

Universidad Mayor de San Simón

Studies on environmental impact and industrial potential of extremophiles

JEANETT DAGA-QUISBERT

Doctoral Thesis in Chemical Technologies

Cochabamba, Bolivia 2023

Studies on environmental impact and industrial potential of extremophiles

JEANETT DAGA-QUISBERT

With due permission of Universidad Mayor de San Simón, the academic Dissertation is submitted for public defense for the Degree of Doctor of Philosophy, Faculty of Science and Technology, Cochabamba.

Doctoral Thesis in Chemical Technologies
UMSS Universidad Mayor de San Simón
Cochabamba, Bolivia 2023

© Jeanett Daga-Quisbert
UMSS Universidad Mayor de San Simón
Faculty of Science and Technology
Center of Biotechnology
Cochabamba, Bolivia 2023

Abstract

Bolivia has many different territories, ranging from salt flats in the highlands to rainforests in the Amazon. These regions encompass abundant water sources. However, they are susceptible to contamination due to industrialization, urbanization, population growth, and climate change. There needs to be more research on the pollution of the Bolivian water resources. Before our studies, only a few molecular analyses based on next-generation sequencing were performed in a salt flat in Bolivia. Microbiome studies provide powerful tools for assessing water sources, providing valuable insights into water quality and its implications for ecological and human health. In this regard, we hypothesized that analyzing the microbiomes and physicochemical characteristics of Bolivian water bodies should lead to a better understanding of their potential chemical and biological contamination. This thesis aimed to comprehensively assess the Bolivian Lake Pastos Grandes in the highlands, Lake Alalay in an urban region, and two Bolivian Amazon rivers, the Sapati and Beni Rivers.

The microbiome of Lake Pastos Grandes did not show a significant influence from anthropogenic actions. It revealed that Burkholderiales and Pseudomonadales dominate river inflows and water ponds, while Halobacteriales and Cyanobacteria can be found in large abundances in the salt flat. Crustacea and Diatomea were also prevalent in the water. Diatom populations previously described in the 70s are still detected in the metagenomes and microscopic observations. In contrast, the Sapati and Beni Rivers microbiomes revealed anaerobic prokaryotes and bacteria typically linked to wastewater, human feces, or intestinal tracts. Additionally, potential pathogens such as *Arcobacter butzleri* and *Acinetobacter baumannii* were detected. Similar sources of contamination were found in Lake Alalay, although Proteobacteria and Cyanobacteria are the main phyla in its saline and alkaline water. Indeed, many alkali and halotolerant genera were distinguished in the lake. Coagulation treatment of Lake Alalay was investigated and effectively reduced the abundance of Cyanobacteria, but the quantity of Proteobacteria and Bacteroidetes increased.

Antimicrobial-resistant genes and bacteria are a severe threat to people and the environment. Therefore, their assessment is prioritized in many countries. The studies conducted in this thesis provide knowledge of such hazards in the Bolivian lakes and rivers. We found that the highest abundances in Lake Pastos Grandes and the Amazon Rivers were related to rifampicin-resistant genes and efflux pumps that may be involved in multi-drug resistance. The high UV radiation in the region likely influences the amount of the former genes in Lake Pastos Grandes. Efflux pumps can be found in metagenomes – not considered a risk – and bacterial pathogens; therefore, their danger to public health depends on their expression in their hosts. Moreover, multidrug-resistant bacteria, such as *Vibrio* and *Acinetobacter*, were isolated from Lake Alalay before and after coagulant treatment. These findings underscore the critical importance of ongoing monitoring and the implementation of effective strategies to deal with biological and chemical contamination in water sources. Furthermore, this research highlights the urgent necessity for improved wastewater sanitation practices and implementing a system for suitable environmental management in Bolivia.

Additionally, this work focused on improving culture conditions for extremophilic microorganisms with biotechnological potential, such as *Geobacillus* sp. LC300 and *Halomonas boliviensis*. The thermophilic bacteria *Geobacillus* sp. LC300 achieved exceptional growth rates on various carbon sources, making it a promising candidate for biorefinery applications. *Halomonas boliviensis* was successfully employed in ectoine production in an air-lift reactor. These findings enhance our understanding of extremophiles and suggest good opportunities for improving biorefinery processes and sustainable biotechnological applications.

Keywords

Lake Alalay, alkaline hypereutrophic lake, urban lake, bioprocess, air-lift reactor, Bolivian Andean region, ectoine, environmental metagenomics, *Geobacillus* sp. LC300, *Halomonas boliviensis*, high-altitude lakes, Lake Pastos Grandes, salt flat.

Resumen

Bolivia cuenta con diversos territorios, que varían desde salares en la región Andina hasta selvas en la Amazonia. Estas regiones tienen fuentes de agua abundantes. Sin embargo, son susceptibles a la contaminación debido a la industrialización, urbanización, el crecimiento poblacional y el cambio climático. Se requiere más investigación sobre la contaminación de los recursos hídricos en Bolivia. Antes de nuestros estudios, sólo se realizaron algunos análisis moleculares basados en secuenciación de nueva generación en un salar en Bolivia. Los estudios del microbioma ofrecen herramientas especializadas para evaluar las fuentes de agua, brindando valiosas perspectivas sobre la calidad del agua y sus implicaciones para la salud ecológica y humana. En este sentido, planteamos la hipótesis de que analizar los microbiomas y las características fisicoquímicas de los cuerpos de agua de Bolivia debería conducir a una mejor comprensión de su posible contaminación química y biológica. Esta tesis tuvo como objetivo evaluar de manera integral el lago Pastos Grandes en la región Andina, la laguna Alalay en una región urbana y dos ríos bolivianos en la Amazonía, esto es los ríos Sapati y Beni.

El microbioma del lago Pastos Grandes no mostró una influencia significativa de las acciones humanas. Reveló que Burkholderiales y Pseudomonadales dominan los afluentes de los ríos y depósitos de agua en la parte norte, mientras que las Halobacteriales y las cianobacterias se encuentran en grandes cantidades en el salar. Los crustáceos y las diatomeas también eran prevalentes en el agua. Las poblaciones de diatomeas descritas previamente en los años 70 todavía se detectan en los metagenomas y observaciones microscópicas. En contraste, los microbiomas de los ríos Beni y Sapati revelaron procariontes anaeróbicos y bacterias típicamente asociadas a aguas residuales, heces humanas o tractos intestinales. Además, se detectaron posibles patógenos como *Arcobacter butzleri* y *Acinetobacter baumannii*. Fuentes similares de contaminación se encontraron en la laguna Alalay, aunque las Proteobacterias y las Cianobacterias son los principales filos en sus aguas salinas y alcalinas. En efecto, se distinguieron muchos géneros alcalinos y halotolerantes en el lago. Se investigó el tratamiento de coagulación de la Laguna Alalay y redujo eficazmente la abundancia de cianobacterias, pero aumentó la cantidad de Proteobacterias y Bacteroidetes.

Los genes y bacterias resistentes a los antimicrobianos son una seria amenaza para las personas y el medio ambiente. Por lo tanto, su evaluación es una prioridad en muchos países. Los estudios realizados en esta tesis proporcionan conocimiento sobre tales riesgos en los lagos y ríos de Bolivia. Encontramos

que las mayores abundancias en el lago Pastos Grandes y los ríos de la Amazonia estaban relacionadas con genes resistentes a la rifampicina y bombas de eflujo que pueden estar involucradas en la resistencia a múltiples medicamentos. La alta radiación UV en la región probablemente influye en la cantidad de los genes mencionados en el lago Pastos Grandes. Las bombas de eflujo se pueden encontrar en metagenomas – no son consideradas un riesgo – y patógenos bacterianos; por lo tanto, su peligro para la salud pública dependerá de su expresión en sus hospedadores. Además, se aislaron bacterias resistentes a múltiples medicamentos, como *Vibrio* y *Acinetobacter*, de la laguna Alalay antes y después del tratamiento de coagulación. Estos hallazgos denotan la importancia crítica del monitoreo continuo y la implementación de estrategias efectivas para abordar la contaminación biológica y química en las fuentes de agua. Además, esta investigación resalta la necesidad urgente de mejorar las prácticas de saneamiento de aguas residuales y la implementación de un sistema de gestión ambiental adecuado en Bolivia.

Además, este trabajo se centró en mejorar las condiciones de cultivo de microorganismos extremófilos con potencial biotecnológico, como *Geobacillus* sp. LC300 y *Halomonas boliviensis*. La bacteria termófila *Geobacillus* sp. LC300 logró tasas de crecimiento excepcionales en diversas fuentes de carbono, lo que lo convierte en un candidato prometedor para aplicaciones en biorefinerías. *Halomonas boliviensis* se empleó con éxito en la producción de ectoína en un reactor “air-lift”. Estos hallazgos mejoran nuestra comprensión de los extremófilos y sugieren buenas oportunidades para mejorar los procesos de biorefinería y aplicaciones biotecnológicas sostenibles.

Palabras clave

Laguna Alalay, laguna hipertrófica y alcalina, laguna urbana, bioprocesos, reactor air-lift, región Andina Boliviana, ectoína, metagenómica ambiental, *Geobacillus* sp. LC300, *Halomonas boliviensis*, lagunas de altitud, laguna Pastos Grandes, salina.

Public defense dissertation

This thesis will be defended on Monday, the 11th of December 2023, at 9:00, Faculty of Sciences and Technology, Calle Sucre y Parque La Torre, Cochabamba.

Respondent:

Jeanett Daga Quisbert,
Center of Biotechnology, Faculty of Science and Technology
Universidad Mayor de San Simón, Bolivia

Faculty opponent:

Ph.D Senior Scientist Tania Pozzo
Ginkgo Bioworks
Boston USA

Evaluation committee:

Professor Ph.D Melina Campero
Unidad de Limnología y Recursos Acuáticos
Universidad Mayor de San Simón, Bolivia

Professor Ph.D María Teresa Arminda Álvarez Aliaga
Instituto de Investigaciones Fármaco Bioquímicas
Universidad Mayor de San Andrés, Bolivia

Professor Ph.D Luis Alejandro Romero Soto
Instituto de Investigación y Desarrollo de procesos Químicos
Universidad Mayor de San Andrés, Bolivia

Respondent's main supervisor:

Professor Ph.D. Jorge Quillaguamán Leytón
Center of Biotechnology,
Faculty of Science and Technology,
Universidad Mayor de San Simón, Cochabamba, Bolivia.

Respondent's co-supervisors:

Professor Ph.D. Antonius Van Maris
Department of Industrial Biotechnology School of Engineering Sciences in Chemistry, Biotechnology and Health
KTH
Royal Institute of Technology, Sweden

Professor Ph.D. Gunaratna Kuttuva Rajarao

Department of Industrial Biotechnology School of Engineering Sciences in Chemistry, Biotechnology and Health

KTH

Royal Institute of Technology, Sweden

List of publications and manuscripts

Paper I

Daga-Quisbert J, Kuttuva RG, Ugarte F, van Maris AJA, Quillaguamán J. Analysis of the microbiome of the Bolivian high-altitude Lake Pastos Grandes. *FEMS Microbiology Ecology*, 2023, <https://doi.org/10.1093/femsec/fiad073>

Paper II

Daga-Quisbert J, Kuttuva RG, van Maris AJA, Romero-Jaldín AM, Mercado-Guzman A, Fernandez CE, Quillaguamán J. Investigating the impact of coagulation treatment on water quality and the microbiome of a hypereutrophic alkaline urban lake. (*Manuscript*)

Paper III

Quillaguamán J, **Daga-Quisbert J**, Cristhian Carrasco, Shawn M. Higdon. Assessing the environmental pollution of two Amazon Bolivian rivers using metagenomic analysis. (*Submitted for publication*)

Paper IV

Ljungqvist E, **Daga-Quisbert J**, van Maris AJA, Gustavsson M. Insights into the rapid metabolism of *Geobacillus* sp. LC300: Unravelling metabolic requirements and optimal growth conditions. *Extremophiles*, 2023. (*Accepted for publication*)

Paper V

Daga-Quisbert J, Kuttuva RG, van Maris AJA, Quillaguamán J. Strategies of ectoine production by *Halomonas boliviensis* using an air-lift reactor. (*Manuscript in preparation*)

Author contributions

Paper I

First author. Performed most experiments (sampling, analysis of physicochemical parameters, and analysis of metagenomes), wrote and revised the manuscript with the co-authors.

Paper II

First author. Performed the sampling, analysis of physicochemical characteristics, and analysis of metagenomes, statistical analysis, and cultivations. Wrote and revised the manuscript with the co-authors.

Paper III

Second author. Performed the laboratory analysis (isolation of DNA, Analysis of metagenomes, physicochemical analysis) and revised the manuscript with the co-authors.

Paper IV

Second author. Contributed to the design of experiments, performed experimental work (screening of vitamins, testing of carbon sources, experiments in bioreactor), and revised the manuscript with the co-authors.

