DETERMINING THE RELATIVE IMPACTS OF NATURAL AND ANTHROPOGENIC CONTAMINATION IN THE TAMBO RIVER BASIN, PERU

by

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ABSTRACT

Peruvian Andean Watersheds are under pressure. Mining, agriculture, urbanization, and natural stressors such as climate chance and geothermal and volcanic activity are causing water quality to deteriorate. Although rapidly declining, little attention has been given to understanding the current state of these watersheds and literature is often limited to grey literature such as academic theses and governmental reports which are difficult to access. Without baseline information on water quality, it can be challenging to properly identify sources of contamination and effectively manage these aquatic ecosystems.

In this context, the water quality of the Tambo River Basin was assessed. Located in Southern Peru, the Tambo River Basin is subject to pressures from a variety of stressors such as a metal mine at its headwaters, geothermal and volcanic activity, agriculture, and urbanization. Water chemistry samples, physicochemical water quality measurements and benthic macroinvertebrates were collected from 15 sites across the basin, representing the different sources of contamination from source to mouth.

Signs of contamination were evident at sites impacted by the mines, where acidic pH was recorded along with high metal concentrations exceeding Peruvian Water Quality standards for both drinking water and the aquatic environment. Sites along geothermal sources of contamination showed high concentrations of arsenic exceeding Peruvian Water Quality standards for both drinking water and the aquatic environment. At the most downstream and urbanized sites, high concentrations of lead were recorded.

To our knowledge this is the first study of benthic macroinvertebrates in the region, thus it provides baseline information on the benthic macroinvertebrate communities of the

Tambo River Basin. Changes in abundance, taxa richness and EPT% were compared at sites with different sources of contamination. More pollution-tolerant taxa dominated the contaminated sites and pollution-sensitive taxa were present at sites that had better water quality. Overall, this study provided baseline information on the water quality of the Tambo River Basin and helped to identify sources of contamination entering the basin contributing to pressures on the water quality and the aquatic environment. The use of benthic macroinvertebrates helped supplement physicochemical and chemistry water quality data which provides information on the conservation and management of the aquatic environment. However, given the complexity of the altitudinal gradient of this basin, this type of study should be repeated during both the dry and rainy seasons to further build upon this database. Efforts should also be directed towards preserving the ecological integrity of high altitudinal wetlands (bofedales) as they possess contaminant filtering capacity.

KEYWORDS: water quality; benthic macroinvertebrates; bioindicator; Peruvian watershed; gold mining; urbanization; agriculture; Andes mountains; high-altitude streams.

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LIST OF ABBREVIATED TERMS

ARD: Acid rock drainage

DEM: Digital elevation model

DO: Dissolved Oxygen

EPT: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)

M.a.s.l.: Meters above sea level

ORP: Oxidation-reduction potential

TDS: Total dissolved solids

TOC: Total Organic Carbon

GENERAL INTRODUCTION

Peruvian Andean streams are under pressure, and watersheds are threatened by natural stressors such as geologically occurring metals and acids, as well as climate change. Further, anthropogenic activities such as agriculture, urbanization and mining are adding to an increasing list of stressors. This is especially true for the Tambo River Basin, Peru. There is an urgent need to understand these complex systems as pressures increase in prevalence and water supplies dwindle (Bebbington & Bury, 2009). The need for baseline data is paramount in the design of effective monitoring programs, developing long-term conservation plans, and prioritizing remediation strategies.

Chapter 1 of this thesis consists of a literature review that introduces the existing knowledge on the water quality of Andean streams, specifically Southern Peruvian Andean streams. It discusses current research themes with a focus on streams that are impacted by anthropogenic and natural sources of metals and acids. Literature is provided on physicochemical water quality studies, the importance of using bioindicators to supplement physicochemical water quality studies, and the use of benthic macroinvertebrates as bioindicators. Emphasis is put on assessing the state of current knowledge of benthic macroinvertebrates in South America as well as their potential use for biomonitoring. The watershed in this study, the Tambo River Basin, is introduced in this chapter as is its need for baseline data to gain an understanding of the current state of the water quality.

Chapter 2 of this thesis provides a detailed description of the sites selected for this study. Furthermore, this information is supplemented with the physicochemical water quality parameters measured along with results from water samples collected from these sites.

Chapter 3 of this thesis incorporates benthic macroinvertebrate data to supplement the water quality assessment at the various sites sampled throughout the basin. The use of benthic macroinvertebrates communities is introduced in this chapter where the presence/absence and abundance of certain taxa are used to further understand the extent of contamination within the Tambo River Basin.

Findings from Chapter 2 and Chapter 3 are combined in Chapter 4 to provide concluding remarks, suggest future directions for research in the Tambo River Basin, as well as discuss how the results from this study will help inform monitoring and remediation plans for the area.

CHAPTER 1: THE USE OF BENTHIC MACROINVERTEBRATES TO ASSESS THE WATER QUALITY IN ANDEAN STREAMS

Peruvian Andean streams provide ecological services, flood mitigation, nutrient recycling, habitat availability for biota and most importantly, irrigation and water for human food resources (Jacobsen & Marín, 2008; Villamarín et al., 2013). Despite their ecological and economic significance, agriculture, livestock, deforestation, urbanization and the mining industry are threatening the ecological integrity of these streams (Prat et al., 2009; A. Ccanccapa-Cartagena et al., 2021). These human-induced pressures along with seasonally variable rain supply and climate change are additional stressors (Bury et al., 2013). Equally important, there is consensus that volcanic and geothermal activity along with natural erosional processes have a notable influence on the concentrations of metals and acids of the region (Gałaś, 2014; Grande et al., 2019; Morales-Simfors, et al., 2020; A. Ccanccapa-Cartagena et al., 2021). Naturally occurring metals and metalloids found in these systems are transported to surface and groundwaters through run-off and infiltration creating environmental problems and hazards to public health (Batayneh, 2010; Jaishankar et al., 2014; Saleem et al., 2019).

Watersheds in Southern Peru are challenging to study. They have inconsistent water supplies due to seasonality, causing highly variable flows at the headwaters, water deficits downstream of the basin during the dry season, and flooding in the coastal valleys during the rainy season (Mortensen et al., 2018; A. Ccanccapa-Cartagena et al., 2021). The varying flows in these rivers along with the deep canyons cutting through the alpine desert landscape, make access difficult.

There are also systemic challenges such as anthropogenic activities including agriculture, mining, and urbanization. These anthropogenic activities have different ecosystem impacts that are co-occurring without integrating background information on biophysical processes, aquatic ecology, and land-use patterns. Therefore, information on pre-impact conditions is limited (Mercado-Garcia et al., 2019). Consequently, the lack of baseline data hinders the ability to distinguish mining impacts from natural sources of metals and acids and to incorporate reference or control streams to compare contaminated sites vs. uncontaminated sites (Mercado-Garcia et al., 2019).

Nonetheless, having monitoring plans and understanding the current state of water quality is becoming increasingly important with climate change and water shortages (Bebbington & Bury, 2009). Many high-altitude ecosystems are currently showing deteriorating water quality, yet high Andean rivers are amongst some of the least-studied ecosystems (Coayla-P et al., 2022). Much of the literature is still limited to grey literature in the form of academic theses or reports by government agencies which leaves a large quantity of valuable information hidden or challenging to access (Arana Maestre et al., 2021).

This is particularly true for the Tambo River Basin. There have been several technical reports released in the region by the Ministry of Energy and Mines (MINEM), the National Water Authority (ANA) and the National Institute of Civil Defense (INDECI) but widely available publications focusing on the water quality of the region remain scarce. A 2021 publication by (A. Ccanccapa-Cartagena et al., 2021) looked at the health risks of dissolved metals in the surface waters of Southern Peru. This study included measurements of elements of concern in groundwater, river water and lake water in multiple basins surrounding the Tambo River Basin. Although this study gives us a good understanding of the general conditions of the area, the Tambo River Basin was not included in the study.

There have been several water emergencies in the region with multiple media articles discussing contamination reported in the Tambo River Basin. Some were as recent as April 2022, where an article claimed that 60,000 residents in the Tambo River Basin were consuming water contaminated with metals such as arsenic, boron, and lead (El Buho, 2022). Contamination from the mine at the headwaters was blamed and was said to have been ongoing for many years. However, specific data and knowledge on the extent of contamination remains limited. The Tambo River Basin is particularly complex and distinguishing the impacts from the mine, volcanic and geothermal activity along the basin mixed with anthropogenic activities such as urbanization, agriculture, and mining, can be challenging. Providing the scientific community with baseline data on the water quality of the Tambo River Basin is essential in understanding where to direct remediation efforts and inform the design of a long-term monitoring plan.

Past studies that assessed the water quality of these rivers have been composed of physicochemical measurements where biological components were not considered (Villamarín et al., 2013). Using a stressor-based approach such as measuring physicochemical water quality parameters focuses on monitoring stressors causing environmental changes. Although useful in informing how to regulate the sources of the stressors, it provides no information on resource management. There is a need to supplement a stressor-based monitoring approach with the assessment of biological responses. By incorporating biological components which allow assessment of how biological communities respond to natural and anthropogenic stressors, and will facilitate the protection and management of these aquatic ecosystems (Roux et al., 1999; Li et al., 2010).

Many anthropogenic activities are conducted without environmental monitoring and the need for assessing the current health of aquatic systems through bioindicators is beginning to become more widely explored (Custodio et al., 2019a). Bioindicators have proven to be useful tool that supplements traditional monitoring (Burger, 2006). Studies on the use of benthic macroinvertebrates as bioindicators for streams have been widely reported in the literature, but work on southern Peruvian Andean benthic macroinvertebrate assemblages is limited (Gerhardt, 2002; Custodio et al., 2019a). Benthic macroinvertebrates are taxonomically diverse, bottom-dwelling invertebrate organisms (Burgess, 2015). They can provide information on environmental conditions as well as indicate sudden changes in streams and rivers since they reflect the overall ecological quality, and integrate the effects of different stressors (Iliopoulou-Georgudaki et al., 2003). They are effective bioindicators as they can be easily identified by a non-specialist, are abundant, are suitable for laboratory experiments, respond sensitively to environmental stressors; and have a high ability for quantification and standardization (Gerhardt, 2002; Li et al., 2010; Villamarín et al., 2013). Benthic macroinvertebrate assemblages are composed of various species across a broad spectrum of environmental tolerances and thus react to pollution in different ways (being more or less sensitive to particular pollutants) resulting in information that can help assess water quality (Li et al., 2010). In streams that are polluted with organic matter, heavy metals, or natural sources of metals and acids, the diversity and abundance of benthic macroinvertebrates is typically reduced (Custodio et al., 2019a).

In South America, most studies that have used benthic macroinvertebrates have focused on the anthropogenic effects on streams such as agriculture, mining, animal grazing and urbanization (Sweeney et al., 2020). Sites where anthropogenic impacts are the most severe have been associated with low macroinvertebrate abundances (Lujan et al., 2013). Multiple factors have been confirmed as influencing the composition of benthic macroinvertebrate communities in South America such as high loads of organic matter in wastewater, fertilized agricultural soils, phosphorous from cattle activities, and metal contamination (Loayza-Muro et al., 2010a; Custodio et al., 2018, 2019a). However, a unique characteristic of the Tambo River Basin is its high altitude. Studies have shown that altitudinal gradients influence physical and chemical changes throughout a watershed that ultimately influence the benthic macroinvertebrate diversity (Lujan et al., 2013). Both physicochemical parameters such as temperature and dissolved oxygen are key factors in the distribution of benthic macroinvertebrate communities in these river systems as well as processes associated with the physical habitat (Custodio et al., 2019a). Loayao-Muro et al., (2010) concluded in their study that several variables indicated that macroinvertebrate change with elevation but that despite the harsh conditions in these environments, the diversity of macroinvertebrates at high-altitude streams is substantial (Loayza-Muro et al., 2010a).

Knowledge of benthic macroinvertebrates in South America lags far behind what is known for vertebrates such as fish. Generally, vertebrates are easier to study than invertebrates as they are large, easy to identify, and low in diversity. Moreover, guides to facilitate taxonomy are widely available. Conversely, macroinvertebrates have high diversity but require a stereomicroscope for identification as they are small in size making identification challenging (Zarges et al., 2019). Most studies on benthic macroinvertebrate communities to date have focused on the high Andean regions in the north of the country but studies in central and southern Peru are minimal (Custodio Villanueva & Chaname Zapata, 2016). There is a clear gap in the knowledge of benthic macroinvertebrates in these central and southern regions which limits their use as local bioindicators.

Habitats in southern Peru differ substantially from other regions of Peru. They are arid to semi-arid while the rest of Peru consists of tropics, shrublands and coastal hills (Arana Maestre et al., 2021). The watersheds of Southern Peruvian basins also have drastic topographical changes ranging from flat to steep lands, resulting in considerable differences in altitudes. The varying altitudinal gradient within a watershed causes changes in vegetation, physicochemical water parameters, hydrological dynamics and ultraviolet exposure which in turn influences benthic macroinvertebrate communities (García-Ríos et al., 2020). There are inconsistencies in the interpretation of benthic macroinvertebrates in some studies which can limit the application of benthic macroinvertebrates as a bioindicator. In a study by Loayza-Muro et al., (2010) overall richness and abundance between reference and polluted sites did not differ significantly, suggesting that these metrics may not be adequate in assessing contamination. Similarly, a study by Sweeney et al. (2020) indicated that in some circumstances it was difficult to differentiate benthic macroinvertebrate communities between control and impacted sites. The lack of studies involving the use of benthic macroinvertebrates in this particular landscape leads to difficulties in determining long-term conservation and management of these watersheds (Acosta et al., 2009; Gauthier et al., 2013; Villamarín et al., 2013).

Although knowledge of the use of benthic macroinvertebrates and their application is relatively new for this region, publications on these aquatic ecosystems in Peru are on the rise. This past decade, 40 publications have been released on benthic macroinvertebrates in the semi-arid to arid aquatic ecosystems of Peru. Within those, rivers were the type of aquatic ecosystem that accounted for most of the publications (Arana Maestre et al., 2021). Sampling was conducted using mainly the Surber and D net methods. With current knowledge, some of the most common families are Chironomidae, Baetidae, Simuliidae, Elmidae, Hydrophilidae, Libellulidae, Physidae, Dytiscidae, Ceratopogonidae, Coenagrionidae, Hydroptilidae, Hydropsychidae, and Tipulidae (Arana Maestre et al., 2021). Multiple studies have taken place in central Peru, specifically at the Cunas River and the Mascon River where water quality was assessed by using benthic macroinvertebrates as bioindicators. There have also been several studies directed toward developing score-based biotic indices using tolerance values that were modified for Andean streams. Considering the unique ecological and geographical features of the Andes, macroinvertebrate indices used in other regions must be adapted. The two main indices adapted for the Andes are the Andean Biotic Index (ABI) and another index designed for high Andean. In recent years, these indices have been incorporated into several studies (Silva-poma et al., 2021; Pimentel et al., 2022; Ríos-Touma et al., 2022).

Increasing the number of studies in the Tambo River Basin will help determine sources of contamination in this basin and help inform how contamination is impacting the aquatic environment. Understanding the distribution of benthic macroinvertebrates communities in the region, and their tolerance to pollution will lead towards understanding basin-specific trends in water quality (Ríos-Touma et al., 2014). If tolerance values are developed that represent taxa sensitivities to the predominant pollutants found in the southern Peruvian Andean streams, it would lead to a better performance of tolerance metrics and indices of biotic integrity, in turn leading to better management and restoration efforts (Yuan, 2004; Chang et al., 2014b). By refining the knowledge of local benthic macroinvertebrate communities, periodic sampling of benthic macroinvertebrates will have the potential of being a useful bioassessment tool for water management, as is used in many countries (Arana Maestre et al., 2021).

To advance our knowledge of benthic macroinvertebrates in the Tambo River Basin, refining sampling techniques that will be successful both during the rainy and dry seasons is important. This is particularly important as with the presence of ephemeral streams, certain rivers may not be accessible with waders while others might be completely dry. Method development should also be explored to establish standard protocols for collecting, sorting, and analyzing benthic macroinvertebrates. It has been repeatedly mentioned in the literature that the need to refine taxonomic keys is substantial in Peru (Arana Maestre et al., 2021; Zarges et al., 2019). Training should be provided in terms of taxonomic identification to have future taxonomic specialists in the region. However, the use of DNA barcoding as an alternative method to traditional morphological taxonomic classification should also be considered.

A subsequent task would be to determine if there are rivers that can be used as reference conditions. Once methods are standardized, sampling in the region increases, and data interpretation is refined there will be a large set of data that can then be used in the development of indices specific to the region for different habitat types.

Therefore, as baseline information on the water quality of the Tambo River Basin is limited, this study will focus on first identifying sources of contamination throughout the basin. Physicochemical measurements along with water chemistry sampling will be conducted at various locations across the basin. Sampling will occur near several sources of contamination as they enter the basin from source to mouth. This includes a mine at the headwaters, volcanic and geothermal sources contamination, agriculture, pasturelands, and urbanization. This will provide information on sources of contamination while working to distinguish natural vs. anthropogenic sources of contamination. To be able to understand the impacts on the aquatic environment, our research will describe the benthic macroinvertebrate community in the Tambo River Basin and explore patterns as they relate to different sources of metals and acids whether anthropogenic or naturally occurring. If more baseline data become available, additional tolerance values can be determined to help create more complete reference datasets.

As the Tambo River Basin has a landscape that is challenging to access and ever-changing water flows, the information gained from this study will also help determine if sampling using the kick and sweep method is possible during the dry season and help establish sampling protocol development for the region. Baseline information on the benthic macroinvertebrate community of the basin will be able to be built upon to further increase our knowledge of benthic macroinvertebrates in the region and increase the robustness of future studies. To our knowledge, this thesis represents the first effort to conduct a study using benthic macroinvertebrates in the Tambo River Basin. This study contributes towards advancing the knowledge of benthic macroinvertebrates in the Southern Peruvian Andes, in watersheds that have stressors such as mines, volcanic and geothermal sources and other anthropogenic stressors such as urbanization and agriculture. Most importantly it will be the first accessible dataset of benthic macroinvertebrates available for this location. Increasing the number of publications can also lead to collaborations with international specialists that have successfully applied standardized methods, such as tolerance values and indices for specific environments. Stressor indices have been developed for other regions such as in Europe, Australia and North America, and they may also be applicable in Andean streams.

CHAPTER 2: WATER QUALITY OF THE TAMBO RIVER BASIN

2.1 ABSTRACT

The Tambo River Basin is exposed to several sources of contamination from source to mouth. Volcanic and geothermal activities as well as anthropogenic activities such as agriculture, urbanization, and mining at the headwaters have led to deteriorating water quality in the basin. In this context, the water quality of the Tambo River basin was assessed to identify sources of contamination. Physicochemical parameters and water chemistry samples were taken at a total of 15 sites across the basin during the 2021 dry season; sampling occurred by the mine headwaters, at sites unimpacted by the mine at the headwaters, near a volcano mid-basin, near hot springs mid-basin and along coastal urbanized areas. Results revealed that the mine-impacted sites had acidic waters with pH, arsenic, boron, nickel, iron, copper and iron all exceeding Peruvian Water Quality Standards. Sites sampled near the geothermal sources had the highest boron, arsenic and lithium levels recorded in the entire basin, which again exceeded Peruvian Water Quality Standards. Urbanized coastal sites had high lead levels compared to what was previously recorded in the basin and phosphorous levels that exceeded Peruvian Water Quality Standards. This data set can serve as a basis for establishing a monitoring program and developing management and mitigation plans to conserve the aquatic environment and ensure its various water uses.

2.2 INTRODUCTION

Water is one of the most important factors that profoundly influences life. Water quality is usually described according to its physical, chemical, and biological characteristics. In many regions of the world, natural and anthropogenic sources of metals and acids are causing heavy and varied pollution in the aquatic environment leading to the deterioration of water quality and depletion of aquatic biota. This is especially true for developing countries such as Peru. Contamination of water can originate from natural processes such as mineral weathering and erosion. However, industrial activities from the dumping of mine waste, pesticides, fertilizers and sewage are equally important sources of contamination responsible for diminishing water quality (Ccanccapa-Cartagena et al. n.d.; Bebbington & Williams, 2008).

The Tambo River Basin's aquatic ecosystem is sensitive and heavily influenced by a variable climate and natural stressors such as geologically occurring metals and acids from source to mouth (A. D. Ccanccapa-Cartagena et al., n.d.). Anthropogenically, the water quality of the Tambo River Basin has been impacted by the mining industry, agriculture, and urbanization. Generally, water resources are limited as they are unevenly distributed across the region due to variation in topography, climate, and hydrology. This variation is quite extreme; there is currently a monthly water supply deficit of 23.65 million cubic meters during the dry season in the region and water supply deficits within a year have reached 1.5 million cubic meters (Carlos Mendoza et al., 2021). Therefore, ensuring that what little supply is available remains uncontaminated is important. The Florencia Tucari mine is located at the headwaters of the Tambo basin. This mine is known to have contributed to significant water-related issues. Since 2016, water pollution due to acid rock drainage has changed the water colour to yellow and orange as a product of mine waste from the mining unit (INDECI, 2021). The middle of the basin is influenced by an elevated population practicing agriculture. Furthermore, currently active geothermal and volcanic activity become apparent mid-basin. In the downstream section of the basin, there is an increase in population along with various industrial activities (Szykulski et al., 2014).

Previous reports by the Alto Tambo Local Water Administration (ALA Tambo), the National Civil Defense Institute (INDECI) and the Agency for Environmental Assessment and Enforcement (OEFA) have shown concentrations of heavy metals (arsenic, aluminum, boron, cadmium, cobalt, iron, nickel, and zinc) exceeding the maximum permissible limits for water quality in the headwaters. This upstream contamination has exposed agriculture, livestock, aquaculture, and human populations to contamination from the mines at the mid-stream Coralaque and Tambo Rivers (Lopez Arisaca, 2018; Garcia Flores de Nieto, 2019; INDECI, 2021). Although numerous reports exist on the water quality of the region, this information is limited to grey literature that is challenging to access. Furthermore, studies were often focused on specific sub-basins or rivers and did not encompass impacts within the entire basin.

One of the factors affecting people's well-being and the health of ecosystems is water quality. In Peru, one of its most pervasive and concerning environmental issues is its steadily declining water quality. In this context, water quality of the Tambo River Basin in a variety of locations impacted by potential sources of contamination were evaluated. This chapter provides; (1) a detailed characterization of sites throughout the watershed along with a current diagnosis of the water quality of the Tambo River Basin during the dry season. Water quality parameters include pH, dissolved oxygen, temperature, conductivity/salinity, and the concentrations of metals and nutrients (phosphorus & nitrogen); (2) A determination of the flows and distribution of natural and anthropogenic sources of heavy metals and nutrients along the watershed; And (3) provides baseline information that can be compared to existing reports and Peruvian Water Quality Standards to identify areas of concern.

2.3 STUDY BASIN

The Tambo River Basin is located in the Southern Peruvian Andes between latitudes 15° 40'S and 17° 15' S, and longitudes 69° 50'W and 72° 00'W and includes the provinces of Sánchez Cerro and Mariscal Nieto in the department of Moquegua, provinces of Arequipa and Islay in the department of Arequipa, and Puno and San Román in the department of Puno (ANA, 2013; Mendoza et al., 2021). In total, the basin is over 13,361 km^2 in area and is 289 km long (Carlos Mendoza et al., 2021). The main tributaries contributing to the Tambo River Basin are the Ichuña, Carurnas, Coralaque and Paltature (Mendoza et al., 2021). The Tambo River itself is the largest watercourse in the region. The Tambo River Basin flows east to west towards the coastal zone, thereby connecting communities from mountain peaks to coastal valleys (Szykulski et al., 2014); Figure 2.1).

Southern Peru has two different seasons. The dry season is from late March to November and the rainy season is between December and February (Garreaud, 2009). The climate of this region is strongly driven by the presence of the Andes, by the Southern Trade winds, the El Nino-Southern Oscillation (ENSO) cycle, and the Humboldt Current System (A. Ccanccapa-Cartagena et al., 2021). The region is known for its varying thermal conditions. According to the Köppen criteria (a method of classifying climate based on seasonal precipitation and temperature patterns), three distinct climatic types can be identified: a very dry semi-warm climate (desert or subtropical arid) with an average annual rainfall of 150 mm and average annual temperatures of 18° C to 19° C; a temperate sub-humid climate (steppe and low inter-Andean valleys) with average annual temperatures surpassing 20° C and annual precipitation below 200 mm; and a cold or boreal climate (Meso-Andean Valleys) characterized by average annual precipitation of 300 mm and annual temperatures less than 12° C (Carlos Mendoza et al., 2021). As a result of the strong seasonality of rainfall, many of the tributaries are intermittent and ephemeral.

The geology of the Tambo River basin shows various events that have caused the deposition of sediments of marine and continental facies, as well as plutonic and volcanic events. The rocks that outcrop the basin range from the Precambrian to the Recent, correspond to clastic and carbonate sedimentary sequences, metamorphic facies, intrusive plutons and a large percentage of lava deposits and volcanic pyroclastic flows associated with significant volcanism during the Paleogene-Neogene. Regarding the sedimentary rocks, there are sandstones, shale, limestone, and conglomerate levels, which in some cases are stratified with levels of volcanic materials. Regarding metamorphic rocks, gneiss (Basal Complex of the Coast) are present. Representative igneous rocks are granitoid in composition. Volcanic and volcanoclastic rocks cover large tracts of terrain and the rocks found in the basin vary from Paleozoic to the Pleistocene (Lopez Catacora, 2020).

The Tambo River Basin is an area with harsh climatic conditions at high altitudes, with treacherous terrain, where snowy mountain peaks and alpine conditions are present year-round (Montesinos-Tubée, 2011). This area is also known for its high tectonic activity and is part of the Quaternary volcanic range which belongs to the Central Volcanic Zone (CVZ) of the Andes. Well-known volcanic features include the Ubinas Volcano, the Ticsani Volcano, the Puente Bello Geyser, and the Meaderos Omate hot springs (Thouret et al., 2005). The rivers of this basin have cut deep canyons through the soft, sedimentary rock, which are part of some of the driest rivers on Earth and extremely susceptible to erosion (A. Ccanccapa-Cartagena et al., 2021). Land uses of the Tambo River Basin are a combination of agricultural land, shrubland, barren and sparsely vegetated zones, as well as arid and barren areas, and water with arid and barren land dominating most of the basin (Carlos Mendoza et al., 2021). The large mammals found most often in the area are the Andean camelids including vicuña, guanaco, llama, and alpaca. They are often kept as livestock and maintained in pastoral regions in surrounding settlements (Jaksic, 2005).

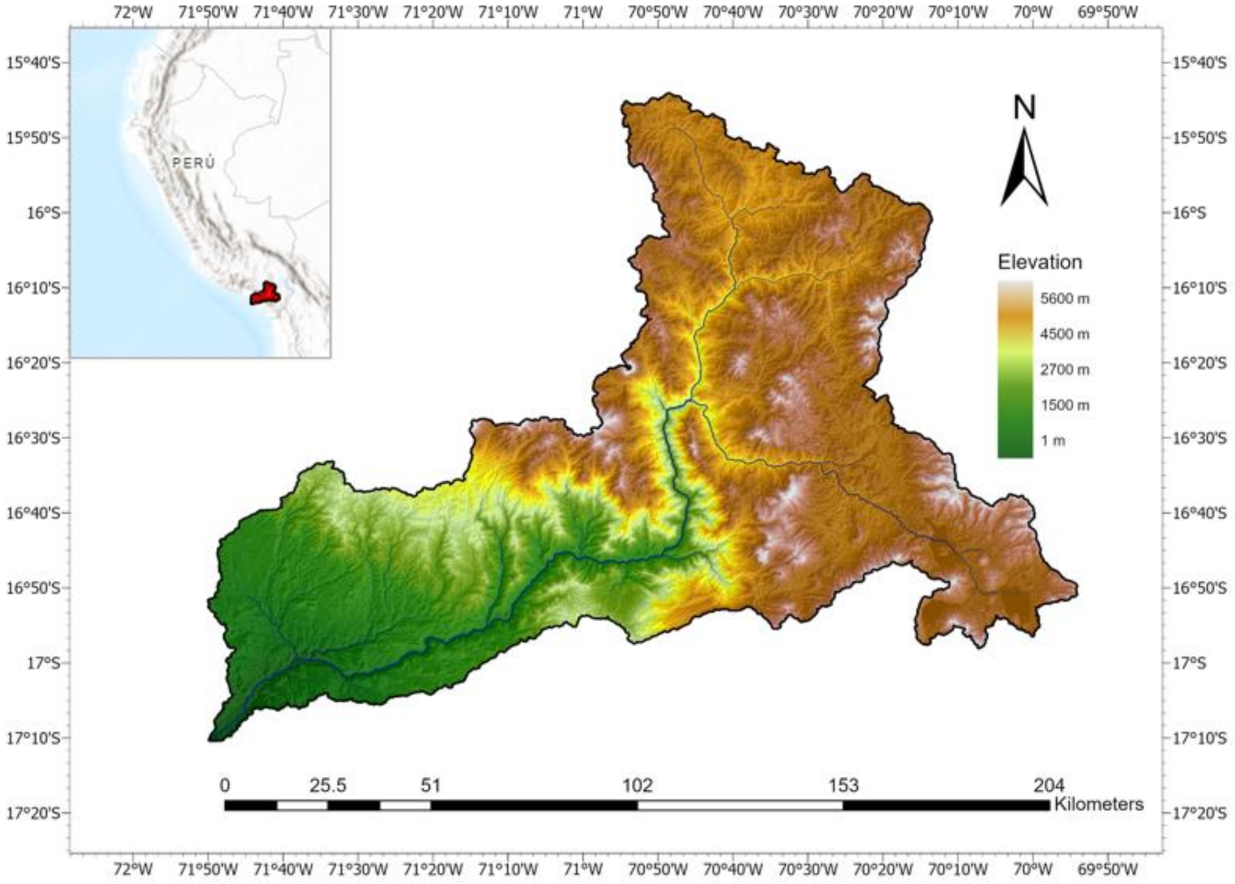


Figure 2.1: Location of the Tambo River Basin in Peru and Digital elevation model (DEM).

2.4 SAMPLING SITES GROUPINGS AND BACKGROUND

Due to the altitudinal gradient and varying stressors throughout the basin from high to low altitudes, sampling sites were divided into six groups. Sites within the same group had similar elevations, and surrounding land uses and were deemed to be exposed or unexposed to the same stressors. For example, sites closest to the mine upstream of the basin were grouped to reflect the potential presence of a mining stressor. For sites near natural sources of metals and acids (volcanic, geothermal activity), the group includes a site upstream of the source to capture background water quality conditions and downstream of the source. These groups will be used to compare the various stressors as they occur in the basin (Figure 2.2). The following groups have been defined: Group 1 (Tucari River, Aruntay River, Titire River) includes the most upstream sites and the sites nearest to the mine, Group 2 (Pacchani River, Vizcachas River) includes sites that are at high elevations but are suspected to be unimpacted by the mine, Group 3 (Coralaque Wetland, Coralaque River) includes sites that are further from the mine and encompasses a wetland that is part of a conservation area and has no known direct source of contamination, Group 4 (Coralaque Huarina, Ubinas River, Alto Tambo) includes sites near the Ubinas Volcano, where the natural source of metals and acid is introduced into the watershed, Group 5 (Tambo-US-Vagabundo, Vagabundo, Tambo-DS-Vagabundo) includes sites near a geothermal source, and Group 6 (Tambo Fiscal, Tambo Costanera) includes the most downstream sites in the most densely populated area (Table 2.1, Figure 2.2, 2.3).

Table 2.1: Characteristics used to determine groups of sampling sites within the Tambo River Basin. Ranges in elevations for the groups are in meters above sea level (m.a.s.l).

Group	Individual Sites	# of sites within group	m.a.s.l	Stressors
1	Tucari, Aruntaya, Titire	3	4358-4456	Mining and field/pasture as their dominating surrounding land uses. Some small villages nearby. Mining is the largest source of impact.
2	Pacchani, Vizcachas	2	4295-4357	The Pacchani River is located near a small village but is unimpacted by the mine. The Vizcachas River is far from any source of known contamination. Its waters originate from a reservoir used as a drinking water source.
3	Coralaque Wetland, Coralaque River	2	3589-4378	At a conservation area and immediately downstream of a conservation area. There is the occasional dwelling.
4	Coralaque Huarina, Ubinas, Alto Tambo	3	2571-2595	Located near the Ubinas Volcano. Agriculture, fields, and pastures are the dominant land uses. This area is more populated than the three most upstream groupings.
5	Tambo-US-Vagabundo, Vagabundo, Tambo DS-Vagabundo	3	1275-1292	Agriculture and pastureland dominated. Geothermal input from geysers and hot springs upstream of the Vagabundo River.
6	Tambo Fiscal, Tambo Costanera	2	21-162	Most urbanized areas. Anthropized riverbanks and open littering. Discharge of untreated wastewater. Increased agriculture.

