potential, dissolved oxygen (DO), temperature, electrical conductivity (EC), and turbidity were measured *in situ* using a Quanta Multimeter (Hach, USA). Water samples were collected 0.1 m below water surface and immediately divided into unfiltered and filtered (through 0.5 µm GF/C; Pall Ltd Metrigard) aliquots. All samples were stored at <4°C prior to analysis.

Aluminum, As, Cd, Cu, Cr, Mn, Pb, Zn in filtered water was analyzed using inductively coupled plasma mass spectrometry (ICP-MS) following USEPA Method 200.8; Fe and Mg were analyzed using ICP (USEPA Method 200.7), and Hg was quantified using cold vapour atomic absorption (CVAA; USEPA Method 245.1,detection limit of 0.0002 mg/L). Dissolved organic carbon was analyzed following USEPA Method 5310B. On unfiltered samples, total Kjeldahl nitrogen (TKN) was analyzed via block digester method (USEPA M351.2); and total phosphorus was analysed with an auto ascorbic acid method (USEPA M365.1). The above samples were airfreighted to ACZ Laboratory in Colorado, USA.

Analysis of ammonia/ammonium (NH_3-N), nitrate/nitrite (NOx-N) and sulfate (SO_4) on filtered water were performed at the Newmont Ghana Ltd. environmental laboratory at Ahafo using a Hach DR 2800 spectrophotometer following APHA (2005) methods 4500B&C, USEPA Method 375.4; and USEPA Method 365.2 respectively.

Data Analysis

Water quality data was ordinated using principal components analysis (PCA) to illustrate patterns in the data, then compared among sites using permutational ANOVA (PERMANOVA). Data were prepared for ordination and analysis by selecting parameters where more than half of the samples were above detection; values below detection were replaced with half the detection limit, missing data were replaced by the average of any other data for that time and treatment, and auto-correlated parameters were reduced to a single representative parameter. Data were also normalized in Primer v6 prior to ordination and analysis. Significance testing of the multivariate data among sites was undertaken on PERMANOVA, using a two-way, unreplicated design with time (fixed) and site (random) as factors, followed by pairwise comparisons between sites. All analyses were performed on Primer v6 (Primer-E; Clarke and Gorley 2006).

RESULTS AND DISCUSSION

Water quality varied among sites (pseudo-F 2.36, $P<0.01$) and over time (pseudo-F 3.92, $P<0.01$), reflecting stochastic events, seasonal trends, and anthropogenic impacts. A PCA of water quality data, separated by month, illustrates the effect of seasonal trends on the data is presented in Figure 3 (note different axis scales). On most occasions, water quality at the headwater sites (1, 2, 3), and minimal land use sites (5, 6) were closely associated with each other, with the exception of site 6 in September and October 2013. Water quality at sites 1, 2 and 6 were not significantly different to each other, but were different to 5 (Table 2). Water from sites 1, 2 and 3 (headwaters) had low EC (0.11-0.35 mS cm^{-1}), turbidity levels generally below the Ghanaian EPA standard of 75 NTU (except on one occasion at site 2, and six occasions for site 3 where turbidity peaked at 247 NTU), and pH levels between 6.03 to 7.81. Water quality at the headwater sites reflects catchment mineralization (NGGL, 2005), with silicate and carbonate mineral weathering, precipitation, and agricultural activities the most significant processes influencing the water quality in the area (Banoeng-Yakubo et al., 2009; Yidana, 2009).

In March 2013, Hg concentrations at sites 1 and 3 exceeded the Ghanaian drinking water standards of 0.002 mg L^{-1}, reaching 0.003 mg L^{-1} – at no other time or site were standards exceeded. Mean (±SE) Fe concentrations declined downstream from 1.07±0.35 at site 1, to 0.25±0.10 by site 9. The Ghanaian drinking water standard for Fe is 0.3 mg L^{-1} and the EPA standard is 1 mg L^{-1}; essentially, exceedances occurred at headwater sites. Manganese exceeded the Ghanaian drinking water standards (0.05 mg L^{-1}) in 93 out of 130 samples, and the EPA standard (0.1 mg L^{-1}) in 67 samples across all sites and times (Figure 4). Although there were exceedances of both standards at sites 1 and 2, at site 3 every sample exceeded the drinking water standard. Sites 4, 5 and 6 (further downstream) had progressively fewer exceedances of the drinking water standard for Mn. Therefore, Hg, Fe, and Mn concentrations in the Surow River do not appear to be directly related to ASGM operations, instead reflecting local geologies.

Sites 5 and 6 were similar to 1, 2, and 3 on most occasions (Figure 3), even during the period of highest ASGM activity (prior to May 2013) at site 4. At sites 5 and 6, EC ranged between 0.14—0.31 mS cm^{-1}, turbidity was 30—247 NTU and pH was 6.57—7.69, with metal concentrations similar to that of the upstream sites, except for Mn concentrations, which were among the highest of all sites at site 5 ranging between 0.03—3.11 mg L^{-1} (mean 0.8±0.3 mg L^{-1}). The similarity of sites above and below site 4, a site of intense ASGM activity, suggests that the impacts of ASGM, as measured, are highly localized.

Site 4 was separated from headwater sites (1-3) in April 2013 at the height of AGSM operations. Pairwise comparisons between all sites (across all times) show no significant differences between the two main ASGM sites 4 and 7, with 5 similar to 7 but not to 4. The start of the wet season in September 2013 altered the relationship among all sites. Although ASGM activity partially returned to site 4 in April 2014, the impact on overall water quality was not pronounced (as indicated by a lack of separation from other sites; Figure 3), possibly due to the heavy rainfall and high flows at this time. Magnitude and timing of flows appeared to have a variable impact on how different site 4 was from the rest of the data set.

At site 4, accumulation of sediment from processing at Kenyase II turned the defined river channel into a broad swamp. The site had a wider range of water temperatures (23.4-31.6 ^{o}C) than other sites - possibly due to its lack of canopy cover. The site had EC similar to the headwater sites (0.11—0.24 mS cm^{-1}) despite its proximity to an ASGM site, although underground mining activities were not significant during the study period (i.e., highly conductive groundwater was not being discharged into the river) Surface mining and ore processing were the main activities, resulting in high turbidity (peak >2000 NTU; mean 277±141 NTU) and sedimentation at site 4 (Figure 4). The number of exceedances of the Ghanaian EPA standard for turbidity was the same as site 3, although values were lower at site 3. Although the impact of ASGM operations on increasing turbidity are clearly visible before April 2013, after this time turbidity was also being generated at site 3. Higher flows during the wet seasons are naturally high in turbidity, with the sedimentation generated at site 3 washed downstream to site 4, and then carried further downstream to site 7. With the exception of Mn, dissolved metal concentrations were also similar at up- and downstream sites (5 and 6). Mean Mn concentrations at site 4 (0.51±0.24 mg L^{-1}) were higher than at site 3 (0.21±0.03 mg L^{-1}), indicating that ASGM activity was a source of the metal. Further, the only times that Mn concentrations were higher at site 4 than at site 3 was during periods of mining activity (April 2013, 2014, and December 2013). Mn is a hematological toxicant in fish, mammals and human (Crossgrove et al., 2004). Over discharge of manganese into aquatic ecosystems may affect the survival of natural fish population (Agrawal et al., 1980).

The occasional spikes seen in nutrient concentrations at site 7 might be related to surrounding farming activities (cocoa plantation and cattle). Alternatively, in a forested stream, following a disturbance such as deforestation in the catchment, vegetative nutrient uptake is reduced while mineralization of organic matter is accelerated, which can result in elevated concentrations of NOx-N, Ca, Mg, K and Na (Webster et al., 1992). Site 7 is a swamp resulting from the deposition of sediment that came with the run-off from the exposed land, elutriation boxes, unregulated tailing, and waste material disposal at Kenyase I site. The size of ASGM operations at Kenyase I (site 7, 8) was larger than Kenyase II (4) this was also reflected in the relative size of the two swamps and mean turbidity values (Figure 4). River sediment is a sink of many pollutants and a medium for bio-geological processes including methylation of mercury; the quality of river water and habitats can strongly be influenced by quality of sediment (Chon et al., 2012; Kehrig et al., 2003).

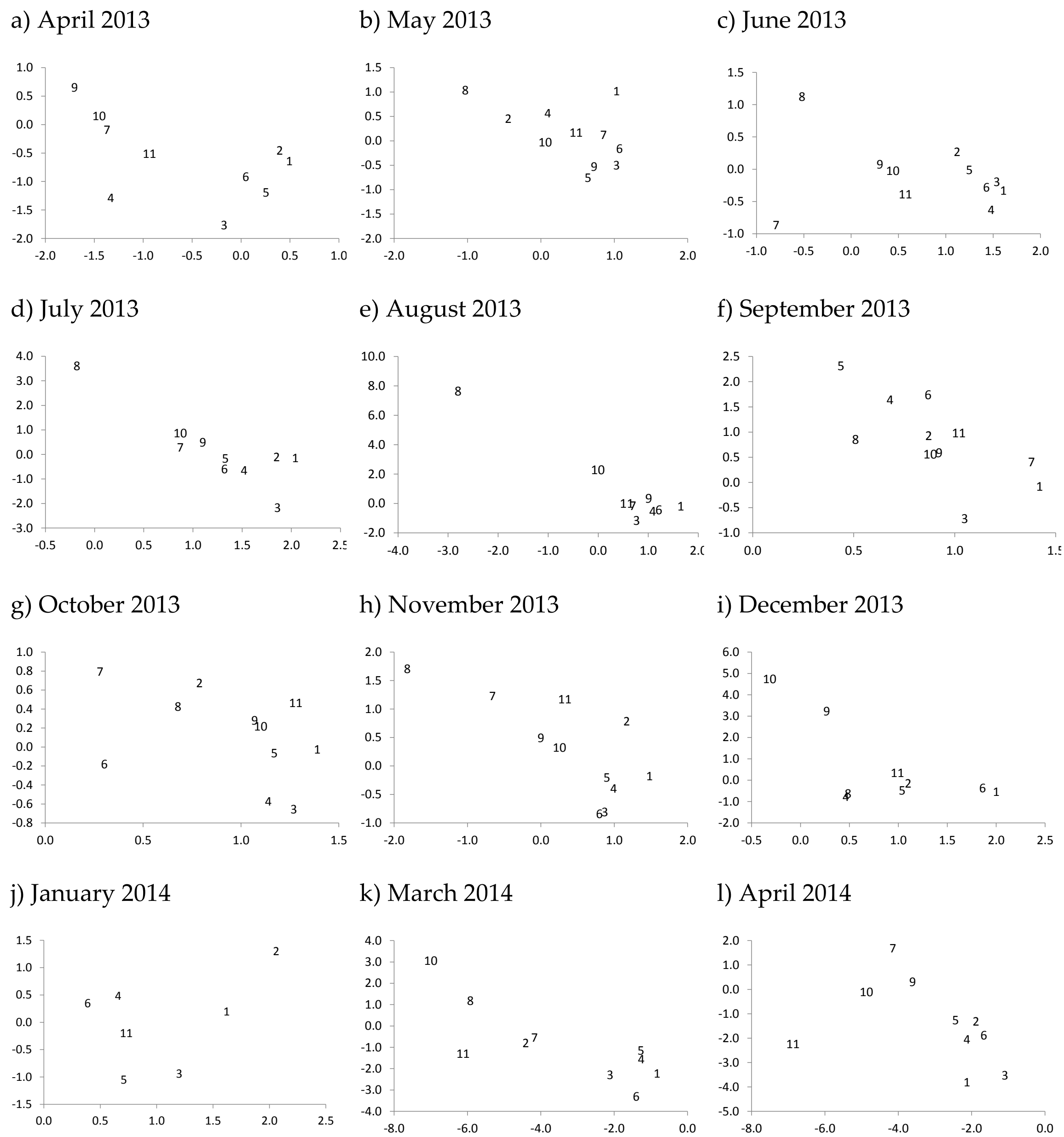
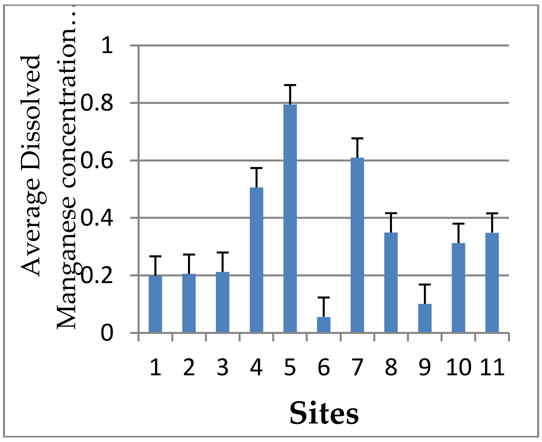


Figure 3. PCA of water quality data per sampling month (a-l) showing each site. Each graph is a subset of a single PCA on all available data. Note different axis scales.

In addition to site 4, sites 7 and 8 were highly impacted by ASGM activities, and tended to separate from other sites, particularly in June, July August, and November 2013 (Figure 3). Site 7 was highly turbid (up to 2000 NTU), particularly in comparison to headwater sites (Figure 4). At site 7, EC ranged from 0.20-0.95 mS cm^{-1}, pH ranged between 6.7—8.2, and occasionally had very high concentrations (and consequently mean concentrations) of NOx-N, TKN, Ca, and P compared to site 6 (site 6 being directly upstream of site 7, and unimpacted by mining). At site 7, metals were similar in concentration to site 6, with the exception of Mg which between December 2013 and April 2014 has concentrations at least an order of magnitude higher than site 6. There was a marginal increase in Mg seen at site 4 during April 2013.

a) Manganese



b) Turbidity

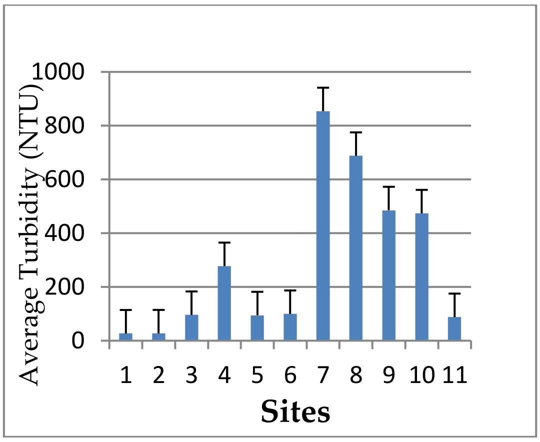


Figure 4 Mean (+SE) of a) manganese and b) turbidity at sites in the Surow River between February 2013 and April 2014. Sites are ordered upstream (1) to downstream (11).

Table 2. Significance of pairwise comparisons between sites from PERMANOVA (ns = P>0.05, t<1.3; s= P<0.05, t>1.3). See Table 1 for site details.

	2	3	4	5	6	7	8	9	10	11
1	ns	s	s	s	ns	s	s	s	s	s
2	-	s	s	s	ns	s	s	s	ns	ns
3	-	-	s	s	s	s	s	s	s	s
4	-	-	-	s	s	ns	s	s	s	ns
5	-	-	-	-	s	ns	s	s	s	ns
6	-	-	-	-	-	s	s	s	s	ns
7	-	-	-	-	-	-	s	s	ns	ns
8	-	-	-	-	-	-	-	s	ns	s
9	-	-	-	-	-	-	-	-	s	ns
10	-	-	-	-	-	-	-	-	-	ns

Site 8 was significantly different to all other sites except 10 (Table 2), and was characterized by high EC (0.58±0.08 mS cm^{-1}; peak 1.02 mS cm^{-1} in April 2013) for the duration of the study, particularly during the height of ASGM operations (February-April 2013). Five out of 13 times EC exceeded the Ghanaian drinking water standard (0.5 mS cm^{-1}) but not the EPA standard of 1.5 mS cm^{-1}. Site 8 received dewatering water from the underground mines at Kenyase I via a drain. Dewatering water at the Kenyase I mine was sampled directly in April 2014 and had an EC of 1.3 mS cm^{-1}, suggesting this as the most likely source of the high EC at site 8. Calcium, Mg and Zn concentrations were generally similar to site 7; and higher than sites 1 to 6, particularly during the dry seasons (although the reason for this pattern was unclear). Site 8 had a pH between 6.7—8.5

(the highest recorded pH at any site during the study); turbidity was high between 19—2000 NTU, with the low turbidities occurring during the wet season when discharge of a large amount of dewatering water occurred.

Sulfate concentrations were substantially higher at site 8 than at all other sites (108.3±46.4 mg L^{-1} compared to <13 mg L^{-1} at sites 1—6), except for in December 2013 when sulfate at site 7 was 281 mg L^{-1}. Sulfate concentrations exceeded the drinking water standard of 250 mg L^{-1} on four occasions. Dewatering water appears to be source of the sulfate obtained from the mineralization of the ores in the area, which mostly contain sulfide composites (NGGL, 2005) typical of the Sefwi belt of the Birimian host rocks, the main source of gold and diamonds that extends across Ghana (Akabzaa et al., 2009). Similarities between sites 7 and 8 were not observed in May, July and August 2013, where site 7 had water quality similar to the headwater sites, possibly reflecting the downturn in ASGM activity at this time.

Site 9 is surrounded by ASGM processors, but no mining activities, and water quality at site 9 was significantly different from all other sites except site 11 (Table 2). Although the high turbidity recorded at sites 7 and 8 also occurred at site 9, values at site 9 were generally lower (Figure 4). The high EC at site 8 did not persist at site 9, and concentration of most metals at site 9 were lower than at sites 7 and 8 –below the Ghanaian drinking water standard with exceptions of Fe (2 occasions) and Mn (8 occasions). Broadly, water quality at sites 9, 10 and 11 tended to be similar (Figure 3, Table 2), with the exception of after December 2013 where 11 separated from 9 and 10 (although water quality overall at site 11 was not significantly different to sites 9 and 10; Table 2). Although overall water quality at sites 9, 10 and 11 tended to be significantly different to upstream sites (Table 2), this distinction was not reflected in the PCA ordinations for May, June, October and November 2013, possibly due to increased hydrological connectivity during the times of highest rainfall. Water quality at site 11 was only significantly different to sites 1, 3 (headwaters) and 8 (ASGM dewatering), likely because as the most downstream site, site 11 represents the cumulative impacts of land use within the catchment.

CONCLUSIONS

Previously, we identified a range of possible environmental impacts of ASGM on riverine systems. In the Surow River, Ghana, ASGM activities increased sedimentation, altered river morphology, and elevated Mn concentrations. As evidenced by high pH across all sites and times, we did not observe acid mine drainage, although the mineralogy of the area made it unlikely because it contains low levels of pyrite. Mercury was only detected in headwater sites, presumably from natural rock sources. Dewatering water discharges were found to substantially increase EC in the river, although, as with most observed parameters, impacts were local. During the wet seasons, we observed that higher flows in the river tended to reduce the differences between sites. Overall, water quality in the river at many sites did not meet the standards set for the environment by the Ghanaian EPA and for drinking water. Future work will investigate the effect of ASGM activities on river ecology.

