# INFLUENCE OF SOIL PROPERTIES ON THE CURCUMINOID CONTENT OF *CURCUMA LONGA* L. CULTIVATED IN CHIANG RAI, THAILAND

**NARONK DUANGKAEW**

**MASTER OF SCIENCE**
**IN**
**APPLIED CHEMISTRY**

**SCHOOL OF SCIENCE**
**MAE FAH LUANG UNIVERSITY**
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**THIS THESIS IS A PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED CHEMISTRY**

**SCHOOL OF SCIENCE**
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**THESIS APPROVAL**

**MAE FAH LUANG UNIVERSITY**

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**Thesis Title:** Influence of Soil Properties on the Curcuminoid Content of *Curcuma Longa* L. Cultivated in Chiang Rai, Thailand

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Naronk Duangkaew

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| **Thesis Title** | Influence of Soil Properties on the Curcuminoid Content of *Curcuma longa* L. Cultivated in Chiang Rai, Thailand |
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## ABSTRACT

Soil properties are major factors that impact Turmeric (*Curcuma longa* L., Zingiberaceae) growth and quality. The Thai Herbal Pharmacopoeia (THP) recommended that dried turmeric should contain not less than 5.0% w/w of total curcuminoids (TC) content. The effect of soil properties on extraction yields and TC contents of turmeric rhizomes was investigated in this study. Twenty-three samples of turmeric rhizome and soils were collected in various cultivation site in Chiang Rai, Thailand. Clustering analysis was used and three group clusters were found; C1, C2, and C3. Two turmeric cultivars; Trang1 (T1) and Trang 84-2 (T84-2) were cultivated in these areas. C1 cultivated both T1 and T84-2 with highest soil pH (4.642±0.802). C2 cultivated exclusively T84-2 with high soil potassium (K) (1821.43 ± 533.63 mg/kg), and C3 cultivated exclusively T1 with high soil calcium (1941.93 ± 686.52 mg/kg). T-test and Principal Component Analysis (PCA) were utilized and found that turmeric qualities between two cultivars was significantly different, with T1 having a higher % yield (p-value = 0.0014), % total curcuminoid (p-value = 0.0131), and % bisdemethoxycurcumin (BDMC) (p-value = 0.001). Pearson correlation analysis was used to find a relationship between soil properties and turmeric qualities. T1 was found having consistent turmeric qualities compared T84-2, which reacted negatively to various soil properties in this study (r<-0.4). This study can be used to understanding the characteristic of turmeric cultivar for managing the extraction

yield of turmeric rhizomes by adjusting the soil properties appropriated for each turmeric cultivar.

**Keywords:** *Curcuma longa* L., Soil Properties, Total Curcuminoids Content, T-Test, Correlation Coefficient

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# ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| BDMC | Bisdemethoxycurcumin |
| C1 | Cluster 1 |
| C2 | Cluster 2 |
| C3 | Cluster 3 |
| CUR | Curcumin |
| DMC | Demethoxycurcumin |
| HPLC | High-performance liquid chromatography |

# CHAPTER 1

# INTRODUCTION

## 1.1 The Background and Importance

Turmeric (*Curcuma longa* L.) is a widely cultivated plant in Southeast Asia, valued for its use as a spice and in traditional medicine. Its rhizomes contain many curcuminoids, and there are three major curcuminoids in its rhizome: curcumin (CUR), bisdemethoxycurcumin (BDMC), and demethoxycurcumin (DMC). These three major curcuminoids are known for their antioxidant, anti-inflammatory, and therapeutic properties (Chainani-Wu, 2003; Niranjan & Prakash, 2008). Among these three, curcumin is the most studied curcuminoid and has the highest superoxide anion scavenging activity but is the least stable, while BDMC is the most stable and most effective in modulating the MDR-1 gene in multidrug-resistant cervical carcinoma cells (Limtrakul et al., 2004). The Thai Herbal Pharmacopoeia (THP) has studied and recommended that dried turmeric should not contain less than 6.0% v/w of turmeric oil content and 5.0% w/w of total curcuminoid contents calculated as curcumin, their method used curcumin content to indicate the standard of curcuminoid content (Thai Herbal Pharmacopoeia, 2024). The quality of turmeric, particularly its curcuminoid content, is a key factor for both culinary and medicinal uses.

In Thailand, turmeric cultivation is expanding due to growing demand for both local consumption and export. Chiang Rai, a northern province with its ideal soil and climate, has become an important area for turmeric cultivation (Horticulture Research Institute, 2023). Important factors for Curcuma longa *L.* growth and quality like soil nutrients, environmental conditions, and altitude significantly influence rhizome yield and curcumin content (Is et al., 2015). Dark-red soil has been associated with higher turmeric yield and curcumin content (Hossain & Ishimine, 2005). Fertilizer management, including the balanced application of nitrogen, potassium, and phosphorus, can further enhance growth, yield, and curcumin content (Akamine et al., 2007; Menon, 2014; Jabborova et al., 2021). While there are substantial amounts of

research on turmeric cultivation globally, there are limited amounts of studies that have focused on the effects of soil in this specific Thailand region. Soil nutrients and properties such as organic carbon, pH, electrical conductivity (EC), and cation exchange capacity (CEC) are known to influence plant growth and secondary metabolite production, but their impact on turmeric quality in Chiang Rai remains unclear.

There are two turmeric cultivars cultivated in Thailand that are officially registered by the Department of Agriculture, Thailand: Trung 1 (T1) and Trung 84-2 (T84-2). In southern Thailand, T1 can give fresh rhizomes about 2.23 tons per rai. It has 10.62% of curcuminoid contents more than Thai Herbal Pharmacopoeia standard, 7.99% of essential oils, and 47% of ar-turmerone. While T84-2 can give fresh rhizomes about 2.59 tons per rai. It has 11.4% of curcuminoid contents more than Thai Herbal Pharmacopoeia standard, 7.78% of essential oils, 47.6% of ar-turmerone and 23.38% of alpha-turmerone. (Medicinal Innovation Center of Mae Fah Luang, 2021)

This study aims to investigate the effects of soil parameters on the extraction yields and curcuminoid content of turmeric grown in different areas of Chiang Rai. By understanding these relationships, we seek to identify optimal soil conditions for improving the quality of *Curcuma longa L.* in this region, contributing to more efficient cultivation practices.

## 1.2 The Objectives

1.2.1 Investigate the curcuminoid content of local *Curcuma longa* L. cultivars from various areas in Chiang Rai

1.2.2 Determine the optimal soil parameters for enhancing *Curcuma longa* L.*'s* curcuminoid yield

## 1.3 The Scope of Work

The study of the effects of soil nutrients on the extraction yields and curcuminoid content of turmeric grown in different areas of Chiang Rai was performed.

Turmeric and soil samples were collected from different areas across Chiang Rai. Curcuminoid contents in turmeric samples such as curcumin (CUR), bisdemethoxycurcumin (BDMC), and demethoxycurcumin (DMC) were determined by spectrophotometric and HPLC methods. Various soil parameters were determined; soil pH, electrical conductivity (EC), cation exchange capacity (CEC), soil organic carbon (SOC), soil macronutrients, and soil micronutrients. Statistical analysis of experiment results was performed by using MetaboAnalyst 6.0 at www.metaboanalyst.ca with statistical methods such as hierarchical clustering analysis for sample grouping, ANOVA, principal component analysis (PCA) and t-test analysis for differential soil parameters of soil samples, and Pearson's correlation coefficient was used to assess the correlation between soil parameters and turmeric qualities.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 The Background of Turmeric and Curcuminoids

One must first understand what turmeric is, its chemical constituents, and biological activity first, to grasp the mechanism of turmeric and curcuminoid content, and to be able to control and increase its quality. The curcuminoid structures were showed in Figure 2.1. The previous studies that investigated this were presented as follows.

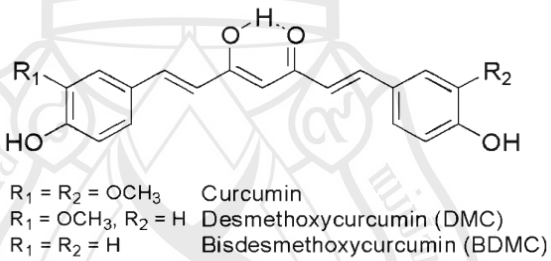



**Figure 2.1** Structures of Curcuminoids

Niranjan and Prakash (2008) studied a broad spectrum of biological activities of turmeric. Tumeric was found to have a potent scavenger of various reactive oxygen species (ROS) and also inhibits ROS-generating enzymes, cyclooxygenase, and lipoxygenase, actively suppressing both COX-I and COX-II. Turmeric extract prevents oxidative damage, and inhibits DNA binding of toxic metabolites. Safety assessments indicate good tolerability at high doses (0.5 to 1.5 g per day). The 3 to 6% curcuminoid content, a mixture of CUR, DMC, and BDMC, constitutes the primary bioactive components. Notably, pure curcumin exhibits stronger superoxide anion scavenging activity compared to its derivatives.

Kita et al. (2008) developed an in vitro turmeric culture system for feeding $^{13}$C-labeled precursors. an $^{13}$C-NMR analysis of unsymmetrical curcuminoid showed that one molecule of acetic acid or malonic acid and two molecules of phenylalanine or

phenylpropanoids, were incorporated into DMC. The incorporation efficiencies of the same precursors into DMC were similar to curcumin with the order of precursors incorporation; malonic acid > acetic acid, and cinnamic acid > p-coumaric acid>>ferulic acid). These findings propose that the curcuminoid biosynthetic pathway utilizes two cinnamoyl CoAs and one malonyl CoA, and that aromatic ring hydroxylation and methoxylation occur subsequent to the formation of the core curcuminoid skeleton, as shown in Figure 2.2.

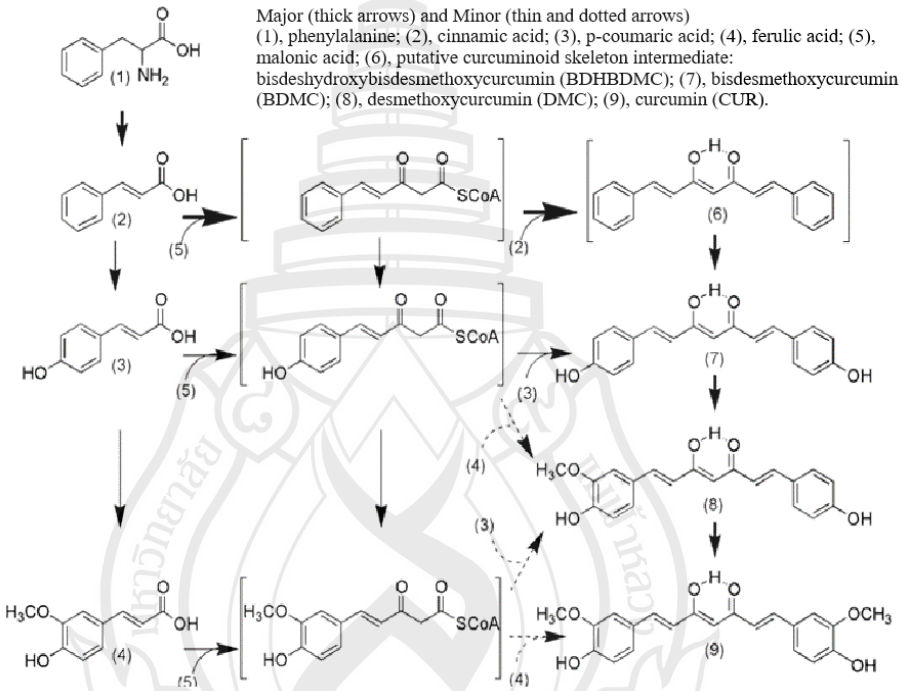



**Figure 2.2** Proposed Curcuminoids Biosynthesis Pathways

Chainani-Wu (2003) investigated numerous reports on curcumin that demonstrated curcuminoids' antioxidants, anti-inflammatory, antiviral, and antifungal properties. Six human trials, involving up to 8000 mg/day curcumin dosages for 3 months, established curcumin's safety. Mechanistically, laboratory studies have identified multiple inflammatory mediators inhibited by curcumin. These findings indicate that curcumin is safe in humans and possesses anti-inflammatory properties, potentially mediated through the inhibition of diverse inflammatory molecules.

From the literature review, it was found that turmeric’s curcuminoids have antioxidants, anti-inflammatory, antiviral, and many other properties. Curcumin was

found to be safe in humans. It also exhibits stronger antioxidants compared to its derivatives and also has potent bioprotectant as well.

## 2.2 The Background of Soil Analysis

Soil analysis is a crucial aspect of successful turmeric (*Curcuma longa* L.) cultivation. Understanding the soil's physical and chemical properties helps determine its suitability for turmeric and guides nutrient management for optimal growth, yield, and curcuminoid content. The previous studies that investigated the effect of soil quality on turmeric were presented as follows.

Is et al. (2015) studied turmeric from various cultivated areas for the effect of factors on turmeric. Turmeric was collected from a high-altitude research station and then planted in 9 different areas. Analyses of soil properties were done up to second generation. The result was turmeric growth in the initial research station showed 5% of curcumin while other areas showed a range of 1.4 to 5% of curcumin.

Hossain and Ishimine (2005) studied the effect on quality of turmeric cultivated with dark-red soil (pH 5.2), gray soil (pH 7.4) and red soil (pH 4.4). 50-cm deep layers of soil were collected. The result was that turmeric cultivated on dark-red soil showed the highest growth, and highest turmeric yield with high curcumin yield compared to other soils.

Choudhury et al. (2017) investigated the impact of pre-harvest soil management and post-harvest processing on the phytochemical properties of turmeric. Pre-harvest soil management was found to significantly ($P \leq 0.001$) affected rhizome phytochemical concentrations. Notably, fresh mother rhizomes exhibited 15-38% higher curcumin compared to fresh finger rhizomes. However, processing fresh rhizomes into dry powder resulted in significant reductions in curcumin (2.0-8.0%). Poultry manuring with liming was found to increase the phytochemical concentrations in the turmeric rhizomes.

Sandeep et al. (2017) investigated the influence of climate, soil, and harvesting phase on curcumin yield and CURS gene expression across two turmeric cultivars, Surama and 'Roma. The author found significant variability in both metrics: curcumin

content ranged from 1.5% to 5.0% in Surama and 1.4% to 5.0% in Roma, while CURS gene expression varied from 0.402-5.584 fold and 0.856-5.217 fold, respectively. Harvesting time was found to be a significant factor, affecting curcumin content at a specific zone (3.95%-4.31% and 3.57%-3.83% in Surama and Roma respectively) and notably influencing CURS expression (7.389-16.882 fold and 4.41-8.342 fold in Surama and Roma respectively). These variations were attributed to the impact of soil and environmental variables. This study showed a positive correlation between CURS gene expression and curcumin content when considering different harvesting times within a given geographical zone.

Shankar et al. (2023) studied the curcuminoid biosynthesis expression levels of MAPK1 and DCS genes, under salinity stress conditions using semi- quantitative PCR. MAPK1 and DCS genes were found to down-regulated under salt stress conditions.

From the literature review, different agroclimatic regions, types of soils and soil management influenced the qualities of turmeric. To achieve the optimal quality of turmeric, these factors must be considered before the cultivation of turmeric.

## 2.3 The Background of Curcuminoids Analysis

The concentration of curcuminoids in turmeric rhizomes is not constant and is influenced by a multitude of factors throughout the plant's lifecycle and post-harvest processing. These factors such as genetics, environment, plant growth and development, and post-harvesting processing. The study for understanding how these factors affecting curcuminoid quality were presented as follows.

Tanaka et al. (2008) developed a rapid, non-destructive method for quantifying major curcuminoids (CUR, BDMC, DMC) in turmeric using near-infrared (NIR) spectroscopy coupled with multivariate statistics. It was found that characteristic curcuminoid absorptions were identified in the second derivatives of NIR spectra at approximately 1700 nm and 2300-2320 nm. Partial least-squares regression (PLS-R) models were constructed from NIR spectra of 34 turmeric samples to quantify individual curcuminoids and total curcuminoid content. The PLS-R predictions

demonstrated high correlation with results obtained from high-performance liquid chromatography (HPLC) quantitation, validating the method's accuracy.

Li et al. (2011) established a sensitive and validated high-performance liquid chromatography coupled with ultraviolet detection and mass spectrometry (HPLC-UV-MS) method for the qualitative and quantitative analysis of curcuminoids in eight herbal medicines. CUR, DMC, and BDMC exhibited excellent linearity ($r > 0.9998$) across concentration ranges of 4.88–625, 4.29–550, and 3.98–510 μg/mL, respectively. The study revealed significant variations in the content of these three major curcuminoids among different herbal medicines.

Papayrata et al. (2024) studied and compared the bioactive compound content and antioxidant activities of three *Curcuma* species (*C. mangga*, *C. zedoaria*, and *C. longa*). HPLC and LC-MS were employed to quantify curcuminoids, phenolics, and flavonoid acids, while DPPH and FRAP assays assessed antioxidant capacity. Colorimetric variations (L*, a*, b*) suggested differing bioactive profiles. *C. longa* demonstrated significantly higher levels ($p < 0.05$) of curcuminoids, compared to *C. mangga* and *C. zedoaria*. Correspondingly, *C. longa* exhibited significantly greater antioxidant activity ($p < 0.05$). These findings reveal substantial interspecies variations in bioactive composition within the *Curcuma* genus, with *C. longa* exhibiting superior levels of key phytochemicals and antioxidant activity.

Singh et al. (2013) studied to find the promising high curcumin genotypes. Cv. Surama of Eastern ghat highland was found yielding high curcumin content (8.8%).

From these literature reviews, it was found that near-infrared spectroscopy, HPLC, and LC-MS can be used to analyze the curcuminoids' quantities.

## 2.4. The Background of Statistical Analysis for Correlation Study

There are various statistical methods that have been used to study the correlation of turmeric quality such as Hierarchical Cluster Analysis (HCA), Principal Component Analysis (PCA), and etc. These methods can be used to analyze the difference in raw data of the study. The statistical analysis methods were presented as follows.

Song et al. (2023). developed a method to differentiate between the rhizomes and tuberous roots, and to identify distinguishing markers. The author used high-performance liquid chromatography (HPLC). Hierarchical Cluster Analysis (HCA), Similarity Analysis (SA), and Principal Component Analysis (PCA) to discriminated between the two sample types, grouping rhizomes and tuberous roots into distinct categories. Quantitative analysis revealed that the content of CUR, BDMC, and DMC was consistently higher in all rhizome samples compared to tuberous roots. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was employed to screen for differential markers, yielding a robust model with excellent predictive capability. Key markers for discriminating samples were identified as DMC and CUR. The study concluded that the combination of HPLC fingerprinting and multivariate statistical analysis provides an effective method for distinguishing between the rhizomes and tuberous roots of *Curcuma longa*.

Jia et al. (2021) employed a three-step strategy to differentiate turmeric samples originating from various Chinese provinces and regions. First, a multiple reaction monitoring (MRM)-based ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was established for comprehensive chemical profiling of curcuminoids in turmeric. Second, response surface methodology was applied to optimize the extraction of target curcuminoids. Finally, multivariate analysis was performed for systematic characterization of 66 curcuminoids. Principal Component Analysis (PCA) and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) successfully categorized 66 turmeric samples into two distinct groups, notably separating those from Sichuan province from other regions, with samples within each group exhibiting similar curcuminoid distribution. Twenty-five differential curcuminoids were identified via OPLS-DA, with most showing higher abundance in Sichuan samples. Furthermore, Hierarchical Cluster Analysis (HCA), utilizing these screened differential curcuminoids, clearly distinguished turmeric samples from different provinces.

Akbar et al. (2016) developed an artificial neural network (ANN) model to predict optimal curcumin content by mapping genome-environment interactions. This model, built and validated using 119 turmeric samples from eight agro-climatic regions in Odisha, exhibited curcumin content ranging from 0.4% to 7.2%. The model was

trained by using 11 soil and climatic parameters as inputs and curcumin content as the output. Sensitivity analysis identified minimum relative humidity, altitude, soil nitrogen content, and soil pH as key environmental factors significantly influencing curcumin accumulation. ANN model provides an efficient tool for predicting and optimizing site-specific curcumin content.

Aarthi et al. (2020) assessed eleven turmeric cultivars grown at various altitudes in India. Statistical analyses revealed significant variations among cultivars, environments, and their effect on yield, and curcumin content. Environmental factors accounted for 70.8% of the variation in fresh yield, whereas genotype significantly influenced curcumin content (17.7%), and dry yield (15.7%). 'Mega Turmeric' demonstrated the highest stability for fresh yield, consistently exhibiting above-average yields across all environments. For curcumin production, 'IISR Kedaram' displayed consistent performance across environments, indicating high stability. These three varieties exhibited high stability for dry yield and represent valuable genetic resources for enhancing yield stability.

From these literature reviews, Hierarchical Cluster Analysis (HCA), Similarity Analysis (SA), Principal Component Analysis (PCA), and artificial neural network (ANN) model were found to had been used for statistical analysis of curcuminoid data.

# CHAPTER 3

# EXPERIMENTAL

## 3.1 Chemical and Materials

In this work, we have used various chemicals and materials; acetic acid (glacial, 100%) was purchased from J.T.Baker (United States), activated charcoal, brucine ($C_{23}H_{26}N_2O_4$), barium chloride (BaCl), diethylenetriamine pentaacetic acid (DTPA)($C_{14}H_{23}N_3O_{10}$), and triethanolamine (TEA) ($(CH_2OHCH_2)_3N$) were purchased from Alfa Aesar/Thermo Scientific Chemicals (United States), ammonium fluoride ($NH_4F$) was purchased from Merck (Germany), ammonium sulfate ($(NH_4)_2SO_4$) was purchased from Daejung (South Korea), ammonium acetate ($NH_4CH_3CO_2$, AR), sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$, AR), sodium chloride (NaCl, AR), and sodium hydroxide (NaOH, AR) were purchased from RCI Labscan Limited (Thailand), calcium chloride dihydrate ($CaCl_2 + 2H_2O$, AR), hydrochloric acid (HCI, 37% AR), potassium dihydrogen phosphate ($KH_2PO_4$, AR), potassium sulfate ($K_2SO_4$, AR), and tin(II) chloride dihydrate ($SnCl_2$ $2H_2O$, AR) were purchased from QReC™ (New Zealand), diammonium molybdate tetrahydrate ($(NH_4)_2MoO_4$) was purchased from Ajax Finechem Pty Ltd. (Australia), gum arabic, mercury(II) iodide ($HgI_2$), and sodium tartrate ($C_4H_4Na_2O_6 \cdot 2H_2O$) were purchased from Acros/Thermo Scientific Chemicals (United States), potassium chloride (KCl) was purchased from Univar Solutions (USA), potassium iodine (KI) was purchased from Pine Chemicals (United States). The deionized water was provided by Mae Fah Luang University (Chiang Rai, Thailand)

The analyzing instruments utilized in this study were: electrical conductivity meter (Seven2Go, Mettler Toledo ,Switzerland), flame photometer (Z-5000 Polarized Zeeman Flame/Graphite Furnace Atomic Absorption Spectrophotometer, Hitachi, Japan), ICP-MS (Agilent 8900 triple quadrupole, Agilent Technologies, Inc., United States), pH meter (Cyberscan pH510, Thermo Fisher Scientific, United States), UV-visible spectrophotometer (USB4000, Ocean Optics, United States) and HPLC system (Agilent Technology HPLC 1260 Infinity II system).