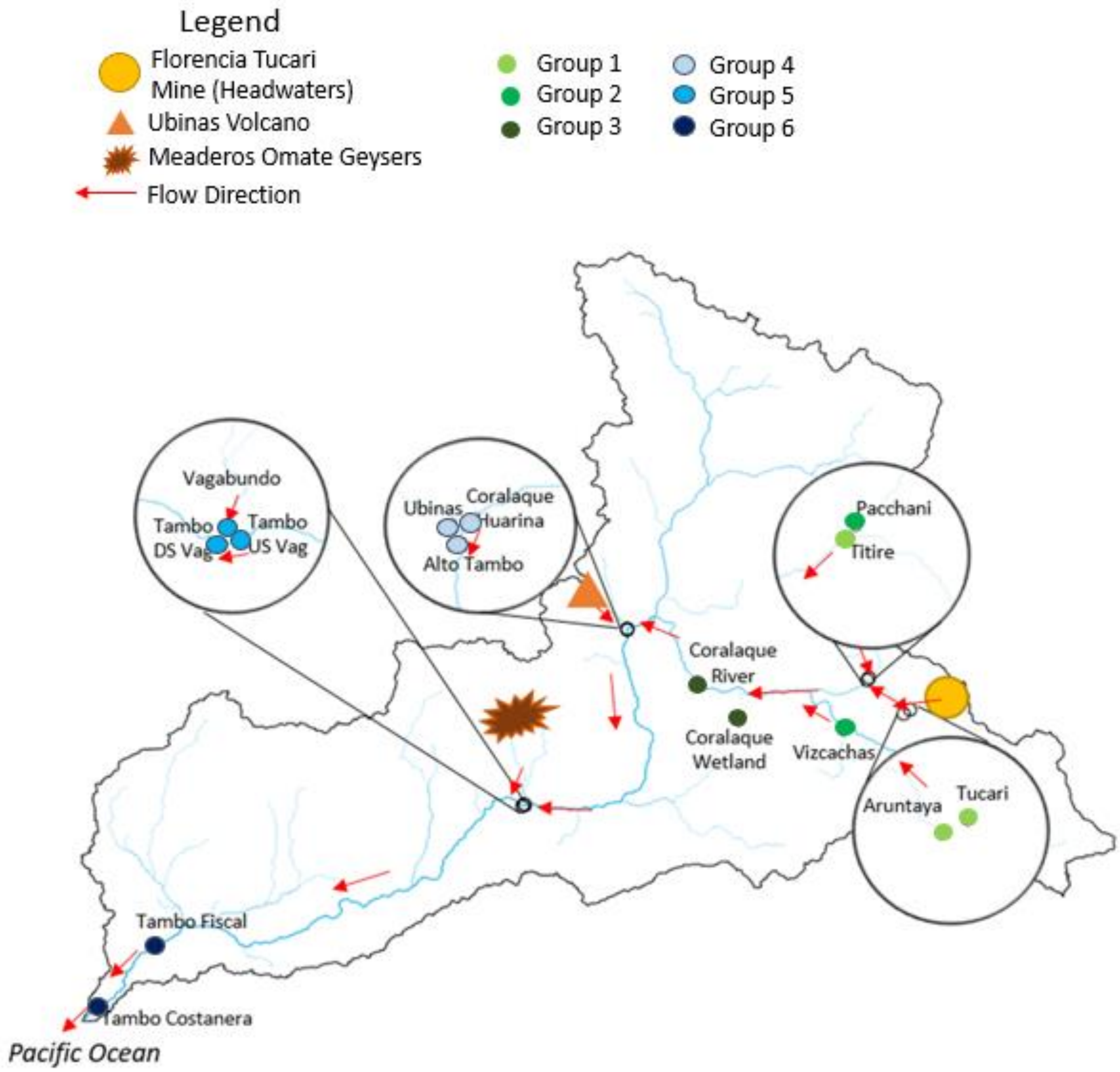
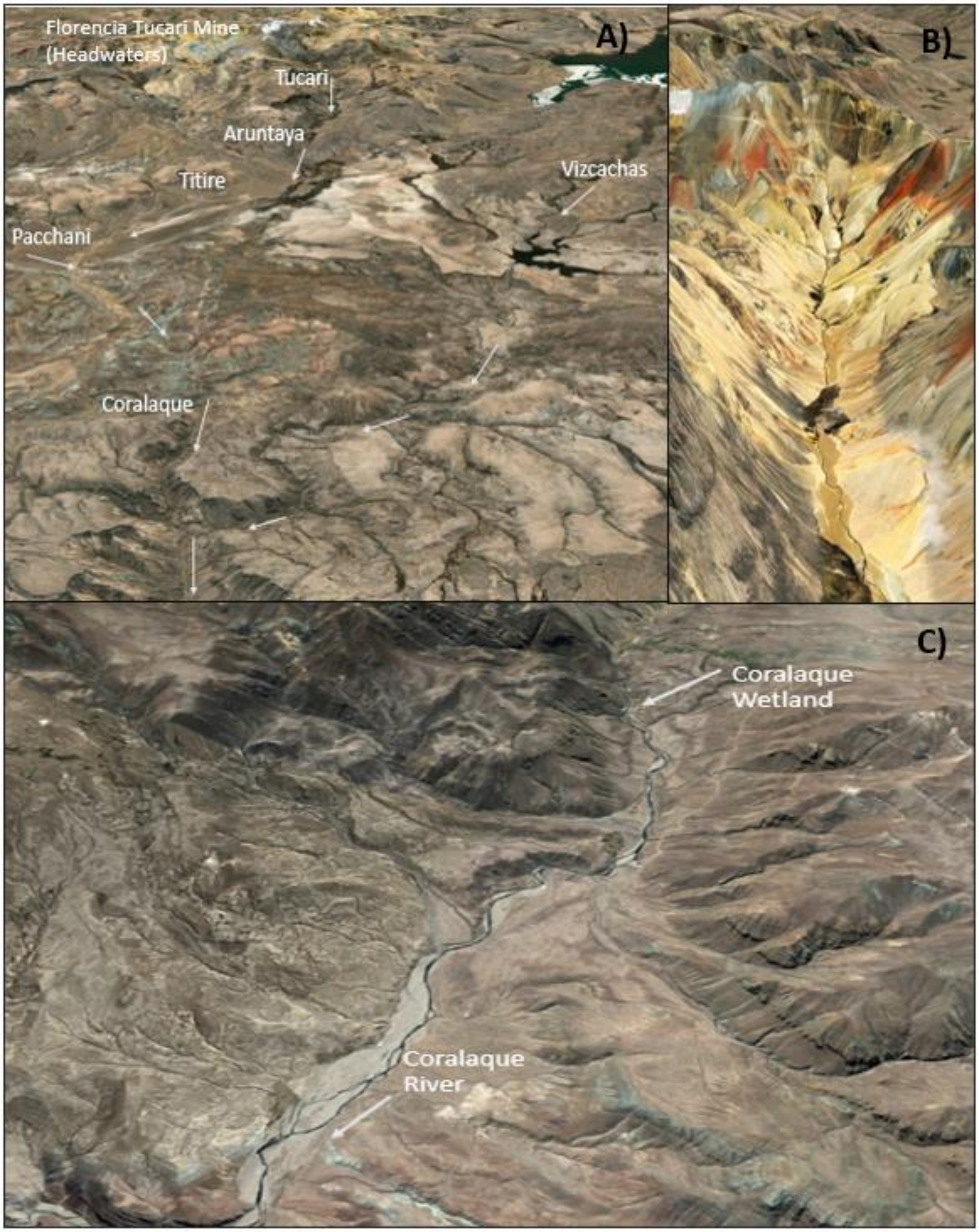


Figure 2.2: Location of sampling sites and sampling site groupings. Arrows indicate direction of flow. Sites belonging to the same group are the same color.


Florencia Tucari Mine
(Headwaters)
A)
Tucari
Aruntaya
Titire
Vizcachas
Pacchani
Coralaque
B)
C)
Coralaque
Wetland
Coralaque
River

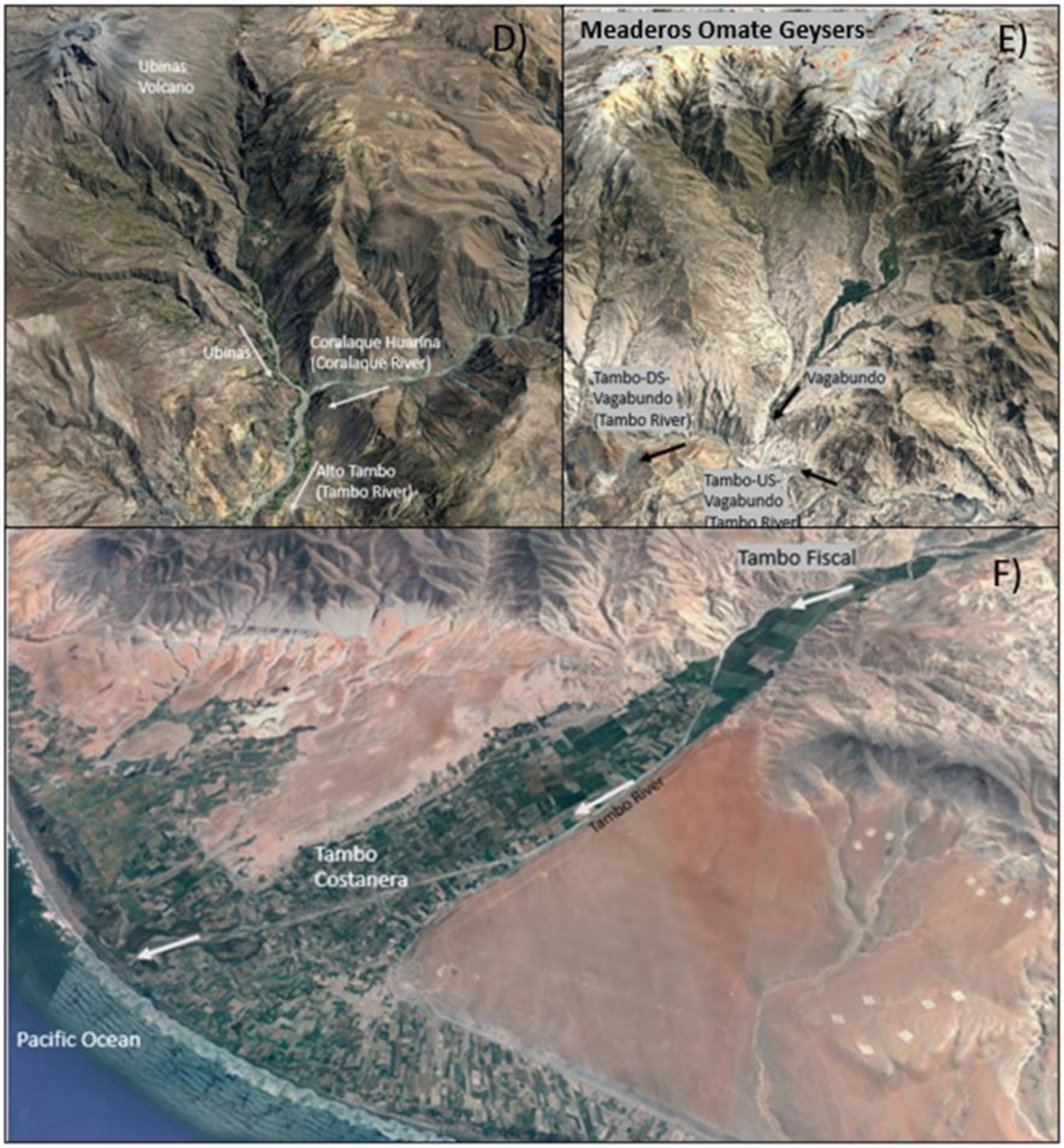


Figure 2.3: Aerial photos of the sites sampled. Arrows indicate the direction of flow. A) Headwaters of the stream where the Florencia-Tucari Mine is located. The Tucari, Aruntaya, Titire, Pacchani, Vizcachas and Coralaque Rivers are shown. B) Aerial photo of the Florencia-Tucari mine. C) Shows the sites sampled along the Coralaque River, the Coralaque Wetland and the Coralaque River site. D) Shows the Ubinas Volcano and the sites sampled in the Ubinas District. E) Shows the sites sampled downstream of the Meaderos Omate Geysers. F) Shows sites sampled in the Tambo Valley.

Group 1 – Tucari, Aruntaya, and Titire Rivers

The Tucari River site is at 4,456 masl. Being the only site on this river, it has the Florencia Tucari Mine at its headwaters and is the closest to a mining stressor. The Margaitani and Apostoloni River tributaries are at the head of the basin where the Florencia Tucari Mine is located. The headwaters drain into the Tucari River and the Aruntaya River. This mine is in the process of being closed but has impacted the surrounding waters since production began in the early 2000s (ANA, 2013). During its operations, the mine extracted gold via open pit mining of the ore body. The ore was then hauled and transported to heap leaching pads and then processed. In the processing plant, the Merrill Crowe process (cyanide leaching to mobilize gold from the ore) was used to obtain a final product of gold concentrates. The Florencia Tucari mine requires large flows of freshwater for its mineral extraction and processing making it a significant contributor to watershed-related issues. The decommissioning of the mine caused discharge to be released from rock outcrops, leachate to form from waste deposits and waste materials, as well as spills and leaks to seep out of uncontained materials. With the mine closure process still ongoing, the release of dissolved metals and acidic waters from the mine pit, leaching pads, and waste rock piles, persists (Lopez Arisaca, 2018).

The Tucari River is above the treeline and has minimal riparian vegetation with only occasional mosses and other mixed grasses and no aquatic macrophytes (Figure 2.4A). The Aruntaya River site is located at 4,418 masl had banks densely vegetated with Ichu (Peruvian feather grass) and is located near the small village of Aruntaya. It discharges into the Titire River, a tributary of the Coralaque River (Figure 2.4B). The Titire River is located at 4,358 masl and adjacent to the village of Titire (ANA, 2013). It had minimal vegetation limited to a mixture of grasses (Figure 2.4C).

Overall, this area was barren or sparsely vegetated (Carlos Mendoza et al., 2021). These high-altitude sites were all above the treeline among mountains with snowy peaks and have mining and field/pasture as their dominating surrounding land uses. The weather is often cold and humid, and the soils are poor resulting primarily in subsistence farming with no commercial agriculture.

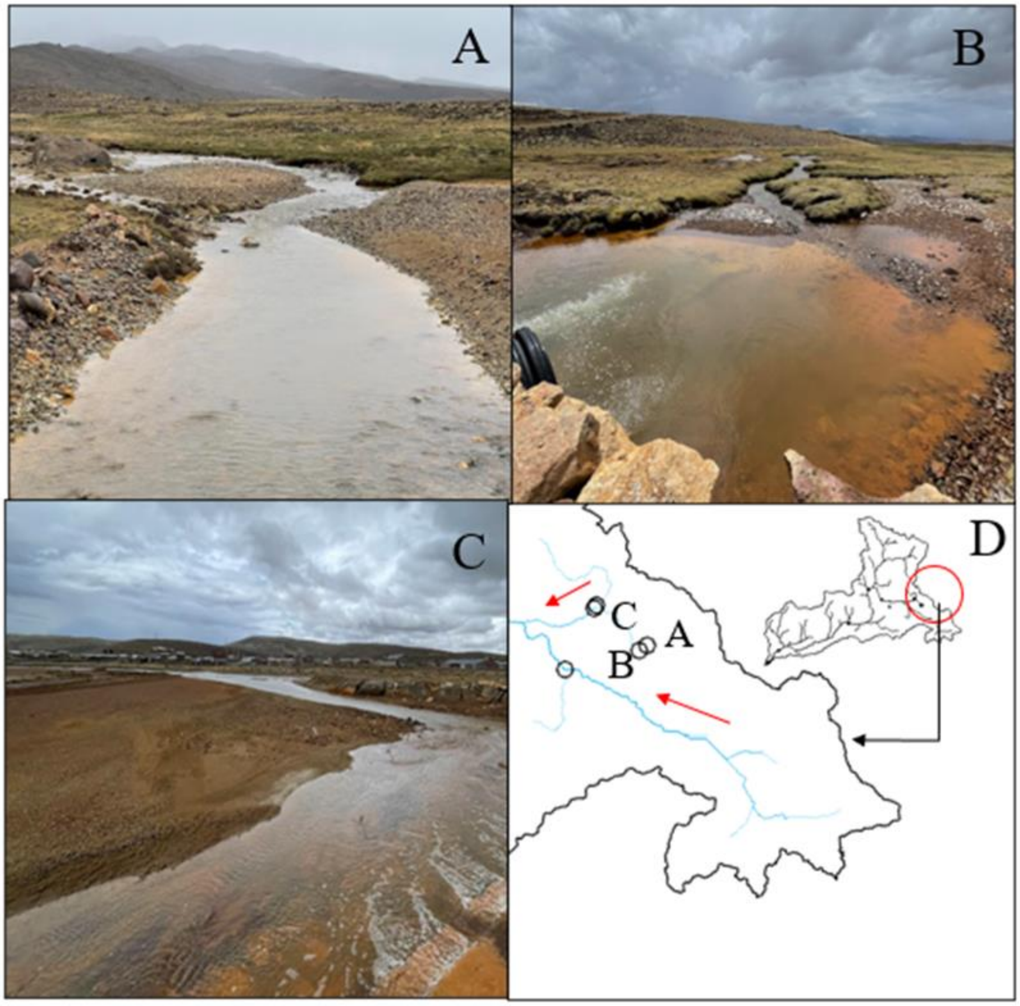


Figure 2.4: Site photos and map reference of group 1 taken during sampling, A) Tucari River, B) Aruntaya River, C) Titire River and D) location of sites.

Group 2 – Pacchani and Vizcachas Rivers

The Pacchani River is located by the village of Titire and runs parallel to the Titire River. However, the headwaters originate from the Pacchani sub-watershed which includes a series of small streams. Upstream of the confluence of the Pacchani and Titire Rivers the landscape is quite rugged, and the soils are poor (ANA, 2013). Recent work by local scientists identified this river as being unimpacted by mining activity. Located at 4,357 masl, the riparian vegetation was limited to grasses and shrubs (Figure 2.5A).

The Vizcachas River waters originate from the Pasto Grande Reservoir which discharges into the Antajarene, Cacachara, Tocco and Patara rivers eventually reaching the Vizcachas River (Figure 4). The landscape is undulating, and the climate is cold. The soils are thin and covered by low-growing grasses, herbs, and shrubs (ANA, 2013). Aside from its proximity to a pumphouse, surrounding lands are natural with no signs of land uses such as the fields and pastures that are found in some highlands. The site was sampled at 4,295 masl and had minimal riparian vegetation and no aquatic macrophytes (Figure 2.5B).

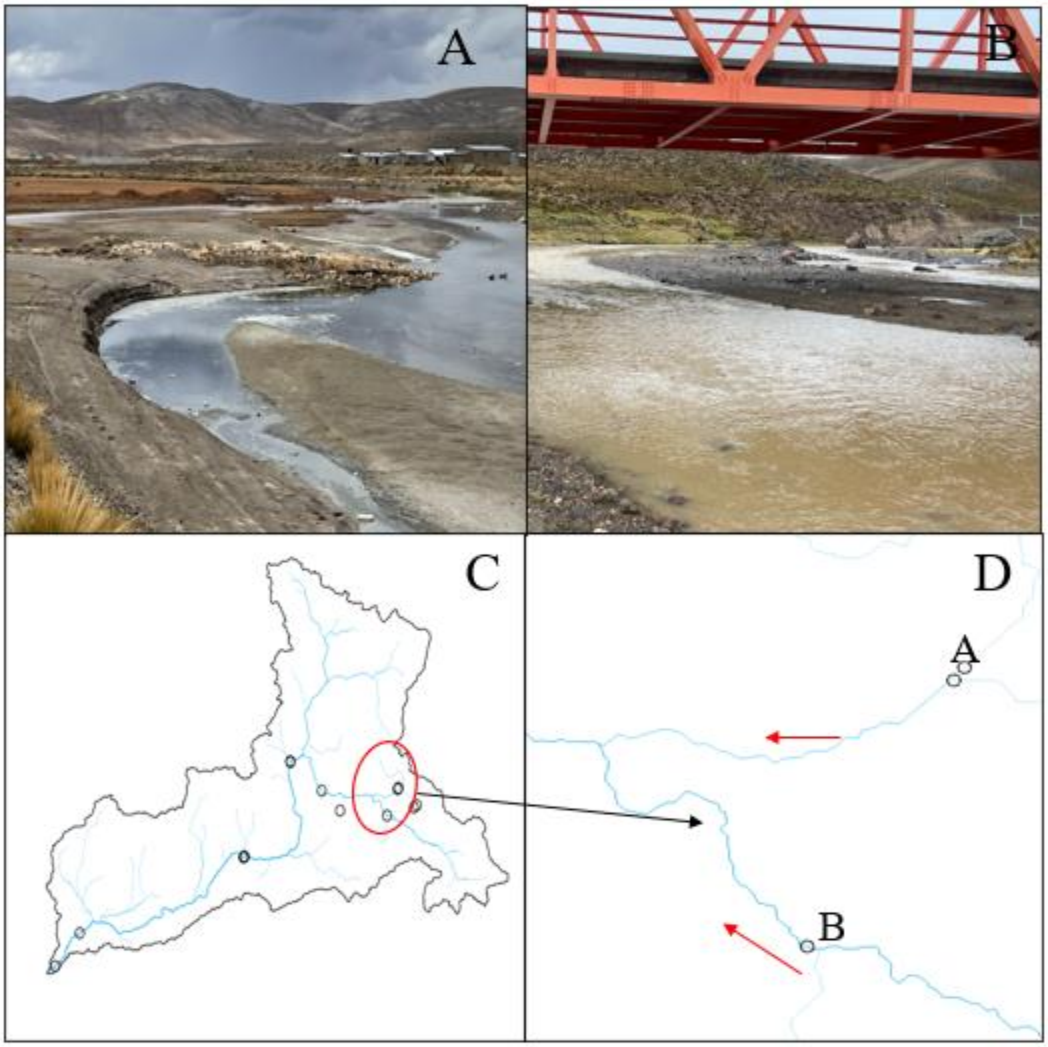


Figure 2.5: Site photos and map reference of group 2 taken during sampling including, A) Pacchani River, B) Vizcachas River, C) Locations of sites and D) Close-up location of sites.

Group 3 – Coralaque Wetland and Coralaque River

Sites sampled at this location included the Coralaque Wetland at 4,378 masl and the first site sampled for the Coralaque River at 3,589 masl. The Coralaque Wetland is part of a conservation area known as the Hatmal Conservation Area. The wetland at this location is known as a bofedales, which is a globally rare type of high-altitude, treeless fen. Bofedales can receive water from glaciers, rivers, lakes and groundwater. Although it is hydrologically connected to the Coralaque River, rain, snow and glacial melt contribute to its significant water storage. They also provide a micro-climate that allows plants to reach their altitude limits in the cold, arid Peruvian Andes (Squeo et al., 2006). This area has natural dips and depressions in the landscape and has slow-growing, hardy ground-covers, and grasses, and also typically contains peat (Fonkén, 2014). The area is minimally populated, and the dominating land uses are fields and pastures. Wild ungulates congregate in this area (Machaca et al., 2018), Figure 2.6A).

The sampling site at the Coralaque River was approximately 15 km downstream of the Coralaque Wetland. This site is located at 3,589 masl and was dominated by field and pasture but was minimally populated with just the occasional family dwelling. The riparian zone was mainly composed of grasses that were few and far between (Figure 2.6B).

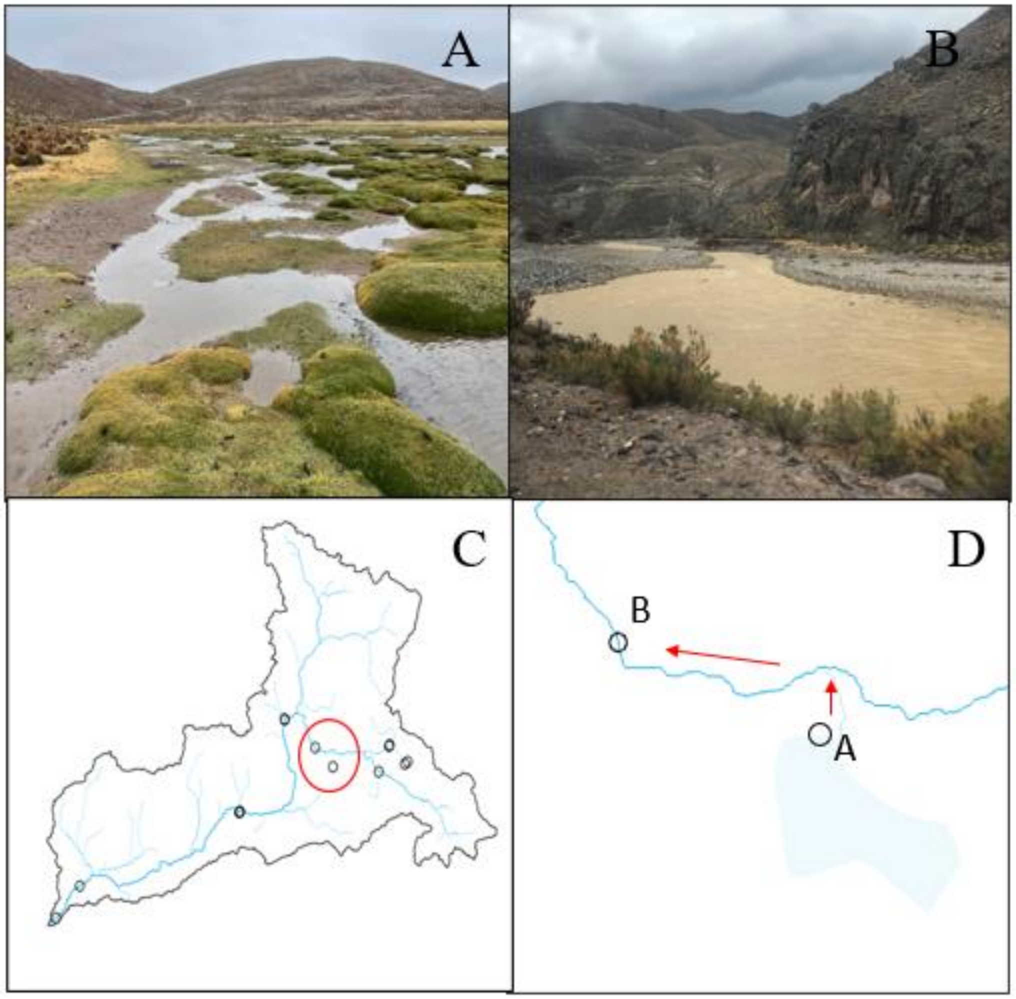


Figure 2.6: Site photos and map reference of group 3 taken during sampling including, A) Coralaque Wetland, B) Coralaque River, C) Locations of sites and D) Close-up location of sites .

Group 4 – Coralaque Huarina, Ubinas and Alto Tambo Rivers

The Ubinas district borders Arequipa to the west and Puno to the north. Three sites were sampled at this location, with the most upstream site sampled at Coralaque Huarina at 2,595 masl, on the Coralaque River. The following site is a tributary of the Tambo River, the Ubinas River site (2,594 masl), and the third site sampled was the Alto Tambo site (Tambo River) at 2,571 masl after its confluence with the Coralaque River and the Ubinas River (Figure 2.7). Upstream of the Coralaque Huarina site (Figure 2.7A), there was minimal subsistence agriculture and no known anthropogenic sources of contamination, whereas the Ubinas River waters originate from Peru's most historically active volcano, the Ubinas Volcano (Samaniego et al., 2020). At 5,672 masl, 1.4 km in height and 65 km^2 in circular surface area, the volcano had its most recent eruptions in 2013 and 2017 (Thouret et al., 2005, Figure 2.7B). Volcanic ash fell during these events onto nearby villages resulting in health problems and evacuations. Ephemeral lakes have previously appeared during the rainy seasons near the volcano, with water collecting over impermeable volcanic materials. The main rivers in the deep Ubinas Valley are fed by meltwater and include the Quebrada Infienillo River, the Volcanmayo River, and the Sacuaya River which becomes the Ubinas River (Carrasco, 2016). The most significant economic activities in the Ubinas Valley are agriculture (subsistence and some commercial) and animal husbandry where the population is approximately 5,000 people (Rivera et al., 2010). Over 1198.62 hectares were cultivated and 70% of agricultural lands were irrigated with local rain (ANA, 2013). The Alto Tambo River is adjacent to the village of Matalaque where the population is estimated to be around 1,000 (Distrito, 2022). Agriculture, fields, and pastures were the dominant land uses of the area. There are approximately 216.59 hectares of partially irrigated cultivated area (ANA, 2013). At the Alto Tambo site, the streamside vegetation was limited to small herbaceous flowering plants (Figure 2.7C).

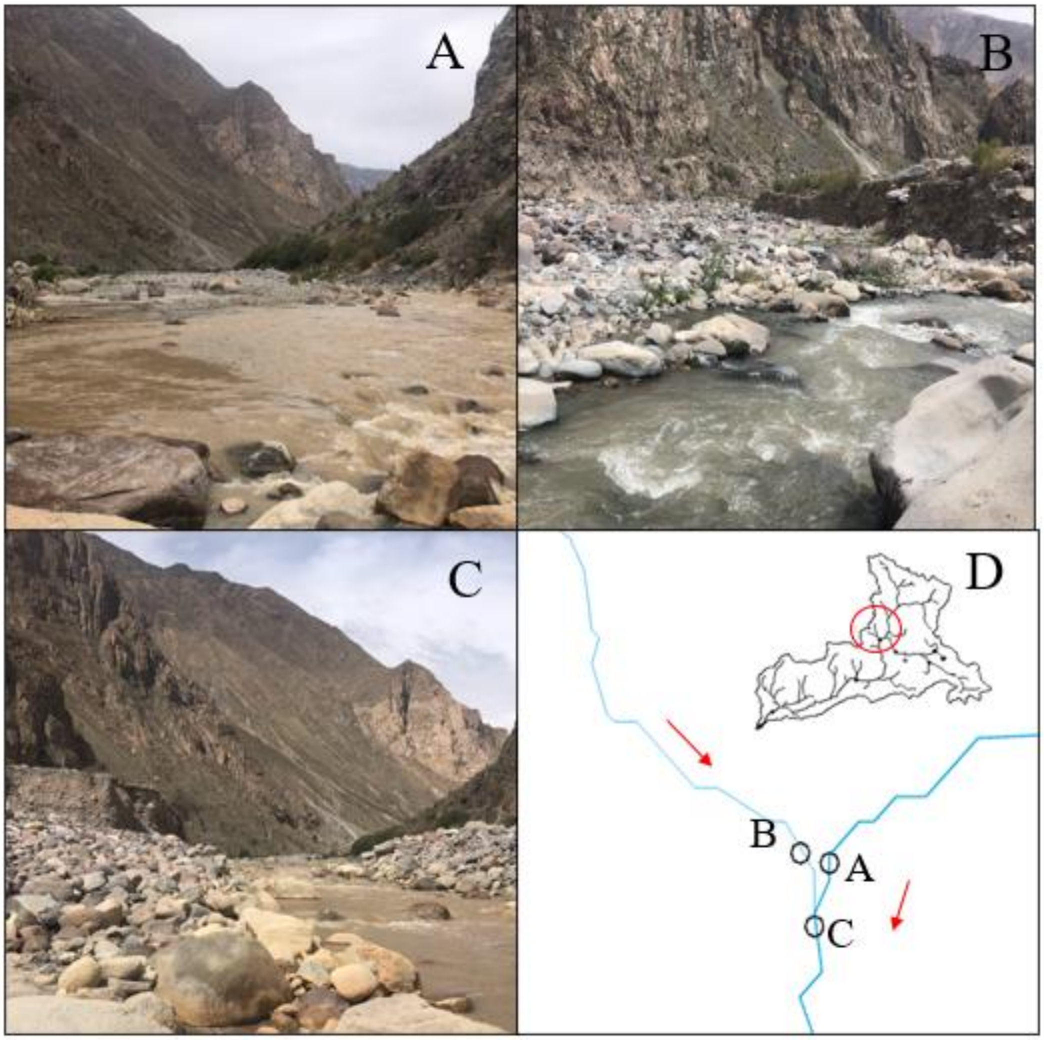


Figure 2.7: Site photos and map reference of group 4 taken during sampling including, A) Coralaque Huarina (Coralaque River), B) Ubinas River, C) Alto Tambo (Tambo River) and D) Site locations.

Group 5 – Tambo-US-Vagabundo (upstream), the Vagabundo River and the Tambo-DS-Vagabundo (downstream)

Tambo-US-Vagabundo was the site sampled before the confluence of the Vagabundo River is located at 1,292 masl and is part of the main river of this system. Its most urban influence was from the Quinistaquillas district located about 15 km upstream (Figure 2.8A). The Quinistaquillas River discharges directly into the Tambo River and was surrounded by 148.28 ha of cultivated land (ANA, 2013). At the site, there were some grasses and small shrubs present. The Vagabundo River is a tributary of the Tambo River and one of the main rivers of the Omate River Sub-Basin (Figure 2.8B). The Vagabundo River site is surrounded by agriculture, fields, and pasturelands. A total of 2,097 hectares was cultivated with local rain being used for irrigation (ANA, 2013). Upstream of the Vagabundo River site, there are also geysers and hot springs known as Meaderos Omate. The third site sampled in this grouping is sampled on the Tambo River (Tambo-DS-Vag) downstream of the Vagabundo River, is located at 1,275 masl and has the same characteristics as Tambo-US-Vagabundo (Figure 2.8C).

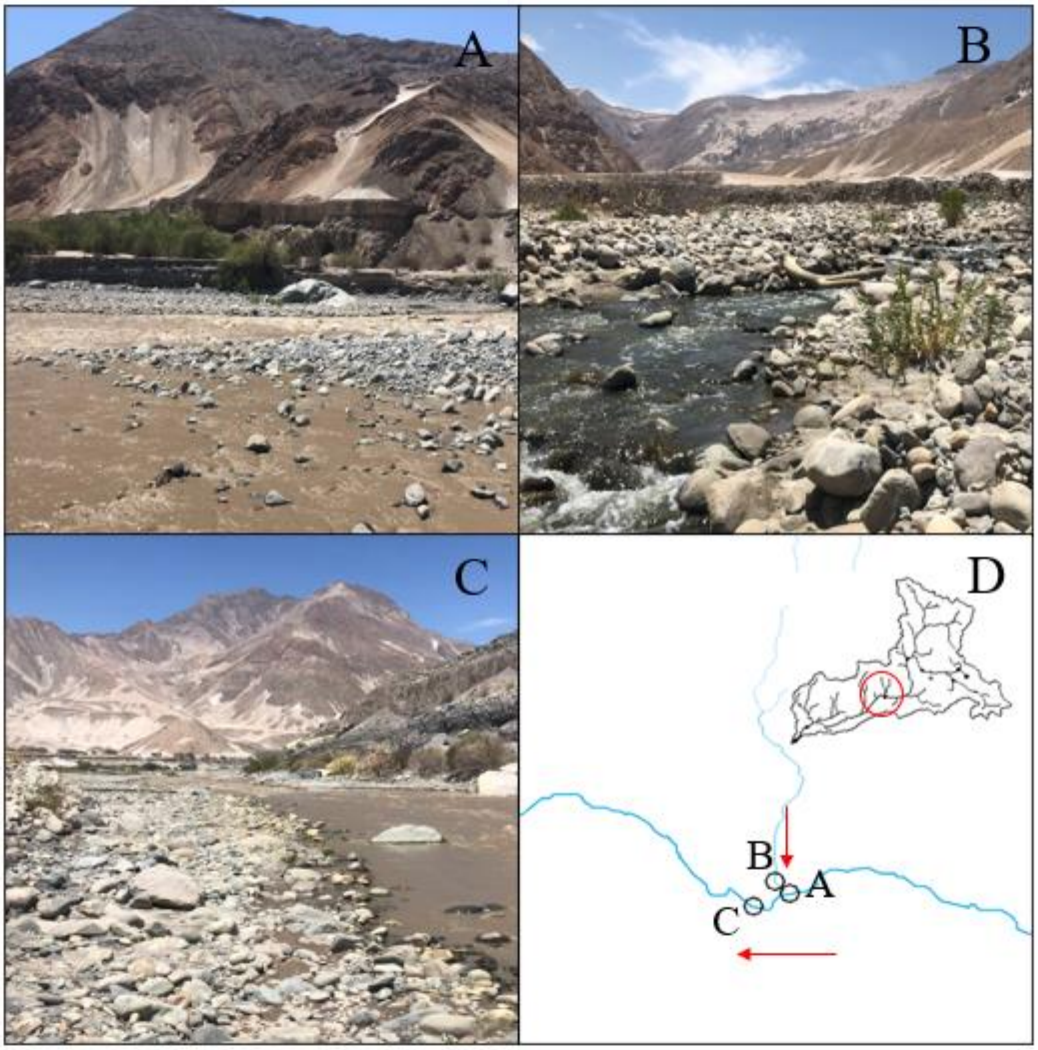


Figure 2.8: Site photos and map reference of group 5 taken during sampling including, A) Tambo-US-Vagabundo, B) Vagabundo River, C) Tambo-DS-Vagabundo and D) Site locations.

Group 6 – Tambo Fiscal River and the Tambo Costanera River

The Tambo Fiscal site and Tambo Costanera sites were the most downstream and urbanized sites sampled in the entire basin and are both sampled on the Tambo River (Figure 2.9). The Tambo Fiscal site is located at the beginning of the Tambo Valley at 162 m.a.s.l. and is surrounded by the town of Ayanquera. This site's riparian zone had grasses and aquatic macrophytes while its floodplain had fields and pastures, as well as small-holder commercial agriculture as dominant land uses (Figure 2.9A). The Tambo Costanera site is located at 21 m.a.s.l. was the most downstream site. It had a well-developed riparian zone with grasses, shrubs, and trees. Like the Tambo Fiscal site, the most dominant land uses were fields, pastures, and small-scale commercial agriculture (Figure 2.9B). This region is known for its rice and garlic production. It also often floods causing erosion and sedimentation. The Cocachacra district which surrounds the area has approximately 8,800 habitants (mindat, 2012).