ACKNOWLEDGEMENTS

The authors wish to acknowledge support and assistance received from various institutions and individuals, including but not limited to: (1) Mr Anthony Loh and Ms Rita Lebene-Tibu of Newmont Ghana Gold Limited (NGGL) at Ahafo for their assistance in airfreighting the samples to be analysed at ACZ in the USA, (2) the NGGL Environment Laboratory staff at Ahafo, (3) Mr. Mumin Zakaria and Mr. Yakubu Ibrahim of Hwidiem, Ghana, (4) Nana Osuodumgya Berima Apiedwaa, Chief of Hwidiem Town (5) Dr Clint McCullough of Golder Associates (Perth) for his contributions to project design, and (6) Kenyasi I Galamsey (ASGM) Committee, Brong Ahafo, Ghana.

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Appendix 3 Variables and score values for riparian health survey

Variables	**Left Bank**	**Rigth Bank**
Canopy cover - trees & tall shrubs >5m height		
<5%	1	1
5-25%	2	2
25-50%	3	3
50-75%	4	4
75-100%	5	5
Canopy Health		
Canopy very sparse / non existent	1	1
Canopy sparse; crown dieback, dead trees common	2	2
Canopy lacking vigour; some dead trees, minor crown dieback	3	3
Canopy slightly irregular and / or with some gaps, no / few dead trees	4	4
Canopy appears intact, no/few dead standing trees	5	5
Understory cover (%). <1.5 m shrubs, sedges, herbs, groundcovers (not grass). Natives & weeds. 5x5 m square		
<5%	1	1
5-30%	3	3
30-100%	5	5
Grass cover - % cover of grass of any height (native and weeds). 5x5 m square		
<5%	1	1
5-30%	2	2
30-60%	3	3
60-80%	4	4
80-100%	5	5
Exposed soil - % cover of exposed soil and ash. Exlude large natural rock formations, boulders, leaf litter and roots. 5x5 m square		
<5%	5	5
5-30%	4	4
30-60%	3	3
60-80%	2	2
80-100%	1	1

Sediment size - dominant on bank		
Clay or silt (0.064 mm)	5	5
Sand (0.064 - 2 mm)	4	4
Gravel (2 - 12 mm)	3	3
Pebbles (12 - 64 mm)	2	2
Cobbles, boulders, bedrock	1	1
Bank slope		
> 70% (or undercut)	5	5
45 - 70% slope	3	3
<45% slope	1	1
Undercutting along 100 transect - combined width		
Undercutting absent	5	5
<5 m	4	4
5 - 10 m	3	3
10 - 20 m	2	2
20 -100 m	1	1
Slumping along 100 m transect		
Slump absent	5	5
<5 m	4	4
5 - 10 m	3	3
10 - 20 m	2	2
20 -100 m	1	1
Gullying along 100 m transect - combined width		
Gullies absent	5	5
<5 m	4	4
5 - 10 m	3	3
10 - 20 m	2	2
20 -100 m	1	1
Animals. All (managed & unmanaged) animal impact. Extent of damage (tree ring barking, trampling, track, grazing)		
0-5 ground damaged	1	1
5-20% ground damaged	3	3
20 - 100% ground damaged	5	5

Appendix 4 Water Physico-chemistry measurement: In situ V.S Grab Sampling

In this study, river water's physico-chemical parameters of pH, oxygen reduction potential (ORP), dissolved oxygen (DO), temperature, electrical conductivity (EC), and turbidity were measured *in situ* using a Quanta Hydrolab (Hach, USA). During extreme weather conditions (river dried up or flooded) or when safety considerations did not permit, grab sampling using a 1 L beaker was used instead of in situ measurement. I performed a small pilot study in early February 2013 to ensure similarity and compatibility of results from the two techniques. In the pilot study, I took physico-chemical characteristics measurement of water samples from 11 randomly selected sites along a stream discharging into the Subri River, Ahafo, Ghana *in situ*. At the same time with each measurement, I took one litre water sample from the same sampling point using a clean 1 L glass beaker and its physicochemical parameters were immediately measured in the beaker using the same probe.

Results (Table A) were subjected to PERMANOVA analysis within the Primer v6 package which showed no statistical difference between *in situ* measurements and grab sample measurements (PERMANOVA $p = 0.978$, $F=0.135$). ANOVA on each variable (Table B) also confirmed the indifference between in situ measurements and grab sample measurements as presented in the following Table.

Table A Water physico-chemical characteristics at 11 sites measured in situ and in 1 L glass beaker (grab sample) using the Quanta Hydrolab (Hach, USA)

Sample	Temp (Celcius)		Conductivity (mS/cm)		Dissolved O (mg/L)		Dissolved O (%)		pH		ORP (mV)		TDS (g/L)		Turbidity (NTU)	
	Insitu	Grab	Insitu	Grab	Insitu	Grab	Insitu	Grab	Insitu	Grab	Insitu	Grab	Insitu	Grab	Insitu	Grab
1	31.56	31.8	0.482	0.48	4.3	4.64	56	58.1	6.68	7.03	172	164	0.3	0.3	75	66.4
2	29.56	29.77	0.439	0.44	7.06	8.18	81.1	96.5	6.9	7.45	225	208	0.3	0.3	25.9	24.4
3	25.78	25.97	0.258	0.259	2.24	2.29	16.9	17.4	6.13	6.5	87	54	0.2	0.2	77.9	77.6
4	37.34	36.88	0.36	0.363	4.28	4.1	59	54.2	6.02	6.42	123	124	0.2	0.2	138	123
5	23.94	24.4	0.173	0.157	7.33	7.36	81.9	81.4	9.65	9.61	46	36	0.08	0.08	152	121
6	29.56	27.77	0.094	0.071	8.69	8.12	108.4	100	8.45	8.6	86	68	0.05	0.04	59.99	36.3
7	32.23	30.77	0.255	0.253	7.03	6.78	85.4	76.2	6.69	7.18	100	88	0.12	0.12	59.99	44.5
8	23.81	24.05	0.399	0.4	4.75	4.84	53.4	58.1	7.34	7.15	209	216	0.3	0.3	599	440
9	25.11	25.03	0.926	0.933	8.64	8.42	103.2	102.5	8.25	8.27	173	171	0.6	0.6	2000	2000
10	24.91	24.75	0.879	0.879	5.21	5.14	60.3	56.2	6.8	7.68	153	137	0.6	0.6	518	484
11	24.5	24.18	0.699	0.697	1.55	1.11	17.1	9.7	7.16	7.36	133	125	0.4	0.4	536	558

Table B. Results of ANOVA between readings from in situ measurements and grab sample measurements distinguishing the indifference between insitu measurements and grab sample measurement.

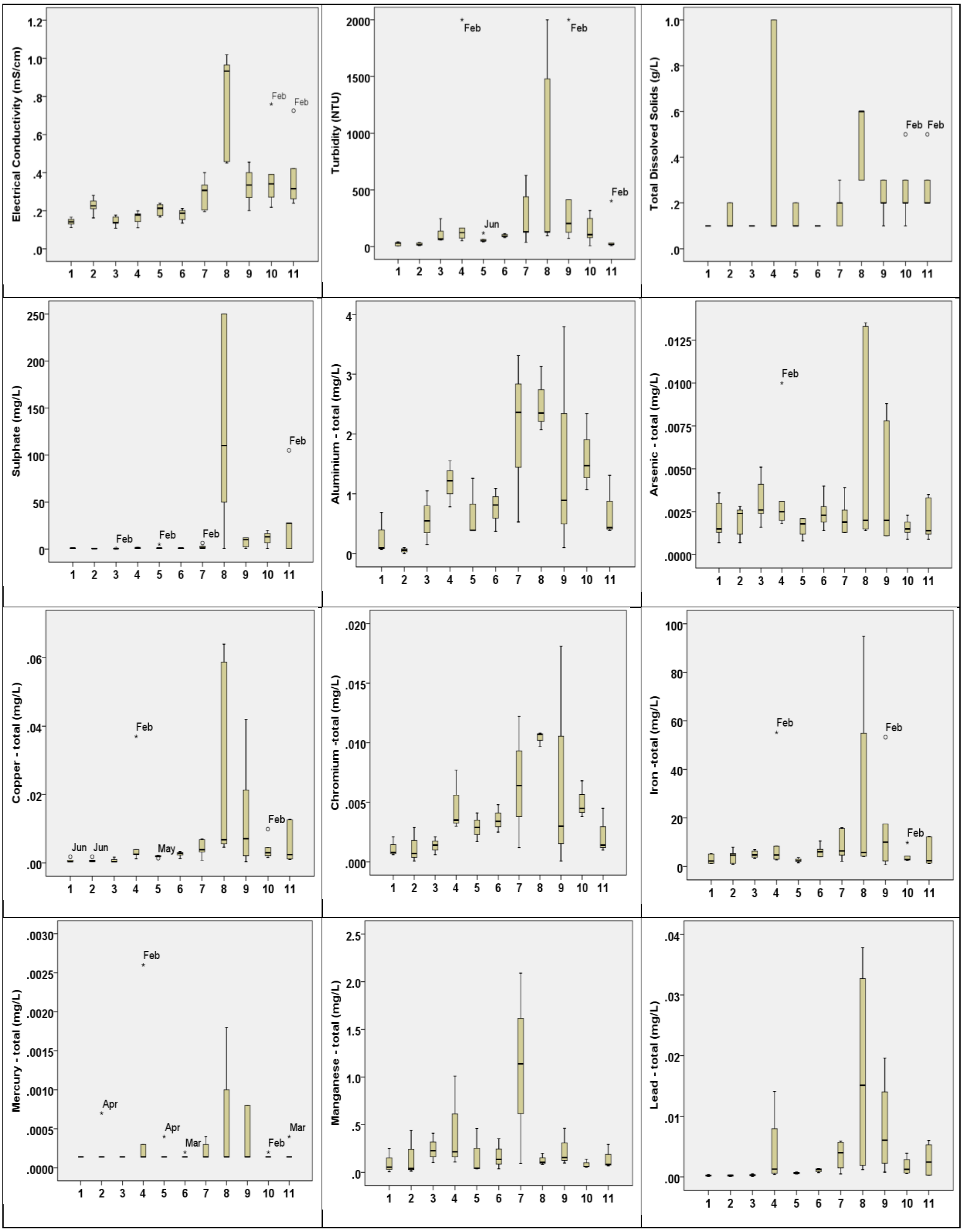
		Sum of Squares	df	Mean Square	F	Sig.
T	Between Groups	0.39	1	0.39	.022	.885
	Within Groups	361.96	20	18.10		
	Total	362.35	21			
EC	Between Groups	0.00	1	0.00	.001	.981
	Within Groups	1.56	20	0.08		
	Total	1.56	21			
DO	Between Groups	6.99	1	6.99	.007	.932
	Within Groups	18809.10	20	940.46		
	Total	18816.09	21			
pH	Between Groups	0.46	1	0.46	.442	.514
	Within Groups	20.81	20	1.04		
	Total	21.27	21			
ORP	Between Groups	611.64	1	611.64	.182	.674
	Within Groups	67176.73	20	3358.84		
	Total	67788.36	21			
TDS	Between Groups	0.00	1	0.00	.000	.991
	Within Groups	0.71	20	0.04		
	Total	0.71	21			
Turb	Between Groups	3230.22	1	3230.22	.010	.923
	Within Groups	6687180.06	20	334359.00		
	Total	6690410.28	21			

Appendix 5 Water quality statistics of the Surow River between February 2013 and April 2014

	N	Minimum	Maximum	Mean	
				Statistic	Std. Error
T (oC)	153	21.48	31.78	25.2175	0.1488
DO (mg/L)	151	0.55	132	4.6759	0.8698
EC (mS/cm)	152	0.108	2.08	0.2878	0.0186
pH	153	6.03	8.63	7.1403	0.0397
TDS (mg/L)	153	0.06	1	0.1865	0.0127
Turbidity (NTU)	153	5.3	2000	290.5503	45.1273
Sulfate (mg/L)	152	0.5	630	24.7299	6.7176
NOx (mg/L)	153	0.01	4.1	0.4988	0.0540
Al - total (mg/L)	33	0.00007	3.79	1.0965	0.1769
As - total (mg/L)	55	0.0007	0.0135	0.0029	0.0004
Cd - total (mg/L)	55	0.00007	0.0007	0.0001	0.0000
Cr -total (mg/L)	33	0.00007	0.0181	0.0042	0.0007
Cu - total (mg/L)	55	0.00035	0.064	0.0067	0.0018
Fe -total (mg/L)	55	0.67	94.9	9.4965	2.2565
Hg - total (mg/L)	55	0.00014	0.0026	0.0003	0.0001
Mn - total (mg/L)	33	0.0079	2.09	0.2754	0.0724
Pb - total (mg/L)	44	0.00007	0.0378	0.0037	0.0011
Zn - total (mg/L)	33	0.00141	0.012	0.0040	0.0004
Al - dissolved (mg/L)	131	0.0021	0.664	0.0432	0.0066
As - dissolved (mg/L)	152	0.00005	0.005	0.0010	0.0001
Cd (mg/L)	153	0.00007	0.04	0.0003	0.0003
Cr (mg/L)	153	0.00035	0.0018	0.0004	0.0000
Cu (mg/L)	143	0.0002	0.0134	0.0019	0.0002
Fe (mg/L)	153	0.00035	3.91	0.5360	0.0534
Hg (mg/L)	153	0.00014	0.003	0.0003	0.0000
Mg (mg/L)	131	0.0072	3.26	0.3377	0.0542
Mn (mg/L)	98	1.4	79.7	8.0449	1.0488
Pb (mg/L)	142	0.00005	0.05	0.0006	0.0004
Zn (mg/L)	120	0.001	0.095	0.0237	0.0023

Appendix 6 Water quality variables at 11 sites on the Surow (numbered 1 to 11 on the horizontal axis) between February and June 2013.

Outliers are indicated along with the time of sampling. Elevated EC, turbidity, pH, sulfate, total arsenic, copper, total iron and lead were recorded at site 8 where the River received ASGM discharges of mine water. Total mercury was only detected at ASGM sites (4, 7, 8, 9) while dissolved mercury was detected in upstream sites with extreme values recorded in March 2013 when ASGM was active.



Appendix 7 Principal Component Analysis of water quality February – June 2013

Twentytwo water quality variables from February to June 2013 data set were analysed for the principal component analysis (PCA). Hierarchical agglomerative cluster analysis on log transformed and normalised data was used to measure similarity between variables and factors by means of the complete linkage using the Euclidian distance (Vega et al., 1998). The distance matrix was then used to form the correlation based Principal Component (PC) ordination, resulting in 22 principal components (PCs) (Clarke et al., 2006).

The 22 principal components (PCs or axis) and their contributions to the total variation expressed as Eigenvalues are given in the table A below. The first 6 PCs contributed 74.85% of the total variance. A scree plot of the eigenvalues, however, shows that after the 5th axis, the curve slope does not change much, indicating declining contributions of the following axis to the total variations. Based on this finding, the first 5 PCs were retained for factor analysis. The PC values were then plotted against original variables. The correlations (Spearman's) between each PC and original variables, termed loadings, which correspond with contributions of variables to the variance (Liu et al., 2003; Vega et al., 1998) are given in Table B.

Table A. Variation explained by individual axis

Axis (PC)	Eigenvalue	Individual%	Cumulative%
1	388.06	32.67	32.67
2	164.4	13.84	46.5
3	107.58	9.06	55.56
4	89.652	7.55	63.11
5	70.249	5.91	69.02
6	69.309	5.83	74.85
7	55.538	4.67	79.53
8	46.45	3.91	83.44
9	37.727	3.18	86.61
10	33.839	2.85	89.46
11	27.113	2.28	91.74
12	24.182	2.04	93.78
13	18.671	1.57	95.35
14	17.31	1.46	96.81
15	12.339	1.04	97.85
16	7.7016	0.65	98.49
17	4.7741	0.4	98.9
18	4.6058	0.39	99.28
19	4.1576	0.35	99.63
20	2.2423	0.19	99.82
21	1.7607	0.15	99.97
22	0.34215	0.03	100

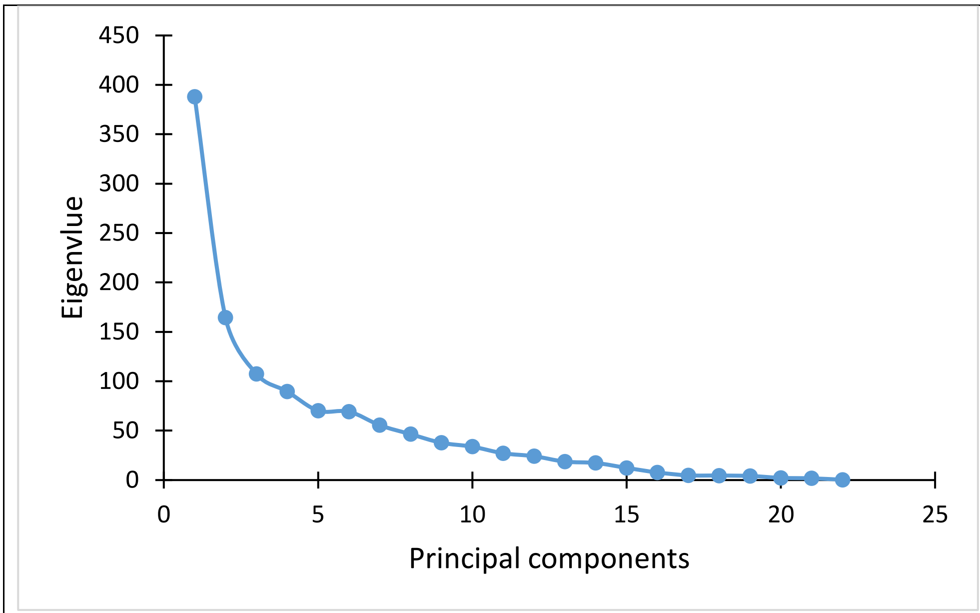


Figure A. Scree plot of the Eigenvalues of each principal component (axes)

Table B. Loadings of 22 experimental variables on five significant principal components for February to June 2013 water quality data. Significant loadings (Spearman's r>│0.4│) are highlighted, indicating significant contributions of variables to the corresponding PCs.