HPLC analysis was performed by using these chemicals and instruments; HPLC system - (Agilent Technology HPLC 1260 Infinity II system) with a quaternary pump operating at 400 bar, temperature-controlled column, auto sampler, and UV-Vis diode array detector. Chromatographic column - Infinity Lab Poroshell 120 EC-C18 column (4.6 * 150 mm, 4 um), Curcumin USP Reference Standard—CAS# 1151855 (Merk, USA), Lot No.: R137E0 Desmethoxycurcumin USP Reference Standard—CAS# 1173100 (Merk, USA) Bisdesmethoxycurcumin USP Reference Standard—CAS# 1075305 (Merk, USA) NIST SRM 3299 *Curcuma longa* L. (Turmeric) Rhizome, NIST-3300 *Curcuma longa* L. (Turmeric) Rhizome Extract. Curcumin Reference Material—DRE-C11780000

The other instruments utilized in this study were mechanical shaker (DAIHAN® Digital Precise Shaking Water Bath "MaXturdy TM", DAIHAN Scientific Co., Ltd., Gangwon-do, Korea). muffled furnace (Carbolite CWF 13/13, Carbolite Gero Ltd., United Kingdom), and universal oven (Memmert UF30, Memmert GmbH + Co.KG, Germany). Whatman filter paper No.1 was utilized in this study (Whatman plc, United Kingdom) 0.22um syringe filter was purchased from (C0000602 Labfil, Zhejiang ALWSCI Technologies Co., Ltd, Zhejiang, China) The deionized water and ashing vessel was provided by Mae Fah Luang University (Chiang Rai, Thailand)

## 3.2 Chemicals Preparation

### 3.2.1 Curcuminoid Content Analysis

3.2.1.1 Total Curcuminoid Content And %Yield Analysis

Total curcuminoid content analysis was performed by using a spectrophotometric method adapted from the Thai Herbal Pharmacopoeia (Thai Herbal Pharmacopoeia 2021).

Standard curcumin solution Dissolved about 300 mg of curcumin reference standard (Curcumin USP Reference Standard—CAS# 1151855 (Merk, USA)), accurately weighed, in sufficient methanol to produce 250.0 mL.

The stock standard was transferred into six 100-mL volumetric flasks, 1, 2, 3, 4, 5, and 6 mL, respectively, and was diluted to volume with methanol, and mixed for standard curcumin calibration curve.

Sample Analysis

The dried turmeric powder sample (300 mg) was extracted with 10 ml of tetrahydrofuran (THF). Set aside at room temperature for 24 hours with frequent shaking from the reciprocating shaker. After that, the supernatant liquid was being filtered by Whatman No. 44 filter paper. The 1.0 mL of clear supernatant liquid was transferred into a 25-mL volumetric flask and diluted to volume with methanol. Finally, the total curcuminoid content was quantified at 420 using a microplate reader (Fluostar/Omega, BMG LABTECH, Germany) at 420 nm.

Calculation

By reference to the standard curve, calculate the Total curcuminoid contents as curcumin in the sample. %Total curcuminoid content in the original samples was calculated as follows:

$$\%\text{Total curcuminoid in original sample} = \frac{C + V}{W} \times D \times 100\% \quad (1)$$

Equation (1) where C = the concentration of analyte from the standard curve (mg/L), V = the extract volume (0.025 L), W = the weight of the test portion (300 g), and D = the dilution factor.

In addition, the solvent in the extractant was being further evaporated, and then dried extractant was weighted to calculate the %Yield as follows.

$$\%\text{Yield} = \frac{\text{weight of dried turmeric powder (g)} - \text{weight of dried extractant (g)}}{\text{weight of dried turmeric powder (g)}} \times 100\% \quad (2)$$

**3.2.2 Curcuminoid Derivative Contents Analysis Preparation**

Analysis of curcuminoid derivative contents was performed by using a High-Performance Liquid Chromatography (HPLC) adapted from Mudge, E. M., et al. (2021) method.

Mixed standard solution

Curcumin (CUR) stock solution, 1000 ug/mL. —Weighed an amount of CUR primary standard (Curcumin USP Reference Standard—CAS# 1151855, Merk, USA) equivalent to 10.0 mg after correction for purity and place in a 10 mL volumetric

flask. Filled to volume with methanol, mixed, and stored in a screw top vial at 20 ºC for up to 4 weeks.

Bisdemethoxycurcumin (BDMC) stock solution, 1000 ug/mL. — Weighed an amount of BDMC primary standard (Bisdesmethoxycurcumin USP Reference Standard—CAS# 1075305, Merk, USA) equivalent to 10.0mg after correction for purity and place in a 10mL volumetric flask. Filled to volume with methanol, mixed, and stored in a screw top vial at 20ºC for up to 4 weeks.

Demethoxycurcumin (DMC) stock solution, 1000 ug/mL. — Weighed an amount of DMC primary standard (Desmethoxycurcumin USP Reference Standard—CAS# 1173100, Merk, USA) equivalent to 10.0 mg after correction for purity and place in a 10mL volumetric flask. Filled to volume with methanol, mixed, and stored in a screw top vial at 20 ºC for up to 4 weeks.

Mixed standard calibration curves — the seven different concentrations of mixed standards (CUR: 5-300 ppm, BDMC: 1-120 ppm, DMC: 1-100 ppm,) using the dilutions in methanol were used to construct standard calibration curves.

## 3.3 Soil and Turmeric Sample Collection

The soil sample was taken from three randomly picked sites at 15 cm depth from various cultivation sites in Chiang Rai, Thailand. Turmeric rhizome was collected at the same site as the soil sampling site. Both samples were taken 7 months after planting in Year 2022. The authenticity of turmeric was determined by comparing them with the herbarium specimen in the Medicinal Plant Innovation Center of Mae Fah Luang University, Chiang Rai, Thailand.

## 3.4 Soil Sample Preparation

The drying of soil samples was achieved by adapting Motsara et al. (2008) method. The samples were dried in the air whose temperature did not exceed 35 °C and relative humidity was about 30–60 percent. Air-dried soil samples were undergoing preparation to achieve a uniform particle size for analysis. Samples were gently crushed

using a mortar and pestle. Pebbles, concretions, and stones were excluded during grinding. The entire processed samples were then sieved through a 2 mm mesh. Materials retained on the sieve (coarse fraction) were reground and re-sieved until all aggregates pass through, minimizing pebbles, organic residues and concretions in the final sample composition.

## 3.5 Preparation of Turmeric Sample

After harvest, fresh turmeric rhizomes were washed with clean water alongside scraping and brushing with (washing) machine and then shade-drying overnight. Shade-dried turmeric rhizomes were then sliced and dried at 55 °C in the oven for 6 hrs.

For turmeric rhizome powder**,** dried turmeric rhizomes were grinded with a grinding machine and filtered with a filter machine to get fine powder.

And for turmeric samples for HPLC analysis, dried turmeric rhizomes were homogenized using a centrifugal mill equipped with a 0.25 mm screen. The ground samples were stored in polypropylene tubes at room temperature, protected from light, and thoroughly mixed before analysis. For each sample, three replicates were prepared. Accurately weighed portions of ground turmeric rhizomes (75 mg) were transferred to 50 mL centrifuge tubes. Methanol (25 mL) was added, and the samples were vortexed for 30 seconds, followed by shaking on a wrist-action shaker for 15 minutes at room temperature. After centrifugation at 4500 g for 5 minutes, 1 mL aliquots were filtered through syringe filters into LC vials and stored at 4°C, protected from light, until analysis. All tincture tests were prepared in triplicate. After gentle inversion to ensure homogeneity, 100 µL of each tincture was diluted with 900 µL of methanol and vortexed. The diluted samples were then filtered through syringe filters into LC vials and stored at 4°C, protected from light, until analysis.

## 3.6 Curcuminoid Analysis by Spectrophotometry and HPLC

The Thai Herbal Pharmacopoeia (2021) method was used to determine curcuminoid content with a spectrophotometer. 300 mg of turmeric powder was extracted with 10 mL THF for 24 hours at room temperature on a shaker. The extract was filtered, and the solvent evaporated. A standard curve was prepared, and curcuminoid content was quantified by measuring the absorbance of a diluted extract at 420 nm using a microplate reader

A high-performance liquid chromatography (HPLC) method was also employed to quantify curcuminoid content. The experimental parameters were adapted from Mudge et al. (2021). The analysis was conducted using a UV detector set at 425 nm, a column temperature of 40°C, an injection volume of 10.0 µL, and a flow rate of 1.0 mL/min. The mobile phase consisted of 45% acetonitrile (ACN) containing 0.1% formic acid and 55% water containing 0.1% formic acid. The total run time was 20 minutes.

To establish a calibration curve, a series of calibration standards (stds 1-7) were injected individually. The peak areas or peak heights corresponding to the analyte of interest were recorded. Subsequently, individual samples were injected, and their peak responses were measured

To ensure the accuracy and precision of the analysis, a quality control measure was implemented. A calibration standard was re-injecting approximately every 10 sample injections and at the end of the analytical run. The peak response of the re-injected standard was compared to its initial value to assess system stability.

The calibration curve was then used to determine the concentration of the analyte in each sample based on its peak response. Statistical analysis was performed to evaluate the accuracy and precision of the results.

Calculations

1. Calibration standards were analyzed to determine peak areas for CUR, DMC, and BDMC. To assess instrument precision and reproducibility, each calibration standard was re-injected and analyzed. The peak areas obtained from the re-injected

standards were required to be within a 5% deviation of the corresponding initial peak areas.

2. To establish a quantitative relationship between analyte concentration and peak area, a plot was constructed with analyte concentration on the x-axis and individual peak areas on the y-axis for CUR, DMS, and BDMC. Least squares regression analysis was employed to determine the equation of the best-fit line for each analyte. This line, characterized by its slope, intercept, and correlation coefficient ($r^2$), provides a mathematical model that can be used to predict the concentration of an unknown sample based on its corresponding peak area.

3. Concentrations of curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) in unknown samples were determined using standard curves and peak area analysis. Extracts with peak areas exceeding standard curve ranges were diluted and reanalyzed. Curcuminoid concentration (mg/L) = curcuminoid peak area - intercept of linear regression slope of linear regression.

4. Total curcuminoid content in the original samples was calculated by using Equation (1).

## 3.7 Soil Properties Analytical Methods

### 3.7.1 The pH Analysis

The pH analysis was adapted from the report of Motsara et al. (2008). The 10.0 g of each soil sample into a 50-ml centrifuge tube, 20 ml of $CaCl_2$ solution was added. The soil was allowed to absorb the $CaCl_2$ solution without stirring, then was stirred thoroughly for 10 seconds using a glass rod. The soil suspension was stirred for 30 minutes, and soil pH was recorded on the calibrated pH meter.

### 3.7.2 The Soil Electrical Conductivity (EC) Analysis

In an adaptation of Motsara et al. (2008), soil electrical conductivity (EC) is determined by weighing 20 g of soil into a 50 mL polyethylene bottle. Deionized water (100 mL) is added, and the capped bottle is shaken horizontally for 60 minutes at 180 rpm on a reciprocating shaker. After settling for 30 minutes, the EC meter's conductivity

cell measures the supernatant without disturbing the sediment. The stable reading is recorded, the electrode rinsed thoroughly, and EC (dS $m^{-1}$) reported at 25 °C.

**3.7.3 The Cation Capacity Exchange (CEC) Analysis**

Following Motsara et al. (2008), CEC was determined by extracting 5 g of soil with 1.0 M sodium acetate (25 mL each, 4 times) followed by ethanol washes. Adsorbed Na was then displaced with ammonium acetate (repeated extraction sequence) and collected in a 100 mL volumetric flask. Flame photometry quantified Na in the final extract using a standard curve (0-10 meq/L Na).

Calculation:

The $NH_4OAc$ extractable Na that is exchangeable Na in meq/100 g soil is calculated as follows.

$$\frac{\text{Na concentration in meq/L} \times 100}{\text{Weight of soil in g (g)}} \times \frac{\text{Volume of extract in ml (100)}}{1000} \quad (4)$$

**3.7.4 The Soil Organic Carbon (SOC) Analysis**

Soil organic carbon (SOC) content can be determined by a gravimetric method called loss-on-ignition (Motsara et al., 2008). 10 g of soil sample in an ashing vessel was oven-dried at 105 °C for 4 hours to remove water. The vessel was removed from the oven and dried in the desiccator. The dried sample is weighed and then ignited in a muffle furnace at 400 °C for 4 hours to combust organic matter. The vessel was removed from the furnace and dried again. After cooling, the final weight is recorded. The SOC content is estimated based on the weight loss after ignition.

Calculation:

The percentage of OM (%OM) is given by:

$$\text{Percent organic matter (OM)} = \frac{(W_1 - W_2)}{W_1} \times 100 \quad (5)$$

Equation (5) where $W_1$ is the weight of soil after oven-dried at 105 °C; $W_2$ is the weight of soil after ignition at 400 °C.

The percent of organic C (%OC) is given by: % OM × 0.58

**3.7.5 The Nitrate and Ammonium Nitrogen Analysis**

Nitrate and ammonium nitrogen were determined following the method of Sahrawat & Prasad (1975). 5 g of soil was briefly shaken with 25 mL of Morgan's reagent (prepared by dissolving 100 g sodium acetate and 30 mL glacial acetic acid in 1 L distilled water, pH adjusted to 4.8) and 0.125 g of activated charcoal in a 50 mL

centrifuge tube. The extract was filtered through Whatman No. 1 paper. For $NO_3$-N determination, 2 mL of extract was treated with 0.2 mL of 2% brucine and 3 mL of sulfuric acid. After color development (red to yellowish-orange, 30 minutes), absorbance was measured at 470 nm with a spectrophotometer. Ammonium determination ($NH_4$-N) utilized another 2 mL of diluted extract (1:1 with water) and involved the addition of 3 mL of tartrate reagent and 7 mL of Nessler's working solution. Absorbance for $NH_4$-N was measured within 5-20 minutes.

Calculation:

1. Concentration of $NO_3$-N ($\frac{mg}{kg}$) is given by:

$$\text{Concentration of NO3} - \text{N}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{C (ppm)} \times \frac{25\text{ mL}}{5\text{ g}} \times \frac{1}{2} \quad (6)$$

Equation (6) is based on 5 g sample of soil, 25 mL of 0.5M $NH_4OAc$ solution, 2 ml aliquot of the filtrate, and ppm to mg/kg = 1. Absorbance reading on UV-visible spectrophotometer = x. Concentration of $NO_3$-N as read from the standard curve for the given absorbance (x) = C (ppm).

2. Concentration of $NH_4$-N ($\frac{mg}{kg}$) is given by adapting Eq. (6):

$$\text{Concentration of NH4} - \text{N}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{C (ppm)} \times \text{D} \times \frac{25\text{ mL}}{5\text{ g}} \times \frac{1}{2} \quad (7)$$

Equation (7) is based on dilution factor (D) = 2. Concentration of $NH_4$-N as read from the standard curve for the given absorbance (x) = C (ppm).

**3.7.6 The Phosphorus (P) Analysis**

Following the method of Bray & Kurtz (1945), 25 mL of ammonium acetate extractant was added to a 5 g soil sample in a 50 mL centrifuge tube. The mixture was shaken for 5 minutes and filtered through Whatman No. 1 filter paper to isolate the extract for phosphorus determination.

Calculation:

Concentration of P is given by adapting Eq. (7):

$$\text{Concentration of P}\left(\frac{\text{mg}}{\text{kg}}\right) = \text{C (ppm)} \times 2 \times \frac{25\text{ mL}}{5\text{ g}} \times \frac{1}{5\text{ mL}} \quad (8)$$

Equation (8) where concentration of P as read from the standard curve for the given absorbance (x) = C (ppm).

**3.7.7 The Potassium (K), Calcium (Ca), Magnesium (Mg) Analysis**

Five grams of soil were weighed into a 50 mL extraction tube and extracted with 33 mL of 1 N ammonium acetate ($NH_4OAc$) solution (pH 7.0) on a reciprocal shaker for 15 minutes. The suspension was then centrifuged and the supernatant filtered through qualitative fine-porous filter paper into a clean 100 mL volumetric flask. This extraction was repeated two times, and the combined filtrates were diluted to 100 mL with ammonium acetate solution and mixed well. The resulting ammonium acetate extract was subsequently analyzed for Ca, Mg, Na, and K by ICP-MS.

Calculation:

Content of $NH_4OAc$ exchangeable cations (mg/kg) is given by:

$$\text{Content of exchangeable cations } \left(\frac{\text{mg}}{\text{kg}}\right) = \text{C (ppb)} \times \frac{100\text{ mL}}{5.0\text{ g}} \times \frac{1}{1000} \qquad (9)$$

Equation (9) is based on soil sample taken = 5.0 g, 1 M $NH_4OAc$ solution used = 100 ml, and ppb to mg/kg = 1/1000. Absorbance reading on ICP-MS of the soil extract being estimated for a particular element = x. Concentration of exchangeable cations as read from the standard curve for the given absorbance (x) = C (ppb).

**3.7.8 The Sulfur (S) Analysis**

As described by Rehm and Caidwell (1968), ten grams of soil were extracted with 25 mL of acidified ammonium acetate solution in a 50 mL centrifuge tube at 200 rpm for 30 minutes. Activated charcoal (0.25 g) was then added to remove interferences, followed by filtration through a sulfate-free filter paper. Sulfur content in the 5 mL aliquot of the filtrate was determined spectrophotometrically at 420 nm after treatment with an acid "seed" solution and barium chloride ($BaCl_2$).

Calculation:

Concentration of extractable S (mg/kg) is given by:

$$\text{Concentration of SO4 (mg)} - \frac{\text{S}}{\text{kg}} \text{ of soil} = \frac{\text{C}\left(\frac{\text{mgS}}{\text{L}}\right) 0.025\text{L}}{0.010\text{ kg soil}} \times \frac{1}{10\text{ mL}} \qquad (10)$$

Equation (10) is based on a 10 g (0.010 kg) of soil sample, 25 ml (0.025L) of 0.5M $NH_4OAc$ solution, and 10 ml aliquot of the filtrate.

**3.7.9 The Micronutrients (Fe, Zn, Cu, Mn) Analysis**

Micronutrients such as iron (Fe), zinc (Zn), copper (Cu) and manganese (Mn) were analyzed by following Motsara et al. (2008). 10 grams of soil were extracted with 20 mL DTPA extract solution in 100 mL polypropylene bottles for 2 hours at room

temperature (25 °C). A blank was included following the same procedure but omitting the soil sample. The resulting extract was used for subsequent micronutrient analysis.

Calculation:

Concentration of DTPA extractable micronutrients (mg/kg) is given by:

$$\text{Content of micronutrient } \left(\frac{\text{mg}}{\text{kg}}\right) = \text{C (ppb)} \times \frac{20\text{ mL}}{10.0\text{ g}} \times \frac{1}{1000} \quad (11)$$

Equation (10) is based on soil sample taken = 10.0 g, DTPA extract solution used = 20 ml, Absorbance reading on ICP-MS of the soil extract being estimated for a particular element = x, Concentration of micronutrients as read from the standard curve for the given absorbance (x) = C (ppb), and ppb to mg/kg = 1/1000

## 3.8 Statistical Analysis

Statistical analyses were performed in MetaboAnalyst 6.0. Soil samples comparisons were analyzed using hierarchical clustering analysis for grouping, ANOVA and t-test analysis was used to find the difference in soil parameters of soil clusters.

Curcuminoid content comparisons between T1 and T84-2 were analyzed using t-tests and Principal Component Analysis (PCA). Correlation analyses between soil parameters and turmeric qualities (yield, total curcuminoids, curcumin, DMC, BDMC) were assessed with Pearson's correlation coefficient.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Geographical and Cultivar Data of Samples

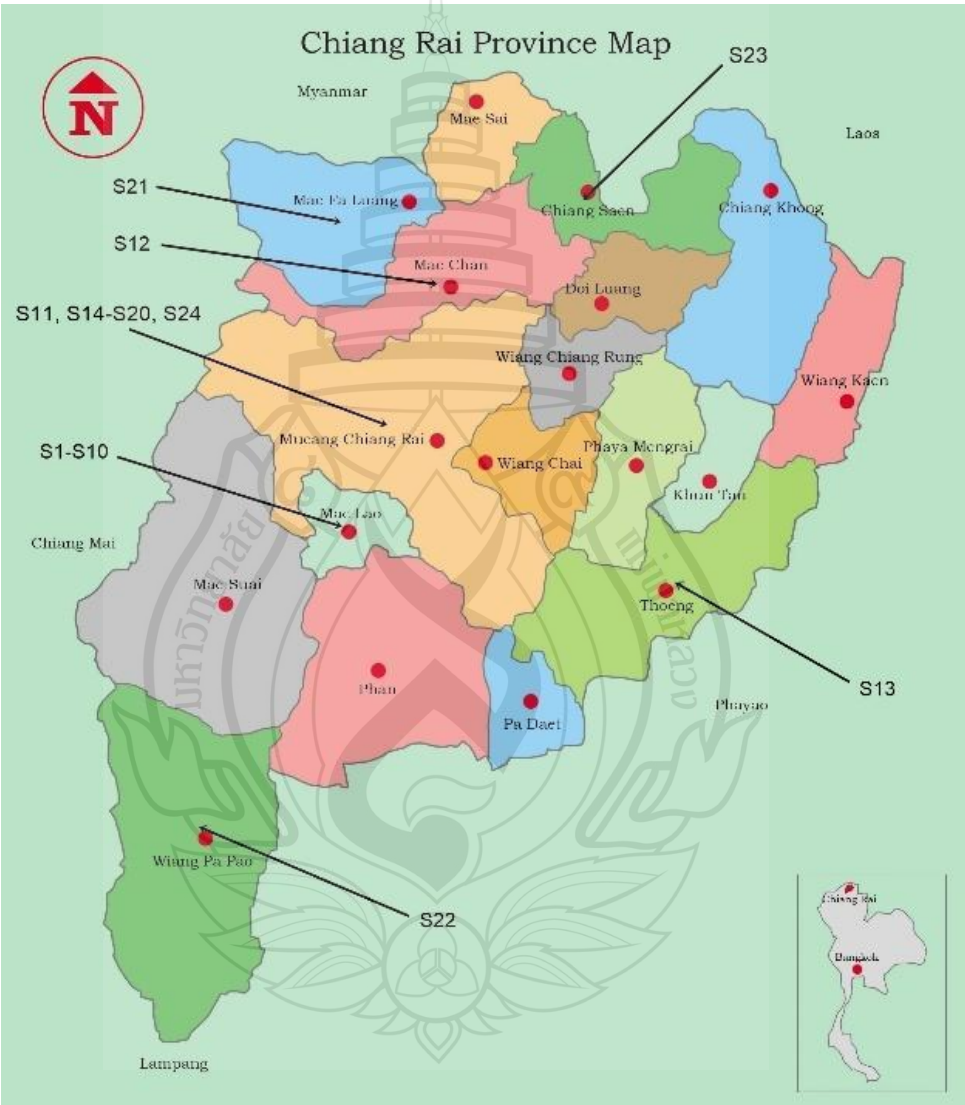
The geographical and turmeric cultivar data of twenty-four samples were obtained, as shown in Table 4.1.