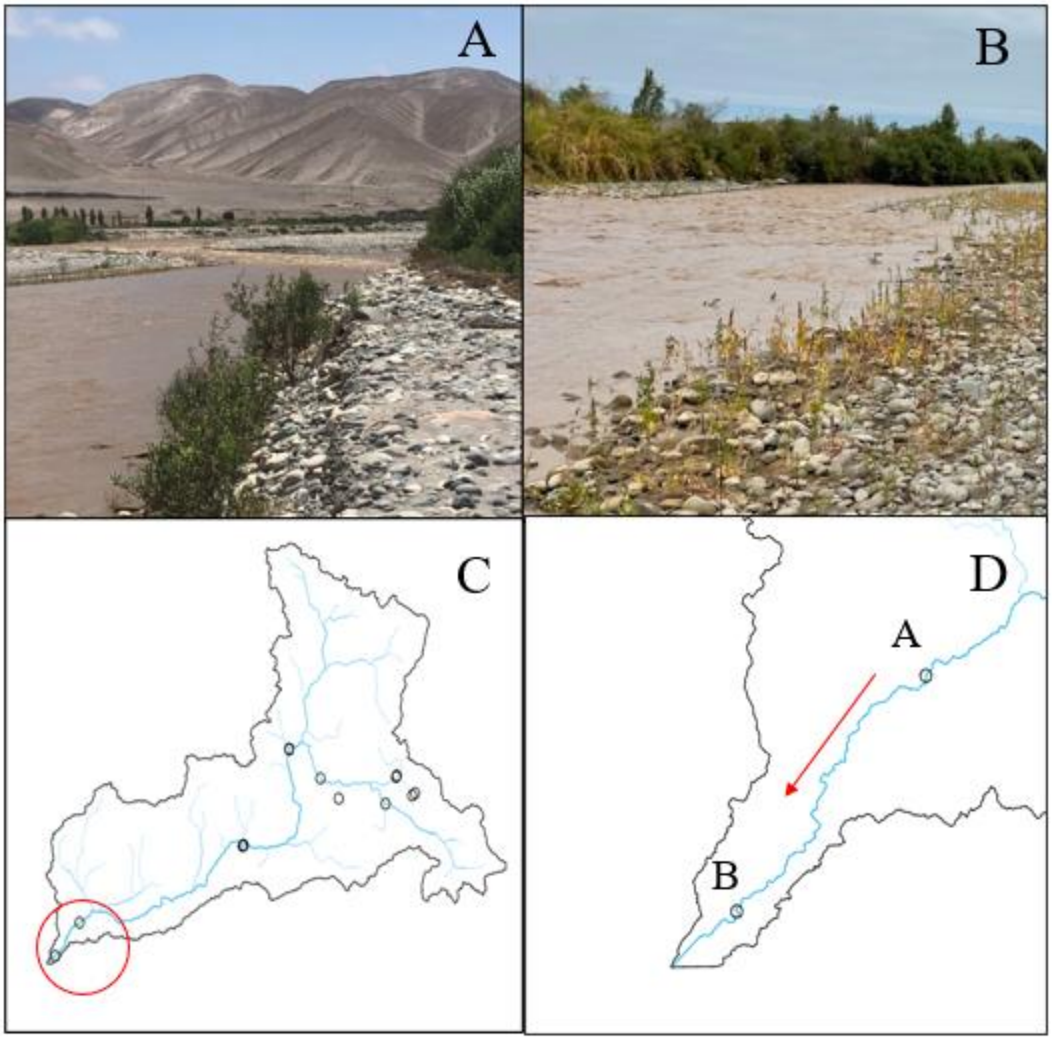


Figure 2.9: Site photos and map reference of group 6 taken during sampling including A) Tambo-US-Vagabundo, B) Vagabundo River, C) Tambo-DS-Vagabundo, and D) Site locations.

2.5 MATERIALS AND METHODS

2.5.1 Field and Laboratory

A total of 15 sites were sampled across the Tambo River Basin in late November of 2021, during the end of the dry season transitioning to the rainy season. This timing allowed samples to be collected at all sampling points as accessibility becomes more restricted during the rainy season due to high flows and limited road access in the mountains.

To cover the steep altitudinal gradient of the Tambo River Basin, sites were selected and grouped across the elevation gradient to represent a variety of potential sources of contamination and reference conditions, as mentioned above. Site selection to determine these groups was done remotely using ArcGIS Scene and Google Earth Pro and was verified and revised by local scientists from the Universidad Nacional de Moquegua (UNAM). With the landscape being challenging to navigate and with limited roads, accessibility also played a large role in determining sampling locations. The digital elevation model (DEM) was produced using ArcGIS pro 3.0.0.

Physicochemical water quality measurements were taken on-site using a YSI ProQuatro. Probes measured temperature, electric conductivity (EC), specific conductance (SC), pH, turbidity, dissolved oxygen (DO), and oxygen-reduction potential (ORP or “redox”). The instrument was calibrated at the beginning of every field day following the ProQuatro user manual. The pH sensor was calibrated using pH buffers at three different midpoints (2, 4, 7) as pH levels have been known to be acidic in waters near the mine. A water volume of 500 mL was collected at every site using clean plastic bottles and were kept cool until sent for laboratory analyses (Figure 2.10). Water samples were analyzed at the ISO accredited S.A.G. (Servicios Analíticos Generales) laboratory (Lima, Peru). A total of 48 metals were analyzed as well as total organic carbon, total phosphorous and total nitrogen. The following standards for preservation and analyses protocols were used: for total metals (EPA Method 200.8), total phosphorous (SMEWW-APHA-AWWA-WEF 4500-P E), total nitrogen (SMEWW-APHA-AWWA-WEF 4500-N C), and total carbon (SMEWW-APHA-AWWA-WEF 5310 C; SAG, 2022). Geocoordinates and altitude were recorded using a Garmin Global Positioning System Device and site characteristics were recorded using a standard field protocol (CABIN, 2012).

Figure 2.10: Collection of water chemistry samples and physicochemical water quality parameters.

2.5.2 Statistical Analysis

A principal component analysis (PCA) based on a Pearson correlation was used to describe the main variation in physicochemical variables collected from the in-situ measurements of the sites. Physicochemical variables used in the PCA were total dissolved solids, conductivity, water temperature, dissolved oxygen, and pH. Before the multivariate analyses, physicochemical variables were tested for normality using the Kolmogorov-Smirnov test using IBM SPSS Statistics version 28. Conductivity and total dissolved solids were log-transformed to meet a normal distribution. Variables were standardized to account for the different scales of the variables included in the PCA. As no major differences were observed, the log-transformed variables were used in the analysis. PCA was also performed for the laboratory water chemistry sample analyses which included metals, nutrients, and total organic carbon.

Before multivariate analysis, these variables were tested for normality using the Kolmogorov-Smirnov test using IBM SPSS Statistics version 28 and those not meeting a normal distribution were log(x+1) transformed which effectively normalized the data. An initial Pearson correlation and a PCA were conducted on the transformed data with all the water sampling data (metals, nutrients and total organic carbon) to reduce the number of variables and multicollinearity by identifying similar pairs of variables that are highly correlated. This initial PCA along with known variables of importance to the region determined which variables were incorporated into the final PCA for the analysis (A. Ccanccapa-Cartagena et al., 2021; A. D. Ccanccapa-Cartagena et al., n.d.; Choque-Quispe et al., 2021; Torres & De-la-Torre, 2022). A minimum of one variable was selected from all major pairs to be used in the analyses to best represent all variables while reducing the number of variables included in the analysis. In the PCA, total organic carbon, total nitrogen, sodium, magnesium, aluminum, potassium, lead, uranium, iron, arsenic, boron, mercury, and zinc were selected. The PCA was performed using XLSTAT Life Science version 2021.5. The Coralaque Wetland was excluded from the multivariate analyses to reduce the possibility of skewing the results as it was the only lentic system.

2.6 RESULTS

2.6.1 Qualitative Site Characteristics

Group 1: Tucari, Aruntaya, Titire Rivers

Water was clear at the Aruntaya River but the Tucari River and Titire Rivers had turbid waters laden with sediment. The streambeds of the latter two rivers were composed of mixed fine-grained sand, fine/medium gravel, and small cobble. The beds were yellow, red, and orange with tailings deposits present in the Tucari River. The Tucari and Titire rivers had occasional grass beyond the stream banks but otherwise the riparian zones were quite barren. Aruntaya River's riparian zone was densely covered in grasses and mosses.

Group 2: Pacchani and Vizcachas Rivers

The streambeds of both sites had fine-grained, organic-rich, mixed sand along with fine/medium gravel. The Pacchani River had visibly suspended organic matter and plant fibres. Waste pipes were suspected to be located nearby. Sediments at the Pacchani River were black, and thick biofilms were also visible. The Vizcachas River had murky water with plant fibres visible. The sediments were mostly yellow and brown with black streaks. The

Pacchani River had the occasional grass and shrubs along the banks while the Vizcachas River had denser vegetation along its banks with a combination of herbs, grasses, and mosses.

Group 3: Coralaque Wetland and Coralaque River

The Coralaque Wetland sediment was composed of fine-grained; organic-rich mixed sand, as well as coarse gravel, very coarse gravel, and small to large cobbles. It was a patchwork of yellow-to-brown with some black areas, and sediments were soft enough for feet to sink about 10-20 cm when sampling. The Coralaque River streambed was different with mixed fine-grained sand, large cobble, and small boulders; sediments were yellow to brown. The water at the wetland was clear but it was highly cloudy in the Coralaque River. Macrophytes and dead plants covered 26-50% of the wetland but were absent in the river. The Coralaque wetland was densely vegetated with grasses, mosses, and shrubs. The Coralaque River had the occasional shrub and grass along its banks.

Group 4: Coralaque Huarina, Ubinas River, Alto Tambo

The Coralaque Huarina and Alto Tambo sites had similar site characteristics. Both had a streambed composed of mixed, fine-grained sand, small cobble, large cobble, and small to large boulders; the colours varied from yellow to brown. Both rivers had turbulent flows that carried high sediment loads. In contrast, the Ubinas River was clear with sediment composed of fine-grained, organic-rich sand mixed with fine to medium gravel, small to large cobbles, and small to large boulders. Organic debris in the form of branches was present at all sites. The Coralaque Huarina site did not have any vegetation however the Ubinas River had the occasional shrub. The Alto Tambo site had the occasion flower present but was otherwise unvegetated.

Group 5: Tambo-US-Vag, Vagabundo, Tambo-DS-Vag

The streambed of the Tambo River upstream of the confluence with the Vagabundo River was composed of mixed, fine-grained sand, small to large cobbles, small to large boulders, and was free of organic material. The Vagabundo River had the same streambed composition. However, while the water of the Tambo River was turbid with high to very high loads of suspended sediment, the Vagabundo River had clear water and was the only site at this location that had plant fibres. All sites, upstream and downstream had yellow to brown sediments. Tambo-US-Vagabundo had some trees and shrubs, Vagabundo was sparsely vegetated with herbs and shrubs. Tambo-DS-Vagabundo was sparsely vegetated with herbs, grasses, and shrubs.

Group 6: Tambo Fiscal, Tambo Costanera

The Tambo Fiscal and Tambo Costanera sites both had highly turbid water with very high loads of suspended sediment. The Tambo Fiscal River had small to large cobbles, and thick layers of biofilm were visible in riffle areas. These were fairly shallow and broad rivers. Macrophytes were present at the Tambo Fiscal River with coverage ranging from 1-25% of the river surface. The Tambo Costanera River had mixed fine sand and small cobble streambed sediments with some biofilm visible. Tambo Fiscal and Tambo Costanera had densely vegetated banks with herbs, grasses, and deciduous trees.

2.6.2 Description of Variations of Physiochemical Water Quality Parameters

Upstream to Downstream Trends

Approximately 40 km downstream of the mining sites, pH values ranged from 7.23-8.83. The average pH value for the basin was 7.4 (Figure 2.1, Table 2.1). Water temperatures fluctuated throughout the sites but were somewhat colder at sites higher in elevation, and were warmer at the downstream sites (Figure 2.1, Table 2.1). Water temperatures ranged from 8.7 °C to 26.8 °C. Generally, dissolved oxygen values increased from upstream to downstream ranging from 4.58 mg/L to 7.05 mg/L, with an average of 5.97 mg/L (Figure 2.1, Table 2.1). Conductivity was at its highest upstream in the basin, specifically at the Tucari, Titire and Pacchani rivers, decreased at Vizcachas and the Coralaque Wetland and slightly increased downstream. Conductivity ranged from 63.1 µS/cm to 7012 µS/cm and the basin average was 2061.24 (µS/cm). TDS followed the same trend as conductivity and ranged from 0.547 mg/L to 5090.5 mg/L and had a basin average of 1335.51 mg/L. The oxidation-reduction potential (ORP) was generally higher at the mine sites, decreased mid-basin, and increased slightly at most downstream sites. Oxygen-reduction potential ranged from 76.9 mV to 465.7 mV (Table 2.2, Figure 2.11).

Group 1: Tucari, Aruntaya and Titire Rivers

Across all groups, there was considerable variation among the study rivers in terms of physiochemical and water chemistry conditions (Figure 2.1). The mine-impacted sites were quite acidic. The Tucari River had a pH of 2.59, the Aruntaya River was at a pH of 4.41, and the Titire River had a pH of 3.48 (Figure 2.1, Table 2.1). The Tucari River was the coldest with a temperature of 12 °C. Dissolved oxygen was at its lowest at 4.58 mg/L which was recorded at the Aruntaya River. The Tucari River had the second highest conductivity value recorded in the basin 4232 (µS/cm). Similarly, total dissolved solids were high at 3289 mg/L. Overall, the oxidation-reduction potential was high at these sites with 465.7 mV at the Tucari River, 428.2 mV at the Aruntaya River and 461 mV at the Titire River (Table 2.2, Figure 2.11).

Group 2: Pacchani and Vizcachas Rivers

There was an increase in pH at the Pacchani River site with a pH of 7.84 and the water temperature was slightly cooler than at the mine sites. The highest salinity values were recorded at the Pacchani River site (5.29 PPT). The water was highly conductive in the Pacchani (7012 µS/cm). Total dissolved solids values were notably higher as well (5090.5 mg/L). Vizcachas had a pH of 8.82 and a temperature that was slightly cool 10.3 °C as well as low conductivity (187 µS/cm). Overall, there was a decrease in the oxydation-reduction potential in comparison to the mining group and the Pacchani River with a value of 360.7 mV (Table 2.2, Figure 2.11).

Group 3: Coralaque Wetland and Coralaque River

The pH of the Coralaque Wetland was 8.18 and the water temperature was at its lowest in the entire basin with a value of 8.7 °C. Dissolved oxygen was comparable to the rest of the basin with a value of 6.31 mg/L. Conductivity and salinity were at their lowest at these sites with a value of 63.1 µS/cm and 0.04 PPT respectively. TDS and ORP were also low at this location. At the Coralaque River, the pH was 7.23 and there was a slight increase in water temperature (13.1 °C). However, there was an increase in TDS, conductivity, salinity and ORP in comparison to the upstream wetland (Table 2.2, Figure 2.11).

Group 4: Coralaque Huarina, Ubinas and Alto Tambo Rivers

The pH at these sites ranged from 8.39 to 8.72 and temperatures were all similar and warmer than all sites upstream ranging from 19.6 to 22 °C. Dissolved oxygen values were close as well ranging with values of 6.3, 6.69 and 6.71 mg/L. The Coralaque Huarina and Alto Tambo sites all had higher values of TDS (1313, 442 and 1287 mg/L) and conductivity (1808, 936 and 1824 μS/cm) and salinity (1.03, 0.49, 1.01 PPTPPT). Oxygen-reduction potential decreased from one site to the next with Coralaque Huarina site having a value of 80.7 mV followed by the Ubinas River with a value of 76.9 mV and the Alto Tambo with a value of 35.6 mV (Table 2.2, Figure 2.11).

Group 5: Tambo-US-Vagabundo, Vagabundo, Tambo-DS-Vagabundo

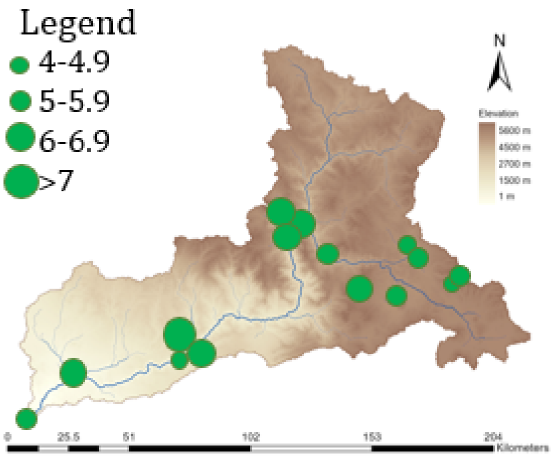
The pH values at these sites were the same (8.66, 8.67 and 8.67). However, the water temperature was noticeably cooler in the Vagabundo River compared to the Tambo River sites at this location. The temperature at the Tambo-US-Vagabundo site was 14.6 °C followed by 26.8 °C at the Vagabundo River and 15°C at the Tambo-DS-Vagabundo site. The Vagabundo River had the highest water temperature recorded in the entire basin. TDS and conductivity were also higher at the Vagabundo River than at the two other sites. Oxygen-reduction potential increased from site to site in this grouping with a value of 123 mV recorded at the Tambo-US-Vagabundo site, 145 mV at the Vagabundo River, and 720 mV at the Tambo-DS-Vagabundo site (Table 2.2, Figure 2.11).

Group 6: Tambo Fiscal and Tambo Costanera

Similar conditions were observed at the Tambo River at both the Tambo Fiscal site and Tambo Costanera site. The pH levels were 8.38 at the Tambo River Fiscal site and 8.83 at the Tambo River Costanera site, with temperatures of 20.5 °C and 23.7 °C, respectively. Dissolved oxygen values were comparable to the rest of the basin with values of 6.55 mg/L for the Tambo Fiscal site and 5.71 mg/L for the Tambo Costanera site. The largest difference at these sites was in TDS where the Tambo Fiscal site had TDS of 547 mg/L and the Tambo Costanera site had TDS of 1631 mg/L (Table 2.2, Figure 2.11).

Table 2.2: Physicochemical measurements from the different sampling sites across the Tambo River watershed.

Sites	Elevation (masl)	Water Temperature (°C)	pH	DO (mg/L)	TDS (mg/L)	Conductivity (μS/cm)	Salinity (PPT)	Oxydation-Reduction Potential (mV)
Tucari	4456	12	2.59	5.84	1521	1764	1.21	465.7
Aruntaya	4418	15.9	4.41	4.58	286.65	370.5	0.21	428.2
Titire	4358	16.6	3.48	5.65	3289	4232	2.74	461
Pacchani	4357	11.5	7.84	4.99	5090.5	7012	5.29	443.2
Vizcachas	4295	10.3	8.82	5.81	171.6	187	0.13	360.7
Coralaque Wetland	4378	8.7	8.18	6.31	59.8	63.1	0.04	295.8
Coralaque River	3589	13.1	7.23	5.57	1053	1252	0.82	352.2
Coralaque Huarina	2594	19.6	8.39	6.71	1313	1808	1.03	80.7
Ubinas	2595	22	8.72	6.3	442	936	0.49	76.9
Alto Tambo	2571	20.8	8.4	6.69	1287	1824	1.01	35.6
Tambo-US-Vag	1292	14.6	8.66	6.88	390.6	1366	0.98	123
Vagabundo	1331	26.8	8.6	7.05	2171	3387	1.74	145.2
Tambo-DS-Vag	1275	15	8.67	4.9	1326	2102	1.04	720
Tambo Fiscal	162	20.5	8.38	6.55	547	2212	1.23	186.1
Tambo Constanera	21	23.7	8.83	5.71	1631	2403	1.29	164.8
Mean	2779.47	16.74	7.41	5.97	1335.51	2061.24	1.28	229.27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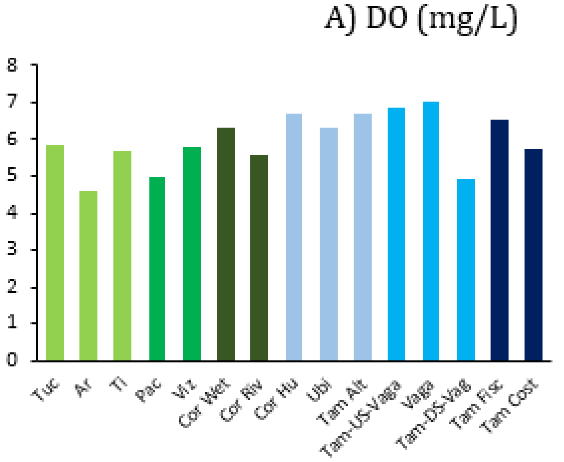
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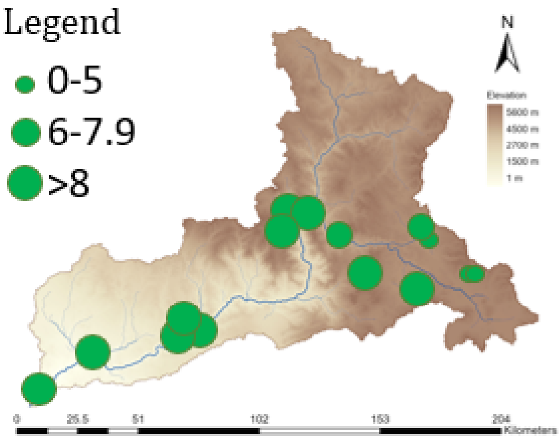
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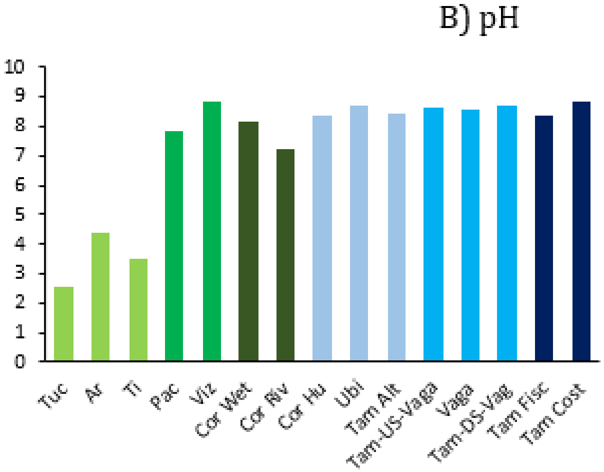
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Ubi
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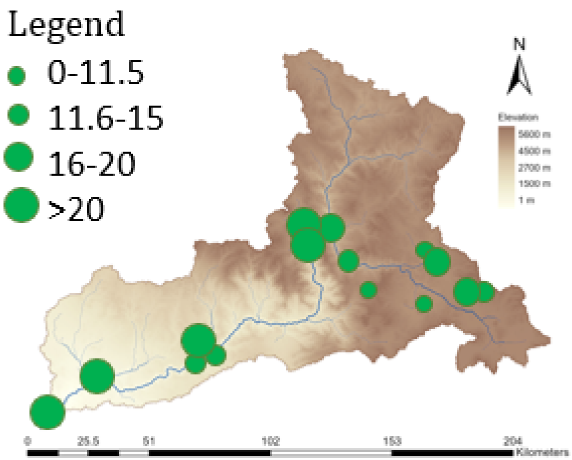
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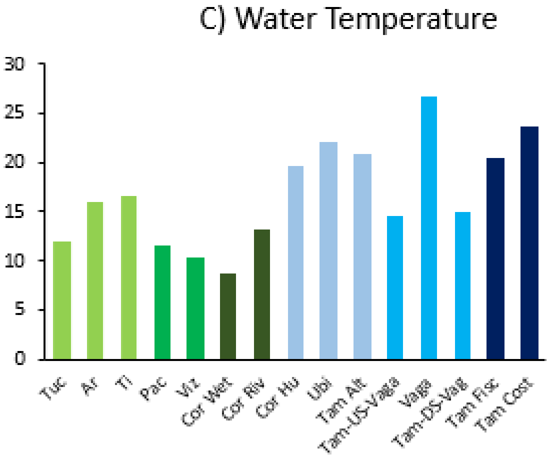
Kilometers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Viz
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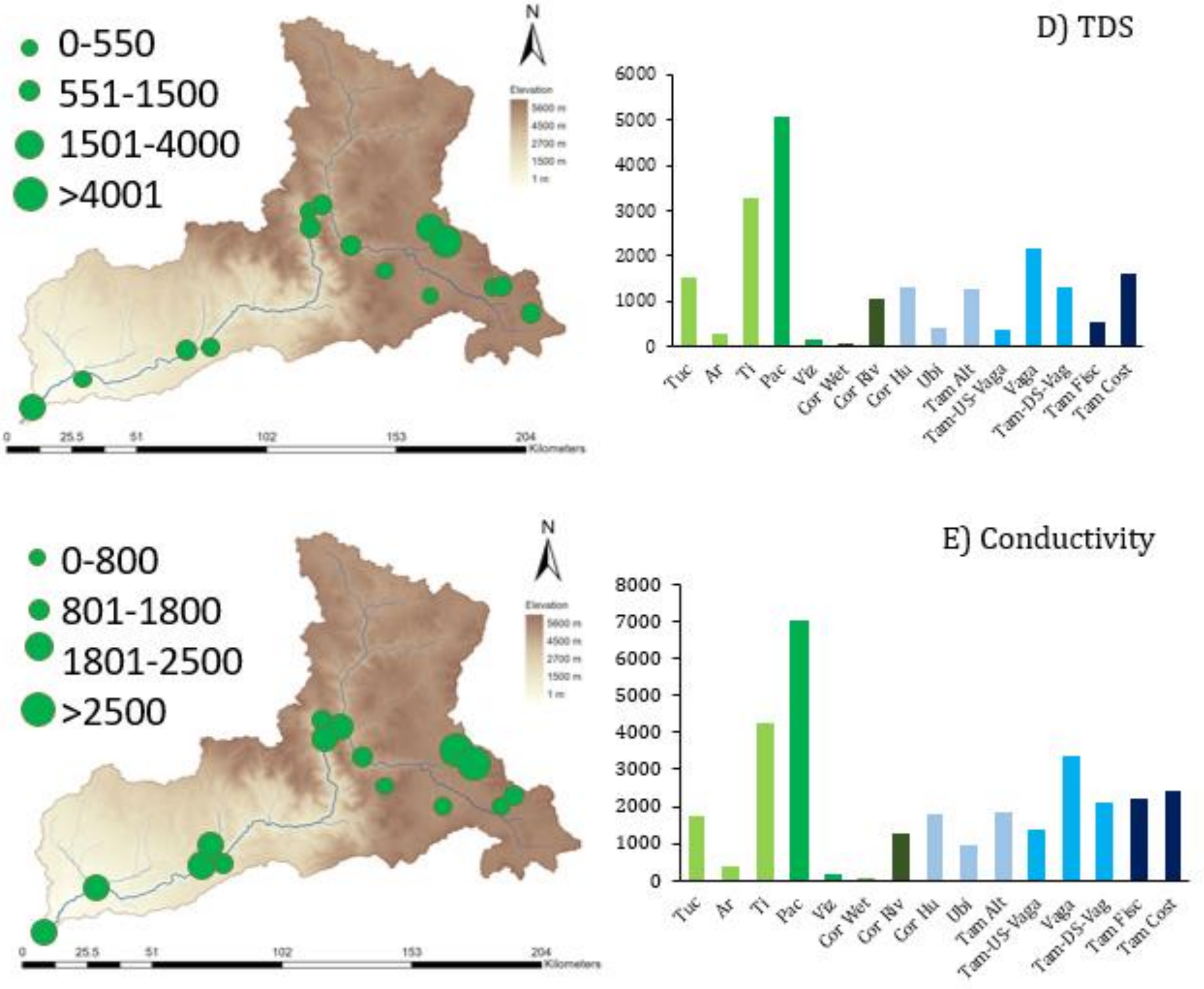


Figure 2.11: Spatial distribution of physicochemical water quality parameters in the Tambo River Basin (A) dissolved oxygen (DO, mg/L), (B) pH, (C) water temperature (°C), (D) Total Dissolved Solids (TDS, mg/L), and (E) Conductivity in µS/cm. Bar graph colours represent the six different groups: group 1 in light green (Tucari, Aruntaya and Titire Rivers), group 2 in medium green (Pacchani and Vizcachas Rivers), group 3 in dark green (Coralaque Wetland and Coralaque River), group 4 in light blue (Coralaque Huarina site, Ubinas River and Alto Tambo), group 5 medium blue (Tambo-US-Vag, Vagabundo, Tambo-DS-Vagabundo), and group 6 in dark blue (Tambo Fiscal and Tambo Costanera).

2.6.3 PCA Correlation Results for Sites & Physiochemical Water Parameters

Pearson's Correlation showed that dissolved oxygen and water temperature were positively correlated and that was the only statistically significant correlation ($r=0.544$, $p<0.05$). Water temperature had weak positive correlations with pH (0.335) and conductivity (0.281) and a weak negative correlation with total dissolved solids (-0.085). For pH, weak negative correlations occurred with conductivity (-0.033) and total dissolved solids (-0.194). Conversely, pH was weakly positively correlated with dissolved oxygen (0.391). Total dissolved solids was weakly positively correlated with conductivity (0.313) and weakly negatively correlated with dissolved oxygen (-0.216, Table 2.4).

Table 2.3: Values for Pearson's Correlation using physicochemical variables measured with a multi-sonde. Bold indicates statistically significant correlations ($p<0.05$).

Variables	Water_Temp.	pH	Log(TDS)	Log (Conductivity)	DO
Water_Temp.	**1**	0.355	-0.085	0.281	**0.544**
pH	0.355	**1**	-0.194	-0.033	0.391
Log(TDS)	-0.085	-0.194	**1**	0.313	-0.216
Log(Conductivity)	0.281	-0.033	0.313	**1**	0.123
DO	**0.544**	0.391	-0.216	0.123	**1**

Values in bold indicate correlations that are significant at $p<0.05$

The first two axes of the PCA explained 66.69% of the total variability, with the first principal component axis (PC1) explaining 39.41% and the second principal component axis (PC2) explaining 27.51%. PC1 had large positive loadings with water temperature (0.808) and dissolved oxygen (0.831). PC2 had a large positive loading with conductivity (0.838) (Table 2.5, Figure 2.12).

Upstream to Downstream Trends

All three mining sites (Group 1) were negatively associated with PC1, water temperature, pH and dissolved oxygen. Within this group, the Titire and Tucari Rivers were associated with higher TDS and conductivity whereas the Aruntaya River was negatively associated with PC2. Surprisingly, The Pacchani River (Group 2, unimpacted by mines) was grouped with the Titire River (Group 1, mine impacted) and was positively associated with PC2. Although grouped with the Pacchani River, the Vizcachas River was negatively associated with both PC1 and PC2. The Coralaque River was negatively associated with PC1 and PC2 as well. Group 4, including the Alto Tambo, Ubinas, and Coralaque Huarina Rivers, was somewhat tightly grouped and mapped positively on PC1. Within this group, the Alto Tambo and Coralaque Huarina Rivers were positively associated with PC2 whereas, the Ubinas River was oppositely associated with PC2. The Tambo-US-Vagabundo site was positively associated with PC1 and negatively associated with PC2. The Vagabundo River was positively associated with PC1 and PC2, whereas the Tambo-DS-Vagabundo site was positively associated with PC2. The downstream Group 6, including the Tambo Fiscal and Tambo Costanera sites were both positively associated with PC1. However, the Tambo Fiscal site mapped negatively on PC2, whereas the Tambo Costanera site was positively associated with PC2 (Figure 2.12).

Table 2.4: Principal component analysis factor loadings. Significant positive loadings are in bold.

	PC1	PC2
Water Temp.	**0.808**	0.261
pH	0.689	-0.211
TDS	-0.326	0.748
Conductivity	0.211	**0.838**
DO	**0.831**	0.002

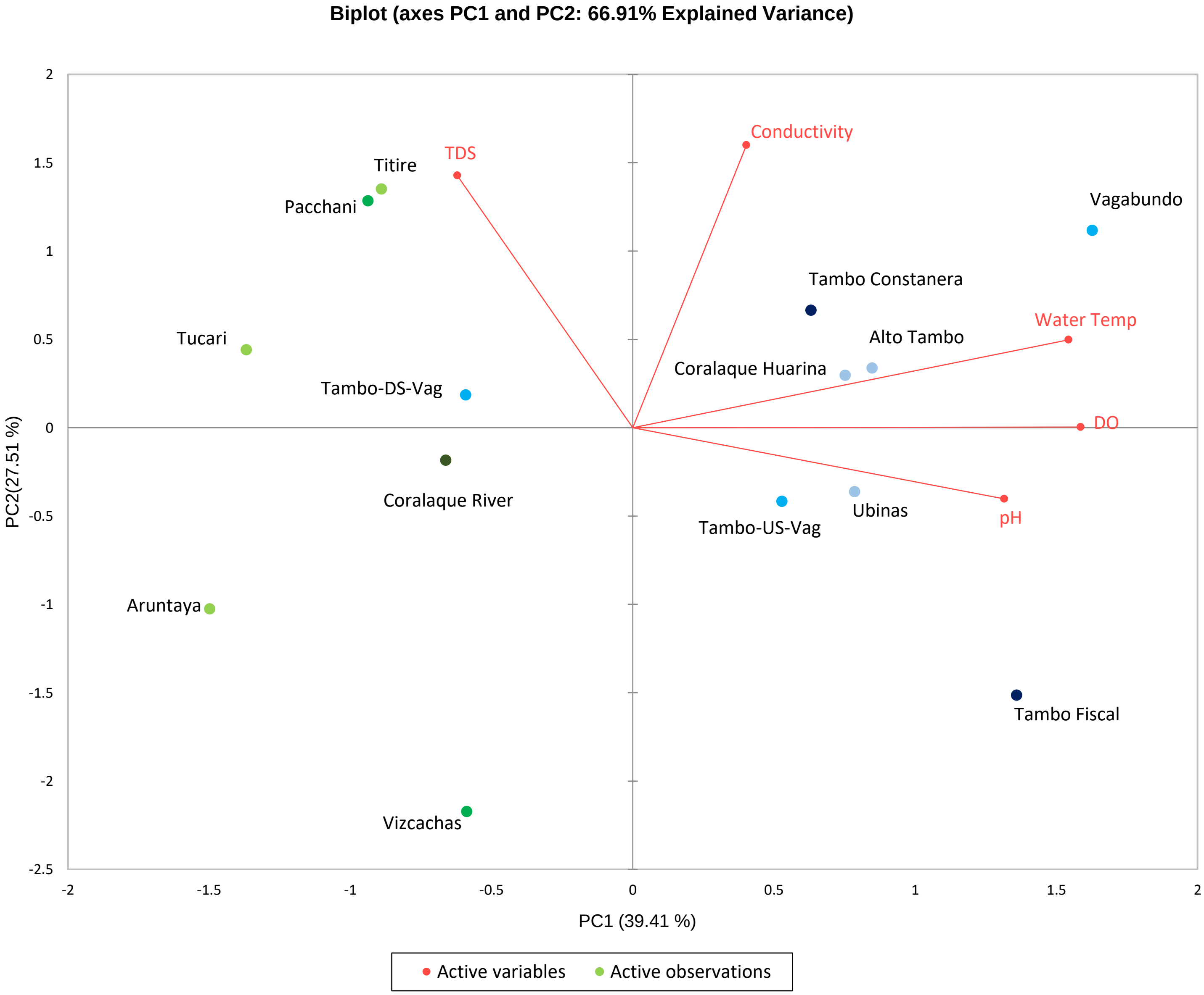


Figure 2.12: Principal Components Analysis (PCA) of physicochemical parameters Dissolved Oxygen (DO), pH, Water Temperature, Conductivity, Salinity, and TDS. Sites within the same group are of the same colour. PC1 explains 39.41% of the variance while PC2 explains 27.51%. The biplot represents 66.91% of the total variance. Group 1 is light green (Tucari, Aruntaya and Titire Rivers), group 2 is medium green (Pacchani and Vizcachas Rivers), group 3 is dark green (Coralaque Wetland and Coralaque River), group 4 is light blue (Coralaque Huarina site, Ubinas River and Alto Tambo), group 5 is medium blue (Tambo-US-Vagabundo site, Vagabundo River, Tambo-DS-Vagabundo site), and group 6 is dark blue (Tambo Fiscal and Tambo Costanera Rivers).

