

**Characterization of Betel Nut (*Areca Catechu*)
and Tobacco (*Nicotiana Tabacum*) of Mizoram**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

LALRINAWMA ZOTE

MZU REGISTRATION NO: 2162 of 2012

Ph.D. REGISTRATION NO.: MZU/Ph.D./1249 of 09.08.2018



**DEPARTMENT OF CHEMISTRY
SCHOOL OF PHYSICAL SCIENCES
AUGUST 2025**

**Characterization of Betel Nut (*Areca Catechu*)
and Tobacco (*Nicotiana Tabacum*) of Mizoram**

By

LALRINAWMA ZOTE

Department of Chemistry

Supervisor

Prof. MUTHUKUMARAN R.

Submitted

**In partial fulfillment of the requirements of the Degree of Doctor of
Philosophy in Chemistry of Mizoram University, Aizawl**

MIZORAM



UNIVERSITY

Department of Chemistry

Muthukumaran, R., Professor

Thesis Certificate

This is hereby certified that the research work for the dissertation entitled “*Characterization of Betel Nut (Areca Catechu) and Tobacco (Nicotiana Tabacum) of Mizoram*” submitted by *Mr. Lalrinawma Zote* to Mizoram University, Tanhril, Aizawl-04, for the award of Doctor of Philosophy (Ph.D.) Degree in Chemistry is a *bona fide* record of the research work carried by Mr. Lalrinawma Zote under my supervision. The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Tanhril, Aizawl-04

MUTHUKUMARAN R.

Date:

Research Supervisor

Tanhril Campus, Aizawl 796 004.

Phone: 94363 52161

Fax: (0389) 233 0834

E-mail:rsic15@gmail.com

DECLARATION
MIZORAM UNIVERSITY
AUGUST, 2025

I **Lalrinawma Zote**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any other University/Institute.

This is being submitted to the Mizoram University for **the Degree of Doctor of Philosophy in Chemistry**.

(LALRINAWMA ZOTE)
Candidate

(Prof. N. MOHONDAS SINGH)
Head

(Prof. MUTHUKUMARAN R.)
Supervisor

ACKNOWLEDGEMENT

To begin with, I thank Almighty God for His infinite mercy, for the strength and good health He graciously bestowed upon me and for empowering me with the resilience to persevere even through the most trying times.

I would like to express my heartfelt gratitude to my supervisor, Prof. Muthukumaran R., Professor, Department of Chemistry, Mizoram University, Tanhril, Aizawl, whose insightful guidance, encouragement, and relentless support have been my driving force behind my accomplishments during my Ph.D journey. His mentorship extended beyond mere academic advice; it was a comprehensive support system that helped me grow as both a researcher and an individual.

I am truly grateful to Prof. N. Mohondas Singh, Head, Department of Chemistry, Mizoram University and other esteemed faculty members, viz., Prof. Diwakar Tiwari, Prof. Zodinpuia Pachuau, Dr. Bimolini Devi, Dr. R. Lalrempuia, Dr. Joydeep Das, Dr. Manjeet Singh, and Dr. G. Rajendro Kumar, for their enduring support and valuable recommendations, which greatly shaped my academic work.

Profound thanks are offered to Dr. Inga Zinikovscaia, Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russia and Prof. Mu-Rong Chao and Prof. Chung-Wen Hu, Chung Shan Medical University, Taichung, ROC whose contributions and valuable discussion have enhanced my research accomplishments.

I would like to express my sincere gratitude to Dr. K. Vanlaldinpuia for introducing me to the field of organic chemistry and for his constant support throughout my journey.

I am immensely thankful to Dr. Prasad P. Phadnis, Bhabha Atomic Research Centre (BARC), Trombay, Mumbai for giving me the opportunity to work in the field of organometallic chemistry, target drug design and delivery, as well as bioimaging that has enhanced my academic growth and research capabilities.

Deep appreciation is extended to my lab-mates, viz., Dr. K. Lalrammawia, Dr. Lalrinhlupuii, Bomngam Kakki, Ananya Buragohain, Malsawmtluangi, Nikrang K.

Marak, Dr. Samson Lalhmangaihzuala and Brilliant N. Marak for their assistance and fun moments in the laboratory which made my research work more valuable.

Closing with this genuine appreciation to my father Vanlalruata Zote and my mother Lalthanpari and my family for their constant support and prayers throughout difficult moments.

Lalrinawma Zote

TABLE OF CONTENTS

	Pages
Certificate	i
Declaration	ii
Acknowledgement	iii - iv
Table of Contents	v - viii
List of Tables	ix - xii
List of Figures	xiii - xv
Abbreviations	xvi - xviii

CHAPTER 1

Pages

1. INTRODUCTION AND REVIEW OF LITERATURE

1.1 Background and Review of Literature	1
---	---

CHAPTER 2

2. MATERIALS AND METHODS

2.1 Samples collected for first set of areca nut.	13
2.2 Samples collected for second set of areca nut.	13
2.3 Samples collected for Tobacco Plants	13
2.4 Soil parameter analysis	17
2.4.1 Soil pH measurement	17
2.4.2 Determination of available nitrogen from soil	17
2.4.3 Determination of available Phosphorous from soil	17
2.4.4 Determination of available Potassium from soil	18
2.4.5 Determination of Soil Organic Matter (SOM)	18

2.5 Neutron Activation Analysis (NAA)	19
2.5.1 Sample Preparation for NAA	20
2.6 Atomic Absorption Spectrometry	22
2.6.1 Sample preparation for AAS	23
2.7 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	24
2.7.1 Sample preparation for analysis of TSNA	24
2.8 Bioaccumulation Index	26
2.9 Pollution Index	30
2.10 Health Risk Assessment	30
2.10.1 Estimated daily intake of elements	30
2.10.2 Non carcinogenic risk	31
2.10.3 Hazard Index	31
2.10.4 Carcinogenic risk	31
2.11 Principal component analysis	32

CHAPTER 3

3. RESULTS AND DISCUSSION

3.1 First set of areca nut	33
3.1.1 Analysis of Soil Parameter	33
3.1.1.1 Soil pH measurement	33
3.1.1.2 Determination of Soil NPK from first set of areca nut	34
3.1.1.3 Determination of soil organic matter (SOM) from first set of areca nut	35
3.1.2 Elemental analysis by Instrumental Neutron Activation Analysis (INAA).	39
3.1.3 Heavy metal analysis by Atomic Absorption Spectrometry (AAS)	41
3.1.4 Discussion	53

3.2 Results of second set of areca nut	57
3.2.1 Analysis of Soil Parameter	57
3.2.1.1 Soil pH measurement	57
3.2.1.2 Determination of Soil NPK from second set of areca nut	57
3.2.1.3 Determination of soil organic matter (SOM) from second set of areca nut	57
3.2.2 Elemental analysis by Instrumental Neutron Activation Analysis (INAA).	61
3.2.3 Discussion	66
3.3 Tobacco Sample	84
3.3.1 Analysis of Soil Parameter	84
3.3.1.1 Soil pH measurement	84
3.3.1.2 Determination of Soil NPK from tobacco plantation	84
3.3.1.3 Determination of soil organic matter (SOM) from tobacco plantation	84
3.3.2. Elemental analysis by Instrumental Neutron Activation Analysis (INAA).	88
3.3.3 Heavy metal analysis by Atomic Absorption Spectrometry (AAS)	88
3.3.4 Determination of carcinogens compounds in smoking product by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)	95
3.3.5 Discussion	105

CHAPTER 4

4. CONCLUSION	120
----------------------	-----

REFERENCES	123
-------------------	-----

BIO- DATA OF THE CANDIDATE

PARTICULARS OF THE CANDIDATE

LIST OF TABLES

Table	Title	Page
2.1	Detail for first set of Areca nut	16
2.1	Detail for second set of Areca nut	16
2.1	Tobacco sample detail	16
2.7.1 (a)	Detailed program of online-SPE coupled with LC separation for determination of nicotine and cotinine	27
2.7.1 (b)	Detailed program of online-SPE coupled with LC separation for determination of NNK, NNN and NNAL	28
2.7.1(c)	MRM parameters used for simultaneous quantification of the nicotine and cotinine	29
2.7.1(d)	MRM parameters used for simultaneous quantification of the NNK, NNN and NNAL	29
3.1.1.1	Measured soil pH from first set of areca nut	36
3.1.1.2	Estimation of available NPK from first set of areca nut	36
3.1.1.3	Observed soil organic matter from first set of areca nut	36
3.1.2 (a)	The concentration of elements determined in areca nut samples (set01) using INAA (in µg/g)	42
3.1.2 (b)	The concentration of elements determined in areca husk samples (set-01) using INAA (in µg/g)	43
3.1.2 (c)	The concentration of elements determined in soil samples collected from areca palm farms (set-01) using INAA (in µg/g)	44

3.1.3 (a)	The concentration of trace elements in areca nut (set-01) using AAS (in µg/kg)	45
3.1.3 (b)	The concentration of trace elements in areca husk (set-01) using AAS (in µg/kg)	45
3.1.3 (c)	The concentration of selected trace elements determined in soil samples collected from areca palm plantation sites (set-01) using AAS (in µg/kg)	45
3.1.4	Correlation between soil pH and the elements in areca husk and areca nut samples.	47
3.1.5	Correlation between Soil Organic Matter (SOM) and the elements in areca husk and areca nut samples.	47
3.1.6	Calculated bioaccumulation index for Areca nut.	49
3.1.7	Calculated bioaccumulation index for Areca Husk	49
3.1.8	Calculated pollution indices for soil samples	51
3.2.1.1	Measured soil pH from second set of areca nut	58
3.2.1.2	Determination of available NPK from second set of areca nut	58
3.2.1.3	Observed soil organic matter from second set of areca nut	59
3.2.2 (a)	The concentration of elements in soil using INAA (in µg/g)	62
3.2.2 (b)	The concentration of elements in areca nut sample using INAA (in µg/g)	63
3.2.2 (c)	The concentration of elements in areca husk sample using INAA (in µg/g)	64
3.2.2 (d)	Element concentration in areca root sample using INAA (in µg/g)	65

3.2.2 (e)	Range, mean and standard deviation of elements in areca nuts, husks, roots and the corresponding soils of NEI using INAA	68
3.2.2 (f)	Range, mean and standard deviation of elements in areca nuts, husks and the corresponding soils of Myanmar	69
3.2.3 (a)	Calculated bioconcentration factor Areca nut	71
3.2.3 (b)	Comparison of elemental contents of areca nut from different countries (mg/kg)	73
3.2.3 (c)	Estimated daily intake (EDI) for different heavy elements in ($\mu\text{g/kg bw/day}$)	80
3.2.3(d)	Calculated Target Hazard Quotient (THQ)for different heavy elements	80
3.2.3 (e)	Calculated Target Carcinogenic Risk (TRC) due to areca nut chewing	80
3.3.1.1	Measured soil pH from tobacco plantation	85
3.3.1.2	Determination of available NPK from tobacco plantation	85
3.3.1.3	Observed soil organic matter from tobacco plantation	86
3.3.2 (a)	The concentration of elements in soil using INAA (in $\mu\text{g/g}$)	90
3.3.2 (b)	The concentration of elements in tobacco root using INAA (in $\mu\text{g/g}$)	91
3.3.2 (c)	The concentration of elements in tobacco stem using INAA (in $\mu\text{g/g}$)	92
3.3.2 (d)	The concentrationof elements in tobacco leaves (Lalrammawia, 2022) for compressional study (in $\mu\text{g/g}$)	93

3.3.3 (a)	Heavy metal concentration levels in soil of tobacco plantation by using AAS (in $\mu\text{g/g}$)	94
3.3.3 (b)	Heavy metal concentration levels of tobacco root by using AAS in ($\mu\text{g/g}$)	94
3.3.3 (c)	Heavy metal concentration levels of tobacco stem by using AAS in ($\mu\text{g/g}$)	94
3.3.4 (a)	Tobacco Specific Alkaloids (TSAs) using LC-MS/MS	102
3.3.4 (b)	Tobacco Specific N-nitrosamines (TSNAs) using LC-MS/MS	103
3.3.4 (c)	Pearson's correlation between pH, Nicotine and cotinine	104
3.3.4 (d)	Pearson's correlation between pH, NNK, NNAL and NNN	104
3.3.5 (a)	Comparison of present data with Indian natural background soil and Indian limit for soil	105
3.3.5 (b)	Calculated bioconcentration and translocation in tobacco	112
3.3.5 (c)	Correlation between elemental concentration and analyzed soil parameters.	115

LISTS OF FIGURES

Fig. No.	Title	Page
1	Report of tobacco used in Northeast India (GATS-2, (2016-17))	4
2.1	Areca palm (<i>Areca catechu</i>) plantation in Mamit district, Mizoram	14
2.2	Tobacco (<i>Nicotiana Tabacum</i>) plant in Mizoram	15
3.1.1.1	Soil pH from first set of areca nut	37
3.1.1.2	Plot of available NPK first set of areca nut	37
3.1.1.3	Estimated soil organic matter from first set of areca nut	38
3.1.2 (a)	Gamma ray spectra of elements in areca palm short lived nuclides	40
3.1.2 (b)	Gamma ray spectra of elements in areca palm long lived nuclides	40
3.1.2 (c)	Gamma ray spectra of elements in areca palm short lived nuclides	41
3.1.6	Bioaccumulation in Areca nut	48
3.1.7	Bioaccumulation in Areca husk	48
3.1.8 (a)	Calculated contamination Factor of sampling site	52
3.1.8(b)	Geoaccumulation (I_{geo}) of Sampling site.	52
3.2.1.1.	Soil pH from second set of areca nut	59
3.2.1.2	Plot of available NPK first set of areca nut	60
3.2.1.3	Estimated soil organic matter from areca nut	60
3.2.3(a)	Bioconcentration and translocation Factor in Areca nu.	72

3.2.3(b)	Bioconcentration and translocation Factor in Areca husk	72
3.2.3(d)	Target hazard quotient comparison of 2 nut and 5 nut per day.	81
2.3.4	PCA Biplot of present study with published element data of areca nut.	83
3.3.1.1.	Soil pH from tobacco plantation	86
3.3.1.2	Plot of available NPK from soil of tobacco plantation.	87
3.3.1.3	Estimated soil organic matter from tobacco plantation	87
3.3.2 (a)	The raw γ -energy decay spectral data (raw spectral data) For tobacco leaves	89
3.3.2 (b)	The raw γ -energy decay spectral data (raw spectral data) for stem	89
3.3.2 (c)	The raw γ -energy decay spectral data (raw spectral data) for root	89
3.3.2 (d)	The raw γ -energy decay spectral data (raw spectral data) for the corresponding soil samples of Mizoram	89
3.3.4 (a)	The chromatogram profiles of alkaloids and nitrosamines in 3R4F reference cigarette using the internal calibration method Nicotine	96
3.3.4 (b)	The chromatogram profiles of alkaloids and nitrosamines in 3R4F reference cigarette using the internal calibration method Cotinine	97

3.3.4 (c)	The chromatogram profiles of alkaloids and nitrosamines in 3R4F reference cigarette using the internal calibration method NNN	98
3.3.4 (d)	The chromatogram profiles of alkaloids and nitrosamines in 3R4F reference cigarette using the internal calibration method NNK	99
3.3.4 (e)	The chromatogram profiles of alkaloids and nitrosamines in 3R4F reference cigarette using the internal calibration method NNAL	100
3.3.4 (a)	Average concentration of Tobacco specific alkaloids (TSAs) With 3R4F reference	101
3.3.4 (b)	Average concentration of Tobacco specific Nitrosamines (TSNAs) with 3R4F reference	101
3.3.4 (c)	Concentration of nicotine from commercially available smoking tobacco	104
3.3.5.1	“Chemical fingerprint” of tobacco plant with respect to “reference plant”	108
3.3.5 (b1)	Bioconcentration and translocation factor in tobacco leaf	111
3.3.5 (b2)	Bioconcentration and translocation factor in tobacco stem	111
3.3.5.2	PCA bi-plot of heavy element in tobacco sample of different countries with the present study.	118
3.3.5.3	HCA of heavy element in tobacco sample of different countries with the present study.	118

Abbreviations

AAR	Age-adjusted incidence rate
ATP	Adenosine triphosphate
AAS	Atomic absorption spectroscopy
APCI	Atmospheric pressure chemical ionization
AT	Average exposure time
ADP	Adenosine diphosphate
BA	Bioaccumulation
BCF	Bioconcentration factor
BW	Body weight
CID	Collision-induced dissociation
CF	Contamination factor
DNA	Deoxyribonucleic acid
EDI	Estimated daily intake
ED	Exposure duration
EI	Electrospray ionization
FCV	Flue-Cured Virginia
FIR	Food ingestion rate
GATS	General agreement on trade in services
HPGe	High-purity germanium
HI	Hazard Index
HRA	Health Risk Assessment
HREE	Higher rare-earth element

HCA	Hierarchical cluster analysis
ICMR	Indian Council of Medical Research
ICMR-NCDIR	ICMR-National Centre for Disease Informatics and Research
IARC	International Agency for Research on Cancer
ICP-MS	Inductively coupled plasma mass spectrometry
INNA	Instrumental neutron activation analysis
INAA	Instrumental Neutron Activation Analysis
JINR	Joint Institute of Nuclear Research
LC	Liquid chromatography
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
LREEs	Lower rare-earth elements
MOHFW	Ministry of Health and Family Welfare
MS/MS	Mass spectrometry
NAA	Neutron Activation Analysis
NEI	North-east India
NIST	National Institute of Standards and Technology
NNAL	Nicotine-derived nitrosaminoaldehyde
NNN	N'-nitrosonornicotine
NNK	Nicotine-derived nitrosaminoketone
OSMF	Oral submucous fibrosis
PAHs	Polycyclic aromatic hydrocarbons
PGNAA	Prompt Gamma Neutron Activation Analysis
PLI	Pollution index

PCA	Principal Component Analysis
ROS	Reactive oxygen species
RNAA	Radiochemical Neutron Activation Analysis
REEs	Rare earth elements
RP	Reference plant
SOM	Soil organic matter
S.D.	Standard deviation
TSNAs	Tobacco-specific nitrosamines
TF	Translocation factor
THQ	Target hazard quotient
TRC	Target Carcinogenic Risk
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
WHO	World Health Organization

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. Background with literature review

Consumption of several types of lifestyle products including smokeless tobacco products in different forms continues to be the preventable induction of cancers at different sites in diverse user populations worldwide (Rumgay et al. 2024; Gomez et al. 2023). Globally smoking tobacco products remain the primary form of tobacco consumption, and it is estimated that more than 15 billion cigarettes are consumed daily worldwide (Reitsma et al., 2021). Manufactured cigarettes (with original and counterfeit products) are extensively available around the world but differ in size and tobacco matter used, whereas the prevalence of hand-rolled cigarettes normally without cellulose acetate-type filters are also still common in few regions across multiple countries. According to World Health Organization (WHO), tobacco use leads to approximately 6 million premature and preventable deaths annually, including those from direct smoking and indirect exposure to secondhand (passive) smoking. It is projected that this number may rise to 7.5 million by 2020 (W.H.O., 2006).

The nationwide consumption of tobacco in India was estimated to be around 30% of the nation population that is 15 years and above. Of these users 47% are men and 14% are women who either smoked or chew tobacco (Bhawna, 2013; Gupta et al. 2016). With respect to Indian Council of Medical Research (ICMR), India new cancer case reports is projected to reached approximately 17.3 lakh by 2020. Further, Northeastern states of India populace accounted for the highest incidence rate of new cancer cases in both females and males. Smokeless tobacco use is very popular, especially among female users in Northeast India (Bhawna, 2013; Gupta et al. 2016). The smoking or smokeless forms else polytobacco use is common among a large proportion of tribal populace in Mizoram (Gupta et al. 2016; Zomawia et al. 2023). It is noteworthy to mention that Aizawl District, Mizoram has been reported having the highest incidence of cancer cases for males in India (ICMR, 2016). Indeed, several epidemiological studies (case-control study) also reported elevated incidence of

tobacco-induced stomach cancer cases among the Mizo population (Phukan et al. 2004, 2005; Zomawia et al. 2023).

Nicotiana tabacum L common name tobacco plant has the capability for sequestering different heavy elements species at varying proportions, predominantly in the leaves along with the vacuoles throughout the plant. Various heavy elemental species are toxic at different concentration levels, whereas certain compounds are non-essential either for both plants and humans, besides some of these heavy elements are exhibiting harmful and carcinogenic even at a trace level of concentration (Lalrammawia et al. 2021; Gomez et al. 2023). In fact, the international agency for research in cancers (IARC) has classified Ni (Ni^{2+}), As, Cd and Cr (Cr^{6+}) as group 1 carcinogens (IARC, 2012)

The tobacco product is consumed mainly by smoking and smokeless tobacco product like khaini, sahdah and zarda. The harvested tobacco plants were dried by us a process called Flue-Cured Virginia (FCV) and for branded cigarette manufacturing is primarily carried out by automated machines, using FCV. And for boosting the crop yield there is an application of herbicides, chemical fertilizers, on tobacco plants before their harvest, which may also be a potential contributing factor for the accumulation of additional toxic elemental content in tobacco leaf matter.

In general, the soil in Mizoram is acidic in nature with pH ranging between 4.5 - 6, due to abundant monsoon rainfall, containing relatively less alkaline and alkali earth ions, high organic carbon content, but low in content of bioavailable phosphorus (Zote et al. 2020; Lalrammawia et al. 2021). In Mizoram, tobacco plants are normally cultivated chemical free without application of any fertilizers or pesticides. Earlier studies have reported increased levels of heavy metals such as mercury (Hg), lead (Pb) and arsenic (As) in tobacco leaves harvested from Mizoram (Lalrammawia et al., 2021). In rural areas of Mizoram, a locally made, hand-rolled cigarette-like tobacco product known as *zozial* is widely used. Unlike commercially manufactured cigarettes, *zozial* is produced using traditional methods. It is typically made on a cottage industry scale, beginning with the collection of mature tobacco leaves. These leaves are manually squashed using bare feet by the farmers. After thorough squashing, the leaves are dried in the sun. Interestingly, the tobacco

residues that accumulate on the workers' feet are scraped off and mixed with the leafy material, as this is believed to enhance the flavour of *zozial*.

The next step involves removing the petiole and main veins from the dried leaves. The deveined leaves are then pounded using a wooden pestle to produce a coarse tobacco powder. This powder serves as the filler for hand-rolling *zozial*. Notably, *zozial* does not include any cellulose acetate-type filter, which means it lacks a mechanism to filter semi-volatile particulate matter released during smoking (Lalrammawia, 2022).

The consumption of the fruit of *Areca catechu* palm commonly called areca nut or betel nut, is a widespread cultural practice among South Asian and Southeast Asian countries. The betel nut is mixed with lime (calcium hydroxide) and flavouring agents/ sweeteners/mouth fresheners and wrapped within a betel vine leaf for chewing. Addition of tobacco-based products along with betel nut are also common. The chewing of betel nut has play an important role in various medical conditions such as tachycardia/arrhythmia (Muthukumaran et al. 2023) and the research reported have indicated high incidence on oral and esophageal cancers in many Oriental countries and in the immigrant Asian communities worldwide (Al-Rmalli et al., 2011; Muthukumaran et al. 2023).

Areca nut cultivation provides a large economic support in the economy as India is the largest producer and consumer of areca nut. In India, areca nut cultivation is primarily concentrated in the states of Karnataka, Kerala and Assam (Vasundhara et al., 2020). The areca nut is either prepared by harvesting the fully ripe mature nuts and drying in sun or produced by harvesting the green areca nut, undergo boiling and subsequently removing the outer husk by peeling.

Production wise, among areca nut producing countries India is the highest producer of areca nut, Karnataka is the largest producer of areca nut in India, Karnataka leads the production both in terms of area and output, contributing nearly 60% of the total national production. Mizoram, Nagaland and Tripura have seen to increase areca nut production in recent times as the consumption of areca nut increases. In Mizoram, areca nuts are mostly produced in an organic manner, i.e., without applying fertilizers and pesticides. In addition, harvested areca palm is not

required to undergo any additional processing as it is normally consumed fresh after their removal of the husk covering the kernel whereas in the neighboring state the areca nut is collected before their maturation and are fermented by burying in the soil or soaked in water before their consumption. The areca or betel nut is locally known as *Kuhva* in Mizoram. Amongst the Mizo people, the offering of *kuhva* is an act of goodwill and a form of welcome gesture. Because of this, chewing of areca nut begins at a very young age leading to an increased prevalence among the young ones. Consumption of areca nuts is reported to be 70.8% and 58.1% in Meghalaya and Mizoram (Singh et al., 2021) showing that out of the total population more than half of the population is consuming these areca or betel nuts in any form on a daily basis. Specific study on epidemiological data on areca nuts is very limited.

The consumption of this lifestyle product is very high among the Northeast region of India. Survey reports on NEI states Mizoram, Meghalaya, Manipur, Tripura, Nagaland, Assam, and Arunachal Pradesh by (GATS-2, 2016-17), on the overall reports with respect to tobacco used in any form is given in Figure 1.

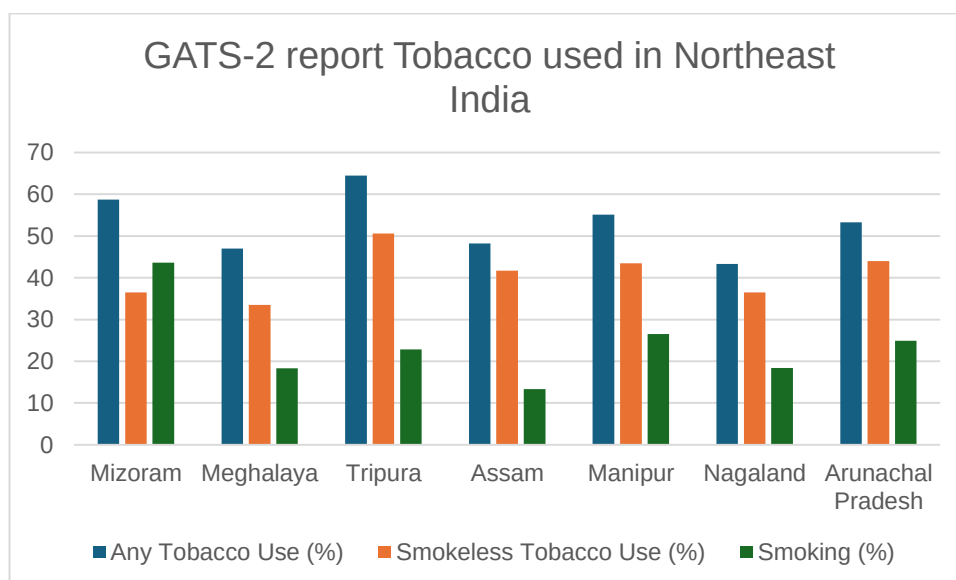


Figure 1: Report of tobacco used in Northeast India (GATS-2, (2016-17))

Besides boosting the economic, intake of areca catechu nuts and tobacco in any form engender detrimental health consequences as they are categorized as Group

1 carcinogens by IARC due to their composition. Chronic tobacco and areca nut use entail significant adverse health risks including oral submucous fibrosis, gastritis, stomach, oral and esophageal cancers (Sinha et al., 2018; Cirilo et al., 2022; Muthukumaran et al., 2023; Zomawia et al., 2023; Mehrotra and Lauby-Secretan, 2025)

Some of the important factors driving cancer rates in the region is the widespread consumption of tobacco products and betelnut along with mixing with tobacco product like gutkha and zarda. From the Global Adult Tobacco Survey (GATS), Northeastern states such as Mizoram and Meghalaya reported to have tobacco usage rates exceeding 50% of the adult population (GATS 2, 2016–17; Zomawia et al., 2023). The consumption of smokeless tobacco, with the combination of areca nut and calcium hydroxide/slaked lime, having a strong correlation with increased risks of oral, esophageal, stomach and lung cancers (GATS 2, 2016–17; Sinha et al., 2018; Cirilo et al., 2022; Muthukumaran et al. 2023; Mehrotra and Lauby-Secretan, 2025). More importantly, tobacco control has become a major concern across the regions around the world, whereas areca nut use remains underrated as a public health issue despite the negative health consequences of rampant and indiscriminate areca nut use.

As mentioned earlier, age-adjusted incidence rate (AAR) in Aizawl district in Mizoram is recorded the highest in India reported on by ICMR-NCDIR (2021), where incidence of cancer is reported as 270.0 per 100,000 males and 186.6 per 100,000 females (Zomawia et al. 2023). Mizoram contributes to substantially higher rate of cancers, viz., esophageal, stomach, liver, nasopharyngeal and lung cancers amongst men whereas cervical, breast, stomach and gallbladder cancers in women. The high prevalence of cancers in Northeast India is strongly linked to the intake of areca nut and tobacco and alcohol, dietary habits and genetic vulnerability (Zomawia et al. 2023).

Adverse health effects of tobacco with or without areca nut consumption

Areca nut along tobacco is widely consumed psychoactive substances, especially in South and Southeast Asia, often in combination with part of betel nut

with leaf called betel quid. Despite their cultural acceptance and long-standing traditional use, both substances are linked with significant negative health impact, especially on the gastrointestinal tract, oral cavity, respiratory system, and overall systemic health. Increasing scientific evidence implicates arecanut and tobacco consumption as major contributors to the global problem of non-communicable diseases, especially cancers and cardiovascular diseases.

The primary active component of arecanut is arecoline, a parasympathomimetic alkaloid that stimulates the central and autonomic nervous systems. Its consumption caused mild euphoria and increased alertness. However, arecoline and its metabolites are also cytotoxic, genotoxic, and carcinogenic. Long-term chewing of arecanut is closely tied with the formation of oral submucous fibrosis (OSMF) a chronic and overtime developing the scarring condition in the oral mucosa leading to rigidity in the oral tissues, difficulty in opening mouth, and significantly increases the risk of advancement to a malignant state as oral squamous cell carcinoma (Tilakaratne et al., 2006; Gupta and Ray, 2004). Moreover, the international agency for research on cancer (IARC) classified areca nut itself as a group 1 carcinogen, signifying adequate evidence for developing cancer in humans (IARC, 2004).

Tobacco, both in smoked and smokeless forms, is another potent carcinogen. It contains thousands of chemicals along with polycyclic aromatic hydrocarbons nicotine, tobacco-specific nitrosamines (TSNAs), many of them were well known carcinogens (Hecht, 2003). Tobacco intake is strongly having a link with a range of cancers, particularly lungs, oral, pharyngeal, esophageal, pancreatic, and bladder cancers. Nicotine, while not directly carcinogenic, contributes to tumor development through its effects on angiogenesis, inflammation, and cellular proliferation (Wang & Hecht, 2021).

When areca nut is consumed in combination with tobacco as is common in many regions including Northeast India the carcinogenic effects are synergistically enhanced. The combined effect has produced higher levels of DNA damage, oxidative stress, and inflammation than either substance alone (Jeng et al., 2001).

Epidemiological studies in South Asia have given that a significantly higher occurrence rates of pharyngeal and oral cancer amid users of areca nut besides with combination of tobacco mixtures. For example, a study reported from India revealed that tobacco and betel quid users had an odds ratio of 8.4 for developing of an oral cancer as compared to non-users (Gupta et al., 1984).

Beyond cancer, chronic use of areca or betel nut and tobacco is linked with a range of systemic effects. Areca nuts have been linked to cardiovascular diseases, with increased blood pressure and heart rate, due to its cholinergic activity. A study led in Taiwan found a strong relation between areca nut chewing and the prevalence of metabolic syndrome, adverse lipid profiles and type 2 diabetes (Yen et al., 2008). Tobacco, in contrast, is a well-known major risk factor like cardiovascular morbidity and mortality, contributing to atherosclerosis, coronary heart disease, and stroke through endothelial dysfunction and pro-inflammatory mechanisms (Benowitz, 2003). Pregnancy are also negatively affected by both substances. Areca nut chewing throughout pregnancy has been correlated to low birth weight, with preterm delivery and neonatal complications (Lee et al., 2005), while tobacco use in any form has been associated with fetal growth restriction, stillbirth, and placental complications.

In Northeast India, where areca nut and tobacco consumption rates are among the highest within the country, the public health burden is significant. The National Family Health Survey data and regional cancer registries report elevated occurrence of oral cancer, especially in states like Mizoram, Manipur, and Meghalaya, correlating with high arecanut and tobacco usage patterns (Thakur et al., 2021). The cultural normalization of chewing these substances often hinders effective public health interventions.

Importance of Element composition in the soil

Soil elemental composition has a foundational role in the growth of plants, and its productivity. The presence of elements and bioavailability of essential elements in soil directly influence plant health, nutrient uptake and yield of the product. Understanding this composition is essential not only for agriculture but also important for ecology, environmental science, and biogeochemical cycling. Plants

require an array of macro- and micronutrients for normal metabolic and physiological processes. These elements are taken up from the soil through the root system, macronutrients which are required in large amounts like nitrogen (N), essential for protein amino acids and chlorophyll. Phosphorus (P) for ATP, DNA, and cell membrane phospholipids. Potassium (K) for osmoregulation, photosynthesis and enzyme activation.

Calcium (Ca), Sulfur (S) and Magnesium (Mg) are required for structural and enzymatic roles. Micronutrients which are required in trace amounts Chlorine (Cl), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Boron (B), and Molybdenum (Mo), are vital for enzyme function, redox reactions, and hormonal regulation in plants for their development.

Tobacco plants are known to actively uptake and translocate metal ions from the soil to their leaves, shoot, and roots. A preliminary study, the analysis of tobacco plants cultivated in the state of Mizoram has revealed elevated concentrations of some heavy elements like arsenic (As), lead (Pb) and mercury (Hg) in the sun-dried tobacco leaves. Unlike other agricultural regions use of fertilizers and pesticides in Mizoram is practically absent when compared to other states. Suggesting the source of relatively elevated concentration levels of these heavy metal species is possibly soil where it is cultivated. Furthermore, for the growth of the areca palm is done in a similar fashion. Implying that the heavy metal species may accumulate into the plant tissues from the soil through the roots and to the leaves and shoots. The elemental characterization of soil in Mizoram has not been explored for studied using detailed scientific approaches. Therefore, conducting a comprehensive analysis of soil samples using different analytical techniques is essential to gain a clear understanding of the soil micronutrient elemental composition in the region.

Neutron Activation Analysis (NAA) is a non-destructive technique and high sensitivity used to determine trace and ultra-trace concentrations of elements in complex matrices. It works by irradiating samples with neutrons, triggering the elements to produce radioactive isotopes. Radio isotopes produced are emitting gamma rays and measured by a gamma detector like HP-Ge to identify and quantify

the elements without chemical preparation. NAA is especially valuable in geological, biological, and environmental studies due to its multi-elemental capability, its precision and low detection limits (Glascock, 2003).

Atomic Absorption Spectroscopy (AAS) is a technique widely employed for quantifying metals and metalloids in various sample types, particularly in environmental, clinical, agricultural, and food analysis. In AAS free atoms produced are absorbing light on their gas phase. Each element absorbs light at a exact wavelength, permitting selective detection. Its high accuracy and cost-effectiveness make it ideal for routine determination of metals like lead, cadmium, and copper (Welz and Sperling, 2008).

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) is a robust technique for detection and quantifying of organic compounds, including pharmaceuticals, toxins, hormones, and metabolites. Combining the separation ability of liquid chromatography along with the advantage of mass spectrometry having high selectivity and high sensitivity. LC-MS/MS is particularly important in biomedical research, environmental monitoring, and food safety because of its capability to identify compounds at picogram levels in complex mixtures (Niessen, 2006).

Together, these analytical methods are important for elemental and compound determination across various scientific disciplines. NAA provides precise multi-element analysis without destroying the sample, AAS offers reliable detection of specific metals, and LC-MS/MS excels in the detection of trace organic compounds, offering unmatched specificity and sensitivity.

Principal component analysis (PCA) works by reducing dimensions from the original data, which is used widely in bioinformatics, chemometrics and data analysis. It helps in transforming the high dimensional data to a lower dimensional data without losing much of the original variance from the data. This technique helps in the reduction of variables and helps with classifying the principal components along the data which varies the most from the original data. It is based on linear algebra concepts like eigenvectors and eigenvalues, covariance matrices. Eigenvalue

and eigenvector from the covariance matrix are used. Eigenvalues measure the variance carried from each principal component, whereas eigenvectors determine the path of new principal components. Principal Component 1 (PC1), that captures the highest variance from the analyzed data, then comes PC2, as the number of principal components increases there will be a decrease in the variance capture from the data set. The components are orthogonal to each other that helps in providing a unique result.

A UK based study analyzing betel quid (a composite of “*Piper betel*” leaves, tobacco, areca nut or flavoured tobacco and lime), by using an inductively coupled plasma mass spectrometry (ICP-MS) detected the presence of Cadmium (Cd), Lead (Pb) and Arsenic (As), where slaked lime contained the highest concentration of Arsenic (As) among all components (Al-Rmalli et al., 2011). Since Cd, As and Pb are identified as some of the most harmful elemental species, they are toxic and carcinogenic even in trace levels. They can disrupt the normal functioning of vital internal organs such as the lungs and kidneys. Presence of heavy metals can also elevate the formation of reactive oxygen species (ROS), potentially leading to harmful effects at both the cellular and organ levels (Beyersmann et al., 2008). Reports on raw tobacco in Switzerland by using neutron activation analysis such that eight elements Mg, Na, Cl, K, Br, Ca, Al, Mn can be determined by purely instrumental means, this results in a high sample throughput per working day and ensures rapid availability of analytical results. In contrast, the determination of heavy metals such as Hg, Cu, Mo, Cd, Zn, Co, As, and Sb require prior chemical separation, which is typically carried out using extractions with various metal-diethyldithiocarbamate reagents (Wyttenbach et al., 1976).

Tobacco has been found to contain approximately 4,000 chemical compounds, both organic and inorganic, which have been categorized by the International Agency for Research on Cancer (IARC) based on their health hazard index. Among these, certain metals such as arsenic, cadmium, nickel and chromium are classified as group 1 carcinogens (IARC, 2007). Although heavy metals are present as minor inorganic constituents in tobacco, they are highly toxic even at low concentrations. While smoking, these metals are readily assimilated into the body

and tend to accumulate in the tissues exposed, posing significant health risks to users (Lin, 1992; Galazyn-Sidorczuk et al., 2008).

The accumulation of heavy metals in tobacco plants results from complex interactions between the soil and the plant. This process is further governed by condition such as soil type, pH levels, the quality of irrigation water, the species of tobacco plant, and the chemical nature of the metals involved (Golia et al., 2008). During tobacco cultivation, farmers often apply large quantities of fertilizers and pesticides, many of which contain high concentrations of heavy metals. This practice contributes significantly to the contamination of both soil and crops (Karaivazoglou et al., 2007; Lecours et al., 2012).

Tobacco plants are known to be rich in toxic heavy metals, which tend to accumulate preferentially in the leaves during growth (Schneider & Krivan, 1993; Golia et al., 2007). The distribution and adsorption of these metals are largely governed by environmental factors like soil pH, type, presence of organic matter (like humic acids), and the solubility of the metals (Noler, 2006).

Cigarettes are composed of tobacco, paper, and numerous additives. In fact, between 600 and 1,400 additives are used in cigarette manufacturing, many of which may introduce additional exposure to toxic heavy metals such as lead, mercury, and cadmium (Nnorom et al., 2005). The use of tobacco products both in smoked and smokeless forms poses direct health risks to users and indirect risks to non-smokers through passive smoking. Tobacco use is thus a major source of human and environmental exposure to toxic metals (Stephens et al., 2005; Galazyn-Sidorczuk et al., 2008; Schneider & Krivan, 1993).

Research on both filtered and unfiltered commercial cigarettes has been conducted to evaluate the effectiveness of cigarette filters in partially reducing semi-volatile particulate matter that contains certain heavy metals. In this study, 27 major and trace elements were analyzed in two cigarette brands, including the tobacco, tobacco ash and both new and used filters. The analyses were performed using Epithermal Neutron Activation Analysis. Findings indicated that tobacco ash retained between 71% and 86% of the original elemental content. However, for more volatile elements like arsenic (As) and cadmium (Cd), only 28–32% of their

initial amounts were found in the ash, reflecting their tendency to volatilize during smoking (Zinicovscaia et al., 2018).

In Mizoram, the cultivation of tobacco and betel nut is generally carried out without the use of fertilizers or pesticides and the processing methods used after harvest differ significantly from those in other parts of India. Typically, tobacco leaves harvested in Mizoram are sun-dried during the less humid winter months to produce *zozial*, whereas in states like Andhra Pradesh and Karnataka, flue-curing is the more common practice.

Betel nut consumption in Mizoram also follows distinct tradition locals usually chew the fresh, soft nut after removing the husk from the tender kernel. In contrast, in many other regions of India, the betel nut kernel is separated from the husk, sun-dried, chopped into varying sizes, and mixing with artificial sweeteners and flavourings before consumption.

In most other parts of India, the cultivation of tobacco and areca nut typically involves the application of chemical fertilizers, herbicides, and pesticides. These agricultural inputs are known to contain toxic trace metal contaminants (Kannan et al., 1992).