**Table 4.1** Geographical Data and Cultivar of Samples

| No. Sample | Location | Cultivar |
|---|---|---|
| S1 - S10 | Pong Phrae, Mae Lao District | Trang 1 |
| S11 | Mueang Chiang Rai District | Trang 84-2 |
| S12 | Mae Jan District | Trang 84-2 |
| S13 | Ngio, Thoeng District | Trang 84-2 |
| S14-S15, S17-S18 | Doi Hang, Mueang Chiang Rai District | Trang 1 |
| S16, S19 | Doi Hang, Mueang Chiang Rai District | Trang 84-2 |
| S20 | Tha Sut, Mueang Chiang Rai District | Trang 84-2 |
| S21 | Mae Salong Nai, Mae Fah Luang District | Trang 1 |
| S22 | Chedi Mai, Wiang Pa Pao District | Trang 84-2 |
| S23 | Chiang San District | Trang 1 |
| S24 | Mae Kao Tom, Muang Chiang Rai District | Trang 1 |

The study examined turmeric samples (*Curcuma longa* L.) collected from various districts in the Chiang Rai province of Thailand. The samples were primarily of two cultivars: Trang 1 (T1) and Trang 84-2 (T84-2). Collection sites included Mae Lao District (specifically Pong Phrae), Muang Chiang Rai District, Mae Jan District, Thoeng District (specifically Ngio), Doi Hang sub-district, Mae Fah Luang District

(specifically Mae Salong Nai), Wiang Pa Pao District (specifically Chedi Mai), Chiang San District, and Mae Kao Tom in Muang Chiang Rai District. The samples were labeled from S1 to S24, with some locations providing multiple samples. The distribution of cultivars showed that both Trang 1 and Trang 84-2 were widely cultivated across different districts, suggesting their adaptability to various locations within the region, as showed in Figure 4.1.




**Source** Mae Hong Son Holidays (n.d.)

**Figure 4.1** Chiang Rai Map with the Locations of Samples

## 4.2 Soil Parameter Data

Sixteen soil parameter data were observed e.g. organic carbon (OC), pH, electrical conductivity (EC), cation exchange capacity (CEC), nitrate-nitrogen ($NO_3$-N), ammonium-nitrogen ($NH_4$-N), phosphorus (P), sodium (Na), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu) from soil samples of each turmeric farming site. The results were shown in Table 4.3.

The samples showed geochemical heterogeneity, as shown by a variety of physicochemical characteristics. The pH values ranged from acidic (~3.7) to slightly alkaline (~6.8), indicating different variables affecting proton activity. Organic carbon content, a critical measure of soil fertility and carbon sequestration, varied greatly, ranging from 1.1% to 5.4%. Electrical conductivity, a measure of soluble salt concentration, demonstrated significant diversity, with sample S13 displaying the highest value of 539 µS/cm. Similarly, potassium values were very varied, with S13 reaching a peak of 681 mg/kg. Calcium (Ca) was the most abundant element, ranging from ~89 mg/kg to > 2900 mg/kg, indicating changes in mineral composition and weathering. Notably, samples S12 and S13 have higher sodium levels compared to the other samples. Zinc (Zn) concentrations ranged from undetectable in S10 to a peak of 206.63 µg/kg in S20, indicating localized enrichment processes. Copper (Cu) contents were typically modest, with the exception of S5 and S11, which exhibited significant enrichment.

**Table 4.2** Soil Parameter Data

| Sample | Soil parameter | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | OC (%w/w) | pH | EC (uS/cm) | CEC (meq/100g) | $NO_3-N$ (mg/kg) | $NH_4$-N (mg/kg) | P (mg/kg) | K (mg/kg) | Na (mg/kg) | S (mg/kg) | Ca (mg/kg) | Mg (mg/kg) | Mn (mg/kg) | Fe (mg/kg) | Zn (ug/kg) | Cu (ug/kg) |
| S1 | 3.65±0.02 | 3.89±0.34 | 61.99±20.29 | 25.0±1.6 | 58.9±3.7 | 46.1±16.9 | 5.9±0.1 | 45.2±0.8 | 3.4±0.3 | 4.1±0.2 | 808.9±12.7 | 114.3±2.9 | 0.038±0.017 | 124.7±18.1 | 83.983±5.035 | 2.239±1.66 |
| S2 | 2.05±0.04 | 4.72±0.86 | 25.55±15.28 | 14.6±0.5 | 0±1.6 | 71.3±3.2 | 2.9±0.3 | 157.3±183.2 | 3.4±3.0 | 2.4±0.4 | 1245.9±1055.0 | 49.9±0.5 | 0.511±0.076 | 105.1±1.1 | 3.403±3.394 | 1.423±0.14 |
| S3 | 2.74±0.13 | 3.71±0.32 | 14.46±0.64 | 16.7±0.3 | 22.1±2.2 | 46.8±2.7 | 9±0.7 | 74.7±3.7 | 0.0±0.0 | 1.6±0.4 | 298.5±7.3 | 154.1±23.2 | 0.092±0.007 | 97.2±19.0 | 0.071±0.122 | 1.093±1.05 |
| S4 | 4.07±0.05 | 6.35±0.33 | 127.08±18.08 | 23.7±0.6 | 32.5±0.7 | 92.6±0.5 | 37.3±0.6 | 498.3±33.6 | 3.8±6.6 | 3.9±0.5 | 1656.0±52.6 | 320.4±31.3 | 1.186±0.157 | 81.3±8.5 | 8.285±3.044 | 1.266±0.43 |
| S5 | 5.45±0.07 | 4.2±0.21 | 13.91±0.68 | 27.5±0.5 | 12±4.1 | 138.8±20.5 | 1.5±0.7 | 109.7±6.4 | 1.4±2.5 | 6.2±0.4 | 441.4±17.2 | 46.6±2.6 | 0.326±0.029 | 70.2±9.2 | 2.724±1.710 | 27.159±1.35 |
| S6 | 4.34±0.12 | 6.25±0.05 | 143.48±150.1 | 27.6±1.3 | 43.8±0.7 | 43±20.9 | 18.6±0 | 262.6±202.7 | 4.8±4.3 | 4.8±0.8 | 1734.2±1314.3 | 136.7±1.5 | 1.614±0.412 | 42.5±31.7 | 97.455±58.952 | 6.208±7.72 |
| S7 | 3.98±0.10 | 4.58±0.20 | 21.8±0.79 | 24.0±1.5 | 25.6±0.5 | 83.8±8.5 | 3.6±0.1 | 85.4±6.7 | 3.3±3.3 | 2.6±1.2 | 744.2±14.4 | 385.8±29.2 | 0.329±0.052 | 60.7±7.2 | 5.899±4.829 | 13.341±7.12 |
| S8 | 3.65±0.59 | 6.12±0.52 | 41.08±12.37 | 23.0±0.4 | 0±0.2 | 52.3±1.4 | 4.6±0.1 | 101.1±8.9 | 39.8±40.5 | 5.2±1.5 | 1926.7±52.2 | 56.5±1.0 | 1.321±0.050 | 60.9±0.9 | 11.873±2.383 | 1.163±0.41 |
| S9 | 3.62±0.10 | 4±0.23 | 25.64±24.66 | 21.1±1.6 | 66.1±6.5 | 40.5±0.7 | 1.5±0.7 | 59.0±4.0 | 5.1±2.9 | 4.9±1.3 | 89.4±9.6 | 220.7±3.9 | 0.105±0.005 | 120.3±3.5 | 1.360±2.356 | 1.501±0.40 |
| S10 | 5.34±0.10 | 4.7±0.98 | 23.7±5.85 | 23.9±2.3 | 4±1.1 | 60±46.6 | 1.2±0.5 | 44.8±0.8 | 3.6±0.8 | 2.4±0.5 | 203.1±2.1 | 260.8±33.3 | 0.254±0.014 | 63.0±2.3 | 0.000±0 | 2.397±0.26 |
| S11 | 2.36±0.07 | 6.5±0.21 | 171.82±30.20 | 18.3±0.2 | 95.3±1.4 | 91.5±19.2 | 15.2±0.5 | 137.8±22.5 | 4.2±1.2 | 5.6±0.3 | 2237.9±59.9 | 233.1±23.8 | 0.791±0.455 | 86.7±33.8 | 18.941±25.989 | 23.724±0 |
| S12 | 3.71±0.06 | 5.99±0.51 | 338.18±257.1 | 19.7±2.0 | 51.6±5.7 | 86.5±5.3 | 9.3±0.9 | 344.7±32.0 | 67.8±5.8 | 4.3±0.4 | 1297.4±21.1 | 138.9±11.2 | 0.291±0.027 | 120.7±13.8 | 6.006±3.031 | 0±0 |
| S13 | 2.65±0.06 | 6.78±0.24 | 539.22±233. | 18.7±0.8 | 45.7±1.1 | 58.4±13.5 | 82±2.1 | 681.0±35.1 | 82.4±5.2 | 5.7±1.6 | 2648.8±168.4 | 320.9±8.3 | 1.414±0.129 | 56.3±4.2 | 35.969±4.770 | 2.873±0.57 |
| S14 | 1.82±0.01 | 5.94±1.03 | 151.86±226.03 | 15.0±1.3 | 11.3±1.3 | 84.7±8.2 | 2.9±0.2 | 43.4±1.8 | 4.4±6.1 | 2.6±1.0 | 834.5±19.5 | 129.6±36.6 | 0.230±0.036 | 109.6±7.5 | 21.941±13.346 | 0.275±0.19 |
| S15 | 1.67±0.04 | 5.69±0.28 | 50.16±11.11 | 15.9±2.7 | 4.3±1.5 | 0±0 | 2.2±0.3 | 75.2±30.5 | 3.3±1.3 | 5.5±1.1 | 1024.9±45.7 | 214.4±161.6 | 0.598±0.028 | 104.7±1.7 | 1.133±1.962 | 0±0 |
| S16 | 2.2±0.03 | 4.46±0.03 | 28.05±14.56 | 13.2±18.8 | 0±2.5 | 72.5±9.4 | 5.1±0.2 | 62.2±0.2 | 1.5±0.7 | 6.1±0.8 | 520.0±7.4 | 165.0±109.9 | 0.819±0.026 | 118.5±6.6 | 0.657±1.139 | 0.184±0 |
| S17 | 2.43±0.05 | 6.75±0.30 | 195.28±119.53 | 18.8±2.1 | 87.2±0.6 | 16.5±9.7 | 205.8±2.5 | 163.3±15.8 | 2.9±1.0 | 8.3±1.1 | 2063.7±86.0 | 138.3±6.4 | 1.080±0.229 | 83.3±10.9 | 29.923±11.503 | 1.771±1.81 |
| S18 | 2.76±0.02 | 6.32±0.01 | 132.55±15.99 | 15.7±1.8 | 16.1±0.8 | 161.9±17.0 | 34.6±1.8 | 232.2±33.7 | 12.1±10.7 | 4±0 | 1300.6±221.7 | 51.1±2.0 | 1.012±0.126 | 115.6±6.6 | 0.227±0.393 | 0.016±0 |
| S19 | 1.11±0.03 | 5.57±0.36 | 22.15±4.10 | 9.5±1.2 | 9.6±0.9 | 9.2±11.5 | 5.4±0.6 | 42.6±1.0 | 0.3±0.6 | 3.7±0.2 | 728.4±15.3 | 120.5±3.1 | 1.010±0.074 | 56.9±0.4 | 2.653±2.196 | 4.055±1.86 |
| S20 | 3.48±0.15 | 5.26±0.38 | 54.83±35.28 | 25.6±0.6 | 5.4±2.0 | 34.4±1.4 | 142.6±6.0 | 84.3±2.3 | 0.7±1.2 | 4.8±0.1 | 1529.0±37.6 | 129.3±7.2 | 0.817±0.047 | 87.1±4.9 | 206.635±13.508 | 2.402±1.92 |

**Table 4.2** (continued)

| Sample | Soil parameter | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | OC (%w/w) | pH | EC (uS/cm) | CEC (meq/100g) | $NO_3-N$ (mg/kg) | $NH_4$-N (mg/kg) | P (mg/kg) | K (mg/kg) | Na (mg/kg) | S (mg/kg) | Ca (mg/kg) | Mg (mg/kg) | Mn (mg/kg) | Fe (mg/kg) | Zn (ug/kg) | Cu (ug/kg) |
| S21 | 2.59±0.06 | 6.52±0.24 | 134.12±33.19 | 15.7±0.9 | 71.7±5.5 | 105±16.2 | 7.8±0.3 | 92.6±4.7 | 9.1±14.8 | 5.8±1.7 | 2970.5±196.0 | 52.7±41.0 | 0.585±0.056 | 90.8±3.1 | 8.814±2.039 | 0.246±0.08 |
| S22 | 3.02±0.10 | 6.42±0.41 | 141.68±56.82 | 16.6±1.3 | 48.1±2.3 | 72.3±10.5 | 47.2±1.0 | 217.2±13.0 | 21.1±5.2 | 4.5±0.6 | 1394.0±29.2 | 276.1±7.5 | 0.697±0.047 | 100.7±15.4 | 71.505±36.131 | 1.078±0 |
| S23 | 1.47±0.02 | 4.81±0.32 | 19.25±0.63 | 10.8±9.0 | 79.2±12.4 | 71.5±71.8 | 5.9±0.3 | 100.2±4.7 | 10.9±2.8 | 5.7±0.3 | 598.1±21.1 | 27.6±2.8 | 0.268±0.013 | 85.1±2.0 | 0±0 | 0.868±0.61 |
| S24 | 3.7±0.16 | 4.08±0.02 | 20.37±1.22 | 16.9±0.3 | 65.1±5.8 | 41±3.3 | 5.7±1.0 | 40.1±11.6 | 10.3±5.9 | 7.2±0.6 | 357.9±244.6 | 111.9±3.7 | 0.191±0.003 | 108.7±17.4 | 2.939±2.024 | 0.494±0.04 |
| **Mean±SD** | **3.08±1.13** | **5.40±1.02** | **104.09±122.30** | **19.1±5.064** | **35.62±30.65** | **28.52±37.58** | **27.41±49.88** | **137.50±16.30** | **12.48±8.28** | **4.66±1.61** | **1721.00±11.10** | **79.29±98.72** | **0.39±0.46** | **20.11±24.09** | **30.82±47.39** | **4.54±7.19** |

## 4.3 Clustering Analysis of Soil Properties across Sampling Locations

Hierarchical cluster analysis was employed with Ward's method and squared Euclidean distance ($p<0.05$). It enabled visualization of relationships between samples through dendrogram plots, highlighting the hierarchical structure of similarity within the dataset. This visualization aided in identifying clusters of samples with similar curcuminoid profiles, providing insights into potential patterns or groupings that may correspond to specific characteristics

The dendrogram resulting from hierarchical cluster analysis, which is based on the soil parameters in each sample depicted in Figure 1 was showed in Figure 4.4.

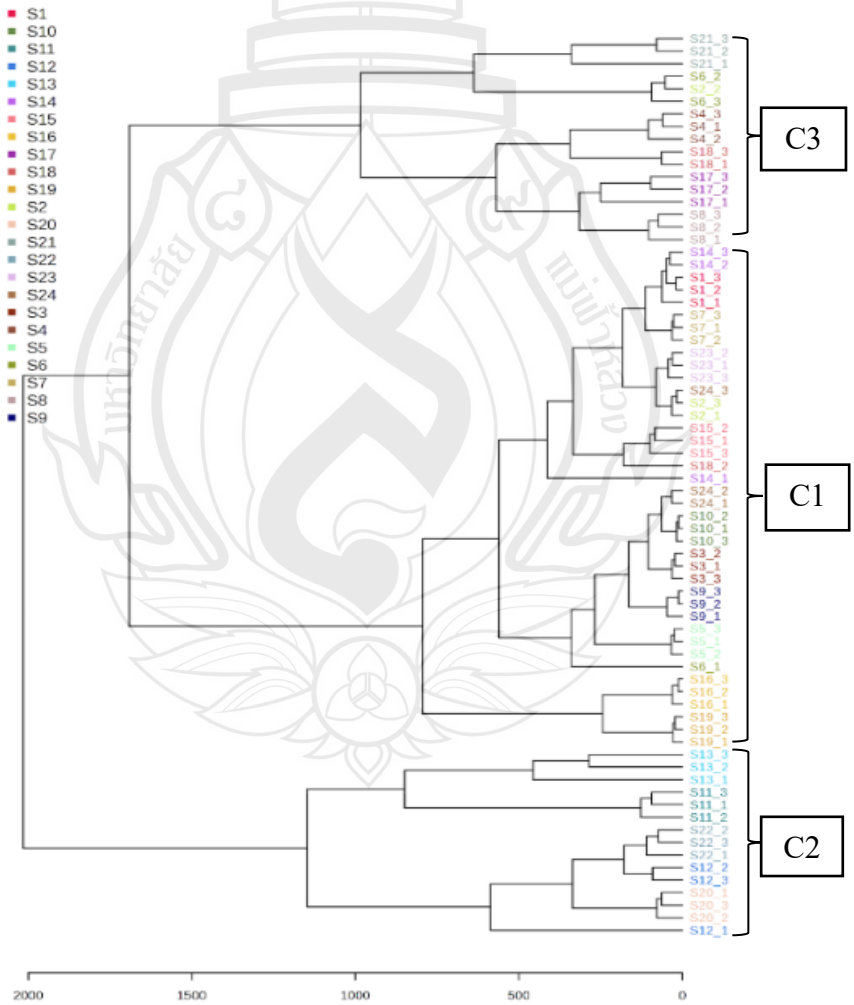



**Figure 4.2** Dendrogram of Soil Sample Clustering

From the analysis, three clusters were observed.

Cluster 1 (C1) contains the largest number of samples with 13 in total. These samples are divided between two categories: T1 and T84-2. The T1 category includes samples S1, S2, S3, S5, S7, S9, S10, S14, S15, S23, and S24, while the T84-2 category contains samples S16 and S19.

Cluster 2 (C2) comprises 5 samples, all belonging exclusively to the T84-2 category. These samples are S11, S12, S13, S20, and S22.

Cluster 3 (C3) contains 6 samples, all found in the T1 category. These samples are S4, S6, S17, S21, S8, and S18.

In total, the study analyzed 24 soil samples, with 17 samples belonging to the T1 category and 7 samples belonging to the T84-2 category. This clustering represents different soil parameter profiles that may influence turmeric growth and quality characteristics, with each cluster potentially representing distinct soil conditions or nutrient compositions.

After the clustering analysis result was obtained, ANOVA analysis was used to find the difference in soil parameters between C1, C2 and C3 soil clusters. The result was shown in Table 4.3.

**Table 4.3** ANOVA Analysis of Soil Parameter between C1, C2 and C3 Soil Clusters

| | Soil cluster | | | P-value | | | |
|---|---|---|---|---|---|---|---|
| **Name** | **C1** | **C2** | **C3** | **C1vsC2vsC3** | **C1vsC2** | **C1vsC3** | **C2vsC3** |
| pH | 4.642±0.802 | 6.192±0.608 | 6.384±0.315 | 1.45E-14* | 1.82E-08* | 9.40E-12* | 0.2967 |
| EC (us/cm) | 36.863±63.081 | 249.145±215.143 | 128.932±79.726 | 5.11E-07* | 1.32E-06* | 0.0001* | 0.0963 |
| CEC (meq Na/100 g) | 17.137±7.146 | 9.890±1.607 | 20.751±4.501 | 6.14E-06* | 0.0004* | 0.3629 | 9.99E-10* |
| Nitrate (mg/kg) | 5.421±5.218 | 49.217±29.156 | 8.389±6.086 | 1.54E-14* | 6.05E-12* | 0.1171 | 3.70E-06* |
| Ammonia (mg/kg) | 16.897±17.851 | 68.633±23.102 | 15.706±9.763 | 3.33E-14* | 1.48E-11* | 0.2135 | 1.18E-09* |
| P (mg/kg) | 3.256±2.598 | 0.003±0.006 | 51.455±70.122 | 2.02E-05* | 1.59E-05* | 0.0004* | 0.0098* |
| K (mg/kg) | 160.264±208.799 | 1821.430±533.632 | 225.019±153.827 | 1.39E-27* | 6.87E-22* | 1.20E-05* | 6.21E-13* |
| Na (mg/kg) | 5.521±4.624 | 19.780±3.213 | 12.087±19.765 | 0.0002* | 7.52E-15* | 0.2944 | 0.0028* |
| Mg (mg/kg) | 85.586±61.199 | 35.249±33.758 | 216.821±99.256 | 1.78E-10* | 0.0047* | 2.74E-06* | 2.54E-07* |
| Ca (mg/kg) | 525.649±442.00 | 251.392±101.657 | 1941.926±686.516 | 1.12E-16* | 0.02346* | 6.30E-11* | 2.49E-10* |

**Table 4.3** (continued)

| | Soil cluster | | | P-value | | | |
|---|---|---|---|---|---|---|---|
| **Name** | **C1** | **C2** | **C3** | **C1vsC2vsC3** | **C1vsC2** | **C1vsC3** | **C2vsC3** |
| Fe (mg/kg) | 80.811±40.232 | 0.802±0.400 | 79.059±25.753 | 4.17E-11* | 6.39E-10* | 0.0274* | 1.27E-12* |
| Mn (mg/kg) | 8.316±19.177 | 292.991±213.802 | 0.637±0.499 | 7.71E-14* | 9.67E-11* | 0.0028* | 3.60E-06* |
| Zn (mg/kg) | 13.500±33.894 | 90.300±25.632 | 0.026±0.039 | 2.90E-14* | 2.67E-10* | 0.0033* | 2.42E-15* |
| Cu (mg/kg) | 0.004±0.008 | 0.068±0.075 | 0.002±0.003 | 1.63E-07* | 4.01E-06* | 0.1289 | 0.0010* |

**Note** * = significantly different at p-value = 0.05

From the analysis, there are significant differences in all of 14 soil parameters except for OC and S on these soil sample clusters, with K being the most prominent among all soil parameters (p-value =1.39E-27), and Ca being the second (p-value =1.12E-16).