2.6.4 Concentrations of Metals, Nutrients, and Total Organic Carbon

Group 1: Tucari, Aruntaya and Titire Rivers

Metal concentrations were notably higher at all mine affected sites in comparison to the rest of the basin. Iron, nickel, silver, cobalt, copper, and aluminum had notably high concentrations. Iron levels ranged from 15.31 mg/L to 73.57 mg/L at these sites. Nickel values ranged from 0.13 mg/L to 0.23 mg/L at these sites. Silver levels reached 0.017 mg/L at the Tucari River and were similarly high at the other two sites. Aluminum values reached 96.01 mg/L at the Tucari River, 67.81 mg/L at the Aruntaya River and 68.423 mg/L at the Tucari River. Cobalt and copper levels at the Tucari, Aruntaya and Titire Rivers were generally higher than the rest of the basin as well. High values of cadmium and mercury were both recorded at the Tucari and Titire River sites. Further, zinc was higher both at the Tucari (3.1 mg/L) and Titire (2.07 mg/L) Rivers than in the rest of the basin. Boron and sodium were noticeably higher at the Titire River than at the Aruntaya and Tucari Rivers. Overall, total nitrogen was at its highest at the most upstream and mining-impacted sites. Total Organic Carbon (TOC) was particularly high at the Aruntaya River (4 mg/L) in comparison to the rest of the basin (Table 2.5, Figure 2.13).

Group 2: Pacchani and Vizcachas Rivers

In the Pacchani River, several elements were the highest concentration recorded across the basin including sodium (1807 mg/L), magnesium (55.96 mg/L) and potassium (74.93 mg/L). Total nitrogen was relatively high with a concentration of 4.46 mg/L. Aluminum, cobalt, copper, iron, manganese, mercury, and nickel to name a few metals were all drastically lower at the Pacchani River and the Vizcachas River compared to Group 1 (the mine-impacted sites, (Tucari, Aruntaya, Titire). The third highest value for total organic carbon was recorded at the Vizcachas River with a value of 2.1 mg/L (Table 2.5, Figure 2.13).

Group 3: Coralaque River and Coralaque Wetland

Midstream of the basin, there was a subtle increase in certain metals in the Coralaque Wetland compared to what was recorded at Group 2 (Pacchani and Vizcachas Rivers). In the Coralaque Wetland site, iron (26 mg/L), nickel (0.024 mg/L), silver (0.00133 mg/L), cobalt (0.0348 mg/L), aluminum (33.411 mg/L), and copper (0.386 mg/L) levels were all greater than at the Pacchani and Vizcachas rivers, and greater than some of the sites downstream. Total phosphorous reached 0.692 mg/L which was the highest concentration in the entire basin. Iron, nickel, silver, cobalt, and aluminum all decreased downstream of the wetland at the Coralaque River site. Concentrations of aluminum and cadmium were at the lowest at this location for the entire basin. The highest concentration of total organic carbon in the entire basin was recorded at the Coralaque River (5 mg/L, Table 2.5, Figure 2.13).

Group 4: Coralaque Huarina, Ubinas and Alto Tambo Rivers

Within this group of sites, the Ubinas River had the third highest concentration of magnesium (41.61 mg/L) which was greater than both upstream and downstream confluence sites at the Coralaque Huarina(19.33 mg/L), and at the Alto Tambo (21.22 mg/L) sites, respectively. The Ubinas River also had higher concentrations of uranium (0.002 mg/L) relative to the two other sites sampled. However, the Ubinas River had lower concentrations of sodium, boron, cobalt, zinc, lead, nickel, iron, and aluminum than the two other sites sampled in this group. Overall, all of these metals were low at all three sites compared to other groups. Total nitrogen was lower at the Ubinas River in comparison to the two other sites (0.35 mg/L); total phosphorous and total organic carbon were comparable to the rest of the basin at all three sites (Table 2.5, Figure 2.13).

Group 5: Tambo-US-Vagabundo, Vagabundo, and Tambo-DS-Vagabundo Rivers

At the Vagabundo River, a tributary of the Tambo River, iron, nickel, silver, cobalt, uranium, zinc, and aluminum concentrations were consistently lower than at the upstream and downstream Tambo River sites sampled within this group. However, the Vagabundo River had the highest concentrations of arsenic (5.31 mg/L) and boron (34.00 mg/L) in the entire basin. Concentrations of arsenic and boron then increased downstream of these sites at the most downstream sites sampled in Group 6 (Tambo Fiscal and Tambo Costanera sites). Sodium was also higher at the Vagabundo River than at the other two sites sampled at this location. Contrastingly, cadmium (0.00006 mg/L) and mercury (0.1 mg/L) were lower at the Vagabundo River. Lead was higher at the Tambo-DS-Vagabundo (0.0173 mg/L), and the Tambo-US-Vagabundo (0.0255 mg/L) sites and lower at the Vagabundo River (0.0005 mg/L). Total phosphorous was higher at the Tambo-Us-Vagabundo and Tambo-DS-Vagabundo sites than at the Vagabundo River (Table 2.5, Figure 2.13).

Group 6: Tambo Fiscal and Tambo Costanera Rivers

Compared to Group 5, aluminum, iron, nickel, zinc, mercury, and cobalt slightly increased from the Tambo River sites sampled upstream however, concentrations were not notably higher. However, lead levels at the Tambo Fiscal site (0.0214 mg/L) and the Tambo Costanera site (0.0521 mg/L) were the highest recorded in the entire basin. Nitrogen increased again slightly relative to the sites sampled upstream on the Tambo River with a concentration of 1.78 mg/L for the Tambo Fiscal site and 2.03 mg/L for Tambo Costanera site. Some of the highest concentrations of total phosphorous in the basin were recorded at the Tambo Fiscal site (0.501 mg/L) and at the Tambo Costanera site (0.636 mg/L, Table 2.5, Figure 2.13).

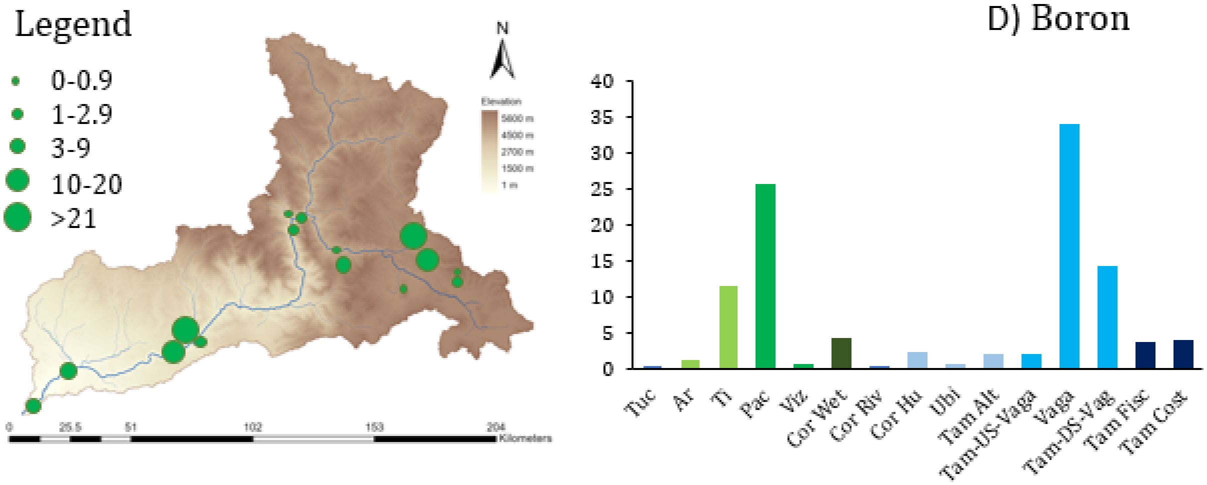
Table 2.5: Water chemistry sample concentrations of the Tambo River Basin. All concentrations are expressed in mg/L. Peruvian Water Quality Standards for drinking water with disinfection and conventional treatment are shown, as well as for the untreated aquatic environments of coastal and mountain rivers.

	1) Production of drinking water		2) Aquatic environment	Sites														
Water Quality Parameters	Potable with disinfection	Potable with conventional treatment	Coastal and Mountain Rivers	Tucari	Aruntaya	Titire	Pacchani	Vizcachas	Coralaque Wetland	Coralaque River	Coralaque Huarina	Ubinas	Tam Alto	Tambo-US-Vag	Vagabundo	Tam-DS-Vag	Tambo Fiscal	Tambo Costanera
Aluminum (Al)	0.9	5	**	96.901	67.810	68.423	0.955	1.949	33.411	0.297	4.908	1.085	3.678	9.134	0.424	6.849	12.802	15.306
Arsenic (As)	0.01	0.01	0.15	0.205	0.017	0.478	0.335	0.089	0.603	0.003	0.052	0.025	0.048	0.126	5.310	1.984	0.346	0.370
Boron (B)	2.4	2.4	**	0.253	1.342	11.636	25.851	0.594	4.412	0.053	2.379	0.625	2.141	2.117	34.007	14.429	3.792	4.077
Calcium (Ca)	**	**	**	102.259	39.078	97.841	227.291	16.480	63.277	8.056	89.102	133.660	94.550	99.505	178.620	134.553	160.048	151.991
Cobalt (Co)	**	**	**	0.285	0.282	0.199	0.002	0.002	0.035	0.001	0.009	0.002	0.009	0.015	0.001	0.010	0.029	0.026
Copper (Cu)	2	2	0.1	1.535	0.063	1.220	0.011	0.010	0.386	0.014	0.040	0.006	0.037	0.068	0.008	0.050	0.213	0.171
Iron (Fe)	0.3	1	**	73.567	15.312	29.449	1.820	2.554	26.461	1.616	4.468	1.602	3.802	11.842	0.581	9.168	13.022	13.567
Lead (Pb)	0.01	0.05	0.0025	0.008	0.002	0.002	0.003	0.002	0.013	0.001	0.009	0.002	0.007	0.026	0.001	0.017	0.021	0.052
Lithium (Li)	**	**	**	0.019	0.086	2.514	5.072	0.065	0.790	0.002	0.519	0.051	0.484	0.350	2.453	1.171	0.613	0.693
Magnesium (Mg)	**	**	**	12.181	98.740	17.457	55.961	4.880	15.058	2.512	19.333	41.609	21.221	21.656	35.629	27.376	25.941	27.437
Manganese (Mn)	0.4	0.4	**	4.761	7.378	3.875	0.671	0.391	1.641	0.058	0.562	0.136	0.476	1.431	0.130	0.841	1.363	1.524
Mercury (Hg)	0.001	0.002	0.0001	0.010	0.000	0.002	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nickel (Ni)	0.07	**	0.052	0.204	0.226	0.132	0.002	0.002	0.024	0.001	0.008	0.002	0.007	0.017	0.002	0.011	0.020	0.019
Potassium (K)	**	**	**	10.215	67.530	43.318	74.933	6.399	17.713	4.508	14.767	10.616	13.700	11.726	65.476	31.966	18.359	21.497
Sodium (Na)	**	**	**	139.472	157.51	746.212	1807.140	28.225	231.192	4.970	285.515	72.317	276.613	161.377	418.472	268.021	320.725	349.218
Total Nitrogen	**	**	**	20.930	4.100	8.410	4.460	1.170	1.590	1.000	1.590	0.350	1.180	0.210	0.220	1.260	1.780	2.030
Total Organic Carbon	**	**	**	0.600	4.000	1.700	0.800	2.100	0.400	5.000	0.900	0.600	1.300	1.700	0.400	1.300	0.900	1.500
Total Phosphorus	0.1	0.15	**	0.266	0.230	0.136	0.220	0.155	0.692	0.102	0.195	0.183	0.182	0.582	0.081	0.420	0.501	0.636
Uranium (U)	0.02	0.02	**	0.005	0.001	0.005	0.002	0.000	0.003	0.000	0.001	0.002	0.001	0.002	0.000	0.001	0.002	0.003
Zinc (Zn)	3	5	0.12	3.101	0.421	2.074	0.021	0.025	0.398	0.004	0.118	0.013	0.104	0.608	0.006	0.325	0.306	0.310

1) *ECA - Surface waters used for the production of drinking water*

2) *ECA-Conservation of the aquatic environment*

*** Parameter not available*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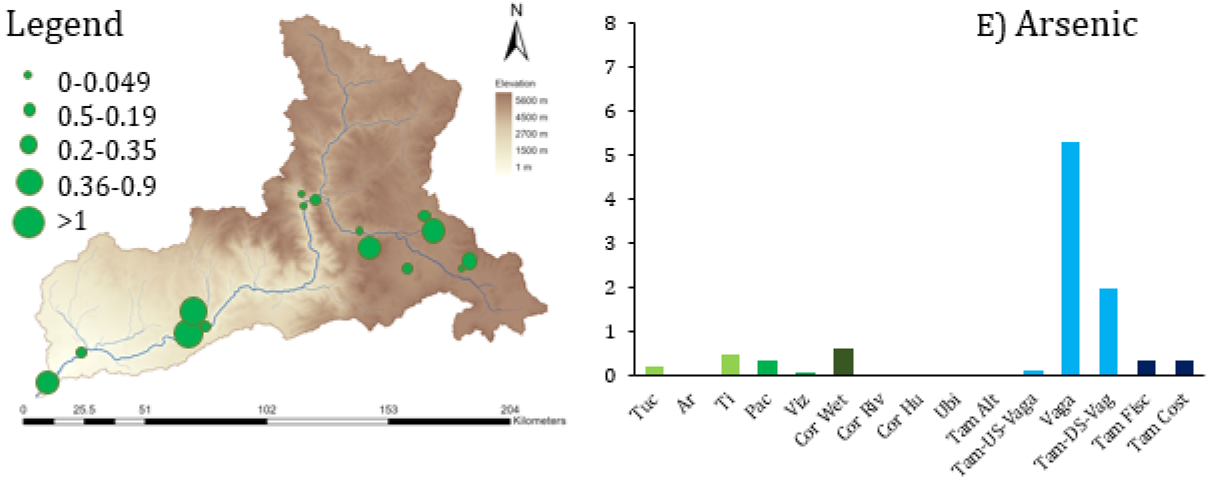
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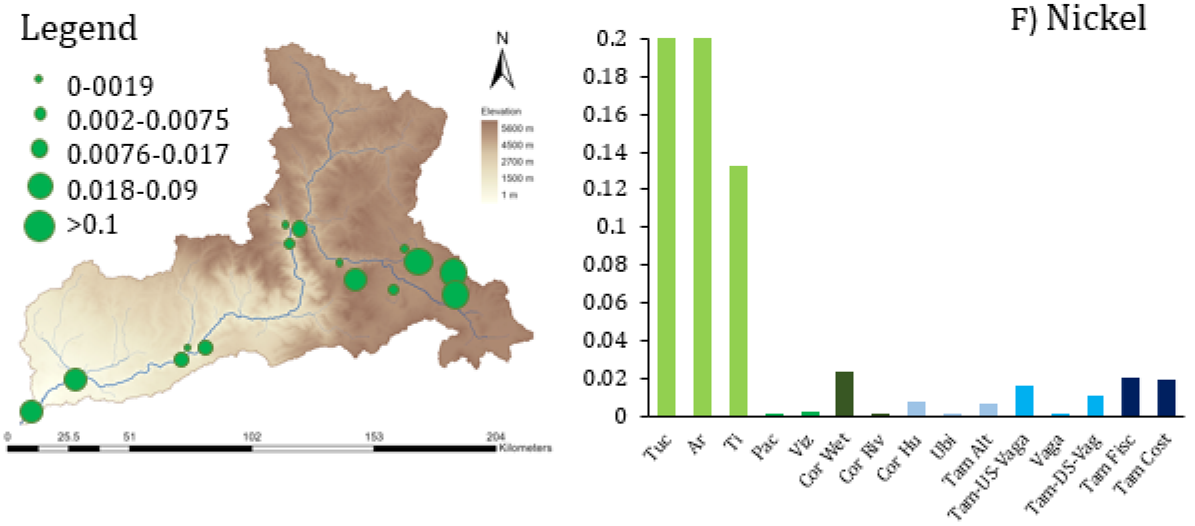
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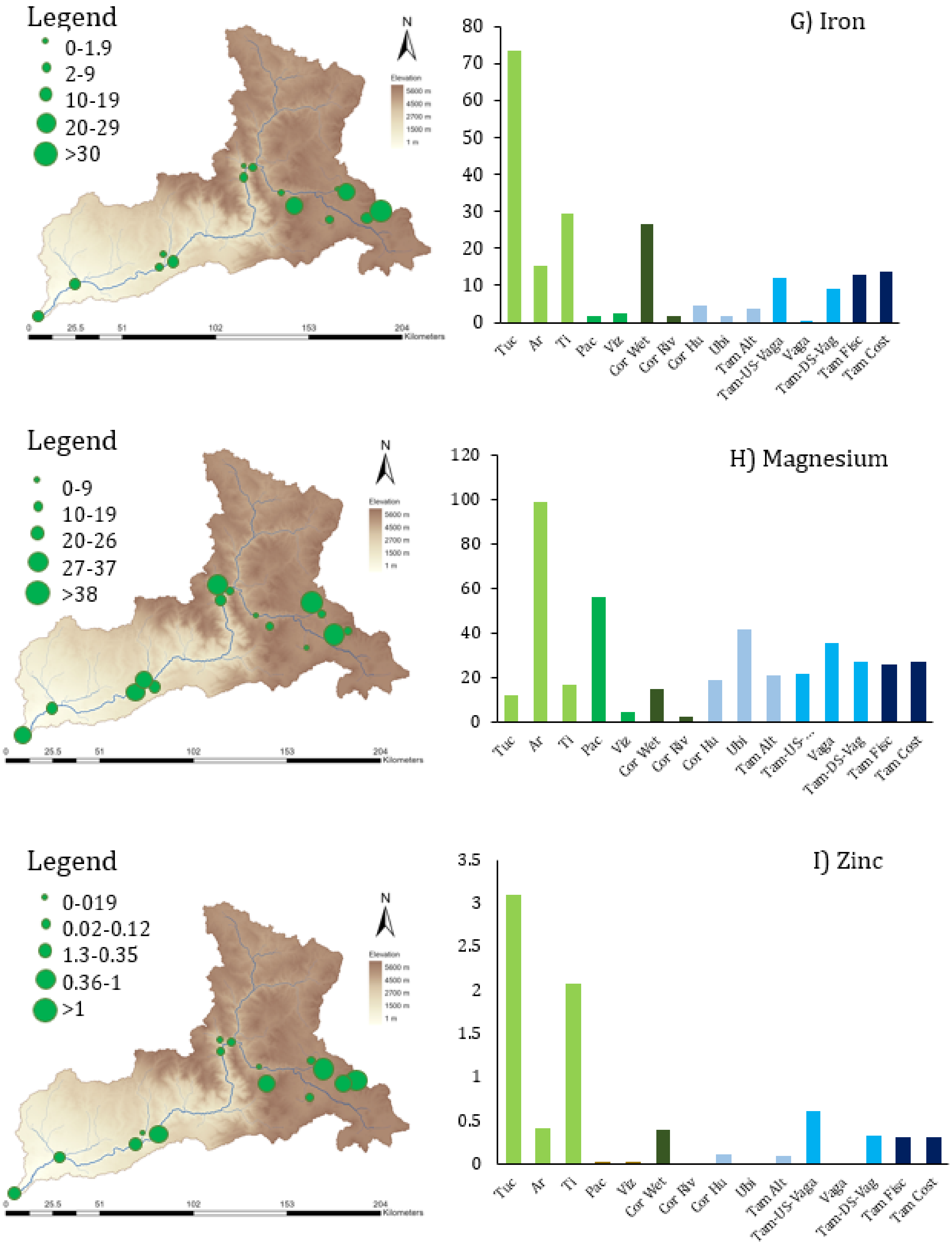Legend
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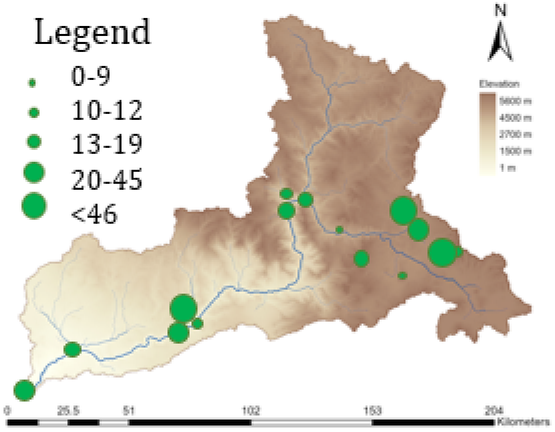
1500 m
1 m
0
25.5
51
102
153
204
Kilometers
G) Iron
80
70
60
50
40
30
20
10
0
Tuc
Ar
Ti
Pac
Viz
Cor Wet
Cor Riv
Cor Hu
Ubi
Tam Alt
Tam-US-Vaga
Vaga
Tam-DS-Vag
Tam Fisc
Tam Cost
Legend
0-9
10-19
20-26
27-37
>38
N
Elevation
5600 m
4500 m
2700 m
1500 m
1 m
0
25.5
51
102
153
204
Kilometers
H) Magnesium
120
100
80
60
40
20
0
Tuc
Ar
Ti
Pac
Viz
Cor Wet
Cor Riv
Cor Hu
Ubi
Tam Alt
Tam-US-...
Vaga
Tam-DS-Vag
Tam Fisc
Tam Cost
Legend
0-019
0.02-0.12
1.3-0.35
0.36-1
>1
N
Elevation
5600 m
4500 m
2700 m
1500 m
1 m
0
25.5
51
102
153
204
Kilometers
I) Zinc
3.5
3
2.5
2
1.5
1
0.5
0
Tuc
Ar
Ti
Pac
Viz
Cor Wet
Cor Riv
Cor Hu
Ubi
Tam Alt
Tam-US-Vaga
Vaga
Tam-DS-Vag
Tam Fisc
Tam Cost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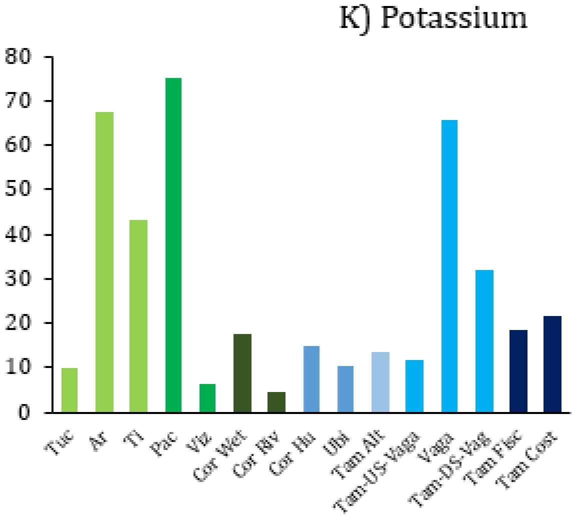
Kilometers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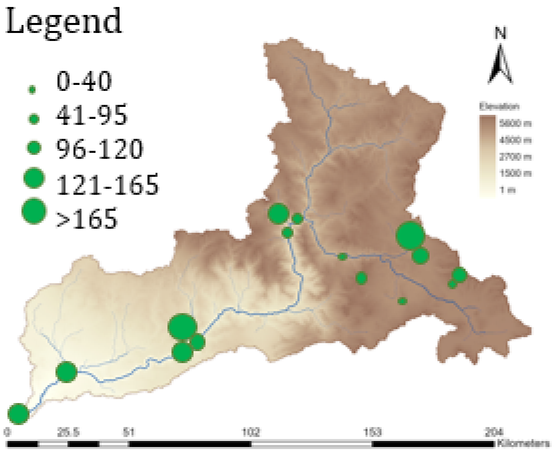
70
60
50
40
30
20
10
0
Tuc
Ar
Ti
Pac
Viz
Cor Wet
Cor Riv
Cor Hu
Ubi
Tam Alt
Tam-US-Vaga
Vaga
Tam-DS-Vag
Tam Fisc
Tam Cost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96-120
121-165
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N
Elevation
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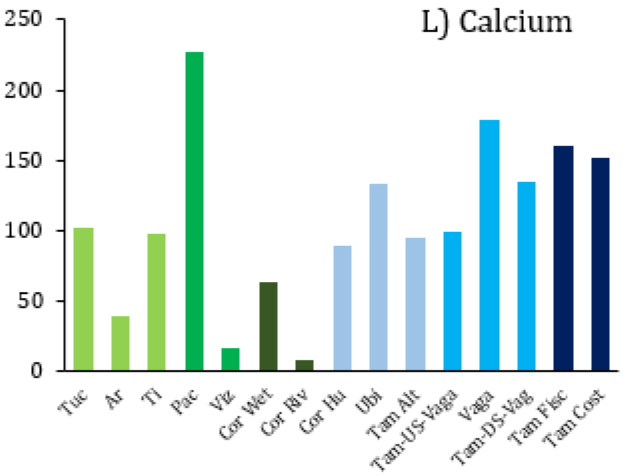
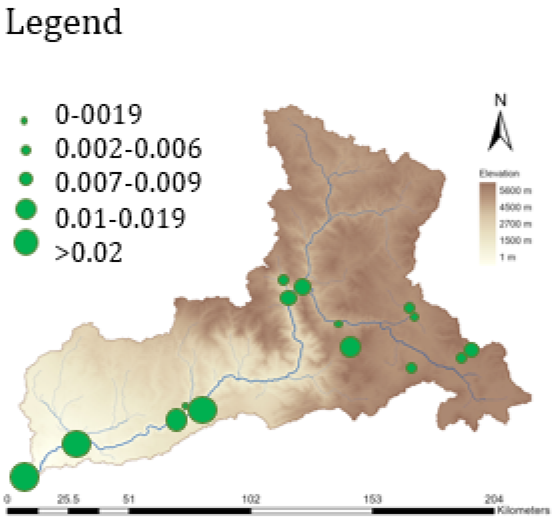
0
25.5
51
102
153
204
Kilometers


L) Calcium
250
200
150
100
50
0
Tuc
Ar
Ti
Pac
Viz
Cor Wet
Cor Riv
Cor Hu
Ubi
Tam Alt
Tam-US-Vaga
Vaga
Tam-DS-Vag
Tam Fisc
Tam Cost


Legend
0-0019
0.002-0.006
0.007-0.009
0.01-0.019
>0.02
N
Elevation
5600 m
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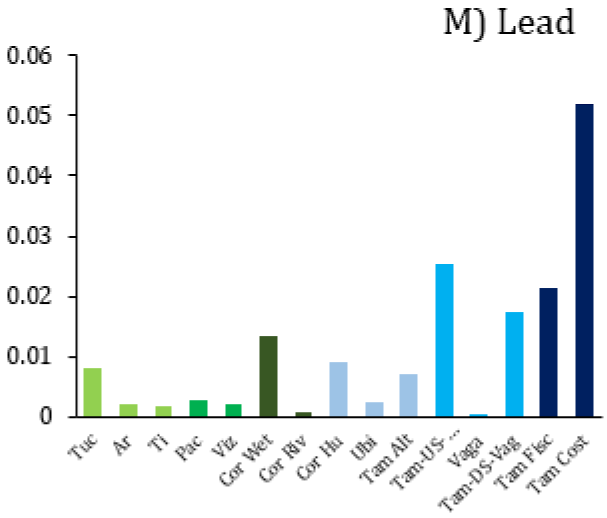
2700 m
1500 m
1 m
0
25.5
51
102
153
204
Kilometers


M) Lead
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0.05
0.04
0.03
0.02
0.01
0
Tuc
Ar
Ti
Pac
Viz
Cor Wet
Cor Riv
Cor Hu
Ubi
Tam Alt
Tam-US-...
Vaga
Tam-DS-Vag
Tam Fisc
Tam Cost

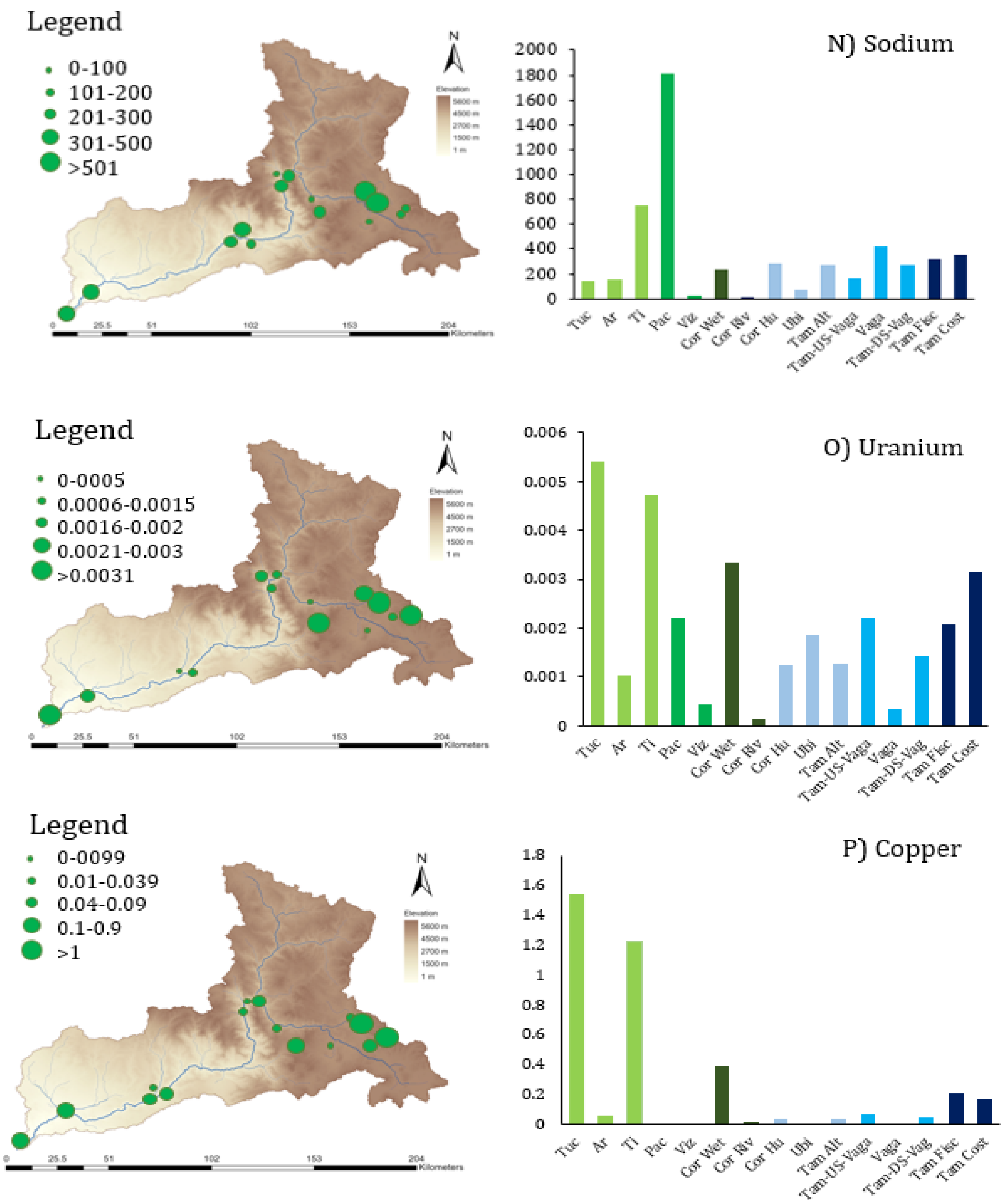

Figure 2.13: Spatial distribution of water chemistry in the Tambo River Basin. Bar graph colours indicate different groups: Group 1 in light green (Tucari, Aruntaya and Titire Rivers), group 2 in medium green (Pacchani and Vizcachas Rivers), group 3 in dark green (Coralaque Wetland and Coralaque River), group 4 in light blue (Coralaque Huarina site, Ubinas River and Alto Tambo River), group 5 medium blue (Tambo-US-Vagabundo, Vagabundo, Tambo-DS-Vagabundo), and group 6 in dark blue (Tambo Fiscal and Tambo Costanera Rivers). All concentrations are in mg/L.

2.6.5 Pearson Correlation Results for Metals, Nutrient and Total Organic Carbon Concentrations

Pearson's Correlation showed several statistically significant (α=0.05) correlations between variables (Table 2.6). Sodium was negatively correlated with total organic carbon (r=-0.584) but positively correlated with potassium (r=0.783) and boron (r=0.774). Magnesium was positively correlated with potassium (r=0.706). Aluminum was positively correlated with uranium (r=0.729), iron (r=0.955), mercury (r=0.598), zinc (r=0.843) and total nitrogen (r=0.734). Potassium was correlated with boron (r=0.810). Uranium was positively correlated with iron (r=0.809), mercury (r=0.740), zinc (r=0.884) and total nitrogen (r=0.768). Similarly, iron was positively correlated with mercury (r=0.693), zinc (r=0.894) and total nitrogen (r=0.732). Arsenic was positively correlated with boron (r=0.754) while mercury was positively correlated with zinc (r=0.837) and total nitrogen (r=0.776). Lastly, zinc was positively correlated with total nitrogen (r=0.797, Table 2.6).

Table 2.6: Pearson correlation matrix of water chemistry parameters. Significant correlations are in bold.

Variables	Total Organic Carbon	Sodium (Na)	Magnesium (Mg)	Aluminum (Al)	Potassium (K)	Lead (Pb)	Uranium (U)	Iron (Fe)	Arsenic (As)	Boron (B)	Mercury (Hg)	Zinc (Zn)	Total Nitrogen
Total Organic Carbon	**1**	**-0.584**	0.092	0.100	-0.219	-0.069	-0.319	0.037	-0.419	-0.432	-0.247	-0.094	-0.029
Sodium (Na)	**-0.584**	**1**	0.360	0.267	**0.783**	0.198	0.406	0.211	0.345	**0.774**	0.001	0.194	0.278
Magnesium (Mg)	0.092	0.360	**1**	0.197	**0.706**	-0.124	-0.133	-0.017	0.029	0.230	-0.209	-0.154	0.078
Aluminum (Al)	0.100	0.267	0.197	**1**	0.200	0.233	**0.729**	**0.955**	-0.210	-0.144	**0.598**	**0.843**	**0.734**
Potassium (K)	-0.219	**0.783**	**0.706**	0.200	**1**	-0.071	0.088	0.033	0.524	**0.810**	-0.158	0.034	0.202
Lead (Pb)	-0.069	0.198	-0.124	0.233	-0.071	**1**	0.261	0.347	-0.024	0.031	-0.050	0.052	-0.109
Uranium (U)	-0.319	0.406	-0.133	**0.729**	0.088	0.261	**1**	**0.809**	-0.151	0.015	**0.740**	**0.884**	**0.768**
Iron (Fe)	0.037	0.211	-0.017	**0.955**	0.033	0.347	**0.809**	**1**	-0.226	-0.187	**0.693**	**0.894**	**0.732**
Arsenic (As)	-0.419	0.345	0.029	-0.210	0.524	-0.024	-0.151	-0.226	**1**	**0.754**	-0.085	-0.086	-0.220
Boron (B)	-0.432	**0.774**	0.230	-0.144	**0.810**	0.031	0.015	-0.187	**0.754**	**1**	-0.276	-0.104	-0.051
Mercury (Hg)	-0.247	0.001	-0.209	**0.598**	-0.158	-0.050	**0.740**	**0.693**	-0.085	-0.276	**1**	**0.837**	**0.776**
Zinc (Zn)	-0.094	0.194	-0.154	**0.843**	0.034	0.052	**0.884**	**0.894**	-0.086	-0.104	**0.837**	**1**	**0.797**
Total Nitrogen	-0.029	0.278	0.078	**0.734**	0.202	-0.109	**0.768**	**0.732**	-0.220	-0.051	**0.776**	**0.797**	**1**

Values in bold are different from 0 with a significance level $p<0.05$

2.6.6 PCA of Metals, Nutrient and Total Organic Carbon Concentrations

The first two factors of the PCA explained 66.31% of the total variability, with PC1 explaining 39.33% and PC2 explaining 26.98%. PC1 contained large positive loadings of aluminum (0.898), uranium (0.925), iron (0.939), mercury (0.828), zinc (0.953) and total nitrogen (0.875). PC2 had a large positive loading of sodium (0.865), potassium (0.911) and boron (0.938) and a moderately large arsenic loading (0.698, Table 7, Figure 12).

