Isolation and characterization of Potassium Solubilizing Bacteria from Mica contaminated soil in Giridih District, development of biofertilizer and its efficacy in crop production

Thesis submitted to Jadavpur University
in partial fulfilment of the requirements for the
degree of Doctor of Philosophy

SAIBAL GHOSH
Index No: – 23/18/Life Sc./25
Indian Statistical Institute
Jadavpur University
Department: Life science & Bio-technology

A tribute to our beloved

Late Mr. Hari Shankar Saw

With pride and gratitude, this thesis is dedicated to my family, teachers, friends, philosophers Dr. Abhishek Mukherjee and Dr. Pradip Bhattacharyya, who always wanted me to do something, but I failed.

Abstract

Index No. 23/18/Life Sc./25

Title: Isolation and characterization of Potassium Solubilizing Bacteria from Mica contaminated soil in Giridih District, development of biofertilizer and its efficacy in crop production

Submitted by: Saibal Ghosh

The state of Jharkhand is rich in mineral resources, including mica and several other minerals.In this study, the effects of long-term mica waste with potentially toxic elements (PTEs-Cr, Ni, Cd, Pb, Cu, and Zn) dumped near mines near agricultural land and water resources are examined, as well as their effects on soil ecosystems and human health.Heavy metals from the mica waste not only deteriorate the soil quality but also results in the uptake metals in crop. The present investigation was conducted to evaluate the effects of different fractions of metals on uptake in rice, soil microbial and biochemical properties in mica waste contaminated soils of Jharkhand, India. From each active mine, soil samples were randomly collected at distances of < 50 m (zone 1), 50-100 m (zone 2), and >100 m (zone 3).The Ni, Cr, Cd and Pb in rice grain were 0.83±0.41, 0.41±0.19, 0.21±0.14 and 0.17±0.08 mg kg^{-1} respectively.The rapid mining activities of mica mines in Giridih district, have led to toxic metal pollution of agricultural soil. This is a key concern for environmental risk and human health.The mean concentration of total and bio-available potentially toxic elements (PTEs - Cr, Ni, Pb, Cu, Zn, and Cd) was higher in zone 1 across three zones.The Positive matrix factorization model (PMF) and Pearson Correlation analysis were used to identify waste mica soils with PTEs. Based on PMF results, Ni, Cr, Cd, and Pb were the most promising pollutants and carried higher environmental risks than the other PTEs. Using the self-organizing map (SOM), zone 1 was identified as a high-potential source of PTEs.Monte Carlo simulations (MCS) model and sensitivity analysis of total carcinogenic risk (TCR), children were more affected by Cr and Ni than adults through ingestion exposure pathways.The wastes from mica mines have the potential to pollute the food chain, degrade the quality of the soil, and harm natural systems over time.

On the other hand, muscovite and biotite are two forms of waste mica, which is a K-bearing mineral that is a waste product of the mica industry; although, it is not recognized that mica contributes to crop production with K.Potassium (K) is an essential component of plant nutrients, performing biological functions to maintain plant growth and yield.To feed the ~ 1.3 billion people, excessive application of chemical fertilizers in India has a negative impact on both the economy and environmental sustainability. It is necessary to introduce sustainable agents that promote evergreen agriculture, such as potassium- solubilizing rhizobacteria (KSB).A total of 30 potassium solubilizing rhizosphere bacteria (KSB) isolates, were collected from different mica-contaminated agriculture fields of Giridih district. Based on K solubilizing capacity, we selected four KSB strains (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) for bio-fertilizer preparation and evaluated their efficiency as K fertilizers on tomatoes grown under Giridih soil (Alfisol).

Abhishek Mukherjee, Ph.D.
Associate Professor
Agricultural and Ecological Research Unit
INDIAN STATISTICAL INSTITUTE
New Barganda, Giridih - 815 301
Jharkhand, India

Signature of Supervisor (1)

Saibal Ghosh.
Signature of Student
12/12/2022

Pradip Bhattacharyya
Pradip Bhattacharyya, PhD
Associate Professor
Agricultural and Ecological Research Unit
Indian Statistical Institute
New Barganda, Giridih-815301
Jharkhand, India

Signature of Supervisor (2)

INDIAN STATISTICAL INSTITUTE

Abhishek Mukherjee, PhD
AssociateProfessor
Biological Science Division
Agricultural & Ecological Research Unit

Rose Villa, New Barganda
Giridih, Jharkhand - 815 301, India
06532-226688 (O), (91) 8902686646 (mobile)
abhi.mukh@yahoo.com

Dated:

Certificate from the supervisor

This is to certify that the thesis entitled **"Isolation and characterization of Potassium Solubilizing Bacteria from Mica contaminated soil in Giridih District, development of biofertilizer and its efficacy in crop production"** submitted by **Mr. Saibal Ghosh** who got his name registered on **7.02.2018** for the award of Ph.D. (Science) degree of **Jadavpur University**, **Department of Life science & Bio-technology**, is absolutely based upon his own work under the supervision of **Dr. Abhishek Mukherjee** and **Dr. Pradip Bhattacharyya**, Indian Statistical Institute, Giridih and that neither this thesis nor any degree / diploma or any other academic award anywhere before.

Supervisor (1),
Abhishek Mukherjee
Associate Professor,
Agricultural and Ecological Research Unit,
Indian Statistical Institute, Giridih.

Abhishek Mukherjee, Ph.D.
Associate Professor
Agricultural and Ecological Research Unit
INDIAN STATISTICAL INSTITUTE
New Barganda, Giridih - 815 301
Jharkhand, India

Pradip Bhattacharyya
Supervisor (2),
Pradip Bhattacharyya
Associate Professor,
Agricultural and Ecological Research Unit,
Indian Statistical Institute, Giridih.

Pradip Bhattacharyya, Ph.D
Associate Professor
Agricultural and Ecological Research Unit
Indian Statistical Institute
New Barganda, Giridih-815301
Jharkhand, India

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled **"Isolation and characterization of Potassium Solubilizing Bacteria from Mica contaminated soil in Giridih District, development of biofertilizer and its efficacy in crop production"** was submitted to the **Life science and Biotechnology Department, Jadavpur University** in partial fulfilment of the award of the degree of Doctor of Philosophy in Life science is a record of research work carried out by me under the Joint-supervision **Dr. Abhishek Mukherjee (Ph.D.)**, Associate Professor, Agriculture, and Ecological Research Unit, Biological Sciences Division, Indian Statistical Institute, Giridih, Jharkhand-815301 and **Dr. Pradip Bhattacharyya (Ph.D.)**, Associate Professor, Agriculture, and Ecological Research Unit, Biological Sciences Division, Indian Statistical Institute, Giridih, Jharkhand-815301.

No part of the thesis has been submitted elsewhere for the award of any other degree.

Saibal Ghosh.

Date: 12/12/2022
Place: Giridih.

(Saibal Ghosh)
Agricultural and Ecological Research Unit,
Biological Sciences Division,
Indian Statistical Institute,
Giridih, Jharkhand-815301.

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Saibal Ghosh.

Date: 12|12|2022

(Saibal Ghosh)

Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION

%	Per cent
@	At the rate of
<	Less than
>	Greater than
° C	Degree centigrade
ANOVA	Analysis of variance
Al	Aluminium
C	Carbon
Ca	Calcium
CA	Coal ash
Cd	Cadmium
CD	Cow dung
CI	Contamination index
CPF	Cancer potency factors
Cu	Copper
DTPA	Diethylenetriaminepentaacetic acid
EC	Electrical conductivity
ERI	Ecological risk index
Fe	Iron
Igeo	Geo-accumulation index
K	Potassium
LSD	Least significant difference
MBC	Microbial biomass carbon
Mn	Manganese
N	Nitrogen
P	Phosphorus

Pb	Lead
PCA	Principal component analysis
PI	Pollution index
PLI	Pollution load index
PTE	Potential toxic element
PTM	Potential toxic metal
SOC	Soil organic carbon
SOC	Soil organic carbon
TOC	Total organic C
TN	Total nitrogen
UV-VIS	Ultra violet- Visible
WHC	Water holding capacity
Zn	Zinc
BOD	Biological oxygen demand
Cfu	Colony forming unit
Cm	Centimetre
DAI	Days after incubation
DAS	Days after sowing
Dsm^{-1}	Decisiemen per meter
e.g.	Example
EM flask	Erlenmeyer flask
et al.	(co-authors)
H_2S	Hydrogen sulphide
HCN	Hydrogen cyanide
i.e.	That is
IAA	Indole acetic acid
KSB	Potassium solubilizing bacteria
KSM	Potassium solubilizing microorganisms
KSR	Potassium solubilizing rhizobacteria

MUB	Modified Universal Buffer
IAA	Indole-acetic acid
OD	Optical density
PGP	Plant growth promoting
PGPR	Plant growth promoting rhizobacteria
SD	Standard Deviation
SE	Standard error
SEm	Standard error mean
Viz.	Namely
WB	Waste Biotite
NEK	Non-exchangeable K
WK	Watersoluble K
ExK	Exchangeable K
WM	Waste Muscovite
CABs	Commercial Alexandrow broth
CEC	Cation exchange capacity
TA	Total acidity
EA	Exchangeable acidity
EA	Extractable acidity
Ca	Calcium
Na	Soil Sodium
M	Molar
N	Normal
GA3	Gibberellic acid
GIS	Geo-stat
NH_3	Ammonia
MAB	Modified Aleksandrow broth

Isolation and characterization of Potassium Solubilizing Bacteria from Mica contaminated soil in Giridih District, development of biofertilizer and its efficacy in crop production

Saibal Ghosh

SYNOPSIS

Jharkhand is rich in mineral resources, including mica and several other minerals, and heavy metals in this mica waste hindered crop production and deteriorated soil quality. The present investigation was conducted to evaluate the effects of long-term dumping of mica waste near mines with agricultural fields on soil microbial and microbial eco-physiological indicators for mica-enriched soils of Giridih district, Jharkhand state, India, as well as biochemical parameters. The present study was conducted to examine the effect of long-term mica waste dumping near the mines with agricultural fields, the effects on different forms of heavy metals as well as Cr, Cd, Cu, Pb, and Ni on soil microbial, microbial eco-physiological indicators, and biochemical parameters of mica enriched soils of Giridih district. The presence of potentially toxic elements (PTEs) in this mica waste hampered crop production, deteriorated soil quality and causes different health issues.

Muscovite and biotite are two forms of waste mica, which is a K-bearing mineral that is a waste product of the mica industry; although, it is not recognized that mica contributes to crop production with K. Potassium (K) is an essential component of plant nutrients, performing biological functions to maintain plant growth and yield. To feed the ~ 1.3 billion people, excessive application of chemical fertilizers in India induces a negative impact on both the economy and environmental sustainability. There is a need to introduce sustainable agents that promote evergreen agriculture, such as potassium solubilizing rhizobacteria (KSB). A total of 30 potassium solubilizing rhizosphere bacteria (KSB) isolates, were collected from different mica-contaminated agriculture fields from the Tisri, Gawan, and Deori blocks of Giridih district, Jharkhand. Based on K solubilizing capacity, we selected four KSB strains (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) for bio-fertilizer preparation and evaluated their efficiency as K fertilizers on tomatoes grown under Giridih soil (Alfisol).

Characterization and impacts of mica-contaminated soil on the environment

Heavy metals from the mica waste not only deteriorate the soil quality but also results in the uptake metals in crop. The present investigation was conducted to evaluate the effects of different fractions of metals on uptake in rice, soil microbial and biochemical properties in mica waste contaminated soils of Jharkhand, India. From each active mine, soil samples were randomly collected at distances of < 50 m (zone 1), 50-100 m (zone 2), and >100 m (zone 3). Sequential metal extraction was used to determine the fractions of different metals (nickel (Ni), cadmium (Cd), chromium (Cr) and lead (Pb)) including water-soluble (Ws) and exchangeable metals (Ex), carbonate-bound metals (CBD), Fe/Mn oxide (OXD) bound metals, organic bound metals (ORG), and residues (RS). The Ni, Cr, Cd and Pb in rice grain were 0.83±0.41, 0.41±0.19, 0.21±0.14 and 0.17±0.08 mg kg-1 respectively. From the variable importance plot of the random forest (RF) algorithm it was observed the Ws fraction of Ni, Cr and Cd and Ex fraction of Pb were the most important predictor for rice grain metal content. Further the partial dependence plots (PDP) give us an insight of the role of two most important metal fractions on rice grain metal content. The microbial and enzyme activity were significantly and negatively correlated with Ws and Ex metal fractions, indicating that water soluble and exchangeable fractions exert a strong inhibitory effect on the soil microbiological parameters and enzyme activities. The wastes from mica mines have the potential to pollute the food chain, degrade the quality of the soil, and harm natural systems over time.

Effects of mica mines and their present potentially toxic elements on soil and human health

The rapid mining activities of mica mines in Giridih district, India, have led to toxic metal pollution of agricultural soil. This is a key concern for environmental risk and human health. 63 top soil samples were collected at a distance of 10m (Zone 1), 50m (Zone 2), and 100m (Zone 3) from near 21 mica mines with agriculture fields. The mean concentration of total and bio-available potentially toxic elements (PTEs - Cr, Ni, Pb, Cu, Zn, and Cd) was higher in zone 1 across three zones. The Positive matrix factorization model (PMF) and Pearson Correlation analysis were used to identify waste mica soils with PTEs. Based on PMF results, Ni, Cr, Cd, and Pb were the most promising pollutants and carried higher environmental risks than the other PTEs. Using the self-organizing map (SOM), zone 1 was identified as a high-potential source of PTEs. Soil quality indexes for PTEs risk zone 1 were found to be higher across three zones. Based on the health risk index (HI), children are more adversely affected than adults. Monte Carlo simulations (MCS) model and sensitivity analysis of total carcinogenic risk (TCR), children were more affected by Cr and Ni than adults through ingestion exposure pathways. Finally, a geostatistical tool was developed to predict the spatial distribution patterns of PTEs contributed by mica mines. In a probabilistic assessment of all populations, non-carcinogenic risks appeared to be negligible. The fact that there is a TCR can't be ignored, and children are more likely to develop it than adults. Mica mines with PTEs contamination were found to be the most significant anthropogenic contributor to health risks based on source-oriented risk assessment.

Temporal dynamics of potassium release from waste mica as influenced by potassium solubilizing bacteria

We have isolated KSB strains using Alexandrov media from waste mica mines of Giridih district of Jharkhand, India. These isolates were evaluated for their potential to dissolve water soluble-K from waste mica (muscovite and biotite). Identity was confirmed based on sequencing of 16S rDNA region of those isolates showing promising water soluble-K dissolving capacity. Strains were found to be different isolates of *Bacillus cereus*, two unconfirmed *Bacillus species* (strain- 6SB1 and GG6), and one each of *B. velezensis* and *Paraburkholderia kururiensis*. Finally, the four most efficient KSBs were selected based on their K-solubilizing capability. The K5B (*B. cereus*) isolate showed the highest K-solubilising capacity in both muscovite and biotite enriched medium. Soil incubation study was conducted using soils of Giridih (Alfisol) with three gradient concentrations of both waste mica tailings and K-solubilising capacity of four KSB isolates (*B. cereus*, strain- K5B, K6, K15; and *Bacillus sp.* GG6- K12) were measured at 4, 7, 14 and 21 days intervals. The K release dynamics in incubated soils indicated that potassium was released from both types of micas to significantly higher water-soluble K (WS-K) and exchangeable K (Ex-K) pools due to the inoculation of KSB isolates. Apart from potassium solubilization, *B. cereus strain* K5B and *Bacillus sp.* GG6 showed capabilities to produce indole acetic acid (IAA) and gibberellic acid (GA). These results suggested that a combination of KSB strain and powdered mica tailings could be a suitable alternative to commercial chemical fertilizers and maintain soil nutrient status for plant uptake.

Pot experiment

Muscovite and biotite are two forms of waste mica, a K-bearing mineral, that are waste products of the mica industry; however, the contribution of K to crop production is not recognized. This study aims to investigate the pattern of K release from waste micas (muscovite and biotite) inoculated with K solubilizing bacterial (KSB) strains like *Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15, as well as their effectiveness as K fertilizers using tomato plants var *Solanum Lycopersicum* and grown under Giridih soil (Alfisol). The application of muscovite (@ 200 mg kg^{-1} soil + *Bacillus cereus* K5B) and biotite (@ 200 mg kg^{-1} soil + *Bacillus cereus* K5B) significantly increased tomato plant biomass, improving fruit quality and yield, while maintaining soil quality and increasing soil availability of K. KSB applied with two types of mica forms on Alfisol soil produced higher fruit yields and K utilization compared to soil fertilized with synthesized K fertilizer and soil without K fertilizer. We observed significant similarities between releasing pattern of K forms in muscovite and biotite during our experiments. It was observed that biomass and tomato yields are positively correlated with K uptake by the tomato plant parts (shoot, root, and fruit) and with different pools of K (Wa.K, Ex.K, and NEK) in the soils tested. The application of bio-intervention (KSB) on muscovite and biotite might be an alternative and effective way to dissolve insoluble forms of K for plants and act as a source of K-fertilizer to maintain soil K levels and crop yields.

Preface of the thesis

The purpose of this thesis is to fulfil a doctoral degree requirement in the department of Life science and biotechnology of Jadavpur University about "Isolation and characterization of Potassium Solubilizing Bacteria from Mica Contaminated Soil in Giridih District, development of biofertilizers and their efficacy in crop production". Hopefully, the results will be of interest to soil scientists, agricultural microbiologists, and environmental scientists for improving soil, crop, and environmental quality and maintaining soil as well as human health.

The work described in the thesis was carried out during (dated) at Life science and biotechnology of Jadavpur University, Kolkata, and Indian Statistical Institute of Giridih district, Jharkhand under the supervision of Dr. Pradip Bhattacharyya Associate Professor and Dr. Abhishek Mukherjee Associate Professor, Agricultural and Ecological Research Unit, Biological Science Division, Indian Statistical Institute of Giridih district, Jharkhand.

This detailed study provided useful information about different forms of mica (muscovite and biotite) and their application in agriculture through biofertilizers technology using KSB isolates.
Chapter 1 predicts the introduction which provides much of the general background and overview of the current situation of mica mines, mining activities, the presence of metal ions in mica mines and its adverse effects on soil and human health, KSB, and its solubilization mechanisms from an unavailable form of K to an available form of K. This chapter justifies the reason for choosing the research topic.

Chapter 2 is comprised of reviews of the literature on relevant work done in the past on the subject and explains the research work carried out by different researchers, both national and international regarding KSB and its mechanisms of solubilization from unavailable form.

Chapter 3 (Objective 1); Effect of metal fractions on rice grain metal uptake and biological parameters in mica mines waste contaminated soils.

Chapter 4 (Objective 2); Source acquainted pollution and health risk assessment of potentially toxic elements in mine tailings contaminated soils in India employing synergistic statistical approaches.

Chapter 5 (Objective 3); Temporal dynamics of potassium release from waste mica as influenced by potassium solubilizing bacteria.

Chapter 6 (Objective 4); Comparative analysis of potassium solubilizing bacterial (KSB) fertilizers on the solubilization of waste micas (muscovite and biotite) and their effect on growth promotion and nutrient acquisition by tomato (*Solanum Lycopersicum L.) plant grown under Alfisol, Giridih district, Jharkhand.*

Keywords: Mica mines, Soil, and human health, PTEs, FIAM, SAMOE, PMF, SOM, GIS, KSB biofertilizers, pot experiment.

CHAPTER-1. INTRODUCTION

1.1. General background: Mica mines and their effects on the environment and human health

Mining activities are one of the leading causes of heavy metal pollution in the environment. It generates a considerable amount of waste tailings and rocks and they are deposited at the surface. In the case of damage to the land surface, wastes such as rocks and tailings are easily unstable, thus polluting the environment. Naked tailings are extremely vulnerable to erosion and often release toxic metals into the soil and water (Shyamsundar et al., 2014). The direct consequences are the loss of agricultural land, forests, or grazing land, and the loss of production; the indirect consequences are pollution of air and water and siltation of rivers. In the long run, these will result in biodiversity loss, amenity deterioration, and economic loss (Wong, 2003). Excessive heavy metal dispersion from mine sites can adversely impact the environment by contaminating water and soil, causing phytotoxicity, and rupturing soil. Agriculture soils and crops near mining areas have been contaminated with heavy metals, which have been considered a serious environmental issue (Zhuang et al., 2009). In these contexts, mica mines are one of the most important sources of heavy metal and metal ions. Mica deposits in India cover a total area of about 3888 sq. km in the districts of Giridih and Koderma in Jharkhand and Munger in Bihar (Huang et al., 2013; Nishanth and Biswas, 2008). The majority of mines in this area are still active right now, but a few have been closed for a number of years. Mica wastes that contain heavy metals have negatively impacted crop production and soil quality, and it is also detrimental to human health. Improving or maintaining soil quality is necessary not only for a good environment but also for sustainable agriculture.

Potentially toxic metals or elements (PTEs) are generally referred to as non-essential toxic elements from a geochemical perspective (Mondal et al. 2017). The sorption-desorption dynamics of such toxicants within the earth's surface heavily influence their migration. Through the application of geostatistical methods, it is possible to better understand the mobility pattern of metals in soil systems (Bhuyan et al. 2015; Mondal et al. 2017). Nature is omnipresent with heavy metals and their compounds, whether it's in the soil, water, or biota. Human beings and other living organisms require metals like Fe, Cu, Mn, and Zn which are essential for proper physiological functioning. These essential metals, however, can also be toxic beyond a limit. Metals like Cr, Cd, As, Co and Pb do not have any known requirements in the human body, and can cause havoc at even very low concentrations (Bruins et al. 2000; Giri et al. 2020).

1.2. Agricultural status in India

In an era in which the global population is growing at an alarming rate, agriculture will face one of the greatest challenges in ensuring world food security. The study assumed the world's population would grow from 7 billion to 8.3 billion in the year 2025, and by 2050 the world will need 70 to 100 percent (%) more food. This is one of the biggest challenges facing agrarian communities in the future, and agriculture will be able to provide this food supply (Bahadur et al., 2016). In India, agriculture is the backbone of the economy, two-thirds of the total population lives in rural areas, linked to their income, and contributes 19% of GDP (Gross Domestic Product)(Srinivasarao et al., 2011). According to the estimation by the NAAS (National Academy of Agricultural Science), India's demand for food grains will reach around 300 million tonnes annually by 2020, with estimates increasing from 218 million tons to 82 million tons Produced in 2009-10 (Kinekar, 2011). As of the year 1952, agriculture was responsible for 52.1% of total dependence; this was down from 72.4% in 1952, largely due to the rapid development of industrial and service sectors. The growth of the agricultural sector has increased in recent years, but there is no substitute for agriculture to meet the needs of the population (Kinekar, 2011). Currently, soil fertility and its assessment are areas that need immediate attention since it is now established that the productivity of different crops in an arrest is due to soil depletion on the one hand, and unbalanced application of plants and other nutrients on the other. As well as these challenging circumstances, lots of effort needs to focus on the soil biological system and the agro-ecosystem as a whole to gain a deeper understanding of the complex processes and efficient interactions between soil and plant microorganisms that control agricultural soil.

1.3. Role of fertilizers in agriculture

On earth, the soil naturally contains many macro and micronutrients such as nitrogen (N), phosphorous (P), potassium (K), and calcium. These nutrients play a vital role in a plant's growth. When crops are harvested; soil nutrients are removed from the soil, and due to the short supply of essential nutrients, plants suffer from nutrient deficiencies and stunted growth. With the imbalance of nutrient levels, the plant cannot function properly and produced the food needed to feed the world’s population requirements. Farmers have applied fertilizers on agricultural land to increase crop yields by providing microelements and microelements required for plant growth and productivity. Plants need a number of essential minerals to grow and develop healthily, but N, P, and K are the most important. In soil, there are 13 mineral nutrients present, and they are classified into two categories (macronutrients and micronutrients) depending on the amount required.

Mineral nutrition and fertilization are the important factors of rice cultivation strongly affecting productivity and quality of the end product. Nitrogen was thought to be the most effective nutrient for crop productivity and quality (Natesan and Ranganathan, 1990). Nevertheless, continuous and long-term application of N alone reduces its efficiency for crop production. This may be explained by unbalanced nutrient supply and availability, accelerated by the depletion and leaching of basic cations like K^+ in soils (Sparks, 2000). K is a major and essential macronutrient for plant growth and development. Phosphorus (P) is another essential macronutrient element for plants, which is a component that is limited in fertilizer management. Without an adequate amount of P, the crop could not reach the top of the economic yield level. P plays a vital role in the plant system; the formation of new cells, and DNA synthesis, promotes root development, particularly of the lateral and fibrous rootlets, hastens leaf development, flowering, fruits, and maintaining seed quality. Rock P is the major source of phosphatic fertilizer in agriculture.

Without the addition of fertilizers, crop yields and agricultural productivity will be significantly reduced. So, it is needed that fertilizer replaces the nutrients that the crop removes from the soil. This is why mineral fertilizers are used as a supplement to nutrient-rich stocks with rich minerals that can be quickly absorbed and used by crops.

1.3.1. Potassium (K) status in Indian agriculture:

As one of the most important macronutrients, Potassium (K) is essential for plant and animal life on Earth and is found in all living things. K is the 7th abundant element in the rocky outer part of the Earth (lithosphere) and chemically active metal in nature. Being a chemically active metal, K is never found in nature in its pure elemental form in the soil (Kinekar, 2011). Plants can absorb K through the soil as water or exchangeable form, soil minerals, and synthetic and organic fertilizers. K consumption was exceeded in India by 264 lakh tons in the following two years (2019 and 2020), and K fertilizers (synthetic and organic) were imported worldwide to meet the agricultural demand, which indicates the harmful application of K fertilizers (FAI, 2020). According to 72% of Indian agricultural soil test results of K fertility status of Indian agricultural soils (representing 266 districts) is classified as three levels low at 21%, medium at 51%, and 28% high. Soil test methods classified as low, medium, and high concentrations of K values need further purification for soil testing/crop response relationship. A serious attitude towards the application of K has still required for farmers. It is a need to educate about nutritional balance and efficiency, peak higher crop yield and quality, and its importance in Indian agriculture (Hasan, 2002).

1.3.2. Role of K in plant system:

Among the essential macronutrients, potassium (K) is 3rd most important nutrient, it is one of the key pillars of balanced fertilizer use after nitrogen (N) and phosphorus (P). It plays a vital role in the synthesis of proteins, cells, starch, cellulose, and vitamins. K also helps plant processes due to the need for activation of at least 60 different types of enzymes involved in plant growth, vital for osmoregulation, cation-ion balance, protein photosynthesis, water balance, habitat loss, disease resistance, resistance to abiotic and biotic stresses and crop yields to improve quality and shelf life (B. B. Basak and Biswas, 2009; Meena et al., 2015; Ramamurthy et al., 2017). K is mainly present in the meristematic tissues of the plant and helps the formation of the strong root. It also helps enhances disease resistance in a plant by strengthening stalks and stems provided to the thicker cuticle and which protects against diseases water loss and controls the turgor pressure within plants to prevent wilting and enhances flavor, texture, and quality (Evans and Sorger, 1966).

1.3.3. Potassium forms present in the soil

Soil potassium (K) availability is influenced by the type of soil and the physicochemical properties of the soil depending on it. In the soil, K exists in four forms: soluble or available, exchangeable, fixed, and structural K. Compared to the total – K present in the soil, the amount of soluble, exchangeable, and non – exchangeable –K in the soil is lower (Sparks and Huang, 1985). The concentration of readily available soluble K in soil is usually very small (1 - 2%) compared to 90 % of K available in the form of insoluble K minerals, such as silicates or micas. In the present study by (Arnold, 1958; Conyers and McLean, 1969) the soil K is divided into water available (WK), NH_4OAc extractable or exchangeable K (EK), HNO_3- extractable or non-exchangeable K (NEK), and structural or mineral K forms. While all four forms of K are taken up directly by plants, the soil amounts are tiny and the soil EK, which is replaced by NH_4 from NH_4 OAC solution, is held by negative charges on organic matter and clay particles and readily absorbed by the plants (Jaiswal et al., 2016). In soils, the exchangeability of K has been well studied; however, the kinetics of exchange has received less attention. Since kinetic reactions are thought to exist between the different phases of soil K, exchange reactions between soil solution and EK are considered to be a major indicator of soil K availability. It is usually assumed that the soil solution and the exchangeable phase react instantly. With the introduction of HYV under intensive crop management, the soil has started depleting from high to medium to low K levels, as evidenced by soil tests. Consequently, crop response has been observed with the application of K, and K sorption capacity is an important soil feature that influences plant response to phosphorus fertilization. Plants respond to potassic fertilizers based on the K adsorption capacity of the soil (Debnath and Majumdar, 1998).

1.4. Forms of micas

In nature, mica (Potassium Aluminum Silicate) occurs as muscovite (granitic pegmatite), biotite (sericite, schists), gneisses, and phlogopite (metamorphosed limestones). Raw mica is classified as flake micas sheet and scrap mica. Scrap and flakes like mica are mainly produced in India, Korea, the United States, and the Soviet Union. Sheet mica is produced in India, Brazil, and a few African countries (Skulberg et al., 2018). In the world, India is blessed to have the largest accumulation of mica mines distributed over a total area of about 3888 sq. km Koderma and Giridih districts of Jharkhand and Munger district of Bihar (Huang et al., 2013; Nishanth and Biswas, 2008). Many mines located in this area are still now active. But there are few which have been closed down for several years. During the dressing of raw micas, large quantities of waste micas (~75% of total mica mines); are generated during the washing of raw micas after their mining which is not used in agriculture as a sole source of K though contains a significant amount of K (8–12% K_2O) and dumped near the mica-mine sides.

Mica was previously used as a filler in pharmaceuticals and cosmetics, but today is used in paints, asphalt, cement, and electrical cables, and the use of mica has increased dramatically over the century from 1900 to 2000). Annual world production was 26001 in 1905, 442001 in 1937, 234 000 in 1974, and 350000 in 1981. Mica dust can cause adverse effects on humans when it is inhaled during mining activities, milling, farming, and factories that process mica products (Skulberg et al., 2018).

Raw micas are one of the huge sources of K, but in micas most of the K is present in unavailable form and not used for plant uptake. These waste materials like muscovite and biotite can effectively be applied as a source for K when it is altered or modified by some biological (bacteria and fungus), and suitable chemicals (phosphoric acid and sulphuric acid) means.

1.5. Role of biofertilizers in soil fertility

The idea of biofertilizer was evolved by (Day and Döbereiner, 1976) with the discovery of nitrogen (N) fixing organism (*Azospirillum*) and (Pikovskaya, 1948) with phosphate solubilizers. The populations of biofertilizers naturally vary in soils and agroclimatic zones, and the populations may not be sufficient for plants to benefit from biofertilizers because their efficiencies vary with strains. The term biofertilizer encompasses a wide range of bio-inoculants, such as nitrogen fixers, phosphorus solubilizers, potassium solubilizers, potassium mobilizers, and growth-promoting rhizospheric microorganisms (Jayaraj et al., 2004).

The use of bio-fertilizers for long-term crops may be limited since most planted crops have some limitations; for example, the use of bio-fertilizers in the early stage of establishing crops can be more effective in nurseries and fields at the stage of growing plants to increase the health of planting stock for the next successful establishment. Crop plantations have been experimentally developed to investigate the use of bio-fertilizers (including organic manures and composts) to increase growth and crop productivity. This performance has been showing good or better than the alternative fertilizers such as inorganic fertilizers. Mineral solubilizers and arbuscular mycorrhizal fungi (AMF) are effective enough for growth improvement to save 25% on P-nutrition of plantation crops like coconut, paper, ginger, cardamom, turmeric, and cashew when grown under field conditions (Nautiyal et al., 2006). Through the application of P-solubilizing bacteria (PSB), the nutrition of inorganic phosphorus cashew plants has been improved with *Bacillus* megaterium and the continued application of AMF, asospirillum, and phosphate solubilizers. (Sharma et al., 2006).

Bacteria and fungi in the soil play a major role in the natural phosphorus (P) and potassium (K) cycles and the solubilizing bacteria (PSB and KSB) in the soil and rhizosphere (Diep and Hieu, 2013). The soil-plant microorganisms have got much importance in recent decades. Microorganisms, especially rhizospheric microbes, are present in the soil, and they play an important role in plant growth and development. The soil contains a large number of microorganisms, especially in the rhizosphere. It is well known that a large number of microorganisms, such as bacteria and fungi, form an overall system with plants. They are able to multiply very easily in a rhizome to promote the growth and yield of the plant (Vessey, 2003).

1.6. Role of biofertilizer in plant growth-promoting hormones

Several types of microorganisms, including N-fixers, and P-solubilizers, are capable of producing plant-growth-promoting hormones, such as auxin, cytokinin, and gibberellin, as well as reducing several plant diseases and parasite infections, and certain types of microbial strains that are capable of bringing about ISR (Induced Systemic Resistance) against diverse pathogens that invade the same host species. Using PGPR strains will be an effective and economical way to protect a wide range of disease-control plants (Bashan and De-Bashan, 2005).

Plant growth-promoting hormones (PGPRs); also known as phytohormones in the rhizosphere, are produced by microbial communities in situ and are derived from root exudates and microorganisms. (Arshad and Frankenberger, 1991) reported inoculation with *Azospirillum* strains shows better growth and higher dry yields in maize and is mainly responsible for the production of N fixation and plant growth regulators. PGPR or phytohormones accelerated plant growth as well as the production of dry matter. PGPR microorganisms produced IAA (indole-3-acetic acid) as the main hormone (Fallik et al., 1989). Compared to other growth-promoting hormones, few researchers found biologically significant levels were different forms of IAA like indole-3- methanol (Crozier et al., 1988), cytokinins, and indole – 3- butyric acid (IBA) (Crozier et al., 1988), cytokinins and indole – 3- butyric acid (IBA) (Fallik et al., 1989), abscissic acid (ABA) (Kolb and Martin, 1985), uncharacterized indole compounds (Hartmann et al., 1983), several gibberellins (Bottini et al., 2004). Few growths promoting strains such as *P. aeroginosa*, *P. cepacia*, *P. putida*, and *P. fluorescens* genera showed on winter wheat increased the plant height, root and shoot mass, and several tillers (Freitas and Germida, 1990). (Radwan et al., 2005) found the increased growth and indole production contents by A. lipoferum Br17 and Azospirillum brasilense Cd inoculants due to moderate aeration. In recent years, more attention has been paid to exploring early root colonizers who have benefited from plant growth, either directly or indirectly. Advantageous root associating soil commonly known as rhizobacteria are generally referred to as PGPR. PGPR belongs to some genera like *Azotobacter*, *Bradyrizobium*, *Azospirillum*, *Rhizobium*, *Bacillus*, *Pseudomonas*, *Erwinia*, *Serratia*, and *Pseudomonads*. Among them, *Pseudomonads* found are the early root colonizers and helps considerably to plant production and protection. PGPR inoculants used as a broad-spectrum disease control are a cost-effective and environmentally friendly way to protect the plant. Using microorganisms in agriculture can reduce the use of chemical fertilizer and yield environmentally friendly crops (Glick, 1995; Requena et al., 1997).

1.7. Role of KSB as a plant growth promoter

Farmers are applying chemical fertilizers in uncontrolled and unbalanced ways for increasing crop production. They are not enlightened about the amount of fertilizer required for plants to vary different crops. There is a huge gap between farmers and researchers. So, most of the farmers only use traditional chemical fertilizers like urea as a source of nitrogen (N), and DI-ammonium phosphate (DAP) as a source of phosphorus, and few farmers are using MOP (muriate of potash) as a source of potassium (K) for crop production. Due to the lack of use of K fertilizers, water-soluble or available forms of K level decrease in soil due to huge utilization by the crop (Meena et al., 2015; Zhang and Kong, 2014).

Some selected bacteria can release K from the insoluble minerals such as micas or feldspar, known as KSB. Researchers have discovered that KSB can provide beneficial effects on growth by suppressing pathogens and improving soil nutrients and structures. The K-solubilizing bacteria can soluble and release K from silicate minerals and secrete bio-available minerals to enhance plant growth. These bacteria are widely used in biological leaching and biological potassium fertilizers (Meena et al., 2015). Few special groups of bacteria are present in rhizospheric soil, colonized the rhizosphere is considered PGPR bacterial species helps the plant growth, plants health, and nutrient uptake, especially under unbalanced nutrition conditions. Some of these PGPR bacteria are capable dissolve K from K-bearing minerals or raw micas by extracting organic acids that could either directly decompose or chelate silicon ions to convert unavailable or no exchangeable K into water-soluble K that could be directly available by plants (Bennett, 1998; Friedrich et al., 1991; Journal et al., 2017; Xiao et al., 2017).

Rhizospheric bacteria assist in soil secretion of soluble compounds, decomposition, and solubilization of soil organic matter and K, accumulation, storage of nutrients, mobilization, and mineralization of nutrients (Zeng et al., 2012). Those bacteria also help in soil nitrogen fixation, phosphate solubilization, nitrification and de-nitrification, sulphate reduction, and potassium solubilization (Diep and Hieu, 2013; Zeng et al., 2012). The usefulness of these microbial organisms in available content K deficient in soils can reduce the risk of environmental pollution hazards (Liu et al., 2012; Requena et al., 1997). KSB had been widely used, as a K-biofertilizer in India and China where soil, where soluble K was deficient in the soil KSB inoculation, has found dozens of examples of improved plant growth. Inoculation with KSB has been reported that KSB has beneficial effects on the growth of cotton, rape, jiggery, peanuts, tea, maize, wheat, and sudhan grass (Xiao et al., 2017). (B B Basak and Biswas, 2009; Singh et al., 2010) also reported that co-inoculation of mize, wheat, and sudhan grass with KSB strains resulted in huge K mobilization from waste mica, which in turn served as a source for plant growth. The mobilization of potassium from relatively hard K minerals like micas (Mons’ hardness index > 3) was too difficult than soft layered formation potassium bearing minerals (Mons’ hardness index < 3). Thus, there have been several reports about KSB regarding the availability of solid K- minerals and soil nutrients and their effects on plant growth (Xiafang and Weiyi, 2002).

Farmers are exposed to the negative environmental impact of excessive use of chemical fertilizers and also increase crop production costs, hence the urgent need to use environmentally friendly and cost-effective agricultural technology to increase crop production. Thus, the use of KSBs is considered to be a sound alternative strategy to improve the productivity of agricultural soils. This novel strategy is also claimed to demonstrate the ability to restore productivity in degraded, marginal productive, and disproportionate agricultural soils. However, the use of KSBs is limited due to a lack of knowledge among farmers and practitioners (Maurya et al., 2014; Meena et al., 2015).

Bacillus mucilaginosus (Sugumaran and Janarthanam, 2007; Zarjani et al., 2013; Zhao et al., 2008), *Bacillus edaphicus* (Sheng, 2005), *Burkholderia, Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, Bacillus edaphicus* (Sheng and He, 2006) *Bacillus circulans,* and *Paenibacillus sp* (Liu et al., 2012; Parmar and Sindhu, 2013)*, Pseudomonas frequentans* and *Clasdosporuim* (de la Torre et al., 1992)*,* and *Paenibacillus giucanolyticus* (Sangeeth et al., 2012) bacterial isolates a wide range of rhizospheric microorganisms and reported as potassium solubilizers or KSB. These KSB strains have the capability to soluble K from K-bearing mines or minerals. Few researchers reported *Bacillus mucilaginosus* and *Bacillus edaphicus* KSB isolates have activity in mobilizing and solubilizing K from minerals and have wide range of applications in mining, bio-fertilizers, crop yield, and metallurgy (Li et al., 2006; Lian et al., 2002; Liu et al., 2012; Zhang and Kong, 2014; Zhao et al., 2008).

1.8. Selection of tomato plant (*Solanum Lycopersicum L.*)

Tomatoes (*Solanum Lycopersicum L.*) are the World's most important and popular horticultural crops worldwide and they have been widely used as a model plant for several fields of plant research. On a global basis, 20 million metric tons of tomatoes are produced every year. The United States, Italy, Spain, and the U.A.R are the top producers of this crop (Monteiro et al., 2012; Salunkhe et al., 1974). Tomatoes, along with other foods, have been recommended as a world-balanced healthy diet. The growing population of the world, including tomatoes, needs stable fruit yields (Daoud et al., 2020). It is also one of the most important protective vegetables or fruits in India and it is produced on 1204 thousand hectares of land and has a productivity of 19, 042 million tons and 21.2 metric ha^{-1} (NHB, 2014) respectively. In India the most tomato producing as Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh, and West Bengal (Nagoni et al., 2017). Tomatoes and their by-products are a huge source of lycopene in the human diet and contain a significant amount of various antioxidant compounds like phenolics, flavonoids, ascorbic acid, and vitamins C and E. The results of epidemiological studies have shown that regular consumption of tomatoes and tomato-based products can help prevent a variety of cancers, especially prostate cancer, heart disease or cardiovascular problems, and metastatic activity, gene function regulation, cell-cell communication, cell cycle arrest, apoptosis, quenching of singlet oxygen and reduction of free radicals, carcinogen metabolism and metabolic pathways involving phase I and phase II drug-metabolizing enzymes (Kanr et al., 2008; Kerkhofs et al., 2005; Toor et al., 2006, 2005; Toor and Savage, 2005). Tomatoes also have β-carotene which is also an antioxidant, is also an important carotenoid in tomatoes which provides to maintain healthy skin and tissue lining and it also plays a wide role as a provitamin a carotenoid, because hypovitaminosis (Meena et al., 2017).

According to some factors like fertilization, nutrient supply has significant effects on the balanced values and quality of tomatoes. K (potassium) and Ca (Calcium) ions are essential nutrients for the growth and quality of tomato fruit (Hernández-Pérez et al., 2020; Sonntag et al., 2019; Weinert et al., 2021). Several researchers have found positive correlations between K-fertilization and environmental stress tolerance like drought, salinity, and cold, as well as resistance to pests and pathogens (Sonntag et al., 2019; Weinert et al., 2021). K also helps maintain fruit size, acidity, soluble solids, the content of sugars, and texture, among others. K forms as K_2O is the nutrient most absorbed by the tomato plant and also helps many physiological processes, such as photosynthesis, enzymatic activation, and synthesis of proteins (Neto et al., 2016; Sonntag et al., 2019).

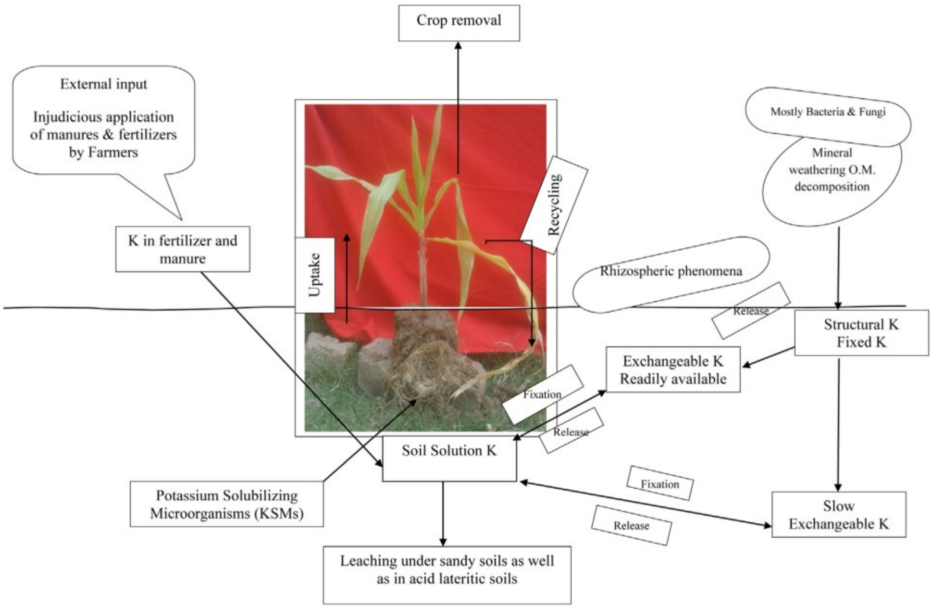


Fig. 1.1. Rhizospheric phenomena between Soil-Microorganism-Plant (SMP) as affected by potassium solubilizing microorganism (KSMs). Sources:(Singh et al., 2016)

Several workers studied the effect of different mines and mining activities on soil microorganisms (Ma et al., 2015; Zhang et al., 2018), the effects of mica mines and available heavy metals in soil, and their effect on microbiological and biochemical indicators of soil quality did not study still now. There is evidence that heavy metal uptake by plants is positively related to the bioavailable concentration of heavy metals in soil.

Although a study published recently (Giri et al. 2021; Giri and Singh, 2022) found the adverse effects of heavy metals and fluoride contamination in groundwater in mica mining areas of Jharkhand states, India; and its effects on human health. The effects of mica mines and mining activities on the soil and the presence of heavy metals in the soil and their effect on human health have not yet been investigated.

Current trends in agriculture mainly focus on reducing the use of toxic chemical pesticides and inorganic fertilizers, and finding alternatives to improve crops in sustainable agriculture. Farmers and researchers are preferred biological fertilizers over chemical fertilizers. A biological approach is not only eco-friendly and economical; it also contributes to soil quality and to preserving natural soil plants and microbial diversity. Environmentally beneficial microbial strains can be used as organic fertilizers as a tool for sustainable agriculture and an alternative to chemical fertilizers and pesticides (Meena et al., 2015; Zhang and Kong, 2014).

Objectives

1) Effect of metal fractions on rice grain metal uptake and biological parameters in mica mines waste contaminated soils.
2) Source acquainted pollution and health risk assessment of potentially toxic elements in mine tailings contaminated soils in India employing synergistic statistical approaches.
3) Temporal dynamics of potassium release from waste mica as influenced by potassium solubilizing bacteria.
4) Comparative analysis of potassium solubilizing bacterial (KSB) fertilizers on the solubilization of waste micas (muscovite and biotite) and their effect on growth promotion and nutrient acquisition by tomato (*Solanum Lycopersicum L.*) plant grown under Alfisol.

CHAPTER – 2. REVIEW OF LITERATURE

2.1. Background knowledge of Mica mines

In Jharkhand and Bihar, Giridih and Koderma districts contain the world's largest mica deposits in an area of about 3888 sq.km (Huang et al. 2013; Nishanth et al. 2008). About 75% of the total mica mine waste is dumped near the mines during the dressing of raw micas. Mining is one of the leading sources of heavy metal pollution in the environment, and it generates substantial amounts of waste tailings and rocks that are deposited on the surface. Whenever the land surface is damaged, wastes such as rocks and tailings become unstable and pollute the environment. A naked tailings pile is extremely vulnerable to erosion and releases toxic metals into the soil and water (Shyamsundar et al., 2014). Direct consequences include the loss of agricultural land, forests, and grazing land, as well as the loss of production; indirect consequences include air- and water-pollution, as well as river silting. This will lead to biodiversity loss, amenity degradation, and economic loss in the long run (Wong, 2003). Since mica wastes are generally fine, loose, and homogeneous, have low bulk density and moisture-holding capacity, and are devoid of nutrients, they not only hinder plant colonization but also pollute the surrounding environment during strong winds and heavy runoff. Mica wastes containing heavy metals have negatively affected crop production and soil quality, as well as human health. Mica wastes containing heavy metals have negatively affected crop production and soil quality, as well as human health. The quality of soil plays an important role in the sustainability of agriculture as well as the preservation of the environment.

2.2. Impact of waste micas on environment

Having heavy metals present in this mica waste hampered crop production and deteriorated soil quality, which may eventually result in the loss of biodiversity, economic opportunities, and resources. Soil quality enhancement or maintenance is essential not only to a healthy environment but also to sustainable agricultural practices. The total metal element in the soil is an effective indicator of soil pollution; they do not provide adequate information about the potential environmental impact. A number of studies have demonstrated that heavy metal uptake by plants is positively related to the bioavailable concentration of soil heavy metals (Monterroso et al., 2014; Xiao et al., 2017). For the testing of heavy metal bioavailability, DTPA, EDTA, $NaNO_3$, and $CaCl_2$ have all been used as extractants because of their simplicity and ease of operation (Xiao et al., 2017). In spite of this, the bioavailable fraction of heavy metals reported by (Prasad, 2004) studies varies due to the slightly different extraction procedures used, which are operational in nature. In this context, sequential chemical extraction methods are more popular methods to use quantification of different forms of metal in soil (Beckett, 1989). The sequential chemical extraction technique breaks down metals into different solubility and mobility forms and can be used to predict the conversion of metals into different forms of metals in soils and the availability of metals in microorganisms (Bhattacharyya et al., 2008).

Several workers studied the effect of different mines and mining activities on soil microorganisms (Ma et al., 2015; Zhang et al., 2018), the effects of mica mines and available heavy metals in soil and their effect on microbiological and biochemical indicators of soil quality did not study still now. There is evidence that heavy metal uptake by plants is positively related to the bioavailable concentration of heavy metals in soil.

2.3. Impact of waste micas on human health

Mica mines and its potentially toxic metals or elements (PTEs) are generally referred to as non-essential toxic elements from a geochemical perspective (Mondal et al. 2017). The sorption-desorption dynamics of such toxicants within the earth's surface heavily influence their migration. The total metal element in the soil is an effective indicator of soil pollution; they do not provide adequate information about the potential environmental impact. According to Dai et al. (2004), many countries use the total metal content of soil samples as a measure of soil contamination to determine heavy metal concentrations in soils. However, this method does not provide information on the ability of the elements to be absorbed by plants, and it cannot predict the transfer of toxic elements through the food chain (Morel 1996). Diethylenetriaminepentaacetic acid (DTPA) is widely used as a method for evaluating the bioavailability of non-essential trace metals (Lebourg et al. 1996).

Nature is omnipresent with heavy metals and their compounds, whether it's in the soil, water, or biota. Human beings and other living organisms require metals like Fe, Cu, Mn, and Zn which are essential for proper physiological functioning. These essential metals, however, can also be toxic beyond a limit. Metals like Cr, Cd, As, Co, and Pb do not have any known requirements in the human body, and can cause havoc at even very low concentrations (Bruins et al. 2000; Giri et al. 2020). The United States Environmental Protection Agency has listed some metals such as Cd, Cr, As, Hg, Pb, Cu, Zn, and Ni as priority control pollutants (USEPA 1997, 2001) because of their potential toxicity. Metals enter the body through many routes including ingestion, inhalation, and dermal contact. Ingestion is the most common route, particularly when drinking water and eating foods (Weissmannová et al. 2017). A systematic assessment of the health risks associated with continuous exposure to toxic metals is a requirement for the populations exposed to toxic metals through groundwater and mines contaminated soils. According to Man et al. (2013), assessment of health risk involves calculating the probability of adverse health effects based on exposure routes, risk sources, and risk receptors. In contrast, uncertainty is intrinsically linked to the assessment of risk, where 'uncertainty' is explained by a lack of knowledge pertaining to the actual value of a variable. (Giri et al. 2020) As a result of the uncertainties, deterministic risk assessments may underestimate or overestimate the risks and should therefore be approached differently (Koupaie et al. 2015). As a result, PRA (probability risk assessment) attempts to characterize the uncertainty and illustrate it through the range and distribution of exposure parameters or variables (Mallongi et al. 2022). According to USEPA, Monte Carlo Simulations can be used for probability risk assessment, which calculates the frequency of occurrences based on probability distributions for each exposure parameter (Giri et al. 2020; Mallongi et al. 2022).

A study published recently (Giri et al. 2021; Giri and Singh, 2022) found the adverse effects of heavy metals and fluoride contamination in groundwater in mica mining areas of Jharkhand states, India; and its effects on human health. The effects of mica mines and mining activities on the soil and the presence of heavy metals in the soil and their effect on human health have not yet been investigated.

2.4. General information on fertilizers

A massive application of chemical fertilizers on Indian soil is negatively affecting the economy and the environment, causing a growing need to return to nature and promote sustainable agriculture (Meena et al., 2015). Agricultural fertilizers with low nutrient efficiency are becoming more expensive, so alternative methods of beneficial and environmentally friendly fertilizers are needed. As part of the soil-plant-microorganism (bacteria and fungi) system, cast soil stores large amounts of nutrients and provides energy for living things (Meena et al., 2016). Due to higher classifications of mineral and biological deposits, pesticides and chemical fertilizers that are hazardous to the environment continue to be used to increase crop production. The current need for alternative and environmentally friendly technologies to enhance organic farming methods includes Plant Growth Promoting Microorganisms (PGPM) and Integrated Pest Management (IPM) (Kumar et al., 2016). In a feasible agricultural ecosystem, rhizospheric microorganisms such as rhizobacteria or fungi are an integral part of decomposing organic matter, converting nutrients, and fostering soil fertility under organic nutrient cycling. Moreover, it controls the flow of nutrients by synthesising them, producing biomass, and binding them organically or biologically. Various chemical transformations of soil are mediated by soil living microorganisms, which influence micro- and micronutrient availability. The use of PGMs organisms helps to increase crop yields as well conventional plant protection (Meena et al., 2016).

Azospirillum, Bacillus subtilis, B. mucilaginosus, B. edaphicus, B. circulans, Paenibacillus spp., Acidithiobacillus ferrooxidans, Pseudomonas, Burkholderia, potassium solubilizers, phosphorous, and zinc solubilizing microorganisms are called SMAT microbes, produced phytohormones, helping plant growth promotion. These microorganisms are also environmentally friendly and maintain an environmental ecosystem. In the earth-crust rhizosphere is a vital part of the soil, which is controlled by plant roots mean.

Potassium ions are essential for plant growth and development; they are involved in immune system function, signalling pathways, and resistance to biotic and abiotic stresses like apoptosis, salinity, drought, and oxidative stress. In addition to improving cell quality, enzyme activation, and photosynthesis, they also help maintain soil potassium levels that have fallen due to erosion, runoff, crop removal, and leaching (Raghavendra et al., 2016; Velázquez et al., 2016; Zahedi, 2016). The current situation of K exhaustion in the soil is gradually increasing due to unavailable form of K present in the soil.

Increasing prices of chemically synthesized potassium fertilizers are a serious problem. Farmers cannot afford to purchase the K they need to grow plants, and this has negative effects on their mechanical stability, nutrition, and pathogen resistance (Jaiswal et al., 2016). Additionally, nutritional imbalances in developing countries and crises in some countries need to be resolved by increasing global production of K fertilizer, and scientists are concerned about soil fertility since India is not self-sufficient in K fertilizer production, and the entire amount of potassic fertilizer required (5 million tonnes) needs to be imported. In order to increase crop yields and enhance soil fertility, it is essential to maintain stable crop-nutrient relationships. Therefore, native sources of K minerals can be considered as alternative sources of cost-effective fertilizers (Saha et al., 2016).

The Earth's crust is one of the largest sources of stored K, with around 98% of the K found in silicate minerals (micas), feldspar, rocks, and orthoclase that are not soluble in water. K concentration in soil exceeds 20,000 ppm, which is initially unavailable to plants. Only 1-2% of the total potassium content is gradually available to plants (Jha and Subramanian, 2016; Rawat et al., 2016; Sharma et al., 2016). In soil, K is present in four forms such as solution or water-soluble K, exchangeable K, unavailable or non-exchangeable K, and minerals or structural K. Some rhizospheric microorganisms such as bacteria (*Pseudomonas, Burkholderia, Acidothiobacillus ferrooxidans, Bacillus mucilaginosus, Bacillus edaphicus, B. circulans, Paenibacillus sp., B. cereus, B. subtilis, B. coagulans, B. amyloliquefaciens, B. megaterium, Enterobacter hormaechei, Flectobacillus spp., and Paenibacillus spp.*) fungal strains (*A. terreus, A. fumigatus, and Aspergillus niger*), and yeast (*Torulaspora globosa*) are capable of K release from K-bearing minerals (feldspar) using extracting organic acids in soils for plant uptake (Liu et al., 2012; Meena et al., 2015; Parmar and Sindhu, 2013; Sheng, 2005; Yadav and Sidhu, 2016).

Potassium solubilizing microorganisms (KSMs) are one of the most promising microorganisms for sustainable agriculture because they promote plant growth. PGP is a complex process that is rarely responsible for a single process because most PGPMs affect plant growth through multiple processes. Almost any rhizospheric microbial agent must interact not only with the plant but also with other organisms in the vicinity of the microenvironment. The potassium solubilizing microorganisms have the capabilities production of plant growth promoting hormones such as IAA production, GA_3 production, HCN, antifungal activity, siderophore production, Ammonia, and K solubilization (Kumar et al., 2016). *Pseudomonas spp. and Bacillus spp.* are the most dominant PGPB of rhizospheric soils, also known as K solubilizing microorganisms. Inoculation of KSMs seems to be committed to promoting plants growth under 2.3 to 3.5 dSm^{-1} salinity concentrations with low availability of phosphorus and potassium. They also protect the plants from injury by salinity stress like lipid peroxidation and increasing plant growth-related physiology. These KSMs help to reduce lipid peroxidation and increase plant cell membrane stability for plant survival under the stress of salt (Jha and Subramanian, 2016). K solubilizers provide an immune boost on plants to fight against pathogens and different environmental stress conditions. The indigenous microbes proved their effectiveness; such microbes suit the environmental conditions in the cropping system for which they are intended. KSMs is one of the best sustainable technologies for agriculture and crop productions, solubilize fixed K to available K for plant uptake. Thus, the biological formulas of potassium-soluble microorganisms as bio-fertilizers suggest an environmentally sustainable approach and also meet the potassium requirements for crop production (Jaiswal et al., 2016).

This review chapter contains broad information of current research work presented in the work topic title is “Isolation and characterization of Potassium Solubilizing Bacteria from Mica contaminated soil in Giridih District, development of biofertilizer, and its efficacy in crop production” and subheadings are described below.

2.4.1. Role of potassium in living organisms

K^+ ions were recognized as an essential nutrient in animals and plants even before the twentieth century. It contributes to nerve and muscle function, heart health, bone health, kidney function, and blood pressure regulation in animals, and it regulates many physiological functions in plants, including plant growth, strength, photosynthesis, immunity to pests and diseases, as well as controlling water conditions and increasing drought tolerance in plants (Zhang and Kong, 2014; Zörb and Peiter, 2013). In the cells, K^+ cation is accountable for protein synthesis, enzyme activation, osmoregulation, ion homeostasis, and manages membrane potential and balance of charge in both plant and animal cells (Fig. 2.4.1) (Marschner, 2011). Generally, cultivated plants absorb potassium by roots as soon as nitrogen (N), although bananas, cotton, and some other species require more potassium than nitrogen. As potassium dissolves in clay solution and mixes with clay and organic colloids, but it can also be part of more complex chemical compounds (Meena et al., 2016; Sheng et al., 2003; Zandonadi et al., 2010).

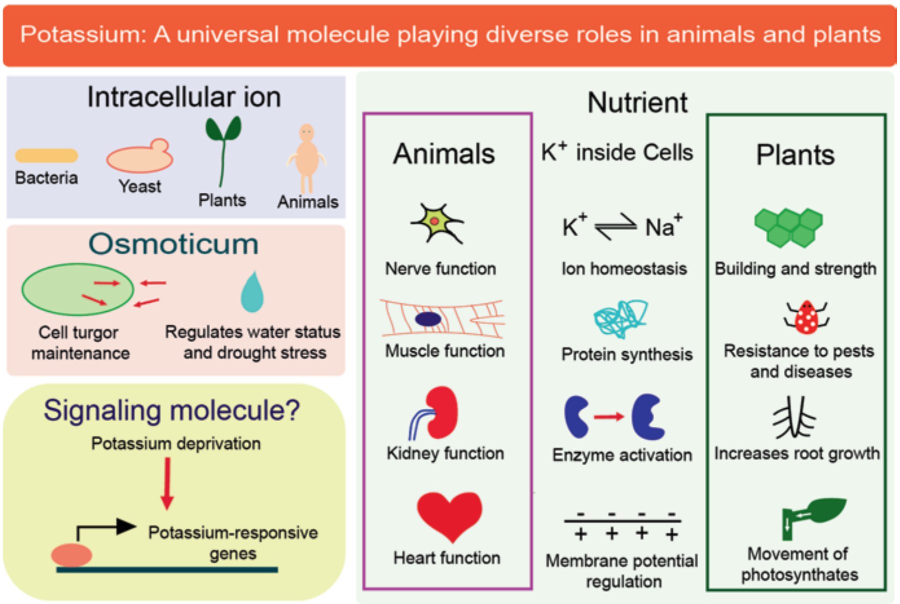


Fig: 2.4.1. Illustration of the role of K ions in plants and animals, and it is responsible at a cellular and physiological level. K is a vital nutrient, osmoticum, and signalling molecule in animals and plants (Pandey and Mahiwal, 2015).

K is the one of the most abundant cations in plant cells, the constant concentration of (80-100 mM) is maintained in the cytoplasm (Dreyer and Uozumi, 2011). K is highly mobile in the plant at all levels, such as tissues, xylem, phloem, cells, and tissues, and is absorbed by the roots and converted into K^+ ions. In contrast with potassium, magnesium and calcium have important structural functions, but only limited mobility in plants. Potassium is vital for regulating water status in plants. K is essential for photosynthesis and meristematic growth, as well as for fruit development and preservation in carbohydrate metabolism (Meena et al., 2016).

The K^+ cation plays a critical role in photosynthesis, involved in enzyme activation and in the production of ATP (adenosine triphosphate), which probably controls the amount of photosynthesis more effectively than stomatal activity. A primary high-energy product is ATP, which is produced when solar energy is combined with CO_2 and water to form sugar; at the point of ATP production, the balance of electrical charge is maintained by the K^+ cation. When plants have a deficiency of K, the rate of photosynthesis are slowdown and the flow of ATP production decrease, and all processes dependent on ATP synthesis. In addition, the respiration by plant is increased, which gives to moderate growth and development (Moran, 2007; Sharma et al., 2016). ATP also helps as an energy source for the plant transport system. When K ions present an inadequate amount, the ATP production also diereses; as a result, the transport system breaks down. This creates photosynthesis in the leaves and reduces the rate of photosynthesis. The general development of energy storage organs such as grains, fruits is considered (Ben-zioni et al., 1970; Sharma et al., 2016).

The production of the crop is significantly affected by biotic stresses and abiotic tresses. It is reported that the highest potential loss weeds production up to 32%, in addition, 18% animal pest, 15%

bacteria and fungi, and 3% viruses. In most cases, potassium deficient plants are more affected by infection compared to those that supply adequate amounts of K ions (Oerke and Dehne, 2004). (Sarwar, 2012) reported that the absence of K supply, the rate of "rice borer infestation" was highest, but K decreased rapidly as the density increased (Holzmueller et al., 2007) had found a similar result with a fungal spp. (*Discula destructiva Redlin*) infection in Cornaceae (*Cornus florida L.*) flowering plant. It also reported that potassium reached fertilizer showed significantly reduce stem rot and AgSS (aggregate sheath spot) disease and found negative correlations between disease inflexibility and potassium percentage (Williams and Smith, 2001). K fertilizer significantly reduces fungal infections (70%), bacteria (69%), mites and insects (63%), viruses (41%), and nematodes (19%) (Sharma et al., 2016). The uses of K^+ cation to reduce biotic stress can express as below (Fig. 2.4.2). In addition to K can control biological stress the effectiveness of plants during abiotic stresses (drought). The uses of K^+ cation plays in abiotic stress reduction are expressed (Fig. 2.4.3).