Among the three clusters, Cluster 1 represented the most acidic soil environment, with a mean pH of 4.64 ± 0.80. Such acidity can negatively affect nutrient solubility, especially phosphorus, and reduce microbial activity essential for organic matter mineralization (Brady and Weil, 2008; Marschner, 2012). This cluster also had the lowest electrical conductivity (36.86 ± 63.08 µS/cm), indicating minimal salinity stress, which is generally favorable for turmeric (Nair et al., 2013).

In contrast, Cluster 2 had a near-neutral pH (6.19 ± 0.61) and the highest EC (249.15 ± 215.14 µS/cm), which may indicate localized salinity concerns. Despite having the lowest CEC (9.89 ± 1.61 meq/100g), this cluster displayed significantly elevated levels of nitrate (49.22 ± 29.16 mg/kg), ammonium (68.63 ± 23.10 mg/kg), and potassium (1821.43 ± 533.63 mg/kg). These values suggest either recent fertilization or active nutrient mineralization (Havlin et al., 2014). However, the phosphorus concentration was nearly undetectable (0.003 ± 0.006 mg/kg), posing a potential limitation to rhizome development, as phosphorus is essential for root and rhizome expansion in turmeric (Ravindran et al., 2007). Micronutrient content was also high, particularly for manganese (292.99 ± 213.80 mg/kg) and zinc (90.30 ± 25.63 mg/kg), which, if excessive, could lead to toxicity or antagonism with other elements such as iron and phosphorus (Kabata-Pendias, 2010). Hence, while Cluster 2 offers high fertility in certain aspects, targeted management, especially phosphorus

supplementation and micronutrient monitoring, is necessary for sustainable turmeric production. Although its cation exchange capacity (CEC) was moderate (17.14 ± 7.15 meq/100g), concentrations of essential macronutrients—nitrate, ammonium, potassium, and phosphorus—were consistently low. Base cations such as calcium (525.65 mg/kg) and magnesium (85.59 mg/kg) were present in moderate levels. However, the overall nutrient profile suggests that Cluster 1 soils would require both pH adjustment and fertilization to become suitable for turmeric cultivation (Sairam et al., 2003).

On the other hand, Cluster 3 demonstrated the most balanced soil profile. With a pH of 6.38 ± 0.32 and moderate EC (128.93 ± 79.73 µS/cm), this cluster falls within the optimal range for turmeric (Nair et al., 2013). Importantly, it had the highest CEC (20.75 ± 4.50 meq/100g), supporting excellent nutrient retention. Phosphorus availability was markedly higher than in the other clusters (51.46 ± 70.12 mg/kg), providing favorable conditions for rhizome development (Sairam et al., 2003). Nitrogen levels were moderate, helping to avoid excessive vegetative growth while supporting overall metabolism and secondary metabolite production such as curcuminoids (Singh et al., 2010). Furthermore, calcium and magnesium were most abundant in this group (1941.93 ± 686.52 and 216.82 ± 99.26 mg/kg, respectively), both of which play crucial roles in cell wall integrity and enzymatic functions (Marschner, 2012). However, this cluster contained the lowest concentrations of zinc and manganese, which may require attention depending on crop demand and bioavailability (Alloway, 2008).

The overview of soil parameter's mean concentration of each cluster was showed in Figure 4.3.

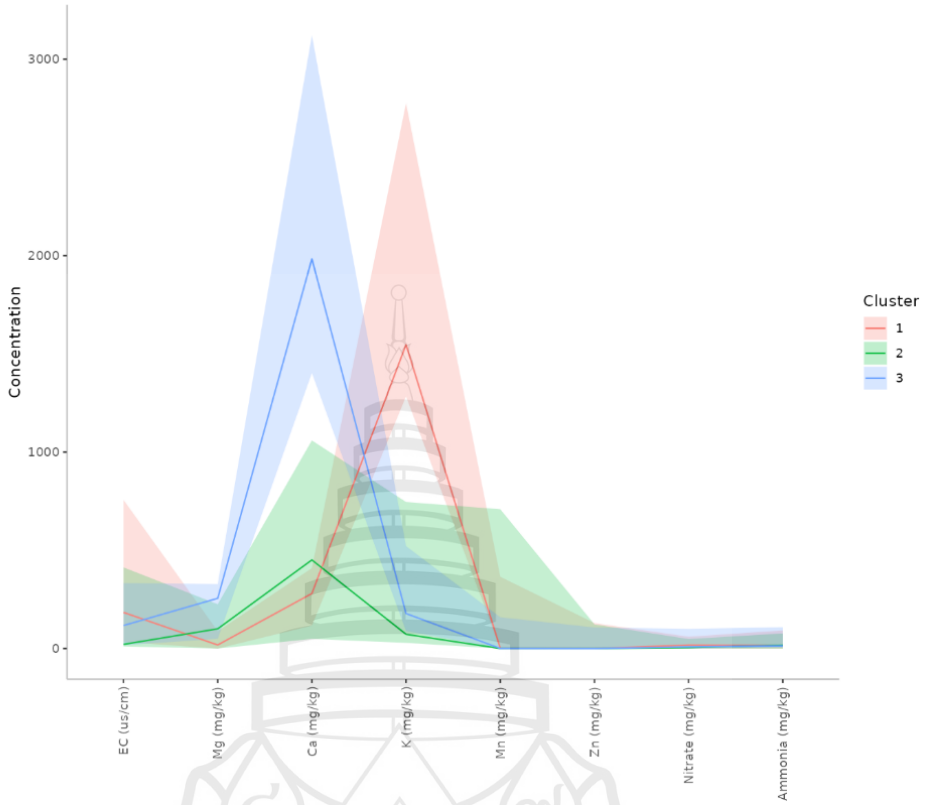



**Figure 4.3** Overview of Soil Parameter's Mean Concentration in each Cluster

## 4.4 Analysis of Curcuminoid Content in Turmeric Cultivars T1 and T84-2

### 4.4.1 Curcuminoid Content in Turmeric Cultivars T1 and T84-2

Five parameters related to turmeric were analyzed: yield, total curcumin (CUR), bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC), and total curcuminoids for both turmeric cultivar T1 and T84-2. The results for these parameters are presented in Table 4.4.

**Table 4.4** Turmeric Parameter Data

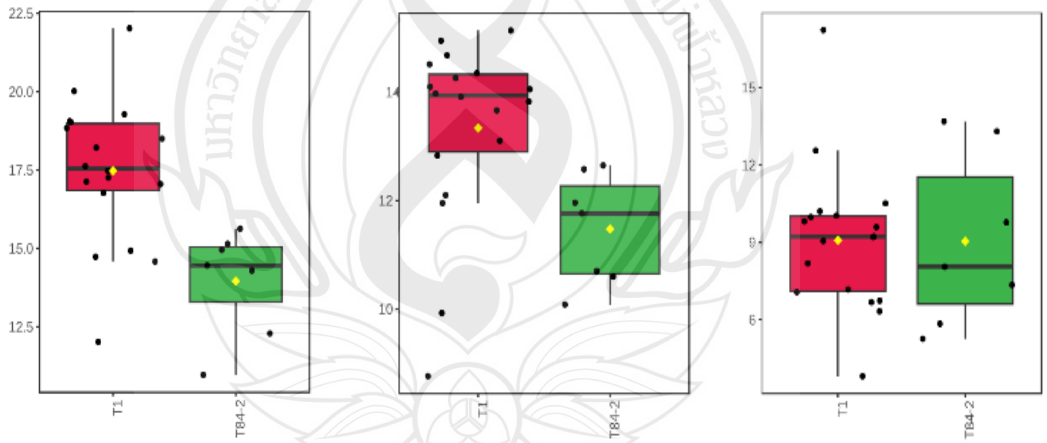
| Sample | Turmeric parameter | | | | | | |
|---|---|---|---|---|---|---|---|
| | Culti var | Yield (%w/w) | CUR (Spectroscopic, %w/w) | CUR (HPLC, %w/w) | BDMC (HPLC, %w/w) | DMC (HPLC, %w/w) | Total Curcuminoids (HPLC, %w/w) |
| S1 | T1 | 17.05±1.0 | 7.18±0.69 | 7.96±0.10 | 2.85±0.07 | 2.33±0.03 | 13.1±0.28 |
| S2 | T1 | 14.73±0.57 | 6.33±0.6 | 7.81±0.05 | 2.71±0.12 | 2.14±0.05 | 12.1±0.30 |
| S3 | T1 | 19.29±0.2 | 9.23±0.73 | 8.37±0.22 | 4.04±0.17 | 2.73±0.08 | 15.14±0.47 |
| S4 | T1 | 17.26±0.19 | 6.73±0.57 | 7.51±0.07 | 3.07±0.05 | 2.22±0.02 | 12.83±0.13 |
| S5 | T1 | 20.02±0.69 | 9.06±0.79 | 8.22±0.11 | 4.02±0.07 | 2.71±0.05 | 14.95±0.22 |
| S6 | T1 | 18.22±0.5 | 9.6±0.86 | 7.60±0.43 | 3.91±0.07 | 2.54±0.13 | 14.05±0.63 |
| S7 | T1 | 22.03±0.59 | 10.21±0.69 | 7.94±0.08 | 3.90±0.04 | 2.76±0.37 | 14.35±0.12 |
| S8 | T1 | 18.51±0.16 | 9.98±0.52 | 8.52±.11 | 3.50±0.06 | 2.50±0.03 | 14.51±0.20 |
| S9 | T1 | 19.03±0.36 | 9.81±0.45 | 7.66±0.23 | 4.06±0.18 | 2.54±0.09 | 14.26±0.50 |
| S10 | T1 | 18.85±0.47 | 10.03±0.2 | 7.77±0.01 | 3.86±0.01 | 2.47±0.01 | 14.10±0.02 |
| S11 | T84-2 | 15.14±0.36 | 7.35±0.24 | 8.06±0.01 | 1.70±0.02 | 2.20±0.00 | 11.96±0.02 |
| S12 | T84-2 | 14.45±0.37 | 13.3±1.07 | 6.24±0.08 | 1.82±0.04 | 2.54±0.05 | 10.60±0.17 |
| S13 | T84-2 | 10.96±0.08 | 5.26±0.39 | 6.38±0.05 | 1.70±0.02 | 2.00±0.01 | 10.08±0.08 |
| S14 | T1 | 12.01±0.12 | 3.81±0.18 | 5.88±0.23 | 1.26±0.09 | 1.62±0.07 | 8.76±0.38 |
| S15 | T1 | 14.93±0.64 | 6.68±0.54 | 7.03±0.12 | 1.16±0.05 | 1.73±0.03 | 9.93±0.19 |
| S16 | T84-2 | 14.96±0.22 | 8.06±0.3 | 7.26±0.13 | 2.20±0.06 | 2.30±0.05 | 11.76±0.23 |
| S17 | T1 | 14.59±0.65 | 8.19±0.48 | 7.14±0.15 | 2.59±0.10 | 2.22±0.05 | 11.95±0.31 |
| S18 | T1 | 17.62±0.20 | 17.23±0.78 | 7.42±0.03 | 4.44±0.01 | 2.82±0.01 | 14.68±0.04 |
| S19 | T84-2 | 15.62±0.28 | 9.78±0.33 | 7.59±0.15 | 2.49±0.07 | 2.58±0.07 | 12.65±0.29 |
| S20 | T84-2 | 14.29±0.38 | 13.69±1.31 | 8.13±.0.10 | 2.06±0.03 | 2.39±0.04 | 12.58±0.17 |
| S21 | T1 | 16.78±0.47 | 9.21±0.25 | 8.29±0.08 | 3.06±0.05 | 2.57±0.03 | 13.91±0.16 |
| S22 | T84-2 | 12.29±0.33 | 5.85±0.56 | 6.48±0.10 | 1.85±0.01 | 2.39±0.04 | 10.7±0.12 |
| S23 | T1 | 17.47±0.07 | 10.51±0.49 | 7.65±0.05 | 3.77±0.03 | 2.55±0.02 | 13.97±0.10 |
| S24 | T1 | 17.14±0.43 | 12.56±1.2 | 7.69±0.07 | 3.38±0.09 | 2.60±0.03 | 13.66±0.18 |
| **Mean±SD** | | **16.49±2.67** | **9.152±0.59** | **7.29±1.32** | **2.92±0.97** | **2.40±0.29** | **12.82±1.71** |

The analysis of 24 turmeric samples from two cultivars; T1 (n = 17) and T84-2 (n =7) revealed significant differences in curcuminoid content and metabolite composition. On average, cultivar T1 exhibited a higher total curcuminoid content (12.82 ± 1.71% w/w) than T84-2. The sample with the highest total curcuminoid level (17.23% w/w) was found in T1, collected from Doi Hang subdistrict (sample S18). T1 also showed higher extract yield and BDMC content than T84-2, suggesting a stronger intrinsic capacity for curcuminoid biosynthesis.

**4.4.1 Comparison of Curcuminoid Content and Derivatives between T1 and T84-2 Cultivars**

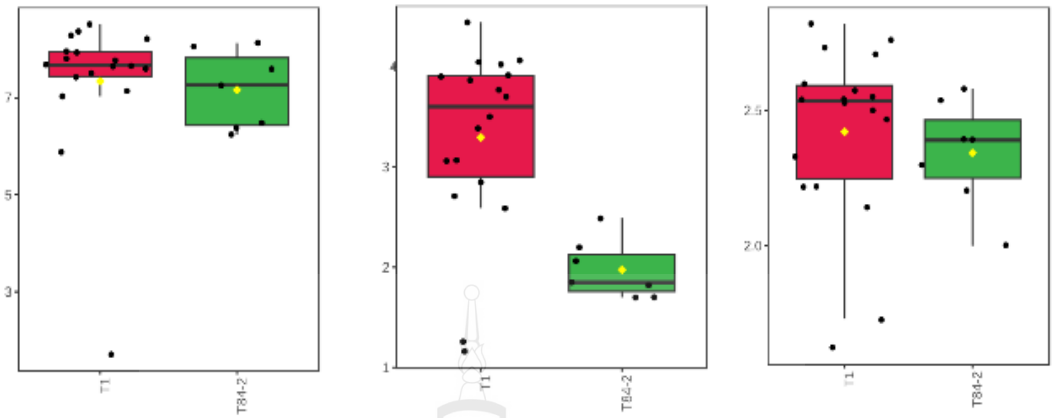
4.4.2.1 T-Test Analysis

T-test analysis of curcuminoid contents between T1 and T84-2 turmeric cultivars revealed significant differences between cultivars T1 and T84-2 in %Yield (p-value = 0.0014), %Total Curcuminoids (p-value = 0.0131), and %BDMC (p-value = 0.001). In contrast, no significant differences were observed between the two cultivars for %CUR (HPLC method), %CUR (Spectroscopic method), and %DMC, as shown in Figure 4.4.




Yield Total Curcuminoids CUR (Spectroscopic method)

**Figure 4.4** T-test of Each Curcuminoid and its Derivatives Contents in T1 and T84-2 Cultivars




CUR (HPLC method) BDMC DMC

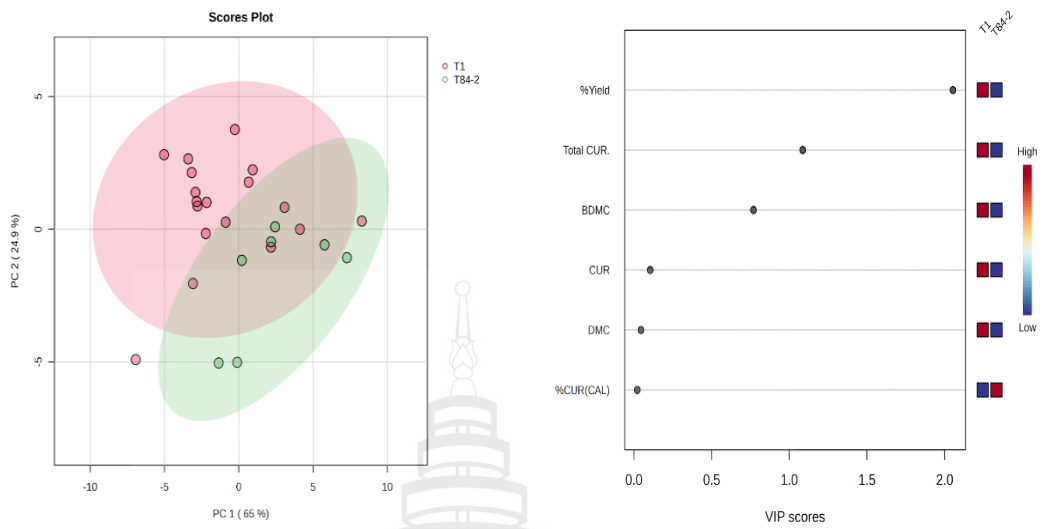
**Figure 4.4** (continued)

4.4.2.2 PCA Analysis

The purpose of running PCA analysis was to study the pattern of data, observe the grouping of samples, group clustering of the data and the differentiation of group data by using data from Table 3.

PCA score plot had been used to study the class pattern of the data. The PCA plotted between the PC1(65%) and PC2(24.9%) of two groups; T1 and T84-2 has been shown in Figure 4.5. Samples can be grouped into two groups; T1 and T84-2 (Figure 4.5A).

Variable importance in projection (VIP) scores was used to differentiate the turmeric quality parameters of T1 (n = 17) and T84-2 (n = 7). VIP scores are derived from PLS-DA analysis. VIP scores ≥ 1.0 were considered significant when selecting metabolites for the final model. The column to the right of the figure displays variations in metabolite peak intensities (Figure 4.5B).




**4.5A** PCA Scores Plot **4.5B** VIP Scores

**Figure 4.5** PCA Plotted between the PC1(65%) and PC2(24.9%) of Two Groups; T1 and T84-2 (4.5A), Variable Importance in Projection (VIP) (4.5B)

From the PCA scores plot figure, there were some overlap results between T1 and T84-2. And from VIP scores, three parameters that distinguish between two groups were %yield, total curcuminoid and BDMC. The %yield and total curcuminoid had VIP score more than 1.0 which means T1 and T84-2 turmeric cultivar had a significant difference in both %yield and total curcuminoid.

These differences are likely linked to genetic variations between the cultivars. Previous reports have confirmed that cultivar background significantly influences curcuminoid production (Limtrakul et al., 2004; Medicinal Innovation Center of Mae Fah Luang, 2021). The elevated levels of BDMC in T1 are noteworthy, given the compound's chemical stability and pharmacological potential, especially in modulating multidrug resistance (Limtrakul et al., 2004).

Statistical comparisons using independent t-tests confirmed that T1 and T84-2 differed significantly in extract yield, total curcuminoid content (Sum HPLC), and BDMC percentage ($p < 0.05$) (Figure 4.4). However, no significant differences were observed for CUR, DMC, or CUR content analyzed by spectrophotometry. These findings align with registration data from the Department of Agriculture, Thailand, which report typical curcuminoid contents for T1 and T84-2 as 10.62% and 11.04%,

respectively (Department of Agriculture, 2023). To further explore patterns in metabolite profiles, Principal Component Analysis (PCA) was conducted using all curcuminoid-related variables. The first two components, PC1 and PC2, explained 65.0% and 24.9% of the total variance, respectively (Figure 4.5A). The PCA plot indicated moderate separation between the two cultivars, with tighter grouping among T1 samples. This trend was further supported by Variable Importance in Projection (VIP) analysis from Partial Least Squares Discriminant Analysis (PLS-DA), which identified extract yield, total curcuminoid content, and BDMC as the top discriminating variables (VIP ≥ 1.0) (Figure 4.5B). T1 not only yields a greater quantity of curcuminoids overall but also produces higher levels of the pharmaceutically relevant derivative BDMC. These traits make T1 a more promising cultivar for both medicinal and commercial applications, particularly in value-added turmeric-based products (Plant Variety and Germplasm Database, 2021).

## 4.5 The Relationship between Soil Clusters and Curcuminoid Parameters of Turmeric Cultivars

### 4.5.1 T1 Turmeric Cultivar on C1 And C3 Soil Clusters

There are seventeen T1 turmeric cultivar samples; C1 has eleven samples (S1, S2, S3, S5, S7, S9, S10, S14, S15, S23, S24) and C3 has six samples (S4, S6, S8, S17, S18, S21). T-test analysis was used to find the difference in curcuminoid parameter of between C1 and C3 soil clusters as shown in Figure 4.6. From the analysis, it was found that there was no significant difference in curcuminoid content between C1 and C3 clusters (p-value <0.05).