Table 2.7: Principal component analysis factor loadings. Strong positive loadings are in bold.

	PC1	PC2
Total Organic Carbon	-0.145	-0.533
Sodium (Na)	0.317	**0.865**
Magnesium (Mg)	-0.021	0.470
Aluminum (Al)	**0.898**	-0.006
Potassium (K)	0.110	**0.911**
Lead (Pb)	0.184	0.033
Uranium (U)	**0.925**	0.075
Iron (Fe)	**0.939**	-0.102
Arsenic (As)	-0.179	**0.698**
Boron (B)	-0.097	**0.938**
Mercury (Hg)	**0.828**	-0.193
Zinc (Zn)	**0.953**	-0.061
Total Nitrogen	**0.875**	0.021

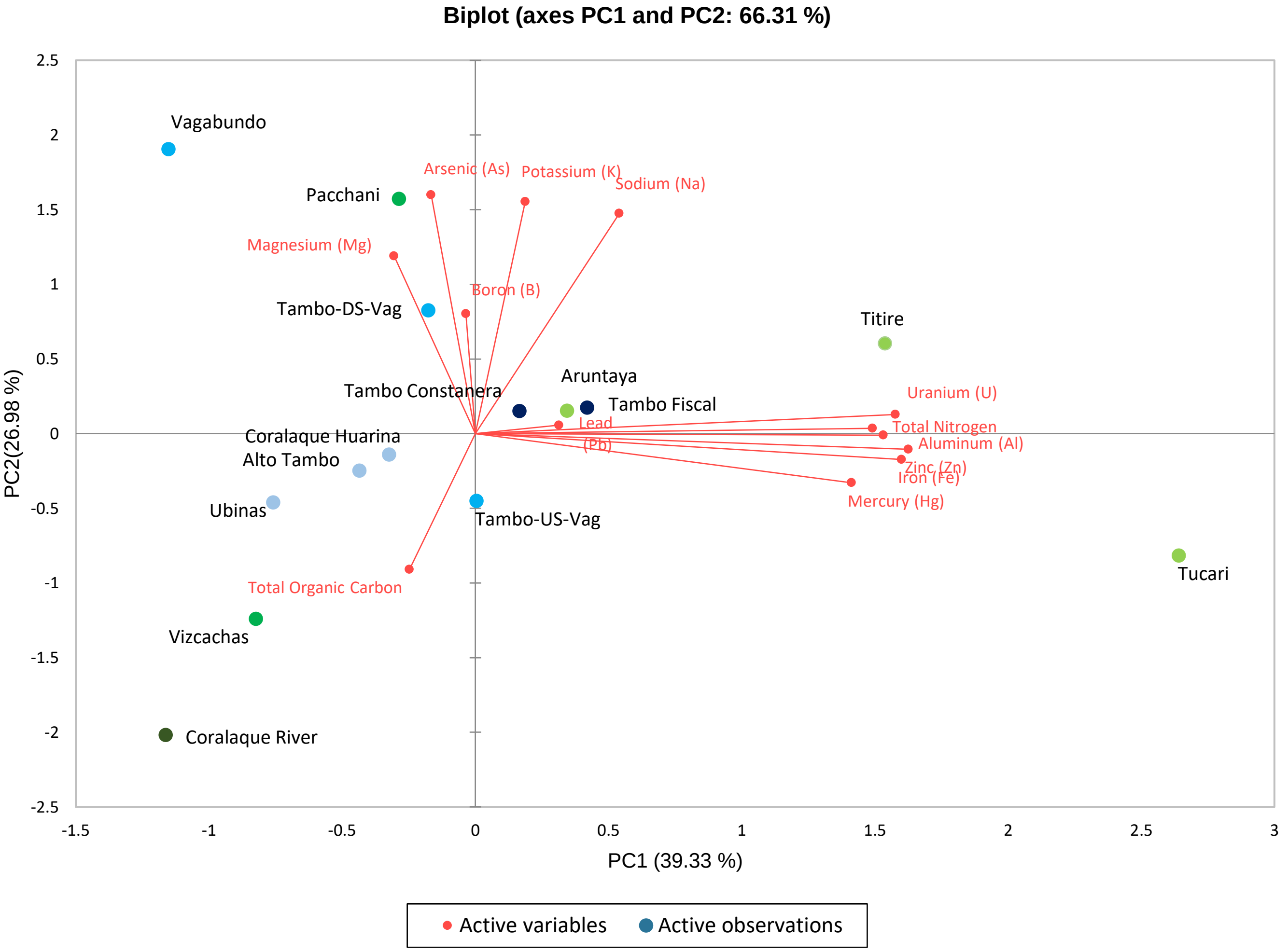

Figure 2.14: Principal Component Analysis (PCA) of metals, nutrients, and total organic carbon. PC1 explains 39.33% of the variance, PC2 explains 26.98%, and both together represent 66.31%. Sites belonging to the same group are in the same colour. Group 1 is light green (Tucari, Aruntaya and Titire Rivers), group 2 is medium green (Pacchani and Vizcachas Rivers), group 3 is dark green (Coralaque Wetland and Coralaque River), group 4 is light blue (Coralaque Huarina site, Ubinas and Alto Tambo Rivers), group 5 is medium blue (Tambo-US-Vagabundo, Vagabundo River, Tambo-DS-Vagabundo sites), and group 6 is dark blue (Tambo Fiscal and Tambo Costanera Rivers).

The mine-impacted sites of Group 1, the Tucari, Aruntaya and Titire rivers were all positively associated with PC1, with the Tucari and Titire rivers being most strongly associated with this factor. The Aruntaya and Titire Rivers were both positively associated with PC2 however, the Tucari River was negatively associated with PC2. The upstream Group 2 that had no mining impacts, the Pacchani and Vizcachas rivers, were both negatively associated with PC1. The Vizcachas River was negatively associated with PC2 where total organic carbon was high, whereas the Pacchani River was positively associated with PC2. Similar to the Vizcachas River, the Coralaque River in Group 3, was negatively associated with both PC1 and PC2, and was associated with higher total organic carbon. Group 4, which included the Coralaque Huarina site, the Ubinas River and the Alto Tambo site, was negatively associated with both PC1 and PC2. For group 5, Tambo-US-Vagabundo was negatively associated with PC2 whereas, the Vagabundo river and the Tambo-DS-Vagabundo site were strongly associated with PC2. For the most downstream group 6, Tambo Fiscal and Tambo Costanera were both positively associated with PC1.

2.7 DISCUSSION

2.7.1 Water Quality of the Tambo River Basin

Mining Contaminated and Mining Unimpacted Sites (Groups 1 and 2)

Since 2016, elevated levels of metals have been measured in the rivers near the Florencia Tucari Mining unit (Aruntani S.A.C.). This mine is an epithermal metal deposit with high sulfidation. Open-pit mining in this region used cyanide to extract the ore and has waste heaps of overburdened rock and spent ore on leaching pads. Acid mine drainage (AMD) from incomplete mine closure and site management has harmed the health of the nearby villagers, flora and fauna (Lopez Catacora, 2020).

My research confirmed the presence of degraded water quality at the mine-impacted river sites (Group 1, the Tucari, Aruntaya, Titire rivers) which is consistent with previous reports showing high concentrations of metals such as arsenic, aluminum, boron and iron present in surrounding waters (Indeci, 2021). These sites had multiple water quality parameters and metal concentrations exceeding Peruvian Water Quality Standards for both drinking water and the conservation of the aquatic environment (MINEM, 2017). Physicochemical measurements differed from the rest of the basin, especially in terms of pH. The water from this group of sites including sites that were in the same general region but known to be unimpacted by mines in Group 2, the Pacchani River and the Vizcachas Rivers, were all notably more acidic than those in the rest of the basin. The pH levels at the mine-affected sites were much lower than the acceptable limits according to Peruvian Water Quality Standards of 6.5-8.5 for potable water with no advanced treatment (defined as waters intended for human consumption and are subject to conventional treatment that includes processes with advanced physical and chemical methods such as pre-chlorination, microfiltration, ultrafiltration, nanofiltration, activated carbon, reverse osmosis or equivalent processes (MINEM, 2017).

One of the most common effects of mining wastes globally is the formation of acid rock drainage (ARD) which is caused by a sequence of complex biogeochemical and mineral dissolution processes originating from sulphidic mine tailings (Nagy et al., 2007). The region's important metals are sulphide-associated ones which are prone to causing ARD when oxidized (Loayza et al., n.d.). Tailings were visible at some of the mine sites, which are known to have produced consequential ground and surface water contamination during both operative and post-operative stages, often resulting from ARD releases of metal-rich and acidic leachate (Dold, 2014). Studies by the Environmental Evaluation and Control Agency (OEFA) in 2016 concluded that Florencia-Tucari leachate from tailings ponds penetrates through the soils contaminating pasturelands and the headwaters of the basin with arsenic, zinc, iron, and copper (INDECI, 2021).

Contamination is known to be prominent in the Tucari, Aruntaya and Titire rivers reaching the Coralaque and Tambo rivers (INDECI, 2021). As shown in this study, this group of sites was associated in the PCA with multiple metals exceeding Peruvian Water Quality Standards with advanced treatment. Similarly, water quality parameters often exceeded the Peruvian Water Quality Standards for the conservation of the aquatic environment with the Tucari River exceeding all standards (Table 2.6, MINEM, 2017).

Notably, in the highest altitude zone, the Titire (mine impacted) and Pacchani (not mine impacted) Rivers, had high levels of TDS. TDS can be linked to precipitation, lithology, volcanic activity, seismic activity and anthropogenic activities (Armijos et al., 2013). In this region, TDS inputs are strongly influenced by the markedly uneven rainfall distribution across the region along with the presence of steep slopes and sparse vegetation making the landscape highly susceptible to erosion and landslides (Armijos et al., 2013). The sedimentary rocks which are primarily silico-clastic in Peru are known to erode at a rapid pace (Moquet et al., 2011). Furthermore, previous studies by Vanacker et al., 2007 have demonstrated that high loads of TDS are often caused by landslides and erosional processes and not necessarily by human activity. However, dissolved loads in mining areas such as ARD can additionally exacerbate weathering processes (Vanacker et al., 2007; Moquet et al., 2018). That said, it is more likely that the high TDS levels at the Titire River weare due to natural causes as the other sites impacted by the mine, the Tucari and Aruntaya Rivers, had notably lower levels of TDS than the Titire River. Although the Titire River showed more evidence of mining contamination than the Pacchani River as anticipated, a distinct separation in site conditions in terms of mining contamination was not obvious due to the high levels of TDS present at both sites.

Nitrogen levels were also highest within groups 1 and 2, at the Tucari, Titire, and Pacchani River sites. Low oxygen conditions in areas of high elevation can lead to the enrichment of nitrogen through denitrification rather than inputs from external sources. Nitrogen has long been an indicator of eutrophication in aquatic ecosystems, and it is possible, that nitrogen levels were also increased due to inputs of wastewater from the local settlements (Aranguren-Riaño et al., 2018). The atmosphere may be another source of nitrogen, as high-altitude areas that have low nutrients and cold temperatures have increased deposition (Catalan & Donato Rondon, 2016; Archundia et al., 2017).

In contrast, water quality was better overall at the Vizcachas River in Group 2. Several water quality parameters that exceeded Peruvian Water Quality Standards for both drinking water and the conservation of the aquatic environment at the mine sites were below Peruvian Water Quality Standards at the Vizcachas river. The low levels of metals especially boron and arsenic, indicated the absence of natural sources of metals and acids along with mining contamination at the Vizcachas River. At the Pacchani River, the same interpretation can be challenging due to its association with parameters from PC2. Although the Pacchani and Vizcachas Rivers did not show signs of mining contamination as expected, Pacchani's high levels of sodium, boron, magnesium, potassium, and nitrogen may suggest some degree of natural or anthropogenic contamination is occurring in this sub-basin.

Mid Basin Coralaque Wetland and Coralaque River (Group 3)

The Coralaque Wetland, being the only wetland sampled during this study had unique characteristics. High Andean wetlands receive water from glaciers, rivers, precipitation and underground aquifers (Fonkén, 2014). This filtering effect can be seen in the higher metal concentrations observed in the wetland compared to downstream. Aluminum, magnesium, iron, arsenic, uranium, nickel, silver, lead, zinc, mercury, sodium and boron are only some of the metals where concentrations were subsequently reduced as water flowed through the wetland and downstream into the Coralaque River. As a result, these metals all had increased concentrations within the wetland when compared to other sites that are unimpacted by the mines such as Pacchani and Vizcachas Rivers. Bofedales act as important sinks for metals, allowing the accumulation, migration and transformation of metals between waterbodies, sediments, soils and biota (Carrillo et al., 2021). Some impacts of mining operations may be localized, but aquatic effects may extend through hydrological processes. However, the concentration of all cations and anions, from natural and human sources, often goes up during the dry season as water evaporates and organic matter decomposes (Custodio et al., 2018). Therefore, it is unclear if the presence of these metals in the wetland can be directly attributed to mining impacts.

The highest phosphorous levels were also recorded in the Coralaque Wetland. Phosphorous can be a limiting factor in primary production, and elevated levels along with other factors such as high residence times and shallow stagnant conditions promote the growth of aquatic plants and algae (Cony et al., 2014). Phosphorous values were about four times greater than the Peruvian Water Quality Standards of 0.15mg/L (Minam, 2017). The more phosphorous there is in a wetland the more deterioration will follow in terms of ecosystem functions such as water purification, sediment and nutrient retention and maintenance of biodiversity to name a few (Yepes & Pérez, 2019). High phosphorous levels are often attributed to anthropogenic activities, however, at this location, it is more probable that the cause is feces from wild camelid species such as the vicuna and guanaco known to use this landscape for grazing, nesting and drinking water(Yepes & Pérez, 2019).

Conductivity was at its lowest in the entire basin in the Coralaque Wetland, as expected given the low levels of cations and anions. The wetland at this location may also have had a larger hydrological input from rainwater, which is mineral-poor compared to river flow and groundwater. Moreover, the high level of organic matter and TOC can complex and sequester cations reducing the water-borne concentration. The greater the amount of organic matter the more ion concentrations are reduced and the less conductive waters become (Asadi & Huat, 2009).

The highest levels of TOC were downstream of the wetland at the Coralaque River site. This suggests a high rate of biological production in the upper levels and enrichment of carbon due to the transport of allochthonous material from the wetland upstream (Sánchez España et al., 2018). In general, at the Coralaque River, water quality was better than at the mine-impacted sites (Group 1, Tucari, Aruntaya, Titire rivers). Physicochemical water parameters and metal levels were notably lower in the Coralaque River. Iron, cobalt, nickel, zinc, boron, aluminum and copper for example were all below Peruvian Water Quality Standards for waters with advanced treatment and for the conservation of the aquatic environment (MINEM, 2017). Notably, there was no sign of contamination due to anthropogenic impacts, mine impacts, or natural sources of metals and acids at this location along the Coralaque River.

Natural Sources of Metals and Acids (Group 4 Ubinas and Vagabundo Sites)

The Tambo River Basin has naturally occurring sources of metals and acids. The basin has five active volcanic areas of Pleistocene age: the Subancaya in the Arequipa region, the Ubinas, the Huaynaputina and Tiscani in the Moquegua region and the Yucamane in the Tacna region. Inputs of volcanic source metals to surface waters includes hot springs, volcanos and geysers (Morales-Simfors, Bundschuh, Herath, Inguaggiato, Caselli, Tapia, Choquehuayta, Armienta, Ormachea, & Joseph, 2020). Fluids originating from geothermal activity and volcanic emissions are known sources of arsenic leading to high concentrations in the surface water and soils (Morales-Simfors et al., 2020). Generally, arsenic, boron and lithium at medium to high concentrations have been reported in previous studies in multiple basins in Peru and South America (Bech et al., 1997; Bundschuh et al., 2012; Tapia et al., 2019, Ccanccapa i-Cartagena et al., 2021).

Arsenic concentrations in the spring waters near the Ubinas district have reached 0.17mg/L in previous studies (Morales-Simfors, Bundschuh, Herath, Inguaggiato, Caselli, Tapia, Choquehuayta, Armienta, Ormachea, & Joseph, 2020). Sites suspected to capture sources of contamination from the Ubinas Volcano (Ubinas River, Alto Tambo) had concentrations of arsenic slightly above Peruvian Water Quality Standards for drinking water but were below levels for the conservation of the aquatic environment. Boron levels were all below Peruvian Water Quality Standards. Therefore, a source of contamination or low/high pH waters coming from the Ubinas River was not captured during this study and the similarity between all sites sampled in this grouping even upstream of the potential source of contamination (Coralaque Huarina) is made evident in the PCA. The ephemeral nature of volcanic activity and pulses could affect the potential source of elements to the basin from this volcanic zone at different times.

The site located nearest to sources of known geothermal activity (Group 5, Vagabundo River and Tambo-DS-Vagabundo) had the highest levels of boron, arsenic and lithium found in the entire basin. Concentrations of arsenic and boron in the Vagabundo River exceeded Peruvian Water Quality Standards for drinking water with advanced treatments and for the conservation of the environment (Minem, 2017). Boron, arsenic, and lithium levels also increased downstream of the confluence of the Tambo and the Vagabundo Rivers in comparison to the site upstream of the Vagabundo River (Tambo-US-

Vagabundo), indicating a point source of naturally occurring contamination entering the basin. The separation between upstream of the source (Tambo-US-Vagabundo), at the source (Vagabundo), and downstream of the source (Tambo-US-Vagabundo) is made evident in the PCA, where Tambo-US-Vagabundo is not associated with sources of boron or arsenic but the two other sites within this grouping are. Arsenic, boron, and lithium are known to originate from natural sources and are often positively correlated with high concentrations of other toxic contaminants such as Fe, Mn, Pb, Zn, and Cd. Despite there being evidence of high concentrations of these metals, their potential environmental effects are often neglected or underestimated (Morales-Simfors, Bundschuh, Herath, Inguaggiato, Caselli, Tapia, Choquehuayta, Armienta, Ormachea, & Joseph, 2020). Similar to the sites sampled in the Ubinas district (Group 4), waters at the sites from Group 5 along the Vagabundo River did not have acidic waters.

Downstream Basin Conditions

The highest levels of lead were recorded at the most downstream Tambo River sites (Group 6, Tambo Fiscal and Tambo Costanera sites). Although they did not exceed Peruvian Water Quality Standards for drinking waters, they did exceed those for environmental conservation. Elevated lead levels along with the high levels of aluminum and iron could have been an indicator of increased anthropogenic activities, as the coastal watersheds were more densely populated and active urban centres. The high levels may also come from natural sources such as soil weathering and erosion of bedrock, volcanos, and long-range atmospheric transport that can widely distribute certain metals (Custodio et al., 2020). The contamination can be particularly concerning as these waters are often used for irrigation and agriculture.

The Tambo River Costanera site had TDS concentrations that exceeded acceptable levels with advanced treatment of 1500 mg/L. Human activity can indirectly cause higher loads of TDS through the construction of roads and ploughing of fields, which leaves sediment unstabilized along with an abundance of exposed and broken calcareous rocks and salt outcrops. The Peruvian Andes are known as the largest source of dissolved material in the Amazon basin (Molina et al., 2008, Armijos et al., 2013).

Phosphorous levels were greater than Peruvian Water Quality Standards of 0.15 mg/L for drinking water. As phosphorous is one of the limiting factors in the growth of phytoplankton, attached algae, and aquatic plants, it determines the eutrophication of water bodies (Custodio et al., 2018). The increase in phosphorous is often attributed to wastewater discharges, such as domestic wastewater and fertilized agricultural land (Custodio et al., 2018). The arid soils of southern Peru can be depleted in nutrients which makes the use of fertilizers for agriculture commonplace. It is unclear what types, how much, and where fertilizer was applied in this region, but overall phosphates, nitrogenous compounds, potash, and sulphur, as well as minor amounts of manganese, boron and certain rare earth elements, are the most essential components used in fertilizers in South America (Harrington et al., 1966; Ramos et al., 2016). Livestock in the region is additionally a potential source of phosphorus through fecal matter contamination (Custodio et al., 2018). Moreover, sediment-bound chemical complexes of phosphorous and iron, when exposed to low levels of dissolved oxygen can cause a release of phosphorous into the water column ("internal phosphorus recycling", Custodio & Peñaloza, 2019). This can be concerning as iron levels were high at these sites, exceeding Peruvian Water Quality Standards and dissolved oxygen levels were generally lower in comparison to the rest of the basin (except for sites high in elevation). Other sites throughout the basin that were high in phosphorous but less populated, could perhaps be attributed to fecal matter from wildlife. High phosphorous concentrations can exert strong pressure on the aquatic environment leading to a shift in the assemblages of fish and invertebrates toward less desirable species, including pollution-tolerant ones, especially when linked to increasing anthropogenic activities (Custodio et al., 2018).

2.8 CONCLUSION

We observed that mining activity had measurable negative effects on the three mining impacted sites in Group 1, the Tucari, Aruntaya and Titire rivers. For the mining unimpacted sites (group 2) the Pacchani river, did not have a mining signal despite its proximity. However, the high levels of sodium, boron, magnesium, and potassium suggest natural sources of these elements occur in the high-altitudes of the basin. Water quality was noticeably better at the other group 2 site, the Vizcachas River. Signals of mining, anthropogenic or natural sources of metals and acids were not obvious at the Coralaque Wetland, however this site had high levels of metals. Contrastingly, metal concentrations were lower at the Coralaque River as expected. Contrastingly, no evident source of natural sources of metals and acids was captured at sites located near the Ubinas Volcano. However, natural sources of contamination from related zones of geothermal activity (active geysers) were detected within Group 5 at the Vagabundo River and were recorded downstream as well at the Tambo-DS-Vagabundo site. Anthropogenic sources of contamination, such as urbanization and agriculture were most apparent at both downstream sites in along the Tambo River in Group 6 (Tambo Fiscal and Tambo Constanera sites) but were not captured anywhere else in the basin.

WATER QUALITY ASSESSMENT OF THE TAMBO RIVER BASIN, PERU USING BENTHIC MACROINVERTEBRATES

3.1 ABSTRACT

The Tambo River Basin has several sources of contamination from source to mouth. Volcanic and geothermal activities and anthropogenic activities such as agriculture, urbanization, and mining may be affecting the water quality. The ecological integrity of the Tambo River Basin is likely to be negatively affected compromising ecosystem services. To complement the physical and chemical data associated with this study, benthic macroinvertebrates were sampled as a biological component to provide information on the water quality of the Tambo River Basin. To capture the potential effects of both anthropogenic and natural contamination, benthic macroinvertebrates were sampled at 15 sites across the basin during the dry season. High Andean headwater sites included several that were potentially mining-affected and others that were unimpacted. Mid basin sites included some that were near to a volcano and others near hot springs. Lower basin sites captured coastal urbanized areas. Principal Component Analysis (PCA) and Canonical Correspondence (CCA) were used to explore relationships between benthic macroinvertebrate metrics (EPT%, taxa richness, Simpson's Index), and water chemistry including metals and physicochemical parameters. A total of nine orders, one class, one phylum and 19 families were found across the basin. On average the basin had five different orders, and 11 families per site. Benthic macroinvertebrate communities were affected the most by geothermal sources of contamination and contamination from the mine upstream. At affected sites, pollution-sensitive families were replaced with pollution-tolerant families and distinct communities were observed at the different sources of

contamination. These findings support the idea that both human-caused and natural environmental stressors that have had an impact on water quality are also impacting aquatic biota.

3.2 INTRODUCTION

High-altitude streams in the Andes are some of the least-studied aquatic ecosystems in the world (Gerhardt, 2002; Custodio et al., 2019a). The watershed in the Andes has uniquely challenging conditions such as low water temperature, low dissolved oxygen concentrations, and exposure to ultraviolet rays which can create harsh conditions for aquatic biota (Loayza-Muro et al., 2010b). These conditions are especially prevalent in the Tambo River Basin. However, the Tambo River Basin is also subject to several sources of contamination both natural and human-caused such as geothermal and volcanic activity, industrial effluents, agriculture, and urbanization. One of the most notable contributors to the metal pollution of the river basin is mining. Mining activity located at the headwaters has resulted in acid-mine drainage and tailing residues which are known to have far-reaching negative impacts on water quality and aquatic biota (A. D. Ccanccapa-Cartagena et al., n.d.).

Despite these pressures, little attention has been given to using biomonitoring, specifically benthic macroinvertebrates, to evaluate ecosystem health. Although limited, several studies have used benthic macroinvertebrates as a measure of evaluating water quality in harsh altitudinal conditions (Custodio Villanueva & Chaname Zapata, 2016; Villanueva et al., 2016; Custodio & Chávez, 2019; Custodio et al., 2019b; Mercado-Garcia et al., 2019).

Benthic macroinvertebrates occur in various aquatic habitats. They are commonly found in riverbeds amongst stones, on submerged aquatic plants, etc. They are a particularly useful tool to indicate environmental quality as they live long enough to reflect water quality conditions in the recent past, are small, easily collected and include species sensitive to particular stressors (Gerhardt, 2002; Kenney et al., 2009). The composition and structures of these communities are heavily influenced by anthropogenic and natural sources of contamination (Custodio et al., 2019b).

Benthic macroinvertebrate organisms have a life span of 1-3 years and reflect cumulative effects as they respond quickly to environmental perturbation (Glozier et al., 2002). Environmental stressors may result in a reduced diversity and abundance of benthic invertebrate populations and influence populations at upstream and downstream reaches. The presence/absence of key species and their relative abundances in contaminated sites compared with the diversity of benthic invertebrates in reference sites can assist in evaluating the current health of the Tambo River Basin (Dabney, 2018). Although studies using benthic macroinvertebrates as an indicator of water quality are very limited in Southern Peru, knowledge of benthic macroinvertebrate fauna is more extensive in the Northern and Central Peruvian Andes. Studies at these locations have used benthic macroinvertebrates as bioindicators of water quality in monitoring programs. The analysis of the composition and structures of the communities can determine how much disturbance a water body has been experiencing (Custodio et al., 2019b). With no known studies in the Tambo River Basin, using benthic macroinvertebrates as bioindicators of the increasing pressures on the Tambo River Basin are needed to assess current water quality.

Considering the lack of information regarding the current health of this basin, our objective was to assess the environmental health of the Tambo River Basin by using benthic macroinvertebrates. This research project assessed the diversity of the benthic macroinvertebrate communities to, (1) identify the presence, absence, and relative abundance of taxa at various locations along the Tambo River Basin. (2) Determine if benthic macroinvertebrate community structure (i.e., total invertebrate density, number of taxa, shifts in the dominant taxa) differed in benthic macroinvertebrate communities in different sources of contamination (geothermal, volcanic, mining, urbanization) relative to sites. And (3), contribute towards providing baseline data on regional benthic macroinvertebrate communities that can be used to design a monitoring plan and establish remediation goals.

3.3 MATERIALS AND METHODS

3.3.1 Field Sampling

The same sampling sites used for water chemistry sampling in Chapter 2 were selected for the sampling of benthic macroinvertebrates, and the same site groupings were used (Groups 1 to 6). Benthic macroinvertebrates were sampled during the lowest dry season flows when the streams could be safely waded. A total of 15 sites were sampled, Sampling protocols followed the Canadian Aquatic Biomonitoring Network (CABIN, 2012).

Samples were collected using the timed (3 minutes) kick and sweep method with a D-net made of 250 μm mesh. Even at low flows, the rivers were too wide and the current too strong to take integrated samples by wading bank-to-bank. Instead, two different habitats were sampled, riffles (erosional zones) and pools (depositional zones). Even so, pools were rare and the depositional zones were mostly eddies along the shoreline. I took three replicate samples from the shallower riffle zones and three from the deeper eddies/pools, for a total of six samples from each study site. The sampling design was: 2 habitat types (riffles, pools) x 15 river sites x 3 replicates. Sediment grab samples were collected near the shoreline of the Coralaque Wetland as the soft, unconsolidated sediment was too thick to sample with the travelling kick and sweep method. Samples were preserved using isopropyl alcohol due to constraints in obtaining high volumes of laboratory-grade ethanol in the region (Figure 3.1A).

3.3.2 Laboratory Processing

Preserved benthic macroinvertebrate samples were reduced in volume and packaged for shipping back to Laurentian University in Greater Sudbury, Ontario. Samples were rinsed using a 250 µm sieve and larger organic and inorganic materials (e.g., twigs and stones) were removed. The remaining samples were placed in WhirlPaks filled with isopropyl alcohol and then packed in coolers for international transport (Figure 3.1B, C). Upon arrival at my Laurentian University Laboratory, samples were refreshed with 70% ethanol and stored until picking. Phloxine-B was added to the samples for 24 h to dye the chitin of invertebrate exoskeletons pink, which facilitated picking. Picking of the samples was performed by eye above a light table, then the entire sample was scanned at 10X magnification under a Leica S6 stereomicroscope to ensure no animals were missed. For sites where benthic macroinvertebrates were abundant, a Marchant box was used to randomly select cells until a total of 300 individuals were picked. Once a count of 300 individuals was achieved, the remainder of the cell was counted and included in the total count. Out of the 15 sites sampled, 10 sites had individual samples that exceed the 300 counts. A plankton splitter wheel was used to subsample a total of 100 individuals in samples that reached 300 individuals. Cells where the 100th count was reached were picked in their entirety. To estimate the absolute abundance of individuals per site from the sites that were subsampled, the portion of the sample that was not subsampled was added to a Marchant box. Individuals were picked from cells until approximately 50 individuals were reached. The average count of individuals per cell was determined in order to estimate the total count of individuals within the portion of the sample that was not subsampled. Counts from the subsamples and the estimated un-subsampled counts were combined to estimate the absolute abundance of the entire sample. Total counts from the subsamples were then scaled to represent the individual family counts for the absolute abundances of the entire site.

Taxonomic identification was performed to either the family level or to the lowest possible taxonomic resolution for individuals where the family level could not be determined. Local keys and a Leica S6 Stereomicroscope were used during the identification process (Domínguez, 2006; Domínguez & Fernández, 2009; Hamada et al., 2018). North American (Dr. Chris Jones, Ontario Ministry of the Environment, Conservation, and Parks) and South American scientists experienced in benthic macroinvertebrate identifications were periodically consulted to verify identifications. Select individuals that could not be identified with available resources or due to missing identification features were excluded from the results.

Figure 3.1: (A) Field sampling of benthic macroinvertebrates. (B) Condensed sample for shipment to Laurentian University Laboratory, Sudbury Ont. (C) Removal of large debris in samples in laboratory to condense samples.

3.3.3 Statistical Analysis

A principal component analysis (PCA) based on Pearson correlation was used to describe the main variation in physicochemical variables collected from the *in-situ* measurements of the sites and the benthic macroinvertebrates. Variables selected in the PCA were total dissolved solids, conductivity, water temperature, dissolved oxygen, and pH. Before the multivariate analysis, physicochemical variables were tested for normality with the Kolmogorov-Smirnov test (IBM SPSS Statistics version 28). Conductivity and total dissolved solids were log-transformed to achieve a normal distribution. Canonical Correspondence Analysis (CCA) was performed based on a Pearson correlation to describe the main variation in water chemistry data (metals, nutrients, and total organic carbon), benthic macroinvertebrates and sampling sites. The CCA used loadings extracted from the PCA (Chapter 2) that included the water sampling data (metals, nutrients, and total organic carbon) as its measured variables. PC1 from Chapter 2 contained large positive loadings of aluminum (0.898), uranium (0.925), iron (0.939), mercury (0.828), zinc (0.953) and total nitrogen (0.875). For PC2 there were large positive loadings of sodium (0.865), potassium (0.911) boron (0.938) and a moderately large arsenic loading (Chapter 2, Table 2.8, Figure 2.14). PC1 is more representative of anthropogenic contamination, specifically mining. Elements such as aluminum, uranium, iron, zinc and copper are all known markers of industrial and mining activities and were found at high concentrations in the mining-impacted sites in Group 1 (Segura et al., 2006; Pizarro et al., 2010; Bianchini et al., 2015; Chowdhury et al., 2016; Ccanccapa-Cartagena et al., 2021; Torres & De-la-Torre, 2022). Whereas, arsenic and boron in the surface waters of Peru have been frequently associated with Andean volcanism and geothermal activities in Peru and South America (Pizarro et al., 2010; Sadloň, 2020; Bundschuh et al., 2021; Morales-Simfors & Bundschuh, 2022; Regis et al., 2022). Furthermore, concentrations of sodium, potassium, arsenic, and boron had a generally higher concentration in sites impacted by natural sources of metals and acids and therefore PC2 can be associated with natural sources of contamination.

As described in Chapter 2, the water sampling data were tested for normality using a Kolmogorov-Smirnov test (IBM SPSS Statistics version 28), and those not meeting a normal distribution were log(x+1) transformed. Relative abundances of benthic macroinvertebrates were used for the multivariate analyses. Canonical Correspondence Analysis (CCA) and Principal Component Analysis (PCA) were both run with and without rare families (defined as taxa at or below a 5% threshold of relative abundance) to determine if rare species skewed the results of the multivariate analyses. As the rare species did not appear to skew the results, the rare taxa were included in the final results of the multivariate analyses. The PCA's and CCA's were performed using XLSTAT Life Science version 2021.5. The Coralaque Wetland was excluded from the multivariate analyses to reduce the possibility of skewing the results as it was the only lentic system. Ephemeroptera, Plecoptera and Trichoptera percentage (EPT%) was calculated as: %EPT=[(E+P+T) *100]/N where, E= number of Ephemeroptera, P = number of Plecoptera, T= number of Trichoptera, and N= total number of individuals in a sample (Fekadu et al., 2022). The non-parametric community structure indices such as taxa richness (R), and Simpson's diversity index (*D*) were calculated, based mostly on the family level (Washington, 1984)**.** All calculations and analyses were based on counts scaled to represent the absolute abundance of the sites.

3.4 RESULTS

3.4.1 General Results

In total, 7,271 individuals from 23 taxa were identified from the sub-samples. A total of eight of the 15 study sites required subsampling where counts were >300 per picked sample. Across the entire study basin, three phyla were present Mollusca, Annelida with most individuals that were identified all belonging to the phylum (Arthropoda) and class (Insecta). There was a total of 9 orders and 19 families. On average each site had five different orders and 11 families. Overall, biodiversity at the level of orders and families did not differ much among sampling sites, ranging from four to seven for orders and 7-15 for families. The orders Diptera and Trichoptera, and the families Chironomidae and Hydroptilidae were present at all sites. Simuliidae was present at 13 of the 15 sites and increased at sites below 3,000 m.a.s.l. Elmidae and Ephydridae were found at 14 of the 15 sites. The three most abundant families were the Chironomidae, Baetidae and Elmidae, (Table 3, 3.2).

3.4.2 Upstream to Downstream Site-Specific Results

Group 1: Tucari, Aruntaya, and Titire Rivers

The Tucari River had four orders and seven families which is the least among all sites sampled in the basin. Dipterans, primarily chironomids, were the most abundant group at these highest-elevation sites. The Tucari River had a count a relative abundance of 67.9% for chironomids, whereas the Aruntaya River had a relative abundance of 53,6% for chironomids, as well as 15.7% for Elmidae and 12% for Baetidae. Tabanids were recovered at the Aruntaya River but were absent at the Tucari and Titire Rivers. The Aruntaya River also had the second-highest abundance of the Plecoptera, Gripopterygidae, out of all sites sampled. The Aruntaya River also had the highest family-level biodiversity in comparison to the Tucari and Titire Rivers with a total of 14 families. At the Tititre River, a total of 141 individuals were recovered, with the sample being mainly composed of Ephydridae (51%) (Table 3.1, 3.2, 3.3 Figure 3.2, 3.3, 3.4). The Tucari River had an EPT% (Ephemeroptera, Plecoptera, and Trichoptera) of 17% which was one of the lowest scores recorded for the whole basin. The Aruntaya River had a somewhat higher EPT score with 28% whereas the Titire River had the lowest score in the entire basin with an EPT% of 9%, respectively. The Tucari and Aruntaya Rivers had the lowest taxa richness and the Tucari, Aruntaya and Titire Rivers had a moderate diversity score (Table 3.4).

