# IMPACT OF ALTITUDINAL VARIATION ON SELECTED SOIL PROPERTIES AND CARBON DYNAMICS IN THE ALPINE WETLANDS OF LESOTHO

**BY**

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# DECLARATION

The work contained in this dissertation was carried out and completed by **Mosiuoa Mochala, 200901543** at the National University of Lesotho Water Institute, National University of Lesotho. I hereby declare that this study constitutes my original work and has never been submitted for the award of a degree or diploma to any University. To the best of my knowledge this dissertation contains no material written by another person except where due reference is made in the dissertation itself.

**Signature…M. Mochala…………………………………………Date…07/10/2025………………………………**

As the candidate’s supervisor, I certify the above statement to be correct to my knowledge and have recommended this dissertation for submission.

**Dr.** Knight Nthebere………………………..**Date**…07/10/2025……………………….........................

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# LIST OF SYMBOLS AND ABBREVIATIONS

| **Symbols and Abbreviations** | | **Description** |
|---|---|---|
| % | : | Percent |
| & | : | And |
| $^{o}$C | : | Degree Celsius |
| = | : | Equal to |
| ANOVA | : | Analysis of variance |
| BD | : | Bulk Density |
| CD | : | Critical difference |
| $C_{VL}$ | : | Very Labile Carbon |
| $C_{L}$ | : | Labile Carbon |
| $C_{LL}$ | : | Less Labile Carbon |
| $C_{NL}$ | : | Non Labile Carbon |
| Cm | : | Centimeter |
| Cmol (p+) kg$^{-1}$ | : | Centimoles of Positive Charge per Kilogram |
| cm hr$^{-1}$ | : | Centimeter per hour |
| FAO | : | Food Agriculture Organization |
| IR | : | Infiltration Rate |
| K*sat* | : | Saturated Hydraulic Conductivity |
| SOC | : | Soil Organic Carbon |
| SQ | : | Soil Quality |
| SQI | : | Soil Quality Index |
| PCA | : | Principal Component Analysis |
| EC | : | Electric Conductivity |
| CEC | : | Cation Exchange Capacity |
| WHC | : | Water Holding Capacity |
| BD | : | Bulk Density |
| SOM | : | Soil Organic Matter |
| P | : | Phosphorus |
| TN | : | Total Nitrogen |
| N | : | Nitrogen |
| LFOC | : | Light Fraction Organic Carbon |
| POC | : | Particular Organic Carbon |
| SMBC | : | Soil Microbial Biomass Carbon |
| WSOC | : | Water Soluble Organic Carbon |
| SOCS | : | Soil Organic Carbon Stock |
| MAOM | : | Mineral-Associated Organic Matter |
| MAOC | : | Mineral-Associated Organic Carbon |
| POM | : | Particular Organic Matter |
| DOC | : | Dissolved Organic Carbon |
| HWSC | : | Hot Water-Soluble Organic Carbon |
| POC | : | Particulate Organic Carbon |
| MBC | : | Microbial Biomass Carbon |
| DHA | : | Dehydrogenase |
| βGaA | : | B- Galactosidase |
| FDA | : | Fluorescein Diacetate Activity |
| | : | Natural Resource Conservation Services |

NRCS : Agro-Ecological Zones
AEZ : Tenesolo
TNL : Percentage Score
PES : Khorong
KRN : Land Use Type
LUT : Khamoqana
KMQ : Khalong-La-Lichelete
KLL : Lets'eng-La- Likhama
LLL : Koting-Sa-Ha Ramosetsana
KSRN : Complete Randomised Block Design
CRBD : Principal Component
PC : Soil Quality Index Calculator
SQI CAL : Minimum Data Set
MDS : Standard Error of the Mean
SE(m) : Above Sea Level
asl : Total Organic Carbon
TOC : Active pool of carbon
$C_{ACT}$ : Passive pool of carbon
$C_{PSV}$ : Calcium
Ca : Magnesium
Mg : Sodium
Na

# ABSTRACT

The study entitled, **“Impact of Altitudinal Variation on Selected Soil Properties and Carbon Dynamics in the Alpine Wetlands of Lesotho”** was conducted in the on-going project entitled, “*Carbon Modelling and Omics Approaches for Screening of Soil Microbes for Climate Change Adaptation in the Alpine Wetlands of Lesotho*,” initiated in November, 2024. The study was designed in blocks (sub-catchments) with six altitudinal variations (from 2500 to 3155 m a.s.l), equivalent to alpine wetlands from three sub-catchments (Senqunyane, Khubelu and Sani) as follows: Khorong (2500-2550 m a.s.l) and Tenesolo (2552-2600 m a.s.l) in Senqunyane; Khamoqana (2839-2880 m a.s.l) and Khalong-la-Lichelete (2891-29950 m a.s.l) in Sani; and Lets’eng-la-Likhama (3040-3080 m a.s.l) and Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) in Khubelu. Each treatment was replicated four times. The soil texture was loam to sandy loam across the alpine wetlands. The alpine wetland soils were slightly acidic and non-saline.

The findings of the study revealed that bulk density (BD) decreased with increasing altitude 0-15 cm soil depth and was significantly lower (1.08 Mg $m^{-3}$) in Koting-Sa- ha Ramosetsana (KSHM) compared to other wetlands. The KSHM also showed significantly higher infiltration rate (IR) of 2.17 cm $hr^{-1}$, maximum water holding capacity (MWHC) of 57.51% and saturated hydraulic conductivity (*Ksat*) of 2.70 cm $hr^{-1}$ *Ksat* at 0-15 cm soil depth whereas, Tenesolo recorded the higher BD and the least IR, MWHC and *Ksat*. Soil organic carbon (SOC) and Calcium (Ca) were significantly higher in KSHM and increased with increasing altitude, except Khorong (KRN) which did not follow this increasing trend, *i.e.*, KRN (2500-2550 m a.s.l) was exhibited with higher contents of SOC and Ca. The electrical conductivity, cation exchange capacity and macronutrients (nitrogen, phosphorus and potassium) availability of the soil were non-significant.

Soil enzyme activities declined significantly with increase in altitude due to lower temperatures at higher elevations, limiting microbial activity. The dehydrogenase, fluorescein di-acetate and β-galactosidase activities were 3.92 and 45.33%, 1.82 and 32.20% and 9.29 and 15.11% lower in KSHM (3087-3155 m a.s.l) compared to Tenesolo (2552-2600 m a.s.l) and Khorong (2500-2550 m a.s.l), respectively. Higher carbon pools *viz.*, very labile ($C_{VL}$), labile ($C_L$), less labile ($C_{LL}$) and non-labile ($C_{NL}$) and total organic carbon (TOC) were recorded under KSHM compared to all other wetlands at varied altitudes. Passive pool of carbon ($C_{PSV}$) was dominant over active carbon pool ($C_{ACT}$) with 75–79% contribution towards TOC. Both $C_{PSV}$ and $C_{ACT}$ were higher in KSHM (higher elevation site).

The Soil Quality Index (SQI) was enhanced (42.54% and 42.51%) at both upper (Koting-sa-ha Ramosetsana) and lower (Khorong) elevation wetlands, indicating that altitude alone does not fully determine soil quality. Instead, wetland condition, vegetation cover, and site-specific environmental factors are critical in shaping soil functionality and regulating carbon processes in alpine wetland ecosystem. Therefore, further research needs to consider synergistic factors including the slope, topography and soil degradation level in addition to the altitude.

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## Chapter-I

# INTRODUCTION

Soil and water management form the basis for a sustainable ecosystem. Wetland ecosystems are characterized by their moist soils, which exhibit unique ecological dynamics and provide essential services to both living organisms and the physical environment (Xiong *et al*., 2023). The alpine wetland is an integral component of mountain ecosystems worldwide, providing essential ecological functions such as water conservation, biodiversity maintenance, climate regulation, and carbon storage and sequestration (Chen *et al*., 2023; Zhao *et al*., 2024). Furthermore, alpine wetlands play a vital role in mediating the exchange of water and energy between the soil and the atmosphere, exerting a significant influence on climate change (Liu *et al*., 2020). The wetlands' multi-functionality is significantly interlinked with rich biodiversity of soil microbes and enzymatic activities being an important component of the ecosystem (Ogola *et al*., 2024). However, the vital natural resources needed for agroecosystems and other agriculture-related endeavors are diminishing due to soil degradation (Nthebere *et al*., 2025).

The major drivers of soil degradation level are soil erosion, overgrazing, climate change and invasive species etc in the alpine wetlands of Lesotho. Heavy grazing by livestock like sheep, goats, and cattle accelerates soil degradation by stripping vegetation that shields the ground from erosion and drying. The problem is compounded by invasive plants, which displace native species and alter soil functions, often thriving in areas disturbed by overgrazing or shifts in water regimes. The wetlands in high altitude and mountainous areas are also more susceptible to climate change and their ecosystem functioning will be altered with climate change (Bentley *et al*.,2019; Lee *et al*., 2015; Mofutsanyana *et al*., 2020). Over 30% of Lesotho's alpine wetlands show signs of soil degradation, mainly due to overgrazing, along-with climate change-related factors (FAO, 2022). The high altitude being the main factor determinants of ecosystem development or decline in terms of wetland health status may affect the water and soil's capability to sustain its ecosystem services in these Wetlands. In all the land uses of Lesotho, the contents of soil organic carbon (SOC) are less, and the Lesotho's alpine wetlands are no exception, demonstrating a predominant issue of potential degradation in the country, which is a considerable solicitude to maintain the wetland soil quality (Mastrojeni, 2019). The situation necessitates a comprehensive evaluation of how high altitude influence soil quality (SQ) -related attributes and carbon dynamics in these wetlands. It is of utmost importance to study the effect of the altitudinal variation on wetland changes via quantitative soil data as to protect wetlands rationally and scientifically.

A number of scientific research were conducted earlier both globally and locally to explore effects of altitudinal variation on carbon dynamics and attributes of the soil related to SQ in distinct land-use types including wetlands (Belay *et al*., 2018). Nevertheless, the research on how high elevation affects carbon dynamics and soil quality is still limited in Lesotho. A significant research gap exists in comprehending how variations in higher elevations in relation to soil impact the soil attributes and carbon dynamics in the alpine wetlands as to inform the policy makers, stakeholders involved (ReNoka, LHDA etc) on wetland management and sustainability, using quantitative soil data. This gap necessitates soil quality indexing to evaluate the influence of higher altitude wetlands in relation to soil and insights on how these variation in higher altitudes translate into specific management practices in the alpine wetlands. Keeping all these in view, the present study is proposed to be undertaken with the following aims;

1. To study the carbon dynamics (soil organic carbon pools) as influenced by altitudinal variation in selected Alpine wetlands.
2. To estimate and analyse the impact of altitudinal variation in selected Alpine wetlands on soil enzyme activities.
3. To assess the soil quality index as influenced by altitudinal variation in selected Alpine wetlands.
4. To study the changes in selected soil properties as influenced by altitudinal variation in selected Alpine wetlands.

**Long-term objective/Goal:**

To investigate how higher altitude impact the selected soil properties, soil enzyme activities, soil organic carbon dynamics (pools) and the overall soil quality in selected Alpine wetlands of Lesotho.

**Hypothesis:**

The soil quality and organic carbon dynamics (pools) will increase with increasing altitude in the alpine wetlands of Lesotho.

**Research Output/ Outcome:**

The quantitative soil data on effects of altitudinal variation on soil attributes and carbon dynamics in the alpine wetlands of Lesotho is anticipated to inform about the recommended management practices to be adopted and provide with an idea about the soil attributes which are potential indicators of soil quality improvement based on PCA soil quality indexing.

## Chapter-II

# REVIEW OF LITERATURE

### 2.1. Overview

High-altitude wetlands significantly provide substantial gains by regulating water flow, reducing floods, and maintaining water quality. They act as major carbon sinks, helping to mitigate climate change. For mankind, they supply freshwater, support grazing and agriculture, and sustain cultural and livelihood practices. To the surrounding regional ecosystem, they conserve biodiversity, provide habitats for unique species, and stabilize fragile mountain landscapes; nevertheless, these possible gains are increasingly threatened unless properly managed (Nthebere *et al*., 2025). These wetlands in high elevation, degrade rapidly mainly due to soil erosion, overgrazing, changes in climate and invasive species etc., thus, affecting the soil hydraulic properties, carbon storage and the soil quality necessitated to maintain the wetland ecosystem services (Padbhushan *et al*., 2022).

At this juncture, the following questions arises;

- Does altitude has an influence on carbon content and its dynamics in the alpine wetlands?
- Does enzyme activities respond rapidly with mountainous altitude in the wetlands?
- Are there any appropriate tools or strategies to use for monitoring and assessing the soil quality (SQ) in the alpine wetlands of Lesotho?
- Which properties of the soil change over space and time due to altitude in the alpine wetlands of Lesotho?

One suitable way to understand and respond to these questions is through analysis of carbon dynamics and responsive attributes of the soil which are related to SQ. The results of several research studies indicate that high altitude has an effect on soil carbon dynamics, physical and hydraulic, chemical, physico-chemical and microbiological attributes which may in turn influence the SQ. In this chapter, an effort has been made to cite relevant and up-to-date review of literature related to the **“Impact of Altitudinal Variation on Selected Soil Properties and Carbon Dynamics in the Alpine Wetlands of Lesotho.”** The literature, thus reviewed, has been presented under the following headings;

I. Impact of altitudinal variation on soil physico-chemical attributes, available macronutrients and cation

   a. pH, Electrical conductivity, organic carbon, Cation Exchange Capacity

   b. Macronutrients availability (nitrogen, phosphorus and potassium) and cations (sodium, calcium and magnesium)

II. Impact of altitudinal variation on physical and hydraulic attributes of the soil.

   a. Soil texture
   b. Soil bulk density
   c. Maximum water holding capacity
   d. Saturated hydraulic conductivity
   e. Infiltration rate

III. Impact of altitudinal variation on carbon dynamics

IV. Impact of altitudinal variation on soil enzyme activity

   a. Dehydrogenase
   b. β- galactosidase
   c. Fluorescein Di-acetate

V. Impact of altitudinal variation on soil quality.

## I. Impact of altitudinal variation on soil physico-chemical attributes, available macronutrients and cations.

Impact of altitude on soil physico-chemical attributes, nutrient and cation distribution is well established and documented (Zhang *et al*., 2021). Variation in altitude, particularly in the alpine wetlands is a crucial topographic feature influencing these soil characteristics (Feng *et al*., 2019). A number of studies were carried-out for studying the influence of altitude on soil parameters. Therefore, it is of great significance to comprehend on how greater altitude affects these properties.

### a. Physico-chemical attributes of the soil

#### pH, electrical conductivity (EC), organic carbon (OC) and cation exchange capacity (CEC).

Altitude, pH and vegetation in the alpine wetlands may be the chief determinants of EC, CEC and spatial distribution of OC (Zhang *et al*., 2021). The research conducted by Charan *et al*. (2013) at cold desert with greater elevation, to study soil physico-chemical properties as influenced by altitudinal variations indicated that significantly higher pH

(8.44) was reported at lower altitude: 10000-11000 meters (m) above sea level (asl), while lowest pH values of 8.21 and 8.25 were recorded at higher altitude of 11000-12000 m asl and >12000 m asl, respectively. Further, the electrical conductivity (EC) was reported to be non-significant with altitude (Charan *et al*., 2013). Kamal *et al*. (2023) examined how altitude influences soil quality within the Swat River catchment. Their findings indicated that soils at lower elevations tended to be slightly alkaline and exhibited higher salt accumulation, whereas soils from higher elevations were mildly acidic and showed reduced electrical conductivity.

The low pH was also observed at higher altitude due to more organic matter, salt reduction and leaching of base cations (Moslehi *et al*., 2019; Ramesh *et al*. 2019). While examining how elevation influences soil properties in China's Fanjing Mountain region, Wang *et al*. (2024) observed that the topsoil layer (0–10 cm) at elevations between 1800 and 2100 meters above sea level had pH values below 5.5, with the most acidic sample registering a pH of 4.09. It was further discovered that increased altitude did not result in significant change in pH and cation exchange capacity (CEC) concentrations (Wang *et al*., 2024). Inconsistent soil physio-chemical attributes (CEC, pH and EC) pattern were reported in previous research studies at various elevations in mountainous regions (Luo *et al*., 2024; Aqeel *et al*., 2024) probably inter-linked with alterations in mountainous natural environments, influencing litter decomposition, the degree of soil breakdown, and various other contributions to the soil over time (Aqeel *et al*., 2024).

The SOC increases with increasing altitude, and this is due to decrease in temperature with increase in altitude (Imtimongla *et al*., 2021). Hence, low temperature with increase in altitude in the alpine wetlands is the chief factor determining the greater SOC with increasing elevation (Kumar *et al*., 2022). An increase in SOC with altitude may also owe to texture of the soil, consistent carbon input losses, and the decrement in biological functionality due to the lower temperatures (Imtimongla *et al*., 2021).

A case study on the effects of altitude on SOC content in the distinct land-uses of Czech Republic by Nozari and Borůvka (2023) demonstrated that SOC increased with increasing altitude, probably as a result synergistic effects of higher leaching at greater elevations and soil acidity resulting in reduced decomposition.

The research undertaken by Teron *et al*. (2024) to explore how the physico-chemical attributes of the soil are influenced by altitude in Eaglenest Wildlife Sanctuary of Arunachal Pradesh in India revealed that SOC levels ranged from 2.57% to 4.16%, with higher values in the sub-tropical zone (800-1800m amsl). Olaleye *et al.* (2022) assessed wetland condition across two distinct agro-ecological regions in Lesotho by analysing soil physico-chemical characteristics. Their results indicated that the mountainous Khalong-la-Lithunya (KHL) wetland, situated at elevations between 2000 and 3484 meters above sea level, contained significantly greater ($p < 0.05$) levels of soil organic matter than the Foot Hills–Ha-Matela (HM) wetland, which lies between 1800 and 2000 meters elevation.

### b. Soil macronutrients (available nitrogen, phosphorus and potassium) and cation availability

Elevation difference significantly impacts availability of soil macronutrients, mainly due to changes in soil processes as well as climatic factors *viz.*, temperature and precipitation in high altitudinal wetlands (Ramzan *et al.*, 2014). The precise impacts vary based on the specific nutrient and ecological zone. However, soils in greater altitude exhibit distinct nutrient dynamics in comparison with that from lower altitude (Sivaranjani *et al.*, 2022).

The availability of soil phosphorus (P) depend upon soil pH such that an increase in altitudinal gradient results in higher P availability. The total nitrogen (TN) is reported to vary in general (Imtimongla *et al.*, 2021). The rise in soil temperature is announced as a key environmental factor which slow-down the nitrogen (N) mineralization, thus impacting the bio-accessibility of N (Zhang *et al.*, 2019). The N inter-linked with soil organic matter (SOM) is not readily mineralized, therefore greater concentration of TN in the soil with an increase in altitudinal gradient could be due to more SOM (Chiriac *et al.*, 2025). In spite the fact that SOC and N concentrations increase with increase in altitude, their bio-accessibility to the living system becomes the chief factor (Chiriac *et al.*, 2025). More of N and P remain bound to the SOM without degrading in less temperatures. Therefore, less soil nutrient availability in higher altitude is due to low temperature which induces the reduction rates of mineralization and decomposition (Zhang *et al.*, 2019).

Studies conducted by Mishra *et al.* (2021) to explore the effect of different altitudes on attributes of the soil in Mon district, Nagaland demonstrated that greatest N concentration was recorded at lower altitude (1000 meters asl) which could be due to warmer temperatures, which facilitate soil organic matter decomposition rate and cycling of available nitrogen in the soil. Further, the kind of vegetation could be another possible factor which contributed towards N accumulation and release in the soil. Sivaranjani *et al.* (2022) reported significantly greater available soil macronutrients and cations (magnesium, manganese, sodium, zinc, phosphorus and potassium contents) at higher altitude (1189m asl) except calcium concentration which was lower, while studying altitudinal impacts on Uludağ fir soil macronutrients in Kastamonu, Turkey. The contents of available soil phosphorus, zinc and copper concentrations increased with an increase in altitude in Takatu mountain range of Quetta in Balochistan, with correlation coefficient of 0.923, 0.995 and 0.999 respectively (Ramzan *et al*., 2014).

### II. Impact of altitudinal variation on physical and hydraulic attributes of the soil

The alpine wetlands are characterized by precipitation, vegetation and low temperatures as a result of high altitude (Vento *et al*., 2024). The altitude being the main factor in these wetlands, influences the soil physical and hydraulic properties (Vento *et al*., 2024). Previous findings indicated that higher altitudes tend to have lower bulk density, and finer particle sizes, while experiencing increased organic matter content, better soil aggregation, maximum water holding capacity, optimum infiltration as well as saturated hydraulic conductivity (Bargués-Tobella *et al*., 2024).

Kamal *et al*. (2023) investigated how altitude influences soil texture, revealing that sand content varied irregularly with elevation. Specifically, the proportion of sand increased between 500 meters and 1300 meters above sea level, rising from 6.43% at the lower elevation to 40.93% at 1300 meters. Beyond this point, however, the sand fraction declined, with a reported value of 30.69% at 1700 meters elevation. The silt fragments decreased significantly with increasing elevation, and was 74.40% at an altitude of 500m while at greater elevation (1700m), the contents were 44.60% (Kamal *et al*., 2023). The proportion of silt exhibited an opposite trend relative to sand, indicating that the parent materials predominantly contained minerals responsible for sand formation (Kumar *et al*., 2022; Kamal *et al*., 2023). Kamal *et al*. (2023) observed significant variation in clay particles distribution among distinct altitudes, showing increasing trend with increase in altitude and were 25.77% at 1700m asl while at lower altitude (500m asl) were 19.00% and this could be due the fact that parent materials found at higher elevations consist primarily of residuum and colluvium, and are largely composed of materials eroded and deposited from lower altitudes (Kamal *et al*., 2023). This process likely contributes to the observed rise in clay content as elevation increases.