Paper V

First author. Designed and performed experiments (ectoine production in air-lift reactor, quantification of parameters), wrote and revised the manuscript together with the co-authors

TABLE OF CONTENTS

ABSTRACT	IV
RESUMEN	VI
PUBLIC DEFENCE DISSERTATION	VIII
LIST OF PUBLICATIONS AND MANUSCRIPTS	X
AUTHOR CONTRIBUTIONS	XI
1. INTRODUCTION	1
1.1. WATER SOURCES AND SCARCITY IN THE WORLD	1
1.2. WATER POLLUTANTS	2
1.2.1. Inorganic contaminants	3
1.2.3. Organic Pollutants	3
1.2.4. Radioactive pollution	3
1.2.5. Biological contaminants	4
1.3. EMERGING POLLUTANTS IN WATER BODIES	4
1.3.1. Antibiotic and antimicrobial resistance (AMR)	4
1.4. MECHANISM OF ANTIBIOTIC RESISTANCE	5
1.4.1. Enzymatic modifications and degradation of the antibiotics	6
1.4.2. Modification of the target site	6
1.4.3. Decrease drug uptake	6
1.4.4. Bacterial efflux systems	7
1.5. SURVEILLANCE OF ANTIMICROBIAL RESISTANCE	7
1.6. TREATMENT OF WATER SOURCES	8
1.6.1 Freshwater water treatment	9
1.6.2 Wastewater treatment	9
1.6.2.1. Primary treatment	9
1.6.2.2. Secondary treatment or biological process	10
1.6.2.3. Tertiary treatment	10
1.7. METHODOLOGIES FOR METAGENOMIC STUDIES	11
1.7.1. 16S rRNA gene amplicon	11
1.7.2. Shotgun metagenomics	12
2. WATER SOURCES IN BOLIVIA	13
2.1. HIGH ALTITUDE SALT FLATS, HYPERSALINE ECOSYSTEMS	13
2.2. URBAN LAKES	14
2.2.1. Lake Alalay	15
2.3. THE AMAZON RIVER BASIN	15
2.3.1. The Beni River	15
2.4. EXTREMOPHILIC MICROORGANISMS	16
3. PRESENT INVESTIGATION	17
3.1. AIM OF THIS THESIS	17
3.2. BIODIVERSITY AND ANTIMICROBIAL RESISTANCE IN AQUATIC ENVIRONMENTS	18

3.2.1. Salt flats microbiome (Paper I)	18
3.2.2. The microbiome of Amazon Rivers in Bolivia (Paper III)	20
3.3. MICROBIOME OF THE POLLUTED LAKE (PAPER II)	21
3.4. INDUSTRIAL POTENTIAL OF EXTREMOPHILES	25
3.4.1. <i>Geobacillus sp. LC300</i> (Paper IV)	25
3.4.2. <i>Halomonas boliviensis</i> (paper V)	26
4. CONCLUSIONS AND FUTURE OUTLOOKS.....	27
5. ACKNOWLEDGEMENTS	29
6. REFERENCES	31

1. INTRODUCTION

1.1. Water sources and scarcity in the world

Water represents one of the most crucial components of biological existence, which is fundamental in supporting biodiversity, human well-being, and economic development. Freshwater sources include lakes, reservoirs, ponds, rivers, streams, or groundwater [1], which are essential and used for agricultural, domestic, and industrial sectors. [2]. **Figure 1** shows the global water consumption, wherein 69% of the world's freshwater is used for agricultural purposes, with the remaining 23% and 8% going to industrial and domestic applications, respectively [3]. In Latin America, 68.7 % of the water is used for agriculture, 11.4 % for industry, and 20.5 % for municipal purposes [4]. It is important to highlight that Latin America is a global food producer, concentrating on generating several grains, fruits, and vegetables [5].

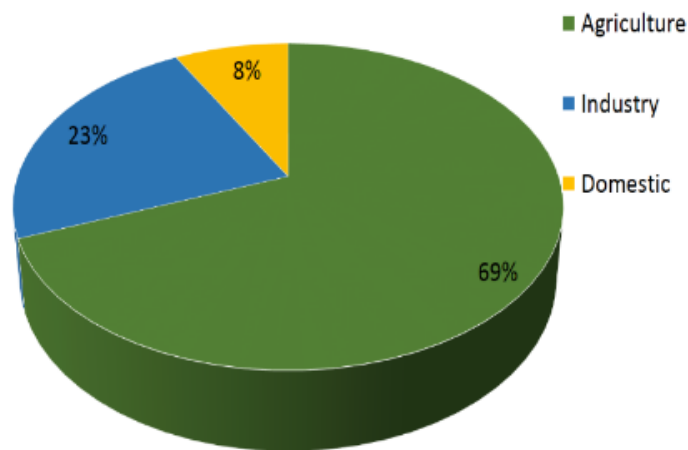


Figure 1: Global water consumption by use [3].

Projections indicate a significant increase in the demand for water by 2050 due to factors such as industrialization, urbanization, population growth, and climate change. This projection suggests that 87 out of 180 countries, particularly low-income countries, are expected to have water scarcity issues [2]. The increasing demand will likely lead to a decline in water quality and availability caused by the continuously discharged wastewater effluent, increasing the concentration of various organic and inorganic pollutants [6,7]. Countries in Latin America have a high renewable internal freshwater;

however, a decrease in the quantity per capita is expected in the coming years compared to other countries (**Figure 2**).

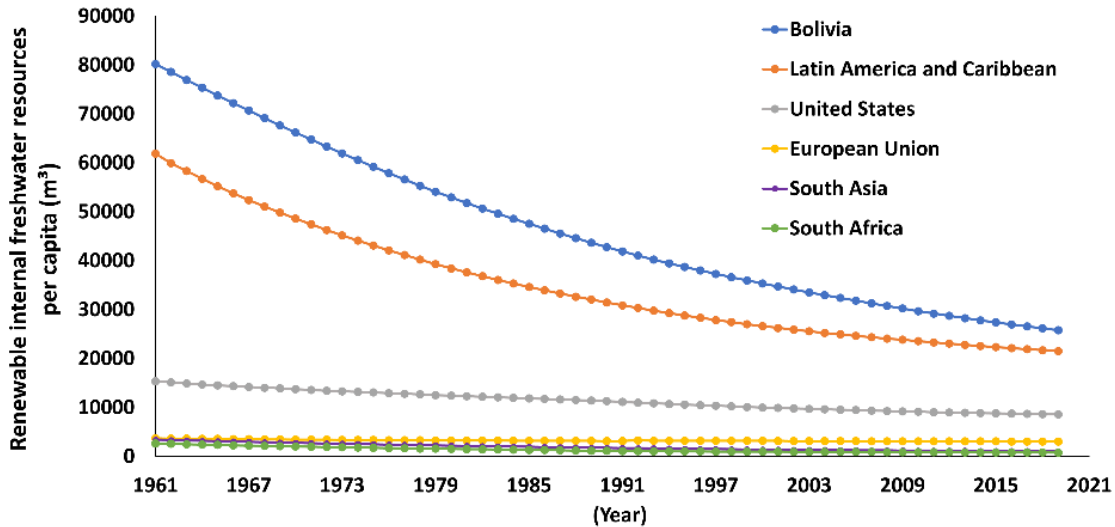


Figure 2. Renewable internal freshwater resources in Bolivia, compared with other continents. [Water Use and Stress - Our World in Data](#)

1.2. Water pollutants

Water contamination is a complex problem that depends on water bodies' characteristics and geographical location. Natural events such as floods and erosion can also contribute to water pollution. The main threats arise from industrial and anthropogenic activities, negatively affecting ecosystems, disrupting aquatic life, and disturbing delicate ecological balances. [3,8]. Currently, a significant portion of wastewater generated on a global scale remains untreated, leading to inevitable water contamination. Approximately 90% of all wastewater is discharged into nearby lakes and rivers without treatment [3,9]. In Bolivia, industrial, agricultural, and municipal wastewater is frequently released into rivers and lakes without adequate treatment. This unprocessed discharge introduces pollutants into these water sources, posing a significant environmental concern. These water pollutants can be inorganic, organic, radiological, or biological contaminants [9], detailed in the following sections.

1.2.1. Inorganic contaminants

Several inorganic compounds can pollute water. The most common are carbonates, metals [10], nitrogen, phosphorous, pharmaceuticals [11], personal care products [12], and pesticides [13]. Oil pollution from human activities, including crude oil, petroleum products, and their derivatives [3], also contributes to water contamination, damaging biodiversity and human health. On the other hand, consuming water contaminated with metals such as arsenic or chromium is associated with cancer risk and other diseases, particularly in children [14–17].

1.2.3. Organic Pollutants

Human activities generate domestic and industrial wastewater, a significant source of organic pollutants in water [18,19]. These organic pollutants comprise pesticides, fertilizers [3], wood, papers, resins, food items, pharmaceuticals, paints, dyes, aromatic compounds, and various biological components such as bacteria, viruses, and protozoa [20].

The significance of these organic pollutants is that they can cause health issues for humans, animals, and the ecosystem. Their accumulation in the environment can persist over a long time, causing health problems, including cancer, digestive problems, compromised immune function, congenital deformities, and disruptions in the reproductive system [20,21]. Managing and mitigating these organic pollutants is essential for preserving water sources.

1.2.4. Radioactive pollution

Water contamination with radioactive substances can originate from natural sources, including soil, sand, minerals, and rocks that come into contact with water [22]. In certain regions, radioactive gases emitted from molten magma can seep into natural water sources [23]. Furthermore, anthropogenic activities play a significant role in this issue. These activities include nuclear accidents, improper handling, and disposal of radioactive waste from nuclear facilities, industrial applications of radioisotopes, mining operations, and medical therapies, among other human-related factors [3,24].

Water is a primary carrier for these radioactive discharges, and their elevated concentrations contribute significantly to environmental pollution [23]. Moreover, ingesting radionuclides through drinking water poses substantial health risks to humans, including a broad spectrum of ailments such as

cancer, leukemia, genetic mutations, bone necrosis, cataracts, and chromosomal damage, among other severe diseases [25].

1.2.5. Biological contaminants

The presence of many different types of microorganisms in aquatic systems occurs naturally. These natural microbial communities are involved in the functioning of the ecosystem, playing a crucial role in maintaining and normalizing its functions, including purification and nutrient cycling [26,27].

Nevertheless, aquatic environments can become contaminated with pathogenic microorganisms, representing an environmental and public health concern [17]. The proliferation and excessive concentration of pathogens can hurt microbial diversity and ecosystem functions [28]. Water contamination by these microorganisms and its consumption contributes to an alarming statistic: approximately 1.4 million preventable childhood deaths occur yearly due to diarrheal diseases linked to unsafe drinking water, inadequate sanitation, and poor hand hygiene [29]. According to World Health Organization statistics, the most common pathogens are *Shigella dysenteriae*, *E. coli*, *Cryptosporidium*, *Giardia lamblia*, viruses such as hepatitis, and Norwalk-type viruses that pose significant public health threats [30]. Maintaining clean and safe drinking water sources helps us to reduce the risk of contracting diseases.

1.3. Emerging pollutants in water bodies

Emerging water pollutants are recognized as a worldwide concern. They are compounds not currently subject to regulatory water supply or wastewater systems monitoring. Still, they can potentially enter aquatic systems and cause adverse effects on human health and the environment [7,31]. Examples of emerging pollutants include pharmaceuticals, pesticides, detergents, flame retardants, and personal care products [5]. Notably, domestic wastewater is recognized as a significant source of these environmental pollutants. Consuming water or being in contact with such contaminants can exhibit mutagenic, teratogenic, and carcinogenic properties for humans and animals [3].

1.3.1. Antibiotic and antimicrobial resistance (AMR)

The discovery of antibiotics revolutionized the treatment of bacterial infections and drastically reduced mortality rates. These drugs have been instrumental in surgical procedures, cancer treatments, and the control of infectious diseases. Nevertheless, the overuse and misuse of antibiotics, some also regarded

as emerging contaminants, have accelerated the development of microorganisms containing antimicrobial resistance genes (AMRGs) [32]. The World Health Organization (WHO) has declared AMRGs one of the greatest threats to human health in the 21st century. If current trends persist, infections with AMR microorganisms could lead to 10 million deaths annually by 2050, surpassing cancer as a leading cause of mortality (**Figure 3**). Understanding the extent and dynamics of antibiotic contamination and AMR dissemination in aquatic ecosystems is essential to apply and improve wastewater treatments.

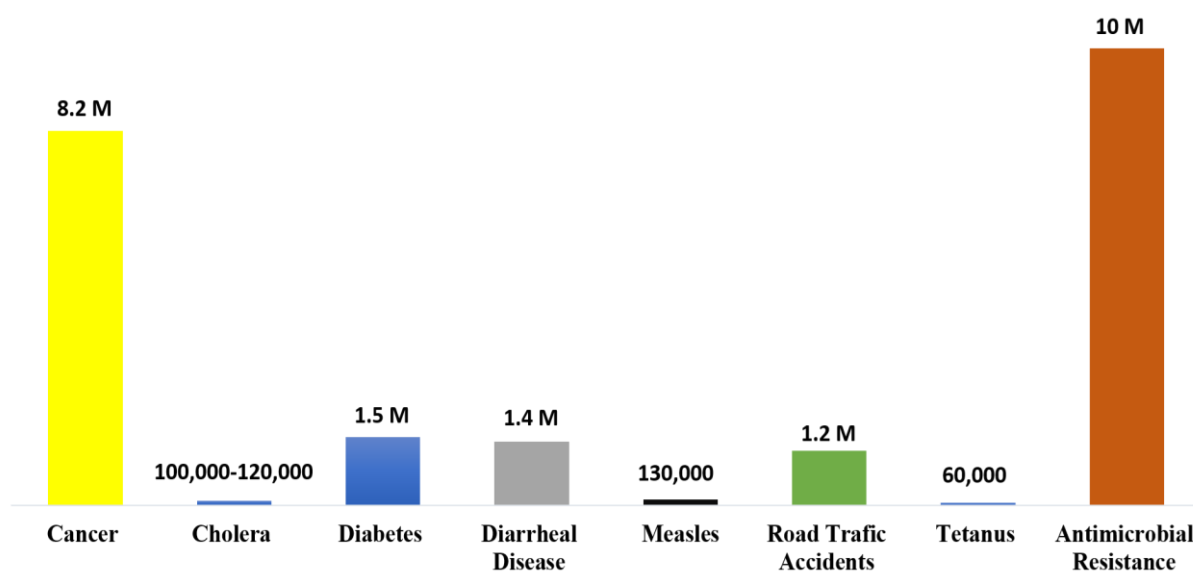


Figure 3. Projected annual deaths due to AMR in comparison to other significant causes of mortality by 2050 (Available on: <https://shorturl.at/mvAVX>)

1.4. Mechanism of antibiotic resistance

When bacteria lose their susceptibility to an antimicrobial agent, they are classified as resistant to that specific antimicrobial substance [33]. This antimicrobial resistance in bacterial species can arise either from their intrinsic ability or through the acquisition of resistance genes from other bacterial species that can grow at high drug levels [34]. The mechanisms bacteria use to become resistant to antibiotics are: 1) enzymatic modifications and degradation of the antibiotics; 2) modification of the target site; 3) decreased antibiotic uptake; and 4) bacterial efflux systems (**Figure 4**).