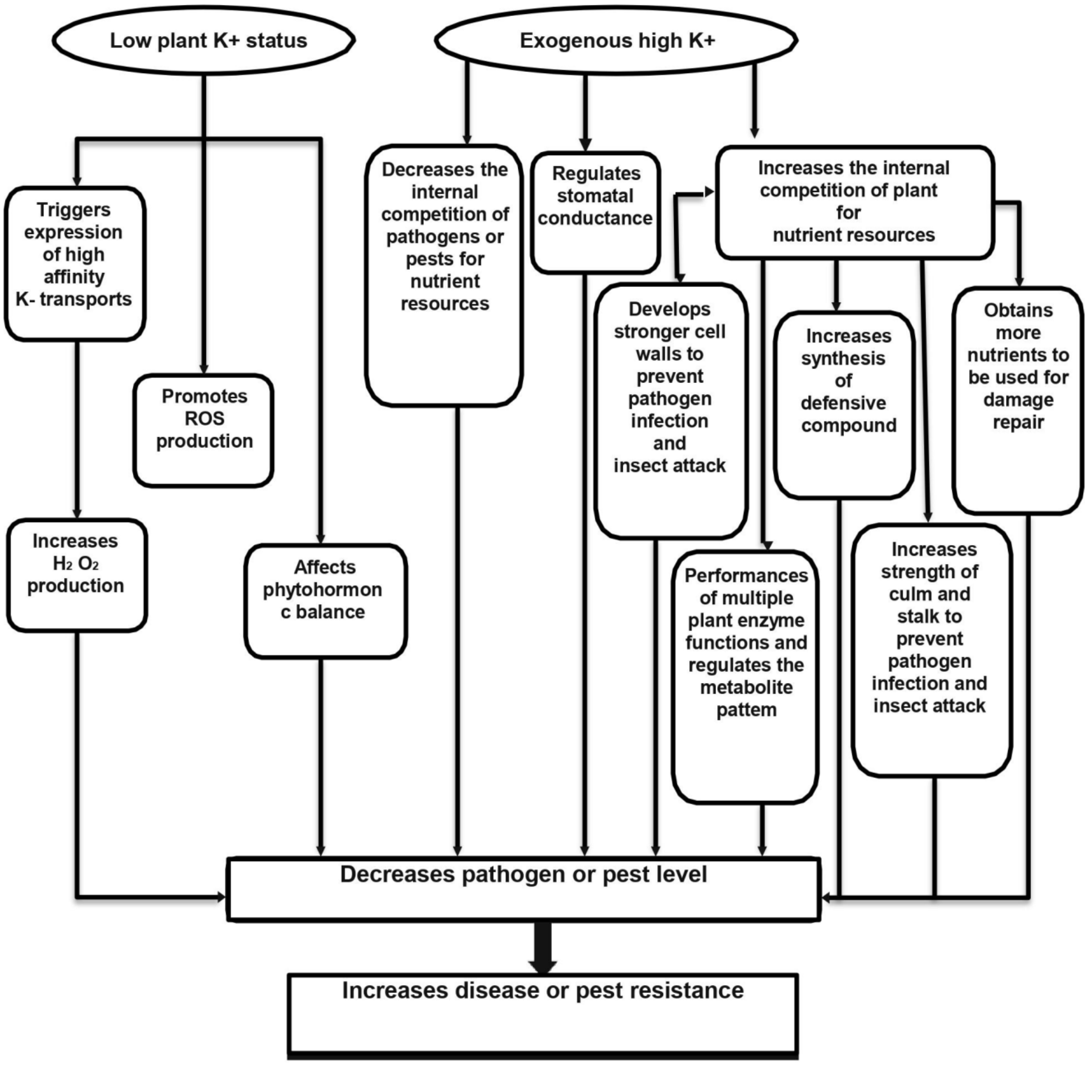


Fig: 2.4.2. The role of K under biotic stress, Sources; (Sharma et al., 2016; Wang et al., 2013)

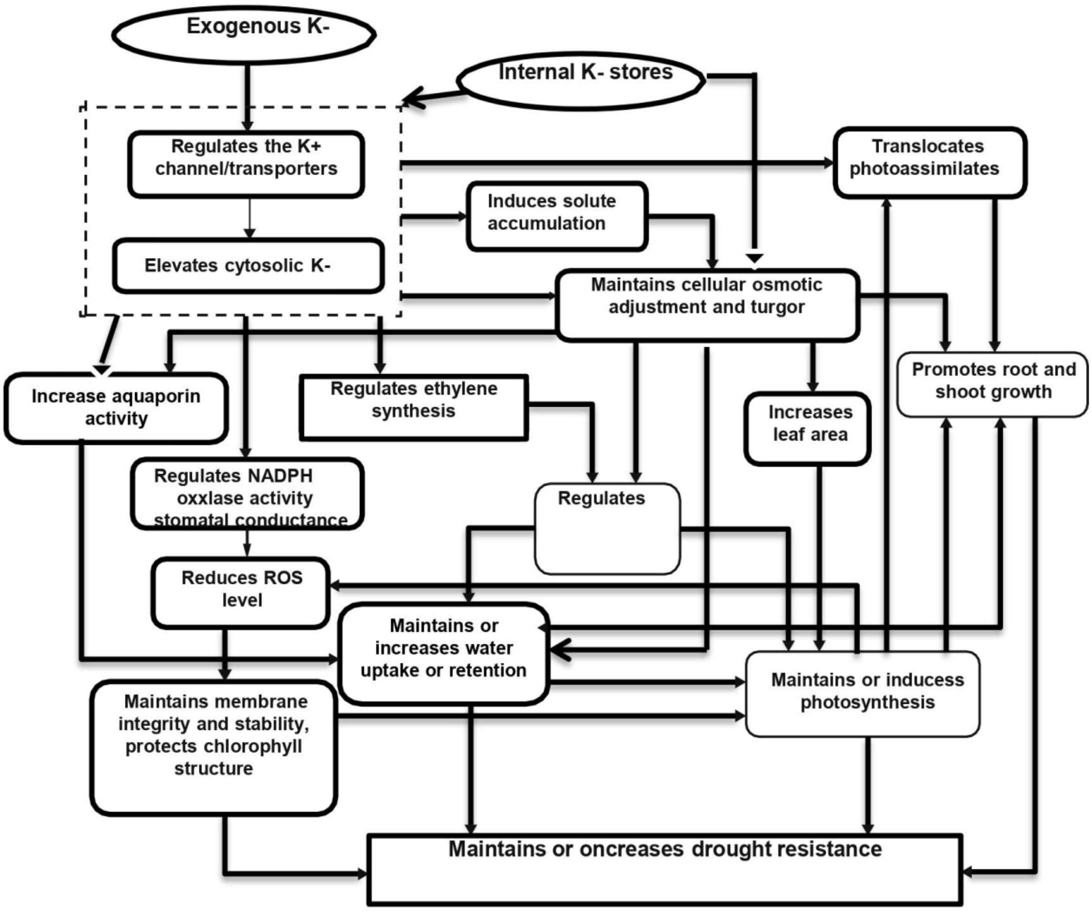


Fig: 2.4.3. The role of K in osmoregulation during drought stress, Sources; (Sharma et al., 2016; Wang et al., 2013)

In order to achieve the potential of the plant, it is necessary to have an insufficient amount of K, along with several factors like grain size and quality, oil content, dry matter, sugar and carbohydrate content, fruit ripening and quality, starch content, and tuber size. The different efficacy of potash in plants is related to physiological conditions and divergent stresses. These functions include diversified and efficient nitrogen and drought tolerance, water use, frost resistance, and pest and disease resistance. Hence, it is not surprising that the lack of K available to plants in the soil results in poor and low-quality crop yields; that damaged during major growing seasons. So, a sufficient amount of K is vital for which it provides some "insurance" against an unfavourable situation in challenging growing seasons (Meena et al., 2016).

2.5. Worldwide potassium demand

It was only in the nineteenth century that K fertilizer was used in agriculture following Justus v. Liebig's discovery that plants need K in distinct quantities and proportions for growth, as well as other vital nutrients such as nitrogen (N), phosphorus (P), and potassium (K) to increase biomass. The key sources of K are soil clay minerals, rocks, micas, and feldspar, in ocean water, rock salt deposits in ocean water, and crystalline minerals in long dry seas (Prakash and Verma, 2016). In the last 40 years, global K utilization has increased 2.5 times. In the year 1960 to 2000, the global utilizes of K fertilizer increased by 9 to 22 metric tons, and 16% of fertilizer use was accounted for total fertilizer. The potash consumption of the developed countries has increased 1.25 times in the past 40 years. Since 1960, the demand for potash has increased 22 times from 0.5 metric tons to 11.3 metric tons in developing countries, and production has increased up to 37 metric tons. Since 1960, the demand for potash has increased 22 times from 0.5 metric tons to 11.3 metric tons in developing countries, and production has increased up to 37 metric tons (Meena et al., 2016). The worldwide consumption of potash in different countries represents following the (Fig. 2.5).

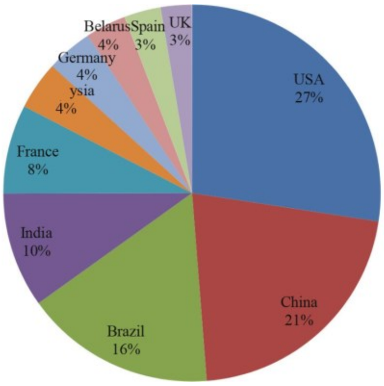


Fig.2.5. The worldwide consumption of potash fertilizers in different countries in year 2000, sources; (Prakash and Verma, 2016).

In global agriculture, KCl or potassium chloride is the key used ore. (Prud'Homme, 1997) reported that KCl accounted for 96% of world potash production with the remaining 4% coming from potassium sources such as potassium sulphate, potassium magnesium salts, and potassium nitrate.

About 28% of the potash produced in 2012 came from Canada, compared to Russia (17%), Belarus (15%), China (8%), Germany (10%), Israel (7%) and Jordan and Chile (3%) respectively (Prakash and Verma, 2016).

2.6. Status of potassium (K) in Indian agricultural soil

India produced 230 million tons of food grains during 2007-08, using 23 million metric tons of NPK, and it was estimated that by 2025, the growing population will need about 45 million metric tons of nutrients (Srinivasarao et al., 2011). It is estimated that India removes over 10 metric tons of N, P, and K every year, and fertilizer application continues to increase. This lack of nutrient removal will result in reduced soil fertility and a negative nutrient balance. The authors (Hasan, 2002; Ramamurthy and Bajaj, 1969) reported that there are about 11 million soil test data in 371 districts, which is split into Low (76 districts), Medium (190 districts), and High (105 districts) with percentage-wise contributions of 21% Low, 51% Medium, and 28% High. To all low and medium K soils, apply 72% K fertilizer, ensuring optimum yield and balanced soil fertility. Addition, removal, and balance are used to provide a state-based picture of nutrition. A state-based picture of nutrition is provided by addition, removal, and balance (Table 2.6.1).

Table 2.6. A state-based picture of nutrition is provided by addition, removal, and balance in Key states of India ('000 t).

State		N			P_2O_5			K_2O		$N+P_2O_5 + K_2O$		
	Add	Rem	Bal	Add	Rem	Bal	Add	Rem	Bal	Add	Rem	Bal
A.P.	1,256	477	779	576	497	79	191	817	-625	2,024	1,791	233
Assam	38	257	-219	15	74	-59	18	294	-277	71	625	-554
Bihar	618	481	137	101	102	-1	54	492	-438	774	1,075	-301
Chhattisgarh	67	156	-89	68	68	-0	13	137	-124	148	360	-212
Gujarat	691	340	351	268	121	147	61	426	-365	1,020	887	123
Haryana	597	362	235	201	145	56	5	490	-485	803	998	-195
H.P.	29	43	-14	5	8	-3	4	25	-21	39	76	-37
Jharkhand	40	165	-125	15	60	-45	5	20	-15	60	245	-185
Karnataka	681	473	209	374	239	135	216	604	-388	1,272	1,315	-43
Kerala	87	149	-62	44	53	-9	87	176	-89	219	377	-158
M.P.	519	696	-177	344	431	-87	24	849	-825	888	1,976	-1,088
Maharashtra	923	1,559	-636	450	608	-158	197	2,096	-1899	1,571	4,262	-2,692
NE States	19	96	-77	5	17	-12	3	84	-81	41	198	-157
Orissa	196	227	-31	56	104	-48	40	282	-242	291	614	-323
Punjab	1,081	589	492	275	279	-4	19	764	-745	3,276	3,580	-304
Rajasthan	547	835	-288	147	235	-88	7	1,068	-1061	1,375	1,631	-256
Tamil Nadu	484	405	79	145	111	34	162	398	-236	791	914	-123
U.P.	2,387	1,497	889	776	305	471	114	1,777	-1664	3,276	3,580	-304
W.Bengal	562	764	-202	297	241	56	226	801	-575	1,085	1,806	-721
All India	10,923	9,613	1,310	4,188	3702	486	1,454	11,657	-10,203	16,564	24,971	-8,406

Add = Additions, Rem = Crop uptake, Bal = Balances. Modified by (Tandon, 2007).

Different types of soils available in Indian agro-ecological zones include medium and deep black soils, alluvial soil, red and lateritic soils. Potassium or K status varies with soil type, parent material, texture, and management method. Generally, deep black type soil has smectite as the governing clay mineral, and soils with high CEC levels show high exchangeable K and moderate to high non-exchangeable K levels (Bakken et al., 1997; Srinivasarao et al., 2011).

As mica is the dominant mineral in the earth's crust, alluvial soils have medium exchangeable K levels and high non-interchangeable K levels, while red and lateritic soils have kaolinite as the dominant mineral and have a lighter soil texture, resulting in less EK and higher NEK levels. The soils EK have low to high concentration status, and K deficiencies are readily apparent in coarse-textured alluvial soils, shallow soils, and red and lateritic soils.

In comparing vertisols, inceptisols and alfisols, vertisols have a higher EK value due to their clay content, and main K depends on the cropping system. Below is a table of K availability under different cropping systems in Indian agriculture (Table. 2.6.2).

Table.2.6.2. Availability of K under different crop system in Indian agriculture, adopted (Sharma et al., 2016)

Cropping system	**Potassium availability (kg ha^{-1})**
Rice-based system	138.8–95.1
Groundnut-based system	129.2–188.8
Soybean-based system	322.2–407.5
Cotton-based system	76.7–272.2
Rabi sorghum system	365.4–500.4
Pearl millet-based system	85.1–163.1
Finger millet-based system	53
Maize-based system	55.6–109.4

2.7. **Potassium (K) dynamics and availability in soils**

This vertical distribution and the different forms of K in the soil may provide useful information about which fertilizers should be considered for long-lasting nutrient availability and efficient crop production, but determining the soil's capacity to supply K from removable or exchangeable and fixed or non-exchangeable forms is difficult due to the reversible transformations that occur between the two forms of K (Sharma et al., 1992). The underlying K status of a soil depends largely on the rate and amount of non-exchangeable K (NEK) release (Deshmukh and Rangacharya, 1992). Crop removal of K exceeds annual additions without any appreciable change in the available K status of soils. This suggests that part of the NEK or lattice K becomes available to plants. The release of NEK occurs when the levels of EK and water-soluble K decrease by crop removal and leaching (Talukdar et al., 1992).

In addition to the release of K ions, soil mineral compositions also influence the availability of K to plants. It involves the uptake of K^+ ions in interlayers of silicates like lattice or illite and vermiculite. Degree of K solubilization depends on the formation of clay minerals and the concentration of their charge, the amount of moisture, the competing ions, and the pH of the soils (Meena et al., 2016;

Sparks, 1987). Furthermore, soil wetting and drying also influence the stability of K; the process of stabilization is relatively quick; on the other hand, the release of stable K is very slow due to the strong bond between potassium and clay minerals present in soil. The soil potassium releases are highly dependent on the K-density of the soil solution (Öborn et al., 2005; Schiavon et al., 2010).

The concentration of H^+ ions in soil solution through soil pH seems to play an influential role in the release of K from soil minerals. Optimizing soil pH can be a way to increase K release. For optimized K fertilizer management practices, it is imperative to understand the factors that control K release from soil NEK pools. This includes the effect of potassium fertilization on the physical properties of the soil. Recent studies have raised the consciousness of the influence of K on soil structure and its water absorption capacity. (Holthusen et al., 2010) reported that; the application of K mineral fertilizers enhances the water holding capacity of the soil and also improves the structural stability of sandy soils. Excessive water preservation plays an important role in producing soil productivity in water-restricted areas. Consequently, more information is required to recognize the physical properties of soil and the effect of K fertilization on soil water holding capacity (Zahedi, 2016). Fig. 2.7, represent the potassium cycle in the soil-plant and animal system.

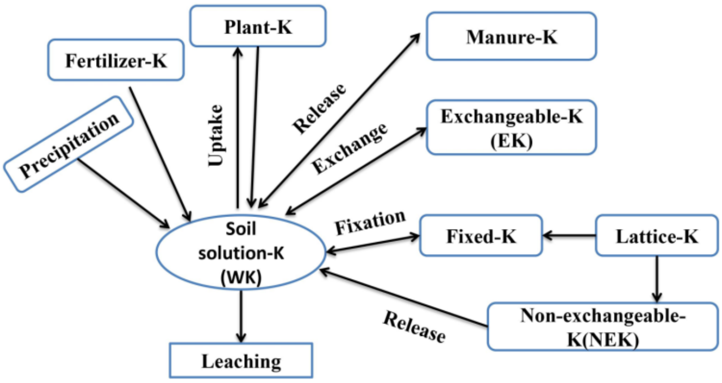


Fig: 2.7. K cycle in plant-soil-animal system, sources; (Syers, 1998).

2.8. Need for fertilizers in soil

Fertilizer uses in Indian agriculture are quite widespread. The uses of imbalanced macronutrients like NPK in agriculture have become very obvious. Fertilizer application efficiency increases to 3 kg/ha. It's universally acknowledged that the application of chemical fertilizers is an integral part of the development of agriculture at a higher level. Research by the Food and Agriculture Organization of the United Nations (FAO) has shown that there is a close relationship between fertilizer consumption levels and average crop yields. The benefits of using nutrients or inorganic fertilizers are immediately available to plants, and the exact amount of any specific ingredient can be measured before feeding the plants. However, commercial fertilizers, especially nitrogen, are easily leaked through rain or irrigation (Meena et al., 2016).

2.8.1. About potassic fertilizers

Potassium is very commonly found in aggregate form as a naturally occurring mineral. Common soils such as granite and ginseng are found in about 3% K. Potassium has been considered a key plant nutrient for many years. Mineral deposits were not discovered until the eighteenth century and were used as fertilizer. The first product used as fertilizer was wood ash and the use of wood ash has existed for many centuries. The content of K in potassic fertilizer is normally known as K-oxide, K_2O also called potash. These fertilizers are produced from ores and minerals. In commercial fertilizers, potassium salts are usually soluble in chlorides and sulfates, so they can be readily absorbed by plants (http://ecoursesonline.iasri.res.in). In 2010-11, potassium fertilizer consumption reached its peak from 100-2000 tons per hectare in 1990-61 to 0.09 kg to 3514000 tons in 2010-15, respectively, at 11.1 and 18.0 kg per hectare (Ramamurthy et al., 2017). There are some countries that use K-fertilizers that contain 60% to 62% K2O - of all the K-fertilizers, Polyhalite (KCl) is the most abundant mineral in commercial deposits. KCl and NaCl (halite) produce common ores called sylvanite (Garrett, 1996). (4 KCl. 4MgSO4.11 H_20) known as kainite ores which are much less common and carnallite ($KCl•MgCl_2•6H_2O$) is present with halite (Eatock, 1985). Another key source of potash is bedded marine evaporate deposits and surface/subsurface K-rich brine. The main ore is a mixture of polyhalite (KCl), sylvanite, and rock salt (NaCl), and India mainly has resources such as gluconate, sylvite, and polyhalite. In addition, there is no commercial utilization here, and the total requirement of potassic fertilizer for direct application as well as production of complex fertilizers is met through import (Prakash and Verma, 2016).

2.8.2. Fertilizers and its impact on Environmental pollution

As fertilizers are necessary for agriculture to yield high yields, differences in the quantity, type, and timing of fertilizer applications, as well as a lack of knowledge in the sector adversely affect the health and environment of animals. As a result of proper fertilization, some problems arise, such as soil salinization, heavy metal accumulation, unbalanced nutrition, negative charges to microbial activity, erosion and nitrate accumulation in water, and the release of gaseous nitrogen and sulfur compounds into the air (https://agris.fao.org/agris-search/search.do?recordID=TR2015300055).

Most fertilizers are safer than harmful pesticides; they show toxic properties in living systems, but not all fertilizers applied to the soil are fully utilized by plants. About 50 percent of the fertilizer applied to the plants is left as a residue. Inorganic fertilizers are usually not toxic or harmful to humans and other life forms, but they have disturbed the ecological balance; nutrients are escaping from agriculture or other fields and are found in excessive quantities in lakes, rivers, and coastal waters, and water algae blooms are high, reducing other aquatic plants in the water. Environmental pollution is caused by the fact that not all fertilizer applied to the crop is taken up by the crop and is not removed from the soil at harvest. This can result in the death of many aquatic inhabitants (Meena et al., 2016). In modern agriculture, fertilizer is essential for maintaining soil fertility, increasing yields, and improving crop quality, but a significant portion has been reduced, increasing agricultural costs, wasting energy, and polluting the environment (Chen et al., 2018).

2.8.3. K availability in soil

In the earth's crust, potassium ranks as the seventh most abundant macro element after N, P, and K, and the concentrations of K nutrients vary widely between 0.04 percent and 3.0 percent (Sparks and Huang, 1985). Most of the potassium reserves in soil are held in organic matter and clay minerals by negative charges, so the binding of K can be strong or weak depending on the conditions in the lattice clay. In the exchangeable form (loosely bound) K^+ ions can be taken up by plants; however, in the non-exchangeable form (strongly bound) ions cannot be taken up by plants. In sandy soils, there are only a few limited amounts of exchangeable K and clay minerals themselves contain K depending on the type of soil clay and the amount of soil organic matter.

2.8.4. Different types of K and availability in soil

K is present in soils in four forms, (1) K in primary minerals or crystal structures; (2) Non-exchangeable-K or secondary minerals; (3) Exchangeable-K or colloidal surfaces; (4) K+ ions present in soil solution and water soluble-K; the concentration of solution-K, exchangeable-K, and non-exchangeable-K levels lower comprise the total-K concentration (Sparks and Huang, 1985). In the order of soil K availability of plants and microbes, the forms of soil K are solution > exchangeable > fixed (non-exchangeable) > mineral (Jaiswal et al., 2016; Sparks, 2000). The solution-K is a form of potassium that is directly absorbed by microbes and plants and is the most common form of leaching form into the soil (Jaiswal et al., 2016). (Saha et al., 2016) reviewed that K content covers about 2.5% of the soils and the actual K content in the soil ranges from 0.04% to 3.0%. About 98% of the total-K is found in NEK or unavailable forms in the different soil types (Fig.2.8.4). Most of the K minerals are insoluble. In soil, micas and feldspars are min minerals that are mostly K containing and common key material for most soils (Meena et al., 2016). Minerals and NEK are seen as important buffer processes for soil solution. The exchangeable form Potassium is the second most abundant plant nutrient and also an irreplaceable resource.

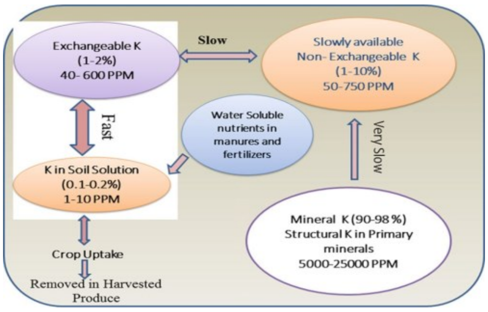


Fig.2.8.4. Represent, K in the soil and its various forms in the soil system, sources (Saha et al., 2016).

2.9. Estimation and Standardization of K supply to Crops

It is well established that essential fertilizers in agriculture provide an adequate amount of soluble potassium to the crop, however, implementation rates and timing are not always based on potassium requirements, and this can cause an excess or deficiency of potassium depending on the crop and soil (Öborn et al., 2005; Zahedi, 2016).

2.9.1. Analysis of potassium (K) present in soil

To recommend specific fertilizers, it is essential to observe the soil storage as well as to determine exchangeable potassium (EK) by using neutral ammonium acetate (1M NH_4OAc), ammonium chloride (NH_4Cl), ammonium fluoride (NH_4F) or Mehlich-3, as well as calcium chloride ($CaCl_2$). That provide the basis for the most commonly used soil tests for K and most K fertilizer suggestion globally (Barbagelata and Mallarino, 2013; McMaster and Wilhelm, 1997). (Luebs et al., 1956) reported that the preparation method involved soil samples being dried at a maximum of 40°C and crushing and passing in a 2 mm sieve and homogeneous mixture to estimate the K content. Traditional extraction methods for K have been pointed out that drying both the air and oven of sample soils can positively affect the concentration of extractable K, but few studies have shown that the concentration increases after soil is dried (Zörb and Peiter, 2013).(Hanway and Scott, 1959) showed that soils in high EK tend to illite or fix K, and low EK tends to K release after drying.

(Zahedi, 2016) reviewed and (Haby et al., 1990) reported that EK testing determined that the effect of drying the sample depends on the balance between the time of sampling and the deviation from the density of K minerals. Illite is another source that seems to release K after drying by stabilizing at a humidity level of more than 4% is related to vermiculite and montmorillonite, including stable K in selected Kentucky lands (Dowdy and Hutcheson Jr, 1963; Zörb and Peiter, 2013). In a greenhouse, pot, and field experiments, another potassium extraction method; is known as a wet-extraction method by natural NH_4OAc extraction of the wet soil. It proposed to establish a better relationship between crop and K-uptake by methods extracting from air-dried soil (Luebs et al., 1956; Zahedi, 2016; Zörb and Peiter, 2013).

It was shown in a recent study by (Barbagelata and Mallarino, 2013) that the method of K extraction by moisture extraction was more effective in predicting crop response to fertilization than the standard method of dry extraction. According to (Mengel and Kirkby, 2012), a few laboratories have selectively applied wet extraction because of inadequate methods such as sieving moist soil. These extraction methods could provide adequate information to recommend fertilizers in light-textured soils without 2:1 clay mineral. In soils containing 2: 1 clay minerals, non-convertible K (NEK) pools typically contribute more than 50% to K cropping (Mengel and Busch, 1982; Zörb and Peiter, 2013). It is most challenging to quantify K for plants available in soils and released from NEK or fixed K reserves, since different forms of K in soil need to be in equilibrium for plants to grow, and there are no routine methods for determining K in soils for plants. In addition, different types of methods are used to estimate the potentially or slowly soluble K in soils like K extraction by 1M nitric acid (HNO_3) with boiling for 10 min (soil and acid ratio = 1:10) (Reitemeier et al., 1948), extraction and claimed by 1M hydrochloric acid (HCl) (Egnér et al., 1960), electro-ultra-filtration (EUF) by (Nemeth, 1980), exchange resins by (Goulding and Loveland, 1986), sodium tetra-phenyl-boron; NaTPB by Jackson's (Andrist-Rangel et al., 2006), and field balances by (Öborn et al., 2005). Natural acid extraction methods, however, remove only a proportion of the K reserves present in the NEK pool. (Øgaard and

Krogstad, 2005) showed that the survey in the grassland compared various methods to evaluate plant availability of soluble K and resulted that K-uptake had better predictions than other acid extraction methods, boiling at 0.5M HNO_3 in lightly textured soils. Furthermore, it was also reported that boiled in 1M HNO_3 is usually available for harvest (Zahedi, 2016; Zörb and Peiter, 2013).

2.9.2. Methods used for potassium solubilizing bacteria and inoculation preparation

Potassium-solubilizing bacterial isolates were cultured by Tryptone Yeast medium (TYMs) (Vincent, 1970), Modified Aleksandrov medium (MAMs) containing (per liter) 5 g glucose, 0.005 g $MgSO_4$ $7H_2O$, 0.1 g $FeCl_3$, 2.0 g $CaCO_3$, 3.0 waste mica (muscovite and biotite) as a potassium mineral (2.0 g used in original media), 2.0 g calcium phosphate (Bajpai, 2015; Hu et al., 2006a; Meena et al., 2013, 2015), sucrose-minimal salts medium (SMSMs) (Xiafang and Weiyi, 2002a), and Nutrient broth medium (NBMs) containing beef extract 3.0 g; peptone 5.0 g; agar 15.0 g; distilled water 1000 ml; pH 6.6–7.0. (Basak and Biswas, 2009; Soils et al., 2010) respectively; and incubated by BOD orbital shaker at 150 rpm for 48 h to & 7 days at 28 ± 2°C, days of incubation is varied growth of different bacterial strains. The bacterial cultured cells in broth were collected by ~ 2822×g for 15 min at 4°C, and the suspension cell was washed with pre autoclaved sterile distilled water. The pelleted KSB cells were re-suspended sterile de-ionized water after that the cells were modified to about 10^8 cells ml^{-1} growth, based on optical density (OD) 620 nm = 0.008 by spectrophotometer (Bhuvaneswari et al., 1980; Zahedi, 2016).

2.10. Type of environmental factors affecting K solubilization in soil

A number of environmental factors affect the mobilization of potassium in soil, including physicochemical properties (soil pH, temperature, exo-polysaccharide, and potassium-bearing minerals), soil aeration, soil texture, soil depth, and liming (Dotaniya et al., 2016; Jaiswal et al., 2016; Meena et al., 2016; Xiafang and Weiyi, 2002b). Microorganisms like rhizospheric bacteria, mycorrhizal fungi, the composition of plant factors, fertilizer, and management practices affect K solubilization and the ability of bacteria to move it. (Jaiswal et al., 2016) reviewed that, few researchers found the efficiency of dissolving potassium bearing minerals by bacteria depends on the characteristics of used minerals (IAKHONTOVA et al., 1987). Some research showed that, variation of extra extracellular polysaccharides found to significantly increase potassium bearing minerals solubility (Welch et al., 1999). The release of K content 35.2 mg/L from KSB strains in 7 days after incubation at 28 ± 2°C at pH (range from 6.5 to 8.0). (Meena et al., 2015) further states that KSB can dissolve potassium in muscovite and biotite. In muscovite, K solubilization ranged from 2.86 to 12.86, 6.30 to 11.40, and 9.26 to 16.20 mg/mL at 7, 14, and 21 days after incubation (DAI). In biotite, range from 8.88 to 13.31, 13.25 to 21.60, and 30.34 to 49.73mg/mL at 7, 14, and 21 DAI. (Sheng et al., 2002) showed release of K content (35.2 mg/L) from KSB strains in 7 days at 28 C at a pH range from 6.5 to 8.0.

A study by (Lian et al., 2008a) found that potassium solubilization was directly related to pH when *Aspergillus fumigates* (a thermophile fungus) and potassium minerals were mixed directly, and that potassium solubilization percentages decreased with increasing volumetric labelling (Lopes-Assad et al., 2010; Renseigné et al., 2006; Ryan et al., 2009). As a result, KSB strains will be very effective for high yields in agriculture under adverse environmental conditions. KSB is an environmentally friendly, affordable, and socially acceptable technology.

2.10.1. Soil factor

The following available soil factors are responsible for the availability and uptake of K

1. Texture of soil:

The soil texture affects the estimation of both readily available K (water-soluble K) and fixed-K (NEK), and topsoil (fine-textured) and coarse-textured soils have mainly significant amounts of both types of K. The presence of K-bearing clay fractions in the significant K fractions of soils will also affect K status, more so in illite dominant or alluvial soils than in smectite dominant black soils and kaolinite dominant red soils, since the amount of available K will vary with clay type (Dotaniya et al., 2016).

2. Depth of soil:

Soil depth is also the vital environmental factor determines the availability of K and K concentration present in the soil. Maximum density of Indian soil; as the depth increases in the form of NEK or WK, it decreases. In India, the Indo Gangetic plain shows more plant uptake able K on top of the surface soil compare to the sub-surface soil, and two types of K forms in Vertisols decrease with depth increasing. (Rosolem et al., 2010) reported a significant amount of K leaching is observed in sandy soils depth 0 to 20 cm when K fertilizer is applied to maintain balance. (Calonego et al., 2005; Rosolem et al., 2007) showed; those organic skeletons that are not bound to K and free of the point mineralization of organic matter and washed directly into the soil system by precipitation.

3. Parent materials:

The parent materials in the soil help develop, type, and amount of minerals in the clay, soil drainage, topography, and duration of soil formation, and soils with a high density of K minerals naturally contain more K than soils with a limited amount of K minerals (Dotaniya et al., 2016).

4. Soil pH, CEC (Cation exchange capacity), and liming:

Soil pH is an essential element for maintaining the soil nutrient cycle; it also affects the availability of nutrients in the soil by plant, biochemical and chemical processes. It regulates the nutritional properties of plants and affects the density of plants in crops. In acidic soils present positive cations (H^+ and Al_3^+; and Ca_2^+ and Mg_2^+) are adversely affected by positive charge carriers K (Dotaniya et al., 2016). As the pH of the soil decreases, the availability of K decreases. The reasons for the weak root growth of the plant due to the presentation of Al^+ and Mn^+ cations are toxicity in the soil acidity and root growth, which prevent K uptake. When confined elements are incorporate into acidic soils, the concentration of exchangeable K increases due to an increase in CEC. Once more, the higher amounts of Ca^+ and Mg^+ cations decrease the saturation of K on cation exchange sites when increasing competition with cations such as Mg^+ and Ca^+. CEC reflects the ability of the K holding with other cations like Mg^+, Ca^+, and Mn^+ deposited in the soil for crop uptake. The available forms of K present in the soil as K+ cation form slowly down by colloids (negatively charged) like primarily clay minerals and organic matter, and it builds CEC of the soil. Naturally, soil with higher CEC has higher storage capacity the ability to K give to the plants (Yadav and Sidhu, 2016). The capacity of CEC in the soil is increased by liming of acid, which in turn increases the absorption of K colloids by soil and decreases the K level in the solution. High Ca^+ cation concentration decreases the uptake of K from the solution soil (Dotaniya et al., 2016).

5. Soil moisture, temperature, and aeration:

Soil moisture helps several functions such as flow of K to the plant root from soil and K diffusion to the roots absorbed by plants roots. With adequate humidity or moisture, disperse occurs more quickly. In addition, it helps reduces drought stress and K availability in soil for root uptake able. Unavailable forms of K make available K faster at higher temperatures because warmer temperatures accelerate the release of K; from K-bearing minerals. Plant physiologically active activities such as roots, plant function, and growth of physiological processes depend on an increase in soil temperature, and improvement of physiological activity leads to higher adaptations of K. The soil needs air to supply oxygen to the soil, the roots need oxygen for respiration, and the plants need it to grow (Yadav and Sidhu, 2016).

2.11. Effects of environmentally friendly biochar on K dynamics and crop uptake

Biochar has been suggested as a possible way to increase soil fertility with potassium (K). However, understanding the effects of biochar in soil dynamics of K is remains limited. It has been proposed as a possible way for increasing soil fertility because of the nutrient and water-holding capacity of the soil and the increase in pH in improved acid soils (Atkinson et al., 2010; Lehmann et al., 2011). A contributing factor to the beneficial effects of biochar may be the presence of nutrients; unlike other components that may be retained as insufficient (e.g., N) or relatively dissolved forms (e.g., M) during pyrolysis, conserved potassium will be converted into highly soluble salts (Wang et al., 2018).

According to Angst & Sohi, (2013), biochar has been proposed as an alternative to conventional K fertilizers, with the rapid release of biochar K making it unlikely to be available after the first year. As reported by (Li et al., 2009), prior experimental results of releasing nutrients were limited since biochar was not mixed with soils or microbes, and the ability of the combined K and crop reactions also depends on the dynamic balance of the soil, which can be affected by soil type, texture, and core K-supply capacity.

Biochar increases the abundance and activity of microbes, which can influence nutrient cycling. Few K-solubilizing bacteria (KSB) such as *Bacillus edaphicus* and *Bacillus mucilaginosus*, emit organic acids or organic anions from K-bearing minerals; that is capable of dissolving kinetic silicon ions or rock K (feldspar and micas)(Wang et al., 2018). Using corn stover biochar (0.6%) as incubation medium, (Liu et al., 2017) showed that *Bacillus mucilaginosus* growth was stimulated by fivefold, and an estimated eighty percent of K-solubilization increased. However, there is no in-situ evidence to support the hypothesis that biochar will help bacteria dissolve K-bearing minerals, and more research is needed to clarify the effects of biochar (Wang et al., 2018).

2.12. Function of K in plant system

While potassium (K) has many functions in plant growth, such as promoting smooth cell division and growth, providing an immune system (increasing disease resistance and drought tolerance), and controlling the transport system (opening and closing of stomata required for osmoregulation), it does not fit the chemical structure of the plant. In addition, potassium is important for photosynthesis, carbohydrate metabolism, and the synthesis of proteins and amino acids. K also facilitates transport during plant development (ontogeny), and one of its most important effects is to boost plant oil production; a decrease in K uptake may cause plant growth problems; a leaf deficiency can produce chlorosis along the leaf margins and can turn yellow and die. There are many reasons why this problem occurs, including inadequate soil K supply, inadequate application of mineral K fertilizers and bio fertilizers, leaching losses, nitrate and phosphorus deficiencies, and complete removal of plant straw (Das and Sen, 1981; Meena et al., 2016; Zahedi, 2016). (Lack and Evans, 2021) reported that in plants, cations K+ are highly water-soluble, highly mobile, and transported through the xylem and through membranes; potassium transport across membranes may be mediated by secondary transporters or electrochemical gradients.

(Jaiswal et al., 2016) reviewed, the dynamics of K in the plant allow affecting most of the features of plant growth. In order to increase crop yields, K stimulates several basic physiological processes including increasing root growth, increasing enzyme activity, decreasing water loss, reducing wilting, helping photosynthesis, maintaining food quality, maintaining turgor, enhancing transport, preventing energy loss, maintaining grain quality, cellulose building, providing immunity and helping retard crop diseases, and reducing lodging. (Wang and Wu, 2015) reported several studies have shown that supplying adequate amounts of K to plants through the application of potassium-soluble microorganisms or natural resources can increase tolerance to various environmental stresses like salt, drought, cold, high light, and pathogens (Fig. 2.12.). A study conducted by (Shabala and Cuin, 2008) showed irrigated land suffers from abiotic stress (salinity) as salt pressure exerts osmotic stress on plant cells, as well as reducing plant stem cell K-up. The excessive expression of the K transporter LNHX2 (Tomato Na^+ / H^+ Exchanger 2) increased salt tolerance in transgenic tomatoes (Huertas et al., 2013). (Xiafang and Weiyi, 2002a) found K application in agriculture, a form of biofertilizer frequently improves crop effectiveness in saline soils.

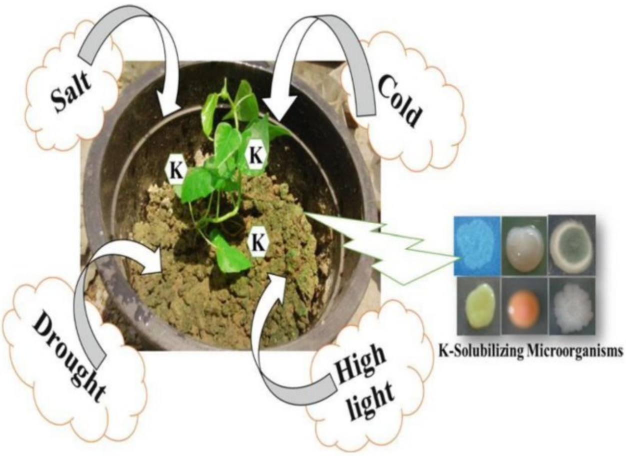


Fig.2.12. Application of (KSM) increases plant tolerance to various environmental stresses, adopted by (Jaiswal et al., 2016).

According to (Egamberdiveya, 2006), KSB (aerobic bacterium) strains are a heterotrophic bacterium that gain their energy and carbon from dead organic matter. In addition, gram-positive KSB bacteria may produce an effective stimulates that stimulates plant growth and inhibits root pathogens. A Gram-positive KSB bacterium may produce an effective stimulating substance that stimulates the growth of plants and inhibits the actions of root pathogens. As mentioned by (Maurya et al., 2014), KSB has important characteristics similar to those of dissolved K from K minerals rock such as micas, illite, and orthoclases, because they are formed by the excretion of various organic. In addition, KSB increases soil K availability and mineral contents in plants.

2.13. K solubilizing rhizospheric microorganisms (KSRMs)

A study by (Gundala et al., 2013) reported that different groups of micro-flora in soil are involved in the process of solubilizing fixed K forms into soluble or available forms so that they are easily absorbed by plants, and that KSRM strains are capable of absorbing K from K bearing rocks and minerals increasing plant growth and crop yield, and are economically sustainable and environmentally friendly. (Muentz, 1890) reported the first evidence that rock potassium is dissolved by microorganisms, and PGPMs have been shown to play a crucial role in the natural cycle of K. The found rooks can be considered as a primitive ecosystem that provides minerals and nutrients to plants by utilizing microbial mineral-induction skills that are controlled through mineral-rich and simple conditions; These microbes are known as pioneer microbes that release inorganic compounds such as nutrients and minerals needed by other organisms (plants). As per Banfield (Banfield et al., 1999), the found rooks are primate ecosystems that provide plants with essential minerals through effective microbial mineral-induction due to their extraordinary adaptability as well as their control of several mineral-rich and simple gene regulation. These microbes are pioneer microbes that release compounds such as nutrients and minerals needed by other organisms. A wide range of rhizospheric microorganisms that are involved in K solubilizing mechanism and transform its insoluble and fixed K forms into available forms that can be easily absorbed by plant roots (Yasin et al., 2016). (Jaiswal et al., 2016; Meena et al., 2016; Yasin et al., 2016) reviewed that, a wide range of KSRMs, namely, *Bacillus mucilaginosus, B. edaphicus, B. circulans, Paenibacillus spp. Acidithiobacillus ferrooxidans, Pseudomonas, and Burkholderia Enterobacter hormaechei, B. mucilaginosus, Paenibacillus mucilaginosus, Sphingomonas spp., Arthrobacter spp., and Paenibacillus glucanolyticus* have been found to K release in an available form from potassium bearing minerals in soils. KSRMs and KSMs were described by (Sindhu et al., 2010) as bacteria and fungi that aid plant growth by dissolving insoluble K forms; these bacteria and fungi are widespread and vary greatly from soil to soil, and they are vital to the efficient dissolution of bound soil minerals. (Sheng et al., 2002) showed a variety of rhizospheric soil microorganisms have to dissolve silicate minerals. KSRMs can soluble; K, silicon, and aluminium in soil from fixed K-bearing minerals by producing organic acids; that are directly soluble rock or chelated silicon to drive K into the solution (Jaiswal et al., 2016; Sheng and He, 2006). Table.2.10. summarizes the list of KSRMs microorganisms in K bearing mineral leaching and solubilization.

Table.2.12. List of potential K solubilizing rhizospheric microorganisms (KSRMs), sources (Raghavendra et al., 2016).

Potassium-solubilizing microorganisms	**References**
Penicillium frequentans, *Cladosporium* spp.	(de la Torre et al., 1992)
Paenibacillus mucilaginosus	(Liu et al., 2012) and (Hu et al., 2006b)
Aspergillus niger, *Penicillium* spp.	(Sperber, 1958)
B. megaterium, *Pseudomonas* spp., *B. subtilis*	(Taha et al., 1969)
B. megaterium, *E. freundii*	(Taha et al., 1969)
Arthrobacter spp., *Bacillus* spp., *B. firmus*	(Bajpai and Rao, 1971)
Aspergillus fumigatus, *Aspergillus candidus*	(Banik and Dey, 1982)
Pseudomonas aeruginosa	(Sheng et al., 2003) and (Badr et al., 2006)
B. mucilaginosus	(Vandevivere et al., 1994), (Welch and Ullman, 1999), (Sheng and He, 2006), and (Zakaria, 2009)
Pseudomonas spp.	(Krishnamurthy, 1989)
Pseudomonas spp., *Burkholderia* spp., *Acidothiobacillicus ferrooxidans*, *Bacillus mucilaginosus*, *Bacillus edaphicus*, and *Bacillus megaterium*	(Sheng et al., 2002)
Bacillus edaphicus	(Sheng and He, 2006)
A. fumigatus	(Teng and Lian, 2007)
Bacillus globisporus	(Sheng et al., 2008)
Pseudomonas, *Burkholderia*, *Acidithiobacillus ferrooxidans*, *Bacillus mucilaginosus*, *Bacillus edaphicus*, *B. circulans* and *Paenibacillus* spp.	(Sheng, 2005), (Lian et al., 2002), (Li et al., 2006), and (Liu et al., 2012)
Pseudomonas chlororaphis and *Bacillus megaterium*	(Yu et al., 2012)
Bacillus altitudinis	(Huang et al., 2013)
Bacillus spp.	(Gundala et al., 2013)
Buttiauxella izardii, *Enterobacter cancerogenus*, *Burkholderia ubonensis*, *E. hormaechei*, and *Burkholderia pyrrocinia*	(Ruangsanka, 2014)

Klebsiella variicola, *Enterobacter cloacae*, *E. asburiae*, *E. aerogenes*, *Pantoea agglomerans*, *Agrobacterium tumefaciens*, *Microbacterium foliorum*, *Myroides odoratimimus*, and *Burkholderia cepacia*	(Zhang and Kong, 2014)

(Meena et al., 2015) reported, scientists from India and China have discovered that near-natural pH gives the best chemical state for dissolving K minerals (micas) to be dissolved by certain bacteria, available to absorb plant roots makes. (Muentz, 1890) was first reported that the rock K solubilization by microorganism. Various types of K-solubilizing bacteria (KSB) have been screened from different agricultural locations and found to capable solubilize fixed K and silicates from unavailable forms of these K bearing minerals and thus play an vital role in nutrient cycle (Table.2.12.a) (Yasin et al., 2016).

Table.2.12.a. Isolated sources of KSRMs from K-bearing minerals and rhizospheric soils of various plants, sources; (Yasin et al., 2016).

Isolation source	**Reference**
Wheat	(Parmar and Sindhu, 2013)
Valencia orange	(Shaaban et al., 2012)
Rice	(Kannan and Raj, 1998)
Tea	(Bagyalakshmi et al., 2012)
Potato	(Abdel-Salam and Shams, 2012)
Black pepper	(Sangeeth et al., 2012)
Chili, sorghum, bajra, and maize	(Archana et al., 2013)
Common bean	(Kumar et al., 2015)
Feldspar	(Sheng et al., 2008)
Potato-soybean cropping sequence	(Biswas, 2011)
Iranian soils	(Keshavarz Zarjani et al., 2013)
Ceramic industry soil	(K.B. Prajapati and Modi, 2012)
Soil, rock, and mineral	(Sugumaran and Janarthanam, 2007)
Soil of Tianmu Mountain, Zhejiang province (China)	(Hu et al., 2006a)
Ha Tien Mountain, Vietnam	(Diep and Hieu, 2013)
Tobacco rhizosphere	(Zhang and Kong, 2014)
Mica core of Andhra Pradesh	(Gundala et al., 2013)
Bio-fertilizers	(Zakaria, 2009)
Tomato, banana, groundnut, cotton, and soybean	(Archana et al., 2012)

2.13.1. K solubilizing bacterial strains (KSB):

A wide range of K-solubilizing bacterial strains was reported as K-solubilizers (Table.2.10), and these strains can be soluble from K-rich minerals. *B. edaphicus* and *B. mucilaginosus* bacterial strains showed a higher capacity for solubilizing and mobilizing K from insoluble or fixed minerals. KSB strains have vast applications in microbial K fertilizer, metallurgy, and mining (Meena et al., 2016).

2.13.2. K solubilizing fungi (KSF):

KSF, also known as arbuscular mycorrhiza fungi (AMF), increases the mineral solubility of K through the release of organic acid anions (oxalate, citrate, and malate) and protons (H^+ and CO_2). It also increases N, P, Ca, and Fe in the plants' fruit and leaves (Yousefi et al., 2011). (Wu et al., 2005) reported *G. intraradices* and *G. mosseae* where two AMF strains was inoclulant in soil on the basis of weight and showed higher K uptake by crop (maize). After 90 days of growth, the plant height and root length, plant dry weight, P and K content, and AM colonization were higher compared to control plant (Alves et al., 2010). (Clark et al., 1999) found, concentration of K uptake as compared to Ca and Mg was significantly in AM controlled grasses grown in acid soils. According to (Alves et al., 2010), UFSC-Pt186 and UFSC-Pt22 AMF isolates (ectomycorrhizal fungi) help to the growing increase of the *Eucalyptus dunnii* seedlings by as sources of P and K. There are several types of KSF, such as *Aspergillus niger* and *Aspergillus terreus*, which were isolated from different K-rich soils. Based on their morphological and colony characteristics, the highest available K in the liquid was found to be mica and feldspar (Meena et al., 2016; K B Prajapati and Modi, 2012). AMF and KSB interactions have subsequent effects on plant growth, and PGPR/KSB interaction influences AMF symbiosis, and their activities demonstrate significant results in K adsorption by plants (Table.2.12.b).

Table.2.12.b. Response interaction between PGPR and AMF on plant tissue potassium (K) concentration, sources, (Priyadharsini and Muthukumar, 2016).

Host plant	**PGPR**	**AMF**	**Tissue K**	**Response**
Acacia senegal	*P. fluorescens*	Natural species	Shoot	Increase
Allium cepa	*A. brasilense*, *A. chorococcum*, *Burkholderia cepacian*	*R. clarus*, *Rhizophagus fasciculatus*	Shoot	Increase
Calendula officinalis	*A. chroococcum*, *P. fluorescens*, *Azospirillum lipoferum*	Efficient indigenous	Leaf/ root	Increase
Capsicum chinense	*A. chroococcum*, *A. brasilense*	Commercial inoculums	Shoot	Increase
Carica papaya	*Bacillus consortium*	*F. mosseae* *G. manihotis*	Stem/ leaves	Increase/ decrease
Casuarina equisetifolia	*Paenibacillus polymyxa*	*Funneliformis geosporum*	Shoot/ root	Increase

Coleus forskohlii	*P. fluorescens*	*Scutellospora* spp.	Shoot	Increase
Dendrocalamus strictus	*P. polymyxa*, *A. brasilense*	*Glomus aggregatum*	Shoot/ rhizome/ root	Increase
Glycyrrhiza glabra	*B. coagulans*	*G. aggregatum*	Root	Increase
Helianthus annuus	*P. fluorescens* strains	*C. etunicatum*, *R. intraradices*	Shoot	Increase
Lycopersicon esculentum	*A. brasilense*, *A. chorococcum*, *Burkholderia cepacian*	*R. clarus*	Shoot	Increase
		R. fasciculatus		
Medicago sativa L. var. Valenciana	*P. aeruginosa*, *B. cepacia*, *Hafnia alvei*, *Enterobacter cloacae*	Consortium of *Glomus* spp.	Shoot	Increase
Musa acuminata Colla AAA cv. “Grande Naine”	*Bacillus* strains	*G. manihotis*	Shoot	Increase
Ocimum basilicum	*B. subtilis*	*R. intraradices*	Leaves	Increase
Solanum viarum	*B. coagulans*, *Trichoderma harzianum*	*G. aggregatum*	Leaves/ root	Increase
Sphaeranthus amaranthoides	*B. subtilis*, *Trichoderma viride*	*Glomus walkeri*	Leaf	Increase
Triticum aestivum	*Pseudomonas* spp.	Indigenous AMF consortium	Grains	Increase/ decrease
Vetiveria zizanioides	*Stenotrophomonas maltophilia*, *Agrobacterium tumefaciens*, *Azospirillum* spp., *B. subtilis*	Commercial inoculums	Shoot	Increase

2.13.3. Application of Earthworms' Gut Microflora (EGM) in mineralization of presenting soil K-minerals

In the ecosystem, earthworms are known as engineers, assisting a vast range of geochemical processes and nutrient cycling in the soil ecosystem. Its activities may increase the rate of silicate mineral weathering; Earthworms' gut microbes are mainly one of the main drivers of degradation of minerals mediated by earthworms, but the diversities of the gut micro-flora that were relevant to mineral weathering are unclear (Liu et al., 2011). (Raghavendra et al., 2016) reviewed earthworms are to be the key component of producing vermicompost. Their intestinal flora contributes to the depletion of complex polysaccharides and the mineralization of soil minerals, and earthworms are ecological engineers, contributing to ecosystem nutrition cycling and geochemical processes.

Based on the 16S rRNA gene sequence of clones obtained from the soil that the earthworm was fed for 10 days, the phylogenetic tree discovered that the library contains homologous similarity matches to 26.86 % *Verrucomicrobia*, 24.30 % *Bacteroidetes*, 12.8 % *Proteobacteria* (including 0.26 % *Alphaproteobacteria*, 0.77 %, *Betaproteobacteria*, 7.42 % *Gammaproteobacteria*, and 4.35 % *Deltaproteobacteria*) and 2.05 % *Firmicutes* and 0.52% *Actinobacteria* are two unidentified species by OTUs (Operation taxonomic unit). Results obtained from the phylogenetic tree and homologues sequence indicate the presence of species such as *Acidobacteria, Acidobacteriales* (*Acidobacteriaceae*), *Verrucomicrobia* (*Opitutae* group), *Bacteroidetes, Sphingobacteria* (*Sphingobacteriales*), and *Proteobacteria*, *Gammaproteobacteria*, *Legionellales*, *Coxiellaceae* (*Aquicella spp.*). These specific groups of organisms, as already mentioned, need to be further evaluated for the mineral weathering in general and the solubilization, especially for the efficacy of the bacteria that produce sidorphora (Liu et al., 2011; Raghavendra et al., 2016).

2.14. Mechanism of K-solubilization by soil rhizospheric microorganism (KSRMs)

K-solubilization by a few prime rhizospheric microorganisms by producing a few organic acids provides K nutrients like nitrogen and phosphorus, which increase plant growth and crop production. It was noted by (Meena et al., 2015) that the primary pH of control (uninoculated) with waste mica added broth was 7.6, which did not affect much during the incubation period; however, pH was slightly altered during the hydrolysis of H^+ ions by K-solubilizing bacteria (KSB) during incubation. As incubation periods increased, such as 14 and 21 days, all rhizobacterial isolates significantly reduced the pH value of the KSB inoculated broth supplemented with waste mica. Decreased pH may be due to the production of a variety of organic and inorganic acids by K-solubilizers.

The release of K from the K bearing minerals was influenced by various factors like pH, oxygen (O_2), and KSB strains (Sheng et al., 2002), and the efficacy of K-solubilization by different KSB strains were observed to vary according to the characteristics of aerobic conditions and K-bearing minerals (Uroz et al., 2007). (Sheng and He, 2006) found *B. edaphicus* KSB strain in liquid media with insoluble

K sources, and the amount of K solubilization was higher than that of illite compared to feldspar. (Sheng and He, 2006) observed the concentration of K nutrient absorbed and accumulated from waste mineral sources in KSR microorganisms is even greater. Silicate bacteria like KSB have been observed to dissolve from soluble minerals such as aluminium (Al), potassium (K), and silica (SiO_2). Soil H^+ ions are directly related to the release of K from K riched minerals. The content of K solubilization was increased by 84.8 to 127.9% in solubilizer inoculated compared to the control treatment. Illite or fixed K by *B. edaphicus* in broth culture compared to K-feldspar. The increasing the level of K solution from bacterial inoculant with insoluble K was recorded as 4.90 mg / L at pH 6.5 to 8.0 by KSB (Badr, 2006). (Sugumaran and Janarthanam, 2007) reported K solubilized at 4.29 mg/L in media supplemented with waste micas (muscovite) as a sole source of soluble K by KSB (*B. mucilaginosus*). The changes of pH affected by K-release by KSB strains, properties of mineral, and aerobic conditions (Lian et al., 2008a). According to (Uroz et al., 2009), he flow of K solubilizing mechanism by that the fixed K and non-exchangeable K, or structurally unavailable K form compounds are synthesized and dissolved in various forms of organic acids that occur during acidolysis and complex exchange reactions. Insoluble K sources such as waste micas (muscovite and biotite, feldspar) are converted soluble or available forms of K by KSB producing organic and inorganic acid, and the net result of increasing K availability to the plants (Meena et al., 2016). (Xiafang and Weiyi, 2002b) reported the production of carbolic acids such as oxalic acid, citric acid, and tartaric acid was associated with the solubilization of the feldspar by KSB like *B. edaphicus* and *B. mucilaginosus*. Very few researchers have isolated diverse fungi from the rhizosphere; that have been able to release K-bearing minerals (mica), metals, and silicate ions from rocks and soils (Burford et al., 2003). (Gadd, 1999) observed, isolates of this fungus produced various types of organic acids like oxalic and citric acid, which helps soluble or decompose silicates and remove metal ions from soils and rocks. (Groudev, 1987) also found that production of exopolysaccharides (EPS) or slime by microorganisms helps to the process of K releasing from silicates. (Liu et al., 2006) showed EPS strongly adsorbed bind organic acids attached to the surface of a mineral, creating a region of high concentration of organic acids near the mineral. It suggested that EPS synthesized silica and affected the equilibrium between the mineral and liquid phases, which caused the silica and K solubilization reaction. (Adeleke et al., 2010) reported redoxolysis / reduction, acidolysis, complication, and metal accumulation are four key mechanisms of fungal leaching. The processes of fungus leaching are mostly directly or indirectly related to the ability of fungi to produce various organic acids and ligands (Burgstaller and Schinner, 1993). (Veresoglou et al., 2011; Yousefi et al., 2011) reported that examined the various effects of Arbuscular Mycorrhizal fungi (AM) on the K-bearing minerals. The release of protons, H^+, or CO_2 and organic acids (oxalate, malate, and citrate) by the AM fungus makes the solubility of K-carrying minerals available, and it helps to increase other protons like N, K, Ca, and Fe in plants. Ectomycorrhiza (EMC) fungi can soluble and K uptake under various conditions such as micas, silicate, vermiculate minerals, and natural E-horizon in soil (Dominguez-Nuñez et al., 2016). Some KSRMs such as *B. megaterium* and *Arthrobacter spp.* can produce mono-hydroxamate (siderophore), which could play an important role in the solubilization mechanisms of K rich content like Si, K, and Fe from the liquid medium with acid leaching soils and micas (Saha et al., 2016).

Few methods are used for KSRMs to solubilization of K from K-rich mineral sources by (a) lowering the concentration of pH, (b) increasing the chelation of K-bound cations, and (c) increasing the acidolysis of the surrounding area of the KSRMs (Fig. 2.12)(Meena et al., 2016).

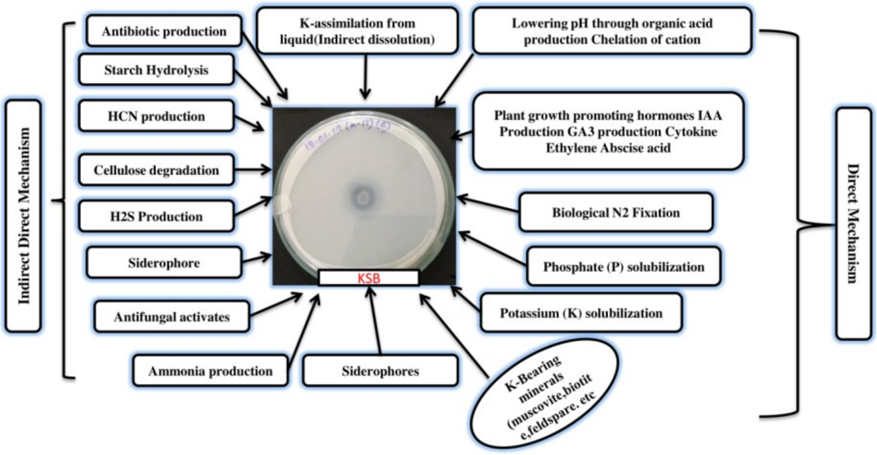
Soluble K, gluconic, oxalic acids (keto-gluconic and succinic citric) is among various organic acids by KRSMs and directly involved solubilizing mechanisms (Table.2.13).

Table.2.13. Represent various production of organic acids by various KSRMs strains; that influence in solubilization of fixed K to soluble K, sources (Ahmad et al., 2016; Dotaniya et al., 2016).

Organism	**Predominant acid produced**
Penicillium frequentans, *Clasdosporium* spp.	Oxalic, citric, gluconic
Paenibacillus mucilaginosus	Tartaric, citric, oxalic
Aspergillus niger, *Penicillium* spp.	Citric, glycolic, sucinnic
B. megaterium, *Pseudomonas* spp.	Lactic, malic, oxalic, lactic
B. megaterium, *E. freundii*	Citric, gluconic
Arthrobacter, *Bacillus* spp.	Lactic, citric
A. fumigatus, *A. candidus*	Oxalic, tartaric, citric, oxalic
P. aeruginosa	Acetate, citrate, oxalate
B. mucilaginosus	Oxalate, citrate
Pseudomonas spp.	Tartaric, citric
Sphingomona, Burkholderia	Acidification, complexation
B. circulans GY92	Lipo-chitooligosaccharides production
B. mucilaginosus	Mica through organic acids
B. edaphicus	Tartaric acid, oxalic acid
B. edaphicus	Production of organic acids
Pseudomonas spp.	Excretion of organic acids
B. mucilaginosus	Illite solubilization by producing IAA
B. mucilaginosus	Organic acids
B. glathei	Siderophores, organic ligands
B. mucilaginosus	Acidification
P. glucanolyticus	Organic acids
P. mucilaginosus	Tartaric, citric, oxalic acids
E. hormaechei	Organic acids

(Archana et al., 2013) showed that the high-performance liquid chromatography and enzymatic methods were used to determine the content of the organic acid produced by KSRMs. The mechanism of dissolution by acidification does not appear to be the sole process. Since in some cases, there is the ability to lower the pH concentration. That is not related to the capability of the minerals to dissolve (Meena et al., 2016; Zhang and Kong, 2014).

Table.2.14. Direct and indirect mechanisms of K solubilizing bacterial strain (KSB) and their K-Solubilizing capability of waste micas (Muscovite and Biotite) on modified Aleksandrow medium, sources (Meena et al., 2016).



According to (Xiafang and Weiyi, 2002b) the chelating capability of the various organic acids is very crucial for plants; and it has been shown that a medium with 0.05 M EDTA has similar dissolving efficacy compared to the *Penicillium bilaii* inoculant.

2.15. Morphological and biochemical characteristics of KSRMs

K-solubilization microorganism like KSB isolates and their cell morph metrics were determined by using optical microscope after staining methods such as gram staining, endospore staining and capsule staining (K B Prajapati and Modi, 2012). Physiological tests, such as Voges-proskauer (VP), Voges-Methyl red (MR), Catalase test, Ornithine utilization, Lysine utilization, H_2S production, Nitrate reduction, Phenylalanine, organic acid utilization, anaerobic growth, acid production from carbohydrates were estimated by using this method (Cappuccino and Sherman, 2011; Chen et al., 2008). The substrate utilization patterns such as salt tolerance and temperature, pH, and salt (Subhashini and Kumar, 2004).

2.16. Molecular mechanisms and transport of K^+ ions in KSRMS

As soil rhizospheric microorganisms such as KSB form extruded polymers such as polysaccharides and primarily proteins, these terms form biofilms (synthesize expolymers) that create a controllable micro-organization surface of microbial cells for weathering. Biofilms on micas (aluminosilicate) enhance the water resident mineral climate compared to bear rocks (surface of mineral), and extracellular polysaccharide (EPS) coatings on mineral grains contribute to maintaining spread pathways as they reduce the possibility of water in soil (Shrivastava et al., 2016). Microbes and plants require potassium (K) to maintain their waste pressure and stimulation of K gaining is the fastest way to respond to osmotic up shock in KSRMs. Potassium is present in almost all intracellular cations, and it plays an important role in bacterial osmoadaptation, pH control, gene expression, and enzyme activation.

The Trk, Kdp, and Kup K+ transporters play an important role in K uptake in plants; bacteria, fungi, and archaea are all capable of uptaking K^+ via Trk and TrkH or TrkG are transmembrane proteins made by Trk. The cytoplasmic-membrane surface protein like TrkA binds to NAD binding protein, required for the system activation (Meena et al., 2016; Sleator and Hill, 2002). (Domínguez-Ferreras et al., 2009) demonstrated that Escherichia coli and some bacteria harbor Kdp, which is an inducible system specific for K+, and the expression of Kdp is significantly controlled at the transcriptional level by KdpE and KdpD regulators. (Epstein and Kim, 1971) reported Bacillus subtilis had a Ktr gene that plays a role in K uptake. Bacterial KUP (TrkD) K^+ transporters are homologous to genes from HAK, KUP, or KT; Escherichia coli has a Km of 0.37 mM for K^+ and shows a similar affinity to those of Rb^+ and Cs^+. Among mycorrhizal fungi, K+ transport and its transporters are found in four families: Trk, Ktr, and HKT; with the exception of PAT ATPases, which were tested in very few studies, the strength and capability of reduction was different in the other three systems and a recent study has identified 62 K+ asset methods for fungal species with available genome sequences, which are mostly non-mycorrhizal species (Dominguez-Nuñez et al., 2016).