The study of Teron *et al*. (2024) which was undertaken in Eaglenest Wildlife Sanctuary, Arunachal Pradesh in India under different ecological zones while investigating the influence of altitude on physical attributes of the soil, revealed that physical attributes varied significantly with altitude and found that the clay (%) fraction was lowest (1.96 ± 0.22%) in the temperate region (1800-2800m asl) at soil depth of 0–20 cm, while the percent of sand and silt were higher (88.38 ± 0.90% and 8.29 ± 0.68%, respectively) in the same region. The water holding capacity (WHC) was also reported greatest (45.54 ± 1.50%) at an altitude of 1800-2800m asl *i.e*., in the temperate region at 0–20 cm soil depth, while the lowest WHC (31.30 ± 2.57%) and the bulk density (0.75 ± 0.01 g cm$^{-3}$) were observed at an altitude of 800-1800m asl in the tropical and 1800-2800m asl in the temperate, respectively (Teron *et al*., 2024).

Badía *et al*. (2016) studied the impact of elevation difference on attributes of the soil soil in South Western, Europe (mountain environment), and discovered that increase in elevation decreases the fine silt-sized particles significantly, while the coarse sand-sized particles increased significantly. Imtimongla *et al*. (2021) and Mangral *et al*. (2023) have announced the decline in bulk density (BD) with increasing altitude mainly attributed to greater amounts of SOM concentration with increasing in altitude. Studies conducted by Kumar *et al*., 2010, also announced reduced BD with increasing altitude.

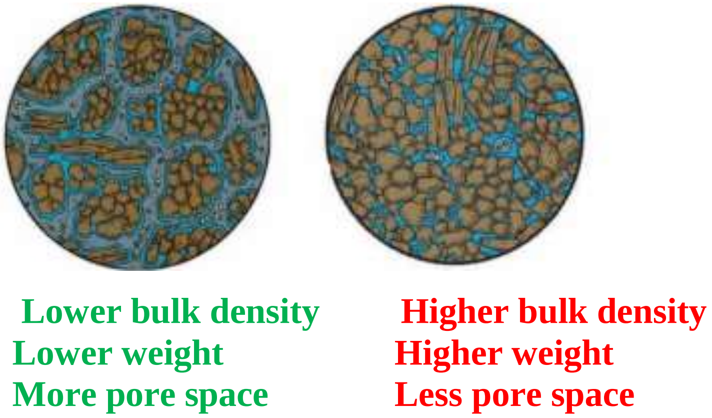

**Fig. 2.1:** Relationship between soil bulk density and other soil physical properties (Rymer, 2017).

Mangral *et al*. (2023) studied the influence of altitudinal gradient physical parameters along the Kashmir Himalaya alpine region in India and the observed results indicated that the mean soil BD decreased (1.11 g cm$^{-3}$) with an increase in altitude (3,220–3,908m asl) at soil surface layer attributed to increased soil organic carbon concentration, ratio of silt and clay content, freeze and thaw phenomenon (Li *et al*., 2017). The infiltration capacity was also reported to increase with increase in altitude, being significantly higher at altitudinal gradient (3,220–3,908m asl) (Mangral *et al*., 2023), being in line with the results of Sun *et al*. (2018). Mangral *et al*. (2023) also announced an increase in the soil infiltration capacity increase in altitude from 3,220–3,908 meters in the alpine regions of Kashmir Himalaya in India.

The voids within the soil structure mainly facilitate the movement and availability of both air and water throughout the soil system (Ramesh *et al*., 2019). Four types of hierarchical pores structure have been identified as 'macro-pores, pore space between macro- aggregates, pores between micro- aggregates but within macro-aggregates, and pores between micro-aggregates in the soil environment' as a result of abiotic (wetting and drying, freezing and thawing *etc*.,) and biotic (burrowing by ice rats, root growth penetration, macro and micro-fauna) factors in the alpine regions. By providing a place for their existence, porosity affects soil biodiversity. Aggregates bind and stabilise SOC, produced by microorganisms within the soil pores, which has an impact on soil carbon sequestration (Ramesh *et al*., 2019).

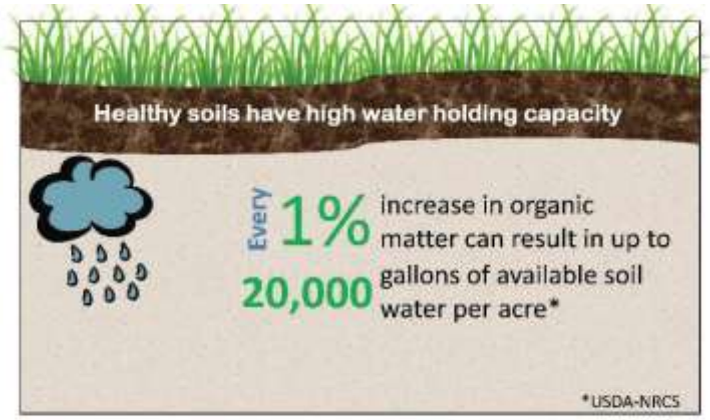

**Figure 2.2:** Soils in wetlands that contain abundant organic matter tend to hold more water effectively (Bhadha *et al*., 2017).

A thin layer of soil covering the Earth's surface supports life by retaining water and essential nutrients for plant uptake. These vital processes primarily take place within the soil's pore spaces, which make up nearly 50% of the total soil volume. However, knowledge about how this volume reacts within wetland ecosystems remains limited (Robinson *et al*., 2022).

Water holding capacity is directly proportional to porosity and porosity is inversely proportional to bulk density (BD) in such a way that the decline in BD, results in optimum soil porosity (Singh *et al.*, 2018). Rana *et al.* (2022) investigated how soil and water conservation practices influenced the hydro-physical properties of meadow soils across four elevation zones on Wugong Mountain in China. Their study examined bulk density, porosity, and water-holding capacity in soils situated between 1600 and 1900 meters above sea level. The results indicated that BD declined noticeably between 1700 and 1900 meters, while both porosity and the soil's capacity to retain water increased significantly at these higher altitudes. Further, they have observed that average infiltration rate ranging from 2.85–27.41 mm min$^{-1}$, and increasing at the highest altitude *i.e.*, 1900m.

## III. Impact of altitudinal variation on soil organic carbon (SOC) dynamics

Soil organic carbon (SOC) plays a vital role in maintaining the health of soils and wetlands. Globally, soils represent the largest reservoir of terrestrial carbon, encompassing diverse carbon fractions that differ in their decomposition rates and stability (Potter & Klooster, 1997). They are an active pool (a weathered facet of root exudates, plant litters), for example; the light fraction organic carbon (LFOC), readily oxidised carbon *i.e.*, particulate organic carbon (POC), water soluble organic carbon (WSOC) and microbial biomass carbon (MBC) (Potter and Klooster., 1997) with a turn over period of few days, which can swiftly be metamorphosed and revived, categorised into labile and very labile carbon; slow pool with a turn over period of up to centuries, which entails the physically stabilized carbon, structural residues of the plant; and passive carbon pool which are SOM stabilized, persisting for over thousands of years (Kavya *et al.*, 2023).

Soil carbon dynamics are driven by several factors such as climate, altitude, the level of soil degradation and soil characteristics etc (Büyük *et al.*, 2020). Wu *et al.* (2022) reported that soil organic carbon (SOC) levels were notably higher at mid to high elevations (2,139–2,500 m) compared to those found at lower elevations (1,380–1,650 m). Similarly, Vibhuti and Bargali (2020) examined the distribution of soil carbon stocks across varying altitudes in the central Indian Himalayas and observed that SOC stock ranged from 22.56 to 81.51 t C ha$^{-1}$, with the highest accumulation (66.01 t C ha$^{-1}$) occurring at higher altitudes, indicating a positive correlation between elevation and SOC content. Baisden *et al.* (2002) also reported increased soil carbon (C) turn-over time with altitude due to decreased average annual temperatures in the Sierra Nevada (California), and deduced that the temperature was the driving factor in soil C dynamics.

The high mountainous soils on poorly weathered rock have greater proportions of labile particulate matter (POM) stabilized via low temperatures, hence slow litter decomposition (Budge *et al*., 2011; Hagedorn *et al*., 2019). Feyissa *et al*. (2023) observed higher SOC stocks in the alpine grasslands in comparison with the forests at decreased elevations. According to Wackett *et al*. (2018), alpine grasslands situated at elevations ranging from 2000 to 2700 meters exhibited the greatest quantities of mineral-associated organic matter (MAOM), with mineral-associated organic carbon (MAOC) contributing substantially to the overall soil organic carbon content.

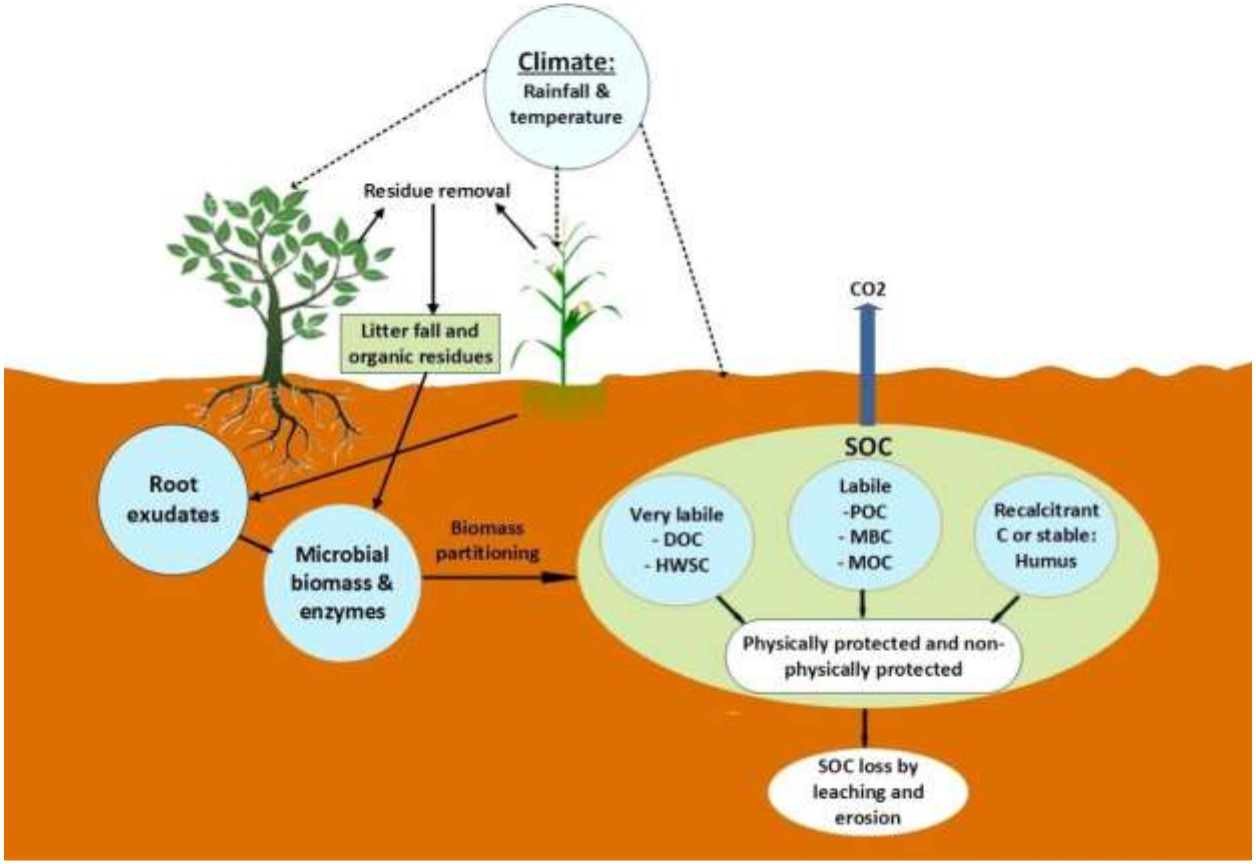

**Figure 2.3:** Illustration depicting the processes involved in soil organic carbon cycling, adapted from Ramesh *et al*. (2019)

DOC: dissolved organic carbon; HWSC: hot water-soluble organic carbon; POC: particulate organic carbon; MBC: microbial biomass carbon; MOC: mineral associated organic carbon: SOC, soil organic carbon.

## IV. Impact of altitudinal variation on soil enzyme activity

The activity of enzymes in soil serves as a critical measure of soil health, soil microbial diversity and ecological restoration, and these enzymes are more susceptible to instant turnover and respond rapidly to elevation variances, any change in soil management practices as well as the natural environmental conditions (Wu *et al*., 2016; Martin-Sanz *et al*., 2018; Zhou *et al*., 2018). A number research findings have demonstrated the effect of altitudinal variances on the activity of enzymes *viz*., dehydrogenase, β- galactosidase and fluorescein diacetate among others in the hilly and mountainous ecological regions of the whole world.

### a. Dehydrogenase (DHA)

Enzyme activity particularly dehydrogenase, is a marker of microbial community and functions. DHA represent changes in biochemical processes and SOM dynamics caused by human-induced changes in abiotic and biotic soil components (Meena and Rao, 2021).

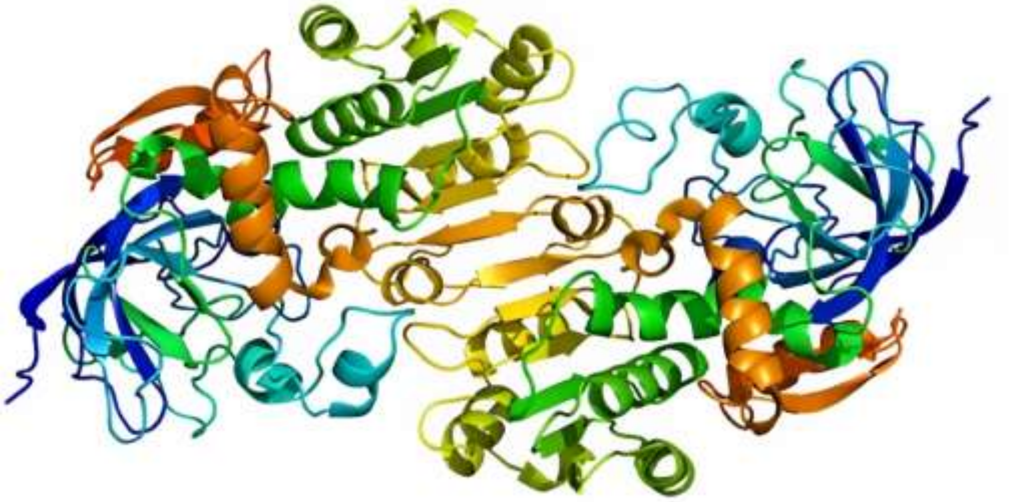

**Fig. 2.4:** Soil Dehydrogenase Model (Adapted from Protein Data Bank: 1m6h; (Sanghani *et al*., 2002).

The activity of these enzyme, is used as an indicator of microbial function in soil (Vasic *et al*., 2022; Ujjainiya *et al*., 2022). The activity of this in soil environment is stabilised by ionic, hydrogen, or covalent interactions with humic components already present in the soil (Rahul *et al*., 2022). Dehydrogenases are most common members of oxidoreductase enzyme class (Gu *et al*., 2009). Moreover, they are inextricably associated with microbial oxido-reduction activities (Wolinska and Zofia, 2012). Therefore, changes in natural environmental conditions brought about by altitudinal variances cause a significant fluctuations on dehydrogenase and its dynamics, which in turn influence the wetland health status in terms of soil biochemical and metabolic processes. Thus, understanding the reactions of dehydrogenase with respect to elevation differences in the alpine wetlands more in detail is critical for assessing the soil quality for better management practices (Maini *et al*., 2020). Massaccesi *et al*. (2020) reported that higher altitudes associated with low soil temperatures (about 2.5 °C) reduced soil microbial community, functions and dehydrogenase activity, thus, promoting soil organic carbon build-up.

### b. β- galactosidase (βGaA)

β- Galactosidase is a hydrolyse enzyme which catalyses the hydrolysis of beta-galactose into carbon or energy source (Singh *et al*., 2023). Such hydrolysis plays a crucial role in enabling soil microorganisms to access energy sources necessary for their survival (Singh *et al*., 2023).

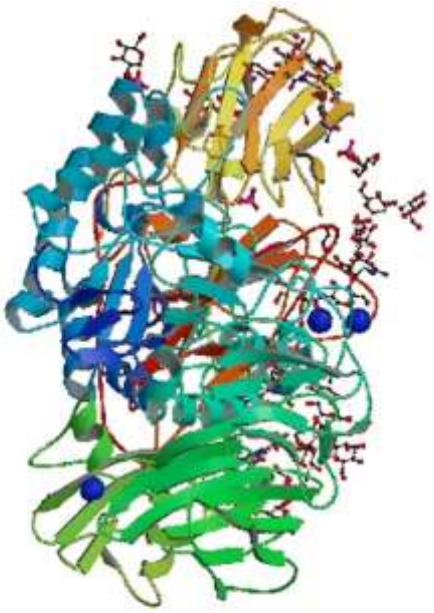

**Figure 2.5:** A simplified β-galactosidase model (Rojas *et al*., 2004).

It holds significant importance in the final disintegration process of complex sugars into simple sugars. βGa enzyme can easily detect any differences in the soil environment, directly linked with quantity of SOM, and is deemed as a promising soil quality indicator to evaluate variations induced by altitude and other climatic factors in the natural ecosystem (Ekenler and Tabatabai, 2003).

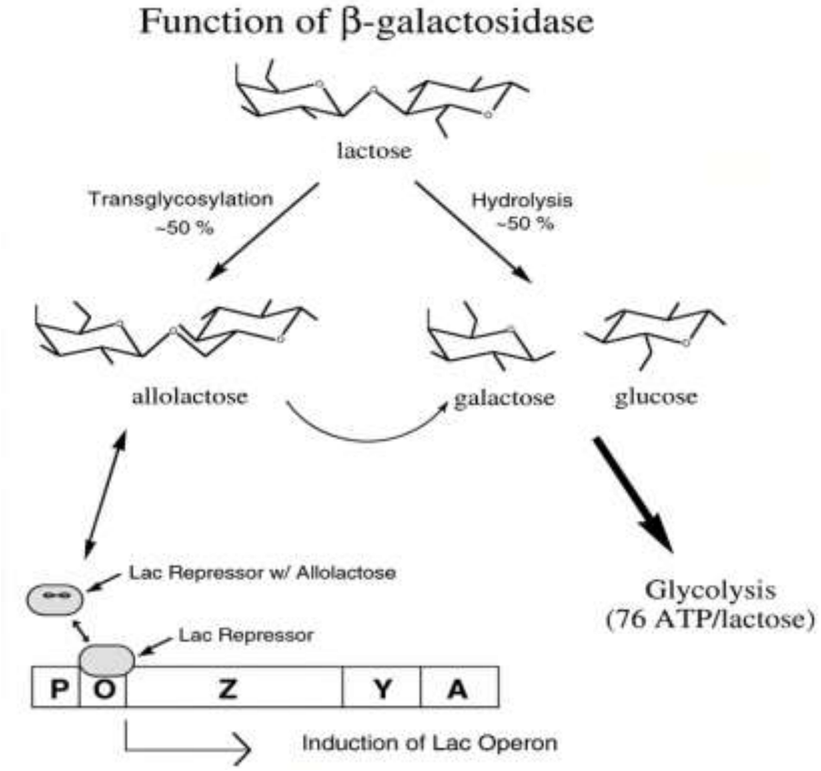

**Figure 2.6:** Schematic precis of the roles played by β- galactosidase in a cell (Adapted from Juers *et al*., 2012).

Fan *et al*. (2021) observed that the degraded wetland has resulted in reduced vegetation biomass, diminishing eco-enzyme activities and inputs of soil nutrient availability. The investigation of Fan *et al*. (2021), in the eastern margin of the Qinghai–Tibet Plateau in South Western China indicated that the altitudes from 3600 to 3800 meters were an ecological transition belt where most of the nutrients and enzyme activities (β-1,4-glucosidase) reached their lowest levels. The results of the study conducted by Wu *et al*. (2022) in Helan Mountains, Northwest China indicated that the relative abundance of the activity of nitrogen-acquiring enzymes, specifically N-Acetyl-β-D-glucosaminidase, in the soil showed an initial rise followed by a decline as altitude increased.

### c. Fluorescein Di-acetate

Fluorescein diacetate (FDA) activity is widely used as an indirect measure to assess the metabolic functions of soil microbial communities (Saikia *et al*., 2019). This method reflects the hydrolytic enzyme actions carried out by soil microbes and serves as an estimate of their total biological activity (Dutta *et al*., 2010). FDA hydrolysis has also been put forward as an essential attribute for soil quality assessment and carries a dormant ability to widely demonstrate enzyme functionality (Shaw and Burns, 2006) as well as the build-up of biological impacts as it is hydrolysed by a number of distinct groups of extracellular enzymes that are linked with microbial decomposition of SOC (Shaw and Burns, 2006). The study of Sun *et al.* (2024) reported a significant impact of altitude on hydrolase enzymes, with the least activities obtained from 2100–2400 m above sea level.

### V. Impact of altitudinal variation on soil quality.

A soil quality index (SQI) represents a streamlined collection of essential soil attributes that, when considered together, yield quantitative insights into how effectively a soil can carry out specific functions (Mukherjee and Lal, 2014). A higher SQI value, indicates high soil quality (Huang *et al*., 2021). A soil quality indicator represents a quantifiable attribute that affects how well soil can carry out a particular role (Acton and Padbury, 1993). Soil quality itself describes the ability of a given soil type to maintain and enhance the growth and survival of plants and animals in both natural and managed settings, all while safeguarding environmental integrity (NRCS, 2012).

Soil quality is threatened by rising human population and by the fact that most of the land is intensively used for different purposes (Paz-Ferreiro and Fu, 2016). Human interventions have notably shaped the ecological condition of the Bayanbulak wetland, at times intensifying or offsetting the impacts of climate variability (Kayumba *et al*., 2021). Grazing activities within alpine meadows have markedly altered vegetation development, overall biomass production, and ecosystem stability, leading to a decline in the soil quality index (SQI) (Jiang *et al*., 2024; Xiang *et al*., 2024; Zhang *et al*., 2002). The better SQI (0.78) was observed at the tree higher altitude site, ascribed to the greater content of SOC (Chandel *et al*., 2018). It is well established and documented that SOC is one of the paramount indicators for maintaining the SQ through carbon and nutrient cycling processes (Chandel *et al*., 2018). However, soil degradation is one of the factors contributing towards the decrement in SQ, estimated by the changes in soil properties (Ganiyu, 2018).