Group 2: Pacchani and Vizcachas Rivers

The Pacchani River samples were mainly composed of Diptera with Ephydridae being the most abundant family (53%) followed by Chironomidae (21%). In contrast, the Vizcachas River samples were mainly composed of Elmidae (36%), followed by Baetidae (27%) and Chironomidae (13%). At the Vizcachas River, Gripopterygidae along with the Tabanidae were at their most abundant at this site. About the same number of insect families were found at the Pacchani (9 families) and Vizcachas River (11 families) sites. Simuliidae were uniquely absent at these two sites, being found at every other site that was sampled (Table 3.1, 3.2, 3.3 Figure 3.2, 3.3, 3.4). The Pacchani River had a low EPT% of 5% however, the Vizcachas River had a greater EPT% than the aforementioned sites with a value of 66%. A similar taxa richness was recorded at both sites, however, the Vizcachas River had a good diversity score whereas the Pacchani River's diversity was moderate (Table 3.4).

Group 3: Coralaque Wetland and Coralaque River

Being the only lentic water site sampled in the basin, the Coralaque Wetland had unique taxa in several ways. While Chironomidae was the most abundant taxon (59%), this site had the highest abundance throughout the basin of Hirudinea, Hyalellidae, and Mollusca. Molluscs were absent at all other sites except for the Pacchani River. The Coralaque Wetland was also the only site in the whole basin from which Elmidae were absent. The wetland had eight different families present while the Coralaque River had the most families recorded (15) in the basin (Table 3.1, 3.2, 3.3 Figure 3.2, 3.3, 3.4). The river also had a high abundance of chironomids with a relative abundance of 80% recorded. The

Coralaque Wetland had an EPT% of 21% whereas the Coralaque River had an EPT% of 7% (Figure 3.7). Moderate taxa richness was recorded at the Coralaque Wetland and at the Coralaque River. However, diversity was lower at the Coralaque River than at the Coralaque Wetland with the Coralaque River having a low diversity score (Table 3.4).

Group 4: Coralaque Huarina, Ubinas, Alto Tambo Rivers

The family Blephariceridae was present in all samples from these three rivers but was absent at all sites upstream (see above). Dipterans dominated at both the Coralaque Huarina and Alto Tambo River sites. At the Coralaque Huarina River site, Simuliidae was the most abundant family (35%) followed by Baetidae (28%) The Ubinas River, an inflowing tributary had ephemeropterans as its most abundant order with Baetidae accounting for most of the individuals (76%) and the highest abundance of the entire basin. The Ubinas River was also the only location where Dystiscidae were recovered (Table 3.1, 3.2, 3.3; Figure 3.2, 3.3, 3.4). At the Alto Tambo River, Chironomidae was the most abundant taxon (36%). EPT% for the three rivers varied from 24% for the Alto Tambo River to 32% for the Coralaque Huarina River, to 81% for the Ubinas River which was the highest EPT% recorded in the entire basin (Figure 3.7). The Ubinas River had one of the highest taxa richness scores recorded in the basin, however, diversity was low. Coralaque Huarina and Alto Tambo had similar taxa richness (12, 10) and relatively good diversity (Table 3.4).

Group 5: Tambo-US-Vagabundo, Vagabundo, Tambo-DS-Vagabundo Rivers

These three sites include the Vagabundo River and the Tambo River both upstream (US) and downstream (DS) of its confluence with the Vagabundo River. The Tambo-US-Vagabundo and Tambo-DS-Vagabundo samples were both mainly composed of Ephemeropterans, specifically Leptohyphidae, with a relative abundance of 46% at the Tambo-US-Vagabundo River and 31% the Tambo-DS-Vagabundo site. Baetidae were also abundant with a relative abundance of 19% at the Tambo-US-Vagabundo and 25% at the Tambo-DS-Vagabundo site. Coleoptera was the most abundant order in the Vagabundo River with Elmidae being the most abundant family in this order. Diptera were also notably higher at the Vagabundo River site in comparison to the two Tambo River sites with Chironomidae being the most abundant family. Generally, there was a reduction in Chironomidae and Diptera in terms of sample composition when compared to all of the sites sampled upstream of this location except for the Vizcachas and Ubinas rivers. There was also an increase in trichopterans, specifically Hydroptilidae, as well as coleopterans, specifically Hydrophilidae when compared to upstream sites. Sites at this location were also where the only odonates were found in the entire basin (Table 3.1, 3.2, 3.3, Figure 3.2, 3.3, 3.4). EPT% at the Tambo-US-Vagabundo site and was 39% at the Tambo-Ds-Vagabundo site. EPT% was 39% at the Vagabundo River and 71% at the Tambo-DS-Vagabundo site. Sites within this grouping had similar taxa richness and good diversity (Table 3.4).

Group 6: Tambo Fiscal and Tambo Costanera Rivers

The Tambo River Fiscal site had the fewest Diptera of the entire basin. Samples were largely composed of Coleoptera with a high relative abundance of Elmidae (50%) and Hydrophilidae (19%). The Tambo River Costanera site had a relative abundance of 33% for Elmidae 25% for chironomids. Uniquely for the entire basin, the family Parastacidae was present at the Tambo Fiscal and Tambo Costanera sites (Table 3.1, 3.2, 3.3, Figure 3.2, 3.3, 3.4). EPT% was 70% was recorded at the Tambo Fiscal site, however, a lower EPT% was recorded at the Tambo Costanera site (14%, Figure 3.7). The Tambo River Costanera site had one of the highest taxa richnesses in the basin (15), whereas the Tambo River Fiscal site had a taxa richness of 11 indicating both sites had good diversity (Table 3.4).

Table 3.1: Subsampled and identified benthic macroinvertebrates (individual per site).

Taxon			Tucari	Aruntaya	Titire	Pacchani	Vizcachas	Coralaque Wetland	Coralaque River	Coralaque Huarina	Ubinas	Alto Tambo	Tambo-US-Vag	Vagabundo	Tambo-DS-Vag	Tambo Fiscal	Tambo Costanera
	Dipteran	Muscidae	0	27	0	7	2	0	1	0	7	2	0	7	0	0	1
		Ephydridae	1	7	72	211	2	2	18	0	1	1	1	5	6	4	22
		Ceratopogonidae	0	0	0	0	3	1	3	1	2	0	0	0	2	0	5
		Chironomidae	49	201	30	92	80	169	461	164	43	227	25	140	78	9	110
		Simuliidae	1	3	8	0	0	1	8	218	51	103	31	13	22	21	75
		Blepheracidae	0	0	0	0	0	0	0	13	3	101	41	1	54	1	1
		Tipulidae	0	1	0	1	0	0	3	2	1	14	0	1	0	0	0
		Tabanidae	0	2	0	0	14	0	2	3	0	0	0	2	0	0	5
		Empididae	0	0	0	0	0	0	2	1	0	0	0	0	0	1	0
	Coleoptera	Dytiscidae	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
		Hydrophilidae	1	1	2	0	0	0	6	1	2	0	36	0	23	125	11
		Elmidae	5	77	11	11	219	0	28	20	8	5	19	212	23	323	145
	Tricoptera	Hydroptilidae	4	9	1	7	22	7	23	19	25	25	64	183	73	22	8
	Hemiptera	Corixidae	0	1	15	5	2	11	3	0	0	0	0	0	0	0	1
	Ephemeroptera	Baetidae	0	56	0	2	166	7	9	178	479	151	126	8	171	92	24
		Leptohyphidae	0	2	1	0	0	0	2	1	1	0	299	10	210	40	20
	Plecoptera	Gripopterygidae	0	28	0	2	74	0	5	0	9	1	0	0	0	0	2
	Odonata		0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Phylum Mollusca		Bivalvia	0	0	0	1	0	20	0	0	0	0	0	0	0	0	0
		Gastropoda	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Subclass		Hirudinae	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0
	Decapoda	Parastacidae	0	0	0	0	0	0	0	0	0	0	0	0	0	5	7
	Amphipoda	Hyalellidae	2	2	1	0	9	53	0	0	1	0	1	1	0	0	0
		Total	63	417	141	339	593	285	574	621	636	630	644	586	662	643	437

Table 3.2: Summary of site replicates, habitats sampled, dominant taxa, subsample counts per habitat, absolute abundance and sampling effort (%).

Site	Habitat Sampled	Most Dominant Taxa	Average count per habitat	Relative abundance of replicates combined	Second Most Dominant Taxa	Average Count Per Habitat	Relative abundance of replicates combined	Third Most Dominant Taxa	Average count per habitat	Relative abundance of replicates combined	Total Counts	Absolute Abundance	Sampling Effort
Tucari	Pool	Chironomidae	21.50	81.13	Hydroptilidae	2.67	7.54	Elmidae	1.00	5.66	81.00	81.00	100%
	Riffle	Chironomidae	4.00	42.85	Elmidae	2.33	25.00	Simuliidae	1.50	10.00			
Aruntaya	Pool	Chironomidae	43.00	57.08	Elmidae	11.67	15.49	Baetidae	7.67	10.18	226.00		
	Riffle	Chironomidae	40.33	50.42	Elmidae	12.67	15.83	Baetidae	16.50	13.75			100%
Titire	Run/Pool Combined	Ephydridae	12.00	51.16	Chironomidae	5.00	21.30	Corixidae	3.75	10.63	141.00	100.00	
	Pool	Ephydridae	68.33	64.00	Chironomidae	28.33	27.00	Elmidae	5.33	5.00	443.00	11019.33	100%
Pacchani	Riffle	Chironomidae	70.00	57.00	Ephydridae	32.00	26.00	Muscidae	7.00	5.00			
Vizcachas	Riffle	Elmidae	36.50	37.00	Baetidae	27.67	28.00	Chironomidae	13.33	13.00	593.00	5246.00	10%
Coralaque Wetland	Pool	Chironomidae	56.33	59.00	Hyalellidae	26.50	19.00	Mollusca	10.00	7.02	285.00	285.00	100%
Coralaque River	Riffle	Chironomidae	82.00	79.87	Elmidae	5.67	6.00	Hydroptilidae	6.00	4.00	308.00	2836.64	16%
	Run	Chironomidae	72.67	81.00	Elmidae	3.67	4.00	Hydroptilidae	3.67	4.00	270.00		
Coralaque Huarina	pool	Baetidae	47.67	46.28	Simuliidae	23.33	22.65	Chironomidae	23.00	22.33	620.00	8600.00	7%
	riffle	Simuliidae	49.33	47.59	Chironomidae	31.67	30.55	Baetidae	11.67	11.25			
Ubinas	Rapids	Baetidae	81.00	76.00	Simuliidae	8.40	8.00	Chironomidae	7.80	7.00	532.00	11057.00	5%
Alto Tambo	Riffle	Chironomidae	45.40	36.00	Baetidae	30.20	24.00	Simuliidae	19.60	16.00	630.00	8202.00	7%
Tambo-US-Vag	Run	Leptohyphidae	57.33	52.00	Baetidae	24.33	11.00	Hydroptilidae	18.00	6.00	644.00	25818.00	2%
	Riffle	Leptohyphidae	42.33	40.00	Baetidae	17.67	17.00	Blepheracidae	12.00	11.00			
Vagabundo	Riffle	Elmidae	35.33	36.00	Hydroptilidae	30.50	31.00	Chironomidae	23.33	24.00	586.00	25526.00	2%
Tambo-DS-Vag	riffle	Leptohyphidae	34.33	31.00	Baetidae	29.00	26.00	Hydroptilidae	13.00	12.00	663.00	10223.00	6%
	run	Leptohyphidae	35.67	32.23	Baetidae	28.00	25.00	Hydroptilidae	11.33	10.00			
Tambo Fiscal	Riffle	Elmidae	59.33	53.00	Hydrophilidae	20.33	18.00	Baetidae	19.00	17.00	642.00	8079.00	8%
	Run	Elmidae	48.33	47.00	Hydrophilidae	21.33	21.00	Baetidae	11.67	11.00			
Tambo Costanera	Run	Elmidae	34.67	48.00	Chironomidae	13.67	19.00	Simuliidae	10.00	14.00	437.00	1837.00	24%
	Riffle	Chironomidae	23.00	31.00	Simuliidae	15.00	21.00	Elmidae	13.67	19.00			

Table 3.3: Relative abundance based on absolute abundance of benthic macroinvertebrates per site (%).

Taxon			Tucari	Aruntaya	Titire	Pacchani	Vizcachas	Coralaque Wetland	Coralaque River	Coralaque Huarina	Ubinas	Alto Tambo	Tambo-US-Vag	Vagabundo	Tambo-DS-Vag	Tambo Fiscal	Tambo Costanera
	Dipteran	Muscidae	0.00	5.79	0.00	1.81	0.34	0.00	0.17	0.00	1.32	0.32	0.00	1.19	0.00	0.00	0.23
		Ephydridae	1.23	1.50	51.06	53.50	0.34	0.70	3.11	0.00	0.19	0.16	0.16	0.85	0.90	0.62	5.03
		Ceratopogonidae	0.00	0.00	0.00	0.00	0.51	0.35	0.52	0.16	0.00	0.00	0.00	0.00	0.30	0.00	1.14
		Chironomidae	67.90	53.65	21.28	34.99	13.49	59.30	80.28	26.45	7.33	36.03	3.88	23.89	11.76	1.40	25.17
		Simuliidae	3.70	0.21	5.67	0.00	0.00	0.35	1.38	35.16	7.89	16.35	4.81	2.22	3.32	3.27	17.16
		Blepheracidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.10	0.56	16.03	6.37	0.17	8.14	0.16	0.23
		Tipulidae	0.00	0.21	0.00	0.23	0.00	0.00	0.52	0.32	0.00	2.22	0.00	0.17	0.00	0.00	0.00
		Tabanidae	0.00	0.43	0.00	0.00	2.36	0.00	0.35	0.48	0.00	0.00	0.00	0.34	0.00	0.00	1.14
		Empididae	0.00		0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00
	Coleoptera	Dytiscidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.00	0.00	0.00	0.00	0.00
		Hydrophilidae	6.17	1.72	0.71	2.26	3.71	2.46	3.98	3.06	3.57	3.97	9.94	31.23	11.01	3.43	1.83
		Elmidae	12.35	15.67	7.80	4.97	36.93	0.00	4.84	3.23	0.75	0.79	2.95	36.18	3.47	50.31	33.18
	Tricoptera	Hydroptilidae	2.47	0.00	1.42	0.00	0.00	0.00	1.04	0.16	0.00	0.00	5.59	0.00	3.47	19.47	2.52
	Hemiptera	Corixidae	0.00	0.86	10.64	1.13	0.34	3.86	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23
	Ephemeroptera	Baetidae	0.00	12.02	0.00	0.45	27.99	2.46	1.56	28.71	76.13	23.97	19.57	1.37	25.79	14.33	5.49
		Leptohyphidae	2.47	0.00	0.71	0.00	0.00	0.00	0.35	0.16	0.19	0.00	46.43	1.71	31.67	6.23	4.58
	Plecoptera	Gripopterygidae	0.00	7.51	0.00	0.45	12.48	0.00	0.87	0.00	1.32	0.16	0.00	0.00	0.00	0.00	0.46
	Odonata		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	0.17	0.00	0.00	0.00
Phylum Mollusca		Bivalvia	1.23	0.00	0.00	0.23	0.00	7.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Gastropoda	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Subclass		Hirudinae	0.00	0.00	0.00	0.00	0.00	4.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Decapoda	Parastacidae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.78	1.60
	Amphipoda	Hyalellidae	2.47	0.43	0.71	0.00	1.52	18.60	0.00	0.00	0.19	0.00	0.16	0.17	0.00	0.00	0.00

Table 3.4: Scores for Percent EPT (%), Taxa richness and Simpson's Diversity across sites. Simpson's Diversity score ranges from 0 to 1, where high scores (close to 1) indicate high diversity and low scores (close to 0) indicate low diversity.

	Percent EPT	Taxa richness	Simpson's Diversity
Tucari	17.28	7	0.53
Aruntaya	28.11	14	0.67
Titire	9.22	9	0.68
Pacchani	5.42	10	0.59
Vizcachas	66.44	11	0.75
Coralaque Wetland	21.05	10	0.61
Coralaque River	7.79	15	0.35
Coralaque Huarina	32.10	12	0.72
Ubinas	81.20	15	0.41
Alto Tambo	24.76	10	0.76
Tambo-US-Vag	29.66	11	0.72
Vagabundo	39.59	14	0.71
Tambo-DS-Vag	71.94	10	0.80
Tambo Fiscal	70.87	11	0.68
Tambo Costanera	14.87	15	0.79

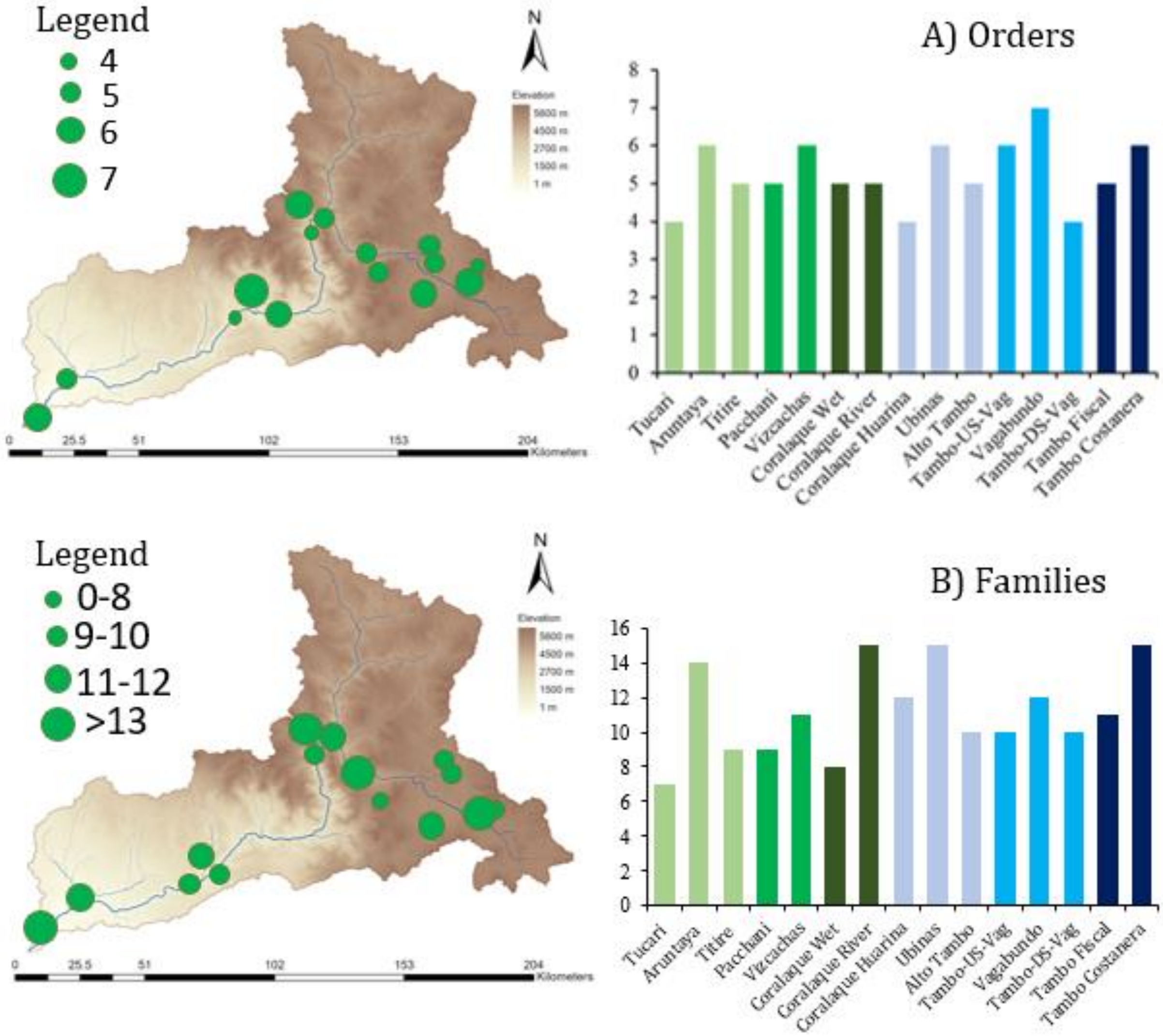

Figure 3.2: Representation of spatial distribution of the number of orders and families present at each site sampled in the Tambo River Basin.

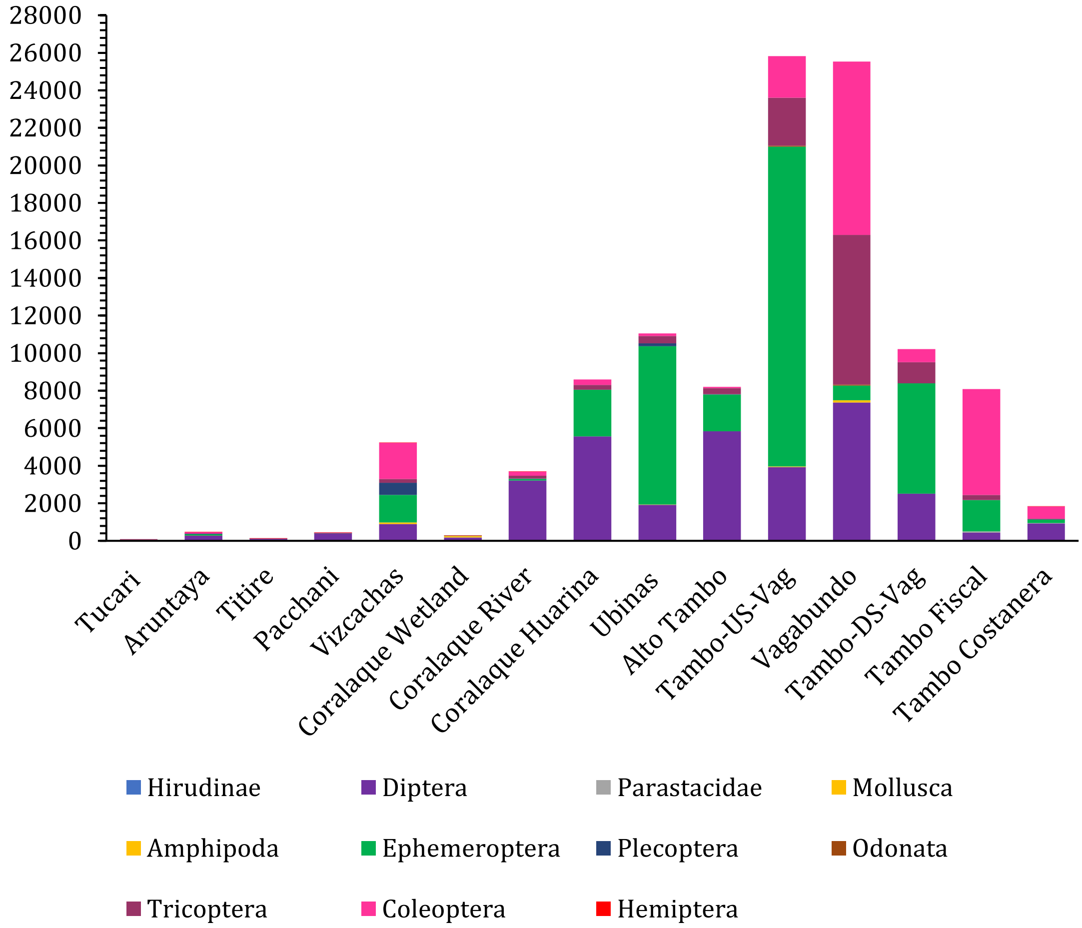

Figure 3.3: Absolute abundance of benthic macroinvertebrate individuals per orders, phylum and class recorded per site.

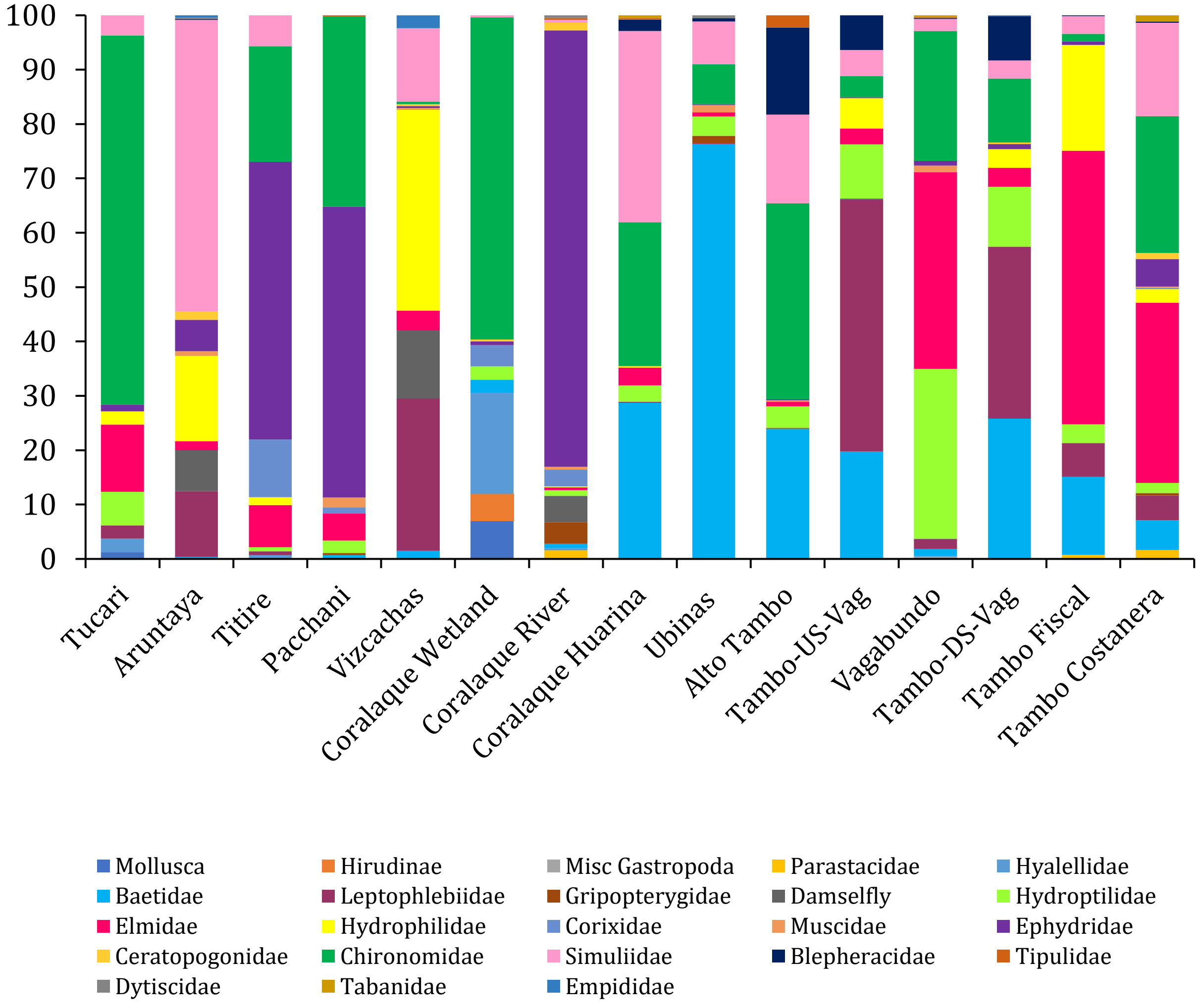

Figure 3.4: Relative abundance of benthic macroinvertebrate individuals per family across sites sampled. Relative abundances are scaled to represent the absolute abundance. Order, phylum and class given for individuals that were not identified to the family level.

3.4.3 Multivariate Statistics

Physicochemical Properties & Benthic Invertebrates

Among the physicochemical variables, Pearson's Correlation results showed positive relationships between water temperature and dissolved oxygen (0.544). No other significant correlations (α=0.05) were observed.

Between the physicochemical variables and benthic macroinvertebrates, water temperature was positively correlated with Gastropoda (0.546), Elmidae (0.558) and Tabanidae (0.550). pH was negatively correlated with Mollusca (-0.620) and Hyalellidae (-0.749). TDS was negatively correlated with Hydrophilidae (-0.636). Conductivity was negatively correlated with Odonata (-0.841) and Empididae (-0.744). Dissolved oxygen was negatively correlated only with Ceratopogonidae (-0,744).

Among the benthic macroinvertebrates, several significant ($p < 0.05$) correlations were observed. Mollusca was positively correlated with Hyalellidae (r=0.927) and Chironomidae (r=0.777, Table 3.5). Gastropoda was positively correlated with Hydroptilidae (r=0.909). Parastacidae was positively correlated with Gripopterygidae (r=0.624) and Ceratopogonidae (r=0.617). Hyalellidae was positively correlated with Chironomidae (r=0.687). Gripopterygidae was strongly positively correlated with Ephydridae (r=0.688) and Dytiscidae (r=0.796). Odonata was positively correlated with Hydrophilidae (r=0.792) and Empididae (0.874). Hydrophilidae with Empididae (r=0.865) and Corixidae was positively correlated with Ephydridae (r=0.644).

Table 3.5: Values for Pearson Correlations using physicochemical variables measured with a multi-sonde and benthic macroinvertebrates. Bold indicates statistically significant correlations (p<0.05).

Variables	Water Temp	pH	TDS	Conductivity	DO	Mollusca	Gastropoda	Parastacidae	Hyalellidae	Baetidae	Leptohyphidae	Gripopterygidae	Odonata	Hydroptilidae	Elmidae	Hydrophilidae	Corixidae	Muscidae	Ephydridae	Ceratopogonidae	Chironomidae	Simuliidae	Blepheracidae	Tipulidae	Dytiscidae	Tabanidae	Empididae
Water Temp	**1**	0.355	-0.067	0.281	**0.544**	-0.366	**0.546**	0.161	-0.304	0.346	-0.373	-0.139	-0.472	0.480	**0.558**	-0.346	-0.138	0.098	-0.385	-0.092	-0.007	0.099	0.100	0.192	0.012	**0.550**	-0.428
pH	0.355	**1**	-0.200	-0.033	0.391	**-0.620**	0.165	0.178	**-0.749**	0.413	0.259	0.077	-0.038	0.233	0.160	0.086	-0.503	0.161	-0.260	-0.119	-0.414	-0.168	0.293	0.177	0.012	0.284	0.134
TDS	-0.067	-0.200	**1**	0.375	-0.211	0.177	0.176	-0.166	0.203	-0.127	-0.210	0.100	-0.217	0.173	-0.479	**-0.636**	0.289	0.270	0.338	0.018	0.509	-0.054	0.112	0.170	-0.034	0.213	-0.219
Conductivity	0.281	-0.033	0.375	**1**	0.123	0.116	0.232	0.072	0.109	-0.104	-0.392	-0.061	**-0.841**	0.306	0.321	**-0.722**	0.328	0.124	0.325	-0.400	0.487	-0.487	0.056	0.099	-0.310	0.197	**-0.744**
DO	**0.544**	0.391	-0.211	0.123	**1**	-0.103	0.408	-0.082	-0.030	0.310	-0.037	-0.132	-0.346	0.451	0.330	-0.131	-0.186	-0.193	-0.390	**-0.544**	0.079	-0.220	0.234	0.294	-0.143	0.145	-0.166
Mollusca	-0.366	**-0.620**	0.177	0.116	-0.103	**1**	-0.091	-0.163	**0.927**	-0.230	-0.171	-0.121	-0.159	-0.001	-0.008	-0.133	-0.107	-0.109	-0.064	-0.210	**0.777**	-0.196	-0.176	-0.116	-0.158	-0.146	-0.113
Gastropoda	**0.546**	0.165	0.176	0.232	0.408	-0.091	**1**	-0.138	-0.051	-0.177	-0.153	-0.121	-0.122	**0.909**	0.441	-0.168	-0.108	0.351	-0.146	-0.178	0.079	-0.184	-0.138	-0.031	-0.134	0.177	-0.096
Parastacidae	0.161	0.178	-0.166	0.072	-0.082	-0.163	-0.138	**1**	-0.119	-0.217	-0.225	**0.624**	0.016	-0.238	0.388	-0.064	0.044	-0.149	0.382	**0.617**	-0.216	-0.139	-0.253	-0.086	0.292	0.513	-0.172
Hyalellidae	-0.304	**-0.749**	0.203	0.109	-0.030	**0.927**	-0.051	-0.119	**1**	-0.220	-0.193	-0.023	-0.169	0.016	-0.027	-0.189	0.173	-0.248	0.042	-0.191	**0.687**	-0.247	-0.216	-0.159	-0.052	-0.183	-0.157
Baetidae	0.346	0.413	-0.127	-0.104	0.310	-0.230	-0.177	-0.217	-0.220	**1**	0.001	0.068	-0.315	-0.033	-0.234	-0.236	-0.268	0.121	-0.370	-0.314	-0.177	-0.001	0.270	0.104	0.465	-0.082	-0.199
Leptohyphidae	-0.373	0.259	-0.210	-0.392	-0.037	-0.171	-0.153	-0.225	-0.193	0.001	**1**	-0.281	0.279	0.102	-0.199	0.429	-0.245	-0.347	-0.339	-0.037	-0.423	-0.044	0.257	-0.275	-0.254	-0.206	0.393
Gripopterygidae	-0.139	0.077	0.100	-0.061	-0.132	-0.121	-0.121	**0.624**	-0.023	0.068	-0.281	**1**	0.146	-0.213	-0.235	-0.253	0.152	0.213	**0.688**	0.491	-0.280	-0.249	-0.183	0.041	**0.796**	-0.081	-0.151
Odonata	-0.472	-0.038	-0.217	**-0.841**	-0.346	-0.159	-0.122	0.016	-0.169	-0.315	0.279	0.146	**1**	-0.319	-0.279	**0.792**	-0.084	0.052	0.059	0.487	-0.476	0.362	-0.250	-0.144	0.181	-0.215	**0.874**
Hydroptilidae	0.480	0.233	0.173	0.306	0.451	-0.001	**0.909**	-0.238	0.016	-0.033	0.102	-0.213	-0.319	**1**	0.375	-0.273	-0.233	0.184	-0.293	-0.334	0.134	-0.317	0.084	-0.038	-0.224	0.094	-0.222
Elmidae	**0.558**	0.160	-0.479	0.321	0.330	-0.008	0.441	0.388	-0.027	-0.234	-0.199	-0.235	-0.279	0.375	**1**	0.126	-0.128	-0.097	-0.244	-0.101	0.027	-0.229	-0.310	-0.226	-0.336	0.427	-0.182
Hydrophilidae	-0.346	0.086	**-0.636**	**-0.722**	-0.131	-0.133	-0.168	-0.064	-0.189	-0.236	0.429	-0.253	**0.792**	-0.273	0.126	**1**	-0.196	-0.177	-0.280	0.127	-0.469	0.215	-0.217	-0.251	-0.169	-0.207	**0.865**
Corixidae	-0.138	-0.503	0.289	0.328	-0.186	-0.107	-0.108	0.044	0.173	-0.268	-0.245	0.152	-0.084	-0.233	-0.128	-0.196	**1**	-0.152	**0.644**	-0.031	-0.013	-0.201	-0.208	-0.101	0.029	-0.153	-0.134
Muscidae	0.098	0.161	0.270	0.124	-0.193	-0.109	0.351	-0.149	-0.248	0.121	-0.347	0.213	0.052	0.184	-0.097	-0.177	-0.152	**1**	0.253	0.026	-0.025	-0.047	-0.267	-0.011	0.381	-0.101	-0.045
Ephydridae	-0.385	-0.260	0.338	0.325	-0.390	-0.064	-0.146	0.382	0.042	-0.370	-0.339	**0.688**	0.059	-0.293	-0.244	-0.280	**0.644**	0.253	**1**	0.296	-0.078	-0.313	-0.292	-0.033	0.358	-0.197	-0.178
Ceratopogonidae	-0.092	-0.119	0.018	-0.400	**-0.544**	-0.210	-0.178	**0.617**	-0.191	-0.314	-0.037	0.491	0.487	-0.334	-0.101	0.127	-0.031	0.026	0.296	**1**	-0.379	0.493	-0.245	-0.142	0.412	0.299	0.103
Chironomidae	-0.007	-0.414	0.509	0.487	0.079	**0.777**	0.079	-0.216	**0.687**	-0.177	-0.423	-0.280	-0.476	0.134	0.027	-0.469	-0.013	-0.025	-0.078	-0.379	**1**	-0.159	0.115	0.268	-0.401	0.160	-0.327
Simuliidae	0.099	-0.168	-0.054	-0.487	-0.220	-0.196	-0.184	-0.139	-0.247	-0.001	-0.044	-0.249	0.362	-0.317	-0.229	0.215	-0.201	-0.047	-0.313	0.493	-0.159	**1**	0.009	0.077	0.019	0.215	0.162
Blepheracidae	0.100	0.293	0.112	0.056	0.234	-0.176	-0.138	-0.253	-0.216	0.270	0.257	-0.183	-0.250	0.084	-0.310	-0.217	-0.208	-0.267	-0.292	-0.245	0.115	0.009	**1**	**0.793**	-0.230	-0.173	-0.151
Tipulidae	0.192	0.177	0.170	0.099	0.294	-0.116	-0.031	-0.086	-0.159	0.104	-0.275	0.041	-0.144	-0.038	-0.226	-0.251	-0.101	-0.011	-0.033	-0.142	0.268	0.077	**0.793**	**1**	-0.083	-0.100	-0.144
Dytiscidae	0.012	0.012	-0.034	-0.310	-0.143	-0.158	-0.134	0.292	-0.052	0.465	-0.254	**0.796**	0.181	-0.224	-0.336	-0.169	0.029	0.381	0.358	0.412	-0.401	0.019	-0.230	-0.083	**1**	-0.217	-0.111
Tabanidae	**0.550**	0.284	0.213	0.197	0.145	-0.146	0.177	0.513	-0.183	-0.082	-0.206	-0.081	-0.215	0.094	0.427	-0.207	-0.153	-0.101	-0.197	0.299	0.160	0.215	-0.173	-0.100	-0.217	**1**	-0.155
Empididae	-0.428	0.134	-0.219	**-0.744**	-0.166	-0.113	-0.096	-0.172	-0.157	-0.199	0.393	-0.151	**0.874**	-0.222	-0.182	**0.865**	-0.134	-0.045	-0.178	0.103	-0.327	0.162	-0.151	-0.144	-0.111	-0.155	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Principal Components Analysis

The PCA included total-dissolved solids, conductivity, water temperature, dissolved oxygen, pH, and every taxon of benthic macroinvertebrate found in the samples. The first two components explained 36.70% of the variability with the first principal component (PC1) explaining 20.27% and the second principal component (PC2) explaining 16.43%. PC1 had high positive loadings with Odonata (0.891) and Hydrophilidae (0.769) and a large negative loading with conductivity (-0.832). PC2 had a positive loading with Ephydridae (0.751) and a moderately large negative loading with pH (-0.732, Table 3.6).