	PC1	PC2	PC3	PC4	PC5
T	.159	**.444****	-.133	.112	-.280*
EC	**.661****	.018	**.451****	**.483****	.137
DO	**.596****	**-.412****	.323*	-.076	-.270*
PH	**.559****	**-.539****	**.553****	.195	-.267*
TDS	**.713****	-.080	.308*	.313*	**.405****
ORP	**.593****	.126	.047	**.493****	**.506****
Turbidity	**.767****	-.050	.053	-.107	.025
TSS	**.819****	-.075	.091	-.008	-.089
As_Total	**.403****	**.751****	**-.490****	.026	.047
Cd_Total	**.437****	-.036	-.291*	.083	-.033
Cu_Total	**.862****	-.184	.231	.122	-.029
Fe_Total	**.615****	**.475****	-.305*	.004	.080
Hg_Total	**.500****	.332*	-.276*	-.085	-.142
As_Diss	-.046	**.875****	**-.403****	.062	.105
Cd_Diss	-.200	-.078	-.306*	.024	.040
Cr_Diss	-.232	**-.420****	.075	.064	-.318*
Cu_Diss	.024	**-.641****	.226	.097	-.187

Fe_Diss	**-.602****	-.190	-.292*	-.787**	.026
Hg_Diss	.057	**.656****	-.210	.427**	-.092
NOx	-.003	**.430****	.172	.308*	**-.428****
FRP	-.207	.368**	-.320*	.075	-.107
Sulfate	**.594****	.209	.349**	**.501****	.339*

Liu et al. (2003) classified loadings into strong loading (>0.75), medium loading (0.5 – 0.75), and weak loading (0.3-0.5). In this study, following (Vega et al., 1998) we took a cut off value of minimum 0.4 loading in assessing the variables. As can be seen in Table B, PC1 is highly participated by all physicochemical parameters but temperature, all total metal concentrations, dissolved Fe and sulfate, making it rather difficult to clearly interpret the nature of water quality and sources of pollution. While EC, TSS, Turbidity, TSS, sulfate and total metal concentrations may be related to anthropogenic impacts, in this case ASGM, DO, ORP and total metal concentrations may also be resulted from natural variability in the system. Similarly, PC2 is highly participated by variables known as of anthropogenic nature including NOx and dissolved Hg as well as natural variables such as temperature, DO, pH and some total and dissolved metals. PC3, however, is highly participated by non-organic variables (EC, pH and As), while PC4 and PC5 are highly participated by organic compounds known to be related to anthropogenic activities (Nitrate and sulfate) as well as EC, TDS and ORP. The factor analysis, however, clearly showed that FRP and dissolved Cd did not participate in the variance, while Temperature, total Cd and dissolved Cr each participate weakly in one of the PCs.

Appendix 8 SIMPER analysis on water quality at control and impact and before and after

Variable	Average values		Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
	Group Control	Group Impact				
pH	-1.02	0.384	3.16	0.95	8.47	8.47
FeD	0.72	-0.27	3.05	0.71	8.18	16.65
HgD	0.351	-0.131	2.87	0.53	7.7	24.35
DO	-0.806	0.302	2.77	0.97	7.43	31.77
AsD	0.471	-0.177	2.76	0.74	7.41	39.18
Turbidit	-0.694	0.26	2.33	0.73	6.25	45.43
CuD	-0.159	5.96E-02	2.17	0.36	5.81	51.24
TSS	-0.683	0.256	2.15	0.59	5.78	57.02
NO_X	-0.163	6.13E-02	1.99	0.82	5.35	62.37
T	-4.81E-02	1.80E-02	1.98	0.68	5.31	67.67
EC	-0.625	0.235	1.94	0.52	5.2	72.87
Sulfate	-0.603	0.226	1.88	0.66	5.03	77.91
TDS	-0.58	0.218	1.84	0.48	4.93	82.84
FeT	-0.374	0.14	1.75	0.56	4.69	87.53
CuT	-0.448	0.168	1.63	0.34	4.36	91.9

Appendix 9 SIMPER analysis on water quality before and after cessation of ASGM

Variable	Average values		Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
	Group Before	Group After				
AsD	0.544	-0.816	2.82	0.8	8.04	8.04
FeT	0.447	-0.67	2.45	0.65	6.97	15.01
NO_X	0.237	-0.356	2.26	0.93	6.44	21.45
Sulfate	0.316	-0.474	2.18	0.71	6.22	27.67
AsT	0.37	-0.555	2.16	0.44	6.15	33.82
T	0.317	-0.476	2.04	0.77	5.83	39.65
EC	0.291	-0.437	2.04	0.54	5.82	45.46
TDS	0.172	-0.257	2.02	0.51	5.74	51.21
DO	-6.10E-02	9.15E-02	2	0.79	5.71	56.91
HgD	0.303	-0.454	1.98	0.46	5.64	62.56
CuD	-0.14	0.21	1.97	0.35	5.61	68.17
Turbidit	0.204	-0.305	1.96	0.68	5.57	73.74
TSS	0.214	-0.321	1.88	0.59	5.37	79.11
PH	-0.18	0.27	1.88	0.84	5.36	84.47
CuT	0.231	-0.346	1.84	0.37	5.24	89.71
HgT	0.23	-0.344	1.83	0.3	5.23	94.94

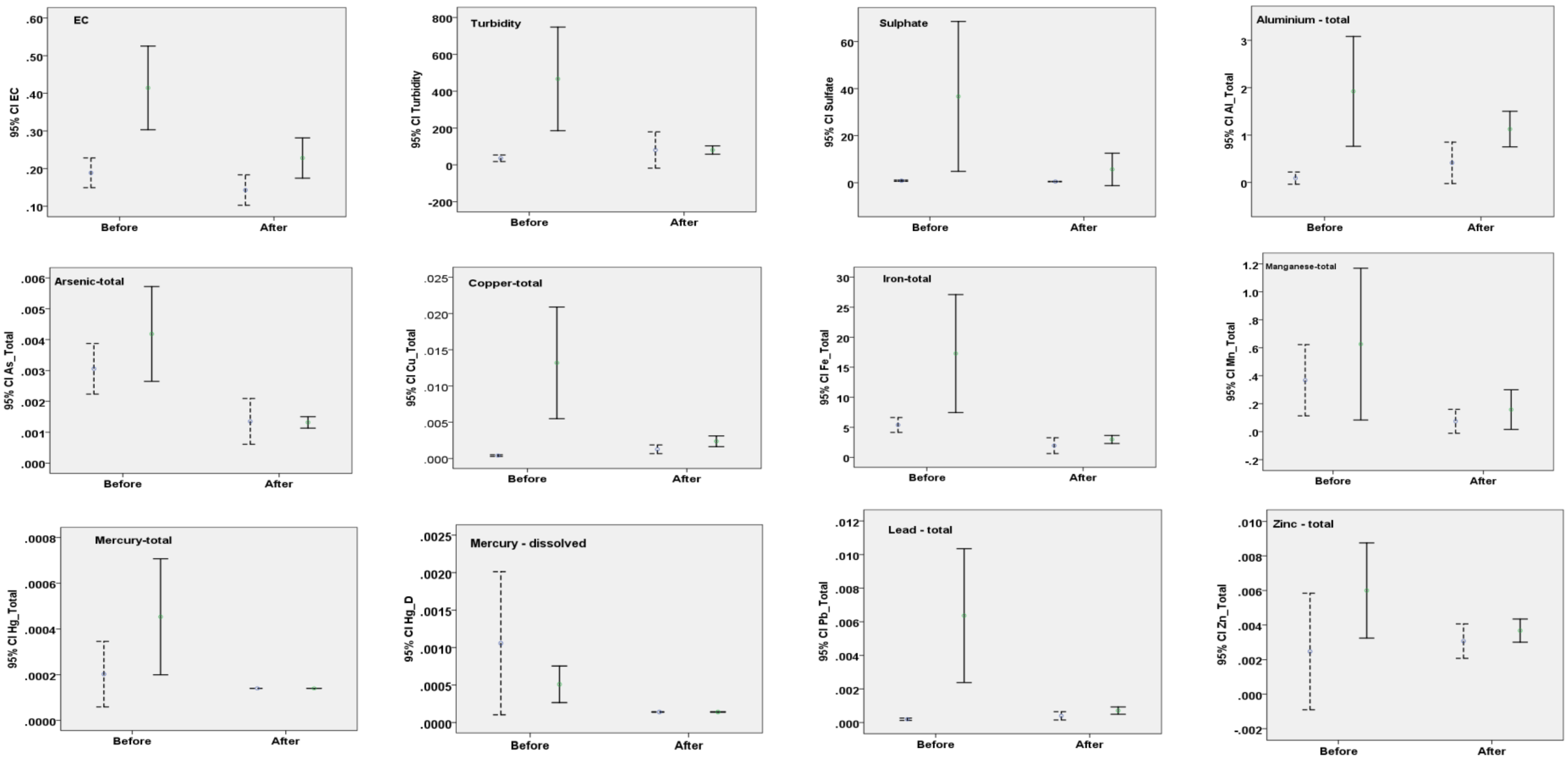
Appendix 10 Two-way PERMANOVA (A) and Pairwise PERMANOVA (B) of water quality to distinguish effect of before and after ASGM cessation (BA) on river water quality at control, medium and high impacted sites
** denotes significance at <0.01 *denotes significance at <0.05

Source	df	SS	MS	Pseudo-F	P(perm)	Unique permutation
Before/After (BA)	1	109.43	109.43	9.0294	0.0001**	9941
Level of impact (Le)	2	126.39	63.196	5.2146	0.0001**	9924
BAxLe	2	49.771	24.885	2.0534	0.028*	9910
Residual	49	593.83	12.119			
Total	54	918				

Level of factor	Groups	t	P(perm)	unique permutation
Control	Before, After	2.2203	0.0005**	4283
Medium	Before, After	1.9299	0.0007**	9537
High	Before, After	2.1601	0.0037**	9532
Before	Control, High	2.9378	0.0001**	9783
	Control, Medium	2.4609	0.0002**	9761
	High, Medium	2.0167	0.0047**	9877
After	Control, High	1.3894	0.0506	2891
	Control, Medium	0.86304	0.653	2881
	High, Medium	0.99166	0.4183	5092

Appendix 11 Error bars representing 95% confidence interval in each water quality variable at control and impact sites to distinguish effect of ASGM cessation (before and after).

Dashed lines represent error values at control and dark lines at impact. The error bars depict how the cessation has improved water quality by reducing EC, turbidity, concentrations of sulfate, total almunium, arsenic, copper, iron, manganese and mercury at impact sites. However, before the cessation, variability in each variable within impact was high due to variability in levels and types of impacts between sites. The cessation did not only lower the levels of impact, but also reducing variabilities within impact. This may also explain the insignificant interaction between before/after and control/impact factors in these variables (Two-way ANOVA) as depicted by the overlapping error bars in the figure bellow.



Appendix 12 Consentrations of elements (mg/kg) in riverine sediment from 11 sampling sites on the Surow River before the cessation of ASGM (Februray 2013) and after the cessation (April 2014)

Site	Ba		Ca		Co		Cr		Cu		Fe		Pb		Mg		Mn		P		K		Sr		S		V		Zn		Hg	
	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14	Feb 13	Apr 14
1	21	1.4	300	520	1.6	0.2	120	4.3	9.6	11	39000	1300	7	6	83	9	90	4.7	330	130	67	7.07	5.2	13	81	17	120	82	8.8	220	0.04	0.04
2	36	1.5	660	500	2.2	0.2	64	4.5	8.2	13	24000	2000	7	14	210	9	77	5.4	210	41	120	7.07	11	16	120	7.07	41	85	15	18	0.04	0.04
3	25	2	660	240	3.6	0.2	130	2.7	13	20	32000	1100	9	10	170	12	140	14	150	57	110	7.07	7.9	16	93	7.07	94	68	17	31	0.2	0.04
4	69	3.7	1200	360	8.1	1.0	29	5.7	17	54	11000	2400	6	19	850	54	310	38	160	77	190	18	24	27	150	7.07	28	140	13	28	0.7	0.1
5	59	3.2	670	240	6.3	0.5	15	1.3	9.3	16	9200	840	6	7	280	19	350	25	170	58	140	7.07	13	17	150	7.07	19	38	16	39	0.4	0.04
6		4.4		170		0.6		9.8		2200		650		7		9		61		73		7.07		18		7.07		23		23		0.04
7	310	6.5	2100	270	27	0.8	87	2.2	31	21	48000	1200	29	17	820	19	1200	35	240	61	540	20	51	28	200	7.07	120	67	18	15	0.9	0.04
8	120	9.8	2300	370	11	1.5	48	1.9	18	24	15000	1300	7	17	1300	33	510	170	250	100	250	18	47	48	400	17	36	71	16	22	0.3	0.04
9	160	4.5	2300	300	23	0.9	80	7.7	22	21	31000	1700	18	15	840	28	850	40	380	60	330	11	42	25	670	7.07	77	79	36	36	0.5	0.04
10	82	1.4	1200	70	11	0.2	43	0.5	20	4.4	17000	160	8	3	1000	8	440	5.9	180	17	230	7.07	29	9	140	7.07	42	10	16	6.8	0.2	0.04
11	87	6.4	920	400	13	0.8	190	5.1	17	28	24000	1500	18	17	480	28	250	20	260	53	240	20	19	37	360	21	69	86	15	30	0.1	0.04

Appendix 13 SIMPER analysis of riverine sediment quality to distinguish variables contributing to the difference between before and after cessation of ASGM (A) and between control and impact (B)

Table A. SIMPER analysis on sediment quality to distinguished

Variable	Average value		Average Squared Distance	Sq.Dist/SD	Contribution %	Cumulative %
	Before	After				
Mg	1.08	-0.853	4.14	1.74	7.93	7.93
S	1.19	-0.75	3.96	2.26	7.58	15.51
K	1.18	-0.73	3.9	2.13	7.46	22.97
Co	1.09	-0.711	3.75	1.52	7.18	30.15
Ba	1.15	-0.688	3.74	1.65	7.15	37.3
Al	1.16	-0.68	3.68	1.64	7.04	44.34
Fe	1.14	-0.619	3.43	1.35	6.57	50.91
Mn	1	-0.595	3.35	1.08	6.41	57.32
Hg	0.838	-0.561	3.35	0.85	6.4	63.72
Cr	1.13	-0.573	3.32	1.36	6.36	70.08
Ca	0.976	-0.518	3.19	0.87	6.11	76.19
P	0.962	-0.643	3.16	0.99	6.05	82.24
Cu	-0.316	0.171	2.04	0.47	3.9	86.14
Zn	-0.483	0.247	1.89	0.62	3.62	89.77
Sr	-0.156	-1.17E-02	1.87	0.8	3.57	93.34

Table B. SIMPER analysis of riverine sediment quality to distinguish variables contributing to the significant difference between control and impact

Variable	Average value		Average Square Distance	Sq.Dist/SD	Contribution %	Cumulative %
	Control	Impact				
Sr	-1.64	0.48	5.26	1.39	26.81	26.81
Hg	-0.33	1.34	4.06	0.99	20.71	47.51
Ca	0.22	1.3	1.56	1.03	7.98	55.49
V	0.51	-0.178	1.54	0.82	7.84	63.33
Co	0.287	1.44	1.47	1.6	7.49	70.82
Pb	-0.572	-2.88E-03	1.28	0.79	6.53	77.35
Mn	0.45	1.24	0.692	1.52	3.53	80.88
Mg	0.545	1.31	0.68	1.43	3.47	84.35
Cu	-0.792	-0.112	0.658	1.13	3.36	87.7
Zn	-0.801	-0.347	0.613	0.6	3.12	90.83

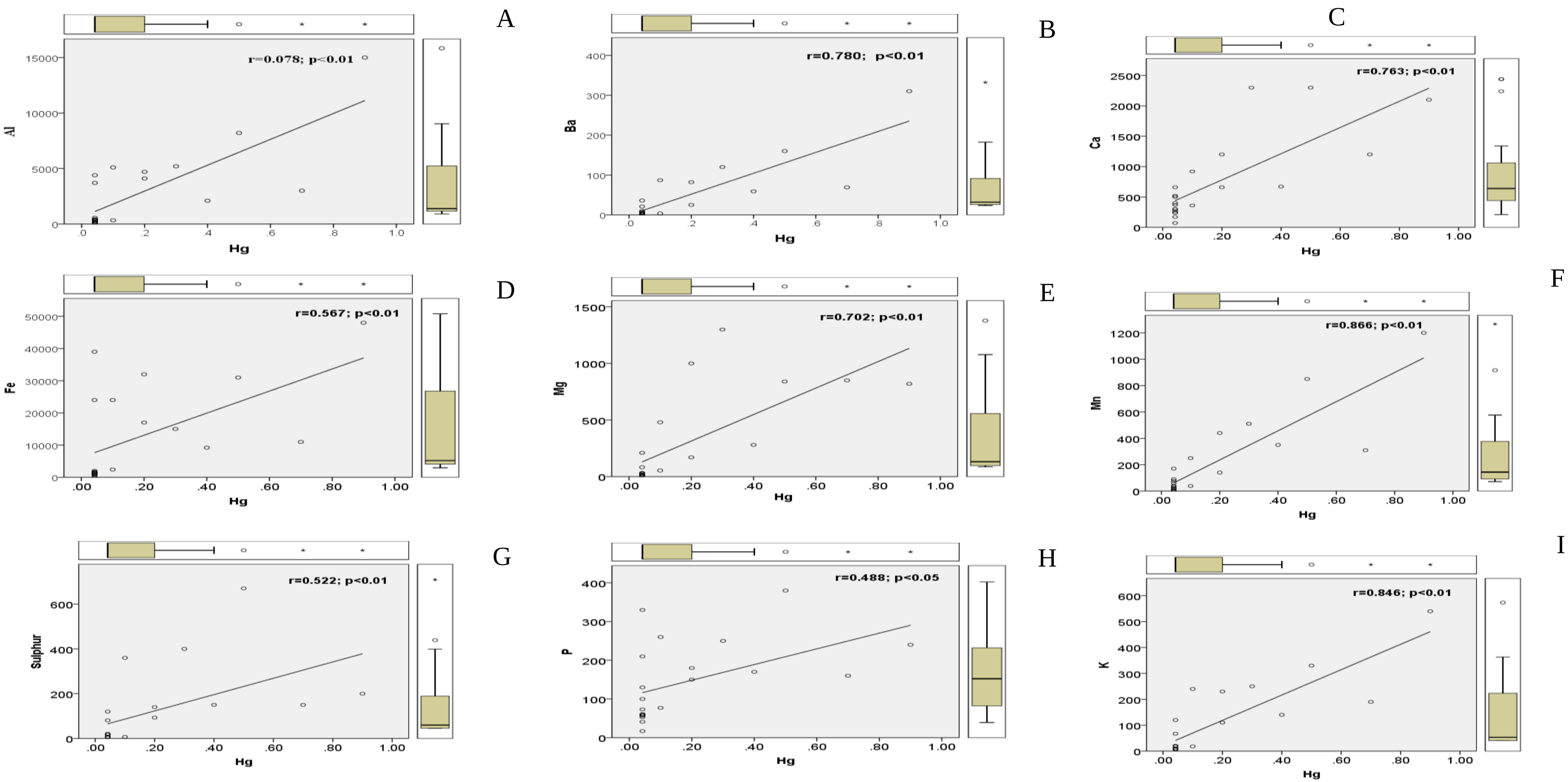
Appendix 14 Two-way ANOVA of sediment metal and metalloid concentrations to distinguish effects of ASGM cessation (before and after; BA) at control and impact (CI)