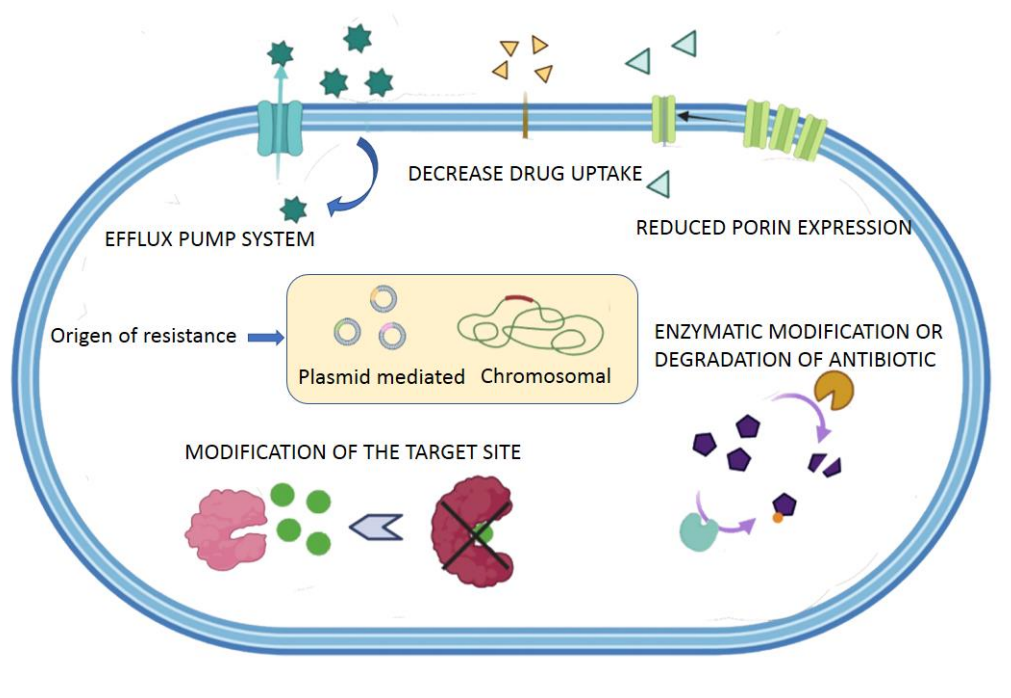


Figure 4. Mechanism of antibiotic resistance

1.4.1. Enzymatic modifications and degradation of the antibiotics

Antibiotics often contain chemically fragile bonds crucial for effectiveness [35]. The degradation or transformation process occurs when bacteria generate one or multiple enzymes that modify the drugs, making them inactive against bacteria [36].

1.4.2. Modification of the target site

Alteration in the target sites of antibiotics is a common mechanism of resistance. Target site changes often result from spontaneous mutation of a bacterial gene on the chromosome in the presence of an antibiotic, acquisition of resistance by mutations in RNA polymerase and DNA gyrase, or by the transfer of resistance genes from other organisms by some form of genetic exchange (conjugation, transduction, or transformation) [37].

1.4.3. Decrease drug uptake

The structure and functions of the lipopolysaccharides layer in Gram-negative bacteria provide a barrier for some types of molecules, limiting antimicrobial agent uptake. This protective feature is less common

in Gram-positive bacteria, although their wall structure naturally resists aminoglycoside antibiotics because polar molecules struggle to penetrate it [38].

1.4.4. Bacterial efflux systems

Transmembrane proteins called efflux pumps help bacteria survive by enabling them to expel antibiotic compounds from the cells [33]. The genes of efflux pumps are located in chromosomes or mobile genetic elements, such as plasmids [39]. Efflux pumps can transport a variety of structurally dissimilar molecules, such as antibiotics from various classes, or they may be specialized for one substrate; in either case, they can be linked to multiple drug resistance [39]. Reygaert (2018) classified efflux pumps into five groups based on their traits, structural properties, and energy sources. These families include the ATP-binding cassette (ABC) family, the major facilitator superfamily, the resistance-nodulation-cell division family, the multidrug and toxic compound extrusion family, and the small multidrug resistance family [38].

Antibiotic resistance in bacteria can be acquired through two distinct evolutionary pathways: vertical evolution, which originates within the bacterial genome due to spontaneous mutations, leading to increased resistance in both the bacterium and its offspring, and horizontal gene transfer, which involves the transfer of resistance genes from a resistant bacterium to a susceptible one [40]. In addition, some transposons can contain specialized regions called integrons that can include different resistant genes, making a bacterial species resistant to various antibiotics. Transposons can be easily transmitted between different bacteria [36].

1.5. Surveillance of antimicrobial resistance

Preventing the spread of antibiotic resistance and developing efficient avoidance and treatment plans for infectious diseases, antimicrobial resistance surveillance is essential for public health [41]. Continuous AMR monitoring and the creation of extensive databases are crucial to this surveillance effort because they help spot new threats and provide priceless information about changing resistance. This entails escalating, recognizing possible worldwide spread, and providing guidance for creating effective public health strategies. Furthermore, to address this urgent global health issue, such monitoring initiatives are crucial for supporting the development of new antimicrobial drugs, diagnostics, and alternative treatment modalities [42].

AMR has become a challenging specialty for clinicians, researchers, epidemiologists, public health agencies, policymakers, and governance [43]. For this reason, the AMR surveillance strategy has a central goal, which is to limit the public demand for unnecessary antibiotics usage in the therapeutic application of antimicrobial agents in farming and aquaculture, and other related sectors, due to the possible increment of AMR which bring consequences for the worldwide economy, especially for developing countries [44].

In 2015, WHO Member States approved the Global Antimicrobial Resistance and Use Surveillance System (GLASS), which “reports official national AMR data for selected bacteria that cause infections in humans: *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Neisseria gonorrhoeae*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, and *Streptococcus pneumoniae*.” Following this, most countries and international organizations have introduced national action plans to combat AMR. Still, Latin American countries such as Bolivia, Venezuela, and Ecuador have not adopted them [43].

The major pollutants in the primary water streams significantly impact the ecosystem and the aquatic environment. Surface water is a substantial source of drinking water, making its quality crucial for human consumption. Contaminants in surface water primarily result from sewage treatment or direct wastewater discharge into nearby water streams [45]. Therefore, it is essential to have an efficient sewage treatment process and controlled release of pollutants.

1.6. Treatment of water sources

Surface water present in rivers and lakes is a vulnerable freshwater resource that serves as accessible reservoirs for ensuring the availability of drinking water supply [46,47]. These water sources are indispensable in various sectors, including industry, housing, healthcare, and agriculture [48,49], leading to wastewater generation. Before using water for many purposes, subjecting it to appropriate treatment is essential. Furthermore, the generated wastewater should also undergo treatment before being discharged into the environment [50], as the inflowing water quality is crucial to maintaining the ecological balance in the ecosystem.

1.6.1. Freshwater water treatment

Freshwater sources, such as rivers and lakes, serve as available drinking water sources. Before using them, it is necessary to subject them to appropriate treatment, which depends on their initial characteristics. A typical sequence of treatment steps includes screening, coagulation-flocculation, sedimentation, filtration, and disinfection. However, depending on the water's characteristics, additional and more complex stages may be required in certain situations, such as pre-oxidation and adsorption [46].

1.6.2. Wastewater treatment

Biologically, physically, chemically, or combining the treatment steps can achieve conventional wastewater treatment technology. However, reducing emerging contaminants is challenging, and it is necessary to study the physical, chemical, and biological strategies to remove all impurities [51]. Several established sewage treatment processes utilize physical, chemical, and biological methods for water sanitation, as detailed in the following sections [52].

1.6.2.1. Primary treatment

In the case of municipal wastewaters, the primary treatment entails the physical separation process, typically beginning with screening, then grit removal, and concluding with sedimentation before commencing the secondary treatment [53]. The effectiveness of using chemical compounds during the primary treatment to remove pollutants depends on the type of water source, operating conditions, and the specific chemicals targeted [54]. Physicochemical separation through coagulation and flocculation is a highly efficient method for addressing emerging pollutants in wastewater, demonstrating its efficacy in removing contaminants from pharmaceutical wastewater [55].

Coagulation, a chemical process leading to the aggregation and settling of colloidal particles over time, is widely recognized as a highly effective pre-treatment method for removing contaminants in wastewater treatment [51]. Cationic coagulants like aluminum Al (III) and iron Fe (III) are commonly employed reagents promoting sedimentation. These coagulants induce the aggregation of unstable particles in water, resulting in the formation of flocs when the appropriate concentration is added [56]. These flocs can then be easily separated through sedimentation or filtration, enabling the removal of

substantial quantities of nitrogen and phosphorus from wastewater [57]. Previous studies have demonstrated the efficiency of aluminum sulfate in coagulation-flocculation processes, achieving an impressive 80% removal of pharmaceuticals such as hydrochlorothiazide, warfarin, and betaxolol [58]. Additionally, it has successfully removed over 78% of personal care products from hospital wastewater [59]. However, it is important to note that certain compounds cannot be effectively removed using metal salt coagulants such as aluminum and ferric sulfate [60]. The ability of coagulation to induce particle aggregation and facilitate the removal of impurities contributes significantly to making our water sources cleaner and safer. However, as water quality challenges continuously change, there is a need for continuous research and innovation in coagulation techniques.

1.6.2.2. Secondary treatment or biological process

The secondary treatment is characterized by biological remediation, which uses plants and microorganisms to eliminate toxic pollutants from water sources and the environment [61]. This method uses the metabolic activity of microorganisms to convert organic pollutants in wastewater into nonthreatening byproducts like CO₂ and water [62]. The biological techniques employed include activated sludge, membrane bioreactors, fixed film processes, composting, and phytoremediation [63]. These natural treatments can function aerobically or anaerobically, having the versatility to effectively remove a wide range of pollutants, including nitrogen, phosphorous, organic carbon, and emerging contaminants such as pharmaceutical compounds. Among them are antibiotics like tetracyclines [64], sulphanilamide [65], fluoroquinolones, β -lactams [66], and macrolides [67]. Biological treatments can emulate natural ecosystems, offering a friendly process that is easy to manage and operate [62]. Improving the water treatments is essential to reduce the release of nitrogen, phosphorous, and organic carbon in the effluent water and have the availability of clean water for human health and the preservation of ecosystems.

1.6.2.3. Tertiary treatment

Additional processes are implemented to diminish organic matter, nutrients, turbidity, nitrogen, phosphorous, heavy metals, bacteria, and viruses. This treatment aims to prepare wastewater for reuse and recycling, enabling its application in activities like irrigation. After purification, the treated water is reintegrated into reservoirs [68]. The assessment of physics and biological characteristics after water

treatment is crucial to determining the efficiency of the treatment process and its suitability for subsequent uses [68].

1.7. Methodologies for metagenomic studies

The microbial communities in an aquatic environment have an important role in the ecosystem health, biogeochemical cycling, and the maintenance of water quality. The ecological interactions between microorganisms, including competition, cooperation, and symbiosis, influence community dynamics and ecosystem stability [69]. The traditional microbiology cultures cover only a fraction of environmental microbial diversity. Also, some traditionally used molecular techniques are inefficient in describing microbiomes [70]. Consequently, metagenomic analysis has emerged as a powerful tool to describe microbial communities [71]. These technique has provided unprecedented insights into microbial populations' diversity, structure, and functional capabilities. The advance of next-generation sequencing (NGS) technologies and computational analysis has enabled a more profound and better resolution of taxonomic characterization, benefiting the disciplines of animal, plant, and environmental microbiome research and microbial interactions in the environment [72,73]. Moreover, the developments and improvements in these technologies enabled us to sequence genomes of species at a relatively low cost and a high speed [74]. Among the most employed metagenome sequencing are rRNA amplicon (16S for prokaryotes or 18S for eukaryotes) and shotgun sequencing of DNA or RNA [73].

1.7.1. 16S rRNA gene amplicon

The 16S rRNA amplicon analysis involves amplifying and sequencing the V3-V4, V5-V6, or V4 hypervariable regions of the 16S ribosomal RNA gene, a universally conserved gene found in all bacteria and archaea [75]. This technique enables identifying and classifying microorganisms based on the genetic signatures in these regions [73]. The DNA reads obtained from a particular environment are compared to those of known microbial species for taxonomic identification. In this respect, various databases containing the reference sequences have been established, among them the Ribosomal Database Project (RDP) [76], Green genes [77], and SILVA [78], which hold 16S rRNA gene information from millions of species. Also, some open-source web apps enable an automatic phylogenic and functional analysis of metagenomes, such as IMG/M, METAREP, CAMERA, and MG-RAST.

1.7.2. Shotgun metagenomics

Shotgun metagenomics provides information on the total metagenomic DNA directly extracted from samples. It can cover the entire microbial community, capturing known and unknown microbiomes if supplied with sufficient sequencing depth [72,73]. Metabolic capabilities of the microbial communities, such as the description of known or novel genes involved in the hydrolysis of polysaccharides or the nitrogen cycle, have been reported [79–81]. Other microbial functions, such as detecting and quantifying antibiotic-resistant genes or virulence factors, are being researched and included in surveillance strategies [82,83]. Furthermore, using shotgun sequencing is important to study communities of rivers and lakes for assessing biodiversity, detecting harmful or pathogen organisms, monitoring ecosystems, and supporting the conservation and restoration of freshwater environments.

2. WATER SOURCES IN BOLIVIA

According to the World Bank, Bolivia has one of the highest freshwater supplies, with 26 733 cubic meters of renewable internal per capita in 2018. Still, renewable internal freshwater resources per capita have decreased in the last few years. They are expected to continue reducing **Figure 2** (World Bank Data, 2018) due to the environmental degradation of the water resources [84]. Bolivia has three prominent hydrographic basins of critical importance: The Amazon, La Plata, and the Altiplano (**Figure 5**). These basins serve as primary water sources, essential in sustaining ecosystems, agriculture, and human populations across Bolivia [85]. However, they face exceptional challenges, including water quality, climate variability, and sustainable management issues.