Table 3.6: Factor loadings for the Principal Components Analysis. Larger loadings are in bold.

	F1	F2
Water Temp	-0.550	-0.527
pH	0.002	-**0.732**
TDS	-0.378	0.463
Conductivity	-**0.832**	0.245
DO	-0.471	-0.551
Mollusca	-0.260	0.509
Gastropoda	-0.463	-0.325
Parastacidae	0.105	0.171
Hyalellidae	-0.270	0.592
Baetidae	-0.117	-0.359
Leptohyphidae	0.413	-0.395
Gripopterygidae	0.195	0.445
Odonata	**0.891**	-0.019
Hydroptilidae	-0.568	-0.401
Elmidae	-0.348	-0.353
Hydrophilidae	**0.769**	-0.341
Corixidae	-0.062	0.569
Muscidae	-0.072	0.085
Ephydridae	0.080	**0.751**
Ceratopogonidae	0.543	0.253
Chironomidae	-0.653	0.375
Simuliidae	0.392	-0.147
Blepheracidae	-0.208	-0.333
Tipulidae	-0.254	-0.127
Dytiscidae	0.288	0.271
Tabanidae	-0.262	-0.208
Empididae	0.740	-0.229

The Tucari and Titire River sites from the mining impacted group (Group1) and the Pacchani River site from the mining unimpacted group (Group 2) were all negatively associated with PC1. Thus, they were all associated with high levels of conductivity and TDS (PC1), and were also positively associated with higher pH, water temperature and low DO (PC2). However, the Aruntaya River site from the mine impacted group and the Coralaque River site were both positively associated with PC1 and PC2. The mining unimpacted site, the Vizcachas River, was positively associated with PC1 but negatively associated with PC2. In contrast, Group 3 including the Coralaque Huarina, Ubinas, Alto Tambo Rivers in the volcanic region, were all negatively associated with PC1 and PC2 and were strongly associated with higher water temperature and DO. In terms of Group 5 (geothermal /geyser group), the Tambo-US-Vagabundo and Tambo-DS-Vagabundo sites were positively associated with PC1 but negatively associated with PC2. However, the Vagabundo River, the site nearest to the geyser, was strongly negatively associated with PC1 and PC2 and had high water temperature, pH, DO, conductivity and TDS. In group 6, the most downstream sites, the Tambo River Fiscal site was positively associated with PC1 but negatively associated with PC2. However, the Tambo River Costanera site was negatively associated with PC1 and PC2 where water temperature, DO, pH, TDS and conductivity were high.

For the benthic macroinvertebrates, Mollusca, Hyalellidae, Corixidae, Muscidae and Chironomidae were associated with low pH, water temperature, DO and high levels of TDS and conductivity. Ephydridae, Gripopterygidae, Dysticidae, Ceratopogonidae and Parasticidae were positively associated with PC1 and PC2 and had high TDS and conductivity but low DO and water temperature. Odonata, Empididae, Hydrophilidae and

Leptohyphidae were positively associated with PC1 and negatively associated with PC2 and had low conductivity and TDS values. Tabanidae, Baetidae, Tipulidae, Blepheracidae, Gastropoda and Hydroptilidae were strongly negatively associated with PC1 and PC2 where DO, pH and water temperature were high (Figure 3.5).

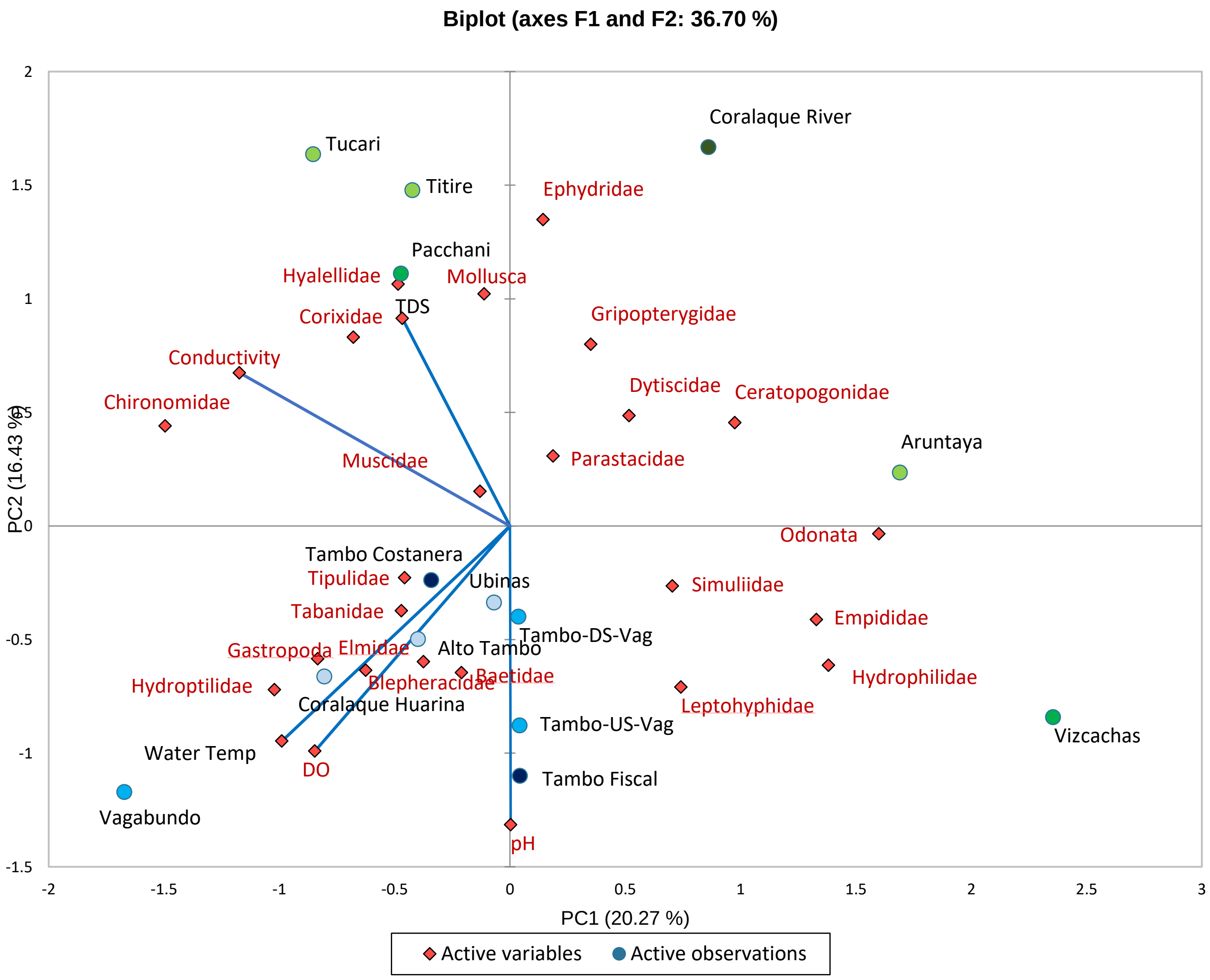

Figure 3.5: Principal component analysis of physicochemical variables (conductivity, pH, DO, TDS) and benthic macroinvertebrates. Vectors represent physicochemical variables. Group 1 is light green (Tucari, Aruntaya and Titire Rivers), group 2 is medium green (Pacchani and Vizcachas Rivers), group 3 is dark green (Coralaque Wetland and Coralaque River), group 4 is

Canonical Correspondence Analysis (CCA) of Metals, Nutrients and Total Organic Carbon Extracted from Chapter 2 (PCA)

The first axis of the CCA explained 67.70% and the second axis explained 32.30% for a total variance of 100% explained. This is expected as all variables were integrated into the model through the incorporation of loadings from the PCA from Chapter 2. The eigenvalues of axis 1 and axis 2 were 0.178 and 0.085, respectively. The total cumulative inertia for axis 1 was 8.25% and 12.18% for axis 2 (Table 3.7).

The main contributors of axis 1 of the CCA included Chironomidae (0.343), followed by Baetidae (0.129) and Elmidae (0.112). The main contributors for axis 2 were Hydroptilidae (0.402), followed by Chironomidae (0.130) and Elmidae (0.113). For the sites, the main contributors to axis 1 were the Coralaque River (0.351) followed by the Titire River (0.170) and the Vizcachas River (0.149). Sites that had the highest contributions to axis 2 were the Tucari River (0.406), the Vagabundo River (0.340), followed by the Pacchani River (0.131, Table 3.8, 3.9).

Table 3.7: Eigenvalues and percentages of inertia for the Canonical Correspondence Analysis.

	Axis 1 (F1)	Axis 2 (F2)
Eigenvalue	0.178	0.085
Constrained inertia (%)	67.698	32.302
Cumulative %	67.698	100.000
Total inertia	8.248	3.936
Cumulative %	8.248	12.184

Table 3.8: Contribution of objects (benthic macroinvertebrates). Large contributions are in bold.

	Axis 1 (F1)	Axis 2 (F2)
Mollusca	0.010	0.040
Gastropoda	0.000	0.014
Parastacidae	0.007	0.004
Hyalellidae	0.013	0.080
Baetidae	**0.129**	0.045
Leptophlebiidae	0.024	0.003
Gripopterygidae	0.057	0.006
Odonata	0.108	0.024
Hydroptilidae	0.020	**0.402**
Elmidae	**0.112**	**0.113**
Hydrophilidae	0.063	0.046
Corixidae	0.030	0.027
Muscidae	0.000	0.028
Ephydridae	0.046	0.012
Ceratopogonidae	0.005	0.003
Chironomidae	**0.343**	**0.130**
Simuliidae	0.001	0.010
Blepheracidae	0.006	0.009
Tipulidae	0.004	0.001
Dytiscidae	0.008	0.001
Tabanidae	0.000	0.002
Empididae	0.014	0.002

Table 3.9: Contribution of sites. Large contributions are in bold.

	Axis 1 (F1)	Axis 2 (F2)
Tucari	**0.140**	**0.406**
Aruntaya	0.009	0.001
Titire	**0.170**	0.025
Pacchani	0.051	**0.131**
Vizcachas	**0.149**	0.009
Coralaque River	**0.351**	0.036
Coralaque Huarina	0.008	0.001
Ubinas	0.054	0.002
Alto Tambo	0.017	0.001
Tambo-US-Vag	0.006	0.008
Vagabundo	0.013	**0.340**
Tambo-DS-Vag	0.013	0.038
Tambo Fiscal	0.004	0.000
Tambo Costanera	0.013	0.002

The Tucari River was associated the strongest with PCA1 (zinc, iron, uranium, aluminum, total nitrogen, and mercury; Figure 3.6). Similarly, the Aruntaya, Tambo Fiscal, and Tambo Costanera River sites were more associated with PCA1 than PCA 2. The Titire River was associated with both PC1 and PCA2. The Vagabundo River, Tambo-DS-Vagabundo and Pacchani Rivers were associated with PCA2 (boron, potassium, sodium and arsenic). The Tambo-US-Vagabundo, Alto Tambo and Coralaque Huarina Rivers, which were not near any known sources of anthropogenic impact were grouped closely together. Surprisingly, the Ubinas River was also negatively associated with both PCA1 and PCA2. The Vizcachas and the Coralaque Rivers were the sites showing the least impacts from the factors contributing to PCA1 and PCA2.

Corixidae, Hyalellidae, and Mollusca were positively associated with PCA1. Elmidae and Chironomidae were affected by factors from both PCA1 and PCA2 (Figure 3.6). Tabanidae, Hydroptilidae and Gastropoda were positively associated with PCA2. Gastropoda, Mollusca, Odonata, Hydroptilidae and Muscidae were all associated with PCA2. Muscidae, Blepheracidae, Tipulidae and Baetidae along with Mollusca and Gastropoda were negatively associated with PCA1. Simuliidae, Ephydridae, Hydrophilidae, Ceratopogonidae, Parastacidae, Odonata, Empididae, Dytiscidae and Gripopterygidae were negatively associated with PCA2.

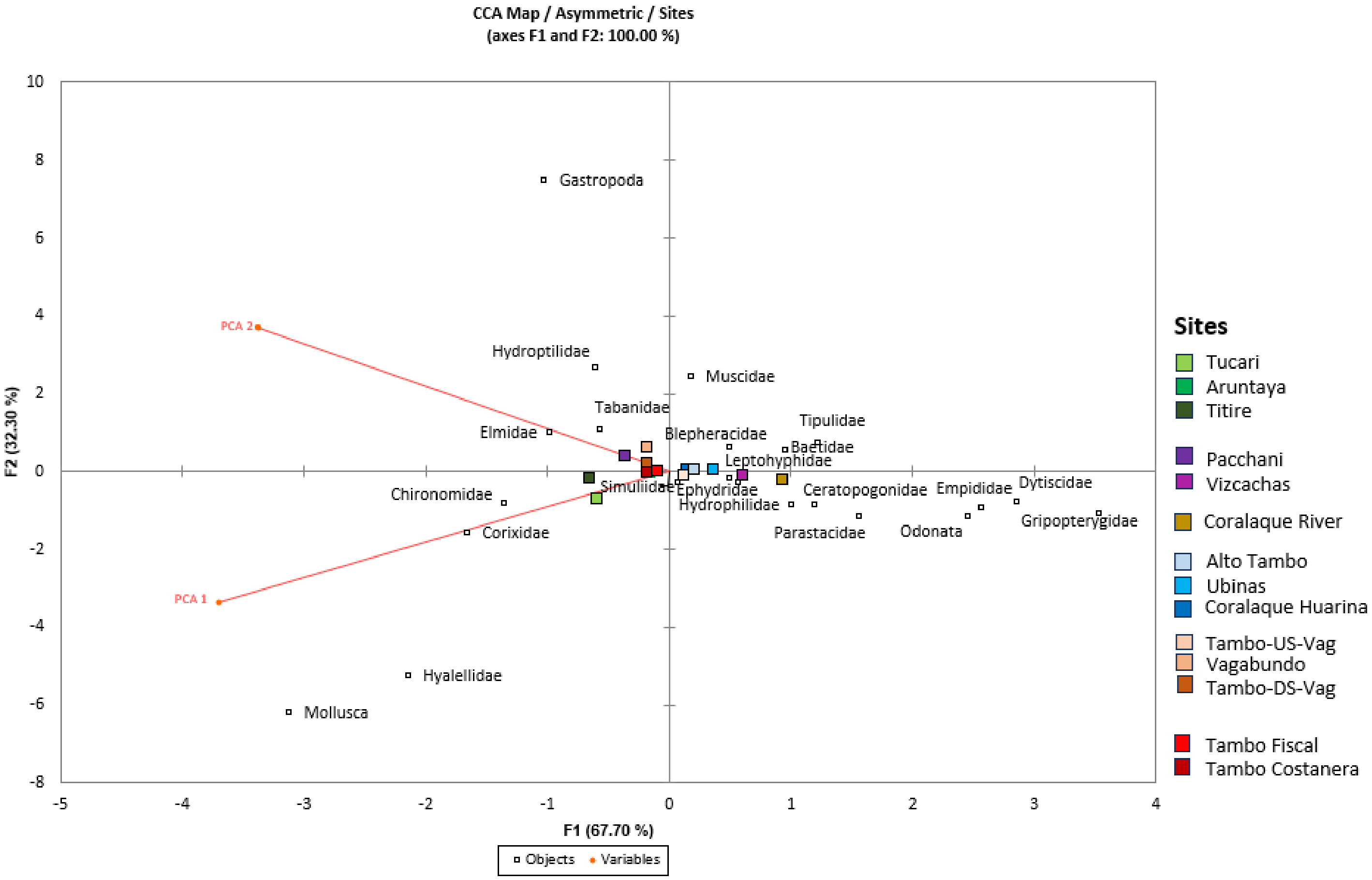

Figure 3.6: Canonical Correspondence Analysis between PCA1 and PCA2 loadings, sites, and benthic macroinvertebrates

3.5 DISCUSSION

Group 1: Tucari, Aruntaya and Titire Rivers

Previous studies around the world have demonstrated that mining activities are a cause of reduced biodiversity and altered species composition in affected streams (Tarras-Wahlberg et al., 2001). Benthic macroinvertebrates are widely regarded as the most sensitive indicators of metal contamination caused by mining drainage (Rosenberg & Resh, 1993). In other parts of the world, studies on the effects of mining drainage on macroinvertebrate communities have revealed changes in community structure, the disappearance of less tolerant species, and the dominance of more tolerant species (Van Damme et al., 2008). Mining activities and improper mining closure practices can additionally lead to a reduction in species richness, growth, density, and production long after a mine ceases production.

This study observed poor water quality at the mining-impacted sites (Group 1) indicated by acidic waters and high levels of metals, often exceeding Peruvian Water Quality Standards for drinking water and conservation of the aquatic environment. Results from benthic macroinvertebrate samples indicate the ecological consequences of poor water quality at these sites with a distinct low biodiversity, taxa richness, as well as the overall absence of sensitive taxa and the dominance of tolerant taxa.

The Tucari River had the lowest abundance of benthic macroinvertebrates found and a low taxa richness and diversity. Diptera, the most abundant family at all three mining-impacted sites indicates poor water quality as this order is a known tolerant species, and their abundance tends to increase with low pH, low dissolved oxygen, and highly toxic concentrations of pollutants (Ríos-Touma et al., 2014). The tailings present at the Tucari River can also have a detrimental effect on aquatic organisms. The orange precipitate from the ARD of the tailings that smothered the streambed was an accumulation of encrusted iron oxyhydroxides. This material limits suitable habitats for benthic macroinvertebrates which can hinder interactions among functional feeding groups (Courtney and Clements, 2000; O'Halloran et al., 2008). The abundance of Chironomidae aligns with previous studies from South America confirming that low oxygen levels and pollution originating from mines, specifically mine tailings and acid mine drainage, create harsh conditions where only resilient taxa can thrive (Acosta & Prat, 2010; De Beethoven et al., 2005; Loayza-Muro et al., 2010; Ríos-Touma et al., 2014). The family Chironomidae dominated environments that had poor water quality. Their presence at higher numbers is associated with their resilience to metal contamination in streams that have been disturbed by metal contamination (Tapia et al., 2018). The family Ephydridae, also abundant at these sites, is also known to be resilient in the polluted waters of South America (Ríos-Touma et al., 2014). The PCA confirmed that Chironomidae was found in habitats with high total dissolved solids and where dissolved oxygen, pH and water temperature were low. However, the relationship between Chironomidae and contaminated sites was not as obvious as Chironomids were the most ubiquitous family throughout the basin. Surprisingly, Gripopterygidae (Plecoptera) were found at the Aruntaya River. This particular family is sensitive to pollutants and has previously been used as an indicator of optimal environmental quality; they also prefer cold water (Oliveira & Callisto, 2010; Ríos-Touma et al., 2014). This is supported by the results of the PCA and CCA where Gripopterygonidae's optimal conditions were where there was minimal pollution and cooler water. Elmidae and Baetidae , both moderately tolerant to pollution, were found equally in the Aruntaya River but were both absent from the Tucari and Titire Rivers. The presence of these families along with slightly greater abundance of total macroinvertebrates recorded suggests that the Aruntaya River may have more suitable water quality during the dry season in comparison to the other mine-impacted sites (Ríos-Touma et al., 2014).

At the Titire River, a clear change in sample composition was observed transitioning from a Chironomid-dominated community to an Ephydridae-dominated community. Ephydridae, are as resilient as chironomids and are known to withstand saline and highly conductive waters as observed at the Titire River (Hamada et al., 2018). The PCA effectively shows this relationship where Ephyrdridae had a strong relationship with the Titire and Pacchani Rivers, with high total dissolved solids and conductivity.

Group 2: Pacchani and Vizcachas Rivers

Our research confirmed that the Pacchani and Vizcachas Rivers, presumably unimpacted by the mines, showed somewhat of a difference in benthic macroinvertebrate communities when compared to the Group 1 described above (Tucari Aruntaya and Titire Rivers). Adjacent to the Titire River, the Pacchani River had a greater abundance of individuals despite having relatively similar physical site characteristics. While both sites were abundant in dipterans, Ephydridae, not Chironomidae was the dominant family at both sites. Nevertheless, the Titire River did not have the sensitive species that were present in other sites and had a low EPT% relative to the rest of the basin. This suggests that the Pacchani River's water quality was better overall compared to the mining-contaminated sites of Group 1, despite its proximity. However, as benthic macroinvertebrate communities were relatively similar at the Titire River and the Pacchani River, it is important to consider that the similarities between channel morphology, water turbulence, and other natural physical parameters may have a stronger effect on the benthic macroinvertebrate communities in these high-altitude sites. These differences can be seen while comparing the PCA with the CCA. The PCA shows that the Pacchani and Titire Rivers have similar physicochemical variables whereas in the CCA, the Titire River is associated with PCA1 “anthropogenic contamination” and the Pacchani

River was moderately associated with PCA2 “natural sources of metals and acids”.

A more remarkable difference in the benthic macroinvertebrate communities was observed at the Vizcachas River. There was a notable difference in family composition and orders recorded. The Vizcachas River was the most high-altitudinal site where the samples were not dominated by dipterans. The presence of Diptera was replaced with Coleopterans and Ephemeropterans which accounted for the majority of the sample composition. There was a notable increase in EPT% (66.4%) and overall good diversity which indicates good water quality.

The Vizcachas River had a high abundance of Elmidae, Baetidae and the highest abundance of Gripoterygonidae and Tabanidae in the entire basin. The reduction in Chironomidae with the replacement by these more tolerant taxa, specifically, Gripopterygonidae indicates good water quality. As supported by the CCA, the Vizcachas River was mapped opposite of both PCA1 and PCA2 effectively demonstrating that it remains unimpacted by mines and has low sources of naturally occurring metals and acids.

This finding supports the results by Smolder et al. (2003), where the presence of chironomids differed at unpolluted and polluted sites at the same altitudes. Significant changes in altitude among sites will undoubtedly have an impact on the composition of the benthic communities.

Of note, the Pacchani and Vizcachas Rivers were at comparable elevations to the mining-impacted sites. Without the confounding factor of elevation, it's highly likely that the Tucari, Aruntaya and Titire River's contamination with evident mine impacts is what caused the domination of Chironomidae in those areas rather than the harsh conditions associated with high altitudes.

Group 3: Coralaque Wetland and Coralaque River

It defies logic that wetlands, particularly those that accumulate peat, could persist in dry areas. Bofedales are close to the hydrological and altitudinal limits for plant life in the cold and dry grasslands of Peru and are present despite their hyper aridity, extreme solar radiation, high-velocity winds, hypoxia, daily frost, and a brief growth season (Squeo et al., 2006). The Coralaque Wetland, being the only wetland sampled in this study has unique characteristics that can have a large influence on the assemblages of macroinvertebrates.

The Coralaque Wetland's unique physicochemical characteristics along with the presence of stagnant pools will have an important role in determining the macroinvertebrate assemblages. These characteristics and the pools can affect the quantity and diversity of taxa as it determines the availability of microhabitats, the quality of the water, hydrological connectivity, and the water exchange rate (Passuni & Fonkén, 2015). As such, I did anticipate that the benthic macroinvertebrates communities at this location would differ from the rest of the basin.

Comparable to upstream sites, there was a lot of chironomids, comprising most of the samples. Multiple studies have reported the presence of chironomids in lentic systems, and they have often been considered a representative family of benthic macroinvertebrates in high-altitude lakes and wetlands. Their strong presence in high-altitude lakes and wetlands has been linked to large amounts of organic matter and low oxygen concentrations since their circulatory systems enable them to withstand these conditions (Coayla-P et al., 2022). However, having relatively high dissolved oxygen levels in comparison to the rest of the basin, their presence is likely due to the large amounts of organic matter which further demonstrates their ability to thrive in different conditions.

Along with the abundance of Chironomids, the Coralaque Wetland had Hirudinea which were absent in the rest of the basin. This tolerant subclass has been previously linked to habitats with submerged vegetation and marshy environments in South America (Gullo, 2009; Ríos-Touma et al., 2014; Zarges et al., 2019). The largest abundance of Hyalellidae was also recorded in the wetland. This moderately tolerant family is often found in areas where mosses are present as they are known to be able to exploit various types of food substrates such as fine and course particulate organic matter (Acosta Rivas & Prat i Fornells, 2011; Zarges et al., 2019). Similarly, the molluscs had the highest abundance at this site which contradicts previous studies where mollusc richness had declined with a rise in altitude. However, most molluscs do not survive in intermittent streams and other factors such as calcium levels and water temperate might explain their unique presence at this site (Dillon, 2000; Maltchik et al., 2010). Elmidae, one of the most abundant families in the basin, were absent at this location, presumably because they prefer a lotic environment (Elliott, 2008).

The unique habitat characteristics of the site, especially the strong presence of organic matter, played an important role in defining the benthic macroinvertebrate community. Wetlands are a sink for many pollutants but with only rudimentary knowledge of the health status of high Andean wetlands, it is challenging to be able to determine what should be defined as good water quality and what should be considered impacted. This further reinforces the importance of using biological communities as indicators to assess the effects of anthropogenic disturbances (Custodio, 2019).

The Coralaque River, immediately downstream of the wetland had the highest abundance of Chironomids. Hyalellidae was the second most abundant family at this location and occurred at the highest abundance of the entire Tambo River system at this location. This family has been associated with mildly impaired streams in South America (Ríos-Touma et al., 2014). The CCA suggests that water quality at this site was indeed high as the site mapped the furthest from both PCA axes (mining contaminated and natural sources of metals and acids).

Group 4: **Coralaque Huarina, Ubinas River and Alto Tambo**

This series of sites is located near the Ubinas Volcano. These sites had a greater abundance of individuals collected than all sites sampled upstream. A change in overall composition was also observed and there was a noticeable shift in terms of chironomid abundance relative to the sites sampled upstream (Coralaque River and Coralaque Wetland).

At the Coralaque Huarina River site, although dipterans were the most abundant order, Simuliidae was the most abundant family. In general, the ecology and the potential for utilizing Simuliidae as bioindicators in South America are poorly understood. However, there is a consensus that they occur in well-oxygenated waters with organic matter but react rapidly to physical and chemical changes, and are absent in heavily polluted waters (Currie & Adler, 2007; Custodio, 2019; Docile et al., 2015). They have mostly been used to describe sites that are moderately impacted and more specifically are an indicator of intermediate urban pollution and agricultural impacts which corresponds with the results from this study (Ríos-Touma et al., 2014; Custodio, 2019). The CCA showed that Simuliidae was not strongly associated with either mining impacts (PCA1) or natural sources of contaminatin (PCA2) and was mainly associated with intermediate impacts, rather than with more severely impacted sites.

Similarly, moderately tolerant families were the most abundant in the Ubinas River where Baetidae was the most abundant family followed by Simuliidae (Ríos-Touma et al., 2014). Although an ephemeropteran, Baetidae is commonly found in polluted waters in South America. The presence of the sensitive family Gripopterygonidae and a higher overall family diversity suggests a slightly better water quality in the Ubinas River than upstream at the Coralaque Huarina River site. Furthermore, this site had the highest EPT% recorded in the basin and had a high taxa richness which indicates good water quality. There may be a slight increase in water quality downstream of the Ubinas River at the Alto Tambo River site as Blepheracidae, another sensitive family, was also recovered at this site. The Alto Tambo River also had the highest abundance of Blepheracidae relative to the rest of the basin.

Generally, the results demonstrate that these sites are moderately impacted by low water quality as supported by the CCA where the Coralaque Huarina, Ubinas and the Alto Tambo Rivers all plotted opposite of both PCA1 and PCA2 suggesting that these sites may reflect the overall typical conditions of the basin and were not impacted by any point source of contamination.

Group 5: Tambo-US-Vagabundo, Vagabundo, and Tambo-DS-Vagabundo Rivers

The previous chapter showed high levels of arsenic originating from the Vagabundo River and increasing downstream in the Tambo River. Arsenic can be found at low concentrations in the environment but in geothermal and mine affected waters arsenic concentrations can become very high (Quenta-Herrera et al., 2021). Upstream of the Vagabundo River are geothermal sources which may influence the presence of macroinvertebrates.

Diptera is often the most common insect group in geothermally influenced streams and aquatic fauna in these systems is typically highly restricted and has low diversity. However, communities may change depending on the micro-habitats present in the vicinity (Boothroyd & Browne, 2006). Thus, instead of Diptera as expected, Ephemeroptera was the most abundant order at these sites, specifically Leptohyphidae. The family Leptohyphidae generally has a low tolerance to pollution but is occasionally present in slightly polluted waters. This particular family was abundant at the Tambo-US-Vagabundo and Tambo-DS-Vagabundo Rivers (Ríos-Touma et al., 2014). Baetidae, a family known to tolerate moderate pollution (Ríos-Touma et al., 2014), was the second most abundant family at these sites. Changes in the community are apparent at the Vagabundo River where arsenic levels were at their highest, with Leptohyphidae being replaced by slightly more tolerant taxa such as Elmidae, Hydroptilidae, and Chironomidae. Previous studies have shown that although some benthic macroinvertebrates can tolerate the extreme chemical conditions associated with geothermal waters, other effects such as temperature and toxic chemicals reduce and even eliminate EOT taxa (Quenta-Herrera et al., 2021).

The CCA confirmed that the Vagabundo and Tambo-DS-Vagabundo sites were associated with PCA2 (natural sources of metals and acids) and that the Vagabundo River may be an important source of contamination, specifically for arsenic and boron. Benthic macroinvertebrate communities responded predictably to the Vagabundo River toxins. Patterns in benthic macroinvertebrates in geothermal streams typically resemble those impacted by heavy metals from mines, as they are dominated by taxa generally classified as being tolerant of anthropogenic stressors (Clements et al., 2011). However, the presence of more tolerant taxa after the confluence of the Vagabundo River suggests that metals and acids originating from the geothermal inputs of the Vagabundo River are not shaping the benthic macroinvertebrate communities downstream. It is also important to note that the presence of particular families might be also dictated by indirect effects caused by geothermal activity. The Vagabundo River had the highest water temperature in the entire basin and also high conductivity. The temperature spike may have caused loss of taxa that could not survive the sudden increase in warming, but studies have shown that sudden changes in conductivity due to geothermal inputs have a minimal influence on benthic macroinvertebrate communities (Quenta-Herrera et al., 2021). Overall, the simultaneous changes in water temperature, conductivity, toxic trace elements, and fine sediments found in the Tambo and Vagabundo Rivers, makes it challenging to assess the ecological effects of the geothermal stressor. However, pollution does appear to be limited to some degree in the Vagabundo River and becomes less prevalent downstream. (Clements et al., 2011).

Group 6: Tambo Fiscal and Tambo Costanera Rivers

Anthropogenic activities such as agriculture, various industries, and urbanization can have a strong influence on the environmental conditions of the water and thus influence the benthic macroinvertebrate communities (Custodio et al., 2019).

The Tambo River Fiscal site had the lowest number of dipterans present in the entire basin. Diptera abundance was replaced by an increase in Coleoptera, with the most abundant family being Elmidae, followed by Hydrophilidae. Elmidae, being a moderately tolerant larva is found in running waters, with sandy and gravelly or submerged bottoms with vegetation and is tolerant of high metals (Loayza-Muro et al., 2010; Zarges et al., 2019). Hydrophilidae, the second most abundant family present is also tolerant and has a wide distribution in South America. However, high mountains in the Andes have been known to be the greatest barrier to their distribution which explains their increase in abundance at lower elevations (Oliva, 2014). Hydrophilidae are generally found where there is detritus, algae, and decaying organic matter which is characteristic of these more productive sites in more urbanized regions and at low altitudes. They are known to be useful bioindicators for heavy element pollution due to their known taxonomy, worldwide distribution, and capacity to live in different environmental conditions (Aydogan et al., 2018). The Tambo Costanera River had an increase in dipteran abundance wherein chironomids were the most abundant family. There was also a reduction in overall benthic macroinvertebrate abundance suggesting that water quality worsened downstream of the Tambo Fiscal River site. The presence of more tolerant families is not surprising due to the high concentrations of anthropogenic sources of metals, and more specifically lead. This relationship is supported in the CCA where both Tambo Fiscal and Tambo Costanera were associated PCA1.

The Tambo Fiscal and Tambo Costanera sites were the only places where, Parastacidae (order Decapoda) was found in the entire Tambo River basin. This family is an essential part of the diet of locals and substantial wild harvests are commercially important, making their ecology especially relevant to (Zarges et al., 2019). At these sites, there was an increase in anthropogenic activities such as agriculture, urbanization, and various industries which have been shown to influence the benthic macroinvertebrate communities. The differences in these sites relative to the rest of the basin may be a reflection of the differences in their surrounding land uses. Due to the ecological importance and the human consumption of Parastacidae, it is highly important to understand the extent of contamination in this area so that sustainable management of aquatic resources can be ensured (Bere et al., 2016).