For the current study, various samples of tobacco plants and betel nuts will be collected from Mizoram. This includes tobacco plants as well as areca fruits (betel nuts) with and without husks. Additionally, soil samples will be collected from a depth of approximately 25–30 cm beneath the surface at the plantation site.

CHAPTER 2

MATERIALS AND METHODS

2.1 Samples collected for first set of areca nut

Areca nut samples were collected during June-July 2019, from Northeast Indian region viz., Mizoram from 5 plantation site where areca nut were grown, two samples from Assam and one samples from areca nut plantation in Mizoram-Tripura border villages. From all the plantation site along with areca nut, the soil was collected from a depth of 20-30 cm from each site. For each location, approximately 15 kg of soil were collected. The collected soil samples were sieved and were packed in a clean polyethylene bag a kept for analysis.

From each plantation 30 fresh and ripened areca nuts were collected and dehusked by using artisanal clippers to separate the husk and nut for elemental analysis. The areca nut and husk were kept in a separate polyethylene bag, all the samples were kept and stored in dry and clean area and away from direct sunlight.

2.2 Samples collected for second set of areca nut

The areca nut samples from within Northeast India as well as from Myanmar bordering the Northeast Indian state of Mizoram were collected during the month of November-December 2019. Within Mizoram 8 samples were taken from various site of areca nut plantation. One sample from the state of Assam, Arunachal Pradesh, Tripura, Meghalaya and two samples from Myanmar. Soil samples were collected within a depth of 25-30 cm near areca palm, along with the soil samples, roots sample of the areca palm was also collected. Later, the husk was dehusked from the collected areca nut by using artisanal clippers. Then they were stored in a clean separate polyethylene bag for analysis.

2.3 Samples collected for Tobacco Plants

Samples of tobacco were taken during the harvesting season December 2019 – February 2020, from different plantation within Mizoram. At every plantation 2-3 tobacco plants (leaf, stem, root) were collected along with the soil samples from 25-30 cm depth. The tobacco sample collected were kept in a clean and dry place kept

away from direct sunlight for natural drying. After it was thoroughly dried it was packed in a clean polyethylene bag for further analysis.



Classification information

Kingdom: Plantae
Phylum: Tracheophyta
Order: Arecales
Family: Arecaceae
Genus: *Areca*
Species: *A. catechu*

Figure 2.1: Areca palm (*Areca catechu*) plantation in Mamit district, Mizoram



Classification information

Kingdom: Plantae
Phylum : Magnoliophyta
Class: Magnoliopsida
Order: Solanales
Family: Solanaceae
Genus: *Nicotiana*
Species: *Nicotiana tabacum*

Figure 2.2: Tobacco (*Nicotiana tabacum*) plant in Mizoram.

Table 2.1: Detail for first set of Areca nut

Sl No.	Place	Identity	Coordinates	
			LATITUDE	LONGITUDE
1	LENGPUI	AS1	23°50'09"N	92°38'01"E
2	LENGTE	AS2	23°46'37"N	92°35'59"E
3	ZAMPUI	AS3	23°56'32"N	92°16'37"E
4	ZAMUANG	AS4	24°06'22"N	92°23'47"E
5	VAIRENGTE	AS5	24°29'28"N	92°45'42.7"E
6	KOLASIB	AS6	24°14'15"N	92°41'10"E
7	Sivasagar	AS7	26°59'15"N	94°38'34"E
8	Golaghat	AS8	26°30'51"N	93°57'57"E

Table 2.2: Detail for second set of Areca nut

Sl No.	Place	Identity	Coordinates	
			LATITUDE	LONGITUDE
1	Dapchhuah	A1	23°46'21"N	92°31'28"E
2	Darlak	A2	23°54'12"N	92°24'53"E
3	Kawrthah	A3	24°00'47"N	92°22'37"E
4	Zamuang	A4	24°06'22"N	92°23'55"E
5	Assam	A5	24°19'59.5"N	92°17'51.0"E
6	Tripura	A6	24°14'22"N	92°17'37"E
7	Kanhmun	A7	24°14'18"N	92°17'53"E
8	Bilkhawthlir	A8	24°20'39.4"NE	92°42'33.8"
9	Vairengte	A9	24°25'25.0"N	92°44'01.9"E
10	Kawrtethawveng	A10	23°51'33"N	92°22'22"E
11	Myanmar (Tahan)	A11	23°12'15"N	94°00'47"E
12	Myanmar	A12		
13	Meghalaya (East Gharo hills)	A13	25°37'17"N	90°38'06"E
14	Arunachal Pradesh	A14	27°48'29"N	95°02'59"E

Table 2.3: Tobacco sample detail

Sl No.	Place	Identity	Coordinates	
			LATITUDE	LONGITUDE
1	Puilo	TS1	23°34'33"N	93°03'20"E
2	Chhawrtui	TS2	23°29'23"N	93°02'59"E
3	Tualte	TS3	23°24'18"N	93°12'56"E
4	Khuangleng	TS4	23°18'19"N	93°17'36"E
5	Bungtlang	TS5	23°11'30"N	92°54'01"E
6	Lungdai	TS6	23°52'53"N	92°44'32"E
7	Hmuntha	TS7	23°28'40"N	92°56'33"E
8	Model	TS8	23°42'44"N	92°42'58"E

2.4 Soil parameter analysis

2.4.1 Soil pH measurement

The soil samples gathered from different plantations were air dried and 50 g air-dried soil samples were put in a 100 mL beaker. Then 50 mL of deionized water was added, and it was then left for 12 h, after which it was stirred for 1 hour and let it settle for 30 min after that it was allowed to stir again after 1 hour. After letting the stirred solution stand undisturbed for 30 min, the supernatant was separated. Then, the pH of supernatant was measured using a pH meter (Eutech 510).

2.4.2 Determination of available nitrogen from soil

The available nitrogen in soil samples was estimated using the alkaline potassium permanganate method (Subbiah and Asija 1956).

For analysis, 20 g of air-dried soil were distilled at a constant rate using a distillation unit with 100 mL of potassium permanganate (KMnO_4) 0.32% and 100 mL of 2.5% sodium hydroxide (NaOH). The ammonia generated during the process was trapped in a conical flask 250 mL which contain 20 mL of 4% boric acid solution and a few drops of a mixed indicator prepared by dissolving 0.5 g bromocresol green and 0.1 g methyl red in 100 mL of 95% ethanol. Distillation was continued for 30 min, after which the distillate collected was then titrated against 0.005 M sulfuric acid (H_2SO_4) using methyl orange for an indicator until a pink endpoint was reached. A blank titration, prepared without soil, was also performed for comparison.

2.4.3 Determination of available Phosphorous from soil

The available phosphorus from the soil was determined following the Bray and Kurtz method (Bray and Kurtz, 2009). Five grams of naturally dried soil were placed in conical flask with Bray's extractant, swirled thoroughly for 5 min, it was filtered by using filter paper Whatman No. 42. An aliquot of 5 mL of the filtrate was taken to a 25 mL volumetric flask, mixing with 5 mL of 1.5% Bray's reagent, which is diluted by distilled water to a total volume of 22 mL. Immediately, 1mL of the diluted solution of SnCl_2 was then added, the mixture was thoroughly homogenized, and the final volume was made up to 25 mL. The intensity of the observed blue color was measured at 660 nm by a spectrophotometer. Blank sample, equipped without soil, was also analysed under the same conditions. The

phosphorus concentration from the soil was determined by referring to a standard calibration curve.

2.4.4 Determination of available Potassium from soil

The available potassium from soil was determined using methods of neutral ammonium acetate extraction as described by Jackson (Jackson 1973). In a 500 mL conical flask, 5 g of air-dried soil were mixed with 25 mL of neutral ammonium acetate solution. The mixture was subsequently filtered by filter paper with Whatman No. 42. The initial few millilitres of the filtrate were excluded, and the potassium concentration in the residual solution was measured by a flame photometer. Blank sample, without soil, was also analysed under identical conditions. Standard calibration curve was made by recording flame photometer readings for potassium chloride (KI) solutions of known concentrations of 2 ppm, 5 ppm, 10 ppm, 15 ppm and 20 ppm, made from a 1000 ppm stock solution made by dissolving 1.907 g of potassium chloride (KI) in 1 L of distilled water.

2.4.5 Determination of Soil Organic Matter (SOM)

The Walkley-Black method was used for the determination of organic carbon from the soil (Walkley, 1934). A 2.00 g portion of air-dried soil was accurately weighed and put in a 250 mL Erlenmeyer flask. Using a pipette, 10 mL of 0.617 M potassium dichromate ($K_2Cr_2O_7$) solution was added, followed by the slowly adding 20 mL concentrated sulfuric acid (H_2SO_4), drop by drop, while gently swirling the flask to ensure thorough mixing. The reaction mixture was allowed to stand undisturbed for 30 min, during which the flask was placed on an insulating pad to minimize heat loss.

After the digestion period, it was diluted by 200 mL of distilled water to obtain a clear suspension suitable for endpoint observation. Subsequently, 10 mL of 85% phosphoric acid (H_3PO_4) and 0.2 g of sodium fluoride (NaF) were added, followed by 10 drops of ferroin indicator. The solution was titrated against 0.5 M ferrous sulfate (Fe^{2+}). Initially, the solution appeared yellow-orange, turning turbid gray just before the endpoint and finally shifting sharply to a wine red (burgundy) color at the endpoint.

A reagent blank was prepared and titrated in the same manner, excluding the soil sample. This blank was used to standardize the Fe^{2+} solution.

2.5 Neutron Activation Analysis (NAA)

Neutron Activation Analysis (NAA) is a sensitive with non-destructive nuclear analytical technique used for the quantitative and qualitative determination of elements present in various matrices. NAA is based on measurement of characteristic gamma (γ) rays emitted from radionuclides formed by a stable isotope due to its interaction with thermal/epithermal neutrons. The whole process of NAA can be divided into three stages viz., irradiation, decay and measurement. The sample is irradiated by a beam of neutrons mostly from the core of a research reactor where the neutron fluxes are high. The irradiation time can differ from a minute to a day (to capture neutrons by the atoms of elements in the sample), with respect to nature of samples. After neutron irradiation, the samples are allowed to decay for a period of time which is known as cooling period to reduce background radiation from some short-lived isotopes. After cooling period, the sample is kept in a gamma ray detector, high-purity germanium (HPGe) detector. HPGe detector measures the intensities of emitted gamma rays, then the observed data were analysed by spectral software (Genie 2000) for identifying (characteristic frequencies corresponding the elements) and quantifying the elements (activity concentration (Bq/kg) $\rightarrow$ concentration levels in ppm (mg/kg)) available in the samples. The benefit of the technique is the ease of sample preparation and versatility of samples that can be analysed. It has found a wide range of applications in environmental science, forensic science, material science, geochemistry, biology and archaeology. There are different types of neutron activation analysis.

- i) Instrumental neutron activation analysis (INNA): This technique does NOT require chemical processing of the sample. It is a non-destructive analysis method, where the neutron-irradiated sample is analysed directly using gamma ray spectroscopy.
- ii) Radiochemical Neutron Activation Analysis (RNAA): This technique involves a chemical separation of element of interest after irradiation of the samples that helps in improving its sensitivity and selectivity for trace elements.

- iii) Prompt Gamma Neutron Activation Analysis (PGNAA): When bombarded with neutrons, it immediately detects within nanoseconds the emitted gamma rays after neutron capture by the sample.

$$A = N \cdot \phi \cdot \sigma \cdot (1 - e^{-\lambda t_i}) \cdot e^{-\lambda t_d}$$

Where,

A = Activity of the product nucleus

N = Number of target atoms

ϕ = Neutron flux

σ = Neutron capture cross-section

λ = Decay constant

t_i = Irradiation time

t_d = Decay time

2.5.1 Sample Preparation for NAA

Neutron activation analysis from REGATA of the reactor IBR-2 was employed for determining the elemental contents of the sample collected from different sites. Further description of the Radioanalytical complex known as the Rigata facility at FLNP, Dubna, is found elsewhere (Zinikovscaia, 2022). All samples, standards and reference materials, were analyzed in triplicate.

Two procedures were applied for samples irradiation.

First procedure: Sample of soil was irradiated for one minute and for areca nut, husk and roots it was irradiated for 3 minutes, and direct measurement was taken after irradiation for the determination of short-lived radionuclide: Al, Mg, Ca, Cl, Si, V, Cu, Ti, Mn and Dy

Second procedure: It was irradiated for 3 days and measurement taken after 4 and 20 days of decay for the determination of Fe, Cr, Sc, K, Na, Zn, Ni, Co, Br, As, Sb, Zr, Sr, Rb, Ba, Cs, Ce, La, Sm, Nd, Tb, Eu, Hf, Yb, T, Ta, Th, U. Gamma spectra of the

analysed sample was measured using HPGe detector. The processing of spectral data and the determination of induced radioactivity of different elements were done by Genie 2000 package. The calculation of elemental concentration levels as a function of the extent of induced radioactivity was performed by using an in-house software developed in Frank Laboratory of Neutron Physics (FLNP), Joint Institute for nuclear research (JINR), Dubna, Russia.

Similarly, for tobacco samples of Northeast India (especially Mizoram), the tobacco leaves, stems, roots and the corresponding soil samples were also subjected to neutron irradiation for NAA as mentioned above for areca palm.

The collected samples for neutron activation analysis (NAA) were dried at 40°C for obtaining a constant weight and were homogenized. For short-term irradiation 0.1 g of soil and 0.3 g of areca nut and husk sample were placed in polyethylene foil and for long term irradiation it was put in an aluminium cup.

Instrumental neutron activation analysis at the Radioanalytical complex, Regata facility of the IBR-2 reactor (Dubna, Russia) was applied to determine the elemental composition of soil and plant samples. The elements converted to short-lived radionuclides upon irradiation, viz., Al, Mg, Si, Cl, Ca, Ti, V, and Mn samples were irradiated for 3 min (plant material) or 1 min (soils) at a thermal neutron flux of $1.1 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$. Immediately after the irradiation, the induced γ -radiation spectra energies were acquired for 15 min. Besides, elements producing long-lived isotopes, viz., Na, K, Sc, Cr, Fe, Co, Ni, Zn, As, Br, Rb, Sr, Zr, Sb, Cs, Ba, La, Ce, Sm, Eu, Tb, Yb, Ta, Hf, W, Th, and U were determined after 4-day irradiations by using the cadmium-screened channel 1 at a neutron flux of $1.8 \times 10^{11} \text{ cm}^{-2} \text{ s}^{-1}$. Subsequently, the induced γ -ray energy decay were measured twice after 4 days as well as 20 days using Canberra HPGe detectors with an efficiency of 70–100% and a resolution of 1.8–2.0 keV at a 1332 keV ^{60}Co total absorption peak. At JINR, typically, the irradiation channel, Channel 1 is Cd-coated to attenuate neutrons with energy lower than 0.55 eV.

For determining the chemical composition of elements in areca/tobacco/soil samples, appropriate reference material with established concentrations of elements

were irradiated under the same conditions as samples. Since, not a single reference material may hold the entire set of requisite elements to be determined, several reference materials can be irradiated, simultaneously. Better option is to average concentration values across multiple standard materials for each element. A collection of few reference materials assembled for this purpose is called a group standard.

For plant and soil samples, a set of reference materials consists of NIST 2709 (San Joaquin Soil), NIST 1547 (Peach Leaves), 1633b (bituminous coal fly ash), NIST 1575a (Trace Elements in Pine Needles), and NIST 1632c (Trace Elements in Coal), were irradiated in tandem with experimental samples (areca plant parts/tobacco plant parts/soil) and to ensure the quality and validation of the analysis. The analysis of raw γ -ray spectra was performed using the Genie2000 software (ver. 4.0, Canberra Genie Spectroscopy Software Suite), and as a result of γ -ray spectral data processing, the activity concentration of elements in Bq/kg are obtained. Subsequently, an in-house software developed at JINR, FLNP, Dubna, Russia, was employed to recalculate the activity concentration as the contents of elements in mg/kg, for different samples.

2.6 Atomic Absorption Spectrometry

Atomic Absorption Spectroscopy (AAS) widely used because of its high sensitivity ideal for used in analytical technique for the quantitative determination of elements in different matrix. The technique relied on the principle such that atoms from the ground were state absorbing light at specific/particular wavelength that are unique for different elements. It is widely used in research fields like environmental monitoring, agriculture, food safety, toxicology, archeology, pharmacology.

The principle is based on the absorption of electromagnetic radiation by free atoms present in its gaseous state. The sample which contain a specific metal is atomized meaning that it is converted in to free atoms, then a light beam of specific wavelength that is characteristics of that metal passed through the vaporized atoms and part of the light is absorbed, where the amount of an absorption is directly proportional to concentration of the element in the analyzed sample, following Beer-Lambert's Law

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon \cdot c \cdot l$$

Where, A - the absorbance

I_0 - incident light intensity

I - transmitted light intensity

ε - molar absorptivity

c - concentration of the analyte

l - path length of the sample

2.6.1 Sample preparation for AAS

0.2 g of areca nut and husk sample were digested in Teflon vessel by using 1 mL of hydrogen peroxide (H_2O_2) along with concentrated nitric acid (HNO_3) 3 mL and for the soil sample 2 mL of HF and 8 mL of HNO_3 was used. For complete digestion the teflon vessel was kept into microwave digestion system (Mars, CEM, USA).

Digestion was performed in two steps:

First step (ramp): Temperature was raised 260°C , within 20 minute and 290-1800 W power supply with pressure of 20 bar

Second step (hold): A temperature of 260°C was hold for 15 minute with power supply of 1800 W and 20 bar pressure.

Then the digested samples were kept into calibrated flasks 50 mL and by using double-distilled water it was made up to mark. All reagents used for this experiment were of analytical grade from Merck (Darmstadt, Germany). The experimental measurement of the concentrations of Cu, Cd and Pb solutions were measured by using Atomic Absorption Spectrometer (AAS) with electrothermal atomization, (Thermo Scientific iCE 3400 series, USA). For calibration standard solutions were prepared from a 1 g/L stock concentration (AAS standard solution; Merck, Germany). All the measurement were done in JINR, FLNP, Dubna, Russia and all samples, standards and reference materials, were analyzed in triplicate.

2.7 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analytical technique that utilized liquid chromatography (LC) for its separation together with the power of a tandem mass spectrometry (MS/MS) for the high sensitivity and specificity, which is extensively used in environmental research analysis, pharmaceutical research, clinical diagnostic research, food safety, forensic and in toxicology research because of its powerful hybrid analytical technique and its ability to detect and quantify compounds in trace level in different matrices.

LCMS/MS works by:

1. Liquid chromatography separation: Samples were dissolved with a suitable solvent, then injected into the column where they get separated on the column by based on their size, polarity and their interaction with the stationary phase.
2. Ionization: Separated by the LC, the analytes are ionized by electrospray ionization (ESI) which is ideal for polar and thermally labile compounds and the second methods atmospheric pressure chemical ionization (APCI) ideal for less polar compounds and semi volatile compounds, leading to the formation of ionized charged particles.
3. Detection MS/MS: The ionized charged particles pass through a series of mass analyser entering the first mass analyser that selects the precursor ions or parent ions of interest, which then collide in the collision cell along with inert gas through collision induced dissociation (CID) and gets fragmented into daughter ions, upon entering in the second mass analyser the fragment ions detected by an ion detector (electron multiplier or a photomultiplier tube). The detected signal is then converted into spectrum and used for quantitative and qualitative analysis.

2.7.1 Sample preparations for analysis of TSNAs

Prior to sample preparation of collected samples (solid tobacco) were kept at ambient temperature for 12 hours for their settlement. Solid tobacco sample of 0.1 g was taken along with 1.5 mL of 100 mM ammonium acetate buffer with pH 6.8 and stirred for one hour at 200 rpm for thorough mixing. The solution obtained was then separated by centrifugation for 5 minutes at 8000 rpm and filtered by Nylon syringe filter of 0.22 μm . From the extract 50 μL was taken and it was mixed with 50 μL internal standard solution of

8 ppb deuterium-labeled 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (d_5 -5'4'-NNAL), 2 ppb Carbon labelled Nitrosonornicotine ($^{13}C_6$ -NNN) and 8 ppb deuterium-labeled nicotine-derived nitrosamine d_3 -NNK. LC-ESI-triple quad MS/MS was used for quantitative analysis. All the measurement were performed at Chung Shan Medical University (CSMU), Taichung, Taiwan and all samples, standards and reference materials, were analyzed in triplicate.

Automated sample purification using on-line SPE and chromatography separation were performed on an Agilent 1100 Series HPLC system. The online SPE consisted of two binary pumps (pump I & II), an autosampler, a two-position ten-port switching valve (VICI Valco Instruments), and a trap column. The trap column used for nitrite and nitrate was Inertsil ODS-3 (33×2.1 mm i.d., 5 μ m, GL Science) and for TSAs and TSNAs was Inertsil ODS-3 (75×2.1 mm i.d., 5 μ m, GL Science). The online SPE process was controlled by the SCIEX control software (Analyst, Applied Biosystems). Following online sample purification, the sample was directly transferred to the main column for analyte separation. For TSAs and TSNAs, an Inertsil ODS-3 column (250×2.1 mm i.d., 5 μ m) was employed.

The targeted monitoring of each analyte was performed using an API 4000 QTRAP tandem mass spectrometer (AB SCIEX) operating in multiple reaction monitoring (MRM) mode with positive ion electrospray ionization (ESI) method. The turbo ion spray voltage was set to 5500 V; the heater gas temperature was set to 450 °C; the gas supply for Gas 1, Gas 2 and curtain gas were set to 50, 50 and 10 psi, respectively, according to the recommended value relative to LC flow rate. The turbo gas temperature and IonSpray voltage were set at 500 °C and 5500 V, respectively. Both Q1 and Q3 were set at a unit mass resolution of 0.7 Da full width at half maximum. The limit of quantitation for each analyte were as following, nicotine: 21.0 μ g/g; cotinine: 4.0 μ g/g; NNK: 2.31 ng/g; NNN: 4.26 ng/g; NNAL: 3.26 ng/g. Detailed column-switching operation and LC gradients used during online purification and analyte separation for each analyte can be found in.

2.8 Bioaccumulation Index

Bioaccumulation (BA) Index was calculated for different elements as the ratio of concentration of an element present in different parts of the plant (C_p) with the determined concentration of element present in soil (C_s) (Kabatia-Pendias, 2011).

$$BA = \frac{C_p}{C_s} \quad (1)$$

Bioconcentration factor (BCF) is determined as the ratio of elemental concentration in plant tissue to that of soil and BCF reflects the plant's ability to accumulate the elemental species in its tissue (nut, husk, and root) from the soil. Similarly, translocation factor (TF) indicates the efficacy of a plant to translocate the elemental contents from the root to its aerial parts (nut, husk) and TF is calculate/derived as the ratio of elemental concentrations in plant tissue to that of root tissue (da Silva et al., 2016).

Table 2.7.1 (a). Detailed program of online-SPE coupled with LC separation for determination of nicotine and cotinine.

Time (min)	Pump I (online SPE)			Pump II (LC separation)			Valve position ^e	Remarks
	S1 ^a (%)	S2 ^b (%)	Flow rate (μ L/min)	A1 ^c (%)	A2 ^d (%)	Flow rate (μ L/min)		
0.0	100	0	200	95	5	200	A	Sample loading and cleanup; equilibration of LC-MS/MS system
1.0			200			200	B	Analyte elution from SPE to LC main column
3.0	100	0	200	95	5	200	A	SPE column cleaning; analyte separation
3.1	0	100	200			200		
4.6			200	65	35	200		
6.6			200	65	35	200		
6.7			200	0	100	200		Cleaning of LC main column
7.0	0	100	200			200		Cleaning of SPE column
7.1	100	0	200			200		Re-equilibration of SPE column
8.7			200	0	100	200		
8.8			200	95	5	200		Re-equilibration of LC column
15.0	100	0	200	95	5	200		

^a 2% (v/v) methanol containing 0.1% trifluoroacetic acid.

^b 75% (v/v) methanol containing 0.1% trifluoroacetic acid.

^c 2% (v/v) methanol containing 0.1% formic acid.

^d 50% (v/v) methanol containing 0.1% formic acid.

^e The valve position was located at A to lead the fluid to waste, while the position at B to lead the fluid into LC-MS/MS.

Table 2.7.1 (b). Detailed program of online-SPE coupled with LC separation for

Time (min)	Pump I (online SPE)			Pump II (LC separation)			Valve position ^e	Remarks
	S1 ^a (%)	S2 ^b (%)	Flow rate (μ L/min)	A1 ^c (%)	A2 ^d (%)	Flow rate (μ L/min)		
0.0	90	10	200	80	20	200	A	Sample loading and cleanup; equilibration of LC-MS/MS system
7.0			200	80	20	200		
8.0			200			200	B	Analyte elution from SPE to LC main column; analyte separation
9.0			200	70	30	200		
10.5	90	10	200			200		
10.6	0	100	200			200		Cleaning of SPE column
11.0			200	70	30	200		
12.5			200			200	A	
13.0			200	0	100	200		Cleaning of LC main column
14.0			200	0	100	200		
14.1			200	80	20	200		Re-equilibration of LC main column
14.5	0	100	200			200		Re-equilibration of SPE column
15.0	90	10	200			200		
19.0	90	10	200	80	20	200		

determination of NNK, NNN and NNAL.

^a 5% (v/v) methanol containing 1 mM ammonium acetate aqueous solution.^b 75% (v/v) methanol containing 1 mM ammonium acetate aqueous solution.^c 5% (v/v) methanol containing 0.1% formic acid.^d 75% (v/v) methanol containing 0.1% formic acid.^e The valve position was located at A to lead the fluid to waste, while the position at B to lead the fluid into LC-MS/MS.

Table 2.7.1(c). MRM parameters used for simultaneous quantification of the nicotine, and cotinine.

Analytes	Q1 ion (<i>m/z</i>)	Q3 ion (<i>m/z</i>)	Dwell time (ms)	DP (V)	EP (V)	CXP (V)	CE (eV)
Nicotine	163	132	100	40	10	6	22
	163	130	100	40	10	6	29
d ₄ -Nicotine	167	136	100	40	10	6	22
Cotinine	177	80	100	50	10	12	35
	177	98	100	50	10	4	29
d ₃ -Cotinine	180	80	100	50	10	12	35

The quantifier ion of each alkaloid was labeled in bold; DP: declustering potential; EP: entrance potential; CXP: collision exit potential; CE: collision energy.

Table 2.7.1(d). MRM parameters used for simultaneous quantification of the NNK, NNN and NNAL.

Analytes	Q1 ion (<i>m/z</i>)	Q3 ion (<i>m/z</i>)	Dwell time (ms)	DP (V)	EP (V)	CXP (V)	CE (eV)
NNK	208	122	100	25	5	8	15
	208	79	100	25	5	4	52
d ₃ -NNK	211	122	100	25	5	8	15
NNN	178	148	100	15	6	9	15
	178	120	100	40	10	5	25
¹³ C ₆ -NNN	184	154	100	15	6	9	15
NNAL	210	180	100	30	5	15	15
	210	93	100	30	5	15	30
d ₃ -NNAL	213	183	100	30	5	15	15

The quantifier ion of each alkaloid was labeled in bold; DP: declustering potential; EP: entrance potential; CXP: collision exit potential; CE: collision energy.

2.9 Pollution Index

Using the equation, single index (CF, I_{geo}) and calculation of integrated pollution index (PLI), respectively.

Contamination factor (CF) is defined as:

$$C_F = \frac{C_M}{C_B} \quad (2)$$

where C_M is the determined concentration of metal at any given site, C_B is the background level for that element. Established Continental Crust data were used as background values (Rudnick and Gao, 2003).

Index of geoaccumulation (I_{geo}) was derived by using the equation 3

$$I_{geo} = \frac{C_M}{1.5C_B} \quad (3)$$

where $\frac{C_M}{C_B}$ is CF.

In order to minimize the outcome of possible variations in the background, a correction factor of 1.5 is applied.

PLI represents the n order geometric mean of an entire set of contamination factor with regards to contaminating elements as follows:

$$PLI = \sqrt[n]{\prod_{i=1}^n C_{F,i}} \quad (4)$$

where n is the total number of contaminating elements. The pollution indices namely CF, I_{geo} and PLI were graded into different classes on the degree of contamination (Salman et al., 2019).

2.10 Health Risk Assessment

2.10.1 Estimated daily intake of elements

Estimated daily intake (EDI), of the elements were calculated using the mean concentration of elements from the areca nut, considering an average of 2 areca nut for normal consumer and 5 areca nut for extensive consumer and an average weight of 65 Kg.

$$EDI = \frac{FIR \times C}{BW} \times 10^{-3} \quad (5)$$

Where food ingestion rate (FIR) given in (g/person/day), C for elements concentration in the areca nut sample and BW body weight.

2.10.2 Non carcinogenic risk

All the target hazard quotient (THQ) was calculated with respect to the standard assumption for an integrated United States Environmental Protection Agency (US EPA) risk analysis as follows (US EPA 1989).

$$THQ = \frac{(Efr \times ED \times FIR \times C)}{(RfD \times BW \times AT)} \times 10^{-3} \quad (6)$$

THQ is the target hazard quotient , EFr for exposure frequency given as 365 days/year, ED exposure duration given as 70 years for adults, consider as equivalent to the average life span of human, FIR is the food ingestion rate in g/person/day , C element concentration in samples (mg/kg), BW the mean body weight (65 kg for an Mizo adult), AT the average time for a non-carcinogens given as 365 days per year × number of exposure years and oral reference dose RfD (mg/kg/day).

2.10.3 Hazard Index

Degree of potential risk with the intake of multiple heavy metal in areca nut given by hazard index (HI) (US EPA 1989, 2011).

$$HI = \sum HQ \quad (7)$$

Where $\sum HQ = HQ_{Cu} + HQ_{Al} + HQ_{As} + HQ_{Cr} + HQ_{Mn} + HQ_{Ni} + HQ_{Co} + HQ_{Sb} + HQ_U$

Where $\sum HQ$ is the summation/addition of hazard quotient of all metals present Cu, Al, As, Cr, Mn, Ni, Co, Sb and U. If the addition of all metals is >1, there existed high potential adverse effect.

2.10.4 Carcinogenic risk

The target cancer risk estimates lifetime cancer risk(US EPA 1989).

$$TCR = \frac{Efr \times ED \times FIR \times C \times CSF_o}{BW \times AT} \times 10^{-3} \quad (8)$$

Carcinogenic risk is the potential of a person to develop cancer due to lifetime exposure of potential carcinogens (US EPA 1989, 2011). Acceptable carcinogen value ranges from 10^{-4} to 10^{-6} . The oral carcinogenic slope factor (CSF_o) from Integrated Risk Information System (US EPA 2021).

2.11 Principal component analysis (PCA)

It is a multivariate, unsupervised statistical technique that helps in simplifying a complex data by transforming the original variables into a new set of variables which is called principal components which are uncorrelated, such that first few captures most of the variation from the original data. The main aim of PCA is reducing the dimensions of data while preserving majority of the variance of the data original data, that helps in making the data easier to analysed, visualised and interpret the data. PCA helps in uncovering the hidden structure from a data. The principle of PCA lies in the concept of variance, which provides a measure of the deviation of data points from the mean giving the important patterns within the data.

In PCA the covariance matrix is computed from the dataset that describes the relation between the variables and then eigenvalues and eigen vector are calculated from the matrix. Such that the eigenvector defines the direction of the new latent variable called principal components and the eigenvalues determine their magnitude, that is the extent to which each principal component captures variability from the original data. The first principal component (PC1) takes the highest variance from data sets, and the second principal component (PC2) captures the maximum variance from the remaining and is orthogonal against PC1. The original data is projected by a new different set of axes defined by the principal component, where majority of the variation from original data are captured by the first few principal components allowing the reduction of the dimensions while preserving the important information.

Chapter 3

Results and Discussion

3. Results

3.1 First set of areca nut

3.1.1 Analysis of Soil Parameter

3.1.1.1 Soil pH measurement

The pH of soil is the fundamental indicator as well as regulator of the soil health. The potential bio-availability of various mineral species, the concomitant fertility of soil depends on the soil pH which controls the essential metabolic processes and the consequent plant growth. The pH of soil influences the solubility and different forms of chemical species required for the growth and development of every plant since the chemical forms of different nutrients has a specific pH range where they are soluble and readily available for the plants from the soil. The nature of soil properties reflects the speciation of different elements found in soil, thus soil pH directly influences the accumulation propensity of plants (Lehoczky and Kiss, 2002). The soil pH < 5.5 is considered as acidic soil, pH with 5.5-6.5 slightly acidic, the range between 6.5-7.5 is considered normal neutral soil, while in the pH range 7.5-8.5, it's alkaline soil and pH > 8.5 are highly alkaline soil not suitable for the soil productivity. As reported, acidic soils causes the accentuated availability and solubility of Al, Cl, Mn, and Fe along with alkali and alkaline earth minerals for the plants. On the other-hand, it decreases the availability of P, Ca, Mg, Mo, K, S and slowing down the mineralization of N (Lindsay, 1979). The optimal availability of N, P, K, Ca, Mg, with most micronutrients excluding Mo occurs in the neutral pH range of 6.5-7.5 (Brandy and Weil, 2008). The bio-availability of Mo normally tends to increase with pH>7, albeit there is a diminished bio-availability of P, Fe, Zn, Cu, Mn, B, thus at this pH range (pH 7.5-8.5), the micronutrient deficiencies are common especially for Mn, Cu, Zn, and Fe. (Marschener, 2012). The toxicity of different elements may be observed with the modulation(s) in the pH of soil. Importantly, Kochian et al. (2005) have reported that the toxicity of Al^{3+} and Mn^{2+} with the low pH conditions of soil, since at those acidic soil conditions, most likely there is an increase in the solubility of available Al^{3+} species from the soil.

The pH of soil samples were measured for 5 soil samples collected from Mizoram, 1 soil samples from Tripura-Mizoram border and 2 soil samples collected in from the villages Assam (near Arunachal Pradesh) during the month of June and July 2019. For the first set of areca nut plantation for the study is depicted in (Table 3.1.1.1). The measured soil pH was in the range between 4.7 -5.92, with mean pH of 5.33, which corresponds to a moderately acidic soil.

3.1.1.2 Determination of Soil NPK from first set of areca nut

Presence of primary macronutrients Nitrogen(N), Phosphorous(P) and Potassium(P) also collectively known as NPK, which are essential for the proper growth of plant, reflecting the fertility of the soil and accounting for the world agricultural productivity. They are the backbone of plant nutrition, influencing the structural integrity, biochemical process, stress tolerance, the crop yield and the quality of the product. Nitrogen is indispensable for the building of chlorophyll the green pigment which is crucial for photosynthesis (Taiz and Zeiger, 2010), they are the core component of the formation of amino acids, nucleic acids, proteins and enzymes (Marschener, 2012). Nitrogen is often taken globally as the most yield limiting nutrient (Vitousek et al., 2009). Deficiency of nitrogen can lead to chlorosis results in lack of chlorophyll leading to yellowing of the leaves. Phosphorous is a fundamental structural component of the primary energy currency of a cell known as adenosine triphosphate (ATP) and adenosine diphosphate (ADP) essential for the reproductive viability of the seeds (Halvin et al., 2016). Phosphorous has low mobility in the soil, and they are readily bound by Al and Fe in acidic soil and by Ca in an alkaline soil result in less availability in plants for their uptake (Brandy and Weil, 2008). Deficiency of phosphorous leads to purpling of leaves, delaying maturity and reduction of flowering. Potassium is required for enzyme activation, they act as a co factor for more than 60 enzymes that are involved in protein synthesis, photosynthesis and in the metabolism of carbohydrates (Amtman et al., 2008) They also help in the transport of water, sugar and important nutrients within the plants and helps in improves the size, flavour colour of the fruits (Cakmak, 2005).

Available NPK of the soil samples were estimated to understand the condition of the fertility of the soil samples. Table 3.1.1.2 gives the available NPK present in the study areas and location provided in Table 2.1.

Depending upon the availability of N present in the soil it is grouped into different categories, for available N if it contains above 450 kg/ha, between 280-450 kg/ha and below 280 kg/ha we categorized into high, medium and low, from the given (Table 3.1.1.2) we can observed that Vairengte, Kolasib, Lengte, Zamuang and Golaghat have a high content of available N, with Vairengte having the highest available N (698.4 kg/ha), and from the study sample we observed that Sivasagar from Assam district have the lowest available N (251.2 kg/ha). For available Potassium when the range is above 90, between 45-90 and below 45 kg/ha, we categorized into high, medium and low, from the table we can observed that Kolasib and Zampui is having a high content of available P, with Kolasib having the highest content of available P with 101.7 kg/ha, while below 45 kg/ha, from the result obtained from the table we can clearly say that Lengpui, lengte, Zamuang and Sivasaghar are having a low content of available P, with Lengpui with the least value of 10.9 kg/ha. And for available Potassium(K) the categories are grouped into High, medium and low when the available K contents are in the range of above 340, between 150-340 and below 150 kg/ha, we can observed that Vairengte, Kolasib, Lengte, Zamuang, Zampui, Golaghat and Sivasaghar are having high content of available K, with Zamuang having the highest with 624.2 kg/ha.

3.1.1.3 Determination of soil organic matter (SOM) from first set of areca nut

Soil organic matter (SOM) covers all the organic materials existing within the soil regardless of their source or the stage of decomposition (Baldock and Skjemstad, 1999). Due to its inherently heterogenous nature SOM lacks a distinct chemical and physical structure but serves as the fundamental basis for healthy and productive soil. Direct measurement of SOM component is challenging, thereby we quantify soil organic carbon (SOC) instead of SOM; to estimate the total SOM content a conversion factor is then applied to the SOC measurement.