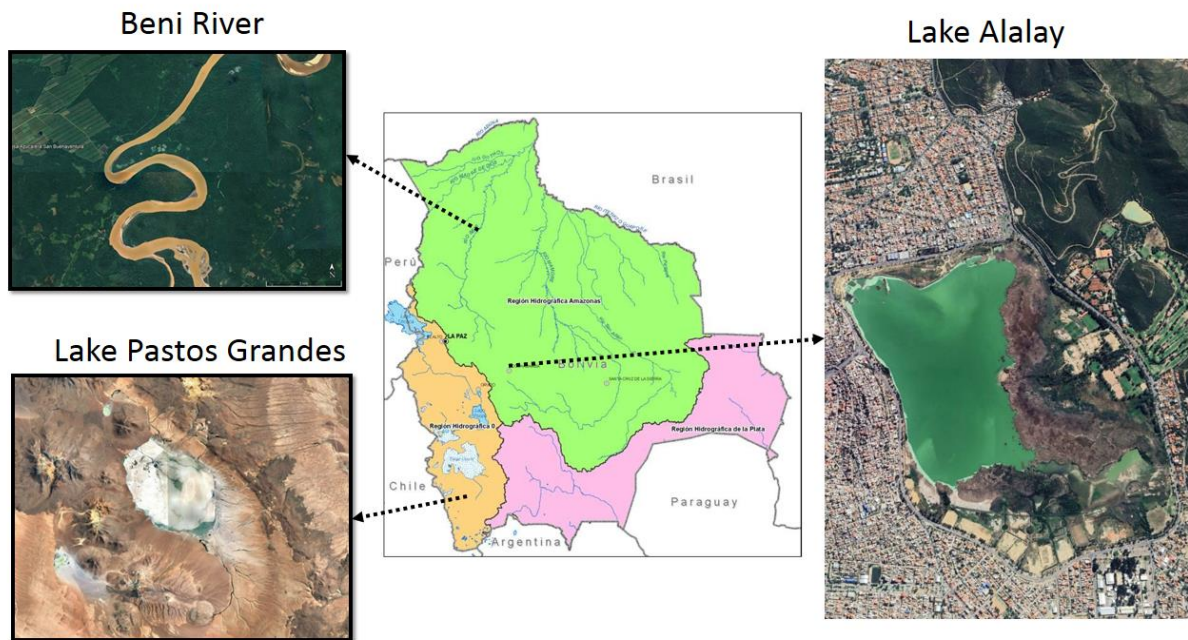


Figure 5. Location of water sources studied. The Sapati, Beni Rivers, and Lake Alalay are part of The Amazon basin. Lake Pastos Grandes belongs to the Altiplano basin.

2.1. High altitude salt flats, hypersaline ecosystems

The mountain chain of the Andes crosses several countries of South America [86,87]; it was formed by the subduction of the Nazca plate, which descends beneath the continental South America plate. Various active volcanoes can be found along with the mountains, making this place an active zone with sporadic

earthquakes [88–90]. This zone contains lakes at high altitudes (≥ 4000 m.a.s.l.) and athallasohaline salt flats [91,92], with extreme environmental conditions such as strong winds, extreme temperature fluctuations between day and night, dryness, low oxygen levels, broad variations in water temperature, and intense solar ultraviolet radiation (UV) levels [93].

The Bolivian Altiplano is a closed basin (200 000 km²) containing 35 salt flats; among the most prominent are Uyuni, Coipasa, and Empexa [94]. The Uyuni salt flat is one of the world's largest crusts (10 000 km²), a source of lithium, boron, and salt extraction. It also has cultural, economic, and environmental values [95]. Some saline lakes close to the salterns have also been investigated. A few novel species were obtained from the lakes and found to produce bioplastic and ectoine products with potential biotechnological applications [96–98].

In the Lipez region, Lake Pastos Grandes (**Figure 5**) is located at an altitude of 4440 m [87,99–101] and is characterized by a short water residence time compared with other Andean lakes, while most of the lake is composed of salt flats [102–104]. The lake is fed by rivers of fresh sweet water, springs water, and hydrothermal springs, with CO₂ degassing causing pH increase and carbonate precipitation; as a result, certain areas of the lake are characterized by elevated temperatures [99,103–107]. Recently, studies reported this lake has particular physicochemical, environmental, and biotic characteristics [102,105,107], which makes it an exciting study area.

2.2. Urban lakes

Urban lakes are a critical component of the global hydrological and biogeochemical cycle because they provide numerous essential services for humans and the ecosystem [108–111]. The lakes are considered one of the most vulnerable freshwater ecosystems in the world (Li et al., 2022; Steele & Heffernan, 2014) due to the continuous reduction in the lake area caused by the expansion of urbanization and anthropogenic activities, which generate eutrophication and pollution [112–116]. The high concentrations of phosphorus and nitrogen promote the eutrophication of the lakes. Therefore, controlling nutrients by reducing phosphorus and nitrogen inflow is critical for recovering such small polluted lakes [117–119].

2.2.1. Lake Alalay

Lake Alalay, located at 2560 meters above sea level in the heart of Cochabamba City, is the largest urban lake in Cochabamba (**Figure 5**). The lake receives wastewater from illegal sewage connections and small industries [120]. On the other hand, there is minimal outflow of both water and the nutrients it contains. Only during exceptional rainfall an overflow channel on the northwest side of the lake allows for water discharge [120]. Consequently, the lake serves as a nutrient repository, intensifying the potential for eutrophication and the proliferation of pathogens, microorganisms, and antimicrobial resistance genes [121].

2.3. The Amazon River basin

The Amazon River basin, originating from numerous rivers in the Andes Mountains, covers nearly 40 percent of the South American continent. It includes the Amazon River, which is the world's second-longest river (6290 kilometers), following the Nile (6670 kilometers) [122]. The Amazon basin has a high biological diversity [123] but, currently, is contaminated by anthropogenic activities, including overexploitation of fish population, degradation of natural habitats, deforestation, construction of hydropower dams, the introduction of invasive species, and various forms of pollution [124–126].

The biodiversity of the Amazon River extends beyond its fauna and flora; it also harbors novel genes with significant potential. For instance, some studies have identified novel genes responsible for encoding proteins involved in the lignin and hemicellulose degradation pathway, offering valuable insights into potential metabolic processes related to the degradation of terrestrial organic matter [127,128]. Moreover, studies have employed metagenomics to gain deeper insights into prokaryotic populations' biogeochemical cycles, strategies, and metabolic capabilities [129,130]. These studies contribute substantially to our understanding of this vital river ecosystem.

2.3.1. The Beni River

The Beni River has one of Bolivia's largest catchments at 282 500 km² [131,132]. It is located within the Amazon region and originates in the Andes (**Figure 5**). Beni River is a tributary of the Madeira River, the most important tributary of the Amazon basin [133]. The Beni River Basin is a reservoir of rich biodiversity [134]. It is a principal migration route and reproduction habitat of various native fish species [135]. However, the region faces significant environmental challenges, primarily driven by anthropogenic activities. Gold mining, an industry in the area, has contaminated some rivers with

mercury [136,137]. Furthermore, constructing the “El Bala” hydropower cascade system has led to erosion, potentially risking biodiversity [138]. Beni River is influenced by a small intermittent river called Sapati River, which is used for fishing. Unfortunately, the deforestation linked to sugar cane mills has adverse effects on the water quality and the impact on ecosystems [135]. In this regard, we saw it necessary to conduct metagenomic studies and their relation with chemical features to understand the sources of the contamination and their impact on the environment.

2.4. Extremophilic microorganisms

Extremophiles include primarily prokaryotes and some eukaryotes. They can be found in habitats characterized by extreme temperatures, pressure, salinity, or pH [139]. These microorganisms hold biotechnological potential thanks to their environmental adaptations. They can produce enzymes and metabolites with industrial applications, including enzymes for biofuel production, extremolytes for pharmaceuticals, and enzymes for bioremediation [140]. Due to the immense benefits of extremophiles to several industries and their market [141], it is essential to continue studying their metabolism and production strategies.

Halophilic microorganisms are studied by their capacity to produce many compounds using biotechnology. Ectoine is an osmolyte that protects the cells against heating, desiccation, UV radiation, and freezing [142,143]; this protective action on biological compounds highlights their potential in biotechnology, pharmacy, and agriculture [144]. *Halomonas boliviensis* is a natural ectoine and polyester producer [145]. It produces high ectoine concentrations in two steps: a fed-batch with high cell density and a high sodium chloride concentration to accumulate ectoine [146]. Nevertheless, other strategies like continuous cultivation have not been studied yet.

On the other hand, thermophilic microorganisms can grow and produce enzymes at high temperatures, being important as biorefinery cell factories due to their ability to produce thermostable enzymes [147]. *Geobacillus* sp. LC300 has grown fast on multiple carbon sources, showing a growth rate of 1.52 h⁻¹ on xylose and 2.15 h⁻¹ on glucose [148]. These extremely high growth rates and accompanying high sugar consumption rates make *Geobacillus* an exciting candidate for further development as a cell factory for converting different sugar sources into value-added products. Products that require free energy to produce would benefit from the high respiratory capacity of *Geobacillus* sp. LC300

3. PRESENT INVESTIGATION

3.1. Aim of this thesis

This thesis aimed to evaluate the microbial and functional diversity within various water sources in Bolivia by applying metagenomic sequencing techniques. Our study aimed to advance our understanding of these ecosystems and explore their potential for long-term conservation efforts, given their crucial role in sustainable aquatic environment management.

In the first section, our focus was on assessing microbial biodiversity, antimicrobial resistance genes, and the impact of physical and chemical characteristics on the biodiversity of Lake Pastos Grandes. This comprehensive analysis allowed us to understand the dominant microorganisms in a pristine lake ecosystem with little anthropogenic influence. It also identified biomarkers that lay the groundwork for future environmental research, providing a holistic perspective on the intricate natural equilibrium of Lake Pastos Grandes (**Paper I**).

Furthermore, we studied an urban, hypereutrophic, alkaline lake with increasing pollution levels over time. Our primary objective was to evaluate the effects of using poly-aluminum chloride (PAC) and iron chloride as coagulants to precipitate phosphate and suspended solids. We also investigated their influence on microbial communities within water samples and suspended solids from Lake Alalay. We utilized studies based on next-generation sequencing of the 16S rRNA amplicon (**Paper II**).

This thesis also investigated the Beni and Sapatí Rivers microbiome in the Bolivian Amazon region, employing shotgun sequencing to gain insights into the microbial communities and antimicrobial resistance genes in these ecosystems. Our findings unveiled instances of biological contamination and the presence of potential pathogens (**Paper III**).

In the second part of our study, we explored extremophilic microorganisms, aiming to understand their biotechnological potential. We examined the metabolism of the thermophilic bacterium *Geobacillus* sp. LC300 uses various carbon sources. We developed a defined culture medium to enhance growth, marking the first step toward optimizing its potential (**Paper IV**). We also researched *Halomonas boliviensis*, which produces bioplastic and ectoine using different bioprocesses, to optimize

ectoine production through three fermentation strategies at varying salt concentrations, employing an air-lift reactor (**Paper V**).

3.2. Biodiversity and antimicrobial resistance in aquatic environments

3.2.1. Salt flats microbiome (Paper I)

Environmental metagenomics involves analyzing DNA sequences extracted from natural environments, providing valuable insights into the evolutionary relationship among microbial communities inhabiting these environments [149,150]. Additionally, shotgun metagenomics enables functional metagenomic analyses to identify and quantify antimicrobial resistance genes (ARGs) in diverse settings and assess their associated risks [151,152]. While studies on the composition of halophilic microbial communities in Salar de Uyuni have been reported [153,154], the microbiome of Lake Pastos in the Bolivian Andes, a lake characterized by extreme environmental conditions, has not yet been studied (**Figure 5**).

Paper I aimed to conduct the first shotgun metagenomic analysis of Lake Pastos Grandes. In this study, we explored the microbial composition and its influence on the physico-chemical features of each sampling point. Then, antimicrobial resistance genes (ARGs) and genes associated with the nitrogen cycle were identified and quantified. The physicochemical characteristics of Lake Pastos Grandes provide information about the alkalinity conditions of water samples and the presence of various ions. Differences in ion concentrations, such as iron, lithium, magnesium, and potassium, were observed among different sampling points (**Table 1**).

Table 1. Physical and chemical characteristics of the water of Lake Pastos Grandes. The numbers in brackets show the standard deviation of the average values. (Table extracted from Paper I)

	Sampling point				
	P1	P2	P3	P4	P5
pH	7.88	7.8	7.74	8.05	7.97
Conductivity (mS/cm)	3.81	0.98	0.61	7.09	1.00
Phosphate (mg/L)	0.47 (± 0.04)	0.29 (± 0.01)	0.26 (± 0.02)	0.47 (± 0.01)	0.11 (± 0.00)
Sulfate (mg/L)	28 (± 0.1)	16.1 (± 0.4)	7.0 (± 0.5)	50 (± 2)	5.95 (± 0.64)
Chloride (mg/L)	1060 (± 85)	135 (± 35)	95 (± 35)	1450 (± 127)	120 (± 28)
Nitrate mg/L (mg/L)	3.4 (± 0.3)	21 (± 1)	< 2.5	16.9 (± 0.9)	16 (± 1)
Iron (mg/L)	< 0.1	< 0.1	< 0.1	0.12 (± 0.07)	< 0.1
Lithium (mg/L)	10.1 (± 0.7)	1.79 (± 0.05)	1.29 (± 0.02)	22.7 (± 0.8)	1.67 (± 0.02)
Magnesium (mg/L)	1.69 (± 0.06)	1.75 (± 0.02)	0.95 (± 0.05)	65 (± 2)	1.34 (± 0.04)
Potassium (mg/L)	76.2 (± 0.9)	17.2 (± 0.6)	9.6 (± 0.0)	208 (± 6)	12.2 (± 0.5)
Sodium (mg/L)	564 (± 13)	117 (± 15)	66 (± 4)	1149 (± 82)	112 (± 11)
Cadmium (ng/mL)	54 (± 3)	9.0 (± 0.2)	3.9 (± 0.3)	112 (± 4)	6.0 (± 0.3)
Lead (ng/ml)	75 (± 4)	14.1 (± 0.9)	8.6 (± 1.2)	237 (± 29)	11 (± 1)
Manganese (ng/ml)	19.3 (± 0.1)	3.5 (± 0.5)	2.6 (± 0.5)	71 (± 5)	2 (± 1)

The biodiversity revealed 13 eukaryotic groups and 27 bacterial orders, whose abundances significantly differed among the sampling points. Some of these microorganisms are shown in **Figure 6**.