The production of phosphate (P) and potassium (K) by organic acids and their various forms is believed to be a key factor in the production of rhizospheric microorganisms (PSB and KSB). This hypothesis has been supported by the cloning of two genes (pqq and gab Y) that are involved in the production of gluconic acids. *Bacillus spp*., *Arthrobacter spp*., *Aspergillus spp*., *Penicillium spp*., and *Pseudomonas spp*. are some of the principal bacterial strains that produce gluconic acid, and sulphydric, carbonic, and nitric acids are alternative chelating substances and inorganic acids that may contribute to K and P solubilization in soil (Meena et al., 2016). The KSB were characterization and identification by 16S rDNA gene (universal primers) and the recombinant plasmids evolved from the correlation between cloned products and strains; and *were* estimated from phylogenetic analysis (Hu et al., 2006a). The families of the transport system have so far been identified in nature, some have been divided into fungi and plants, and others have been exclusively identified within these groups.

2.17. Role of K-solubilizing microorganisms (KSMs) on plant growth and crop yielding

In sustainable agriculture, KSMs could replace a potential technology with soluble K bearing minerals like waste muscovite and biotite (forms of mica) and release K from micas for plants available as nutrients that are efficiently used as a source of K nutrients to maintain crop production and soil potassium (Bahadur et al., 2016). When treated with KSMs on plant seed and seedlings, the percentage of germination, seedling strength, plant growth, yield, and K+ uptake by plant roots under controlled greenhouse conditions was significantly increased (Meena et al., 2016). Researchers have observed that KSMs have improved the growth of several crops such as mustard and cotton (Sheng, 2005), cucumber and pepper (Han and Lee, 2006), sorghum (Badr, 2006), banana (Shaaban et al., 2012), tomato (Lian et al., 2008b), sudan grass (Basak and Biswas, 2010), chilli (Ramarethinam and Chandra, 2005). The first reported by Alexandrov (1958) was the application of organominerals to a combination of silicate bacteria to increase plant growth and corn and wheat yields. (Xie, 1998) research conducted on field-level experimental crops like wheat, maize, forage crop, and sudangrass has suggested that the application of KSMs could significantly reduce the usage of synthetic fertilizers such as chemical or organic fertilizers. Few studies have found that enhanced nutrient growth of K^+ may be caused by stimulating root growth / the extension of roots by rhizospheric microbes, and therefore there is no direct effect on soil exchangeable K^+ (Sindhu et al., 2014). (Basak and Biswas, 2012; Subhashini and Kumar, 2004) assert that the efficiency of the use of K in agricultural fields can be modified effectively through KSM inoculation periods, and that is a sustainable and integrated approach to soil nutrient management for agricultural crop production. The KSMs are also believed to boost the immune system and growth of plants by acting as N-fixers and P solubilizers. The effect of *B. mucilaginosus, A. chroococcum,* and *Rhizobium spp.* inoculants on K mobilizations from waste micas by hydroponics study with wheat and maize as the test crops under a phytotron growth chamber (Singh et al., 2010). Inoculations of *B. mucylaginosus* rhizobium and *A. crococcum* showed a significant number of K+ supplements. Research on K-mobilization by NBT wild-type strains of *B. edaphicus* has been conducted with a pot experiment in low available K, yellow-brown brown-yellow color. After inoculation of KSB isolates, the growth of root and shoot of the wheat plant was significantly increased and the macronutrients (NPK) of plant components was higher than that of un-inoculated components. A study by (Badr, 2006) reported that phosphorus and potassium bearing minerals inoculated with sorghum resulted in increased dry matter yield (48 %, 65 %, and 58 %), phosphorus (71 %, 110 %, and 116 %), and potassium (41 %, 93 %, and 79 %) uptake. Sandy, calcareous, and clay are three different types of soil. A study conducted by Sugumaran & Janarthanam, (2007) showed that KSMs can help groundnut yield by inoculating one KSB strain (*B. mucilaginosus*) into the soil as a result of dry matter increasing by 25% and available P and K increasing by 6.24 to 9.28 mg/kg and 86.57 to 99.60 mg/kg respectively when compared to controls (un-inoculated). (Liu et al., 2012) found that by enriching microbial species and genes as microbial fertilizers, it is essential to isolate more species of mineral soluble bacteria, which will be very beneficial to the environmental development of agriculture.

2.18. Future prospect of KSMs in sustainable agriculture

K-solubilizing microorganisms (KSMs) and their vital role play in plant growth and nutrition. That increases plant acquisition through soil that promotes activate growth promotion and contribution a key role as to bio-fertilization of crops yield. Consistently, further investment is required to improve the production and utilization of KSMs as beneficial microbes. More attention needs to be paid to the study and application of new efficient combinations of KSMs and growth-promoting microorganisms (PGMs) of other plants for better results (Meena et al., 2016). Most of the incubation experiments were conducted in the laboratory. Very little information has been found on the field application of such methods, which is likely to be problematic in inoculating agricultural soil under field conditions. To evaluate the effectiveness of such applications for agricultural yielding systems, the researcher needs more field studies that assess their impact on both soil properties and crop production. A better apprehension of the mechanisms behind the release of K^+ from K-bearing minerals is critical to developing effective novel approaches to sustainable agriculture. It is extremely difficult to estimate the contribution of non-exchangeable K (NEK) and the origin of K^+ adsorbed by plans due to a lack of satisfactory methods for field and microbial experiments. Few researchers concluded that there is a difference in the ability of crops to convert to soluble K^+ from NEK and limited K^+ concentration areas. Selecting KSM species that are effective in K^+ solution through K efflux should have a considerable potential to increase their resource utilization efficiency (Saha et al., 2016).

CHAPTER-3. OBJECTIVE 1

"Effect of metal fractions on rice grain metal uptake and biological parameters in mica mines waste contaminated soils."

3.1. Introduction

In the modern world, soil and agricultural pollution brought on by mining operations is a significant problem (Candeias et al., 2014). Mica deposits in India cover a total area of about 3888 sq. km in the districts of Giridih and Koderma in Jharkhand and Munger in Bihar (Nishanth and Biswas, 2008). The majority of mines in this area are still active right now, but a few have been closed for a number of years. Mica wastes are typically fine, loose, and homogeneous, have a low bulk density and are moisture-holding capacity, and lower nutrients, which not only inhibit plant colonization but also contaminate the surrounding areas during strong winds and heavy runoff during monsoon (Kumar and Maiti, 2015). Around 75% of raw mica mine waste is disposed of nearby the mines during the dressing process, and as a result, these mine wastes wash into the fields and rivers, contaminating land and water resources (Basak and Biswas, 2009; Meena et al., 2015). Rice is the major crop in the mica mine waste contaminated soils in that region. In India daily consumption of milled rice is high, approximately 103 per capita year^{-1} (Mandal et al.,2021) Rice is prone to absorb some toxic heavy metals such as cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb), which is a major threat to food security and human health (Zhao and Wang, 2020). Increased accumulation of Cd in rice as a result of extensive Cd contamination in paddy soils brought on by mining, smelting, and other industrial operations have been reported by Huang et al, (2021). Lead contamination in rice due to mining activities have been previously reported from Zamfara state, Nigeria (Mandal et al.,2022). Although, the total metal content in the soil is an effective indicator of soil pollution; it does not provide adequate information about the potential environmental impact. A number of studies have demonstrated that heavy metal uptake by plants is positively related to the bioavailable concentration of soil heavy metals (Monterroso et al., 2014; Xiao et al., 2017). Sequential chemical extraction methods are more popular to use quantification of different fractions of metal in soil (Beckett, 1989). The sequential chemical extraction technique breaks down metals into different solubility and mobility forms and can be used to predict the conversion of metals into different fractions of metals in soils and the availability of metals in microorganisms (Bhattacharyya et al., 2005). Several workers studied the effect of different metal fractions on soil microorganisms and metal uptake by rice plant in mining affected soils (Ma et al., 2015; Zhang et al., 2018).

Further due to the presence of heavy metals in this mica waste there is a decline in soil quality which ultimately leads to the loss of biodiversity, business possibilities, and resources. Microorganisms play a vital role in the energy driving process under increased stress conditions. Enhancing or maintaining soil quality is crucial for both sustainable agriculture practises and a healthy ecosystem. Multiple soil microbiological parameters, such as microbial biomass carbon (MBC), metabolic quotient (respiration/biomass ratio), respiratory quotient (basal soil respiration/substance-induced respiration ratio), and microbial enzyme activity quality, are more pertinent than single parameter measurements when determining the soil environmental quality (Doran and Parkin, 2015). To evaluate the ecological stress level, some microbiological parameters viz. soil enzyme activity, biomass carbon, microbial respiration are found to be most reliable. The basal soil respiration (BSR) is one of the oldest and still the most widely used parameters for measuring microbial activity in the soil (Huang et al., 2013; Tripathy et al., 2014). The metabolic quotient or qCO_2 is a sensitive indicator of the expression of heavy metal toxicity under stressed conditions. Soil enzyme activities are widely used as biological indicators for soil health (Zhou et al., 2017). They play a significant role in nutrient turnover and are influenced by a number of biotic and abiotic factors. β-D glucosidase, phosphatase and urease are a few of the key enzymes in the major biogeochemical cycle i.e., C, N, P. Dehydrogenase, an oxidoreductase group enzyme often acts as an indicator of the microbial redox system.

To our knowledge no information is available on the effect of mica mine waste on distribution of metals in soil and rice plant and their effect on microbiological and biochemical indicators of soil quality. This study assess the relationship between metal components and their bioavailability in soil in addition to their effect on soil microbiological and biochemical properties in tropical soils contaminated by mica waste. In this study we measured the microbial biomass, respiration and various enzymes such as glucosidase, urease, phosphatase, which are related with the cycling of carbon, nitrogen and phosphorous respectively. This study will allow us to evaluate how different metal fractions in soil contaminated by mica waste negatively impact the soil environment. The accumulation of heavy metals in rice grains from mica waste contaminated agricultural fields were also determined. We also predicted the rice grain metal content in relation to the different fractions of the metals in with the help of variable importance plot and partial dependence plots (PDP) using the random forest (RF) machine learning algorithm. The PDPs will shed light on how certain metal fractions governs its uptake in rice.

3.2. Materials and methods

3.2.1. Study site

The Jharkhand state is the world's largest deposit of mica mines in India. Koderma and Giridih are two of the best mica-producing districts in India, both of which belong to the state of Jharkhand. Geographically Giridih (4941 sq. Km) is bounded by 24.18°N / 86.3°E, and is situated in the northern region of the state. As for the climate of the Giridih, it is dry, and the average annual rainfall is 1350 mm; the maximum and minimum temperatures of this district are 42 °C and 10 °C, respectively. Within the Giridih district, there are three types of soil: Entisols, Inceptisols, and Alfisols. The percentages of the three types of soil are respectively 63.6%, 18.4%, and 16.9% (https://giridih.nic.in/geography/). In the Giridih district, there are twelve blocks, and three of them - Gawan, Tisri, and Deori - are particularly contaminated with heavy metals and waste mica.

3.2.2. Sample collection and processing

Mica waste amendment soil and rice samples were collected during the maturity phase of rice (*Oryza sativa* L.) from near mica mines from three blocks (Deori, Gawnan, and Tisri) of Giridih district of Jharkhand, India in September, 2016 (Fig.1). Mica mines are located mainly in these three blocks of the district in Giridih. Rice fields were collected near mica mines, where surface run-off water is naturally stored because of lower altitudes due to leaching out of mines. From each active mine, three soil samples were randomly collected at distances of < 50m (zone 1), 50-100m (zone 2), and >100m (zone 3). Therefore, in total 63 soil samples (= sampling site) were collected from 21 mica mines. At each sampling site, soil and rice samples were collected from three nearby rice fields using the W-pattern sampling method. Finally, ~1 Kg soil/site was collected following the subsampling method. Surface soil was removed before the collection of rhizospheric soil from a depth of ~10 cm. Along with, the rice plants were removed from the ground with all of their roots remaining intact. Plant roots were separated from the rhizosphere soil by shaking gently with hands. The samples (soil and rice) were preserved separately in sterile plastic bags, labelled properly, and brought back to the soil laboratory at the Indian Statistical Institute, Giridih, within the same day and stored at 4 °C before analysis. Samples of processed within seven days of collection. In the laboratory the rice grains were seperated from the ears, followed by dehusking, then drying it to a constant weight in an oven and finally milling the grain into powder for the purpose of determining heavy metal concentrations. In addition, each soil sample was air-dried, then sieved through a 2mm sieve for further analysis. In microbiological analyses, moist soil was used, while in physicochemical analyses, air-dried soil was used. Chemical and microbiological characteristics were calculated on a moisture-free basis.

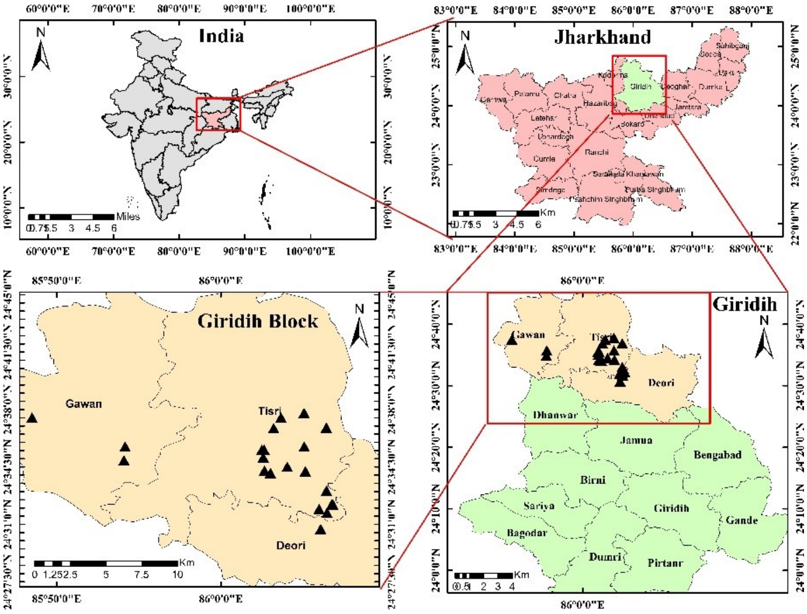


Fig. S1. Map showing the range of mica mines contaminated soil samples collection sites in Giridih District, Jharkhand, India. Samples were collected from nearly 21 active mica mines sites in the Deori, Gawan, and Tisri blocks of Giridih Districts respectively. Each state's sampling zones were illustrated using the geographic co-ordinates obtained using GPS at each location.

3.2.3. Physicochemical analysis of mica waste soils:

The physicochemical analyses were carried out with (2mm and 0.2mm) air-dried samples by standard protocol (Page et al.,1982). The pH (H_2O) was measured in 1:2.5 soil water suspension using a calibrated electrode of Systronics Digital pH meter 335 whereas electrical conductivity (EC) of the samples suspensions was measured by a Conductivity Bridge; using 1:5 ratio of sample-water suspension. Organic C was estimated by reacting the samples with potassium dichromate and sulphuric acid and the excess potassium dichromate was titrated by ferrous ammonium sulphate. Total nitrogen content in the samples was measured by Kjeldahl method. In brief, soil was digested by concentrated sulphuric acid with the mixture of potassium sulphate and copper sulphate. After digestion, ammonium ion content was measured by distillation of the digested material with sodium hydroxide in N-auto analyzer. CEC was measured by titration method. Briefly, 200mg soil was treated with 0.05N HCl, followed by an hour of barium acetate soaking and finally titrated with 0.05N NaOH. Available phosphorous of samples was estimated by Olsen extraction method. Exchangeable Na, Ca and Mg concentrations in the samples were determined from the neutral 1(N) ammonium acetate extracts (1:5 ratio) and measured by a Flame Photometer and Atomic Absorption Spectrophotometer respectively. Available Ni, Cr, Cd and Pb concentrations in the samples were determined from the DTPA extracts and measured by Atomic Absorption Spectrophotometer.

3.2.4. Sequential extraction of heavy metals:

Metals (Cr, Ni, Pb and Cd) were sequentially extracted from soil following the method of (Tessier et al., 1979). For water-soluble fractions, 1.0 g of air-dried soil samples were extracted sequentially with 50 ml deionized water, shaking (120 rpm) for 30 minutes at room temperature; 0.5 M $Mg(NO_3)_2$ in 50 ml with shaking (120 rpm) for 30 min at room temperature for exchangeable fraction; with 1M NaOAc, shaking (120 rpm) 5h at room temperature for bound to carbonate fraction; with 0.08 M NH_2OH HCl, shaking (120 rpm) 6h at 96 °C temperature for bound to Fe and Mn – oxide phase metal; for bound to organic matter fraction 1g soil samples with 0.02M HNO_3 and H_2O_2 shaking (120 rpm) 2h and 3 h at 85 °C temperature and sulphides phase fraction, with 3.2 M NH_4OAc shaking (120 rpm) 30 min at room temperature, and finally with concentred HNO_3 at 105 °C for mineral matrix fraction (Jackson, 1958). The metal concentrations in the extracts were measured by an atomic absorption spectrometer (AAS – 816, Systronics, India).

For calibration, standard solutions of the respective metals were prepared from stock solutions (1000 mg L^{-1}, Merck grade) in 1% (v/v) HNO_3. Certified reference material SRM 2710 and blank extract were used for quality assurance. The reference material was then digested in ultrapure $HF/HNO_3/HClO_4/H_2O_2$ to confirm the accuracy of the metals analytical method. The results were compared with certified SRMs. The results indicate that the analytical method was accurate for all metals.

3.2.5. Estimation of total metals from rice grain:

The total metal concentration of Cr, Ni, Pb and Cd in rice grains was determined in a sample of 1g rice digested in a 4:1 di-acid mixture (HNO_3:$HClO_4$) in a beaker placed on an electric heating plate at 190 C for 1 hour until the solution turned white (Li et al., 2018). We estimated the metal concentration in these digests to be similar to what we described in the previous section (*Section 2.4*). A quality assurance and quality control (QA/QC) study of metal content in rice samples was conducted by determining the metal content of blank and duplicate samples along with certified reference materials (SRM-2710).

3.2.6. Microbiological analysis:

Microbial biomass carbon (MBC) was determined by fumigation extraction followed by KCl extractable carbon determination, and the difference between fumigated soil and unfumigated soil was divided by the calibration factor (K_{EC} - 0.38). microbial biomass carbon per gram of dry sample was calculated; where K_{EC} is the difference between KCl extractable carbon content of the fumigated and unfumigated soils (Joergensen et al., 2011). In order to quantify soil respiration, CO_2 was measured when soil was incubated with glucose in a closed system and trapped in NaOH solution, then titrated with HCl (Alef and Nannipieri, 1995). Urease enzyme activity in soils was assayed by the buffer methods (Tabatabai, 1994). The method involved the resolution of ammonia (NH_3) released by urease activities when the soil was incubated with Tris (hydroxymethyl) aminomethane (THAM) buffer at optimal pH (pH - 9.0) with and without toluene and 0.2 (M) urea solution at 37 °C for 2 hours. The activity of acid phosphatase and β-D-glucosidase enzymes was determined using the method described by the method (Tabatabai, 1994), following incubation of moist soil with p-nitrophenyl phosphate (PNP) and p-nitrophenyl glucopyranoside in buffer for 1 hour at 37 °C. The product p-nitrophenol (PNP) was determined calorimetrically at 420 nm. Estimation of fluorescein diacetate hydrolysis activity in soils the methods as described by Schnürer and Rosswall (1982). The release of fluorescein when 1g moist soil was incubated with sodium phosphate buffer (pH 7.6) and fluorescein diacetate solution at 25 ^{0}C for 3h. Dehydrogenase enzyme activity of the moist sample was measured as described by the method of Casida (1964). The determination of DHG was based on the estimation of the triphenyl tetrazolium chloride (TTC) reduction rate to triphenyl formazan (TPF) in soils after incubation at 37°C for 24 h. The microbial metabolic quotient and respiratory quotient were calculated using values of microbial biomass C, basal soil respiration, and substrate-induced respiration through the use of equations A and B (Alef and Nannipieri, 1995; Chakraborty et al., 2022).

$$Microbial\ metabolic\ quotient\ (qCO_2) = \frac{CO_2{-}cc\ from\ basal\ respiration}{Microbial\ biomass\ C} \tag{A}$$

$$Respiratory\ quotient = \frac{Basal\ respiration}{Substrate\ induced\ respiration} \tag{B}$$

3.2.7. Statistical analysis

The statistical analysis was done using R-Studio (*Version 1.3.1093 2.3.1*). The violin plots were prepared using the '*ggpubr'* (version 0.40) package. The correlation plots were made using the *'correlation' (version 0.8.0), 'corrplot' (version 0.84) and 'psych' (version 2.0.12).* Principal component analysis (PCA) was performed using the *'princomp' (version 4.0.3) and 'factoextra'(version 1.0.7).* The linear correlation between the two variables was assessed through a Spearman correlation analysis. The random forest models were developed considering metal content in rice grain as the dependent variable and the soil fractions of Ni, Cr, Cd and Pb as the predictor variable for each metal. Random Forest is a supervised machine learning algorithm for classification and regression that is based on the recursive partitioning principle (Breiman, 2001) and is independent of the assumption of functional relationships between the response and predictor variables. Random Forest analysis, in a nutshell, ensembles multiple regression trees by a technique known as "bootstrap aggregation" or "bagging." To provide an average prediction for the response variable, a random subset of the data space is taken (with replacement) to grow a tree to its full length, and each node of the tree group's observations is described by particular requirements on the predictor variables. Two-third of the bootstrapped data is used in each tree-growing process, and one-third of the observations (out-of-bag data, OOB) is used to estimate prediction errors. Second, each node split in a tree takes into account a random subset of predictor variables, which is usually the square root of the entire number of predictor variables. To produce final projections, the forecasts from all of the trees are summed. The Random Forest algorithm's variable significance function ranks predictor variables based on the increase in model error by randomly permuting the predictor variables' values. (James et al., 2013; Sengupta et al.,2021). The difference in OOB- MSE before and after random permutation of a predictor variable is averaged over all trees to compute variable importance. The models were developed using the '*randomForest' (version 4.6-14) package* with a *ntree=500* and *mtry*=2. For variable importance plot *'vip' (version 0.3.2)* package was used. The partial dependence plot from the random forest was prepared using the *'pdp' (version 0.7.0)* package.

3.3. Results and Discussion

3.3.1 Chemical and Biological properties of the study site

Table 3.1 represents the chemical and biological properties of the soils of the study sites (Zone 1, 2 and 3). The pH and EC of the soil ranged from 4.78-8.30 and 1.60-27.00 mSm cm^{-1} with a mean of 6.01 and 5.01 mSm cm^{-1} respectively across all the zones of sampling. The mean total organic carbon (TOC) content of the soil was 0.61 with a range of 0.22-1.08 %. The cation exchange capacity (CEC) of the soil ranged from 2.98-16.10 meq 100g^{-1} with a mean value of 7.84. The mean microbial biomass carbon (MBC) was 99.14 mg kg^{-1} and ranged from 23.79-396.82 mg kg^{-1}. The mean FDA, DHG, APS, Urease and BetaD were 63.55 µg fluorescein g^{-1} soil h^{-1}, 16.15 µg TPF g^{-1} h^{-1}, 419.86 µg p-nitrophenolg^{-1} soil h^{-1}, 26.66 µg urea hydrolysed g^{-1} soil h^{-1} and 30.99 µg p-nitrophenolg^{-1} soil h^{-1} respectively. The available and total N of the soil was 127.76 mg kg^{-1} and 0.24 % and ranged from 50.96-221.20 mg kg^{-1} and 0.03-0.77 % respectively. The mean exchangeable P, Ca, Mg and Na were 1.04, 2921.30, 307.82 and 19.63 mg kg^{-1} respectively. The mean DTPA extractable Ni, Cr, Cd and Pb were 3.27, 3.96, 0.05 and 1.31 mg kg^{-1} respectively and ranged from 0.83-5.67, 1.30-5.50, 0.007-0.148 and 0.19-6.47 mg kg^{-1} respectively. The mean rice grain content of Ni, Cr, Cd and Pb were 0.83, 0.41, 0.21, 0.17 mg kg^{-1} respectively.

Table 3.1. Descriptive statistics of the soil parameters and heavy metal content in rice grain (n=63)

Parameters	Mean	Range (Min-Max)	Median	$^{*}Q_3$-Q_1
pH	6.0	4.7-8.3	5.8	6.4-5.3
EC (mSm cm^{-1})	5.0	1.6-27.0	4.1	5.7-3.0
TOC (%)	0.6	0.2-1.0	0.61	0.73-0.49
CEC (meq 100g^{-1})	7.8	2.9-16.1	7.6	9.4-6.0

MBC (mg kg^{-1})	99.1	23.7-396.8	88.6	120.4-60.9
FDA (µg fluorescein g^{-1} soil h^{-1})	63.5	15.1-171.7	60.1	80.6-41.3
DHG (µg TPF g^{-1} h^{-1})	16.1	3.1-45.8	14.9	21.5-9.2
APS (µg p-nitrophenol g^{-1} soil h^{-1})	419.8	44.7-1462.2	351.9	552.4-221.7
Urease (µg urea hydrolysed g^{-1} soil h^{-1})	26.6	3.3-89.4	23.6	32.0-16.8
BetaD (µg p-nitrophenol g^{-1} soil h^{-1})	30.9	16.0-61.2	29.6	39.5-20.2
BSR	14.3	9.5-21.6	14.3	15.2-13.3
SIR	81.8	39.2-118.7	88.2	101.2-56.6
qCO$_2$	0.05	0.009-0.136	0.045	0.06-0.03
QR	0.19	0.08-0.38	0.16	0.22-0.14
Available N (mg kg^{-1})	127.7	50.9-221.2	127.6	144.9-108.3
Total N (%)	0.2	0.03-0.7	0.1	0.2-0.1
Exchangeable P (mg kg^{-1})	1.0	0.1-5.2	0.4	1.0-0.3
Exchangeable Ca (mg kg^{-1})	2921.3	796.9-6076.6	2415.7	4457.8-1606.3
Exchangeable Mg (mg kg^{-1})	307.8	68.7-893.7	268.7	402.5-181.8
Exchangeable Na (mg kg^{-1})	19.6	1.84-213.8	5.5	18.9-3.6
Available Ni (mg kg^{-1})	3.2	0.8-5.6	3.1	3.6-2.8
Available Cr (mg kg^{-1})	3.9	1.3-5.5	4.3	4.9-3.1
Available Cd (mg kg^{-1})	0.05	0.007-0.148	0.04	0.07-0.03
Available Pb (mg kg^{-1})	1.31	0.19-6.47	1.23	1.78-0.96
Rice Ni (mg kg^{-1})	0.83±0.41	0.05-2.00	0.80	1.10-0.52
Rice Cr (mg kg^{-1})	0.41±0.19	0.06-0.95	0.40	0.55-0.25
Rice Cd (mg kg^{-1})	0.21±0.14	0.05-0.60	0.20	0.27-0.10
Rice Pb (mg kg^{-1})	0.17±0.08	0.05-0.40	0.15	0.20-0.10

*Q$_3$-Q$_1$: Represents the inter quantile range, between 3rd Quantile (Q$_3$) and 1st Quantile (Q$_1$)

3.3.2. Nickel, chromium, cadmium and lead content in rice grain

The violin plots in (Fig. 3.1) represents the heavy metal content in the rice grain across the different sampling zones. The non-parametric Kruskal-Wallis test revealed that a significant difference

($p < 0.05$) in rice grain metal content was observed across the different zones. All the heavy metals in rice grain followed the trend of zone 1 > zone 2 > zone 3. The mean Ni content in zone 1 was 1.25 mg kg^{-1} followed by 0.72 mg kg^{-1} in zone 2 and minimum of 0.51 mg kg^{-1} at zone 3. The Cr content was maximum in zone 1 (0.57 mg kg^{-1}) followed by zone 2 (0.39 mg kg^{-1}) and minimum at zone 3 (0.28 mg kg^{-1}). The highest Cd content was 0.32 mg kg^{-1} at zone 1 followed by 0.19 mg kg^{-1} (zone 2) and 0.12 mg kg^{-1} (zone 3). Zone 1 had the maximum Pb content of 0.24 mg kg^{-1} followed by 0.17 mg kg^{-1} and 0.10 mg kg^{-1} at zone 2 and 3 respectively. However irrespective of the zones of sampling the rice grain Cd content was less than the maximum tolerable concentration of 0.4 mg kg^{-1} as per the codex recommendation (JEFCA, 2017). The Pb content in rice grain in zone 1 was higher than the codex recommended value of 0.2 mg kg^{-1}. In zone 2 and 3 the Pb content in rice was less than the codex recommended maximum tolerable concentration. The concentrations of water-soluble and exchangeable metal fractions were positively correlated with the total metal content of rice grain; Ws-Ni (r = 0.57**) and Ex-Ni (r = 0.41**), Ws-Cr (r = 0.45**) and Ex-Cr (r = 0.39**), Ws-Cd (r = 0.49**) and Ex-Cd (r = 0.24), Ws-Pb (r = 0.26**) and Ex-Pb (r = 0.50**), respectively. In DTPA extractable fraction of metals Ni (r = 0.56**), Cd (r=0.28**) and Pb (r = 0.55**) found highly positive correlated with rice grain compared to other metals (Fig. S2).

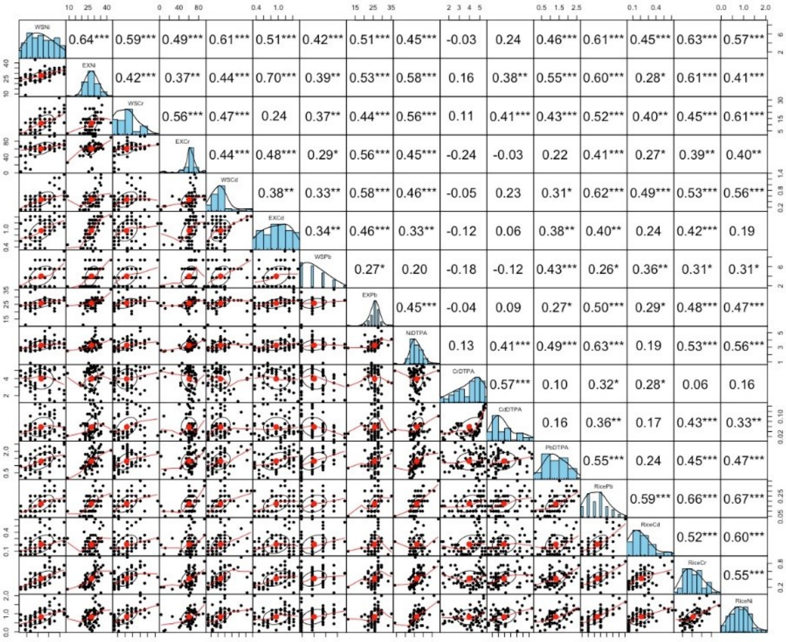


Fig. S2. Spearman correlation between metal content in rice grain and the different fractions of the metals in soil.

These findings are well corroborated with the previous findings by (Zhou et al., 2014).

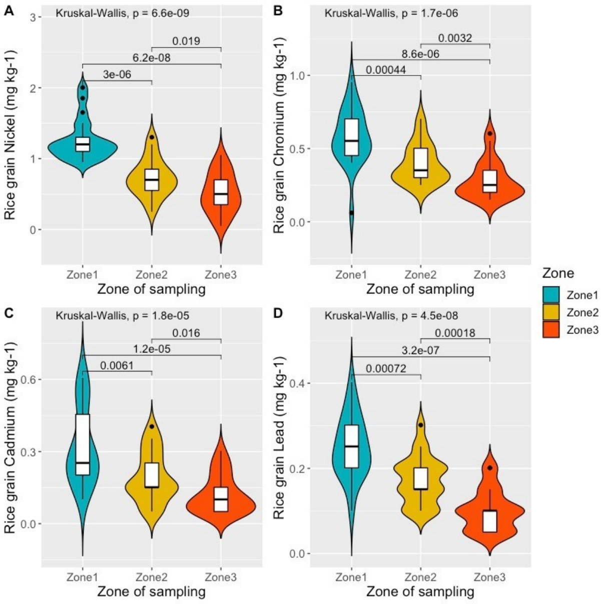


Fig. 3.1. Violin Plots representing the comparison among the 3 zones in terms of heavy metal content in rice grain.

3.3.3. Importance of the fractions of nickel, chromium, cadmium and lead

The variable importance plot from the random forest models for each metal can be observed in (Fig. 3.2). The % IncMSE shows how much the model accuracy decreases if we leave out that variable. The IncNodePurity is the measure how pure the nodes are at the end of the tree without each variable. More useful variables achieve higher increases in node purities, that is to find a split which has a high inter node variance and a small intra node variance. For Ni it was observed that the water soluble (WSNi) and the exchangeable (ExNi) were the two most important variable. For Cr it was water soluble fraction followed by the carbonate bound Cr (CBD Cr) and exchangeable Cr. In case of Cd the water-soluble fraction (WSCd) contributed the most followed by oxidisable Cd (OXD Cd), exchangeable Cd (EXCd) and residual Cd (RSCd). The exchangeable fraction of Pb (EXPb) and carbonate bound Pb (CBDPb) were the only important fraction governing the uptake of Pb in rice grain. Variable Importance plot shows how worst the model performs without each variable. In our study for Ni (WSNi and EXNi), for Cr (WSCr, CBDCr and EXCr), for Cd (WSCd, OXDCd, EXCd and RSCd) and for Pb (EXPb and CBDPb) are the important variables in absence of which the model will have the worst performance irrespective of the zones. A comparison of the metal fractions with respect to the zone of sampling can be observed in Fig. S3 (A and B).

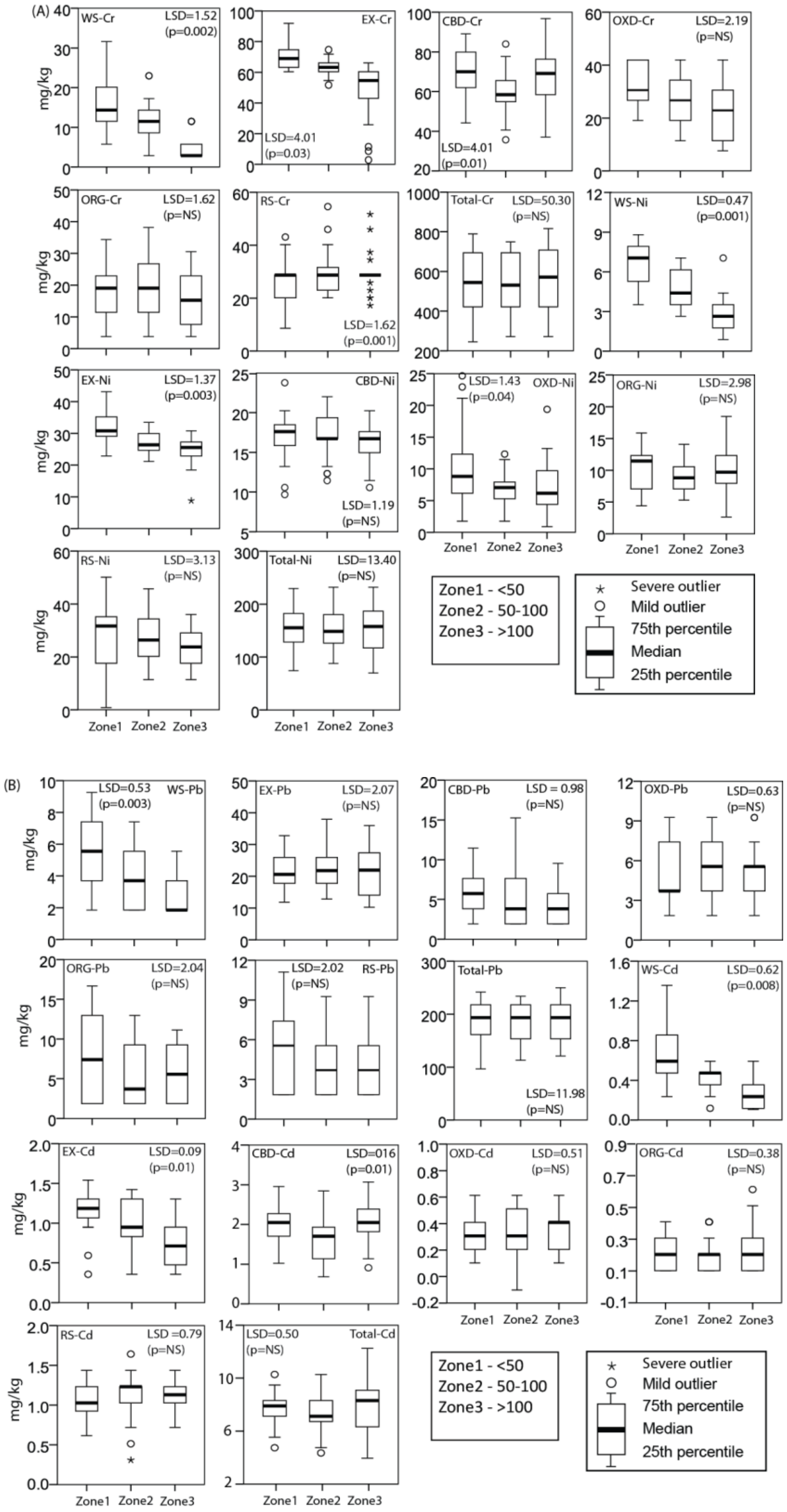


Fig. S3 (A and B). Boxplot demonstrating the concentration of total and fractions of various metals of the mica waste amended soils of three sample zones (LSD-Least significant difference).

All metal fractions were varied significantly (*P* < 0.05) higher in between the mica waste contaminated soils of zone 1 as compared to zone 2 and 3. Significant variations existed in the metal concentrations among the mica amended contaminated soil due to the diversity in the waste materials dumped at the mine sites over the years. Throughout soils, metals can be found in a variety of different fractions, including those that are easily leached and those that are reacting, as a result of interactions with diverse soil components.

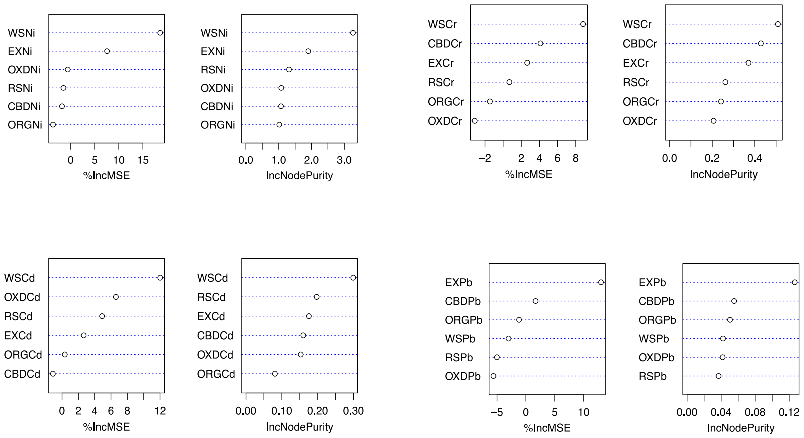


Fig. 3.2. Variable Importance plot from Random Forest representing the importance of different fractions of Ni, Cr, Cd and Pb in soil influencing the uptake of metals in rice grain. (%IncMSE: Mean decrease in accuracy, IncNodePurity: Mean Decrease Gini).

Fig. 3.3 depicts the three dimension (3D) partial dependence plot of variation of rice grain metal content for each metal with the two most important variables derived from the variable importance plots of random forest models. It can be observed that The ExNi and the WSNi both have significant contributions to grain Ni content. The grain Ni content increased when the ExNi was more than 30 mg kg^{-1} and the WSNi crossed 4 mg kg^{-1}. For Cr the CBDCr of above 70 mg kg^{-1} and WSCr above 10 mg kg^{-1} resulted in highest uptake of Cr in rice grain. The WSCd above 0.4 mg kg^{-1} and OXDCd above 0.1 mg kg^{-1} resulted in significant uptake of Cd in rice grain. The EXPb fraction above 25 mg kg^{-1} and the CBDPb above 2 mg kg^{-1} resulted in the maximum uptake of Pb in rice grain. The partial dependence plot shows the marginal effect one or two features have on the predicted outcome of a machine learning model. The partial dependence plot depicts the marginal impact of one or two features on a machine learning model's predicted outcome (Friedman 2001). A partial dependence plot can be used to determine whether the relationship between the target and a feature is linear, monotonic, or complex. PDPs show the link between a subset of variables (usually 1-3) and the response while accounting for the average effect of the other predictors in the model. Partial dependence reveals the relationship between the variables in a model we're interested in and the expected outcome by marginalising the model output over the distribution of the predictor variables.

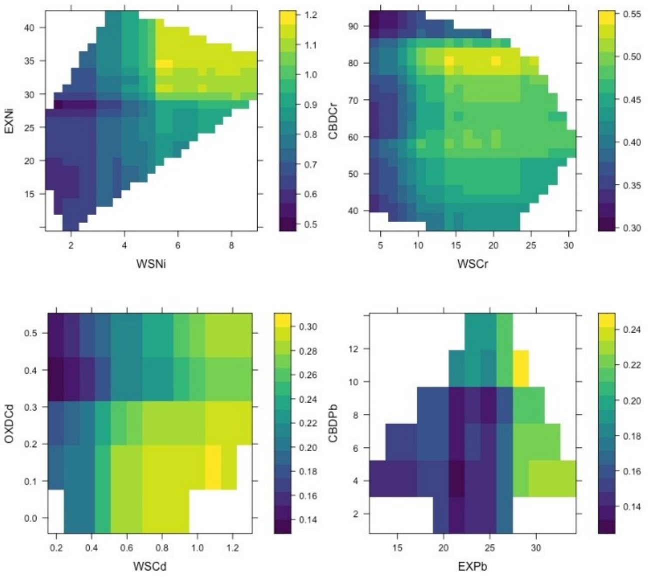


Fig. 3.3. Partial dependence plot from random forest model showing the marginal effect of two most important fractions of soil Ni, Cr, Cd and Pb affecting the content of the metals in rice grain (represented by values and colour intensity at right side of each plot). All the parameters have the unit of mg kg-1.

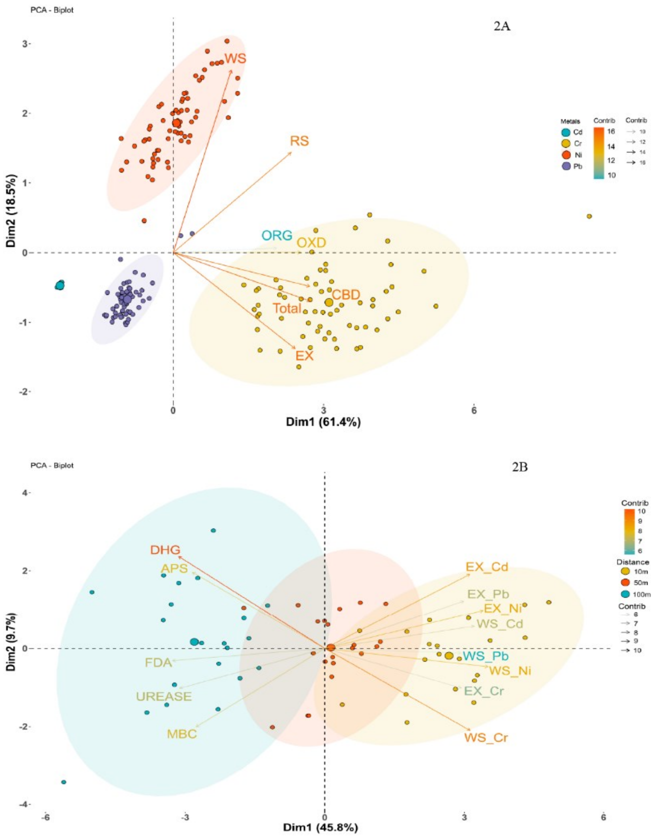


Fig. 3.4 (A and B). Principal component analysis (PCA) of different fractionations of heavy metals in the study area. Each metal has been colour coded and contribution of different fractionations

was represented with gradient colours of orange to blue. The number in parenthesis of Dim-1 and Dim-2 represent % explained variance to total variance.

Principal component analysis (PCA) revealed that first two dimensions (Dim-1, Dim-2) of PCA conserved 79.9% of total variation (Fig. 3.4). Dim-1 and Dim-2 possess an eigen values of 3.37 and 1.42 explaining 61.4% and 18.5% of total explained variance respectively (Fig. 3.4A). PCA biplot revealed highest positive coordinate of total, carbonate bound, exchangeable and oxide bound fractions with a contribution of 23.07%, 22.82%, 21.06% and 16.68% respectively to Dim-1. This result shows that most of the heavy metals were in carbonate bound form following exchangeable, oxide bound and other fractions. Lowest contribution of water-soluble form to Dim-1 indicated lowest abundance of this fraction. The PCA in Figure 3.4B represents the different fractions of the metals along with the soil biological properties. The first two dimensions explained 55.5% variation of the data (Dim-1: 45.8% and Dim-2: 9.7%). It was observed that WS-Pb, Ni and Cr and EX-Cr were found to be in positive coordinate to Dim-1. Other fraction of metals was found in negative coordinate in respect to both Dim-1 and Dim-2. All the biological parameters were also found in negative coordinate in respect to both Dim-1 and Dim-2. It also implies a negative correlation between them. The abundance of both fractions of heavy metals decreases with an increase in distance from mica mines, while a reverse trend was observed in the case of microbial parameters. The metal concentration in soil does not provide a reliable indicator for predicting the effects of metals on soil microorganisms and their enzyme activity (Bhattacharyya et al., 2008; Xu et al., 2019). The controlling metal fractions were those bound to carbonate. Using PCA is also a good way of ensuring that as much variation in the data is covered as possible (Mondal et al., 2017; Mandal et al., 2022).

3.3.4 Soil chemical and biological properties across 3 zones

From the non-parametric Kruskal-Wallis test as can be observed from the violin plots (showing the distribution of the data) in Fig. 3.5 revealed that all the chemical properties of soil, pH, EC, TOC, CEC, total and available N, exchangeable P, Ca, Mg and Na were statistically non-significant ($p > 0.05$) in terms of zones of sampling from the source of contamination.

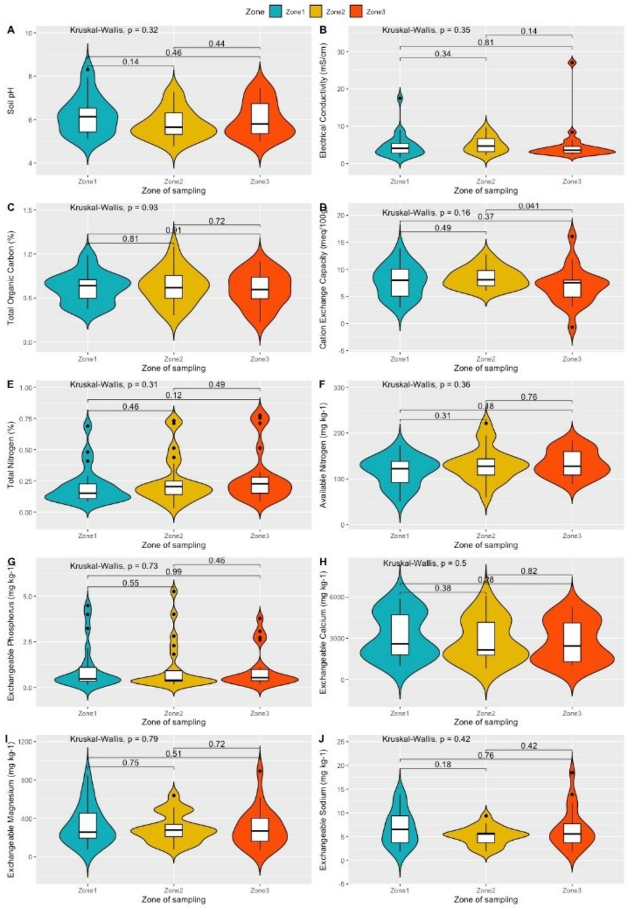


Fig. 3.5. Violin Plots representing the comparison among the 3 soil sampling zones in terms of chemical properties. (Zone 1- <50, Zone 2- 50-100 and Zone 3- >100).

The tested soils were found natural to slightly acidic in nature zone 3 and 2 based on pH values. The range of pH values between three zones were (6.32 to 8.3), (5.5 to 6.31), and (4.78 to 5.44) for zones 1, 2, and 3 respectively. The lowest pH value was found in zone 3 due to the production of organic acids by potassium solubilizing bacteria (KSB); it has previously been reported that KSB bacteria can change soil pH by producing organic acids (acidolysis) (Meena et al., 2015; Saha et al., 2016). The results of these studies are found to be similar, to the earlier findings (Ciarkowska et al., 2017). pH and CEC values was found to vary significantly (*P* < 0.05) across all the three sample sites (zone 1, 2, and 3) (Fig. Figure 5). There was a lower CEC capacity in zone 3 (mean = 4.86 Cmol/Kg) as compared to zone 1 (mean = 10.51 Cmol/Kg) and 2 and (mean = 7.66 Cmol/Kg) respectively, which may be the result of a lack of inorganic colloids and low organic carbon in this mica contaminated soil (Shyamsundar et al., 2014; Chen et al., 2021). The concentration of organic C was significantly (*P* < 0.05) higher in zone 3 (mean = 0.81%) as compared to zone 2 (mean = 0.62) and zone 1(mean = 0.40) respectively. In available N, zone 3 (mean = 163.00 mg/Kg) was found significantly (*P* < 0.05) higher concentration of N as compared to zone 2 (mean = 127.79 mg/Kg) and zone 1 (mean = 92.47 mg/Kg) respectively. The higher organic C content of zone 3 (>100m) soil than the zone 2 (50-100m) and zone

1 (<50m) was due to the presence of high amounts of organic materials present in rice agricultural field. It was previously reported that, there was a higher concentration of organic C present in soils containing a greater amount of organic matter (Tripathy et al., 2014). The low level of nitrogen available in the samples is related to the low organic matter contribution (due to the samples' acidity, resulting in low positive charge). These studies found similar results to earlier ones (Park et al., 2011; Shyamsundar et al., 2014). The available P was higher in sample zone 3 (mean = 5.95 mg/Kg) compared to zone 1 (mean = 4.68 mg/Kg) and zone 1 (mean = 3.79 mg/Kg) respectively (Fig. 5). Phosphorus availability is influenced by organic matter, and when organic matter is present, phosphorus availability increases. In addition, organic molecules will compete with phosphate adsorbing on soil surfaces, thereby reducing phosphorus retention, thereby increasing phosphorus availability.

3.3.5. Soil microbial biomass carbon and metabolic quotient

Microbial biomass is an integral part of soil organic matter and a labile pool for plant nutrients (Tang et al., 2019; Bhattacharyya et al., 2008). Therefore, the suppression of the soil microbial biomass could often lead to a decrease in the rate of nutrient recycling and the volume of the labile nutrient pool. The MBC value in the mica waste soils was markedly higher in zone 3 (mean = 140.80 mg/kg) than in zone 2 (mean = 91.64 mg/kg) and zone 1 (mean = 64.95 mg/kg) and varied significantly ($p < 0.05$) at each of the three sites (Fig. 3.6). In nearby mines side soils (zone 1), the concentration of MBC was lower due to a low organic matter content. The ranged of MBC was zone 3 (74.83 mg/kg to 396.81 mg/kg) medium zone (53.24 mg/kg to 195.20 mg/kg), and lower in zone 1 (23.78 mg/kg to 142.17 mg/kg) respectively. According to Tripathy et al. (2014), the presence of higher organic matter in soil is correlated with higher MBC. The variation in MBC in the landfill soils are related to the interplay of physicochemical properties as well as bioavailable metals in these soils.

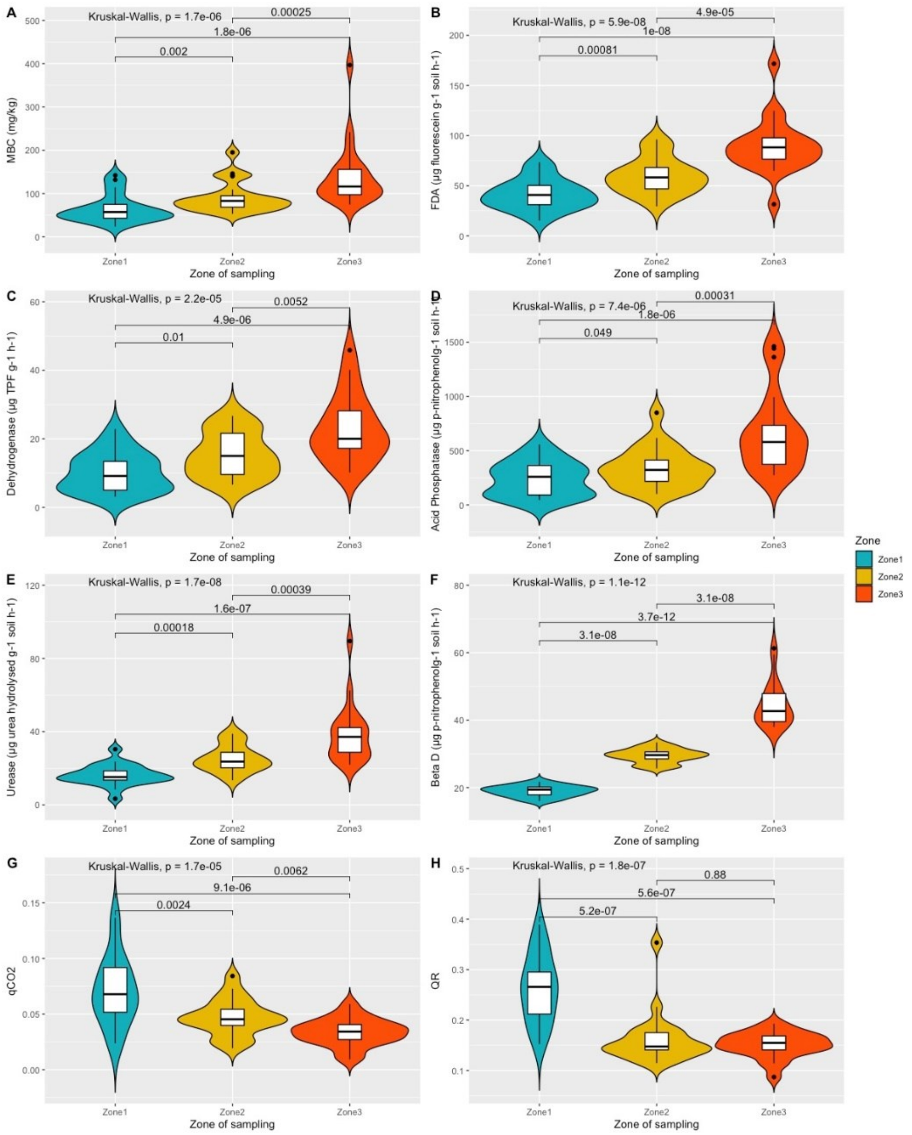


Fig. 3.6. Violin Plots representing the comparison among the 3 soil sampling zones in terms of microbiological properties. (Zone 1- <50, Zone 2- 50-100 and Zone 3- >100)

The microbial activity in mica waste amended soils was assessed using three parameters, like basal respiration (BR), and FDA hydrolyzing activities. qCO_2, or microbial metabolic quotient, is an indicator of microbial response to disturbance, based on the ratio of basal respiration to microbial biomass (Dinesh & Chaudhuri, 2013; Tripathy et al., 2014). qCO_2 measures the efficiency of the soil MBC in utilizing C resources, with qCO2 value higher for zone 1 (mean = 0.07) than zone 2 (mean = 0.17) and zone 3 (mean = 0.03) respectively (Fig. 3.6). Increasing qCO_2 showed shifting of energy by microbial biomass from growth to maintenance, pointing to metal-induced stress on the soil as a consequence of microorganisms spending more energy to survive in mica waste contaminated soils, resulting in faster respiration and less efficient incorporation of fresh substrate into new microbial biomass (Zhang et al., 2010; Xiao et al., 2017). Chander et al. (2001) stated that metals disturb the biological division of energy between growth and maintenance in soil microorganisms, which then require greater quantities of C for maintenance, reducing the amount of C incorporated into the biomass. In addition, the high qCO_2 value indicates that microbial respiration occurred faster than their biomass growth, which indicates that most of the biomass energy was used for survival rather than growth (Chakraborty et al., 2022). These results clearly indicate that zone 3 (agricultural field) was more favourable site for microbial proliferation compared to the zone 1 and 2.

3.3.6. Soil microbial respiration quotient (QR)

A microbial respiration quotient is a ratio of basal soil respiration (BSR) to substrate-induced respiration (SIR), which indicates how microbial communities respond to environmental perturbations. BSR is metabolically inherent, whereas SIR pertains to metabolically active populations (Tripathy et al., 2014; Amoakwah et al., 2022). BSR and SIR are dependent on two groups of microorganisms in soil: the K-strategists, which are autochthonous microbial populations, and the R-strategists, which are zymogenous microorganisms (Dilly, 2005). It was found that the QR value in zone 1 soil (mean = 0.26) was considerably higher than in zone 2 (mean = 0.16), and zone 3 (mean = 0.15) (Fig. 6). It reflects that, dormant populations of metabolically active organisms are suppressed. Hence, the suppressive effects of soil acidity seem to be specific to metabolically active zymogenous populations, which indicates the synthesis of biomass is less efficient under heavy metal loadings in tailings, and the reduction of biomass in heavy metal contaminated soils is mainly due to inefficient biomass synthesis. Higher concentrations of QR suggest a shift from energy-based enhancement to ecosystem maintenance (Grodnitskaya et al., 2022). An extended QR ratio also indicates a shift from an increase in energy to the maintenance of the ecosystem (Brookes, 1995; Bhattacharyya et al., 2008).

3.3.7. Fluorescein diacetate hydrolyzing activity (FDA)

Among three sample zones, FDA value was found significantly ($p<0.05$) higher in zone 3 (mean = 89.53) as compared to zone 2 (mean = 59.69) and zone 1 (mean = 41.42) respectively (Fig. 6). FDA concentrations in zone 1 were lower due to heavy metal contamination in mica tailing soils, decreased uptake by active microbes, and a reduction in microbial biomass carbon availability under heavy metal stress, and inefficient biosynthesis contributes to the reduction of biomass in heavy metal contaminated soils (Atakpa et al., 2022; de Aguiar Santiago et al., 2022). FDA activity has long been regarded as a potential biological indicator to determine soil microbial activity. It is a non-fluorescent, non-polar derivative that is easily transported to the cell, where it has been hydrolysed into polar fluorescein by several enzymes viz. lipase, esterase, protease, that are involved in organic matter decomposition (Tripathy et al. 2014; Nannipieri et al., 2003). Active cells are capable of converting the FDA into fluorescein. When the cells are unable to hold the fluorescein, it eventually gets out of the cell and is measured spectrophotometrically (Dzionek et al., 2018). The finding results indicated that presences of higher concentration of heavy metals may be inhibited the production of the enzymes in the mines side soils (zone 1).

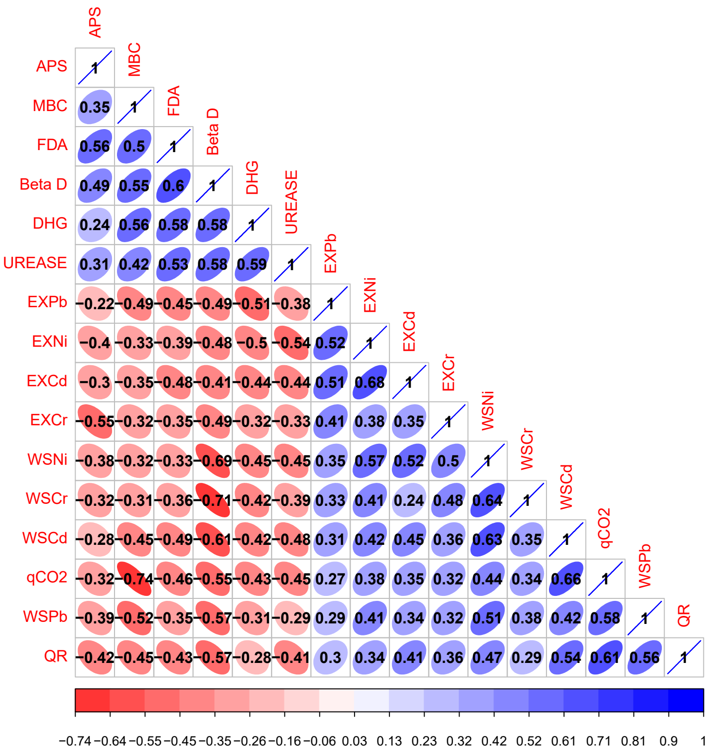
3.3.8. Soil enzyme activities

The assay of enzyme activities is important for estimating the effects of metal pollution on the soil environment (Dick, 1997). Soil enzyme activity is a sensitive indicator of the effect of environmental factors on microbial functions. Dehydrogenase, urease, phosphatase, and beta-D glucosidase activities of the mica waste contaminated soils varied significantly ($p<0.05$) between all three samples zones (zone 1, 2, and 3) (Fig. 6). The values of dehydrogenase, urease, phosphatase, and beta-D glucosidase activities were higher in zone 3 (mean = 23.01, 678.38, 38.51, and 44.57 mg/kg, respectively) as compared to zone 2 (mean = 15.24, 341.34, 25.24, and 29.46 mg/kg, respectively) and zone 3 (mean = 10.19, 239.88, 16.22, and 18.94 mg/kg, respectively). In soils in zone 3 (>100m) there were higher enzyme activity levels, as a result of the presence of organic matter. The enzyme activity levels were also stabilized by the enzyme-organic matter complex (Tripathy et al., 2014; Dick, 1997). Inhibition of dehydrogenase activity might be due to the reduced microbial biomass and the activity in the soils, because the activity is derived from the intracellular enzymes (Dick, 1997).

The microbial enzyme activities in soil are used to estimate the adverse effects of pollutants on soil quality (Warman and Munroe, 2010; Niemeyer et al., 2012; Tripathy et al., 2014). Microbes are the chief controlling agent of enzymatic regulations; thus, they have the potential to respond to any environmental changes and provide detailed information on enzyme catalytic reactions associated with the biological process. They serve as an indicator of soil health quality (Dick, 1997; Taylor et al., 2002).

3.3.9. Metal removal interactions and microbial attributes: A correlation-based analysis

The heat plot (Fig. 7) represents the interaction (spearman correlation) of the different fractions of metals (Ni, Cd, Cr and Pb) with the soil biological properties. The soil biological properties APS, MBC, FDA, Beta D, Urease and DHG were positively correlated with each other at ($p < 0.05$). All the bioavailable fractions of Ni, Cr, Cd and Pb were negatively correlated with the soil biological properties at $p < 0.05$. On the other hand, a significant positive correlation was observed between the different fractions of metals (Ni, Cd, Cr and Pb). However, it is difficult to determine whether cadmium, chromium, lead and nickel strongly affected the soil microbial properties individually because there were highly positive correlations ($P < 0.05$) among all the metals studied and the changes in the concentration of the metals in the field were similar to each other (Fig. 7). It is suggested that the metals affected microbial properties by behaving synergistically or additively with each other (Tripathy et al., 2014). The water-soluble and exchangeable metal fractions are usually the most toxic than other fractions because they can be easily released into the water as metal ions. That can easily and direct affect soil microbes (Ghosh et al., 2004; Bhattacharyya et al., 2005). Metal ions such as Cr, Cd, Ni and Pb are thought to react with sulfhydryl groups to inactive enzymes, a reaction similar to the formation of metallic sulfide. In enzymes, the sulfhydryl group acts as an integral part of the catalyst activating site or as an involved group to maintain the proper structural relationship of the enzyme protein (Juma and Tabatabai, 1977; Kamaludeen et al., 2003). Metal can also reduce microbial enzyme activity by interacting with enzyme-substrate complexes, using denaturing the enzyme proteins, or interacting with protein-active groups (Dick, 1997; Tripathy et al., 2014). Thus, we expect that the higher the level of water-soluble and exchangeable metals, the lower activity of soil microorganisms.