Table 3.1.1.1: Measured soil pH from first set of areca nut

Sample ID	Soil pH
Lengpui	5.4
Lengte	5.54
Zampui	5.66
Zamuang	5.34
Vairengte	5.09
Kolasib	5.92
Sivasaghar	5.24
Golaghat	4.7

Table 3.1.1.2: Estimation of available NPK from first set of areca nut

Sample ID	N(kg/Ha)	P(kg/Ha)	K(kg/Ha)	pH
Vairengte	698.4	89.4	418.4	5.09
Kolasib	583	101.7	491.7	5.92
Lengpui	443.6	10.9	159.1	5.40
Lengte	486.8	12.9	335.3	5.54
Zamuang	520.5	26.4	624.2	5.34
Zampui	342.6	97.8	340.9	5.66
Golaghat	486.8	58.7	352.2	4.7
Sivasaghar	251.2	42.3	381.8	5.24

Table 3.1.1.3: Observed soil organic matter from first set of areca nut

SAMPLE ID	% of Soil Organic Matter
S1	3.04
S2	4.00
S3	4.30
S4	4.40
S5	5.16
S6	4.23
S7	3.9
S8	5.0

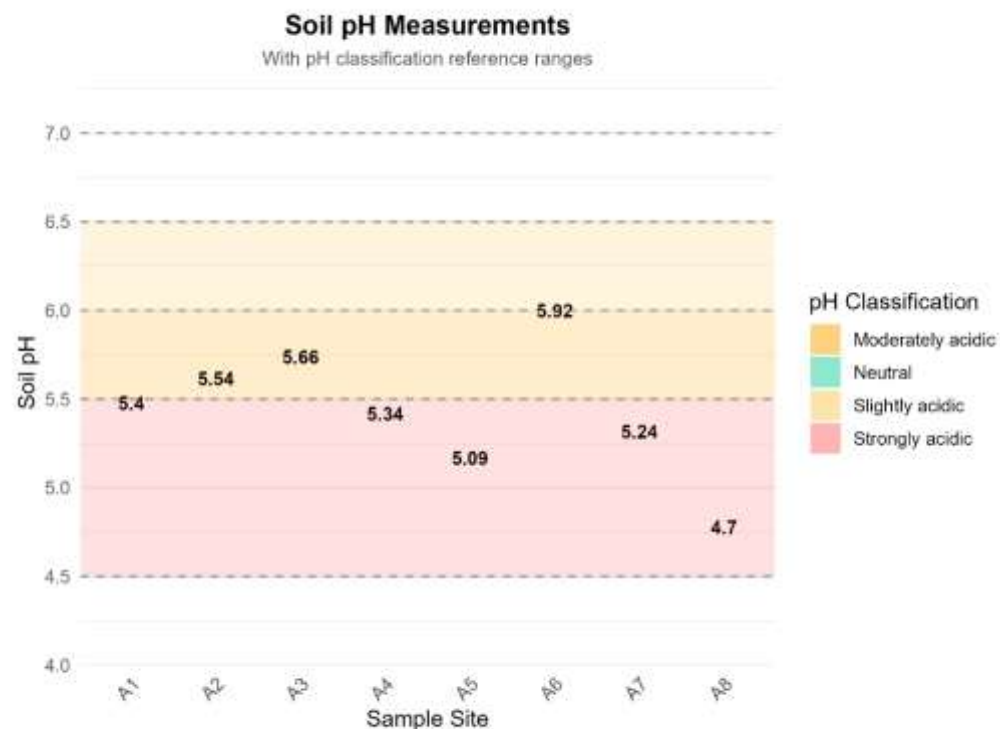


Figure 3.1.1.1: Soil pH from first set of areca nut

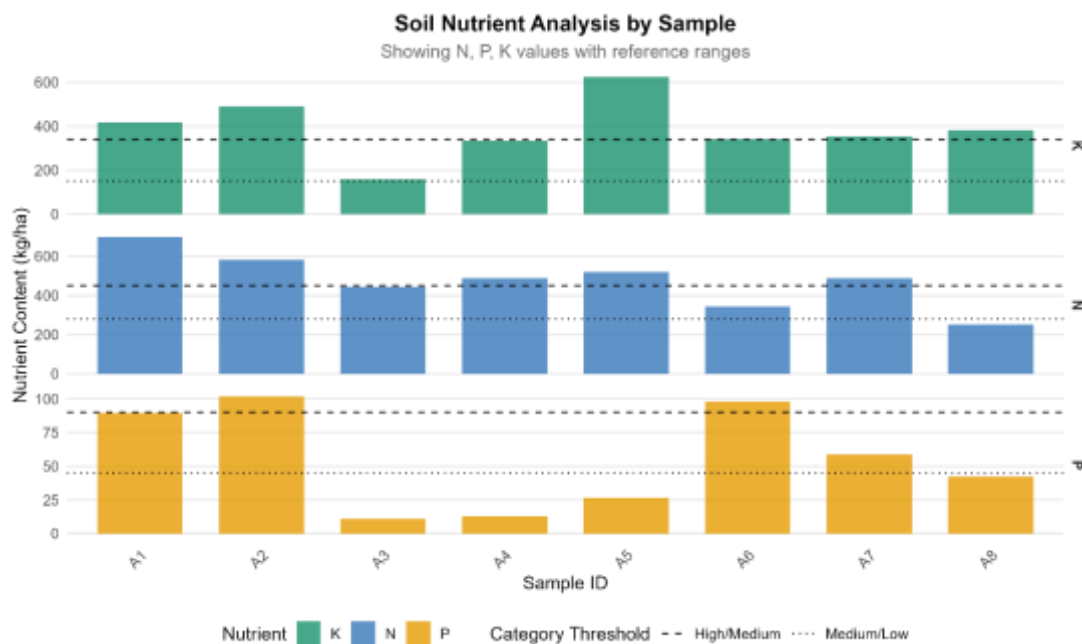


Figure 3.1.1.2: Plot of available NPK first set of areca nut

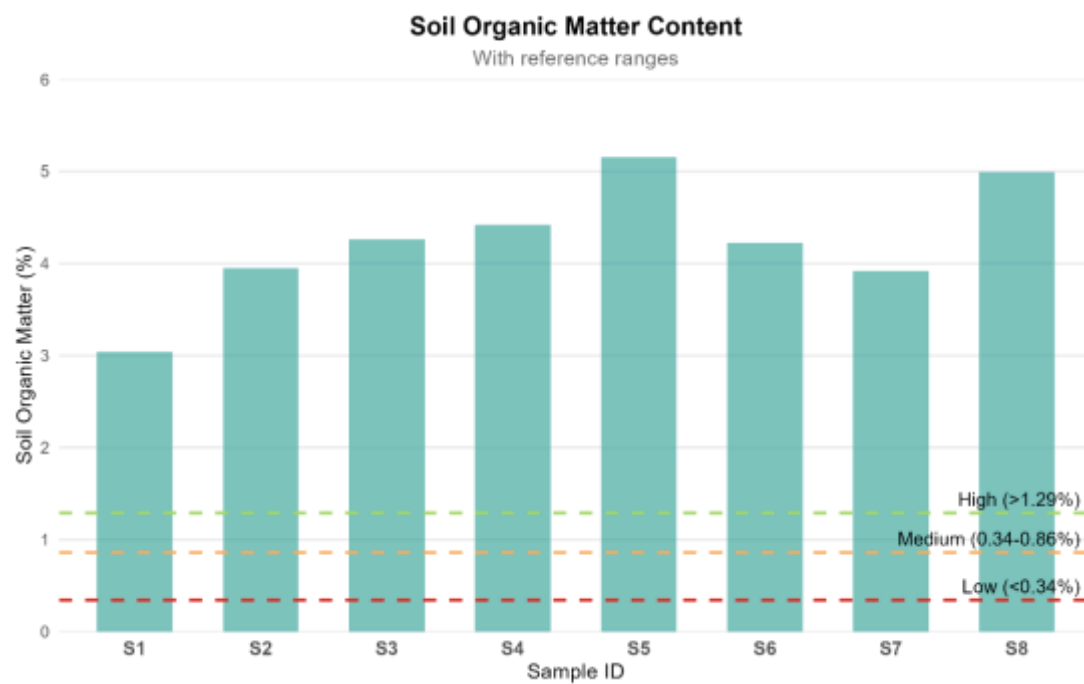
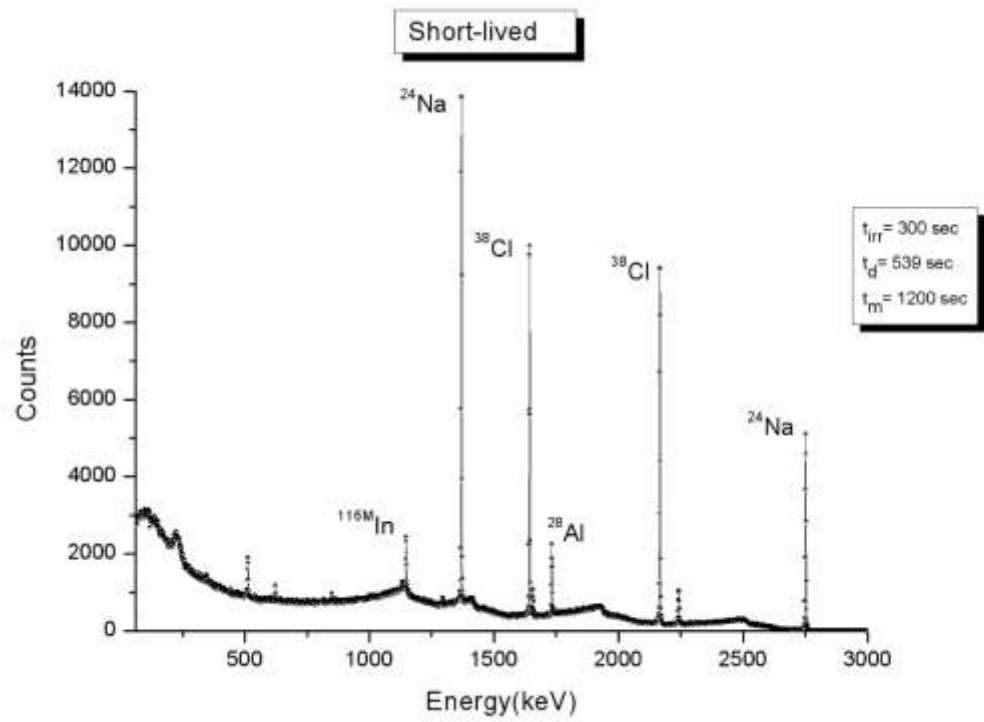


Figure 3.1.1.3: Estimated soil organic matter from first set of areca nut

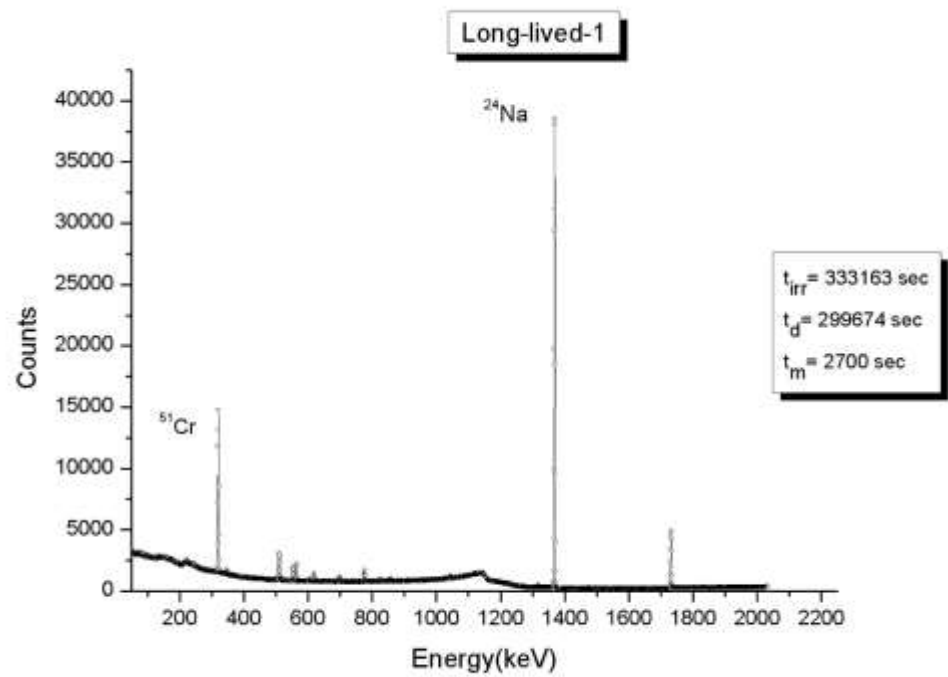
3.1.2 Elemental analysis by Instrumental Neutron Activation Analysis (INAA).

To the best of our knowledge, it is for the first time, the chemical composition of areca nut cultivated in Northeast India was determined in the present study. Using INAA methods, the elemental distribution of macro, micro trace and non-essential elements in areca palm were determined. The presence of 14 elements As, Ca, Cd, Cl, Co, Cu, K, Mg, Na, Rb, Sb and Zn were determined in the areca nut and areca husk at ppm(ug/g) level. Besides, 31 elements including rare earth elements were detected by using INAA, whereas none of the rare earth elements (REEs) were detected in the edible part of areca nut. The elemental concentration measured from the areca nut samples collected can be arranged in the order of $K > Cl > Ca > Mg > Na > Mn > Zn > Al > Rb > Co > Sb > As$. Besides, we also observed that areca nut (endosperm) is containing lesser quantities of Al, As, Ca, Cl, K, Mg, Na, and Rb than in areca palm husk (pericarp), although Zinc (Zn), Tin (Sb) and Manganese (Mn) contents are comparable both in areca palm husk and areca nut. The concentrations of Sb and Mn in the husk are lower than those in the areca nut with a factor 0.62 and 0.64, respectively. The contents of K, Na, Rb, Ca, Mg, Zn, Cl and As in the areca husk were higher than in the areca nut with the increasing order, ranging from 1.1 to 4.76 respectively. Additionally, the elemental concentration of Al was significantly elevated in the husk by a factor of 43.6 times higher compared to the areca nut.

It is interesting to note that even though, the essential micronutrient element, iron (Fe) present was found in large amounts in the tested soil sample (acidic soils), most likely in an inorganic form despite the fact that it was not detected in the areca nut and areca husk except in one husk sample, on the other hand other micronutrient elements in the areca nut and husk such as Mn and Zn, in addition to the trace element, Cobalt (Co) in areca nut were detected. Moreover, inessential element, Si, is only detected in the husk even though it is not detected in the edible areca nut. Despite of the fact that, the contents of Ba, Ni, Cr and Hg in the areca nut, areca palm husk or the soil samples, in our study, were not detected in all tested samples as their contents were below the detection limits of the applied techniques.



(a)



(b)

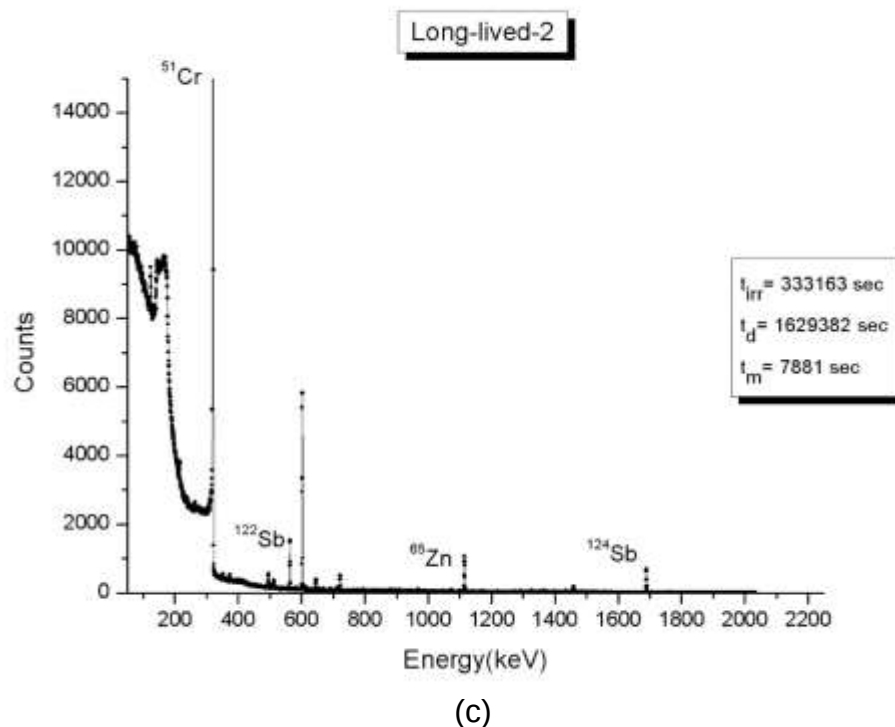


Figure 3.1.2 Gamma ray spectra of elements in areca palm (a) short lived nuclides, (b) long lived-1 nuclides, (c) long lived-2 nuclides

3.1.3 Heavy metal analysis by Atomic Absorption Spectrometry (AAS)

The concentration levels of some heavy elements, viz., Cd, Cu, and Pb from areca nut, areca husk and collected soil sample were quantitatively determined by using atomic absorption spectrometry (AAS) methods. The mean concentration levels of Cd in areca nut, areca husk and from collected soil samples were determined to be 0.06 $\mu\text{g/kg}$, 0.05 $\mu\text{g/kg}$, and 0.07 $\mu\text{g/kg}$, respectively, whereas the mean concentration levels of Cu detected were 14.0 $\mu\text{g/kg}$ in areca nut, 14.5 $\mu\text{g/kg}$ in areca husk and 22.7 $\mu\text{g/kg}$ in soil samples, respectively (Table 3.1.3 (a), (b), (c)). The determined concentration levels of lead (Pb) in areca nut, areca husk and soil samples were 0.67 $\mu\text{g/kg}$, 0.87 $\mu\text{g/kg}$, and 20.06 $\mu\text{g/kg}$, respectively. Both in the areca husk and areca nut, Cu, Cd, and Pb contents are comparable. The concentrations of Cu, Cd and Pb are slightly elevated in nut by the factors, 1.2, 1.03 and 1.3, respectively.

Table3.1.2 (a): The concentration of elements determined in areca nut samples (set01) using INAA (in µg/g)

Elements	Aerca nut(n=8)	
	Range	Mean±SD
Na	44-164	89 ± 44
Mg	325-929	667 ± 197
Al	4.9-19.7	12.80 ± 4.55
Cl	1160-2920	1989 ± 657
K	5090-9270	6979 ± 1667
Ca	513-818	676 ± 104
Mn	18-75	39 ± 22.6
Co	0.46-1.83	0.98 ± 0.42
Zn	8.2-15.5	13.4 ± 3.0
As	0.044-0.07	0.065 ± 0.02
Rb	5.1-18.7	11.3 ± 4.8
Sb	0.2-0.46	0.36 ± 0.1
W	0.34	0.34

Table 3.1.2 (b): The concentration of elements determined in areca husk samples (set-01) using INAA (in µg/g)

Elements	Aerca husk (n=8)	
	Range	Mean±SD
Na	113-1040	424 ± 351
Mg	728-2170	1202 ± 515
Al	88-2310	558 ± 752
Si	6290-9470	7880 ± 2249
Cl	3230-11300	5223 ± 2663
K	10200-12500	15888 ± 4459
Ca	872-1770	1221 ± 330
Ti	229 [♦]	229 [♦]
V	1.18-3.34	2.3 ± 1.5
Mn	11.8-28.4	25 ± 13.4
Fe	1160 [♦]	1160 [♦]
Co	0.43-0.61	0.52 ± 0.13
Zn	6-23.0	15 ± 6.7
As	0.054-0.35	0.18 ± 0.1
Rb	16-38	23.14 ± 7.9
Sr	9.9-23.5	14.9 ± 5.1
Sb	0.06-0.23	0.2 ± 0.1
Cs	0.32 [♦]	0.32 [♦]
La	0.72 [♦]	0.72 [♦]
Sm	0.086-0.21	0.13 ± 0.07
Th	0.3 [♦]	0.3 [♦]
U	0.033 [♦]	0.033 [♦]

[♦]Detected only in one sample

Table 3.1.2 (c): The concentration of elements determined in soil samples collected from areca palm farms (set-01) using INAA (in µg/g)

Elements	Soil (n=8)	
	Range	Mean±SD
Na	1540-5510	3086 ± 1510
Mg	1105-6474	3821 ± 2054
Al	50300-68800	60738 ± 6662
Si	96700-209000	154450 ± 44037
Cl	22-303	96.8 ± 86.6
K	10100-21400	15125 ± 4532
Ca	438-2080	1292 ± 577
Sc	6-14.6	11 ± 2.6
Ti	4070-6170	4743 ± 626
V	75-137	105 ± 19
Mn	165-1140	605 ± 323
Fe	15900-42100	30875 ± 7506
Co	8.2-16.7	14 ± 3
Zn	45-150	81 ± 34
Ga	8-19.4	15.22 ± 4.02
As	1.9-15.2	8.5 ± 5.7
Rb	64-141	101 ± 28
Sr	22.5-35	56 ± 24
Zr	52-186	151 ± 59
Sb	0.37-0.93	0.66 ± 0.19
Cs	2.4-8.3	5.6 ± 1.8
La	23.3-37.6	32.4 ± 5.3
Ce	57.9-91	68 ± 11
Sm	4.9-9	6.6 ± 1.4
Tb	0.33-0.89	0.63 ± 0.2
Dy	4.0-8.1	5.7 ± 2.5
Hf [†]	6.2-8.7	7.2 ± 0.9
Ta	1.0-1.7	1.3 ± 0.6
W	1.2-2.9	2.0 ± 0.6
Th	8.6-17.2	13.6 ± 3.1
U	2.13-2.33	2.8 ± 0.4

Table 3.1.3 (a): The concentration of trace elements in areca nut (set-01) using AAS (in µg/kg)

Elements	Areca nut (n=8)	
	Range (µg/g)	Mean ±SD
Cd	0.028-0.11	0.056 ±0.028
Cu	8.85-19.6	14.01 ± 3.21
Pb	0.14-2.33	0.76 ±0.69

Table 3.1.3 (b): The concentration of trace elements in areca husk (set-01) using AAS (in µg/kg)

Elements	Areca husk (n=8)	
	Range (µg/g)	Mean ±SD
Cd	0.03-0.082	0.051±0.022
Cu	7.6-22.6	14.53±4.44
Pb	0.11-3.4	0.87±1.01

Table 3.1.3 (c): The concentration of selected trace elements determined in soil samples collected from areca palm plantation sites (set-01) using AAS (in µg/kg)

Elements	Areca husk (n=8)	
	Range (µg/g)	Mean ±SD
Cd	0.02-0.14	0.07±0.04
Cu	13.41-27.06	22.7±5.0
Pb	16.6-23.9	20.1±3.0

The physicochemical features of the soil can influence the translocation and distribution of elements in plants (Jin et al., 2005; Zeng et al., 2011). Accordingly, a correlation analysis was performed to assess the relationship between soil properties and contents of all elements in various parts of the areca nut and areca husk from the collected samples. The concentration levels of Potassium (K) in areca nut have a positive correlation trend with the soil pH, whereas Al, Cl and Mg contents are having a negative correlation trend with soil pH albeit statistical significance was not observed (Table 3.1.4). Other elements like Rb, Ca, Na, As, Mn, and Sb have displayed no correlation with soil pH. Besides, in areca husk, concentration levels of K showed significant and positive correlation with soil pH ($p < 0.05$), while Sb levels displayed significant and negative correlation ($p < 0.01$).

Subsequently, the correlations between SOM and the amount of elements in areca palm were also explored. Sb levels ($p < 0.05$) showed significantly positive correlation with SOM. However, statistically nonsignificant strong and positive correlation between Na and Al levels in areca nut and SOM were observed. Non-significant strong negative correlation between Mg, Cl, as well as Mn contents and SOM and non-significant weak negative correlation between K levels and SOM were observed (Table 3.1.5). In husk samples, weak negative correlations without statistical significance between Zn, Rb, As, Al, Mn, K, and Mg contents and SOM were observed, while Na levels showed a weak positive and non-significant correlation, whereas no correlations were observed for other elements (Cl, Ca, and Sb) no correlation were observed.

Table 3.1.4: Correlation between soil pH and the elements in areca husk and areca nut samples.

Soil pH	Na	Mg	Al	Cl	K	Ca	Mn	Zn	As	Rb	Sb
Husk	0.32	-0.44	0.14	0.53	0.72*	0.34	-0.39	-0.17	0.31	0.26	-0.85**
Areca Nut	-0.02	-0.28	-0.59	-0.21	0.505	0.17	0.013	0.04	-0.015	-0.2	0.012

** - Correlation is significant at $p < 0.01$ level (2-tailed).

* - Correlation is significant at $p < 0.05$ level (2-tailed).

Table 3.1.5: Correlation between Soil Organic Matter (SOM) and the elements in areca husk and areca nut samples.

SOM	Na	Mg	Al	Cl	K	Ca	Mn	Zn	As	Rb	Sb
Areca nut	0.58	-0.49	0.33	-0.37	-0.21	0.21	-0.3	0.01	0.17	0.02	.77*
Husk	0.26	-0.2	-0.38	0.17	-0.32	0.1	-0.34	-0.60	-0.35	-0.37	0.11

* - Correlation is significant at $p < 0.05$ level (2-tailed).

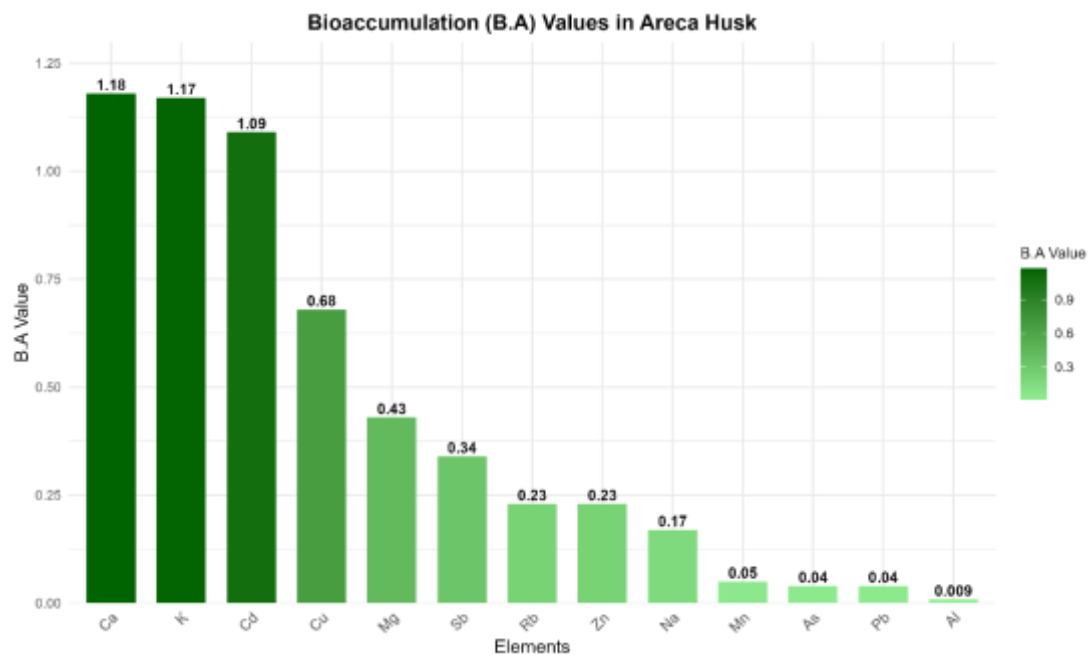


Figure 3.1.6: Bioaccumulation in Areca nut

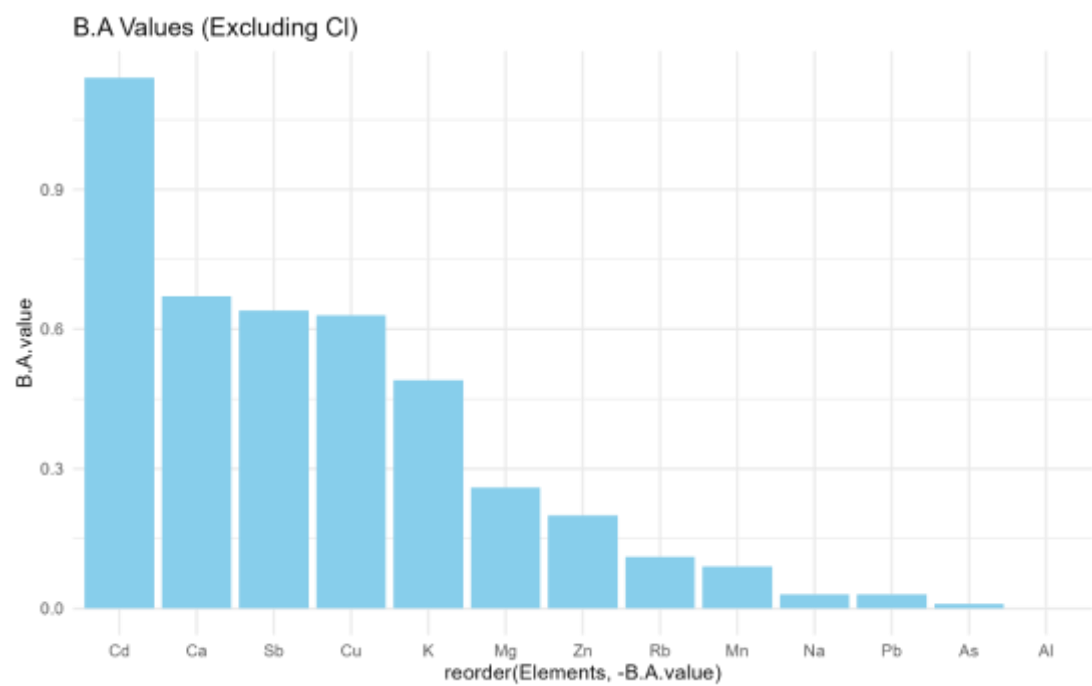


Figure 3.1.7: Bioaccumulation in Areca husk

Table 3.1.6: Calculated bioaccumulation index for Areca nut.

Elements	B.A value*
Na	0.03 ± 0.02
Mg	0.26 ± 0.22
Al	0.0002 ± 0.0001
Cl	39.81 ± 45.70
K	0.5 ± 0.21
Ca	0.67 ± 0.44
Mn	0.09 ± 0.08
Zn	0.2 ± 0.10
As	0.01 ± 0.02
Rb	0.11 ± 0.04
Sb	0.64 ± 0.36
Cu	0.63 ± 0.11
Cd	1.14 ± 0.84
Pb	0.03 ± 0.02

* Average ± Standard deviation

Table3.1.7: Calculated bioaccumulation index for Areca Husk.

Elements	B.A Value*
Na	0.17 ± 0.16
Mg	0.43 ± 0.36
Al	0.01 ± 0.008
Cl	78.1 ± 52.2
K	1.17 ± 0.6
Ca	1.18 ± 0.65
Mn	0.05 ± 0.04
Zn	0.23 ± 0.17
As	0.04 ± 0.05
Rb	0.23 ± 0.06
Sb	0.34 ± 0.18
Cu	0.7 ± 0.3
Cd	1.1 ± 0.82
Pb	0.04 ± 0.04

* Average ± Standard deviation

The bioaccumulation index was calculated for areca nut and husk for each plantation site, Table (Table 3.1.6 and 3.1.7) gives the calculated mean and standard deviation of BA index. The bioaccumulation index value provides the information on the extent of uptake and translocation of heavy elements by plant from the soil (Kabata-Pendias, 2011). The calculated BA index values of areca nut samples were in the range of 0.27 – 2.65 for Cd; 0.2 - 1.25 for Sb; 0.42- 0.74 for Cu; 0.05 - 0.3 for Zn; 0.03 - 0.2 for Mn; 0.043-0.15 Co; 0.007 – 0.09 for Pb; 0.002-0.05 for As, respectively. In addition, for husk samples, the calculated BA index values were in the range of 0.23 – 2.50 for Cd; 0.33 – 1.2 for Cu; 0.1-0.6 for Sb; 0.04-0.5 for Zn; 0.007-0.2 for As; 0.005 – 0.14 for Pb; 0.01-0.1 for Mn; and 0.043-0.052 (Co), respectively.

Similarly, in order to assess the degree of anthropogenic contamination in soil due to heavy elements, CF, I_{geo} and PLI were calculated for the following elements normally considered as the environmental pollutants: V, Co, Zn, As, Sb, U, Cd, Cu and Pb. The Pollution Indices are summarized in Table 3.1.8.

Table 3.1.8: Calculated pollution indices for soil samples.

Elements	Lengpui	Lengte	Zampui	Zamuang	Vairengte	Kolasib	Sibsagar	Golaghat
CF								
V	1	1.4	1.1	1.1	0.9	0.8	1.3	1.1
Co	0.6	0.8	0.9	0.9	0.9	0.5	1	0.8
Zn	0.7	0.9	2.2	0.8	1.4	1	1.3	1.4
As	4	3.2	0.4	1.6	1.5	0.9	1.5	1.2
Sb	2	2.3	2	1.8	1.6	0.9	1.6	1.1
U	1.2	1.2	1.1	1.1	1.1	0.8	0.9	0.9
Cu	0.6	0.8	1	0.8	1	0.5	1	0.8
Cd	0.2	0.3	0.9	1.1	0.8	0.4	1.5	0.9
Pb	1	1.1	1.4	1.1	1.4	1	1.4	1.1
I _{geo}								
V	0.69	0.94	0.72	0.72	0.63	0.52	0.86	0.71
Co	0.41	0.53	0.62	0.58	0.63	0.32	0.64	0.55
Zn	0.45	0.6	1.49	0.54	0.94	0.64	0.86	0.97
As	2.64	2.11	0.26	1.07	0.97	0.58	0.97	0.81
Sb	1.32	1.55	1.33	1.17	1.03	0.61	1.08	0.72
U	0.79	0.81	0.73	0.74	0.7	0.53	0.58	0.62
Cu	0.4	0.52	0.64	0.55	0.69	0.32	0.64	0.56
Cd	0.14	0.2	0.6	0.76	0.5	0.24	1.01	0.61
Pb	0.65	0.74	0.92	0.72	0.94	0.66	0.94	0.73
PLI								
	0.26	0.26	0.27	0.36	0	0.75	0.15	0.26

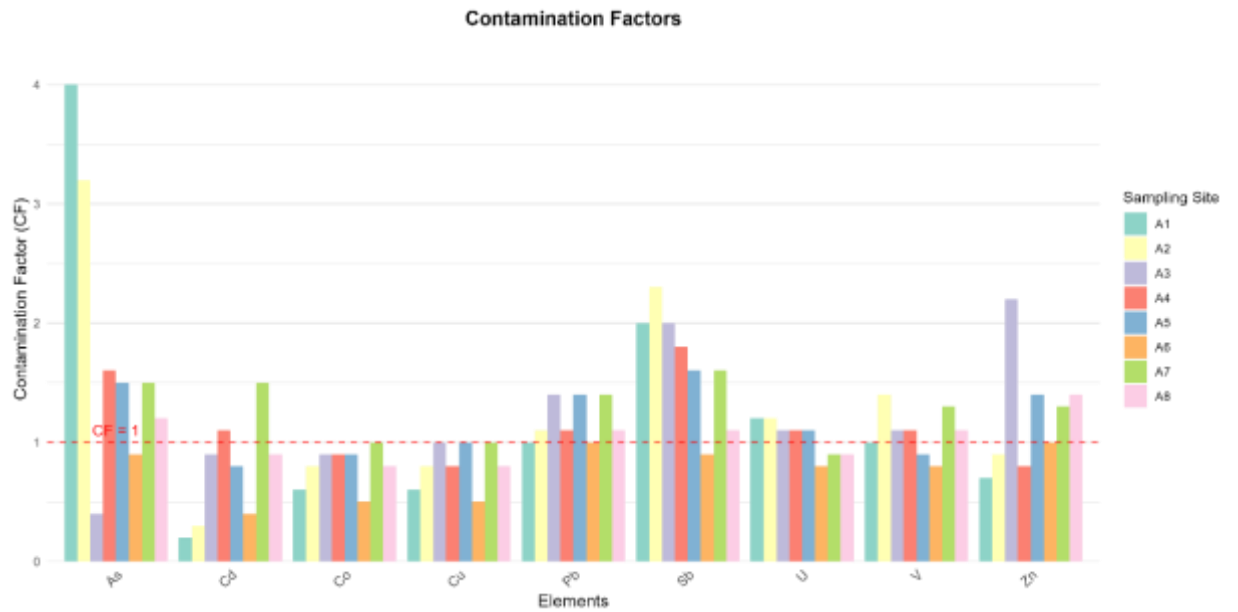


Figure 3.1.8 (a): Calculated contamination Factor of sampling site

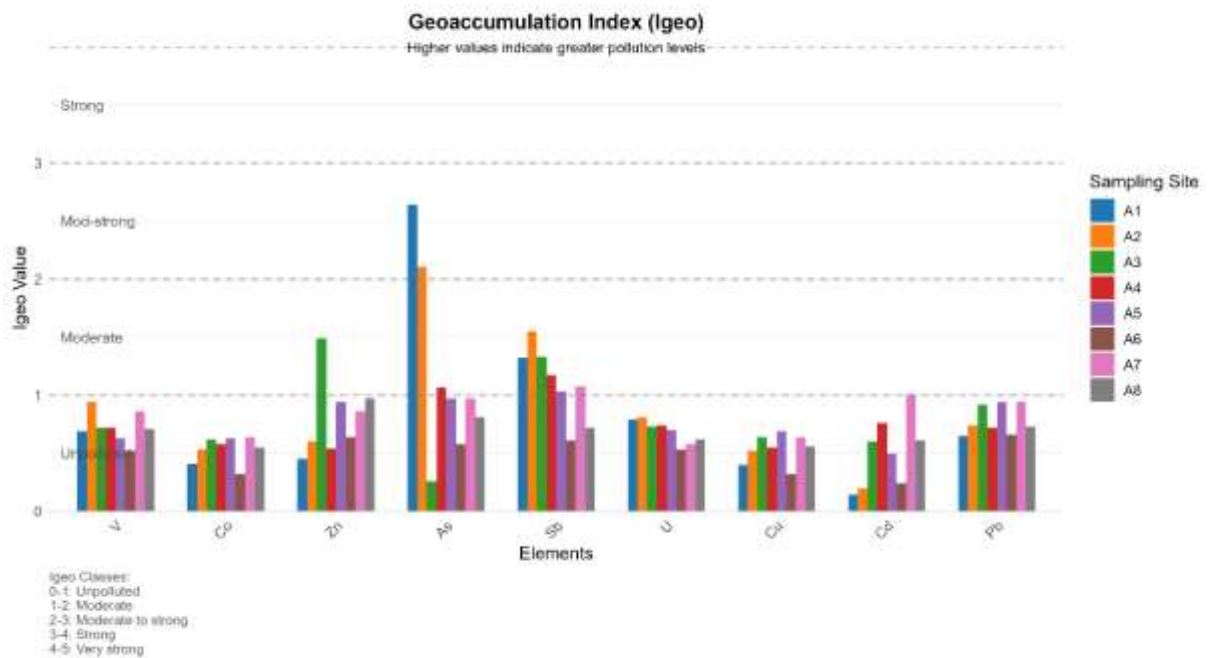


Figure 3.1.8(b): Geoaccumulation (I_{geo}) of Sampling site

3.1.4 Discussion

The detected elemental profile of soil is most likely a consequence of natural geochemical background features of the soil. Besides, in this study the natural trace element background concentrations may be in moderate levels at the plantation sites in Mizoram and Assam. Moreover, areca catechu palm trees are raised in an 'organic' manner as small-scale households across NEI grow their plantation crops without the application of any commercial fertilisers or insecticides and fungicides (Baruah et al., 2013) implying that anthropogenic inputs for these trace elemental species may be minimal or negligible.

Below pH 6, soil acidity entails highly ion exchangeable Al^{3+} as well as lower ion exchangeable alkaline earth mineral elements such Mg^{2+} and Ca^{2+} (Natesan, 1999, Di Meo et al., 2003). However, as a non-acidophilic plant species, areca catechu tended to uptake and transport through roots to other parts of the plant, less amount of inessential Al^{3+} and more amounts of essential micronutrient elements Ca^{2+} and Mg^{2+} , which is reflected in the mineral distributions of nut and husk samples.

The detected elemental profile of the soil is most likely a consequence of natural geochemical background features of the soil. Besides, in this study the natural trace element background concentrations may be in moderate level at the plantation site in Mizoram and Assam.

In most cases, BA index can also be related to the nature of the soil and approximate geographical background besides the ability of a specific plant species to translocate heavy elements from soil to the plant tissues (Kabata-Pendias, 2011, Zhuang et al., 2009). When $\text{BA index} > 1.0$, then, the plant species not only can absorb the heavy elements but also can accumulate heavy elements in the aerial parts of the plant including edible parts such as fruits, whereas for $\text{BA index} < 1.0$, the plant species can absorb the heavy elements albeit they may not accumulate them in the aerial parts (Zhuang et al., 2009; Sonu et al., 2019). In the present study, presumably except for Cd with mean BA index = 1.09 ± 0.82 , areca palm grown in acidic soils may not be able to accumulate other heavy elements in the edible areca nut.

In all the collected soil sample, except for Vairengte and Kolasib sites, the CF values of vanadium were between 1 and 2 indicating moderately polluted soils. In case of Co, except Sibsagar site, all soil samples can be considered as uncontaminated. Soils

collected in Zampui, Vairengte, Sibsagar and Golaghat are moderately contaminated with zinc, whereas Lengpui and Lengte sites are considerably contaminated with arsenic. Based on CF values, Sb, U, Cu, Cd and Pb contents in analyzed soils can be classified as low or moderately contaminated (Table 3.1.8). I_{geo} values for V, Co, Zn, U, Cu, Cd and Pb were lower than 1.0 indicating uncontaminated to moderately contaminated soil. For all soil samples, essentially I_{geo} values for As and Sb ranged from uncontaminated to moderately contaminated. I_{geo} value of As in soils has suggested that Lengpui and Lengte sites are moderately to significantly contaminated soils (Table 3.1.8). PLI values for all elements were less than 1.0 indicative of unpolluted soils (Salman et al., 2019).

In the present study, the amount of As present in the areca nut samples were in the range of 0.043-0.107 μgg^{-1} , which is higher than the mean As concentration level of 0.013 μgg^{-1} present in betel nuts of Bangladesh (Al-Rmalli et al., 2011), whereas it is considerably less than the As content present in betel nuts (0.34 μgg^{-1}) of Pakistan (Zaidi et al., 2002). This observation also suggests that the As content in areca nut samples of NEI are significantly less than the USFDA permissible levels of As in fruits and vegetables, viz., 1.4 μgg^{-1} (Roshila et al., 2007).

Moreover, the Cd content with a mean concentration of 0.06 μgg^{-1} and the Pb species with a mean concentration of 0.67 μgg^{-1} present in the areca nut samples, which are higher than the Cd and Pb contents in the areca nut samples available in Bangladesh (Al-Rmalli et al., 2011) Furthermore, the observed Cd levels in areca nut samples is comparatively lower than FAO/WHO's permissible levels of Cd, viz., 0.2 μgg^{-1} , whereas the Pb content is relatively higher than FAO/WHO's safe levels of Pb in fruits and vegetables, viz., 0.3 μgg^{-1} (Elbagermi et al., 2012) It is also pertinent to note that cadmium and lead exhibit strong bioaccumulation potential due to their long biological half-lives in mammals (Gairola and Wagner, 1991; Mussalo-Rauhamaa et al., 1986) Chronic exposure to and the concomitant bioaccumulation of these heavy elements in various organs may entail adverse health effects.