## Chapter-III

# MATERIALS AND METHODS

The current study, entitled ***“Impact of Altitudinal Variation on Selected Soil Properties and Carbon Dynamics in the Alpine Wetlands of Lesotho”***, forms part of the ongoing project led by Dr. Knight Nthebere, *“Carbon Modelling and Omics Approaches for Screening of Soil Microbes for Climate Change Adaptation in the Alpine Wetlands of Lesotho.”* This project, initiated in November 2024, is being implemented across six alpine wetlands located within three sub-catchments; two in Khubelu, Senqunyane, and Sani, beginning in March 2025, funded by the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) and the German Government, with financial management coordinated by the WaterNet Trust. This chapter outlines the materials utilized and the procedures implemented throughout the course of the research.

### 3. 1 Description of the study area

The present study was undertaken within Lesotho at elevations ranging from 2500 to 3089 meters above sea level in the mountain agro-ecological zones (AEZs) of Mokhotlong and Ha-Mohale in three sub-catchments, namely, Khubelu, Senqunyane and Sani with the Upper Senqu main catchment in Lesotho. In each catchment, the two alpine wetlands were deemed for the current research, resulting in six alpine wetlands.

In the Khubelu catchment, the Lets’eng-la-Likhama and Koting-sa-ha Ramosetsana wetlands were selected, whereas in the Senqunyane catchment, the Khorong and Tenesolo wetlands were chosen. In the Sani catchment, the Khamoqana and Khalong-la-Lichelete wetlands were selected. In Lesotho’s mountainous agro-ecological regions, shrubs are among the primary plant types thriving at higher elevations. The geographical map of Lesotho with ten districts within the African continent and the study sites (alpine wetlands) is illustrated in Figure 3.1. The GPS map showing the main catchment (Upper Senqu) in which the three sub-catchments (Khubelu, Senqunyane and Sani) falls under and wetlands is shown in Figure 3.2.

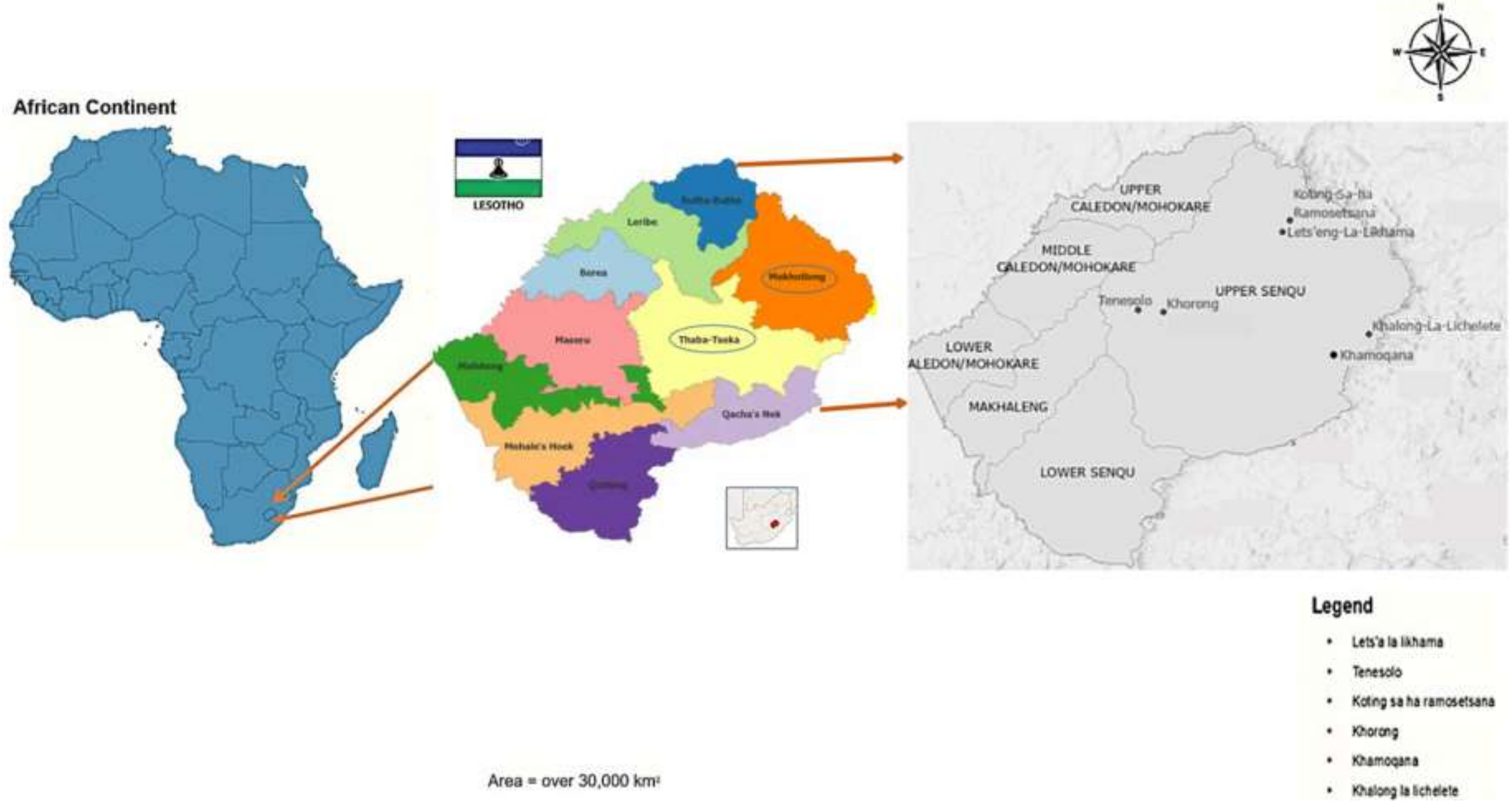

**Figure 3.1:** Geographical map of Lesotho with ten districts within the African continent and the study sites (alpine wetlands). The circled districts (Mokhotlong and Thaba-Tseka) were the districts where the study was undertaken. The black dots indicate the alpine wetland.

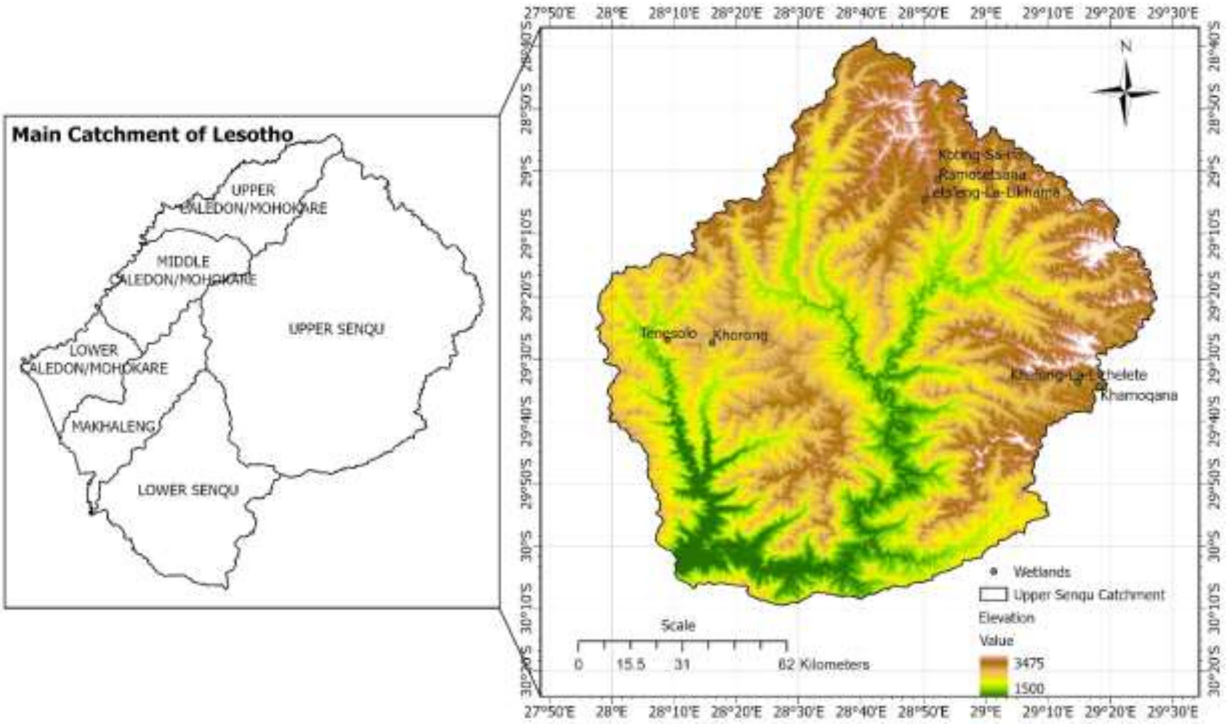

**Figure 3.2:** The global positioning system (GPS) map showing the main catchment (Upper Senqu) in which the three sub-catchments (Khubelu, Senqunyane and Sani) falls under and wetlands.The map is generated in Arc GIS software (Version 3.5.x).

### 3.1.1 Tenesolo wetland

Tenesolo (TNL) is an alpine wetland, situated in the mountain agro-ecological zone (AEZ) in Senqunyane catchment area (Figure 3.3). It is positioned at an elevation of 2552- 2600 meters asl, and at points latitude: -29.457168 S and Longitude: 28.268082 E The geological composition of this region is characterized by formations identified as Lesotho genesis (Schmitz and Rooyani, 1987). The population is too small in this site, as it is utilized only by human-beings living in the cattle posts; nevertheless, there is a remarkable destruction done to the wetland caused by burning of the vegetation, previous overgrazing of the land and burrowing by the ice rats. Using the WET-Health version 2.0- An advanced collection of methods designed to evaluate the current condition and extent of degradation within wetland ecosystems, the percentage score (PES) for soil degradation level of this wetland was 45%, indicating "extensively altered and alterations in ecological functions accompanied by the disappearance of natural habitats and native species." as fully described in Table 3.1. The average annual rainfall often recorded for this area is 1000 mm (Malebajoa, 2010).

**Figure 3.3:** Overview of Tenesolo alpine wetland

### 3.1.2 Khorong wetland

Khorong (KRN) is an alpine wetland located in the mountain agro-ecological zone (AEZ) in Senqunyane catchment area at an altitude between 2500 to 2550 meters asl, at points; Latitude: -29.449256 S and longitude 28.149214 E (Figure 3.4).

**Figure 3.4:** Overview of Khorong alpine wetland

The geology of this area is Lesotho's genesis (Schmitz and Rooyani, 1987). The type of land use (LUT) around this place is cropping, and the level of soil degradation was 80% assessed using WET-Health version 2.0 (Table 3.1).The mean annual rainfall often recorded for this area is 1000 mm (Malebajoa, 2010).

### 3.1.3 Khamoqana wetland

Khamoqana (KMQ) is an alpine wetland, situated in the mountain agro-ecological zone (AEZ) in Sani catchment area (Figure 3.5). It is positioned at an altitude of 2839-2880 meters a.s.l, and at point's latitude -29.457178 S and longitude 28.268094 E. The geological composition of this region is characterized by formations identified as Lesotho genesis (Schmitz and Rooyani, 1987). The population is too small in this area. There is burrowing by ice rats contributing towards the formation of holes, soil erosion within the wetland area due to running water, sparse and shallow rooted vegetation. The percentage score (PES), assessed with WET Health version 2.0 for soil degradation level of this wetland, was 30% indicating "seriously modified, the change in ecosystem processes, great loss of natural habitat and biota but some remaining natural habitat features still being recognized" as shown in Table 3.1. The average annual rainfall often recorded for this area is 1044 mm (Malebajoa, 2010).

**Figure 3.5:** Overview of Khamoqana alpine wetland

### 3.1.4 Khalong-La- Lichelete wetland

Khalong-La-Lichelete (KLL) is an alpine wetland, located in the mountain agro-ecological zone (AEZ) in Sani catchment area (Figure 3.6). It is at an altitude of 2891-

2995 meters a.s.l, and at point's latitude: -29.563552 S and longitude: 29.247207 E. The geological composition of this region is characterized by formations identified as Lesotho genesis (Schmitz and Rooyani, 1987). The population is too less in this area, as it is used by the human-beings staying in the cattle posts. The vegetation surrounding the wetland is deep rooted (Figure 3.4). The percentage score (PES), assessed with WET Health version 2.0 for soil degradation level of this wetland, was 90%, indicating unmodified natural wetland as illustrated in Table 3.1. The average annual rainfall often recorded in this place is 1044 mm (Malebajoa, 2010).

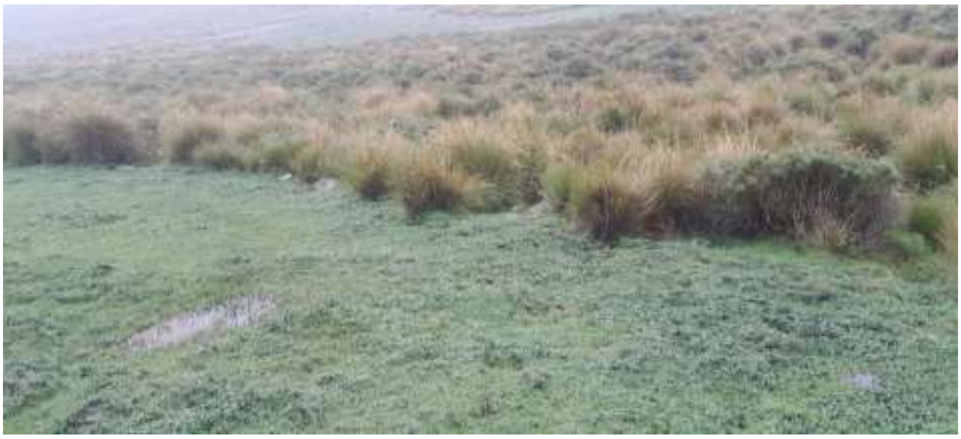

**Figure 3.6:** Overview of Khalong-La- Lichelete alpine wetland

### 3.1.5 Lets'eng -La- Likhama

Lets'eng-La- Likhama (LLL) is an alpine wetland, positioned in the mountain agro-ecological zone (AEZ) in Khubelu catchment area (Figure 3.7). It is at an altitude of 3040-3080 m a.s.l, and at point's latitude: -29.076355 S and longitude: 28.836095 E. The geological composition of this region is characterized by formations identified as Lesotho genesis (Schmitz and Rooyani, 1987). The area is only used by the people living in the animal posts. There is less and shallow grass, soil erosion due to less vegetation cover and overgrazing practices as indicated by the sheeps within the wetland.

**Figure 3.7:** Overview of Lets'eng-La- Likhama alpine wetland

The percentage score (PES), assessed with WET Health version 2.0 for soil degradation level of this wetland, was 40% *i.e*., "largely modified with a large change in ecosystem processes and loss of natural habitat, and biota has occurred" as shown in Table 3.1. The average annual rainfall often recorded for this site is 1044 mm (Malebajoa, 2010).

### 3.1.6 Koting-Sa-ha Ramosetsana wetland

Koting-Sa-ha Ramosetsana (KSRN) is an alpine wetland, positioned in the mountain agro-ecological zone (AEZ) in Khubelu catchment area at an altitude of 3087-3155 meters a.s.l, and at point's latitude: -29.022686 S and longitude: 28.871324 E (Figure 3.8). The geology of this area is Lesotho genesis (Schmitz and Rooyani, 1987). The area is only used by the people living in the animal posts. The vegetation surrounding the wetland is dominated by the shrub. The grass is within the wetland. The percentage score (PES) for soil degradation level of this wetland, obtained with WET Health version 2.0 was 85%, indicating "largely natural with few modifications and a slight change in ecosystem processes being discernible and a small loss of natural habitats and biota may have taken place" as fully described in Table 3.1. The average annual rainfall often recorded for this area is 1044 mm (Malebajoa, 2010).

**Figure 3.8:** Overview of Koting-Sa-ha Ramosetsana alpine wetland.

**Table 3.1: Ecological Class utilised for percentage score (PES) evaluations of inland aquatic ecosystems in South Africa, along-with the applicable range of PES Scores for each Class (Kleynhans, 1996; Macfarlane *et al*., 2009) [colour-coding is as per the River Eco-Status Monitoring Programme (REMP) of DWS].**

| ECOLOGICAL CLASS | INTERPRETATION | PES SCORE (%) |
|---|---|---|
| **A** | Unmodified, natural. | 90-00 |
| **B** | Largely natural with few modifications. A slight change in ecosystem processes is discernible and a small loss of natural habitats and biota may have taken place. | 80-89 |
| **C** | Moderately modified. A moderate change in ecosystem processes and loss of natural habitats has taken place but the natural habitat remains predominantly intact. | 60-79 |
| **D** | Largely modified. A large change in ecosystem processes and loss of natural habitat and biota and has occurred. | 40-59 |
| **E** | Seriously modified. The change in ecosystem processes, loss of natural habitat and biota is great but some remaining natural habitat features are still recognizable. | 20-39 |
| **F** | Critically modified. Modifications have reached a critical level and ecosystem processes have been modified completely with an almost complete loss of the natural habitat and biota. | 0-19 |

## 3.2 Climate

Lesotho's climate is primarily shaped by its location at the heart of the Southern African Plateau. It can be described as sub-humid to temperate-cool, characterized by warm, rainy summers and cold, dry winters (Grundling *et al*., 2015). During winter, the average minimum temperatures typically hover around 0 °C in June, which is the coldest month. In the lowland regions, winter temperatures often fall between –1 °C and –3 °C, while in the highlands, they can drop further to about –6 °C to –8.5 °C (Malebajoa, 2010). Mean annual temperatures are approximately 15.2 °C in the lowlands and about 7 °C in the highlands.

January usually records the country's highest average maximum temperatures, with values reaching up to 32 °C in the lowlands and around 20 °C in the highlands. Annual rainfall varies across the country, ranging from roughly 500 mm to 1,200 mm, particularly higher in the northern and eastern areas. Most precipitation is around 85% and falls between October and April. In the mountainous regions, winter frequently brings frost and snowfall.

## 3.3 Study methods

### 3.3.1 The design and treatments

A block design was employed in which altitudinal variation across alpine wetlands was grouped into blocks (catchments) with comparable features, particularly regarding their condition (degraded versus healthy) and elevation range, to minimize variability. By accounting for these characteristics, the treatment effects (altitudinal variation equivalent to alpine wetlands) were accurately measured. The altitude range was treated as a factor, represented by six alpine wetlands selected from three sub-catchments: Khubelu, Senqunyane, and Sani, *i.e.*, two wetlands from each catchment. The sub-catchments with treatment details (altitude equivalent to alpine wetlands) are shown in Table 3.2.

### 3.3.2 Wetlands selection criteria

The study area covered altitudes ranging from 2,500 to 3,155 meters above sea level (asl). Alpine wetland's selection was done based on the altitude of ≥3000 m asl, ≥2500 m asl and ≥2800 m asl in Khubelu, Senqunyane and Sani sub-catchment areas, respectively, considering one degraded (being the control) and healthy from each sub-catchment to make a total of six alpine wetlands, equivalent to altitudinal variations. The assessment of each wetland health status was achieved with WET HEALTH version 2.0 (Kleynhans, 1996; Macfarlane *et al*., 2008).

### 3.3.3 Selection of sampling points

The sampling points in each wetland were selected via a stratified random sampling method, and the points within each wetland were selected via a grid line pattern to select a representative sample that meets the soil sampling objectives (Paulsen *et al*., 1991). Each wetland was subdivided into four equal grids to obtain four replications.

### 3.2: Treatment details within the sub-catchments (blocks)

| Sub-Catchments (Blocks) | Treatment(s) | |
|---|---|---|
| | **Alpine Wetlands** | **Altitude (m) asl** |
| Senqunyane | Khorong | 2500-2550 |
| | Tenesolo | 2552-2600 |
| Sani | Khamoqana | 2839-2880 |
| | Khalong-La-Lichelete | 2891-2995 |
| Khubelu | Lets'eng- La-Likhama | 3040-3080 |
| | Koting-Sa-ha Ramosetsana | 3087-3155 |

### 3.4 Characteristics of study

### 3.4.1 Soil Sample(s) Collection and preparation

Soil sampling was carried out in February 2025 (autumn season, *i.e.*, wet season) through a stratified random sampling method in each wetland, by collecting soil from 0 to 15 cm depth at multiple locations to make 10 sub-samples, for each replication resulting in a composite mixture. There were four replications taken per site. The soil was sampled at depth of 0–15 cm. The gathered soil samples were combined to form composites, air-dried in the shade, gently ground, and sieved through 2.0 mm and 0.5 mm mesh screens. After proper labelling, the samples were stored in polyethylene bags. Subsequent analyses of their physical, chemical, physico-chemical, and biological characteristics were performed using established standard methods.

### 3.5 Parametric analysis for soil

### 3.5.1 Physico-chemical attributes of the soil

### 3.5.1.1 Soil reaction (pH)

The soil pH was measured by preparing a 1:2.5 ratio suspension of soil to water, allowing it to equilibrate for half an hour with intermittent stirring, and then recording the pH using a glass electrode pH meter (Jackson, 1973).

### 3.5.1.2 Electrical Conductivity

Electrical conductivity (EC) was assessed using a 1:2.5 ratio of soil to water suspension. After allowing the mixture to stabilize and settle until a clear supernatant formed, the EC of the solution was measured using a conductivity meter, following the method described by Jackson (1973).