	APS	MBC	FDA	Beta D	DHG	UREASE	EXPb	EXNi	EXCd	EXCr	WSNi	WSCr	WSCd	qCO2	WSPb	QR
APS	1															
MBC	0.35	1														
FDA	0.56	0.5	1													
Beta D	0.49	0.55	0.6	1												
DHG	0.24	0.56	0.58	0.58	1											
UREASE	0.31	0.42	0.53	0.58	0.59	1										
EXPb	−0.22	−0.49	−0.45	−0.49	−0.51	−0.38	1									
EXNi	−0.4	−0.33	−0.39	−0.48	−0.5	−0.54	0.52	1								
EXCd	−0.3	−0.35	−0.48	−0.41	−0.44	−0.44	0.51	0.68	1							
EXCr	−0.55	−0.32	−0.35	−0.49	−0.32	−0.33	0.41	0.38	0.35	1						
WSNi	−0.38	−0.32	−0.33	−0.69	−0.45	−0.45	0.35	0.57	0.52	0.5	1					
WSCr	−0.32	−0.31	−0.36	−0.71	−0.42	−0.39	0.33	0.41	0.24	0.48	0.64	1				
WSCd	−0.28	−0.45	−0.49	−0.61	−0.42	−0.48	0.31	0.42	0.45	0.36	0.63	0.35	1			
qCO2	−0.32	−0.74	−0.46	−0.55	−0.43	−0.45	0.27	0.38	0.35	0.32	0.44	0.34	0.66	1		
WSPb	−0.39	−0.52	−0.35	−0.57	−0.31	−0.29	0.29	0.41	0.34	0.32	0.51	0.38	0.42	0.58	1	
QR	−0.42	−0.45	−0.43	−0.57	−0.28	−0.41	0.3	0.34	0.41	0.36	0.47	0.29	0.54	0.61	0.56	1

Fig. 3.7. Interactions between metal bioavailability and microbial activity are based on spearman correlation ($p<0.05$).

3.4. Conclusions

Particularly concerning is the contamination of fractional metals labelled as being water-soluble and exchangeable fractions. The sequential fractionation scheme appears to be a very useful method for assessing the chemical nature of contaminated soils. According to the results, metal contamination is most critical in zone 1 soils since it contains significantly more bioavailable metals than those in other studied soils such as zone 2 and zone 3. Chromium (Cr), nickel (Ni), and led (Pb) were found to be the most abundant heavy metals in the mica contaminated paddy soil samples. The results indicate that the water-soluble and exchangeable metal fractions inhibit the biological properties of soil, despite occupying only a small portion of the total metal concentration in the soil. It is possible that unplanned and inadvertently dumped mica mine wastes will contaminate the food chain and affect the soil quality and ecological systems in the long run.

CHAPTER-4. OBJECTIVE 2

"Source acquainted pollution and health risk assessment of potentially toxic elements in mine tailings contaminated soils in India employing synergistic statistical approaches."

4.1. Introduction

In the Giridih and Koderma districts of Jharkhand, India has the largest mica deposit in the world, spanning about 3888 sq. km (Huang et al., 2013; Nishanth and Biswas, 2008). During the dressing of raw mica, large amounts (~ 75%) of waste are dumped near the mines. Muscovite (or white mica) and biotite (or black mica) wastes are typically homogeneous, loose, and fine; have a low moisture-holding capacity and bulk density, and lack nutrients, inhibiting plant growth and contaminating the environment during monsoons and strong winds (Kumar and Maiti, 2015). Muscovite mica is the most commonly mined and used type of mica in Giridih. The presence of potentially toxic metals (PTEs: Ni, Cd, Zn, Mn, Pb, Cu, and Cr) in this mica-contaminated groundwater and nearby agricultural fields is generally related to mica minerals such as muscovite and biotite (Ghosh et al., 2022; Giri et al., 2021a). Mica mine waste that contains PTEs has negatively impacted crop production and soil quality and is also detrimental to soil health (Ghosh et al., 2022). In addition to being widespread components of concern in mica waste soils, these PTEs also pose a significant threat to human health through long-term exposure through ingestion, inhalation, and contact with the dermal (Huang et al., 2021). Previously observed by (Huang et al., 2021; Lin et al., 2018) the persistence and biotoxicity of PTEs can cause a variety of diseases and even total potential cancer. In addition to being persistent, PTEs also pose a health risk when their concentration exceeds permissible limits due to their inability to degrade or compost over time.

A systematic assessment of the human health risks associated with continuous exposure to waste mica with PTEs is a requirement for populations exposed to toxic metals through groundwater and mine-contaminated soils (Weissmannová and Pavlovský, 2017). According to Streets et al. (2018); Zn, Ni, Cr (VI), As, Cd, Hg, Cu, and Pb are common PTEs inputs from anthropogenic activities, and heavy metal pollution is almost irreversible. PTEs such as Cd, As, Pb, and Hg can affect the key functions of the kidneys, lungs, or other human organs of the body. This can cause cerebrovascular diseases and skeletal disorders (Yang et al., 2019). In order to ensure the safety of human health, it is important to understand the pollution levels of soil and the health risks associated with PTEs, particularly for children who practice hand-to-mouth behaviours and are more likely to ingest PTEs accidentally (Huang et al., 2021). An assessment of the health risks posed by waste mica contamination in soil PTEs requires identifying and quantifying the sources of pollution, especially those caused by humans. Using self-organizing map (SOM), which are unsupervised learning algorithms, it is possible to solve problems that are nonlinear (Wang et al., 2020). For this reason, SOM is widely used as a tool for recognizing soil PTE patterns. In general, positive matrix factorization (PMF) has been widely recognized and endorsed as a powerful tool for source allocation, particularly for air pollutants by the USEPA (2014). On the other hand, very few researchers have used this method for analysing water quality (Goswami and Kalamdhad, 2022; Zhang et al., 2020), and soils (Huang et al., 2021). In this statistical model, each data point is calculated to determine its non-negative source contribution (Sun et al., 2020; Tian et al., 2018). According to Li et al. (2020), PMF model relies primarily on researchers' interpretations of background data of the study area, making it limited in scope and accuracy. To improve the accuracy of source identification, the PMF model was combined with Pearson correlation analysis.

As opposed to uncertainty, which is intrinsically tied to assessing risk, uncertainty is explained by a lack of knowledge about variables' actual values (Giri et al., 2020). Due to uncertainties, deterministic risk assessments may underestimate or overestimate risks, and therefore should be approached differently (Koupaie and Eskicioglu, 2015). As a result, probability risk assessment (PRA) attempts to characterize the uncertainty and illustrate it through the range and distribution of exposure parameters or variables (Mallongi et al., 2022). Based on the United States Environmental Protection Agency (USEPA. 2021), Monte Carlo Simulations (MCS) can be used for probability risk assessment, which calculates the frequency of occurrences based on probability distributions for each exposure parameter (Giri et al., 2020; Mallongi et al., 2022).

PTEs are generally referred to as non-essential toxic elements from a geochemical perspective (Chabukdhara et al., 2017; Mondal et al., 2017). Through the application of geostatistical methods, it is possible to analyzed the mobility pattern of metals in soil systems (Bhuyan et al., 2015; Mondal et al., 2017). According to Burgos et al. (2006), geostatistics describes spatial distributions of elements through the theory of regionalized variability. By referencing spatial coordinates, homogeneous groups of samples can be formed that are spatially autocorrelative. Kriging is the most popular estimation approach among various approaches (Strano, 2008, 2006). The Kriging method does not only estimate any sampled locations but also calculates probabilistic models of the uncertainty of undefined predicted values. The results of such assessments can be analyzed visually, and the map reveals the spatial variability of targets over the land surfaces (Mondal et al., 2017).

A study published recently by Giri et al. (2021b) and Giri and Singh. (2022) found the adverse effects of heavy metals and fluoride contamination in groundwater in mica mining areas of Jharkhand states, India; and its effects on human health. Hence the key objectives of this investigation are (a) to investigate soil PTEs pollution characteristics in soils contaminated with waste mica (b) to assess the potential pollution sources of PTEs using the SOM and PMF model (c) to predict PTEs impacts on soil quality, geostatistical tools were applied, and (d) to determine the risk of cancer and the most influential factors using Monte Carlo simulations. Our study provided important information on improving soil quality policies and estimating the presence of major carcinogenic- and noncarcinogenic-related health risks.

4.2. Materials and methods

4.2.1. Study site

Jharkhand is home to the largest deposit of mica in India. Koderma and Giridih are two of the top mica-producing districts in Jharkhand. Geographically, Giridih (4941 sq. km) lies in the northern part of the state, bounded by 24.18°N / 86.3°E. Giridih typical climate is dry, and it receives an average of 1350 mm of rainfall annually. Summer temperatures reach 42°C, and winter temperatures reach 10°C. Three of the twelve blocks in Giridih district - Gawan, Tisri, and Deori - are particularly rich in waste mica and potentially toxic metals contamination.

4.2.2. Soil and mica collection, preparation, and analysis

At the beginning of October 2018, waste mica and mica-contaminated rhizospheric soils were collected from the field near potassium (K) bearing mines in Gawan, Tisri, and Deori blocks of Giridih district, Jharkhand. Using a W pattern statistical sampling method, 63 soil (8 to 10 cm depth) samples were collected at a distance of 10m (Zone 1), 50m (Zone 2), and 100m (Zone 3) from near 21 mica mines with agriculture fields. For further analysis, samples were brought into the laboratory in labelled and sealed polythene bags. Each soil sample was carried out with air-dried and later sieved through a 2 mm sieve for physicochemical and metal analyses. The soil pH and electrical conductivity (EC), organic carbon (OC), soil texture, and total PTEs (Cr, Ni, Pb, Cu, Cd, and Zn) content were measured using a standard protocol derived from (Page et al., 1982). Estimation of bio-availability of PTEs was done in a single extraction using the 0.005 (M) diethylene triamine penta-acetic acid (DTPA) method (Lindsay and Norvell, 1978).

4.2.3. Assessment of instrumentation and quality control for PTEs

In order to prepare the acid digest, and DTPA extractable soil samples, we followed the methods outlined by Sparks et al. (2020), and Lindsay and Norvell. (1978) respectively. PTEs content in the extractants were estimated by an atomic absorption spectrometer and are expressed on a moisture-free basis. For calibration, standard solutions of the respective PTEs were prepared from stock solutions (1000 mg L^{-1}, Merck grade) in 1% (v/v) HNO_3. For quality check, SRM 2710 was used along with a blank extract, which was digested in ultrapure $HF/HNO_3/HClO_4/H_2O_2$ to ensure the accuracy of the PTEs analytical method. As part of a quality check and quality control (QC/QC) study, blank and soil samples, along with certified reference materials (SRM-2710), were analyzed for PTEs content. Results indicate that all PTEs were accurately analysed using the analytical method.

4.2.4. Allocation of sources by positive matrix factorization

There are many ways of allocating sources, but one of the most widely used is the positive matrix factorization (PMF) receptor model Deng et al. (2022). For the PMF model, the algorithms minimize Q, which is calculated as follows Goswami and Kalamdhad. (2022).

$$X = FG + E \tag{1}$$

When the species content (i.e. the concentration of PTEs (C_s)) was greater than the standard deviation (SD), the uncertainty (Unc.) of PTEs was calculated Eq. (3);

$$Unc. = 0.1 \times C_s + \frac{SD}{3} \quad (2)$$

$$Q = \sum_{i=1}^{n} \sum_{j=1}^{m} (\frac{e_{ij}}{s_{ij}})^2 \quad (3)$$

Where, e_{ij}; represent sum of squared differences between the original matrix data (X), and output of PMF (GF), and s_{ij}; represent calculated uncertainties of PTEs. EPA PMF 5.0 was used for the investigation. Q was used to estimate the optimal number of factors. As a result, the model is able to fit the data well. For each model run, a global minimum was obtained by varying the seed value from 1 to 20 times Goswami and Kalamdhad. (2022).

4.2.5. Self-Organizing Map (Kohonen's)

An artificial neural network, Self-organizing maps (SOM), developed by Kohonen and Honkela. (2007), has been successfully used for complex data analyses such as coordination, evaluation, and prediction Bhuiyan et al. (2021). SOM was introduced in this work for zone wise pattern recognition of PTEs in mica contaminated soil samples. In this study, a neuron number was selected using the following heuristic equation (Wang et al., 2020).

$$m = 5 \times \sqrt{n} \quad (4)$$

Where m denotes the abundance of SOM map nodes, and n represents for the size of input data. The data were trained using Visualizing using the Kohonen package for R-programming language (V.4.2.2).

4.2.6. Assessment of soil quality indexes through potential metal pollution

We computed soil quality indexes through PTEs using the formula below (5), the Pollution Index (PI) was calculated to assess the potential toxicity of mica-contaminated soils (Ferreira et al., 2022).

$$Pollution\ Index\ (Pi) = \frac{\frac{Cr}{100} + \frac{Ni}{100} + \frac{Pb}{100} + \frac{Cu}{100} + \frac{Cd}{100} + \frac{Zn}{300}}{6} \quad (5)$$

Mica-contaminated soils were also assessed using the contamination index (CI) and ecological risk index (ERI), with CI explaining the gross metal enrichment as well as the background metal concentration. CI was calculated following Weissmannová and Pavlovský. (2017) as below:

$$Contamination\ index\ (CI) = \frac{C_n}{B_n} \quad (6)$$

As for the ERI, it was calculated from the sum of changes in metals compared with reference background values taking toxicological factors into account (Ferreira et al. 2022), as shown below:

$$Ecological\ risk\ index\ (ERI) = \sum \frac{T_i.C_n}{B_n} \tag{7}$$

Here, T_i represents the toxic response factor for a given PTEs, for Cr: 2, Ni: 2, Pb: 5, Cu: 5, Cd: 30, and Zn: 1.

Additionally, Biswal et al. (2022) enumerated the geo-accumulation index (I_{geo}) in order to estimate the probable potential toxic metal accumulation patterns of various PTEs in the near future.

$$geo - accumulation\ index\ (I_{geo} = \ log_2 \frac{C_n}{1.5_{B_n}}) \tag{8}$$

For mica-contaminated soils, C_n, represents the metal concentration in the soil, B_n, represents the PTEs concentration in the nearby soil, and 1.5 represents the variation in the metal concentration caused by anthropogenic activities. The reference values for B_n of different PTEs were taken from the latest report of the Soil and Land Use Survey of India Soil and Land Use Survey of India (SLUSI Annual Report, 2019–20). Classification of I_{geo} index was obtained by Sun et al. (2019).

4.2.7. Probabilistic assessment of non-carcinogenic risk to human health

4.2.7.1. Calculation of the average daily dose (ADD) of different exposure pathways

In the present work, the non-cancer risk was assessed based on human exposure to the soil through three different exposure pathways like $\text{Intake}_{\text{inhalation}}$, $\text{Intake}_{\text{ingestion}}$, and $\text{Intake}_{\text{dermal}}$ (Weissmannová and Pavlovský 2017). The average daily dose (*ADD*) is calculated separately for each exposure pathway (USEPA 1997). The given equations are used to estimate intake via each of the exposure pathways:

$$ADD_{ingestion} = \frac{C \times IngR \times EF \times ED}{BW \times AT} \times 10^{-6} \tag{9}$$

$$ADD_{inhalation} = \frac{C \times InhR \times EF \times ED}{PEF \times BW \times AT} \tag{10}$$

$$ADD_{dermal} = \frac{C \times SA \times SAF \times ABS_{dermal} \times EF \times ED}{BW \times AT} \times 10^{-6} \tag{11}$$

Here, *C* is metal concentration, *IngR* is ingestion rate (mg/day) for adult = 100 mg/day child = 200 mg/day, *InhR* is inhalation rate (mg/day) Adult: 20; child: 7.6, *SA* is skin surface area available for daily contact expressed in cm^2 for adult:0 .5700; child: 0.2800, *SAF* is skin adherence factor for soil. For adult: 0.07 $mg/cm^2/hr$; Child: 0.2$mg/cm^2/hr$, ABS dermal is 0.001 (for all metal), *EF* is exposure frequency expressed in days/ year (350 days/ year), *ED* is exposure duration (days/year), adult: 24 and child: 6, *BW* is body weight, adult: 60 kg and child: 15 kg, *AT* is average time, (*ED* x 365), and *PEF* is partial emission fraction (m^3/kg) =1.36×10^9.

4.2.7.2. Human health probabilistic risk assessment for non-carcinogenic PTEs

PTEs exposure can be evaluated for its noncarcinogenic effects on human health by calculating the hazard quotient (HQ) and hazard index (HI), respectively (Weissmannová et al. 2017; USEPA, 2004).

$$HQ_{ingestion} = \frac{Intake_{ingestion}}{Reference\ dose\ (R_f\ D_{ingestion})} \quad (12)$$

$$HQ_{inhalation} = \frac{Intake_{inhalation}}{Reference\ dose(R_f D_{inhalation})} \quad (13)$$

$$HQ_{dermal} = \frac{Intake_{dermal}}{Reference\ dose\ (R_f\ D_{dermal})} \quad (14)$$

The HI was introduced to evaluate non-carcinogenic risks resulting from multiple exposure pathways, which is the sum of all HQs (Saha et al., 2017).

$$HI = \sum HQ_{ex}P = HQ_{ingestion} + HQ_{inhalation} + HQ_{dermal} \quad (15)$$

HI > 1 indicates that PTEs may cause adverse health effects or that further study is needed, and HI < 1 indicates that PTEs do not cause adverse health effects (USEPA, 2004).

4.2.7.3. An assessment of PTEs carcinogenic risk (CR) and total carcinogenic risk (TCR)

CR is a measure used to determine a contaminant's likelihood of causing cancer. PTEs (Ni, Cr, Pb, and Cd) were classified as potential carcinogens in this study. The CR of exposure was estimated as follows (USEPA, 2004):

$$Carcinogenic\ risk\ (CR) = ADD \times SF \times ADAF \quad (16)$$

Here, ADD is the average daily dose (mg/ kg/day), SF is the cancer slope factor of desired metals like Cr (0.5), Pb (0.042), Cd (0.38), and Ni (0.91) and ADAF is the age-dependent adjustment factor (adult = 1 and child = 4.5).

The value of TCR is measured as the probability of an individual developing cancer over time (Saha et al. 2017; USPEA, 2004):

$$TCR = \sum CR \quad (17)$$

The acceptable range of CR by the USEPA varies from 10^{-4} to 10^{-6}; if risks exceeding 10^{-4} are considered unacceptable, while risks below 10^{-6} are unlikely to cause significant health problems (Li et al., 2020).

4.2.7.4. Analyzing uncertainty through MCS

Human health risk assessment is subject to uncertainty due to the variation in environmental conditions and individual characteristics (Giri et al., 2020). Among the various mathematical methods for assessing probabilistic risk, MCS is one (Kalantary et al., 2022). We performed MCS and sensitivity analyses using Crystal Ball (V. 11.1.1.1, Oracle, Inc., USA).

4.2.8. Geostatistical approach

Using ArcGIS version 10.4, Kriging interpolation was used to project the spatial distribution of PTEs and ecological risk zones. For showing the PTEs in the study area, the PMF scores were plotted using Kriging and spatial interpolation (Goovaerts, 1997).

4.2.9. Statistical analysis

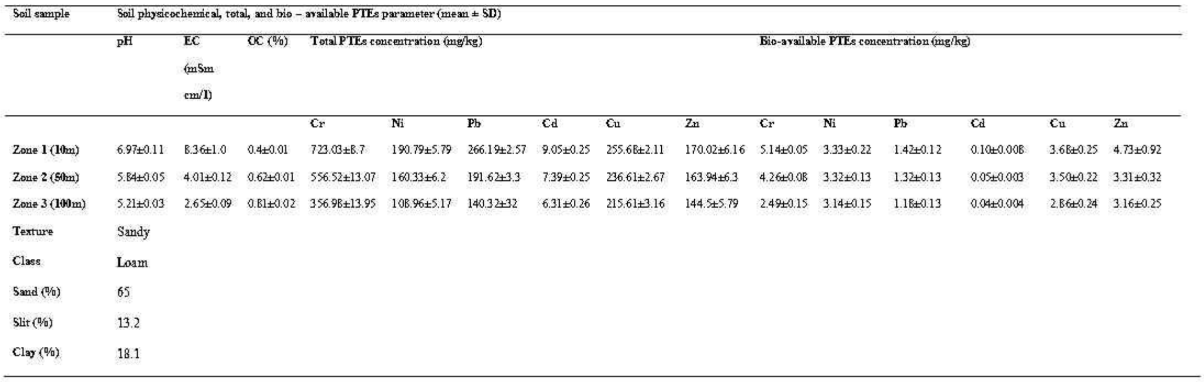
The least significant difference (LSD – ANOVA), and correlation analysis were carried out using statistical software SPSS statistical package version 26.

4.3. Results and discussion

4.3.1. Characteristics of the experimental site and total and available PTEs

Table 1, represents the pH, EC, OC, soil texture, mica properties, and total and bioavailable (DTPA) PTEs such as Cd, Cr, Cu, Ni, Pb, and Zn of the study sites (Zone 1, 2, and 3) are presented. The mean soil pH, EC, and OC of the three zones are as follows: zone 1 (pH - 6.97, EC – 8.36 mSm cm^{-1}, and OC – 0.4%), zone 2 (pH - 5.84, EC – 4.01 mSm cm^{-1}, and OC – 0.62%), and zone 3 (pH - 5.21, EC – 2.65 mSm cm^{-1}, OC – 0.81 %) respectively and was found to be statistically significant. The texture of the three zones of soil was sandy, class–loam, and the mechanical properties of each of the soil properties were sand (65%), slit (13.2%), and clay (18.1). The texture of the three zones of soil was sandy, class – loam, and the mechanical properties of each of the soil properties were sand (65%), slit (13.2%), and clay (18.1%). The mean total and bioavailable (DTPA) soil PTEs content (mg/kg) across three zones; zone 1 soil samples were statistically significantly higher than that of zones 2 and 3(Table 1).

Table 1. Descriptive statistics of the soil parameters and total and bio-available potentially toxic elements on mica contaminated soil samples. Here, least significant differences (LSD) and significant level (p) of pH (LSD = 0.11; $p = 0.001$); EC (LSD = 0.35; $p = 0.001$); OC (LSD = 0.25; $p = 0.001$), total PTEs as Cr (LSD = 17.17; $p = 0.001$); Ni (LSD = 8.11; $p = 0.001$); Pb (LSD = 4.87; $p = 0.001$), Cd (LSD = 0.36; $p = 0.004$); Cu (LSD = 3.79; $p = 0.001$), and Zn (LSD = 8.65; $p = 0.005$), and bio-available PTEs as Cr (LSD = 0.37; $p = 0.005$); Ni (LSD = 0.18; $p = 0.007$); Pb (LSD = 0.16; $p = 0.02$), Cd (LSD = 0.011; p = NS); Cu (LSD = 0.28; $p = 0.004$), and Zn (LSD = 0.76; $p = 0.004$). T = Traces.



Soil sample	Soil physicochemical, total, and bio – available PTEs parameter (mean ± SD)														
	pH	EC (mSm cm/l)	OC (%)	Total PTEs concentration (mg/kg)						Bio-available PTEs concentration (mg/kg)					
				Cr	Ni	Pb	Cd	Cu	Zn	Cr	Ni	Pb	Cd	Cu	Zn
Zone 1 (10m)	6.97±0.11	8.36±1.0	0.4±0.01	723.03±8.7	190.79±5.79	266.19±2.57	9.05±0.25	255.68±2.11	170.02±6.16	5.14±0.05	3.33±0.22	1.42±0.12	0.10±0.008	3.68±0.25	4.73±0.92
Zone 2 (50m)	5.84±0.05	4.01±0.12	0.62±0.01	556.52±13.07	160.33±6.2	191.62±3.3	7.39±0.25	236.61±2.67	163.94±6.3	4.26±0.08	3.32±0.13	1.32±0.13	0.05±0.003	3.50±0.22	3.31±0.32
Zone 3 (100m)	5.21±0.03	2.65±0.09	0.81±0.02	356.98±13.95	108.96±5.17	140.32±32	6.31±0.26	215.61±3.16	144.5±5.79	2.49±0.15	3.14±0.15	1.18±0.13	0.04±0.004	2.86±0.24	3.16±0.25
Texture	Sandy														
Class	Loam														
Sand (%)	65														
Slit (%)	13.2														
Clay (%)	18.1														

4.3.2. PTEs concentration characteristics and source apportionment by PMF

Table S1, presents descriptive statistics for PTEs in waste mica-contaminated soil samples. As compared, the maximum concentrations of all PTEs except Cd exceeded their respective soil quality standards when compared to soil baseline values. The mean concentrations of Cr, Ni, Cd, Pb, Cu, and Zn were 545.51, 153.37, 7.59, 186.38, 235.97, and 159.50 mg/kg respectively (Table S1). In particular, the mean concentrations of Cr, Ni, Pb, Zn, Cu and Cd were 14.86, 3.87, 3.82, 2.03, and 0.15 times greater than their respective BV.PTEs, respectively. The proportions of Cr, Cd, Ni, Pb, Zn, and Cu above the baseline in all samples were 87.39%, 74.00%, 58.96%, 58.51%, 40.16%, and 34.05%, respectively. This indicates that these PTEs may be the main enriched toxic pollutants in waste mica contaminated soils. According to the classification criteria for the degree of variation (Hu et al., 2011; Huang et al., 2021), PTEs were 0.82% for Cr, 2.05% for Ni, 7.25 for Cd 3.81% for Pb, 3.28% for Cu, and 3.32% for Zn. Among them, Cd, Pb, Zn, and Cu showed the greatest special variation. The high diversification may be due to the high concentrations in mica-contaminated soils, indicating that Cd, Pb, Zn, and Cu may be resulting from key sources of toxic pollution.

Table S1. Descriptive statistical summary of waste mica contaminated soil potentially toxic element concentrations (mg/kg) for mica mines in Jharkhand.

Total soil PTEs	**Min**	**Max**	**Mean**	**SD**	**CV%**	**BV.PTEs**
Cr	244.90	816.33	545.51	4.49	0.82	36.7
Ni	69.82	231.98	153.37	3.14	2.05	39.6
Cd	3.95	12.26	7.59	0.55	7.25	50.8
Pb	96.77	250.00	186.38	7.11	3.81	48.78
Cu	189.44	279.10	235.97	7.75	3.28	116.1
Zn	95.30	235.60	159.50	5.29	3.32	68.1

An effective apportionment of natural and anthropogenic sources of PTEs was determined by Pearson correlation analysis of concentrations of PTEs. Then, to apportion and quantify the potential PTEs sources, the PMF model was used (Fig. 1a and 2b). In addition, the PMF model's factor contributions were also correlated with the Pearson correlation coefficients of PTEs in order to verify source apportionment results (Fig. 4.1c).

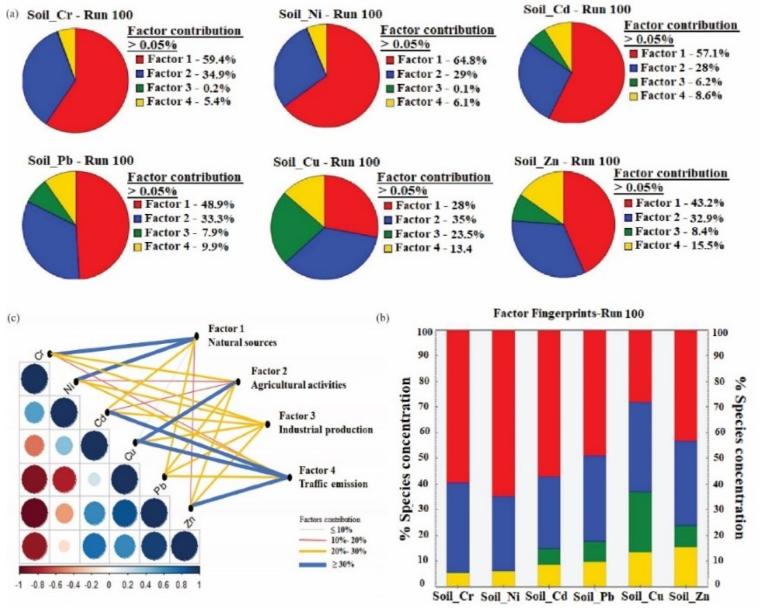


Fig. 4.1. Source apportionment of potentially toxic metals (PTEs) in mica mines contaminated soils of the study area (a) Positive matrix factorization (PMF) model contribution percentage for each Factor. (b) The factor profile of PTEs in mica contaminated soils derived from the PMF model for 63 samples. (c) Combine Pearson correlation analysis with PMF modelling to identify correlations between PTEs. A color gradient indicates the correlation coefficient for pairwise comparisons of PTEs. PMF contributions were related to PTEs. The width of the edges corresponded to the proportion of factor loading, and the color of the edges corresponded to the range of factor loadings.

In the PMF model, the residual matrix was estimated based on a minimum objective function Q (Hsu et al., 2017; Manousakas et al., 2017). Four factors were then determined as the appropriate number. PTEs with a strong signal-to-noise ratio (S/N) were considered strong. The correlation coefficients (r^2) between observed concentrations and predicted concentrations are shown in (Fig. S1).

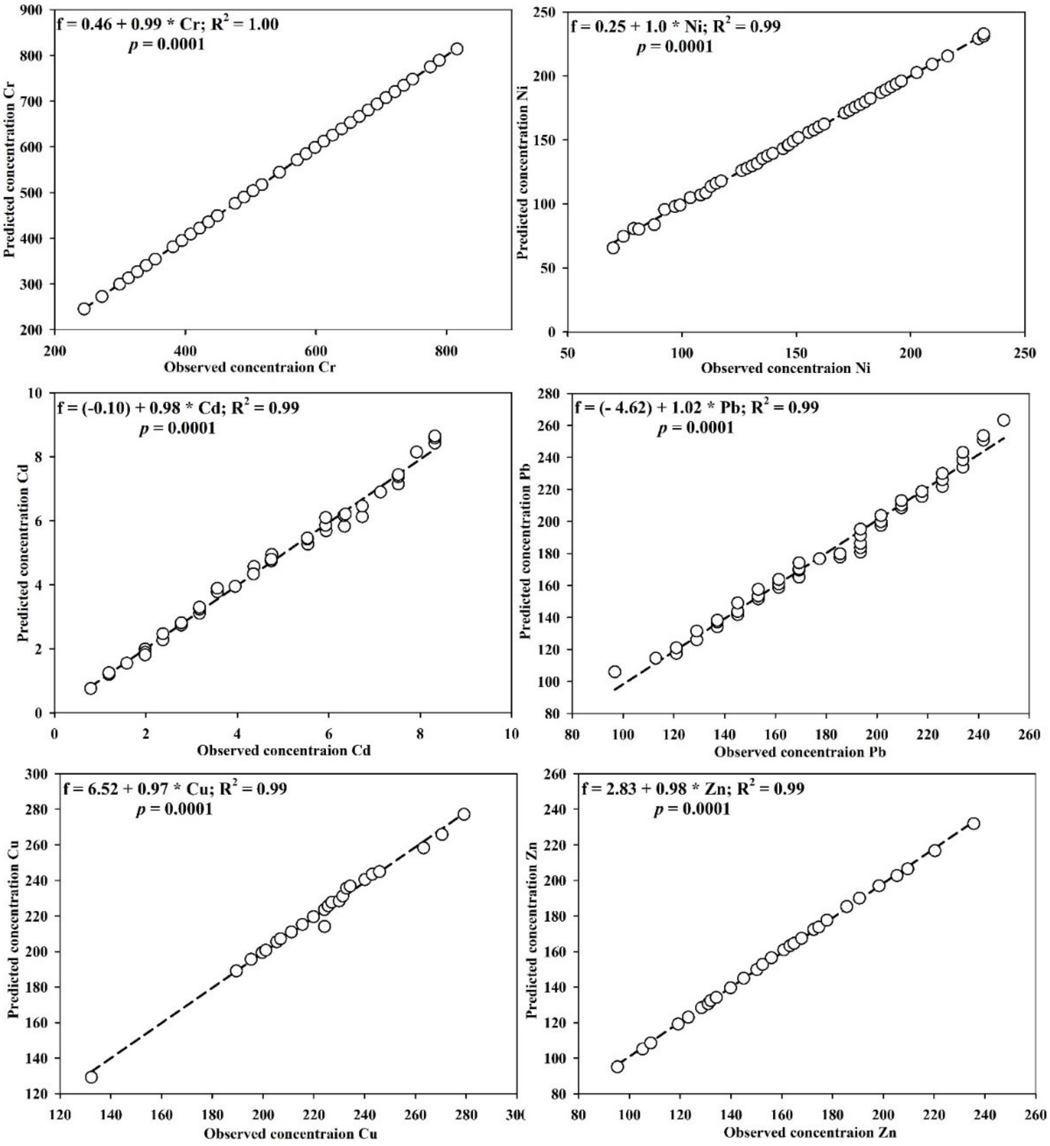


Fig. S1. Regression of observed concentrations and predicted concentrations of potentially toxic elements by PMF model.

The (r^2) values of all PTEs were greater than 0.95, suggesting a strong relationship between predicted and observed values. A detailed Factor-profile and contribution of each PTEs to the PMF model is shown in (Fig. 4.2).

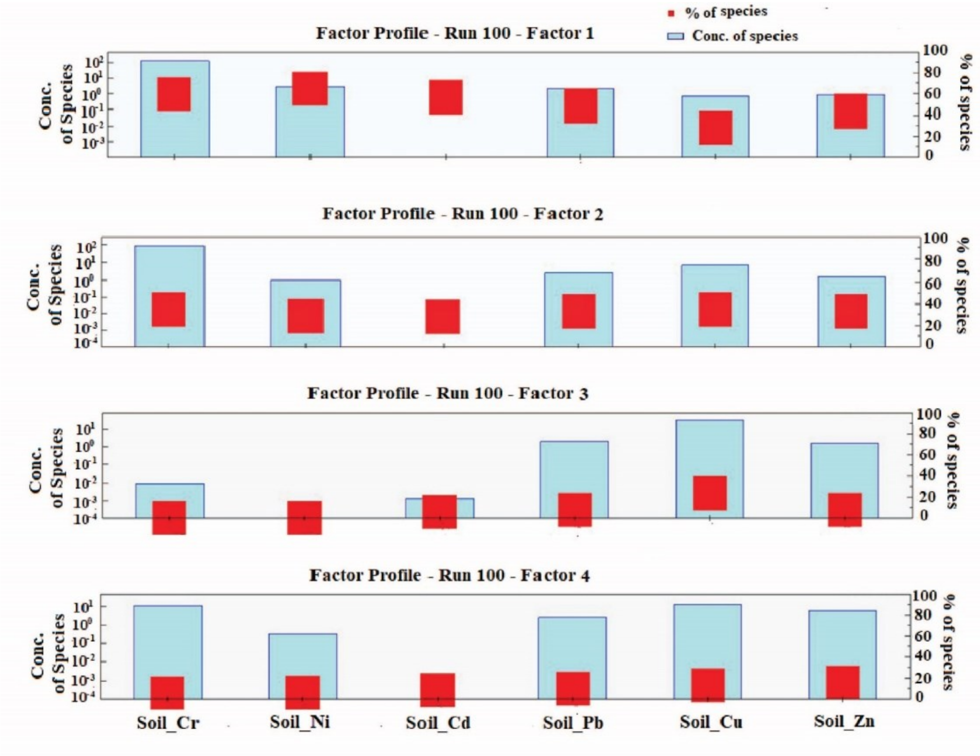


Fig. 4.2. Potentially toxic metals (PTEs) relative contribution (%) to the four factors derived from PMF.

In pairwise comparisons of soil PTEs within them and soil PTEs with four factors (Fig. 1c), a strong correlation was observed (r > 0.6), which indicated a possible common source of PTEs. (Huang et al., 2021). Based on the PMF model, four factors were extracted (called factor 1, 2, 3, and 4 respectively), and their contribution to each PTE percentage was shown in (Fig. 1a). The PMF results reveal that factor 1 was loaded on Ni, Cr, Cd; factor 2 was governed by Cu, Cr, Pb, and Zn; factor 3 was characterized by Cu; and factor 4 was governed by Zn, Cu, and Pb (Fig. 1b). It is supported by the findings of (Goswami and Kalamdhad, 2022; Huang et al., 2021). Factor 1, which accounted for 59.4% (Cr), 64.8% (Ni), 57.1% (Cd), 48.9% (Pb), 28.0% (Cu), and 43.2% (Zn) of the contribution rate (Fig. 1a), was strongly correlated with all PTEs in waste mica-contaminated soils (Fig. 1c). These PTEs are generally closed to soil parent materials of lithogenic origin. Natural sources are mainly responsible for factor 1 rather than anthropogenic sources. It can therefore be said that factor 1 reflects the soil parent material that was the natural source (Chai et al., 2021). Natural background concentrations of Cd were recognized because its average concentration in soils was lower than its local BV.PTEs (Table S1). Ni and Cr are also correlated with natural sources since both are present in soil parent materials and have been confirmed as abundant in pedogenic processes (Hou et al., 2020; R. Zhang et al., 2020). The PMF model indicates that Ni, Cr, Cd, Pb, and Zn can mainly be attributed to anthropogenic sources such as nearby mica mines. Thus, factor 1 relates to anthropogenic contamination. As for factor 2, it was observed that only Cu, Cr, Zn, and Cd contributed. Similarly, the contributions of PTEs as well as Cu, Zn, Pb, and Cr were observed in factor 3 and factor 4 (Fig. 2). These PTEs have been reported to originate from natural or lithogenic deposits in northeast India throughout literature (Goswami and Kalamdhad, 2022). Therefore, anthropogenic contamination can be attributed to factor 1 based on source apportionment analysis using PMF. Contrary to this, factor 2, 3, and 4 are related to contamination caused by natural or lithogenic sources.

4.3.3. Analysing samples using SOM

As a result of its strong classification capability, the SOM can expose significant information to interpret results that would otherwise remain obscure with traditional approaches. This includes hierarchical clustering and principal component analysis (PCA) (Wang et al., 2020). The purpose of the SOM in this study is to identify patterns of PTEs, develop a quantitative estimation method to show the zone-wise variation of PTEs in mica waste soil, and establish a scientific keystone for the category and treatment of various PTEs sources. A unified distance matrix (U-matrix) is made up of the distance between the weight vectors of each neuron and its neighbours. Component planes provide a graphical representation of the relationships between variables. In component planes, similar gradients indicate a positive correlation, whereas antiparallel gradients indicate a negative correlation. Samples with shorter hexagonal distances have more similar characteristics. Fig. 4.3(a - c) illustrates the component planes of SOM analysis output, where each variable corresponds to the one shown in (Fig. 3a) and Fig. 3b, presents a zone-wise view.

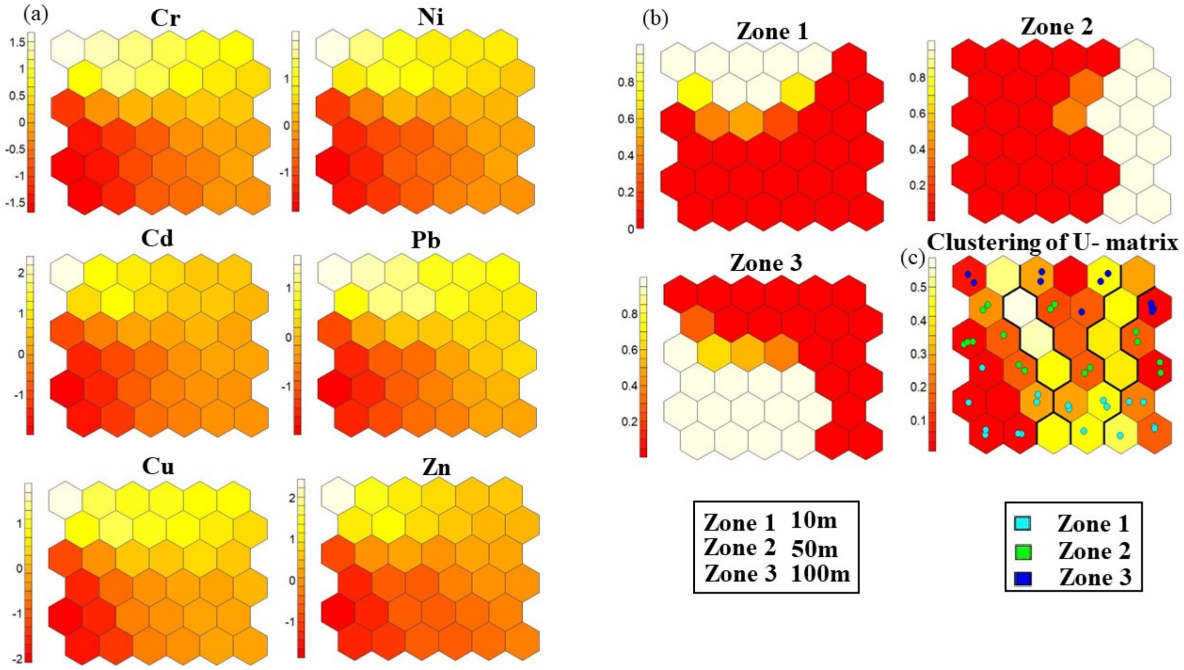


Fig. 4.3. (a) Self-organizing map (SOM) of concentration of Potentially toxic metals (PTEs) in mica mines contaminated soils (b) Zone-wise PTEs concentration distribution maps (c) U-matrix clustering represents Self-organizing map (SOM) of the sampling site.

In order to illustrate the significance of delivered variables for each SOM unit, SOM planes were constructed in color ranks. Pb and Cr concentrations are higher in the upper left corner neurons, whereas Cu and Ni concentrations are moderately high in the upper left corner neurons (Fig.4.3a). Cd and Zn showed a similar concentration pattern and ranked inside all PTEs: Pb > Cr > Cu > Ni > Cd and Zn. PTEs concentrations in three different sample zones showed that the neurons in the upper left corner to middle corner to lower left corner of zone 3 were higher than those in zone 2 (upper right to lower right of the maps) and zone 1 (left corner to right corner of the maps) (Fig. 4.3b). Finally, the SOM algorithm was used to prepare a U-matrix clustered by zone-wise sample location (Fig. 4.3c). On the basis of cluster maps most of the neurons belong to zone 1 (11 neurons) with respect to zone 2 (8 neurons) and zone 3 (5 neurons). It appears that soil contaminated with PTEs can be characterized by regional characteristics based on the differences in PTEs emissions associated with zone 1.

4.3.4. Assessment of potential metal pollution

The concentration of potentially toxic elements (PTEs) was substantially higher in the waste mica mines side (zone 1) as compared to zone 2 and zone3; that is eventually disposed of on land, posing a serious negative effect on the environment as well as human health. Most of the active mica mines are situated near agricultural fields and mica with toxic metals contaminates land and water resources over time. The issue was addressed by assessing the pollution index (PI) and human health hazards (Fig. S2).

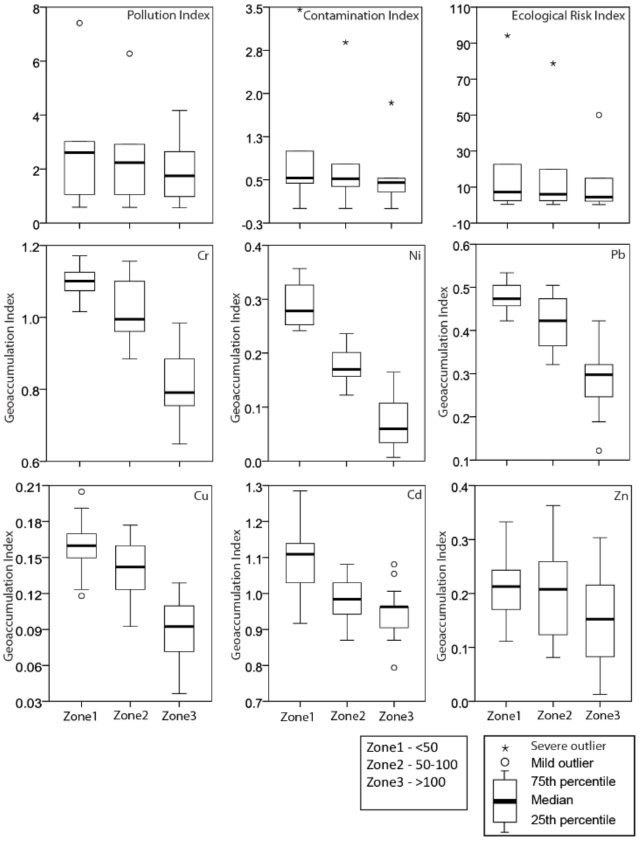


Fig. S2. Boxplot demonstrating the comparison among the 3 zones in terms of pollution index, ecological risk index, contamination index, and geoaccumulation index of waste mica contaminated soils.

The pollution index determines the potential for contamination with multiple elements, and a value > 1 indicates that the substance in soils nearby mica mines are heavily contaminated with gross metals content above the permissible limits (1 ≤ PI ≤ 3 indicates moderate to high polluted), thus requiring remediation (Mondal et al., 2017; Weissmannová and Pavlovský, 2017). Hence, PI in zone 1(mean = 2.38) was found higher as compared to zone 2 (mean = 2.05) and zone 3 (mean = 1.47) (Fig. S2). The contamination index value for zone 1 samples varied from 0.002 to 3.45 with an average of 0.99; while the contamination index ranged for zone 2 and 3 samples between 0.002 to 2.88 and 0.002 to 1.83, with an average of 0.84 and 0.59 respectively (Fig. S2). The level of contamination by PTEs was higher in soils near mica mines (zone 1) than in the other two zones. The ERI values for the mica-contaminated soils are presented in (Fig. S2). The ERI values were found low to moderate ecological risk (ERI < 300) in our studies, due to the occurrence of PTEs. Among the three zones, the highest ERI values were observed in zone 1 (mean = 23.34) as compared to zone 2 (mean = 18.87) and (mean =12.71) respectively posing a moderate ecological risk for the nearby mica mine ecosystems (Weissmannová et al. 2017). Using I_{geo} and contamination level, the mica-contaminated soil samples were categorized into six classes (Fig. S2). The contaminated soil samples of the study zones can be categorized into unpolluted to moderately polluted ($0 \leq I_{geo} \leq 1$) and moderately polluted ($1 \leq I_{geo} \leq 2$)

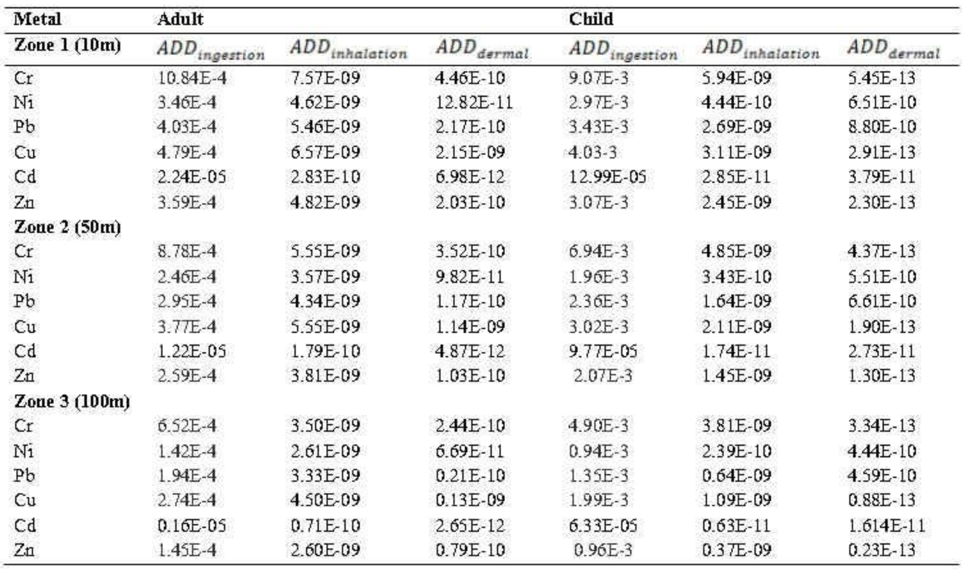
respectively, due to the occurrence of Cr, Cd, Pb, Ni, Zn, and Cu (Weissmannová et al. 2017). Mondal et al. (2017) reported that PTEs have an antagonistic effect on soil ecosystems and human health.

4.3.5. Assessing the risk to human health from non-carcinogenic substances

The results of the health risks associated with non-carcinogenic and carcinogenic metals exposures in mica-contaminated soils are shown in (Supplementary Tables 2-4), via different human body pathways (ingestion, dermal, and inhalation). Children are more vulnerable to non-carcinogenic effects resulting from exposure to soil contaminated with mica via three-body pathways like ingestion, dermal absorption, and inhalation by PTEs (Cr, Ni, Pb, Cu, Cd, and Zn) (Table S2). Across three different zones, zone 1 showed significantly the highest non-carcinogenic risk as compared to zone 2 and zone 3 over three exposure pathways of both adult, and child (Table S4). Based on this result, ingestion of soil by children and adults is the main route of exposure to waste mica-contaminated soils that are associated with health risks for PTEs followed by inhalation and dermal contact.

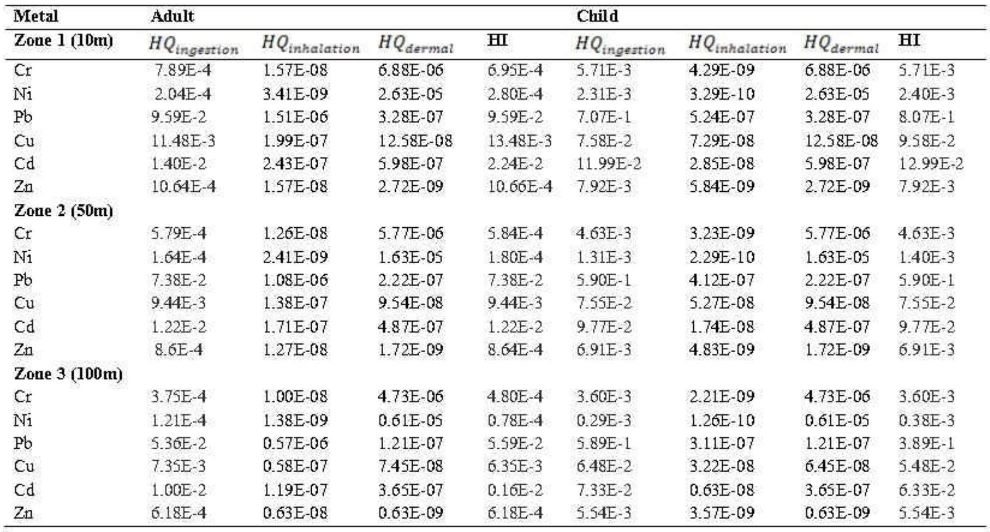
According to the hazard quotient (HQ), children have a higher negative health impact than adults in terms of ingestion (mean child $HQ_{ingestion}$: 1.35E-01; mean adult $HQ_{ingestion}$: 1.94E-02) only, whereas adults have the highest HQ for inhalation (mean adult $HQ_{inhalation}$: 2.32E-07; mean child $HQ_{inhalation}$: 8.23E-08) (Table S3).

Table S2. Non carcinogenic risk (three exposure pathway) values on adult and children.



Metal	**Adult**			**Child**		
Zone 1 (10m)	$ADD_{ingestion}$	$ADD_{inhalation}$	ADD_{dermal}	$ADD_{ingestion}$	$ADD_{inhalation}$	ADD_{dermal}
Cr	10.84E-4	7.57E-09	4.46E-10	9.07E-3	5.94E-09	5.45E-13
Ni	3.46E-4	4.62E-09	12.82E-11	2.97E-3	4.44E-10	6.51E-10
Pb	4.03E-4	5.46E-09	2.17E-10	3.43E-3	2.69E-09	8.80E-10
Cu	4.79E-4	6.57E-09	2.15E-09	4.03-3	3.11E-09	2.91E-13
Cd	2.24E-05	2.83E-10	6.98E-12	12.99E-05	2.85E-11	3.79E-11
Zn	3.59E-4	4.82E-09	2.03E-10	3.07E-3	2.45E-09	2.30E-13
Zone 2 (50m)						
Cr	8.78E-4	5.55E-09	3.52E-10	6.94E-3	4.85E-09	4.37E-13
Ni	2.46E-4	3.57E-09	9.82E-11	1.96E-3	3.43E-10	5.51E-10
Pb	2.95E-4	4.34E-09	1.17E-10	2.36E-3	1.64E-09	6.61E-10
Cu	3.77E-4	5.55E-09	1.14E-09	3.02E-3	2.11E-09	1.90E-13
Cd	1.22E-05	1.79E-10	4.87E-12	9.77E-05	1.74E-11	2.73E-11
Zn	2.59E-4	3.81E-09	1.03E-10	2.07E-3	1.45E-09	1.30E-13
Zone 3 (100m)						
Cr	6.52E-4	3.50E-09	2.44E-10	4.90E-3	3.81E-09	3.34E-13
Ni	1.42E-4	2.61E-09	6.69E-11	0.94E-3	2.39E-10	4.44E-10
Pb	1.94E-4	3.33E-09	0.21E-10	1.35E-3	0.64E-09	4.59E-10
Cu	2.74E-4	4.50E-09	0.13E-09	1.99E-3	1.09E-09	0.88E-13
Cd	0.16E-05	0.71E-10	2.65E-12	6.33E-05	0.63E-11	1.614E-11
Zn	1.45E-4	2.60E-09	0.79E-10	0.96E-3	0.37E-09	0.23E-13

Table S3. Calculation of hazards quotients (HQ) and hazards indexes (HI) for adults and children.

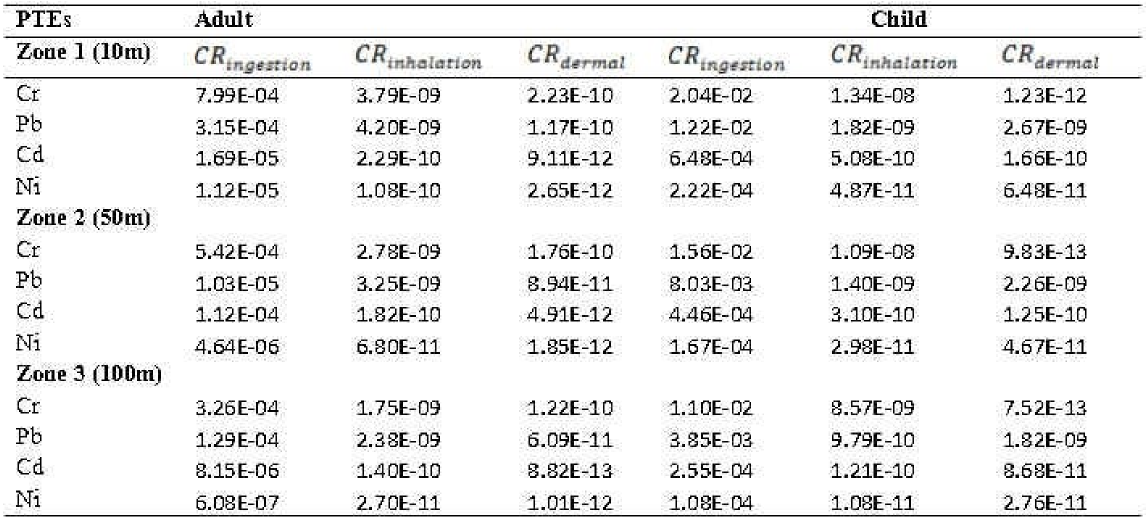


Metal	Adult				Child			
Zone 1 (10m)	$HQ_{ingestion}$	$HQ_{inhalation}$	HQ_{dermal}	HI	$HQ_{ingestion}$	$HQ_{inhalation}$	HQ_{dermal}	HI
Cr	7.89E-4	1.57E-08	6.88E-06	6.95E-4	5.71E-3	4.29E-09	6.88E-06	5.71E-3
Ni	2.04E-4	3.41E-09	2.63E-05	2.80E-4	2.31E-3	3.29E-10	2.63E-05	2.40E-3
Pb	9.59E-2	1.51E-06	3.28E-07	9.59E-2	7.07E-1	5.24E-07	3.28E-07	8.07E-1
Cu	11.48E-3	1.99E-07	12.58E-08	13.48E-3	7.58E-2	7.29E-08	12.58E-08	9.58E-2
Cd	1.40E-2	2.43E-07	5.98E-07	2.24E-2	11.99E-2	2.85E-08	5.98E-07	12.99E-2
Zn	10.64E-4	1.57E-08	2.72E-09	10.66E-4	7.92E-3	5.84E-09	2.72E-09	7.92E-3
Zone 2 (50m)								
Cr	5.79E-4	1.26E-08	5.77E-06	5.84E-4	4.63E-3	3.23E-09	5.77E-06	4.63E-3
Ni	1.64E-4	2.41E-09	1.63E-05	1.80E-4	1.31E-3	2.29E-10	1.63E-05	1.40E-3
Pb	7.38E-2	1.08E-06	2.22E-07	7.38E-2	5.90E-1	4.12E-07	2.22E-07	5.90E-1
Cu	9.44E-3	1.38E-07	9.54E-08	9.44E-3	7.55E-2	5.27E-08	9.54E-08	7.55E-2
Cd	1.22E-2	1.71E-07	4.87E-07	1.22E-2	9.77E-2	1.74E-08	4.87E-07	9.77E-2
Zn	8.6E-4	1.27E-08	1.72E-09	8.64E-4	6.91E-3	4.83E-09	1.72E-09	6.91E-3
Zone 3 (100m)								
Cr	3.75E-4	1.00E-08	4.73E-06	4.80E-4	3.60E-3	2.21E-09	4.73E-06	3.60E-3
Ni	1.21E-4	1.38E-09	0.61E-05	0.78E-4	0.29E-3	1.26E-10	0.61E-05	0.38E-3
Pb	5.36E-2	0.57E-06	1.21E-07	5.59E-2	5.89E-1	3.11E-07	1.21E-07	3.89E-1
Cu	7.35E-3	0.58E-07	7.45E-08	6.35E-3	6.48E-2	3.22E-08	6.45E-08	5.48E-2
Cd	1.00E-2	1.19E-07	3.65E-07	0.16E-2	7.33E-2	0.63E-08	3.65E-07	6.33E-2
Zn	6.18E-4	0.63E-08	0.63E-09	6.18E-4	5.54E-3	3.57E-09	0.63E-09	5.54E-3

With respect to the HQ_{dermal} parameter, adults and children showed the same results (mean adult HQ_{dermal}: 3.81E-06; mean child HQ_{dermal}: 3.81E-06). Among three different zones, zone 1 showed significantly the highest hazard quotient (HQ) of different exposure pathways over zone 2 and zone 3 (Tables S3). A health risk that is considered acceptable or tolerable by an act on USEPA is 1.0E-6 to 1.0E-4 (Saha et al., 2017; Tong et al., 2019). According to the results, the hazard quotient (HQ) could be a health hazard to consumers (both adult and child) if its PTE content is above (HQ > 1.0E-4), resulting in a lifetime health risk.

The total health risk index (HI) values indicate all of the zones from mica mines are highly alarming for the welfare of the human health of the workers and agricultural farmers in the areas and the health of children living in these zones (Table S3). Here are the mean HI values of zones 1 (adult = 2.23E-2; child = 1.75E-01), zone 2 (adult = 1.62E-02; child = 1.29E-01), and zone 3 (adult = 1.08E-02; child = 8.61E-02), respectively. As measured by the HI index, children (mean HI_{child} = 1.30E-01) are more adversely affected by mica-contaminated soils than adults (mean HI_{adult} = 1.64E-02). In terms of carcinogenic risk assessment (CR) of the exposure pathways of child (mean, $CR_{ingestion}$ = 6.08E-03; $CR_{inhalation}$ = 3.17E-09; CR_{dermal} = 6.06E-10) over adult (mean, $CR_{ingestion}$ = 1.90E-04; $CR_{inhalation}$ = 1.58E-09; CR_{dermal} = 6.74E-11) (Table S3).

Table S4. Carcinogenic risk (three exposure pathway) values on adult and children.



PTEs	Adult			Child		
Zone 1 (10m)	$CR_{ingestion}$	$CR_{inhalation}$	CR_{dermal}	$CR_{ingestion}$	$CR_{inhalation}$	CR_{dermal}
Cr	7.99E-04	3.79E-09	2.23E-10	2.04E-02	1.34E-08	1.23E-12
Pb	3.15E-04	4.20E-09	1.17E-10	1.22E-02	1.82E-09	2.67E-09
Cd	1.69E-05	2.29E-10	9.11E-12	6.48E-04	5.08E-10	1.66E-10
Ni	1.12E-05	1.08E-10	2.65E-12	2.22E-04	4.87E-11	6.48E-11
Zone 2 (50m)						
Cr	5.42E-04	2.78E-09	1.76E-10	1.56E-02	1.09E-08	9.83E-13
Pb	1.03E-05	3.25E-09	8.94E-11	8.03E-03	1.40E-09	2.26E-09
Cd	1.12E-04	1.82E-10	4.91E-12	4.46E-04	3.10E-10	1.25E-10
Ni	4.64E-06	6.80E-11	1.85E-12	1.67E-04	2.98E-11	4.67E-11
Zone 3 (100m)						
Cr	3.26E-04	1.75E-09	1.22E-10	1.10E-02	8.57E-09	7.52E-13
Pb	1.29E-04	2.38E-09	6.09E-11	3.85E-03	9.79E-10	1.82E-09
Cd	8.15E-06	1.40E-10	8.82E-13	2.55E-04	1.21E-10	8.68E-11
Ni	6.08E-07	2.70E-11	1.01E-12	1.08E-04	1.08E-11	2.76E-11

Here, zone 1 and intake ingestion pathways had the greatest negative impact on both adults and children across all three zones and exposure pathways. This was over zone 2 and zone 3 and intake inhalation and ingestion. According to Chabukdhara and Nema (2013), adults are at a higher risk of developing cancer than children. HI was generally found higher in children than in adults for all studied PTEs. Based on HI values, Pb > Cd > Cu > Zn > Cr > Ni showed high HI in three zones. High HI (< 1) was computed for children in three zones over the adult. Mondal et al. (2017) reported that HI for all the examined metals was higher in children than in adults. The high intake of Pb can interfere with children's development and cause progressive neurological conditions (Ferreira-Baptista and de Miguel, 2005).

4.3.6. Monte Carlo simulation and sensitivity analysis

Based on the combined pathways of human health risk attributes such as ingestion, inhalation, and dermal contact, Monte Carlo Simulation (MCS) was used to evaluate total cancer risk (TCR). The simulation model predicted 100.000 trials. According to the simulations of ingestion, inhalation, and dermal contact in (Fig. S3: a-c'). The 95-percentile values of TCR for adults and children were (adult = 1.00E-8 and child = 1.28E-8), (adult = 6.82E-1 and child = 2.45+1), and (adult = 8.97E-5 and child = 2.31E-9) respectively. Using these results, we found that the estimated cancer risk of ingestion was an unacceptable range of the USEPA - 10^{-4} to 10^{-6} (Li et al., 2020) and poses health hazards, whereas the estimated cancer risk of inhalation was no threat to health, and in dermal contact, only adults showed above the level of acceptable exposure, whereas children showed no carcinogenic threats from PTEs (Cr, Ni, Pb, and Cd) exposure.