Available experimental studies, all of them from South India, have indicated that because of the unendurable agricultural practices, Cu content in areca nut and the soil were significantly higher (Khan et al., 2015; Alexander et al., 2019). Typically, in South India, copper is administrated, prior to the monsoon rains, as a fungicidal spray to the fruit bearing areca palm trees known as Bordeaux mixture which can be freshly prepared as an aqueous mixture of copper sulphate and slaked lime at an appropriate ratio (Mathew et al., 2015). Moreover, in Indian made cigarettes the copper content was also found to be significantly higher possibly due to the application of a variety of Cu sprays employed in tobacco crop protection in India (Mishra et al., 1986). Nearly all heavy elemental species sequestered in tobacco plant tissues were attributed to either atmospheric depositions or the amendment of fertilizers else sewage water /sewage sledge irrigations (Mann et al., 2011) The observed Cu, Cd, and Pb contents in NEI soils were well below the range of permissible limits for these trace elements in soils as prescribed by Indian standards for agricultural soils, viz., 135-270 mg/kg (Cu); 3-6 mg/kg (Cd); and 250-500 mg/kg (Pb) (Awasthi, 2000) as well as the Soil guideline values of Canada, China, and Poland (Kumar et al., 2019) Besides, these observed heavy elements contents were also relatively below the range of heavy elements concentrations detected in uncontaminated soils, viz., 2-100 mg/kg (Cu); 0.01-0.7 mg/kg (Cd); and 2-200 (Pb) (Bowen, 1966) However, the Cu content is within the limits, whereas Pb content in NEI soils is exceeded the standard Indian natural soil background level, viz., 56.5 mg/kg (Cu), and 13.1 mg/kg (Pb) (Kumar et al., 2019). Since, in Mizoram, due to the prohibitive cost of transportation in the rural region, “Organic” cultivation practices are followed and the computed pollution index, contamination factor and geoaccumulation reflect the geological attributes of the region of soil sample.

The observed Cu content in areca nut samples is significantly less than FAO/WHO’s permissible levels for Cu in fruits and vegetables, viz., $40 \mu\text{g g}^{-1}$ (Elbagermi et al., 2012) One reasonable possibility is that in NEI, rural small landscape holders, in general, hardly apply intensive agricultural methods (Baruah et al., 2013) which are reflected by the elemental distributions in areca nut and husk samples. In this study, the concentration levels of heavy elements (As, Cd, and Cu) except Pb are well within the permissible limits in biomatrices that may be attributed to edaphic and biogeochemical

features of the soil in addition to the rudimentary ‘organic’ agricultural practices that may be employed (Barbosa et al., 2014).

Uranium and thorium as well as few REEs were detected sporadically, only in some of the assayed areca palm husk samples. The natural radioactive elements, thorium and uranium are widely distributed in soils and rocks of earth’s crust besides under certain environmental conditions these elements can be accumulated in plant matrices. In the tested soil samples, the amount of uranium and thorium were present in the range, 2.13-3.3 μgg^{-1} and 8.6-17.2 μgg^{-1} , respectively. The manifestation of their radioactivity normally observed as decay products, viz., radon or thoron, have been detected at multiple sites in India including the NEI (Singh et al., 2007; Singh et al., 2016; Zoliana et al., 2016).

The results presented in this study provide the detailed information available to date on the distribution of macro, micro and trace elements at different parts (endosperm and pericarp) of the ‘mature and fresh’ unprocessed areca palm fruit. More akin to other palm species, areca catechu fruit also germinates in soil when both an endosperm (nut) that contains embryo and a fibrous pericarp (husk), are intact (Davis, 1978). Moreover, in this study we have observed that fibrous husk contained higher amount of various elements than present in the areca nut. It is likely that the pericarp of areca palm fruit tends to act as a reservoir of nutrient mineral elements, in addition perhaps, sequesters trace amounts of heavy elements.

It should be noted that areca palm husks are commercially processed and consumed as a masticatory article in Hunan province of China, besides in Taiwan, Indonesia, Philippines, Papua New Guinea and few Micronesian islands (Papke et al., 2020), normally young tender unripe fresh areca palm fruit with husk is consumed (Lee et al., 2011). Hence, the population which consumes the areca palm husk as a masticatory article may be potentially exposed to higher contents of trace elements in addition to areca alkaloids. It has been suggested that higher amount of areca alkaloids (Srimany et al., 2016; Yuan et al. 2018) in addition to relatively higher heavy metal contents present in the pericarp (husk) of areca palm may potentiate the carcinogenic risks of areca husk chewing (Khan et al., 2015; Yuan et al., 2018).

3.2 Results of second set of areca nut

3.2.1 Analysis of Soil Parameter

3.2.1.1 Soil pH measurement

The soil samples collected from 8 soil samples from Mizoram, sample each from the state of Assam, Arunachal Pradesh, Tripura, Meghalaya and two samples from Myanmar during the month of November and December 2019 for analysis. The pH measured value is given in the (Table 3.2.1.1), the soil pH value ranges from 4.29 to 6.5 with a mean pH 5.06 indicating the soils of areca nut plantation were acidic in nature.

3.2.1.2 Determination of Soil NPK from second set of areca nut

From the table we can see that the estimated available nitrogen in the soil sample range from 192.7 kg/ha-591.6 kg/ha, with A3 plantation site having the highest available nitrogen 591.6 and the available phosphorous range from 10.0 kg/ha – 320.7 kg/ha. The determined available potassium range was 34.7 kg/ha – 220.6 kg/ha.

3.2.1.3 Determination of soil organic matter (SOM) from second set of areca nut

The abundance of soil organic matter was estimated and range from 2.27 % - 5.58%, with A3 having the highest soil organic matter content 5.58%.

From the estimated available N, P, K and soil organic matter shows that the areca nut plantations were mostly grown under organic conditions without the application of fertilizers and pesticides.

Table 3.2.1.1: Measured soil pH from second set of areca nut

SAMPLE ID	Soil pH
A1	5.23
A2	4.82
A3	5.11
A4	4.85
A5	4.69
A6	5.78
A7	4.54
A8	4.69
A9	4.94
A10	5.14
A11	6.51
A12	5.82
A13	4.29
A14	4.44

Table 3.2.1.2 : Determination of available NPK from second set of areca nut

SAMPLE ID	N kg/ha	P kg/ha	K kg/ha
A1	394.4	35.8	118.7
A2	426	13.8	105.3
A3	591.6	19.9	103.0
A4	308.6	16.0	220.7
A5	371.8	28.4	121.0
A6	338.7	10.0	97.4
A7	338.7	278.5	155.7
A8	394.4	18.6	118.7
A9	252.9	10.3	38.1
A10	321.8	15.5	60.5
A11	192.7	11.8	71.7
A12	260.4	320.7	58.3
A13	492.3	20.4	109.8
A14	269.5	13.0	34.7

Table 3.2.1.3: Observed soil organic matter from second set of areca nut

SAMPLE ID	% of Soil Organic Matter
A1	3.1
A2	2.9
A3	5.6
A4	3.0
A5	3.3
A6	2.7
A7	3.3
A8	3.9
A9	3.0
A10	3.3
A11	2.3
A12	3.6
A13	4.7
A14	3.1

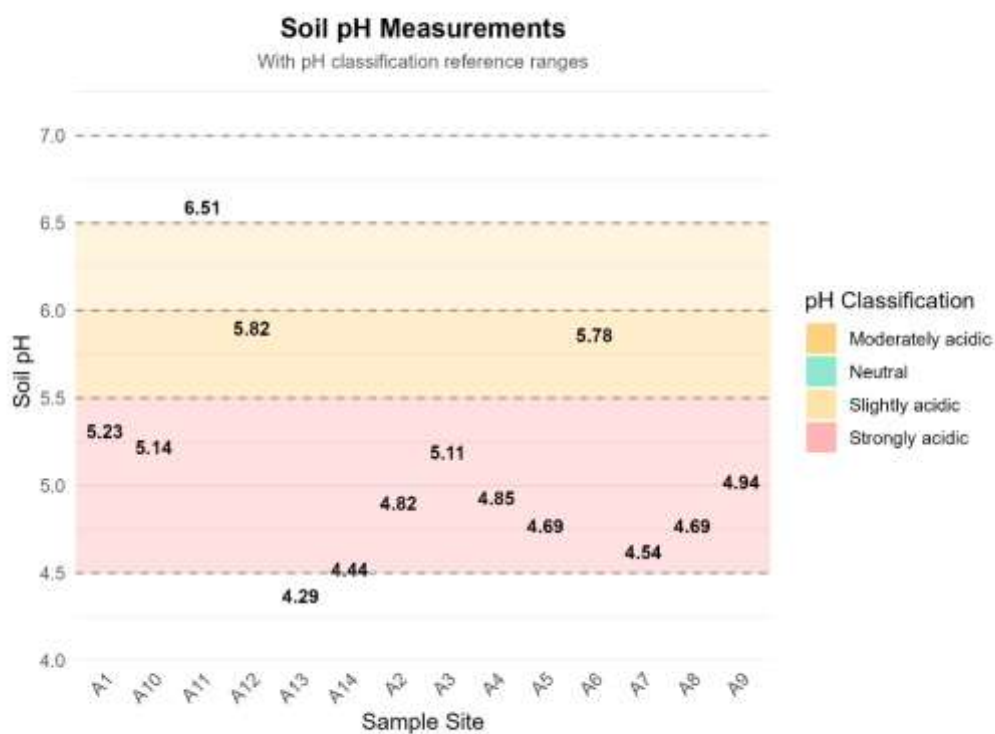


Figure 3.2.1.1: Soil pH from second set of areca nut

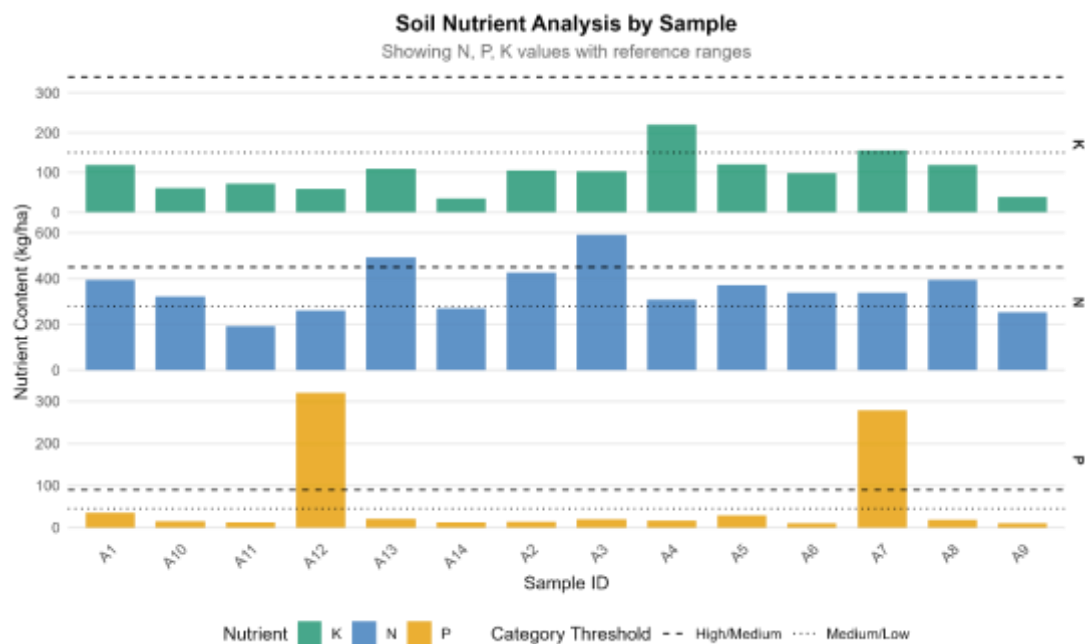


Figure 3.2.1.2: Plot of available NPK second set of areca nut

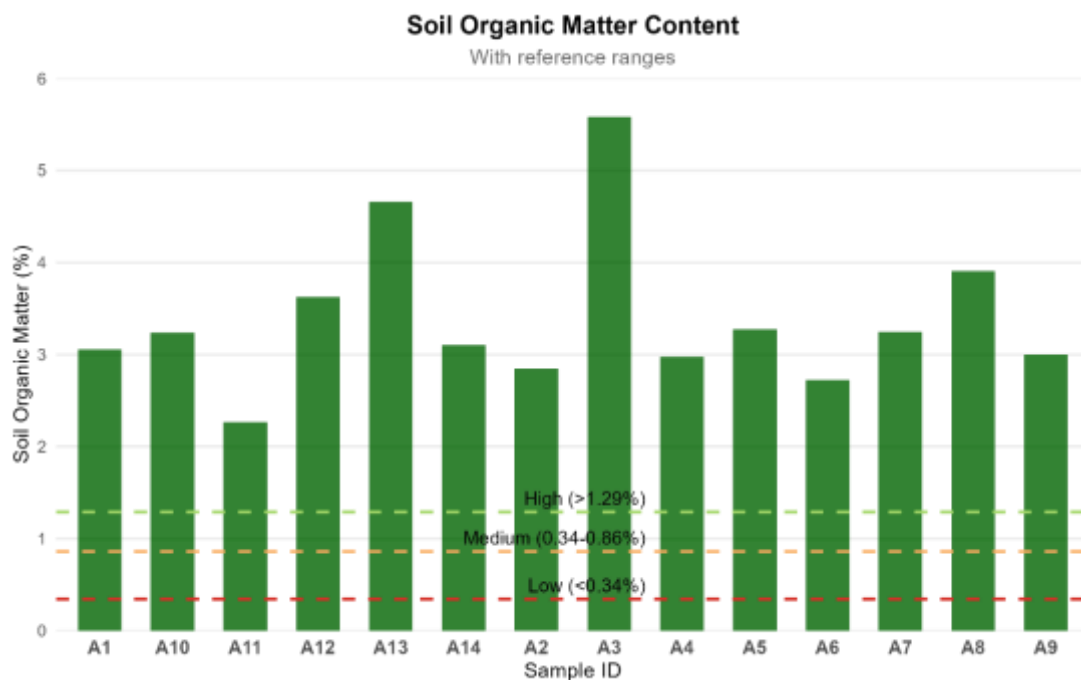


Figure 3.2.1.3: Estimated soil organic matter from areca nut plantation

3.2.2 Elemental analysis by Instrumental Neutron Activation Analysis (INAA).

In this study multielement concentration was determined by using instrumental neutron activation analysis (INAA), essential minerals, trace elements and rare earth metals were detected from the samples collected from areca nut, husk, root and soil. The determination of elemental composition of soil along with areca nut collected from Northeast India sample is very rare, as far as we can ascertain this is the first time it has been performed and same with Myanmar samples no record of such analysis has been performed before.

The mean concentrations of the detected elements were given in the table below (Table 3.2.2 (a), (b), (c)). The mean concentration of elements present in areca nut follows the order K> Si> Cl> Mg> Ca> Al> Na> Ti> Fe> Mn> Rb> Zn> Br> Cu> Sr> Zr> Ni> Ba> V> Nd> Cr> Ce> Dy> Ar> W> La> Co> Yb> Hf> U> Sm> Th> Tb> Ta. From the result given in the table (Table 3.2.2 (b)), we observed that the elemental concentration showed a trend in decreased of the concentration as follows from soil>root>husk>nut.

The soil pH value ranges from 4.29 to 6.5 with a mean pH 5.06 indicating the soils were acidic in nature. The soil may be classified as sandy soil according to grain size distribution. The estimated available N, P, K and soil organic matter shows that the areca nut plantations were mostly grown under organic conditions without the application of fertilizers and pesticides.

The overall mean concentration of K is highest in the husk>root>soil>nut, also Cl is highest in the husk>areca nut>root>soil. Comparing the areca nut and husk except for V (1.33), Ce (0.453) and Nd (0.929), all the other mean elements concentration were higher in the husk than in the areca nut, within the elements present in the areca nut K has the highest concentration and Ta has the lowest concentration.

Pearson's correlation was applied and did not give a significant correlation ($p<0.05$ and $p<0.01$) between the soil pH and the elemental concentrations of areca nut, husk, root and soil. Although a weak correlation between soil pH and husk were observed.

Table 3.2.2 (a): The concentration of elements in soil using INAA (in µg/g)

ELEMENTS	Soil (n=14)	
	Range	Mean±SD
Na	1190-9110	4746 ±3085
Mg	10900-38000	19414±6359
Al	40200-86400	64664±13437
Si	221000-562000	316071±96713
Cl	71-247	115.6±44.1
K	5300-17600	12400±3745
Ca	112-5100	2263±1575
Sc	7.0-17	12±2.7
Ti	2700-5650	4412±896
V	54-149	89.2±26.6
Cr	55-218	106.4±46.5
Mn	232-1080	560±240
Fe	19800-44300	30329±7164
Co	6.0-21	12.5±4.1
Ni	23-137	50±32
Cu	77-300	216 ±55
Zn	40-95	65.5±19.1
As	4.0-11	7.0±2.1
Br	1.0-14	6.4±4.0
Rb	62-163	105±35
Sr	33-128	72.6±27.0
Zr	93-330	163.2±57.0
Sb	0.24-0.62	0.47±0.12
Cs	3.0-9.0	5.5±2.3
Ba	167-439	327.5±91
La	22-80	38.2±14.2
Ce	45-174	79.1±31.4
Nd	18-48	30.6±8.0
Sm	4-10.1	6.3±1.7
Eu	0.6-1.18	0.9±0.2
Tb	0.46-1.3	0.8±0.2
Dy	3.0-9	5.0±1.6
Yb	1.5-4.2	2.8±0.6
Hf	4.0-15	7.7±2.7
Ta	0.57-1.43	1.0 ±0.3
W	0.8-2.8	1.8±0.7
Th	8.0-46	16.6±9.0
U	1.7-8.2	2.9±1.6

Table 3.2.2 (b): The concentration of elements in areca nut sample using INAA (in $\mu\text{g/g}$)

ELEMENTS	Aerca nut (n=14)	
	Range	Mean $\pm$ SD
Na	46-297	149.8 $\pm$ 97.5
Mg	49-2740	999.2 $\pm$ 578.6
Al	4.0-6590	500 $\pm$ 1753
Si	1440-23000	5902 $\pm$ 5056
Cl	96-15200	3194 $\pm$ 3650
K	5910-17800	12681 $\pm$ 3493
Ca	34-2030	831 $\pm$ 455
Sc	0.005-0.021	0.01 $\pm$ 0.005
Ti	11-492	60 $\pm$ 125
V	0.03-17	1.4 $\pm$ 4.6
Cr	0.72-1.1	0.8 $\pm$ 0.1
Mn	3-294	44.3 $\pm$ 72.7
Fe	17-107	50.3 $\pm$ 27.5
Co	0.03-0.11	0.06 $\pm$ 0.021
Ni	1.3-3.4	2.0 $\pm$ 0.7
Cu	2.0-31	13.4 $\pm$ 6.9
Zn	10.0-19	14.7 $\pm$ 2.7
As	0.04-0.28	0.13 $\pm$ 0.08
Br	1.0-29	14.2 $\pm$ 9.7
Rb	3.0-59	22 $\pm$ 15.6
Sr	2.0-8	5.5 $\pm$ 1.6
Zr	2.0-3	2.21 $\pm$ 0.43
Sb	0.002-0.020	0.01 $\pm$ 0.005
Cs	0.003-0.083	0.024 $\pm$ 0.02
Ba	1.1-2.2	1.6 $\pm$ 0.34
La	0.02-0.08	0.05 $\pm$ 0.02
Ce	0.4-0.5	0.45 $\pm$ 0.03
Nd	0.8-1.05	0.93 $\pm$ 0.06
Sm	0.002-0.02	0.008 $\pm$ 0.005
Eu	0.014-0.017	0.015 $\pm$ 0.01
Tb	0.002-0.003	0.002 $\pm$ 0.0003
Dy	0.08-0.19	0.17 $\pm$ 0.10
Yb	0.027-0.034	0.03 $\pm$ 0.002
Hf	0.013-0.02	0.016 $\pm$ 0.002
Ta	0.0007-0.0018	0.001 $\pm$ 0.0003
W	0.051-0.15	0.09 $\pm$ 0.03
Th	0.005-0.024	0.007 $\pm$ 0.005
U	0.011-0.022	0.015 $\pm$ 0.004

Table 3.2.2 (c): The concentration of elements in areca husk sample using INAA (in $\mu\text{g/g}$)

ELEMENTS	Aerca husk (n=14)	
	Range	Mean $\pm$ SD
Na	117-949	448 $\pm$ 247
Mg	770-2720	1585 $\pm$ 699
Al	72-5460	770 $\pm$ 1449
Si	8380-27700	12628 $\pm$ 4751
Cl	402-11700	6232 $\pm$ 3197
K	2950-299000	40525 $\pm$ 86152
Ca	576-3250	1875 $\pm$ 851
Sc	0.0096-1.5	0.180 $\pm$ 0.4
Ti	44-440	91 $\pm$ 101
V	0.2-3	1.16 $\pm$ 2.5
Cr	0.83-24.3	3.15 $\pm$ 6.1
Mn	20-117	45.2 $\pm$ 29.4
Fe	50-1460	557.6 $\pm$ 1017.6
Co	0.06-2.17	0.3 $\pm$ 0.5
Ni	1.62-15	3.33 $\pm$ 3.4
Cu	10-25.4	14.6 $\pm$ 5
Zn	17-54	24.45 $\pm$ 10
As	0.09-0.71	0.34 $\pm$ 0.2
Br	4-115	49.5 $\pm$ 36.7
Rb	8.0-82	40.3 $\pm$ 23.3
Sr	5.0-36	20.25 $\pm$ 9.7
Zr	3.0-22	5.9 $\pm$ 4.8
Sb	0.02-0.11	0.05 $\pm$ 0.028
Cs	0.02-0.49	0.14 $\pm$ 0.13
Ba	2.0-29	8.3 $\pm$ 8.2
La	0.07-2.5	0.46 $\pm$ 0.68
Ce	0.51-5	2.4 $\pm$ 1.3
Nd	0.81-1.03	0.93 $\pm$ 0.075
Sm	0.013-0.540	0.086 $\pm$ 0.143
Eu	0.016-0.1	0.03 $\pm$ 0.02
Tb	0.002-0.068	0.011 $\pm$ 0.01
Dy	0.21-0.67	0.33 $\pm$ 0.1
Yb	0.038-0.25	0.09 $\pm$ 0.05
Hf	0.02-0.19	0.08 $\pm$ 0.13
Ta	0.002-0.062	0.017 $\pm$ 0.011
W	0.11-0.35	0.24 $\pm$ 0.08
Th	0.01-0.82	0.15 $\pm$ 0.24
U	0.02-0.20	0.08 $\pm$ 0.05

Table 3.2.2 (d): Element concentration in areca root sample using INAA (conc. in $\mu\text{g/g}$)

ELEMENTS	Aerca nut (n=14)	
	Range	Mean $\pm$ SD
Na	239-1610	618.5 $\pm$ 365.2
Mg	1480-3700	2922.5 $\pm$ 649.4
Al	3510-13700	7695 $\pm$ 2539.7
Si	21500-68800	37683.3 $\pm$ 14583
Cl	120-5370	1492.5 $\pm$ 1625
K	2950-64000	26087.5 $\pm$ 23999
Ca	634-4340	2498 $\pm$ 1390
Sc	0.7-2.8	1.9 $\pm$ 0.7
Ti	190-793	479 $\pm$ 158.8
V	4.0-14	10.2 $\pm$ 2.8
Cr	4.0-20	12.5 $\pm$ 4.15
Mn	75-167	108 $\pm$ 25.24
Fe	1940-6750	4381.7 $\pm$ 1365
Co	0.9-3.9	2.67 $\pm$ 0.9
Ni	3.0-13	8.3 $\pm$ 3.4
Cu	26.3-46	33.8 $\pm$ 6.6
Zn	12-264	39 $\pm$ 71
As	0.5-2.2	1.22 $\pm$ 0.5
Br	2.2-25	10.1 $\pm$ 7.7
Rb	11.0-74	25 $\pm$ 16.8
Sr	6.0-41	26.75 $\pm$ 10.7
Zr	6.0-59	23.5 $\pm$ 14.2
Sb	0.05-0.23	0.1 $\pm$ 0.05
Cs	0.34-1.2	0.9 $\pm$ 0.2
Ba	30-96	65 $\pm$ 20.7
La	2.0-18	8.9 $\pm$ 5.5
Ce	5.0-42	16.1 $\pm$ 10.2
Nd	1.0-14	7.1 $\pm$ 4
Sm	0.4-3.3	1.5 $\pm$ 0.9
Eu	0.09-0.57	0.25 $\pm$ 0.13
Tb	0.05-0.38	0.19 $\pm$ 0.1
Dy	0.39-1.46	0.8 $\pm$ 0.4
Yb	0.14-0.9	0.58 $\pm$ 0.25
Hf	0.26-2.13	0.9 $\pm$ 0.5
Ta	0.04-0.28	0.14 $\pm$ 0.06
W	0.17-0.53	0.29 $\pm$ 0.1
Th	0.74-8.6	2.7 $\pm$ 2
U	0.15-1.6	0.5 $\pm$ 0.31

3.2.3 Discussion

The determined soil pH is indicative of acidic soils due to copious rainfall in the region. As indicated in the earlier studies, the anthropogenic inputs for areca palm cultivation may be minimal or negligible at the plantation sites as reflected in the observed elemental profile of the soil samples (Zote et al., 2021; Lalrammawia et al., 2021). The present study showed the mean values for Cr, Fe, Co in soil (Northeast India and Myanmar) were within the limit of Indian natural soil background values (Kumar et al., 2019). Besides, the mean values of Mn, Ni, Cu, Zn exceeds Indian natural soil background limits, while the mean soil concentration of Ni, Cu, and Zn were less than the standards of Indian permissible limit for soil (Kumar et al., 2019). The present study showed that mean values for Cr, Fe, Co in soil were within the limit of Indian natural soil background values. Besides, the mean values of Mn, Ni, Cu, Zn exceeds Indian natural soil background limits, while the mean soil concentration of Ni, Cu, and Zn were less than the values of Indian permissible limit for soil. When the multi-element concentrations of Northeast Indian and Myanmar soils were compared, moderate differences were observed for most elements; Myanmar soils are comparatively enriched in mineral and micro-elements, while Northeast Indian soils are relatively enriched in REEs. Moreover, the observed differences indicate the areca palm's highly adaptive potential of tolerating various levels of potential toxic elements, in addition reflecting the wide variations of elemental contents occurring in soils (Singh et al., 2005) pertaining to the prevailing edaphic and pedogenic conditions of the hilly terrain.

The observed elemental contents in areca nut and areca husk samples did not vary significantly between Northeast India and Myanmar. Trace levels of rare earth elements (REEs) observed in Northeast India and Myanmar areca palm fruits (nut and husks). However, K concentration in nuts and husks is much higher than that of Na with the K/Na ratio of areca nut is 3 times higher in Northeast India (K/Na=105.6) than Myanmar (35.3); The concentration of Al in nuts of Myanmar is 3 times more than that of Northeast India and the content of As in nuts is ~ 2.8 times higher in Northeast India than Myanmar. A perusal of elemental contents data (Table 3.2.2 (e), (f)) also shows that S.D. values for most of the elements in nut and husk

samples are rather large suggesting significant variation may possibly be due to difference in the edaphic factors and geochemical features of different plantation sites of Northeast India and Myanmar which are mostly >60 km away from each other.

Table 3.2.2 (e): Range, mean and standard deviation of elements in areca nuts, husks, roots and the corresponding soils of NEI using INAA.

Elements	Nuts		Husks		Roots		Soils	
	Range (mg/kg)	Mean±Std	Range (mg/kg)	Mean±Std	Range (mg/kg)	Mean±Std	Range (mg/kg)	Mean±Std
Na	46-297	129.6±87	117-711	394±203	239-1610	618±383	1290-9110	4311±2721
Mg	49-1210	855±322	810-2720	1467±575	1480-3680	2852±631	10900-24700	17446±3629
Al	4.0-53	23.4±12	72-2100	447.5±591	3510-9780	7149±1778	40200-84800	61482±11799
Si	1440-6140	4610±1320	8380-14900	11805±2017	21500-58700	34855±11327	221000-562000	300818±95387
Cl	96-4080	2361±1298	1300-11300	6552±2407	120-5370	1595±1663	52-247	114±49
K	5910-17800	13673.6±3161	2950-170000	22259±49202	2950-64000	28168±24008	7200-17600	13437±3256
Ca	34-1010	682±290	576-2980	1723±715	814-4340	2668±1322	821-5100	2091±1364
Sc	0.0021-0.0214	0.008±0.005	0.0096-0.49	0.09±0.15	0.7-2.8	1.81±0.63	7.0-17	12±2.8
Ti	11.0-35	8±6.8	52-106	66±14.7	190-694	450±130	2700-5650	4276±956
V	0.03-0.14	0.10±0.03	0.2-3.0	0.59±0.80	4.0-14	9.93±2.8	54-123	85±23
Cr	0.72-0.9	0.8±0.05	1.15-4	1.6±0.8	4.0-20	12.1±4.3	55-109	94±17
Mn	3.0-46.0	29±12.5	18-117	45.3±28	84-167	110.9±24.1	247-1080	582.8±250
Fe	17-93	47±23.2	50-1460	328±415	1940-6750	4343±1425	19800-36700	29818±6267
Co	0.034-0.112	0.05±0.02	0.06-0.6	0.16±0.15	0.9-3.9	2.7±0.93	7.0-17	12.2±3
Ni	1.3-3.4	2.13±0.7	1.62-4.4	2.52±0.93	3.0-13	8.61±3.43	23-59	39±12
Cu	2.0-19	12.3±4.9	13-23	13.9±4.1	26.3-46	32.7±5.9	77-260	200±49
Zn	10.0-19	14.8±2.9	17-32	21.7±4.9	12.0-264	40.4±74.3	40-95	64±18
As	0.04-0.28	0.14±0.09	0.45-0.65	0.35±0.20	0.5-2.2	1.13±0.57	4.0-11	7.2±2.3
Br	5.0-29	16.3±9.2	13-115	59.2±35.1	3.0-25	10.43±7.90	3.0-14	6.6±3.1
Rb	7.0-44	21.4±11.2	12.0-80	40.72±20.24	11.0-74	25.05±17.57	62-163	114±34
Sr	4.0-8.0	5.93±1.3	7.0-36	20±9	14-41	28.7±8.6	47-128	73±27
Zr	2.0-3.0	2.2±0.40	3.0-7.0	4.61±1.0	6.0-37	20±9.0	102-190	155±29
Sb	0.003-0.020	0.009±0.005	0.019-0.11	0.04±0.03	0.05-0.23	0.10±0.05	0.24-0.63	0.49±0.12
Cs	0.003-0.060	0.02±0.01	0.02-0.23	0.08±0.07	0.34-1.2	0.80±0.27	3.0-9	5.92±2.4
Ba	1.1-2.2	1.6±0.4	2.0-21	7.5±6	30-96	67±20	217-439	361±69
La	0.03-0.07	0.05±0.01	0.07-1.0	0.32±0.37	2.0-16	8.0±4.9	23-47	37±6.6
Ce	0.41-0.5	0.46±0.03	0.5-2.0	0.82±0.60	5.0-25	13.7±6.4	49-100	76±14
Nd	0.85-1.05	0.93±0.06	0.8-1.0	0.90±0.08	1.0-14	6.6±3.8	34-38	31±6
Sm	0.002-0.021	0.008±0.005	0.009-0.21	0.06±0.07	0.4-3.3	1.5±0.8	4.0-8.0	6.21±1.2
Eu	0.014-0.0165	0.015±0.0007	0.016-0.04	0.02±0.005	0.09-0.57	0.25±0.14	0.59-1.18	0.91±0.2
Tb	0.002-0.003	0.002±0.0003	0.003-0.027	0.007±0.008	0.05-0.38	0.2±0.10	0.46-0.96	0.80±0.14
Dy	0.08-0.19	0.16±0.03	0.25-0.35	0.3±0.03	0.39-1.46	0.8±0.34	3.0-7.0	4.8±1.0
Yb	0.028-0.034	0.03±0.002	0.038-0.07	0.05±0.009	0.14-0.9	0.51±0.23	1.5-3.3	2.9±0.5
Hf	0.014-0.02	0.02±0.002	0.02-0.19	0.05±0.06	0.26-1.4	0.71±0.33	4.0-9	7.3±1.7
Ta	0.0007-0.0018	0.002±0.0003	0.002-0.04	0.007±0.01	0.04-0.19	0.13±0.04	0.57-1.28	1±0.2
W	0.058-0.148	0.10±0.03	0.14-0.35	0.24±0.07	0.17-0.46	0.27±0.08	0.8-2.6	1.9±0.6
Th	0.005-0.0236	0.007±0.006	0.01-0.48	0.10±0.15	0.74-3.4	2.2±0.8	10.0-19	15.5±2.4
U	0.011-0.022	0.02±0.004	0.02-0.10	0.04±0.03	0.15-0.64	0.40±0.14	1.7-3.2	2.6±0.5

Table 3.2.2 (f): Range, mean and standard deviation of elements in areca nuts, husks and the corresponding soils of Myanmar

Elements	Nuts		Husks		Soils	
	Range (mg/kg)	Mean±Std	Range (mg/kg)	Mean±Std	Range (mg/kg)	Mean±Std
Na	126-353	240±161	708-949	829±170	8730-9110	8920±269
Mg	780-1070	925±205	2560-2720	2640±113	20200-38000	29100±12586
Al	24-122	73±69	161-5460	2810.5±3747	59400-86400	72900±19092
Si	4440-4480	4460±28	9580-27700	18640±12813	297000-481000	389000±130108
Cl	1260-2290	1775±728	402-11700	6051±7989	113-142	128±21
K	6720-10200	8460±2461	3200-20300	11750±12091	8700-11800	10250±2192
Ca	794-1310	1052±365	3050-3250	3150±141	3790-4780	4285±700
Sc	0.0058-0.021	0.01±0.01	0.03-1.5	0.74±1.0	10.0-16	13±3.6
Ti	24-25	24.5±0.71	67-440	255±263	4450-5080	4765±446
V	0.095-0.23	0.16±0.09	0.194-9.0	4.80±6.5	89-149	119±42
Cr	0.76-1.1	0.93±0.24	0.83-24.3	12.57±16.6	194-218	206±17
Mn	14-16	14.8±1.5	22-94	58±50.91	555-642	599±62
Fe	63-107	85±31.1	282-3850	2066±2523	30700-44300	37500±9617
Co	0.029-0.073	0.05±0.03	0.10-2.17	1.14±1.5	15-21	18±4
Ni	1.0-2.0	1.5±0.71	2.2-15	8.6±8.9	96-137	117±29
Cu	6.0-15	10.6±6.5	18-25.4	21.5±5.6	244-300	272±40
Zn	12.0-16	14.2±2.6	32-54	43±15.6	82-94	88±9
As	0.04-0.06	0.05±0.02	0.14-0.71	0.43±0.40	6.0-7.0	6.4±0.71
Br	1.0-2.0	1.65±0.32	4.0-9.0	6.58±3.71	1.0-2.0	1.6±1.2
Rb	3.0-9.0	6.23±4.35	8.0-25	16.85±11.95	65-81	73±11.3
Sr	4.0-6.0	5.2±1.41	27-30	28.5±2.12	82-100	91±13
Zr	2	2±0	4.0-22	13.15±12.94	93-160	127±47
Sb	0.010-0.012	0.01±0.001	0.035-0.098	0.07±0.04	0.38-0.43	0.41±0.03
Cs	0.012-0.029	0.02±0.01	0.15-0.49	0.32±0.24	3.0-5.0	4±0.9
Ba	1.2	1.2±0	2.0-29	16±19	203-246	225±30
La	0.02-0.08	0.05±0.04	0.08-2.5	1.3±1.7	22-25	23.5±2.1
Ce	0.4-04.5	0.43±0.04	1.0-5.0	3.0±3.3	45-53	49±6
Nd	0.8-0.95	0.88±0.11	0.94-1.0	0.97±0.04	19-25	22±4
Sm	0.003-0.013	0.008±0.007	0.017-0.54	0.28±0.37	4.0-5.0	4.6±0.6
Eu	0.0116-0.015	0.01±0.002	0.025-0.1	0.06±0.05	0.59-0.6	0.60±0.007
Tb	0.002	0.002±0	0.003-0.07	0.04±0.05	0.56-0.65	0.61±0.06
Dy	0.14	0.14±0	0.34-0.67	0.51±0.23	3.0-5.0	3.9±1.2
Yb	0.027-0.03	0.03±0.002	0.044-0.25	0.14±0.14	2.1	2.12±0.03
Hf	0.013-0.015	0.01±0.001	0.02-0.49	0.26±0.33	5.0-7.0	5.8±1.4
Ta	0.0016-0.0018	0.002±0.0001	0.003-0.062	0.03±0.04	0.66-0.67	0.67±0.007
W	0.051-0.052	0.05±0.0007	0.11-0.12	0.12±0.007	1.1-1.2	1.15±0.04
Th	0.005-0.01	0.01±0.007	0.01-0.82	0.42±0.57	8.0-9.0	8.4±0.6
U	0.011	0.01±0	0.02-0.20	0.11±0.12	1.7-1.9	1.8±0.12

The transport and deposition of heavy elements absorbed from the soil by plants to the aerial parts, especially the edible parts of plant could act as one of the main routes of toxic heavy elements ingestion into human body via food stuff and/or lifestyle products (Zeng et al., 2011; Lalrammawia et al., 2021). Cl has higher BCF and TF values which suggests that Cl^- ion can be absorbed and translocated easily from the acidic soil by areca palm. BCF of Br^- , Rb, and K have high BCF in the following order: husk>nut>root. TF values of Br, Rb, and K are also high in husk, followed by nut. Ca, Zn, Na, Mg, Si, Mn, and Cu are translocated in moderate level to the aerial parts (husk> nut). All REEs and heavy elements are observed to have both BCF and $\text{TF} < 1$, while Al, Cr, V, and Sb have modest BCF and TF values

Table 3.2.3 (a): Calculated bioconcentration factor for Areca nut

Element	Arecanut		Husk		Root
	BCF	TF	BCF	TF	BCF
Na	0.03	0.24	0.09	0.72	0.13
Mg	0.05	0.34	0.08	0.54	0.15
Al	0.01	0.06	0.01	0.10	0.12
Si	0.02	0.16	0.04	0.34	0.12
Cl	27.6	2.14	53.9	4.2	12.9
K	1.02	0.49	3.27	1.55	2.10
Ca	0.37	0.33	0.83	0.75	1.10
Sc	0.00	0.00	0.02	0.09	0.16
Ti	0.01	0.12	0.02	0.19	0.11
V	0.02	0.14	0.01	0.12	0.11
Cr	0.01	0.07	0.03	0.27	0.11
Mn	0.08	0.41	0.08	0.42	0.19
Fe	0.00	0.01	0.02	0.13	0.14
Co	0.00	0.02	0.02	0.12	0.21
Ni	0.04	0.24	0.07	0.40	0.17
Cu	0.06	0.40	0.07	0.43	0.16
Zn	0.22	0.38	0.37	0.63	0.
As	0.02	0.12	0.05	0.31	0.16
Br	2.20	1.40	7.73	4.90	1.58
Rb	0.21	0.88	0.4	1.62	0.24
Sr	0.08	0.21	0.28	0.75	0.37
Zr	0.01	0.09	0.04	0.25	0.14
Sb	0.02	0.09	0.08	0.40	0.20
Cs	0.01	0.04	0.02	0.15	0.15
Ba	0.00	0.02	0.03	0.13	0.20
La	0.00	0.01	0.01	0.06	0.23
Ce	0.01	0.03	0.00	0.01	0.20
Nd	0.03	0.13	0.03	0.13	0.23
Sm	0.00	0.01	0.01	0.06	0.24
Eu	0.02	0.06	0.03	0.13	0.27
Tb	0.00	0.01	0.01	0.06	0.25
Dy	0.04	0.23	0.06	0.38	0.16
Yb	0.01	0.06	0.02	0.12	0.17
Hf	0.00	0.02	0.01	0.09	0.12
Ta	0.00	0.01	0.01	0.08	0.14
W	0.05	0.30	0.11	0.67	0.17
Th	0.00	0.00	0.01	0.06	0.16
U	0.01	0.03	0.02	0.10	0.17