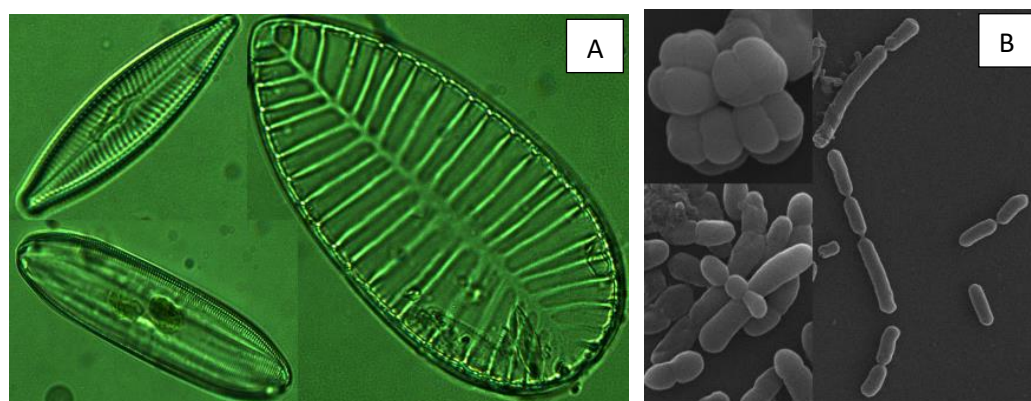


Figure 6. Microorganisms that were observed in Lake Pastos Grandes (Sampling point P4). The figures show **A)** diatoms and **B)** bacteria.

The functional analysis of Lake Pastos Grandes (**Paper I**) found 39 different antimicrobial resistance genes **Table 2**. The harm of these genes to living organisms will depend on the expression in their hosts [152,155].

Table 2. Antimicrobial resistance genes are found in Lake Pastos Grandes and Amazonas Bolivian rivers.

ARGs found in Lake Pastos Grandes		
<i>amrB, ugd, OprN, novA, ceoB, otr(A), arnA, smeB, smeE, TriC, OpmH, MexB, MexD, MexE, MexF, mexI, mexJ, mexK, mexN, mexQ, mexY, mexW, CpxR, OpmB, emrE, OprM, MuxB, MuxC, QepA2, OprZ, PmpM, AxyY, rpoB2, rpoB, RanA, YajC, rsmA, mupA, mupB</i>		
Beni and Sapati Rivers		
ARGs	Gene cassettes	
<i>MuxB, rpoB, ugd, rpoB2, MuxC, MexB, AxyY, mexK, smeE, adeK, CpxR, OprM, MexA, bcr-1, adeJ, MexD, novA, MexF, mexY, PmpM, cmcB, mupB, arnA, mexI, otr(A), mexN, triE, smeB, ileS, mupA, mdtB, OpmB, amrB, mexW, acrB, ceoB, mexQ, mdtC</i>	<i>aacA4, aadA6, qnrVC1, aacA28, qacK, cmlA1, catB6, dfrA17, oxa20, smr2, arr2, dfrA16, dfrA21, fosE, linF, dfrA7, aadA1a, oxa2, aadA2, cmlA2, gim1, vim2, qacG</i>	

Moreover, the genes identified genes in the nitrogen cycle in the samples that were related to dissimilatory and assimilatory nitrate reduction, denitrification, and environmental nitrogen assimilation. The results offer valuable insights into the lake ecosystem and establish baseline data for future environmental studies.

3.2.2. The microbiome of Amazon Rivers in Bolivia (Paper III)

Studies on the Amazon River's microbial composition revealed noteworthy variations. While genes related to the degradation of organic matter were identified [156], there were also genes associated with the expression of biogeochemically significant processes in the carbon, nitrogen, and phosphorus cycles [157–159]. Metagenomic research on ARGs in the Amazon basin is limited, with no prior studies focused on the Amazon Bolivian Rivers. The objective of **Paper III** was to perform a comprehensive analysis of the rivers' microbiome using shotgun metagenomics. To achieve this, we collected samples from three distinct locations: two examples from the Sapati River and one piece from the Beni River, used as a comparative reference.

Our metagenomic investigations provided insights into the overall diversity of the samples, encompassing prokaryotic orders and eukaryotic groups. Through statistical analysis, we could identify organisms whose abundance levels exhibited significant differences between the Sapati River and the reference site, the Beni River. The Sapati River has a high abundance of archaea groups, Methanobacteriales, Methanosarcinales, and orders of microaerophilic or anaerobic microorganisms such as Bacteroidales, Clostridiales, Desulfobacterales, Methanobacteriales, and Desulfovibrionales. The bacterial species with the highest abundance were *Sulfurospirillum cavolei*, followed by *Arcobacter butzleri* and *Limmnohabitans* sp. Furthermore, the ARGs detected were related to efflux pumps, which can lead to multidrug resistance; these ARGs are listed in **Table 2**. Integrons carrying ARGs were also identified on the Sapati and Beni Rivers.

3.3. Microbiome of the polluted lake (Paper II)

Antibiotic resistance is a growing concern in environmental science, with water bodies like lakes being critical players in the spread of antibiotic-resistant bacteria (ARB). The dynamics of this phenomenon, influenced by various factors, including human activities and water quality, are of significant interest. Lakes and other water bodies are crucial reservoirs and transport routes for ARB within our environment [160]. Previous studies have highlighted the substantial impact of human activities, elevated nutrient levels, heavy metals' presence on pollution levels, and the prevalence of ARGs in lakes [161].

Lake Alalay is an alkaline hypereutrophic lake, an overly nutrient-rich lake housing sewage-related microorganisms, antibiotic-resistant, and virulence genes [121]. We aimed to explore the lake's chemical characteristics and how they affect the bacterial community. To achieve this, we conducted a series of experiments, both small and large-scale, to investigate the prolonged impact of introducing iron chloride and poly-aluminum chloride (PAC) coagulants into the lake's water. These experiments relied on the analysis of 16S rRNA amplicon sequences. The bacterial composition of Lake Alalay, before applying coagulants for treatment, displayed Proteobacteria as the dominant species at all sampling points, followed by Cyanobacteria. *Arthrospira* and *Roseobacter* were consistently found across all sampling points. Various genera were identified, including *Desulfobacter*, *Desulfomicrobium*, *Sulfurospirillum*, *Hydrogenophaga*, and *Flavobacterium* (**Paper II**). It's noteworthy that sampling point 2 has a low level of dissolved oxygen and orthophosphate (**Figure 7**); these features led to a significantly higher abundance of the Desulfobacterota species than other phyla.

This physicochemical characteristic revealed Lake Alalay is characterized as an alkaline and saline body of water with elevated levels of orthophosphate, sulfate, and ammonium (**Figure 7**). Furthermore, research on suspended solids on the lake exhibited distinct two groups of bacteria, one associated with sewage-related environments, including *Bacteroides*, *Enterococcus*, *Clostridium*, *Arcobacter*, *Acetobacterium*, *Lactococcus*, and *Lactobacillus*, and the other with alkaline and saline water bodies, encompassing bacterial genera such as *Arthrospira*, *Desulfomicrobium*, *Mongoliitalea*, *Haloimpatiens*, *Porphyrobacter*, *Sphaerochaeta*, and *Wandonia*.



Figure 7. Physical and chemical characteristics of sampling points in Lake Alalay. Data extracted from **Paper II**.

A series of experiments, both on a small and large scale, investigated the long-term effects of introducing coagulants, iron chloride, and PAC. These experiments were made using 16S rRNA amplicon sequencing. Moreover, antimicrobial resistance bacteria before and after the treatment of the lake's water (**Figure 8**).

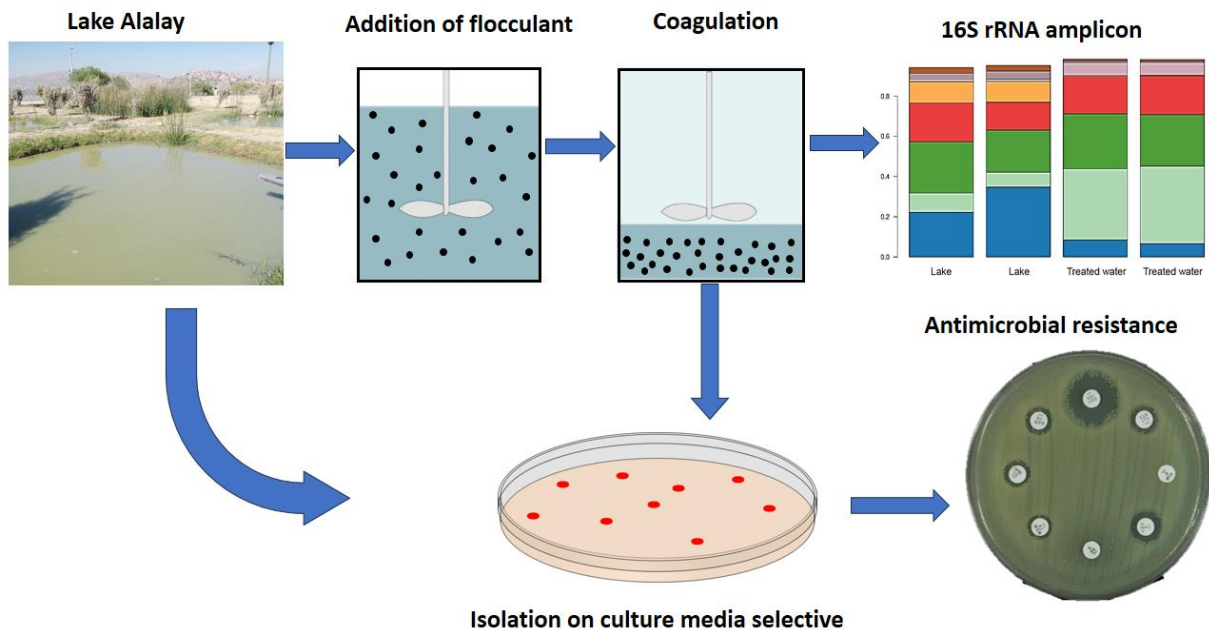


Figure 8. Experimental strategy used in **Paper II**. Biodiversity and antimicrobial resistance bacteria were observed in Lake Alalay before and after poly-aluminum and iron chloride treatment.

On a laboratory scale, we used 600 ml of water sample to use poly-aluminum chloride and iron chloride as coagulants to precipitate phosphate and suspended solids. The concentration of iron chloride (0.6 Kg/m^3) and PAC (0.3 Kg/m^3) used in the water samples resulted in semi-transparent water, and phosphate concentration was not detected; the abundance of Cyanobacteria was also reduced. However, Proteobacteria and Bacteroidota increased their relative abundance, and the presence of Firmicutes was noticed.

For a large-scale experiment, we used 320 liters of water sample from sampling point P4 (**Figure 7**). This water sample was treated with iron chloride (0.98 Kg/m^3) and PAC (0.49 Kg/m^3). In the lake, before treatment with coagulants, the Proteobacteria and Cyanobacteria were the species with the highest relative abundance and were followed by Verrucomicrobiota, Firmicutes, and Bacteroidota. Later, samples were taken after four weeks, and the suspended solids were precipitated with the coagulants. As observed in the laboratory scale experiments, the relative abundance of Cyanobacteria decreased notoriously, while the quantity of Proteobacteria and Bacteroidota increased. Furthermore, the beta diversity revealed that the overall bacterial communities significantly differed between the lake and its

treated water. The treated water was still alkaline, although its conductivity increased. Among metals in the water, sodium concentration decreased, and sulfate, potassium, magnesium, and iron concentrations increased. Additionally, the concentration of ammonium increased more than threefold. Finally, we isolated bacteria before and after treatment in selected media (**Figure 9**). We randomly separated two purple colonies on EMB medium, identified as *Acinetobacter* spp. Two red-colored colonies were isolated on Chromagar medium and were identified as *Vibrio* spp. All strains showed resistance to beta-lactam antibiotics (ampicillin, amoxicillin, and ceftriaxone), and only the *Acinetobacter* strains resisted the glycopeptide vancomycin.

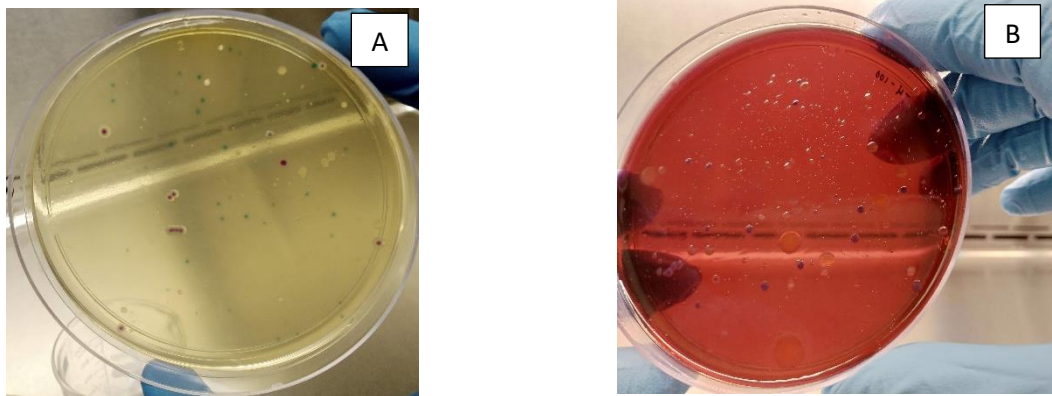


Figure 9. Bacterial colonies on **A)** Chromagar medium commonly used to obtain pathogens, and **B)** EMB medium to isolate Gram-negative bacteria.

In conclusion, physicochemical features play a significant role in influencing microbial biodiversity. Coagulation treatment can decrease the cyanobacteria population while increasing the abundance of Proteobacteria and Bacteroidota. Furthermore, antimicrobial-resistant bacteria were isolated before and after the coagulant treatment. These isolates demonstrated resistance to antibiotics. Multidrug-resistant bacteria carrying antimicrobial resistance genes pose a rapid and potentially significant risk to public health [162].