3.6 CONCLUSION

The impacts of mining activities on water quality and benthic macroinvertebrate communities were clear in both abundance and diversity measures. In terms of sources of contamination from volcanic and geothermal sources, sites near the Ubinas Volcano showed no obvious signs of impact, however, at the Vagabundo River there was a clear reduction in EPT% taxa and a shift from pollution-sensitive families to pollution-tolerant families. Contrastingly, taxa richness, EPT%, diversity, and the abundance of benthic macroinvertebrate communities were generally higher at sites unimpacted by mining (Groups 2-6). The collection and analysis of benthic macroinvertebrate communities at various locations across the basin that had exposures to different stressors indicate that they could be used as potential bioindicators for the management of the aquatic environment of the Tambo River Basin. Benthic macroinvertebrate species can be used to establish ecological criteria to classify the river ecosystem as being healthy or polluted. This information is very important as a baseline study for the area to inform regulators of the basin conditions and how the use of bioindicators can assist in environmental monitoring and management of the area. Since this is the first dataset on benthic macroinvertebrates in the Tambo River Basin it will be a useful body of information to be able to conduct future studies using benthic macroinvertebrates.

CHAPTER 4: CONCLUSION AND FUTURE RESEARCH

This study of physical and biological measures along the Tambo River Basin effectively captured the impacts of anthropogenic activities such as mining, agriculture, and urbanization as well as natural contamination coming from volcanic and geothermal sources. It identified river sites that are relatively unimpacted by assessing physicochemical properties, water chemistry, and benthic macroinvertebrate communities. Physicochemical measurements, water chemistry and the benthic macroinvertebrate communities showed clear deterioration at the headwaters near the former Tucari mine with, the Tucari River having the worst water quality in the entire basin. The Tucari River had severe biological impairment and all mining-impacted sites had multiple water chemistry measurements that exceeded Peruvian Water Quality Standards for drinking water and the conservation of the aquatic environment. The poor biological quality at the high-altitude mine sites, the Tucari and Titire rivers was mostly influenced by the contamination from the mine. High altitude creates harsh river conditions that are a potential confounding factor. However, the substantial presence of benthic macroinvertebrates at sites at comparable altitudes but unaffected by mining, suggests a direct negative relationship between high levels of mining contamination and a low presence of macroinvertebrates. The benthic macroinvertebrates surviving in mine-impacted sites thus needed to adapt to effects of both high altitude and mining pollution (Mercado-Garcia et al., 2019). Mining impacts were the most severe stressor in the entire basin and mining-related effects were not seen at any other sites aside from the Tucari, Aruntaya and Titire Rivers. In the past, mining contamination had apparently reached as far as the Coralaque River but this was not evident in this study. Perhaps the upper reaches of the Coralaque River that were not sampled in this study, would have also shown the effects of mining.

In terms of volcanic and geothermal sources of metals and acids, our results did not capture a volcanic signal at the sites in group 4 near the Ubinas Volcano: the Coralaque Huarina, Ubinas, Alto Tambo Rivers. However, a strong volcanic and geothermal signal was captured at the Vagabundo River where concentrations of arsenic and boron were at the highest levels in the entire basin. The final group along the Tambo River at the Tambo Fiscal and Tambo Costanera sites did show signs of anthropogenic contamination with high levels of lead, aluminum and phosphorous. As Parastacidae (crayfish) found at these sites are consumed by local residents, it would be important to investigate if they are safe for consumption.

Before this study, our understanding was that no reference site would provide good background conditions due to the complexity of the landscape, however, locals suspected that the Pacchani River would be the most suitable site to compare mining vs. non-impacted sites. Although this study showed that the Pacchani River site is useful as a reference for a mining-impacted river (Titire River), it cannot be used as a reference site for the rest of the basin as it still showed evidence of some contamination and was likely also affected by high altitude and potentially the adjacent community. A preferable reference site indicated by this study is the Vizcachas River, and more research should be directed toward evaluating this idea as this site was one of the least impacted anthropogenically and naturally.

Furthermore, benthic macroinvertebrates were successfully collected from all sites. That is a significant finding because it confirms that, even in a multi-stressor basin with extreme altitudinal gradients such as the Tambo River Basin, using benthic macroinvertebrates as bioindicators is still an effective approach that can help discern environmental differences along the entire basin.

Although sampling using the D-Net kick and sweep method was successful in this study, it was conducted in the dry season when flows were at their lowest (but certain sites were still not easily accessible). Further studies should focus on sampling both in the dry and rainy seasons to capture seasonal variability of benthic macroinvertebrates. Seasonal variability can influence both taxonomic and biological trait composition and different taxonomic communities may appear in different seasons, particularly in intermittent streams (Bêche et al., 2006). Water chemistry data and physicochemical water quality parameters should also be measured in both seasons to better understand how water quality may change with an increase in flows from rain and glacial melt.

An important limitation of this study is that the stressor areas overlapped, meaning that multiple stressors or sources of contamination were sometimes present in one area. These confounding effects could not be avoided in the study design. As a result, the findings from this research are largely explorative and qualitative. Therefore, conclusions based on high levels of statistical rigour cannot be drawn from this baseline dataset. Moreover, a substantial confounding factor is the altitudinal gradient and understanding the effects of this factor on benthic macroinvertebrate communities is critical for rigorous analyses. Altitudinal gradients cause quite large longitudinal differences in water chemistry, flow characteristics, sediment loads, light penetration, primary production, and organic carbon sources. Understanding the effects of the altitudinal gradient on the benthic macroinvertebrates in the Tambo River Basin is important so that these effects can be contrasted with those of other stressors.

Future studies should additionally focus on providing training in field methods, laboratory sorting of samples and taxonomic identification to ensure that there are local experts able to conduct studies like this, which will increase our understanding of the benthic macroinvertebrate communities in the basin. As the data set on benthic macroinvertebrates for this area grows, it will help identify site-specific bioindicators that are associated with specific hydrological, geochemical and physiochemical patterns. This information will enhance the ability to capture a variety of different impacts.

Certain knowledge gaps do remain. There was limited background information available for this study, and in general high-mountain experts are rare therefore local expert knowledge on the area was minimal. This study could also have benefitted from better local taxonomic keys and local expertise to identify macroinvertebrates to the genus or species level. Less granular taxonomic resolution limited our study as there may be differences in pollution tolerance at the genus or species level (Mazzoni et al., 2014). The need to refine taxonomic keys is substantial in Peru, especially in terms of pupae which we were not able to identify in this study (Zarges et al., 2019; Arana Maestre et al., 2021).

Overall, this study will serve as an important and valuable building block for understanding ecological impacts in the Tambo River Basin. Supplementing physicochemical and water quality parameters with a biological component is crucial in understanding complex changes in an multi-stressor system especially when impacts are constantly in flux due to the different impacts associated with varying anthropogenic influences and overall seasonality (Mercado-Garcia et al., 2019). The present study contributes to increasing knowledge about the bioindicator potential of benthic macroinvertebrates and advances the use of biomonitoring for assessing the environmental quality of mountain streams of Southern Peru. A water quality monitoring program should take into account the recommendations listed in this section. Most importantly, it should be noted that, (1) sites with the worst deterioration were located near the mine, (2) the Vizcachas River is an suitable un-impacted reference for basin-wide studies, (3) the Vagabundo River is an important source of naturally occurring metals and acids that should not be overlooked, and (4) the Tambo River Fiscal and Tambo Costanera sites are showing signs of pollution due to urbanization that differ from upstream inputs. Of additional importance is the globally rare and ecologically unique habitat of the bofedales. Efforts should be directed towards preserving the ecological integrity of the Coralaque Wetland as it is likely serving as a natural filter for mining contamination from the upstream regions. Lastly, that use of a basin-wide monitoring program that incorporates a multivariate approach will be valuable in establishing targets for remedial actions, allow for measures of improvement, and provide the ability to detect seasonal and temporal changes in the Tambo River Basin of Southern Peru.

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APPENDIX

Figure 5.1: Tucari River streambed.

Figure 5.2: A) Water samples, B) Condensed benthic macroinvertebrate samples, C) samples stored in Peruvian laboratory.

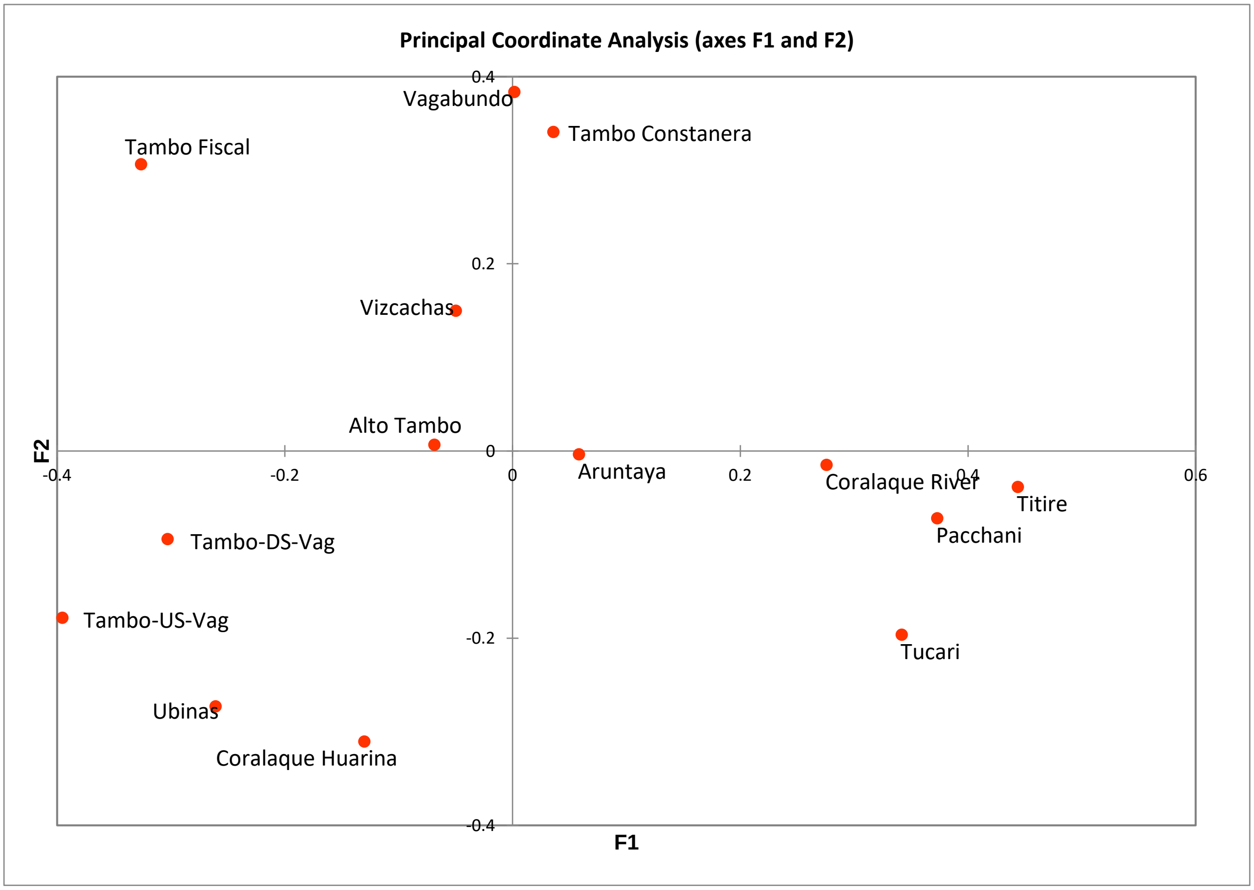

Figure 5.3: Principal coordinate analysis (PCoA) using Bray-Curtis as the underlying distance coefficient to assess the differences in the arrangement in sites (PCA vs. PCoA).

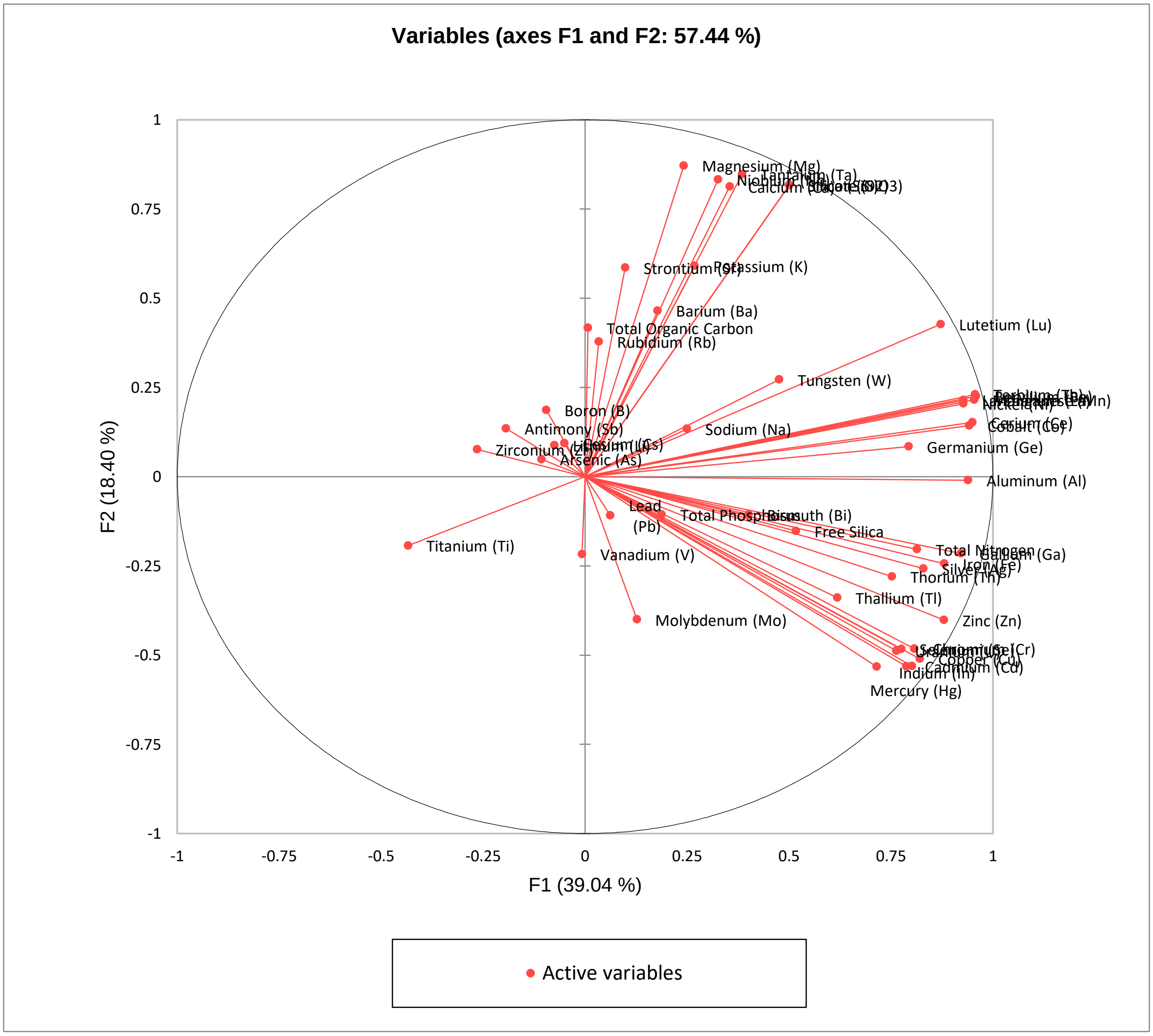

Figure 5.4: PCA including all of the water chemistry data. Used in combination with the Pearson Correlation identify similar pairs of variables.

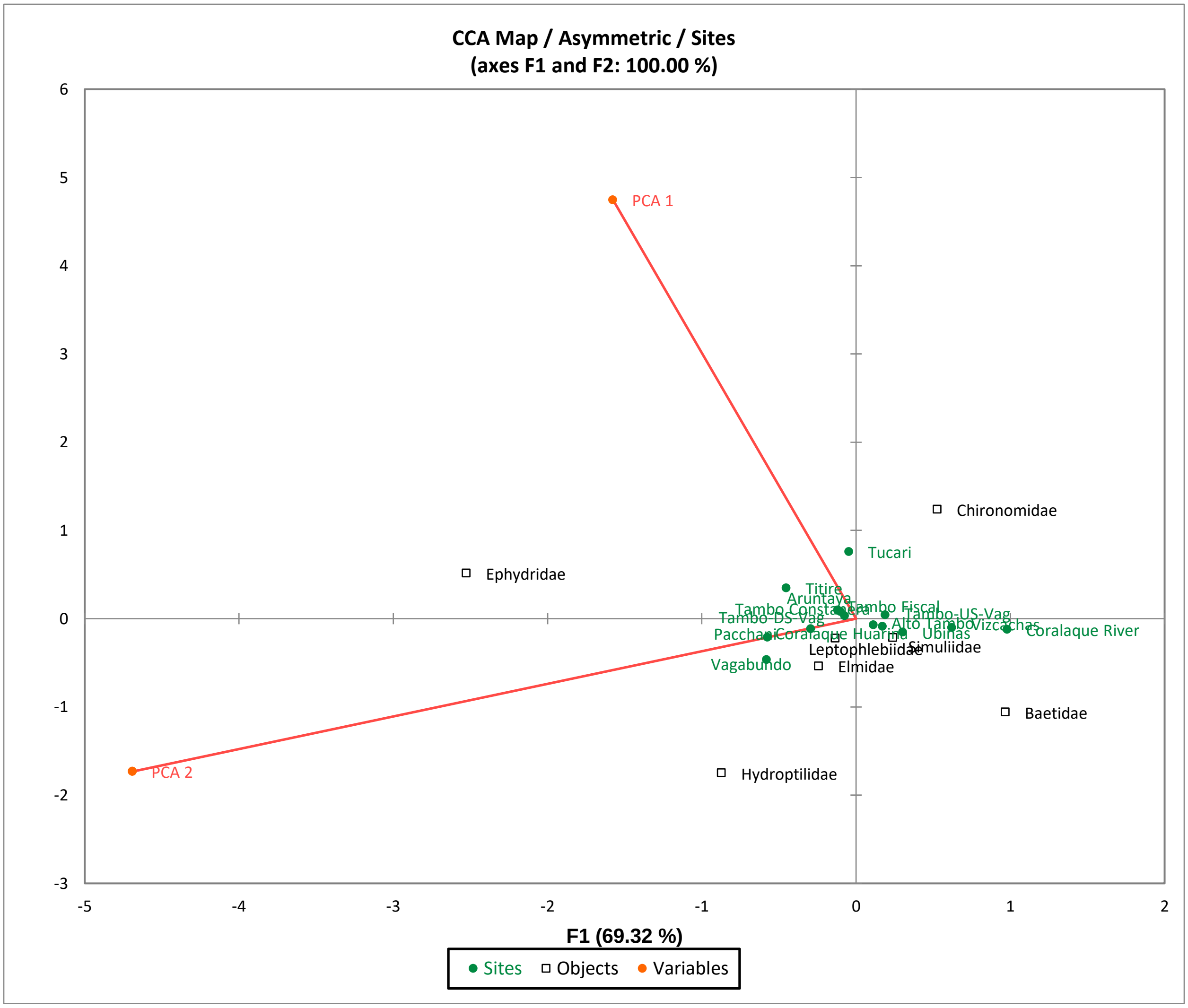

Figure 5.5: CCA performed without the rare taxa.

Table 5.1: Sampling dates, site coordinates and elevation (m).

Site	Sampling Date	Latitude	Longitude	Elevation (m)
Tucari River	November 26th, 2021	16°35'31.54"S	70°17'17.55"W	4456
Aruntaya River	November 26th, 2021	16°35'38.78"S	70°17'55.56"W	4418
Titire River	November 26th, 2021	16°31'54.44"S	70°21'38.92"W	4358
Pacchani River	November 26th, 2021	16°31'50.26"S	16°31'50.26"S	4357
Vizcachas River	November 26th, 2021	16°37'33.46"S	70°24'28.31"W	4295
Coralaque Wetland	November 27th, 2021	16°36'24.10"S	70°36'26.45"W	4378
Coralaque River	November 27th, 2021	16°32'14.88"S	70°41'0.46"W	3589
Coralaque Huarina	November 30th, 2021	16°26'14.85"S	70°48'39.88"W	2594
Ubinas River	November 30th, 2021	16°26'15.26"S	70°48'50.65"W	2595
Alto Tambo	November 30th, 2021	16°26'25.21"S	70°48'45.26"W	2571
Tambo-US-Vag	November 29th, 2021	16°46'9.77"S	71° 0'20.81"W	1292
Vagabundo River	November 29th, 2021	16°45'48.56"S	71° 0'21.21"W	1331
Tambo-DS-Vag	November 29th, 2021	16°45'48.69"S	71° 1'7.07"W	1275
Tambo Fiscal	November 28th, 2021	17° 1'47.85"S	71°41'27.79"W	162
Tambo Costanera	November 28th, 2021	17° 8'43.92"S	71°47'45.50"W	21

Table 5.2: Site characteristics recorded during sampling.

Site	Surrounding Land Use	Dominant Land Use	Habitat Types	Canopy Coverage (%)	Macrophyte Coverage (%)	Streamside Vegetation	Dominant Streamside Vegetation	Periphyton Coverage	Habitat Sampled
Tucari River	mining, field/pasture	mining	riffle, straight run, pool/back eddy	0%	0	ferns/grasses	grasses	1	riffles, pools
Aruntaya River	field/pasture, mining, alpaca farm	mining	riffle, pool back eddy	0	0	ferns/grasses, shrub	ferns/grasses, mosses	2	riffles, pools
Titire River	field/pasture, mining, alpaca farm, residential	residential	run, pool	0	0	grasses	grasses	2	run, pool
Pacchani River	field/pasture, residential/urban	field/pasture	riffle. Straight run	0	0	shrubs	grasses	1	riffles, straight run
Vizcachas River	unknown	unknown	riffle	0	0	unknown	unknown	unknown	riffles
Coralaque (wetland)	conservation area	forest/conservation area	riffle, run	0	26-50	ferns/grasses	mosses	3 (floating algae)	grab net in wetland water
Coralaque River	field/pasture	field/pasture/old smelter area	riffle, straight run	0	0	ferns/grasses, shrubs	ferns/grasses	1	riffles, straight run
Coralaque Huarina	field/pasture, agriculture	field/pasture	riffle, rapids, straight run, pool/back eddy	0	0	none	none	1	riffle, pool
Ubinas River	field/pasture, agriculture	field/pasture	rapids	0	0	shrubs	shrubs	3	rapids
Alto Tambo	field/pasture, agriculture	field/pasture	riffle, rapids	0	0	none	none, small flowers	1	riffle
Tambo-US-Vag	field/pasture	field/pasture	riffle, rapids, straight run	0	0	ferns/grasses	shrubs	2	riffle, straight run
Vagabundo River	unknown	unknown	rapids, straight run	0	0	ferns, grasses	shrubs	3	rapids
Tambo-DS-Vag	unknown	unknown	riffle, rapids, straight run	0	0	ferns/grasses, shurbs	shrubs	2	run, riffle
Tambo Fiscal	field/pasture, agriculture	residential/urban	straight run	0	(01-25)	ferns/grasses, shrubs, deciduous trees	shrubs	1	straight run
Tambo Costanera	field/pasture, agriculture, residential/urban	field/pasture	riffle, rapids, straight run	0	0	ferns/grasses, shrubs	ferns/grasses	1	riffle, run

Table 5.3: Water sampling site observations.

Site	Water Clarity	Pollution Sources	Scum	Dominant Surrounding Land Use	Biological Structures	Debris	Shoreline Erosion	Sediment Colour
Tucari River	suspended	mine/animals	no	field/pasture	none	none	yes, slight	yellow to brown, red, sulfide rock tailings visible
Aruntaya River	solid/murky, slightly turbid	mine/animals	no	field/pasture	none	none	yes	red
Titire River	clear but with some material suspended	mining	no	field/pasture, nearby village, alpaca farms	none	none	yes	red/orange
Pacchani River	suspended, organics visible	waste pipes maybe	yes	field/pasture and urban	none	plant fibres	yes	black
Vizcachas River	solid/murkey	none	no	unknown	none	plant fibres	no	Yellow to brown, some black
Coralaque Wetland	clear	none	no	hatmal conservation area	macrophytes	plant fibres	no	Yellow to brown, some black
Coralaque River	highly cloudy	used to be around an old smelter	no	conservation area upstream	none	none	yes	yellow to brown
Coralaque Huarina	highly cloudy	volcano	no	field/pasture	none	branches	no	yellow to brown
Ubinas River	clear	volcano	no	field/pasture	none	branches	no	yellow to brown
Alto Tambo	highly cloudy	volcano	no	field/pasture	none	branches	no	yellow to brown, brown
Tambo-US-Vag	highly cloudy	unknown	no	TBD	none	none	yes	yellow to brown
Vagabundo River	clear	unknown	no	TBD	none	plant fibres	no	yellow to brown
Tambo-DS-Vag	highly cloudy	unknown	no	TBD	none	none	no	yellow to brown
Tambo Fiscal	highly cloudy	urban area, agriculture, land clearing	yes (thick)	field/pasture (extensive agriculture)	none	none	yes	brown
Tambo Costanera	highly cloudy	possible waste pipes	yes	field/pasture, extensive agriculture	none	none	no (some areas, large and medium cobble deposits)	brown

Table 5.4: Supplemental physicochemical parameters.

Site	Specific Conductance (uS/cm)	Salinity (PPT)
Tucari River	2339	1.21
Aruntaya River	442.2	0.21
Titire River	5054	2.74
Pacchani River	9446	5.29
Vizcachas River	265	0.13
Coralaque Wetland	91.5	0.04
Coralaque River	1619	0.82
Coralaque Huarina	2015	1.03
Ubinas River	993	0.49
Alto Tambo	1981	1.01
Tambo-US-Vag	1332	0.98
Vagabundo River	3338	1.74
Tambo-DS-Vag	2038	1.04
Tambo Fiscal	2381	1.23
Tambo Costanera	2510	1.29

Table 5.5: Complete water chemistry dataset from water samples collected across the Tambo River basin. All concentrations are in mg/L.

Parameters	Tucari	Aruntaya	Titire	Pacchani	Vizcachas	Coralaque Wetland	Cor River	Coralaque Huarina	Ubinas	Alto Tambo	Tam-US-Vagabundo	Vagabundo	Tam-DS-Vagabundo	Tambo Fiscal	Tambo Costanera
Free Silica	80.51	64.16	84.02	39.91	51.36	96.17	34.53	48.32	73.74	48.44	72.1	72.8	83.08	78.65	96.87
Total Phosphorus	0.266	0.23	0.136	0.22	0.155	0.692	0.102	0.195	0.183	0.182	0.582	0.081	0.42	0.501	0.636
Total Nitrogen	20.93	4.1	8.41	4.46	1.17	1.59	1	1.59	0.35	1.18	0.21	0.22	1.26	1.78	2.03
Total Organic Carbon	0.6	4	1.7	0.8	2.1	0.4	5	0.9	0.6	1.3	1.7	0.4	1.3	0.9	1.5
Lithium	0.01859	0.086	2.51447	5.07171	0.06477	0.79033	0.00158	0.5187	0.0508	0.48438	0.35006	2.45304	1.17143	0.61314	0.69269
Beryllium	0.00376	0.0048	0.00317	0.00017	0.00022	0.00225	0.00007	0.00034	0.00015	0.00038	0.00106	0.00008	0.00062	0.00116	0.00178
Boron	0.2528	1.342	11.6357	25.8506	0.5936	4.4121	0.0525	2.3791	0.6246	2.1407	2.1174	34.0067	14.4288	3.7922	4.0767
Sodium	139.472	157.51	746.212	1807.14	28.225	231.192	4.97	285.515	72.317	276.613	161.377	418.472	268.021	320.725	349.218
Magnesium	12.181	98.74	17.457	55.961	4.88	15.058	2.512	19.333	41.609	21.221	21.656	35.629	27.376	25.941	27.437
Aluminum	96.901	67.81	68.423	0.955	1.949	33.411	0.297	4.908	1.085	3.678	9.134	0.424	6.849	12.802	15.306
Silicon	35.831	28.637	38.311	17.23	23.062	43.342	15.036	21.068	32.96	21.096	32.033	32.286	36.961	34.975	43.442
Silica	76.679	61.283	81.986	36.872	49.353	92.752	32.178	45.085	70.535	45.145	68.55	69.092	79.096	74.846	92.966
Silicate	97.1	77.61	103.82	46.69	62.5	117.46	40.75	57.09	89.32	57.17	86.81	87.5	100.16	94.78	117.73
Potassium	10.215	67.53	43.318	74.933	6.399	17.713	4.508	14.767	10.616	13.7	11.726	65.476	31.966	18.359	21.497
Calcium	102.259	39.078	97.841	227.291	16.48	63.277	8.056	89.102	133.66	94.55	99.505	178.62	134.553	160.048	151.991
Titanium	0.00431	0.0061	0.00639	0.02138	0.06788	0.07242	0.02161	0.07669	0.02159	0.05867	0.04842	0.00989	0.04444	0.05211	0.06405
Vanadium	0.00648	0.0026	0.00529	0.00604	0.00739	0.02501	0.00271	0.01077	0.00684	0.00919	0.02301	0.00229	0.01669	0.0195	0.02555
Chromium	0.0101	0.002	0.0068	0.0004	0.0006	0.0058	0.0003	0.0013	0.0003	0.0011	0.0053	<0.0002	0.0039	0.0033	0.0042
Manganese	4.76123	7.37816	3.87523	0.67062	0.39148	1.64084	0.05794	0.56241	0.13642	0.47632	1.430557	0.12978	0.84147	1.36331	1.5238
Iron	73.5668	15.3116	29.4492	1.82027	2.554	26.46116	1.61589	4.46761	1.6016	3.80229	11.84158	0.58071	9.16768	13.0218	13.567
Cobalt	0.28527	0.28243	0.19932	0.00192	0.00203	0.034833	0.00061	0.00949	0.00202	0.00856	0.014824	0.00097	0.00951	0.02894	0.02559
Nickel	0.20414	0.2259	0.13237	0.00179	0.0021	0.02383	0.0014	0.00776	0.00166	0.00708	0.01657	0.00164	0.01074	0.02002	0.01916
Copper	1.5347	0.063	1.2202	0.0107	0.0098	0.3864	0.0141	0.0404	0.006	0.037	0.0684	0.0077	0.0497	0.213	0.1706
Zinc	3.10097	0.4207	2.07432	0.02108	0.02462	0.39789	0.00432	0.11754	0.01257	0.10432	0.6078	0.00563	0.32482	0.30605	0.31041
Gallium	0.00673	0.0038	0.00404	0.00027	0.00067	0.0047	0.00011	0.00117	0.00035	0.00088	0.0026	0.00007	0.00182	0.0022	0.00354
Germanium	0.00294	0.0029	0.00411	0.00235	0.00035	0.00331	0.00004	0.00083	0.00021	0.00071	0.0014	0.00214	0.00166	0.00206	0.00226
Arsenic	0.20528	0.0173	0.4784	0.33503	0.08924	0.60324	0.00331	0.05179	0.02473	0.04811	0.12624	5.3098	1.98405	0.34632	0.36997
Selenium	0.0174	0.002	0.0058	<0.0002	<0.0002	0.0011	<0.0002	0.0005	0.0011	0.0005	0.0006	0.0005	0.0005	0.001	0.001
Rubidium	0.02772	0.2339	0.33794	0.34666	0.0229	0.11072	0.00777	0.07957	0.02274	0.06711	0.06867	0.7373	0.31251	0.10951	0.14163
Strontium	0.38473	3.1391	1.65466	5.49036	0.19645	0.97046	0.07102	1.62843	0.89313	1.39147	1.24379	1.7394	1.42906	1.86904	2.10387
Zirconium	0.0003	0.0011	0.00029	0.00095	0.00227	0.00174	0.00042	0.00128	0.00089	0.00117	0.00207	0.00022	0.00158	0.00138	0.00184
Niobium	0.00003	0.0006	0.00024	0.00007	0.00019	0.00011	0.00011	0.00016	0.0002	0.00014	0.00013	0.00014	0.00008	0.00008	0.00018
Molybdenum	0.00471	0.0008	0.00199	0.00457	0.00151	0.00207	0.00038	0.00246	0.00372	0.00249	0.00176	0.00296	0.00206	0.00247	0.00209
Silver	0.01703	0.0063	0.00509	0.00064	0.00086	0.00133	0.00055	0.00101	0.00111	0.00069	0.00069	0.00197	0.00068	0.00116	0.00088
Cadmium	0.05881	0.0012	0.03896	0.00013	0.00013	0.00574	0.00003	0.0013	0.00008	0.00112	0.00323	0.00006	0.00177	0.00353	0.00326
Indium	0.00293	0.0002	0.00126	<0.00002	<0.00002	0.00075	<0.00002	<0.00002	<0.00002	<0.00002	0.00004	<0.00002	0.00003	0.00013	0.00012
Tin	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004	<0.0004
Antimony	0.003	0.001	0.0048	0.0012	0.001	0.0011	<0.0001	0.0005	0.0005	0.0004	0.0017	0.1752	0.0349	0.0046	0.0044
Cesium	0.00663	0.0097	0.41619	0.28517	0.00901	0.09612	0.00018	0.05159	0.00693	0.04171	0.04877	0.73786	0.29525	0.10078	0.12372
Barium	0.02085	0.03295	0.05153	0.13503	0.08371	0.28408	0.02076	0.14034	0.05321	0.11231	0.22064	0.07016	0.16796	0.27202	0.41902
Lanthanum	0.02846	0.03671	0.02175	0.00153	0.00295	0.02154	0.00047	0.00509	0.00174	0.00411	0.014176	0.0003	0.0091	0.01064	0.01907
Cerium	0.07419	0.08661	0.06148	0.00369	0.00666	0.05484	0.00114	0.01266	0.00395	0.01031	0.033906	0.00064	0.0211	0.02721	0.04703
Terbium	0.00171	0.0021	0.00121	0.00004	0.00007	0.0008	<0.00001	0.00015	0.00004	0.00012	0.00045	<0.00001	0.00027	0.00043	0.00055
Lutetium	0.00052	0.00094	0.00038	1.7E-05	3.1E-05	0.00028	5E-06	5.5E-05	1.4E-05	4.3E-05	0.000153	6E-06	0.00009	0.00013	0.00018
Tantalum	0.00002	0.00003	0.00008	0.00003	0.00003	0.00003	0.00004	0.00004	0.00008	0.00004	0.00003	0.00008	0.00002	0.00001	0.00004
Tungsten	0.00009	0.0002	0.00026	<0.00002	0.00006	<0.00002	<0.00002	0.00003	0.00007	<0.00002	0.00015	0.00013	0.00014	0.00026	0.00021
Mercury	0.00989	0.0004	0.00191	0.00005	0.00009	0.00052	0.00005	0.00006	0.00006	0.00006	0.00032	0.00004	0.00026	0.00027	0.00043
Thallium	0.00566	0.0008	0.0039	0.00047	0.00017	0.00105	<0.00002	0.00049	0.00022	0.00036	0.00072	0.00436	0.00187	0.00089	0.00093
Lead	0.0081	0.002	0.0018	0.0027	0.0022	0.0133	0.0008	0.0091	0.0023	0.0071	0.0255	0.0005	0.0173	0.0214	0.0521
Bismuth	<0.000004	<0.000004	0.00049	<0.000004	<0.000004	<0.000004	<0.000004	<0.000004	<0.000004	<0.000004	<0.000004	<0.000004	<0.000004	<0.000004	<0.000004
Thorium	0.00343	0.00165	0.00321	0.00033	0.00033	0.00479	0.00021	0.0007	0.00058	0.00058	0.001347	0.00048	0.00111	0.00075	0.002
Uranium	0.0054	0.00104	0.00472	0.00219	0.00044	0.003342	0,00014	0.00124	0.00187	0.00127	0.002198	0.00036	0.00142	0.00209	0.00317