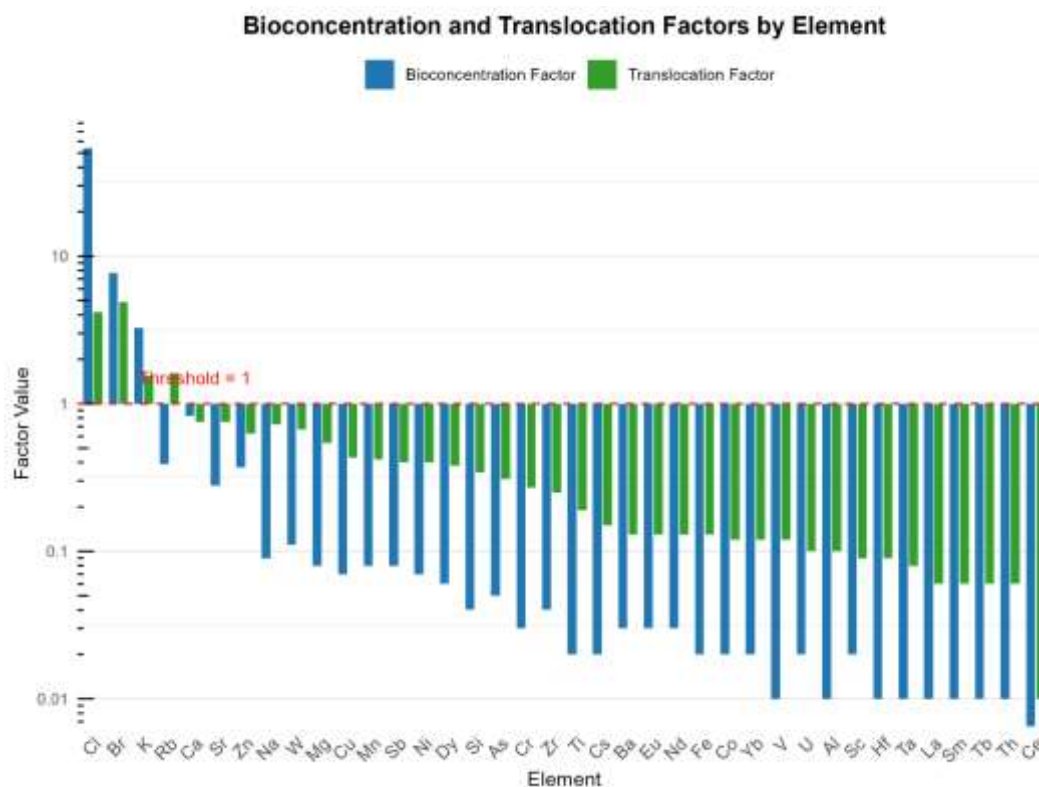


Figure 3.2.3(a): Bioconcentration and translocation Factor in Areca nut

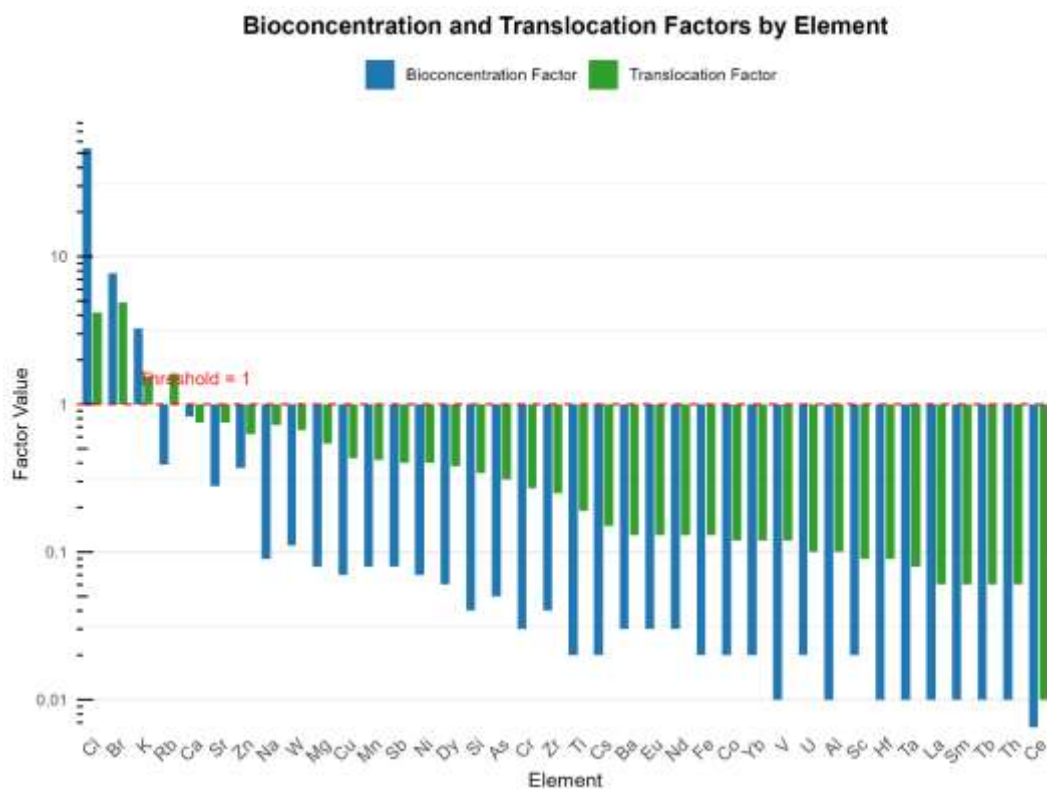


Figure 3.2.3(b): Bioconcentration and translocation Factor in Areca husk

Table 3.2.3 (b): Comparison of elemental contents of areca nut from different countries (mg/kg)

Elements	Present study	Pakistan	Bangla. nuts in UK	Taiwan Spring	Taiwan Summer
Na	149.8	127	181	385.3	382
Mg	999.2	2.8	587	3270	3015
Al	23.5	2.9	23	14	9.8
Si	5902	-	-	-	-
Cl	3194	1500	1590	5175	8250
K	12681	4300	-	17925	13950
Ca	831	1.2	575	9418	5368
Sc	0.009	0.018	-	-	-
Ti	59.5	0.014	-	-	-
V	1.34	12	-	1.69	0.84
Cr	0.81	0.5	-	-	-
Mn	44.3	47	11	148.8	247.5
Fe	50.3	75	-	1212.5	975
Co	0.05	0.027	-	-	-
Ni	2.01	-	-	-	-
Cu	13.4	-	5	127.8	49.3
Zn	14.7	5	-	29	39.9
As	0.13	0.34	0.013	-	-
Br	14.1	7.2	-	21.3	50.8
Rb	22	57	-	16.7	23.7
Sr	5.51	-	-	-	-
Zr	2.21	-	-	-	-
Sb	0.009	0.013	-	-	-
Cs	0.024	0.25	-	-	-
Ba	1.53	1.7	-	-	-
La	0.05	0.044	-	-	-
Ce	0.45	0.24	-	-	-
Nd	0.93	0.01	-	-	-
Sm	0.008	0.023	-	-	-
Eu	0.014	0.005	-	-	-
Tb	0.002	0.01	-	-	-
Dy	0.18	0.012	-	-	-
Yb	0.03	0.008	-	-	-
Hf	0.02	0.018	-	-	-
Ta	0.001	0.007	-	-	-
W	0.09	-	-	-	-
Th	0.007	-	-	-	-
U	0.015	-	-	-	-

Limited studies have analysed element concentrations in areca nuts (Table 3.2.3 (b)) and mostly they have focused on few elements (Al-Rmalli et al., 2011; Zaidi et al., 2002; Wei and Chung, 1997). Wei and Chung (1997) compared the seasonal elemental profile in areca nut harvested during spring and summer seasons. In the Taiwanese study, areca nut samples harvested during spring contained higher mean concentrations for Na (385 µg/g), Mg (3270 µg/g), Al (14 µg/g), K (17945 µg/g), Ca (9417 µg/g), V (1.69 µg/g), Fe (1212 µg/g), and Cu (128 µg/g) compared to areca nut samples harvested in summer, whereas Taiwanese areca nut samples harvested in summer contained higher mean concentrations for Cl (8250 µg/g), Mn (247 µg/g), Zn (40 µg/g), Br (51 µg/g), and Rb (24 µg/g) than those found in areca nuts harvested during spring (Wei and Chung, 1997). The contents of these elements except Al in areca nuts from Taiwan were significantly higher than areca nuts from Bangladesh (23 µg/g), Pakistan (2.9 µg/g), and in our study (23.5 µg/g). Compared with the previous studies, in the current study, the concentration levels of Al is in slightly higher concentration levels in Myanmar (73 µg/g) samples. The mean arsenic (As) concentration observed in this study was considerably higher than the mean As concentrations of areca nuts from Bangladesh (0.013 µg/g) available in the UK and Myanmar samples (0.05 µg/g) albeit it was ~ 2.6 times lower than found in Pakistan samples.

The mean Cu level in areca nuts from Bangladesh was lower than found in our study albeit both these values were markedly lower than those from Taiwan. The mean concentration of Mn in the current study was found to be less than Taiwanese areca nuts, while comparable with areca nuts from Pakistan, albeit ~ 4-times higher than areca nuts of Bangladesh available in the UK. The mean Fe concentrations were quite comparable between areca nuts of Pakistan and the present study; however, these values were markedly lower than those found in areca nuts from Taiwan. Ca and Mg contents in areca nuts of the present study were quite similar to those from Bangladesh but significantly higher than those from Pakistan. In addition to the present study, (Zaidi et al., 2002) also reported the presence of REEs in Pakistani areca nut at trace levels. It may be evoked that the concentration levels of different elements in plants are determined not only by the edaphic factors and pedogenic

processes of terrestrial environments, especially the composition of soils in principle, but also by the inherent nature of plant species (Carpenter et al., 2019).

Extensive dietary aluminium intake induces aging process, and it also impairs the mitochondrial energy homeostasis of brain. The impaired mitochondrial functions are implicated in metallo-dyshomeostasis in general, iron dyshomeostasis in particular (Raven et al., 2013). Concomitantly, the deranged oxidative stress response along with oxidative stress induced neurotoxicity in brain entails Alzheimer's disease (Wu et al., 2012; Ayton et al., 2013). Chromium ion is an essential nutrient required by the human body at trace levels to potentiate the action of insulin which is essential for the glucose, fat and protein metabolism (Beyersmann and Hartwig, 2008). Although chromium ion is a micro-nutrient, yet chronic exposure to Cr species at relatively high levels can have negative health consequences such as dermatitis, bronchial asthma, ulceration in nasal septum and lung cancers. The toxicity of Cr is most likely due to the adduct forming ability of Cr(III) with bio-macromolecules (Zhitkovich, 2005).

Intriguingly, *in vitro* studies revealed that copper can potentiate the cytotoxicity of areca nut extract most likely due to the synergetic interaction of copper with organic components of areca nut. Indeed, Cu^{2+} species present areca nut extract, under alkaline conditions can relatively easily form coordination complexes with the areca nut alkaloids and these copper complexes were shown to exasperate reactive oxygen species generation (Khan et al., 2015). In a previous study that measured Cu content in areca nuts, the Cu concentrations were in moderate levels yet according to that report the concentrations of Cu in areca nut could be correlated significantly to the incidence of oral submucous fibrosis in areca nut users (Trivedy et al., 1997). Exposure to nickel species through oral ingestion induces allergic dermatitis, neurotoxicity, kidney diseases and mitochondrial energy homeostasis dysfunctions. Although the putative molecular mechanisms are not clearly understood, the impaired mitochondrial membrane potential, attenuated ATP content, and increased mtDNA damage most likely cause nickel-induced mitochondrial dysfunctions (Zhao et al., 2009; Zambelli and Ciurli, 2013). By virtue of the similarity in ionic radii, Ni^{2+} (0.69 Å) can be a surrogate of Mg^{2+} (0.72 Å),

interfering with the Mg^{2+} ion binding proteins' functions. In addition, International Agency for research on cancers has classified metallic nickel and nickel-containing alloys as group 2B carcinogen (possibly carcinogenic to humans), whereas both insoluble and soluble nickel compounds are classified as group 1 carcinogens (proven human carcinogens) (IARC, 2012).

Chronic antimony exposure manifests as pleural adhesions possibly through the inflammation of the pleural cavity, nephritis, pancreatitis, dermatitis, and cardiovascular diseases. In general, antimony exist as a co-contaminant with arsenic and it may possibly cause damage through interaction with sulfhydryl groups of proteins and the concomitant dysfunctions of proteins in general, antioxidant systems in particular. Chronic arsenic exposure poses increased risks of diabetes mellitus, dermatitis, peripheral neuropathy, inflammation of liver, chronic kidney disease, and cancers of kidney, bladder, and skin. Cancers associated with long-term exposure to arsenic probably involves impaired DNA repair capacity, altered DNA methylation patterns, and increased DNA damage linked to inhibitions of DNA damage mechanisms through persistent interactions of As with sulfhydryl groups of DNA repair proteins (Beyersmann and Hartwig, 2008; Ng, 2005).

Even though as a metal-cofactor of vitamin B_{12} , Cobalt (II) species is essential in trace levels, beyond critical concentrations, through dietary factors, Co^{2+} can be toxic (Hartwig and Schwerdtle, 2002). Co^{2+} (0.75 Å) ions at higher concentrations, can substitute Zn^{2+} (0.74 Å) as both ions are having near identical ionic radii and as a result, Co^{2+} can interferes with the Zn^{2+} containing proteins, especially proteins of DNA machinery (DNA transcription factors and DNA repair proteins) in nucleus (Kasten et al., 1997). Moreover, Co^{2+} can facilitate the generation of ROS from pro-oxidant like H_2O_2 by Fenton-type reactions and thus cobalt compounds are capable of inducing oxidative DNA damage in kidney liver and lung tissues (IARC, 2006). Although Co^{2+} species does not exhibit any known genotoxicity, it can potentiate the mutagenicity of genotoxic agents (Kopera et al., 2004).

Chromium ion is an essential nutrient required by the human body at trace levels to potentiate the action of insulin which is essential for the glucose, fat and protein metabolism. Although chromium ion is a micro-nutrient, yet chronic

exposure to Cr species at relatively high levels can have negative health consequences such as dermatitis, bronchial asthma, ulceration in nasal septum and lung cancers. The toxicity of Cr is most likely due to the adduct forming ability of Cr(III) with bio-macromolecules (Zhitkovich, 2005).

Health Risk Assessment (HRA)

Health risk assessment is an important scientific tool which is used to evaluate the potential adverse health effect of human exposure to a hazardous substance such as trace elements, heavy metals through exposure from sources like water, soil, food and environmental exposure due to occupation. Trace elements can accumulate in the plant tissue and can enter the human body through dietary intake, consumption of lifestyle products, inhalation or through dermal contact. Assessing the level of exposure and the risk associated with consumption of trace element, heavy metals is crucial for protecting public health.

In this study, the non-carcinogenic and carcinogenic health risk values for toxic elements in areca nut were evaluated using equations 5-8.

Estimated daily intake (EDI)

The estimated daily intake (EDI) of ingested heavy elements for adult population based on their mean concentration values and consumption rate (normal or extensive users), followed in the decreasing order: Mn>Al>Cu>Ni>Cr>As>Co>U>Sb. Our results indicated that most likely no serious health risks posed by exposure to heavy elements through areca nut chewing. In addition, targeted hazard quotient (THQ) and hazard index (HI) values were calculated (Slezakova et al., 2014) for assessing the potential health risks associated with heavy elements exposure due to chronic areca nut chewing.

Target Hazard Quotient (THQ)

THQ is a non-carcinogenic health risk index that provides an estimate of daily oral exposure to human population in comparison with a standard reference dose, known as oral reference dose. The computed THQ values were ranging from 1.36×10^{-4} (Cr) to 1.2×10^{-1} (As) for normal areca nut chewers and ranging from 3.4×10^{-4} (Cr) to 3.01×10^{-1} (As) for extensive areca nut users. The contribution of different elements to the total THQ is summarized in the decreasing order: As>Cu>Mn>Co>Ni>U>Al>Sb>Cr. In the present study, for normal areca nut chewers no obvious non-carcinogenic health risks can be expected with prolonged heavy elements exposure. Whereas, for extensive areca nut users, the total THQ value was close to 1.0 ($\Sigma_{\text{THQ}} = 9.02 \times 10^{-1}$), indicative of a moderate non-carcinogenic health risks possibly from the

contributions of As (3.01×10^{-1}) and Cu (1.97×10^{-1}). However, the collective and interactive adverse health effects of a mixture of few heavy elements calculated as hazard index (HI) suggest that modest hazard (HI = 0.36) is expected for normal areca nut users, while for extensive areca nut chewers, moderate health hazard (HI = 0.9) is expected because of ingestion of these heavy elements via chronic areca nut chewing habits.

Target Carcinogenic Risk (TRC)

Similarly, carcinogenic health risks as estimated target carcinogenic risks (TCR) due to heavy elements exposure through areca nut chewing were calculated. For normal areca nut users, the estimated TCR values for Cr, and As, were below the threshold value of 1×10^{-4} , while for Ni ($\text{TCR}_{\text{Ni}} = 9.33 \times 10^{-4}$) a modest level of risk may be possible. However, for extensive areca nut users, daily ingestion of Cr (1.36×10^{-4}) would cause moderate carcinogenic risks, while Ni ($\text{TCR}_{\text{Ni}} = 2.33 \times 10^{-3}$) ingestion would elicit adverse carcinogenic risks because of persistent areca nut chewing (Kumar, 2019; Jeng et al., 2021; Chen et al., 2017; Sharan et al., 2012). It is worthwhile to note that the potential toxic elements uptake through other lifestyle products use in addition to areca nut chewing such as smoking tobacco products (Indian cigarettes, Myanmarese cigarettes, indigenous hand-rolled cigarettes, viz., *zozial*) use and smokeless tobacco products use (gutkha, khaini, tuibur) or nut-c (tobacco leaves aqueous extract dipped betel quid) use were not taken into consideration in addition to consumption coffee or tea. Thus, if other lifestyle products use is also considered for the calculation of THQ, then the putative risk values would be presumably different than the calculated values.

Table 3.2.3 (c): Estimated daily intake (EDI) for different heavy elements
in ($\mu\text{g}/\text{kg bw}/\text{day}$)

Elements	EDI 2 nuts	EDI 5 NUTS
Cu	3.15×10^{-3}	7.9×10^{-3}
Al	6.02×10^{-3}	0.015
As	3.61×10^{-5}	9.02×10^{-5}
Cr	2.04×10^{-4}	5.1×10^{-4}
Mn	7.2×10^{-3}	0.018
Ni	5.5×10^{-4}	1.37×10^{-3}
Co	1.29×10^{-5}	3.22×10^{-5}
Sb	2.32×10^{-6}	5.8×10^{-6}
U	5.15×10^{-6}	1.29×10^{-5}

Table 3.2.3(d): Calculated Target Hazard Quotient (THQ) for different heavy elements

Elements	THQ 2 nuts	THQ 5 nuts
Cu	0.079	0.197
Al	6.02×10^{-3}	0.015
As	0.12	0.301
Cr	1.36×10^{-4}	3.4×10^{-4}
Mn	0.051	0.13
Ni	0.027	0.07
Co	0.043	0.11
Sb	5.8×10^{-3}	0.015
U	0.026	0.064

Table 3.2.3 (e): Calculated Target Carcinogenic Risk (TRC) due to areca nut chewing

Elements	TCR - 2 nuts	TCR - 5 nuts
Cr	5.41×10^{-5}	1.36×10^{-4}
As	1.02×10^{-5}	2.54×10^{-5}
Ni	9.33×10^{-4}	2.33×10^{-3}

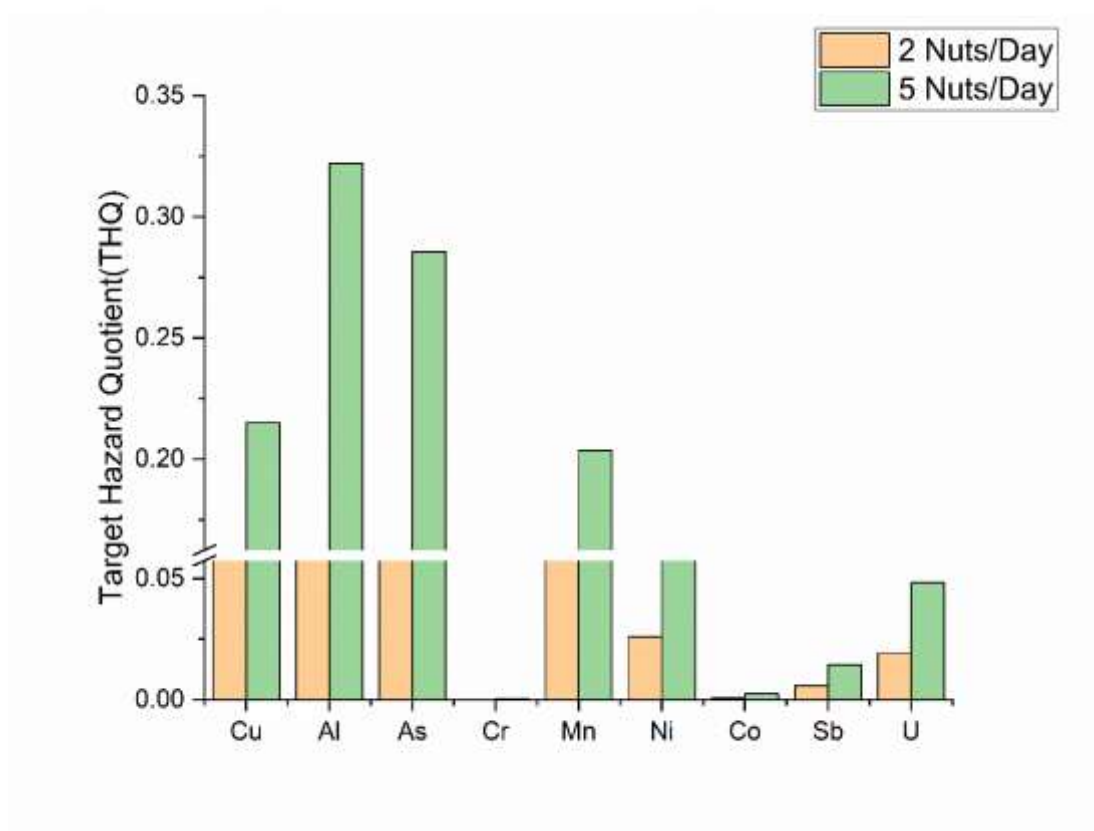


Figure 3.2.3(d): Target hazard quotient comparison of 2 nut and 5 nut per day

Principal component analysis was performed to identify the hidden patterns within elemental compositions of areca nuts (Al, Ca, Mg, Mn, Na, Rb and Zn) cultivated in different Asian countries and possibly distinguish the elemental content of areca nuts based on their geographical origin. In the biplot, PC1 represents 58.2% of the total variance while PC2 represents 14.2% of the total variance. In the PCA plot the vector represents the influence of variables (loadings) on the samples (scores) location in the plot. Compared to other previously published data on areca nut samples of Asia, it can be seen that Mn, Zn, Ca, and Mg are the main component of PC1, where Ca and Mg exert moderate influence on Northeast India and Myanmar samples, while Mn, and Zn lie in the positive side, and they also exert strong influence on Taiwan samples. Besides, with PC2 we observed that Rb, being on the positive side, has a significant influence on Pakistan sample, while Al on the negative side exerts modest influence on Myanmar sample. It is clear that by applying PCA, in the biplot we can distinctly classify the areca nut samples into groups, in terms of their geographical origins since the absorption and transfer of different elements by plants from the soil is primarily influenced by soil profile such as soil pH, geochemical attributes, pedogenic factors, and clay contents (Kashem and Singh, 2001; Zeng et al., 2011; Pepi and Vaccaro, 2018).

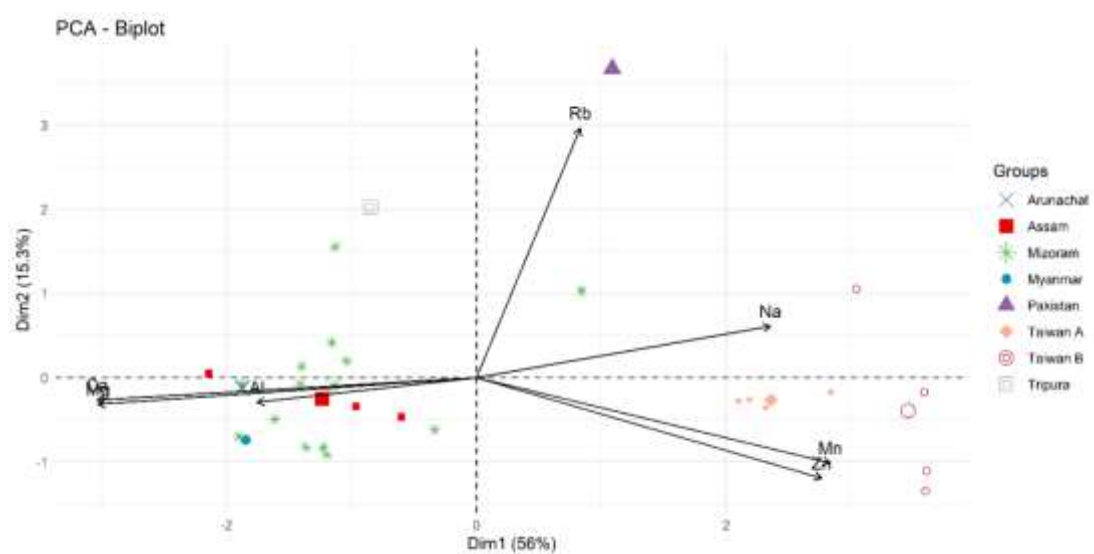


Figure 2.3.4: PCA Biplot of present study with published element data of areca nut

3.3 Tobacco Sample

3.3.1 Analysis of Soil Parameter

3.3.1.1 Soil pH measurement

The pH of the soil table (Table 3.3.1.1) collected from tobacco plantation was measured and ranges from almost neutral with a pH of 6.93 to strongly acidic with pH of 4.3, indicating that the tobacco were mostly grown in an acidic soil.

3.3.1.2 Determination of Soil NPK from tobacco plantation

From the obtained soil available NPK was determined, the available Nitrogen values range from 281.5 kg/ha – 534.4 kg/ha, by comparing with the reference range we can observed that T6 is having high abundance of available nitrogen 534.4 kg/ha, whereas the lowest available nitrogen content was observed in T1 with 281.5 kg/ha. We can observe that with compared to the NPK reference range the available nitrogen in all the samples were within high and medium range. The Available phosphorous ranges from 9.11kg/ha - 275.8 kg/ha, showing that T3 soil is having very less available Phosphorous as compared to the reference range. The observed available Potassium ranges from 84 kg/ha - 209.4 kg/ha, reflecting that the K content is below average with respect to the reference range. This reflects that the tobacco was grown without any addition of fertilizers.

3.3.1.3 Determination of soil organic matter (SOM) from tobacco plantation

The soil organic matter ranges from 3.2 – 5.9 %, with T6 plantation site having the highest content of soil organic matter while T5 having the lowest range of 3.2 %. We can observe that the overall soil organic matter content was above average implicating a good soil microbial activity

Table 3.3.1.1: Measured soil pH from tobacco plantation

SAMPLE ID	Soil pH
T1	6.93
T2	5.28
T3	5.25
T4	5.54
T5	5.0
T6	5.19
T7	4.7
T8	4.38

Table 3.3.1.2: Determination of available NPK from tobacco plantation

Sample ID	N (kg/ha)	P (kg/ha)	K (kg/ha)
T1	281.5	79.1	146.7
T2	325.2	28.9	135.5
T3	379.4	9.1	161.3
T4	379.4	21.9	86.3
T5	454.6	169.5	84
T6	534.4	17.9	209.5
T7	408	201.7	136.7
T8	465.2	275.8	93

Table 3.3.1.3: Observed soil organic matter from tobacco plantation

SAMPLE ID	% of Soil Organic Matter
T1	4.4
T2	3.3
T3	4.3
T4	5.0
T5	3.2
T6	5.9
T7	3.6
T8	3.5

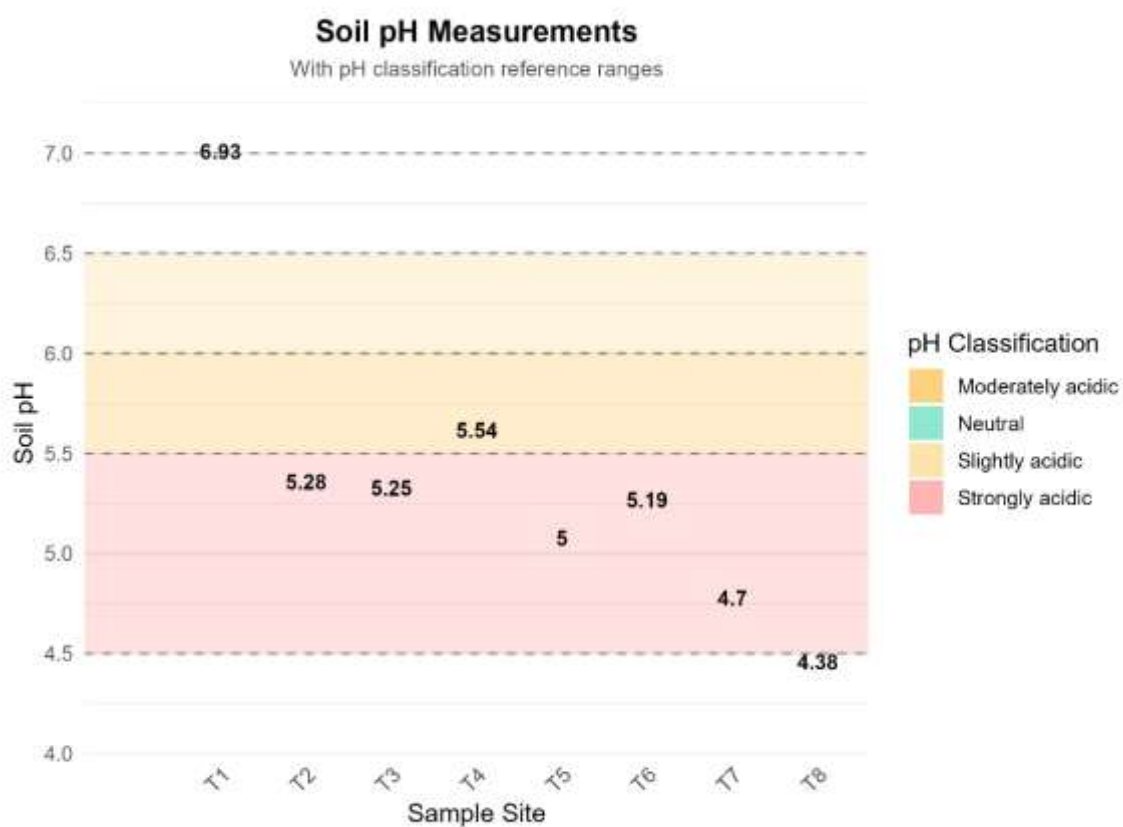


Figure 3.3.1.1: Soil pH from tobacco plantation

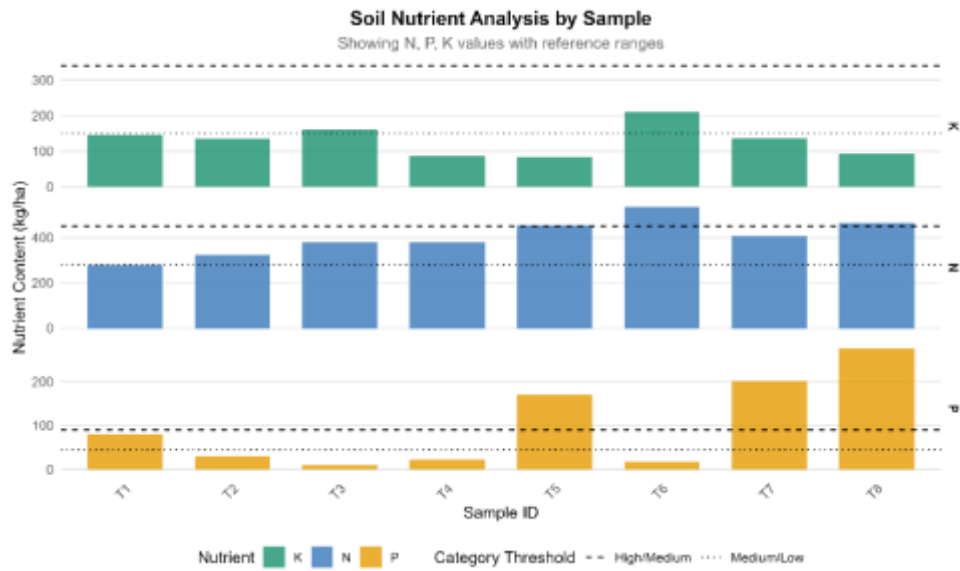


Figure 3.3.1.2: Determined available NPK from tobacco plantation

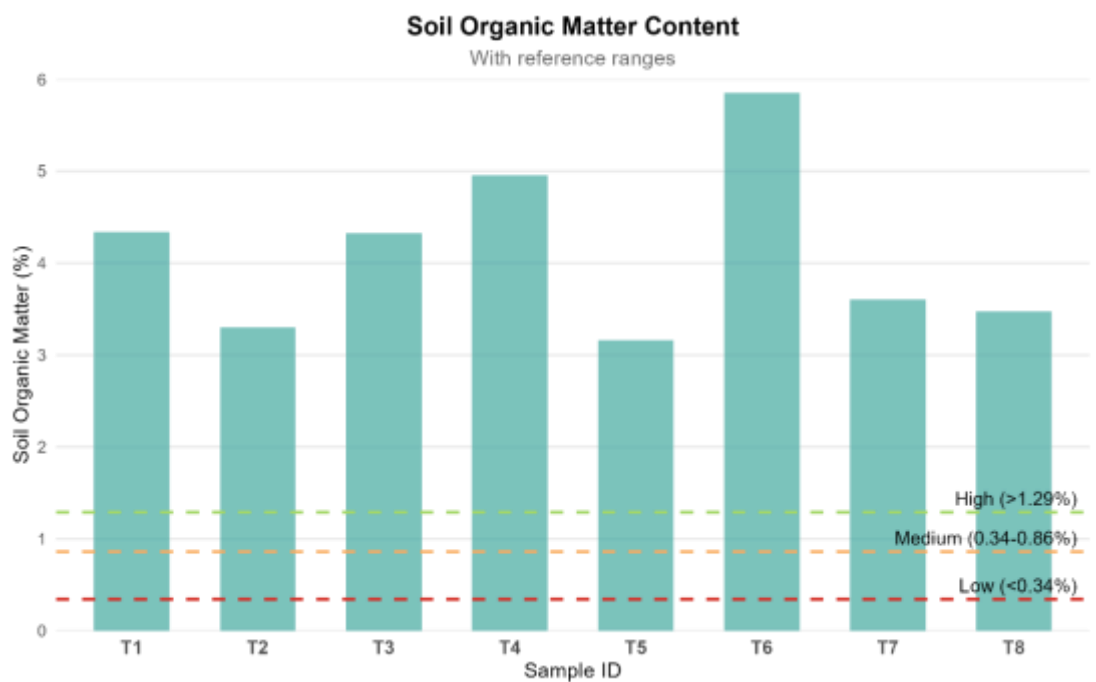


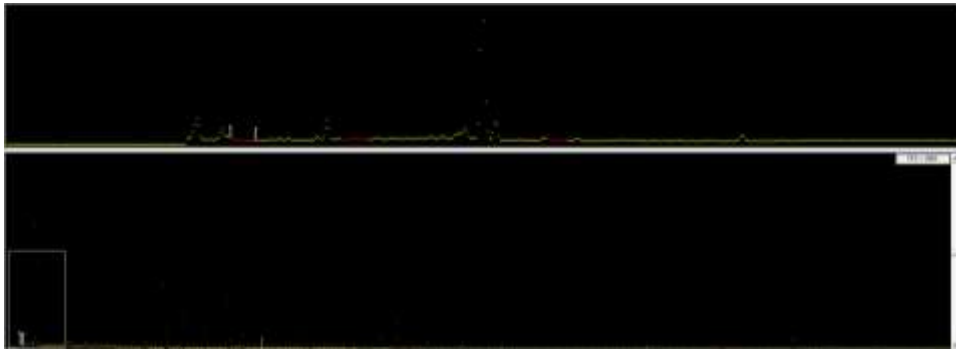
Figure 3.3.1.3: Estimated soil organic matter from tobacco plantation

3.3.2. Elemental analysis by Instrumental Neutron Activation Analysis (INAA).

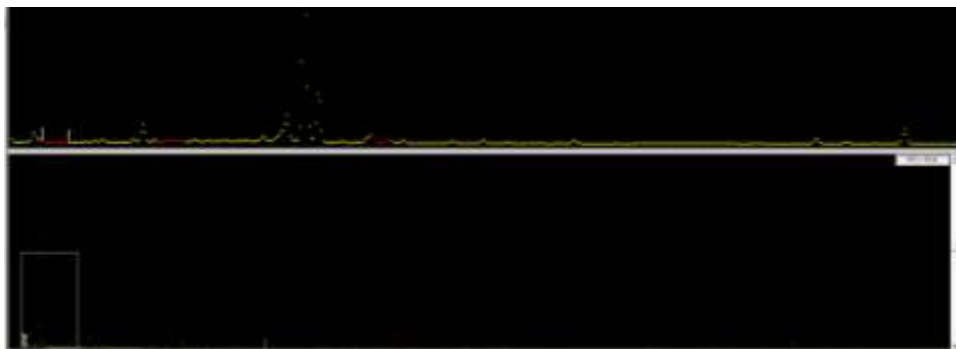
By using INAA, we have performed extensive soil elemental analysis of Tobacco grown in Mizoram, India for the first time. A total of 37 elements were determined besides, essential mineral elements, as well as nonessential trace elements along with the rare-earth elements (REEs) are broadly distributed in soil see (Table 3.3.2 (a)). In the acidic soils of Mizoram, the quantitative mean mineral and non-essential elemental contents are present in the following order: Si > Al > Fe > Mg > K > Ti > Na > Ca > Mn > Ba > Zr > Cu > Rb > Cr > V > Cl > Zn > Ce > Sr > Ni > La > Nd > Co > Th > Sc > Pb > As > Hf > Br > Sm > Cs > Dy > Yb > U > W > Eu > Ta > Tb > Sb > Cd. Among the studied elements determined in the soil samples, Cl exhibited the highest coefficient of variance, followed by Ca, Cu, and Br, respectively, whereas Ti, Pb, and REEs had the lowest coefficient of variance. For comparison studies we have taken the data of elemental concentration of tobacco leaf from the thesis (Lalrammawia 2022)

3.3.3 Heavy metal analysis by Atomic Absorption Spectrometry (AAS)

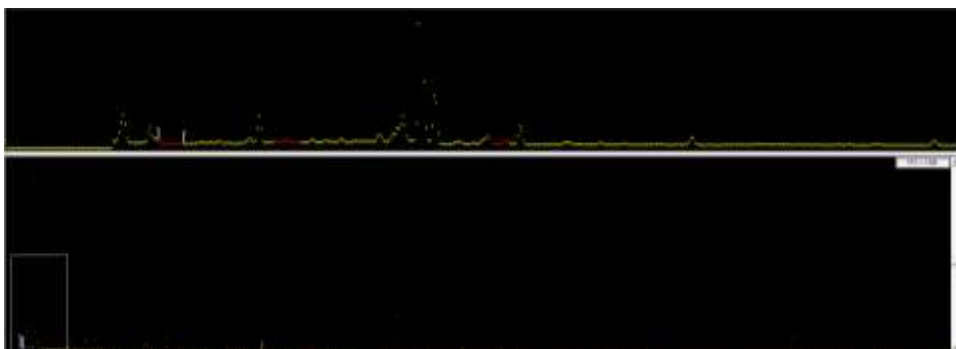
AAS method was applied for determining the heavy metals viz., Cu, Cd and Pb from the soil of tobacco plantation as well as from the root and stem of tobacco samples. The concentration ranges were given in the Table. From the AAS data, presence of cadmium (Cd) at a concentration ranging between 0.018-0.079 µg/g, copper (Cu) 7.99-21.98 µg/g, lead (Pb) 9.14-13.185 µg/g in the soil samples with mean value of cadmium 0.05 µg/g, copper (Cu) 13.368 µg/g and lead (Pb) 11.245 µg/g. From the elemental analyzed copper is the most abundant followed by lead and cadmium the least abundant in the soil of tobacco plantation site. The heavy metal concentration was also assessed in the root and stem of the tobacco plant which ranges viz., cadmium 0.039-0.119 µg/g, copper 4.86-12.25 µg/g, lead 0.279-1.818 µg/g for the roots and cadmium 0.043-0.204 µg/g, copper 2.51-15.43 µg/g, lead 0.292-2.073 µg/g for the stem, it can be seen from the (Table 3.3.3 (b), (c)) that the mean concentration of cadmium and copper from root and stem is higher in the stem by a factor of 1.31 and 1.1, respectively



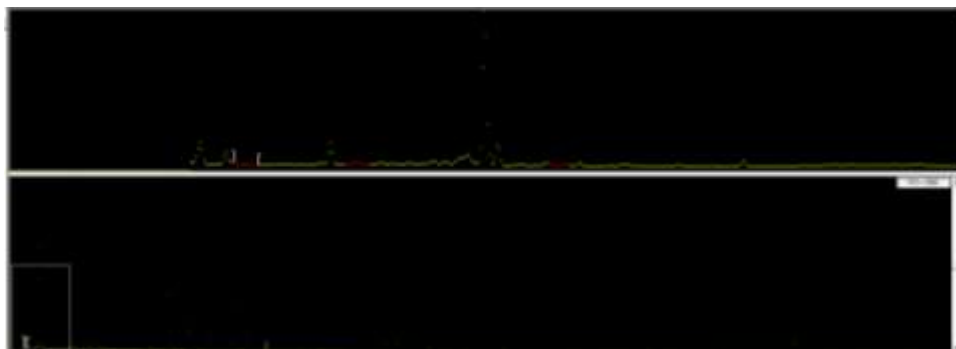
(a)



(b)



(c)



(d)