3.4. Industrial potential of extremophiles

3.4.1. *Geobacillus* sp. LC300 (Paper IV)

This section explores our study on *Geobacillus* sp. LC300. This thermophile exhibits remarkable adaptability and resilience, thus making it an ideal candidate for various biotechnological applications. The culture medium employed for the growth of *Geobacillus* sp. LC300 in previous studies contained ten different vitamins and yeast extract, constituting a non-defined culture medium [148,163–165]. This medium facilitated exceptionally high growth rates, especially when utilizing glucose (2.15 h^{-1}) and xylose (1.52 h^{-1}) as carbon sources[148]. Notably, the specific xylose utilization rate was three times higher than that of the best metabolically engineered organism [148]. Making it an intriguing focus for our research. In alignment with previous research, the primary objective of **Paper IV** was to formulate a well-defined culture medium optimized for the optimal growth of *Geobacillus* LC300. This involved several steps: (1) screening ten vitamins used in previous studies to determine their essentiality, coupled with adjustments in the concentrations of phosphorus, nitrogen, iron, and other minerals based on metabolic requirements predicted by the genome-scale model [163], (2) evaluating temperature and pH factors on growth rates via batch fermentation using a bioreactor, (3) examining fifteen distinct carbon sources, including monosaccharides, oligosaccharides, polysaccharides, and organic acids, using the optimized culture medium, allowing us to explore the diversity of carbon substrates these bacteria can metabolize.

Geobacillus sp. LC300 demonstrated its ability to grow optimally in a defined culture medium without requiring all ten vitamins. It exhibited auxotrophy for biotin and vitamin B12, achieving an impressive growth rate of 2.18 h^{-1} when glucose served as the carbon source. Remarkably, *Geobacillus* sp. LC300 exhibited robust growth rates when exposed to other saccharides, including glycerol (**Figure 10**). Furthermore, the optimal growth temperature for *Geobacillus* sp. LC300, maintained at a neutral pH of 6.6 - 6.9, was identified to range between 62-64 °C, representing a lower temperature range than previously reported [148]. These findings provide a foundational basis for further physiological investigations aimed at unraveling the metabolism of this thermophilic bacterium, with potential applications in biotechnology under extreme conditions in the future and as a potential host microorganism.

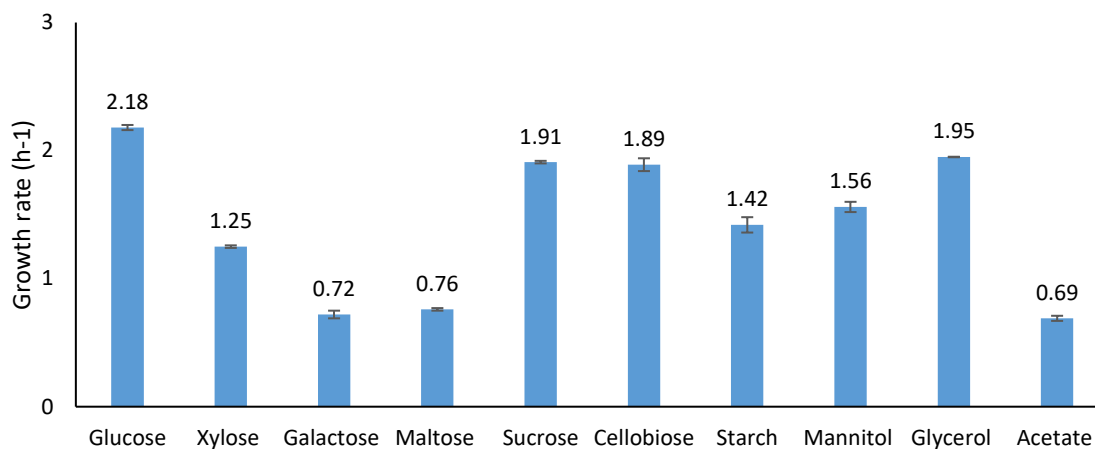


Figure 10. Growth rates on different carbon sources. Values are the mean of triplicate on shake flask cultivation. Glycerol data come from duplicates of bioreactor cultivation. Data collected from **Paper IV**.

3.4.2. *Halomonas boliviensis* (paper V)

Ectoine is a naturally occurring molecule produced by halophilic microorganisms in response to high salt concentrations. *Halomonas boliviensis* is a proficient ectoine producer, capable of synthesizing up to 6 g/L of ectoine with 18.5 % (w/v) NaCl salinity, using a combined two-step fed-batch culture and milking process [146]. In **Paper V**, three strategies for ectoine production in an air-lift reactor were assessed. The first involved a fed-batch approach with 5 % (w/v) NaCl to induce cell growth, followed by sodium chloride addition for ectoine production, yielding a maximum productivity of 0.79 g L⁻¹ d⁻¹ at 12.5 % (w/v) NaCl. A second fed-batch with replenishing culture media during growth was conducted using 12.5 % (w/v) NaCl, which improved intracellular ectoine productivity to 0.84 g L⁻¹ d⁻¹. Finally, a continuous culture strategy at $D=0.0514$ h⁻¹ produced high ectoine levels at 8.5 and 10.5 % (w/v) NaCl, achieving productivities of 2.92 g L⁻¹ d⁻¹ and 3.12 g L⁻¹ d⁻¹, respectively. These strategies at lower salt concentrations have potential benefits, including reduced reactor corrosion and lower production costs. Investigating alternative ectoine production methods proved crucial for enhancing efficiency, sustainability, and cost-effectiveness.

4. CONCLUSIONS AND FUTURE OUTLOOKS

This thesis has contributed to understanding Bolivia's microbiomes of diverse water sources using advanced sequencing techniques. The current microbiome composition and physicochemical characteristics of these water sources provide crucial insights into contamination levels and the presence of hazardous compounds. This knowledge is fundamental for effective water resource management, restoration efforts, and preserving ecosystem equilibrium, addressing critical concerns for public health.

In **Paper I** and **Paper III**, we employed shotgun sequencing techniques to explore the microbiomes of Lake Pastos Grandes and two Amazon Bolivian Rivers. Lake Pastos Grandes is a unique natural salt flat characterized by its extreme environmental conditions and the presence of various metal ions. Interestingly, our investigations did not reveal evidence of human contamination. ARGs found in the lake are not considered a hazard when found in metagenomes. Nevertheless, the expression of ARGs in pathogenic bacteria may pose a risk. The Amazon Bolivian Rivers (**Paper III**) that we studied contained bacterial species associated with wastewater, human feces, or intestinal tracts, which can potentially cause diseases with symptoms similar to those caused by various pathogens, including *Arcobacter butzleri*. Additionally, we detected antimicrobial resistance genes related to efflux pumps. Therefore, it is imperative to implement appropriate measures for wastewater treatment before its discharge into these rivers. The water treatment using iron chloride and PAC in Lake Alalay reduced the relative abundance of Cyanobacteria but increased the quantity of Proteobacteria and Bacteroidota (**Paper II**). Moreover, multi-drug-resistant bacteria were not removed from the water. Therefore, further research is needed to explore alternative treatment methods to eradicate pathogens effectively. Given the expected expansion of agriculture and industrialization in the coming years, monitoring antimicrobial resistance genes in water sources is critical to prevent their environmental spread and the emergence of multidrug-resistant pathogens. Moreover, examining ecological factors, including antibiotic concentrations, metal ion levels, the viability of pathogenic microorganisms, and physicochemical parameters within aquatic ecosystems, is vital.

This study implies that human activities around lakes and rivers have a significant impact, leading to water source contamination. Therefore, Bolivian authorities should implement appropriate treatments to mitigate pollution before discharging it into aquatic environments to prevent the proliferation of contaminants. Additionally, future studies should prioritize the isolation of new

microorganisms from halophilic environments, expanding our knowledge of extremophiles and their biotechnological potential. Such isolates could add to the portfolio of extremophile studies and thereby expand the industrial potential of extremophilic microorganisms (**Papers IV and V**).

5. ACKNOWLEDGEMENTS

I would like to begin by expressing my sincere gratitude to my supervisor, Professor Jorge Quillaguamán Leytón, who trusted me and allowed me to pursue this Ph.D. Thank you very much for your constant support, patience, and guidance throughout these years. I am deeply grateful for your mentorship, expertise, and invaluable assistance during my Ph.D. journey.

I thank my co-supervisor, Professor Ton van Maris, for engaging in scientific discussions, sharing knowledge, and supporting me during my time at KTH. I am also grateful to my co-supervisor, Gunaratna Rajarao, for her motivating words, unwavering support, and encouraging advice.

I thank the coordinators of the Chemical Technology program in Bolivia, Dr. Héctor Guzmán and Dr. Edwin Escalera, for their support and assistance with administrative tasks.

A heartfelt thank you to Emil Ljungqvist for your friendship, patience in teaching me to use lab equipment, and always being willing to help.

Martin Gustavsson, thank you for your patience during the metabolic engineering course and your kindness in discussions.

To Johan, thank you for your kindness and support with computer tasks during my time in Sweden.

I would like to thank all the individuals at the Centro de Investigación y Tecnología Aplicada (CITA) who helped me with equipment repairs at the Planta Piloto de Bioprocesos and for creating a pleasant work environment.

Special thanks to Marcelo Via, Ing. Marco Aranciabia, and colleagues from my home university for their unwavering support over the years.

To my friends Patty, Ana, Virginia, Danitza, and Daniela, thank you for your support.

To my friends in Sweden, Laura, Beatrice, Loris, Ha Nie, thank you for the joyful moments and unforgettable memories, even the attempts to learn not-so-polite words in different languages. I cherished every moment spent with you.

I would like to show my appreciation to Jeroen and Meeri for their kindness and friendship.

To my family, my parents Inés and Enrique, my sister Mery, and my brothers Rolando, Jhony, and Jorge, your unwavering support sustained me during the most challenging phases of this journey.

A big thank you to my Ph.D. colleagues: Jenny, Rocio, Jhony, Neysa, Nancy, Maria Ester.

6. REFERENCES

1. Schweitzer L, Noblet J (2018) Chapter 3.6 - Water contamination and pollution, In: Török B, Dransfield T (Eds.), *Green Chemistry*, Elsevier, 261–290.
2. Baggio G, Qadir M, Smakhtin V (2021) Freshwater availability status across countries for human and ecosystem needs. *Science of The Total Environment* 792: 148230.
3. Kuchangi SN, Mruthunjayappa MH, Sanna Kotrappanavar N (2023) Chapter 4 - An overview of water pollutants in present scenario, In: Pandey JK, Manna S, Patel RK, et al. (Eds.), *3D Printing Technology for Water Treatment Applications*, Elsevier, 83–105.
4. Rodríguez C, García B, Pinto C, et al. (2022) Water context in Latin America and the Caribbean: Distribution, regulations and prospects for water reuse and reclamation. *Water* 14.
5. Souza MCO, Rocha BA, Adeyemi JA, et al. (2022) Legacy and emerging pollutants in Latin America: A critical review of occurrence and levels in environmental and food samples. *Science of The Total Environment* 848: 157774.
6. Boretti A, Rosa L (2019) Reassessing the projections of the World Water Development Report. *npj Clean Water* 2: 15.
7. Saquib S, Yadav AK, Prajapati KB (2021) Chapter 19 - Emerging pollutants in water and human health, In: Ahamad A, Siddiqui SI, Singh P (Eds.), *Contamination of Water*, Academic Press, 285–299.
8. Schweitzer L, Noblet J (2018) Chapter 3.6 - Water Contamination and Pollution, In: Török B, Dransfield T (Eds.), *Green Chemistry*, Elsevier, 261–290.
9. Lamastra L, Suciú NA, Trevisan M (2018) Sewage sludge for sustainable agriculture: contaminants' contents and potential use as fertilizer. *Chemical and Biological Technologies in Agriculture* 5: 10.
10. Sharma S, Bhattacharya A (2017) Drinking water contamination and treatment techniques. *Applied Water Science* 7: 1043–1067.
11. Cizmas L, Sharma VK, Gray CM, et al. (2015) Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. *Environmental Chemistry Letters* 13: 381–394.
12. Kim E, Jung C, Han J, et al. (2016) Sorptive removal of selected emerging contaminants using biochar in aqueous solution. *Journal of Industrial and Engineering Chemistry* 36: 364–371.

13. Álvarez-Martín A, Rodríguez-Cruz MS, Andrades MS, et al. (2016) Application of a biosorbent to soil: a potential method for controlling water pollution by pesticides. *Environmental Science and Pollution Research* 23: 9192–9203.
14. Cantor KP (1997) Drinking Water and Cancer. *Cancer Causes & Control* 8: 292–308.
15. Gatseva PD, Argirova MD (2008) High-nitrate levels in drinking water may be a risk factor for thyroid dysfunction in children and pregnant women living in rural Bulgarian areas. *International Journal of Hygiene and Environmental Health* 211: 555–559.
16. Parvizishad M, Dalvand A, Mahvi A, et al. (2017) A Review of adverse effects and benefits of nitrate and nitrite in drinking water and food on human health. *Health Scope* In Press.
17. Lin L, Yang H, Xu X (2022) Effects of water pollution on human health and disease heterogeneity: A review. *Frontiers in Environmental Science* 10.
18. Karpińska J, Kotowska U (2019) Removal of organic pollution in the water environment. *Water* 11.
19. Wild SR, Jones KC (1991) Organic contaminants in wastewaters and sewage sludges: Transfer to the environment following disposal, In: Jones KC (Ed.), *Organic Contaminants in the Environment: Environmental Pathways & Effects*, Dordrecht, Springer Netherlands, 133–158.
20. Ojha A, Tiwary D (2021) Chapter 16 - Organic pollutants in water and its health risk assessment through consumption, In: Ahamad A, Siddiqui SI, Singh P (Eds.), *Contamination of Water*, Academic Press, 237–250.
21. Boudh S, Singh JS, Chaturvedi P (2019) Chapter 19 - Microbial resources mediated bioremediation of persistent organic pollutants, In: Singh JS (Ed.), *New and Future Developments in Microbial Biotechnology and Bioengineering*, Elsevier, 283–294.
22. He R, Liaw S, Zhou M, et al. (2022) Environmental evaluation of radioactivity levels and associated radiation hazards in groundwater around the WIPP site. *Ecotoxicology and Environmental Safety* 242: 113849.
23. Rajkhowa S, Sarma J, Rani Das A (2021) Chapter 15 - Radiological contaminants in water: pollution, health risk, and treatment, In: Ahamad A, Siddiqui SI, Singh P (Eds.), *Contamination of Water*, Academic Press, 217–236.
24. Hussain CM, Keçili R (2020) Chapter 1 - Environmental pollution and environmental analysis, In: Hussain CM, Keçili R (Eds.), *Modern Environmental Analysis Techniques for Pollutants*, Elsevier, 1–36.