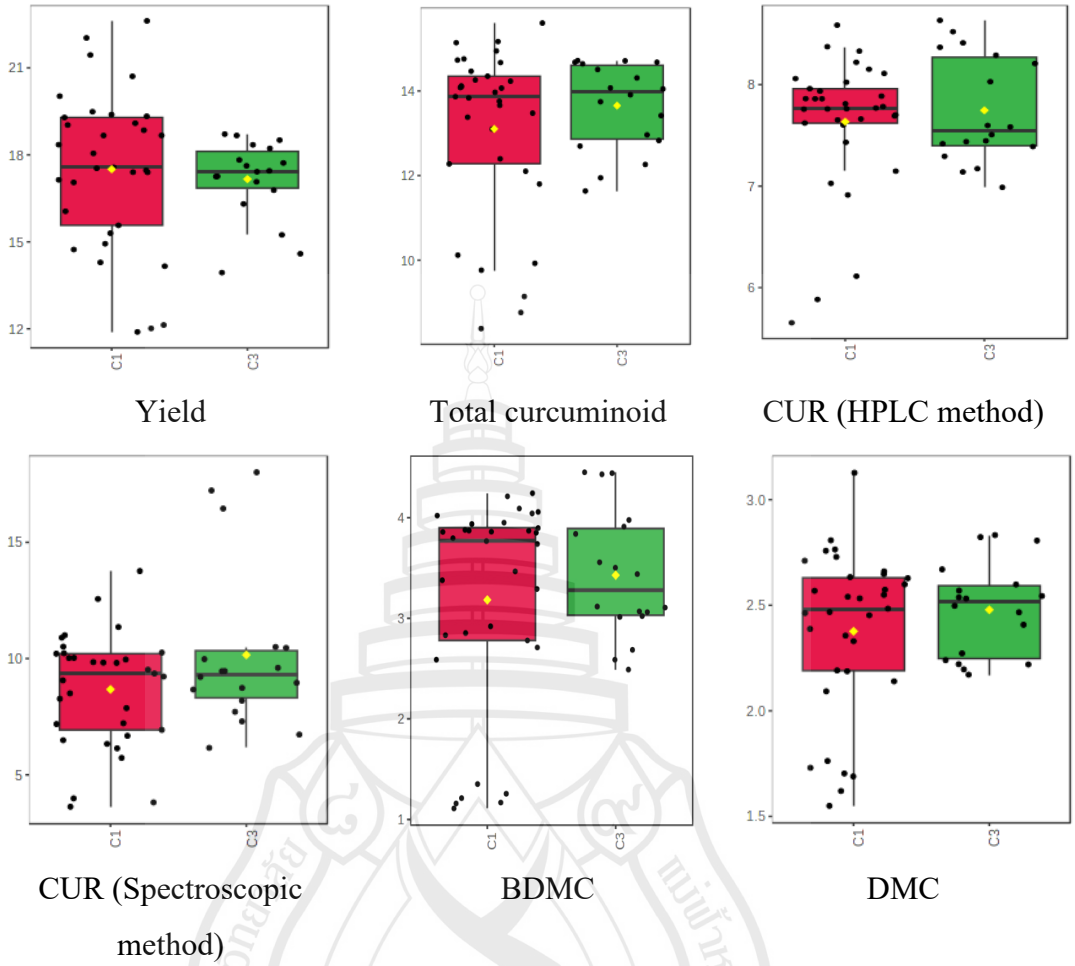



**Figure 4.6** T-test Analysis of C1, C3 Soil Clusters and Curcuminoid Parameters of T1 Turmeric Cultivar

### 4.5.2 T84-2 Turmeric Cultivar on C1 And C2 Soil Clusters

There are seven T84-2 samples; C1 soil cluster has two samples (S16, S19) and C2 soil cluster has five samples (S11, S12, S13, S20, S22). T-test analysis was used to find the difference of curcuminoid parameters between C1 and C2 soil sample clusters, as shown in Figure 4.7.

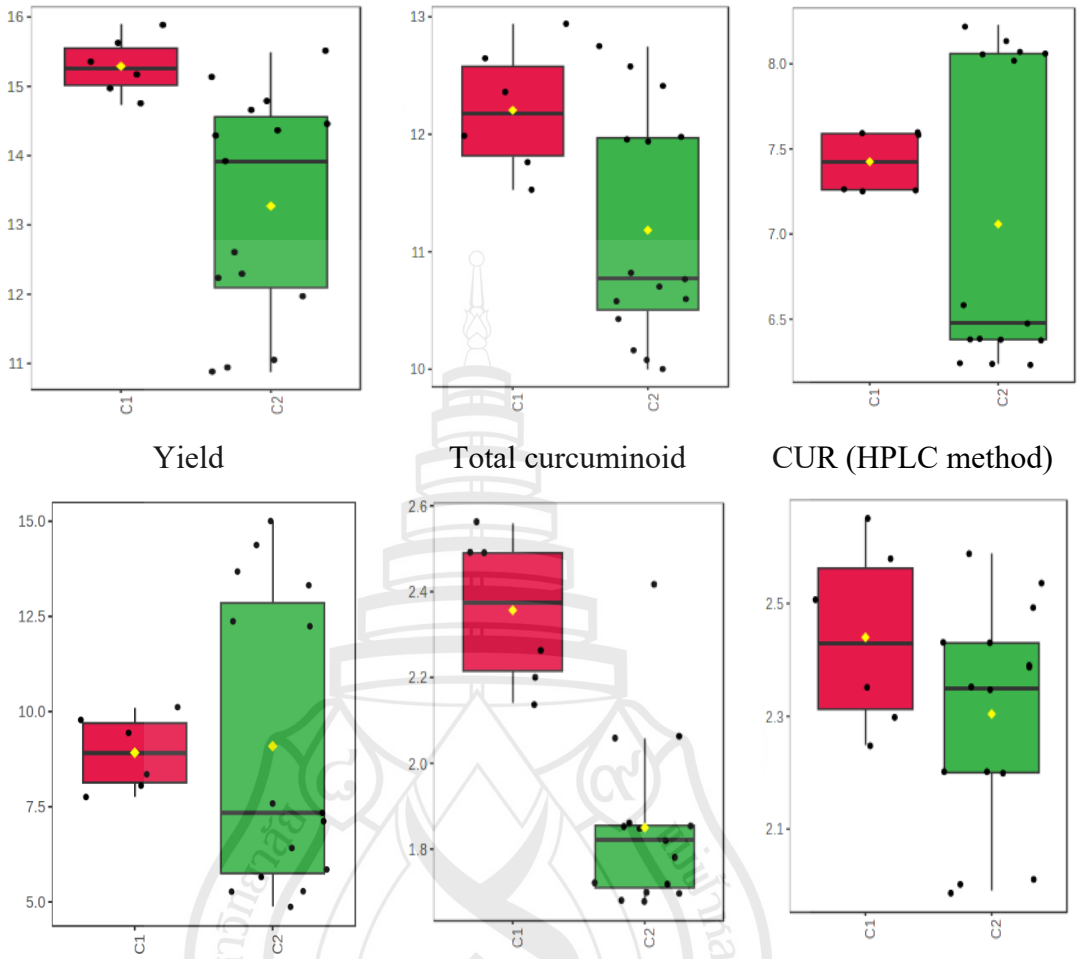



**Figure 4.7** T-test Analysis of C1, C2 Soil Clusters and Curcuminoid Parameters of T84-2 Turmeric Cultivar

From the analysis, it was found that there is a significant difference in T84-2 turmeric's curcuminoid content between C1 and C2 clusters. The T-test results revealed significant differences in %yield ($p = 0.00032$), %total curcuminoids ($p = 0.0449$), and %BDMC ($p = 0.0012$) between clusters. Specifically, turmeric grown in C1 had significantly higher curcuminoid content than in C2, as shown in Table 4.5.

**Table 4.5** Curcuminoid Contents for T84-2 found in C1, C2 Soil Clusters

| Name | Mean (SD) of C1 | Mean (SD) of C2 | P-value |
|---|---|---|---|
| %Yield | 15.290 (0.426) | 13.273 (1.625) | 0.0003* |
| %Total CUR (HPLC) | 12.205 (0.541) | 11.184 (0.971) | 0.0449* |
| %Total CUR (Spectro) | 8.920 (0.983) | 9.092 (3.857) | 0.5187 |
| %CUR | 7.425 (0.181) | 7.058 (0.882) | 0.5045 |
| %BDMC | 2.357 (0.178) | 1.850 (0.199) | 0.0012* |
| %DMC | 2.440 (0.163) | 2.304 (0.195) | 0.1488 |

**Note** * = significantly different at p-value = 0.05

T-test analysis of soil parameters between C1 and C2 soil clusters that cultivated T84-2 were further performed to find the difference in soil parameters, as shown in Table 4.6.

**Table 4.6** Analysis Result for Each Variable between C1, C2 Soil Clusters that Cultivated Turmeric T84-2 and Soil Properties

| Name | Suitable Range (SD) | Critical level | Sample range | Reference | Mean (SD) of C1 | Mean (SD) of C2 | P-value |
|---|---|---|---|---|---|---|---|
| **%OC** | 1.9–2.2 | 1.9 | 1.0-3.7 % | Musinguzi et al., 2016 | 1.626 (0.582) | 2.972 (0.502) | 0.00004* |
| **pH** | ~6.2 | | 4.4-6.9 | Akbar et al., 2016 | 5.013 (0.652) | 6.192 (0.629) | 0.0011* |
| **EC (us/cm)** | 0-200 | <200 | 18-757 uS/cm | Motsara et al., 2008 | 25.100 (10.101) | 249.145 (222.694) | 0.0002* |
| **CEC (meq Na/100 g)** | depend on soil texture | - | 4.0-13.0 meq Na/100 g | - | 11.346 (2.287) | 19.780 (3.326) | 0.00004* |
| **Na (mg/kg)** | 0-640 | <640 | 0-90.0 mg/kg | Horneck D.A. et al. 2011 | 0.977 (0.779) | 35.274 (34.916) | 0.00888* |
| **Mg (mg/kg)** | 60-300 | 300 | 49.0-409.0 mg/kg | Horneck D.A. et al. 2011. | 93.287 (47.539) | 251.392 (105.225) | 0.0084* |

**Table 4.6** (continued)

| Name | Suitable Range (SD) | Critical level | Sample range | Reference | Mean (SD) of C1 | Mean (SD) of C2 | P-value |
|---|---|---|---|---|---|---|---|
| S (mg/kg) | 5-20 | >10 | 3.4-7.4 mg/kg | Tandon, 1991 | 4.877 (1.424) | 4.990 (0.894) | 0.6683 |
| Ca (mg/kg) | >300 | 300 | 510-2775 mg/kg | Sathish, A. et al. 2018 | 624.184 (114.641) | 1821.430 (552.362) | 0.00004* |
| K (mg/kg) | 250-800 | 800 | 41.0-710.0 mg/kg | Horneck D.A. et al. 2011 | 52.377 (10.729) | 292.991 (221.306) | 0.00004* |
| Mn (mg/kg) | 1.2-32 | <1.2 | 0.18-1.55 mg/kg | Lindsay and Norwell W A 1978 | 0.620 (0.430) | 0.802 (0.414) | 0.3809 |
| Fe (mg/kg) | >4.5 | 4.5 | 47.6-130.0 mg/kg | Lindsay and Norwell W A 1978 | 87.687 (33.988) | 90.300 (26.532) | 0.9096 |
| Zn (mg/kg) | >0.8 | 0.8 | 0-0.22 mg/kg | Lindsay and Norwell W A 1978 | 0.001 (0.002) | 0.068 (0.077) | 0.0009* |
| Cu (mg/kg) | >0.2 | 0.2 | 0-0.023 mg/kg | Lindsay and Norwell W A 1978 | 0.002 (0.002) | 0.003 (0.006) | 0.7152 |
| Nitrate (mg/kg NO3-N of soil) | >8.0 ppm | 8.0 | 0-100.0 mg/kg | Horneck D.A. et al. 2011 | 4.882 (6.144) | 49.217 (30.179) | 0.0027* |
| Ammonia (mg/kg) | 2-10 ppm | 10 | 0-109.0 mg/kg | Horneck D.A. et al. 2011 | 41.800 (34.378) | 68.633 (23.912) | 0.0541 |
| P (mg/kg) | 40-100 | 100 | 4.8-149.0 mg/kg | Horneck D.A. et al. 2011 | 5.203 (0.440) | 59.250 (50.855) | 0.0005* |

**Note** * = significantly different at p-value = 0.05

From this table, it was found that there were significant differences in many soil parameters at p-value < 0.05 e.g. %OC (0.00004), pH (0.0011), EC (0.00015), CEC (0.00004), Na (0.00888), Mg (0.00837), Ca (0.00004), K (0.00004), Zn (0.00092), Nitrate (0.00269) and P (0.00053).

Soils with Fe >4.5 ppm are not expected to show Fe-deficiency (Lindsay & Norvell, 1978). Slightly acidic soil (pH value ~6.2) with low altitude favored curcumin content. Curcumin content is favored by acidic pH (~6.2) (Akbar et al., 2016). Salt free soil (EC 0-200 uS/cm) has almost no impact on most crops. Only extremely salt-sensitive crops might be affected (Motsara, 2015). Non-linear regression models predicted critical SOC for optimal yields to be 2.204% at the 50 kgN $ha^{-1}$ rate. (Musinguzi, 2016)

With these soil parameters, another t-test analysis was performed to find the correlation between soil parameters of C1, C2 soil clusters and curcuminoid parameters of T84-2 turmeric cultivar, as shown in Table 4.7.

**Table 4.7** T-test Analysis between Soil Properties of C1, C2 Soil Clusters and Curcuminoid Parameters of T84-2

| **Name** | **Yield** | | **Total curcuminoid** | | **BDMC** | |
|---|---|---|---|---|---|---|
| | **P-value** | **R** | **P-value** | **R** | **P-value** | **R** |
| %OC | 0.0467* | -0.4381 | 0.0458* | -0.4402 | 0.0140 | -0.5274 |
| pH | 0.0049* | -0.5893 | 0.0084* | -0.5594 | 0.0015 | -0.6486 |
| EC (us/cm) | 0.0053* | -0.5854 | 0.0004* | -0.6989 | 0.0045 | -0.5943 |
| CEC (meq Na/100 g) | 0.2197 | -0.2796 | 0.5760 | -0.1294 | 0.0487 | -0.4351 |
| Nitrate (mg/kg $NO_3$-N of soil) | 0.3203 | -0.2279 | 0.0742 | -0.3977 | <0.001* | -0.7872 |

**Table 4.7** (continued)

| Name | Yield | | Total curcuminoid | | BDMC | |
|---|---|---|---|---|---|---|
| | **P-value** | **R** | **P-value** | **R** | **P-value** | **R** |
| P (mg/kg) | 0.0771 | -0.3941 | 0.8615 | 0.0406 | 0.7404 | -0.0769 |
| K (mg/kg) | <0.001* | -0.8270 | <0.001* | -0.8456 | 0.0014 | -0.6504 |
| Na (mg/kg) | <0.001* | -0.7560 | <0.001* | -0.8649 | 0.0041 | -0.5989 |
| Mg (mg/kg) | <0.001* | -0.7932 | <0.001* | -0.8519 | 0.0016 | -0.6441 |
| Ca (mg/kg) | 0.0059* | -0.5795 | 0.0383* | -0.4548 | <0.001* | -0.7566 |
| Zn (mg/kg) | 0.5876 | -0.1256 | 0.2249 | 0.2766 | 0.6890 | 0.0928 |

**Note** * = significantly different at p-value = 0.05

It was found that T84-2 curcuminoid parameters e.g. %Yield, %Total curcuminoids and %BDMC were negatively influenced by some soil parameters at p-value <0.05.

OC, EC, K, Na, Mg, and Ca had a negative influence on %Yield, %Total curcuminoid and %BDMC. CEC and Nitrate had a negative influence on %BDMC. This finding is in support of the finding of Samarakoon et al. (2006) that High EC can reduce plant yield. Liu et al. (2023) found that salt stress inhibited ginger growth with increased osmoregulatory substances, increased reactive oxygen species, increased Na, ion content, and enhanced antioxidant enzyme activity. Akbar et al. (2016) who found that pH had negative correlation with curcumin content of turmeric rhizome at $r = -0.16$ ($P < 0.05$). Dixit and Vastava (2000) found that too much phosphate in acid soil could also induce iron deficiency.

But many of these findings contrast with many previous findings. For example, the findings of Ma et al. (2023) that increasing soil OC with currently available management practices would increase crop production by only 0.7% once other production constraints have already been addressed. Akamine et al. (2007) found that K alone increased the curcumin content in rhizomes but did not increase turmeric yield. Menon (2014) found that the application of Mg along with the recommended package of practices could produce 29.27% increase in yield over the recommended package of practices alone. Menon (2014) stated that "the higher yielding treatments were associated with higher contents of Ca in the shoots.

## 4.6. The Effect of Soil Properties on Curcumin Contents and Its Derivatives

The selected parameters were individually analyzed to identify those influencing the variation in curcumin contents, BDMC and DMC of turmeric. Pearson correlation analysis of soil properties displayed the impact of OC, EC, CEC, pH, mineral composition (P, Na, K, S, Ca, Mg, Mn, Fe, Zn Cu) on %CUR, %BDMC and %DMC from turmeric T1 and T84-2 (Table 4.8 and 4.9 respectively).

**Table 4.8** Soil Properties Range and Turmeric Qualities's Correlation Data of T1 Sample

| Soil Nutrient | Range Parameter | Pearson Correlation (R) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Yield | Total curcumi-noid | CUR (HPLC) | CUR (Spectro) | BDMC (HPLC) | DMC (HPLC) |
| OC | 1.4-5.5 % | **0.642**[a] | **0.525**[b] | **0.437**[b] | 0.148 | **0.510**[b] | **0.434**[b] |
| pH | 3.5 to 6.8 | **-0.412**[c] | -0.308 | -0.327 | -0.032 | -0.293 | -0.277 |
| EC | 10.0 - 415.0 uS/cm | -0.376 | -0.275 | -0.358 | -0.089 | -0.229 | -0.219 |
| CEC | 13.0-28.0 meq Na/100 g | **0.493**[b] | 0.326 | 0.309 | -0.023 | 0.307 | 0.236 |
| Nitrate | 0-18.0 mg/kg | -0.028 | 0.095 | 0.035 | 0.100 | 0.069 | 0.162 |
| Ammonia | 0-32.0 mg/kg | 0.190 | 0.290 | 0.081 | 0.324 | 0.361 | 0.345 |
| P | 0.9-210.0 mg/kg | -0.290 | -0.157 | -0.231 | -0.008 | -0.127 | -0.099 |
| K | 29.0-525.0 mg/kg | 0.0025 | 0.054 | -0.046 | 0.022 | 0.110 | 0.017 |
| S | 1.0-10.0 mg/kg | -0.100 | 0.016 | 0.024 | 0.181 | -0.061 | 0.071 |
| Na | 0-27.0 mg/kg | 0.058 | 0.143 | 0.188 | 0.205 | 0.090 | 0.104 |
| Ca | 200.0-3200.0 mg/kg | -0.257 | -0.096 | 0.062 | -0.068 | -0.195 | -0.093 |
| Mg | 25.0-330.0 mg/kg | -0.108 | -0.071 | -0.136 | 0.054 | -0.015 | -0.079 |
| Mn | 0.03-2.55 mg/kg | 0.260 | 0.211 | 0.106 | 0.118 | 0.250 | 0.191 |
| Fe | 9.8-143.1 mg/kg | **-0.396**[c] | -0.297 | -0.245 | -0.020 | -0.285 | -0.232 |
| Zn | 0-0.16 mg/kg | -0.109 | -0.096 | -0.089 | -0.200 | -0.077 | -0.113 |
| Cu | 0-0.03 ug/kg | **0.490**[b] | 0.309 | 0.255 | 0.004 | 0.304 | 0.279 |

**Note** a = high positive linear correlation; b = moderate positive linear correlation; c= moderate negative linear correlation

**Table 4.9** Soil Properties Range and Turmeric Qualities's Correlation Data of T84-2 Sample

| Soil nutrient | Range parameter | Pearson Correlation (R) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Yield | Total curcuminoid | CUR (HPLC) | CUR (Spectro) | BDMC (HPLC) | DMC (HPLC) |
| OC | 1.0-3.7 % | **-0.438**[b] | **-0.440**[b] | -0.329 | 0.375 | **-0.527**[b] | -0.0423 |
| pH | 4.4-6.9 | **-0.589**[b] | **-0.652**[c] | -0.356 | **-0.436**[b] | **-0.649**[c] | -0.394 |
| EC | 18-757 uS/cm | **-0.585**[b] | **-0.700**[c] | **-0.564**[b] | -0.226 | **-0.594**[b] | **-0.467**[b] |
| CEC | 4.0-13.0 meq Na/100 g | -0.280 | -0.130 | 0.094 | **0.399**[b] | **-0.435**[b] | -0.230 |
| Nitrate | 0-100.0 mg/kg | -0.228 | **-0.397**[b] | -0.132 | -0.342 | **-0.787**[c] | -0.352 |
| Ammonia | 0-109.0 mg/kg | -0.221 | **-0.530**[b] | -0.332 | -0.242 | **-0.742**[c] | -0.332 |
| P | 4.8-149.0 mg/kg | -0.394 | 0.041 | 0.196 | 0.197 | -0.077 | -0.292 |
| K | 41.0-710.0 mg/kg | **-0.827**[d] | **-0.846**[d] | **-0.701**[c] | -0.353 | **-0.650**[c] | **-0.586**[b] |
| S | 3.4-7.4 mg/kg | -0.205 | -0.220 | 0.042 | -0.387 | -0.361 | **-0.678**[c] |
| Na | 0-90.0 mg/kg | **-0.756**[c] | **-0.865**[d] | **-0.809**[d] | -0.137 | **-0.600**[c] | -0.342 |
| Ca | 510-2775 mg/kg | **-0.580**[b] | **-0.455**[b] | -0.103 | -0.387 | **-0.757**[c] | **-0.877**[d] |
| Mg | 49.0-409.0 mg/kg | **-0.793**[c] | **-0.852**[d] | **-0.815**[d] | -0.241 | **-0.644**[c] | -0.277 |
| Mn | 0.18-1.55 mg/kg | 0.303 | 0.051 | 0.262 | **-0.516**[b] | 0.030 | **-0.550**[b] |
| Fe | 47.6-130.0 mg/kg | 0.192 | -0.124 | -0.191 | 0.287 | -0.122 | 0.264 |
| Zn | 0-0.22 mg/kg | -0.126 | 0.276 | 0.371 | 0.376 | 0.092 | -0.001 |
| Cu | 0-0.023 mg/kg | 0.114 | 0.200 | 0.325 | -0.128 | -0.066 | -0.156 |

**Note** a = moderate positive linear correlation; b = moderate negative linear correlation; c = high negative linear correlation; d = very high negative linear correlation

This study analyzed the relationship between soil properties and curcuminoid content in two turmeric cultivars, T1 and T84-2, to assess how soil nutrients influence yield and bioactive compound synthesis. The findings demonstrate that specific soil characteristics significantly impact curcuminoid profiles, with differences in each cultivar's response to nutrient availability.