Figure 3.3.2: The raw γ -energy decay spectral data (raw spectral data) for (a) tobacco leaves, (b) stem, (c) roots and (d) the corresponding soil samples of Mizoram

Table 3.3.2 (a): The concentration of elements in soil using INAA (in µg/g)

Elements	Soil (n=8)	
	Range (µg/g)	Mean ±SD
Na	2730-5680	3911.3 ± 1283
Mg	17000-30200	21900 ± 4901
Al	59000-103000	72175 ± 15108
Si	40400-395000	339500 ± 68197
Cl	43-275	98.5 ± 76
K	11800-25500	18600 ± 4530
Ca	633-2870	1885 ± 1052
Sc	10-16	13.5 ± 2.4
Ti	3670-5620	4864 ± 623
V	80-149	104± 25
Cr	75-142	105 ± 20
Mn	503-1700	1047 ± 387
Fe	31500-54400	41788 ± 7463
Co	15-23	20.3 ± 7.2
Ni	36-62	49 ± 8.4
Zn	58-124	88 ± 24.4
As	6.0-12	8.8 ± 2.0
Br	2.0-15	7.7 ± 3.9
Rb	89-171	134 ± 29
Sr	45-90	68.5 ± 14
Zr	160-170	225 ± 41
Sb	0.36-0.71	0.57 ± 0.1
Cs	4.0-9.0	6.6 ± 1.9
Ba	338-520	446 ± 73
La	32-44	40.6 ± 4.3
Ce	64-91	83.6 ± 8.7
Nd	23-42	34 ± 6.2
Sm	5.0-8.0	6.8 ± 1.2
Eu	0.74-1.44	1.23 ± 0.24
Tb	0.57-1.03	0.9 ± 0.17
Dy	3.0-7.0	4.8 ± 1.3
Yb	2.2-3.5	3.1 ± 0.4
Hf	6.0-9.0	7.8 ± 1.2
Ta	0.94-1.25	1.15 ± 0.09
W	1.8-2.3	2.1 ± 0.2
Th	15-18	16.1 ± 1.3
U	2.7-3.2	2.96 ± 0.17

Table 3.3.2 (b): The concentration of elements in tobacco root using INAA (in $\mu\text{g/g}$)

Elements	Tobacco root (n=8)	
	Range	Mean $\pm$ SD
Na	290-2500	846 $\pm$ 862
Mg	990-4060	2524 $\pm$ 853
Al	944-2940	2680.5 $\pm$ 2016
Si	4950-29700	14306 $\pm$ 7071
Cl	1360-14000	6073 $\pm$ 4955
K	20200-64000	33725 $\pm$ 14866
Ca	6080-14100	9248 $\pm$ 3228
Sc	0.3-1.7	0.7 $\pm$ 0.48
Ti	49-259	186 $\pm$ 128
V	1 - 10	4 $\pm$ 3
Cr	03 - 12	6.8 $\pm$ 3.8
Mn	24-100	62.8 $\pm$ 28
Fe	713-4490	1798 $\pm$ 1235
Co	0.7-2.2	1.196 $\pm$ 0.5
Ni	02 - 06	4.9 $\pm$ 1.5
Zn	17-115	43 $\pm$ 32
As	0.19-1.1	0.42 $\pm$ 0.3
Br	05 - 40	21.2 $\pm$ 13
Rb	09 - 31	17.6 $\pm$ 8.4
Sr	54-146	91 $\pm$ 30
Zr	03 - 27	11.4 $\pm$ 8.3
Sb	0.003-0.16	0.071 $\pm$ 0.04
Cs	0.15-0.87	0.36 $\pm$ 0.23
Ba	54-126	77.7 $\pm$ 32.1
La	1.0-5.3	2.4 $\pm$ 1.5
Ce	02 - 10	4.4 $\pm$ 3
Nd	1 - 4	1.7 $\pm$ 1.18
Sm	0.1-1	0.4 $\pm$ 0.28
Eu	0.03-0.18	0.07 $\pm$ 0.05
Tb	0.02-0.13	0.05 $\pm$ 0.04
Dy	0.21-0.69	0.36 $\pm$ 0.15
Yb	0.07-0.51	0.2 $\pm$ 0.15
Hf	0.09-1.01	0.44 $\pm$ 0.33
Ta	0.02 - 0.13	0.06 $\pm$ 0.04
W	0.1-0.24	0.15 $\pm$ 0.06
Th	0.33-1.9	0.82 $\pm$ 0.6
U	0.05-0.38	0.17 $\pm$ 0.11

Table 3.3.2 (c): The concentration of elements in tobacco stem using INAA
(in µg/g)

Elements	Tobacco stem (n=8)	
	Range	Mean ±SD
Na	78-1890	561± 680
Mg	1180-6100	3054 ± 1732
Al	421-3780	1379 ± 1104
Si	6100-20100	11908 ± 5015
Cl	776-26300	11666 ± 10132
K	25600-117000	49900 ± 29717
Ca	3930-13500	7517 ± 3604
Sc	0.12-0.93	0.4 ± 0.3
Ti	31-248	108 ± 66
V	0.59-5.9	2.0 ± 1.7
Cr	1.58-10.3	3.8 ± 2.8
Mn	17.5-91	46 ± 26
Fe	870-3660	1052 ± 1091
Co	0.54-1.22	0.7 ± 0.3
Ni	3.25-7.7	4.2 ± 1.9
Zn	26.5-155	54.7 ± 43.8
As	0.32-0.9	0.31 ± 0.23
Br	12.4-98	42.3 ± 32.6
Rb	8.2-56	20 ± 15.4
Sr	68-130	79.2 ± 26.5
Zr	6.8-17	6.8 ± 4.9
Sb	0.048-0.23	0.06 ± 0.07
Cs	0.188-0.43	0.17 ± 0.12
Ba	61-165	98 ± 44
La	1.34-3.26	1.5 ± 0.87
Ce	2.36-6.2	2.7 ± 1.72
Nd	1-1.6	1.1 ± 0.23
Sm	0.28-0.48	0.32 ± 0.13
Eu	0.042-0.11	0.06 ± 0.03
Tb	0.028-0.06	0.033 ± 0.02
Dy	0.23-0.41	0.31 ± 0.09
Yb	0.1-0.27	0.12 ± 0.07
Hf	0.3-0.63	0.42 ± 0.19
Ta	0.041-0.1	0.06 ± 0.03
W	0.16-0.43	0.3 ± 0.13
Th	0.51-1.23	0.8 ± 0.36
U	0.092-0.23	0.1 ± 0.06

3.3.2 (d): The concentration of elements in tobacco leaves (Lalrammawia, 2022) for comparison study (in µg/g)

Elements	Tobacco leaves (n=8)	
	Range	Mean ±SD
Na*	129-753	411 ± 268
Mg*	4900-11600	8563 ± 2117
Al*	599-3140	1446 ± 829
Si*	15900-64600	37500 ± 15303
Cl*	643-42100	16927 ± 15476
K*	33400-64000	49050 ± 11310
Ca*	18600-35900	26050 ± 7336
Sc*	0.1-0.8	0.34 ± 0.22
Ti*	61-172	114.3 ± 35
V*	0.9-1.8	1.7 ± 1.3
Cr*	2.0-8.0	4.3 ± 2.1
Mn*	107-681	215 ± 191
Fe*	442-3390	1184 ± 951
Co*	0.8-3.5	1.32 ± 0.9
Ni*	2.0-10.0	5.8 ± 2.5
Zn*	42-265	150 ± 71
As*	0.2-0.9	0.4 ± 0.2
Br*	25-129	62 ± 35
Rb*	7.0-31.0	21 ± 9.3
Sr*	130-221	173.3 ± 34
Zr*	4.0-13.0	7.3 ± 3
Sb*	0.05-0.20	0.1 ± 0.06
Cs*	0.07-0.30	0.16 ± 0.08
Ba*	107-241	159 ± 45
La*	2.0-5.0	3.1 ± 1.1
Ce*	2.0-7.0	3.5 ± 1.7
Nd*	1.0-4.0	1.8 ± 1
Sm*	0.3-0.8	0.4 ± 0.2
Eu*	0.04-0.16	0.07 ± 0.04
Tb*	0.02-0.09	0.04 ± 0.02
Dy*	0.31-0.75	0.5 ± 0.2
Yb*	0.31-0.75	0.1 ± 0.1
Hf*	0.19-0.52	0.3 ± 0.12
Ta*	0.013-0.072	0.03 ± 0.02
W*	0.2-0.42	0.3 ± 0.1
Th*	0.17-1.03	0.43 ± 0.3
U*	0.04-0.23	0.1 ± 0.06
Cd [†]	0.182-0.751	0.4 ± 0.2
Cu [†]	06.5-43.2	20 ± 15
Pb [†]	0.6-2.1	1.2 ± 0.5

*INAA ; [†]AAS

Table 3.3.3 (a): Heavy metal concentration levels in soil of tobacco plantation by using AAS (in $\mu\text{g/g}$)

Elements	Soil (n=8)	
	Range ($\mu\text{g/g}$)	Mean $\pm$ SD
Cd	0.02-0.08	0.05 $\pm$ 0.03
Cu	8 - 22	14 $\pm$ 5
Pb	9.2-13.2	11.5 $\pm$ 1.5

Table 3.3.3 (b): Heavy metal concentration levels of tobacco root by using AAS (in $\mu\text{g/g}$)

Elements	Tobacco root (n=8)	
	Range ($\mu\text{g/g}$)	Mean $\pm$ SD
Cd	0.04-0.12	0.07 $\pm$ 0.03
Cu	4.9-12.3	7.7 $\pm$ 4.5
Pb	0.28-1.8	0.8 $\pm$ 0.5

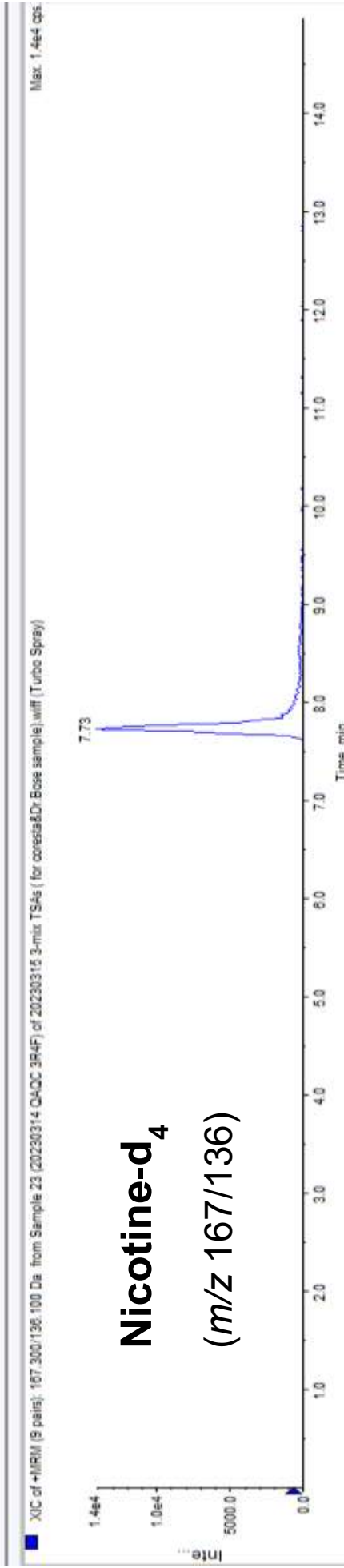
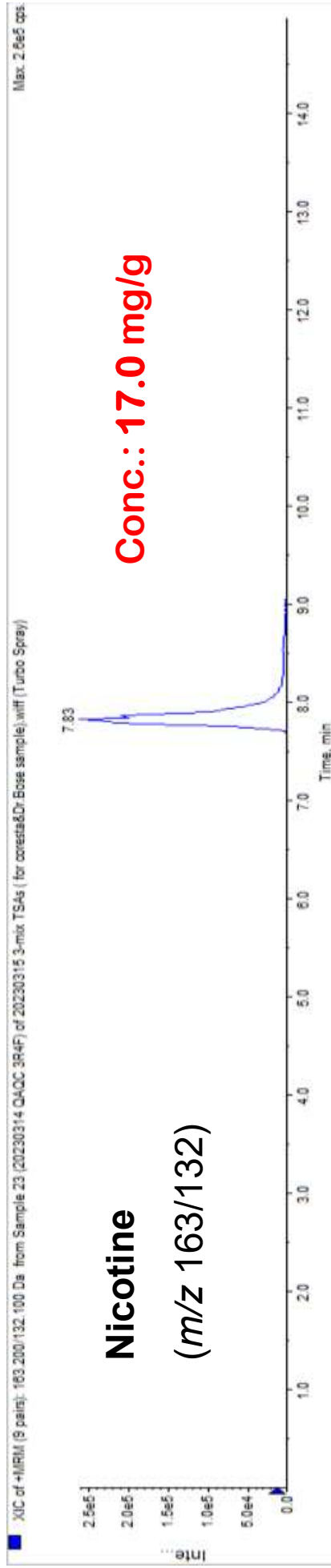
Table 3.3.3 (c): Heavy metal concentration levels of tobacco stem by using AAS (in $\mu\text{g/g}$)

Elements	Tobacco stem (n=8)	
	Range ($\mu\text{g/g}$)	Mean $\pm$ SD
Cd	0.04-0.2	0.09 $\pm$ 0.06
Cu	2.5-15.4	8.4 $\pm$ 6.1
Pb	0.3-2.1	0.7 $\pm$ 0.6

3.3.4 Determination of carcinogens compounds in smoking product by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

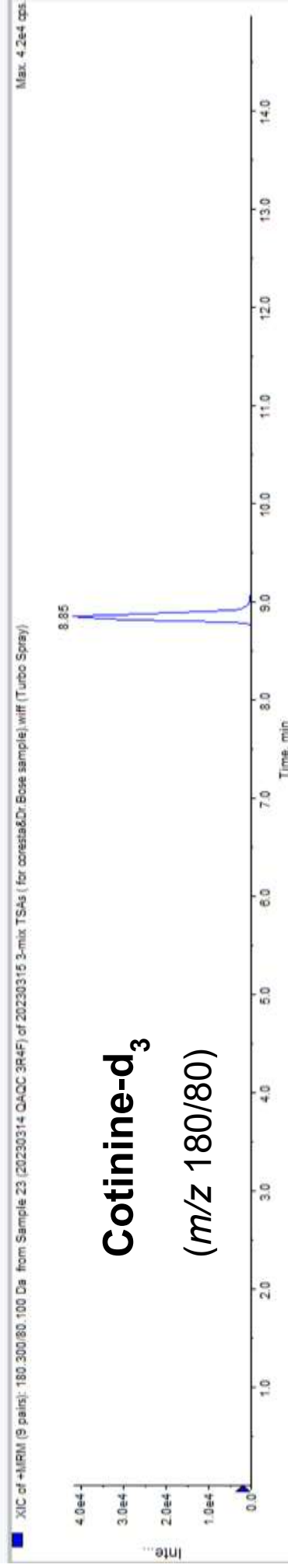
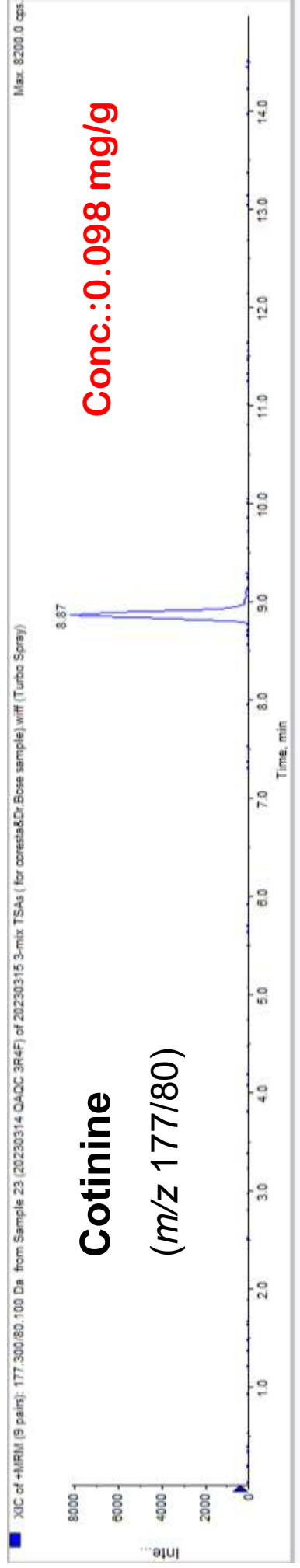
LC-MS/MS method is used for determining the tobacco specific alkaloids (TSAs) and tobacco specific *N*-nitrosamines (TSNAs) present in commercially available tobacco smoking products like zozial, beedi, commercial Indian and Myanmar cigarette, tobacco leaf filler and hookah by using 3R4F as reference. TSNAs like Nicotine and Cotinine along with TSNAs viz., N-nitrosonornicotine (NNN), Nicotine-derived nitrosamine ketone (NNK) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were detected by LC-MS/MS from the sample analyzed and are given in the table below (Table 3.3.4 (a), (b), (c)).

Tobacco alkaloids; **Conc.: 200 ng/mL (a Mixture of Standards)**



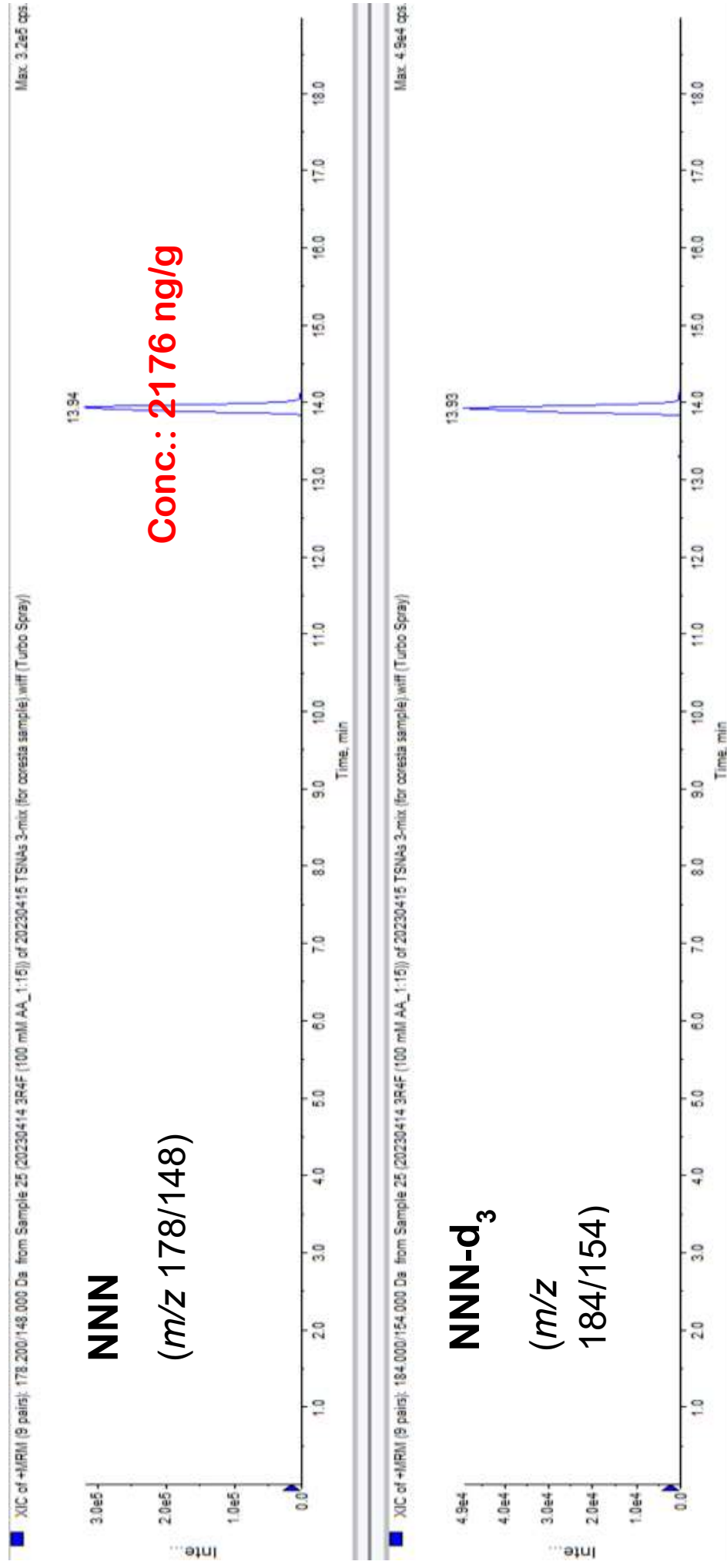
(a)

Tobacco alkaloids; **Conc.: 200 ng/mL (a Mixture of Standards)**



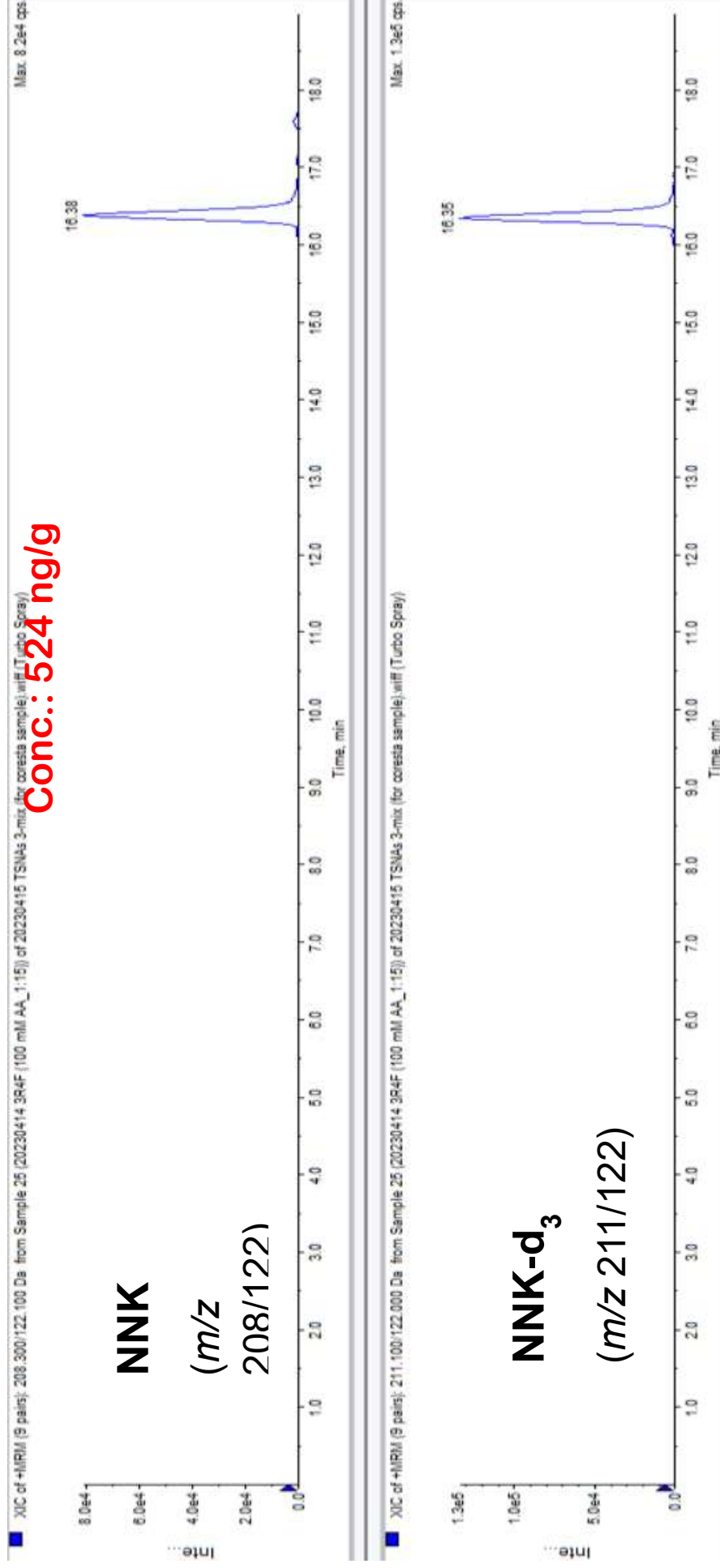
(b)

Tobacco-specific nitrosamines; **Conc.: 0.05 ng/mL (a Mixture of Standards)**



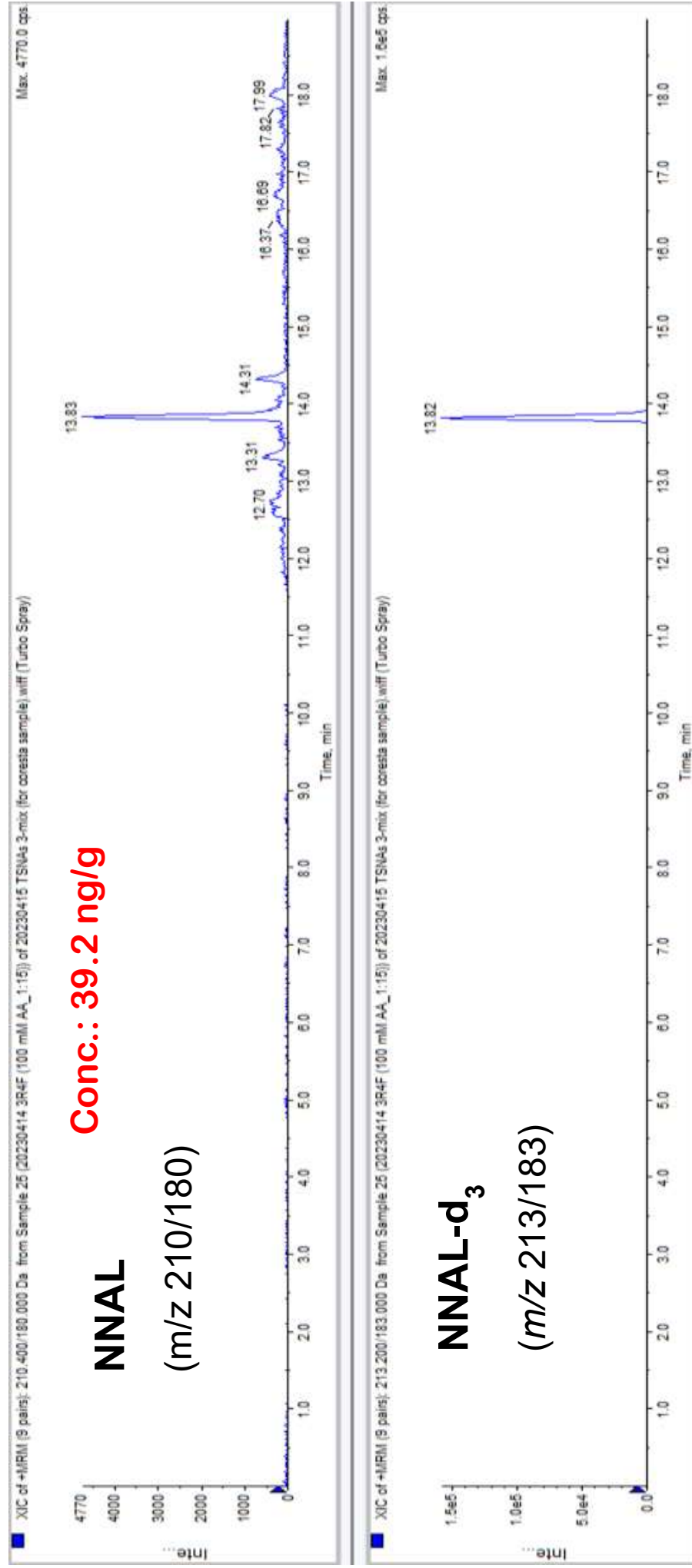
(c)

Tobacco-specific nitrosamines; **Conc.: 0.05 ng/mL (a Mixture of Standards)**



(d)

Tobacco-specific nitrosamines; **Conc.: 0.05 ng/mL (a Mixture of Standards)**



(e)

Figure 3.3.4 : The chromatogram profiles of alkaloids and nitrosamines in 3R4F reference cigarette using the internal calibration method (a) Nicotine, (b) Cotinine, (c) NNN (d) NNK , (e) NNAL

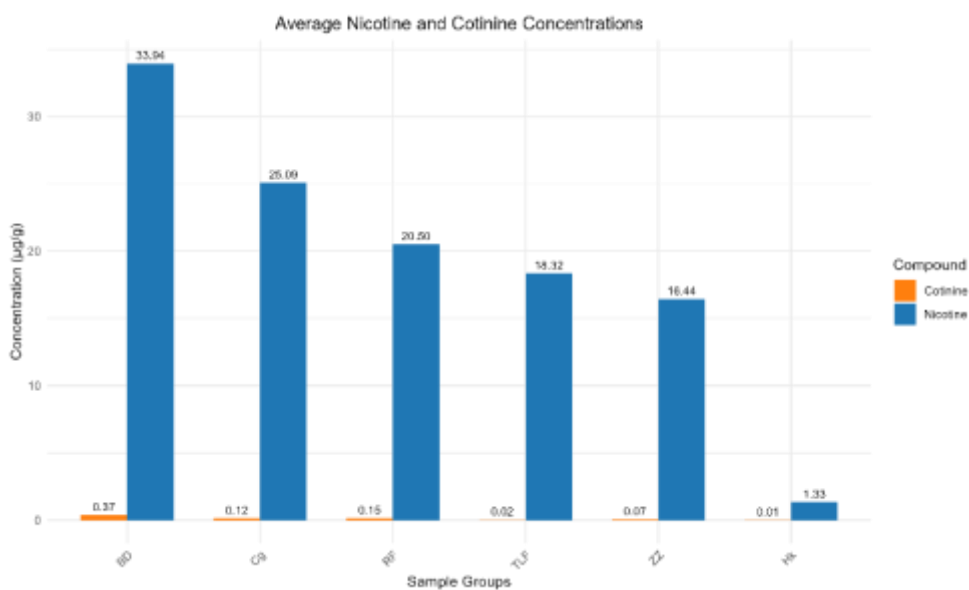


Figure 3.3.4 (a): Average concentration of Tobacco specific alkaloids (TSAs) with 3R4F reference

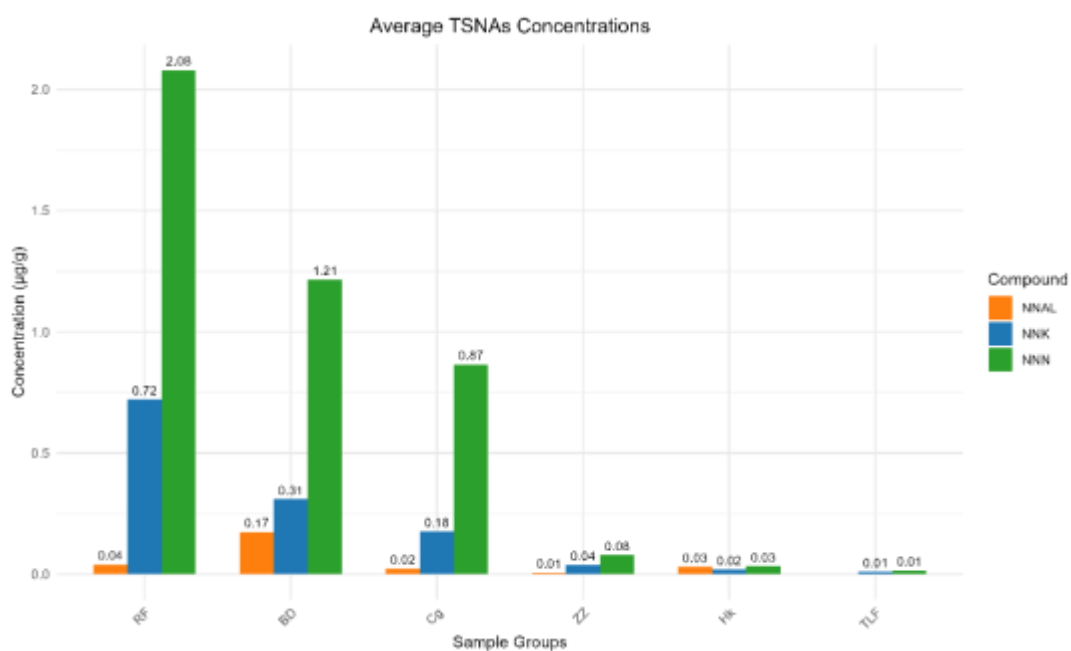


Figure 3.3.4 (b): Average concentration of Tobacco-Specific Nitrosamines (TSNAs) with 3R4F reference

Table 3.3.4 (a): Tobacco Specific Alkaloids (TSAs) using LC-MS/MS

SampleID	Tobacco Specific Alkaloids (TSAs) ($\mu\text{g/g}$)	
	Nicotine	Cotinine
ZZ1	20.8	0.06
ZZ2	11.5	0.11
ZZ3	18.4	0.03
ZZ4	18.5	0.05
ZZ5	13.0	0.08
BD1	38.5	0.44
BD2	36.4	0.31
BD3	21.1	0.16
BD4	33.2	0.32
BD5	43.1	0.42
BD6	28.0	0.5
BD7	37.3	0.5
Cg1	24.0	0.15
Cg2	25.8	0.10
Cg3	24.2	0.1
Cg4	26.4	0.1
Cg5	23.4	0.16
Cg6	27.7	0.14
Cg7	24.3	0.2
Cg8	24.9	0.06
TLF1	17.8	0.02
TLF2	20.7	0.02
TLF3	15.8	0.02
TLF4	19.0	0.03
Hk1	2.4	0.03
Hk2	0.8	0.002
Hk3	0.8	0.003
3R4F	20.5	0.15

Table 3.3.4 (b): Tobacco Specific N-nitrosamines (TSNAs) using LC-MS/MS

SampleID	Tobacco Specific N-nitrosamines (TSNAs) (µg/g)		
	NNK	NNAL	NNN
ZZ1	0.03	0.004	0.1
ZZ2	0.04	0.01	0.15
ZZ3	N. D	N. D	0.02
ZZ4	0.02	0.004	0.04
ZZ5	0.06	0.01	0.1
BD1	0.6	0.07	1.6
BD2	0.20	0.02	0.5
BD3	0.13	0.02	0.7
BD4	0.73	0.13	1.6
BD5	0.42	0.04	1.3
BD6	0.03	0.47	1.3
BD7	0.06	0.47	1.5
Cg1	0.18	0.02	4.4
Cg2	0.11	0.06	0.5
Cg3	0.07	0.01	0.3
Cg4	0.06	0.01	0.4
Cg5	0.32	0.03	0.3
Cg6	0.34	0.03	0.5
Cg7	0.3	0.02	0.5
Cg8	0.04	0.002	0.03
TLF1	N. D	N. D	0.01
TLF2	N. D	N. D	0.01
TLF3	0.01	N. D	0.02
TLF4	0.01	N. D	0.02
Hk1	0.05	0.06	0.1
Hk2	0.003	0.001	0.004
Hk3	0.004	N. D	0.002
3R4F	0.72	0.04	2.1

N.D – Not Detected

Table 3.3.4 (c): Pearson's correlation between pH, Nicotine and cotinine

	pH	Nicotine	Cotinine
pH	1		
Nicotine	-0.306 *	1	
Cotinine	0.345 **	0.575 **	1

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3.3.4 (d): Pearson's correlation between pH, NNK, NNAL and NNN

	pH	NNK	NNAL	NNN
pH	1			
NNK	0.024	1		
NNAL	0.427 **	0.653 **	1	
NNN	0.383 **	0.737 **	0.918 **	

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.01 level (2-tailed).

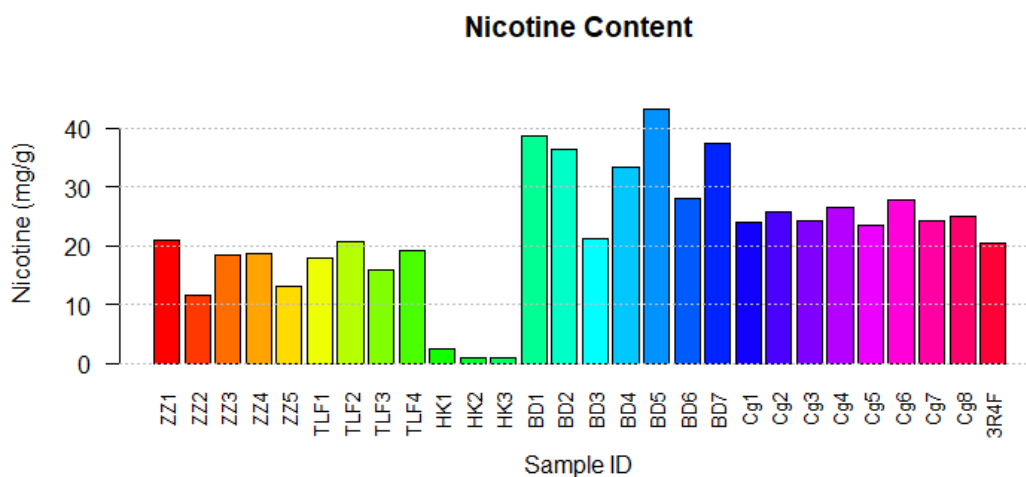


Figure 3.3.4 (c): Concentration of nicotine from commercially available smoking tobacco

3.3.5 Discussion

The data obtained from this study shows that the mean values for As, Cd, Cu, Ni, and Pb content in Mizoram soils do not exceed the permissible limits of agricultural soil of Indian Standards (Awashthi, 2000). Additionally, the mean values of As, Cd, Co, Cr, Cu, Ni, Pb and Zn was within the limit of Indian natural soil background values. These values were consistent with the values indicated by the soil guidelines of Poland, however the mean value of Fe, and Mn of Mizoram soils exceed Indian natural soil background limits (Kumar et al. 2019). In addition, the mean soil concentration levels were significantly less than the guideline values of natural soils specified by Canada, China, and Czech Republic as well as the Swedish limits in polluted soils (Michálková et al., 2017; Kumar et al., 2019). It is apparent that acidic

	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Indian natural soil background	114	209	32015	15.2	27.7	56.5	22.1	-	13.1
Indian limit for soil	-	-	-	60-110	75-150	135-270	300-600	3.0-6.0	250-500
Minima (Present Study)	75	503	31500	15	36	8	58	0.02	9.14
Maxima (Present Study)	142	1700	54400	37	62	22	124	0.8	13.2
Mean (Present Study)	105	1047	41788	20.3	49	13.4	88	0.14	11.3

soils of tobacco cultivation sites were enriched with Fe and Al.

Table 3.3.5 (a): Comparision of present data with Indian natural background soil and Indian limit for soil.

From our studies, we believe that the determined elemental composition of the soil samples depends on the prevailing natural geochemical background features of the soil in the region (Zote et al., 2021). Conversely, studies have reported that heavy elements in soil are distributed in numerous chemical forms with differential degree of mobility and bioavailability (Roberts et al., 2005; Alloway, 2013). They are essentially available in soil fractions as in the form of inorganic such as soluble, insoluble forms and also as exchangeable fractions adsorbed on the surface of mineralized metal oxides present in the soil, in addition to bound to humic acids as the organometallic coordination complex forms exhibiting various degrees of

dissolubility and mobility (Spěváčková and Kučera 1989; Orroño and Lavado, 2009). Studies have revealed that tobacco can differentially uptake various heavy elements through their roots, translocate them and simultaneously sequester them in the leaves (Bi et al., 2011; da Silva et al., 2016). Likewise, tobacco plant can also absorb heavy elements possibly which are present from the soil or from application of fertilizers, manure or through the application of pesticides and fungicides (Chauhan and Chauhan, 2014). Thus, the levels of trace elements in tobacco can increase when grown in soils, which contain high concentrations of heavy elements and by growing them in a contaminated soil (Fässler et al., 2010). The uptake of heavy metals in tobacco plant can be also influenced by agricultural practices like topping and de-suckering, employed for enhancing leaf and nicotine yield, which in turn lets the tobacco plant to absorb such as the nutrient elements and heavy elements including REEs, from the soil where it grows (Tso, 1990).

The mean concentration of elements is as follows, Sb (0.15), Cd (0.57), As (0.7), Th (0.86), V (0.9), Pb (1.52), and Co (2.7) along with REEs vary within a narrow range by a factor of ≤ 4.5 . Elemental concentration range of Cr (6.0), Ni (8.0) and Zr (9.0) exhibits moderate variation. The concentration range of elements such as Cu (36.7), Br (104), Ti (111), Zn (223), and Mn (574) display large variations whereas contents of Al (2541), Fe (2948), and Mg (6700) show a greater dispersion of concentrations. However, the range of concentrations of Ca (17,300), K (30,600), Cl (41,457), and Si (48,700) in Northeast tobacco leaves vary greatly.

