Chapter 1
INTRODUCTION

The term "heavy metal" refers to elements occurring naturally in the Earth's crust that have a relatively high atomic density (>5g/cm^3) and weight (Singh *et al.*, 2011). With regard to their role within biological systems, they are classified as essential and non-essential. Some of these elements are required for the regulation and survival of organisms, but only in modulated or trace amounts. Excess presence of heavy metals may, in turn, cause severe harm to the same organism. Examples of such elements include iron (Fe), copper (Cu), cobalt (Co), zinc (Zn), vanadium (V), manganese (Mn), chromium (Cr), nickel (Ni), arsenic (As), molybdenum (Mo) and selenium (Se). Other heavy metals that are less abundantly distributed in the natural environment are therefore also less likely to be nutritionally essential; for example, cadmium (Ca), tin (Sn), lead (Pb), tungsten (W), etc., are rarely required for growth or development. These are thus non-essential in biological context but. Upon introduction into biological systems, they may be highly detrimental (Sharma & Agrawal, 2005). The occurrence of these heavy metals above the threshold level in the environment is of grave concern as it can increase the chances of exposure to them. High incidence of heavy metals within organisms can cause irreversible damage and can even be lethal in some cases. Heavy metals can bioaccumulate within an organism, i.e., they can become concentrated inside cells and persist following uptake. They also go through biomagnification, which is the process of transfer of the concentration of the metal contained within an organism of a lower trophic level to subsequent higher trophic levels in a food web, leading to a higher concentration of the metal being present in the apex predator (Ali & Kha, 2019). The cumulative effect of these traits can cause severe harm to organisms exposed to these metals.

Of all the hazardous heavy metals, a few, such as arsenic (As) , cadmium (Ca), lead (Pb) and especially mercury (Hg), demand special attention. This is due to the sheer magnitude of damage they can inflict upon their hosts, and their unyielding persistence in ecosystems, once leached. Mercury is unique among these heavy metals. The extreme toxicity of mercury to living cells has earned it the third-place position in the "priority list of hazardous substances" (Bansal *et al.*, 2013). It has many different forms, broadly categorized into elemental mercury, inorganic and organic mercury (Langford & Ferner, 1999). Organic mercury is the form it takes when it combines with carbon and is thought to constitute some of the most toxic mercury compounds. Alternating between the different forms allows mercury to navigate through the environment into soil, air and water. Its mobility and biotransformation rely heavily on the microbial population of a region (Trevors, 1986). The elemental form of mercury can also volatilize in warmer climates (vapour pressure of 0.167 Pa at 25°C) and undergo long-range transcontinental transport (Loewen *et al.*, 2005). As such, the biogeochemical cycling of this pollutant is particularly relevant.

The source of mercury contamination of an environment may be natural/geo-genic (volcanic eruptions or weathering of rocks) or anthropogenic (mining, burning of fossil fuel, hospital waste, agricultural waste, etc.) (Pavithra *et al.*, 2022). Mercury pollution is on the rise globally in direct correlation with the rise in urbanization as a consequence of increased anthropogenic interference (Selin, 2009). The risks associated with such pollution are not only towards plant, animal and human life but can cause the breakdown of entire functioning ecosystems. This was unfortunately exemplified in the Minamata Bay disaster in Japan that killed over 900 people and negatively impacted the health of around 2 million (Kudo *et al.*, 1998). The implications of increased exposure to mercury in humans continue to be studied extensively and have been reported to aggravate several diseases, including neurological, metabolic, renal, and cardiovascular conditions. The degree of toxicity exerted is dictated by the form mercury is present in (Tchounwou *et al.*, 2003).

Multiple mechanisms of toxicity govern the vast array of detrimental health effects it may give rise to. On the cellular level, mercury can cause cell membrane disruptions, oxidative stress within cells, inflammation, autoimmunity and protein/enzyme inactivation via its high affinity to sulfhydryl (–SH) groups in cysteine (Cys) residues (Bernhoft, 2012). Regional and global distribution patterns of mercury and its correlation with physical, chemical and biological factors are being studied thoroughly to understand the full scope of the issue. A convention dedicated to understanding and alleviating mercury pollution and the associated health and environmental risks has been established. The "Minamata Convention" was adopted in 2013, with India entering into this agreement in 2018 (Kessler, 2013).

According to recent reports, mercury is being detected increasingly in very remote locations, generally thought of as pristine and unpolluted, viz. Mountains (Szopka *et al.*, 2011). This may be attributed to increased mercury emissions in corresponding low-lying areas with high incidences of anthropogenic activity. This phenomenon has been observed in many parts of the world. Most relevant is its occurrence in the Tibetan plateau, also known as the "third pole" of the world. The accumulation of mercury in and over the Tibetan plateau is significant as it is surrounded by some of the biggest contributors to the global load of atmospheric mercury, i.e., East and South Asia (47%). South Asia alone emits a further 16% mercury into the atmosphere, and emissions are only increasing with the passing years (GMA, 2018). As such, this vast area is set ideally to bear the consequences of these extreme emission trends. Moreover, the Tibetan plateau is home to the Himalayan ranges, which provide a perfectly positioned global sink for this volatile heavy metal (Pokhrel *et al.*, 2016).