For T1, soil organic carbon (OC) and cation exchange capacity (CEC) showed moderate positive correlations with %Yield (r = 0.642 and r = 0.493, respectively), indicating that these properties contribute modestly to increased yield. OC also showed a moderate correlation with %BDMC (r = 0.510), suggesting that higher organic content slightly enhances BDMC concentration. Other soil factors, including pH, EC, and micronutrients, exhibited low to very low correlations with curcuminoid levels, indicating that T1 is relatively stable across varying soil conditions. This resilience suggests T1 can maintain consistent curcuminoid content despite soil variability, making it suitable for diverse growing environments.

In contrast, T84-2 displayed moderate to very strong negative correlations between curcuminoid parameters and several soil nutrients, such as OC, pH, EC, CEC, nitrate, ammonia, K, S, Na, Ca, Mg, and Mn. Yield was affected negatively from highest to lowest by Cu, Zn, Fe, Mn, Mg, Ca, Na (-0.827, -0.793, -0.756, -0.589, -0.585, -0.58, -0.438, respectively). Total curcuminoid was affected negatively by OC, pH, EC, Nitrate, Ammonia, K, Na, Ca, and Mg (-0.440, -0.652, -0.700, -0.397, -0.530, -0.846, -0.865, -0.455, -0.852, respectively). Total curcumin detected by HPLC was affected negatively by EC, K, Na, and Mg (-0.564, -0.701, -0.809, -0.815, respectively).
CUR detected by spectrophotometry method was affected positively by CEC (0.399) but affected negatively by pH, and Mg (-0.436, -0.516, respectively). BDMC was affected negatively by OC, pH, EC, CEC, nitrate, ammonia, K, Na, Ca, and Mg (-0.527, -0.649, -0.594, -0.435, -0.787, -0.742, -0.650, -0.600, -0.757, -0.644, respectively). DMC was affected negatively by EC, K, S, Ca, and Mn (-0.467, -0.586, -0.678, -0.877, -0.550, respectively).

The present study revealed that both nitrate and potassium fertilization exerted negative effects on curcuminoid content in turmeric (Curcuma longa). This result contrasts with several earlier reports that generally associated balanced potassium supply with enhanced rhizome yield and curcumin concentration (Rahman et al., 2013;

Karmakar et al., 2015; Sujatha et al., 2017). One possible explanation lies in the growth–differentiation balance (GDB) and carbon–nutrient balance (CNB) frameworks, which posit that abundant nutrient supply, particularly nitrogen in nitrate form, promotes vigorous vegetative growth but suppresses the biosynthesis of secondary metabolites, as carbon skeletons are preferentially allocated toward primary metabolism (Herms & Mattson, 1992). Consequently, excessive nitrate availability may downregulate curcuminoid biosynthesis despite increasing biomass.

Similarly, although potassium is known to enhance carbohydrate translocation, osmoregulation, and enzymatic activity in turmeric (Marschner, 2012; Rahman et al., 2013) This imbalance could explain the reduced curcuminoid accumulation under high K conditions observed in the present study. Furthermore, the source of potassium, such as potassium chloride (MOP), may also influence metabolite synthesis through chloride-associated salinity stress, which has been reported to affect quality traits in spice crops (Hamza et al., 2004).

Taken together, the results highlight the importance of fine-tuning nutrient management in turmeric production. Moderate application of nitrogen and potassium is essential for rhizome development and yield, but excessive inputs may compromise metabolite concentration and quality. Future studies should therefore focus on optimizing N form (nitrate vs. ammonium), the timing of K application, and the use of alternative sources such as potassium sulfate, as well as monitoring cation balance (K:Ca:Mg) to maintain both yield and curcuminoid quality.

The greater sensitivity of T84-2 to soil composition suggests that nutrient-rich conditions, especially with elevated nitrate, potassium, and phosphorus, could substantially improve its curcuminoid profile. T1's stable yield across various soil types, on the other hand, highlights its adaptability and potential for cultivation in a range of soil conditions with minimal amendment requirements. This adaptability could make T1 a more versatile option, particularly in settings with limited resources for intensive soil management.

The distinct responses of T1 and T84-2 to soil nutrients underscore the importance of cultivar-specific soil management strategies. For T1, stable curcuminoid contents across various soil types suggest reduced reliance on nutrient modifications, supporting cost-effective production with consistent bioactive compound levels. T84-

2, however, would benefit from targeted soil enhancements to optimize yield and curcuminoid content, especially in soils enriched with nitrate and potassium.

This study indicated that tailored soil management can effectively optimize curcuminoid yields in turmeric cultivars. Intensive nutrient management for T84-2 may enhance its curcuminoid synthesis and yield, adding to its therapeutic and commercial value. Conversely, T1's stability across soil conditions makes it a promising candidate for resource-efficient cultivation with reliable bioactive compound levels. These findings offer valuable insights for optimizing turmeric cultivation based on specific soil and cultivar interactions, improving yield and quality for targeted agricultural and commercial applications.

The identified stable genotypes for the curcumin and its analogues may have direct impact on nutraceutical and pharmaceutical industries (Aarthi et al., 2020).
The present report deals with 'Surama', a high yielding turmeric cultivar that was cultivated at nine agroclimatic zones. It was found that for curcumin content, the most sensitive factors were the altitude, soil pH, nitrogen and Potassium content. In contrast, the temperature and phosphorus content were found critical for leaf essential oil while nitrogen, phosphorus and potassium contents were the key quantitative and qualitative determinants for rhizome essential oil. The change in curcumin and essential oil content of turmeric at different agroclimatic zones may not be due to any individual factors but may be more due to a combined effect of soil and environmental factors (Sandeep et al., 2016).

Eleven cultivars were evaluated for fresh yield (10 environments), curing per cent, curcumin and dry yield (five environments) across India, four each in North and South India and two in Northeast India, ranging from 43 to 893 m above mean sea level. Combined analyses showed significant differences among cultivars, environments, and cultivar by environment interactions for yield, curing per cent and curcumin contents. A large proportion (70.8%) of variation on fresh yield was attributed to environments; however, for curing per cent, curcumin content and dry yield, genotype effect accounted for 31.2%, 17.7% and 15.7% of variation, respectively. (Anandaraj et al., 2014).

This study demonstrated that each turmeric cultivar may have different reactions to the amount of soil nutrient/ soil parameter in this study, turmeric cultivar T1 had stable curcuminoid yields when cultivated with various soil nutrient/ soil

parameter conditions compared to turmeric cultivar T 84/2 which had more dependency on soil conditions. Moreover, T1 was found to have more BDMC compared to T84-2 as well.

To improve turmeric quality at sites within Cluster 2, several soil amendments are recommended. Application of gypsum ($CaSO_4$) can displace exchangeable $Na^+$ and improve soil structure and permeability, while incorporation of elemental sulfur or acidic organic matter (e.g., composted pine bark) can gradually reduce pH to the optimal range of 5.5–6.0 (Motsara, 2015). At phosphorus-deficient sites such as S13, supplementation with rock phosphate or monoammonium phosphate is advised. Correlation analysis also indicated that soil nitrate and available phosphorus were positively associated with curcuminoid yield, particularly in T84-2, which is consistent with studies emphasizing the role of N and P in turmeric metabolite biosynthesis (Aarthi et al., 2020; Anandaraj et al., 2014). Soils in Cluster 1 were optimal for T84-2. These soils possessed moderate acidity (pH ~5.5–5.8), high organic carbon, and low EC, all of which favored curcuminoid synthesis—especially BDMC. To preserve these favorable conditions, farmers should avoid excessive liming and maintain soil fertility by recycling organic residues and applying sulfur-containing fertilizers if necessary. Notably, T84-2 showed strong positive response to nitrate and phosphorus availability suggests that targeted N and P fertilization could be used as a lever to improve curcuminoid quality, particularly in marginal sites with suboptimal baseline fertility (Musinguzi et al., 2016). Precision soil management that integrates both cluster-based soil classification and cultivar-specific nutritional needs can support higher curcuminoid yields and consistent product quality, particularly important for the functional food and pharmaceutical industries.

# CHAPTER 5

# CONCLUSION

## 5.1 Conclusion

This study's objectives are to investigate the curcuminoid content of local *Curcuma longa* L. cultivars from various areas in Chiang Rai and determine the optimal soil parameters for enhancing *Curcuma longa* L. 's curcuminoid yield. The result of this study indicates that each turmeric cultivar; Trang 1 (T1) and Trang 84-2 (T84-2) reacted to soil parameters differently, T1 had more consistent curcuminoid yields and had more BDMC compared to T84-2. Using Hierarchical Clustering Analysis, 3 clusters were found: C1, C2, and C3. C1 was cultivated mixed with T1 and T84-2, C2 was cultivated T84-2 exclusively, and C3 was cultivated T1 exclusively. There was no significant difference in curcuminoid quality between C1 and C3 that cultivated T1 whereas there was significant difference between C1 and C2 that cultivated T84-2 such as %yield, %total curcuminoids, and %BDMC. Further analysis was found that C1 and C2 had various difference in soil parameters, however T84-2 had mostly negative correlations on those soil parameters such as OC, EC, K, Na, Mg, Ca, CEC and nitrate.

In conclusion, Soil nutrients have an impact on the quality of *Curcuma longa* L. T1 turmeric cultivar may have more BDMC and react to soil properties more stable compared to T84-2. This study provides more information on two turmeric cultivars characteristics; T1 and T84-2 and can be used to predict and make improvements on soil nutrients for better *Curcuma longa* L. yield.

## 5.2 Suggestion

A larger sample study is needed to provide more insight into the effect of soil parameters on *Curcuma longa* L. quality.

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# APPENDIX A

# SOIL pH AND EC EVALUATION

In this study, soil pH and EC was rated by following Motsara et al. (2008), as shown in Table A1 and A2.

**Table A1** Soil pH Reaction Ratings

| pH Range | Soil Reaction Rating |
|---|---|
| < 4.6 | Extremely acidic |
| 4.6-5.5 | Strongly acidic |
| 5.6-6.5 | Moderately acidic |
| 6.6-6.9 | Slightly acidic |
| 7.0 | Neutral |
| 7.1-8.5 | Moderately alkaline |
| > 8.5 | Strongly alkaline |

**Table A2** Chemical Characteristics of Saline, Non-saline Sodic and Saline Sodic Soils

| Soil | EC (dS/m) | Exchangeable Sodium Percentage | pH |
|---|---|---|---|
| Saline | > 4.0 | < 15 | < 8.5 |
| Sodic (non-saline) | < 4.0 | > 15 | > 8.5 |
| Saline sodic | > 4.0 | > 15 | < 8.5 |

# APPENDIX B

## $NO_3$-N, $NO_4$-N, P, AND S STANDARD CALIBRATION GRAPH

$NO_3$-N, $NO_4$-N, and S were analyzed by using UV-visible spectrophotometer. The standard calibration graphs of them were obtained. Soil extractable $NO_3$-N was observed at 470 nm absorbance and the standard calibration graphs were shown in Figure B1. Soil extractable $NO_4$-N was observed at 425 nm absorbance, the calibration graphs of the standard were shown in Figure B2. Soil extractable P was observed at 660 nm absorbance and the standard calibration graph was shown in Figure B3. Soil extractable S was observed at 420 nm absorbance and the standard calibration graph were shown in Figure B4.

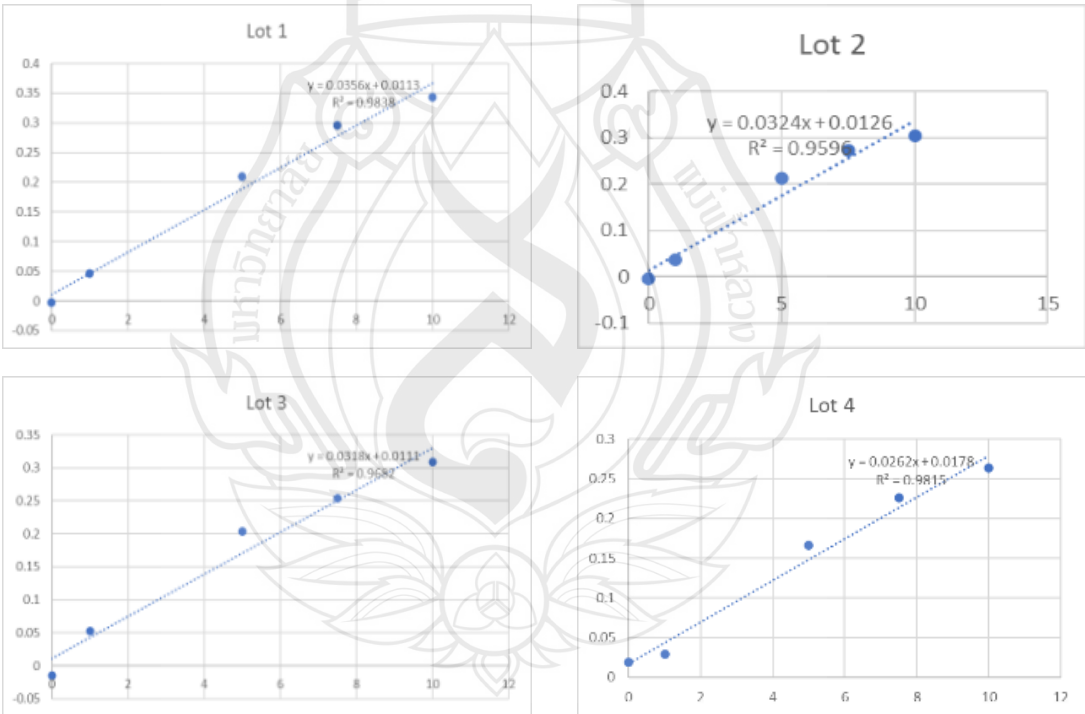



**Figure B1** Standard Calibration Graphs of Standard $NO_3$-N (Lot 1-7)

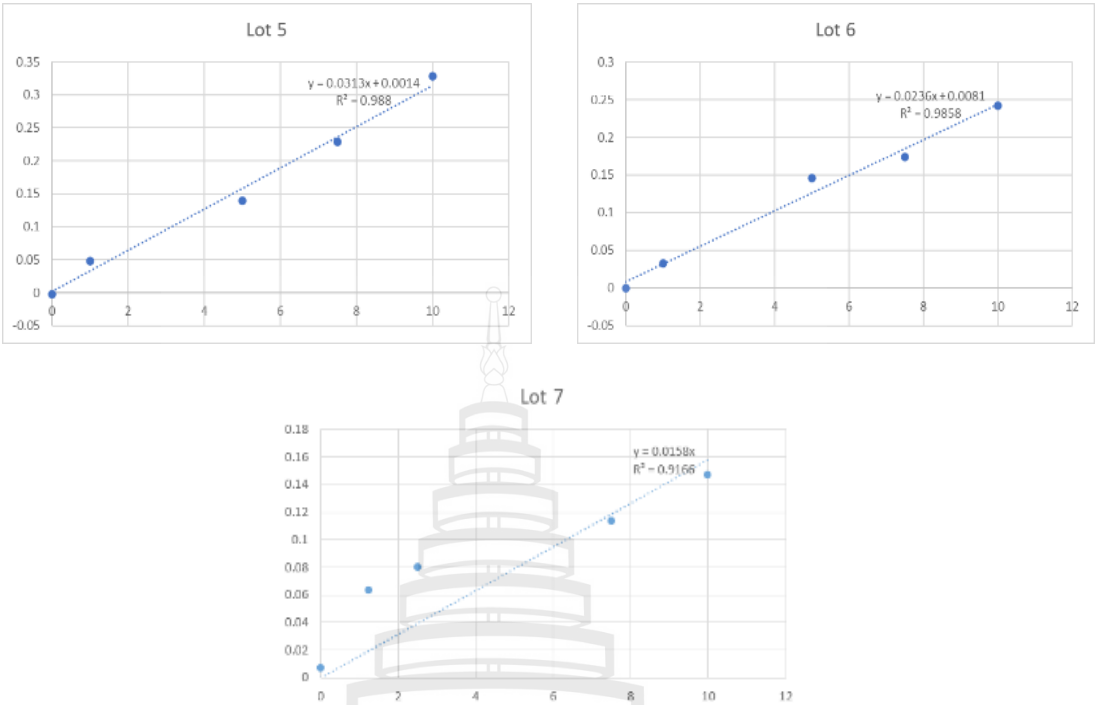



**Figure B1** (continued)

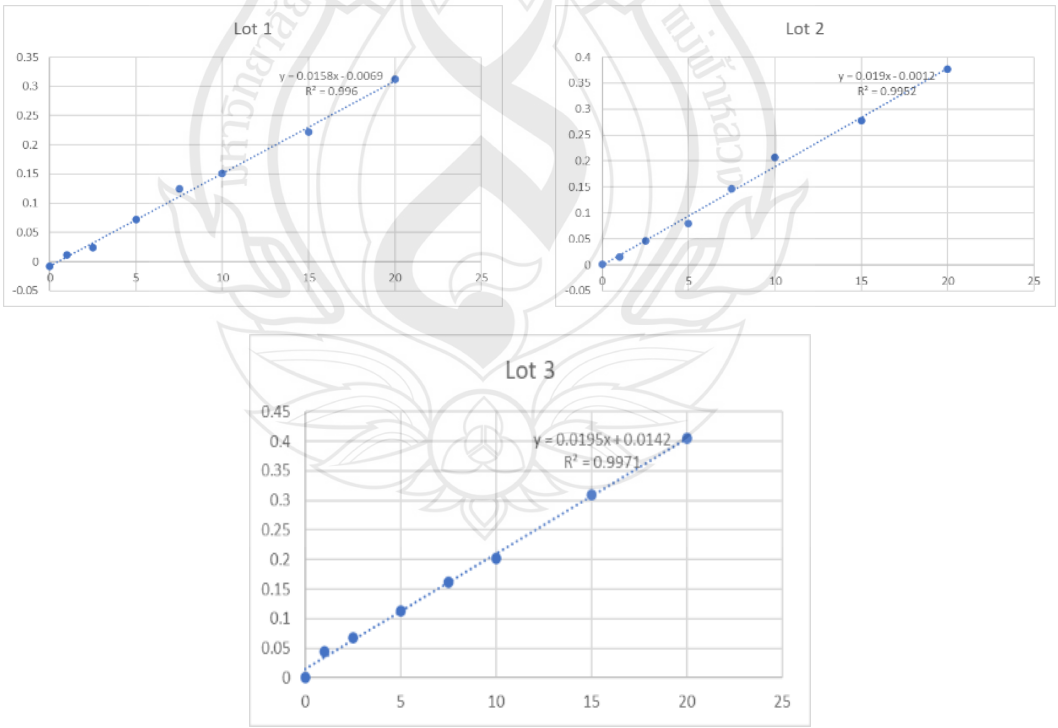



**Figure B2** Standard Calibration Graphs of Standard $NO_4$-N (Lot 1-3)

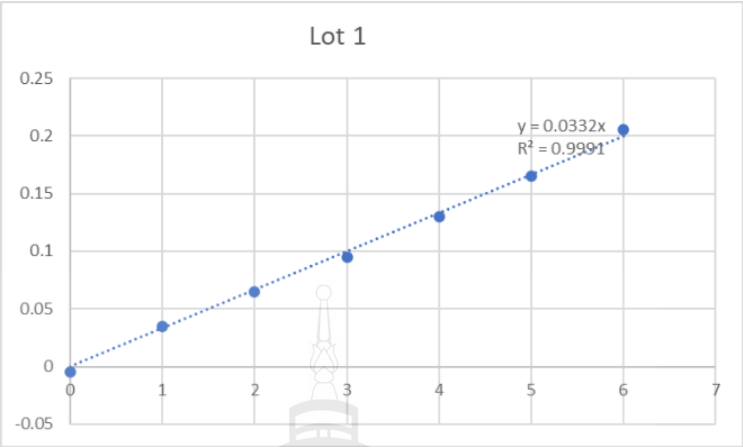



**Figure B3** Calibration Graph of Standard Phosphorus

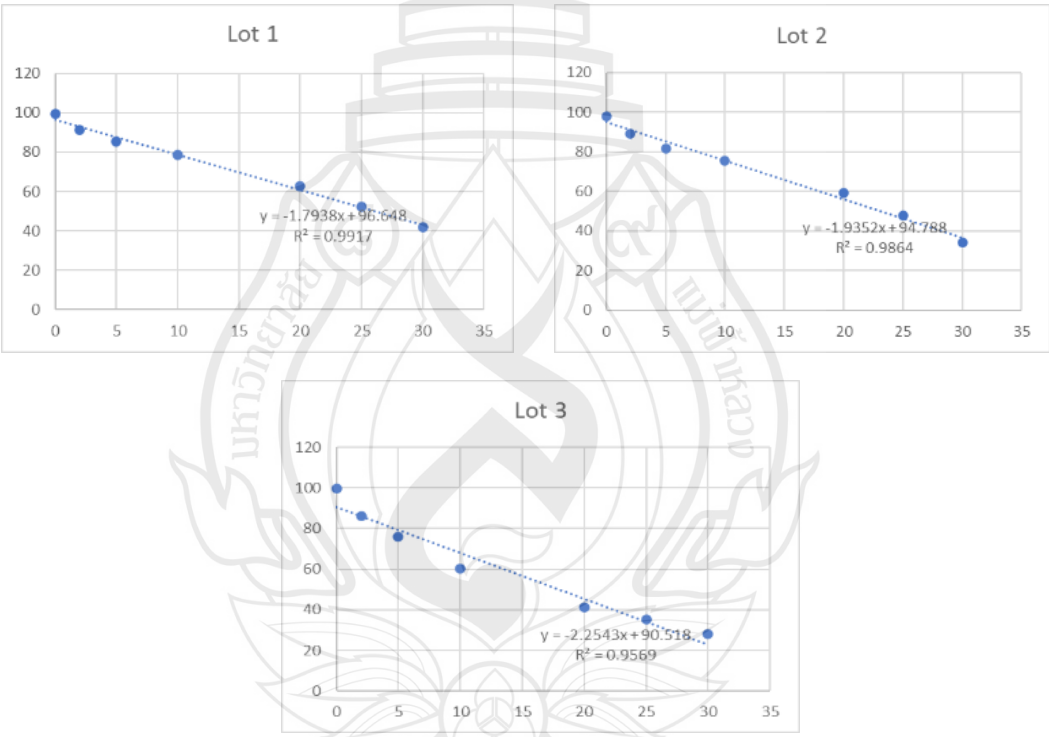



**Figure B4** Calibration Graph of Standard Sulphur (Lot 1-3)

# APPENDIX C

# THE CONDITION OF THE FLAME PHOTOMETER FOR CEC AND K

Cation exchange capacity (CEC) and extractable potassium (P) were analyzed and quantified by using flame photometer. The condition for determine CEC was shown in Table C1, and the condition for determine K was shown in Table C2. The standard calibration graph of K was shown in Figure C1.