For healthy development of plants essential nutrients play a major role (Kumar et al 2021). The elements that are naturally required in concentration higher than >1 ppm are considered as macronutrients, while the element which are normally required in a lower concentration <1 ppm are termed as micro-elements (Pandey, 2018). According to this N, K, Mg, Ca, S, Si and P falls under the classification of macronutrients and Na, B, Cl, Mn, Fe, Ni, Cu, Zn and Mo fall under micronutrients (Taiz et al., 2015). N and S play an important role in the development of plant growth, they serve as the major constituents of many co-enzymes and biological macromolecule. Different macro and micronutrients are taken up by the plants for their various metabolism requirements. Mg, Mn, Ca and Cl are important for the survival of plant. Similarly, elements like As, Al, Cd, Cr, Se, Ba and Pb can be accumulated and stored in the

tissue of plants and whether they are beneficial for plants are not known and are considered as non-essential elements. Likewise, tobacco plants are known to accumulate non-essential elements in their aerial parts of the plant (Taiz et al., 2015; Sharma, 2006).

As established by Markert (Markert, 1992 and Markert, 2015), the elemental composition of plants growing in uncontaminated environments can serve as reference data, which is termed the “reference plant” (RP). Plant species can be classified based on by their elemental composition, determined by their characteristic patterns of element accumulation or exclusion relative to the “reference plant”. Thereby using RP value, we can assess the “chemical fingerprint” (Markert, 2015) of any plantation growing in any kind of soil.

The relative deviation in elemental concentrations of tobacco compared to the “reference plant” (RP) is presented in the figure which illustrates the relative concentration of various inorganic elements in the NEI tobacco plant. Notably, the levels of Mn, Sb, Cs, Rb, and Pb in NEI tobacco especially in the leaves were found to be quite similar to those in the RP. Furthermore, the relative concentration of elements such as Cd, Cl, Cu, Zn, and Mg was significantly higher in the leaves than in the roots when compared to the RP. In particular, the levels of Cd and Cl in NEI tobacco leaves were at least seven times greater than those in the RP. Some elements exhibited negative relative concentrations, indicating lower concentrations in tobacco compared to RP. Interestingly, the tobacco plant did not appear to restrict the uptake of elements such as Al, Ti, Cr, Co, Ni, As, Ba, and Zr, which were found in considerably higher concentrations compared to the RP. This suggests that analysing both leaves and roots of tobacco plants could serve as a useful tool for monitoring the mobility of these elements in soil, depending on local edaphic (soil-related) conditions.

Additionally, the relative deviations in higher rare-earth element (HREE) concentrations showed a distinct pattern: HREEs were approximately twice as concentrated in roots compared to leaves, and overall levels were considerably higher than in the RP. In contrast, the lower rare-earth elements (LREEs) exhibited

greater relative deviations in both leaves and roots, with average concentrations at least five times higher than those in the RP.

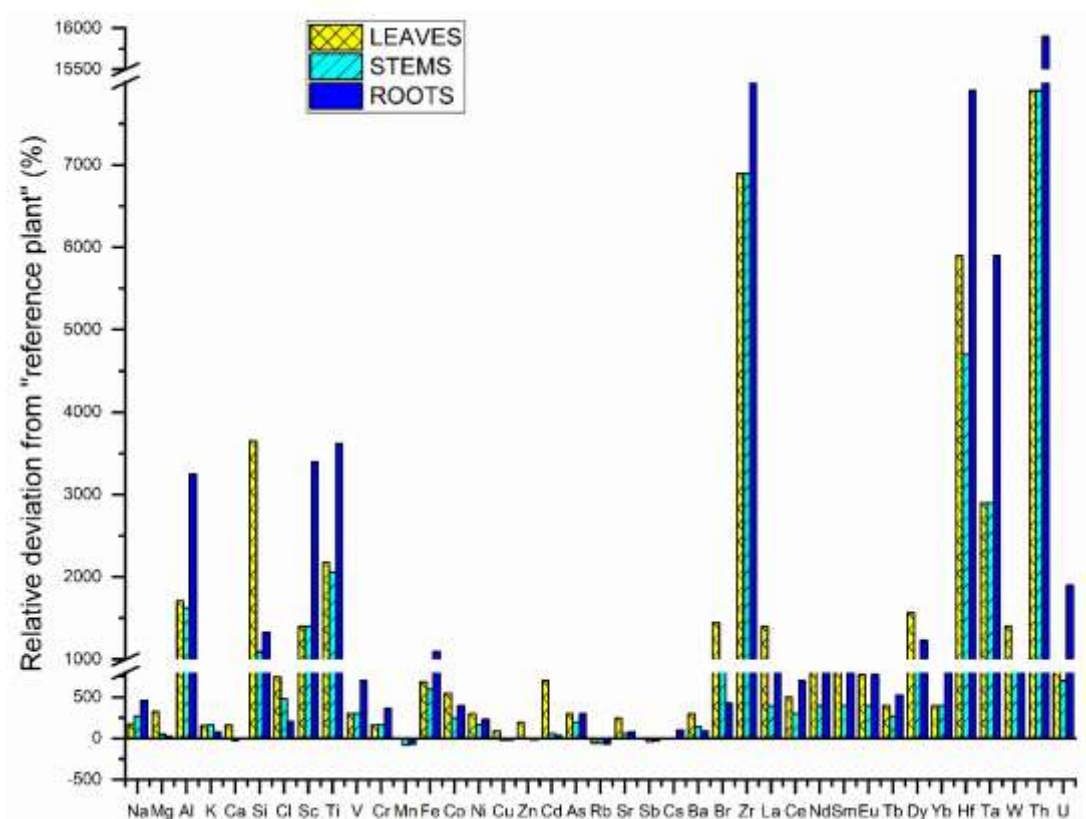


Figure 3.3.5.1: “Chemical fingerprint” of tobacco plant with respect to “reference plant”

Even though, the uptake of trace elements from the soil and the concomitant accumulation in the leaves by the tobacco plant is well known fact, however, the distribution of the heavy elements, vary among the leaves also depending on the stalk position (Ekren et al., 2012). Study have shown that higher concentration values of metal species were observed in the first priming leaves compared to the other stage of priming. The observed variation in the bioaccumulation of elements across different priming is more likely limited the mobility of metal species within tobacco plants (Golia et al., 2007). Therefore, the determined elemental contents of tobacco leave in the present study are probably at higher levels because of its location (bottom) at the plant.

Bioaccumulation of the essential and non-essential elements from the soil to the plant tissues depends on the uptake capacity of plant species in addition to the bioavailability of the metals. Therefore, the bioavailability of various elements is related to the physico-chemical and rhizosphere attributes of soil, along with the influence of soil properties on the bioavailable heavy elements is also varied significantly between the sites (Saha et al., 2016). Since, all elements detected in soils were also detected at various parts of tobacco plant exhibiting differential concentration ranges of these elemental species is indicative of the accumulative potential of tobacco plant (da Silva et al., 2016).

Bioconcentration factor is the ability of a plant to enrich the elemental species in plant tissues by extracting from the soil and it is estimated as plant tissue-to-soil quotient. Similarly, the elemental translocation factor depends on the partition of elemental content between aerial plant tissues like leaf, stem and in roots as it is calculated as the plant's shoot (leaf or stem)-to-root quotient. Earlier studies have indicated that tobacco plant can be a potential bioaccumulator of heavy elements (Fässler et al., 2010; da Silva et al., 2016). Because of the hilly terrains in Mizoram, certainly there is lack of water stagnation and low water holding capacity of soil that can leads to acidic nature of soil. Nevertheless, low pH conditions in soil would lead to higher mobility of Cl^- ions. The substantial bioconcentration factor and high translocation factor values of Cl suggested that Cl^- ion was absorbed and translocated by tobacco plant with great ease in acidic soils (Tso, 1990). It is

important to know that all REEs have their bioconcentration factor values in leaves, stem and roots are < 0.1 indicative of the fact that tobacco plant is one of the very weak accumulators of REEs as the plant may restrict the translocation of REEs into the aerial parts significantly. The lower translocation factor value for REEs in acidic soils is also indicative of higher adsorption capacity of soil colloidal solutions (Alloway and Ayres, 1997; Alloway, 2013). The calculated values of BCF are given in the (Table 3.3.5 (b)).

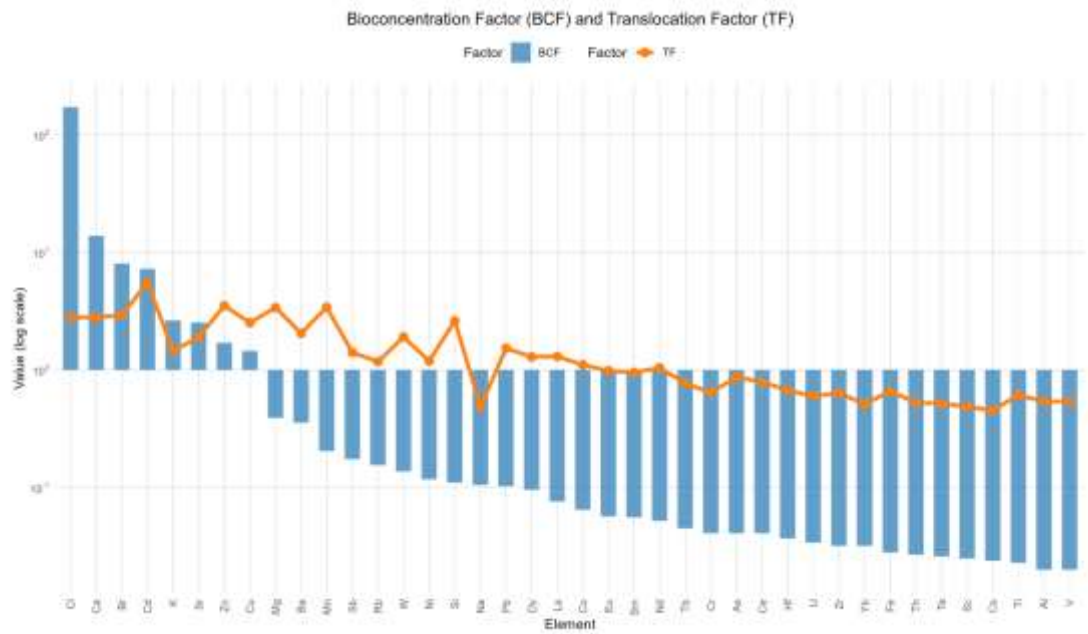


Figure 3.3.5 (b1): Bioconcentration and translocation factor in tobacco leaf

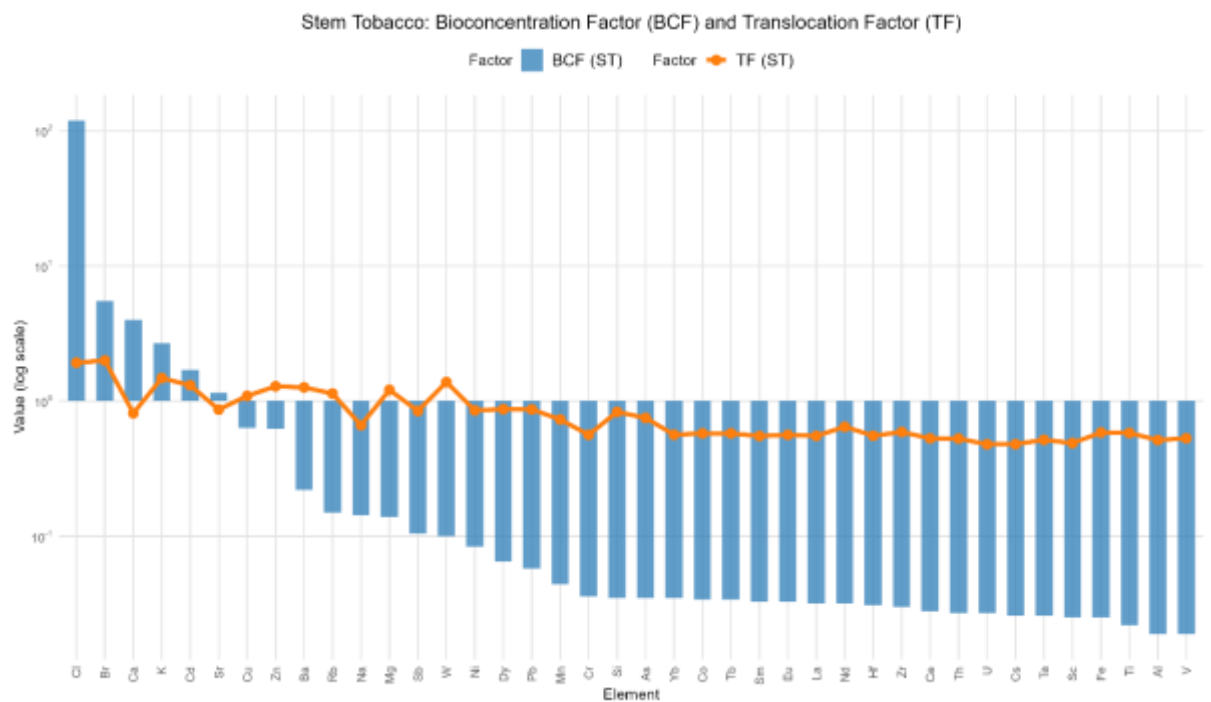


Figure 3.3.5 (b2): Bioconcentration and translocation factor in tobacco stem

Table 3.3.5 (b): Calculated bioconcentration and translocation in tobacco.

	BCF (L)	TF (L)	BCF (ST)	TF (ST)	BCF(R)
Na	0.11	0.5	0.14	0.67	0.22
Mg	0.4	3.4	0.14	1.21	0.12
Al	0.02	0.54	0.02	0.52	0.04
Si	0.11	2.6	0.04	0.8	0.04
Cl	172	2.8	119	1.9	61.7
K	2.7	1.5	2.7	1.5	1.8
Ca	13.82	2.82	4	0.8	4.9
Sc	0.03	0.5	0.025	0.5	0.05
Ti	0.023	0.61	0.022	0.6	0.04
V	0.02	0.54	0.02	0.531	0.04
Cr	0.04	0.64	0.04	0.6	0.064
Mn	0.21	3.42	0.044	0.73	0.06
Fe	0.03	0.7	0.03	0.6	0.043
Co	0.07	1.1	0.034	0.6	0.06
Ni	0.12	1.2	0.084	0.86	0.1
Zn	1.7	3.52	0.63	1.29	0.5
As	0.04	0.9	0.035	0.75	0.05
Br	8.0	2.9	5.5	2	2.8
Rb	0.16	1.2	0.15	1.14	0.13
Sr	2.5	1.9	1.2	0.9	1.34
Zr	0.032	0.64	0.03	0.6	0.051
Sb	0.18	1.41	0.105	0.85	0.125
Cs	0.024	0.45	0.026	0.48	0.054
Ba	0.36	2.05	0.220	1.27	0.18
La	0.08	1.30	0.032	0.56	0.06
Ce	0.041	0.8	0.03	0.53	0.052
Nd	0.052	1.045	0.032	0.65	0.050
Sm	0.056	0.952	0.033	0.55	0.06
Eu	0.057	1.0	0.033	0.57	0.06
Tb	0.045	0.77	0.034	0.58	0.06
Dy	0.096	1.3	0.065	0.871	0.074
Yb	0.032	0.513	0.035	0.57	0.062
Hf	0.037	0.67	0.031	0.56	0.056
Ta	0.026	0.52	0.026	0.52	0.050
W	0.14	1.9	0.1	1.4	0.072
Th	0.027	0.53	0.027	0.54	0.050
U	0.034	0.6	0.027	0.48	0.056
Cd	7.24	5.58	1.7	1.31	1.3
Cu	1.45	2.6	0.63	1.09	0.58
Pb	0.10	1.53	0.06	0.87	0.07

BCF - Bioconcentration Factor, TF - Translocation Factor, L – Leaf, ST – Stem,
R- Root

From the table, we observed that Cl, K, Ca, Zn, Br, Sr, Cd and Cu showed higher bioconcentration in the leaves and Cl, K, Ca, Br, Sr and Cd showed higher bioconcentration in the stems. Mg, Cl, K, Zn, Br, Rb, Ba, W, Cd, Cu and Pb showed higher translocation in the stems while Mg, Si, Cl, K, Ca, Mn, Co, Ni, Zn, As, Br, Rb, Sr, Sb, Ba, La, Ce, Nd, Sm, Eu, Tb, Dy, W, Cd, Cu and Pb showed higher translocation in the leaves.

Various factors such as edaphic characteristics, agro-climatic conditions, agricultural practices, and plant cultivars can modulate the efficacy of non-essential heavy elements absorption and translocation in tobacco plants (Lugon-Moulin et al., 2004; Lugon-Moulin et al., 2008). Some of the heavy metals such as iron, manganese, zinc and copper are important micronutrients which may accentuate plant growth and yield. However, other metals such as lead, cadmium, nickel and chromium are not essential for the plant growth albeit they can cause serious tissue injury and adverse growth effects (Wagner and Yeargan, 1986). The analysis of the partitioning of heavy elements between plant tissues suggest that it is more likely that Cd is preferentially and effectively absorbed and translocated in leaves. However, it was found that under soil acidification conditions, Zn was more efficiently and strongly mobilized than Cd or Cu in soil solutions. Nevertheless, in acidified soil with fertilizer amendments, Cd was absorbed and translocated in tobacco with better efficacy than Zn (Fässler et al., 2010). In contrast, when low pH soils treated with fertilizers and subsequently tobacco was grown, it was found that Zn was exhibiting comparable bioconcentration and translocation values with Cd (da Silva et al., 2016).

The soil parameters obtained from soil like pH, concentrations of available NPK, the soil organic matter (SOM) were used for performing statistical analysis to measure the correlation between the soil and the leaf samples. The performed correlation analysis between the elemental concentration with the corresponding soil parameters indicates there exist correlation between the tobacco and the soil where it grows. Table shows the detailed correlation between the elemental concentrations and the soil parameters. Pearson correlation shows a weak positive correlation between the pH of the soil along with concentrations of elements such as K, Ni, Zr and Eu indicating that as increase in the soil pH the concentrations of these elements tends to increases and vice versa, all the other elemental concentrations show weak

negative correlation with pH indicating that as the pH of the soil is inversely proportional to the concentrations of the elements. A significant positive correlation exists between the nitrogen in soil and Cl, phosphorus in soil and Na, Cs at $p < 0.01$ level. Significant positive correlation at $p < 0.05$ level was also found to exist between N (in soil) and Si, Ca, Cr and Dy. Phosphorus (soil) and Al and Sb also exhibit a significant negative correlation at p value ($p < 0.05$) level. A significant negative correlation ($p < 0.05$) was also found between K (in soil) and Tb. The concentrations of elements such as Na, Al, Sc, V, Zn, Cs, Ta, W and Th show significant negative correlation with the soil parameter of soil organic matter at $p < 0.05$ level.

Table 3.3.5 (c): Correlation between elemental concentration and analyzed soil parameters

Elements	Soil parameters				
	pH	N	P	K	SOM
Na	-0.53	0.27	0.93**	-0.49	-0.72*
Mg	-0.4	0.4	0.3	0.16	-0.26
Al	-0.4	0.2	0.72*	-0.61	-0.77*
Si	-0.7	0.81*	0.51	-0.25	-0.23
Cl	-0.51	0.85**	0.39	-0.09	0.033
K	0.12	-0.45	0.32	-0.43	-0.7
Ca	-0.51	0.81*	0.64	0.084	0.06
Sc	-0.3	0.12	0.62	-0.7	-0.75*
Ti	-0.041	0.3	0.51	0.002	-0.3
V	-0.38	0.242	0.65	-0.6	-0.7*
Cr	-0.53	0.73*	0.33	0.043	-0.16
Mn	-0.16	0.3	0.18	-0.34	-0.36
Fe	-0.31	0.24	0.58	-0.6	-0.65
Co	-0.19	0.42	0.15	-0.25	-0.26
Ni	0.03	0.28	-0.005	0.3	-0.12
Cu	-0.42	0.07	-0.17	0.1	-0.422
Zn	-0.48	0.014	0.475	-0.06	-0.714*
As	-0.27	0.26	0.56	-0.51	-0.58
Br	-0.33	-0.25	-0.071	-0.127	-0.65
Rb	-0.3	-0.22	0.24	-0.24	-0.7
Sr	-0.29	0.67	0.19	0.443	0.38
Zr	0.026	-0.003	0.2	-0.23	-0.33
Sb	-0.51	0.24	0.81*	-0.48	-0.67
Cs	-0.5	0.18	0.84**	-0.6	-0.82*
Ba	-0.4	0.4	-0.11	0.18	0.037
La	-0.2	0.247	-0.14	-0.4	-0.1
Ce	-0.42	0.39	0.37	-0.44	-0.47
Nd	-0.3	0.16	0.2	-0.65	-0.53
Sm	-0.23	0.3	0.36	-0.62	-0.41
Eu	0.063	0.02	0.1	-0.51	-0.4
Tb	-0.25	0.2	0.44	-0.8*	-0.5
Dy	-0.50	0.74*	0.44	-0.177	-0.18
Yb	-0.22	0.35	0.27	-0.35	-0.4
Hf	-0.2	0.042	0.37	-0.36	-0.54
Ta	-0.24	0.1	0.56	-0.54	-0.72*
W	-0.46	-0.2	0.14	-0.24	-0.77*
Th	-0.32	0.18	0.6	-0.61	-0.71*
U	-0.25	0.14	0.6	-0.45	-0.66
Cd	-0.26	-0.11	0.18	-0.08	-0.3
Cu	-0.11	-0.35	-0.4	0.36	-0.23
Pb	-0.66	0.62	0.7	-0.5	-0.5

** Correlation is significant at $p < 0.01$ level (2-tailed).

* Correlation is significant at $p < 0.05$ level (2-tailed).

It is also noteworthy that absorption and translocation attributes of tobacco to accumulate Cd can vary significantly between plant varieties, viz., *N. rustica* and *N. rotundifolia* were primarily high root and low foliar accumulators, while *N. tabacum* (Burley and Kentucky cultivars) was shown to be a high leaf and moderate-root accumulator (Wagner and Yeargan, 1986). In marginally alkaline soils amended with fertilizers and spiked with different concentrations of Cd solutions, and tobacco was grown in pots, it was demonstrated that in a concentration dependent manner Cd was selectively enriched than Zn, Cu or Ni with higher Cd bioconcentration factor values. However, beyond critical concentrations, at higher contamination levels of Cd, impediment of Cd uptake was also observed (Liu et al., 2016). Tobacco plant has a greater propensity to adopt higher accumulation potential when exposed to higher heavy element contents present in contaminated soils, while exposed to lower concentration levels of heavy elements present in fields, tobacco plant more akin to other crop plants exhibit relatively lower accumulation propensity (Angelova et al., 2003).

In the present study, under acidic soil and natural rhizosphere conditions, without any land preparation as practiced during shifting cultivation, it was observed that soil Cd and Zn contents were found to be lower as compared with those levels present in plant tissues reflecting tobacco plant ability to absorb, translocate and sequester nutrient and non-essential elements from the soil.

The calculated mean bioconcentration and translocation factor values of Cd in tobacco leaves were considerably higher than those values of Zn, reflecting tobacco plants selective bioaccumulate capacity which corresponds with earlier studies (Bi et al., 2011; Liu et al., 2016). A distinctive characteristic of Northeast Indian tobacco is its significant enrichment with Mg, K, Ca, and Cl, with concentrations varying by two orders of magnitude or greater. While these elements are essential for plant growth, their bioavailability - particularly for Ca, Mg, and Mo - decreases markedly when soil pH falls below neutral levels (Barber et al., 1963).

Elements like Fe, Zn, Cu, Mn, Ni, Cr, Cd, and Pb and 140 samples including standard tobacco samples' elemental data set, were used for performing principal component analysis. The two principal components cumulatively explained 54.2% of

the variance within the metal mass concentration. The PC1 contains the highest variances (29.3%), while the PC2 explains (24.9%) of total variance. Fe, Cu, and Ni vectors cluster more tightly together along the negative side of PC1 exerting a strong influence on NEI tobacco. Different brands of Indian cigarettes are well confined to a narrow region in the exploratory bi-plot. The modest influence of heavy elements on Indian cigarettes suggests that different brands of Indian cigarettes consist of similar element concentration. PC2 is separating the illicit cigarettes from UK and Pakistani tobacco products. In addition, Pakistani cigarettes are interspersed with Turkish cigarettes. Moreover, Turkish cigarettes are interspersed with UK cigarettes and tobacco products imported in Pakistan. More akin to UK cigarettes, different brands of Indian cigarettes, available in both Indian and Turkish markets, are closely clustered in the bi-plot within a smaller area near the origin, most likely due to the use of high-quality FCV tobacco. UK cigarettes and Indian cigarettes are also subtly discerned, along PC2. Mainly accounted by Mn, Cd, and Pb, illicit cigarettes seized in the UK are also scattered, so widely and extensively in the bi-plot in accordance with the previous reports (Özcan et al., 2019). This is a consequence of large variations in metal mass fractions found in those illicit cigarettes, more likely blending different types of tobacco, perhaps to obscure the originating region (Stephens et al., 2005).

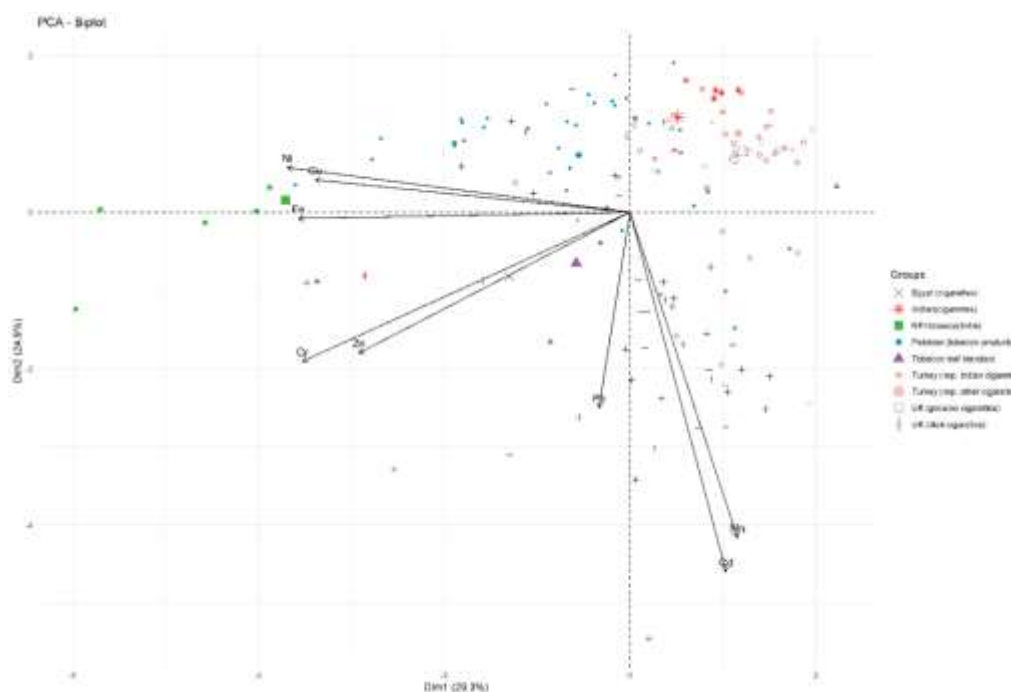


Figure3.3.5.2: PCA bi-plot of heavy element in tobacco sample of different countries with the present study

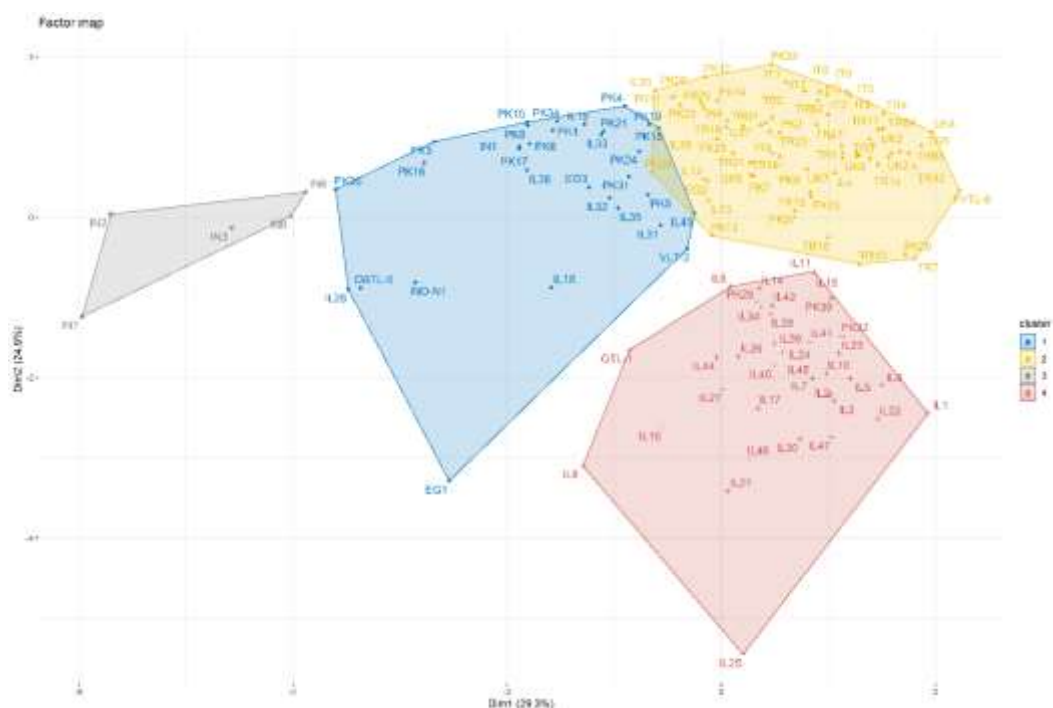


Figure 3.3.5.3: HCA of heavy element in tobacco sample of different countries with the present study

Hierarchical cluster analysis (HCA) was used by applying k-means method, for confirming the clustering observed in the principal component analysis methods as shown in the Figure 3.3.5.3. Thus, the results obtained using HCA confirm the discrimination of tobacco samples according to the type of tobacco and according to their geographical origin. It should be noted that some overlap of sub-clusters can be attributed to the similarities in the mass fractions of macro- and micronutrient elements of the tobacco samples

Chapter 4

Conclusion

The present study highlights the determination of different elements in areca nut and tobacco leaf samples as well as in the soil from the agricultural fields in which they were cultivated. To the best of our knowledge, this is first time the chemical analysis (especially the distribution of several REEs) of soil samples collected from different parts of Mizoram and Meghalaya was performed using INAA methods. The relative distribution of various elements in soil samples collected from Mizoram, Meghalaya, Assam and Arunachal Pradesh have indicated several geological features (geoaccumulation, contamination factor, and pollution), while the soil pH and SOM have suggested the mobility and the bioavailability of macro-, micro-nutrient and toxic heavy elements. Notably, Mizoram soils are low or moderately contaminated with Sb, U, Cu, Cd, and Pb.

The elemental profiling of areca nut (betel nut) revealed that areca palm husk may be acting as a reservoir of essential mineral elements for the germination of areca palm, whereas areca nut hardly contain REEs. Besides, Areca nut also contains toxic heavy elements, viz., As, Cd, Co, Pb, and Sb in considerable levels. More importantly, Areca palm tree has the ability to accumulate Cd, and/or Cu species in notable levels even though areca palm cultivation is practiced in Northeast India without any fertilizers, herbicides and copper ion-based fungicides as agricultural inputs.

The chemical analysis of tobacco plant reveals the significant concentration levels of both essential and toxic elements predominantly present in tobacco leaves, contributing to severe health risks upon chronic consumption. Indeed, the heavy elements distribution in the aerial parts of plant has reflected the robust accumulation propensity of tobacco plant for Cl, Br, and Cd. Since, tobacco leaves which are rich in chloride ion tends to have relatively lower combustion propensity, the smoking tobacco product, zozial, produced by using Mizoram tobacco leaves also often exhibits poor smouldering combustion attributes.

In addition, for the first time, to best of our knowledge, the concentration of nicotine, cotinine and carcinogenic tobacco-specific nitrosamines were determined, by internal calibration (with the addition of isotopically enriched standards) using LC-MS/MS methods, in the tobacco exclusively cultivated in Mizoram for the production of an indigenous smoking tobacco product, locally known as *zozial* in comparison with Indian bidi and hookah tobacco samples. Therefore, the findings of present study underscore the urgent need for stricter regulatory policies.

The chronic use of areca nut combined with tobacco, commonly present in products like gutkha and zarda exacerbates adverse health risks. Moreover, In Mizoram, most of the chronic betel quid users are also known to regularly consume different tobacco products. The consumption of areca nut in association with tobacco products causes the ingestion of a complex mixture of biologically active compounds into the human body. Long-term exposure to several of these toxic chemical species is causally linked to a spectrum of adverse health outcomes ranging from chronic obstructive pulmonary disease (COPD), cardiovascular disease, gastritis to cancers at different sites in chronic users.

The addition of slaked lime (calcium hydroxide) to betel quid enhances the bioavailability of toxic elements and organic compounds, facilitating their absorption into systemic circulation. Besides, the altered alkalinity of saliva induces oxidative stress, DNA damage, and cellular mutations, significantly increasing the risk of oral submucous fibrosis (OSF), leucoplakia, and squamous cell carcinoma. Furthermore, chronic exposure to toxic heavy elements and other xenobiotic chemical species in different lifestyle products have been linked to the development of various disease conditions including cardiovascular diseases (hypertension, atherosclerosis), neurotoxicity (Pb induced cognitive impairment), renal dysfunction (Cd species accumulation in kidneys), digestive disorders, and induction of cancer at different sites.

Since, the smoking tobacco product, produced using tobacco cultivated in Mizoram, namely *zozial*, which is popular among rural communities remains unregulated. It is pertinent to spread the public awareness campaigns educating consumers on the risks of areca nut and tobacco, particularly among the youth. In conclusion, the elemental

distribution in areca nut and tobacco poses detrimental health consequences for the tribal and rural populace of the Northeast India in general and Mizoram in particular. Therefore, based on the elemental distribution as well as carcinogenic TSNAs evaluated in the Mizoram tobacco, it may be prudent to incorporate pictorial warnings in the *zozial* packs similar to branded cigarette packets available in the market. These kind of pictorial warnings may possibly create an increased awareness among the rural population of Mizoram.

The chronic consumption of lifestyle products such as *zozial*, betel quid (with or without tobacco) diminishes the quality of life, which may be monitored with cost-effective biomarker studies such as micronuclei frequency in oral epithelial cells, protein carbonyl content in the blood plasma, antioxidant capacity of saliva/blood, FRAP assay, etc. These types of informative biomarkers studies maybe performed in future on a periodic basis to provide the much needed data for the policy makers, health professionals and social workers so that regulatory mechanisms can be further strengthened along with the regulations for the sale of lifestyle products such as *zozial*, *kuhva*, *Zarda*, etc.

References

- Ajab, H., Yaqub, A., Malik, S. A., Junaid, M., Yasmeen, S., & Abdullah, M. A. (2014). Characterization of toxic metals in tobacco, tobacco smoke, and cigarette ash from selected imported and local brands in Pakistan. *The Scientific World Journal*, 2014, 413614. <https://doi.org/10.1155/2014/413614>
- Alexander, A. J., Ramani, P., Sherlin, H. J., & Gheena, S. (2019). Quantitative Analysis of Copper Levels in Areca Nut Plantation Area – A Role in Increasing Prevalence of Oral Submucous Fibrosis: An In vitro Study, *Indian J Dent Res.* 30:261-6.https://doi.org/10.4103/ijdr.IJDR_431_17
- Alloway, B.J., & Ayres D.C. (1997). *Chemical principles of environmental pollution.* Blackie Academic & Professional, London
- Alloway, B. J. (2013) Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. *Environ. Pollut.* 22, Springer, Dordrecht. <https://doi.org/10.1007/978-94-007-4470-7>
- Al-Rmalli, S. W., Jenkins, R. O., & Haris, P. I. (2011). Betel quid chewing elevates human exposure to arsenic, cadmium and lead. *Journal of Hazardous Materials*, 190(1–3), 69–74. <https://doi.org/10.1016/j.jhazmat.2011.02.068>
- Angelova, V., Ivanov, K., & Ivanova, R. (2004). Effect of Chemical Forms of Lead, Cadmium, and Zinc in Polluted Soils on Their Uptake by Tobacco. *J. Plant Nutr.* 27:757–773. <https://doi.org/10.1081/PLN-120030609>
- Awashthi, S. K. (2000). *Prevention of Food Adulteration Act no 37 of 1954.* Central and State Rules as Amended for 1999, (3rded.) Ashoka Law House, New Delhi.
- Ayton, S., Lei, P., & Bush, A. I. (2013). Metallostasis in Alzheimer's disease. *Free radical biology & medicine*, 62, 76–89. <https://doi.org/10.1016/j.freeradbiomed.2012.10.558>.
- Barber S. A., Walker J.M., & Vasey, E.H. (1963). Mechanisms for Movement of Plant Nutrients from Soil and Fertilizer to Plant Root, *J. Agric. Food Chem.*, 11(3): 204–207. <https://doi.org/10.1021/jf60127a017>
- Barbosa, R. M., Batista, B. L., Varrique, R. M., Coelho, V. A., Campiglia, A. D., & Barbosa, F. (2014). The use of advanced chemometric techniques and trace element levels for controlling the authenticity of organic coffee. *Food*

- Research International, 61, 246–251.
<https://doi.org/10.1016/j.foodres.2013.07.060>
- Baruah, B. K., Das, B., Medhi, C., & Misra, A. K. (2013). Fertility status of soil in the tea garden belts of Golaghat District, Assam, India. *Journal of Chemistry*, 2013. <https://doi.org/10.1155/2013/983297>
- Benowitz, N. L. (2003). Cigarette smoking and cardiovascular disease: Pathophysiology and implications for treatment. *Progress in Cardiovascular Diseases*, 46(1), 91–111. [https://doi.org/10.1016/S0033-0620\(03\)00079-8](https://doi.org/10.1016/S0033-0620(03)00079-8)
- Beyersmann, D., & Hartwig, A. (2008). Carcinogenic metal compounds: Recent insights into molecular and cellular mechanisms., *Arch. Toxicol.* 82:493-512.
- Bi, R., Schlaak, M., Siefert, E., Lord, R., & Connolly, H. (2011). Influence of electrical fields (AC and DC) on phytoremediation of metal polluted soils with rapeseed (*Brassica napus*) and tobacco (*Nicotiana tabacum*), *Chemosphere*, 83: 318-26.
<https://doi.org/10.1016/j.chemosphere.2010.12.052>
- Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic and available forms of phosphorus in soils. *Soil Science*, 59(1), 39-45.
<https://doi.org/10.1097/00010694-194501000-00006>
- Carpenter, D., Boutin, C., Allison, J.E., Parsons, J.L., Ellis, D.M. (2019). Uptake and Effects of Six Rare Earth Elements (REEs) on Selected Native and Crop Species Growing in Contaminated Soils, *PLoS One* 10(6):e0129936.
<https://doi.org/10.1371/journal.pone.0129936>
- Chauhan. P., & Chauhan R.P. (2014). Measurement of fertilizers induced radioactivity in tobacco plants and elemental analysis using ICAP–AES. *Radiat.Meas.* 63:6–11. <https://doi.org/10.1016/j.radmeas.2014.02.006>
- Chen, P. H., Mahmood, Q., Mariottini, G. L., Chiang, T. A., & Lee, K. W. (2017). Adverse health effects of betel quid and the risk of oral and pharyngeal cancers. *BioMed research international*. vol. 2017, Article ID 3904098, 25 pages. <https://doi.org/10.1155/2017/3904098>
- Chu, N. S. (1993). Cardiovascular responses to betel chewing. *Journal of the Formosan Medical Association*, 92(9), 835–837.
- da Silva, C. P., de Almeida, T. E., Zittel, R., de Oliveira Stremel, T. R., Domingues,

- C. E., Kordiak, J., & de Campos, S. X. (2016). Translocation of metal ions from soil to tobacco roots and their concentration in the plant parts. *Environmental Monitoring and Assessment*, 188(12), 663. <https://doi.org/10.1007/s10661-016-5679-3>
- Davis, T. A., Ghosh, S. S., & Ghose, M. (1978). Morphology of juvenile areca palm. *Journal of the Indian Botanical Society*. 57: 219-227.
- Di Meo, V., Michele, A., Paola, A., & Pietro, V. (2003). Availability of potassium, calcium, magnesium, and sodium in “bulk” and “rhizosphere” soil of field-grown corn determined by electro-ultrafiltration. *Journal of Plant Nutrition*, 26(6), 1149–1168. <https://doi.org/10.1081/PLN-120020362>
- Ekren, S., Gokcol A. & Sonmez, C. (2012). Heavy Metal Contents of Aegean Region Tobaccos According to Quality Groups and Stalk Position, *Bulg. J. Agric. Sci.*, 18 (1): 92-99.
- Elbagermi, M. A., Edwards, H. G. M., & Alajtal, A. I. (2012). Monitoring of Heavy Metal Content in Fruits and Vegetables Collected from Production and Market Sites in the Misurata Area of Libya. *ISRN Analytical Chemistry*, 2012, 1–5. <https://doi.org/10.5402/2012/827645>
- Fässler, E., Robinson, B. H., Stauffer, W., Gupta, S. K., Papritz, A., & Schulin, R. (2010). Phytomanagement of metal-contaminated agricultural land using sunflower, maize and tobacco. *Agriculture, Ecosystems & Environment*, 136(1), 49–58. <https://doi.org/10.1016/j.agee.2009.11.007>
- Gairola, C. G., & Wagner, G. J. (1991). Cadmium accumulation in the lung, liver and kidney of mice and rats chronically exposed to cigarette smoke. *Journal of Applied Toxicology*, 11(5), 355–358. <https://doi.org/10.1002/jat.2550110510>
- Gluscock, M. D. (2003). Neutron activation analysis. In C. R. Pollard & H. A. Orna (Eds.), *Chemical analysis in archaeology* (pp. 15–21). American Chemical Society. <https://doi.org/10.1021/bk-2003-0843.ch002>
- Global Adult Tobacco Survey (GATS-2). (2016–2017). India. Ministry of Health and Family Welfare. <https://ntcp.nhp.gov.in>
- Golia, E.E., Dimirkou, A., & Mitsios, I.K. (2007). Accumulation of Metals on Tobacco Leaves (Primings) Grown in an Agricultural Area in Relation to Soil, *Bull Environ Contam Toxicol*, 79:158–162 <https://doi.org/10.1007/s00128->

007-9111-0

- Gomez, F., Ayo-Yusuf, O., Yershova, K., Jain, V., Alcheva, A., Hatsukami, D. K., Parascandola, M., & Stepanov, I. (2023). Heterogeneity of harmful constituent profiles in smokeless tobacco products from five African countries. *Chemical Research in Toxicology*, 36(12), 1901–1911. <https://doi.org/10.1021/acs.chemrestox.3c00181>
- Gupta, P. C., & Ray, C. S. (2003). Smokeless tobacco and health in India and South Asia. *Respirology*, 8(4), 419–431. <https://doi.org/10.1046/j.1440-1843.2003.00507.x>
- Gupta, P. C., & Ray, C. S. (2004). Epidemiology of betel quid usage. *Annals of the Academy of Medicine, Singapore*, 33(4 Suppl), 31–36. <https://annals.edu.sg/pdf200404/Ray.pdf>
- Gupta, P. C., Arora, M., Sinha, D., Asma, S., & Parascandola, M. (2016). Smokeless tobacco and public health in India. Ministry of Health and Family Welfare, Government of India.
- Gupta, P. C., Mehta, F. S., Daftary, D. K., Pindborg, J. J., & Bhonsle, R. B. (1984). Intervention study for primary prevention of oral cancer among 36,000 Indian tobacco users. *The Lancet*, 324(8412), 1235–1239. [https://doi.org/10.1016/S0140-6736\(84\)92737-3](https://doi.org/10.1016/S0140-6736(84)92737-3)
- Hartwig, A., & Schwerdtle, T. (2002). Interactions of carcinogenic metal compounds with DNA repair processes: toxicological implications. *Toxicol Lett* 127:47–54
- Hecht, S. S. (2003). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nature Reviews Cancer*, 3(10), 733–744. <https://doi.org/10.1038/nrc1190>
- IARC (The International Agency for Research on Cancer) (2012). Nickel and nickel compounds. *IARC Monogr. Eval. Carcinog. Risk Hum.*, 100C: 169–218.
- ICMR-NCDIR. (2021). Profile of cancer and related health indicators in the North East Region of India – 2021.
- Zinicovscaia, I. (2022). Impact of some metals determined by neutron activation analysis on the quality of the environment, PhD Thesis, Institute of Chemistry, Republic of Moldova.