### 3.5.1.3 Cation Exchange Capacity and Cation Availability

Five grams of soil sample was taken into a beaker to which little NH4OAc was added, stirred to swirl and allowed to remain stationary for about ten minutes. A 250ml Erlenmeyer flask was mounted and filtration was done with a Whatman No.1 filter paper. The washing of the soil mixture was done onto the filter paper in the funnel with

NH4OAc, the filtrate was collected; the volume was adjusted upto 100 ml with NH4OAc, and stored (filtrate one). The soil residual was washed with about 100 mL of the NaOAc solution to saturate all the exchange sites with $Na^+$ and the filtrate was thrown away. The $Na^+$ in excess was washed with about 100 ml of (~4 full funnels) of pure alcohol and the filtrate was then discarded. The adsorbed $Na^+$ was replaced by washing the soil with about 100 ml of NH4OAc, collecting the filtrate and made up the volume to 100 ml with NH4OAc and stored (filtrate two). The cations ($Ca^{++}$, $Mg^{++}$, $K^+$ and $Na^+$) in filtrate one and CEC as $Na^+$ in filtrate 2 were read on the Atomic Absorption Spectrometer (Thomas, 1982).

### 3.5. 1.4 Soil Organic Carbon

The concentration of Soil Organic Carbon was estimated using the method originally described by Walkley and Black (1934). For this analysis, soil samples were sieved to obtain particles passing through an 80–100 mesh screen. A measured 1.0 g portion of the sieved soil was placed in an Erlenmeyer flask, to which 10 ml of potassium dichromate solution and 20 ml of concentrated sulphuric acid were added. The mixture was left undisturbed for 30 minutes to allow digestion to proceed. After this period, 100 ml of distilled water was incorporated into the flask, followed by a small quantity of sodium fluoride and several drops of diphenylamine indicator. The resulting violet-colored solution was then titrated with 0.5N ferrous ammonium sulphate until a green endpoint was reached.

### 3.5.1.5 Available Soil Nitrogen

The alkaline permanganate procedure outlined by Subbiah and Asija (1956) was employed for determination of available soil nitrogen.

### 3.5.1.6 Available Soil Phosphorous

The concentration of available phosphorus in the soil was assessed following the procedure described by Olsen and colleagues (1954), which utilizes a 0.5 M sodium bicarbonate solution for extraction. The phosphorus content was subsequently measured with a double-beam spectrophotometer set to a wavelength of 660 nm.

### 3.5.1.7 Available Soil Potassium

The concentration of exchangeable potassium in the soil samples was assessed by extracting with a neutral 1N ammonium acetate solution, followed by measurement on a flame photometer as outlined by Jackson (1973).

### 3.5.2 Soil Physical properties

### 3.5.2.3 Soil texture

The texture of the soil was analysed using hydrometer method (Bouyoucos 1927).

### 3.5.2.4 Bulk density

The core sampler method was employed to estimate the bulk density (Blake and Hartge, 1986).

### 3.5.2.5 Saturated Hydraulic conductivity

The saturated hydraulic conductivity (Ksat) was assessed employing a constant head approach derived from Darcy's law principles. Intact soil cores were prepared by saturating them in the laboratory. This was accomplished by positioning the samples on fabric-covered perforated plates within a tray approximately 25 cm deep, maintaining a water depth of 5 cm to achieve full saturation. Once saturated, each soil core was attached to an additional core placed above it. Siphon tubes linked to a constant head apparatus were used to maintain a steady water level of 0.05 meters over the soil column. The quantity of water infiltrating through the sample was recorded at specified time intervals.

Finally, the saturated hydraulic conductivity was computed following the procedure described by Klute and Driksen (1986), applying the formula under;

$$K_S = \frac{Q.L}{t.A.\Delta H}$$

$K_s$= saturated hydraulic conductivity (m $s^{-1}$)
Q = volume of water flowing out in time 't' (liters $s^{-1}$)
L= length of soil column (m)
A = cross sectional area ($m^2$)
$\Delta H$ = difference in head at inlet and outlet (m)
t = time(s)

### 3.5.2.6 Maximum water holding capacity

A clean and dry Keen's cup was prepared, and a circular filter paper, trimmed to fit the base of the cup precisely, was placed inside. The combined weight of the empty Keen's cup and filter paper was recorded. Finely sieved soil (passed through a 0.2 mm mesh) was gently added into the cup, tapping lightly to ensure uniform packing until the container was filled. Any surplus soil was leveled off using a spatula or glass rod. The cup was then positioned in a tray containing water, with the water level maintained approximately 2 cm below the bottom of the cup to allow for capillary saturation over 24 hours.

After the 24-hour period, any water that rose above the soil due to swelling was carefully removed using a sharp blade. The following day, the cup was removed from the tray and left to drain naturally for 30 minutes. The weight of the cup containing the saturated soil was then recorded. The soil-filled cup was subsequently placed in a drying oven set at 105°C and kept for 24 hours or until the sample achieved a constant weight. After drying, the final weight of the cup with oven-dried soil was measured. Additionally, the internal diameter and height of the Keen's cup were recorded using Vernier calipers.

The soil's maximum water holding capacity was determined using the Keen-Raczkowski method (1921) and calculated according to the formula given below;

$$\text{Maximum water holding capacity (MWHC)} = \frac{(W_{bds} - W_b) \times 100}{(W_{bds} - W_b)}$$

Weight of the Keen cup + filter paper = $W_b$ (g)
Weight of the Keen cup + filter paper + oven- dried soil = $W_{bds}$ (g)

#### 3.5.2.7 Infiltration rate

A double-ring infiltrometer setup, comprising an inner ring 30 cm in diameter and an outer ring 60 cm in diameter, each standing 30 cm tall, was utilized to measure the water infiltration rate during the study (Bouwer, 1986). Before starting the experiment, the soil's initial moisture level was assessed. The rings were driven into the soil to a depth of approximately 5 cm at the center of a uniform, nearly level plot using a hammer. Water was then added to both rings, maintaining a ponded depth between 20 and 25 cm.

The fall of water level in the inner ring was recorded at 2, 5, 10, 15, 30, 60, 120 minutes and thereafter on hourly basis till the water infiltration was constant. More water was added into the rings when the water level fell by 4-5 cm in order to check the drastic water level fluctuations. Using the scale, the water level and time just before and after response were recorded. The time interval was kept short as mentioned above in order to avoid errors caused by intake during the refilling period. Finally, the values of infiltration rate and cumulative infiltration were plotted as integral of time.

### 3.6 Carbon dynamics (pools of Soil organic carbon)

Carbon dynamics (fractions of soil organic carbon) were quantified using a modified version of the Walkley and Black procedure outlined by Chan *et al*. (2001). To separate the carbon pools, 5, 10, and 20 ml of concentrated sulphuric acid were applied, generating acid-to-water ratios of 0.5:1, 1:1, and 2:1. These corresponded to acid normalities of 12.0N, 18.0N, and 24.0N, respectively (Table 3.3). This approach enabled the partitioning of total soil organic carbon into distinct fractions.

**Table 3.3: Carbon dynamics**

| **S. No** | **Pools of soil organic carbon Chan *et al*. (2001)** | |
|---|---|---|
| 1 | Very labile carbon (Pool-I) | Organic C oxidizable with 12.0 N $H_2SO_4$ |
| 2 | Labile carbon (Pool-II) | SOC extracted with 12.0 N $H_2SO_4$ minus SOC extracted with 18.0 N |
| 3 | Less labile carbon (Pool-III) | SOC extracted with 18.0 N $H_2SO_4$ minus SOC extracted with 24.0 N |
| 4 | Non-labile carbon (Pool-IV) | Obtained by subtraction of Pool-I, Pool-II and Pool-III from TOC |
| 5 | Total organic carbon (TOC) Jha *et al*. (2014) | $\log_{10}$ TOC= 0.725×$\log_{10}$ (Walkley-black carbon) + 0.198 × $\log_{10}$ (silt + clay) – 0.0759 × $\log_{10}$ (mean annual rainfall) + 0.015 |

## 3.7 Soil enzyme activity

The moisture content of the soil samples was assessed following the procedure outlined by Monteiro and Frighetto (2000). The resulting data were then applied to calculate the measured enzyme activities on an oven-dry weight basis.

### 3.7.1 Dehydrogenase activity

Approximately one gram of soil was accurately weighed into screw-cap test tubes. To each sample, 50 milligrams of calcium carbonate were incorporated, followed by the addition of 2.5 millilitres of distilled water and 1 millilitre of a 3% solution of triphenyl tetrazolium chloride (TTC). The mixture was gently swirled to ensure thorough mixing and then left to incubate at ambient temperature for a period of 24 hours. Upon completion of incubation, several millilitres of methanol were introduced to the tubes, and the mixture was shaken to aid in dissolving the red-coloured precipitate formed. The solution was subsequently filtered, and the final volume was adjusted to 25 millilitres using methanol. The absorbance of the developed red colour was recorded at a wavelength of 485 nanometers as described by Casida and colleagues (1964).

### 3.7.2 Fluorescein Di-acetate activity

One gram of soil sample was weighed into a test tube to which 50 ml THAM buffer was added, followed by 0.5 ml FDA substrate and 0.5 ml of acetone to controls. The mixture was incubated for 3 hours at 37 °C. Swirlingwas done to terminate the FDA hydrolysis. After hydrolysis, the substrate was added to controls. The contents were mixed and filtered. The color intensity was measured at 490 nm (Green *et al*., 2006).

### 3.7.3 β- Galactosidase activity

A soil sample weighing one gram was placed into a flask, to which 0.2 ml of toluene was introduced. The mixture was left inside a fume hood for approximately 15 minutes.

Subsequently, 4 ml of modified universal buffer (MUB) adjusted to pH 6 and 1 ml of p-nitrophenyl-β-D-galactopyranoside solution were added. The preparation was incubated at 37°C for one hour. Upon completion of the incubation period, 1 ml of 0.5 M calcium chloride solution was incorporated, followed by the addition of 4 ml of 0.1 M THAM buffer at pH 12 to all samples. After the reaction was stopped, the substrate was introduced into the blank controls. The contents were mixed thoroughly and filtered. Yellow colour intensity was measured at 405 nm (Eivazi and Tabatabai, 1988).

### 3.8 Statistical Analysis

The collected soil data underwent statistical evaluation through analysis of variance (ANOVA), adhering to the procedure for a single-factor completely randomized design, as described by Panse and Sukhatme (1989). To assess the significance of treatment means at the 5% probability threshold, the critical difference was calculated using the "R" statistical software. Additionally, Pearson correlation coefficients were computed to explore associations among soil parameters. Principal component analysis (PCA) was also conducted to identify key indicator variables and to derive the minimum dataset (MDS). These analyses were carried out with the help of the SQI-CAL tool, developed by Mohanty (2020).

### 3.9 Quantification of Soil Quality Index (SQI)

The influence of variations in altitude on soil quality was assessed using a weighted index approach (Mukherjee and Lal, 2014), implemented through the SQI CAL software— a soil health evaluation tool that employs principal component analysis (PCA) techniques. This application performs PCA to distill a minimal dataset from the measured soil parameters (Thakur *et al.*, 2024). The derived principal components (PCs) integrate contributions from all evaluated soil variables and are sequenced so that the initial PCs account for most of the variance present within the dataset. PCs displaying higher eigenvalues were regarded as more representative of the variability in soil properties. Therefore, only those PCs with eigenvalues exceeding one were retained in subsequent analysis.

Within each PC, every soil variable received a factor loading indicating its relative influence on that component. For constructing the soil quality index (SQI), only variables with substantial factor loadings were selected. If multiple parameters with high loadings appeared within a single PC, multivariate correlation analysis was applied to determine their interrelationships and identify any redundancy, potentially excluding closely associated variables from the SQI (Nthebere *et al.*, 2025). Where highly weighted variables showed no significant correlation, all were preserved as relevant contributors.

In cases where strong correlations were observed among variables, the parameter with the highest absolute factor loading was chosen for inclusion in the index (Nthebere *et al.*, 2025). Weights for each selected parameter were determined based on the proportion of variance explained by the respective PC (Nthebere *et al.*, 2025). The variance percentages were normalized so that their total equaled one, thereby assigning appropriate weights to each parameter within a component (Nthebere *et al.*, 2025). To prepare for SQI estimation, input data were compiled in CSV format and uploaded into SQI CAL, where PCA was performed following the workflow presented in Figure 3.9.

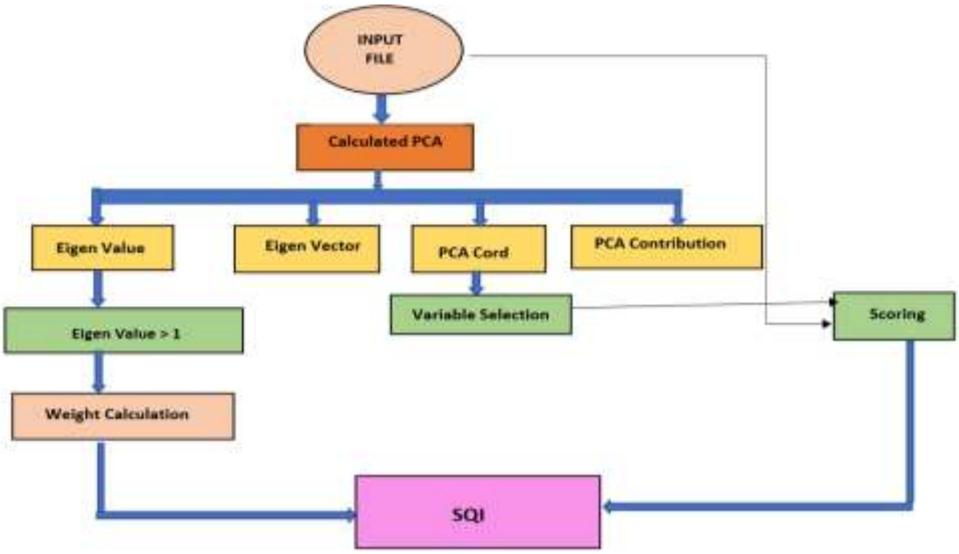

**Figure 3.9:** Schematic flow diagram of soil quality indexing by SQI CAL (Mohanty, 2020).

PCs with eigenvalues greater than one were individually extracted. Based on the computed eigen values, factor loadings for each component, and observed correlations among soil properties, parameters were chosen from the PCA outputs (with a tolerance range of ±10%) to form the minimum data set (MDS) while minimizing redundancy (Singh *et al.*, 2013; Panwar *et al.*, 2022). The selected parametric or variable indicators were normalized through homothetic linear transformations, guided by three evaluative criteria: (a) less is better, (b) more is better, and (c) optimum is better (Nthebere *et al.*, 2025) (Table 3.4). Each indicator was assigned a weight derived from the proportion of variance it contributed within its respective principal component, relative to the total cumulative variance of all principal components retained for variable and minimum data set (MDS) selection (Nthebere *et al.*, 2025). Finally, the Soil Quality Index based on PCA

($SQI_{PCA}$) was determined in accordance with Equation 1, incorporating the revised weights and the scoring results outlined below;

$$SQI_{PCA} = \sum_{i=1}^{n} SixWi$$

In this context, *Si* indicates the linear value attributed to each indicator and Wi corresponds to the computed weight factor.

**Table 3.4: Grading curve of distinct soil parameters considered for calculation of soil quality index (Nthebere *et al*., 2025)**

| S. No | Indicators | Grading curve |
|---|---|---|
| 1 | Sand | Optimum is better |
| 2 | Silt | More is better |
| 3 | Clay | More is better |
| 4 | Bulk Density | Less is better |
| 5 | Saturate hydraulic conductivity | Optimum is better |
| 6 | Infiltration rate | Optimum is better |
| 7 | Maximum water holding capacity | More is better |
| 8 | pH | Optimum is better |
| 9 | Electrical Conductivity | Less is better |
| 10 | Cation Exchange Capacity | More is better |
| 11 | Magnesium | More is better |
| 12 | Calcium | More is better |
| 13 | Sodium | Less is better |
| 14 | Soil organic carbon | More is better |
| 15 | Available soil nitrogen | More is better |
| 16 | Available soil phosphorus | More is better |
| 17 | Available soil potassium | More is better |
| 18 | Dehydrogenase activity | More is better |
| 19 | Fluorescein Di-acetate | More is better |
| 20 | β-Galactosidase | More is better |
| 21 | Active carbon pool | More is better |
| 22 | Passive carbon pool | More is better |
| 23 | Total organic carbon | More is better |

## Chapter-IV

# RESULTS

The findings obtained from the current study entitled, **"Impact of Altitudinal Variation on Selected Soil Properties and Carbon Dynamics in the Alpine Wetlands of Lesotho"** are presented in this chapter. The soil data was statistically analysed and results were presented in the form of tables and graphs and interpretations were made only for significant findings based on the statistical parameters. These results are discussed critically with the cause and relationship effect under the these sections;

4.1 Impact of altitudinal variation on physical and hydraulic attributes

4.2 Impact of altitudinal variation on soil physico-chemical attributes

4.3 Impact of altitudinal variation on macronutrients (nitrogen, phosphorus and potassium) and cation availability

4.4 Impact of altitudinal variation on soil enzyme activity (dehydrogenase, β-galactosidase and fluorescein di-acetate activity)

4.5 Impact of altitudinal variation on soil carbon dynamics

4.6 Impact of altitudinal variation on soil quality index

## 4.1 Impact of altitudinal variation on soil physical and hydraulic attributes

### 4.1.1 Soil particle size distribution (PSD) and texture

The soil PSD and texture were significantly influenced by altitudinal variation of selected alpine wetlands. The PSD and texture results are presented in Table 4.1.

The proportion of sand, silt and clay particles ranged from 39.79 – 64.98%, 21.92–35.72% and 10.45 – 25.19%, respectively. There was a decreasing trend in sand content with increasing altitude across the alpine wetlands. The significantly higher sand content was observed at Khorong, Tenesolo and Khalong-La-Lichelete, while significantly lower sand proportions were recorded at Lets'eng-La-Likhama and Koting-sa- ha Ramosetsana (Table 4.1). Silt and Clay contents showed increasing trend with altitude, with clay percentage increase from 10.45% to 25.19% in Tenesolo to Koting-sa-ha Ramosetsana (Table 4.1). The soil texture was sandy loam in Khorong, Tenesolo and Lets'eng-la-Likhama (Table 4.1). The texture was categorized into loam in wetlands with altitude higher than others in this study.

**Table 4.1: Impact of Altitudinal Variation on soil particle size distribution and texture**

| **Treatment(s)** | | **Sand** | **Silt** | **Clay** | **Textural Class** |
|---|---|---|---|---|---|
| **Wetlands** | **Altitude (m) asl** | **(%)** | | | |
| Khorong | 2500-2550 | 64.98 | 23.67 | 11.36 | Sandy loam |
| Tenesolo | 2552-2600 | 60.63 | 28.92 | 10.45 | Sandy loam |
| Khamoqana | 2839-2880 | 52.82 | 34.04 | 13.14 | Loam |
| Khalong-La- Lichelete | 2891-2995 | 64.43 | 21.92 | 13.65 | Sandy loam |
| Lets'eng- La-Likhama | 3040-3080 | 46.38 | 35.72 | 17.90 | Loam |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 39.79 | 35.02 | 25.19 | Loam |
| **SE (m)±** | | 0.279 | 0.140 | 0.245 | |
| **CD (P<0.05)** | | 0.868 | 0.307 | 0.764 | |

CD (P<0.05) = Critical Difference at less than 5% probability level; SE(m) = Standard Error of the mean;

### 4.1.2 Soil bulk density

The altitudinal variation in the alpine wetlands resulted in significant changes in soil bulk density (BD) in the surface layer (0-15 cm). The BD ranged from 1.09 – 1.52 Mg m$^{-3}$ across the altitudinal wetlands. BD decreased with increasing altitude and was significantly lower (1.09 Mg m$^{-3}$) and higher (1.52 Mg m$^{-3}$) at Koting-Sa-ha Ramosetsana and Tenesolo, respectively. Data on BD is presented in Table 4.2 and figure 4.1a.

### 4.1.3 Maximum water holding capacity

The results on maximum water holding capacity (MWHC) as influenced by altitude in the wetlands demonstrated significant difference (Table 4.2 and Figure 4.1b).

The MWHC variation was from 43.90-57.51% across the whole wetlands elevation. The significantly higher MWHC (57.51%) was pronounced under Koting-Sa-ha Ramosetsana (KSHM): 3087-3155 m a.s.l, while the least MWHC (43.90%) was recorded at Tenesolo (TNL): 2552-2600 m a.s.l in comparison with other altitudes in all the wetlands under the study. There was no consistent pattern observed on MWHC as affected by the altitudinal variation within the wetlands.

### 4.1.4 Saturated hydraulic conductivity (*Ksat*)

The impact of altitudinal variation in the alpine wetlands, assessed in this study had illustrated significant influence on *Ksat* in the soil surface (0-15 cm) as presented in Table 4.2 and Figure 4.1c.

The *Ksat* varied from 1.28- 2.70 cm hr$^{-1}$ across the altitudes in the alpine wetlands with significantly higher *Ksat* (2.70 cm hr$^{-1}$) under Koting-Sa-ha Ramosetsana (KSHM): 3087-3155 m a.s.l, followed by Letseng-la- Likhama (LLL): 3040-3080 m a.s.l, Khorong (KRN): 2500-2550 m a.s.l and lower (1.28 cm hr$^{-1}$) at Tenesolo (TNL): 2552-2600 m a.s.l among all other altitudes in the wetlands (Table 4.2 and Figure 4.1c). There was no trend observed on *Ksat*.

### 4.1.5 Infiltration rate

The infiltration rate (IR) was significantly impacted by altitude difference of the alpine wetlands. The IR ranged from 1.20- 2.17 cm hr$^{-1}$. The results are presented in Table 4.2 and Figure 4.1d. The significantly higher rate of infiltration (2.17 cm hr$^{-1}$) was observed under Koting-Sa-ha Ramosetsana (KSHM): 3087-3155 m a.s.l in over all other alpine wetlands (on the basis of altitude).