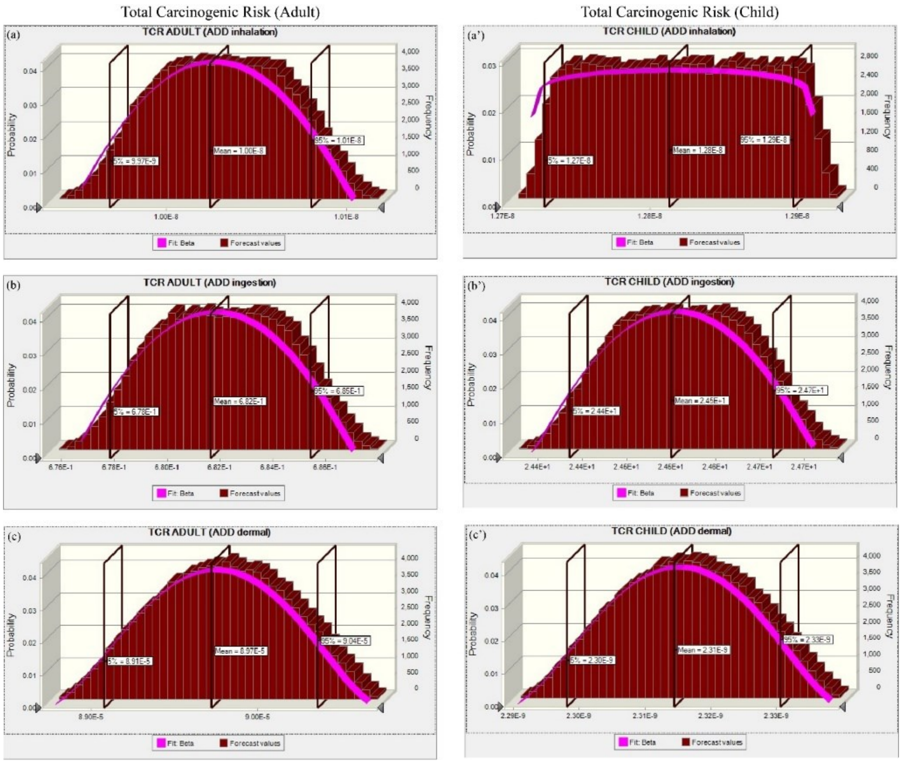


Fig. S3 (a-c'). The Monte Carlo simulation probability of TCR average daily dose by ingestion, inhalation, and dermal from the combined pathway.

As a result of MCS, sensitivity analyses were carried out to classify the most effective input parameters for the TCR as a result of exposure to PTEs (Fig 4.4: a-c'). According to our findings, the TCR exposure pathways of $ADD_{inhalation}$ in both adults and children were extremely sensitive to Cr (adult, $ADD_{inhalation}$ = 82%; child, $ADD_{inhalation}$ = 98.4%) followed by Ni (adult, $ADD_{inhalation}$ = 18%; child, $ADD_{inhalation}$ = 1.5%). The TCR of $ADD_{ingestion}$ in both adults and children was very sensitive to Cr (adult, $ADD_{ingestion}$ = 81.6%; child, $ADD_{ingestion}$ = 56%) followed by Ni (adult, $ADD_{ingestion}$ = 18.3%; child, $ADD_{ingestion}$ = 44%) and Pb ($ADD_{ingestion}$ = 0.1%) only in the child. When it comes to the ADD_{dermal} exposure pathway, Ni (adult and child, $ADD_{ingestion}$ = 100%) is more responsible than Cr, Pb, and Cd for causing dermal diseases. As far as Cd is concerned, no sensitive issues have been observed through three exposure pathways, including that of adults and children. Monte Carlo simulation and sensitivity analysis correlate well; in our case, MCS was used for interpreting the TCR of different exposure pathways of human beings (adult and child). Where a sensitivity analysis can be used to explain the contribution (percentage) of each toxic element to different exposure pathways. According to Mallongi et al. (2022), Cr concentration is a significant factor contributing to cancer in both adults and children. Despite the fact that PTEs exposure risk assessment appears to be an acceptable method, it is complicated by revealing uncertainties, human exposure variables, and changes in risk factors from day to day and area to area (Mohammadi et al., 2022). Hossain and Patra. (2020) confirmed that toxic metals were the most influential factor in carcinogenic hazard calculation. Children are more likely to develop cancer when discrete exposure events occur more frequently in mica mines, mining activities, mines' side water resources, and agricultural land. Therefore, children should avoid consuming contaminated food and water as well as reduce their activities near mining sites. It was reported that children accumulate more metal than adults due to their body ratio (Mallongi et al. 2022).

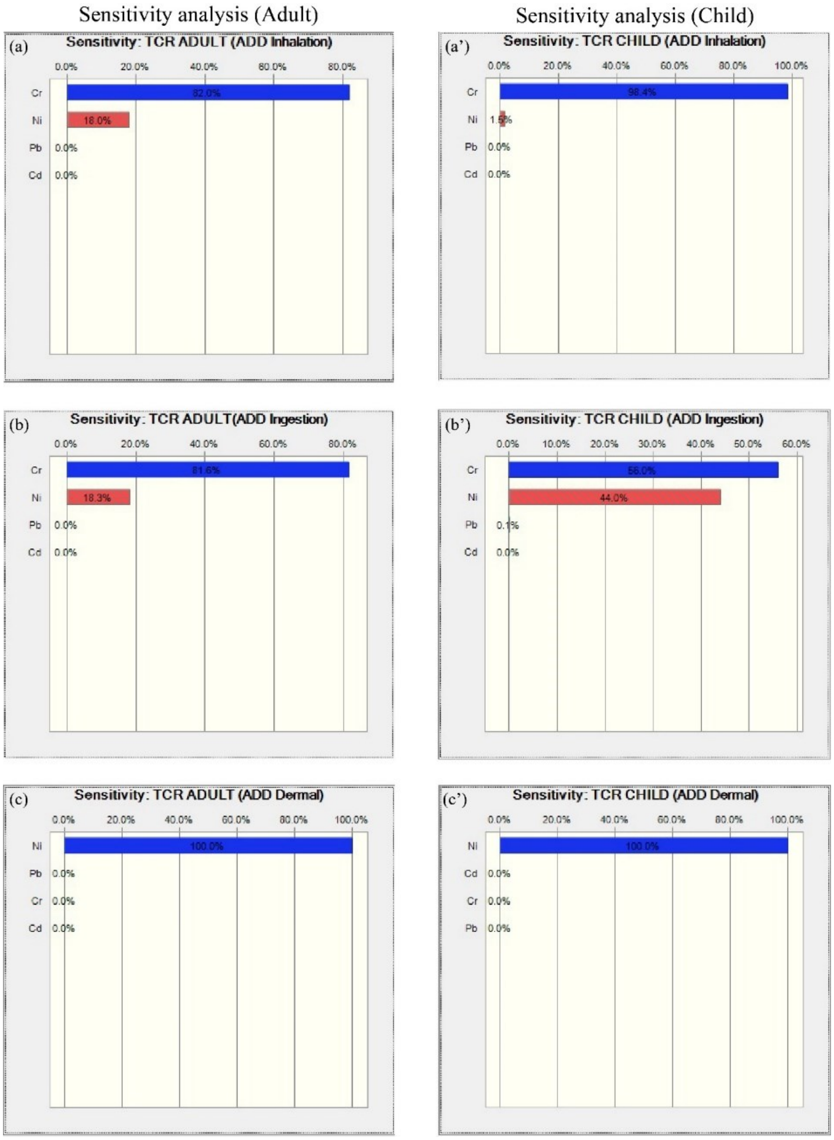


Fig. 4.4 (a-c'). Sensitivity analysis for a contribution of main PTEs on TCR in adults and children.

4.3.7. Spatial distribution of PTEs

Geostatistical methods were applied to estimate the spatial distribution of PTEs (Cr, Ni, Cd, Pb, Cu, and Zn) values in mica-contaminated soils in non-sampled sites (Fig. S4), based on the values obtained from the field samples. The values that were closest to each other were more similar than those that were farther apart. In order to determine the spatial distribution of metal content, ordinary kriging was used. The best-fitted semi-variogram models of metal content in the studied area are shown in Table S5. As a result of the semi-variogram assessment, the nugget values characterize the variability of all parameters observed at zero distance, which is positive in this study. This study also used the most appropriate fit model with the lowest Akaike Information Criterion (AIC).

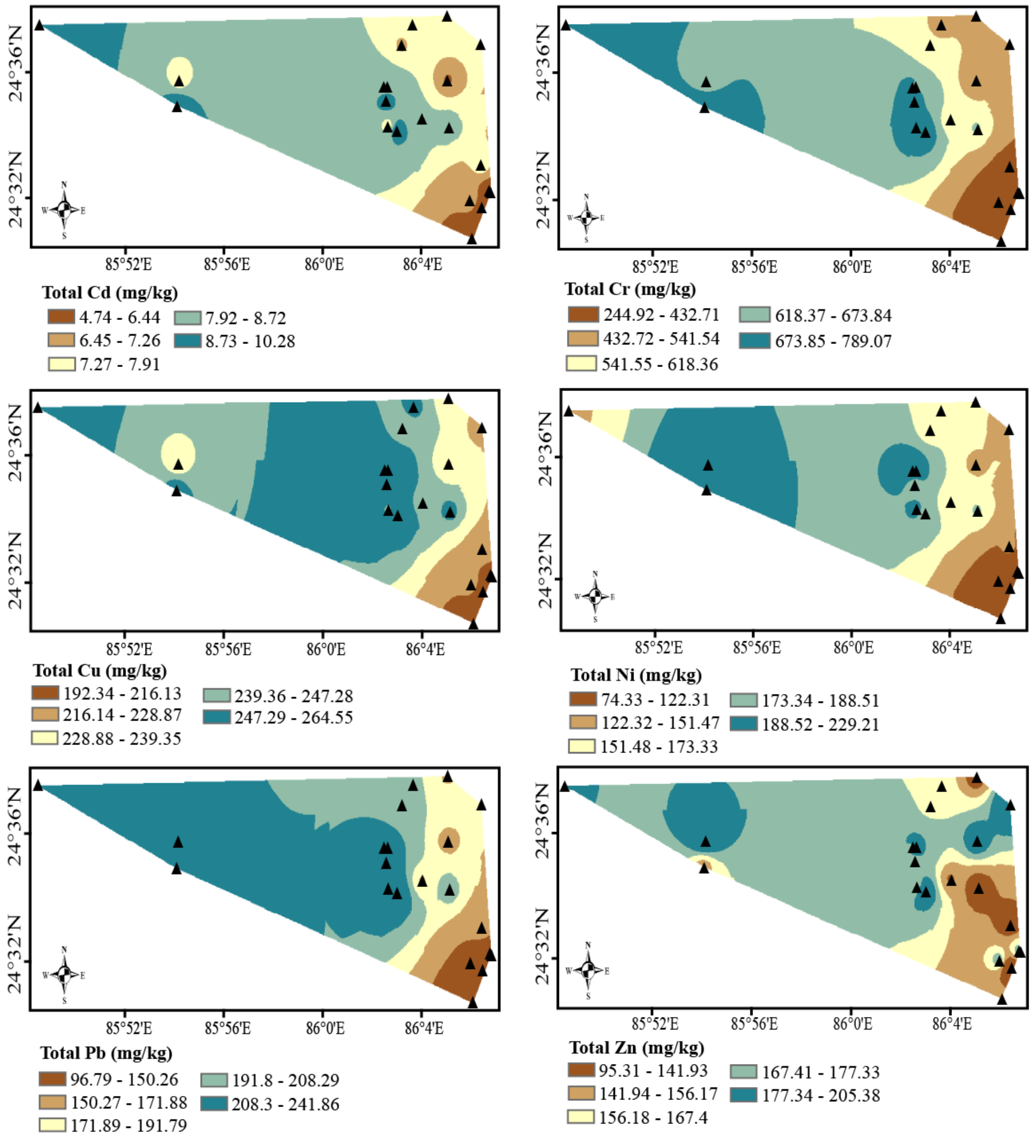


Fig. S4. Spatial distribution maps of total metals (Cd, Cr, Cu, Ni, Pb, and Zn) in waste mica contaminated soils.

Additionally, the sill and nugget represent the maximum variance between data pairs as well as regional differences. Here, the semi-variograms exhibited a lower nugget effect on Cd. This effect was found in other metals such as Cr, Cu, Pb, and Ni, except for Zn. This indicates that the sampling density was adequate to explain the spatial structure of the data (Burgos et al., 2006; Grath et al., 2004).

The semi-variograms for PTEs contents in the studied soils were analyzed using the exponential model (Table S5). For Cu, Cr, and Ni content, the Gaussian model was employed, whereas for Cd and Pb, the spherical model was applied. Despite the strong spatial dependence on Zn, the nugget-to-sill ratio for all variables (except Zn) was moderate to strong. The higher the nugget-to-sill ratio (25%–75% or more), the weaker the spatial dependence (Cambardella et al., 1994). As well, the model's fitness was substantiated by close root mean square error (RMSE) and average standard error (ASE) values for

PTEs in mica. For all metals except Zn, the G value exceeded zero, which indicates that spatial prediction using semi-variogram parameters is superior to assuming the mean of observed values at any unsampled location.

Table S5. Characteristics parameters of variogram models used in the geostatistical modelling of the total metal content. AIC – Akaike Information Criterion; ME – mean error; MSE – mean square error; RMSE – root mean square error; RMSSE - root mean square standard error ASE – average standard error; G – Goodness-of-prediction.

Total Metal	Nugget	Partial sill	Nugget/Sill*100	Spatial dependency	Model	AIC	RMSE	MSE	RMSSE	ASE	G
Cr	32.87	32870	0.10	Strong	Gaussian	7.38	71.95	0.04	1.22	88.59	80.28
Cd	0.62	1.56	39.74	Moderate	Spherical	54.38	1.15	0.0019	1.03	1.11	28.65
Cu	104.35	544.64	19.16	Strong	Gaussian	50.94	12.1	0.0118	0.89	14.37	63.09
Ni	448.63	3010	14.90	Strong	Gaussian	55.58	21.47	0.045	0.95	29.38	74.57
Pb	46.38	2082	2.23	Strong	Spherical	28.98	21.47	0.039	1.06	24.31	68.77
Zn	728.79	87.08	836.92	Weak	Exponential	76.37	30.92	-0.03	1.03	29.99	-22.05

Furthermore, semi-variogram parameters derived from experimental semi-variogram data can accurately describe spatial variation. A Kriging interpolation analysis of the factor scores showed that factor 1 was heavily loaded in the northeast part of the mica mines (Fig. 4.5a), where most of the agricultural lands are located. The factor 2 load was located mostly in the middle and north west sections of the mica mines (Fig. 4.5b), which are mainly occupied by mines and a few inhabited by agriculture. Consequently, the mica mines in the south and west had a high factor 3 score (Fig. 4.5c). An agricultural area to the southwest of the mica mines was found to be a pollution hotspot in factor 4 (Fig. 5d).

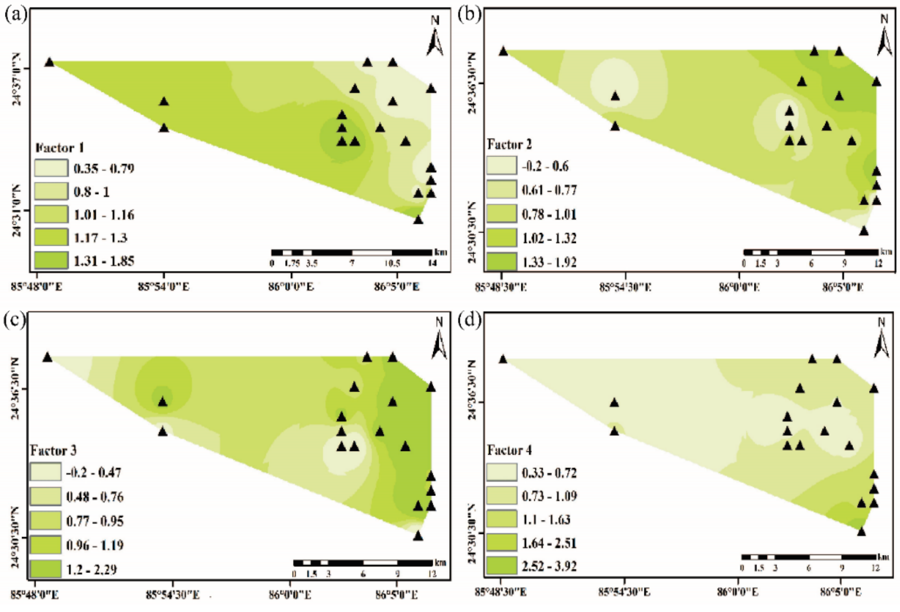


Fig. 4.5 (a-d). Spatial variation with GIS within the mica mines area forms the basis of the normalized contributions of four factors from PMF model.

4.4. Conclusions

The total and bioavailable forms of PTEs in soils were found in mines side (zone 1) soils across three zones. Based on PMF results, Ni, Cr, Cd, and Pb were the most serious toxic pollutants and showed higher ecological risks than the other PTEs. SOM maps recognizing clusters of similar metal content in soil were able to categorize the level of pollution by distinguishing groups of high and low polluted sites. The soil quality indexes based on PI, CI, ERI, and I_{geo} of PTEs indicate that the contamination of PTEs was significantly above the permissible limit in zone 1. The distribution map revealed clear spatial patterns of PTEs contents, as did the soil sampling density. The Kriged map predicted metals spatially better than assuming the mean of the observed values for an unsampled location by using semi-variogram parameters. When it comes to non-carcinogenic PTEs exposed to soil with mica contaminated by adult or child, children are more susceptible to non-carcinogenic effects resulting from exposure via three-body pathways – ingestion, dermal absorption, and inhalation. In terms of HQ and total health risk index (HI) values indicate that all of the zones surrounding mica mines are highly alarming for the well-being of the workers and farmers in the area, as well as children. For children in three zones, HI < 1 values were higher than those of adults. Based on the MCS, the TCR of children via ingestion was significantly higher than that of adults due to their body ratios. The sensitivity analysis for TCR showed that the contribution of soil Cr and Ni elements on the three exposure pathways of adults and children outweighed Pb and Cd elements. The results of all human health risk assessments show that intake ingestion is a key risk factor for the development of cancer.

CHAPTER-5. OBJECTIVE 3

“Temporal dynamics of potassium release from waste mica as influenced by potassium solubilizing bacteria.”

5.1. Introduction

Mineral nutrition and fertilization are the two most important factors for crop production and productivity. Among the plant nutrients, nitrogen (N) is regarded as the most effective nutrient for crop productivity and quality. Although, the continuous and long-term application of N alone reduces the efficiency of crop production (Natesan and Ranganathan, 1990). This might be due to an unbalanced nutrient supply, which is accelerated by depletion and leaching of basic cation like potassium in soils (Sparks, 2002). To meet the food grain production in recent years, primarily focus shifted towards the use of nitrogen and phosphorus; while the use of potassium is often neglected.

After nitrogen (N), Potassium (K) is the second most abundant nutrient in plant leaves and also the most abundant cation in the plant cell (Sardans and Peñuelas, 2015). It is one of the crucial macronutrients after nitrogen (N) and phosphorus (P) for plant growth and maintenance of grain quality. It plays a major role in the synthesis of protein, enzyme activation, starch production, cellulose, and vitamins (Etesami et al., 2017). Additionally, it influences photosynthesis at multiple levels and participates in nutrient transport and uptake. Potassium also imparts resistance against different biotic and abiotic stresses, which helps in increasing crop production and provides immunity to plant diseases (Maqsood et al., 2013; Meena et al., 2015). The deficiency of potassium leads to poor root development, stunted growth, and decreased immunity resulting in a significant reduction in yield. There are several reports available regarding the deficiency of potassium in Indian soil due to the rapid development of the agricultural sector. However, in comparison to nitrogenous or phosphatic fertilizers application of potassic fertilizers are often neglected. According to a report by Meena et al. (2016); out of 371 districts (~11 million soil test data), potassium fertility status was found to be low in 21% of districts, medium in 51% of districts, and high in 28% of districts. Moreover, potassium is required by plants in large amounts, but marginal farmers in India couldn’t afford potassic fertilizers due to its high cost (Hoa et al., 2006). This situation is further complicated by the absence of potassium-bearing minerals for the production of potassic fertilizers in the country, which results in a huge amount of import from other countries (Meena et al., 2015).

Potassium in the soil is available to plants by three means; dissolved in soil water (water-soluble K), adsorbed by clay or organic matter (non-exchangeable K), and held within a different crystal structure like mica or feldspar. Potassium held in organic matter is easily leached out due to its high solubility. Water-soluble K, available directly to plants shares only 0.1 – 0.2% of total soil potassium. Both water-soluble and exchangeable fractions of potassium comprised only 1 - 2% and the remaining 96 – 99% remain as soil unavailable fractions (Huo-Yan et al., 2010; Britzke et al., 2012). The availability of K to plants depends upon several factors like availability of other forms of potassium (solution, exchangeable and non-exchangeable) and weathering of minerals like feldspar and micas (Sparks, 1987). Some soil microorganisms have been reported to have the capacity of solubilizing insoluble or fixed forms of potassium from minerals like micas, illite, and orthoclase (Basak and Biswas, 2009). They are often regarded as potassium solubilizing microorganisms (KSM).

KSM including both bacteria and fungi influences the availability of soil minerals, thus playing a central role in ion cycling and maintaining soil fertility (Meena et al., 2016). They produce some organic acids which help to dissolve rock-bound potassium or chelate silicon ions to bring potassium into the solution, thus make available to plants (Bennett et al., 1998). There are certain bacteria, which have been reported to release a certain amount of potassium from minerals like aluminosilicate (Basak and Biswas, 2009). Several bacterial species like *Bacillus mucilaginosus*, *B. edaphicus*, *B. circulans*, *Paenibacillus* spp., *Acidithiobacillus ferrooxidans*, *Pseudomonas*, and *Burkholderia* have been reported as efficient potassium solubilizing bacteria (KSB). Fungal species like *Aspergillus terreus* and *A. niger* were isolated from potassium-rich soil showed a great potential of solubilizing insoluble potassium in a liquid medium in presence of insoluble sources of potassium like feldspar and potassium aluminium silicate (Prajapati & Modi, 2012). While several fungal species including arbuscular mycorrhizal fungi have been reported to solubilize potassium, the KSBs are known for their efficiency for solubilising unavailable K and applications as microbial fertilizers, mining, and metallurgy (Zhang and Kong, 2014). Apart from potassium solubilization, they have also been reported to release plant growth regulating substances, antibiotic production, organic matter biodegradation, and nutrient recycling (Meena et al., 2013; A. Zhang et al., 2013). Therefore, using these microorganisms as biofertilizers could be an alternative option to chemical fertilizers along with maintaining soil fertility, agricultural improvement, and environmental sustainability.

India is the top producer of mica distributed over a total area ~ 38882 km in Munger district of Bihar and Koderma and Giridih districts of Jharkhand. During the processing of mica production, about 75% of total mined mica is generated as waste contains a significant amount of potassium (8 – 12 % K_2O), which could be a potential source of potassium for agricultural uses (Nishanth and Biswas, 2008). These waste mica tailings are categorized as muscovite mica, can effectively be used as sources of potassium if modified or altered by some chemical or biological processes (Basak and Biswas, 2009). To this end, the use of potassium solubilizing bacteria can be an effective solution. However, the success of each KSB depends upon the identification of the efficient bacterial strain. Native bacterial strain in high potassium enriched soil might have more efficient potassium solubilization capability. Meena et al. (2015) isolated twelve potassium solubilizing rhizobacteria from some Kharif crops of potassium enriched mica mines from the Koderma district of Jharkhand, India. Two strains, namely *A. tumefaciens* OPVS 11 and *Rhizobium pusense* OPVS6 showed the highest potassium solubilizing ability. However, to date, there is no report of KSB isolated from Giridih district of Jharkhand, India which is the second-largest producer of mica next to Koderma. There are several active mines in Giridih, Jharkhand, where mining activities such as crushing, grinding, washing, smelting result in the generation of a huge amount of mica tailings. These bare tailings are very prone to erosion and cause environmental toxicity. Therefore, the combined use of native KSB and waste mica tailings could be a potential solution to inorganic chemical potassic fertilizer and environmental contamination. Moreover, there are little information available on KSB in rice agroecosystem and their potential as potassium solubilizer.

In view of the above discussion, the specific research objectives of this study were (i) isolation and characterization of promising KSB isolates from mica contaminated agricultural fields of rice; (ii) evaluation of potassium solubilising capacity of selected KSB isolates using both qualitative and quantitative approaches; (iii) assessing the ability of KSB isolates in producing plant growth promoting substances and other macro-nutrients (P-solubilization and N-fixation).

5.2. Materials and methods

5.2.1. Soil sampling

Rhizospheric soil samples were collected during the maturity phase of rice (*Oryza sativa* L.) from three blocks (Deori, Gawan, and Tisri) of Giridih district of Jharkhand, India in September 2016. The majority of active mica mines in Giridih are distributed within these three blocks of the district. Samples were collected from rice fields near mica mines where leached out and surface run-off water from mines is stored naturally due to lower altitude. From each mine, three soil samples were collected randomly. Therefore, in total 63 soil samples (= sampling site) were collected from 21 mica mines. Each sampling site consisted of soil samples collected from three nearby rice fields following the W-pattern sampling method. Finally, ~1 Kg soil/site was collected following the subsampling method. Surface soil was removed before the collection of rhizospheric soil from a depth of ~10 cm (Meena et al., 2015). Samples were kept in sterile plastic bags, properly labelled, and brought back to the soil laboratory of the Indian Statistical Institute, Giridih within the same day, and stored at 4 °C before processing. Samples of processed within seven days of collection.

Along with soil collection, waste mica tailings were also collected during sampling. These mica tailings were dumped near mines during the processing of mica. From each mine, ~1 kg mica tailings were collected, labelled properly, and stored in a plastic jar for further experiment. The pure form of both muscovite and biotite was obtained from the Geological Research Unit, Indian Statistical Institute, Kolkata, India. These forms were used for the potassium solubilization capacity test of the selected bacterial strain.

5.2.2. Potassium (K) content in waste mica

Both muscovite and biotite were grounded using a mixture grinder and passed through a 2mm sieve for further experimental use. Waste powder mica was immersed in sterile distilled water for 48 hours to eliminate water-soluble potassium. Total potassium content in waste mica was determined by following the hydrogen fluoride digestion method and measured by using a flame photometer (Systronics-130) (Jackson, 1958). The initial concentration of potassium in muscovite and biotite were found to be 26.98 mg/L and 27.98 mg/L respectively.

5.2.3. Isolation of potassium solubilizing bacteria (KSB)

Commercial Aleksandrow broth medium (CAB- HiMedia Laboratories, M-1997) was used for screening of KSB. The enrichment technique followed by a serial dilution technique (in 0.87 % normal sterile saline solution) was used for the isolation. In the enrichment process, 5 g of soil was inoculated into sterile CAB broth media and incubated at 28 ± 2 °C under shaking conditions for seven days. After incubation, serially diluted (10^{-6}) enriched KSB isolates were plated on CAB media with agar-agar (3%) followed by incubation at 28 ± 2 °C for seven days (Hu et al., 2006; Meena et al., 2015). The pure culture of the colonies forming a clear halo zone was further established in Aleksandrow broth media (Sugumaran & Janarthanam, 2007). Screened KSB isolates were stained (gram-stain) for approximate detection of purity of each of KSB isolates, and KSB pure colonies ($n = 30$) were transferred to sterile slants on nutrient agar medium (HiMedia) and sterile glycerol stock medium for long term preservation at -20 ° C (Meena et al., 2015).

5.2.4. Potassium solubilizing ability of screened KSB isolates (first layer screening)

The potassium solubilization capacity of KSB isolates were measured in both qualitative and quantitative approaches. In the case of the qualitative approach, pure KSB isolates were plated in modified CAB agar media as previously described and the zone of solubilization was measured using a digital calliper (Mitutoyo) after 7 days of incubation. Measurements were taken as described by Meena et al. (2015). For morphological characterization of pure KSB isolates, a standard phenotypic technique was followed as described by Holt et al. (1994).

For quantitative assessment, 30 pure KSB isolates were selected which showed prominent halo zone formation during the qualitative assay. Those isolates were raised to a population of 10^{-8} CFU/ML/ml under overnight shaking (150 rpm, 28 ± 2 °C, OD_{600} 0.5) condition in 30 ml of commercial Aleksandrow broth (CAB) media. Uninoculated CAB served the purpose of control. These isolates (replications = 3) were incubated in a shaker incubator (150 rpm, 28 ± 2 °C) for 7, 14, and 21 days as described by Meena et al. (2015). Finally, temporal changes of available potassium content were measured using the flame-photometric method (Systronics-130). This experiment was repeated thrice for statistical analysis.

5.2.5. Quantification of soluble potassium and pH in modified Aleksandrow broth (second layer screening)

In the second layer of screening, 10 pure KSB isolates were selected based on their performance in the first layer of screening. These isolates were raised in manually prepared modified Aleksandrow broth media (MAB - per litter, 5.0 g glucose, 0.005 g $MgSO_4.7H_2O$, 0.1 g $FeCl_3$, 2.0 g $CaCO_3$, and 3 g of either powdered muscovite or biotite as a source of potassium) (Saha et al., 2016). Incubation conditions and period of incubation were similar as described in the previous section (section- *2.4*). For quantification of soluble potassium, KSB inoculated broth (MAB) solutions (30 ml) were vortexed for 10 minutes followed by centrifugation at 5000 rpm for 30 minutes to separate the supernatant from grown bacterial cells and waste mica (both muscovite and biotite). Potassium concentration and pH of supernatant were measured by flame-photometry and pH meter respectively. Finally, a comparison was made to uninoculated control. To prepare a standard curve, we followed the protocol as described by Meena et al. (2015). This experiment was repeated three times with three replications and the result was presented as mean ± SD with pooled data.

5.2.6. Physiological characterization of KSB isolates

For physiological characterization, the survival ability of KSB isolates was tested in a wide range of temperature, pH and saline conditions Atlas et al. (1991). For temperature tolerance, selected four KSB isolates (100 µl of 10^{-8} CFU/ML/ml) were grown in sterile 30 ml commercial Aleksandrow broth medium (CAB) and incubated at varying temperature ranges (20, 25, 30, 35, and 45 $^{\circ}$C) for 14 days with three replications. At 14 days after incubation (DAI), the turbidity of broth media was measured by optical density (OD) at 530 nm using a UV spectrophotometer. Relative growth of bacterial culture at varying temperature ranges was determined by using the OD data (Saha et al., 2016). Similarly, pH tolerance was measured by adjusting the pH level (4.5, 5.5, 6.5, 7.5, 8.5, and 9.5) of CAB using 0.1 N HCl and NaOH and incubated for 14 days at 30 ± 2 $^{\circ}$C. To assess the salt tolerance, KSB isolates were grown in CAB, supplemented with different concentrations of NaCl (1, 2, 4, 6, 8, and 10%), and incubated as described in the case of pH tolerance.

5.2.7. Plant growth-promoting attributes of KSB isolates

5.2.7.1. Indole acetic acid (IAA) production

KSB isolates (10^{-8} CFU/ml) were grown in CAB supplemented with L-Tryptophan (100 mg/L) in triplicates and incubated in a shaker incubator (150 rpm, 28 ± 2 °C) for 7 days. After 7 DAI, cultures were centrifuged at 5000 rpm for 30 minutes to remove bacterial cells and excess insoluble elements of media. IAA production was measured by a spectrophotometric method using Salkowski reagent (50 ml, 35% Perchloric acid, and 1 ml 0.5 M $FeCl_3$ solution) (Restu et al., 2019). IAA production was measured as described by Saha et al. (2016).

5.2.7.2. Gibberellic acid (GA) production

Gibberellic acid (GA) production was estimated by the 2, 4- Dinitrophenyl hydrazine (DNPH) method (Graham and Thomas, 1961). Briefly, KSB isolates (10^{-8} CFU/ml) were grown in CAB (10 ml) for 10 days followed by centrifugation as described earlier (see section *2.7.1*). An equal volume of Cell-free supernatant and ethyl acetate was mixed by vigorous vortex (10 minutes) and left for separation of ethyl acetate layer (repeated thrice). After complete evaporation of the ethyl acetate layer, the left-over supernatant was dissolved in an equal volume of absolute alcohol (Zeigler et al., 1980). 1 ml of DNPH was added to this mixture (2ml) followed by subsequent hot (100 °C for 5 min in the hot water bath) and cold incubation at room temperature. 5 ml of 10% KOH was added to this suspension and left of red wine colour development. The concentration of GA was measured using a spectrophotometer at 430 nm against the standard GA (HiMedia) as described by (Banerjee et al., 2019).

5.2.7.3. Hydrogen cyanide (HCN), Ammonia, and Siderophore production

To identify whether selected KSB isolates can produce HCN, they were streaked on a Kings's B agar media supplemented with 0.4% (w/v) glycine. A Whatman (No.-1) filter paper soaked with alkaline picric acid (2% Na_2CO_3 in 0.5% picric acid) was placed on the lid of the Petri plate and incubated (28 ± 2 °C) for 7 days. The Colour change of filter paper from red-brown to yellow indicated HCN production (Miller and Higgins, 1970).

Ammonia production ability was confirmed by a growing single colony of KSB isolates in 10 ml peptone broth (Peptone - 10 g/L; NaCl - 5 g/L) and incubated (28 ± 2 °C) for 3 days. At 3DAI, Nessler's reagent was added to the broth, and the change of colour from brown to yellow indicated ammonia production (Cappuccino and Sherman, 1992, 2011).

Siderophore production; was determined by the CAS (Chrome Azurol S) assay (Schwyn and Neilands, 1987; Alexander and Zuberer, 1991). The pure bacterial colonies were stricken on CAS plates and incubated at 28 ± 2 °C for 4 days. The orange-yellow halo around the growing colonies indicated siderophore production.

5.2.7.4. Phosphate solubilizing ability of KSB isolates

The phosphate solubilization ability was confirmed by raising (28 ± 2 °C for 3 days) selected KSB isolates in Pikovskaya's agar media-producing halo zone around a grown-up colony (Pikovskaya, 1948). The diameter of the halo zone provided a qualitative assessment. For, quantitative assessment, KSB isolates (10^{-8} CFU/ml) were grown in 30 ml of Pikovskaya's broth in a shaker incubator (28 ± 2

°C for 7 days, 150 rpm). Uninoculated Pikovskaya's broth acted as the control. Soluble phosphorus of cell-free supernatant (centrifuge at 5000 rpm for 30 minutes) was measured by a modified Olsen method (Olsen, 1954).

5.2.7.5. Nitrogen-fixing ability of KSB isolates

Nitrogen-free Jensen agar media was used to grow (at 30 °C for 3 days) the KSB isolates and colony growth was measured for qualitative assessment of nitrogen-fixing ability. For quantitative assessment, bacterial isolates were grown in Jensen broth media as previously discussed. The concentration of fixed nitrogen was measured by digestion and subsequent estimation by the modified Kjeldahl method (Kizilkaya, 2009).

5.2.8. Soil incubation study

Soil incubation study was conducted at Agricultural experimental farm of ISI-Giridih, Jharkhand. 5 kg of sub-surface soil was autoclaved thrice for complete sterilization. Grounded waste micas (muscovite or biotite) were mixed thoroughly with 300 g sterilized soil at different concentrations (see Supplementary Table 1 for treatments detail). This mixture was kept in UV sterilized plastic container and brought to 60% water holding capacity, incubated with KSB (10^{-8} CFU/ml) isolates for 4, 7, 14, 21 days at 28 ± 2 °C. Water-soluble and exchangeable potassium was measured at different DAI was measured following a standard protocol (Grewal and Kanwar, 1966).

5.2.9. Molecular characterization of KSB isolates

For confirmatory molecular identification, genomic DNA was isolated from a single colony of KSB isolates using a DNA isolation kit (NucleoSpin Microbial DNA, MACHEREY- NAGEL). Genomic DNA was stored at -20 °C for further use. Amplification of 16S ribosomal DNA was carried out using bacterial universal primers sets, forward- 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse-1492r (5′-TACGGTTAC CTTGTTACGACTT-3′). For PCR amplification, 25 µl reaction mix contained 45 ng of genomic DNA, 5U/µl of Taq polymerase (Takara Bio Inc.), 2.5 µl 10 × buffer, 2.5 mM dNTP Mixture, 1.5 mM $MgCl_2$, and 10 pmol/µl of each primer. PCR reaction was performed using the following conditions: initial denaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing temperature at 54 °C for 1min, extension at 72 °C for 1min, and a final extension at 72 °C for 7 min. To confirm amplification, PCR amplified product (5 µl) was visualized using 1% agarose gel electrophoresis and visualized in a gel documentation system (Bio-Rad). A 100 bp plus ladder (GeNetBio Corpo, Korea) was used to determine the product size. Once confirmed, amplified PCR products were gel purified using a PCR purification kit following the manufacturer's protocol (Macherey-Nagel) and kept at -20 °C for further analysis (Meena et al., 2015).

Gel purified samples were sent to AgriGenome Labs Pvt. (Hyderabad, India) for sequencing of 16S ribosomal DNA. The obtained sequence was analyzed using BioEdit (version-7.2.5) and then the BLAST tool was used for similarity search in NCBI (www.ncbi.nlm.nih.gov). The sequences were submitted to NCBI GenBank and finally, a phylogenetic tree was constructed using the Maximum-Likelihood method with 1000 bootstrap in Molecular Evolutionary Genetics Analysis Software (MEGA version-X).

5.2.10. Statistical analysis

All data obtained in the laboratory experiments were subjected to statistical analysis as ANOVA (analysis of variance), assessed by Least significant difference (LSD) with a probability $p < 0.05$, by using R statistical software (version- 4.0.2). The regression equation for the quadratic response for physiological characterization of KSB isolates was also conducted using the same software. For the soil incubation study, we have performed three-way ANOVA to identify the significance of potassium solubilization across doses, bacterial strains and time points.

5.3. Result and discussions

5.3.1. Isolation and characterization of KSB isolates

During the initial isolation of KSB, a total of 95 bacterial colonies were found to grow in CAB media. Out of these, 30 bacterial isolates were found to produce a clear halo zone indicating the capacity to solubilize potassium (Altomare et al., 1999). The diameter of the zone of solubilization ranged between 0.23 – 1.12 cm, varied significantly across KSB isolates (LSD = 0.16, $p < 0.05$). Out of 30 isolates, nine showed high (0.63 – 1.12 cm), nine moderate (0.47 – 0.61 cm) and rest 12 as low (0.18 – 0.44 cm) solubilization zone. Bacterial isolates K5B and K6 showed the highest diameter of potassium solubilization (Fig. 5.1).

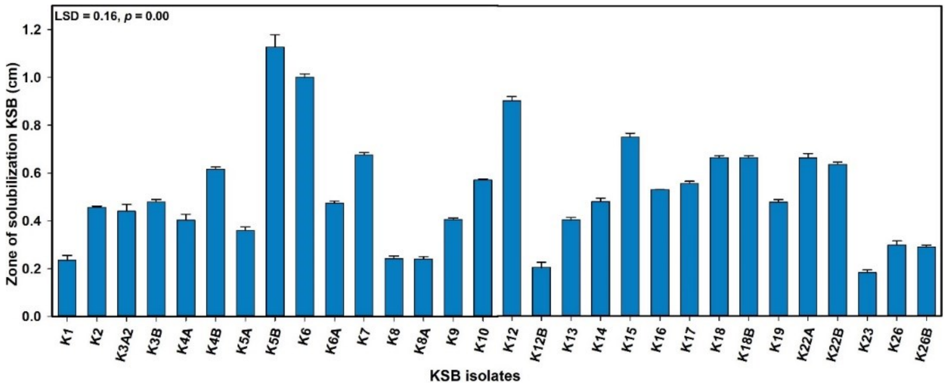
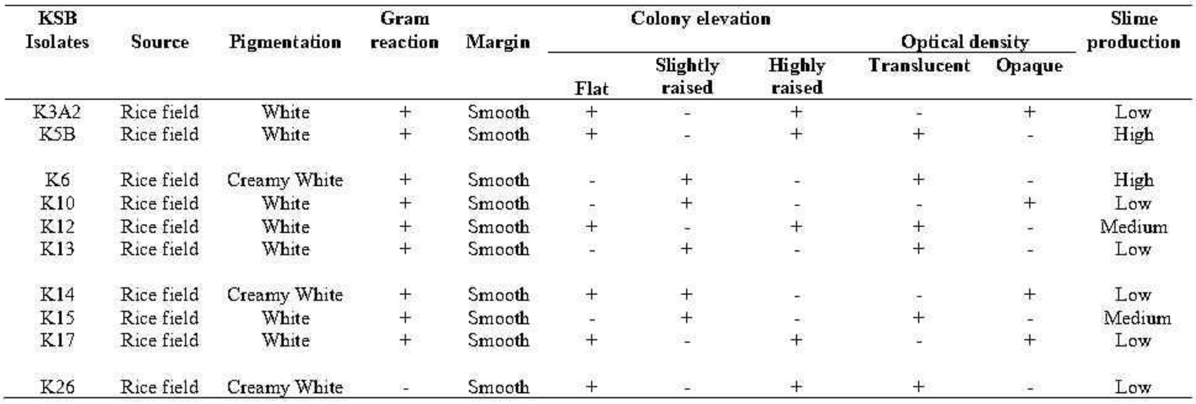


Fig. 5.1. Potassium solubilization of thirty K-solubilizing bacteria grown in commercial Alexandrow agar plate based on zone of solubilization (qualitative assay). Each bar represent mean ± SD from three replications used in this experiment for each KSB isolate. LSD (least significant difference) signifies the statistical differences in colony diameter across KSB isolates.

After the second layer of screening, 10 isolates were selected which showed the highest potassium solubilizing capacity. All the KSB isolates were found to produce slime with varying magnitude, while the K5B and K6 isolates showed the highest slime production (Table 1).

Table 1

Colony characteristic of K-solubilizing bacteria isolated from mica enriched rhizospheric soils of rice fields. Observations of colony morphology was taken at seven days of incubation grown in CAB agar plate.



KSB Isolates	Source	Pigmentation	Gram reaction	Margin	Colony elevation			Optical density		Slime production
					Flat	Slightly raised	Highly raised	Translucent	Opaque	
K3A2	Rice field	White	+	Smooth	+	-	+	-	+	Low
K5B	Rice field	White	+	Smooth	+	-	+	+	-	High
K6	Rice field	Creamy White	+	Smooth	-	+	-	+	-	High
K10	Rice field	White	+	Smooth	-	+	-	-	+	Low
K12	Rice field	White	+	Smooth	+	-	+	+	-	Medium
K13	Rice field	White	+	Smooth	-	+	-	+	-	Low
K14	Rice field	Creamy White	+	Smooth	+	+	-	-	+	Low
K15	Rice field	White	+	Smooth	-	+	-	+	-	Medium
K17	Rice field	White	+	Smooth	+	-	+	-	+	Low
K26	Rice field	Creamy White	-	Smooth	+	-	+	+	-	Low

Isolates named K12 and K15 showed moderate slime production ability, while the rest six KSB isolates produces less slime (Meena et al., 2015). In the case of colony morphology, seven isolates were found to produce white pigmentation, while the rest three produces creamy whitish pigmentation. All isolates showed smooth circular to round colony margin. In terms of colony elevation, K3A2, K5B, K12, K17, and K26 produced highly raised colonies, while the rest five KSB isolates slightly elevated colonies. Similar colony elevation morphology was observed by several authors (O. P. Meena et al., 2013; Prajapati & Modi, 2012). Six KSB isolates (K5B, K6, K12, K13, K15, and K26) appeared to be translucent, while the rest four were found to be opaque. All KSB isolates except K26 were found to be gram +ve, rods as described in previous studies on KSB (Archana et al., 2012). The K26 isolate belonged to the genus *Paraburkholderia*, which showed gram -ve reaction is also capable of potassium solubilization (Mahmud *et al.*, 2021a). Several studies reported the occurrences of different KSB isolates in a wide range of crops belonging to cereals, oilseeds, fruits, and ornamental crops as observed in our study (Friedrich et al., 1991). However, a study of KSB by Meena et al. (2015) observed that bacterial strains isolated from cereals like maize showed a greater zone of solubilization than isolates from oilseed crops. As all the KSB isolates in this study have been isolated from rice grown in a mica enriched zone might show promising responses as potassium solubilizers.

5.3.2. Screening of KSB isolates

During first layer screening of quantitative assessment in CAB, potassium solubilization of KSB isolates (n=30) varied between 0.49 – 7.03 µg/ml; 1.07 to 9.66 µg/ml and 1.70 to 12.02 µg/ml at 7, 14 and 21 DAI respectively (Fig. 2). KSB isolate K5B showed the highest K-solubilization potential across all three time periods, while K6 showed the second-highest K-solubilization potential at 7 DAI

(5.5 ± 0.45 µg/ml) and 14 DAI (7.28 ± 0.12 µg/ml). At 21 DAI, both K6 (10.76 ± 0.31 µg/ml) and K12 (10.74 ± 0.06 µg/ml) isolate showed higher potassium solubilization after K5B (Supplementary Figure 5.2). Finally,10 isolates were selected (K3A2, K5B, K6, K10, K12, K13, K14, K15, K17 and K26) based on cumulative solubilization potential after three observations for further assessment with both muscovite and biotite (MAB).

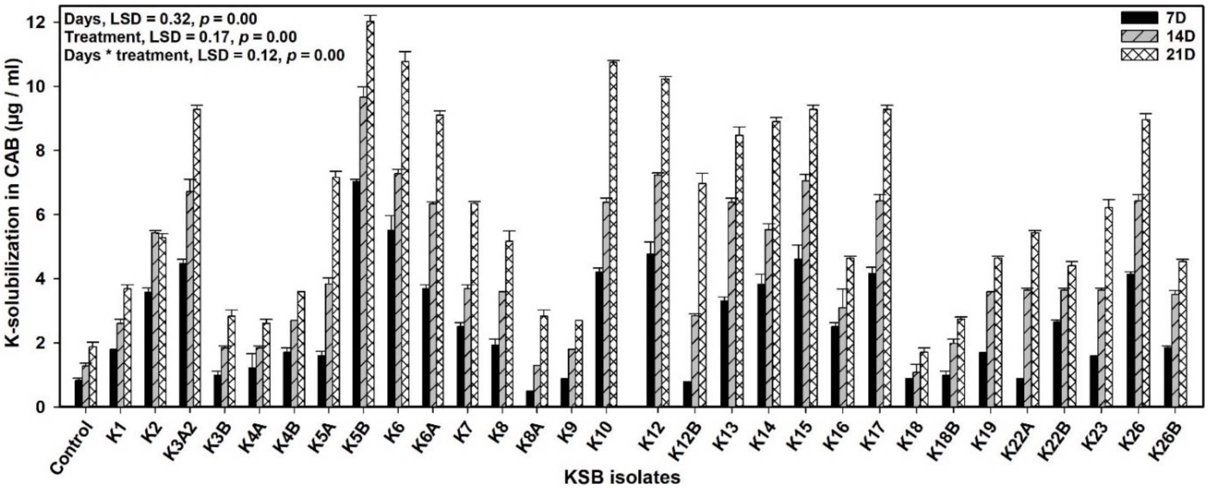


Fig. 5.2. Quantitative assessment of potassium solubilization (µg/ml) potential of thirty KSB isolates grown in CAB broth in three (7, 14 and 21 days) different time points. Each bar represent mean ± SD values of K-solubilization from three technical replicates. Pattern inside bar represent the days of observation. LSD (least significant difference) values signifies the statistical differences in potassium solubilizing ability both across KSB isolates (treatments) and time points (days) using Post Hoc multiple comparison of observed means.

During the second layer of screening, a very minute amount of potassium content was observed in uninoculated control broth could be attributed to the structural disturbance in waste mica during shaking condition of incubation resulted in the release of potassium by hydrolysis (Liu et al., 2012; Meena et al., 2015). However, inoculation of KSB in MAB resulted in a significant release of potassium than control. The amount of potassium solubilization showed an increasing trend with the incubation period. All 10 KSB isolates were found to solubilize potassium from both muscovite (Mus) and biotite (Bio), but their ability of solubilization varied significantly both across time period (LSD_{Mus} = 0.38, LSD_{Bio} = 0.34) and isolates (LSD_{Mus} = 1.28, LSD_{Bio} = 1.08) (Fig. 5.3A & 5.3B).

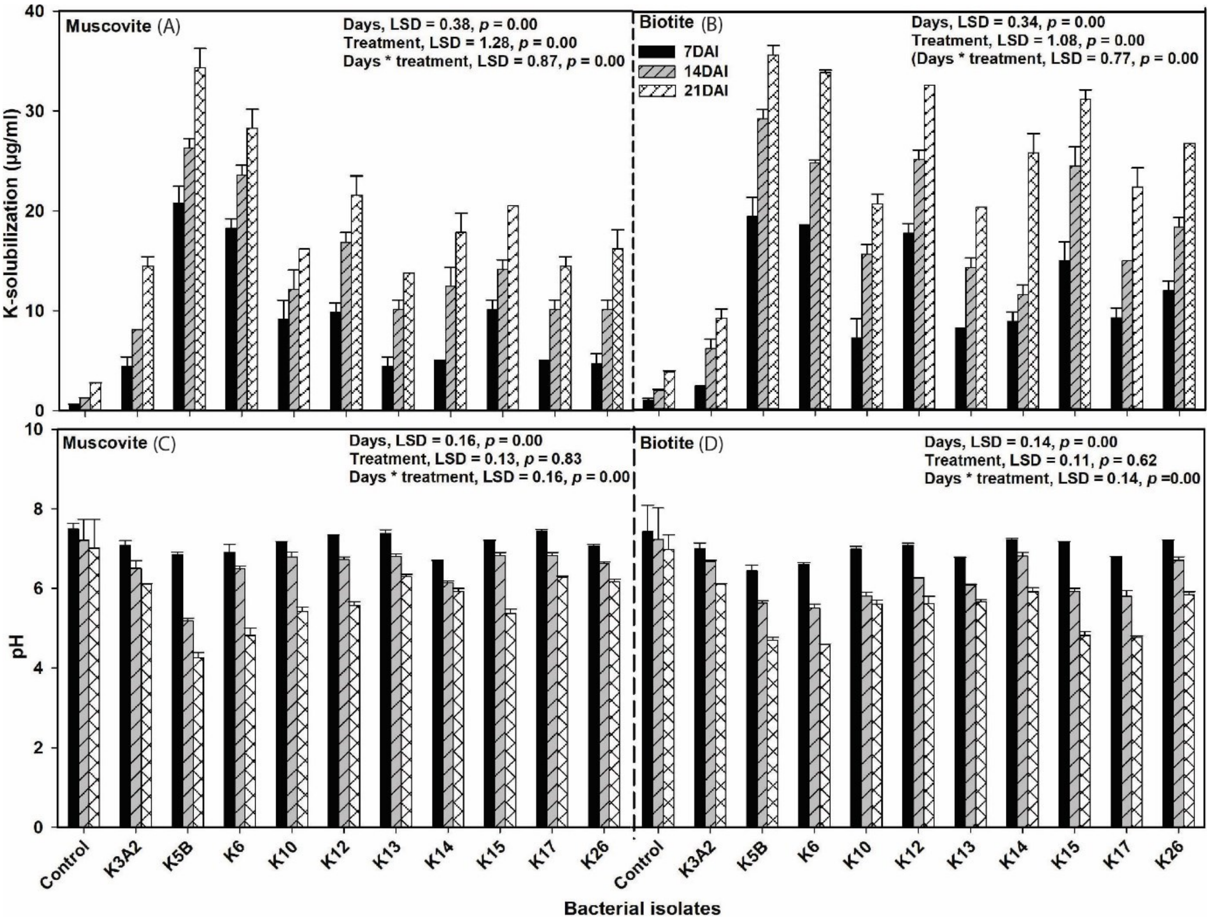


Fig. 5.3. Quantitative second layer screening (A & B) of selected KSB isolates in modified Aleksandrow broth media using waste mica (both muscovite and biotite) as source of potassium incubated for three different time periods (7, 14 and 21 DAI). Dynamics of pH changes with time period was measured across bacterial isolates (C & D). Each bar represent mean ± SD values of observed variables from three technical replicates. Patterns inside bar represent different days of incubation.

In the case of waste muscovite, potassium solubilization ability varied from 4.41 to 20.80, 10.11 to 26.30 and 13.79 to 34.32 µg/ml at 7, 14 and 21 DAI (Fig. 3A). In waste biotite, potassium solubilization ranged between 7.14 to 19.28, 11.46 to 29.0 and 20.18 - 35.35 µg/ml at three different time periods respectively (Fig. 3B). Overall, potassium solubilization of KSB isolates from waste biotite was found to be higher than waste muscovite, a similar trend was observed by (Meena et al., 2015). Across all KSB isolates, K5B showed the highest potassium solubilization potential across time periods and the type of waste mica used as a potassium source. Apart from K5B; K6, K12 and K15 showed a considerable amount of potassium solubilization in both mica at 21 DAI (Fig. 3A and 3B). Therefore, an increase in potassium content in MAB than uninoculated control could be due to the production of organic acids by KSB isolates (Saha et al., 2016). Organic acids produced by KSB isolates might have played a significant role in destabilizing the crystal surface complex structure of waste mica or by complexion metals in solution (Stillings et al., 1996). After the second layer of screening, four KSB isolates (K5B, K6, K12 and K15) were selected for further biochemical, physiological characterization and soil incubation studies based on cumulative potassium solubilization potential.

5.3.3. Impact of KSB isolates on pH dynamics of MAB

The initial pH of MAB was 7.6; which didn't change significantly with the incubation period. A slight decrease in pH of uninoculated MAB could be due to the production of H^+ ion during mechanical disturbance of waste mica. A similar observation was also reported by (Binbin and Bin, 2011). The pH of KSB inoculated MAB decreased significantly over uninoculated control across all isolates with increase in incubation period ($LSD_{Mus} = 0.16$, $LSD_{Bio} = 0.14$) (Fig. 3C & 3D). Our findings corroborate with previous studies by Meena et al. (2015) and Saha et al. (2016), who also found a similar trend. In the case of muscovite and biotite, K5B showed a significant decrease in pH at 7, 14 and 21 DAI compared to other KSB isolates (Fig. 3C and 3D). At 21 DAI, the pH of MAB inoculated with K5B decreased to 4.26 in waste muscovite and 4.69 in waste biotite; while in the case of K6, it decreased to 4.82 and 4.59 in muscovite and biotite respectively (Fig. 3C). As described in previous findings, KSB isolates produce mono-, di- and tri- organic acids like oxalic, citric, gluconic, fumaric, acetic, citric, and tartaric acids during incubation with waste might result in a decrease in pH of the media (Han & Lee, 2006; Meena et al., 2013; Mo & Lian, 2011; Stiillings et al., 1996). Production of these organic acids leads to solubilization of crystal waste mica, which increases the abundances of Si^{4+} and K^+ ions resulting in lowering the pH of inoculated MAB (Maurya et al., 2014).

5.3.4. Physiological characterization

The highest bacterial growth of the isolate K6 was observed at pH 7.5, followed by K15, K12 and K5B. Bacterial cell growth showed an increasing trend with an increase in pH level up to 7.5, afterwards, a decreasing growth pattern was observed. Significantly lowest bacterial growth was observed at pH 4.5 (Fig. 5.4A).

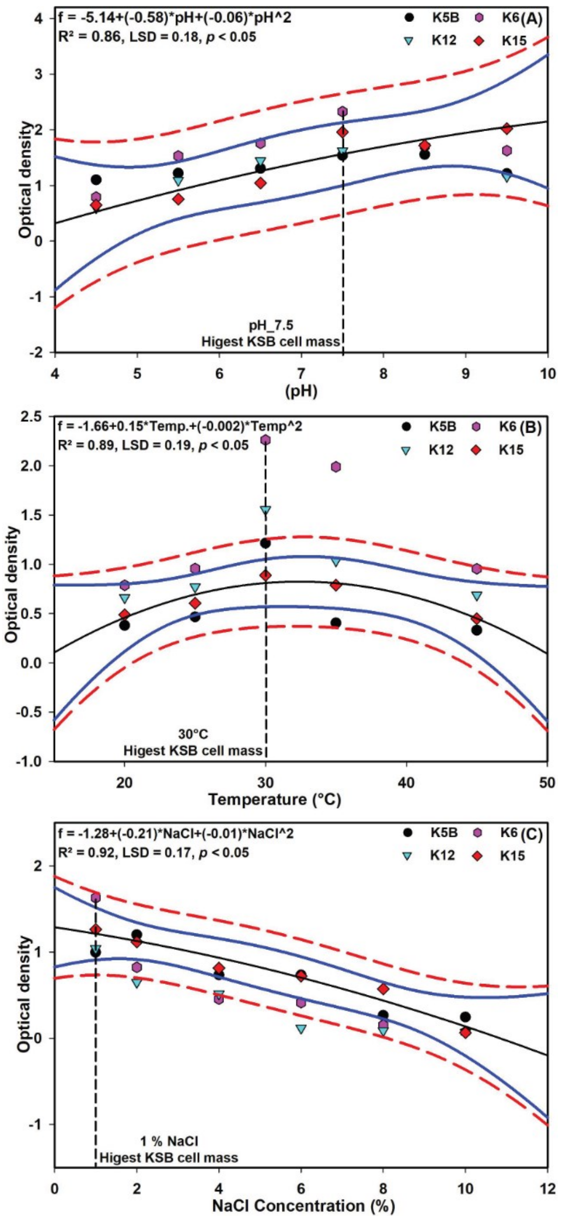


Fig. 5.4. Response of bacterial cell mass was observed under varying pH (A), temperature (B) and salt concentrations (C) range. Different colour and shaped points in plot represent four different KSB isolates, while black vertical dotted line signifies the optimum pH, temperature and salt concentrations for growth of KSB. Blue and red line represent 95% confidence and prediction band respectively.

This result showed at all KSB isolates performed best in the neutral pH range, but can withstand both acidic and alkaline conditions up to a certain extent and can be applied to both soil conditions (Saha et al., 2016; Son et al., 2006). Relationship between pH range and bacterial cell growth showed a quadratic response that fits with following equation: $f = -5.1405 + (-0.5894) \times pH + (-0.0609) \times pH^2$, $R^2 = 0.8655$.

In the case of temperature tolerance, the highest bacterial cell growth was observed at 30 °C in the case of all KSB isolates. Bacterial isolate K6 showed significantly higher cell growth than other isolates. In most of the cases, optimum bacterial growth was observed in the temperature range between 30 – 35 °C. The lowest bacterial growth was observed at 20 °C while decreasing growth pattern was observed at 40 °C (Saha et al., 2016). The regression equation $f = -1.6699 + 0.1539 \times \text{Temp.} + (-0.0024) \times \text{Temp.}^2$, $R^2 = 0.8951$ showed the relationship between bacterial cell growth and temperature (Fig. 5.4B). Cell growth of KSB isolates was also altered by the salt concentration of the media. The highest growth was observed at the lowest salt concentration of 1%, furthermore, a decreasing pattern of cell growth was observed with increasing concentrations of NaCl (Fig. 5.4C). The isolate K6 showed the highest cell growth at 1% NaCl concentration, followed by K15, K12 and K5B. A quadratic response was found between salt concentrations and bacterial cell growth, can be inferred through following equation: $f = -1.2885 + (-0.2123) \times \text{NaCl} + (-0.0133) \times \text{NaCl}^2$, $R^2 = 0.9259$. However, these results showed that all four KSB isolates can survive in extreme pH, temperature and salinity range and can be employed in diverse environmental situations (Song and Huang, 1988; Kulkarni and Nautiyal, 1999).

5.3.5. Plant growth promotion attributes of KSB isolates

All four KSB isolates showed their ability to produce different plant growth promotion substances, like indole-3-acetic acid (IAA), gibberellic acid (GA), hydrogen cyanide (HCN), ammonia and siderophores (Fischer et al., 2007). The Highest IAA production (10.82 ± 0.6 µg/ml) was observed by KSB isolate K5B (Fig. 5.5A). No significant variation was observed in IAA production ability across KSB isolates (LSD = 0.36, *p* = NS). According to (Saha et al., 2016), *Bacillus spp.* showed the higher potential of IAA production, which corroborate with our findings, as all four isolates in our study also belong to the genus *Bacillus spp.* Significant variation in GA production was observed across KSB isolates (LSD= 0.59, $p<0.05$) (Fig. 5B). Highest GA production was observed in K12 (1.4 ± 0.12 µg/ml), followed by K15 (1.2 ± 0.11 µg/ml), K5B (1 ± 0.18 µg/ml) and K6 (0.49 ± 0.06 µg/ml) (Supplementary Figure 3). KSB isolates belonging to genera *Pseudomonas*, *Azotobacter* and *Bacillus* have been reported to produce GA (Desai, 2017; Biswas et al., 2018).

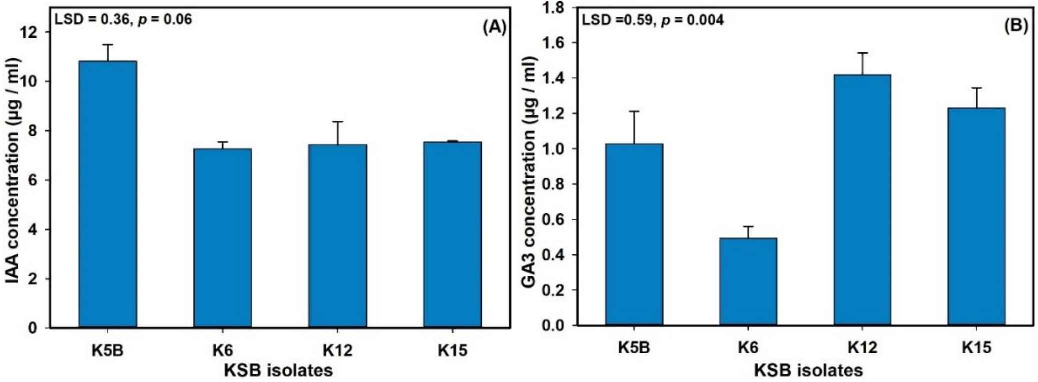


Fig. 5.5. Indole-3-acetic acid (A) and gibberellic (B) acid production ability of fours selected KSB isolates. Each bar represent mean ± SD values of measured parameters from three technical replicates. LSD (least significant difference) values signifies the statistical differences in IAA and GA3 production ability across KSB isolates.

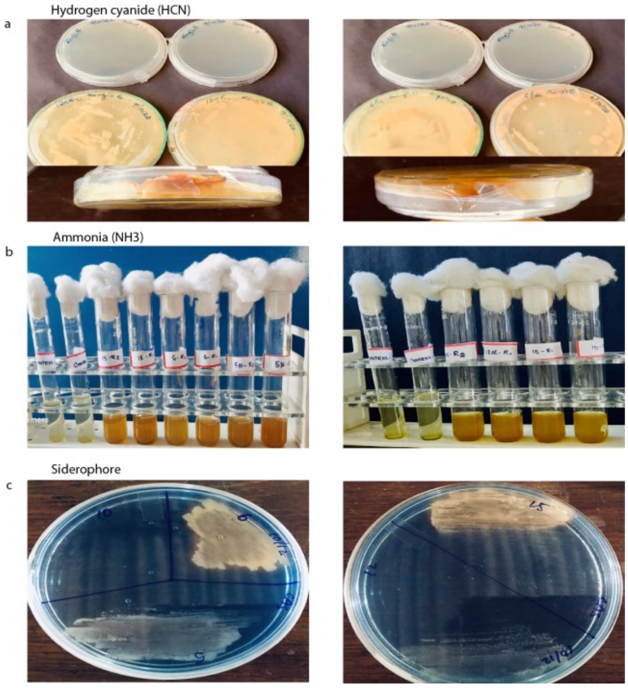


Fig. 5.6. Plant growth promoting attributes of selected KSB isolates. Hydrogen cyanide production was indicated by turning o yellow picrate filter paper into red-brown (a). Change of peptone broth colour from yellow to brown indicated the ammonia production ability (b). Siderophore production was evident from orange yellow halo zone in CAS agar plate (c).