The Himalayas are a natural and cultural treasure, boasting some of the world's highest peaks, supporting diverse ecosystems and unique indigenous populations that depend entirely on the biogeology and orography of these mountains. In such cases, indigenous human and wildlife populations become critically vulnerable to the dangers of heavy metal pollution. Such trends are already being studied among the wildlife and Inuit populations of the Antarctic, recently having been discovered to be victimized by the global cycling of mercury and the consequent pollution problem (Dewailly *et al.*, 2001).

Several different approaches have been explored to alleviate mercury pollution. The main challenge with heavy metal remediation is that heavy metals cannot be degraded or mineralized like organic pollutants but can only be transformed into less toxic, less persistent forms and, ideally, captured to restrict their mobilization into the food chain. Physico-chemical methods tend to produce large amounts of mercury sludge, the disposal of which is neither environmentally friendly nor economically sustainable (Jan *et al.*, 2009). Bioremediation of heavy metal-contaminated spaces often proves to be the best solution. Bioremediation is the use of living organisms like tolerant microbes for the removal of toxicants of various kinds from the environment (Dash & Das, 2012). Detoxification in this way is preferred as it can be cost-effective, far more efficient and the most sustainable approach.

Microbes, especially bacteria, have a propensity to adapt to adverse conditions. Major stressors like the continued presence of excess mercury in the environment induce mutations within bacterial communities, facilitating various mechanisms of adaptation to the stress in order to survive. In doing so, these communities become proficient in not only handling the stress themselves but sometimes also in ridding the environment itself of the toxic contaminant as a byproduct of their survival strategies. Such bacteria may be deemed as “tolerant” or “resistant” with respect to the contaminant and can be employed in bioremediation after a thorough assessment of their remediation capabilities and relationship with the environment (Priyadarshanee *et al.*, 2022). Bacterial cultures are easy to manage, control, and tweak to our specific advantage. Moreover, bacteria can be relatively easily genetically modified for the introduction of desired characteristics or to increase efficiency of expression by up-regulation as per requirement. As bacteria are naturally intertwined with the cycling of mercury in the environment and responsible for its bio-transformations, they make good candidates for the exploration of bioremediation potential.

This work focuses on the exploration of mercury-tolerant bacterial populations from the soil of locations in and around Darjeeling that could harbour such a resilient microbiome adept in dealing with high levels of mercury stress. Soil is one of the most significant reservoirs of mercury and is also fundamentally responsible for the sustenance of a habitable ecosystem. Soil health, in turn, is directly governed by a healthy microbiome (Chaparro *et al.*, 2012). The accumulation of excess mercury in soil can devastate soil health by complete annihilation of beneficial microflora. Mountain soil is thought to be especially vulnerable to being subjected to such pollutants as it may harbour a microbiome far less exposed to such stressors (Wang *et al.*, 2022). While a significant number of studies have looked into the effect of mercury in aquatic ecosystems due to the immediate threat of mercury exposure water bodies can pose via the consumption of fish and shellfish, very few studies have been conducted on its effects on terrestrial ecosystems.

The implications of mercury pollution in soil and the involvement of microbes in the cycling of terrestrially deposited mercury are only now beginning to be understood. Mountains, in particular, are being regarded as a critical and much-overlooked sink zone in the biogeochemical cycle of this heavy metal. No studies of this nature have thus far been focused on the Indian Himalayas. As such, this study attempted to explore the terrestrial microbiome of the Darjeeling hills and some surrounding high-altitude areas of Sikkim and Nepal for a comparative analysis of the ramifications of mercury on mountain microbiomes.

This study was designed around the biochemical and molecular characterization of mercury-tolerant bacterial isolates for assessment of their potential as bioremediation candidates. There are several factors to consider, apart from its mercury tolerance ability, before a bacterial strain may be considered for bioremediation. An understanding of the metabolic characteristics of the bacterium and its preferences for nutrient sources, for example, can be crucial to formulating the most sustainable bioremediation plan. The interactions such a bacterial strain may have with its environment, and the implications of its presence in a microbiome are of great significance not only to bioremediation strategies but also to understanding the impact these strains have had on their place of origin. Information on the growth pattern of the isolates is vital to the design and implementation of bioremediation plans. Moreover, bacterial strains showcasing tolerance to additional toxicants like other heavy metals can ensure their survival to a much greater degree in contaminated sites, leading to successful remediation operations. Such displays, along with antibiotic susceptibility profiles, can help elucidate the possibility of particular resistance mechanisms. It can also give us insight into the spread of antibiotic resistance among these microbiomes that experience significantly less exposure to such drugs. Additionally, beneficial traits like plant growth promotion or commercially valuable metabolites can help us draw further benefits from the bacterial strains beyond their mercury bioremediation potential. With this in mind, this study has been accomplished via categorical analysis of their biochemical traits, plant growth promotion (PGP) abilities, tolerance abilities towards mercury and other relevant heavy metals, assessment of their susceptibility towards clinical and natural antibiotics, and an investigation into their metabolome through GC-MS analysis of their metabolites.