**Table C1** The Condition of the Flame Photometer for Cation Exchange Capacity (CEC) Determination

| **Instrument:** | **Analytical Method** |
|---|---|
| 1-Na | 1-Na |
| Signal mode: BKG Corr. | Atomizer: Standard |
| Measurement Mode: Integral | Flame Type: Air-C2H2 |
| Wavelength(nm): 589.0 | Fuel Flow(l/min): 2.2 |
| Slicing Height (%): – | Oxidant(l/min).: 15.0 |
| Determine the W.L.: Auto | Oxidant(kPa): 160 |
| Slit Width(nm): 0.4 | Burner Height(mm): 7.5 |
| Time Constant(s): 1.0 | Delay Time(s): 5 |
| Lamp Current(mA): 12.0 | Measurement Time(s): 5.0 |
| PMT Voltage(V): 0 | Standard Corr. Coef.: 0.9969 |

**Table C2** The Condition of the Flame Photometer (ICP-OES) for Potassium Determination

| **Instrument** | **Analytical Method** |
|---|---|
| 1-K | 1-K |
| Signal mode: BKG Corr. | Atomizer: Standard |
| Measurement Mode: Integral | Flame Type: Air-C2H2 |
| Wavelength(nm): 766.5 | Fuel Flow(l/min): 2.4 |

**Table C2** (continued)

| Instrument | Analytical Method |
|---|---|
| Slicing Height (%): – | Oxidant(l/min).: 15.0 |
| Determine the W.L.: Auto | Oxidant(kPa): 160 |
| Slit Width(nm): 1.3 | Burner Height(mm): 7.5 |
| Time Constant(s): 1.0 | Delay Time(s): 5 |
| Lamp Current(mA): 12.0 | Measurement Time(s): 5.0 |
| PMT Voltage(V): 0 | Standard Corr. Coef. :0.9885 |

Element : K STD Unit : ug/mL UNK Unit : ug/mL

| Sample ID | Sample Name | Conc. | RSD(%) | ABS | REF |
|---|---|---|---|---|---|
| STD1 | | 0.00 | 300.00 | 0.0001 | 0.0000 |
| STD2 | | 0.50 | 0.15 | 0.0679 | 0.0077 |
| STD3 | | 1.00 | 0.52 | 0.1163 | 0.0123 |
| STD4 | | 1.50 | 0.41 | 0.1694 | 0.0143 |
| STD5 | | 2.00 | 1.09 | 0.2117 | 0.0186 |

Coefficient : K3=--
K2=--
K1=1.049400E-001
K0=8.140000E-003
Corr.Coef. : 0.9967

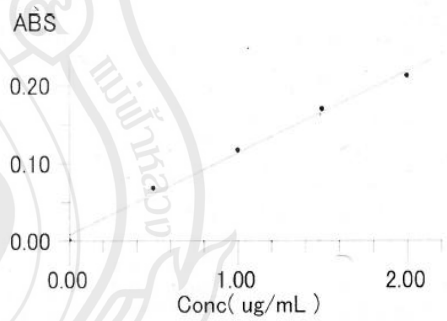
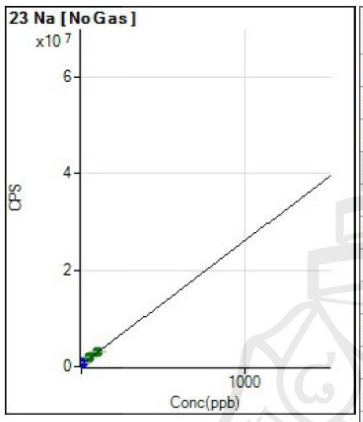



**Figure C1** Calibration Graph of Potassium Determination

# APPENDIX D

# AMMONIUM ACETATE EXTRACTED-SOIL NUTRIENTS DETERMINATION

The result of calibration graphs of soil nutrients that extracted by using ammonium acetate were shown in Figure D1-D6.

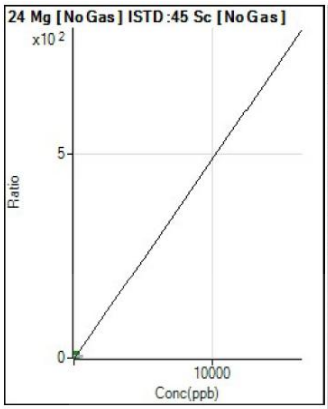



y = 25605.0676 * x + 596733.1667
R = 0.9966
%RSE = 3874.0
DL = 0.3278 ppb
BEC = 23.31 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 596733.17 | | P | 0.5 | |
| 2 | ☐ | 0.100 | 8.760 | 821021.52 | | P | 1.0 | 8659.5 |
| 3 | ☐ | 0.500 | 1.449 | 633826.54 | | P | 0.1 | 189.7 |
| 4 | ☐ | 1.000 | 2.160 | 652029.86 | | P | 0.2 | 116.0 |
| 5 | ☐ | 5.000 | 7.221 | 781614.68 | | P | 0.2 | 44.4 |
| 6 | ☐ | 10.000 | 10.984 | 877986.42 | | P | 0.5 | 9.8 |
| 7 | ☐ | 50.000 | 53.501 | 1966618.51 | | A | 5.2 | 7.0 |
| 8 | ☐ | 100.000 | 98.015 | 3106420.79 | | A | 4.1 | -2.0 |
| 9 | ☐ | | | 717130.15 | | P | 0.3 | |
| 10 | ☐ | | | 687341.58 | | P | 0.4 | |
| 11 | ☐ | | | 651643.99 | | P | 0.4 | |
| 12 | ☐ | | | 637997.65 | | P | 0.9 | |
| 13 | ☐ | | | 638075.77 | | P | 0.6 | |
| 14 | ☐ | | | 641689.72 | | P | 0.5 | |
| 15 | ☐ | | | 666702.02 | | P | 0.9 | |
| 16 | ☐ | | | 481016.29 | | P | 0.7 | |
| 17 | ☐ | | | 500612.18 | | P | 1.3 | |
| 18 | ☐ | | | 536116.83 | | P | 0.4 | |
| 19 | ☐ | | | 495052.81 | | P | 0.6 | |
| 20 | ☐ | | | 531997.55 | | P | 1.0 | |
| 21 | ☐ | | | 504025.60 | | P | 0.7 | |
| 22 | ☐ | | | 515214.89 | | P | 1.6 | |

**Figure D1** Na (No Gas) Calibration Curve

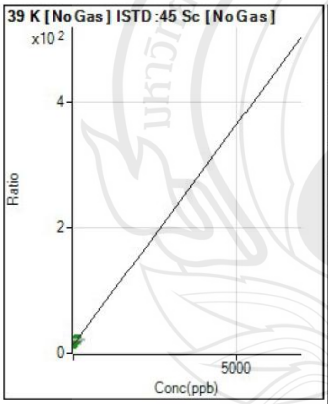



y = 0.0490 * x + 0.0892
R = 0.9999
%RSE = 413.1
DL = 0.246 ppb
BEC = 1.82 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 32892.41 | 0.0892 | P | 4.5 | |
| 2 | ☐ | 0.100 | 1.023 | 50345.50 | 0.1394 | P | 0.7 | 923.3 |
| 3 | ☐ | 0.500 | 0.625 | 43326.67 | 0.1199 | P | 1.0 | 25.0 |
| 4 | ☐ | 1.000 | 1.142 | 53033.10 | 0.1452 | P | 1.0 | 14.2 |
| 5 | ☐ | 5.000 | 5.071 | 125428.36 | 0.3378 | P | 2.6 | 1.4 |
| 6 | ☐ | 10.000 | 9.904 | 209667.31 | 0.5748 | P | 0.4 | -1.0 |
| 7 | ☐ | 50.000 | 49.161 | 904752.64 | 2.4993 | P | 0.4 | -1.7 |
| 8 | ☐ | 100.000 | 100.422 | 1774530.65 | 5.0123 | A | 6.4 | 0.4 |
| 9 | ☐ | | | 30135.53 | 0.0827 | P | 2.8 | |
| 10 | ☐ | | | 23996.01 | 0.0690 | P | 7.4 | |
| 11 | ☐ | | | 30937.57 | 0.0918 | P | 4.8 | |
| 12 | ☐ | | | 26200.25 | 0.0750 | P | 1.2 | |
| 13 | ☐ | | | 38945.92 | 0.1095 | P | 0.5 | |
| 14 | ☐ | | | 36866.55 | 0.1022 | P | 1.2 | |
| 15 | ☐ | | | 41594.64 | 0.1183 | P | 4.5 | |
| 16 | ☐ | | | 25271.67 | 0.0688 | P | 4.0 | |
| 17 | ☐ | | | 27162.26 | 0.0745 | P | 4.7 | |
| 18 | ☐ | | | 38260.49 | 0.1035 | P | 1.0 | |
| 19 | ☐ | | | 40761.55 | 0.1127 | P | 1.7 | |
| 20 | ☐ | | | 22710.26 | 0.0606 | P | 2.3 | |
| 21 | ☐ | | | 32250.62 | 0.1012 | P | 11.0 | |
| 22 | ☐ | | | 29320.29 | 0.0823 | P | 0.5 | |

**Figure D2** Mg (No Gas) Calibration Curve

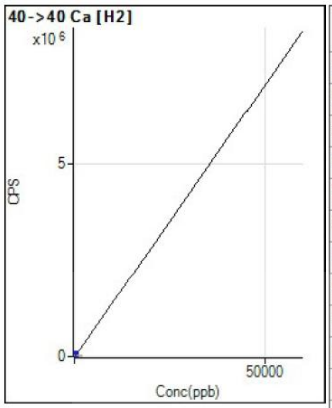



y = 0.0700 * x + 15.1331
R = 0.9933
%RSE = 4547.5
DL = 5.693 ppb
BEC = 216.1 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 5581471.17 | 15.1331 | A | 0.9 | |
| 2 | ☐ | 0.100 | 10.206 | 5722831.16 | 15.8477 | A | 1.6 | 10105.6 |
| 3 | ☐ | 0.500 | 6.089 | 5624476.79 | 15.5595 | A | 1.5 | 1117.8 |
| 4 | ☐ | 1.000 | 2.616 | 5590848.67 | 15.3163 | A | 3.9 | 161.6 |
| 5 | ☐ | 5.000 | 5.369 | 5759032.41 | 15.5091 | A | 1.2 | 7.4 |
| 6 | ☐ | 10.000 | 6.073 | 5674940.54 | 15.5584 | A | 2.0 | -39.3 |
| 7 | ☐ | 50.000 | 51.070 | 6772235.73 | 18.7089 | A | 0.7 | 2.1 |
| 8 | ☐ | 100.000 | 99.785 | 7832413.42 | 22.1197 | A | 5.2 | -0.2 |
| 9 | ☐ | | | 5669444.91 | 15.5523 | A | 3.5 | |
| 10 | ☐ | | | 5544528.46 | 15.9254 | A | 2.8 | |
| 11 | ☐ | | | 5586399.92 | 16.6139 | A | 8.8 | |
| 12 | ☐ | | | 5519187.21 | 15.8061 | A | 0.4 | |
| 13 | ☐ | | | 5552074.08 | 15.6032 | A | 0.9 | |
| 14 | ☐ | | | 5413330.96 | 15.0058 | A | 0.7 | |
| 15 | ☐ | | | 5550852.63 | 15.7876 | A | 2.0 | |
| 16 | ☐ | | | 5431911.59 | 14.7805 | A | 3.9 | |
| 17 | ☐ | | | 5311269.19 | 14.5682 | A | 1.7 | |
| 18 | ☐ | | | 5272669.09 | 14.2604 | A | 0.7 | |
| 19 | ☐ | | | 5271709.50 | 14.5824 | A | 3.7 | |
| 20 | ☐ | | | 5404346.17 | 14.4205 | A | 1.0 | |
| 21 | ☐ | | | 5224996.48 | 16.3792 | A | 9.7 | |
| 22 | ☐ | | | 5092708.68 | 14.2904 | A | 1.1 | |

**Figure D3** K (No Gas) Calibration Curve

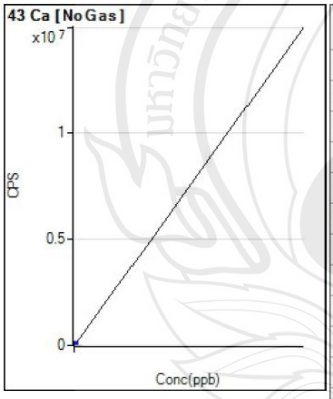



y = 141.2362 * x + 5574.5767
R = 0.7973
%RSE = 16164.6
DL = 5.806 ppb
BEC = 39.47 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 5574.58 | | P | 4.9 | |
| 2 | ☐ | 0.100 | 34.810 | 10491.05 | | P | 3.3 | 34710.3 |
| 3 | ☐ | 0.500 | 43.338 | 11695.42 | | P | 1.3 | 8567.5 |
| 4 | ☐ | 1.000 | 53.638 | 13150.20 | | P | 5.5 | 5263.8 |
| 5 | ☐ | 5.000 | 41.070 | 11375.16 | | P | 1.7 | 721.4 |
| 6 | ☐ | 10.000 | 30.039 | 9817.20 | | P | 1.7 | 200.4 |
| 7 | ☐ | 50.000 | 52.126 | 12936.62 | | P | 2.6 | 4.3 |
| 8 | ☐ | 100.000 | 94.354 | 18900.83 | | P | 3.7 | -5.6 |
| 9 | ☐ | | | 11044.85 | | P | 2.2 | |
| 10 | ☐ | | | 8576.27 | | P | 8.0 | |
| 11 | ☐ | | | 11068.21 | | P | 0.9 | |
| 12 | ☐ | | | 7865.81 | | P | 4.5 | |
| 13 | ☐ | | | 11158.31 | | P | 1.5 | |
| 14 | ☐ | | | 12686.39 | | P | 0.1 | |
| 15 | ☐ | | | 17395.39 | | P | 1.1 | |
| 16 | ☐ | | | 8826.43 | | P | 4.4 | |
| 17 | ☐ | | | 11838.93 | | P | 2.5 | |
| 18 | ☐ | | | 11465.23 | | P | 5.2 | |
| 19 | ☐ | | | 6965.28 | | P | 4.7 | |
| 20 | ☐ | | | 7328.81 | | P | 4.1 | |
| 21 | ☐ | | | 10998.16 | | P | 2.8 | |
| 22 | ☐ | | | 7869.15 | | P | 2.9 | |

**Figure D4** Ca ($H_2$) Calibration Curve

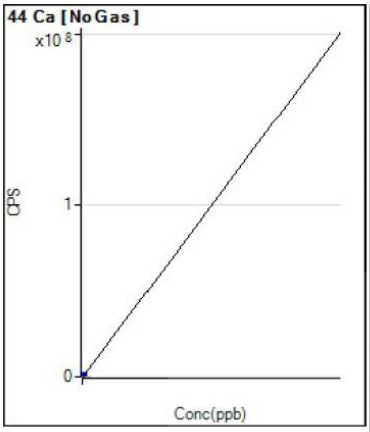



y = 97.9427 * x + 8346.1200
R = 0.9013
%RSE = 12111.9
DL = 6.314 ppb
BEC = 85.21 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 8346.12 | | P | 2.5 | |
| 2 | ☐ | 0.100 | 25.987 | 10891.38 | | P | 2.2 | 25887.2 |
| 3 | ☐ | 0.500 | 35.048 | 11778.86 | | P | 4.4 | 6909.7 |
| 4 | ☐ | 1.000 | 40.260 | 12289.33 | | P | 2.8 | 3926.0 |
| 5 | ☐ | 5.000 | 25.102 | 10804.64 | | P | 2.0 | 402.0 |
| 6 | ☐ | 10.000 | 25.953 | 10888.05 | | P | 1.9 | 159.5 |
| 7 | ☐ | 50.000 | 50.856 | 13327.06 | | P | 2.9 | 1.7 |
| 8 | ☐ | 100.000 | 96.381 | 17785.89 | | P | 4.3 | -3.6 |
| 9 | ☐ | | | 10250.85 | | P | 5.0 | |
| 10 | ☐ | | | 8919.83 | | P | 2.3 | |
| 11 | ☐ | | | 10444.33 | | P | 2.4 | |
| 12 | ☐ | | | 8869.82 | | P | 6.7 | |
| 13 | ☐ | | | 10334.27 | | P | 3.1 | |
| 14 | ☐ | | | 11398.53 | | P | 3.5 | |
| 15 | ☐ | | | 13760.84 | | P | 1.3 | |
| 16 | ☐ | | | 10948.11 | | P | 3.4 | |
| 17 | ☐ | | | 15502.85 | | P | 1.2 | |
| 18 | ☐ | | | 24076.26 | | P | 2.1 | |
| 19 | ☐ | | | 34497.05 | | P | 0.6 | |
| 20 | ☐ | | | 47890.68 | | P | 1.0 | |
| 21 | ☐ | | | 62731.97 | | P | 2.9 | |
| 22 | ☐ | | | 85071.62 | | P | 1.1 | |

**Figure D5** Ca (No Gas 1) Calibration Curve




y = 1349.9969 * x + 77332.3733
R = 0.8473
%RSE = 14636.6
DL = 2.184 ppb
BEC = 57.28 ppb

Weight: <None>
Min Conc: 0

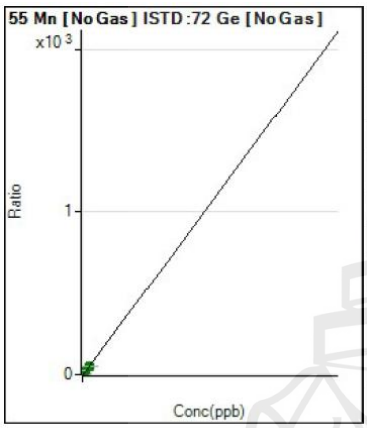
| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 77332.37 | | P | 1.3 | |
| 2 | ☐ | 0.100 | 31.493 | 119847.98 | | P | 0.3 | 31393.1 |
| 3 | ☐ | 0.500 | 40.448 | 131937.67 | | P | 1.1 | 7989.7 |
| 4 | ☐ | 1.000 | 47.239 | 141105.46 | | P | 2.0 | 4623.9 |
| 5 | ☐ | 5.000 | 35.682 | 125502.62 | | P | 0.5 | 613.6 |
| 6 | ☐ | 10.000 | 25.510 | 111771.28 | | P | 0.7 | 155.1 |
| 7 | ☐ | 50.000 | 53.209 | 149163.97 | | P | 1.9 | 6.4 |
| 8 | ☐ | 100.000 | 94.617 | 205065.02 | | P | 1.9 | -5.4 |
| 9 | ☐ | | | 116483.96 | | P | 2.9 | |
| 10 | ☐ | | | 94330.44 | | P | 0.9 | |
| 11 | ☐ | | | 119703.93 | | P | 1.2 | |
| 12 | ☐ | | | 91425.75 | | P | 1.7 | |
| 13 | ☐ | | | 114246.82 | | P | 1.2 | |
| 14 | ☐ | | | 128499.16 | | P | 1.1 | |
| 15 | ☐ | | | 170679.73 | | P | 1.6 | |
| 16 | ☐ | | | 117684.40 | | P | 0.5 | |
| 17 | ☐ | | | 165778.43 | | P | 1.1 | |
| 18 | ☐ | | | 234668.91 | | P | 0.1 | |
| 19 | ☐ | | | 309085.88 | | P | 1.9 | |
| 20 | ☐ | | | 432317.73 | | P | 0.1 | |
| 21 | ☐ | | | 574477.90 | | P | 1.0 | |
| 22 | ☐ | | | 751191.60 | | P | 1.8 | |

**Figure D6** Ca (No Gas 2) Calibration Curve

# APPENDIX E

## DTPA EXTRACTED-SOIL NUTRIENTS DETERMINATION

The result of calibration graphs of soil micronutrients that extracted by using DTPA were shown in Figure E1-E6.