25. Ogundare FO, Adekoya OI (2015) Gross alpha and beta radioactivity in surface soil and drinkable water around a steel processing facility. *Journal of Radiation Research and Applied Sciences* 8: 411–417.
26. Tyson GW, Chapman J, Hugenholtz P, et al. (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* 428: 37–43.
27. Shade A, Peter H, Allison S, et al. (2012) Fundamentals of microbial community resistance and resilience. *Frontiers in Microbiology* 3.
28. Gomes IB, Maillard J-Y, Simões LC, et al. (2020) Emerging contaminants affect the microbiome of water systems—strategies for their mitigation. *npj Clean Water* 3: 39.
29. World Health Organization (2015).
30. World Health Organization (2019).
31. Morin-Crini N, Lichtfouse E, Liu G, et al. (2022) Worldwide cases of water pollution by emerging contaminants: a review. *Environmental Chemistry Letters* 20: 2311–2338.
32. Koch N, Islam NF, Sonowal S, et al. (2021) Environmental antibiotics and resistance genes as emerging contaminants: Methods of detection and bioremediation. *Current Research in Microbial Sciences* 2: 100027.
33. Chawla M, Verma J, Gupta R, et al. (2022) Antibiotic potentiators against Multidrug-Resistant Bacteria: Discovery, development, and clinical relevance. *Frontiers in Microbiology* 13.
34. Jian Z, Zeng L, Xu T, et al. (2021) Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *Journal of Basic Microbiology* 61: 1049–1070.
35. Wright GD (2005) Bacterial resistance to antibiotics: Enzymatic degradation and modification. *Advanced Drug Delivery Reviews* 57: 1451–1470.
36. Alanis AJ (2005) Resistance to Antibiotics: Are We in the Post-Antibiotic Era? *Archives of Medical Research* 36: 697–705.
37. Lambert PA (2005) Bacterial resistance to antibiotics: Modified target sites. *Advanced Drug Delivery Reviews* 57: 1471–1485.
38. Reygaert WC (2018) An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology* 4: 482–501.
39. Webber MA, Piddock LJV (2003) The importance of efflux pumps in bacterial antibiotic resistance. *Journal of Antimicrobial Chemotherapy* 51: 9–11.
40. Laws M, Shaaban A, Rahman KM (2019) Antibiotic resistance breakers: current approaches and future directions. *FEMS Microbiology Reviews* 43: 490–516.

41. Kuzmenkov AY, Trushin IV, Vinogradova AG, et al. (2021) AMRmap: An interactive web platform for analysis of antimicrobial resistance surveillance data in Russia. *Frontiers in Microbiology* 12.
42. Fuhrmeister AS, Jones RN (2019) The importance of antimicrobial resistance monitoring worldwide and the origins of SENTRY antimicrobial surveillance program. *Open Forum Infectious Diseases* 6: S1–S4.
43. Bobate S, Mahalle S, Dafale NA, et al. (2023) Emergence of environmental antibiotic resistance: Mechanism, monitoring and management. *Environmental Advances* 13: 100409.
44. Iskandar K, Molinier L, Hallit S, et al. (2021) Surveillance of antimicrobial resistance in low- and middle-income countries: a scattered picture. *Antimicrobial Resistance & Infection Control* 10: 63.
45. Walker DB, Baumgartner DJ, Gerba CP, et al. (2019) Chapter 16 - Surface water pollution, In: Brusseau ML, Pepper IL, Gerba CP (Eds.), *Environmental and Pollution Science (Third Edition)*, Academic Press, 261–292.
46. Chiavola A, Di Marcantonio C, D'Agostini M, et al. (2023) A combined experimental-modeling approach for turbidity removal optimization in a coagulation–flocculation unit of a drinking water treatment plant. *Journal of Process Control* 130: 103068.
47. Cosgrove WJ, Loucks DP (2015) Water management: Current and future challenges and research directions. *Water Resources Research* 51: 4823–4839.
48. Chai WS, Cheun JY, Kumar PS, et al. (2021) A review on conventional and novel materials towards heavy metal adsorption in wastewater treatment application. *Journal of Cleaner Production* 296: 126589.
49. Shabir M, Yasin M, Hussain M, et al. (2022) A review on recent advances in the treatment of dye-polluted wastewater. *Journal of Industrial and Engineering Chemistry* 112: 1–19.
50. Nishat A, Yusuf M, Qadir A, et al. (2023) Wastewater treatment: A short assessment on available techniques. *Alexandria Engineering Journal* 76: 505–516.
51. Kumar R, Qureshi M, Vishwakarma DK, et al. (2022) A review on emerging water contaminants and the application of sustainable removal technologies. *Case Studies in Chemical and Environmental Engineering* 6: 100219.
52. Kesari KK, Soni R, Jamal QMS, et al. (2021) Wastewater treatment and reuse: a Review of its applications and health implications. *Water, Air, & Soil Pollution* 232: 208.

53. Englande Jr A, Krenkel P, Shamas J (2015) Wastewater treatment & water reclamation. *Reference module in earth systems and environmental sciences*.
54. Shanmuganathan R, Sibtain Kadri M, Mathimani T, et al. (2023) Recent innovations and challenges in the eradication of emerging contaminants from aquatic systems. *Chemosphere* 332: 138812.
55. Ahmed MB, Zhou JL, Ngo HH, et al. (2017) Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: A critical review. *Journal of Hazardous Materials* 323: 274–298.
56. El-taweel RM, Mohamed N, Alrefaey KA, et al. (2023) A review of coagulation explaining its definition, mechanism, coagulant types, and optimization models; RSM, and ANN. *Current Research in Green and Sustainable Chemistry* 6: 100358.
57. Ali TU, Kim D-J (2016) Phosphorus extraction and sludge dissolution by acid and alkali treatments of polyaluminum chloride (PAC) treated wastewater sludge. *Bioresource Technology* 217: 233–238.
58. Huerta-Fontela M, Galceran MT, Ventura F (2011) Occurrence and removal of pharmaceuticals and hormones through drinking water treatment. *Water Research* 45: 1432–1442.
59. Suarez S, Lema JM, Omil F (2009) Pre-treatment of hospital wastewater by coagulation–flocculation and flotation. *Bioresource Technology* 100: 2138–2146.
60. Adams C., Wang Y., Loftin K., et al. (2002) Removal of Antibiotics from Surface and Distilled Water in Conventional Water Treatment Processes. *Journal of Environmental Engineering* 128: 253–260.
61. González-González RB, Flores-Contreras EA, Parra-Saldívar R, et al. (2022) Bio-removal of emerging pollutants by advanced bioremediation techniques. *Environmental Research* 214: 113936.
62. Liu B, Zhang S, Chang C-C (2018) Emerging Pollutants - Part II: Treatment. *Water Environment Research* 90: 1792–1820.
63. Kumar R, Qureshi M, Vishwakarma DK, et al. (2022) A review on emerging water contaminants and the application of sustainable removal technologies. *Case Studies in Chemical and Environmental Engineering* 6: 100219.
64. Paulus GK, Hornstra LM, Alygizakis N, et al. (2019) The impact of on-site hospital wastewater treatment on the downstream communal wastewater system in terms of antibiotics and antibiotic resistance genes. *International Journal of Hygiene and Environmental Health* 222: 635–644.

-
65. He Y, Nurul S, Schmitt H, et al. (2018) Evaluation of attenuation of pharmaceuticals, toxic potency, and antibiotic resistance genes in constructed wetlands treating wastewater effluents. *Science of The Total Environment* 631–632: 1572–1581.
 66. You X, Wu D, Wei H, et al. (2018) Fluoroquinolones and β -lactam antibiotics and antibiotic resistance genes in autumn leachates of seven major municipal solid waste landfills in China. *Environment International* 113: 162–169.
 67. Pazda M, Kumirska J, Stepnowski P, et al. (2019) Antibiotic resistance genes identified in wastewater treatment plant systems – A review. *Science of The Total Environment* 697: 134023.
 68. Koul B, Yadav D, Singh S, et al. (2022) Insights into the Domestic Wastewater Treatment (DWWT) regimes: A review. *Water* 14.
 69. Hui C, Li Y, Zhang W, et al. (2022) Modelling structure and dynamics of microbial community in aquatic ecosystems: The importance of hydrodynamic processes. *Journal of Hydrology* 605: 127351.
 70. Desai C, Pathak H, Madamwar D (2010) Advances in molecular and “-omics” technologies to gauge microbial communities and bioremediation at xenobiotic/anthropogen contaminated sites. *Bioresource Technology* 101: 1558–1569.
 71. Zeglin LH (2015) Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Frontiers in Microbiology* 6.
 72. Li B, Yan T (2023) Chapter Two - Metagenomic next generation sequencing for studying antibiotic resistance genes in the environment, In: Makowski GS, Sariaslani S (Eds.), *Advances in Applied Microbiology*, Academic Press, 41–89.
 73. Mukherjee A, D’Ugo E, Giuseppetti R, et al. (2023) Chapter 5 - Metagenomic approaches for understanding microbial communities in contaminated environments: Bioinformatic tools, case studies and future outlook, In: Kumar V, Bilal M, Shahi SK, et al. (Eds.), *Metagenomics to Bioremediation*, Academic Press, 103–156.
 74. Pervez MT, Hasnain MJ ul, Abbas SH, et al. (2022) A comprehensive review of performance of Next-Generation Sequencing platforms. *BioMed Research International* 2022: 3457806.
 75. Woo PCY, Lau SKP, Teng JLL, et al. (2008) Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clinical Microbiology and Infection* 14: 908–934.
 76. Cole JR, Wang Q, Fish JA, et al. (2014) Ribosomal Database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Research* 42: D633–D642.

-
77. DeSantis T, Philip H, Larsen N, et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology - AEM* 72: 5069–5072.
 78. Quast C, Pruesse E, Yilmaz P, et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41: D590–D596.
 79. Bhosle A, Wang Y, Franzosa EA, et al. (2022) Progress and opportunities in microbial community metabolomics. *Current Opinion in Microbiology* 70: 102195.
 80. Datta S, Rajnish N, Samuel M, et al. (2020) Metagenomic applications in microbial diversity, bioremediation, pollution monitoring, enzyme and drug discovery. A review. *Environmental Chemistry Letters*.
 81. Mosley OE, Gios E, Close M, et al. (2022) Nitrogen cycling and microbial cooperation in the terrestrial subsurface. *The ISME Journal* 16: 2561–2573.
 82. Ko KKK, Chng KR, Nagarajan N (2022) Metagenomics-enabled microbial surveillance. *Nature Microbiology* 7: 486–496.
 83. Yang C, Zhao Y, Cao W, et al. (2022) Metagenomic analysis reveals antibiotic resistance genes and virulence factors in the saline-alkali soils from the Yellow River Delta, China. *Environmental Research* 214: 113823.
 84. Haider L, Boonstra W, Peterson G, et al. (2017) Traps and sustainable development in rural areas: A review. *World Development* 101.
 85. Ureña J, Saavedra O, Kubota T (2021) The development of a combined satellite-based precipitation dataset across Bolivia from 2000 to 2015. *Remote Sensing* 13.
 86. Espinoza JC, Garreaud R, Poveda G, et al. (2020) Hydroclimate of the Andes Part I: Main Climatic Features. *Frontiers in Earth Science* 8.
 87. Risacher F, Fritz B (1991) Geochemistry of Bolivian salars, Lipez, southern Altiplano: Origin of solutes and brine evolution. *Geochimica et Cosmochimica Acta* 55: 687–705.
 88. de Silva S (1990) Altiplano-Puna volcanic complex of the central Ande. *Geology* 17.
 89. de Silva SL, Self S, Francis PW, et al. (1994) Effusive silicic volcanism in the Central Andes: The Chao dacite and other young lavas of the Altiplano-Puna Volcanic Complex. *Journal of Geophysical Research: Solid Earth* 99: 17805–17825.
 90. Molina V, Dorador C, Fernández C, et al. (2018) The activity of nitrifying microorganisms in a high-altitude Andean wetland. *FEMS Microbiology Ecology* 94: fiy062.