- International Agency for Research on Cancer (IARC) (2006). Cobalt in hard metals and cobalt sulfate, gallium arsenide, indium phosphide and vanadium pentoxide. IARC monographs on the evaluation of carcinogenic risks to humans, vol 86. Lyon, pp 119–237.
- International Agency for Research on Cancer (IARC). (2004). Betel-quid and Areca-nut Chewing and Some Areca-nut-derived Nitrosamines (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 85). Lyon, France. <https://monographs.iarc.who.int/wp-content/uploads/2018/06/mono85.pdf>
- International Agency for Research on Cancer. (2012). A review of human carcinogens: Arsenic, metals, fibres, and dusts (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 100C). World Health Organization.
- Jackson, M. L. (1958). Soil chemical analysis. Prentice-Hall.
- Jeng, J. H., Chang, M. C., & Hahn, L. J. (2001). Role of areca nut in betel quid-associated chemical carcinogenesis: current awareness and future perspectives. *Oral oncology*, 37(6), 477-492. [https://doi.org/10.1016/S1368-8375\(01\)00003-3](https://doi.org/10.1016/S1368-8375(01)00003-3)
- Jeng, J. H., Chang, M. C., & Hahn, L. J. (2001). Role of areca nut in betel quid-associated chemical carcinogenesis: Current awareness and future perspectives. *Oral Oncology*, 37(6), 477–492. [https://doi.org/10.1016/S1368-8375\(01\)00011-9](https://doi.org/10.1016/S1368-8375(01)00011-9)
- Jin, C. W., Zheng, S. J., He, Y. F., Zhou, G. Di., & Zhou, Z. X. (2005). Lead contamination in tea garden soils and factors affecting its bioavailability. *Chemosphere*, 59(8), 1151–1159. <https://doi.org/10.1016/j.chemosphere.2004.11.058>
- Kabata-Pendias, A. (2011). Trace elements in soils and plants. Boca Raton, Florida: Taylor & Francis Group. <https://doi.org/10.1201/b10158>
- Kaličanin, B., & Velimirović, D. (2012). Potentiometric stripping analysis of zinc, cadmium, and lead in tobacco leaves (*Nicotiana Tabacum* L.) and soil samples. *International Journal of Electrochemical Science*, 7, 313–323.
- Kashem, M.A., & Singh, B.R., (2001). Metal availability in contaminated soils: I. Effects of flooding and organic matter on changes in Eh, pH and solubility of

- Cd, Ni and Zn. *Nutrient Cycl. Agroecosys.*, 61: 247-255.
- Kasten, U., Mullenders, L.H.F., & Hartwig, A. (1997). Cobalt(II) inhibits the incision and the polymerization step of nucleotide excision repair in human fibroblasts. *Mut Res* 383: 81–89.
- Khan, I., Pant, I., Narra, S., Radhesh, R., Ranganathan, K., Rao, S. G., & Kondaiah, P. (2015). Epithelial atrophy in oral submucous fibrosis is mediated by copper (II) and arecoline of areca nut. *Journal of Cellular and Molecular Medicine*, 19(10), 2397–2412. <https://doi.org/10.1111/jcmm.12622>
- Kopera, E., Schwerdtle, T., Hartwig, A., & Bal, W. (2004) Co(II) and Cd(II) substitute for Zn(II) in the zinc finger derived from the DNA repair protein XPA, demonstrating a variety of potential mechanisms of toxicity. *Chem. Res. Toxicol.*, 17: 1452–1458.
- Kumar, S., Kumar, S., & Mohapatra, T. (2021). Interaction Between Macro- and Micro-Nutrients in Plants. *Front. Plant Sci.* 12:665583. doi: 10.3389/fpls.2021.665583
- Kumar, P. (2019). Betel Quid Chewing: A Possible Source of Heavy Metal Exposure to the People in Khulna, Bangladesh. *Journal of Materials and Environmental Sciences* ISSN : 2028-2508
- Kumar, V., Sharma, A., Kaur, P., Singh Sidhu, G. P., Bali, A. S., Bhardwaj, R., Thukral, A. K., & Cerda, A. (2019). Pollution assessment of heavy metals in soils of India and ecological risk assessment: A state-of-the-art. *Chemosphere*, 216, 449–462. <https://doi.org/10.1016/j.chemosphere.2018.10.066>
- Lalrammawia, K. (2022). Physico-chemical characterization and the evaluation of oxidative potential of Zozial, the indigenous hand-rolled cigarettes of Mizoram [Doctoral dissertation, Mizoram University].
- Lalrammawia, K., Buragohain, A., Kakki, B., Zote, L., Marak, N.K., Lalmuanpuii, R., Kumar, N.S., Jahau, L., Sudarshan, M., Chaligava, O. & Yushin, N., (2021). Determination of multi elements in tobacco plant of northeast India by neutron activation analysis and atomic absorption spectrometry. *Biological Trace Element Research*, pp.1-16 . <https://doi.org/10.1007/s12011-021-03040-2>

- Lee, C. H., Ko, A. M. S., Warnakulasuriya, S., Yin, B. L., Sunarjo., Zain, R. B., Ibrahim, S. O., Liu, Z. W., Li, W. H., Zhang, S. S., Kuntoro, Utomo, B., Rajapakse, P. S., Warusavithana, S. A., Abdul Razak, I., Abdullah, N., Shrestha, P., Kwan, A. L., Shieh, T. Y., ... Ko, Y. C. (2011). Intercountry prevalences and practices of betel-quid use in south, southeast and eastern asia regions and associated oral preneoplastic disorders: An international collaborative study by asian betel-quid consortium of south and east Asia. *International Journal of Cancer*, 129(7), 1741–1751. <https://doi.org/10.1002/ijc.25809>
- Lee, C. H., Ko, A. M., Warnakulasuriya, S., Yin, B. L., Sunarjo, Sekhar, K., ... & Ko, Y. C. (2005). Betel-quid chewing-associated disorders: A neglected global health problem. *Bulletin of the World Health Organization*, 83(10), 807–815. <https://www.scielo.org/article/bwho/2005.v83n10/807-815/>
- Liu, H., Wang, H., Ma, Y., Wang, H., & Shi, Y. (2016). Role of transpiration and metabolism in translocation and accumulation of cadmium in tobacco plants (*Nicotianatabacum*L.), *Chemosphere*, 144: 1960-65. <http://dx.doi.org/10.1016/j.chemosphere.2015.10.093>
- Lugon-Moulin, N., Martin, F., Krauss, M.R., Ramey, P.B., & Rossi, L. (2008). Arsenic concentration in tobacco leaves: a study on three commercially important tobacco (*Nicotiana tabacum* L.) types. *Water Air Soil Pollut.* 192: 315-319. <https://doi.org/10.1007/s11270-008-9658-3>
- Lugon-Moulin, N., Zhang, M., Gadani, F., Rossi, L., Koller, D., Krauss, M., & Wagner, G. J. (2004). Critical Review of the Scienceand Options for Reducing Cadmium in Tobacco (*Nicotiana Tabacum* L.) and Other Plants. *Advances in Agronomy*. [https://doi.org/10.1016/S0065-2113\(04\)83003-7](https://doi.org/10.1016/S0065-2113(04)83003-7)
- Mann, R. M., Vijver, M. G., & Peijnenburg, W. J. G. M. (2011).Metals and Metalloids in Terrestrial Systems: Bioaccumulation,Biomagnification and Subsequent Adverse Effects, Ch. 03 in *Ecological Impacts of Toxic Chemicals*,Sánchez-Bayo F, van den Brink PJ, Mann RM (Eds),(pp 43-62). Bentham Science:Sydney,
- Markert, B. (1992). Establishing of ‘Reference Plant’ for inorganic characterization of different plant species by chemical fingerprinting. *Water Air Soil Pollut*

64:533–538. <https://doi.org/10.1007/BF00483363>

- Markert, B., Fränzle, S., & Wünschmann, S. (2015). The biological system of the elements. Chemical evolution. Springer, Cham
- Mathew, P., Austin, R., Varghese, S., & Manojkumar, A. (2015). Effect of copper-based fungicide (bordeaux mixture) spray on the total copper content of areca nut: Implications in increasing prevalence of oral submucous fibrosis. *Journal of International Society of Preventive and Community Dentistry*, 5(4), 283. <https://doi.org/10.4103/2231-0762.161755>
- Mehrotra, R., & Lauby-Secretan, B. (2025). Smokeless tobacco is a major risk factor for oral cancer in South and Southeast Asia. *Oral Diseases*, 31(5), 1473–1478. <https://doi.org/10.1111/odi.15241>
- Micháľková Z, Martínez-Fernández D, & Komárek, M. (2017). Interactions of two novel stabilizing amendments with sunflower plants grown in a contaminated soil. *Chemosphere* 186:374–380. <https://doi.org/10.1016/j.chemosphere.2017.08.009>
- Mishra, U. C., Shaikh, G. N., & Sadasivan, S. (1986). Trace elements in tobacco and tobacco smoke by X-ray fluorescence technique. *Journal of Radioanalytical and Nuclear Chemistry Articles*, 102(1), 27–35. <https://doi.org/10.1007/BF02037947>
- Mussalo-Rauhamaa, H., Salmela, S. S., Leppanen, A., & Pyysalo, H. (1986). Cigarettes as a source of some trace and heavy metals and pesticides in man. *Archives of Environmental Health*, 41(1), 49–55. <https://doi.org/10.1080/00039896.1986.9935765>
- Muthukumaran, R. B., Bhattacharjee, P., Bhowmick, P., Zote, L., Malsawmtluangi, Kumar, N. S., Jahau, L., Cooke, M. S., Hu, C.-W., & Chao, M.-R. (2023). Genetic and epigenetic instability induced by betel quid associated chemicals. *Toxicology Reports*, 10, 223–234. <https://doi.org/10.1016/j.toxrep.2023.02.001>
- Natesan, S. (1999). Tea Soils, In *Global advances in Tea Science* (Ed. V. K. Jain) N Delhi: Aravali Publishers Pvt Ltd., 519-532.
- Ng, J.C. (2005). Environmental contamination of arsenic and its toxicological impact on humans, *Environ. Chem.*, 2: 146-160.

- Niessen, W. M. A. (2006). *Liquid chromatography-mass spectrometry* (3rd ed.). CRC Press.
- Orroño, D.I., & Lavado, R.S. (2009). Distribution of extractable heavy metals in different soil fractions, *Chemical Speciation & Bioavailability*, 21:4, 193-198. <https://doi.org/10.3184/095422909X12473204137916>
- Özcan, M.M., Aljuhaimi, F, Uslu, N et al. (2019) Distribution of heavy metal and macroelements of Indian and imported cigarette brands in Turkey. *Environ Sci Pollut Res* 26:28210–28215. <https://doi.org/10.1007/s11356-019-05978-2>
- Pandey, N. (2018). Role of Plant Nutrients in Plant Growth and Physiology. In: Hasanuzzaman M., Fujita M., Oku H., Nahar K., Hawrylak-Nowak B. (eds) *Plant Nutrients and Abiotic Stress Tolerance*. Springer, Singapore. https://doi.org/10.1007/978-981-10-9044-8_2
- Papke, R. L., Bhattacharyya, I., Hatsukami, D. K., Moe, I., & Glatman, S. (2020). Betel Nut (areca) and Smokeless Tobacco Use in Myanmar. *Substance Use and Misuse*, 55(9), 1385–1394. <https://doi.org/10.1080/10826084.2019.1624774>
- Pepi S, & Vaccaro, C. (2018). Geochemical fingerprints of "Prosecco" wine based on major and trace elements, *Environ Geochem Health*, 40(2):833-847. <https://doi.org/10.1007/s10653-017-0029-0>.
- Phukan, R. K., Somalia, E., Narain, K., Hazarika, N. C., & Mahanta, J. (2005). Tobacco use and stomach cancer in Mizoram, India. *Cancer Epidemiology, Biomarkers & Prevention*, 14(8), 1892–1896. <https://doi.org/10.1158/1055-9965.EPI-05-0033>
- Rani, M., Bonu, S., Jha, P., Nguyen, S. N., & Jamjoum, L. (2003). Tobacco use in India: Prevalence and predictors of smoking and chewing in a national cross-sectional household survey. *Tobacco Control*, 12(4), e4. <https://doi.org/10.1136/tc.12.4.e4>
- Raven ,E.P., Lu, P.H., Tishler, T., Heydari, P., & Bartzokis, G. (2013). Increased Iron levels and decreased tissue integrity in hippocampus of Alzheimer's disease detected in vivo with magnetic resonance imaging, *J Alzheimer's dis.*, 37: 127-136.

- Reitsma, M. B., Flor, L. S., Mullany, E. C., Gupta, V., Hay, S. I., & Gakidou, E. (2021). Spatial, temporal, and demographic patterns in prevalence of smoking tobacco use and initiation among young people in 204 countries and territories, 1990-2019. *The Lancet Public Health*, 6(7), e472-e481. [https://doi.org/10.1016/S2468-2667\(21\)00102-X](https://doi.org/10.1016/S2468-2667(21)00102-X)
- Roberts, D., Nachtegaal, M., & Sparks, D.L. (2005). Speciation of metals in soils. In M.A. Tabatabai and D.L. Sparks (eds.) *Chemical Processes in Soils*. No. 8, Soil Science Society of America, MadisonWI, p.619-654.
- Roshila, M., Andrew, K., & Sreekanth, B. J. (2007). Elemental composition and chemical characteristics of five edible nuts (almond, Brazil, pecan, macadamia and walnut) consumed in Southern Africa. *Journal of Environmental Science and Health - Part B Pesticides, Food Contaminants, and Agricultural Wastes*, 42(5), 585–591. <https://doi.org/10.1080/03601230701391591>
- Rumgay, H., Nethan, S. T., Shah, R., Vignat, J., Ayo-Yusuf, O., Chaturvedi, P., Guerra, E. N., Gupta, P. C., Gupta, R., Liu, S., Magnusson, C., Parascandola, M., Paulino, Y. C., Rezaei, N., Siddiqi, K., Warnakulasuriya, S., Lauby-Secretan, B., & Soerjomataram, I. (2024). Global burden of oral cancer in 2022 attributable to smokeless tobacco and areca nut consumption: A population attributable fraction analysis. *The Lancet Oncology*, 25(11), 1413–1423. [https://doi.org/10.1016/S1470-2045\(24\)00458-3](https://doi.org/10.1016/S1470-2045(24)00458-3)
- Saha, N. M., Rahman, S., Jolly, Y.N., Atiqur, R. A., Sattar, M.A., & Hai, M.A. (2016). Spatial distribution and contamination assessment of six heavy metals in soils and their transfer into mature tobacco plants in Kushtia District, Bangladesh, *Environ Sci Pollut Res.*, 23, 3414–3426. <https://doi.org/10.1007/s11356-015-5575-3>
- Salman, S. A., Zeid, S. A. M., Seleem, E.-M. M., & Abdel-Hafiz, M. A. (2019). Soil characterization and heavy metal pollution assessment in Orabi farms, El Obour, Egypt. *Bulletin of the National Research Centre*, 43(1). <https://doi.org/10.1186/s42269-019-0082-1>
- Sharan, R. N., Mehrotra, R., Choudhury, Y., & Asotra, K. (2012). Association of betel nut with carcinogenesis: revisit with a clinical perspective. *PLoS ONE*

- 7(8): e42759. <https://doi.org/10.1371/journal.pone.0042759>
- Sharma, C.P. (2006). Plant micronutrients, 1st Edition. CRC Press, Boca Raton 273. <https://doi.org/10.1201/9781482280425>
- Singh, A.N, Zeng, D.H., & Chen, F.S. (2005). Heavy metal concentrations in redeveloping soil of mine spoil under plantations of certain native woody species in dry tropical environment, India. *J Environ. Sci.*, 1: 168-174.
- Singh, P. K., Yadav, A., Singh, L., Mazumdar, S., Sinha, D. N., Straif, K., & Singh, S. (2021). Areca nut consumption with and without tobacco among the adult population: A nationally representative study from India. *BMJ Open*, 11(6), e043987. <https://doi.org/10.1136/bmjopen-2020-043987>
- Singh, S., Jaishi, H. P., Tiwari, R. P., & Tiwari, R. C. (2016). A study of variation in soil gas concentration associated with earthquakes near Indo-Burma Subduction zone. *Geoenvironmental Disasters*, 3(1), 0–7. <https://doi.org/10.1186/s40677-016-0055-8>
- Singh, S., Sharma, D. K., Dhar, S., Kumar, A., & Kumar, A. (2007). Uranium, radium and radon measurements in the environs of nurpur area, Himachal Himalayas, India. *Environmental Monitoring and Assessment*, 128(1–3), 301–309. <https://doi.org/10.1007/s10661-006-9313-7>
- Slezakova K, Morais S, Pereira M.C. (2014). Trace metals in size-fractionated particulate matter in a Portuguese hospital: exposure risks assessment and comparisons with other countries, *Environ Sci Pollut Res Int.*, 21(5): 3604-20. <https://doi.org/10.1007/s11356-013-2316-3>
- Sonu, K., Chandra Yadav, I., Kumar, A., & Devi, N. L. (2019). Dataset on assessment of heavy metals contamination in multi-environmental samples from Patna, India. *Data in Brief*, 25. <https://doi.org/10.1016/j.dib.2019.104079>
- Srimany, A., George, C., Naik, H. R., Pinto, D. G., Chandrakumar, N., & Pradeep, T. (2016). Developmental patterning and segregation of alkaloids in areca nut (seed of *Areca catechu*) revealed by magnetic resonance and mass spectrometry imaging. *Phytochemistry*, 125, 35–42. <https://doi.org/10.1016/j.phytochem.2016.02.002>
- Stephens, W.E, Calder, A., & Newton, J. (2005). Source and health implications of high toxic metal concentrations in illicit tobacco products. *Environ Sci*

- Technol 39:479–488. <https://doi.org/10.1021/es049038s>
- Subbiah, B. V., & Asija, G. L. (1956). A rapid procedure for the estimation of available nitrogen in soils. *Current Science*, 25, 259-260.
- Taiz, L., Zeiger, E., Moller, I.M, & Murphy, A .(2015). *Plant physiology and development*, 6th Edition. 888, Sinauer Associates, Sunderland, CT
- Thakur, J. S., Paika, R., Narain, J. P., & Tripathy, J. P. (2021). Burden of cancers and their management in the North-East Region of India: Challenges and ways forward. *Indian Journal of Cancer*, 58(1), 103–108. https://doi.org/10.4103/ijc.IJC_534_20
- Tilakaratne, W. M., Klinikowski, M. F., Saku, T., Peters, T. J., & Warnakulasuriya, S. (2006). Oral submucous fibrosis: Review on aetiology and pathogenesis. *Oral Oncology*, 42(6), 561–568. <https://doi.org/10.1016/j.oraloncology.2005.08.005>
- Trivedy, C., Baldwin, D., Warnakulasuriya, S., Johnson, N., & Peters, T. (1997). Copper content in Areca catechu (betel nut) products and oral submucous fibrosis. *Lancet*, 349: 1447
- Tso, T.C. (1990). *Production, physiology, and biochemistry of tobacco plant*. IDEALS, Beltsville, Md.
- United States Environmental Protection Agency (EPA). (1989). Risk assessment guidance for Superfund: Volume I—Human health evaluation manual (Part A) (EPA/540/1-89/002). https://www.epa.gov/sites/default/files/2015-09/documents/rags_a.pdf
- United States Environmental Protection Agency (EPA). (2005). Guidelines for carcinogen risk assessment (EPA/630/P-03/001F). https://www.epa.gov/sites/default/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf
- United States Environmental Protection Agency (EPA). (2011). Exposure factors handbook: 2011 edition (EPA/600/R-09/052F). <https://www.epa.gov/sites/default/files/2014-05/documents/efh-frontmatter.pdf>
- Vasundhara, R., Prakash, N. B., Anil Kumar, K. S., & Hegde, R. (2020). Characterisation and classification of arecanut-growing soils of Karnataka,

- India. *Journal of Plantation Crops*, 48(2), 91-102.
<https://doi.org/10.25081/jpc.2020.v48.i2.6367>
- Verma, S., Yadav, S., & Singh, I. (2010). Trace metal concentration in different Indian tobacco products and related health implications. *Food and Chemical Toxicology*, 48(8–9), 2291–2297. <https://doi.org/10.1016/j.fct.2010.05.062>
- Wagner, G.J., & Yeargan, R. (1986) Variation in Cadmium Accumulation Potential and Tissue Distribution of Cadmium in Tobacco, *Plant Physiol.*, 82(1): 274–279. <https://doi.org/10.1104/pp.82.1.274>
- Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 37(1), 29-38.
<https://doi.org/10.1097/00010694-193401000-00003>
- Wang, Y., & Hecht, S. S. (2021). Metabolism of tobacco-specific nitrosamines and its relevance to human cancer. *Journal of Clinical Investigation*, 131(3), e136438. <https://doi.org/10.1172/JCI136438>
- Wei, Y.Y., & Chung, C. (1997). Elemental analysis of Taiwanese areca nut and limes with INAA, *J Radioanal Nucl Chem.*, 217(1): 45-51.
- Welz, B., & Sperling, M. (2008). *Atomic absorption spectrometry* (3rd ed.). Wiley-VCH.
- World Health Organization (WHO). (2006). A global epidemic of addiction and disease. Tobacco: Deadly in any form or disguise. WHO Tobacco Free Initiative: World No Tobacco Day.
- Wu, Z., Du, Y., Xue, H., Wu, Y., & Zhou, B. (2012). Aluminum induces neurodegeneration and its toxicity arises from increased iron accumulation and reactive oxygen species (ROS) production, *Neurobiol. Aging*, 33: 199.e1–199.e12.
- Yen, A. M., Chiu, Y. H., Chen, L. S., Wu, H. M., Huang, C. C., Boucher, B. J., & Chen, T. H. (2008). A prospective community-population-registry-based study of the association between betel-quid chewing and cardiovascular disease in men. *The American Journal of Clinical Nutrition*, 87(1), 70–78.
<https://doi.org/10.1093/ajcn/87.1.70>
- Yuan, H., Cao, M., Yi, P., Xie, Q., Jian, Y., Li, B., Qin, Y., Peng, C., Wu, H., Tan, D.,

- Qin, Y., & Wang, W. (2018). Determination of alkaloids and phenols in the chewable husk products of *Areca catechu* L. Using HPLC-UV and UHPLC-MS/MS. *Journal of Liquid Chromatography and Related Technologies*, 41(10), 612–620. <https://doi.org/10.1080/10826076.2018.1486326>
- Zaidi, J. H., Arif, M., Fatima, I., & Qureshi, I. H. (2002). Radiochemical neutron activation analysis for trace elements of basic ingredients of pan. *Journal of Radioanalytical and Nuclear Chemistry*, 253(3), 459–464. <https://doi.org/10.1023/A:1020481822492>
- Zambelli B, Ciurli S (2013) Nickel and human health. *Met. Ions Life Sci.*, 13: 321–357.
- Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., & Zhang, G. (2011). The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environmental Pollution*, 159(1), 84–91. <https://doi.org/10.1016/j.envpol.2010.09.019>
- Zhao J, Shi X, Castranova V, Ding, M. (2009). Occupational toxicology of nickel and nickel compounds. *J. Environ. Pathol. Toxicol. Oncol.*, 28: 177–208.
- Zhitkovich A. (2005). Importance of chromium-DNA adducts in mutagenicity and toxicity of chromium(VI). *Chemical research in toxicology*, 18(1), 3–11. <https://doi.org/10.1021/tx049774+>
- Zhuang, P., McBride, M. B., Xia, H., Li, N., & Li, Z. (2009). Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. *Science of the Total Environment*, 407(5), 1551–1561. <https://doi.org/10.1016/j.scitotenv.2008.10.061>
- Zinicovscaia, I. (2022). Impact of some metals determined by neutron activation analysis on the quality of the environment, PhD Thesis, Institute of Chemistry, Republic of Moldova.
- Zoliana, B., Rohmingliana, P. C., Sahoo, B. K., Mishra, R., & Mayya, Y. S. (2016). Measurement of radon concentration in dwellings in the region of highest lung cancer incidence in India. *Radiation Protection Dosimetry*, 171(2), 192–195. <https://doi.org/10.1093/rpd/ncw056>
- Zomawia, E., Zami, Z., Vanlallawma, A., Kumar, N. S., Zothanzama, J., Tlau, L., Chhakchhuak, L., Pachuau, L., Pautu, J. L., & Hmangaihzuai, E. V. (2023).

Cancer awareness, diagnosis and treatment needs in Mizoram, India: Evidence from 18 years trends (2003-2020). *The Lancet Regional Health - Southeast Asia*, 17, 1-14. <https://doi.org/10.1016/j.lansea.2023.100281>

Zote, L., Lalrammawia, K., Buragohain, A., Lalrinhlupuii, Kakki, B., Lalmuanpuii, R., Pachuau, Z., Vanlalhruaia, J., Muthukumaran, R. B., Kumar, N. S., Jahau, L., Sudarshan, M., Yushin, N., Nekhoroshkov, P., Grozdov, D., Sergeeva, A., & Zinicovscaia, I. (2021). Macro-, micro-, and trace element distributions in areca nut, husk, and soil of northeast India. *Environmental Monitoring and Assessment*, 193(2), 65. <https://doi.org/10.1007/s10661-021-08859-9>

BIO-DATA

1. **Name** : Lalrinawma Zote
2. **Date of Birth** : 19th March 1993
3. **Father's Name** : Vanlalruata Zote
4. **Permanent Address** : Chanmari West, Aizawl
5. **Email Address** : nanau009@gmail.com
6. **Educational Qualifications**

Examination Passed	Year of Passing	Board/ University	Class/ Division	% of Marks	Subjects
HSLC	2010	MBSE	I	65.3	English, Mizo, Science, Mathematics, Social Sciences
HSSLC	2012	MBSE	I	60.6	English, Mizo, Chemistry, Physics, Biology
B.Sc Chemistry	2015	MZU	I	72.33	Chemistry, Zoology, Botany
M.Sc Chemistry	2018	MZU	I	7.5	Chemistry

LIST OF PUBLICATION

1. **Zote, L.**, Lalrammawia, K., Buragohain, A., Lalrinhlupuii, Kakki, B., Lalmuanpuii, R., ... Zinicovscaia, I. (2021). Macro-, micro-, and trace element distributions in areca nut, husk, and soil of northeast India. *Environmental Monitoring and Assessment*, 193(2). <https://doi.org/10.1007/s10661-021-08859-9>
2. **Zote, L.**, Kakki, B., Buragohain, A., Lalrammawia, K., Lalrinhlupuii, Malsawmtluangi, Marak, N., Muthukumaran, R. B., Jahau, L., Khiangte, B., Ralte, J., Yushin, N., Nekhoroshkov, P., Grozdov, D., Zinicovscaia, I., & Dului, O. G. (2025). Essential and presumably contaminating trace elements in Indian Areca (*Areca catechu* L.) nut and their potential health effect. *Romanian Reports in Physics*, 77, 701. <https://doi.org/10.59277/RomRepPhys.2025.77.701>
3. Muthukumaran, R. B., Bhattacharjee, P., Bhowmick, P., **Zote, L.**, Malsawmtluangi, Kumar, N. S., Jahau, L., Cooke, M. S., Hu, C.-W., and Chao, M.-R. (2023). Genetic and epigenetic instability induced by betel quid associated chemicals. *Toxicology Reports*, 10, 223–234. <https://doi.org/10.1016/j.toxrep.2023.02.001>
4. Lalrammawia, K., Buragohain, A., Kakki, B., **Zote, L.**, Marak, N.K., Lalmuanpuii, R., Kumar, N.S., Jahau, L., Sudarshan, M., Chaligava, O. and Yushin, N., 2021. Determination of multi elements in tobacco plant of northeast India by neutron activation analysis and atomic absorption spectrometry. *Biological Trace Element Research*, pp.1-16. <https://doi.org/10.1007/s12011-021-03040-2>
5. Phadnis, P.P., Suman, S.K., Kumar, C., **Zote, L.**, Kumar. A., Bahadur. J., Rakshit, S. and Chakraborty, A., (2026). Development of ⁶⁸Ga labeled silver nanoparticles based multimodal formulation for *in-vivo* diagnosis of *Staphylococcus aureus* infection in Wistar rats. *J Radioanal Nucl Chem*. <https://doi.org/10.1007/s10967-026-10816-0>

PRESENTATIONS

1. National Seminar On Conservation of Biodiversity (NSCB 2022), Oral presentation, 'Determination of elemental profile of areca nut grown in NorthEast India using Neutron Activation Analysis' 2-3 September 2022, North-Eastern Hill University, Tura Campus, Meghalaya.
2. Mizoram Science Congress (2022), Poster Presentation, 'Distribution of rare earth elements in areca palm', 24-25, November, 2022. Aijal Club, Aizawl, Mizoram.
3. International Conference on Science and Technology for Innovative and Sustainable Development (STISD-2023). Oral Presentation, 'Essential Mineral and Other Trace Elements in Northeast Indian Areca Nut and their health consequences', 28-30 June, 2023, Mizoram University, Aizawl, Mizoram.

WORKSHOP/CONFERENCES ATTENDED

1. Workshop on Computer Aided Drug Design (Hands on Training). 14-20 April, 2023. By Centre for Advanced Computational Chemistry Studies, New Delhi, India
2. International Workshop on "Computational Genomics with R: Hands on course on NGS Data Analysis". 22-29 March, 2023. Organized by Department of Zoology, Mizoram University.
3. Workshop on Biomedical Statistics. 27 February-4 March, 2023. Organized by Technological Enabling Centre (DST Funded), Mizoram University.
4. Hands on Training on Synthesis and Characterization of Ferroelectrics (HTSCF). 10-17 February, 2023. Organized by Department of Basic Science, Humanities & Social Sciences (Physics), NIT Aizawl, Mizoram.
5. School on 'Know Your Elements (KYE-2019)', 4-10 August, 2019. Chemical Sciences Division, Saha Institute of Nuclear Physics, Kolkata, India.
6. International conference on Chemistry & Environmental Sustainability (ICCES-2019) 19-22 February, 2019. Department of Chemistry, Mizoram University.

7. One Week National School On Computer Assisted Spectroscopic Data Interpretation Techniques (NSCADIT-2019), 7-11, January 2019, Organised by Department of Physics, R.T.M. Nagpur.

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE:	LALRINAWMA ZOTE
DEGREE:	Doctor of Philosophy (Ph.D)
DEPARTMENT:	Chemistry
TITLE OF THESIS:	Characterization of Betel Nut (<i>Areca Catechu</i>) And Tobacco (<i>Nicotiana Tabacum</i>) of Mizoram
DATE OF ADMISSION:	9 th AUGUST 2018
APPROVAL OF RESEARCH PROPOSAL	
1. DRC:	16.04.2019
2. BOS:	2.0.04.2019
3. SCHOOL BOARD:	08.05.2019
4. MZU REGN. NO.:	2162 of 2012
5. Ph.D REGISTRATION NO. & DATE:	MZU/Ph.D./1249 of 09.08.2018
6. EXTTENSION (IF ANY):	No.16-2/MZU(Acad)/24/14-15
Dtaed: 3 rd July, 2024	

Head
Department of Chemistry

ABSTRACT

**Characterization of Betel Nut (*Areca Catechu*)
and Tobacco (*Nicotiana Tabacum*) of Mizoram**

**AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

LALRINAWMA ZOTE

MZU REGISTRATION NO: 2162 of 2012

Ph.D. REGISTRATION NO.: MZU/Ph.D./1249 of 09.08.2018



**DEPARTMENT OF CHEMISTRY
SCHOOL OF PHYSICAL SCIENCES
AUGUST 2025**

**Characterization of Betel Nut (*Areca Catechu*)
and Tobacco (*Nicotiana Tabacum*) of Mizoram**

By

LALRINAWMA ZOTE

Department of Chemistry

Supervisor

Prof. MUTHUKUMARAN R.

Submitted

**In partial fulfillment of the requirements of the Degree of Doctor of
Philosophy in Chemistry of Mizoram University, Aizawl**

ABSTRACT

The consumption of areca nut and tobacco represents one of the most prevalent lifestyle habits worldwide, ranking only behind alcohol and caffeine in terms of global usage. These products hold deep-rooted cultural, social, and ceremonial significance, particularly in regions like Mizoram, where their consumption is widespread. However, despite their cultural acceptance, they pose severe health risks, including addiction and a heightened incidence of cancer. Mizoram, in fact, reports the highest cancer rates in India, with oral and lung cancers being the most predominant, directly linked to the extensive use of locally grown areca nut and tobacco.

Given that these crops are primarily cultivated through organic farming methods in Mizoram, it becomes crucial to analyze their elemental composition, especially since they are consumed with minimal processing. To investigate this, samples of locally produced areca nut, tobacco, and the soil in which they are cultivated were subjected to detailed analytical testing. Instrumental Neutron Activation Analysis (INAA) was employed due to its precision and minimal sample preparation requirements, allowing for the detection of trace elements at concentrations as low as $\mu\text{g/g}$. In areca nut samples, we identified essential elements like sodium (Na), magnesium (Mg), potassium (K), calcium (Ca) trace metals like aluminum (Al), silicon (Si), chlorine (Cl), scandium (Sc), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), toxic elements arsenic (As), bromine (Br), rubidium (Rb), strontium (Sr), zirconium (Zr), antimony (Sb), cesium (Cs), barium (Ba) rare earth elements like lanthanum (La), cerium (Ce), neodymium (Nd), samarium (Sm), europium (Eu), terbium (Tb), dysprosium (Dy), ytterbium (Yb) also from the actinides hafnium (Hf), tantalum (Ta), tungsten (W), thorium (Th), uranium (U)

Tobacco samples contained also contains major elements sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), trace elements like rubidium (Rb), cesium (Cs), strontium (Sr), barium (Ba), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), zirconium (Zr), rare earths, lanthanum (La), cerium (Ce), neodymium (Nd), samarium (Sm), europium

(Eu), terbium (Tb), dysprosium (Dy), ytterbium (Yb), heavy metals like hafnium (Hf), tantalum (Ta), tungsten (W), thorium (Th), uranium (U)

Complementary analysis via Atomic Absorption Spectroscopy (AAS) confirmed the presence of cadmium (Cd), copper (Cu), and zinc (Zn) at $\mu\text{g/kg}$ levels. Notably, several detected elements particularly arsenic (As), nickel (Ni), chromium (Cr), and cadmium (Cd) are established carcinogens, while copper (Cu) and zinc (Zn) exhibit toxicity at elevated concentrations.

The heavy element concentration of tobacco is found to be weakly correlated to different soil parameters like soil pH, soil organic matter, and available NPK presents in the soil. The bioconcentration factor values indicate that the heavy elements Cd (7) are selectively absorbed and more efficiently in the leaves as compared to Zn (1.7), Cu (1.5), Ni (0.12) and Pb (0.1). The soil analysis was towards an acidic soil, under this condition Cl^- ion is absorbed and translocated into the stem more easily than the rare earth elements. The soil analysis showed that they were rich in organic soil matter. The characteristic of the soil also played a role in the uptake and transport of elements from soil to various parts of the plant as observed that correlation analysis performed on the various soil parameter, in areca nut the K concentration level have a positive correlation trends along with the pH of the soil, on the other hand Al, Mg and Cl contents have negative correlation with the soil pH. The calculated bioaccumulation on areca nut shows that the level of Cl and Cd are higher as compared to the other elements showing that these two elements are translocated more in the acidic soil of areca nut plantation.

From the calculation of pollution indices for metal pollutants: V, Co, Zn, As, Sb, U, Cd, Cu, and Pb from the results it shows that As is having the highest contaminant, moderate contamination shown by Zn and Pb whereas the other metal pollutant mostly lies below the threshold value i.e, below 1.

The health risk assessment shows that consumption of areca nuts daily may have led to an adverse health effect in long term consumption of the product. Additionally, the consumption of areca nut and tobacco products may lead to accelerating the health of a person who consumed the product since both the products have a synergistic effect upon consumption.

Tobacco-specific nitrosamines (TSNAs) and tobacco-specific aromatic amines (TSAS) are among the most hazardous carcinogens found in tobacco products. These compounds form during the curing, fermentation, and combustion of tobacco leaves and are strongly associated with oral, esophageal, and lung cancers. Liquid chromatography tandem mass spectrometry (LCMS/MS) has emerged as the gold-standard analytical technique for quantifying trace levels of tobacco-specific nitrosamines (TSNAs) and tobacco-specific aromatic amines (TSAS) due to its exceptional sensitivity and selectivity. The presence of these compounds in commercially available product were also analyse by using this technique, which detects the presence of TSNAs N- nitrosornicotine (NNN), 4- methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK) and its metabolite 4- (methylnitrosamino)-4-(3-pyridyl)-butanal (NNAL) along with tobacco alkaloids such as Nicotine and Nornicotine in the present study which are classified as Group 1 carcinogens by IARC.

Principal component analysis (PCA) was also applied in order to see the distribution pattern of the elements to verify the origin of the product that can be used as a geographical origin of a product based on their elemental contents. In our study it clearly distinguishes the study samples from the samples of different country origin from available published literature, showing the capabilities of using PCA for finding their geographical origin of a product.

In the resent study, it has been observed that ionic heavy elements and carcinogenic nitrosamines are present in the tobacco used in an indigenous smoking tobacco product, called zozial, which remain unregulated, and it would be prudent to regulate the sale of zozial and a pictorial warning is included in the packs of zozial. Furthermore, consumption of betel quid (with or without tobacco) is group 1 carcinogens, and in the present study, we have observed along with ionic heavy elements in themature areca nut relatively lower concentration of Cu species, which is known to elicit ROS production, it would be prudent to regulate the sale of betel quid, locally known as kuhva.