**Table 4.2: Impact of Altitudinal Variation on physical and hydraulic attributes of the soil at 0-15 cm soil**

| **Treatment(s)** | | **Bulk Density (Mg m$^{-3}$)** | **Hydraulic conductivity** | **Infiltration Rate** | **Water Holding Capacity (%)** |
|---|---|---|---|---|---|
| **Wetlands** | **Altitude (m) asl** | | **cm hr$^{-1}$** | | |
| Khorong | 2500-2550 | 1.30 | 2.60 | 2.11 | 50.04 |
| Tenesolo | 2552-2600 | 1.52 | 1.28 | 1.20 | 43.90 |
| Khamoqana | 2839-2880 | 1.28 | 1.50 | 1.30 | 46.20 |
| Khalong-La- Lichelete | 2891-2995 | 1.26 | 2.63 | 2.16 | 47.00 |
| Lets'eng- La-Likhama | 3040-3080 | 1.27 | 1.52 | 1.19 | 51.82 |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 1.09 | 2.70 | 2.17 | 57.51 |
| **SE (m)±** | | 0.016 | 0.111 | 0.085 | 1.814 |
| **CD (P<0.05)** | | 0.049 | 0.345 | 0.266 | 5.651 |

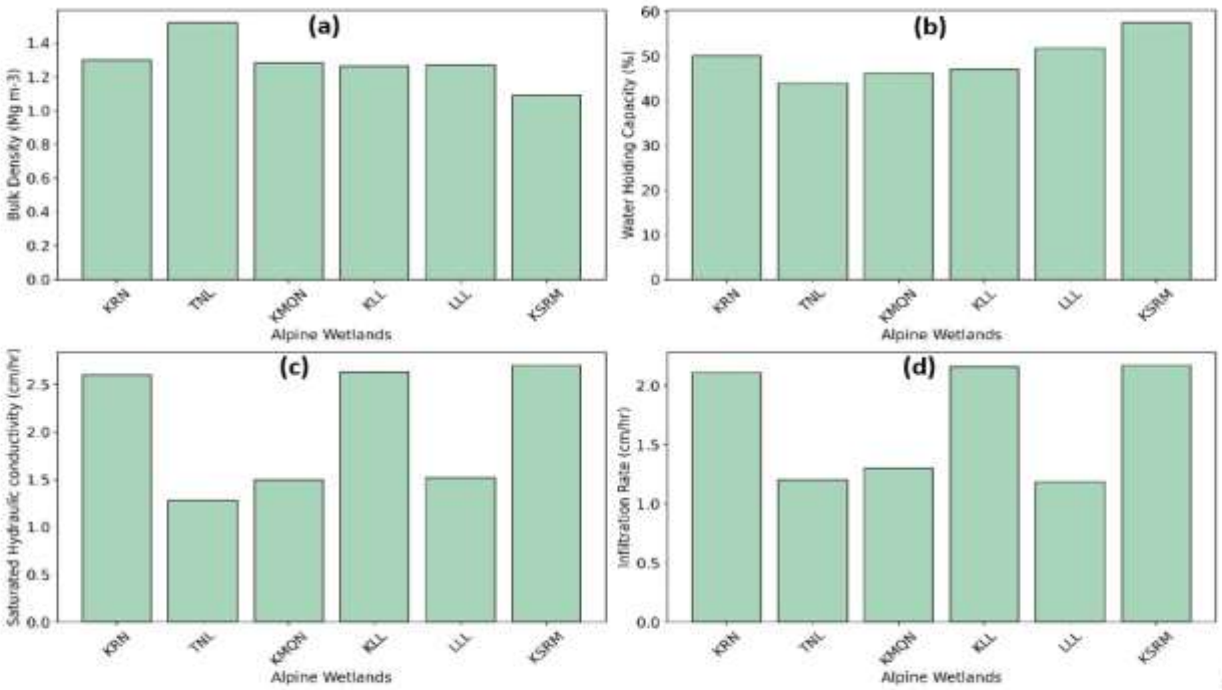

**Figure 4.1:** Impact of altitudinal variation on bulk density at 0-15 cm **(a)**, water holding capacity at 0-15 cm **(b)**, saturated hydraulic conductivity **(c)** at 0-15 cm soil depth and infiltration rate **(d)**. KRN= Khorong (2500-2550m asl); TNL= Tenesolo (2552-2600m asl); KMQN= Khamoqana (2839-2880m asl); KLL= Khalong-la-Lichelete (2891-2995m asl); LLL= Lets'eng-la-Likhama (3040-3080m asl); KSRM=Koting-sa-ha Ramosetsana (3087-3155m asl).

## 4.2 Impact of altitudinal variation on soil physico-chemical attributes

### 4.2.1 Soil pH

Soil pH was significantly impacted by altitude in the alpine wetlands. pH varied from 5.53-6.04 with the lower unit (5.53) and higher unit (6.04) under Lets'eng-la- Likhama (LLL): 3040-3080 m a.s.l and Khorong (KRN): 2500-2550 m a.s.l, respectively. There was no consistent trend in soil pH values in relation to altitudinal variation. The pH values indicated slightly acidic soil condition across the whole wetlands. The data is presented in Table 4.3 and Figure 4.2a.

### 4.2.2 Electrical conductivity

Soil electrical conductivity (EC) was not significantly influenced by the variation in altitude of the alpine wetlands depicted in Table 4.3 and Figure 4.2b. The soil EC varied from 0.29- 0.35 dS $m^{-1}$ across the wetlands, indicating low salinity in selected wetland soils.

### 4.2.3 Cation exchange capacity

Cation Exchange Capacity measures a soil's ability to hold and exchange positively charged ions. The cation exchange capacity (CEC) varied from 21.50- 31.04

(cmol) ($P^+$) $kg^{-1}$ across the alpine wetlands. However, the influence of altitude in these wetlands on CEC was non-significant (Table 4.3 and Figure 4.2c). CEC among wetlands was not non-significant statistically. Numerically, CEC was 31.04 (cmol) ($P^+$) $kg^{-1}$ higher at 3087-3155 m a.s.l *i.e.*, Koting-Sa-ha Ramosetsana (KSHM) and lower (21.50 (cmol) ($P^+$) $kg^{-1}$) at 2552-2600 m a.s.l *i.e.*, Tenesolo (TNL).

### 4.2.4 Organic carbon

The status of organic carbon (OC) varied significantly with altitudinal variation in the alpine wetlands with ranges from 69.14- 95.80 g $kg^{-1}$. The SOC was significantly higher (95.80 g $kg^{-1}$) at Koting-Sa-ha Ramosetsana (KSHM): 3087-3155 m a.s.l, compared to all other alpine wetlands at different elevations. The Tenesolo (TNL): 2552-2600 m a.s.l was observed with the least SOC (69.14 g $kg^{-1}$) among all wetlands. In general, the observations indicated that SOC increased with increasing altitude across the wetlands. The SOC data is presented in Table 4.3 and Figure 4.2d.

**Table 4.3: Impact of altitudinal variation on Physico-chemical properties of the soil**

| **Treatment(s)** | | **CEC (cmol) ($P^+$) $kg^{-1}$** | **pH** | **EC (dS $m^{-1}$)** | **SOC (g $kg^{-1}$)** |
|---|---|---|---|---|---|
| **Wetlands** | **Altitude (m) asl** | | | | |
| Khorong | 2500-2550 | 28.94 | 5.76 | 0.34 | 84.67 |
| Tenesolo | 2552-2600 | 21.50 | 6.04 | 0.35 | 69.14 |
| Khamoqana | 2839-2880 | 26.45 | 5.98 | 0.35 | 73.24 |
| Khalong-La- Lichelete | 2891-2995 | 27.93 | 5.80 | 0.33 | 80.27 |
| Lets'eng- La-Likhama | 3040-3080 | 26.10 | 5.53 | 0.29 | 94.34 |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 31.04 | 6.01 | 0.32 | 95.80 |
| **SE (m)±** | | 1.939 | 0.072 | 0.014 | 8.28 |
| **CD (P<0.05)** | | NS | 0.223 | NS | 18.24 |

CD ($P< 0.05$) = Critical Difference at less than 5% probability level; SE(m) = Standard Error of the mean; asl= Above sea level; CEC= Cation exchange capacity; EC= Electrical conductivity; SOC=Soil organic carbon; NS= Non-significant.

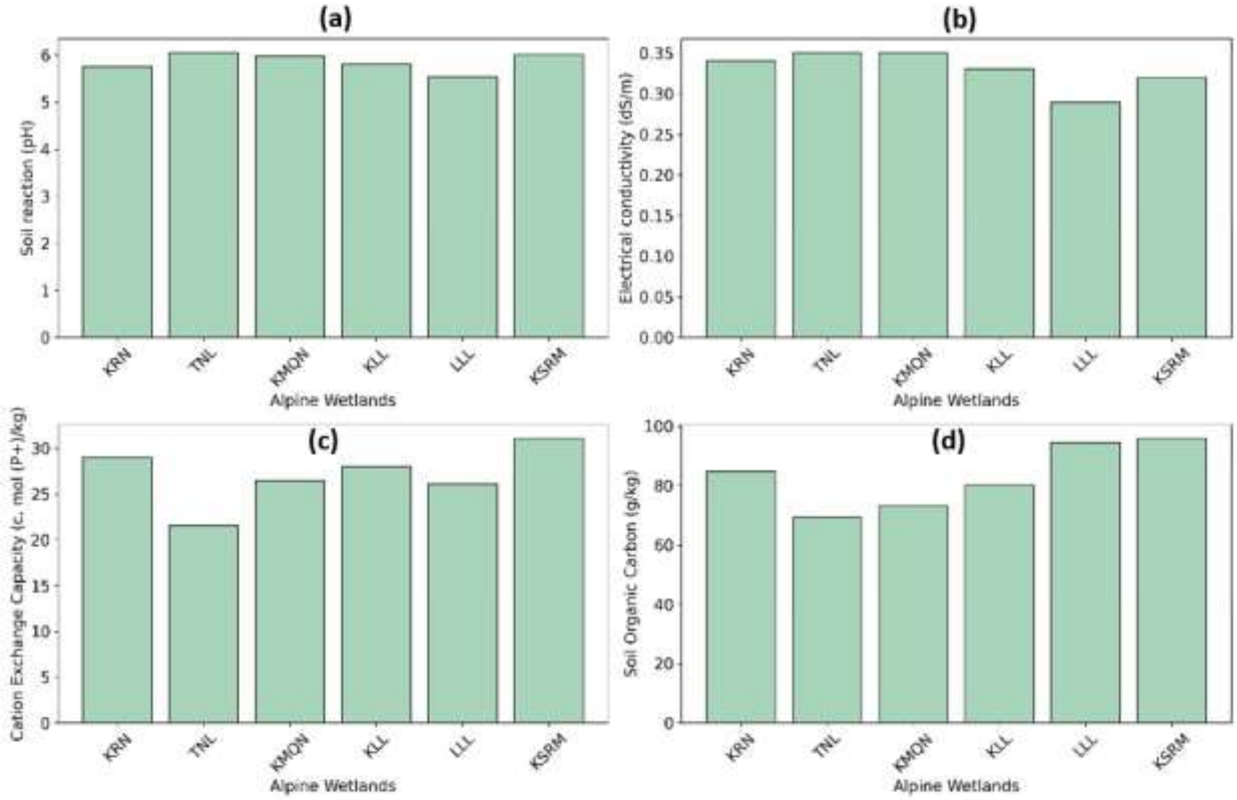

**Figure 4.2:** Impact of altitudinal variation on pH (a), electrical conductivity (b), cation exchange capacity (c) and soil organic carbon (d) at 0-15 cm soil depth. KRN= Khorong (2500-2550m asl ); TNL= Tenesolo (2552-2600m asl); KMQN= Khamoqana (2839-2880m asl); KLL= Khalong-la-Lichelete (2891-2995m asl); LLL= Lets'eng-la-Likhama (3040-3080m asl ); KSRM=Koting-sa-ha Ramosetsana (3087-3155m asl).

## 4.3 Impact of altitudinal variation on soil macronutrient and cation availability

### 4.3.1 Soil macronutrient availability (Nitrogen, Phosphorus and Potassium)

The variation of altitude in the alpine wetlands did not result in any significant influence on soil macronutrient availability *viz*., nitrogen (N), Phosphorus (P) and potassium (K) (Table 4.4 and Figure 4.3a, b, c). The available soil N, P and K varied from 373.65-878.08 kg $ha^{-1}$, 132.63-196.27 g $kg^{-1}$ and 190.88-302.68 mg $kg^{-1}$, respectively across the altitudes within the wetlands.

**Table 4.4: Impact of altitudinal variation on the soil macronutrient availability**

| **Treatment(s)** | | **Nitrogen (kg $ha^{-1}$)** | **Phosphorus (g $kg^{-1}$)** | **Potassium (mg $kg^{-1}$)** |
|---|---|---|---|---|
| **Wetlands** | **Altitude (m) asl** | | | |
| Khorong | 2500-2550 | 873.08 | 188.93 | 299.38 |
| Tenesolo | 2552-2600 | 373.65 | 132.63 | 190.88 |
| Khamoqana | 2839-2880 | 595.84 | 186.09 | 196.62 |
| Khalong-La- Lichelete | 2891-2995 | 864.44 | 192.62 | 202.38 |
| Lets'eng- La-Likhama | 3040-3080 | 627.20 | 148.53 | 215.17 |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 878.08 | 196.27 | 302.68 |
| **SE (m)±** | | 188.799 | 25.339 | 34.160 |
| **CD (P<0.05)** | | NS | NS | NS |

CD (P<0.05) = Critical Difference at less than 5% probability level; SE(m) = Standard Error of the mean; NS= Non-significant.

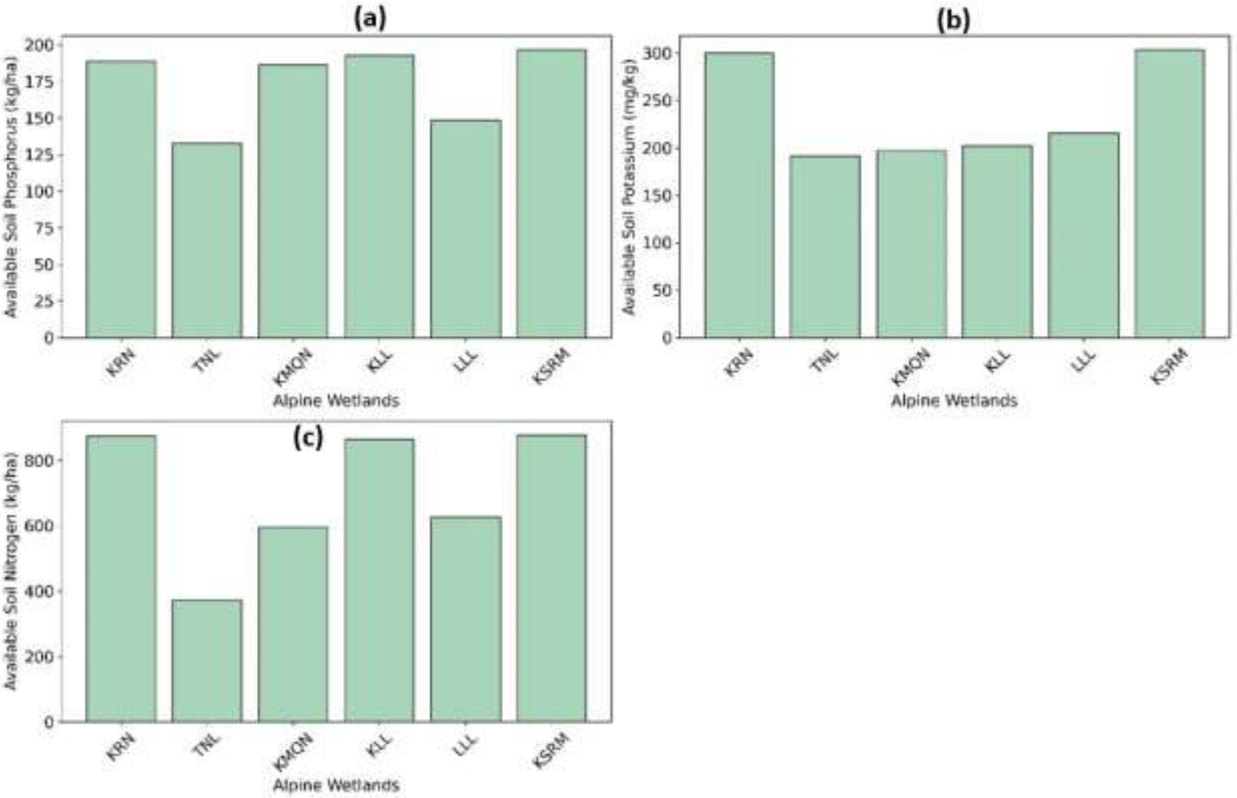

**Figure 4.3:** Impact of altitudinal variation on Phosphorus **(a)**, Potassium **(b)** and Nitrogen **(c)** at 0-15 cm soil depth. KRN= Khorong (2500-2550m asl ); TNL= Tenesolo (2552-2600m asl); KMQN= Khamoqana (2839-2880m asl); KLL= Khalong-la-Lichelete (2891-2995m asl); LLL= Lets'eng-la-Likhama (3040-3080m asl ); KSRM=Koting-sa-ha Ramosetsana (3087-3155m asl).

### 4.3.2 Soil cation availability (Calcium, Magnesium and Sodium)

The cation availability was not significantly influenced by altitudinal variation in the alpine wetlands except calcium (Ca). The variation for calcium (Ca), magnesium (Mg) and sodium (Na) availability was from 4190.60-4901.39 mg kg$^{-1}$, 779.62-901.38 mg kg$^{-1}$ and 59.52-85.70 mg kg$^{-1}$, respectively across the altitudes within the wetlands. The soil cation availability results are presented in Table 4.5 and Figure 4.4a, b, c.

**Table 4.5: Impact of altitudinal variation on the Cation Availability**

| **Treatment(s)** | | **Ca** | **Mg** | **Na** |
|---|---|---|---|---|
| **Wetlands** | **Altitude (m) asl** | **mg kg$^{-1}$** | | |
| Khorong | 2500-2550 | 4795.36 | 891.69 | 82.28 |
| Tenesolo | 2552-2600 | 4190.60 | 779.62 | 59.52 |
| Khamoqana | 2839-2880 | 4532.20 | 839.46 | 76.17 |
| Khalong-La- Lichelete | 2891-2995 | 4793.00 | 870.00 | 81.48 |
| Lets'eng- La-Likhama | 3040-3080 | 4723.05 | 864.20 | 77.31 |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 4901.39 | 901.38 | 85.70 |
| **SE (m)±** | | 97.004 | 128.346 | 7.156 |
| **CD (P=<0.05)** | | 302.210 | NS | NS |

CD (P< 0.05) = Critical Difference at less than 5% probability level; SE(m) = Standard Error of the mean; asl= Above sea level; Ca= Calcium; Mg= Magnesium; Na= Sodium; NS= Non-significant.

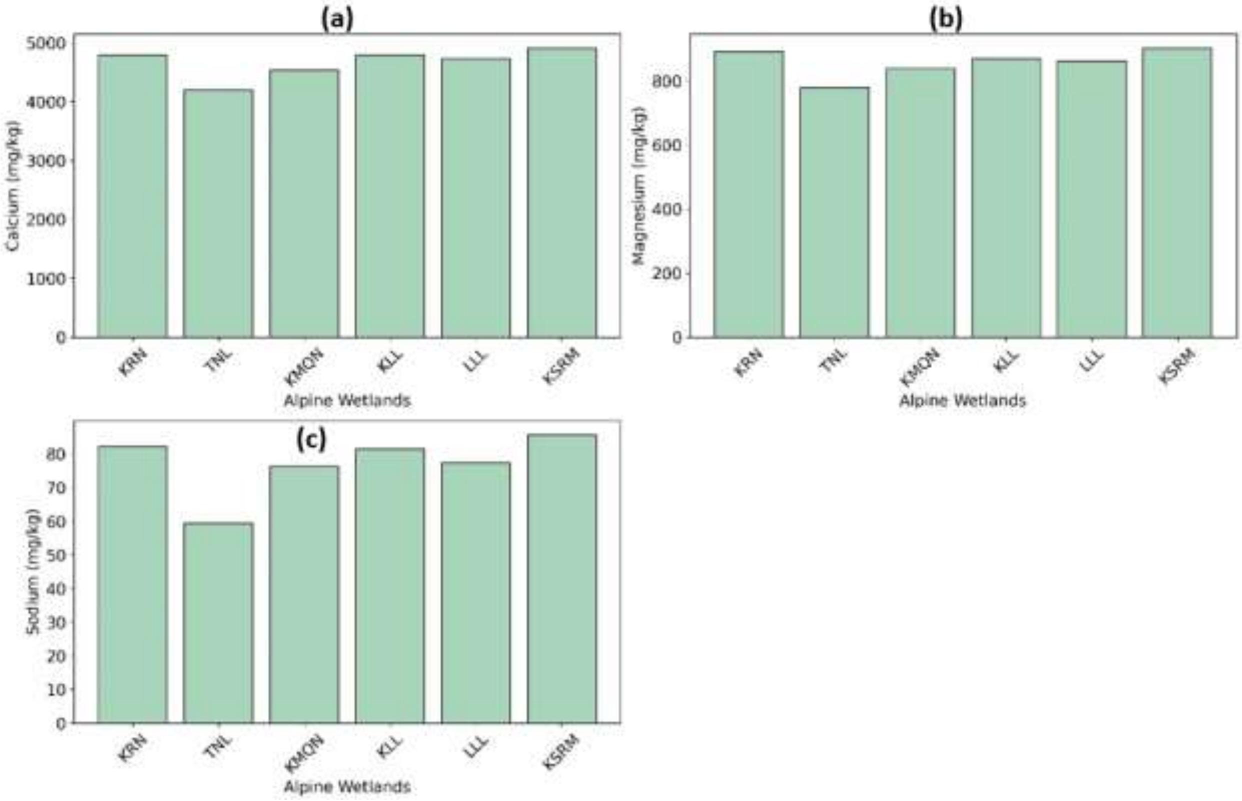

**Figure 4.4:** Impact of altitudinal variation on calcium **(a)**, Magnesium **(b)** and Sodium **(c)** at 0-15 cm soil depth. KRN= Khorong (2500-2550m asl ); TNL= Tenesolo (2552-2600m asl); KMQN= Khamoqana (2839-2880m asl); KLL= Khalong-la-Lichelete (2891-2995m asl); LLL= Lets'eng-la-Likhama (3040-3080m asl ); KSRM=Koting-sa-ha Ramosetsana (3087-3155m asl).