91. Fornari M, Risacher F, Féraud G (2001) Dating of paleolakes in the central Altiplano of Bolivia. *Palaeogeography, Palaeoclimatology, Palaeoecology* 172: 269–282.
92. Risacher F, Fritz B (2008) Origin of salts and brine evolution of Bolivian and Chilean salars. *Aquatic Geochemistry* 15: 123–157.
93. Aguilera X, Lazzaro X, Coronel JS (2013) Tropical high-altitude Andean lakes located above the tree line attenuate UV-A radiation more strongly than typical temperate alpine lakes. *Photochem Photobiol Sci* 12: 1649–1657.
94. López Steinmetz RL, Salvi S (2021) Brine grades in Andean salars: When basin size matters A review of the Lithium Triangle. *Earth-Science Reviews* 217: 103615.
95. Saccò M, White NE, Harrod C, et al. (2021) Salt to conserve: a review on the ecology and preservation of hypersaline ecosystems. *Biological Reviews* 96: 2828–2850.
96. Guzmán D, Quillaguamán J, Muñoz M, et al. (2010) *Halomonas andesensis* sp. nov., a moderate halophile isolated from the saline lake Laguna Colorada in Bolivia. *International Journal of Systematic and Evolutionary Microbiology* 60: 749–753.
97. Quillaguamán J, Hatti-Kaul R, Mattiasson B, et al. (2004) *Halomonas boliviensis* sp. nov., an alkalitolerant, moderate halophile isolated from soil around a Bolivian hypersaline lake. *International Journal of Systematic and Evolutionary Microbiology* 54: 721–725.
98. Quillaguamán J, Delgado O, Mattiasson B, et al. (2004) *Chromohalobacter sarecensis* sp. nov., a psychrotolerant moderate halophile isolated from the saline Andean region of Bolivia. *International Journal of Systematic and Evolutionary Microbiology* 54: 1921–1926.
99. Ballivian O, Risacher F (1981) Los salares del Altiplano boliviano: metodos de estudio y estimacion economica.
100. Jones B, RENAUT R (2006) Crystal fabrics and microbiota in large pisoliths from Laguna Pastos Grandes, Bolivia. *Sedimentology* 41: 1171–1202.
101. Bougeault C, Vennin E, Durllet C, et al. (2019) Biotic–abiotic influences on modern Ca–Si-rich hydrothermal spring mounds of the Pastos Grandes Volcanic Caldera (Bolivia). *Minerals* 9.
102. Bougeault C, Christophe D, Vennin E, et al. (2020) Variability of Carbonate Isotope Signatures in a Hydrothermally Influenced System: Insights from the Pastos Grandes Caldera (Bolivia). *Minerals* 10.
103. Lizeca JL, Moon W, Hutton C, et al. (1999) Investigation of Pastos Grandes (Bolivia) volcanic features with RADARSAT. *IEEE 1999 International Geoscience and Remote Sensing*

- Symposium. IGARSS'99 (Cat. No.99CH36293)*, Hamburg, Germany, 1999, pp. 803-805 vol.2, doi: 10.1109/IGARSS.1999.774446.
104. Risacher F, Eugster HP (1979) Holocene pisoliths and encrustations associated with spring-fed surface pools, Pastos Grandes, Bolivia. *Sedimentology* 26: 253–270.
 105. Bougeault C, Vennin E, Durllet C, et al. (2019) Biotic–Abiotic Influences on Modern Ca–Si-Rich Hydrothermal Spring Mounds of the Pastos Grandes Volcanic Caldera (Bolivia). *Minerals*.
 106. de Silva S (1990) Altiplano-Puna volcanic complex of the central Ande. *Geology* 17.
 107. Muller E, Gaucher EC, Durllet C, et al. (2020) The origin of continental carbonates in Andean salars: A multi-tracer geochemical approach in Laguna Pastos Grandes (Bolivia). *Geochimica et Cosmochimica Acta* 279: 220–237.
 108. Jingying Z, Song C, Wang J, et al. (2020) China’s inland water dynamics: The significance of water body types. *Proceedings of the National Academy of Sciences* 117: 13876–13878.
 109. Mitroi V, Maleval V, Deroubaix J-F, et al. (2022) What urban lakes and ponds quality is about? Conciliating water quality and ecological indicators with users’ perceptions and expectations about urban lakes and ponds quality in urban areas. *Journal of Environmental Policy & Planning* 24: 1–18.
 110. Vilbaste S, Järvalt A, Kalpus K, et al. (2016) Ecosystem services of Lake Vörtsjärv under multiple stress: a case study. *Hydrobiologia* 780: 145–159.
 111. Reynaud A, Lanzanova D (2017) A global meta-analysis of the value of ecosystem services provided by Lakes. *Ecological Economics* 137: 184–194.
 112. Domínguez D, Chacón L, Wallace J (2021) Anthropogenic Activities and the Problem of Antibiotic Resistance in Latin America: A Water Issue. *Water* 13: 2693.
 113. Liu C, Zhong J, Wang J, et al. (2016) Fifteen-year study of environmental dredging effect on variation of nitrogen and phosphorus exchange across the sediment-water interface of an urban lake. *Environmental Pollution* 219: 639–648.
 114. Peng F, Guo Y, Isabwe A, et al. (2020) Urbanization drives riverine bacterial antibiotic resistome more than taxonomic community at watershed scale. *Environment International* 137: 105524.
 115. Spänig S, Eick L, Nuy J, et al. (2021) A multi-omics study on quantifying antimicrobial resistance in European freshwater lakes. *Environment International* 157: 106821.
 116. Zhu L, Li R, Yan Y, et al. (2022) Urbanization drives the succession of antibiotic resistome and microbiome in a river watershed. *Chemosphere* 301: 134707.

117. Betiku OC, Sarjeant KC, Ngatia LW, et al. (2021) Evaluation of microbial diversity of three recreational water bodies using 16S rRNA metagenomic approach. *Science of The Total Environment* 771: 144773.
118. Schindler D (2012) The dilemma of controlling cultural eutrophication of lake. *Proceedings Biological sciences / The Royal Society* 279: 4322–4333.
119. Lewis WMJr, Wurtsbaugh WA, Paerl HW (2011) Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of Inland waters. *Environ Sci Technol* 45: 10300–10305.
120. Ayala R, F A, Mooij W, et al. (2007) Management of Laguna Alalay: A case study of lake restoration in Andean valleys in Bolivia. *Aquatic Ecology* 41: 621–630.
121. Quillaguamán J, Guzmán D, Campero M, et al. (2021) The microbiome of a polluted urban lake harbors pathogens with diverse antimicrobial resistance and virulence genes. *Environmental Pollution* 273: 116488.
122. Sioli H (1984) Introduction: History of the discovery of the Amazon and of research of Amazonian waters and landscapes, In: Sioli H (Ed.), *The Amazon: Limnology and landscape ecology of a mighty tropical river and its basin*, Dordrecht, Springer Netherlands, 1–13.
123. Ghai, R. (2015). Rivers, Metagenomics of. In: Highlander, S.K., Rodriguez-Valera, F., White, B.A. (eds) *Encyclopedia of Metagenomics*. Springer, Boston, MA..
124. Castello L, McGrath DG, Hess LL, et al. (2013) The vulnerability of Amazon freshwater ecosystems. *Conservation Letters* 6: 217–229.
125. Castello L, Macedo MN (2016) Large-scale degradation of Amazonian freshwater ecosystems. *Global Change Biology* 22: 990–1007.
126. Hurd LE, Sousa RGC, Siqueira-Souza FK, et al. (2016) Amazon floodplain fish communities: Habitat connectivity and conservation in a rapidly deteriorating environment. *Biological Conservation* 195: 118–127.
127. Santos Júnior C, Sarmiento H, Miranda F, et al. (2020) Uncovering the genomic potential of the Amazon River microbiome to degrade rainforest organic matter. *Microbiome* 8, 151
128. Santos Júnior Célio Dias, Toyama Danyelle, de Oliveira Tereza Cristina Souza, et al. (2019) Flood season microbiota from the Amazon Basin Lakes: Analysis with metagenome sequencing. *Microbiology Resource Announcements* 8: 10.1128/mra.00229-19.
129. Satinsky BM, Zielinski BL, Doherty M, et al. (2014) The Amazon continuum dataset: quantitative metagenomic and metatranscriptomic inventories of the Amazon River plume, June 2010. *Microbiome* 2: 17.

130. Satinsky BM, Smith CB, Sharma S, et al. (2017) Patterns of bacterial and archaeal gene expression through the lower Amazon River. *Frontiers in Marine Science* 4.
131. Gautier E, Brunstein D, Vauchel P, et al. (2007) Temporal relations between meander deformation, water discharge and sediment fluxes in the floodplain of the Rio Beni (Bolivian Amazonia). *Earth Surface Processes and Landforms* 32: 230–248.
132. Rivera AR, Heredia MW (2023) Application of Delft3D computational model to estimate the influence of El Bala run-of-the-river dam on the morphological activity of Beni River, Bolivia. *IOP Conference Series: Earth and Environmental Science* 1136: 012030.
133. Molina-Carpio J, Espinoza JC, Vauchel P, et al. (2017) Hydroclimatology of the Upper Madeira River basin: spatio-temporal variability and trends. *Hydrological Sciences Journal* 62: 911–927.
134. Smith L, Magdalena C, Przelomska N, et al. (2022) Revised species delimitation in the Giant water Lily Genus *Victoria* (Nymphaeaceae) confirms a new species and has implications for its conservation. *Frontiers in Plant Science* 13: 883151.
135. Miranda-Chumacero G, Mariac C, Duponchelle F, et al. (2020) Threatened fish spawning area revealed by specific metabarcoding identification of eggs and larvae in the Beni River, upper Amazon. *Global Ecology and Conservation* 24: e01309.
136. Maurice-Bourgoin L, Quiroga I, Guyot JL, et al. (1999) Mercury pollution in the upper Beni River. *Ambio* 28: 312.
137. Maurice-Bourgoin L, Quiroga I (2002) Total mercury distribution and importance of the biomagnification process in rivers of the Bolivian Amazon. *The ecohydrology of South American rivers and wetlands* 6: 49–67.
138. Rivera AR, Heredia MW (2023) Application of Delft3D computational model to estimate the influence of El Bala run-of-the-river dam on the morphological activity of Beni River, Bolivia. *IOP Conference Series: Earth and Environmental Science* 1136: 012030.
139. Arora NK, Panosyan H (2019) Extremophiles: Applications and roles in environmental sustainability. *Environmental Sustainability* 2: 217–218.
140. Rathinam N, Sani R (2018) Bioprospecting of extremophiles for biotechnology applications, *Extremophilic Microbial Processing of Lignocellulosic Feedstocks to Biofuels, Value-Added Products, and Usable Power*, 1–23.
141. Kochhar N, I.K K, Shrivastava S, et al. (2022) Perspectives on the microorganism of extreme environments and their applications. *Current Research in Microbial Sciences* 3: 100134.

142. Jörg Kunte H, Galinski EA, Trüper HG (1993) A modified FMOC-method for the detection of amino acid-type osmolytes and tetrahydropyrimidines (ectoines). *Journal of Microbiological Methods* 17: 129–136.
143. Kuhlmann A, Bursy J, Gimpel S, et al. (2008) Synthesis of the compatible solute ectoine in *Virgibacillus pantothenicus* is triggered by high salinity and low growth temperature. *Applied and environmental microbiology* 74: 4560–4563.
144. Pastor JM, Salvador M, Argandoña M, et al. (2010) Ectoines in cell stress protection: Uses and biotechnological production. *Biotechnology Advances* 28: 782–801.
145. Guzmán H, Van-Thuoc D, Martín J, et al. (2009) A process for the production of ectoine and poly(3-hydroxybutyrate) by *Halomonas boliviensis*. *Applied Microbiology and Biotechnology* 84: 1069–1077.
146. Van-Thuoc D, Guzmán H, Quillaguamán J, et al. (2010) High productivity of ectoines by *Halomonas boliviensis* using a combined two-step fed-batch culture and milking process. *Journal of Biotechnology* 147: 46–51.
147. Atalah J, Cáceres-Moreno P, Espina G, et al. (2019) Thermophiles and the applications of their enzymes as new biocatalysts. *Bioresource Technology* 280: 478–488.
148. Cordova LT, Long CP, Venkataramanan KP, et al. (2015) Complete genome sequence, metabolic model construction and phenotypic characterization of *Geobacillus* LC300, an extremely thermophilic, fast growing, xylose-utilizing bacterium. *Metabolic Engineering* 32: 74–81.
149. Sjöling S, Cowan D (2003) High 16S rDNA bacterial diversity in glacial meltwater lake sediment, Bratina Island, Antarctica. *Extremophiles : life under extreme conditions* 7: 275–282.
150. Yang J, Li W, Teng D, et al. (2022) Metagenomic insights into microbial community structure, function, and salt adaptation in saline soils of arid land, China. *Microorganisms* 10.
151. Martínez JL, Coque TM, Baquero F (2015) What is a resistance gene? Ranking risk in resistomes. *Nat Rev Microbiol* 13: 116–123.
152. Zhang A-N, Gaston JM, Dai CL, et al. (2021) An omics-based framework for assessing the health risk of antimicrobial resistance genes. *Nature Communications* 12: 4765.
153. Haferburg G, Gröning JAD, Schmidt N, et al. (2017) Microbial diversity of the hypersaline and lithium-rich Salar de Uyuni, Bolivia. *Microbiological Research* 199: 19–28.
154. Pérez-Fernández CA, Iriarte M, Rivera-Pérez J, et al. (2019) Microbiota dispersion in the Uyuni salt flat (Bolivia) as determined by community structure analyses. *International Microbiology* 22: 325–336.

-
155. Zhang Z, Zhang Q, Wang T, et al. (2022) Assessment of global health risk of antibiotic resistance genes. *Nature Communications* 13: 1553.
 156. Santos-Júnior CD, Sarmiento H, de Miranda FP, et al. (2020) Uncovering the genomic potential of the Amazon River microbiome to degrade rainforest organic matter. *Microbiome* 8: 151.
 157. Hilton JA, Satinsky BM, Doherty M, et al. (2015) Metatranscriptomics of N₂-fixing cyanobacteria in the Amazon River plume. *The ISME journal* 9: 1557–1569.
 158. Satinsky BM, Crump BC, Smith CB, et al. (2014) Microspatial gene expression patterns in the Amazon River Plume. *Proceedings of the National Academy of Sciences* 111: 11085–11090.
 159. Satinsky BM, Fortunato CS, Doherty M, et al. (2015) Metagenomic and metatranscriptomic inventories of the lower Amazon River, May 2011. *Microbiome* 3: 39.
 160. Liguori K, Keenum I, Davis BC, et al. (2022) Antimicrobial resistance monitoring of water environments: A framework for standardized methods and quality control. *Environmental Science and Technology* 56: 9149–9160.
 161. Yang Y, Xu C, Cao X, et al. (2017) Antibiotic resistance genes in surface water of eutrophic urban lakes are related to heavy metals, antibiotics, lake morphology and anthropic impact. *Ecotoxicology (London, England)* 26.
 162. Serwecińska L (2020) Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. *Water* 12.
 163. Ljungqvist E, Gustavsson M (2022) Genome-scale reconstruction and metabolic modelling of the fast-growing thermophile *Geobacillus* sp. LC300. *Metabolic Engineering Communications* 15: e00212.
 164. Cordova LT, Cipolla RM, Swarup A, et al. (2017) ¹³C metabolic flux analysis of three divergent extremely thermophilic bacteria: *Geobacillus* sp. LC300, *Thermus thermophilus* HB8, and *Rhodothermus marinus* DSM 4252. *Metabolic Engineering* 44: 182–190.
 165. Cordova LT, Antoniewicz MR (2016) ¹³C metabolic flux analysis of the extremely thermophilic, fast growing, xylose-utilizing *Geobacillus* strain LC300. *Metabolic Engineering* 33: 148–157.