Variable	Source of variance	Type III Sum of Squares	df	Mean Square	F	Sig.
Aluminium	BA	99,596,365.67	1	99,596,365.67	14.93	.001**
	CI	4,887,713.78	1	4,887,713.78	0.73	.404
	BA* CI	4,722,940.96	1	4,722,940.96	0.71	.412
	Error	113,399,430.76	17	6,670,554.75		
	Total	431,888,584.00	21			
Barium	BA	23,257.47	1	23,257.47	8.52	.010*
	CI	11,293.99	1	11,293.99	4.14	.058
	BA * CI	9,867.21	1	9,867.21	3.61	.074
	Error	46,406.05	17	2,729.77		
	Total	161,249.96	21			
Calcium	BA	2,022,038.35	1	2,022,038.35	11.23	.004**
	CI	754,395.04	1	754,395.04	4.19	.056*
	BA * CI	1,377,615.42	1	1,377,615.42	7.65	.013*
	Error	3,062,092.86	17	180,123.11		
	Total	21,385,300.00	21			
Cobalt	BA	262.68	1	262.68	12.20	.003**
	CI	162.21	1	162.21	7.54	.014*
	BA * CI	133.14	1	133.14	6.19	.024*
	Error	365.94	17	21.53		
	Total	1,800.79	21			
Chromium	BA	29,787.29	1	29,787.29	21.72	.000**
	CI	1,232.58	1	1,232.58	0.90	.356
	BA * CI	1,297.58	1	1,297.58	0.95	.344
	Error	23,318.06	17	1,371.65		
	Total	90,954.25	21			
Copper	BA	84,652.31	1	84,652.31	0.35	.563
	CI	90,180.12	1	90,180.12	0.37	.551
	BA * CI	79,438.09	1	79,438.09	0.33	.576
	Error	4,144,582.24	17	243,798.96		
	Total	4,849,285.25	21			
Iron	BA	2,799,924,943.98	1	2,799,924,943.98	38.51	.000**
	CI	101,580,145.68	1	101,580,145.68	1.40	.253
	BA * CI	91,504,307.04	1	91,504,307.04	1.26	.278
	Error	1,236,048,506.55	17	72,708,735.68		
	Total	7,703,723,700.00	21			
Mercury	BA	0.21	1	0.21	7.29	.015*
	CI	0.14	1	0.14	4.63	.046*
	BA * CI	0.12	1	0.12	4.26	.055*

	Error	0.50	17	0.03		
	Total	1.92	21			
Lead	BA	4.03	1	4.03	0.09	.768
	CI	72.41	1	72.41	1.62	.220
	BA * CI	7.95	1	7.95	0.18	.678
	Error	759.02	17	44.65		
	Total	3,745.00	21			
Magnesium	BA	896,466.97	1	896,466.97	22.51	.000**
	CI	460,670.80	1	460,670.80	11.57	.003**
	BA * CI	420,178.01	1	420,178.01	10.55	.005**
	Error	677,149.60	17	39,832.33		
	Total	5,185,935.00	21			
Manganese	BA	389,741.84	1	389,741.84	9.03	.008**
	CI	264,919.07	1	264,919.07	6.14	.024*
	BA * CI	184,210.84	1	184,210.84	4.27	.054*
	Error	734,008.59	17	43,176.98		
	Total	2,969,126.06	21			
Kalium	BA	133,073.94	1	133,073.94	21.67	.000**
	CI	35,337.06	1	35,337.06	5.75	.028*
	BA * CI	30,507.22	1	30,507.22	4.97	.040*
	Error	104,411.55	17	6,141.86		
	Total	662,060.46	21			
Strontium	BA	0.94	1	0.94	0.01	.935
	CI	1,327.51	1	1,327.51	9.56	.007**
	BA * CI	180.76	1	180.76	1.30	.270
	Error	2,360.01	17	138.82		
	Total	15,915.66	21			
Sulphur	BA	149,067.97	1	149,067.97	10.92	.004
	CI	41,692.39	1	41,692.39	3.05	.099
	BA * CI	41,967.43	1	41,967.43	3.07	.098
	Error	232,055.18	17	13,650.30		
	Total	874,132.28	21			
Vanadium	BA	3.19	1	3.19	0.00	.962
	CI	1,999.42	1	1,999.42	1.50	.238
	BA * CI	242.68	1	242.68	0.18	.675
	Error	22,677.02	17	1,333.94		
	Total	117,625.00	21			
Zink	BA	7,277.87	1	7,277.87	4.62	.046
	CI	3,816.39	1	3,816.39	2.42	.138
	BA * CI	5,192.96	1	5,192.96	3.30	.087
	Error	26,764.18	17	1,574.36		
	Total	58,843.68	21			

Appendix 15 Correlations between concentration of mercury and select metals and metalloids in sediment.

Concentration of mercury in sediment significantly and positively correlated with sediment concentration of aluminium (a), barium (b), cobalt (c), iron (d), magnesium (e), manganese (f), sulphur (g), phosphorus (h), and kalium (i)



Appendix 16 Correlations (Pearson's r) between concentrations of dissolved select metals in water unless otherwise indicated. Dissolved metal in water correlates negatively and significantly with concentrations of almost all sediment metals and metalloids

** denotes significant correlation at the P<0.01 level and * at the P<0.05 level (2 tailed)

	As	Cd	Cu	Fe	Hg	Mg	Mn	Pb
As	1	.125	-.075	.473*	.397	-.351	.040	.252
Fe	.473*	-.115	-.308	1	.489*	-.417	-.196	.683**
Hg	.397	.156	-.285	.489*	1			.032
Pb	.252	.022	.142	.683**	.032	.001	.118	1
Ca	-.342	.077	-.107	-.402	?	.999**	-.255	-.035
DOC	.518	-.383	-.115	.745**	?	-.394	-.077	.030
TKN	.378	-.099	.112	.699*	?	-.804**	.094	.152
Al _ sediment	-.256	-.141	-.091	-.020	-.608**	.396	.036	.128
Ba_Sediment	-.314	-.128	-.101	-.223	-.661**	.444	-.085	-.098
Ca_Sediment	-.327	-.116	.038	-.251	-.747**	-.137	.105	-.056
Co_Sediment	-.359	-.138	-.040	-.287	-.725**	.271	.101	-.133
Cr_Sediment	-.132	-.153	-.087	.232	-.431	-.174	-.114	.547*
Fe_Sediment	-.146	-.171	-.071	.311	-.449*	-.055	.304	.462*
Mg-Sediment	-.384	-.152	.012	-.280	-.781**	.032	.529	-.030
Mn-Sediment	-.358	-.148	-.024	-.274	-.679**	.033	-.164	-.185
P-sediment	-.121	-.208	-.021	.272	-.469*	-.167	.021	.403
K-sediment	-.339	-.149	-.045	-.206	-.761**	.570	.148	.004
Sr_Sediment	-.293	.215	.108	-.494*	-.322	.293	.034	-.394
S_Sediment	-.290	-.130	.047	-.178	-.671**	-.050	-.022	.062
Hg-Sediment	-.342	-.135	-.017	-.297	-.693**	-.212	.578	-.257

Appendix 17 ANOVA of water quality variables distinguishing variability between time of sampling (months)

Variables and Sources of Variance		Sum of Squares	df	Mean Square	F	Significance
T	Between Groups	268.329	13	20.641	11.639	.000**
	Within Groups	246.503	139	1.773		
	Total	514.833	152			
DO	Between Groups	1,321.429	13	101.648	.881	.575
	Within Groups	15,814.035	137	115.431		
	Total	17,135.464	150			
EC	Between Groups	1.113	13	0.086	1.738	.060
	Within Groups	6.795	138	0.049		
	Total	7.908	151			
pH	Between Groups	4.807	13	0.370	1.618	.087
	Within Groups	31.773	139	0.229		
	Total	36.580	152			
TDS	Between Groups	0.482	13	0.037	1.588	.095
	Within Groups	3.246	139	0.023		
	Total	3.728	152			
Turbidity	Between Groups	5,544,114.246	13	426,470.327	1.418	.158
	Within Groups	41,816,191.496	139	300,835.910		
	Total	47,360,305.742	152			
Sulfate	Between Groups	140,869.391	13	10,836.107	1.671	.074
	Within Groups	894,858.395	138	6,484.481		
	Total	1,035,727.786	151			
NOx	Between Groups	29.507	13	2.270	8.239	.000**
	Within Groups	38.295	139	0.276		
	Total	67.802	152			
Ammonia	Between Groups	0.406	10	0.041	.567	.838
	Within Groups	9.244	129	0.072		
	Total	9.650	139			
TKN	Between Groups	32.556	11	2.960	3.057	.001**
	Within Groups	115.217	119	0.968		
	Total	147.773	130			
TP	Between Groups	0.751	11	0.068	2.349	.012*
	Within Groups	3.461	119	0.029		
	Total	4.212	130			
DOC	Between Groups	1,410.432	10	141.043	11.999	.000**
	Within Groups	1,222.431	104	11.754		
	Total	2,632.863	114			

As_Dis solved	Between Groups	0.000	13	0.000	6.666	.000**
	Within Groups	0.000	139	0.000		
	Total	0.000	152			
Hg_Dis solved	Between Groups	0.000	13	0.000	36.957	.000**
	Within Groups	0.000	139	0.000		
	Total	0.000	152			
Al_Dis solved	Between Groups	0.174	11	0.016	3.468	.000**
	Within Groups	0.544	119	0.005		
	Total	0.718	130			
Fe_Dis solved	Between Groups	11.940	11	1.085	2.462	.008**
	Within Groups	52.459	119	0.441		
	Total	64.399	130			
Mn_Di ssolved	Between Groups	10.702	11	0.973	2.951	.002**
	Within Groups	39.241	119	0.330		
	Total	49.943	130			
Zn_Dis solved	Between Groups	0.001	11	0.000	.172	.999
	Within Groups	0.077	109	0.001		
	Total	0.078	120			

Appendix 18 Principal component and factor analysis of water quality variables

A principal component analysis (PCA) was used to draw a general pattern of the river's water quality using all of the 45 research variables listed in 0 on a set of water quality data acquired from 2005 to 2014 (N=241). The PCA is also used to identify variables contributing most significantly to the variance in water quality along the river over time.

A scree plot of all 45 Principal Components (PC) against their Eigenvalues shows that after the first five components that contribute 43.31% to the total variance, the slope drops significantly, suggests a declining contribution of the other PCs to the total variance. However, we explored the first 15 PCs which accounted for 71% of total variance to determine the variables loadings. Table A lists the first 15 PC's contributions to the total variance in the form of Eigenvalues.

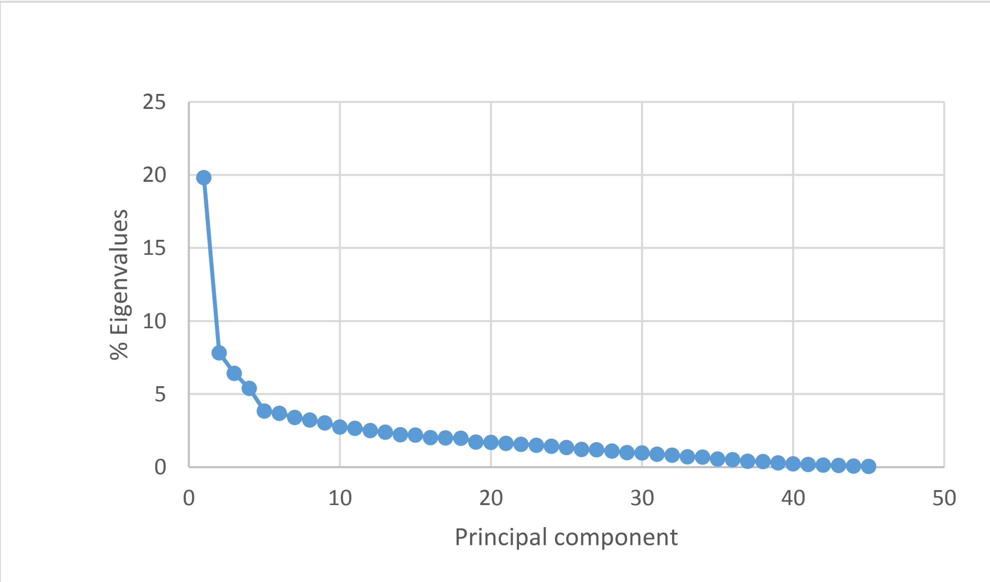


Figure A Scree plot of principal components and their contributions to the river water quality variability showing that after the 5th PC the slopes dramatically change.

Table A. The Eigenvalue and contributions of principal components of the Subri River water quality pattern and characteristics

Axis (PC)	Eigenvalue	Individual%	Cumulative%
1	2140.3	19.82	19.82
2	844.95	7.82	27.64
3	693.44	6.42	34.06
4	583.8	5.41	39.47
5	414.93	3.84	43.31
6	398.46	3.69	47
7	366.75	3.4	50.39
8	348.49	3.23	53.62
9	327.64	3.03	56.66
10	297.83	2.76	59.41
11	288.47	2.67	62.08

12	271.66	2.52	64.6
13	260.41	2.41	67.01
14	240.41	2.23	69.24
15	237.59	2.2	71.44

The variables loadings were determined by plotting the PCs against the original research variables with Spearman's correlation test(Table B). Significant correlation values (Rho) between variables and the PCs are the proxies for variable loadings on each PCs. Variable loading values of >0.75, 0.75 – 0.50, and 0.5 – 0.30 often classified as "Strong", "Moderate", and "Weak" loadings respectively (Liu et al., 2003). In this study we use a cut off loading value of 0.4 in selecting significant variables (Vega et al., 1998). Out of the initial 45 research variables, only 28 variables participated in the PCs with loading values >0.4 and only 9 PCs, namely PC1 to 6, PC8, PC9 and PC10 were contributed by variables with significant loadings. The first principal component (PC1), which accounted for 19.82% of the total variance, was participated positively pH, EC, TDS, alkalinity, Ca, Mg, Na, nitrates and sulfate and total Se. It is also participated negatively by total Fe, total Sb, dissolved Fe and dissolved Al. PC2 was participated positively by F, but negatively by K, Cl, total arsenic and dissolved arsenic. PC3 (accounted for 6.42% to total variance) was participated positively by Cl and dissolved As, and negatively by F, total Cu and total Pb. PC4 was participated positively by total Al and Se, and negatively by total Al and total Sb; whilst PC5 was participated positively by K and dissolved Sb but negatively by pH. PC6, 8, 9 and 10 were each participated by single significant variable, namely DO, dissolved Sb, K and temperature. PC11 and onwards are not participated by any variable with significant loadings.

A follow up multivariate analysis was performed on 241 water samples data set using the 28 variables with significant loadings listed in Table B. This time, the first 8 PCs contributed to 75.8% of total variance so we plotted the first 8 PC against the original data corresponding to the variables, resulting in correlations (Spearman's) or loadings as presented in Table C. Variables with loading values > 0.4 are selected as having significant contributions (in bold).

Table B. Loading of 45 variables on the first 15 PCs contributing to the variations in water quality data set.
Bold values represent medium to strong loadings.** denotes significant Spearman's correlation at $p<0.01$ * denotes significant Spearman's correlations at $p<0.05$

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15
pH	***.482*****	.296**	-.010	.156*	***-.409*****	.330**	-.052	.120	.083	-.094	.078	-.134*	-.092	.016	-.062
T	.112	-.005	-.327**	-.099	.025	-.194**	.196**	.395**	.191**	**.419****	.040	.108	-.235**	.204**	-.274**
Turb	**-.587****	-.330**	-.232**	.348**	-.198**	-.024	-.201**	-.232**	.012	-.111	-.190**	-.141*	-.198**	.002	.284**
TSS	**-.544****	-.288**	-.306**	.307**	-.197**	-.038	-.125	-.144*	.048	-.067	-.265**	-.124	-.225**	.094	.199**
EC	**.889****	-.189**	-.162*	-.030	-.004	.079	.138*	-.182**	-.132*	-.323**	.232**	-.030	-.093	.062	-.225**
TDS	**.872****	-.202**	-.156*	-.003	.001	.067	.119	-.201**	-.123	-.332**	.234**	-.039	-.106	.068	-.202**
DO	.146*	**.423****	.151*	.073	-.319**	**.464****	-.212**	.094	-.035	-.036	.145*	-.321**	.227**	.117	.096
Alk	**.673****	-.001	-.309**	-.208**	-.072	.358**	.077	.005	-.026	-.161*	.169*	-.374**	.048	.099	-.042
Ca	**.919****	-.117	-.138*	-.040	-.057	.120	.209**	-.086	-.045	-.308**	.197**	-.042	-.083	.132*	-.193**
Mg	**.928****	-.114	-.117	.018	-.062	.072	.211**	-.099	-.059	-.307**	.181**	.016	-.097	.170**	-.222**
Na	**.711****	-.315**	.036	-.049	.021	.139*	-.232**	-.117	-.231**	-.319**	.268**	-.120	-.133*	.029	-.189**
K	.140*	**-.489****	-.118	.029	**.416****	-.090	-.018	.036	**-.497****	-.023	.387**	.300**	-.149*	-.106	-.297**
Cl	-.094	**-.676****	**.499****	.115	-.003	.102	**-.410****	-.152*	-.308**	-.133*	.200**	.064	-.291**	-.034	-.126
N	**.672****	-.093	-.109	**.418****	.009	.069	.205**	-.090	-.023	-.325**	.013	.034	-.043	.116	-.122
SO4	**.891****	-.036	-.212**	.143*	-.093	.047	.232**	-.108	.057	-.279**	.171**	.044	-.092	.133*	-.212**
F	.187**	**.401****	**-.704****	**-.452****	.304**	.036	.080	-.009	-.127	.059	.223**	-.144*	.242**	-.099	-.011
Fe_T	**-.795****	**-.418****	.050	.150*	-.049	-.014	-.132*	-.110	-.080	.047	-.080	.002	-.110	-.002	.074
Mn_T	-.390**	-.277**	-.038	**-.505****	-.112	.037	-.168**	-.084	-.017	.129*	.038	-.179**	.113	-.033	.110
Cu_T	-.190**	.238**	**-.751****	-.204**	.195**	.033	-.014	.036	-.015	.182**	.139*	-.167**	.235**	-.087	-.003
Zn_T	-.203**	-.017	-.270**	-.034	-.006	-.009	.064	.027	-.069	.274**	.225**	-.001	.299**	-.298**	-.128*
Pb_T	.387**	.137*	**-.499****	-.064	.049	.349**	**.417****	-.187**	.059	.003	.240**	.179**	.115	.142*	-.247**
Hg_T	.098	.040	-.090	-.110	-.043	-.139*	-.009	-.112	.132*	-.080	-.103	-.090	-.149*	-.152*	.092
Cr_T	.060	-.243**	-.066	.136*	-.072	-.151*	-.253**	-.043	.131*	.029	-.132*	.070	.152*	.192**	-.160*
As_T	-.098	**-.555****	.220**	.180**	-.214**	.281**	.022	-.153*	-.098	-.225**	.112	-.021	-.365**	.163*	.061