The Ca availability was observed to be significantly higher (4901.39 mg kg$^{-1}$) under Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l), followed by Khorong (2500-2550 m a.s.l) with 4795.36 mg kg$^{-1}$ being statistically on par with Khalong-la-Lichelete (2891-2995 m a.s.l) with 4793.00 mg kg$^{-1}$. The availability was significantly lower (4190.60 mg kg$^{-1}$) under Tenesolo (2552-2600 meters a.s.l) based on treatment comparison. However, the availability for both Ca and Mg was in high range, whereas that of Na was in low range as per the ratings.

### 4.4 Impact of altitudinal variation on soil enzyme activity (dehydrogenase, β-galactosidase and fluorescein di-acetate activity).

Soil dehydrogenase activity (DHA), β-galactosidase activity (β-GaA) and fluorescein di-acetate activity (FDA**)** was significantly influenced by altitudinal variation in the alpine wetlands. The activity of DHA, β-GaA and FDA varied from 29.03-49.63 µg TPF g$^{-1}$ dry soil day$^{-1}$, 126.49- 173.22 nmol *p*-nitrophenol g$^{-1}$ dry soil hr$^{-1}$ and 119.01- 227.72 µg. Fluorescein g$^{-1}$ dry soil 3hr$^{-1}$, respectively. The results are presented in Table 4.6 and Figure 4.5a, b, c.

**Table 4.6: Impact of altitudinal variation on dehydrogenase (DHA), β-Galactosidase (β-GaA) and fluorescein di-acetate (FDA) activity**

| Treatment(s) | | DHA ($\mu$g TPF g$^{-1}$ dry soil day$^{-1}$) | β-GaA (nmol *p*-nitrophenol g$^{-1}$ dry soil hr$^{-1}$) | FDA ($\mu$g.Fluorescein g$^{-1}$ dry soil 3hr$^{-1}$) |
|---|---|---|---|---|
| **Wetlands** | **Altitude (m) asl** | | | |
| Khorong | 2500-2550 | 49.63 | 173.22 | 227.72 |
| Tenesolo | 2552-2600 | 35.49 | 153.23 | 188.27 |
| Khamoqana | 2839-2880 | 36.70 | 140.00 | 187.60 |
| Khalong-La- Lichelete | 2891-2995 | 39.82 | 151.44 | 220.64 |
| Lets'eng- La-Likhama | 3040-3080 | 29.03 | 126.49 | 119.01 |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 34.15 | 150.48 | 172.26 |
| **SE (m)±** | | 2.068 | 7.633 | 16.157 |
| **CD (P<0.05)** | | 6.442 | 23.779 | 50.335 |

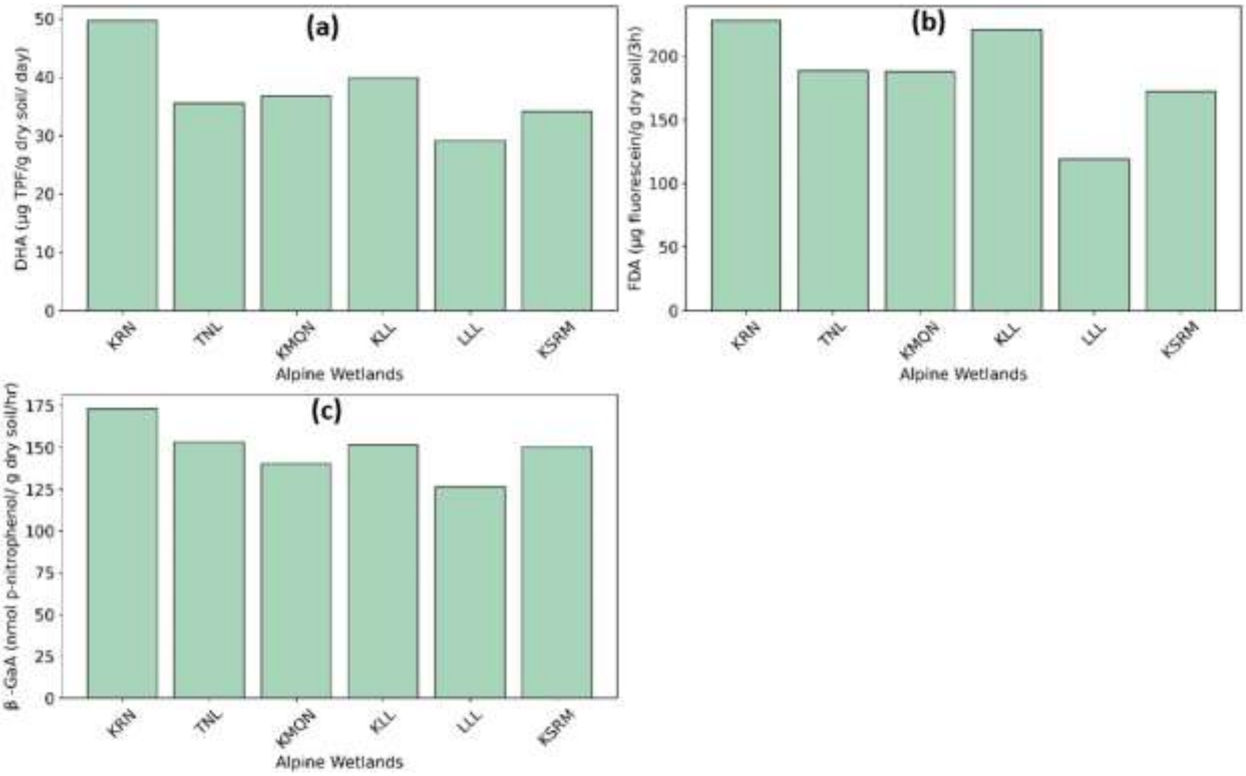

**Figure 4.5:** Impact of altitudinal variation on Dehydrogenase activity: DHA **(a)**, Fluorescein di-acetate activity: FDA **(b)** and β-Galactosidase activity: β-GaA **(c)** at 0-15 cm soil depth. KRN= Khorong (2500-2550m asl ); TNL= Tenesolo (2552-2600m asl); KMQN= Khamoqana (2839-2880m asl); KLL= Khalong-la-Lichelete (2891-2995m asl); LLL= Lets’eng-la-Likhama (3040-3080m asl ); KSRM=Koting-sa-ha Ramosetsana (3087-3155m asl).

A significant decrease on DHA, β-GaA and FDA was observed with an increase altitude. Even though the activity of these examined decreased in these alpine wetlands, based on treatment comparison, significantly higher DHA, β-GaAand FDA was observed with Khorong (2500-2550 m a.s.l), while the least was pronounced under Lets’eng-la-Likhama (3040-3080 m a.s.l) followed by Koting-sa- ha Ramosetsana (3087-3155 m a.s.l) among all the alpine wetlands under the present study.

## 4.5 Impact of altitudinal variation on carbon dynamics

### 4.5.1 Soil carbon pools

The Very labile carbon ($C_{VL}$), labile carbon ($C_L$), less labile ($C_{LL}$), non-labile ($C_{NL}$) pools and total organic carbon (TOC) were significantly influenced by altitudinal variation in the alpine wetlands. The carbon pools and TOC data is presented in Table 4.7 and Figure 4. 4.6.

Significantly higher $C_{VL}$ (21.57 g kg$^{-1}$), $C_L$ (4.46 g kg$^{-1}$), $C_{LL}$ (28.81 g kg$^{-1}$), $C_{LL}$ (54.85 g kg$^{-1}$) and TOC (109.69 g kg$^{-1}$) were observed under Koting-sa ha Ramosetsana (3087-3155 meters a.s.l) while lower $C_{VL}$ , $C_L$, $C_{LL}$, $C_{LL}$ and TOC were recorded under Tenesolo (2552-2600 meters a.s.l) among all other treatments. Although the $C_{VL}$ and $C_L$ were significantly higher in wetlands positioned at higher elevation (Koting-sa-ha Ramosetsana and Lets’eng-la-Likhama), there was no consistent trend because Khorong (2500-2550 meters a.s.l) recorded significantly higher $C_{VL}$ and $C_L$ in comparison with Khalong-la-Lichelete (2891-2995 meters a.s.l) and Khamoqana (2839-2880 meters a.s.l) (Table 4.7 and Figure 4.6) indicating better bio-physical conditions occurring at Khorong (KRN) wetland probably due to natural health status of the wetland assessed with Wet Health version 2.0 assessment tool, regardless of altitude in KRN.

**Table 4.7: Impact of altitudinal variation on soil organic carbon pools**

| **Treatment(s)** | | **$C_{VL}$** | **$C_L$** | **$C_{LL}$** | **$C_{NL}$** | **TOC** |
|---|---|---|---|---|---|---|
| **Wetlands** | **Altitude (m) asl** | **g kg$^{-1}$** | | | | |
| Khorong | 2500-2550 | 18.19 | 1.25 | 25.23 | 41.94 | 86.61 |
| Tenesolo | 2552-2600 | 14.34 | 1.14 | 19.10 | 39.43 | 74.01 |
| Khamoqana | 2839-2880 | 17.04 | 1.64 | 23.27 | 44.45 | 86.40 |
| Khalong-La- Lichelete | 2891-2995 | 17.09 | 3.35 | 26.16 | 44.66 | 91.26 |
| Lets'eng- La-Likhama | 3040-3080 | 19.19 | 4.08 | 25.39 | 45.95 | 94.61 |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 21.57 | 4.46 | 28.81 | 54.85 | 109.69 |
| **SE (m)±** | | 1.070 | 0.027 | 5.899 | 3.001 | 4.263 |
| **CD (P<0.05)** | | 3.334 | 0.084 | NS | NS | 13.282 |

CD (P< 0.05) = Critical Difference at less than 5% probability level; SE(m) = Standard Error of the mean; NS= Non-significant.

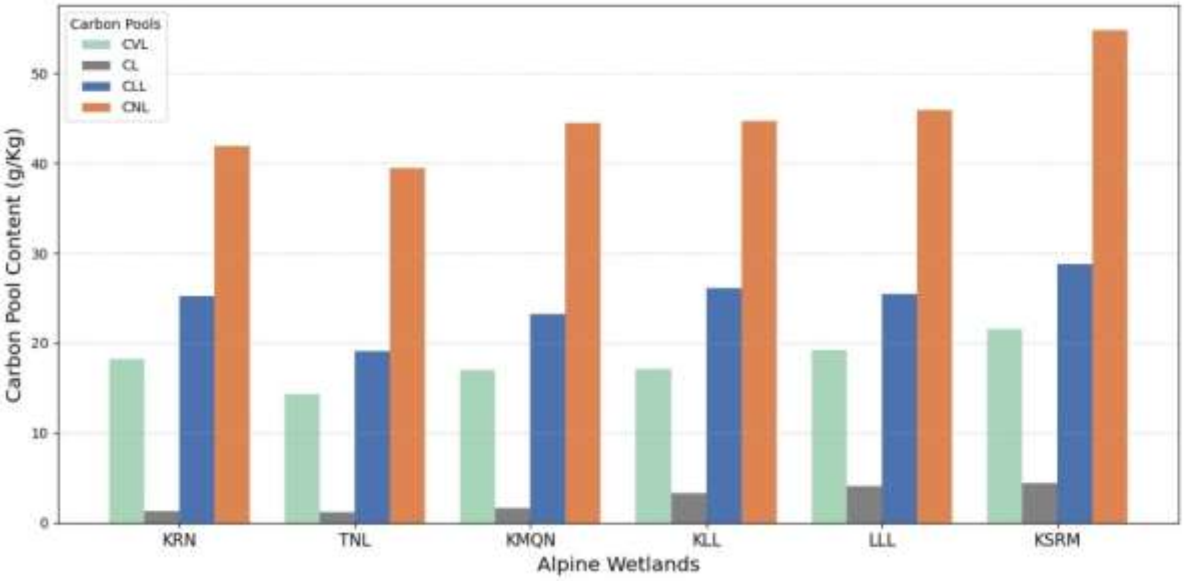

**Figure 4.6:** Impact of altitudinal variation on soil organic carbon pools *viz*., $C_{VL}$= very labile carbon; $C_L$ =labile carbon; $C_{LL}$= less labile carbon; $C_{NL}$= non-labile carbon. KRN= Khorong (2500-2550m asl); TNL= Tenesolo (2552-2600m asl); KMQN= Khamoqana (2839-2880m asl); KLL=Khalong-la-Lichelete (2891-2995m asl); LLL=Lets'eng-la-Likhama (3040-3080m asl); KSRM=Koting-sa-ha Ramosetsana (3087-3155m asl).

### 4.5.2 Active and passive pools of carbon

The active carbon ($C_{ACT}$) and passive carbon ($C_{PSV}$) pool results are presented in Table 4.8. The results indicated that both $C_{ACT}$ and $C_{PSV}$ were higher (26.04 and 83.66 g kg$^{-1}$) under Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l), respectively and lower (15.48 and 58.53 g kg$^{-1}$) in Tenesolo (2552-2600 m a.s.l), respectively. There was an increase in $C_{PSV}$ with increasing altitude across the wetlands.

### 4.5.3 Percent contribution of active and passive pools of carbon to total organic carbon

Passive carbon ($C_{PSV}$) pool was the main contributor to total organic carbon (TOC) compared to active carbon ($C_{ACT}$) pool as influenced by altitudinal variation in the alpine wetlands (Figure 4.7). The $C_{PSV}$ and $C_{ACT}$ pools contributed 75-79% and 21-25% to TOC, respectively.

The higher percent contribution of $C_{ACT}$ pools to TOC was 24-25% observed under Lets'eng- La-Likhama (3040-3080 m a.s.l) and Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) among all other wetlands (Figure 4.7), indicates that wetlands at high elevation can act as carbon-sink and storage.

**Table 4.8: Impact of altitudinal variation on the active ($C_{ACT}$) and passive ($C_{PSV}$) pools of carbon**

| **Treatment(s)** | | **$C_{ACT}$** | **$C_{PSV}$** |
|---|---|---|---|
| **Wetlands** | **Altitude (m) asl** | **g kg$^{-1}$** | |
| Khorong | 2500-2550 | 19.44 | 67.17 |
| Tenesolo | 2552-2600 | 15.48 | 58.53 |
| Khamoqana | 2839-2880 | 18.68 | 67.72 |
| Khalong-La- Lichelete | 2891-2995 | 20.44 | 70.82 |
| Lets'eng- La-Likhama | 3040-3080 | 23.27 | 71.34 |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 26.04 | 83.66 |

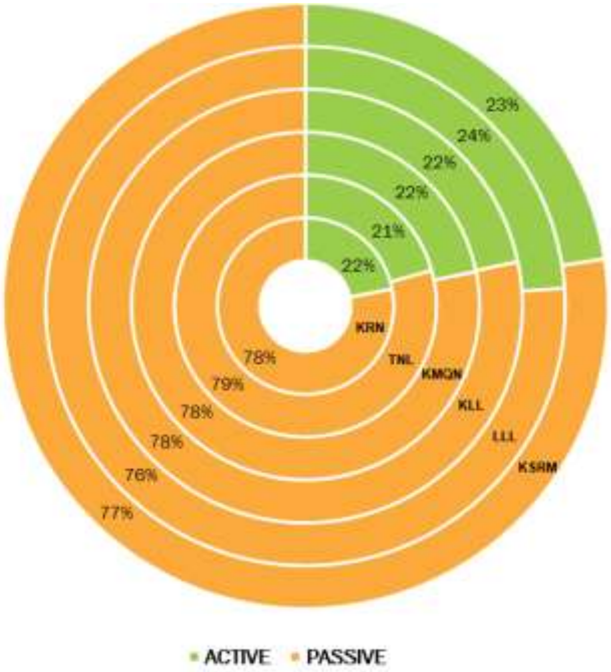

**Figure 4.7:** Impact of altitudinal variation on active and passive pools of carbon. KRN= Khorong (2500-2550m asl ); TNL= Tenesolo (2552-2600m asl); KMQN= Khamoqana (2839-2880m asl); KLL= Khalong-la-Lichelete (2891-2995m asl); LLL= Lets’eng-la-Likhama (3040-3080m asl ); KSRM=Koting-sa-ha Ramosetsana (3087-3155m asl).

## 4.6 Impact of altitudinal variation on soil quality

### 4.6.1 Identification of key indicators

After evaluating how changes in altitude affect soil quality parameters, the gathered data was used to compute soil quality indices in order to evaluate how well the treatments (representing altitudes similar to alpine wetlands) preserved soil quality. For this purpose, 23 soil quality indicators were chosen and analysed using principal component analysis (PCA). Out of these, 14 parameters showed strong positive correlations (Figure 4.8) with soil quality and exhibited significantly high factor loadings in their respective principal components (PCs).

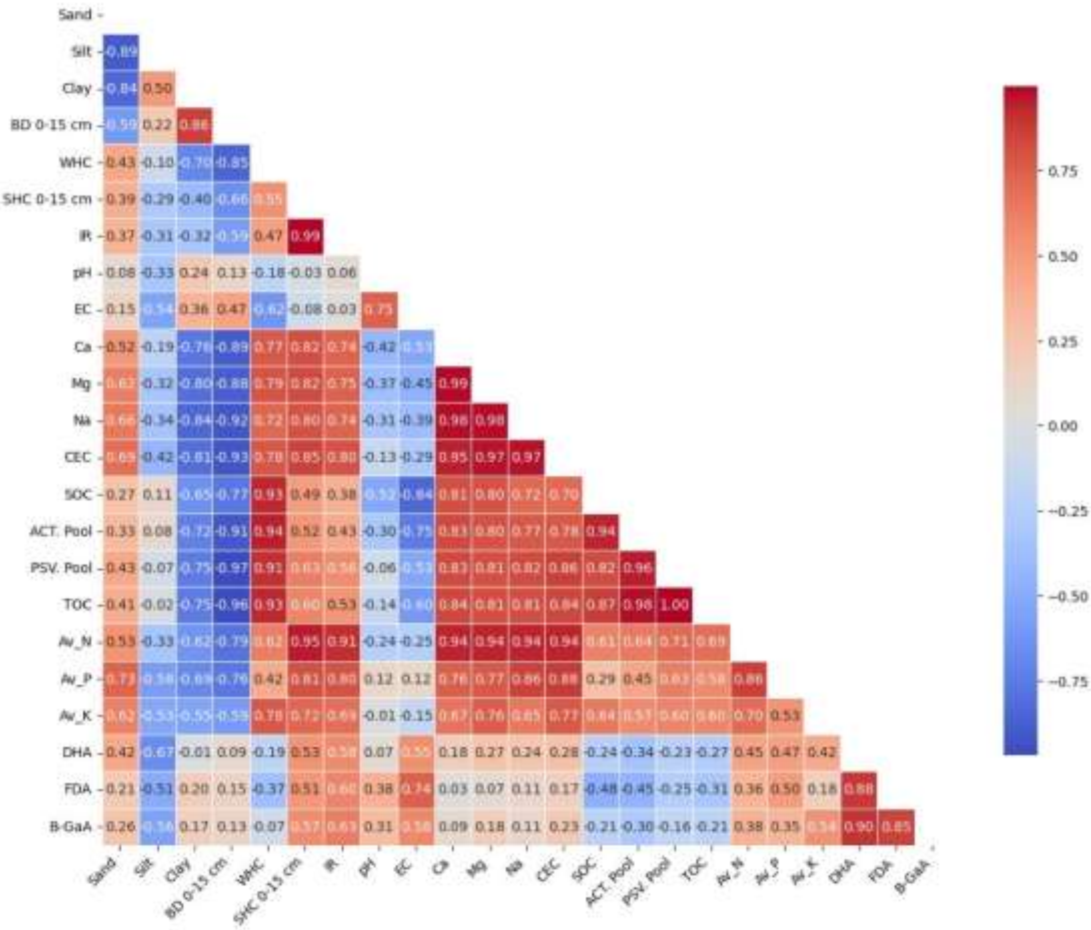

**Figure 4.8:** Graph showing correlation matrix of all variables for SQI computation as influenced by altitudinal variation in the alpine wetlands. BD 0-15 cm= Bulk density at 0-15 cm; IR= Infiltration rate; WHC=Water holding capacity; SOC= Soil organic carbon; ACT. Pool= Active pool of carbon; PSV. Pool= Passive pool of carbon; Ca=Calcium; Mg= Magnesium; Na= Sodium; EC= Electrical conductivity; SHC 0-15 cm= Saturated hydraulic conductivity at 0-15 cm; CEC=Cation exchange capacity; PSV. Pool= Passive pool of carbon; TOC= Total organic carbon; Av_N= Available soil nitrogen; Av_P= Available soil phosphorus; Av_K= Available soil potassium; DHA= Dehydrogenase; FDA= Fluorescein Di-acetate; β-GaA= β-galactosidase activity.

### 4.6.2 Data Pre-processing for Soil Quality Index (SQI) Evaluation

For the evaluation of soil quality indices, the dataset was prepared and imported in CSV format into the SQI CAL program developed by Mohanty (2020). Principal Component Analysis (PCA) was then performed. Among the five principal components extracted, four components with Eigenvalues exceeding 1 were retained, collectively accounting for 95.71% of the total variance in the dataset, as detailed in Table 4.9.

Principal Component 1 (PC1) explains 57.42% of the total variance, which is quite significant. PC2 adds 24.82% more variance, bringing the cumulative total to 82.24%. This suggests that the first two components together capture a large majority of the information (variance) in the data. PC3 contributes an additional 7.96%, taking the total explained variance to 90.20%. Hence, including PC3 can be useful if a higher explanation of variance is desired. Finally, PC4 explains 5.59%, pushing the cumulative explained variance to 95.79% (Table 4.9).