Hydrogen cyanide (HCN) production ability was observed among KSB isolates K5B, K6 and K12 indicated by turning of yellow picrate filter paper into red-brown (Fig. 5.6A). In the case of ammonia production ability, all four isolates showed positive results by changing the yellow colour of peptone broth into brown (Fig. 5.6A). Selected KSB isolates showed siderophore production ability by orange halo zone production around bacterial colonies in blue CAS agar plate. KSB isolates have been previously reported to produce all these growth-promoting substances (Kotasthane et al., 2017; Gupta and Pandey, 2019; Verma et al., 2020). Production of HCN, ammonia and siderophores might play important role in the uptake of nutrients like iron (through siderophore production) and suppresses the growth of pathogenic fungi by the accumulation of ammonia (Swamy et al., 2016; Agrawal et al., 2017; Richard et al., 2018). Therefore, in addition to potassium solubilization, all these isolates showed their potential in the production of plant growth promotion substances which might help in better plant growth and vigour.

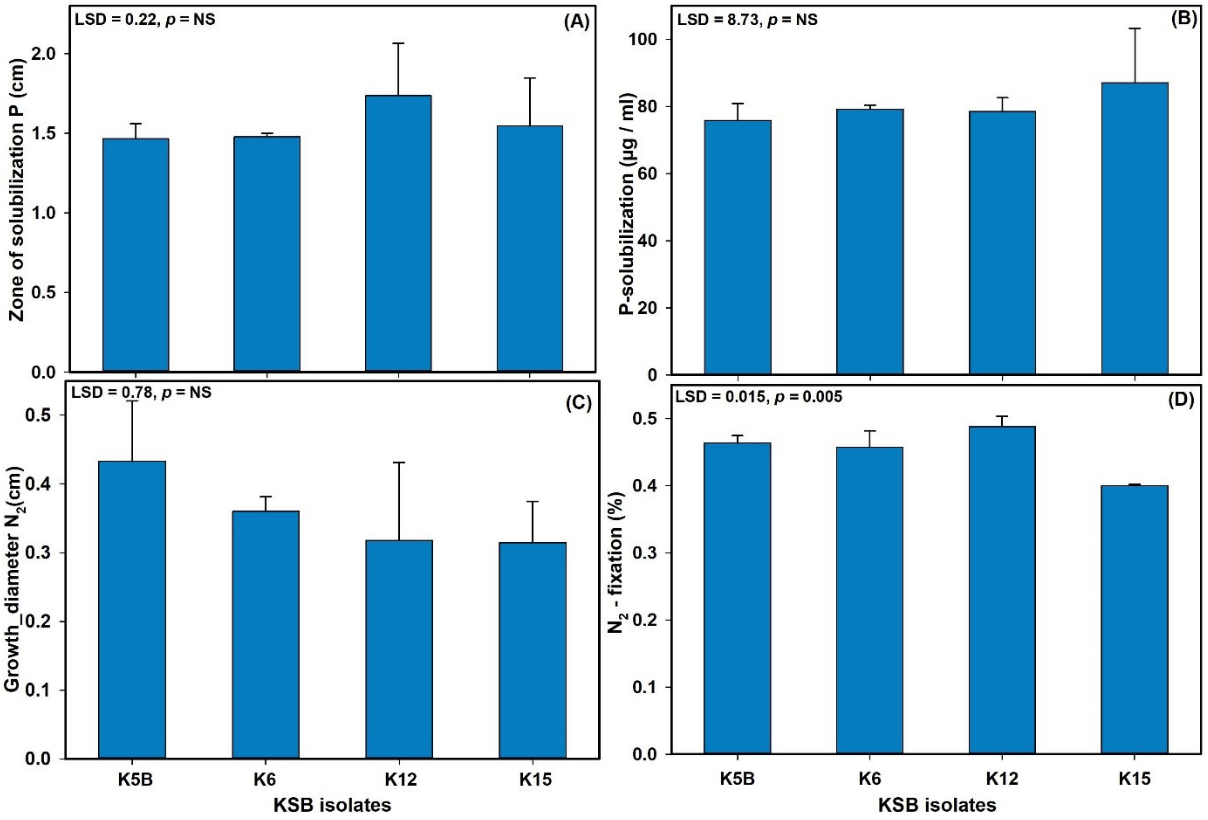


Fig. 5.7. Phosphorus solubilization (A & B) and nitrogen fixation (C & D) ability of four selected KSB isolates using both qualitative and quantitative assays in Pikovskaya and Jenson media respectively. Each bar represent mean ± SD values of measured parameters from three technical replicates. LSD (least significant difference) values signifies the statistical differences in measured parameters across KSB isolates.

Apart from potassium solubilization, all four KSB isolates showed their potential in phosphate solubilization and nitrogen fixation. Previous studies also reported the beneficial effect of KSB on nutrient uptake and plant growth through different mechanisms like nitrogen fixation, the transformation of nutrient elements like phosphorus and iron when applied to seeds incorporated into the soil (Herridge et al., 2008; Sindhu et al., 2010, 2016). In our study, the K12 isolate showed the largest (1.73 cm) clear halo zone in Pikovskaya's agar plate followed by K15, K6 and K5B respectively indicating their potential of phosphate solubilization (Fig. 5.7A). Though K12 performed well during the qualitative assay, K15 showed the highest (87.03 mg/kg) phosphate solubilization potential during the quantitative assay (Fig. 5.7B). No significant variation was observed across KSB isolates in phosphate solubilization during both qualitative (LSD= 0.22, *p* = NS) and quantitative assays (LSD = 8.73, *p* = NS). K5B showed the highest colony diameter in Jansen agar media, indicating nitrogen-fixing potential (Fig. 5.7C). Other isolates also showed their nitrogen-fixing capacity, though no significant variation was observed in terms of colony diameter across KSB isolates. During the quantitative assay, K12 showed the highest nitrogen fixation of 0.4%, followed by K5B, K6 and K15 respectively (Fig. 5.7D). A recent study by (Mali and Attar, 2021) showed both nitrogen fixation and phosphorus solubilization capability of KSB isolate belonging to *Enterobacter hormaechei* corroborate our findings. In our study, all KSB isolated belonged to genus *Bacillus* has been previously reported to show both N-fixation and P-solubilization ability supports our observations (Hino and Wilson, 1958; Vafadar et al., 2014; Cherif-Silini et al., 2016).

5.3.6. Soil incubation study

A soil incubation study was performed to assess the potassium solubilization potential of KSB isolates when incubated in soil. The lowest potassium solubilization was observed in the case of control treatment where no waste mica was added across both water-soluble (WS-K) and exchangeable potassium (Ex-K) and mica types (Fig. 8). The amount of potassium solubilization showed an increasing trend with increasing days and amount of waste mica (both muscovite and biotite) used. KSB isolate K5B showed the highest K-solubilization ability, followed by K6, K12 and K15 in both types of micas and potassium measured (WS-K & Ex-K) (Fig. 4). In case of WS-K, significant variation was observed in case of muscovite across bacterial isolates ($F_{3,192} = 125.8$, $p < 0.05$), time points ($F_{3, 192} = 221.9$, $p < 0.05$), doses ($F_{3, 192} = 377.7$, $p < 0.05$) and also their interaction effect ($F_{27, 192} = 1.85$, $p < 0.05$). Similarly, significant variations of all these factors were observed in the case of biotite, but their interaction showed no significant variation ($F_{27, 192} = 1.1$, p = NS). Exchangeable potassium varied significantly in both mica across KSB isolates, time points and doses. However, their interaction effect showed significant variation in case of muscovite ($F_{27, 192} = 5.5$, $p < 0.05$), but not in biotite ($F_{27, 192} = 1.34$, p = NS). In microcosm study by (Pramanik et al., 2019) showed that inoculation of *Bacillus pseudomycoides*, a KSB strain isolated from tea soil increased the K-availability from 47 ± 7.1 mg/kg to 104.9 ± 7.1 mg/kg after 105 days of incubation. In another study by (Xiao et al., 2017) showed the inoculation of KSB strains belonging to *Mesorhizobium sp., Paenibacillus sp. And Arthrobacter sp.* isolated from rape rhizospheric soil not only increased the available potassium in the soil but also increased growth, vigour and biomass yield of ryegrass in potassium deficient soil. These results showed that our KSB isolates, specifically K5B isolates could potentially be used as biological fertilizer.

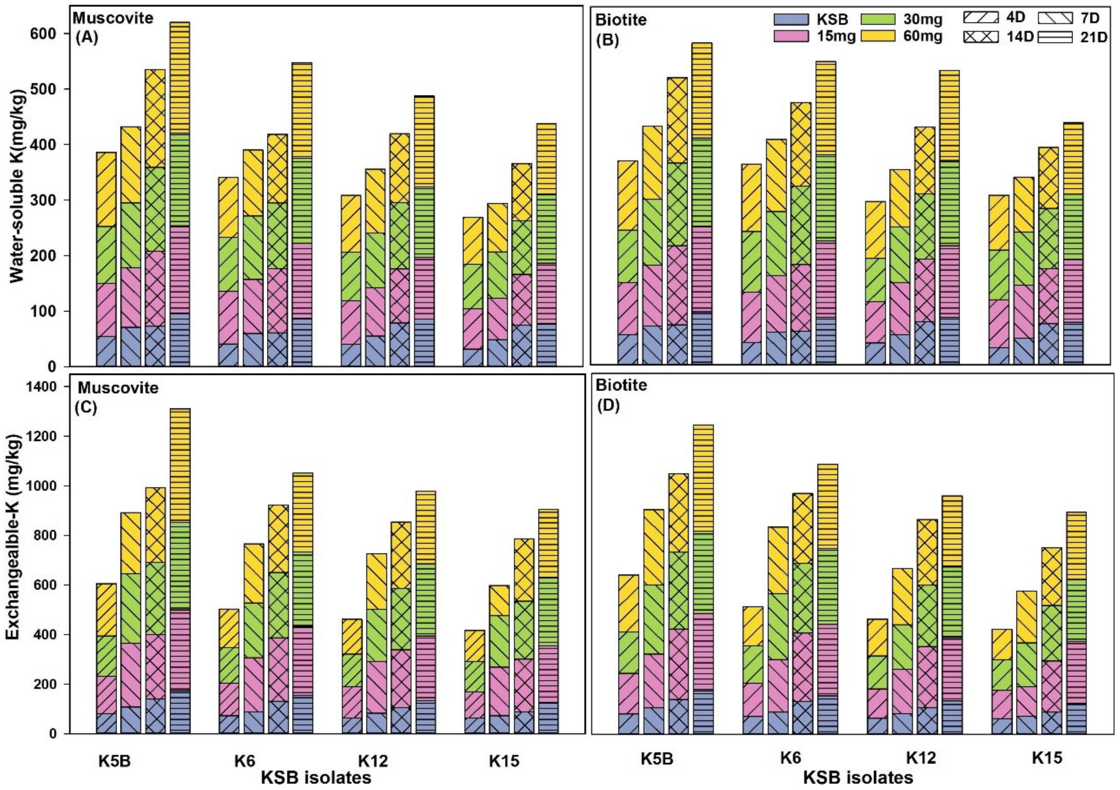


Fig. 5.8. Potassium solubilization (both water soluble and exchangeable) potential of four KSB isolates from waste mica (both muscovite and biotite) inoculated in sterilized soil across treatments (for details, see Supplementary Table 1) and time points (7, 14 and 21 DAI) has been visualized using a stack bar. Each stack in bars represent the amount of solubilized potassium (WS-K or Ex-K) by a KSB isolate at certain dose of waste mica. Patterns in each bar represent days of incubations, while fill colour of each stack in a bar represent doses of waste mica.

3.7. Phylogenetic analysis of KSB isolates

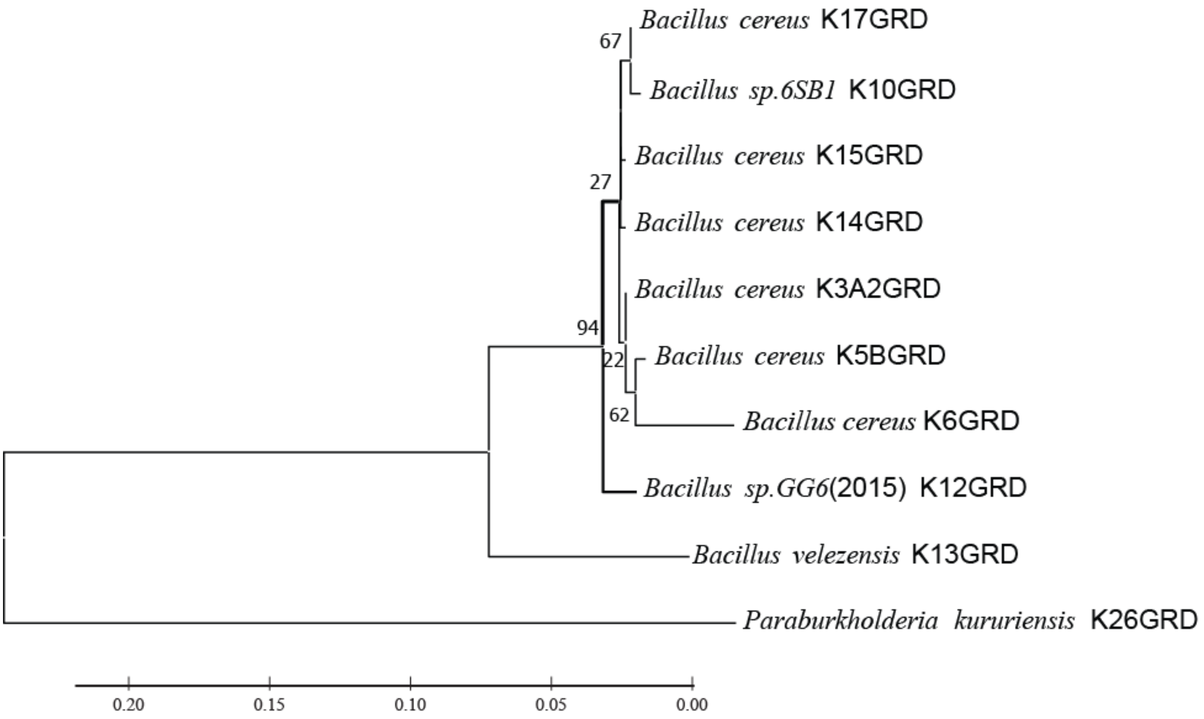


Fig. 5.9. Phylogenetic relationships of ten KSB isolates based on16S rDNA sequences of KSB isolates using maximum-likelihood. Numbers at each branch point represent the bootstrap values. The scale bar denotes 0.05 substitutions per nucleotide position.

The 16S rDNA of 10 KSB isolates when compared to the know 16S bacterial sequence in NCBI formed two major clusters (Fig. 9). In a single cluster, 9 out of 10 isolates belonged to genus *Bacillus*, while in another cluster one single KSB isolate was found belonging to genus *Paraburkholderia*. All KSB isolates showed 97 – 100% similarity with the available sequences of GenBank (Table 2).

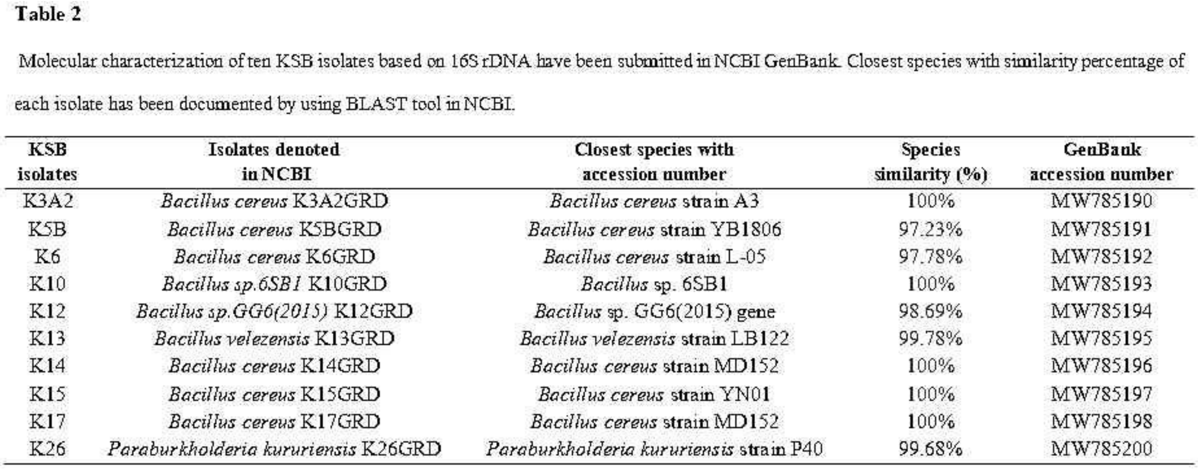


Table 2

Molecular characterization of ten KSB isolates based on 16S rDNA have been submitted in NCBI GenBank. Closest species with similarity percentage of each isolate has been documented by using BLAST tool in NCBI.

KSB isolates	Isolates denoted in NCBI	Closest species with accession number	Species similarity (%)	GenBank accession number
K3A2	*Bacillus cereus* K3A2GRD	*Bacillus cereus* strain A3	100%	MW785190
K5B	*Bacillus cereus* K5BGRD	*Bacillus cereus* strain YB1806	97.23%	MW785191
K6	*Bacillus cereus* K6GRD	*Bacillus cereus* strain L-05	97.78%	MW785192
K10	*Bacillus sp.6SB1* K10GRD	*Bacillus* sp. 6SB1	100%	MW785193
K12	*Bacillus sp.GG6(2015)* K12GRD	*Bacillus* sp. GG6(2015) gene	98.69%	MW785194
K13	*Bacillus velezensis* K13GRD	*Bacillus velezensis* strain LB122	99.78%	MW785195
K14	*Bacillus cereus* K14GRD	*Bacillus cereus* strain MD152	100%	MW785196
K15	*Bacillus cereus* K15GRD	*Bacillus cereus* strain YN01	100%	MW785197
K17	*Bacillus cereus* K17GRD	*Bacillus cereus* strain MD152	100%	MW785198
K26	*Paraburkholderia kururiensis* K26GRD	*Paraburkholderia kururiensis* strain P40	99.68%	MW785200

Among *Bacillus*, six isolates were found to be *B. cereus*, while two were unidentified species and the rest belonged to *B. velezensis*. *Bacillus cereus* is well-known species having K-solubilization ability

(Ali et al., 2021). Both *Bacillus* and *Pseudomonas* are the two most widely studied genera having potassium solubilization property supports our observations (Sugumaran & Janarthanam, 2007; Zhou et al., 2006). Though the genus *Paraburkholderia* is known for phosphate solubilizing ability, it has also been reported to solubilize potassium from soil and promote plant nutrition in potassium deficient soil (Gao et al., 2019; Mahmud et al., 2021).

5.4. Conclusions

The present study demonstrated that the application of KSB strains isolated from rice rhizospheric mica enriched soil can solubilize waste mica bound potassium into water-soluble and exchangeable forms, which are readily available to plants. After preliminary screening, 10 isolates showed potassium solubilizing potential among which four isolates (K5B, K6, K12 and K15) showed the most promising response. Among these four isolates, K5B identified as *Bacillus cereus* was found to show the highest potassium solubilization capacity during both quantitative and soil incubation assays. In all four isolates, the pH of broth was found to decrease with time indicating the production of organic acids (acidolysis) which could be a potential mechanism of solubilization of crystal bound potassium. All these four isolates showed their survival ability in wide temperature, pH and salinity range indicating that these isolates could be used across a wide range of climatic and edaphic situations. Apart from K-solubilization, these isolates showed their potential in the production of growth-promoting substances like IAA, GA, HCN, ammonia and siderophores and thus might play a significant role in plant growth promotion. Moreover, phosphate solubilization and nitrogen-fixing ability of KSB isolates might also increase the availability of nutrients to plants. Therefore, these KSB isolates showed their potential as biofertilizers to reduce the huge requirement of import of potassic fertilizers for ecologically benign and economically sustainable crop production. However, joint academic and industrial collaboration is required to develop large scale production of site-specific, climate-resilient active formulations of potassium solubilizing bacteria.

CHAPTER-6. OBJECTIVE 4

"Influence of potassium solubilizing rhizobacteria strains and two types of waste micas (muscovite and biotite) on potassium uptake dynamics by tomato (*Solanum Lycopersicum L.)* plant *grown under Alfisol, Giridih district, Jharkhand."*

6.1. Introduction

India is an agricultural-dependent country and agriculture is the backbone of the Indian economy. Two-thirds of the total population lives in rural areas, associated with their income and contributing to 19% of GDP (Gross Domestic Product) (Srinivasarao et al., 2011). According to estimation by the NAAS (National Academy of Agricultural Science), India's demand for food grains will reach around 300 million tonnes annually by 2020, with estimates increasing from 218 million tons to 82 million tons produced in 2009-10 (Kinekar, 2011). At present status the dependency on agriculture is to 52.1% from 72.4% in the year 1952. Due to the fast-growing of industrial and other service sectors, the growth of the agricultural sector has increased in recent times. But there is no alternative to agriculture to meet the needs of a growing population (Kinekar, 2011). Soil fertility and its assessment is a field that needs immediate attention as it is now established that the productivity of different crops in an arrest is due to soil depletion on the one hand and unbalanced application of plants and other nutrients.

In nature, potassium (K) is a chemically active metal that is a vital macronutrient for all kinds of plants and animals. It is the 7th most abundant element in the rocky outer part of the Earth (lithosphere), and it is a vital macronutrient for plants and animals. Despite being a chemically active metal, potassium is never found in its pure form in nature (Kinekar, 2011). In terms of essential macronutrients, potassium is the third most important nutrient and one of the key ingredients in balanced fertilizer use after nitrogen (N) and phosphorus (P). It plays a crucial role in the synthesis of proteins, cells, starch, cellulose, and vitamins. As part of plant processes, K is needed to activate at least 60 different types of enzymes that contribute to growth, osmoregulation, cation balance, photosynthesis, maintaining water balance, disease resistance, resistance to abiotic and biotic stresses, and crop yields to improve quality and shelf life (V. S. Meena et al., 2015; Ramamurthy et al., 2017).

Potassium consumption increased by 264 lakh tons in India in the following two years (2019 and 2020), and synthetic and organic potassium fertilizers were imported globally to meet agriculture demand, indicating harmful application of potassium fertilizers (FAI, 2020). It has been reported that 72% of Indian agricultural soil testing results (representing 266 districts) classify the soil K fertility in three levels; low in 21%, medium in 51%, and high in 28%, and farmers still need to adopt a serious attitude towards potassium application (Hasan, 2002). K is present in soil in four forms: water-soluble K (Wa.K), available or exchangeable K (Ex.K), non-exchangeable K (NEK), and mineral K. Of these four types of K, water-soluble and exchangeable K contribute 1-2% to plant uptake, while non-exchangeable K can also contribute 1-10%(Basak & Biswas, 2009; Sparks & Huang, 1985). The release of NEK to EK form occurs when exchangeable and water-soluble K are decreased by crop removal (leaching) and possibly when microbial activity is increased (Basak & Biswas, 2009; Sparks, 1987). Because there is no potassium-bearing mineral reserve in India for the production of commercial potassium fertilizers, most potassium-fertilizers, like muriate of potash and sulphate of potash, are imported (Nishanth & Biswas, 2008). As a result of farmers adding potassium to the soil in the form of potassic fertilizers, India ranks fourth globally in the consumption of potassium fertilizers (FAI 2007). It was necessary to find alternative indigenous sources of potassium fertilizer to meet the needs of and maintain the status of potassium in soils to sustain crop production.

As it turns out, India is fortunate to have the world's highest accumulatin of mica mines located in the Munger district of Bihar and the Koderma and Giridih districts of Jharkhand, covering an area of about 3888 sq. km. The dressing of raw mica after its mining generates large quantities of waste mica (~75% of the total amount of mica mined) that are not used in agriculture as a sole source of potassium though they contain a large amount of potassium (8–12% K_2O) and are dumped near the mica mine sites. As with waste mica forms (muscovite and biotite), these waste materials can also be used as a source of potassium when modified biologically (bacteria and fungi) and chemically (phosphoric acid and sulfuric acid).

Few special groups of microorganisms in rhizospheric soil are capable to solubilize NEK forms of potassium-bearing minerals like micas, illite, orthoclase, and K- feldspars by extracting organic acids like indole acetic acid, gibberellic acid. That directly soluble in rock potassium or chelating silicon ions to bring the potassium into solution (Basak & Biswas, 2009, 2010; Zhang & Kong, 2014). These bacteria are also known as potassium solubilizing rhizosphere bacteria (KSB), silicate dissolving bacteria, and biological potassium biofertilizer (BKF)(Basak & Biswas, 2010; Bennett, 1998). KSB, a bio-trigger that dissolves insoluble potassium in mica into a plant-available pool, can also be used effectively as a source of potassium fertilizer for maintaining and sustaining crop production and soil potassium (Zhang & Kong, 2014). A broad range of KSB like *Bacillus mucilaginosus, B. circulans, B. edaphicus, Bacillus sp.GG6 (2015)*, *Bacillus sp.6SB1, Paraburkholderia kururiensis Acidithiobacillus ferrooxidans, Pseudomonas, Burkholderia,* and *Paenibacillus sp,* have been reported to release K insoluble or available form from K-bearing minerals in soils (A. Kumar & Maiti, 2015) and those are able of decomposing micas (aluminosilicate minerals) and releasing a part of the K contained in that (Basak & Biswas, 2009). Some KSB microorganisms have been reported to that supplementary suitable beneficial on the growth of *Gossypium hirsutum* (cotton) and *Brassica napus* (rape), *Triticum* (wheat), *Sorghum × drummondii* (Sudan grass), and *Cucumis sativus* (cucumber), and *Piper nigrum* (G. Singh et al., 2010). The application of potassium dissolved rhizospheric bacteria is a promising method to increase the availability of K in the soil.

Tomatoes (*Solanum Lycopersicum L.*) are widely regarded as one of the most important and popular horticultural crops in the world and their use as model plants is widespread (Monteiro et al., 2012; Salunkhe et al., 1974). Tomatoes are included in a world-balanced healthy diet, and the increasing population of the world, including tomatoes, requires stable fruit yields (Daoud et al., 2020). Moreover, it is one of the most important vegetables or fruits in India, grown on 1204 thousand hectares, with a productivity of 19, 042 million tons and 21.2 metric tons per hectare (NHB, 2014); In India, the most tomato crops are grown in Bihar, Karnataka, Uttar Pradesh, Orissa, Andhra Pradesh, Maharashtra, Madhya Pradesh and West Bengal (Nagoni et al., 2017). Tomatoes and their by-products contain a significant amount of lycopene and other antioxidant compounds such as phenolics, flavonoids, ascorbic acid, and vitamins C and E. A variety of epidemiological studies have demonstrated that the consumption of tomatoes and tomato-based products can prevent cancers, including prostate cancer, heart disease, and cardiovascular problems, as well as gene function regulation, cell-to-cell communication, cell cycle arrest, apoptosis, quenching of singlet oxygen and reducing free radicals, carcinogen metabolism, and metabolic pathways involving phase I and phase II drug-metabolizing enzymes (Kanr et al., 2008; Toor et al., 2005, 2006). Tomatoes also have β-carotene which is also an antioxidant, is also an important carotenoid in tomato which provides to maintain healthy skin and tissue lining and it also plays a wide role as a provitamin a carotenoid, because of hypovitaminosis (M. Meena et al., 2017).

There are several factors that affect fertilization and nutrient supply on the balanced values and quality of tomatoes, and K and Ca ions are essential nutrients for their growth and quality (Hernández-Pérez et al., 2020; Weinert et al., 2021). There is a positive correlation between K-fertilization and environmental tree tolerance, including drought, salinity, and cold as well as resistance to pests and pathogens (Sonntag et al., 2019; Weinert et al., 2021). Additionally, K helps maintain fruit size, acidity, soluble solids, sugar content, and texture, among others. K_2O is the nutrient most readily absorbed by tomatoes plants, and it aids many physiological processes, such as photosynthesis, enzyme activation, and protein synthesis. (Neto et al., 2016; Sonntag et al., 2019). Therefore, the application of potassium-solubilizing bacteria in the rhizosphere could provide a promising method for increasing the availability of potassium in the soil. However, the objectives of this study were (i) to find out potassium release from waste micas like muscovite and biotite treated soil as governed by potassium solubilizing rhizospheric bacterial biofertilizers (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.GG6* (2015) K12, and *Bacillus cereus* K15) and (ii) to find out its effectiveness as potassium fertilizer using tomato plant (SL-120) as test crop grown under Giridih soil (Alfisol).

6.2. Materials and methods

6.2.1. K-bearing waste micas

We obtained potassium-bearing minerals from the waste mica tailings near mica mines located at Tisri, Gawan, Deori blocks Giridih district of Jharkhand, India. Most of the active mica mines in Giridih are distributed within these three blocks of the district. Most of the mica tailings were found as a form of muscovite. A pure form of biotite was obtained from the Geological Research Unit, Indian Statistical Institute, Kolkata, India. Micas are generally mined as a raw mica block which is cleaned before using as an electrical insulator, navigation compasses, optical fibbers pyrometers, etc. During the dressing of raw micas, about 75% of the waste materials are dumped near the mines area and are not used in agriculture. The white flake-like structures are known as muscovite $(OH)_4K_2(Si_6Al_2)Al_4O_{20}$ and black color micas as biotite $K(Mg, Fe)_3AlSi_3O_{10}(F, OH)_2$. Micas was ground in a mixture grinder and passed through a 2 mm sieve before further use. The initial amount of water-soluble K (30.0 mg kg^{-1} and 33.89 mg kg^{-1}), exchangeable K (157.5 mg kg^{-1} and 167.67 mg kg^{-1}), and non-exchangeable K (260.0 mg kg^{-1} and 278.77 mg kg^{-1}) in muscovite and biotite respectively. These forms of micas were used for the pot experiments as a potassium source for the plant.

6.2.2. Bacterial culture and inoculants preparation

K-solubilizing rhizobacterial inoculants (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.GG6* (2015) K12, and *Bacillus cereus* K15) were obtained from the Agricultural and Ecological Research (AERU) unit, Biological Science Division (BSD), Indian Statistical Institute (ISI), Giridih, Jharkhand, India. These four types of KSB inoculants were characterized at Soil laboratory (AERU), ISI, Giridih, Jharkhand, India, and used as K-solubilizing bio-fertilizers. Selected isolates were multiplied using Aleksandrow broth medium (HiMedia Laboratories Pvt. Ltd.) and subsequently multiplied (1.64×10^8 cfu) for further use. The KSB strains were maintained on nutrient agar slants in a refrigerator at 4°C. For prepared KSB broth inoculant fertilizers, take each loop of bacterial strains was inoculated into sterile (autoclaved at 120°C and 0.1 MPa for 20 min) nutrient broth and incubated at 30°C for 3days, and the culture was multiplied up to (1.45×10^8-1.64×10^8 cfu).

6.2.3. Collection of soil

Rhizospheric soil samples (at 0-10 cm depth), were collected from the agricultural research farm of the AERU unit, Indian Statistical Institute, Giridih, Jharkhand, India. The latitude and longitude are N24°11.701' and E086°18.015'. These soils were selected during the period the variable amounts of different pools of K, especially the potassium available (K-deficiency) as well as their various mineralogical compositions are also low. It belongs to the Alfisol family with illite and montmorillonite as the dominant clay minerals.

6.2.4. Soil analysis

6.2.4. A. Estimation of Physico-chemical properties during pot experiment and benefit ratio

The bulk soil samples were air-dried and passed through a 2 mm and 0.2 mm sieve before Physico-chemical properties were analyzed by the standard method of Page et al., (1982). The particle size was determined by the hydrometer method of Bouyoucos., (1962) after dispersing the soil samples in sodium hexametaphosphate-$(NaPO_3)_6$ solution. The changes in temporal soil reaction pH and electrical conductivity (EC) were estimated in soil: water ratios 1:2.5 and 1:5 respectively (M. L. Jackson, 1973). Total organic carbon (TOC), free mineralizable nitrogen (N) (Av.N), available phosphorus (Av.P), exchangeable potassium (Ex. K), and water-soluble potassium (Ws.K) of sample soils were estimated following the protocol of Page et al., (1982) and non-exchangeable K was determined by the method as described by M. L. Jackson., (1967). Analytical grade (more than 90% purity) was used in all physicochemical analyses exempted according to the general quality control guidelines.

The performance of the four KSB bacterial strains biofertilizer was estimated to evaluate the potential benefits. The favorable effect was reckoned for variables such as pH, EC, easily mineralizable, or Av. N, Av. P, and Av. K. The outcomes for each variable were computed at the start of treatment (T = 0 days) and the end of treatment (T = 90 days) for each experiment by the following equation Hussain (et al., 2016); Sahariah et al., (2015).

Benefit ratio (BR) for N, P, K, pH, and EC

$$= \frac{Average\ concentration\ (90\ d) - Average\ concentration\ (0\ d)}{Average\ concentration\ (0\ d)} \tag{1}$$

Benefit ratio (BR) for TOC

$$= \frac{Average\ concentration\ (0\ d) - Average\ concentration\ (90\ d)}{Average\ concentration\ (90\ d)} \tag{2}$$

Some important physicochemical properties of the initial experimental of the initial experimental soil are of texture - sandy, class – loam, and mechanical properties as sand, slit, and clay percent wise: 65 (%), 13.2 (%), and 18.1 (%) respectively; pH 6.3; EC 0.06; organic C, 0.49 (%); available N, 44.14 (g/kg); available P, 0.25 (mg/kg); available K, 51.71 (mg/kg); water-soluble K, 25.63 (mg/kg); and non-exchangeable K, 481.43 (mg/kg) respectively.

6.2.4. B. Estimation of microbial-enzymatic properties

The temporal changes in soil microbial-enzymatic parameters were estimated using moist soil. Soil microbial biomass carbon (MBC) was estimated by the fumigation extraction method followed by the determination of KCl extractable C and calculated by multiplying constant values of Ec with 2.64, where Ec is the difference between KCl extractable C of the fumigated and unfumigated sample soils (Joergensen et al., 1995). The basal soil respiration (BSR) and substrate-induced respiration (SIR) were measured by the estimation of CO_2 evolved during the incubation of soil with glucose in a closed system, and trapping of CO_2 in NaOH solution, which was then titrated with HCl (Alef & Nannipieri, 1995). The determination of DHG was based on the reduction rate of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) in the soil after 24 hours of incubation at 37°C (Alef & Nannipieri, 1995; Thalmann, 1968). The enzyme activity of FDA in soil was measured by Thalmann., (1968), which involved measuring how much fluorescein was released when moist soil was incubated with phosphate buffer solution (pH 7.6) and fluorescein di acetate solution at 25°C. Based on the values of the MBC, basal respiration, and substrate-induced respiration, we were able to calculate the microbial metabolic quotient and respiratory quotient using the formula (3) and (4).

$$Microbial\ metabolic\ quotient\ (qCO_2) = \frac{CO_2{-}cc\ from\ basal\ respiration}{Microbial\ biomass\ C} \tag{3}$$

$$Respiratory\ quotient = \frac{Basal\ respiration}{Substrate\ induced\ respiration} \tag{4}$$

6.2.5. Crop

Tomatoes (*Solanum Lycopersicum L.*), and verity SL-120 were chosen as test crops for the pot experiment. This crop has been chosen because tomatoes retain their fast-growing nature. Thus, there is a possibility to accumulate potassium in more of its requirements depending on the available potassium present in the soil, and potassium is added through potassium fertilizers. Tomato is one of the most popular horticultural and beneficial crops in the world.

6.2.6. Experimental setup (pot experiment)

The pot experiment was carried out in a greenhouse (originally an open net house) in the ‘rabi’ season, from December 2019 to February 2020, at the Indian Statistical Institute farm, Giridih, Jharkhand located at N24°11.701’ latitude and E086°18.015’ longitude of 788ft above sea level. The normal climatic condition of the study side is dry, and the average annual rainfall is ~ 1350 mm. The average maximum and minimum temperatures of this district; are 42° C in summer and 10° C in winter respectively. The pot experimental plan was based on eighteen treatments. These were T1, negative control [only soil (S) no micas, no bacterial culture, and no basal doses were added only borax-B was used]; T2, positive control [only added standard basal doses (Kg/ha @ N:P: K = 200:150:100) or fertilizers (F)]; T3 and T4, muscovite (M) at 50 mg kg^{-1} and 200 mg kg^{-1} soil + *Bacillus cereus* K5B; T5 and T6, muscovite (M) at 50 mg kg^{-1} and 200 mg kg^{-1} soil + *Bacillus cereus* K6; T7 and T8, muscovite (M) at 50 mg kg^{-1} and 200 mg kg^{-1} soil + *Bacillus sp.GG6* (2015) K12; T9 and T10, muscovite (M) at 50 mg kg^{-1} and 200 mg kg^{-1} soil + *Bacillus cereus* K15; T11 and T12, biotite (B) at 50 mg kg^{-1} and 200 mg kg^{-1} soil + *Bacillus cereus* K5B; T13 and T14, biotite (B) at 50 mg kg^{-1} and 200 mg kg^{-1} soil + *Bacillus cereus* K6; T15 and T16, biotite (B) at 50 mg kg^{-1} and 200 mg kg^{-1} soil + *Bacillus sp.GG6* (2015) K12; T17 and T18, biotite (B) at 50 mg kg^{-1} and 200 mg kg^{-1} soil + *Bacillus cereus* K15 respectively. The experiment was kept in a completely random design. Each treatment was replicated three times. Processed 12 kg soil (~ 5 mm particle size) was placed on a clean baby plastic tray. Two types of required quantities of waste micas and 44.44 ml (1.45×10^8 to 1.64×10^8 cfu) each of KSB bacterial inoculants were added to soil as per the treatment and mixed uniformly. A basal doses ration in Kg/ha (N:P:K = 200:150:100) of urea-N (2.33 g / 12 kg soil), single superphosphate-SSP (5g / 12 Kg soil), muriate of potash-MOP (924 mg / 12Kg soil), and borax-B (80 mg / 12Kg soil) were used all treatments except negative control (T1). A suitable amount of water was added to increase the moisture content of the soil to pot capacity. Waste muscovite and biotite treated with four types of KSB bio-fertilizers were eventually placed in cement pots with a diameter of 30 cm higher, 22 cm lower, and depth of 23 cm. A 500 tomato seeds were soaked in water for 2 hours and then kept for 7 days for germination on 26th November. After germination; one healthy tomato plant was shown to each pot and grown for 90 days to ensure enough biomass production and removal of nutrients from the soil. During this period the pots were kept weed-free and maintained in an optimal soil moisture system, with about 60% water holding capacity factor of the soil during testing with regular crop irrigation to ensure almost no limiting factors.

For physicochemical and microbial analysis of the sample, 1 kg of soil was taken from the rhizospheric surface (0-0.2 m) at random from each plot on different days (0D, 30D, 60D, and 90D) of interval. One part of each of the soil samples was processed by standard protocol (Page et al., 1982) for physicochemical analysis; and the other part of the moist soil samples was kept in a refrigerator at 4°C for microbial analysis. Following each harvest, the plant parts both root and shoot were cleaned and biomass was recorded after drying the sample at 65±2°C until a constant weight was obtained. The oven-dried plant (root and shoot) and fruit samples were ground by a mechanical mixer grinder at (~5 mm size) and desiccators till analyses. The changes in physicochemical (pH, EC, TOC, Av. N, Av. P, Ex. K, NEK, and DTPA extraction of heavy metals) and microbial properties (MBC, BSR, SIR, DHG, and FDA) of fertilizers treated soil samples were analyzed followed by different standard methods that we have already described previously section *1.4. A* and *1.4.B*. The ground shoot, root and fruit samples were analyzed for nutrients such as N, P, and K uptake by plant parts (shoot and root) and fruits by the standard method (Cottenie et al., 1982; J. H. Jackson, 1958). The percent (%) potassium (K) recovery by tomatoes plant parts (shoot and root) and fruit were calculated by the relation as given below (Basak & Biswas, 2009);

$$\text{Percent K recovery} = 100 \times (UKt - UKc)/A \tag{5}$$

Where UKt= Uptake of K in KSB treated fertilizer pot (mg K pot^{-1}); UKc= Uptake of K in control pot (mg K pot^{-1}); A= Amount of K applied in soil (mg K pot^{-1}).

6.2.7. Enumeration of microbial growth (CFU- (colony forming unit)

1g of soil samples (T1-T18) from both control and fertilizers (soil + KSBs) were suspended in 10 ml of sterile de-ionized water and allowed to vortex for 15 min, then 100 µl (0.1 ml) of each aliquot was serially diluted from 10^{-1} to 10^{-6} respectively and inoculated in Aleksandrov agar plates following spread plate technique for enumeration of KSB bacterial population. Then, the inoculated plates were incubated at 28 ± 2°C for 7 days (Hussain, Singh, Saha, Venkata, et al., 2016). After incubation KSB bacterial colonies grown on the agar plates were recorded with the help of a colony counter. The whole experiment was replicated thrice and CFU ml^{-1} was calculated following the formula given below,

$$\text{Number of bacterial cell ml}^{-1} = \frac{Number\ of\ colonies \times dilution\ of\ samples}{amount\ (volume) of\ plate} \tag{6}$$

6.2.8. Determination of agronomic parameters

6.2.8.1. Growth parameters

Growth parameters of plants such as plant root and shoot height (cm) were recorded at 90 days of harvesting from the date of planting. The height of the plant was measured from ground level to the growing tip of the main stem was calculated 90 days after planting (DAP). Determination of leaf area (cm^2); after harvesting five mature photo-synthetically active leaves were taken during vegetative growth stages from the plant of all the treatments. The area of a leaf was measured and plant dry mass (gm/plant) by the standard method (R. Singh et al., 2007). The modified indexes such as leaf area index, fertility ratio, agronomic efficiency, physiological efficiency, apparent nutrient recovery efficiency, and nutrient uptake efficiency were computed by standard formula (R & Reddy, 2007).

$$Leaf\ area\ index\ (LI) = \frac{Total\ leaf\ area}{Grownd\ area} \tag{7}$$

$$Fertility\ ratio\ (FR) = \frac{Total\ number\ of\ fruits/plant}{Total\ number\ of\ flowers/plant} \tag{8}$$

$$Agronomic\ efficiency\ (AE) = \frac{(Fruit\ yield\ of\ fertilized\ crop - Fruit\ yield\ of\ unfertilized\ crop)}{Quantity\ of\ fertilizer\ applied} \tag{9}$$

$$Physiological\ efficiency\ (PE) = \frac{(Totl\ drymatter\ yield\ in\ fertilized\ plant - Totl\ dry\ matter\ yield\ in\ un-fertilized\ plant)}{(Nutrient\ uptake\ by\ fertilized\ plant - Nutrient\ uptake\ by\ unfertilized\ plant)} \tag{10}$$

$$Apparent\ nutrient\ recovery\ efficiency\ (ANRE) = \frac{(Nutrient\ uptake\ by\ fertilized\ plant - Nutrient\ uptake\ by\ unfertilized\ plant)}{Quantity\ of\ fertilizer\ applied} \tag{11}$$

$$Nutrient\ use\ efficiency\ (NUE) = Physiological\ efficiency \times Recovery\ efficiency \tag{12}$$

6.2.8.2. Determination of flowering, yield, quality, and colour of the fruit

The total number of fully mature flowers per plant was calculated from the date to the first flower opening from each treatment. The yield of tomato fruits started 60 days after transplanting and continued till the end of the experiment. The total number of tomatoes and fruits harvested per plant from each pot, treatment, and replication wise was calculated until the final crop of all treatments was harvested, and added and the average yield per plant was finished and expressed in kilogram (kg). Determination of tomato fruit quality such as fruit diameter (mm), fruit weight (gm), and volume (cc) of the fruit was estimated followed by standard methods (Nagoni et al., 2017), and determination of percentage (%) of fruit set calculated by the given formula (Nagoni et al., 2017);

$$Percent\ fruit\ set\ (\%) = \frac{No.of\ fruits\ set\ among\ the\ tagged\ flowers}{No.of\ flowers\ tagged} \times 100 \tag{13}$$

6.2.9. Determination of chlorophyll and carotenoid

The chlorophyll and carotenoid content of tomato fruit has been analyzed following the methods (NAGATA & YAMASHITA, 1992). All the pigments in the treated samples were extracted at once with the ratio of acetone-hexane (4:6), and then the OD (optical density of the supernatant was measured at 663nm, 645nm, 505nm, and 453nm by a UV spectrophotometer at the same time. From these values, the content of chlorophyll (a, and b), lycopene, and car-carotene is calculated given by the given formula;

$$Chlorophyll\ a\ (mg/100ml) = 0.999A663 - 0.0989A645 \tag{14}$$

$$Chlorophyll\ b\ (mg/100ml) = -0.328A663 + 1.77A645 \tag{15}$$

$$Lycopene\ (mg/100ml) = -0.0458A663 + 0.204A645 + 0.372A505 - 0.0806A453 \tag{16}$$

$$\beta\text{-}Carotene\ (mg/100ml) = 0.216A663 - 1.22A645 - 0.304A505 + 0.452A453 \tag{17}$$

A663, A645, A505 and A453 are absorbance at 663nm, 645nm, 505nm and 453nm each other.

6.2.10. Statistical analysis

All data obtained in the greenhouse (pot) experiments were subjected to a statistical analysis of variance (ANOVA) appropriate to the experimental design with three replicates using IBM-SPSS 26.0

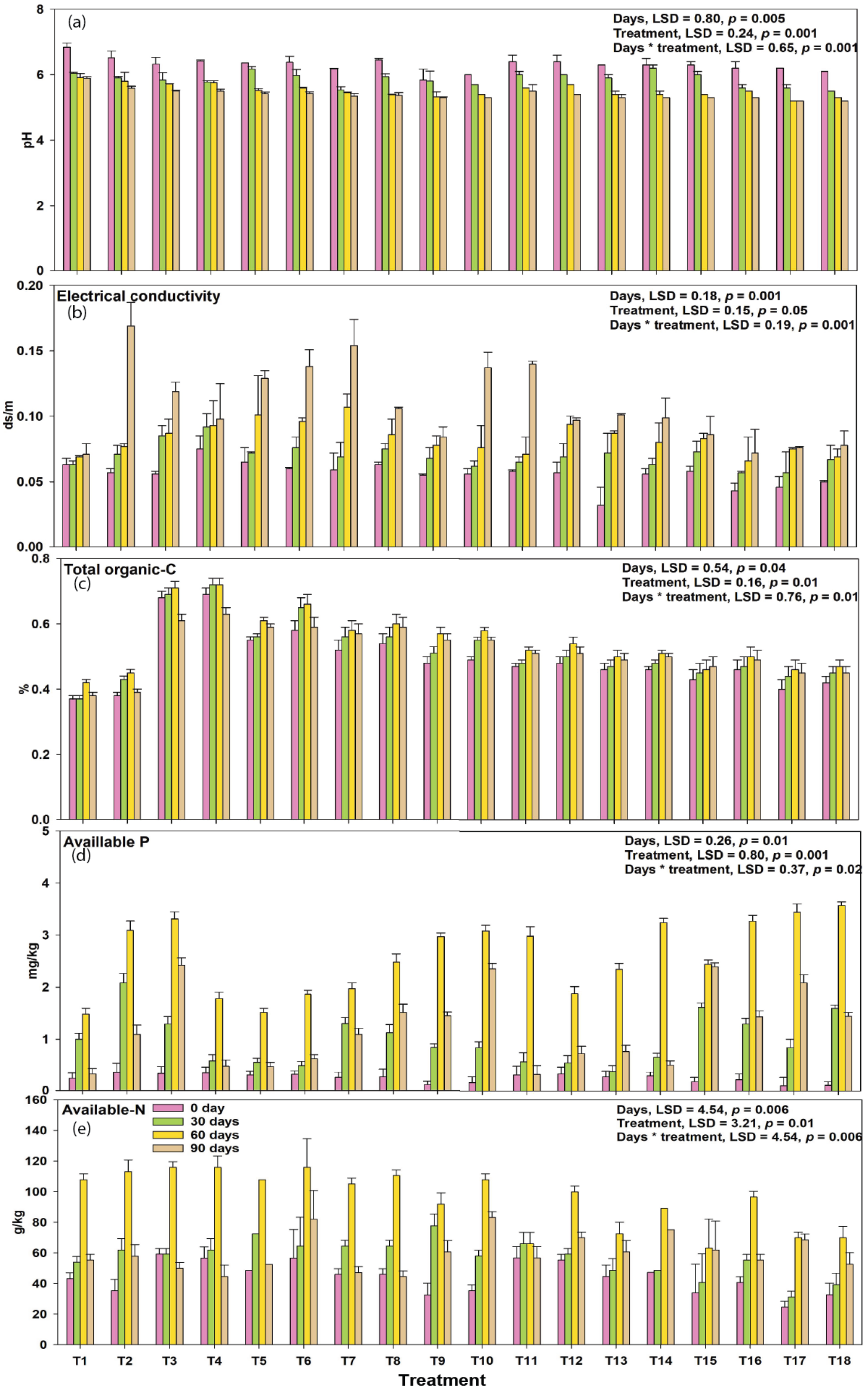
statistic software. F-test was carried out to test the significance of the differences in treatment and the Least significant difference (LSD at $p = 0.05$) was assessed. Pearson's correlation matrix between plant biomass yield, fruit production, K uptake by the plant, and different pools of potassium (Wa. K, Ex. K, K, and NEK) in soils was assessed by the IBM-SPSS 26.0 statistic software.

6.3. Results and discussions

6.3.1. Temporal changes in physico-chemical properties and benefit ratio of the KSB biofertilizers treated soils during the greenhouse experiments

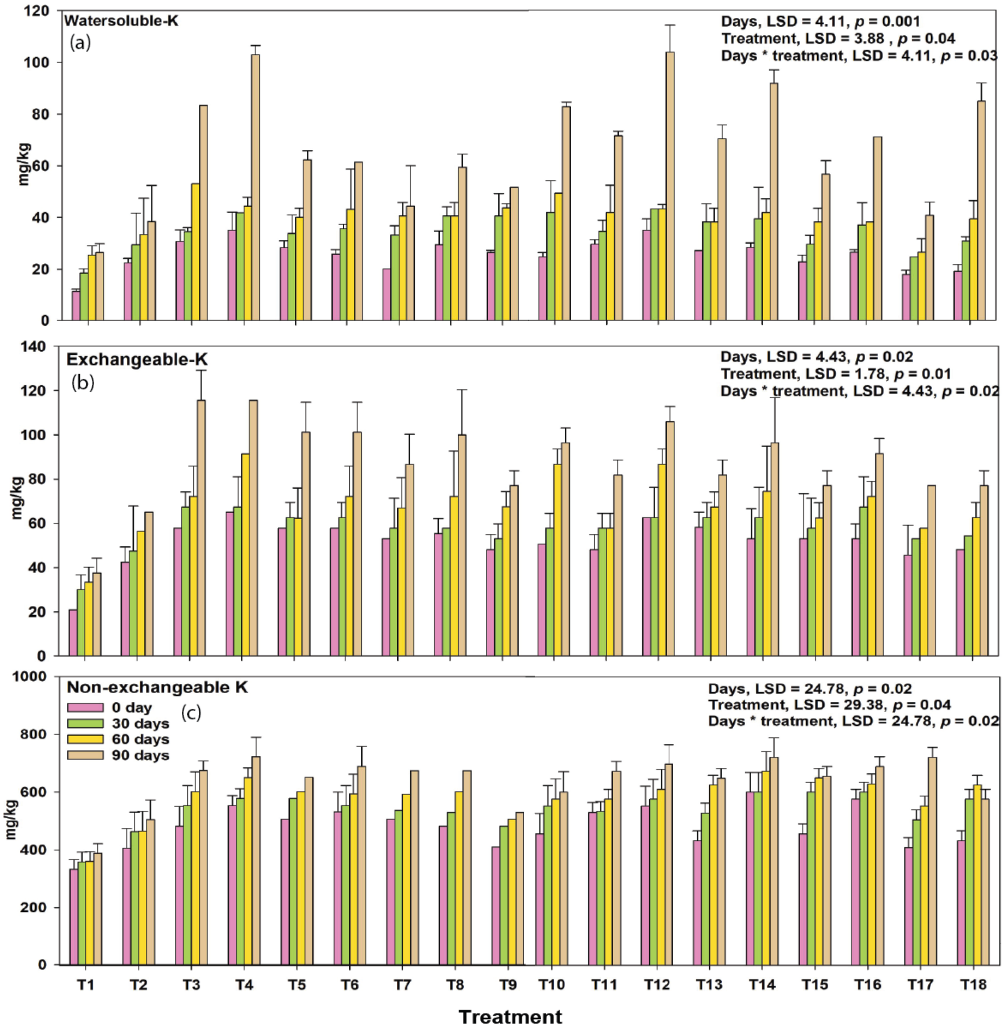
The temporal changes in physicochemical properties such as pH, EC, total organic C, available N and P, and exchangeable or available K in the KSB fertilizers treated soils have been presented in Fig. 1(a-e) and Fig. 2 (a). We used the benefit ratio (BR) as a measure of the performance of the four KSB bacterial isolates (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) in the composting process with two types of waste micas (muscovite and biotite) Fig.3.

Temporary changes in pH of KSB treated soils appear to be lower in 90 days as compared to 0 days, and EC values of tasted soil were higher in 90 days as compared with different incubation periods of four KSB bacterial strains and different concentrations (50mg/kg and 200mg/kg) of muscovite and biotite treated soils Fig. 1(a) and (b). In general, the pH and EC of the muscovite and biotite treated soils substantially decreased and increased average pH (muscovite = 1.15 and biotite = 1.18); EC (muscovite = 0.52 and biotite = 0.55) folds as compared to the initial alkaline value after 90 days of incubation (p for treatment pH = 0.001; LSD = 0.24; p for treatment EC = 0.05; LSD = 0.15) respectively. pH was significantly decreased in T9 and T10, @ (M) 50mg and 200mg kg-1 soil + *Bacillus cereus* K15, compared to the other treatments, and EC was significantly higher in T2 (Soil + Basal dose) and T7 (@, (M) 50 mg kg-1 soil + *Bacillus sp.GG6* (2015) K12, compared to the other treatments during the incubation period.



T1-S; T2-S+F; T3-M(50mg/kg)+K5B; T4-M(200mg/kg)K5B; T5-M(50mg/kg)+K6; T6-M(200mg/kg)K6; T7-M(50mg/kg)+K12; T8-M(200mg/kg)K12; T9-M(50mg/kg)+K15; T10-M(200mg/kg)K15; T11-B(50mg/kg)+K5B; T12-B(200mg/kg)K5B; T13-B(50mg/kg)+K6; T14-B(200mg/kg)K6; T15-B(50mg/kg)+K12; T16-B(200mg/kg)K12; T17-B(50mg/kg)+K15; T18-B(200mg/kg)K15.

Fig. 6.1. Temporal variation of soil physicochemical properties (pH, EC, organic-C, Available P, and Available N) during pot experiments with various concentrations of muscovite and biotite @ 50 and 200 mg K kg-1 soil, with four different types of K solubilizing bacterial strains on Alfisol at different times of incubation.



T1-S; T2-S+F; T3-M(50mg/kg)+K5B; T4-M(200mg/kg)K5B; T5-M(50mg/kg)+K6; T6-M(200mg/kg)K6; T7-M(50mg/kg)+K12; T8-M(200mg/kg)K12; T9-M(50mg/kg)+K15; T10-M(200mg/kg)K15; T11-B(50mg/kg)+K5B; T12-B(200mg/kg)K5B; T13-B(50mg/kg)+K6; T14-B(200mg/kg)K6; T15-B(50mg/kg)+K12; T16-B(200mg/kg)K12; T17-B(50mg/kg)+K15; T18-B(200mg/kg)K15.

Fig. 6.2. Temporal changes in potassium pools vis, Water-soluble, exchangeable, and non-exchangeable K (mg kg-1) on Alfisol and application of various concentrations (@ 50 and 200 mg K kg-1 soil) of muscovite and biotite as influenced by four K solubilizing bacterial strains on Alfisol during pot experiments.

The pH values were significantly (p for days = 0.005 and LSD = 0.80) lower in 90 days due to the presence of potassium solubilizing bacterial strains in the soil and those strains are able to produce organic acids such as oxalic, citric, gluconic, fumaric, acetic, and tartaric acids that then help solubilize the K-mineral (V. S. Meena et al., 2015) and enhance significantly (p for days = 0.001 and LSD = 0.18) salt content in incubated soil at 90 days (Saha et al., 2016a). Control treatments (T1 = only soil; T2 = fertilized treated soil) slightly decreased pH with incubation time and may be due to the production of H^+ during the hydrolysis of some presence of salt and added salt fertilizers respectively (Mo & Lian, 2011). A significant (p for treatment = 0.001 and LSD = 0.21) reduction in pH was observed in D3 (biotitle @ 200 mg kg^{-1} soil with *Bacillus cereus* K6) and in EC was significant (p for treatment = 0.05

and LSD = 0.15) higher in D2 (biotite @ 50 mg kg^{-1} soil with *Bacillus cereus* K6). Fig.3 (a) and (b). The reduction of pH in tested soils indicated the production of various forms of organic acids and anions such as PO_4^{3-} and NO^{3-} etc. (Deka et al., 2011; Hussain, Singh, Saha, Kumar, et al., 2016).

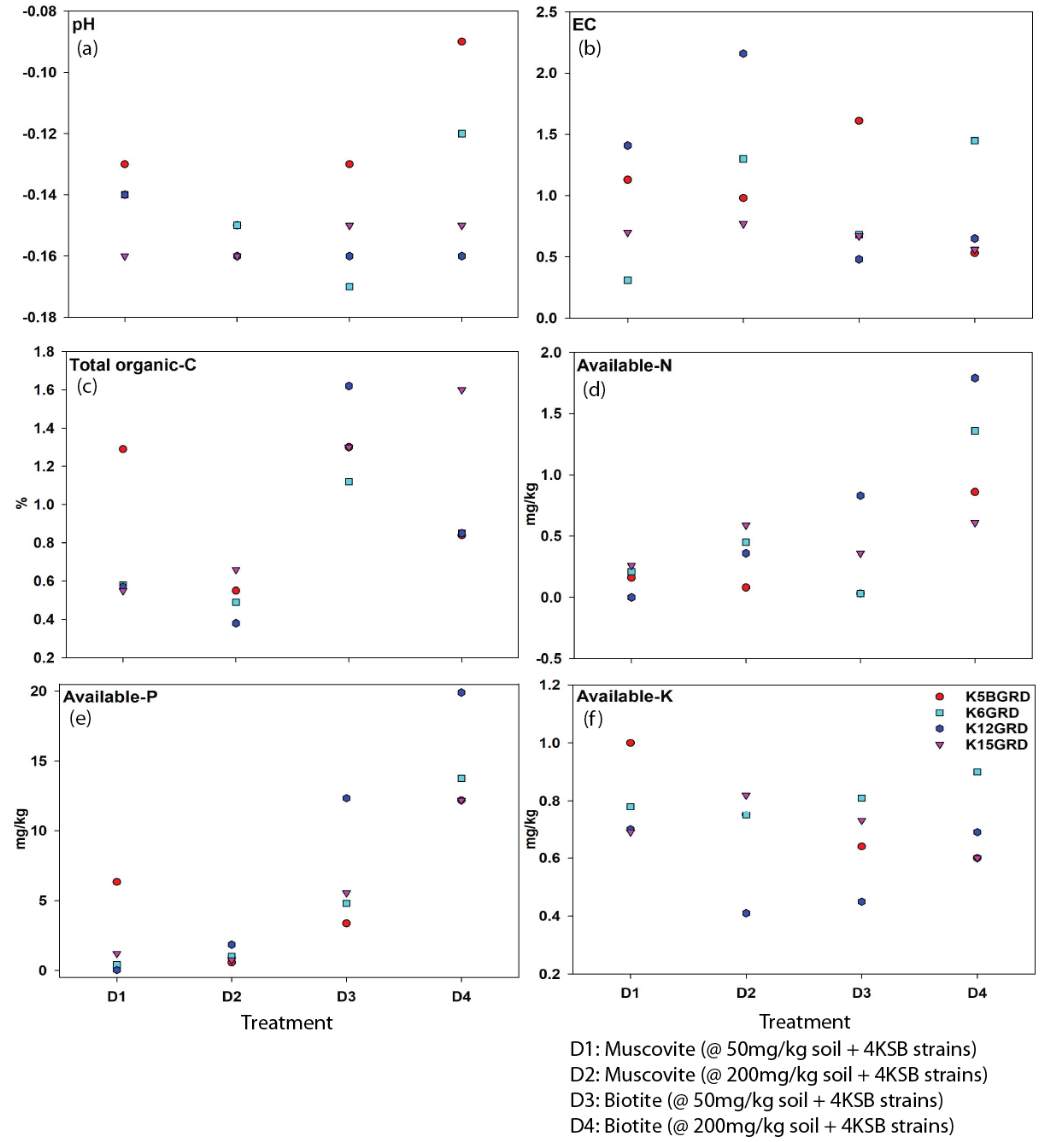


Fig. 6.3. Performance of the four bio-composites produced with Bacillus cereus K5B, Bacillus cereus K6, Bacillus sp.GG6 (2015) K12, and Bacillus cereus K15 on Alfisol and application of various concentrations (@ 50 and 200 mg K kg-1 soil) of muscovite and biotite during pot experiments at different days of incubation (0d, 30d, 60d, and 90d) expressed as Benefit ratio.

The total organic C was initially low at all treatments during the incubation period, but the concentration was significantly (*p* for days = 0.04 and LSD = 0.54) higher at 60 days, then slightly decreased at 60 days Fig. 1(c). There was a significant difference (*p* for treatment = 0.01 and LSD = 0.16) in TOC concentration in muscovite treated soils (50 mg kg^{-1} and 200 mg kg^{-1} soil) and compared to biotite soils (50 mg kg^{-1} and 200 mg kg^{-1} soil) with four KSB treated soils. Both muscovite and biotite treated with four KSB treated soils, T4 (@ (M) 200 mg kg^{-1} soil + *Bacillus cereus* K5B) and T3 (@ (M) 50 mg kg^{-1} soil + *Bacillus cereus* K5B) showed significant (*p* for days × treatment = 0.01 and LSD = 0.76) best results. The highest BR ratio was observed in D4 (@ (B) 200 mg kg^{-1} soil) and *Bacillus sp.GG6* (2015) K12 bacterial strain treated soils Supply metal Fig.1. The TOC content after 60 days in the different KSB biofertilizers and mica waste mixture was in order: *Bacillus sp.*GG6 (2015) K12 > *Bacillus cereus* K15 > *Bacillus cereus* K5B > *Bacillus cereus* K6; D4 > D1 > D3 > D2. A significant reduction in TOC levels after 60 days of incubation periods significantly increases the rate of organic matter mineralization (Hussain, Singh, Saha, Kumar, et al., 2016).