Ni_T	-.092	-.164*	.049	.007	-.314**	-.102	.341**	.313**	.153*	.242**	-.117	.188**	.083	-.139*	-.330**
Cd_T	-.066	-.006	-.132*	-.032	.162*	.088	-.129*	.188**	.178**	-.192**	-.012	.099	-.011	.162*	.056
Al_T	-.384**	-.077	-.182**	**.625****	-.298**	-.036	-.043	-.079	.215**	-.073	-.175**	.036	-.223**	.142*	.065
Ag_T	-.205**	.216**	.108	-.168*	-.171**	-.059	.215**	-.175**	-.204**	-.217**	.213**	.122	-.188**	.056	-.005
Sb_T	**-.602****	.342**	-.145*	**-.448****	.142*	.161*	-.262**	.208**	-.067	.383**	-.005	-.288**	.121	-.201**	.294**
Co_T	.088	-.101	.104	-.130*	-.286**	.063	-.278**	.175**	-.263**	-.012	-.012	.215**	.145*	-.102	.141*
Se_T	**.641****	-.251**	-.067	**.426****	-.008	.114	.257**	-.133*	.019	-.270**	.055	.122	-.090	.165*	-.205**
Fe_Diss	**-.775****	-.190**	.306**	.024	.047	-.106	-.062	.105	.005	.284**	-.141*	.118	-.049	-.173**	.001
Mn_Diss	-.093	-.351**	.311**	-.036	-.274**	-.065	-.087	-.078	.270**	-.050	-.209**	-.017	-.059	.039	.079
Cu_Diss	.043	-.130*	-.249**	-.047	.171**	-.292**	.196**	-.009	-.329**	-.252**	-.215**	-.278**	.284**	-.013	.266**
Zn_Diss	.060	.063	.072	.074	-.124	.123	-.073	-.146*	.266**	-.026	.292**	.186**	.027	-.173**	-.004
Pb_Diss	-.044	.150*	-.168**	.064	.180**	.188**	-.067	-.186**	-.054	-.007	-.189**	.188**	-.038	.121	-.007
Hg_Diss	.115	.074	.134*	.070	-.116	-.130*	.164*	-.192**	-.183**	.190**	.009	-.122	.077	-.125	.015
Cr_Diss	.011	.011	-.058	.149*	-.136*	-.040	.114	-.073	.128	-.145*	-.153*	.057	.149*	-.153*	-.071
Ni_Diss	.172**	-.189**	.030	.181**	.058	.091	-.115	.166*	.164*	.043	-.142*	-.137*	.130*	-.223**	.245**
As_Diss	.107	**-.481****	**.427****	.168**	-.084	.143*	.202**	.104	.115	-.001	-.042	.143*	-.225**	.085	-.144*
Cd_Diss	.020	.054	-.102	-.089	-.112	.109	-.112	.111	-.112	.103	-.107	.112	-.099	-.100	-.085
Al_Diss	**-.520****	-.110	.083	.384**	-.116	-.196**	-.112	.061	.140*	.225**	.033	.152*	-.167**	-.125	-.040
Ag_Diss	.050	.025	-.114	-.007	-.023	.066	.112	-.093	.104	.066	.093	.114	.113	.111	.114
Sb_Diss	-.044	.111	-.180**	-.282**	**.488****	.344**	-.171**	**.433****	.017	.283**	.088	-.229**	.133*	-.203**	-.090
Co_Diss	.016	-.212**	.125	-.004	-.217**	-.303**	.000	.070	.192**	-.186**	.211**	.014	.073	.122	.227**

Table C Loadings of 28 selected variables

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
pH	**.484****	.254**	-.004	.270**	**-.423****	-.114	.151*	.015
T	.115	.077	-.327**	-.167**	.205**	.195**	-.122	**-.515****
Turb	**-.593****	-.320**	-.338**	.267**	-.121	-.252**	.003	-.036
TSS	**-.548****	-.260**	**-.405****	.241**	-.098	-.177**	-.042	-.098
EC	**.888****	-.172**	-.226**	-.149*	.046	-.032	-.094	-.013
TDS	**.872****	-.189**	-.223**	-.128*	.060	-.037	-.088	-.014
DO	.184**	.346**	.219**	.247**	**-.495****	-.309**	.312**	.135*
Alk	**.685****	.072	-.283**	-.287**	-.251**	-.121	.000	-.080
Ca	**.922****	-.088	-.198**	-.110	-.040	.060	-.093	-.053
Mg	**.927****	-.102	-.184**	-.059	.007	.071	-.132*	-.049
Na	**.707****	-.299**	-.002	-.154*	.050	-.333**	.139*	-.120
K	.130*	**-.482****	-.180**	-.246**	**.474****	.081	.206**	.204**
Cl	-.101	**-.742****	.372**	.035	.070	-.349**	.216**	-.058
Nitrates as N	**.677****	-.151*	-.215**	.284**	.046	.218**	.025	.202**
SO4	**.893****	-.040	-.273**	.054	.033	.125	-.111	-.055
F	.183**	**.511****	**-.544****	**-.537****	.064	-.124	-.042	.242**
FeTotal	**-.799****	**-.409****	-.030	.091	-.045	-.093	.084	-.016
MnTotal	-.395**	-.149*	-.006	**-.479****	-.158*	-.326**	-.127*	-.252**
CuTotal	-.170**	.379**	**-.638****	-.339**	.010	-.221**	.017	.263**
PbTotal	**.403****	.179**	**-.476****	-.245**	-.209**	.248**	-.178**	**.424****
AsTotal	-.132*	**-.612****	.058	.096	-.222**	-.002	.096	-.117
AlTotal	-.388**	-.133*	-.340**	**.614****	-.114	.007	.104	-.061
SbTotal	**-.508****	**.400****	.002	-.385**	-.159*	-.252**	.256**	-.005
SeTotal	**.562****	-.275**	-.172**	.234**	.079	.199**	.027	.145*
FeDissolved	**-.777****	-.215**	.296**	.059	.050	.123	.137*	-.033
AsDissolved	.102	**-.514****	.267**	.075	-.055	**.409****	.058	-.178**
AlDissolved	**-.524****	-.155*	.002	.394**	.131*	.106	.233**	-.144*
SbDissolved	-.137*	.182**	-.065	**-.467****	-.001	.012	**.527****	-.117

Appendix 19 SIMPER (Pairwise) analysis of the Surow river's water quality to identify variables distinguishing the significant differences between mine site and impact (IM) after the mine commenced

Variable	After Impact	After Mine	Av.Sq. Dist	Sq.Dist / SD	Contrib (%)	Cum (%)
N	0.0289	1.03	3.38	0.88	9.44	9.44
TDS	0.0403	0.788	2.94	0.6	8.22	17.66
Co-T	0.0975	0.0193	2.85	0.21	7.95	25.61
Mn-T	-0.134	0.0251	2.47	0.38	6.91	32.52
Mg	0.246	0.937	2.46	0.82	6.86	39.38
Ca	0.306	0.853	2.12	0.86	5.93	45.31
TSS	-0.316	0.128	2.07	0.56	5.79	51.1
F	0.388	-0.289	2.05	0.77	5.74	56.84
K	-0.271	0.321	2.01	0.53	5.62	62.46
SO4	0.359	0.782	1.95	0.84	5.44	67.89
pH	0.419	0.115	1.74	0.61	4.86	72.75
Na	0.0929	0.75	1.71	0.65	4.78	77.53
Fe-T	-0.548	-0.178	1.57	0.5	4.39	81.92
Al-T	-0.21	0.0221	1.55	0.46	4.33	86.25
Ni-T	-0.013	-0.0915	1.45	0.26	4.06	90.31

Appendix 20 The Subri River water characteristics (December 2006 – April 2014) and applicable water quality guidelines for aquatic ecosystem protection (the Ghana and US EPA) and Water Company guidelines for raw drinking water quality

Parameter	Statistics (mg l^{-1})			Ghana EPA standard for aquatic	Ghana Water Company guideline	USEPA water standard (chronic)
	Min	Max	Mean ±SE (N=241)			
pH	5.92	8.80	7.37 ± 0.025	6-9	6.5 - 8.5	6.5-9
T	18.7	86.9	25.96 ± 0.29	Ambient < 3	n/a	
Turbidity (NTU)	1.06	1000.0	42.72 ± 5.38	75	n/a	
TSS (mg l^{-1})	1.00	1650.0	43.23 ± 7.67	50	n/a	
EC (µS/cm)	116.00	2709.0	612.08 ± 32.65	1500	n/a	
TDS (mg l^{-1})	6.40	1438.0	317.43 ± 17.66	1000	1000	
DO (mg l^{-1})	0.30	8.5	4.22 ± 0.11	n/a	n/a	
Alkalinity (mg l^{-1})	0.30	414.1	113.078 ± 3.93	n/a	n/a	>20
Ca^{2+} (mg l^{-1})	1.15	335.0	65.04 ± 4.43	n/a	n/a	
Mg^{2+} (mg l^{-1})	2.90	344.6	23.29 ± 2.07	n/a	n/a	
Na^{+} (mg l^{-1})	2.00	105.0	24.38 ± 0.82	n/a	n/a	
K^{+} (mg l^{-1})	1.30	43.0	7.19 ± 0.27	n/a	n/a	
Cl^{-} (mg l^{-1})	2.50	52.4	17.57 ± 0.53	250	250	230
NO_3^{-} (mg l^{-1})	0.01	165.0	8.13 ± 1.24	16	13	
Sulfate (mg l^{-1})	0.30	940.0	133.80 ± 13.02	300	250	
Fluoride (mg l^{-1})	0.07	1.30	0.39 ±0.02	10	1.5	
Cyanide (WAD)	0.0021	0.00	0.002 ± 0	0.2	0.2	
Fe-Total (mg l^{-1})	0.0071	54.0	3.02 ± 0.30	n/a	0.3	
Mn-Total (mg l^{-1})	0.0050	9.63	0.40 ± 0.06	n/a	0.1	
Cu-Total (mg l^{-1})	0.0006	0.05	0.005 ± 0	n/a	1	
Zn-Total (mg l^{-1})	0.0069	0.08	0.01 ± 0.001	n/a	3	
Pb-Total (mg l^{-1})	0.0001	0.03	0.006 ± 0	n/a	0.01	
Hg-Total (mg l^{-1})	0.0004	0.003	0.0007 ± 0	n/a	0.001	
Cr-Total (mg l^{-1})	0.0071	0.03	0.007 ± 0	0.5	n/a	
As-Total (mg l^{-1})	0.0006	0.027	0.0023 ± 0	0.5	0.01	
Ni-Total (mg l^{-1})	0.0009	0.0228	0.0074 ± 0	n/a	0.02	
Cd-Total (mg l^{-1})	0.0001	0.015	0.0071 ± 0	n/a	0.003	
Al-Total (mg l^{-1})	0.04	25.13	1.08 ± 0.14	n/a	0.2	
Ag-Total (mg l^{-1})	0.00007	0.0071	0.007 ± 0	n/a	n/a	
Sb-Total (mg l^{-1})	0.00050	0.0707	0.06 ± 0.002	n/a	0.005	
Co-Total (mg l^{-1})	0.0071	0.04	0.008 ± 0	n/a	n/a	
Se-Total (mg l^{-1})	0.0002	0.01	0.0013 ± 0	n/a	0.01	0.005
Fe-Diss. (mg l^{-1})	0.0071	8.14	0.56 ± 0.06	n/a	n/a	1
Mn-Diss (mg l^{-1})	0.004	7.04	0.19 ± 0.047	0.1	n/a	
Cu-Diss (mg l^{-1})	0.0006	0.01	0.007 ± 0	2.5	n/a	
Zn-Diss (mg l^{-1})	0.0071	0.1	0.008 ± 0	5	n/a	0.12

Pb-Diss (mg l^{-1})	0.0003	0.01	0.007 ± 0	0.1	n/a	0.0025
Hg-Diss (mg l^{-1})	0.0007	0.0022	0.0007 ± 0.000009	0.005	n/a	0.00077
Cr-Diss (mg l^{-1})	0.0071	0.02	0.007 ± 0.00005	n/a	n/a	0.0072
Ni-Diss (mg l^{-1})	0.0071	0.04	0.007 ± 0.00002	0.5	n/a	0.052
As-Diss (mg l^{-1})	0.0004	0.0121	0.001 ± 0.00001	0.1	n/a	0.15
Cd-Diss (mg l^{-1})	0.0071	0.01	0.007 ± 0	0.1	n/a	0.00025
Al-Diss (mg l^{-1})	0.02	2.7	0.08 ± 0.015	5	n/a	0.087
Ag-Diss (mg l^{-1})	0.0002	0.0072	0.007 ± 0.00003	0.1	n/a	
Sb-Diss (mg l^{-1})	0.0004	0.011	0.002 ± 0.00005	1.5	n/a	
Co-Diss (mg l^{-1})	0.007	0.02	0.007 ± 0.0001	n/a	n/a	

Appendix 21 SIMPER (Pairwise) analysis of the Surow river's water quality to identify variables distinguishing the significant differences between before and after (BA), control and impact (CI), and the significant interaction between BA and CI.

Variable	Before Impact	Before Control	Av.Sq. Dist	Sq.Dist / SD	Contrib (%)	Cum. (%)
Hg-T	-0.153	4.27	29	1.27	47.04	47.04
Zn-T	1	0.352	5.17	0.42	8.39	55.43
As-T	-0.0693	1.24	4.74	0.69	7.7	63.13
Na	-0.212	-1.85	4.7	0.87	7.63	70.76
Cl	-1.25	-1.86	2.65	0.95	4.3	75.06
Al-T	0.337	-0.0415	2.35	0.6	3.82	78.88
pH	0.0285	-0.999	2.29	0.86	3.71	82.59
K	0.186	0.975	2.19	0.79	3.56	86.15
TSS	0.0183	-0.0456	1.63	0.7	2.64	88.78
EC	-1.41	-1.9	1.36	0.64	2.2	90.99

Variable	After Impact	After Control	Av.Sq. Dist	Sq.Dist / SD	Contrib %	Cum.%
Alk	0.324	-0.904	4.23	0.59	10.17	10.17
Ni-T	-0.013	0.142	3.32	0.31	7.97	18.15
F	0.388	-0.29	2.98	0.86	7.17	25.32
SO4	0.359	-1.05	2.91	1.15	7	32.32
pH	0.419	-0.572	2.6	0.74	6.26	38.57
Fe-T	-0.548	0.701	2.52	0.88	6.05	44.63
Co-T	0.0975	-0.0158	2.35	0.19	5.64	50.27
As-T	-0.254	0.199	1.98	0.28	4.76	55.03
Mn-T	-0.134	0.263	1.94	0.39	4.66	59.69
Ca	0.306	-0.69	1.93	0.79	4.64	64.33
K	-0.271	-0.0207	1.88	0.48	4.51	68.84
TDS	0.0403	-0.565	1.84	0.64	4.43	73.27
Mg	0.246	-0.729	1.82	0.63	4.37	77.65
TSS	-0.316	0.262	1.64	0.69	3.95	81.6
Na	0.0929	-0.331	1.53	0.53	3.69	85.29
Al-T	-0.21	-0.0411	1.33	0.61	3.19	88.47
N	0.0289	-0.607	1.26	0.59	3.02	91.49

Appendix 22 t-Test on water quality variables to distinguish differences between Control and Impact after mining

	t-test for Equality of Means		
Variables	t	df	Sig. (2-tailed)
pH	-7.73	199.00	.000**
Turb	5.56	197.00	.000**
TSS	3.34	199.00	.001**
EC	-6.08	199.00	.000**
TDS	-5.73	199.00	.000**
Alk	-7.76	184.00	.000**
Ca	-6.93	198.00	.000**
Mg	-4.01	198.00	.000**
Na	-3.76	194.00	.000**
K	1.71	198.00	.089
Cl	8.43	199.00	.000**
N	-4.00	197.00	.000**
SO4	-6.68	199.00	.000**
F	-3.68	189.00	.000**
FeT	10.46	199.00	.000**
MnT	0.74	199.00	.458
CuT	-1.71	199.00	.089
ZnT	-1.13	195.00	.259
PbT	-6.53	199.00	.000**
HgT	0.87	199.00	.386
AsT	3.29	187.00	.001**
NiT	0.88	199.00	.382
AlT	1.65	199.00	.100
CoT	-0.65	188.00	.519
SeT	-4.01	187.00	.000**

Appendix 23 t Test on water quality variables to distinguish differences between Mine site and Impact Site on the Subri River

	t-test for Equality of Means		
Variables	t	df	Sig. (2-tailed)
pH	-1.805	219	.072
Turb	3.207	196	.002**
TSS	2.493	219	.013*
EC	6.485	219	.000**
TDS	6.137	219	.000**
Alk	1.696	208	.091**
Ca	5.249	219	.000**
Mg	4.496	219	.000**
Na	5.826	215	.000**
K	3.150	218	.002**
Cl	10.080	217	.000**
N	7.201	217	.000**
SO4	5.469	219	.000**
F	-4.748	208	.000**
FeT	2.066	219	.040*
MnT	.660	219	.510
CuT	-1.904	219	.058
ZnT	-2.340	216	.020*
PbT	6.013	219	.000**
HgT	-.666	219	.506
AsT	1.422	208	.156
NiT	-.671	218	.503
AlT	1.111	219	.268
CoT	-.152	211	.879
SeT	10.086	208	.000**

Appendix 24 Concentrations of metal and metalloids in the Subri River sediment across 7 sites in April 2014 and February 2013 with sediment quality guidelines for protection of

Site	Al	As	Ba	Co	Cr	Cu	Fe	Hg	Mn	Pb	S	Sb	Se	Sr	Ti	U	Va	Zn	P	Ca	Mg	K
NSW9	220	1.41	1.7	0.2	5.0	12.	2000	0.042	9.7	8.0	7.1	2.12	2.12	19.0	1.6	0.04	62	35	610	55	12	7.1
KSW16	230	1.41	1.6	0.2	0.8	11.	690	0.042	10.0	4.0	12.	2.12	2.12	19.0	0.6	0.04	25	18	470	56	12	7.1
KSW3	490	1.41	2.8	0.7	4.5	30.	1600	0.042	18.0	10.0	22.	2.12	2.12	82.0	1.5	0.10	90	28	360	120	76	17.0
KSW13	150	1.41	2.6	0.3	1.3	0.35	700	0.042	24.0	0.7	7.1	2.12	2.12	0.2	1.5	0.04	0	0	2	61	35	7.1
NSW6	65	1.41	1.0	0.2	0.2	2.3	190	0.042	5.5	2.0	7.1	2.12	2.12	9.4	0.5	0.04	9	9	150	18	8	7.1
NSW8	120	1.41	1.7	0.3	0.4	6.7	250	0.042	15.0	2.0	40.	2.12	2.12	53.0	0.8	0.04	13	15	160	62	24	7.1
KSW2	140	1.41	9.7	1.8	6.6	14.	800	0.042	73.0	18.0	7.1	2.12	2.12	38.0	3.3	0.04	52	17	170	52	29	7.1
KSW2	1200	1.41	35.0	2.5	12.0	3.7	3300	0.042	78.0	2.0	130.			17.0	2.8		10	7	120	480	220	78.0
KSW3	8100	1.41	68.0	12.	130.0	21.	39000	0.042	370.0	10.0	660.			74.0	14.		94	20	350	3000	1700	300.0
KSW13	2700	1.41	36.0	4.6	45.0	5.7	16000	0.042	370.0	4.0	65.			15.0	5.4		33	10	290	990	760	230.0
Mean	1341.5	1.4	16.0	2.3	20.6	10.7	6453	0.0	97.3	6.1	95.7	2.1	2.1	32.7	3.2	0.1	38.8	15.9	268.2	489.4	287.6	66.7
StErr	794	0.0	7.2	1.2	12.9	2.9	3915	0.0	46.2	1.7	63.9	0.0	0.0	8.9	1.3	0.0	10.8	3.2	57.8	295.3	173.1	34.2
Sediment Quality Guidelines																						
TEL		5.9			37.3	35.7		0.174		35								123				
LEL		6			26	16		0.2		31								120				
MET		7			55	28		0.2		42								150				
ERL		33			80	70		0.15		35								120				
TEL_HA28		11			36	28		NG		37								98				
ERL (Aust)		8.2			81	34		0.15		46.7								410				