Within the first principal component (PC1), eight variables met the qualification criteria. These included bulk density measured at the 0–15 cm depth (BD_0–15 cm), calcium (Ca), magnesium (Mg), sodium (Na), cation exchange capacity (CEC), the passive carbon pool (PSV Pool), total organic carbon (TOC), and available nitrogen in the soil (Av_N). All of these variables demonstrated strong positive correlations, with factor loadings ranging between 0.89 and 0.99. In contrast, PC2 comprised three qualifying variables; dehydrogenase activity (DHA), fluorescein di-acetate activity (FDA), and β-galactosidase activity (β-GaA), each showing factor loadings from 0.89-0.96.The sand and silt proportions were qualified in PC3 with factor loadings of 0.61 and 0.57, respectively. In PC4, only pH qualified with the factor loading value of 0.75. The higher weighted values (0.60) was observed in PC1, followed by PC2 with 0.26 in a dataset (Table 4.10). The lowest weighted values (0.08 and 0.06) were found in PC3 and PC4, respectively (Table 4.10).

**Table 4.9: The eigenvalues, the proportion of variance explained, and the cumulative variance were computed through principal component analysis (PCA), considering only components with eigen values exceeding one**

| PC | Eigen values | Variance percent | Cumulative variance percent |
|---|---|---|---|
| 1 | 13.21 | 57.42 | 57.42 |
| 2 | 5.71 | 24.82 | 82.24 |
| 3 | 1.81 | 7.96 | 90.2 |
| 4 | 1.29 | 5.59 | 95.79 |

PC= Principal Component; The total number of rows shows the total no of PCs.

**Table 4.10: Weight values from respective Principal Components (PC's).**

| PC | Weighted Values |
|---|---|
| 1 | 0.60 |
| 2 | 0.26 |
| 3 | 0.08 |
| 4 | 0.06 |

PC= Principal Component; Row number indicates PC.

$$\text{Weightage values} = \frac{\text{Cumulative variance percentage}}{\text{Variance percentages}}$$

**Table 4.11: Selection of variables derived from the computed principal component analysis (within a 10% range) was utilized to estimate the soil quality index**

| S.NO | Principal Component | column | Variable | Column_For_Scoring | Value |
|---|---|---|---|---|---|
| 1 | 3 | 3 | Sand | 2 | 0.61 |
| 2 | 3 | 3 | Silt | 3 | 0.57 |
| 3 | 1 | 1 | BD_0.15cm | 5 | 0.94 |
| 4 | 4 | 4 | pH | 9 | 0.75 |
| 5 | 1 | 1 | Ca | 11 | 0.97 |
| 6 | 1 | 1 | Mg | 12 | 0.98 |
| 7 | 1 | 1 | Na | 13 | 0.97 |
| 8 | 1 | 1 | CEC | 14 | 0.99 |
| 9 | 1 | 1 | PSV. Pool | 17 | 0.90 |
| 10 | 1 | 1 | TOC | 18 | 0.89 |
| 11 | 1 | 1 | Av_N | 19 | 0.92 |
| 12 | 2 | 2 | DHA | 22 | 0.91 |
| 13 | 2 | 2 | FDA | 23 | 0.96 |
| 14 | 2 | 2 | β-GaA | 24 | 0.89 |

Value= Factor loading; BD= Bulk density; Ca= Calcium; Mg= Magnesium; Na= Sodium; CEC=Cation exchange capacity; PSV. Pool= Passive pool of carbon; TOC= Total organic carbon; Av_N= Available soil nitrogen; DHA= Dehydrogenase activity; FDA= Fluorescein Di-acetate activity; β-GaA= β-galactosidase activity.

### 4.6.3 Soil quality index (SQI)

Soil quality index computed from different scores and weighted values obtained from respective principal components (PC's) was significantly higher (42.54 % and 42.51%) under Koting-Sa-ha Ramosetsana (3087-3155 meters a.s.l) and Khorong (2500-2550 meters a.s.l), respectively compared to all other alpine wetlands (equivalent to the altitudes) depicted in Table 4.12 and Figure 4.9. The lowest SQI (34.37%) was notable under Tenesolo (2552-2600 meters a.s.l) (Table 4.12 and Figure 4.9).

SQI distribution reached significantly maximal value of 42.54 % and 42.1 % under Koting-Sa-ha Ramosetsana (3087-3155 meters a.s.l) and Khorong (2500-2550 meters a.s.l), respectively (Table 4.13 and Figure 4.9). The median SQI was 39.63 %. SQI was significantly distributed at a minimum of 34.37%. In general, the average SQI value was 39.31 (Table 4.13 and Figure 4.9). All other descriptive statistics for SQI are presented in Table 4.13.

**Table 4.12: Impact of altitudinal variation on soil quality index (SQI)**

| **Treatment(s)** | | SQI (%) |
|---|---|---|
| **Wetlands** | **Altitude (m) asl** | |
| Khorong | 2500-2550 | 42.51 |
| Tenesolo | 2552-2600 | 34.37 |
| Khamoqana | 2839-2880 | 37.49 |
| Khalong-La- Lichelete | 2891-2995 | 42.13 |
| Lets'eng- La-Likhama | 3040-3080 | 36.92 |
| Koting-Sa-ha Ramosetsana | 3087-3155 | 42.54 |

asl= Above sea level.

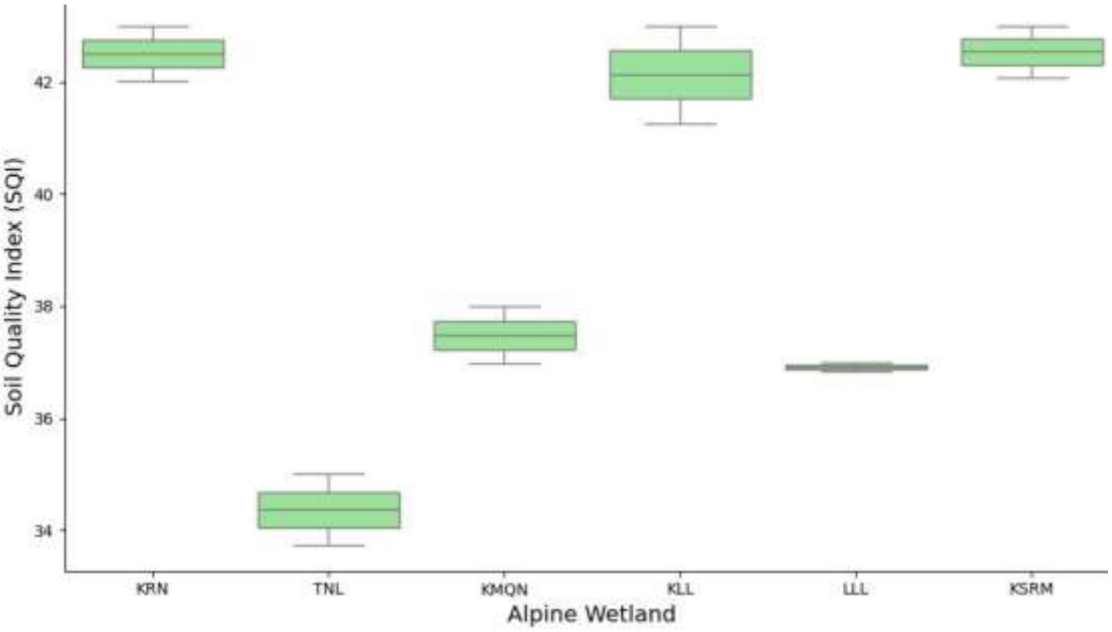

**Figure 4.9:** Box plot of soil quality index (%) by wetland as influenced by altitudinal variation. KRN= Khorong (2500-2550m asl); TNL= Tenesolo (2552-2600m asl); KMQN= Khamoqana (2839-2880m asl); KLL=Khalong-la-Lichelete (2891-2995m asl); LLL=Lets'eng-la-Likhama (3040-308 asl); KSRM=Koting-sa-ha Ramosetsana (3087-3155m asl).

**Table 4.13: Statistical summary of SQI (%) box -plot across the alpine wetlands.**

| **Descriptive statistics** | |
|---|---|
| Mean | 39.31 |
| Standard Error | 3.34 |
| Quartile 1 | 36.93 |
| Median | 39.63 |
| Quartile 3 | 42.41 |
| Kurtosis | -1.59 |
| Skewness | -0.28 |
| Minimum | 33.74 |
| Maximum | 42.54 |

## Chapter-V

# DISCUSSIONS

The discussions in this chapter are drawn from the results obtained from the present study entitled, **“Impact of Altitudinal Variation on Selected Soil Properties and Carbon Dynamics in the Alpine Wetlands of Lesotho.”** The results are discussed critically with the cause and relationship effect under the following headings;

5.1 Impact of altitudinal variation on soil physical and hydraulic attributes
5.2 Impact of altitudinal variation on soil physico-chemical attributes
5.3 Impact of altitudinal variation on soil macronutrients (nitrogen, phosphorus and potassium) and cation availability
5.4 Impact of altitudinal variation on soil enzyme activity (dehydrogenase,β-galactosidase and fluorescein di-acetate activity)
5.5 Impact of altitudinal variation on soil carbon dynamics
5.6 Impact of altitudinal variation on soil quality index

### 5.1 Impact of altitudinal variation on soil physical and hydraulic attributes

The predominant soil texture identified was sandy loam, which reflects favourable drainage properties. In wetland areas situated at higher elevations, the soils were classified as loam according to the USDA soil taxonomy, suggesting a more balanced composition capable of enhanced water and nutrient retention. The silt and clay contents increased with increasing altitude in the alpine wetlands. The trend could be due to large influence by sediment transport and deposition processes. At higher elevations within the mountain catchments, higher organic carbon contents, slower water movement and localized depositional settings promote the settling of finer particles, whereas lower areas tend to accumulate coarser materials (sand particles) carried further downstream. These findings align with observations by Eyayu *et al.* (2009), who documented a greater proportion of clay in loam soils at elevated sites, likely attributable to increased organic carbon levels. In a similar way, Reichert *et al.* (2022) reported a gradual reduction in sand fractions accompanied by relatively higher clay content as altitude increased.

Bulk density (BD) gives an idea of soil compaction and porosity. Lower values generally indicate better soil structure and higher porosity. The significantly lower BD value, higher infiltration rate, maximum water holding capacity and saturated hydraulic conductivity recorded in Koting-Sa-ha Ramosetsana, indicate loosely packed soil with optimum porosity probably due to higher soil organic carbon contents in that altitude higher than any other altitudes in the wetlands, while Tenesolo was observed with significantly higher BD, suggesting more compacted soil. Generally, BD decreased with increasing altitude, which aligns with a number of studies (Teron *et al.*, 2024), in which they found that higher altitudes often exhibit low BD, higher soil organic carbon content, greater water holding capacity, and infiltration rate. Therefore, it may be assumed that lower temperatures and reduced microbial activity at higher altitudes lead towards slower organic matter decomposition and better soil aggregation, thus reducing BD.

Soils with low BD have better-connected pores that enhance water transmission and entry into the soil, hence optimum hydraulic conductivity and infiltration capacity. Koting-Sa-ha Ramosetsana also exemplifies this pattern, reinforcing the idea that porous soils at higher altitudes are more permeable. Conversely, compacted soils like Tenesolo restrict water movement.

Soils rich in organic carbon and fine particles (clay, silt) hold more water. Loosely and porous soils with lower BD also enhance water retention. Hence, maximum water holding capacity (MWHC) mirrors the trends in BD and infiltration, further emphasizing altitude-related differences with which Teron *et al.* (2024) observed the highest MWHC at 1800–2800 m asl (higher elevation site)**,** supporting the trend of improved hydro-physical properties (MWHC and infiltration rate) at higher elevations. In congruence with the results obtained from the present study, Singh *et al.* (2018) showed that direct proportionality between MWHC and porosity, reinforcing the inverse relationship between BD and MWHC.

### 5.2 Impact of altitudinal variation on soil physico-chemical attributes

The soil pH was slightly acidic across the wetlands. This trend is consistent with several other studies (Charan *et al.*, 2013; Kamal *et al.*, 2023) who have observed that soils at higher altitudes exhibit acidity, likely due to increased leaching of base cations and greater accumulation of organic matter. The study of Ramesh *et al*. (2019) and Wang *et al*. (2024) also revealed lower pH values (< 5.5) at high elevations in mountain top soils. This pattern is likely the result of lower microbial activity and slower decomposition at cooler places, especially higher elevations, which causes organic acids to accumulate, thus increasing acidity. Slightly acidity may influence nutrient availability (especially P, Ca, and Mg), which may require liming to optimize the pH for productive vegetation.

Soil organic carbon (SOC) plays a crucial role in maintaining and enhancing the health of wetland soils. In general, the higher SOC recorded in the present study in all the wetlands is attributed to acidic condition of the soil and lower temperatures prevailing at higher elevation as in the mountainous regions, slowing down the rate of mineralization, hence more of SOC content. These findings are in congruence with that of Imtimongla *et al*. (2021) and Kumar *et al*. (2019) who reported that lower temperatures at higher altitudes slow decomposition, lead towards greater SOC accumulation. Similar results were reported by Nozari and Borůvka (2023) in Czech Republic, who have discovered increased leaching and reduced microbial activity at higher altitude, contributing tow increased leaching and reduced microbial activity, contributing towards SOC accumulation. Olaleye *et al*. (2022) also reported higher soil organic carbon concentration in high-altitude wetlands of Lesotho, reinforcing this in the regional context. Higher SOC is critical for wetland health, as it enhances water retention, nutrient supply, and soil structure, which supports biodiversity and ecosystem services.

Higher CEC and SOC at higher altitudes suggest better nutrient retention and organic matter content, beneficial for wetland productivity. Slightly acidic soils pronounced in this study, may affect nutrient availability; thus, liming could be recommended for correction of the pH. However, lower electrical conductivity (EC) values recorded across the alpine wetlands, indicate minimal salinity stress on wetland vegetation.

### 5.3 Impact of altitudinal variation on soil macronutrients (available nitrogen, phosphorus, and potassium) and cation availability

The overall alpine wetlands appeared to have contained highest nitrogen (N) contents, suggesting robust nutrient cycling and possibly greater biological productivity. In contrast to that, Tenesolo recorded less than half the nitrogen levels of these top sites, indicating possibility for low nitrogen content, less vegetation cover and microbial activity. This disparity underscores the strong spatial heterogeneity in nutrient distribution across sites. Interestingly, no consistent trend with altitude emerged, as both the highest and lowest N values are recorded at similar elevations (~873–878 m a.s.l) (Kumar *et al*. 2022). This suggest that altitude alone does not dictate nutrient richness in these wetlands. This is somewhat counter to the findings by Lamma *et al*. (2022), who reported higher macronutrient and cation availability at higher altitudes in Turkish fir forests. The divergence may stem from ecosystem-specific responses and climatic or vegetation differences, as nutrient dynamics are influenced by more than one factor, including land use history, degradation status, and organic matter content not only the elevation.

Phosphorus (P) availability showed a similar pattern. Koting-Sa-ha Ramosetsana (196.27 g kg$^{-1}$), Khalong-la- Likhama and Khorong exhibited better phosphorus (P) levels, although P contents recorded in this study were in medium range as per the rating. This is likely a reflection of better fertile and less degraded conditions, conducive for wetland vegetation species and productivity. Conversely, Tenesolo recorded the lowest P, numerically which reinforce poor nutrient status and possibly degraded site. The trend was similar for potassium (K). This consistency across nutrient dynamics suggests a systemic nutrient deficiency at Tenesolo, likely interlinked with soil degradation level, low soil organic carbon content coupled with reduced microbial activity. This is supported by Shasha *et al*. (2020) who announced that lower temperatures found at higher altitudes, reduce decomposition and mineralization rates, which in turn limit nutrient release from soil organic matter (SOM). While Lamma *et al*. (2022) observed higher nutrient levels at elevated sites in Turkey, these findings may reflect regional variation in soil formation, parent material, and climatic context.

It is also plausible that in some systems, accumulation of undecomposed organic matter at higher altitudes results in greater total nutrient content, but less availability due to slow mineralization — a nuance that may reconcile the conflicting patterns. It has been observed that there was a high content of calcium at higher elevations (Koting-sa-Ha Ramoseletsana), ascribed to buffering soil acidity. Higher concentrations of this cation help to maintain a more neutral to slightly alkaline pH, which is generally conducive for nutrient availability and microbial activity, despite the potential for organic acid accumulation in wetlands (Brady and *et al*., 2008).

### 5.4 Impact of altitudinal variation on soil enzyme activity

All three enzyme activities, being dehydrogenase activity (DHA), β-galactosidase activity (βGaA), and fluorescein di-acetate activity (FDA), showed a declining pattern with increasing altitude, supporting the concept that lower temperatures, reduced vegetation cover, and limited substrate inputs at higher elevations dampen microbial functioning. Khorong (2500-2550 m a.sl) exhibited higher DHA, suggesting robust microbial respiration and metabolic activity. The Lets’eng-La-Likhama (3040-3080 meters a.s.l), along with Tenesolo (2552-2600 meters a.s.l) and Khamoqana (2839-2880 meters a.s.l), presents significantly lower DHA, indicating reduced microbial metabolism likely due to harsher environmental conditions or lower organic matter inputs. This pattern aligns with the outcomes of Fan *et al*. (2021), who reported that higher altitudes with lower temperatures (~2.5 °C) suppress microbial activity, which resulted in the reduction of DHA and promoted organic carbon accumulation due to slower decomposition rates.

The β-galactosidase activity (βGaA) reflects microbial capacity to degrade carbohydrates, particularly lactose and structurally similar polysaccharides. With regard to that, Khorong demonstrated the higher βGaA, indicating the possibility for active microbial populations and higher amounts of organic matter turnover. The Lets'eng-La-Likhama had the lower βGaA, and there was an overall decline in βGaA with increase in elevation, suggesting that microbial carbohydrate decomposition is altitude-sensitive. Similarly, Fan *et al*. (2021) observed the decline in β-enzymatic activity due to wetland degradation and lower nutrient input at high altitudes, while Ekenler and Tabatabai (2003) emphasized that βGaA is interlinked with soil organic matter (SOM) and environmental sensitivity.

Despite the fact that Koting-Sa-ha Ramosetsana had conducive bio-physical conditions (lower bulk density, optimum infiltration and higher maximum water holding capacity), it recorded lower fluorescein di-acetate activity (FDA), indicating that good physical traits do not always predict higher biological functionality. These points to other limitations, such as temperature stress, low organic inputs, or restricted microbial diversity. Khalong-La-Lichelete defied the trend with higher FDA despite the altitude, suggesting unique local conditions that may buffer against elevation effects. Wang *et al*. (2024) and Wu *et al*. (2022) both reported altitude-dependent enzymatic shifts, reinforcing this study’s findings.

FDA is highly sensitive to management practices, environmental shifts, and restoration efforts, making it an essential tool for ecological monitoring. Nevertheless, the present findings must be interpreted with caution since vegetation type, productivity, and cover factors known to strongly influence microbial communities and enzymatic activities were not included in this study. Additionally, because soil sampling was restricted to the wet season, seasonal microbial fluctuations that affect enzyme activity were not captured.

## 5.5 Impact of altitudinal variation on soil organic carbon dynamics

The observed increase in very labile carbon ($C_{VL}$) *i.e.*, coarse visible litter and Total Organic Carbon (TOC) with altitude, peaking at Koting-sa- ha Ramosetsana, suggests a strong positive correlation between altitude and accumulation of organic carbon in wetland soils. Conversely, less labile ($C_{LL}$) and non-labile ($C_{NL}$) fractions showed fluctuations across wetlands, but generally exhibited higher values at greater elevations. The trend of increasing $C_{VL}$ and TOC with elevation may be attributed to several ecological and environmental factors associated with higher altitudes:

The decrease in temperatures with an increase in altitude slow-down microbial decomposition rates, allowing more organic residues (like leaf litter and root biomass) to accumulate (Imtimongla *et al*., 2021; Kumar *et al*., 2022). This reduces the turnover of organic matter and contributes to higher TOC and $C_{VL}$ content. The greater soil organic carbon (SOC) at higher altitudes is attributed to reduced decomposition probably due to lower temperatures, alongside increased leaching and soil acidity (Nozari and Borůvka's, 2023). These processes inhibit microbial breakdown of organic inputs, leading to greater OC accumulation in soil. The high-altitude wetlands typically support unique alpine vegetation with slower decomposition rates and higher litter input (Feyissa *et al*., 2023), which goes hand in hand with Olaleye *et al*. (2022) showing significantly higher soil organic matter (SOM) in mountainous agro-ecological zones in Lesotho compared to lower-lying wetlands, affirming that elevation is a key driver of organic carbon enrichment. This could explain the consistency of higher $C_{VL}$ values at more elevated sites. However, soil parent material and land use pressure, both not considered in the present work, may also account for differences in SOC pools between wetlands. For example, the degraded conditions and lower SOC recorded in Tenesolo may reflect historical land use pressure and soil degradation rather than solely elevation effects.

The total organic carbon (TOC) showed significant variation among wetlands, with the higher values recorded at higher altitudes *viz*., Koting-Sa- ha Ramoseletsana and Letseng-La- Likhama. The results were unexpected because Koting-Sa-Ha Ramoseletsane was initially scored 85% healthy, while Letséng La Likhama was scored 40% on the basis of soil degradation level, which could be due to more of the soil organic carbon (SOC) in the form of passive pool of carbon and carbon storage in soils being accumulated over centuries. Therefore, the altitude influenced OC pools in these wetlands, with higher elevated wetlands having greater OC pools, suggesting that wetland conservation and management strategies should consider altitudinal gradients for better soil health maintenance and carbon sequestration.

The increase in passive pool of carbon ($C_{PSV}$) with increasing altitude observed, across the wetlands and being the dominant contributor of SOC to TOC, is ascribed to recalcitrance coupled with the lack of treatment imposition in the wetland environment. The active pool of carbon ($C_{ACT}$) is the most responsive pool and is readily influenced by management practices and environmental changes, *i.e.*, $C_{ACT}$ pools disintegrate rapidly and oxidize readily with changes in management practices (Sahoo *et al*., 2019; Dinesh *et al*., 2023). The $C_{PSV}$ pools are recalcitrant, form organic-mineral compounds, and breakdown at a slow pace through microbiological activities (Sahoo *et al*., 2019). In the present study, the significantly greater $C_{ACT}$ proportion in the Koting-Sa-ha Ramosetsana wetland was attributed to the naturally undisturbed nature of the wetland and increased soil organic matter (SOM), which likely increased biomass production. These $C_{ACT}$ pools have been recognized as early indicators of soil quality because of their rapid response to management techniques (Sahoo *et al*., 2019). The extent of the $C_{ACT}$ and $C_{PSV}$ pools in this study may also be linked to vegetation inputs, hydrological dynamics, and disturbance history, which were not explored but could strongly influence carbon partitioning in alpine wetlands.