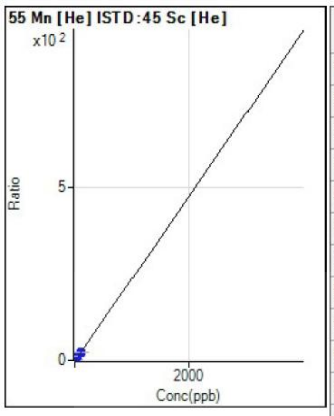



y = 0.5266 * x + 0.1025
R = 0.9998
%RSE = 30.9
DL = 0.013 ppb
BEC = 0.1946 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 13333.76 | 0.1025 | P | 2.2 | |
| 2 | ☐ | 0.100 | 0.169 | 23932.83 | 0.1914 | P | 2.8 | 68.8 |
| 3 | ☐ | 0.500 | 0.529 | 47486.26 | 0.3813 | P | 1.4 | 5.9 |
| 4 | ☐ | 1.000 | 1.016 | 81315.39 | 0.6378 | P | 0.8 | 1.6 |
| 5 | ☐ | 5.000 | 4.922 | 340996.26 | 2.6948 | P | 1.6 | -1.6 |
| 6 | ☐ | 10.000 | 9.737 | 658834.07 | 5.2301 | P | 1.2 | -2.6 |
| 7 | ☐ | 50.000 | 48.353 | 3250679.22 | 25.5662 | A | 0.9 | -3.3 |
| 8 | ☐ | 100.000 | 100.853 | 6246086.99 | 53.2142 | A | 4.9 | 0.9 |
| 9 | ☐ | | | 17358.68 | 0.1322 | P | 1.9 | |
| 10 | ☐ | | | 17138.37 | 0.1363 | P | 2.5 | |
| 11 | ☐ | | | 16537.59 | 0.1337 | P | 7.0 | |
| 12 | ☐ | | | 15649.78 | 0.1206 | P | 2.3 | |
| 13 | ☐ | | | 16364.02 | 0.1260 | P | 3.2 | |
| 14 | ☐ | | | 16277.25 | 0.1254 | P | 3.4 | |
| 15 | ☐ | | | 18183.18 | 0.1432 | P | 1.3 | |
| 16 | ☐ | | | 13794.26 | 0.1106 | P | 2.1 | |
| 17 | ☐ | | | 13240.33 | 0.1087 | P | 1.6 | |
| 18 | ☐ | | | 14208.05 | 0.1164 | P | 0.8 | |
| 19 | ☐ | | | 13126.91 | 0.1086 | P | 2.4 | |
| 20 | ☐ | | | 13670.81 | 0.1093 | P | 2.9 | |
| 21 | ☐ | | | 14671.93 | 0.1341 | P | 7.4 | |
| 22 | ☐ | | | 14194.74 | 0.1163 | P | 0.7 | |

**Figure E1** Mn (No Gas) Calibration Curve

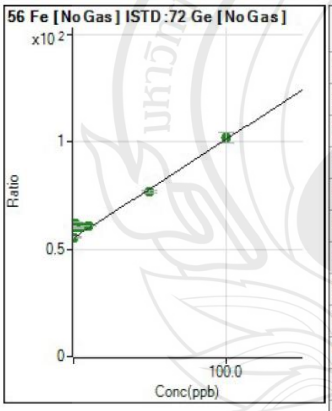



y = 0.2393 * x + 0.0456
R = 0.9999
%RSE = 21.8
DL = 0.2828 ppb
BEC = 0.1906 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 40.00 | 0.0456 | P | 49.4 | |
| 2 | ☐ | 0.100 | 0.069 | 66.67 | 0.0620 | P | 69.2 | -31.3 |
| 3 | ☐ | 0.500 | 0.345 | 130.00 | 0.1282 | P | 26.4 | -31.0 |
| 4 | ☐ | 1.000 | 1.096 | 313.34 | 0.3079 | P | 34.9 | 9.6 |
| 5 | ☐ | 5.000 | 5.852 | 1423.42 | 1.4459 | P | 24.6 | 17.0 |
| 6 | ☐ | 10.000 | 10.746 | 2500.26 | 2.6171 | P | 8.7 | 7.5 |
| 7 | ☐ | 50.000 | 49.244 | 12356.12 | 11.8292 | P | 15.8 | -1.5 |
| 8 | ☐ | 100.000 | 100.261 | 24179.97 | 24.0372 | P | 3.6 | 0.3 |
| 9 | ☐ | | | 26.67 | 0.0287 | P | 40.4 | |
| 10 | ☐ | | | 23.33 | 0.0248 | P | 49.8 | |
| 11 | ☐ | | | 26.67 | 0.0317 | P | 86.6 | |
| 12 | ☐ | | | 30.00 | 0.0378 | P | 44.8 | |
| 13 | ☐ | | | 26.67 | 0.0259 | P | 66.7 | |
| 14 | ☐ | | | 23.33 | 0.0256 | P | 173. | |
| 15 | ☐ | | | 20.00 | 0.0228 | P | 56.3 | |
| 16 | ☐ | | | 20.00 | 0.0214 | P | 41.2 | |
| 17 | ☐ | | | 16.67 | 0.0165 | P | 121. | |
| 18 | ☐ | | | 16.67 | 0.0183 | P | 42.2 | |
| 19 | ☐ | | | 3.33 | 0.0036 | P | 173. | |
| 20 | ☐ | | | 30.00 | 0.0322 | P | 45.7 | |
| 21 | ☐ | | | 23.33 | 0.0227 | P | 67.4 | |
| 22 | ☐ | | | 20.00 | 0.0225 | P | 90.7 | |

**Figure E2** Mn (He) Calibration Curve

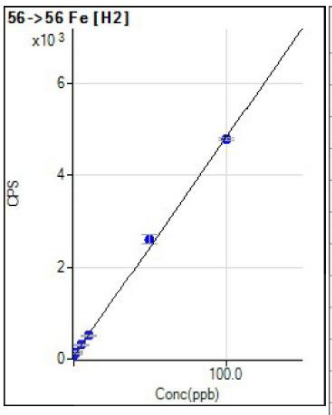



y = 0.4597 * x + 55.4842
R = 0.9882
%RSE = 5192.8
DL = 5.421 ppb
BEC = 120.7 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 7218599.06 | 55.4842 | A | 1.5 | |
| 2 | ☐ | 0.100 | 11.361 | 7591869.26 | 60.7072 | A | 1.5 | 11260.9 |
| 3 | ☐ | 0.500 | 13.756 | 7696526.34 | 61.8084 | A | 3.0 | 2651.3 |
| 4 | ☐ | 1.000 | 10.888 | 7712072.59 | 60.4896 | A | 1.0 | 988.8 |
| 5 | ☐ | 5.000 | 9.681 | 7584240.51 | 59.9347 | A | 1.5 | 93.6 |
| 6 | ☐ | 10.000 | 11.912 | 7678877.39 | 60.9603 | A | 0.5 | 19.1 |
| 7 | ☐ | 50.000 | 46.451 | 9769799.23 | 76.8393 | A | 1.4 | -7.1 |
| 8 | ☐ | 100.000 | 101.173 | 11972016.90 | 101.9965 | A | 5.0 | 1.2 |
| 9 | ☐ | | | 7344328.23 | 55.9270 | A | 1.1 | |
| 10 | ☐ | | | 7461769.06 | 59.3763 | A | 3.7 | |
| 11 | ☐ | | | 7468490.51 | 60.3892 | A | 5.8 | |
| 12 | ☐ | | | 7441439.47 | 57.3413 | A | 1.9 | |
| 13 | ☐ | | | 7516984.26 | 57.8854 | A | 1.6 | |
| 14 | ☐ | | | 7276989.47 | 56.0505 | A | 3.7 | |
| 15 | ☐ | | | 7436573.64 | 58.5492 | A | 2.5 | |
| 16 | ☐ | | | 7083385.73 | 56.7970 | A | 2.0 | |
| 17 | ☐ | | | 6824628.86 | 56.0182 | A | 2.5 | |
| 18 | ☐ | | | 6799639.69 | 55.6874 | A | 0.4 | |
| 19 | ☐ | | | 6824216.15 | 56.4565 | A | 1.8 | |
| 20 | ☐ | | | 6938811.56 | 55.4674 | A | 2.9 | |
| 21 | ☐ | | | 6809494.90 | 62.3632 | A | 10.5 | |
| 22 | ☐ | | | 6773083.44 | 55.4916 | A | 1.2 | |

**Figure E3** Fe (No Gas) Calibration Curve

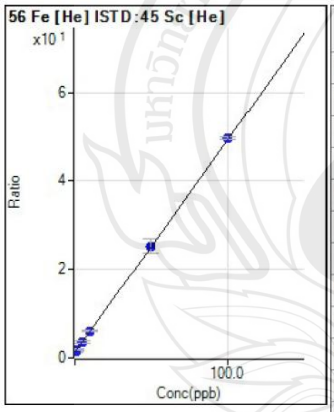



y = 47.5695 * x + 90.0000
R = 0.9993
%RSE = 206.4
DL = 1.892 ppb
BEC = 1.892 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 90.00 | | P | 33.3 | |
| 2 | ☐ | 0.100 | 0.561 | 116.67 | | P | 24.7 | 460.6 |
| 3 | ☐ | 0.500 | 0.631 | 120.00 | | P | 16.7 | 26.1 |
| 4 | ☐ | 1.000 | 1.051 | 140.00 | | P | 50.0 | 5.1 |
| 5 | ☐ | 5.000 | 4.555 | 306.67 | | P | 7.5 | -8.9 |
| 6 | ☐ | 10.000 | 8.829 | 510.01 | | P | 9.0 | -11.7 |
| 7 | ☐ | 50.000 | 52.911 | 2606.95 | | P | 7.9 | 5.8 |
| 8 | ☐ | 100.000 | 98.682 | 4784.26 | | P | 0.9 | -1.3 |
| 9 | ☐ | | | 106.67 | | P | 5.4 | |
| 10 | ☐ | | | 193.33 | | P | 13.0 | |
| 11 | ☐ | | | 93.33 | | P | 27.0 | |
| 12 | ☐ | | | 86.67 | | P | 24.0 | |
| 13 | ☐ | | | 76.67 | | P | 19.9 | |
| 14 | ☐ | | | 90.00 | | P | 29.4 | |
| 15 | ☐ | | | 193.33 | | P | 13.0 | |
| 16 | ☐ | | | 110.00 | | P | 32.8 | |
| 17 | ☐ | | | 83.33 | | P | 45.4 | |
| 18 | ☐ | | | 86.67 | | P | 37.1 | |
| 19 | ☐ | | | 83.33 | | P | 70.3 | |
| 20 | ☐ | | | 96.67 | | P | 48.9 | |
| 21 | ☐ | | | 136.67 | | P | 11.2 | |
| 22 | ☐ | | | 56.67 | | P | 36.7 | |

**Figure E4** Fe ($H_2$) Calibration Curve

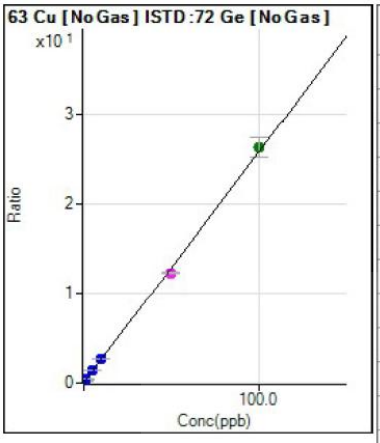



y = 0.4804 * x + 1.4627
R = 1.0000
%RSE = 107.6
DL = 0.9776 ppb
BEC = 3.045 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 1310.07 | 1.4627 | P | 10.7 | |
| 2 | ☐ | 0.100 | -0.123 | 1566.77 | 1.4035 | P | 10.1 | -223.3 |
| 3 | ☐ | 0.500 | 0.057 | 1506.76 | 1.4899 | P | 9.2 | -88.7 |
| 4 | ☐ | 1.000 | 0.920 | 1960.16 | 1.9047 | P | 16.0 | -8.0 |
| 5 | ☐ | 5.000 | 4.472 | 3560.52 | 3.6108 | P | 22.8 | -10.6 |
| 6 | ☐ | 10.000 | 9.675 | 5841.38 | 6.1100 | P | 8.1 | -3.3 |
| 7 | ☐ | 50.000 | 49.332 | 26344.35 | 25.1594 | P | 13.2 | -1.3 |
| 8 | ☐ | 100.000 | 100.396 | 50009.48 | 49.6881 | P | 1.1 | 0.4 |
| 9 | ☐ | | | 1103.39 | 1.1426 | P | 14.9 | |
| 10 | ☐ | | | 1403.42 | 1.4875 | P | 6.7 | |
| 11 | ☐ | | | 1010.04 | 1.2067 | P | 4.8 | |
| 12 | ☐ | | | 956.71 | 1.1724 | P | 10.2 | |
| 13 | ☐ | | | 993.38 | 1.0517 | P | 25.0 | |
| 14 | ☐ | | | 950.04 | 1.0609 | P | 12.2 | |
| 15 | ☐ | | | 2080.18 | 2.2946 | P | 9.9 | |
| 16 | ☐ | | | 1150.06 | 1.2721 | P | 14.0 | |
| 17 | ☐ | | | 816.70 | 0.8299 | P | 5.2 | |
| 18 | ☐ | | | 923.37 | 0.9895 | P | 11.4 | |
| 19 | ☐ | | | 926.71 | 1.0577 | P | 3.5 | |
| 20 | ☐ | | | 930.05 | 0.9503 | P | 13.9 | |
| 21 | ☐ | | | 1023.38 | 0.9891 | P | 12.1 | |
| 22 | ☐ | | | 806.69 | 0.8858 | P | 11.4 | |

**Figure E5** Fe (He) Calibration Curve

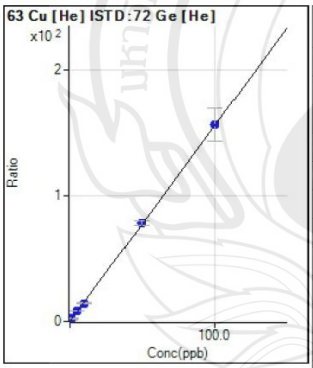



y = 0.2574 * x + 0.1782
R = 0.9994
%RSE = 39.8
DL = 0.03624 ppb
BEC = 0.6924 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 23188.17 | 0.1782 | P | 1.7 | |
| 2 | ☐ | 0.100 | 0.189 | 28355.49 | 0.2267 | P | 3.2 | 88.5 |
| 3 | ☐ | 0.500 | 0.487 | 37823.87 | 0.3037 | P | 1.7 | -2.5 |
| 4 | ☐ | 1.000 | 0.979 | 54843.35 | 0.4302 | P | 2.7 | -2.1 |
| 5 | ☐ | 5.000 | 4.808 | 179148.90 | 1.4157 | P | 1.1 | -3.8 |
| 6 | ☐ | 10.000 | 9.607 | 333939.72 | 2.6510 | P | 0.3 | -3.9 |
| 7 | ☐ | 50.000 | 46.996 | 1560771.07 | 12.2750 | M | 0.4 | -6.0 |
| 8 | ☐ | 100.000 | 101.551 | 3084787.35 | 26.3175 | A | 8.7 | 1.6 |
| 9 | ☐ | | | 233335.15 | 1.7771 | P | 2.9 | |
| 10 | ☐ | | | 210070.27 | 1.6718 | P | 4.1 | |
| 11 | ☐ | | | 196792.26 | 1.5898 | P | 3.1 | |
| 12 | ☐ | | | 183445.12 | 1.4136 | P | 3.2 | |
| 13 | ☐ | | | 179142.41 | 1.3794 | P | 1.7 | |
| 14 | ☐ | | | 165892.52 | 1.2780 | P | 3.7 | |
| 15 | ☐ | | | 166965.80 | 1.3144 | P | 2.2 | |
| 16 | ☐ | | | 19695.54 | 0.1579 | P | 0.8 | |
| 17 | ☐ | | | 20600.35 | 0.1691 | P | 4.4 | |
| 18 | ☐ | | | 22613.81 | 0.1852 | P | 2.6 | |
| 19 | ☐ | | | 21331.55 | 0.1764 | P | 1.8 | |
| 20 | ☐ | | | 20346.60 | 0.1626 | P | 2.6 | |
| 21 | ☐ | | | 19234.82 | 0.1765 | P | 13.3 | |
| 22 | ☐ | | | 17215.21 | 0.1411 | P | 2.9 | |

**Figure E6** Cu (No Gas) Calibration Curve

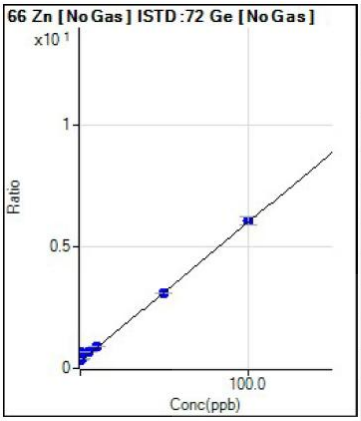



y = 1.5566 * x + 0.9350
R = 0.9999
%RSE = 17.8
DL = 0.1844 ppb
BEC = 0.6006 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 693.36 | 0.9350 | P | 10.2 | |
| 2 | ☐ | 0.100 | 0.092 | 960.04 | 1.0789 | P | 9.5 | -7.5 |
| 3 | ☐ | 0.500 | 0.683 | 1260.06 | 1.9985 | P | 18.3 | 36.6 |
| 4 | ☐ | 1.000 | 0.978 | 1683.45 | 2.4578 | P | 21.7 | -2.2 |
| 5 | ☐ | 5.000 | 4.789 | 6064.82 | 8.3893 | P | 8.5 | -4.2 |
| 6 | ☐ | 10.000 | 8.737 | 11218.39 | 14.5351 | P | 5.3 | -12.6 |
| 7 | ☐ | 50.000 | 49.996 | 53470.76 | 78.7600 | P | 4.9 | 0.0 |
| 8 | ☐ | 100.000 | 100.138 | 105398.54 | 156.8142 | P | 16.4 | 0.1 |
| 9 | ☐ | | | 4184.05 | 5.9006 | P | 19.3 | |
| 10 | ☐ | | | 3637.21 | 4.9935 | P | 12.5 | |
| 11 | ☐ | | | 3407.14 | 4.7134 | P | 11.9 | |
| 12 | ☐ | | | 3373.80 | 5.1981 | P | 6.6 | |
| 13 | ☐ | | | 3090.40 | 4.3385 | P | 15.8 | |
| 14 | ☐ | | | 2983.70 | 4.5035 | P | 14.6 | |
| 15 | ☐ | | | 3153.75 | 4.2385 | P | 18.8 | |
| 16 | ☐ | | | 576.68 | 0.9703 | P | 30.4 | |
| 17 | ☐ | | | 586.69 | 0.9109 | P | 7.8 | |
| 18 | ☐ | | | 680.02 | 0.9528 | P | 17.4 | |
| 19 | ☐ | | | 633.35 | 0.8213 | P | 8.5 | |
| 20 | ☐ | | | 540.01 | 0.8017 | P | 18.0 | |
| 21 | ☐ | | | 576.68 | 0.8084 | P | 0.9 | |
| 22 | ☐ | | | 466.68 | 0.6957 | P | 11.6 | |

**Figure E7** Cu (He) Calibration Curve

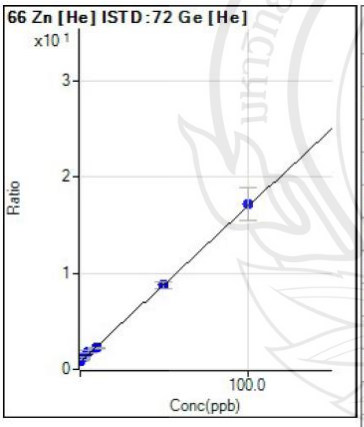



y = 0.0572 * x + 0.3230
R = 0.9982
%RSE = 2652.4
DL = 0.4916 ppb
BEC = 5.647 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 42017.31 | 0.3230 | P | 2.9 | |
| 2 | ☐ | 0.100 | 6.030 | 83528.37 | 0.6679 | P | 2.0 | 5930.2 |
| 3 | ☐ | 0.500 | 0.966 | 47112.02 | 0.3782 | P | 1.3 | 93.2 |
| 4 | ☐ | 1.000 | 1.231 | 50150.42 | 0.3934 | P | 2.0 | 23.1 |
| 5 | ☐ | 5.000 | 5.898 | 83558.38 | 0.6603 | P | 1.2 | 18.0 |
| 6 | ☐ | 10.000 | 9.817 | 111418.02 | 0.8845 | P | 0.8 | -1.8 |
| 7 | ☐ | 50.000 | 48.490 | 393723.80 | 3.0965 | P | 0.4 | -3.0 |
| 8 | ☐ | 100.000 | 100.718 | 713955.96 | 6.0838 | P | 5.5 | 0.7 |
| 9 | ☐ | | | 49042.80 | 0.3735 | P | 2.0 | |
| 10 | ☐ | | | 43870.84 | 0.3488 | P | 0.5 | |
| 11 | ☐ | | | 45596.74 | 0.3678 | P | 2.5 | |
| 12 | ☐ | | | 46068.28 | 0.3550 | P | 1.5 | |
| 13 | ☐ | | | 48072.39 | 0.3702 | P | 4.3 | |
| 14 | ☐ | | | 52155.37 | 0.4018 | P | 2.0 | |
| 15 | ☐ | | | 52215.62 | 0.4111 | P | 2.5 | |
| 16 | ☐ | | | 42666.22 | 0.3420 | P | 1.9 | |
| 17 | ☐ | | | 45097.94 | 0.3702 | P | 0.5 | |
| 18 | ☐ | | | 45599.85 | 0.3735 | P | 1.3 | |
| 19 | ☐ | | | 39669.65 | 0.3283 | P | 4.5 | |
| 20 | ☐ | | | 45546.34 | 0.3640 | P | 2.4 | |
| 21 | ☐ | | | 38957.71 | 0.3550 | P | 2.7 | |
| 22 | ☐ | | | 39606.07 | 0.3245 | P | 2.4 | |

**Figure E8** Zn (No Gas) Calibration Curve




y = 0.1617 * x + 0.9065
R = 0.9994
%RSE = 1139.5
DL = 0.6528 ppb
BEC = 5.607 ppb

Weight: <None>
Min Conc: 0

| | Rjct | Conc. | Calc Conc. | CPS | Ratio | Det. | RSD | %RE |
|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | 0.000 | 0.000 | 670.02 | 0.9065 | P | 3.9 | |
| 2 | ☐ | 0.100 | 2.643 | 1186.73 | 1.3338 | P | 4.9 | 2543.4 |
| 3 | ☐ | 0.500 | 1.251 | 700.02 | 1.1087 | P | 18.1 | 150.1 |
| 4 | ☐ | 1.000 | 1.256 | 766.70 | 1.1096 | P | 19.7 | 25.6 |
| 5 | ☐ | 5.000 | 5.852 | 1343.41 | 1.8525 | P | 9.6 | 17.0 |
| 6 | ☐ | 10.000 | 8.328 | 1736.79 | 2.2529 | P | 8.1 | -16.7 |
| 7 | ☐ | 50.000 | 48.812 | 5968.11 | 8.7979 | P | 7.8 | -2.4 |
| 8 | ☐ | 100.000 | 100.710 | 11502.00 | 17.1883 | P | 19.7 | 0.7 |
| 9 | ☐ | | | 686.69 | 0.9721 | P | 23.8 | |
| 10 | ☐ | | | 580.01 | 0.7871 | P | 0.5 | |
| 11 | ☐ | | | 750.02 | 1.0371 | P | 10.6 | |
| 12 | ☐ | | | 736.69 | 1.1325 | P | 6.1 | |
| 13 | ☐ | | | 680.02 | 0.9559 | P | 17.6 | |
| 14 | ☐ | | | 780.03 | 1.1758 | P | 10.9 | |
| 15 | ☐ | | | 826.70 | 1.1059 | P | 13.8 | |
| 16 | ☐ | | | 650.02 | 1.0995 | P | 35.5 | |
| 17 | ☐ | | | 703.36 | 1.0913 | P | 8.6 | |
| 18 | ☐ | | | 653.35 | 0.9217 | P | 24.2 | |
| 19 | ☐ | | | 643.35 | 0.8358 | P | 14.4 | |
| 20 | ☐ | | | 690.02 | 1.0481 | P | 23.6 | |
| 21 | ☐ | | | 650.02 | 0.9115 | P | 11.1 | |
| 22 | ☐ | | | 566.68 | 0.8578 | P | 18.9 | |

**Figure E9** Zn (He) Calibration Curve

# CURRICULUM VITAE

| | |
|---|---|
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