Appendix 25 SIMPER analysis identifying variables contributed to the significant difference between sediment quality in February 2013 and April 2014

Variable	Average value		Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
	Group Apr-14	Group Feb-13				
K	-0.894	1.2	4.55	2.81	8.56	8.56
S	-0.733	1.22	4.49	1.37	8.45	17.02
Ca	-0.817	1.21	4.45	1.87	8.38	25.39
Al	-0.831	1.14	4.34	1.64	8.16	33.56
Cr	-0.732	1.17	4.26	1.4	8.01	41.57
Fe	-0.805	1.06	4.06	1.45	7.64	49.21
Ba	-0.875	1.03	3.87	2.02	7.28	56.49
Co	-0.749	0.987	3.83	1.06	7.21	63.7
Ti	-0.8	0.72	3.11	1.04	5.85	69.56
Mg	-0.879	0.734	2.84	1.86	5.35	74.9
Va	-0.106	0.555	2.14	0.66	4.03	78.93
Mn	-0.839	0.493	2.07	1.46	3.89	82.82
P	-0.191	0.234	1.98	0.47	3.73	86.56
Pb	-7.20E-02	0.391	1.95	0.8	3.67	90.23

Pairwise SIMPER analysis identifying variables contributed to the significant difference between sediment quality in the mine site and in impact sites downstream

Variable	Average value		Av.Sq.Dist	Sq.Dist/SD	Contrib%	Cum.%
	Group Mine	Group Impact				
Fe	0.949	-0.869	4.51	1.19	9.32	9.32
Al	1.05	-0.76	4.5	1.21	9.31	18.63
Cr	1.07	-0.603	4.22	1.1	8.72	27.36
Ca	1	-0.629	4.08	1.11	8.43	35.78
S	1.16	-0.324	3.94	1.05	8.15	43.93
Co	0.958	-0.481	3.69	0.99	7.63	51.56
Ti	0.749	-0.672	3.39	1.04	7	58.56
K	0.836	-0.587	3.27	1.16	6.75	65.32
Ba	0.636	-0.424	2.79	0.99	5.77	71.09
Pb	1.08	-0.23	2.79	1.21	5.76	76.85
Cu	1.3	-0.145	2.42	1.43	5.01	81.86
Va	1.19	-0.257	2.41	1.71	4.99	86.85
Mg	0.596	-0.689	2.35	1.15	4.86	91.71

Appendix 26 Multiple comparison of mean concentration of metal and metalloid in sediment in control, mine and impact sites on the Subri River.
*. The mean difference is significant at the 0.05 level.

Dependent Variable			Mean Difference	Std. Error	Significance
Al	Control	Impact	443.75	2152.36	1.000
		Mine	-6038.33	2324.81	.095
	Impact	Control	-443.75	2152.36	1.000
		Mine	-6482.08	2324.81	.071
	Mine	Control	6038.33	2324.81	.095
		Impact	6482.08	2324.81	.071
Ba	Control	Impact	-1.38	26.28	1.000
		Mine	-59.79	28.39	.205
	Impact	Control	1.38	26.28	1.000
		Mine	-58.42	28.39	.221
	Mine	Control	59.79	28.39	.205
		Impact	58.42	28.39	.221
Co	Control	Impact	0.13	8.40	1.000
		Mine	-18.24	9.08	.238
	Impact	Control	-0.13	8.40	1.000
		Mine	-18.36	9.08	.233
	Mine	Control	18.24	9.08	.238
		Impact	18.36	9.08	.233
Cr	Control	Impact	8.22	44.45	1.000
		Mine	-115.14	48.01	.130
	Impact	Control	-8.22	44.45	1.000
		Mine	-123.36	48.01	.099
	Mine	Control	115.14	48.01	.130
		Impact	123.36	48.01	.099
Cu	Control	Impact	0.59	4.31	1.000
		Mine	-22.07083*	4.65	.004*
	Impact	Control	-0.59	4.31	1.000
		Mine	-22.65833*	4.65	.004*
	Mine	Control	22.07083*	4.65	.004*
		Impact	22.65833*	4.65	.004*
Fe	Control	Impact	3712.50	9819.48	1.000
		Mine	-26019.17	10606.25	.119
	Impact	Control	-3712.50	9819.48	1.000
		Mine	-29731.67	10606.25	.069
	Mine	Control	26019.17	10606.25	.119
		Impact	29731.67	10606.25	.069
Mn	Control	Impact	60.55	228.56	1.000
		Mine	-425.91	246.87	.368
	Impact	Control	-60.55	228.56	1.000
		Mine	-486.46	246.87	.253

	Mine	Control	425.91	246.87	.368
		Impact	486.46	246.87	.253
Pb	Control	Impact	-1.83	4.93	1.000
		Mine	-11.16	5.33	.209
	Impact	Control	1.83	4.93	1.000
		Mine	-9.33	5.33	.354
	Mine	Control	11.16	5.33	.209
		Impact	9.33	5.33	.354
S	Control	Impact	-23.25	210.35	1.000
		Mine	-604.53	227.20	.086
	Impact	Control	23.25	210.35	1.000
		Mine	-581.28	227.20	.101
	Mine	Control	604.53	227.20	.086
		Impact	581.28	227.20	.101
Sr	Control	Impact	-16.05	11.92	.645
		Mine	-53.0308333*	12.87	.010*
	Impact	Control	16.05	11.92	.645
		Mine	-36.98	12.87	.062
	Mine	Control	53.0308333*	12.87	.010*
		Impact	36.98	12.87	.062
Ti	Control	Impact	0.43	5.90	1.000
		Mine	-14.23	6.37	.168
	Impact	Control	-0.43	5.90	1.000
		Mine	-14.65	6.37	.152
	Mine	Control	14.23	6.37	.168
		Impact	14.65	6.37	.152
Va	Control	Impact	9.14	17.28	1.000
		Mine	-77.9125000*	18.66	.009*
	Impact	Control	-9.14	17.28	1.000
		Mine	-87.0500000*	18.66	.005*
	Mine	Control	77.9125000*	18.66	.009*
		Impact	87.0500000*	18.66	.005*
Zn	Control	Impact	3.71	6.88	1.000
		Mine	-9.29	7.43	.740
	Impact	Control	-3.71	6.88	1.000
		Mine	-13.00	7.43	.355
	Mine	Control	9.29	7.43	.740
		Impact	13.00	7.43	.355
P	Control	Impact	193.05	114.34	.389
		Mine	3.05	123.50	1.000
	Impact	Control	-193.05	114.34	.389
		Mine	-190.00	123.50	.487
	Mine	Control	-3.05	123.50	1.000
		Impact	190.00	123.50	.487
Ca	Control	Impact	137.50	619.49	1.000
		Mine	-1716.17	669.13	.100

	Impact	Control	-137.50	619.49	1.000
		Mine	-1853.67	669.13	.073
	Mine	Control	1716.17	669.13	.100
		Impact	1853.67	669.13	.073
Mg	Control	Impact	134.50	390.75	1.000
		Mine	-1020.58	422.06	.126
	Impact	Control	-134.50	390.75	1.000
		Mine	-1155.08	422.06	.077
	Mine	Control	1020.58	422.06	.126
		Impact	1155.08	422.06	.077
K	Control	Impact	38.00	76.82	1.000
		Mine	-142.84	82.98	.370
	Impact	Control	-38.00	76.82	1.000
		Mine	-180.84	82.98	.183
	Mine	Control	142.84	82.98	.370
		Impact	180.84	82.98	.183

Appendix 27 Geo-accumulation index of sediment elements across sites on the Subri River

Site	Sampling date	Geo-accumulation Index (I_geo)																		
		Al	As	Ba	Co	Cr	Cu	Fe	Hg	Mn	Pb	S	Sr	Ti	Va	Zn	P	Ca	Mg	K
NSW9	Apr-14	10.4	0.3	0.7	-3.5	3.7	4.6	14.8	-6.7	4.1	3.8	3.5	5.5	0.5	7.8	6.7	12.4	7.6	4.6	3.5
KSW16	Apr-14	10.4	0.3	0.6	-3.5	1.9	4.5	13.7	-6.7	4.2	3.1	4.0	5.5	-0.4	6.9	6.0	12.2	7.6	4.6	3.5
KSW3	Apr-14	11.2	0.3	1.2	-2.3	3.6	5.5	14.6	-6.7	4.8	4.0	4.6	6.9	0.5	8.2	6.5	11.9	8.4	6.4	4.4
KSW13	Apr-14	10.0	0.3	1.1	-3.2	2.3	1.0	13.7	-6.7	5.0	1.3	3.5	1.0	0.5	2.7	2.1	6.8	7.7	5.6	3.5
NSW6	Apr-14	9.2	0.3	0.1	-3.5	0.5	2.9	12.4	-6.7	3.6	2.4	3.5	4.8	-0.6	5.9	5.3	11.0	6.5	4.2	3.5
NSW8	Apr-14	9.8	0.3	0.7	-3.2	1.2	4.0	12.7	-6.7	4.6	2.4	5.2	6.5	-0.2	6.3	5.9	11.1	7.7	5.3	3.5
KSW2	Apr-14	9.9	0.3	2.4	-1.4	4.0	4.7	13.9	-6.7	6.2	4.6	3.5	6.2	1.3	7.7	6.0	11.1	7.6	5.4	3.5
KSW2	Feb-13	12.1	0.3	3.7	-1.0	4.6	3.4	15.3	-6.7	6.2	2.4	6.4	5.4	1.1	6.0	5.1	10.8	9.8	7.5	5.9
KSW3	Feb-13	14.0	0.3	4.3	0.5	6.9	5.1	17.8	-6.7	7.8	4.0	8.0	6.8	2.7	8.3	6.1	11.9	11.6	9.5	7.3
KSW13	Feb-13	12.9	0.3	3.7	-0.4	5.9	3.8	16.9	-6.7	7.8	3.1	5.7	5.2	1.8	7.2	5.4	11.7	10.5	8.7	7.0
Mean			0.3	2.1	-1.8	3.8	4.1	14.9	-6.7	5.7	3.3	5.2	5.5	1.0	6.9	5.6	11.1	8.8	6.5	4.8
StError			0.0	0.5	0.5	0.7	0.4	0.6	0.0	0.5	0.3	0.5	0.5	0.4	0.5	0.4	0.4	0.5	0.6	0.5

Appendix 28 Enrichment Factor (EF) of elements in the Subri River sediment across 7 sites

Site	Sampling Date	Enrichment Factor (EF)																		
		Al	As	Ba	Co	Cr	Cu	Fe	Hg	Mn	Pb	S	Sr	Ti	Va	Zn	P	Ca	Mg	K
NSW9	Apr-14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
KSW16	Apr-14	1	1.0	0.9	1.0	0.2	0.9	0.3	1.0	1.0	0.5	1.6	1.0	0.4	0.4	0.5	0.7	1.0	1.0	1.0
KSW3	Apr-14	1	0.5	0.7	1.5	0.4	1.1	0.4	0.5	0.8	0.6	1.4	1.9	0.4	0.7	0.4	0.3	1.0	2.8	1.1
KSW13	Apr-14	1	1.5	2.2	2.1	0.4	0.0	0.5	1.5	3.6	0.1	1.5	0.0	1.4	0.0	0.0	0.0	1.6	4.3	1.5
NSW6	Apr-14	1	3.4	2.0	3.4	0.1	0.6	0.3	3.4	1.9	0.8	3.4	1.7	1.1	0.5	0.8	0.8	1.1	2.3	3.4
NSW8	Apr-14	1	1.8	1.8	2.6	0.1	1.0	0.2	1.9	2.8	0.5	10.3	5.1	0.9	0.4	0.8	0.5	2.1	3.7	1.8
KSW2	Apr-14	1	1.6	9.0	13.5	2.1	1.8	0.6	1.6	11.8	3.5	1.6	3.1	3.2	1.3	0.8	0.4	1.5	3.8	1.6
KSW2	Feb-13	1	0.2	3.8	2.2	0.4	0.1	0.3	0.2	1.5	0.0	3.4	0.2	0.3	0.0	0.0	0.0	1.6	3.4	2.0
KSW5	Feb-13	1	0.0	1.5	4.0	0.9	0.1	0.5	0.0	2.3	0.1	3.1	0.0	0.4	0.0	0.0	0.0	1.0	2.9	0.8
KSW3	Feb-13	1	0.0	1.1	1.6	0.7	0.0	0.5	0.0	1.0	0.0	2.5	0.1	0.2	0.0	0.0	0.0	1.5	3.8	1.1
KSW13	Feb-13	1	0.1	1.7	1.8	0.7	0.0	0.7	0.1	3.1	0.0	0.7	0.1	0.3	0.0	0.0	0.0	1.5	5.2	2.6
Mean		1	1.0	2.3	3.1	0.6	0.6	0.5	1.0	2.8	0.7	2.8	1.3	0.9	0.4	0.4	0.4	1.3	3.1	1.6
StError		0	0.3	0.7	1.1	0.2	0.2	0.1	0.3	0.9	0.3	0.8	0.5	0.3	0.1	0.1	0.1	0.1	0.4	0.2

Appendix 29 Analysis of variance (ANOVA) between water quality at site KSW13 and NSW8 distinguishing the difference between water quality at mine site and that of discharged from ECD

Variables and sources of variations		Sum of Squares	df	Mean Square	F	Significance
pH	Between Groups	.738	1	.738	1.166	.283
	Within Groups	58.228	92	.633		
	Total	58.966	93			
Temperature	Between Groups	1.049	1	1.049	1.731	.192
	Within Groups	55.746	92	.606		
	Total	56.795	93			
Turbidity	Between Groups	11.997	1	11.997	10.300	.002**
	Within Groups	107.166	92	1.165		
	Total	119.164	93			
TSS	Between Groups	9.823	1	9.823	7.930	.006**
	Within Groups	113.966	92	1.239		
	Total	123.790	93			
EC	Between Groups	3.587	1	3.587	4.523	.036*
	Within Groups	72.963	92	.793		
	Total	76.550	93			
TDS	Between Groups	3.485	1	3.485	4.682	.033*
	Within Groups	68.470	92	.744		
	Total	71.955	93			
DO	Between Groups	.119	1	.119	.112	.739
	Within Groups	97.865	92	1.064		
	Total	97.984	93			
Alk	Between Groups	.009	1	.009	.063	.802
	Within Groups	12.632	92	.137		
	Total	12.641	93			
Ca	Between Groups	1.393	1	1.393	1.683	.198
	Within Groups	76.140	92	.828		
	Total	77.533	93			
Mg	Between Groups	2.593	1	2.593	2.903	.092
	Within Groups	82.182	92	.893		
	Total	84.774	93			
Na	Between Groups	9.157	1	9.157	9.227	.003**
	Within Groups	91.300	92	.992		
	Total	100.457	93			
K	Between Groups	4.390	1	4.390	6.188	.015*
	Within Groups	65.271	92	.709		
	Total	69.661	93			
Chloride	Between Groups	31.874	1	31.874	56.744	.000**
	Within Groups	51.678	92	.562		
	Total	83.553	93			
Nitrates	Between Groups	17.461	1	17.461	16.659	.000**

	Within Groups	96.433	92	1.048		
	Total	113.894	93			
Sulfate	Between Groups	.389	1	.389	.664	.417
	Within Groups	53.947	92	.586		
	Total	54.336	93			
Flouride	Between Groups	10.769	1	10.769	20.657	.000**
	Within Groups	47.960	92	.521		
	Total	58.728	93			
Fe_Total	Between Groups	12.343	1	12.343	14.033	.000**
	Within Groups	80.923	92	.880		
	Total	93.267	93			
Mn_Total	Between Groups	.648	1	.648	.443	.507
	Within Groups	134.508	92	1.462		
	Total	135.156	93			
Cu_Total	Between Groups	.231	1	.231	.145	.704
	Within Groups	146.466	92	1.592		
	Total	146.696	93			
As_Total	Between Groups	1.442	1	1.442	7.250	.008**
	Within Groups	18.293	92	.199		
	Total	19.734	93			
Al_Total	Between Groups	5.683	1	5.683	4.877	.030*
	Within Groups	107.198	92	1.165		
	Total	112.881	93			
Sb_Total	Between Groups	4.090	1	4.090	2.778	.099
	Within Groups	135.443	92	1.472		
	Total	139.534	93			
Se_Total	Between Groups	37.849	1	37.849	24.037	.000**
	Within Groups	144.865	92	1.575		
	Total	182.714	93			
Fe_Dissolved	Between Groups	.539	1	.539	2.222	.140
	Within Groups	22.308	92	.242		
	Total	22.847	93			
As_Dissolved	Between Groups	.764	1	.764	7.162	.009**
	Within Groups	9.818	92	.107		
	Total	10.582	93			
Al_Dissolved	Between Groups	.744	1	.744	.451	.503
	Within Groups	151.725	92	1.649		
	Total	152.469	93			
Sb_Dissolved	Between Groups	.791	1	.791	.773	.382
	Within Groups	94.216	92	1.024		
	Total	95.007	93			

Appendix 30 Macroinvertebrate identities, abundance, diversity and occurences

In total, 198 samples consisting of 39,965 individuals were collected; 126 samples containing 24,906 individuals were from the Surow River; and 72 samples containing 15,059 individuals were from the Subri River. Eighty eight taxa, distributed among 22 orders, were identified mostly to family levels. Seventy eight taxa were found in the Surow River and 80 taxa were found in the Subri River. The taxa, their number of occurrences and total abundance in each river are listed in Table A.Although identification of organisms in this study was intended to stop at the family level, specimens from eight samples from the first sampling period were identified to lowest practical taxa by Dr Godwin Amungbe, for exploratory purposes, with 39 families yielding 77 taxa, as presented in Table B.. These were samples corresponding to sites 6, 4 and 9 on the Surow and site KSW 13 on the Subri.