The higher percent contribution of $C_{ACT}$ pools to TOC observed under Lets'eng-La-Likhama and Koting-Sa-ha Ramosetsana, indicates that wetlands at high elevation can act as carbon-sink and storage. $C_{ACT}$ is the prime source of nutrients and can be easily harnessed by soil microbes; thus, the content of the $C_{ACT}$ pool fluctuates rapidly in comparison with that of the $C_{PSV}$ pool (Zhou *et al*., 2022). Nevertheless, the $C_{PSV}$ pool is very resistant to microbial attack and can be preserved as organic–mineral complexes and difficult to access (Von-Lützow *et al*., 2007), which may increase its relative fraction within the TOC (Zhou *et al*., 2022). The better biophysical conditions because of little or no disturbance and lower temperatures at higher altitudes (Koting-Sa-ha Ramosetsana wetland) coupled with the complete natural conditions of the wetland with few modifications, a slight change in ecosystem processes and a small loss of natural habitats and biota might have influenced the decomposition rates, resulting in higher $C_{ACT}$ turnover.

### 5.6 Impact of altitudinal variation on soil quality index (SQI)

Soil quality is inherently dependent on both the soil type and its specific location, and it tends to fluctuate based on key influencing factors, including the soil's natural characteristics (Kamal *et al*., 2023). SQI was improved in Koting-Sa-ha Ramosetsana wetland positioned at higher altitude (3087-3155 meters a.s.l), followed by Khorong

(2500-2550 meters a.s.l) indicating that SQI does not depend only on the altitude alone. This is evidenced by the SQI value under Khorong (KRN) which was on par with the one from Koting-Sa- ha Ramosetsana (KSHM) at different elevations. This could be due to favourable bio-physical environmental conditions due to healthy status of the wetland, assessed with Wet Health tool. The Tenesolo (TNL) recorded the lower SQI attributed to lower altitude and degraded wetland. All the examined soil quality related parameters were also enhanced by KSHM followed by KRN, while TNL wetland recorded low.

The present investigation highlighted the importance of total organic carbon (TOC) as a central indicator for assessing soil quality across diverse land use systems, including wetlands situated within various agro-ecological zones. Organic carbon plays a pivotal role in influencing and enhancing numerous soil functions, such as nutrient biogeochemical cycling, enzyme-mediated processes, soil aggregation, and the capacity to retain and supply nutrients. In this context, TOC demonstrated significant positive associations with cation availability, available nitrogen levels, enzyme activities, passive carbon pools, particle size fractions (sand and silt), and soil pH, with correlations significant at the 0.05 to 0.01 probability levels (Panwar *et al*., 2022; Zeraatpisheh *et al*., 2020). The results indicate that relatively undisturbed condition of wetlands at higher elevations, such as Koting-Sa-ha Ramosetsana, as well as the sustained health of wetlands at comparatively lower altitudes like Khorong, both contributed to enhanced soil quality relative to other alpine wetland areas. The following soil parameters were retained in principal component (PC) 1; calcium, magnesium, sodium; total organic carbon, passive pool of carbon, cation exchange capacity, soil available nitrogen and PC2; soil enzyme activities (SEAs). Attributed to the results of this study, SEAs are potential soil quality indicators across the mountainous agro-ecological regions due to strong correlation among them as indicated by higher factor loadings and weighed values. In PC3, sand and silt particles were retained, because they serves as fundamental indicators of soil physical structur, influencing crucial functions such as water drainage, aeration and nutrient retention. Despite being coarse, less chemically reactive, sand provides provides a baseline for understanding the wetland’s unique hydrological and physical characteristics, which are distinct from other ecosystems. In PC4, pH was included probably due its significance in controlling the key biological, chemical, and physical functions in these sensitive ecosystems (alpine wetlands).

## Chapter-VI

# SUMMARY AND CONCLUSIONS

The present study was conducted to assess the **"Impact of Altitudinal Variation on Selected Soil Properties and Carbon Dynamics in the Alpine Wetlands of Lesotho"** in six alpine wetlands from three different sub-catchments; two in khubelu, Senqunyane and Sani from February, 2025 in an on-going research project of Dr. Knight Nthebere entitled, " *Carbon Modelling and Omics Approaches for Screening of Soil Microbes for Climate Change Adaptation in the Alpine Wetlands of Lesotho*," initiated from November, 2024. This study was carried-out with these objectives:

1. To study the carbon dynamics (soil organic carbon pools) as influenced by altitudinal variation in selected Alpine wetlands.
2. To estimate and analyse the impact of altitudinal variation in selected Alpine wetlands on soil enzyme activities.
3. To assess the soil quality index as influenced by altitudinal variation in selected Alpine wetlands.
4. To study the changes in selected soil properties as influenced by altitudinal variation in selected Alpine wetlands.

The study was conducted in blocking design with six altitudinal variations equivalent to alpine wetlands from three sub-catchment areas (Senqunyane, Khubelu and Sani) under the upper Senqu main catchment as follows; Khorong (2500-2550 m a.s.l) and Tenesolo (2552-2600 m a.s.l) in Senqunyane catchment; Khamoqana (2839-2880 m a.s.l) and Khalong-la-Lichelete (2891-29950 m a.s.l) in Sani catchment; Lets'eng-la-Likhama (3040-3080 m a.s.l) and Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) in Khubelu catchment.

**The salient findings obtained from the present study are summarized as under:**

- ✓ Soil physical and hydraulic properties (soil texture, bulk density, infiltration rate, maximum water holding capacity and saturated hydraulic conductivity) were significantly influenced by higher altitude of the alpine wetlands.
- ✓ The bulk density (BD) was inversely proportional to the altitude *i.e.*, BD decreased with increase in altitude and increased at lower altitude. The BD was 13.49-16.15% and 28.29% lower in Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) compared to Khamoqana (2839-2880 m a.s.l), Khalong-la-Lichelete (2891-29950

m a.s.l), Lets'eng-la-Likhama (3040-3080 m a.s.l), Khorong (2500-2550 m a.s.l) and Tenesolo (2552-2600 m a.s.l) alpine wetlands, respectively.

- ✓ The soil texture varied significantly and was loam and sandy loam across the whole altitudes within the alpine wetlands.
- ✓ Infiltration rate (IR), maximum water holding capacity (MWHC) and saturated hydraulic conductivity (SHC) increased with an increase in altitude. Significantly higher IR, MWHC & SHC were observed under Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) in comparison with all other alpine wetlands under the study.
- ✓ Physico-chemical properties of the soil *viz.*, pH and soil organic carbon were significantly influenced by the altitudinal variation of the alpine wetlands. The pH was in acidic range across the whole altitudes within the wetlands. Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) was observed with significantly SOC content in comparison with all other wetlands under study. The electrical conductivity and cation exchange capacity were non-significant.
- ✓ Passive pool ($C_{PSV}$) was dominant over Active pool of carbon ($C_{ACT}$) and the main contributor to total organic carbon, with 75-79%. The 24-25% of $C_{ACT}$ was observed in Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) and Lets'eng-la-Likhama (3040-3080 m a.s.l), which are the wetlands with higher altitude in comparison with others under the study. The 21% of $C_{ACT}$ was observed with Tenesolo (2552-2600 m a.s.l)
- ✓ Lets'eng-la-Likhama (3040-3080 m a.s.l) also outperforms Khorong (2500-2550 m a.s.l) in total organic carbon by 8.46%, reinforcing the elevation–carbon link.
- ✓ Soil enzymatic activity (β-galactosidase, fluorescein di-acetate and dehydrogenase activity) decreased with increasing altitude, suggesting a detrimental impact of higher altitudes on the functional activity of these enzymes due to low temperatures at higher elevations. Khorong (2500-2550 m a.s.l) exceeded Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) by 24.35% of FDA and 31.19% of DHA.
- ✓ The observed increase in $C_{PSV}$ relative to the $C_{ACT}$ carbon pool with rising altitude, coupled with the decline in soil enzymatic activity, has important implications for climate change outcomes. These trends influence soil carbon storage, with high-altitude regions functioning as substantial, though comparatively less dynamic, carbon reservoirs.
- ✓ Soil quality (SQ) was higher (42.54%) at Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l), and was on par (42.51%) with Khorong (2500-2550 m a.s.l), while it was

lower (34.39%) in Tenesolo (2552-2600m a.s.l). These observations on SQ suggest that SQ is not only based on the altitude differences but also on the wetland health status in terms of the soil degradation level.

## CONCLUSIONS

On the basis of the present study on **“Impact of Altitudinal Variation on Selected Soil Properties and Carbon Dynamics in the Alpine Wetlands of Lesotho”** the following conclusions are drawn:

- ✓ Altitude significantly influenced the soil physical and hydraulic properties in which the higher infiltration rate (IR), saturated hydraulic conductivity (SHC), maximum water holding capacity and lower bulk density (BD) with loam textured soil dominated by higher proportion of clay content were observed under Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) in comparison with all other wetlands under the study. Tenesolo (2552-2600 m a.s.l) was observed with higher BD, low SHC and IR with lower clay and higher sand contents, categorized into sandy loam.
- ✓ Soil organic carbon showed significant variation among wetlands with the higher SOC content observed under Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l).
- ✓ The biological properties in terms three enzyme activities *viz*., dehydrogenase activity (DHA), β-galactosidase activity (β-GaA), and fluorescein di-acetate activity (FDA) showed a declining pattern with increasing altitude suggesting the negative impact of higher altitude associated with lower temperatures on the biological functions of the soil enzymes.
- ✓ All pools of soil organic carbon viz., very labile, labile, less labile, non-labile and total organic carbon (TOC) were higher under Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l) compared to all other wetlands. Passive pool ($C_{PSV}$) was dominant over Active pool of carbon ($C_{ACT}$) with 75-79% and the main contributor to TOC.
- ✓ Soil quality (SQ) was higher (42.54%) at Koting-Sa-ha Ramosetsana (3087-3155 m a.s.l), and was on par (42.51%) with Khorong (2500-2550 m a.s.l). There was no consistent linear relationship between altitude and SQI.
- ✓ SQI fluctuated across altitudes within the wetlands, suggesting that factors other than altitude alone (e.g., local environmental conditions, land use,

vegetation, management practices, or soil type) significantly influence soil quality in these wetlands.

## LIMITATIONS OF THE STUDY

### Temporal Variability Not Considered

- ✓ The season of sampling (wet vs. dry season) can affect soil moisture, microbial activity, and nutrient availability
- ✓ Sampling depth (0-15cm) cannot give full details on the distribution of the examined soil parameters.

### Lack of Vegetation Data

- ✓ The type of vegetation, cover, and productivity, not explored in the present study can significantly affect organic matter input, microbial communities, and nutrient cycling.

### Environmental Variables

- ✓ Variables such as land use history, vegetation type, hydrological dynamics, human disturbance, and soil parent material may independently influence soil properties.
- ✓ Tenesolo's nutrient-poor status might be a result of soil degradation level or land-use pressure other than the altitude.

## RECOMMENDATIONS AND FUTURE LINE OF WORK

## RECOMMENDATIONS

### Conservation efforts should prioritize high-altitude wetlands for their superior soil health and carbon sequestration potential.

- ✓ High-altitude wetlands such as Koting-Sa-Ha Ramoseletsana should be designated as priority conservation zones due to their rich SOC reserves.
- ✓ It is recommended to encourage vegetation conservation, limit disturbances, and consider controlled grazing or reforestation to maintain and increase carbon stocks.

### Addressing Site-Specific Nutrient Deficiencies.

- ✓ Tenesolo stands out as severely nutrient-depleted (N, P, and K). Implement site rehabilitation strategies.
- ✓ It is recommended to foster minimal disturbance practices such as contour farming near the wetlands.

## FUTURE LINE OF WORK

- ✓ Further research should consider sampling attitudinally in a wider range *i.e.*, from the lowlands to the alpine wetlands zones.
- ✓ Research on soil metagenomics should be carried out in the alpine wetlands.
- ✓ Temporal variability in terms of multi-season and long-term monitoring of the examined soil variables in the present study should be considered with future research.
- ✓ Modelling of soil physical and hydraulic properties should be carried out in the alpine wetlands.
- ✓ Modelling of soil carbon dynamics should be attempted in wetlands studies.
- ✓ Vegetation isotopic studies should be undertaken for better identification of the plant species composition, diversity and variation in the alpine wetlands.

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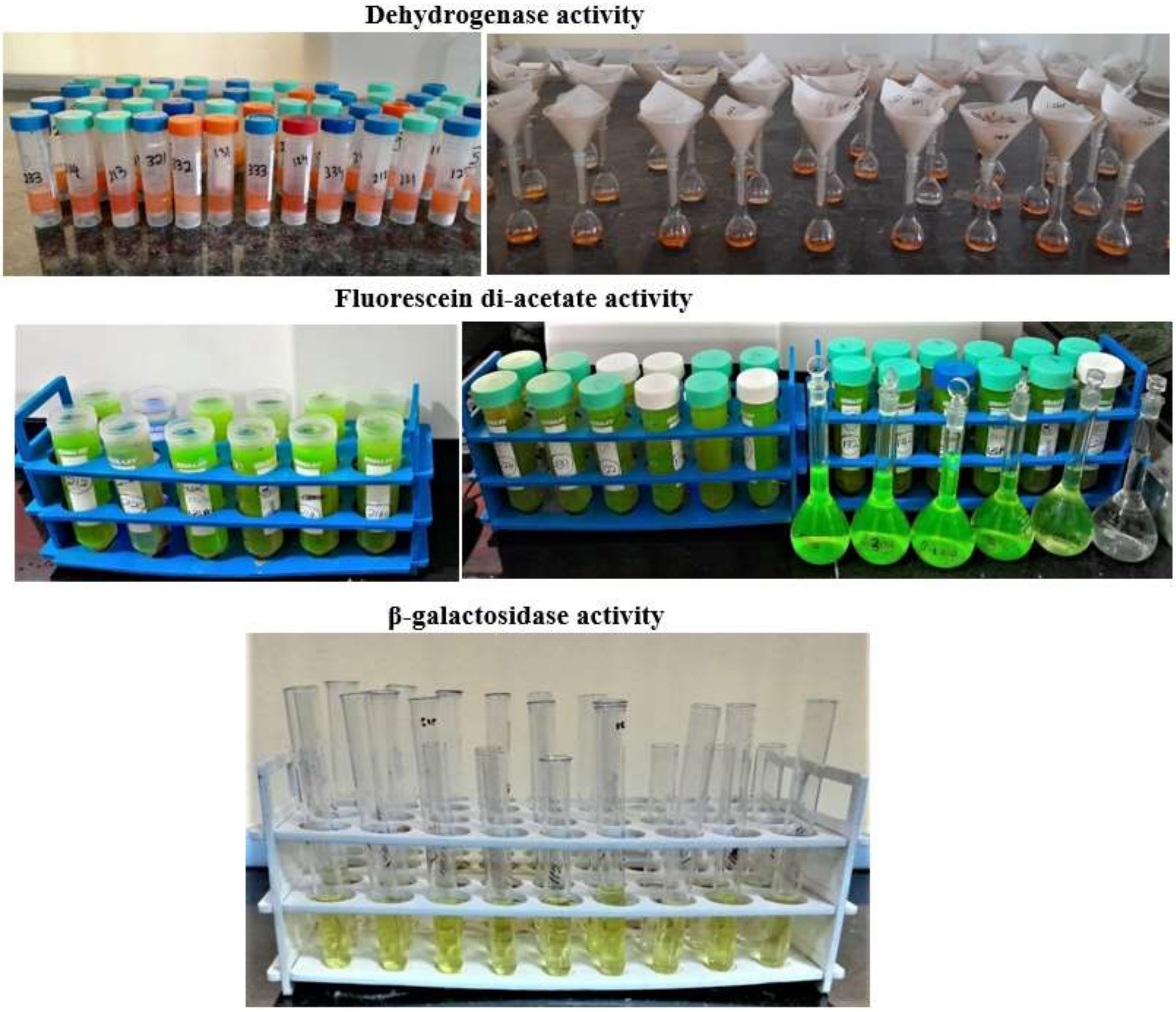
# APPENDICES



Dehydrogenase activity

Fluorescein di-acetate activity

β-galactosidase activity

**Appendix 1:** Plates showing the development of colours following the specified incubation time for assaying of soil enzyme activity

**Appendix 2: Supplementary table showing correlation matrix of all variables for SQI computation as influenced by altitudinal variation in the alpine wetlands**

| | Sand | Silt | Clay | BD | WHC | SHC | IR | pH | EC | Ca | Mg | Na | CEC | SOC | ACT.Pool | PSV.Pool | TOC | Av_N | Av_P | Av_K | DHA | FDA | BGA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sand** | 1 | | | | | | | | | | | | | | | | | | | | | | |
| **Silt** | -0.891 | 1 | | | | | | | | | | | | | | | | | | | | | |
| **Clay** | -0.84 | 0.5018 | 1 | | | | | | | | | | | | | | | | | | | | |
| **BD** | -0.592 | 0.2217 | 0.8627 | 1 | | | | | | | | | | | | | | | | | | | |
| **WHC** | 0.429 | -0.099 | -0.699 | -0.846 | 1 | | | | | | | | | | | | | | | | | | |
| **SHC** | 0.394 | -0.292 | -0.403 | -0.66 | 0.5499 | 1 | | | | | | | | | | | | | | | | | |
| **IR** | 0.366 | -0.314 | -0.322 | -0.592 | 0.4705 | 0.992 | 1 | | | | | | | | | | | | | | | | |
| **pH** | 0.081 | -0.328 | 0.2371 | 0.1299 | -0.184 | -0.034 | 0.064 | 1 | | | | | | | | | | | | | | | |
| **EC** | 0.149 | -0.542 | 0.3633 | 0.4661 | -0.623 | -0.081 | 0.031 | 0.748 | 1 | | | | | | | | | | | | | | |
| **Ca** | 0.52 | -0.189 | -0.764 | -0.894 | 0.7736 | 0.816 | 0.742 | -0.419 | -0.526 | 1 | | | | | | | | | | | | | |
| **Mg** | 0.624 | -0.325 | -0.801 | -0.882 | 0.7941 | 0.822 | 0.75 | -0.371 | -0.455 | 0.986 | 1 | | | | | | | | | | | | |
| **Na** | 0.655 | -0.343 | -0.839 | -0.917 | 0.7216 | 0.803 | 0.738 | -0.313 | -0.39 | 0.979 | 0.978 | 1 | | | | | | | | | | | |
| **CEC** | 0.693 | -0.425 | -0.813 | -0.926 | 0.7832 | 0.855 | 0.804 | -0.127 | -0.287 | 0.945 | 0.966 | 0.973 | 1 | | | | | | | | | | |
| **SOC** | 0.274 | 0.1069 | -0.65 | -0.775 | 0.9325 | 0.487 | 0.383 | -0.517 | -0.838 | 0.812 | 0.802 | 0.719 | 0.705 | 1 | | | | | | | | | |
| **ACT.Pool** | 0.327 | 0.0848 | -0.724 | -0.911 | 0.9425 | 0.518 | 0.427 | -0.304 | -0.746 | 0.828 | 0.799 | 0.775 | 0.778 | 0.939 | 1 | | | | | | | | |
| **PSV.Pool** | 0.435 | -0.069 | -0.746 | -0.968 | 0.9091 | 0.628 | 0.563 | -0.063 | -0.53 | 0.831 | 0.81 | 0.82 | 0.86 | 0.821 | 0.9566 | 1 | | | | | | | |
| **TOC** | 0.405 | -0.022 | -0.746 | -0.959 | 0.9282 | 0.599 | 0.526 | -0.139 | -0.603 | 0.838 | 0.814 | 0.813 | 0.842 | 0.866 | 0.9792 | 0.9958 | 1 | | | | | | |
| **Av_N** | 0.532 | -0.325 | -0.624 | -0.789 | 0.6225 | 0.951 | 0.912 | -0.237 | -0.245 | 0.941 | 0.941 | 0.941 | 0.94 | 0.612 | 0.6382 | 0.7099 | 0.694 | 1 | | | | | |
| **Av_P** | 0.728 | -0.585 | -0.687 | -0.762 | 0.4195 | 0.808 | 0.802 | 0.12 | 0.118 | 0.756 | 0.774 | 0.856 | 0.879 | 0.293 | 0.4494 | 0.6322 | 0.581 | 0.863 | 1 | | | | |
| **Av_K** | 0.619 | -0.529 | -0.546 | -0.594 | 0.7773 | 0.723 | 0.69 | -0.007 | -0.15 | 0.668 | 0.763 | 0.653 | 0.77 | 0.644 | 0.5668 | 0.6018 | 0.596 | 0.703 | 0.535 | 1 | | | |
| **DHA** | 0.424 | -0.666 | -0.011 | 0.0916 | -0.189 | 0.531 | 0.579 | 0.071 | 0.554 | 0.184 | 0.269 | 0.244 | 0.279 | -0.24 | -0.34 | -0.228 | -0.266 | 0.455 | 0.474 | 0.424 | 1 | | |
| **FDA** | 0.214 | -0.506 | 0.1978 | 0.148 | -0.366 | 0.513 | 0.599 | 0.377 | 0.744 | 0.029 | 0.07 | 0.107 | 0.166 | -0.48 | -0.448 | -0.249 | -0.314 | 0.36 | 0.497 | 0.176 | 0.882 | 1 | |
| **BGA** | 0.261 | -0.558 | 0.1701 | 0.1305 | -0.072 | 0.566 | 0.633 | 0.315 | 0.584 | 0.085 | 0.178 | 0.109 | 0.228 | -0.21 | -0.297 | -0.165 | -0.208 | 0.38 | 0.352 | 0.541 | 0.901 | 0.852 | 1 |