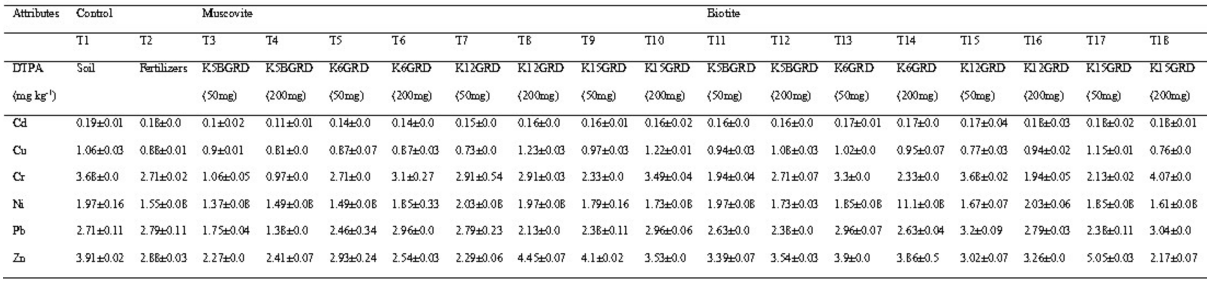
In general, the availability of easily mineralizable or available N (AN) in our tested soils significantly increased (*p* for days = 0.006, LSD = 4.54) gradually after incubation of all treatments, but decreased at 90 days Fig. 1(e). Comparatively, to biotite treated with four KSB strains in soils and both positive (T1) and negative (T2) controls, AN content was higher in muscovite treated with KSB strains in soil. For muscovite and biotite with 200mg kg^{-1} soil concentration, significant results (*p* for treatment = 0.01 and LSD = 3.21) showed the best results as compared to 50mg kg^{-1} soil and both positive (T1) and negative (T2) controls. Among all the treatments, the highest concentrations of AN were found in muscovite and biotite at T6 (@ (M) 200 mg kg^{-1} soil + *Bacillus cereus* K6), T4 (@ (M) 200 mg kg^{-1} soil + *Bacillus cereus* K5B), and T3 (@ (M) 50 mg kg^{-1} soil + *Bacillus cereus* K5B), respectively, even higher than in fertilizer treated soil (T2) (*p* for days × treatment = 0.006 and LSD = 4.54). N availability was higher at 60 days and constant at 90 days after incubation; this may have been caused by the combination of KSB microorganisms and waste micas and; the production of nitrogenous organic compounds by rhizospheric bacteria (Deka et al., 2011; Hussain, Singh, Saha, Kumar, et al., 2016). According to (Biswas et al., 2009) report that the increase in available N has primarily resulted from the total loss of dry matter like TOC (total organic carbon) as CO_2 and the evaporation of water during composting. Furthermore, the nitrogen-fixing ability of KSB bacterial strains can also increase available N during the composting process or incubation period. Similarly, the concentration of available P was significantly higher in 60 days during the incubation period (p for days = 0.01 and LSD = 0.26) for all treatments Fig. 1(d). In comparison to the different concentrations (@ 50 and 200 mg kg^{-1} soil) of muscovite and biotite treated soils, biotite (200 mg kg^{-1} soil) concentration showed significantly (p for treatment = 0.001 and LSD = 0.80) best results as compared to the rest of the all treatments, even muscovite (200 mg kg^{-1} soil) concentration showed the best results among all muscovite treatments. T18 (@ (B) 200 mg kg^{-1} soil + *Bacillus cereus* K15), T17(@ (B) 50 mg kg^{-1} soil + *Bacillus cereus* K15), and T3(@ 50 mg kg^{-1} soil + *Bacillus cereus* K5B) showed best results (*p* for days × treatment = 0.02 and LSD = 0.37) during the incubation periods. The availability of K (EK) content was significantly (*p* for days = 0.02 and LSD = 4.43) increased during the increasing incubation period was increased at 90 days. As compared to muscovite and biotite treated soils muscovite treated soils were showed the best EK content over biotite treated soils and concentration wise @ 200 mg kg^{-1}

soil, showed the best concentration as compared to the concentration of @ 50 mg kg^{-1} soil (*p* for treatments = 0.01 and LSD = 17.8) Fig. 2(b). In muscovite treated soils T3 (@ 50 mg kg^{-1} soil + *Bacillus cereus* K5B) and T4 (@ 200 mg kg^{-1} soil + *Bacillus cereus* K5B); and in biotite treated soils T11 (@ 50 mg kg^{-1} soil + *Bacillus cereus* K5B) and T12 (@ 200 mg kg^{-1} soil + *Bacillus cereus* K5B) were showed best results as compared to all treatments (*p* for days × treatments = 0.02 and LSD = 4.43). A significant increase in exchangeable K was found in waste muscovite and biotite, inoculated with K-solubilizing bacteria, because some organic acid was produced by the KSB isolate during the 90 days. (Basak & Biswas, 2009, 2010) found a similar pattern of exchangeable K in their results. At the end of the study, the BR ratios of N and P in D3 (@ (B) 50 mg kg^{-1} soil with 4 KSB isolates) and K in D2 (@ (M) 200 mg kg^{-1} soil with 4 KSB isolates) were higher, Fig.3(d), (e), and (f). During the incubation periods, *Bacillus sp.*GG6 (2015) K12 bacterial strains for N and P and *Bacillus cereus* K5B bacterial strains for K produced the best results when compared to other KSB strains. In both muscovite and biotite-treated soils (50 and 200 mg kg-1), different types of bioavailable heavy metals were found to be significantly higher than different types of controls (T1 and T2).

DTPA extractable metals, such as Cd, Cu, Cr, Ni, Pb, and Zn content in tested or compost soil were below the detectable Table 1. In general, all bio-available heavy metal (Cd, Cu, Cr, Ni, Pb, and Zn) ions were found to be higher in controls T1 (only soil) and T2 (soil + F) as compared to muscovite and biotite with four KSB bacterial strains treated soils. As compared to muscovite and biotite treated soils, muscovite + 4 KSB showed the lowest heavy metal (Cd, Cu, Cr, Ni, Pb, and Zn) content as compared to biotite + 4 KSB isolates. T4 (@ (M) 200 mg kg^{-1} soil + *Bacillus cereus* K5B) and T3 (@ (M) 50 mg kg^{-1} soil + *Bacillus cereus* K5B) showed significantly lowest bioavailable heavy metal (Cd, Cu, Cr, Ni, Pb, and Zn) content overall all treatments during the incubation periods. (Henagamage et al., 2021) demonstrated that *Bacillus sp*. as *B. subtilis* showed high tolerance to metal ions such as Cr^{3+}, Cd^{2+}, and Pb^{2+} ions. According to (Čásová et al., 2009) suggest that heavy metals or metal ions are not biodegradable, therefore they persist in the environment for long periods. There is evidence to suggest that soil microorganisms can detoxify and bioremediate heavy metals such as Pb^{2+} and Cd^{2+}.

Supplemental table 1

Temporal changes of available heavy metals vis. Cd, Cu, Cr, Ni, Pb, and Zn during the pot experiment treated with various concentration (@ 50 and 200 mg kg^{-1} soil) of muscovite and biotite and four KSBs.



Attributes	Control		Muscovite								Biotite							
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18
DTPA ($mg\ kg^{-1}$)	Soil	Fertilizers	K5BGRD (50mg)	K5BGRD (200mg)	K6GRD (50mg)	K6GRD (200mg)	K12GRD (50mg)	K12GRD (200mg)	K15GRD (50mg)	K15GRD (200mg)	K5BGRD (50mg)	K5BGRD (200mg)	K6GRD (50mg)	K6GRD (200mg)	K12GRD (50mg)	K12GRD (200mg)	K15GRD (50mg)	K15GRD (200mg)
Cd	0.19±0.01	0.18±0.0	0.1±0.02	0.11±0.01	0.14±0.0	0.14±0.0	0.15±0.0	0.16±0.0	0.16±0.01	0.16±0.02	0.16±0.0	0.16±0.0	0.17±0.01	0.17±0.0	0.17±0.04	0.18±0.03	0.18±0.02	0.18±0.01
Cu	1.06±0.03	0.88±0.01	0.9±0.01	0.81±0.0	0.87±0.07	0.87±0.03	0.73±0.0	1.23±0.03	0.97±0.03	1.22±0.01	0.94±0.03	1.08±0.03	1.02±0.0	0.95±0.07	0.77±0.03	0.94±0.02	1.15±0.01	0.76±0.0
Cr	3.68±0.0	2.71±0.02	1.06±0.05	0.97±0.0	2.71±0.0	3.1±0.27	2.91±0.54	2.91±0.03	2.33±0.0	3.49±0.04	1.94±0.04	2.71±0.07	3.3±0.0	2.33±0.0	3.68±0.02	1.94±0.05	2.13±0.02	4.07±0.0
Ni	1.97±0.16	1.55±0.08	1.37±0.08	1.49±0.08	1.49±0.08	1.85±0.33	2.03±0.08	1.97±0.08	1.79±0.16	1.73±0.08	1.97±0.08	1.73±0.03	1.85±0.08	11.1±0.08	1.67±0.07	2.03±0.06	1.85±0.08	1.61±0.08
Pb	2.71±0.11	2.79±0.11	1.75±0.04	1.38±0.0	2.46±0.34	2.96±0.0	2.79±0.23	2.13±0.0	2.38±0.11	2.96±0.06	2.63±0.0	2.38±0.0	2.96±0.07	2.63±0.04	3.2±0.09	2.79±0.03	2.38±0.11	3.04±0.0
Zn	3.91±0.02	2.88±0.03	2.27±0.0	2.41±0.07	2.93±0.24	2.54±0.03	2.29±0.06	4.45±0.07	4.1±0.02	3.53±0.0	3.39±0.07	3.54±0.03	3.9±0.0	3.86±0.5	3.02±0.07	3.26±0.0	5.05±0.03	2.17±0.07

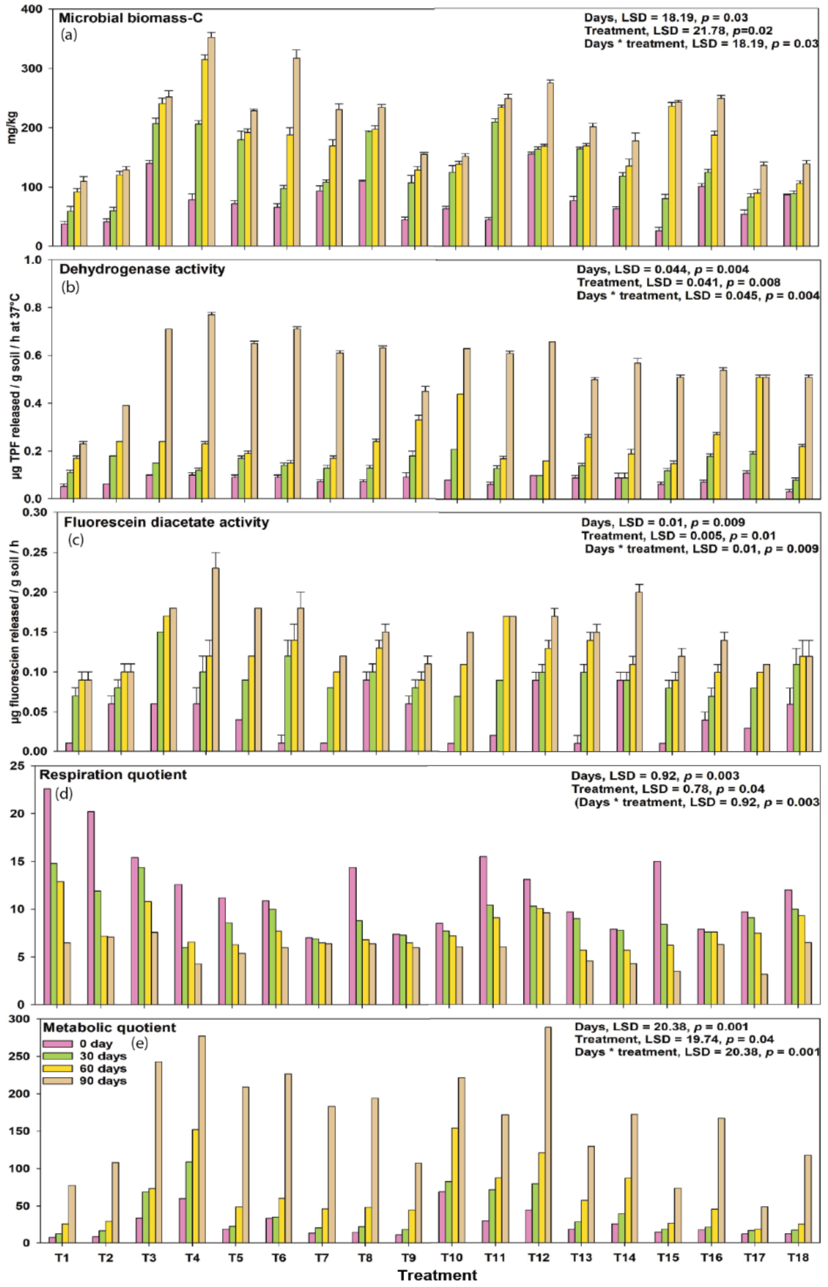
6.3.2. Dynamics of K in the soil during incubation

Temporal changes of water-soluble K and non-exchangeable K were found to be higher at 90 days in different concentrations (@ 50 and 200 mg kg^{-1} soil) of both muscovite and biotite within four KSBs bacterial strain treated soils after an incubation period during the pot experiments Figs. 2(a) and (c). On average both WK and NEK were found in lower T1 (negative control, where no K was added) and T2 (positive control, soil + F), where K fertilizer was added as compared to all treatments. A significant (*p* for days (WK) = 0.001 and LSD = 4.11; *p* for days (NEK) = 0.02 and LSD = 24.78) increase in WK and NEK content was observed at 90 days in the pot experiment. Concentration wise @ (M) 50 mg kg^{-1} soil for WK and @ (B) 200 mg kg^{-1} soil for NEK showed best result during the pot experiment and T4 (@ (M) 200 mg kg^{-1} soil + *Bacillus cereus* K5B) and T3 (@ (M) 50 mg kg^{-1} soil + *Bacillus cereus* K5B); T12 (@ (B) 200 mg kg^{-1} soil + *Bacillus cereus* K5B) and T11 (@ (B) 50 mg kg^{-1} soil + *Bacillus cereus* K5B) showed significantly (*p* for days × treatment (WK) = 0.03 and LSD = 4.11; *p* for days × treatment (NEK) = 0.02 and LSD = 24.78) best results as compared rest of the all treatments during the different incubation period. We described before section 2.1 the forms of K that are exchangeable or available in experimental soils.

During the experiments, WK and EK formed by releasing K from non-exchangeable pools of K with the help of four KSBs and releasing organic acids. Compared to soil treated with fertilizers, waste muscovite and biotite treated with KSB fertilizers exhibited higher WK, EK, and NEK. The findings of (Basak & Biswas, 2009, 2010) showed similar results, which supported our findings. The effectiveness of mica or silicate rock powder has previously been reported to increase when soils have low levels of nutrients (N, P, and K) (Basak & Biswas, 2009; Hinsinger et al., 1995). In addition, WK, as well as EK, significantly increased after the inoculation of two types of waste micas with KSB strains in Giridih (Alfisol) soil. This can be attributed to the solubilization of structural and NEK through the production of different types of organic acids such as citric, ferulic, coumaric, oxalic, and malic acids. The results of the present work supported the findings of other researchers (Barker et al., 1998; O. P. Meena et al., 2013; V. S. Meena et al., 2015; Saha et al., 2016). Several studies have shown that mineral K, mostly WK and EK, can be absorbed directly by crops (Basak & Biswas, 2009, 2010). As shown in (Wang et al., 2000), in continuous cropping systems, the losses of soil K caused by crops far exceed consumption from the available soil and NEK pool, which indicates that the minerals release a percentage of the K taken up by crops. (Sekhon, 1992) explains that differences in total soil K content and NEK can be attributed to differences in sand, clay, and silt contents and their mineral compositions. In addition to the release of non-exchangeable K from feldspar, (Moritsuka et al., 2004) attributed this to a release of acids such as citric acid and oxalic acid from the roots. The ratio of soil-clay minerals (2:1) impacts soil K availability. In addition, several studies have shown that even supportive amounts of such minerals present in clay increase soil K availability (Basak & Biswas, 2009). According to (Barré et al., 2008), illitic-clay minerals contain a renewable potassium reserve. The K reservoir in the soil plays a significant role in the K cycle (Basak & Biswas, 2009). The most obvious effect of such clay layers is that they provide short-term K for plant needs and protect the productivity of long-term ecosystems by reducing K leaching. The special importance of clay layers lies in the ability to absorb and release K ions from a 2: 1 clay mineral interlayer or lattice K position (Hinsinger, 2002).

6.3.3. Temporal changes in microbial properties of KSB treated compost soils during the greenhouse experiments

The MBC in all the four KSB with waste muscovite and biotite treated soils during the incubation considerably increased with time Fig. 4. (a). Significant (*p* for days = 0.03 and LSD = 18.19) variations in MBC in different treatments were observed between 0 to 90 days. The MBC levels in KSB fertilizer treated soils were determined at the end of the incubation period with muscovite treated soils showing the best results compared to biotite treated soils. The concentration of muscovite and biotite at 200 mg kg^{-1} soil showed significant differences (*p* for treatment = 0.02 and LSD = 21.78) compared to 50 mg kg^{-1} soil. MBC was significantly higher for T4 and T3 treatments (*p* for days × treatment = 0.03 and LSD = 18.19) compared with the other treatments. (Chakraborty et al., 2022) reported a similar pattern of results findings their experiments. The estimation of MBC; by the fumigation-extraction method is dependable; measure for acidic soils (Bhattacharyya et al., 2005; Hussain, Singh, Saha, Kumar, et al., 2016).



T1-S; T2-S+F; T3-M(50mg/kg)+K5B; T4-M(200mg/kg)K5B; T5-M(50mg/kg)+K6; T6-M(200mg/kg)K6; T7-M(50mg/kg)+K12; T8-M(200mg/kg)K12; T9-M(50mg/kg)+K15; T10-M(200mg/kg)K15; T11-B(50mg/kg)+K5B; T12-B(200mg/kg)K5B; T13-B(50mg/kg)+K6; T14-B(200mg/kg)K6; T15-B(50mg/kg)+K12; T16-B(200mg/kg)K12; T17-B(50mg/kg)+K15; T18-B(200mg/kg)K15.

Fig. 6.4. Changes of temporal variation of microbial properties vis. Microbial biomass-C, fluorescein diacetate, dehydrogenase activity, respiratory quotient and metabolic quotient during the pot experiments of various concentrations (@ 50 and 200 mg K kg-1 soil) of muscovite and biotite with four types of K solubilizing rhizospheric bacterial strains (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) at different days of incubation on Alfisol.

The metabolic quotient (qCO_2) measures the efficiency of microorganisms in utilizing carbon and their tolerance to toxic elements (Chakraborty et al., 2022). However, the microbial metabolic quotient was significantly (p for days = 0.001 and LSD = 20.38) higher in waste muscovite treated soils than in biotite treated soils, and concentration wise, at 200 mg kg^{-1} soil, the qCO_2 levels were higher than those at 50 mg kg^{-1} soil Fig. 4(e) (*p* for treatment = 0.04 and LSD = 19.74). After 90 days of incubation, qCO_2 levels of all treatments were found to be higher. T4 and T3 in muscovite and T12 and T11 in biotite showed high qCO_2 levels during the pot experiment (*p* for days × treatment = 0.001 and LSD = 20.38). Based on the high qCO_2 value, it appears that the rate of microbial respiration was higher than the rate of biomass growth, which indicates most of the accumulated energy in the microbial biomass was spent on survival rather than growth (Chakraborty et al., 2022). According to (Pal et al., 2007) microbial respiratory quotient (RQ) is an indicator of the stress in the immediate environment. Here, metabolically dormant microorganisms are reflected as basal respiration, which is largely dependent on autochthonous microbial populations. In general, the respiratory quotient was reduced in all the KSB fertilizers with muscovite and biotite treated soils 90 days after the incubation period Fig.4 (d) (*p* for days = 0.003 and LSD = 0.92). RQ levels were highest in T1 (negative control) and T2 (S + F), respectively (*p* for treatment = 0.04 and LSD = 0.78), and lowest in T4 and T3 at muscovite and T12 and T11 at biotite treated soils (*p* for days × treatment = 0.003 and LSD = 0.92). (Rusinowski et al., 2019) reported that the autochthonous microbes effectively counteract the metal toxicity, but they inhibit plant growth. A substrate-induced compost respiration profile, on the other hand, represents the respiration profile of metabolically active and plant-growing microorganisms (Dilly, 2005).

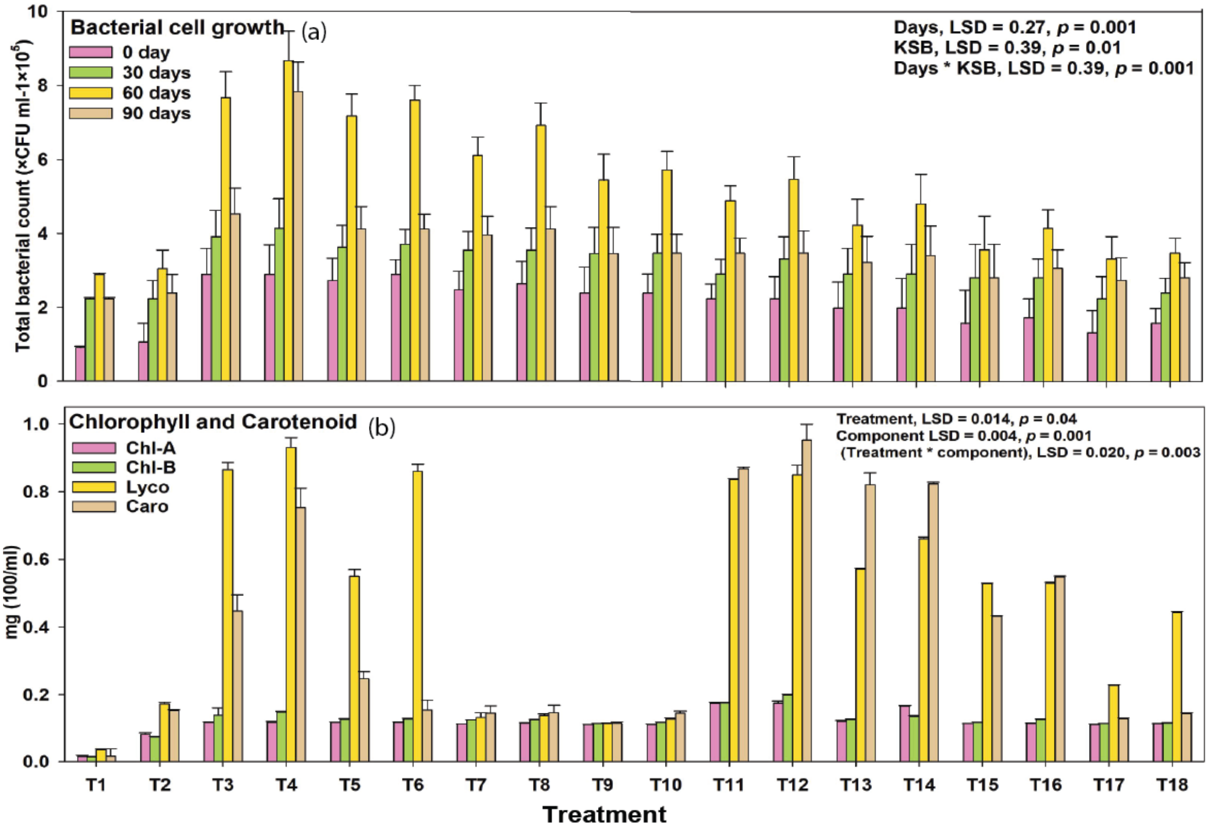
In order to determine the overall biological activity of different KSB fertilizers with muscovite and biotite-treated soils, FDA hydrolysis profiles were determined Fig. 4 (c). (Tian et al., 2015) report that several microbial enzymes (esterase, protease, and lipase) hydrolyze FDA when conditions are favourable. The FDA hydrolysis assay indicates an overall increase in fungal and bacterial activity. As such, FDA hydrolysis assay was significantly muscovite with KSB treated soils as compared to biotite with KSB treated soils (*p* for treatment = 0.01 and LSD = 0.005) and FDA level was found to be higher at 90 days during the incubation period (*p* for days = 0.01 and LSD = 0.009). In muscovite T4 and T3 and biotite T12 and T11 showed significantly (*p* for days × treatment = 0.01 and LSD = 0.009) greater results as compared rest of the treatments during the pot experiments. A group of enzymes (urease, acid and alkaline phosphatases, sulphatases, and glycosidase) can be used to measure the total hydrolase activity of soil microflora treated with biofertilizers and thus, hydrolase enzymes are largely responsible for regulating nutrients (N, P, K, S, and C) (Chakraborty et al., 2022).

Dehydrogenase (DHG) is a test used to assess soil microbial activity in situ; in fact, starvation believes that this test is a reliable indicator of soil microbial activity (Casida Jr et al., 1964). Muscovite with KSB fertilizer treated soils had a higher DHG activity level than biotite with KSB fertilizer treated soils (*p* for treatment = 0.008 and LSD = 0.041). @ 200 mg kg^{-1} soil concentration of both muscovite and biotite showed the best results as compared to the concentration of @ 200 mg kg^{-1} soil. After 90 days of soil incubation, DHG levels were higher (*p* for days = 0.004 and LSD = 0.045). The best DHG activities were observed during the pot experiments during times T4 and T3 for muscovite (*p* for days

× treatment = 0.004 and LSD = 0.045). Although there is no relationship between DHG and bacterial numbers unless organic amendments have been made to the soil, DHG is the endogenous respiration of the soil (Casida Jr et al., 1964). Dehydrogenase activity determines the total microbial activity, and this enzyme has sufficient sensitivity to indicate perturbations caused by microbial inoculation (del Carmen Rivera-Cruz et al., 2008).

6.3.4. Microbial growth during the incubation: A comparison

Fig. 6.5 (a). shows the growth of the bacterial community that solubilizes K. During the treatment, the growth of the bacterial community was estimated. The total bacterial count (colony-forming unit-cfu) was significantly (*p* for treatment = 0.01 and LSD = 0.39) higher in soil treated with muscovite (200 and 50 mg kg^{-1}) than in soil treated with biotite and controls (T1 and T2). In the beginning, bacterial cell growth was low at 0 days, but it gradually increased at 60 days; after 90 days, bacterial cell growth decreased (*p* for treatment = 0.01 and LSD = 0.39). For muscovite T4 and T3 and biotite T12 and T11, the most significant growth occurred during incubation in soil (p for days × treatment = 0.001 and LSD = 0.39). The bacterial population in muscovite and biotite-treated soil with the four KSB biofertilizers was in the following order: *Bacillus cereus* K5B > *Bacillus cereus* K6 > *Bacillus sp.GG6* (2015) K12 > *Bacillus cereus* K15 respectively. (Zhang & Kong, 2014) observed that KSB growth increases the decomposition of silicate minerals such as mica (muscovite and biotite) and K feldspar, which transform solid or non-exchangeable K in soil into an available form of K that can be directly absorbed by plants. Under invitro conditions, bacteria such as *B. subtilis* sp. and *B. megaterium* sp. were highly capable of solubilizing K and producing exopolysaccharides (Anjanadevi et al., 2016). (Muthuraja & Muthukumar, 2021), show that *A. violaceofuscus*, *A. niger*, and *A. terreus* from saxum soil solubilize K in Alfisol and Vertisol amended with mica.

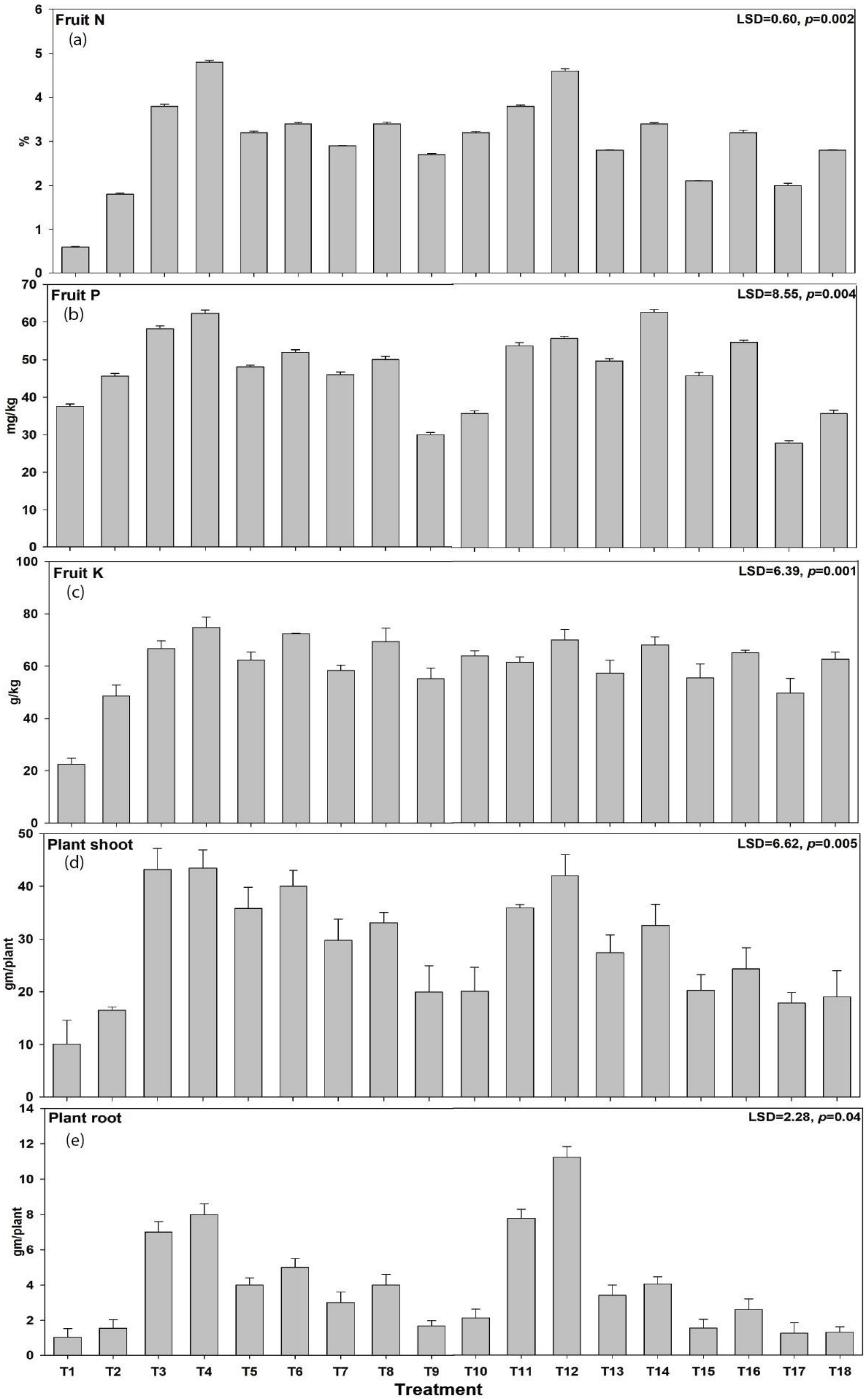


T1-S; T2-S+F; T3-M(50mg/kg)+K5B; T4-M(200mg/kg)K5B; T5-M(50mg/kg)+K6; T6-M(200mg/kg)K6; T7-M(50mg/kg)+K12; T8-M(200mg/kg)K12; T9-M(50mg/kg)+K15; T10-M(200mg/kg)K15; T11-B(50mg/kg)+K5B; T12-B(200mg/kg)K5B; T13-B(50mg/kg)+K6; T14-B(200mg/kg)K6; T15-B(50mg/kg)+K12; T16-B(200mg/kg)K12; T17-B(50mg/kg)+K15; T18-B(200mg/kg)K15.

Fig. 6.5. (a) the temporal changes of microbial proliferation (cfu) in various concentration of (@ 50 and 200 mg K kg-1 soil) of muscovite and biotite as influenced by four KSB bacterial strains (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) at different days of incubation on Alfisol; S(2b) The changes of chlorophyll (chlorophyll-A and B) and carotenoids (Lycopene and β-carotene) content of tomatoes in various concentration of muscovite and biotite as influenced by KSB bacterial strains grown in Alfisol.

6.3.5. Biomass accumulation by a plant during the incubation periods

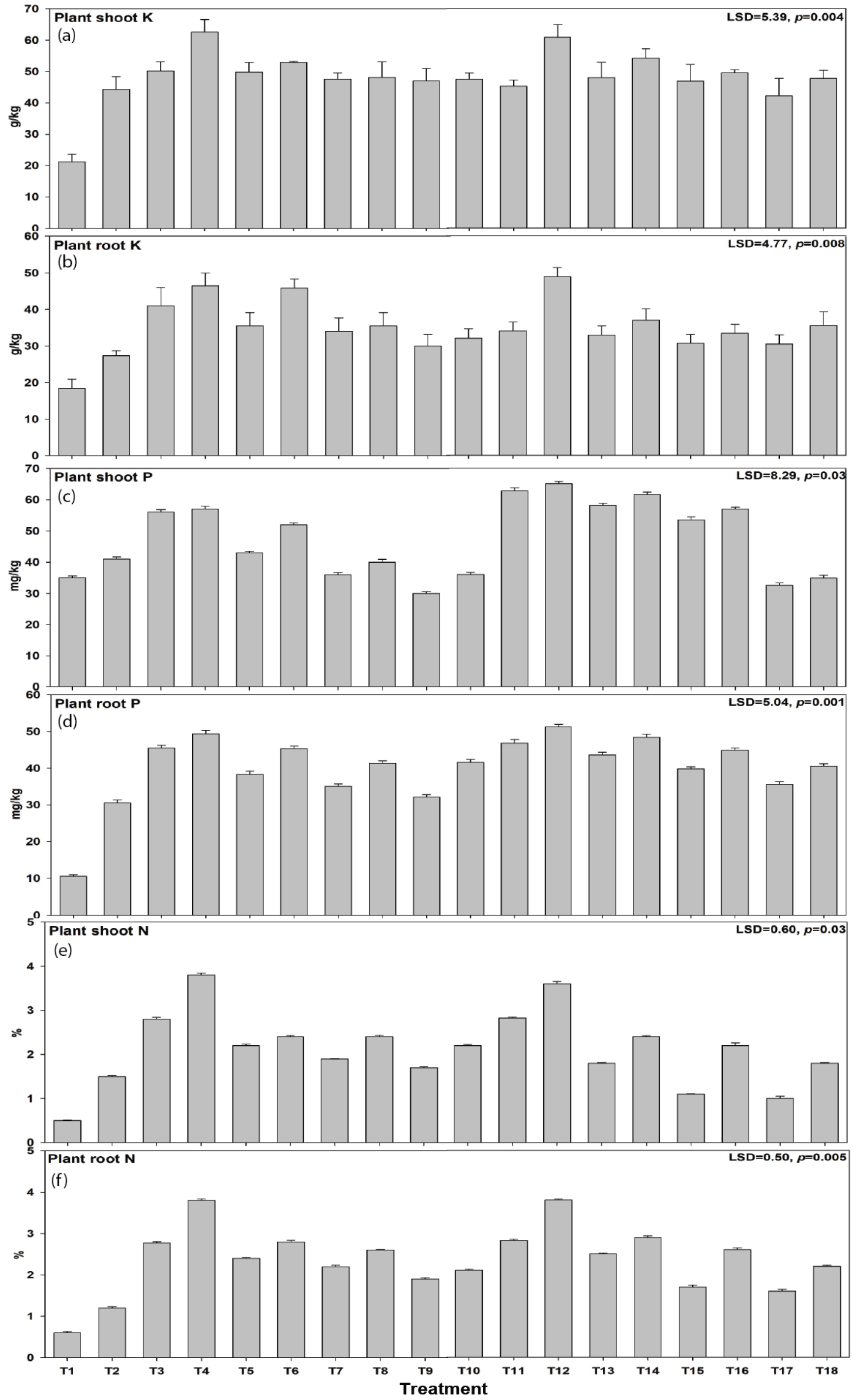
Biomass accumulation (sum of six cuttings) by tomato plants parts (shoot and root) was significantly increased with the addition of muscovite with KSB fertilizers treated soils, compared to both negative and fertilizers treated soils and biofertilizers with KSB fertilizers treated soils (*p* for shoot = 0.005 and LSD = 6.62; *p* for root = 0.04 and LSD = 2.28, respectively) Figs. 6 (d and e). Both muscovite and biotite were best for pant biomass accusation by shoot and root at 200 mg kg^{-1} soil concentration than at 50 mg kg^{-1} soil concentration. *Bacillus cereus* K5B bacterial fertilizers showed that KSB fertilizers outperformed the rest of the three KSB fertilizers. Inoculation with KSBs and mineral K (muscovite and biotite) have both increased biomass compared to control. Therefore, KSB fertilizers had increased biomass of tomato plant yield either directly through the ability of KSB to solubilize soil K reserves and convert them into available K through biological processes or indirectly by stimulating or producing plant-growth-promoting hormones through their promoting activities. Our data is supported by previous findings (Basak & Biswas, 2009, 2010). There was a significant increase in biomass yield due to the inoculation of four KSB strains, which occurred as a result of bacterial strains emitting oxalic, citric, and tartaric acids, resulting in the mobilization of K (Malinovskaya et al., 1990; V. S. Meena et al., 2015). The combination of low-molecular-weight ligands and polymers might affect the weathering of minerals by bacteria like *B. mucilaginosus* (Basak & Biswas, 2010; Malinovskaya et al., 1990). The production of organic acids by K solubilizers facilitates the weathering of minerals by dissolving K from rock and metal-organic complexes like mica, forming a chelate with silicon ions to bring the K into solution (Bennett, 1998). Solubilizing bacteria such as *Bacillus mucilaginosus* and *Bacillus edaphicus* produce carboxylic acids and capsular polysaccharides that are associated with the solubilization of feldspar (X.-F. Sheng et al., 2002). The growth-promoting activity of KSB such as *Bacillus mucilaginosus* strain may also be attributed to the ability to accumulate biomass, which is able to convert unavailable K into available through biological processes (Basak & Biswas, 2009). According to (Vessey, 2003) the positive effect of the KSB strains on tomato biomass accumulation is due primarily to the production of growth-promoting substances, such as their ability to saturate P and fix nitrogen. In the case of nutrients such as available P, the primary mechanism of solubilizing rock P or insoluble P is via H^+ ions excretion and organic acid production (Hinsinger et al., 2003) reported that plant roots exude H^+ ions into the rhizosphere from a higher uptake of cations than anions. It is known that P-solubilizing microorganisms produce organic acids, including citric, oxalic, tartaric, lactic, gluconic, acetic α-ketogluconic acid, etc. These acids are capable of dissolving rock P and converting it into a plant-available form. In addition to reducing pH, organic acid anions can solubilize rock P through a chelating reaction (Basak & Biswas, 2009; Reyes et al., 2007). (Ding et al., 2021) reported that humic acid combined with K-solubilizing bacteria (*Bacillus circulars*) is soluble in a form of K that plants can easily absorb from sandy loam soil. Bio-inoculants such as *Mesorhizobium sp., Paenibacillus sp.*, and *Arthrobacter sp.* inoculated with waste micas increased the concentration of available K after incubation times (Xiao et al., 2017).



T1-S; T2-S+F; T3-M(50mg/kg)+K5B; T4-M(200mg/kg)K5B; T5-M(50mg/kg)+K6; T6-M(200mg/kg)K6; T7-M(50mg/kg)+K12; T8-M(200mg/kg)K12; T9-M(50mg/kg)+K15; T10-M(200mg/kg)K15; T11-B(50mg/kg)+K5B; T12-B(200mg/kg)K5B; T13-B(50mg/kg)+K6; T14-B(200mg/kg)K6; T15-B(50mg/kg)+K12; T16-B(200mg/kg)K12; T17-B(50mg/kg)+K15; T18-B(200mg/kg)K15.

Fig. 6.6. (a-c), Effects of waste muscovite and biotite inoculated with K-solubilizing bacterial strains (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) on nutrient (N,P, and K) acquisition by tomato fruit in an Alfisol; Fig 4 (d-e), Effects of waste muscovite and biotite inoculated with K-solubilizing bacterial strains (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) on biomass yield (gm/pot) of tomato plant root and shoot (sum of six cuttings) after final harvesting in an Alfisol soil.

6.3.6. K uptake during incubation



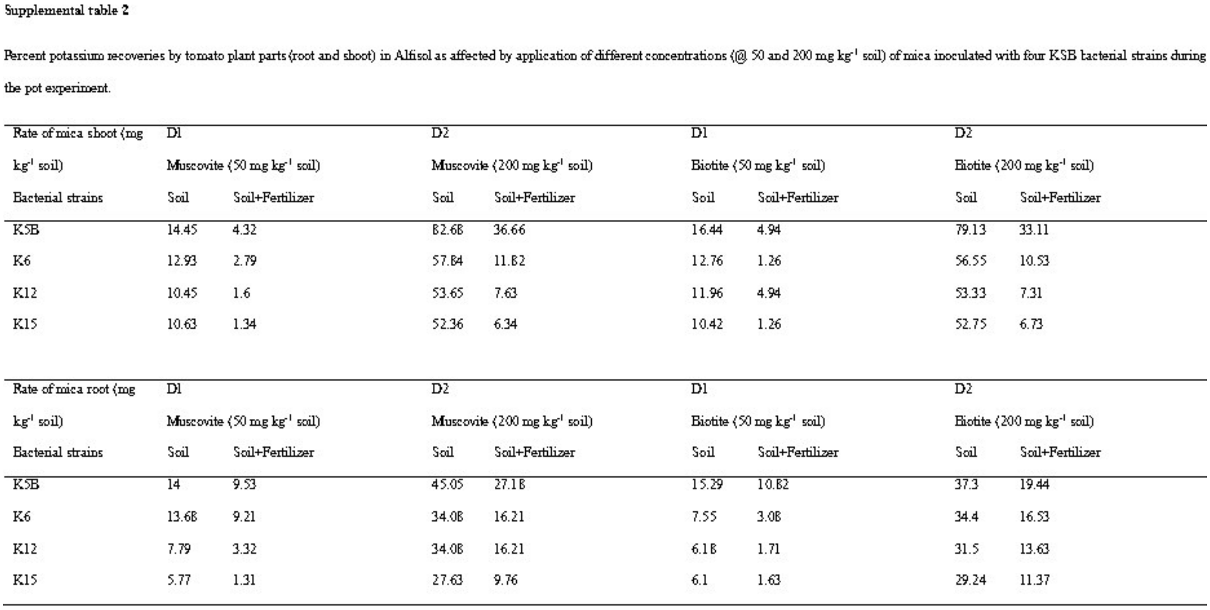
T1-S; T2-S+F; T3-M(50mg/kg)+K5B; T4-M(200mg/kg)K5B; T5-M(50mg/kg)+K6; T6-M(200mg/kg)K6; T7-M(50mg/kg)+K12; T8-M(200mg/kg)K12; T9-M(50mg/kg)+K15; T10-M(200mg/kg)K15; T11-B(50mg/kg)+K5B; T12-B(200mg/kg)K5B; T13-B(50mg/kg)+K6; T14-B(200mg/kg)K6; T15-B(50mg/kg)+K12; T16-B(200mg/kg)K12; T17-B(50mg/kg)+K15; T18-B(200mg/kg)K15.

Fig. 6.7 (a-f). Effects of waste muscovite and biotite inoculated with K-solubilizing bacterial strains (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) on K uptake and nutrient (N and P) acquisition by tomato fruit in an Alfisol.

Root and shoot K uptake (sum of six cuttings) from tomato plants in pot experiments and application of different concentrations of muscovite and biotite treated with four KSB fertilizers resulted in a significantly higher uptake of total K than soil with fertilized soil (*p* for root = 0.008 and LSD = 4.77; *p* for shoot = 0.004 and LSD = 5.39) Fig. 7 (a and b). @ 200 mg kg^{-1} soil best concentration for K uptake by both root and shoot during the pot experiment over @ 50 mg kg^{-1} soil. In muscovite and biotite *Bacillus cereus* K5B fertilized soil showed the best results over the three KSB fertilizers. There is a significant increase in K uptake by tomato plants in Giridih soil (Alfisol) when bio-intervention waste mica is introduced with KSBs. (Xiafang & Weiyi, 2002), also found similar results that confirmed our result; they reported higher total K uptake by K-bearing minerals such as waste micas. K-feldspar was inoculated and incubated with K solubilizing bacteria. In addition, due to the use of illite (K-bearing minerals) infused with *Bacillus edaphicus* NBT strains (KSB), there was a significant increase in root and shoot dry mass yields for cotton and rape. (Basak & Biswas, 2009, 2010) also reported a significant increase in the mass yield of Sudan grass when inoculated with a K-solubilizing bacterial strain *Bacillus mucilaginosus* and incubated with micas. In a study by (Ding et al., 2021) it was shown that vegetative growth of faba bean plants was significantly enhanced in sandy soil with humic acid and inoculation with a K-solubilizing bacteria strain (*Bacillus circulars*). Some research has shown that the application of KSB such as *P. ananatis*, *Enterobacter sp*., and *R. aquatilis* to rice plants can increase yielding mass production (Bakhshandeh et al., 2017). K-solubilizing bacteria strain *B. pseudomycoides* demonstrated significantly higher K uptake by tea plants in K deficient soil at Tyroontea Estate, Titabor, Assam, India (Pramanik et al., 2021). On a rape rhizospheric soil, (Xiao et al., 2017) reported that *Mesorhizobium sp., Paenibacillus sp*., and *Arthrobacter sp.* improved K mobilization from K-bearing minerals and the uptake and growth of ryegrass. Increasing the population of rhizobacteria in the root and rhizosphere soil could be attributed to the higher solubilization of available K from inaccessible forms of K minerals such as muscovite and biotite micas, and this is due to the inoculation with four types of K solubilizing bacteria (KSBs). A few researchers have reported that bacterial inoculation resulted in higher K concentrations in plant parts and increased growth promotion. (Egamberdiyeva & Höflich, 2003).

6.3.7. K content recovery (%)

According to Table 2, the percentage of K varies by root and shoot of tomato plants grown in Giridih soil (Alfisols) with muscovite and biotite and four KSBs. Both plant shoots and roots of tomato plants recovered a higher amount of K from the treatment of 200 mg kg^{-1} soil (D2) than from 50 mg kg^{-1} soil (D1) during the pot experiments. The percent K recovery rate also increased with increased soil incubation time in combination with high concentrations of muscovite and biotite with four KSB biofertilizers. *Bacillus cereus* K5B biofertilizer treated soils showed the best percent K recovery over the other three KSB fertilizers and they were ranked as *Bacillus cereus* K5B > *Bacillus cereus* K6 > *Bacillus sp.*GG6 (2015) K12 > *Bacillus cereus* K15. When KSB biofertilizer treaded soils were compared to negative controls (only soil) and positive controls (S+F), the percent of K recovered was higher on the negative control. A higher biomass accumulation can be attributed to the soil having a greater number of different K pools, including water-soluble K, exchangeable K, non-exchangeable K, and total K, as well as available P and N in the initial soil. These results supported the findings of earlier research that Sudan grass produces a greater percent K recovery when inoculated with KSB strain (*Bacillus mucilaginosus*) (Basak & Biswas, 2009).



Supplemental table 2

Percent potassium recoveries by tomato plant parts (root and shoot) in Alfisol as affected by application of different concentrations (@ 50 and 200 mg kg^{-1} soil) of mica inoculated with four KSB bacterial strains during the pot experiment.

Rate of mica shoot (mg kg^{-1} soil)	D1		D2		D1		D2	
	Muscovite (50 mg kg^{-1} soil)		Muscovite (200 mg kg^{-1} soil)		Biotite (50 mg kg^{-1} soil)		Biotite (200 mg kg^{-1} soil)	
Bacterial strains	Soil	Soil+Fertilizer	Soil	Soil+Fertilizer	Soil	Soil+Fertilizer	Soil	Soil+Fertilizer
K5B	14.45	4.32	82.68	36.66	16.44	4.94	79.13	33.11
K6	12.93	2.79	57.84	11.82	12.76	1.26	56.55	10.53
K12	10.45	1.6	53.65	7.63	11.96	4.94	53.33	7.31
K15	10.63	1.34	52.36	6.34	10.42	1.26	52.75	6.73

Rate of mica root (mg kg^{-1} soil)	D1		D2		D1		D2	
	Muscovite (50 mg kg^{-1} soil)		Muscovite (200 mg kg^{-1} soil)		Biotite (50 mg kg^{-1} soil)		Biotite (200 mg kg^{-1} soil)	
Bacterial strains	Soil	Soil+Fertilizer	Soil	Soil+Fertilizer	Soil	Soil+Fertilizer	Soil	Soil+Fertilizer
K5B	14	9.53	45.05	27.18	15.29	10.82	37.3	19.44
K6	13.68	9.21	34.08	16.21	7.55	3.08	34.4	16.53
K12	7.79	3.32	34.08	16.21	6.18	1.71	31.5	13.63
K15	5.77	1.31	27.63	9.76	6.1	1.63	29.24	11.37

6.3.8. Nutrient acquisition by a tomato plant and fruit

In pot experiments, root, shoot, and fruits of tomato plants were measured for nutrient acquisition Figs 6. (a, b, and c) and figs 7. (c, d, e, and f). Nutrient uptake was highest in muscovite with KSB fertilizer treated soils over biotite with KSB fertilizer treated soils as well as with both positive and negative controls for nitrogen and phosphorus uptake. It was found that for phosphorus uptake by plants, biotite provided the best results in comparison to muscovite when it was planted on KSB-treated soils and when it was planted without fertilizers. The highest nutrient acquisition by tomato plant parts was observed at 200 mg kg^{-1} soil of both muscovite and biotite over 200 mg kg^{-1} soil. With muscovite and biotite, biofertilizers such as *Bacillus cereus* K5B showed significantly ($p < 0.05$) the best results for improving nutrient uptake by tomato roots, shoots, and fruits over the other KSB fertilizers. *Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15GRD strains have also provided evidence that K solubilizers can dissolve P and fixed N during the incubation period (my second paper). (Ding et al., 2021) found that bio-inoculation with K-solubilizing bacteria as *Bacillus circulars* applied with humic acid (HA) increased the availability of N and P. In the studies (Basak & Biswas, 2010) conclude that co-inoculation of waste mica with two types of bacterial strains (*B. mucilaginosus* and *A. chroococcum* A-41) can enhance nutrient uptake by Sudan grass. As well as mobilizing K from waste mica due to secretion of organic acids by K solubilizers, thus enhancing and developing plant growth and greater uptake of nutrients. (Bennett et al., 1998; Friedrich et al., 1991) found that the organic acids produced by K-solubilizers solute the weathering of minerals by bringing directly soluble K from rocks or micas into the solution. In a greenhouse experiment, it was suggested that co-inoculation of biofertilizer with N fixer (*A. chroococcum*), P solubilizer (*B. megatherium*), K solubilizer (*B. mucilaginosus*), and arbuscular mycmycorhizae fungi (*Glomus mosseae* and *G. intradices*) significantly increased the uptake of minerals (N, P, and K) by maize (S. C. Wu et al., 2005). Recent studies found that K solubilizers could increase the uptake of nutrients such as N, P, and K by faba bean (Abdel-Salam & Shams, 2012; Labib et al., 2012). Recent studies found that K solubilizers could increase the uptake of nutrients such as N, P, and K by faba bean (Abdel-Salam & Shams, 2012; Labib et al., 2012). The application of bio-fertilizers (*Bacillus cereus*) on potato plants significantly increased nutrients such as N, P, and K, as reported by (Ali et al., 2021).

6.3.9. Agronomical characteristics of tomato plant

The use of waste muscovite and biotite with four KSBs significantly increased the growth, fruit yield, and quality of the plant compared to that of the control-treated plant ($p > 0.05$). In present studies, initially plant height increased slowly up to one month (30 days) after transplanting (DAT) and then increased rapidly during final harvesting (90 days) DAT (Table 1). All the treatments followed almost similar fashions due to the application of different fertilized and unfertilized treatments on plants. It was found that when muscovite or biotite treated soil was applied, plant growth such as plant height was higher at 200 mg K kg^{-1} soil concentration over 50 mg K kg^{-1} soil, and on an average plant significantly (p for plant height = 0.03 and LSD = 21.29) height was observed for muscovite treated with four KSB fertilizers as opposed to biotite treated with four KSB fertilizers and both negative (soil only) or positive controls (soil and fertilizer) respectively. It was observed that the leaf area of tomato plants of KSB fertilizers with muscovite and biotite treated soils was significantly (p for leaf area = 0.03 and LSD = 1.13) higher at 90 days after the pot experiment as compared to both negative (only soil) and positive (S + F) control. Biotite (@ 200 and 50 mg kg^{-1} soil + *Bacillus cereus* K5B) treated plants had a greater leaf diameter than those treated with muscovite (@ 200 and 50 mg kg^{-1} soil + *Bacillus cereus* K5B) Fig.7 (a). Due to the integrated application of muscovite and biotite with four KSB biofertilizers, the total number of flowers, fruits, and an average weight of tomato fruits per treated plant significantly increased (p for flower = 0.006 and LSD = 19.92; p for fruit = 0.002 and LSD = 8.82; p for the weight of fruit = 0.04 and LSD = 2.28) Fig. 8(b-d) The treatments T4 and T3 for muscovite and T12 and T11 for biotite were successful. It has been determined that *Bacillus cereus* K5B fertilizers have the best effects on tomato flowering and fruit, and they were ranked *Bacillus cereus* K5B > *Bacillus cereus* K6 > *Bacillus sp.*GG6 (2015) K12 > *Bacillus cereus* K15. The different treatment data were computed significantly influences the percent of fruit per set. Among the treatments, muscovite (@ 200 and 50 mg kg-1 soil) had the highest percent fruit set while biotite (@ 200 and 50 mg kg^{-1} soil), T2 (S + F), and T2 (only soil) had the lowest percent fruit set. A biofertilizer treated with *Bacillus cereus* K5B yielded the highest fruit yield compared to a biofertilizer treated with *Bacillus cereus* K6.

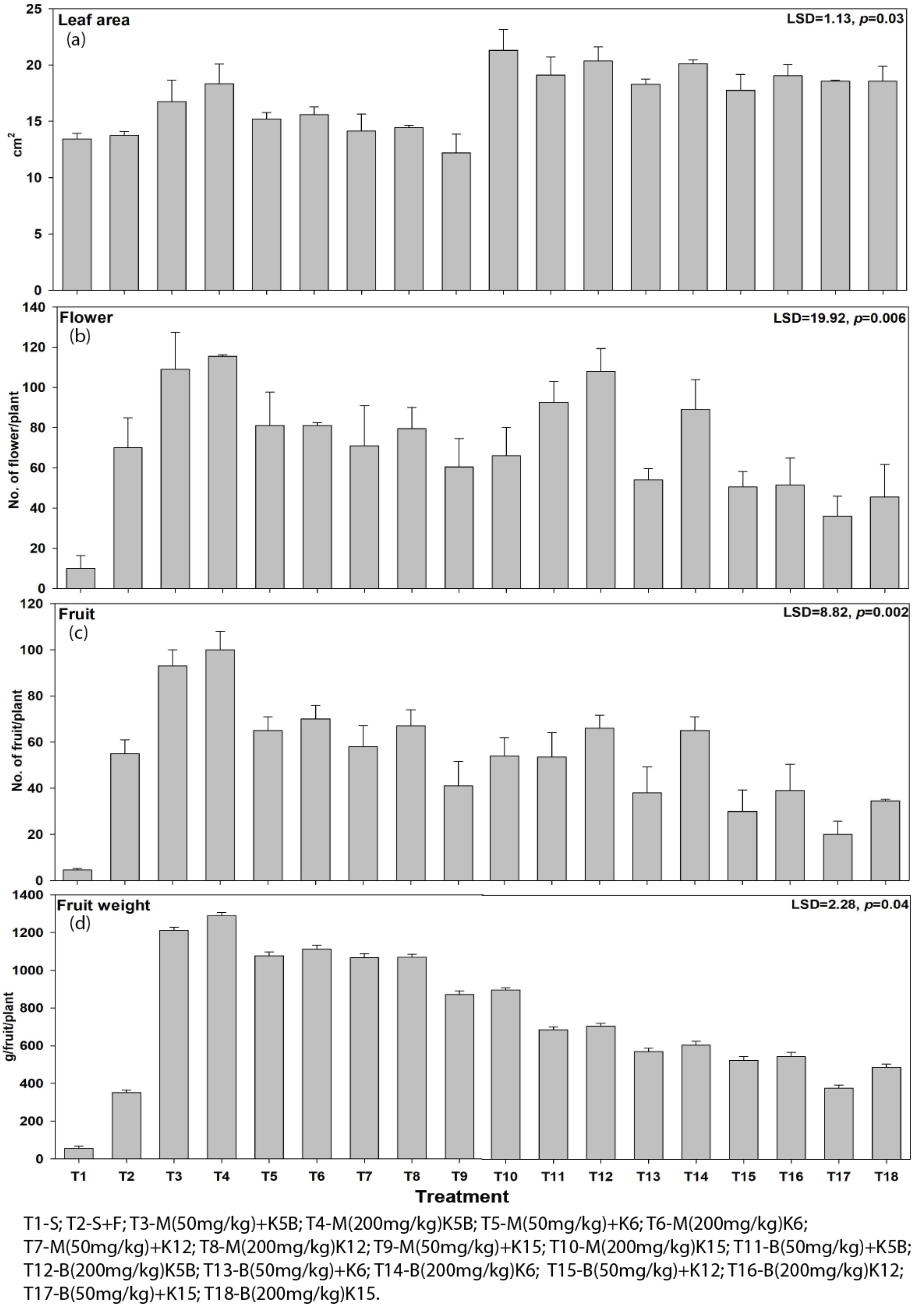
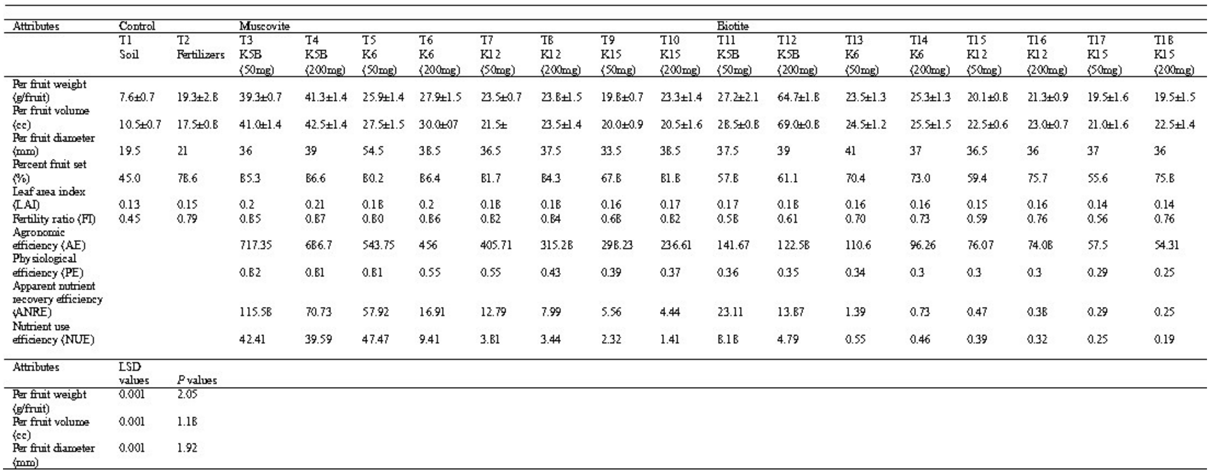


Fig. 6.8 (a-d) Effects of waste muscovite and biotite inoculated with K-solubilizing bacterial strains (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, and *Bacillus cereus* K15) on leaf area, number of flowers per plant, number of fruits per plant, and fruit weight in an Alfisol.

We calculated the following fruit quality attributes after harvest: yielding fruit weight (g/fruit), yielding fruit volume (cc), and yielding fruit diameter (mm). Combined application of muscovite and biotite with four KSB biofertilizers resulted in significant ($p > 0.05$) differences in fruit quality when compared to the controls (T1 and T2) Table3.

Table 1

Effects of different concentration (@ 50 and 200 mg K kg^{-1} soil) of muscovite and biotite as influenced by KSB bacterial strains on various agronomical attributes by tomato plants grown in Alfisols (Mean ± SD).



Attributes	Control		Muscovite								Biotite							
	T1 Soil	T2 Fertilizers	T3 K5B (50mg)	T4 K5B (200mg)	T5 K6 (50mg)	T6 K6 (200mg)	T7 K12 (50mg)	T8 K12 (200mg)	T9 K15 (50mg)	T10 K15 (200mg)	T11 K5B (50mg)	T12 K5B (200mg)	T13 K6 (50mg)	T14 K6 (200mg)	T15 K12 (50mg)	T16 K12 (200mg)	T17 K15 (50mg)	T18 K15 (200mg)
Per fruit weight (g/fruit)	7.6±0.7	19.3±2.8	39.3±0.7	41.3±1.4	25.9±1.4	27.9±1.5	23.5±0.7	23.8±1.5	19.8±0.7	23.3±1.4	27.2±2.1	64.7±1.8	23.5±1.3	25.3±1.3	20.1±0.8	21.3±0.9	19.5±1.6	19.5±1.5
Per fruit volume (cc)	10.5±0.7	17.5±0.8	41.0±1.4	42.5±1.4	27.5±1.5	30.0±07	21.5±	23.5±1.4	20.0±0.9	20.5±1.6	28.5±0.8	69.0±0.8	24.5±1.2	25.5±1.5	22.5±0.6	23.0±0.7	21.0±1.6	22.5±1.4
Per fruit diameter (mm)	19.5	21	36	39	54.5	38.5	36.5	37.5	33.5	38.5	37.5	39	41	37	36.5	36	37	36
Percent fruit set (%)	45.0	78.6	85.3	86.6	80.2	86.4	81.7	84.3	67.8	81.8	57.8	61.1	70.4	73.0	59.4	75.7	55.6	75.8
Leaf area index (LAI)	0.13	0.15	0.2	0.21	0.18	0.2	0.18	0.18	0.16	0.17	0.17	0.18	0.16	0.16	0.15	0.16	0.14	0.14
Fertility ratio (FI)	0.45	0.79	0.85	0.87	0.80	0.86	0.82	0.84	0.68	0.82	0.58	0.61	0.70	0.73	0.59	0.76	0.56	0.76
Agronomic efficiency (AE)			717.35	686.7	543.75	456	405.71	315.28	298.23	236.61	141.67	122.58	110.6	96.26	76.07	74.08	57.5	54.31
Physiological efficiency (PE)			0.82	0.81	0.81	0.55	0.55	0.43	0.39	0.37	0.36	0.35	0.34	0.3	0.3	0.3	0.29	0.25
Apparent nutrient recovery efficiency (ANRE)			115.58	70.73	57.92	16.91	12.79	7.99	5.56	4.44	23.11	13.87	1.39	0.73	0.47	0.38	0.29	0.25
Nutrient use efficiency (NUE)			42.41	39.59	47.47	9.41	3.81	3.44	2.32	1.41	8.18	4.79	0.55	0.46	0.39	0.32	0.25	0.19

Attributes	LSD values	*P* values
Per fruit weight (g/fruit)	0.001	2.05
Per fruit volume (cc)	0.001	1.18
Per fruit diameter (mm)	0.001	1.92

It was found that the weight, volume, and diameter of a single fruit per plant were higher in T12 and T11 for biotite (@ 200 and 50 mg kg^{-1} soil + *Bacillus cereus* K5B) and in T4 and T3 for muscovite (@ 200 and 50 mg kg^{-1} soil + *Bacillus cereus* K5B). In this study, agronomical indices such as leaf area index, fertility ratio, agronomic efficiency, physiological efficiency, apparent nutrient recovery efficiency, and nutrient use efficiency were calculated after harvest. During the pot experiment, muscovite (@ 200 mg kg^{-1} soil + *Bacillus cereus* K5B) and biotite (@ 200 mg kg^{-1} soil + *Bacillus cereus* K5B) showed the best results from the agronomical indexes with the four KSBs treated soil. It was found that different muscovite treatments were performed in the following order: (T4 - T3) followed by (T6 - T5), (T8 - T7), and (T10 -T9), respectively; similarly, biotite treatments were performed in the following order: (T12 - T11) followed by (T14 - T13), (T16 - T15), and (T18 - T17). In the case of general fertility, a higher proportion was found in the application of muscovite-treated soils than in biotite-treated.