Diptera were the most abundant in the Surow River, with Chironomidae and Ceratopogonidae being the most dominant, accounting for 48% of the river's total macroinvertebrate abundance; this compared to 19% in the Subri River. On the contrary, Gastropods were most abundant in the Subri River, contributing 27% to the total abundance, compared to the 8.7% in the Surow River. Other dominant families in both rivers were from the orders Ephemeroptera, Coleoptera, Hemiptera, Decapoda (Atydae) and Odonata (Figure A)

Across rivers, Chironomidae was the most frequently occurring family, found in 83% of all samples, Planorbidae (67%), Coenagrionidae (64%) and Dytiscidae (62%). The families of Barbarochthidae, Psychomyidae, Policentropodidae (Trichoptera), Trichortythidae, Policentrophlebidae (Ephemeroptera), and Protoneuridae (Odonata) were represented by single specimen.

In the Surow River, Chironomidae were the most frequent taxa found in 96% of all samples, followed by Baetidae (80%), Dytiscidae (65%), Planorbidae and Coenagrionidae (59% each). Although found in the Subri River, Calamoceridae, Psychomydae, Barbarochtidae (Tricoptera), Trychortidae, Oligoneuridae (Ephemeroptera), Athericidae, Muscidae (Diptera) and Porifera were absent from the Surow River. In the Subri River, Thiaridae was found in 80% of allsamples, making it the most frequently encountered taxa, followed by Chironomidae (75%), Planorbidae (70%), Coenagrionidae (64%) and Elmidae (58%).

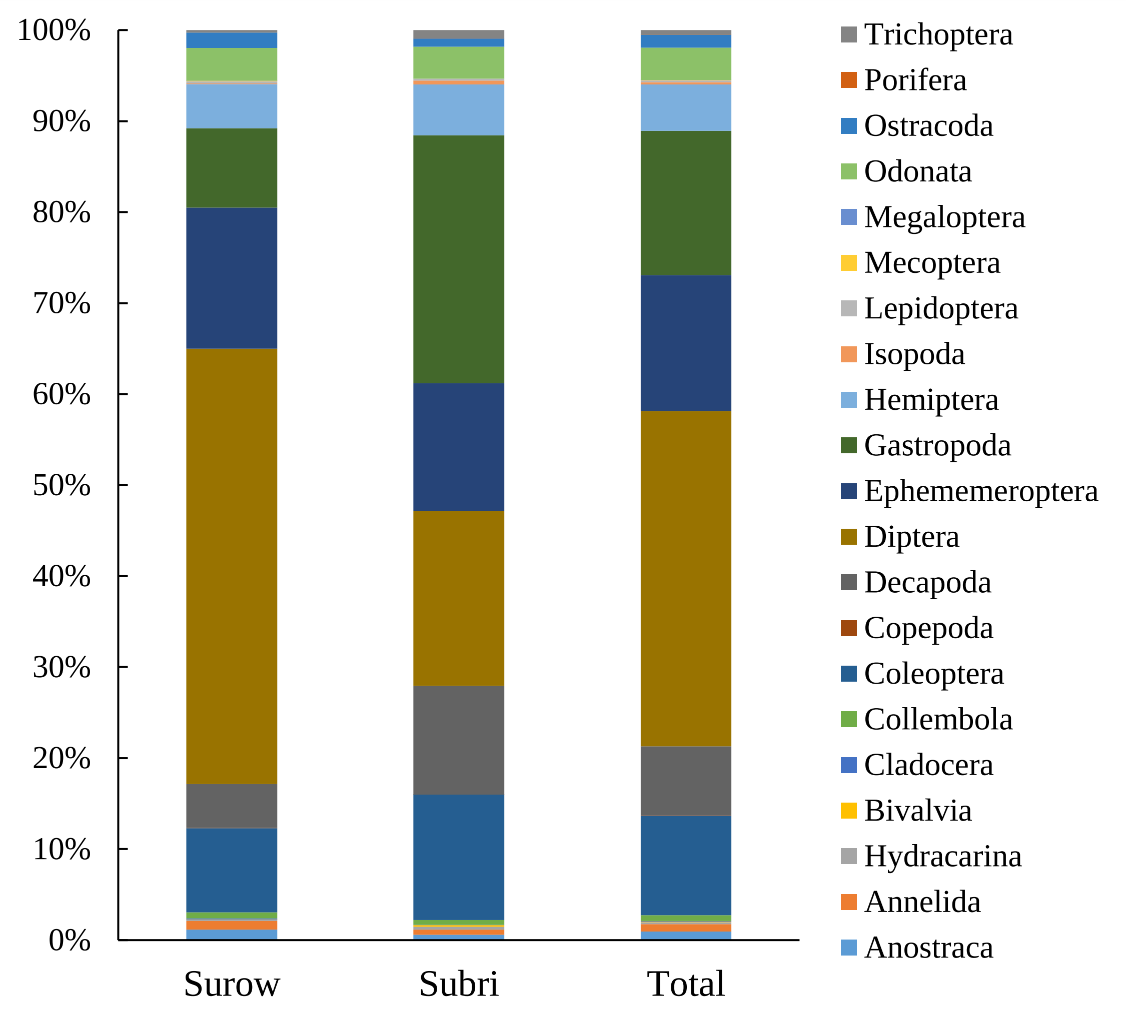


Figure A. Relative abundance of macroinvertebrate groups (order level) in samples collected from the Surow and Subri rivers between July 2013 and April 2014.

Table A Lists of taxa (family level or higher) identified in all samples collected from the Surow and Subri rivers 2013 – 2014 and their numbers of occurrence and abundance

Phylum/ Order / Class	Family	Surow River (N = 126; 11 sites)		Subri River (N = 72; 7 sites)	
		Frequency of occurrence	Total abundance	Frequency of occurrence	Total abundance
Anostraca		5	287	1	90
Hirudinea		33	62	12	23
Oligochaeta		48	155	21	68
Turbellaria		11	16	2	2
Hydracarina		21	47	19	44
Bivalvia	Pisidiidae	1	1	5	20
	Sphaeriidae	1	1	3	4
	Unionidae	0	0	2	6
Cladocera		14	23	3	5
Collembola		52	167	24	81

Coleoptera	Curculionidae	2	3	2	2
	Dytiscidae	84	915	40	503
	Elmidae	49	256	51	854
	Haliplidae	18	65	10	15
	Gyrinidae	21	199	17	272
	Hydraenidae	29	113	18	103
	Hydrochidae	14	36	5	8
	Hydrophilidae	72	492	25	286
	Hygrobidae	17	38	5	39
	Psephenidae	10	16	2	2
	Scirtidae	35	165	18	60
	Ptilodactylidae	6	8	2	3
Copepoda		5	9	1	1
Decapoda	Atydae	72	1191	41	1860
	Potamonautidae	3	3	3	4
Diptera	Athericidae	0	0	5	6
	Ceratopogonidae	69	4516	24	356
	Chironomidae	103	7107	62	2352
	Chaoboridae	1	2	2	2
	Culicidae	39	176	22	192
	Dixidae	1	2	4	4
	Dolichopodidae	1	1	1	1
	Emphypidae	1	1	1	1
	Muscidae	0	0	1	1
	Psychodidae	9	13	5	8
	Sciomyzidae	2	3	0	0
	Simuliidae	7	45	6	23
	Stratyomiidae	8	8	8	19
	Syrphidae	4	4	1	1
	Tabanidae	7	7	7	9
	Tipulidae	14	23	15	24
Ephememeroptera	Baetidae	101	3104	58	1081
	Caenidae	50	686	45	383
	Heptagenidae	14	52	23	175
	Leptophlebiidae	5	18	8	14
	Policentrophlebidae	0	0	1	1
	Trichorythidae	1	2	0	0
Gastropoda	Ampullaridae	9	12	3	3
	Ancylidae	11	24	14	50
	Bithyniidae	5	12	0	0
	Hydrobiidae	5	9	0	0
	Lymnaeidae	44	185	19	38
	Physidae	59	736	27	494
	Pilidae	4	6	6	11
	Planorbiidae	74	277	59	799

	Thiaridae	56	905	67	2854
	Viviparidae	6	19	0	0
Hemiptera	Belostomatidae	44	236	21	104
	Corixidae	18	131	9	79
	Gerridae	20	100	33	154
	Hydrometridae	9	13	3	4
	Mesoveliidae	23	36	22	36
	Nepidae	14	47	5	7
	Naucoridae	12	15	8	10
	Notocnetidae	33	303	24	262
	Pleidae	20	61	17	74
	Veliidae	24	259	33	141
Isopoda		3	25	2	67
Lepidoptera	Pyralidae	26	60	13	30
Mecoptera	Mecoptera	4	12	0	0
Megaloptera	Corydalidae	2	2	0	0
Odonata	Gomphidae	18	56	6	13
	Calopterygidae	1	2	4	5
	Coenagrionidae	75	403	54	373
	Corduliidae	6	18	2	4
	Libellulidae	71	406	48	150
	Lestidae	1	2	2	2
	Protoneuridae	1	2	0	0
Ostracoda		27	421	21	146
Porifera		0	0	5	6
Trichoptera	Barbarochthonidae	0	0	1	1
	Calamoceratidae	0	0	3	4
	Ecnomidae	1	3	2	2
	Hydropsychidae	10	31	6	25
	Psychomyidae	0	0	1	1
	Hydroptilidae	2	11	4	22
	Leptoceridae	18	28	27	79
	Policentropodidae	0	0	1	1
Total			24906		15059

Table B Taxa identified by Dr. Godwin Amugbe, Macroinvertebrate Laboratory, Ghana CSIR, Accra to genus and species levels in eight randomly selected samples from sites 6, 4, 9, and KSW13 in July 2013.

Class/Order	Family	Species/Genus
Hirudinea		*Hirudinea sp.*
Oligochaeta		*Oligochaeta sp.*
Turbelaria		*Nais sp.*
Hydracarina		*Hydracarina sp.*
Bivalvia	Pisidiidae	*Pisidiidae sp.*

	Sphaeriidae	*Pelecypoda sp.*
Cladocera		*Daphnia sp.*
Collembola		*Collembola sp.*
Coleoptera	Dytiscidae	*Laccophilus sp.*
		Hyphydrus sp
		Neptosternus guignot.
	Elmidae	*Elmidae sp.*
		Potamodytes sp.
		Omotonus sp.
	Haliplidae	*Haliplidae sp.*
		Aulonogyrus sp.
	Gyrinidae	*Orectogyrus vagus*
	Scirtidae	*Scirtes sp.*
Decapoda	Atydae	*Atyia sp*
Diptera	Athericidae	*Atherix sp.*
	Ceratopogonidae	*Ceratopogonidae sp.*
	Chironomidae	*Chirinomus sp*
		Chironomus formosipennis
		Clinotanypus claripennis
		Polypedilum sp.
		Stictochironomus puripennis
		Polypedilum griseoguttatum
		Nilodorum brevipalpis
	Culicidae	*Culex sp.*
	Psychodidae	*Psychodidae*
	Simuliidae	*S. damnosum*
	Syrphidae	*Eristalis sp.*
	Tipulidae	*Antocha sp.*
Ephememeroptera	Baetidae	*Centroptilum sp.*
		Cloeon sp.
	Caenidae	*Caenomedea sp.*
	Heptagenidae	*Afronurus sp.*
		Notourus sp.
	Leptophlebiidae	*Thraulus sp.*
Gastropoda	Ancylidae	*Ferrissia sp.*
	Bithyniidae	*Gabbiella africana*
	Hydrobiidae	*Hydrobia accrensis*
	Physidae	*Aplexa waterlotti*
	Planorbiidae	*Gyraulus sp*
		Bulinus globosus
		Bulinus forskalii
		Planorbella sp
		Segmentorbis angustus
	Thiaridae	*Potadoma sp.*

		Potadoma freethi.
Hemiptera	Belostomatidae	*Diplonychus sp.*
	Corixidae	*Micronecta sp.*
	Gerridae	*Limnogonus chopardi*
		Gerridae sp.
	Nepidae	*Ranatra sp.*
	Notocnetidae	*Anisops sp.*
	Pleidae	*Plea sp*
	Veliidae	*Rhagovelia Reitteri sp.*
		Veliide sp.
Lepidoptera	Pyralidae	*Pyralidae sp.*
Odonata	Gomphidae	*Phyllogomphus aethiops*
	Cholorocypidae	*Chlorocypha sp.*
	Coenagrionidae	*Ceriagrion sp.*
		Coenagriidae sp.
		Pseudagrion sp.
	Corduliidae	*Phyllomacromia sp*
	Libellulidae	*Libellulidae sp.*
		Zygonyx torrida
		Zygonyx sp.
Ostracoda		*Ostracoda sp.*
Tricoptera	Hydropsychidae	*Cheumatopsyche falcifera*
	Hydroptilidae	*Protomacronema sp*
	Leptoceridae	*Parasetodes sp.*
		Ceraclea sp
		Setodes sp.

Appendix 31 Results of two-way ANOVA on macroinvertebrate taxa richness (S), total abundance (N), Margalef's diversity (d) and Shannon-Wienner diversity indices with rivers and control and impacts as factors

Descriptive analysis

N: macroinvertebrate abundance

CI	River	Mean	Std. Deviation	N
Control	Surow	293.5	236.561	38
	Subri	288.607	252.954	28
	Total	291.424	241.742	66
Impact	Surow	154.528	205.994	89
	Subri	155.067	101.131	45
	Total	154.709	177.369	134
Total	Surow	196.11	223.944	127
	Subri	206.288	185.795	73
	Total	199.825	210.399	200

S: taxa richness

CI	River	Mean	Std. Deviation	N
Control	Surow	13.3421	5.39407	38
	Subri	19	6.7714	28
	Total	15.7424	6.59909	66
Impact	Surow	14.6854	5.57102	89
	Subri	14.8444	6.64337	45
	Total	14.7388	5.92805	134
Total	Surow	14.2835	5.53187	127
	Subri	16.4384	6.95019	73
	Total	15.07	6.15912	200

D= Margaleff diversity index

CI	River	Mean	Std. Deviation	N
	Surow	2.3112	0.91124	38
Control	Subri	3.3508	1.14019	28
	Total	2.7522	1.13171	66
	Surow	3.0019	0.97874	89
Impact	Subri	2.9132	1.06811	44
	Total	2.9725	1.00599	133
	Surow	2.7952	1.00683	127
Total	Subri	3.0834	1.10972	72
	Total	2.8995	1.05164	199

Shannon diversity index

CI	River	Mean	Std. Deviation	N
Control	Surow	1.4488	0.55539	38
	Subri	1.9591	0.60571	28
	Total	1.6653	0.62654	66
Impact	Surow	1.8905	0.53204	89
	Subri	1.7051	0.59866	45
	Total	1.8282	0.55998	134
Total	Surow	1.7583	0.57402	127
	Subri	1.8025	0.60997	73
	Total	1.7745	0.58626	200

Analysis of Variance

N: macroinvertebrate abundance

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[d]
Intercept	Hypothesis	8327017	1	8327016.747	41937.9	0.003	41937.893	1
	Error	198.556	1	198.556[a]				
CI	Hypothesis	777718.7	1	777718.712	2517.35	0.013	2517.349	1
	Error	308.943	1	308.943[b]				
River	Hypothesis	198.556	1	198.556	0.643	0.57	0.643	0.065
	Error	308.943	1	308.943[b]				
CI * River	Hypothesis	308.943	1	308.943	0.008	0.931	0.008	0.051
	Error	7982329	196	40726.169[c]				

S: taxa richness

	Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[d]
Intercept	Hypothesis	40090.14	1	40090.14	113.14	0.06	113.135	0.596
	Error	354.36	1	354.356[a]				
CI	Hypothesis	82.83	1	82.825	0.26	0.699	0.262	0.056
	Error	316.66	1	316.660[b]				
River	Hypothesis	354.36	1	354.356	1.12	0.482	1.119	0.075
	Error	316.66	1	316.660[b]				
CI * River	Hypothesis	316.66	1	316.66	8.88	0.003	8.882	0.843
	Error	6987.66	196	35.651[c]				

d: Margaleff diversity index

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[d]
Intercept	Hypothesis	1396.23	1	1396.23	148.201	0.052	148.201	0.66
	Error	9.421	1	9.421[a]				
CI	Hypothesis	0.667	1	0.667	0.05	0.86	0.05	0.051
	Error	13.264	1	13.264[b]				
River	Hypothesis	9.421	1	9.421	0.71	0.554	0.71	0.067
	Error	13.264	1	13.264[b]				
CI * River	Hypothesis	13.264	1	13.264	12.985	0	12.985	0.948
	Error	199.18	195	1.021[c]				

Shannon diversity index

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[d]
Intercept	Hypothesis	513.666	1.00	513.666	464.624	0.03	464.624	0.909
	Error	1.106	1.00	1.106[a]				
CI	Hypothesis	0.369	1.00	0.369	0.073	0.832	0.073	0.052
	Error	5.067	1.00	5.067[b]				
River	Hypothesis	1.106	1.00	1.106	0.218	0.722	0.218	0.055
	Error	5.067	1.00	5.067[b]				
CI * River	Hypothesis	5.067	1.00	5.067	16.019	0	16.019	0.978
	Error	61.998	196.00	.316[c]				

Appendix 32 Results of pairwise SIMPER analysis between macroinvertebrate community compositions in impact and control on the Surow and Subri rivers.

Family	Av.Abundance at Surow Impact	Av.Abundance at Surow Control	Av.Diss	Diss/SD	Contrib %
Chironomidae	4.01	8.85	9.15	1.28	12.75
Ceratopogonidae	2.16	5.51	7.57	0.88	10.55
Baetidae	2.61	5.54	6.03	1.25	8.39
Atydae	2.2	1.29	3.77	0.93	5.25
Thiaridae	1.71	0.46	2.81	0.65	3.92
Dytiscidae	1.56	2.22	2.73	1.13	3.8
Caenidae	0.94	1.91	2.71	0.92	3.78
Physidae	1.34	1.58	2.52	1.04	3.51
Coenagrionidae	1.18	1.39	2.08	1.16	2.89
Hydrophilidae	1.16	1.36	1.96	1.11	2.74
Planorbiidae	0.8	1.5	1.74	1.1	2.42
Elmidae	0.76	0.76	1.66	0.78	2.31
Ostracoda	0.55	1.13	1.63	0.72	2.28
Libellulidae	1.23	0.66	1.63	0.86	2.28
Belostomatidae	0.51	1.26	1.58	1	2.21
Oligochaeta	0.51	0.83	1.37	0.82	1.91
Lymnaeidae	0.66	0.66	1.34	0.79	1.86
Collembola	0.76	0.45	1.28	0.79	1.78
Scirtidae	0.37	0.86	1.27	0.79	1.77
Culicidae	0.61	0.58	1.2	0.81	1.67
Notocnetidae	0.64	0.52	1.09	0.7	1.51
Veliidae	0.62	0.09	0.96	0.42	1.33
Hydraenidae	0.45	0.27	0.79	0.61	1.11
Gyrinidae	0.43	0.15	0.78	0.37	1.09
Corixidae	0.27	0.39	0.76	0.55	1.06
Hirudinea	0.34	0.35	0.69	0.75	0.96
Pyralidae	0.3	0.23	0.63	0.56	0.88
Hydracarina	0.17	0.4	0.62	0.61	0.87
Gerridae	0.36	0.17	0.61	0.46	0.85
Pleidae	0.21	0.39	0.6	0.61	0.83
Haliplidae	0.24	0.26	0.52	0.53	0.73
Gomphidae	0.29	0.03	0.48	0.42	0.67
Hygrobidae	0.26	0	0.42	0.41	0.58

Appendix 33 Results of pairwise SIMPER analysis between macroinvertebrate compositions in impact and control on the Subri River

Species	Av.Abundance Subri Impact	Av.Abundance Subri Control	Av.Diss	Diss/SD	Contrib%	Cum.%
Atydae	2.82	3.67	5.3	0.93	7.79	7.79
Thiaridae	5.87	3.36	4.87	1.09	7.17	14.96
Chironomidae	3.49	4.82	4.81	0.74	7.08	22.03
Baetidae	1.92	4.2	3.42	1.21	5.03	27.06
Planorbiidae	2.47	2.53	3.14	0.74	4.63	31.69
Elmidae	2.96	1.03	3.05	1.1	4.49	36.17
Physidae	0.44	2.52	2.53	0.78	3.73	39.9
Caenidae	1.52	1.56	2.5	0.82	3.68	43.58
Dytiscidae	0.78	2.39	2.29	0.86	3.37	46.95
Notocnetidae	0.27	2.04	2.25	0.92	3.32	50.27
Coenagrionidae	1.97	1.24	2	1.14	2.95	53.22
Hydrophilidae	0.34	1.83	1.79	0.85	2.63	55.84
Ceratopogonidae	0.64	1.47	1.69	0.71	2.49	58.33
Gerridae	0.66	1.26	1.55	0.95	2.27	60.61
Culicidae	0.26	1.42	1.42	0.68	2.09	62.7
Veliidae	0.66	1.14	1.4	0.95	2.06	64.76
Gyrinidae	0.85	0.31	1.36	0.41	2.01	66.77
Heptagenidae	0.84	0.58	1.33	0.76	1.96	68.73
Belostomatidae	0.25	1.19	1.3	0.96	1.92	70.65
Libellulidae	0.93	1.36	1.28	1.22	1.88	72.53
Ostracoda	0.43	0.92	1.14	0.64	1.67	74.2
Leptoceridae	0.52	0.66	1.09	0.82	1.6	75.8
Hydraenidae	0.33	0.83	0.97	0.75	1.42	77.23
Collembola	0.64	0.35	0.89	0.74	1.32	78.54
Oligochaeta	0.52	0.34	0.8	0.74	1.18	79.72
Scirtidae	0.3	0.59	0.8	0.7	1.18	80.9
Pleidae	0.19	0.78	0.76	0.71	1.11	82.02
Hydracarina	0.28	0.53	0.67	0.77	0.98	83
Mesoveliidae	0.33	0.47	0.65	0.86	0.96	83.96
Corixidae	0.13	0.67	0.63	0.53	0.93	84.89
Lymnaeidae	0.41	0.24	0.61	0.7	0.9	85.79
Pisidiidae	0.03	0.34	0.57	0.41	0.84	86.63
Ancylidae	0.42	0.13	0.57	0.54	0.83	87.47
Pyralidae	0.24	0.25	0.45	0.54	0.66	88.13
Hygrobidae	0.02	0.48	0.44	0.43	0.65	88.78
Tipulidae	0.3	0.17	0.44	0.65	0.64	89.42
Hirudinea	0.15	0.34	0.43	0.56	0.64	90.05

Appendix 34 Results of pairwise SIMPER analysis between macroinvertebrate community compositions on impact in the Surow Rand Subri rivers

Species	Av.Abund at Surow Impact	Av.Abundance at Subri Impact	Av.Diss	Diss/SD	Contrib %	Cum. %
Thiaridae	1.71	5.87	7.51	1.2	10.47	10.47
Chironomidae	4.01	3.49	5.31	1.11	7.4	17.87
Atydae	2.2	2.82	4.5	1.02	6.27	24.14
Elmidae	0.76	2.96	3.83	1.13	5.34	29.47
Baetidae	2.61	1.92	3.33	1.14	4.64	34.12
Planorbiidae	0.8	2.47	3.14	1.12	4.38	38.49
Ceratopogonidae	2.16	0.64	3.07	0.66	4.28	42.77
Coenagrionidae	1.18	1.97	2.55	1.13	3.55	46.33
Caenidae	0.94	1.52	2.27	1.09	3.17	49.5
Dytiscidae	1.56	0.78	2.21	0.83	3.08	52.58
Gyrinidae	0.43	0.85	1.9	0.4	2.65	55.22
Libellulidae	1.23	0.93	1.85	0.89	2.58	57.81
Physidae	1.34	0.44	1.83	0.8	2.55	60.36
Hydrophilidae	1.16	0.34	1.62	0.88	2.27	62.62
Collembola	0.76	0.64	1.59	0.8	2.21	64.83
Veliidae	0.62	0.66	1.57	0.61	2.19	67.03
Heptagenidae	0.27	0.84	1.33	0.64	1.85	68.87
Gerridae	0.36	0.66	1.2	0.75	1.67	70.54
Lymnaeidae	0.66	0.41	1.16	0.73	1.62	72.17
Oligochaeta	0.51	0.52	1.16	0.81	1.61	73.78
Ostracoda	0.55	0.43	1.05	0.59	1.47	75.25
Culicidae	0.61	0.26	1.02	0.69	1.42	76.67
Notocnetidae	0.64	0.27	0.95	0.64	1.33	78
Hydraenidae	0.45	0.33	0.93	0.59	1.29	79.29
Leptoceridae	0.2	0.52	0.84	0.8	1.17	80.46
Belostomatidae	0.51	0.25	0.83	0.67	1.16	81.62
Scirtidae	0.37	0.3	0.74	0.61	1.04	82.66
Mesoveliidae	0.27	0.33	0.67	0.69	0.94	83.59
Pyralidae	0.3	0.24	0.66	0.62	0.93	84.52
Ancylidae	0.16	0.42	0.66	0.57	0.92	85.44
Gomphidae	0.29	0.12	0.61	0.46	0.84	86.28
Hirudinea	0.34	0.15	0.6	0.61	0.84	87.12
Hydracarina	0.17	0.28	0.59	0.56	0.83	87.95
Tipulidae	0.15	0.3	0.55	0.59	0.76	88.71
Haliplidae	0.24	0.15	0.5	0.47	0.69	89.4
Hygrobidae	0.26	0.02	0.49	0.41	0.68	90.08

Appendix 35 SIMPER analysis on rare microbial communities and microbial communities identified to the Phyla level, comparing mine dewatering factors (no-discharge and discharge) in the Surow River

Rare Phyla	Average abundance		Average dissimilarity	Dissimilarity/SD	Contribution of each phyla to the difference between communities at Discharge and No-dischargeof mine water	Cumulative contribution (%)
	No-discharge	Discharge				
Fusobacteria	0.99	0.21	2.59	1.49	9.44	9.44
OP11	0.2	0.98	2.58	1.76	9.41	18.86
Deinococcus-Thermus	0.63	0.82	2.27	1.3	8.29	27.15
Spirochaetes	0.4	0.83	2.18	1.23	7.97	35.12
BRC1	1.21	0.71	1.82	1.16	6.66	41.78
Chlorobi	1.82	1.36	1.79	0.92	6.53	48.31
WS3	0.93	0.82	1.76	1.08	6.42	54.73
Gemmatimonadetes	1.86	2.3	1.71	1.4	6.22	60.96
Unclassified;Other	1.45	1.52	1.67	1.1	6.09	67.05
Armatimonadetes	1.68	1.87	1.38	1.32	5.03	72.08
Crenarchaeota	1.19	1.29	1.33	0.96	4.85	76.93
SR1	0.45	0	1.28	0.77	4.69	81.61
Fibrobacteres	0.08	0.34	1.12	0.75	4.08	85.69
TM7	0.08	0.35	1.07	0.74	3.9	89.59
Chlamydiae	1.81	1.83	0.98	1.22	3.58	93.17

Phyla	Average abundance		Average dissimilarity	Dissimilarity / Standard Deviation	Contribution of each phyla to the difference between communities at Discharge and No-discharge of mine water	Cummulative Contribution (%)
	No discharge	Discharge				
Cyanobacteria/Chloroplast	0.76	0.78	0.78	1.5	5.92	5.92
Fusobacteria	0.43	0.11	0.77	1.64	5.88	11.8
Nitrospira	0.77	1.03	0.69	1.45	5.27	17.07
Spirochaetes	0.11	0.34	0.67	1.46	5.15	22.22
Bacteria;OP11	0.1	0.34	0.66	1.4	5	27.22
Deinococcus-Thermus	0.23	0.23	0.59	1.13	4.48	31.71
Bacteroidetes	1.38	1.56	0.57	1.12	4.35	36.06
Bacteria;WS3	0.31	0.23	0.57	1.19	4.34	40.4
Euryarchaeota	1	0.76	0.57	1.05	4.32	44.72
Chloroflexi	1.35	1.11	0.56	1.83	4.27	49
Gemmatimonadetes	0.67	0.82	0.55	1.56	4.22	53.22
Chlorobi	0.65	0.46	0.52	0.91	3.97	57.19
Bacteria;Other	2.26	2.04	0.5	1.66	3.78	60.97
Unclassified;Other	0.56	0.52	0.43	1.24	3.27	64.24
Bacteria;SR1	0.19	0	0.42	0.88	3.17	67.41
Crenarchaeota	0.48	0.45	0.41	1	3.1	70.51
Actinobacteria	1.34	1.34	0.4	1.39	3.07	73.59
Firmicutes	1.47	1.48	0.4	1.22	3.02	76.61
Verrucomicrobia	1.27	1.22	0.38	1.21	2.91	79.52
Armatimonadetes	0.56	0.62	0.36	1.43	2.76	82.28
Chlamydiae	0.62	0.63	0.3	1.42	2.28	84.56
Proteobacteria	2.53	2.65	0.28	1.34	2.13	86.69
Planctomycetes	0.94	0.94	0.27	1.33	2.06	88.75
Bacteria;BRC1	0.41	0.38	0.27	1.05	2.05	90.8

Appendix 36 Relationships between dbRDA coordinate axes and orthonormal water quality variables (multiple partial correlations) in the Surow and Subri rivers in April 2014. Strong correlations are in bold.

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA5	dbRDA6	dbRDA7	dbRDA8	dbRDA9	dbRDA10
pH	0.056	-0.323	-0.042	0.02	-0.088	-0.307	0.176	-0.056	**-0.416**	0.225
T	**0.45**	-0.197	-0.342	0.187	-0.087	-0.126	**-0.478**	-0.005	0.081	-0.16
Turb	-0.23	**-0.472**	-0.053	-0.208	0.177	0.267	-0.356	0.067	**-0.373**	-0.196
EC	0.229	-0.03	0.203	-0.063	-0.296	0.235	0.165	-0.323	0.183	-0.16
TDS	0.046	**0.345**	-0.032	-0.072	-0.174	0.059	-0.217	-0.296	-0.198	0.049
DO	0.4	-0.012	0.273	-0.294	0.189	-0.231	-0.049	0.182	-0.262	0.059
ORP	0.155	0.094	-0.083	0.273	-0.115	**0.358**	0.036	-0.097	-0.241	0.286
TKN	0.049	0.285	-0.121	-0.193	0.354	0.001	0.198	-0.278	-0.05	-0.121
NH4	-0.025	0.206	0.263	**0.482**	0.14	0.077	-0.019	-0.084	-0.187	-0.5
NOx	0.009	-0.092	0.18	0.26	**0.629**	-0.066	0.06	-0.287	-0.137	0.174
SO	**0.439**	**0.387**	-0.193	-0.014	0.011	-0.052	0.032	0.234	-0.324	0.055
FRP	-0.286	0.2	**-0.496**	-0.144	0.211	-0.247	0.033	-0.093	0.124	0.109
Ca	0.042	0.002	-0.02	-0.116	-0.146	-0.263	-0.006	-0.282	-0.107	-0.159
Mg	-0.091	0.023	0.063	-0.192	-0.123	-0.332	-0.019	-0.217	-0.168	**-0.482**
Al	0.117	-0.113	0.017	-0.026	-0.032	-0.263	**0.327**	0.181	0.177	-0.165
As	0.265	-0.113	-0.114	-0.22	0.251	0.353	**0.355**	0.25	0.078	-0.299
Cu	0.181	-0.284	-0.256	**0.453**	0.066	-0.21	0.161	-0.133	0.169	-0.066
Fe	-0.268	0.148	**-0.342**	0.227	-0.105	0.042	0.147	**0.345**	**-0.307**	-0.263
Mn	0.012	0.231	0.189	0.072	0.235	-0.161	**-0.434**	0.291	**0.304**	-0.06
Pb	-0.163	0.044	**0.345**	0.18	-0.174	-0.244	0.148	0.29	-0.114	0.105

Appendix 37 Correlations between coordinate axes of MDS of microbial community assemblage and log water quality variables in the Surow and Subri River, April 2014

** Correlation is significant at the 0.01 level (two tailed). * correlation is significant at the 0.05 level (two tailed)

	MDS	T	Turb	EC	NH	Nox	Sulfate	FRP	Al	As	Fe	Mn	Pb
Pearson's r	MDS1	.389	-.103	.398	.100	.202	.654**	-.342	-.066	.308	-.287	.158	-.124
Sig. (2-tailed)		.100	.674	.092	.685	.407	.002	.152	.787	.199	.234	.517	.614
N		19	19	19	19	19	19	19	19	19	19	19	19
Pearson's r	MDS2	-.121	-.621**	.282	-.177	-.116	.376	.298	.184	.232	.089	.084	.063
Sig. (2-tailed)		.622	.005	.242	.468	.635	.113	.216	.451	.338	.717	.733	.797
N		19	19	19	19	19	19	19	19	19	19	19	19
Pearson's r	MDS3	-.382	.103	.196	-.339	-.256	-.158	-.469*	-.186	-.156	-.525*	.056	.302
Sig. (2-tailed)		.107	.675	.422	.156	.291	.518	.043	.446	.522	.021	.820	.209
N		19	19	19	19	19	19	19	19	19	19	19	19

Appendix 38 Relationships between dbRDA coordinate axes and orthonormal river water quality variables (multiple partial correlations) in the Surow River on April 2014. Strong correlations are in bold.

Variable	dbRDA1	dbRDA2	dbRDA3	dbRDA4	dbRDA5	dbRDA6	dbRDA7	dbRDA8	dbRDA9	dbRDA10
pH	-0.33	-0.259	-0.022	-0.107	-0.008	0.236	**0.438**	-0.265	-0.133	0.266
T	-0.214	-0.36	-0.258	0.194	-0.185	**0.494**	**-0.314**	0.056	0.031	-0.204
Turb	0.036	-0.35	0.365	-0.075	**-0.534**	-0.017	-0.16	-0.109	0.205	-0.32
EC	**-0.318**	0.21	0.179	0.05	**0.314**	0.129	-0.184	0.257	**0.342**	0.041
TDS	-0.144	0.247	-0.006	0.029	0.055	0.302	0.109	0.223	**0.387**	-0.067
DO	**-0.428**	0.273	0.104	-0.044	-0.161	-0.164	**0.319**	-0.321	0.127	-0.138
ORP	-0.083	0.097	-0.128	0.098	0.212	0.063	0.061	-0.091	0.119	**-0.576**
TKN	0	0.003	-0.084	0.082	0.037	-0.079	0.065	-0.119	0.186	0.018
NH	-0.284	-0.344	0.057	0.192	0.073	**-0.341**	0.138	0.359	-0.044	-0.184
Nox	0.153	0.062	0.131	0.536	-0.011	-0.186	0.029	0.024	-0.161	-0.194
SO	-0.213	0.184	**-0.313**	-0.054	-0.137	-0.02	-0.139	-0.399	-0.215	-0.187
FRP	0.254	-0.137	**-0.379**	-0.184	-0.076	0.271	**0.412**	0.27	0.079	-0.085
Ca	-0.041	0.076	0.111	-0.196	0.034	0.169	0.14	0.22	**-0.457**	-0.244
Mg	0.023	0.126	0.143	-0.121	0.12	0.064	0.001	0.179	-0.46	-0.235
Al	-0.077	-0.13	0.021	-0.13	0.228	-0.101	0.238	-0.055	0.088	**-0.356**
As	**-0.438**	-0.092	**-0.334**	-0.302	-0.019	**-0.341**	**-0.333**	0.257	-0.107	0.093
Cu	-0.205	-0.245	-0.057	**0.532**	0.099	0.01	0.207	0.005	-0.138	0.217
Fe	0.232	-0.044	**-0.491**	0.126	0.253	-0.163	-0.097	-0.212	0.074	-0.127
Mn	-0.098	**0.457**	-0.21	0.305	**-0.497**	0.072	0.059	0.228	-0.122	0.031
Pb	-0.112	0.004	0.195	0.101	0.295	0.373	-0.277	-0.256	-0.221	0.027

Appendix 39 Correlations between coordinate axes of MDS of microbial community assemblage and log water quality variables in the Surow in April 2014

** Correlation is significant at the 0.01 level (two tailed). * correlation is significant at the 0.05 level (two tailed)

	MDS axis	pH	T	Turb	EC	TDS	DO	ORP	TKN	NH	NOx	Sulfate	FRP	Ca	Mg	Al	As	Cu	Fe	Mn	Pb
Pearson's r	MDS1	-0.46	-0.31	-0.02	-0.51	-0.47	-.72**	0.04	0.00	-0.21	-0.06	-.576*	.56*	-0.22	-0.19	0.18	-0.43	-0.25	0.34	-0.30	0.11
Sig		0.12	0.30	0.95	0.08	0.11	0.01	0.91	0.99	0.49	0.85	0.04	0.05	0.47	0.55	0.56	0.15	0.41	0.26	0.32	0.73
N		13	13	13	13	13	13	13	13.	13	13	13	13	13	13	13	13	13	13	13	13
Pearson's r	MDS2	0.06	0.41	0.48	-0.45	-0.35	-0.37	-0.13	0.02	0.34	0.25	-0.23	0.18	-0.10	-0.10	0.06	-0.12	0.29	0.15	-0.38	-0.15
Sig		0.84	0.17	0.10	0.13	0.24	0.22	0.67	0.94	0.25	0.41	0.45	0.56	0.74	0.73	0.84	0.69	0.33	0.63	0.20	0.62
N		13	13.00	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
Pearson's r	MDS3	0.18	0.03	.770**	-0.33	-0.23	0.33	-0.28	-0.24	0.26	0.48	-0.04	-0.45	0.06	0.03	-0.23	-0.27	0.29	-.56*	0.27	-0.11
Sig		0.55	0.92	0.00	0.27	0.45	0.27	0.36	0.44	0.39	0.10	0.89	0.12	0.84	0.91	0.45	0.38	0.33	0.05	0.36	0.73
N		13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13