Agricultural skills create a short-term indicator of the effect of applied nutrients on productivity. Phytohormones or plant hormones are responsible for increasing plant height because they increase cell division and ensure proper propagation of the stems, which produces a prolonged plant in the tomato (Islam et al., 2013). Few researchers find out a similar trend of results; in tomato plants (Islam et al., 2013; Uddain et al., 2009; C. W. Wu et al., 1983). However, other studies have shown that plant height decreased with increased concentrations of plant hormones that were different from ours (Chhonkar & Ghufran, 1968). According to (Gosavi et al., 2010), the combined application of organic and inorganic fertilizers may increase leaf area by enhancing the synthesis and translocation of metabolically active enzymes, in turn promoting cell division and proliferation through the combined effects of zinc acids and auxin (a growth-promoting compound). Few studies have shown that K-solubilizing bacteria can produce growth-promoting hormones like auxin, gibberellic acid, hydrogen cyanide, ammonia, and siderophore that can help promote plant growth (V. S. Meena et al., 2016). As a result of the application of waste mica (muscovite and biotite) with KSBs, the reproductive parameters like the number of flowers and fruit per plant showed the best results. After transplanting, the number of flowers per plant gradually increases up to 60-75 days, then decreases. During the months of February and March, the potency of the flowers was reduced with the increase in the temperature, and the higher flowering that was obtained was the result of the use of K solubilizers, which are essential macro elements for plant growth and flowering (V. S. Meena et al., 2015, 2016). The number of total fruits produced during the pot treatment was higher when the plants received adequate nutrients during the growing period. It was gradually increased until the final harvest (90 days), but with the decreased, perhaps lower production of productive flowers, the number of fruits per plant decreased. Based on the use of an integrated combination of organic fertilizer, chemical fertilizer, and poultry manure, higher flowers were obtained per treated plant (Farhad et al., 2009; Islam et al., 2013). According to (Molla et al., 2012; Yeptho et al., 2012), the application of bio-fertilizers and their effects on plant growth by improving the availability of nutrients were the keys to yielding more flowers per plant and more fruits.

Various parameters, such as total fruit weight per plant, per single fruit weight, fruit volume, and fruit diameter, tended to be higher when soils were treated with waste micas (muscovite and biotite) and four types of KSBs. Many workers have reported that they have found higher-yielding parameters in tomato plants during the experiments. As part of a trial experiment, capsicum was grown under a poly house with 50 percent fertigation and 50 percent foliar-applied according to growth properties (Kaur et al., 2017).

(Bihari et al., 2018) concluded that the recommended dose of fertilizer with 15 MT ha^{-1} organic matter increased tomato crop growth and yield as well as nutrient concentration and absorption (recommended) levels over the control (which is in the ground). P and K together promote the production of auxins, which increase the permeability of cell membranes, contributing to tomato fruit's growth and size. Furthermore, auxins stimulate the synthesis of specific DNA-dependent mRNA and specific enzymatic proteins to increase cell flexibility and ultimately lead to the proliferation of cells. In addition, the increased size of fruits may result from plants assimilating more nutrients and producing more food when using organic fertilizers (Chatterjee & Bandyopadhyay, 2014; Kanwer et al., 2014). Using plant growth-promoting rhizobacteria with arbuscular mycorrhizal fungi (AMF) to increase the K content of lycopene, antioxidant activity, and shoots and fruits of the tomato plant may influence the plant's growth (Ordookhani et al., 2010). Using plant growth-promoting rhizobacteria with arbuscular mycorrhizal fungi (AMF) to increase the K content of lycopene, antioxidant activity, and shoots and fruits of the tomato plant may influence the plant's growth (Ordookhani et al., 2010). Various indexes like the leaf area index (LAI), the fertility ratio (FR), agronomic efficiency (AE), physiological efficiency (PE), apparent nutrient recovery efficiency (ANRE), and nutrient use efficiency (NUE) play an important role in crop production systems; all of those parameters can be significantly impacted by fertilizer management as well as by soil-plant water management. (Dobermann, 2007) reviewed NUE measurements and calculations as well as their application and limitations according to different crop systems. LAI is an important amount that controls the physical and biological processes of the plant canopy. In general, LAI relates to radiation resistance and is the total one-sided leaf area per unit of ground surface area (Chen & Black, 1992). According to (R & Reddy, 2007), the plant fertility ratio is a measure of the capacity of each plant to produce fruit based on its total flower production. Using the nutrient increase in yield per unit as a measure of the effectiveness of K-feldspar blended with organic matter and dissolved bacteria (Badr, 2006), the efficacy is calculated. This more closely reflects the direct production effect of applied fertilizer and is directly related to the economic return. (Chuan et al., 2013) found higher AE was higher in N, P, and K-treated soil. N acceptance and AE use of high-yielding varieties of wheat and barley have been tested with rates of 0, 140, and 210 kg ha-1 and 0, 80, and 140 kg N^{-1}, respectively (Delogu et al., 1998). Physiological efficiency (PE) is defined as the increase in yield associated with the increase in crop nutrient uptake in the topsoil of plants. For AE and RE, a plot without the application of the nutrients of interest must be set up; the nutrient density must also be measured in crops and it is mostly used for scientific research. A plant's apparent recovery efficiency (RE) is one of the more complex ways of expressing NUE and is defined as the difference between nutrient uptake between a plant and a soil crop compared to the amount typically applied. NUE is often the preferred expression for scientists studying grain nutrient responses (Ordookhani et al., 2010) showed that inoculating tomato roots with PGPR and AMF improves fruit quality. When K-feldspar is combined with organic materials and silicate or insoluble K dissolving bacteria as well as organic materials and organic materials combined with K-feldspar, the fruit and shoots contained higher K content and uptake 150 days after transplanting (Badr, 2006).

6.3.10. Correlation matrix

The data were analyzed using Pearson's correlation matrix in Giridih soil (Alfisol) treated with muscovite and biotite with four KSB bacterial fertilizers. During the soil incubation periods of the pot experiments, we observed that tomato plant parts (root, shoot, and fruit) accumulated the most biomass and accumulated the most potassium from muscovite and biotite-treated with KSB biofertilizers soils Table 4. Using Pearson's correlation analysis, we have analyzed the relationship between different forms of K within them, biomass and fruit yield, and K uptake by root, shoot, and fruit of tomato plants. As a result of the pot experiment, muscovite with four KSB soils, biomass yielded by tomato plants (shoots and roots), and total fruit yield were positively and significantly related to water-soluble, exchangeable, and non-exchangeable K levels [r : biomass yield (shoot) : Wa.K = 0.72*, Ex.K = 0.67, and NEK = 0.64; r : biomass yield (root) : Wa.K = 0.56, Ex.K = 0.78*, and NEK = 0.80**; r : tomato fruit yield : Wa.K = 0.64, Ex.K = 0.81**, and NEK = 0.78*respectively]. Biotite with four KSB treated soils, biomass yielded by tomato plants (shoot and root), as well as total fruit yield were highly positive and significantly correlated with Wa.K and Ex.K [r : biomass yield (shoot) : Wa.K = 0.88**, Ex.K = 0.93**, and NEK = 0.21; r : biomass yield (root) : Wa.K = 0.85**, Ex.K = 0.85*, and NEK = 0.14; r : tomato fruit yield : Wa.K = 0.94**, Ex.K = 0.83**, and NEK = 0.05 respectively].

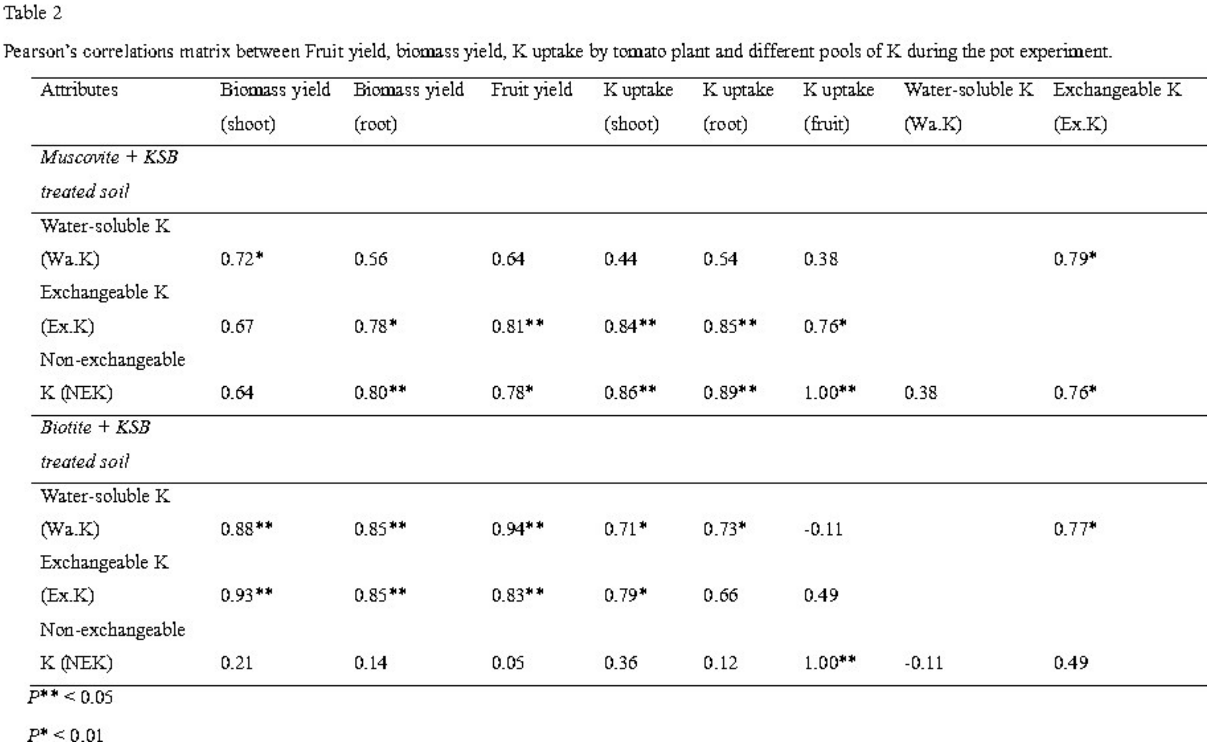


Table 2

Pearson's correlations matrix between Fruit yield, biomass yield, K uptake by tomato plant and different pools of K during the pot experiment.

Attributes	Biomass yield (shoot)	Biomass yield (root)	Fruit yield	K uptake (shoot)	K uptake (root)	K uptake (fruit)	Water-soluble K (Wa.K)	Exchangeable K (Ex.K)
Muscovite + KSB treated soil								
Water-soluble K (Wa.K)	0.72*	0.56	0.64	0.44	0.54	0.38		0.79*
Exchangeable K (Ex.K)	0.67	0.78*	0.81**	0.84**	0.85**	0.76*		
Non-exchangeable K (NEK)	0.64	0.80**	0.78*	0.86**	0.89**	1.00**	0.38	0.76*
Biotite + KSB treated soil								
Water-soluble K (Wa.K)	0.88**	0.85**	0.94**	0.71*	0.73*	-0.11		0.77*
Exchangeable K (Ex.K)	0.93**	0.85**	0.83**	0.79*	0.66	0.49		
Non-exchangeable K (NEK)	0.21	0.14	0.05	0.36	0.12	1.00**	-0.11	0.49

$P^{**} < 0.05$

$P^{*} < 0.01$

The total potassium uptake by the tomato plant parts (root, shoot, and fruit) from muscovite with four KSB bacterial biofertilizer soils was highly correlated with exchangeable and non-exchangeable forms of K from mica treated soils over water-soluble forms [r : biomass yield (shoot) : Wa.K = 0.44, Ex.K = 0.81**, and NEK = 0.86**; r : biomass yield (root) : Wa.K = 0.54, Ex.K = 0.85*, and NEK = 0.89; r : tomato fruit yield : Wa.K = 0.38, Ex.K = 0.76*, and NEK = 1.00** respectively]. Following application of biotite with four KSB biofertilizers, K uptake by tomato plant shoots was strongly positive and significantly correlated with Wa.K and Ex.K over NEK, and K uptake by plant roots was significantly correlated with Wa.K than Ex.K and NEK. A positive correlation was found between Wa.K, Ex.K, and NEK during the pot experiment [r : biomass yield (shoot) : Wa.K = 0.71*, Ex.K = 0.79*, and NEK = 0.36; r : biomass yield (root) : Wa.K = 0.73*, Ex.K = 0.66, and NEK = 0.12; r : tomato fruit yield : Wa.K = -0.11, Ex.K = 0.49, and NEK = 1.00** respectively].

In between three forms of K of muscovite treated soils, Wa. K, Ex. K, and NEK, Wa. K was significantly and positively correlated with Ex. K (r = 0.79*) then NEK (r = 0.38); the Ex. K form of K was significantly and positively correlated with NEK (r = 0.76*). In biotite-treated soils, Wa. K was positively and significantly correlated to Ex. K (r = 0.77*) and negatively related to NEK (-0.11); with Ex. K, from was positively related to NEK forms of K (r = 0.49).

It was found that there was a significant correlation ($p > 0.05$) between total biomass accumulation, total tomato fruit yields, and K uptake with water-soluble, exchangeable, and non-exchangeable K in muscovite and biotite. Few researchers have reported that water-soluble K is depleted in the soil after crop uprooting, and it's replaced by exchangeable K. Moreover, non-exchangeable K is converted into exchangeable K forms, and then this exchangeable form is converted into available or soluble K poos (Basak & Biswas, 2009). Different forms of K pools may emerge due to harvesting from the available pool of K, because some of the non-convertible K of the crop emphasized may be converted into exchangeable form. The amount of non-exchangeable forms of K obtained in both muscovite- and biotite-treated soils were significantly and positively correlated with the amount of exchangeable forms of K. According to previous research, a complex interaction in soil between different types of minerals and biological processes can enhance the availability of structural K (Basak & Biswas, 2009; Hinsinger, 2002). There was a significant association between the cumulative K uptake by plants and the release of soluble potassium from clay minerals both exchangeable and non-exchangeable (Basak & Biswas, 2009; Bhattach; Jalali, 2005).

6.3.11. Chlorophyll and carotenoid content in mature tomato fruit

The use of muscovite and biotite, the primary sources of potassium, with four types of KSB biofertilizers had a significant impact on after-harvest tomato fruit chlorophyll (chlorophyll-a and chlorophyll-b) carotenoid (lycopene and β-carotene). During the pot experiment, our results revealed that the pigmentation concentration of chlorophyll-a, chlorophyll-b, lycopene, and β - carotene was higher when biotite (200 and 50 mg kg^{-1} soil) and four KSB biofertilizers were applied rather than muscovite (200 and 50 mg kg^{-1} soil) and four KSB biofertilizers even both negative (T1, only soil) and positive (T2, S+F) controls Fig.5(b) Muscovite and biotite both performed best with a concentration of @ 200 mg kg^{-1} soil, compared to a concentration of @ 50 mg kg^{-1} soil. Among the harvest of biotite with KSB biofertilizer soil T12 (@ 200 mg kg^{-1} soil + *Bacillus cereus* K5B) and T11 (@ 50 mg kg^{-1} soil + *Bacillus cereus* K5B); and in muscovite with KSB biofertilizer soil T4 (@ 200 mg kg^{-1} soil + *Bacillus cereus* K5B) and T3 (@ 50 mg kg^{-1} soil + *Bacillus cereus* K5B) was found highest concentration of tomato fruit chlorophyl and carotenoid [Biotite: (T4-T3) > (T6-T5) > (T8-T7) > (T10-T9) respectively; Muscovite: (T12 - T11) > (T14 - T13) > (T16 - T15) > (T18 - T17) respectively]. There was a lower concentration of chlorophyll-a and chlorophyll-b in the controls not treated with KSB biofertilizers. (Dorais, 2005) reviewed the relationship between K content and lycopene content; when K is increased in the nutrient solution, tomato fruit contains more lycopene. The study (Dorais, 2005) reported that tomatoes contain a wide range of health compounds, such as antioxidants, vitamins, carotenoids, and flavonoids, and they are also a good source of potassium and fiber. A concentration increase of up to 8 meq of lycopene is observed after potassium supplementation (Dorais, 2005). According to (Dorais, 2005), different concentrations of K were tested in soil-free culture, and the tomato plants with the highest concentration of K had the highest lycopene content. Microorganisms that solubilize K, such as KSB and AMF, help to separate soluble K from insoluble K present in the soil, and that soluble K can help plant growth, yield, and fruit quality (V. S. Meena et al., 2016).

6.4. Conclusion

The results of the study conclude that muscovite and biotite applied in combination with four types of K solubilizing microbes (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.GG6* (2015) K12, *Bacillus cereus* K15) significantly improve biomass yield, K uptake, and recovery, nutrient acquisition, fruit quality (chlorophyll-a, b, lycopene, and -carotene content) and yield of tomato plants grown under Alfisols with waste mica and KSB due to higher K solubilization. The combination of waste micas (muscovite and biotite) with K solubilizers showed the best results when compared to traditionally used fertilizers (N, P, and K). The combination of muscovite and biotite at 200 mg kg^{-1} soil showed the best results in developing plant growth, fruit yields, and maintaining fruit quality. A combination of different KSB biofertilizers with waste muscovite and biotite enhances soil K pools (water-soluble, exchangeable, and non-exchangeable K), increasing soil K availability for plant uses. In general, the application of *Bacillus cereus* K5B biofertilizer demonstrated the best K solubility from different concentrations of muscovite and biotite treated soils than the other three KSB biofertilizers. Furthermore, KSB biofertilizers demonstrated significant improvements in soil health such as the enrichment of macronutrients (N, P, and K) and microbial enzyme activity in the soil. Bio-intervention with muscovite and biotite (sources of insoluble K) can be a viable alternative and effective technology for dissolving insoluble K in soluble form. In addition, it can be used as a source of K fertilizer to maintain crop production and maintain potassium levels in the soil. To a great extent, further studies are required to see whether these types of bacteria have any beneficial effects on the mobilization of K-bearing micas in the field.

CHAPTER-7. SUMMARY & CONCLUSION

7.A. Characterization of waste mica and mica contaminated agricultural soils

Physical, physicochemical, chemical, biochemical, and microbiological methods were used to evaluate the maturity and stability of waste mica tailings from Gawan, Tisri, and Deori blocks of Giridih district, Jharkhand.

Mica was fine, loose, homogenous, had low bulk density and moisture-holding capacity, and was devoid of nutrients. The amount of water-soluble, exchangeable, non-exchangeable, and total K was determined using standard procedure and the concentration of ground waste mica had 10.0% of total K, 30.0 mg kg−1 of WK, 157.5 mg kg−1 of EK, and 260.0 mg kg−1 of NEK.

The soil samples were predominantly Alfisols (63%). The mean sand, slit, and clay percentages were 45.35 %, 34.5 %, and 19.4 respectively. The sample possessed a moderate water holding capacity (63%). The samples were slightly acidic (pH, 5.5 – 6.06). The sample contains low organic-C (0.61%) and low nitrogen (0.24%) respectively. The CEC of the samples was low.

Different forms of K, as well as mean total and non-exchangeable K pools, were higher on the mica tailings side and exchangeable and available forms of K were higher farther from mica tailings.

The total heavy metal concentration, bioavailable forms of metals (DTPA extractable), water, and forms of metal (Cd, Cr, Cu, Ni, Pb, and Zn) content were higher in mica mines site (zone 1) soil samples as compared to zone 2 and zone 3 (agricultural soil).

There was an increase in biochemical parameters like urease, acid phosphatase, MBC, FDA, DHG, APS, and β-D glucosidase concentration at 100 meters, which means further away from mines. All the biological parameters were higher in the agricultural field (zone3) soil samples as compared to mine side (zone 1) soils. Soil microbes were found to be affected by higher concentrations of different forms of heavy metal ions.

According to the results, metal contamination is most critical in zone 1 soils since it contains significantly more bioavailable metals than those in other studied soils such as zone 2 and zone 3. According to the results, metal contamination is most critical in zone 1 soils. This is because it contains significantly more bioavailable metals than those in other studied soils such as zone 2 and zone 3. The most abundant heavy metals in mica-contaminated paddy soil samples were chromium (Cr), nickel (Ni), and lead (Pb), contributing to the highest bio-accessibility Zn concentrations in paddy grains, followed by Ni, Cu, Cr, Cd, and Pb.

7.B. Source acquainted pollution and health risk assessment of potentially toxic elements in mine tailings contaminated soils in India employing synergistic statistical approaches

The presence of potentially toxic elements (PTEs) in this mica waste hampered crop production, deteriorated soil quality, and causes different health issues. The soil quality was evaluated using four different indexing methods such as metals pollution index (PI), contamination index (CI), ecological risk index (ERI), and geoaccumulation index (Igeo). A carcinogenic (cancer) and noncarcinogenic risk to the mines workers was determined by estimating the levels of PTEs through ingestion, inhalation, and dermal contact.

Metal contamination zones in mica soils were mapped using geostatistical analysis, Monte Carlo simulations, sensitivity analysis, and DTPA extractable metals.

The values of PTE concentrations were higher in mine side zone samples (zone 1) as compared to zone 2 and 3. As a result of this, mine workers and children may suffer from several health problems caused by PTEs (Cr, Ni, Pb, Cu, Cd, and Zn). According to the soil quality index based on the heavy metal pollution index, contamination index, and ecological risk index, contamination was significantly more pronounced in mine-side soil samples (zone 1), possibly due to the presence of a high concentration of total and bioavailable metals.

Using the Monte Carlo simulation model, TCR was higher in children than in adults because of their body ratio. Both adults and children were significantly affected by Cr, Cd, and Ni concentrations in the sensitivity analysis. Inhalation and dermal contact significantly increase the risk of cancer in adults and children when Cr and Ni concentrations are high.

7.C. Isolation of K solubilizing bacterial isolates from rhizospheric soils of Giridih district, Jharkhand

A total of 63 agricultural soil samples were collected from 21 mica mines in 3 blocks of Giridih district, Jharkhand.

7.C.1. Isolation and screening of KSB

A total of 95 KSB isolates were isolated from the rhizospheric soil of the Giridih district. On a solid Aleksandrw agar medium, isolates were tested for solubilization by growth and production of a zone of solubilization. KSB isolates with a clear zone of solubilization were selected for quantitative testing of K solubilizing capacity. Based on K solubilizing capacity on commercial Aleksandrw broth medium a total of 10 KSB isolates were selected for K solubilizing capacity on modified Aleksandrw broth medium with two forms of mica, muscovite and biotite.

K solubilizing capacity of selected isolates was studied in-vitro using waste muscovite and biotite supplemented broth. Choose four KSB isolates for biochemical and soil incubation studies based on their K solubilization.

Based on 16S phylogeny, out of 10 isolates confirmed species of KSB, nine KSB isolates (90 %) were found to be closely phylogenetically related to Bacillus genus, and out of nine, six isolates (66.66 %) were found closely phylogenetically related to Bacillus cereus. All six isolates showed about 97.78 – 100 % similarity in their 16S rDNA sequences. The most common Bacillus strain is Cereus (60 %), which accounts for 6 out of 10 isolates, followed by *Bacillus spp.* 6SB1, *Bacillus sp.* GG6B1 (2015), and *Bacillus velezensis* K13 strains (10 %, 1 in 10 isolates).

7.C.2. Characterization of KSB

Out of 10 KSB isolates, 8 KSB isolates were gram-positive and the rest of the two isolates were gram-negative in reaction. The zone of solubilization was significantly higher with K4A (2.25 cm) and K14 (2.0 cm) respectively.

Among ten KSB isolates, four (K5B, K6, K12, and K15) were selected for biochemical analysis; K12 and K5B strains produced significant amounts of IAA and GA3. As a result of K5B, K6, and K12, HCN was produced. K5B, K6, K12, and K15 strains produced NH_3 and siderophore.

Four KSB isolates significantly influenced K release and pH dynamics in broth culture of waste muscovite and biotite at 7, 14, and 21 days after incubation (DAI) out of 10 KSB isolates.

The dynamics of K release from two forms of waste micas were significantly affected by K5 and K6 compared with the rest of the strains in KSB.

In comparison to other treatments, pH 7.5, 1% NaCl concentration, and 30°C significantly increased cell growth.

7.C.3. Soil incubation studies

An experiment was done to determine the water-soluble and exchangeable forms of K after the addition of waste muscovite and biotite to four KSB isolates were incubated at 30°C for 4, 7, 14, and 21 DAI.

A higher concentration of water-soluble and exchangeable K was observed after 21 DAI. A K5B isolate showed the highest K-solubilizing capacity (163.11 mg/kg, soil containing 15 mg waste muscovite), followed by K6 (173.82 mg/kg, soil containing 30 mg waste biotite); in water-solubilized and exchangeable K, K6 showed the greatest K-solubilizing capacity (452.21 mg/kg, soil containing 60 mg waste muscovite, and 313.52 mg/kg,

7.D. Pot experiments

The pot experimental plan was based on eighteen treatments. These were T1, negative control [only soil (S) no micas, no bacterial culture, and no basal doses were added only borax-B was used]; T2, positive control [only added standard basal doses (Kg/ha @ N:P: K = 200:150:100) or fertilizers (F)]; T3 and T4, muscovite (M) at 50 mg kg-1 and 200 mg kg-1 soil + Bacillus cereus K5B; T5 and T6, muscovite (M) at 50 mg kg-1 and 200 mg kg-1 soil + Bacillus cereus K6; T7 and T8, muscovite (M) at 50 mg kg-1 and 200 mg kg-1 soil + Bacillus sp.GG6 (2015) K12; T9 and T10, muscovite (M) at 50 mg kg-1 and 200 mg kg-1 soil + Bacillus cereus K15; T11 and T12, biotite (B) at 50 mg kg-1 and 200 mg kg-1 soil + Bacillus cereus K5B; T13 and T14, biotite (B) at 50 mg kg-1 and 200 mg kg-1 soil + Bacillus cereus K6; T15 and T16, biotite (B) at 50 mg kg-1 and 200 mg kg-1 soil + Bacillus sp.GG6 (2015) K12; T17 and T18, biotite (B) at 50 mg kg-1 and 200 mg kg-1 soil + Bacillus cereus K15 respectively.

7.D.1. Physico-chemical properties

Temporary changes in pH of KSB treated soils appear to be lower in 90 days as compared to 0 days, and EC values of tasted soil were higher in 90 days as compared with different incubation periods of four KSB bacterial strains and different concentrations (50mg/kg and 200mg/kg) of muscovite and biotite treated soils. The total organic C was initially low at all treatments during the incubation period, but the concentration was significantly (*p* for days = 0.04 and LSD = 0.54) higher at 60 days, then slightly decreased at 90 days. In general, the availability of easily mineralizable or available N (AN) in our tested soils significantly increased (*p* for days = 0.006, LSD = 4.54) gradually after incubation of all treatments, but decreased at 90. N availability was higher at 60 days and constant at 90 days after incubation; this may have been caused by the combination of KSB microorganisms and waste micas and; the production of nitrogenous organic compounds by rhizospheric bacteria. The availability of K (EK) content was significantly (*p* for days = 0.02 and LSD = 4.43) increased during the increasing incubation period was increased at 90 days. As compared to muscovite and biotite treated soils muscovite treated soils were showed the best EK content over biotite treated soils and concentration wise @ 200 mg kg^{-1} soil, showed the best concentration as compared to the concentration of @ 50 mg kg^{-1} soil (*p* for treatments = 0.01 and LSD = 17.8). During the incubation periods, *Bacillus sp.*GG6 (2015) K12 bacterial strains for N and P and *Bacillus cereus* K5B bacterial strains for K produced the best results when compared to other KSB strains. DTPA extractable metals, such as Cd, Cu, Cr, Ni, Pb, and Zn content in tested or compost soil were below the detectable.

7.C.2. Biochemical chemical properties

The MBC in all the four KSB with waste muscovite and biotite treated soils during the incubation considerably increased with time. Significant (*p* for days = 0.03 and LSD = 18.19) variations in MBC in different treatments were observed between 0 to 90 days. MBC was significantly higher for

T4 and T3 treatments (*p* for days × treatment = 0.03 and LSD = 18.19) compared with the other treatments. However, the microbial metabolic quotient was significantly (*p* for days = 0.001 and LSD = 20.38) higher in waste muscovite treated soils than in biotite treated soils, and concentration wise, at 200 mg kg^{-1} soil, the qCO_2 levels were higher than those at 50 mg kg^{-1} soil. After 90 days of incubation, qCO_2 levels of all treatments were found to be higher. T4 and T3 in muscovite and T12 and T11 in biotite showed high qCO_2 levels during the pot experiment. Microbial respiratory quotient (RQ) is an indicator of the stress in the immediate environment. Here, metabolically dormant microorganisms are reflected as basal respiration, which is largely dependent on autochthonous microbial populations. In general, the respiratory quotient was reduced in all the KSB fertilizers with muscovite and biotite treated soils 90 days after the incubation period.

FDA when conditions are favorable. The FDA hydrolysis assay indicates an overall increase in fungal and bacterial activity. As such, FDA hydrolysis assay was significantly muscovite with KSB treated soils as compared to biotite with KSB treated soils (*p* for treatment = 0.01 and LSD = 0.005) and FDA level was found to be higher at 90 days during the incubation period (*p* for days = 0.01 and LSD = 0.009). Dehydrogenase (DHG) is a test used to assess soil microbial activity in situ; in fact, starvation believes that this test is a reliable indicator of soil microbial activity. Muscovite with KSB fertilizer treated soils had a higher DHG activity level than biotite with KSB fertilizer treated soils (*p* for treatment = 0.008 and LSD = 0.041). @ 200 mg kg^{-1} soil concentration of both muscovite and biotite showed the best results as compared to the concentration of @ 200 mg kg^{-1} soil. After 90 days of soil incubation, DHG levels were higher (*p* for days = 0.004 and LSD = 0.045).

7.D. Crop

7.D.1. Biomass accumulation by plant

Biomass accumulation (sum of six cuttings) by tomato plants parts (shoot and root) was significantly increased with the addition of muscovite with KSB fertilizers treated soils, compared to both negative and fertilizers treated soils and biofertilizers with KSB fertilizers treated soils (*p* for shoot = 0.005 and LSD = 6.62; *p* for root = 0.04 and LSD = 2.28, respectively). Both muscovite and biotite were best for pant biomass accusation by shoot and root at 200 mg kg^{-1} soil concentration than at 50 mg kg^{-1} soil concentration. *Bacillus cereus* K5B bacterial fertilizers showed that KSB fertilizers outperformed the rest of the three KSB fertilizers.

7.D.2. Nutrient acquisition by a tomato plant and fruit

Nutrient uptake was highest in muscovite with KSB fertilizer treated soils over biotite with KSB fertilizer treated soils as well as with both positive and negative controls for nitrogen and phosphorus uptake. It was found that for phosphorus uptake by plants, biotite provided the best results in comparison to muscovite when it was planted on KSB-treated soils and when it was planted without fertilizers. The highest nutrient acquisition by tomato plant parts was observed at 200 mg kg^{-1} soil of both muscovite and biotite over 200 mg kg^{-1} soil.

The use of waste muscovite and biotite with four KSBs significantly increased the growth, fruit yield, and quality of the plant compared to that of the control-treated plant ($p > 0.05$). In present studies, initially plant height increased slowly up to one month (30 days) after transplanting (DAT) and then increased rapidly during final harvesting (90 days) DAT.

All the treatments followed almost similar fashions due to the application of different fertilized and unfertilized treatments on plants. It was found that when muscovite or biotite treated soil was applied, plant growth such as plant height was higher at 200 mg K kg^{-1} soil concentration over 50 mg K kg^{-1} soil, and on an average plant significantly (p for plant height = 0.03 and LSD = 21.29) height was observed for muscovite treated with four KSB fertilizers as opposed to biotite treated with four KSB fertilizers and both negative (soil only) or positive controls (soil and fertilizer) respectively.

7.D.3. Dynamics of K in soil

Temporal changes of water-soluble K and non-exchangeable K were found to be higher at 90 days in different concentrations (@ 50 and 200 mg kg^{-1} soil) of both muscovite and biotite within four KSBs bacterial strain treated soils after an incubation period during the pot experiments. On average both WK and NEK were found in lower T1 (negative control, where no K was added) and T2 (positive control, soil + F), where K fertilizer was added as compared to all treatments.

During the experiments, WK and EK formed by releasing K from non-exchangeable pools of K with the help of four KSBs and releasing organic acids. Compared to soil treated with fertilizers, waste muscovite and biotite treated with KSB fertilizers exhibited higher WK, EK, and NEK.

Overall, my thesis takes a holistic approach to examining mica mines with potentially toxic elements and their adverse effects on closely bound agricultural soil fields, food systems, and human health (adult and children) through three body exposure pathways (inhalation, ingestion, and dermal contact). On the other hand, mica is one of the alternative sources of potassium. On the other hand, mica is one of the alternative sources of potassium. The combination of muscovite and biotite with K-solubilizing microbes (*Bacillus cereus* K5B, *Bacillus cereus* K6, *Bacillus sp.*GG6 (2015) K12, *Bacillus cereus* K15) significantly improve tomato plant biomass yield, plant growth, fruit quality and yields etc. as compared to traditionally used fertilizers (N, P, and K). Overall *Bacillus cereus* K5B with different concentration of muscovite and biotite showed best results as compared to others. A combination of mica with KSB isolates can be used as an alternative source of potassium fertilizer for plant growth and yield.

PICTURES

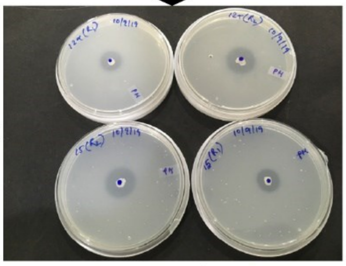

Mica mines


Soil samples


Muscovite & Biotite


Pot preparation


Broth preparation


KSB isolares


Vegetative stage


First fruit


Agronomical data collection


Final harvesting


After harvesing


Before harvesting

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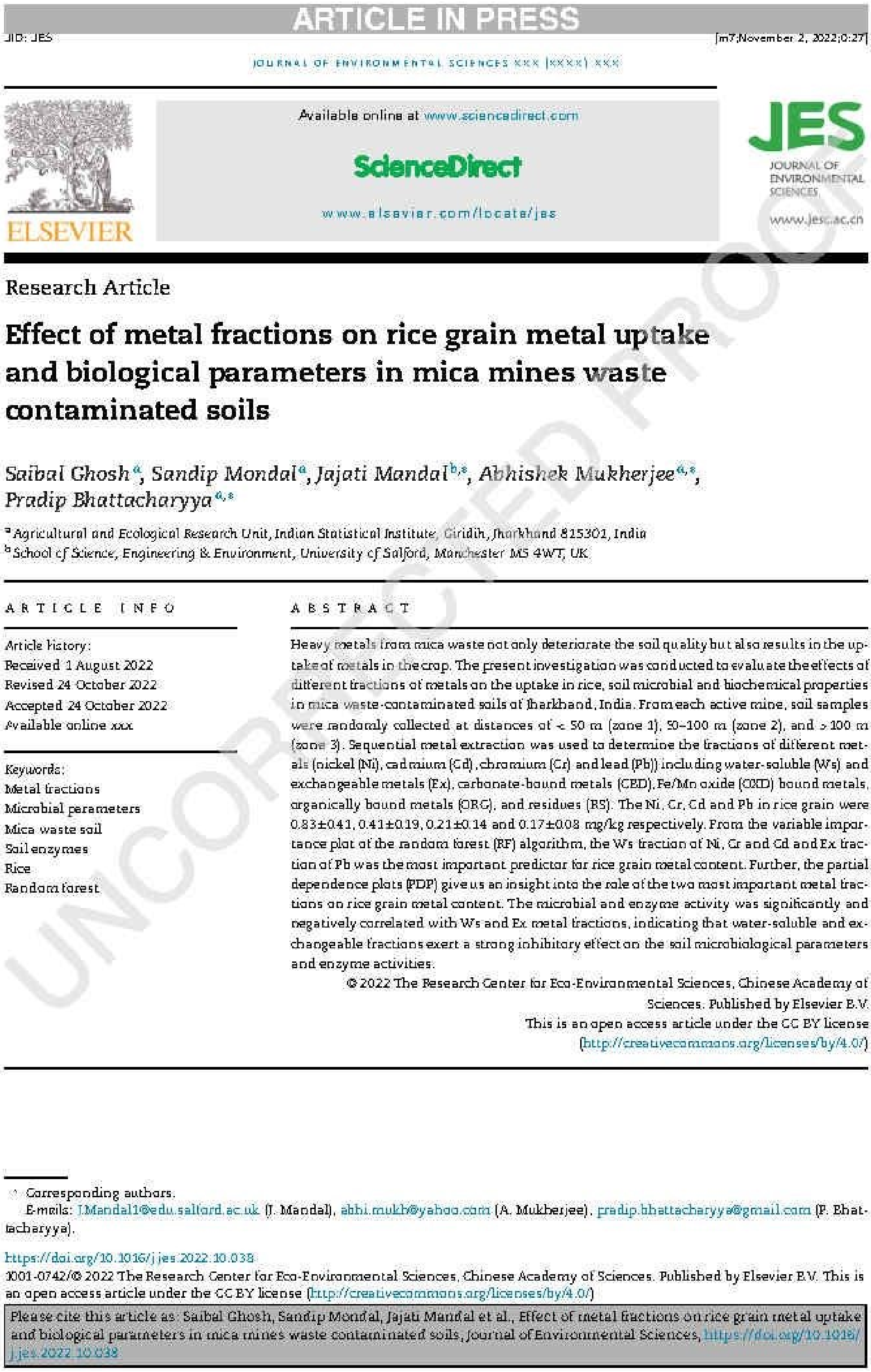
PUBLICATIONS

Publications from Thesis:



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Research Article

Effect of metal fractions on rice grain metal uptake and biological parameters in mica mines waste contaminated soils

Q1 *Saibal Ghosh*[a], *Sandip Mondal*[a], *Jajati Mandal*[b,*], *Abhishek Mukherjee*[a,*], *Pradip Bhattacharyya*[a,*]

[a] *Agricultural and Ecological Research Unit, Indian Statistical Institute, Giridih, Jharkhand 815301, India*
[b] *School of Science, Engineering & Environment, University of Salford, Manchester M5 4WT, UK*

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ABSTRACT

Heavy metals from mica waste not only deteriorate the soil quality but also results in the uptake of metals in the crop. The present investigation was conducted to evaluate the effects of different fractions of metals on the uptake in rice, soil microbial and biochemical properties in mica waste-contaminated soils of Jharkhand, India. From each active mine, soil samples were randomly collected at distances of < 50 m (zone 1), 50–100 m (zone 2), and > 100 m (zone 3). Sequential metal extraction was used to determine the fractions of different metals (nickel (Ni), cadmium (Cd), chromium (Cr) and lead (Pb)) including water-soluble (Ws) and exchangeable metals (Ex), carbonate-bound metals (CBD), Fe/Mn oxide (OXD) bound metals, organically bound metals (ORG), and residues (RS). The Ni, Cr, Cd and Pb in rice grain were 0.83±0.41, 0.41±0.19, 0.21±0.14 and 0.17±0.08 mg/kg respectively. From the variable importance plot of the random forest (RF) algorithm, the Ws fraction of Ni, Cr and Cd and Ex fraction of Pb was the most important predictor for rice grain metal content. Further, the partial dependence plots (PDP) give us an insight into the role of the two most important metal fractions on rice grain metal content. The microbial and enzyme activity was significantly and negatively correlated with Ws and Ex metal fractions, indicating that water-soluble and exchangeable fractions exert a strong inhibitory effect on the soil microbiological parameters and enzyme activities.

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[*] Corresponding authors.
E-mails: J.Mandal1@edu.salford.ac.uk (J. Mandal), abhi.mukh@yahoo.com (A. Mukherjee), pradip.bhattacharyya@gmail.com (P. Bhattacharyya).

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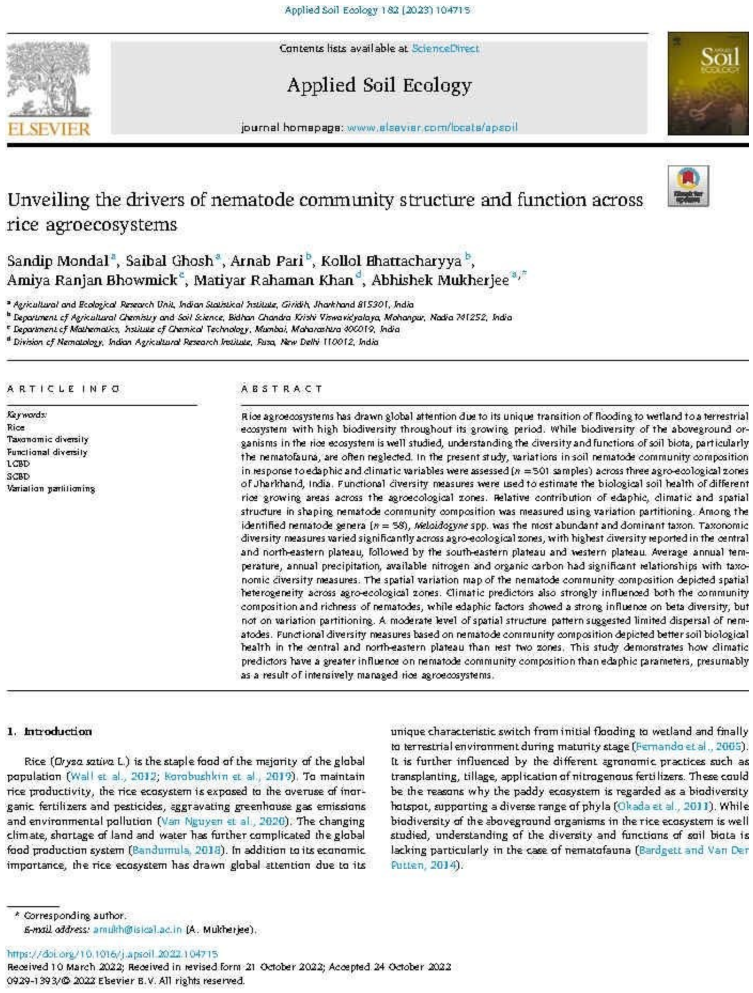

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Unveiling the drivers of nematode community structure and function across rice agroecosystems

Sandip Mondal[a], Saibal Ghosh[a], Arnab Pari[b], Kollol Bhattacharyya[b], Amiya Ranjan Bhowmick[c], Matiyar Rahaman Khan[d], Abhishek Mukherjee[a,*]

[a] Agricultural and Ecological Research Unit, Indian Statistical Institute, Giridih, Jharkhand 815301, India
[b] Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia 741252, India
[c] Department of Mathematics, Institute of Chemical Technology, Mumbai, Maharashtra 400019, India
[d] Division of Nematology, Indian Agricultural Research Institute, Pusa, New Delhi 110012, India

A R T I C L E I N F O

Keywords:
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A B S T R A C T

Rice agroecosystems has drawn global attention due to its unique transition of flooding to wetland to a terrestrial ecosystem with high biodiversity throughout its growing period. While biodiversity of the aboveground organisms in the rice ecosystem is well studied, understanding the diversity and functions of soil biota, particularly the nematofauna, are often neglected. In the present study, variations in soil nematode community composition in response to edaphic and climatic variables were assessed ($n = 501$ samples) across three agro-ecological zones of Jharkhand, India. Functional diversity measures were used to estimate the biological soil health of different rice growing areas across the agroecological zones. Relative contribution of edaphic, climatic and spatial structure in shaping nematode community composition was measured using variation partitioning. Among the identified nematode genera ($n = 58$), *Meloidogyne* spp. was the most abundant and dominant taxon. Taxonomic diversity measures varied significantly across agro-ecological zones, with highest diversity reported in the central and north-eastern plateau, followed by the south-eastern plateau and western plateau. Average annual temperature, annual precipitation, available nitrogen and organic carbon had significant relationships with taxonomic diversity measures. The spatial variation map of the nematode community composition depicted spatial heterogeneity across agro-ecological zones. Climatic predictors also strongly influenced both the community composition and richness of nematodes, while edaphic factors showed a strong influence on beta diversity, but not on variation partitioning. A moderate level of spatial structure pattern suggested limited dispersal of nematodes. Functional diversity measures based on nematode community composition depicted better soil biological health in the central and north-eastern plateau than rest two zones. This study demonstrates how climatic predictors have a greater influence on nematode community composition than edaphic parameters, presumably as a result of intensively managed rice agroecosystems.

1. Introduction

Rice (*Oryza sativa* L.) is the staple food of the majority of the global population (Wall et al., 2012; Korobushkin et al., 2019). To maintain rice productivity, the rice ecosystem is exposed to the overuse of inorganic fertilizers and pesticides, aggravating greenhouse gas emissions and environmental pollution (Van Nguyen et al., 2020). The changing climate, shortage of land and water has further complicated the global food production system (Bandumula, 2018). In addition to its economic importance, the rice ecosystem has drawn global attention due to its unique characteristic switch from initial flooding to wetland and finally to terrestrial environment during maturity stage (Fernando et al., 2005). It is further influenced by the different agronomic practices such as transplanting, tillage, application of nitrogenous fertilizers. These could be the reasons why the paddy ecosystem is regarded as a biodiversity hotspot, supporting a diverse range of phyla (Okada et al., 2011). While biodiversity of the aboveground organisms in the rice ecosystem is well studied, understanding of the diversity and functions of soil biota is lacking particularly in the case of nematofauna (Bardgett and Van Der Putten, 2014).

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[*] Corresponding author.
E-mail address: amukh@isical.ac.in (A. Mukherjee).

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ORIGINAL ARTICLE

Application of biochar and vermicompost against the rice root-knot nematode (*Meloidogyne graminicola*): an eco-friendly approach in nematode management

Sandip Mondal[1] · Saibal Ghosh[1] · Abhishek Mukherjee[1]

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Abstract

The rice root-knot nematode (*Meloidogyne graminicola*) is one of the key pests of rice (*Oryza sativa* L.) in irrigated and upland rice ecosystems inflicting about 20–80% yield loss. While chemical nematicides are widely used to combat this pest, they have a negative impact on the environment as well as on animal and human health. The present study evaluated the efficacy of organic amendments like biochar and vermicompost in managing the rice root-knot nematode (RRKN) at 0.6, 1.2, 2.5, and 5% (w/v) concentrations. Our result showed that biochar and vermicompost exudate neither exhibit any direct killing ability nor altered the infectivity of RRKN second-stage juveniles (J2). Second-stage juvenile hatching was, however, reduced in two higher doses (2.5% and 5%) of vermicompost exudates. When final nematode population was normalized with root weights at 21 days' post inoculation, a reduction in population build-up was observed with increased doses of vermicompost. A reverse trend was observed for biochar treatments. Overall, our study showed that application of 1.2% biochar and 5% vermicompost could be helpful in mitigating the RRKN stress in rice. Hence, application of biochar and vermicompost could be an effective alternative to toxic chemical nematicides and recommended as eco-friendly management options against *M. graminicola* in rice.

Keywords Biochar · *Meloidogyne graminicola* · Reproduction factor · Vermicompost

Introduction

One of the major challenges to the present agriculture and food system is to ensure food security of the projected nine billion people by 2050 (FAO 2017). Balancing between population expansion and food production would require the optimal utilization of finite resources (Sundquist 2007). Global food security is highly influenced by rice (*Oryza sativa*) production in Asia, specifically, in India and China, which together contribute 49% of global rice production (Bandumula 2018). Among the several biotic constraints of rice production, plant-parasitic nematodes (PPN) constitute one of the greatest threats (Mantelin et al. 2017; Kumar et al. 2020).

The rice root-knot nematode (RRKN, *Meloidogyne graminicola*) is one such PPN that is considered as the key pest in the irrigated and upland rice ecology in Asia. It has also been designated as a pest of international importance (Cabasan et al. 2012; Jain et al. 2012). Field-based studies reported an estimated yield loss between 20 and 80% due to the attack by the RRKN (Soriano and Reversat 2003; Padgham 2004). In India, RRKN infestations cause an annual loss of about Rs. 23.27 billion (Kumar et al. 2020).

M. graminicola can complete its lifecycle within 15 days at 27–37 °C enabling rapid population build-up within a growing season (Jaiswal and Singh 2010). Besides, its obligate sedentary endo-parasitic nature, wide adaptability to environmental conditions and egg-laying inside the roots limit the options to control RRKN (Cabasan et al. 2012; Mantelin et al. 2017). Traditional methods of RRKN management include soil flooding and applications of nematicides like carbofuran for seed treatment or in the seedbed (Tandingan et al. 1996; Jain et al. 2012). However, the recent

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✉ Abhishek Mukherjee
abhi.mukh@yahoo.com

[1] Indian Statistical Institute, Giridih, Jharkhand 815301, India

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Research Article

Effect of metal fractions on rice grain metal uptake and biological parameters in mica mines waste contaminated soils

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[a] *Agricultural and Ecological Research Unit, Indian Statistical Institute, Giridih, Jharkhand 815301, India*
[b] *School of Science, Engineering & Environment, University of Salford, Manchester M5 4WT, UK*

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* Corresponding authors.
E-mails: J.Mandal1@edu.salford.ac.uk (J. Mandal), abhi.mukh@yahoo.com (A. Mukherjee), pradip.bhattacharyya@gmail.com (P. Bhattacharyya).

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Introduction

In the modern world, soil and agricultural pollution brought on by mining operations is a significant problem (Candeias et al., 2014). Mica deposits in India cover a total area of about 3888 km^2 in the districts of Giridih and Koderma in Jharkhand and Munger in Bihar (Nishanth and Biswas, 2008). The majority of mines in this area are still active right now, but a few have been closed for several years. Mica wastes from these mines (active and closed) are typically fine, loose, and homogeneous, have a low bulk density and moisture-holding capacity, and lower nutrients, which not only inhibit plant colonization but also contaminate the surrounding areas during strong winds and heavy runoff during monsoon (Kumar and Maiti, 2015). Around 75% of raw mica mine waste is disposed of nearby the mines during the dressing process, and as a result, these mine wastes wash into the fields and rivers, contaminating land and water resources (Basak and Biswas, 2009; Meena et al., 2015). Rice is the major crop in the mica mine waste-contaminated soils in that region. In India, daily consumption of milled rice is high, approximately 103 per capita/per year (Mandal et al., 2021). Rice is prone to absorb some toxic heavy metals such as cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb), which is a major threat to food security and human health (Zhao and Wang, 2020). Increased accumulation of Cd in rice as a result of extensive Cd contamination in paddy soils brought on by mining, smelting, and other industrial operations has been reported by Huang et al. (2021). Lead contamination in rice due to mining activities have been previously reported in Zamfara state, Nigeria (Mandal et al., 2022). Although the total metal content in the soil is an effective indicator of soil pollution, it does not provide adequate information about the potential environmental impact. Many studies have demonstrated that heavy metal uptake by plants is positively related to the bioavailable concentration of soil heavy metals (Monterroso et al., 2014; Xiao et al., 2017). Sequential chemical extraction methods are more popular to use quantification of different fractions of metal in soil (Beckett, 1989). The sequential chemical extraction technique breaks down metals into different solubility and mobility forms and can be used to predict the conversion of metals into different fractions of metals in soils and the availability of metals in microorganisms (Bhattacharyya et al., 2005). Several workers studied the effect of different metal fractions on soil microorganisms and metal uptake by rice plants in mining-affected soils (Ma et al., 2015; Zhang et al., 2018).

Further due to the presence of heavy metals in this mica waste there is a decline in soil quality which ultimately leads to the loss of biodiversity, business possibilities, and resources. Microorganisms play a vital role in the energy-driving process under increased stress conditions. Enhancing or maintaining soil quality is crucial for both sustainable agriculture practices and a healthy ecosystem. Multiple soil microbiological parameters, such as microbial biomass carbon (MBC), metabolic quotient (respiration/biomass ratio), respiratory quotient (basal soil respiration/substance-induced respiration ratio), and microbial enzyme activity quality, are more pertinent than single parameter measurements when determining the soil environmental quality (Doran and Parkin, 2015). To evaluate the ecological stress level, some microbiological parameters viz. soil enzyme activity, biomass carbon, and microbial respiration are found to be most reliable. Basal soil respiration (BSR) is one of the oldest and still the most widely used parameters for measuring microbial activity in the soil (Huang et al., 2013; Tripathy et al., 2014). The metabolic quotient or qCO_2 is a sensitive indicator of the expression of heavy metal toxicity under stressed conditions. Soil enzyme activities are widely used as biological indicators for soil health (Zhou et al., 2017). They play a significant role in nutrient turnover and are influenced by several biotic and abiotic factors. β-D glucosidase, phosphatase and urease are a few of the key enzymes in the major biogeochemical cycle i.e., C, N, P. Dehydrogenase, an oxidoreductase group enzyme often acts as an indicator of the microbial redox system.

To our knowledge, no information is available on the effect of mica mine waste on the distribution of metals in soil and rice plants and their effect on microbiological and biochemical indicators of soil quality. This study assesses the relationship between metal components and their bioavailability in the soil in addition to their effect on soil microbiological and biochemical properties in tropical soils contaminated by mica waste. In this study, we measured the microbial biomass, respiration and various enzymes such as glucosidase, urease, and phosphatase, which are related to the cycling of carbon, nitrogen and phosphorous respectively. This investigation will provide insight to assess how different metal fractions in soil contaminated by mica waste negatively impact the soil environment. Further, the accumulation of heavy metals in rice grains from mica waste-contaminated agricultural fields was also determined. We also predicted the rice grain metal content in relation to the different fractions of the metals with the help of variable importance plots and partial dependence plots (PDP) using the random forest (RF) machine learning algorithm. The PDPs will shed light on how certain metal fractions govern the metal uptake in rice.

1. Materials and methods

1.1. Study site

The Jharkhand state is the world's largest deposit of mica mines in India. Koderma and Giridih are two of the best mica-producing districts in India, both of which belong to the state of Jharkhand. Geographically Giridih (4941 km^2) is bounded by 24.18°N / 86.3°E, and is situated in the northern region of the state. As for the climate of the Giridih, it is dry, and the average annual rainfall is 1350 mm; the maximum and minimum temperatures of this district are 42 °C and 10 °C, respectively. Within the Giridih district, there are three types of soil: Entisols, Inceptisols, and Alfisols. The percentages of the three types of soil are respectively 63.6%, 18.4%, and 16.9% (https://giridih.nic.in/geography/). In the Giridih district, there are twelve blocks, and three of them - Gawan, Tisri, and Deori - are particularly contaminated with heavy metals and waste mica.

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1.2. *Sample collection and processing*

Mica waste amendment soil and rice samples were collected during the maturity phase of rice (*Oryza sativa* L.) from near mica mines in three blocks (Deori, Gawnan, and Tisri) of Giridih district of Jharkhand, India in September 2016 **(Appendix A Fig. S1)**. Mica mines are located mainly in these three blocks of the district in Giridih. Rice fields were selected near mica mines, where surface run-off water is naturally stored because of lower altitudes due to leaching out of mines. From each active mine, three soil samples were randomly collected at distances of < 50 m (zone 1), 50–100 m (zone 2), and >100 m (zone 3). Therefore, in total 63 soil samples were collected, 21 from each zone. At each sampling site, soil and rice samples were collected from three nearby rice fields using the W-pattern sampling method. Finally, ~1 kg soil/site was collected following the subsampling method. Surface soil was removed before the collection of rhizospheric soil from a depth of ~10 cm. The rice plants were collected from the field with all of their roots remaining intact. Plant roots were separated from the rhizosphere soil by shaking gently with hands. The samples (soil and rice) were preserved separately in sterile plastic bags, labelled properly, and brought back to the soil laboratory at the Indian Statistical Institute, Giridih, within the same day and stored at 4 °C before analysis. Samples were processed within seven days of collection. In the laboratory, the rice grains were separated from the ears, followed by dehusking, then drying to a constant weight in an oven and finally milling the grain into powder for the purpose of determining heavy metal concentrations. In addition, each soil sample was air-dried and then sieved through a 2 mm sieve for further analysis. In microbiological analyses, moist soil was used, while in physicochemical analyses, air-dried soil was used. Chemical and microbiological characteristics were calculated on a moisture-free basis.

1.3. *Physicochemical analysis of mica waste soils*

The physicochemical analyses were carried out with (2 mm and 0.2 mm) air-dried samples by standard protocols. The pH was measured in 1:2.5 (m/V) soil water suspension using a calibrated electrode of Systronics Digital pH meter 335 whereas electrical conductivity (EC) of the samples suspensions was measured by a Conductivity Bridge; using 1:5 ratio of sample-water suspension. Soil organic carbon was estimated by reacting the samples with potassium dichromate and sulphuric acid and the excess potassium dichromate was titrated by ferrous ammonium sulphate (Walkley and Black, 1965). The total nitrogen content in the samples was measured by Kjeldahl method. In brief, the soil was digested by concentrated sulphuric acid with a mixture of potassium sulphate and copper sulphate. After digestion, ammonium ion content was measured by distillation of the digested material with sodium hydroxide in an N-auto analyzer. The cation exchange capacity (CEC) of the soil was determined by extracting the soil with the buffer $BaCl_2$ solution at pH 8.1, adjusted with triethanolamine, following the method of Bascomb (1964) and modified by Dewis and Freitas (1984). The available phosphorous (P) of samples was estimated by 0.5 mol/L $NaHCO_3$ as suggested by Olsen and Sommers (1982) and estimated through a UV–vis spectrophotometer. Exchangeable Na, Ca and Mg concentrations in the samples were determined from the neutral 1 mol/L ammonium acetate extracts (1:5, m/V) and measured by a flame Photometer (Knudsen et al., 1983). Available Ni, Cr, Cd and Pb concentrations in the samples were determined from the DTPA extracts and measured by atomic absorption spectrophotometer (Lindsay and Norvell, 1978).

1.4. *Sequential extraction of heavy metals*

Metals (Cr, Ni, Pb and Cd) were sequentially extracted from soil following the method of (Tessier et al., 1979). For water-soluble fractions, 1.0 g of air-dried soil samples were extracted sequentially with 50 mL deionized water, shaking (120 r/min) for 30 min at room temperature; 0.5 mol/L $Mg(NO_3)_2$ in 50 mL with shaking (120 r/min) for 30 min at room temperature for exchangeable fraction; with 1 mol/L NaOAc, shaking (120 r/min) for 5 h at room temperature for bound to carbonate fraction; with 0.08 mol/L NH_2OH HCl, shaking (120 r/min) for 6 h at 96°C temperature for bound to Fe and Mn – oxide phase metal; for bound to organic matter fraction 1 g soil samples with 0.02 mol/L HNO_3 and H_2O_2 shaking (120 r/min) for 2 and 3 h at 85 °C temperature and sulphides phase fraction, with 3.2 mol/L NH_4OAc shaking (120 r/min) for 30 min at room temperature, and finally with concentred HNO_3 at 105 °C for mineral matrix fraction (Jackson, 1958). The metal concentrations in the extracts were measured by an atomic absorption spectrometer (AAS – 816, Systronics, India).

For calibration, standard solutions of the respective metals were prepared from stock solutions (1000 mg/L, Merck grade) in 1% (*V/V*) HNO_3. Certified reference material SRM 2710 and blank extract were used for quality assurance. The reference material was then digested in ultrapure $HF/HNO_3/HClO_4/H_2O_2$ to confirm the accuracy of the metals analytical method. The results were compared with certified SRMs. The results indicate that the analytical method was accurate for all metals.

1.5. *Estimation of total metals from rice grain*

The total metal concentration of Cr, Ni, Pb and Cd in rice grains was determined in a sample of 1 g rice digested in a 4:1 (*V/V*) di-acid mixture (HNO_3:$HClO_4$) in a beaker placed on an electric heating plate at 190 C for 1 h until the solution turned white (Li et al., 2018). We estimated the metal concentration in these digests to be similar to what we described in the previous section (Section 1.4). Quality assurance and quality control (QA/QC) study of metal content in rice samples was conducted by determining the metal content of blank and duplicate samples along with certified reference materials (SRM-2710).

1.6. *Microbiological analysis*

Microbial biomass carbon (MBC) was determined by fumigation extraction followed by KCl extractable carbon determination, and the difference between fumigate soil and unfumigated soil was divided by the calibration factor (K_{EC}= 0.38). MBC per gram of dry sample was calculated; where K_{EC} is the difference between KCl extractable carbon content of the fumigated and unfumigated soils (Joergensen et al., 2011). In order to quantify soil respiration, CO_2 was measured when

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the soil was incubated with glucose in a closed system and trapped in NaOH solution, then titrated with HCl (Alef and Nannipieri, 1995). Urease enzyme activity in soils was assayed by the buffer methods (Tabatabai, 1994). The method involved the determination of ammonia (NH_3) released by urease activities when the soil was incubated with Tris (hydroxymethyl) aminomethane (THAM) buffer at optimal pH (pH 9.0) with and without toluene and 0.2 mol/L urea solution at 37 °C for 2 h. The activity of acid phosphatase (APS) and β-D-glucosidase (Beta D) enzymes was determined using the method described by the method (Tabatabai, 1994), following incubation of moist soil with p-nitrophenyl phosphate (PNP) and *p*-nitrophenyl glucopyranoside in the buffer for 1 h at 37 °C. The product *p*-nitrophenol (PNP) was determined calorimetrically at 420 nm. Estimation of fluorescein diacetate hydrolysis activity (FDA) in soils the methods described by Schnürer and Rosswall (1982). The release of fluorescein when 1 g of moist soil was incubated with sodium phosphate buffer (pH 7.6) and fluorescein diacetate solution at 25 °C for 3 h. The dehydrogenase enzyme activity of the moist sample was measured as described by the method of Casida (1964). The determination of Deydrogenase (DHG) was based on the estimation of the triphenyl tetrazolium chloride (TTC) reduction rate to triphenyl formazan (TPF) in soils after incubation at 37 °C for 24 h. The microbial metabolic quotient and respiratory quotient were calculated using values of microbial biomass C, basal soil respiration, and substrate-induced respiration using Eqs. (1) and (2) (Alef and Nannipieri, 1995; Chakraborty et al., 2022).

$$qCO_2 = (Rb - CO_2)/C \tag{1}$$

$$qr = Rb/Rs \tag{2}$$

where, qCO_2 is the microbial metabolic quotient, $R_{b\text{-}CO2}$ is the CO_2-cc from basal respiration, C is the microbial biomass carbon, q_r is the respiratory quotient, R_b is the basal respiration, R_s is the substrate induced respiration.

1.7. *Statistical analysis*

The statistical analysis was done using R-Studio (Version 1.3.1093 2.3.1). The violin plots were prepared using the '*ggpubr*' (version 0.40) package. The correlation plots were made using the '*correlation*' (version 0.8.0), '*corrplot*' (version 0.84) *and* '*psych*' (version 2.0.12). Principal component analysis (PCA) was performed using the '*princomp*' (version 4.0.3) and '*factoextra*' (version 1.0.7). The linear correlation between the two variables was assessed through a Spearman correlation analysis. The random forest models were developed considering metal content in rice grain as the dependent variable and the soil fractions of Ni, Cr, Cd and Pb as the predictor variable for each metal. Random forest is a supervised machine learning algorithm for classification and regression that is based on the recursive partitioning principle (Breiman, 2001) and is independent of the assumption of functional relationships between the response and predictor variables. Random Forest analysis, in a nutshell, ensembles multiple regression trees by a technique known as "bootstrap aggregation" or "bagging." To provide an average prediction for the response variable, a random subset of the data space is taken (with replacement) to grow a tree to its full length, and each node of the tree group's observations is described by particular requirements on the predictor variables. Two-thirds of the bootstrapped data is used in each tree-growing process, and one-third of the observations (out-of-bag data, OOB) is used to estimate prediction errors. Second, each node split in a tree takes into account a random subset of predictor variables, which is usually the square root of the entire number of predictor variables. To produce final projections, the forecasts from all of the trees are summed. The Random Forest algorithm's variable significance function ranks predictor variables based on the increase in model error by randomly permuting the predictor variables values. (James et al., 2013; Sengupta et al., 2021). The difference in Mean Squared Error (MSE) before and after random permutation of a predictor variable is averaged over all trees to compute variable importance. The models were developed using the '*randomForest*' (version 4.6-14) package with *ntree*=500 and *mtry*=2. For the variable importance plot, '*vip*' (version 0.3.2) package was used. The partial dependence plot from the random forest was prepared using the '*pdp*' (version 0.7.0) package.

2. Results and discussion

2.1. *Chemical and Biological properties of the study site*

Table 1 represents the chemical and biological properties of the soils of the study sites (Zones 1, 2 and 3). The pH and EC of the soil ranged from 4.78 to 8.30 and 1.60 to 27.00 mSm/cm with a mean of 6.01 and 5.01 mSm/cm respectively across all the zones of sampling. The mean total organic carbon (TOC) content of the soil was 0.61 with a range of 0.22–1.08%. The cation exchange capacity (CEC) of the soil ranged from 2.98 to 16.10 meq/100g with a mean value of 7.84. The mean microbial biomass carbon (MBC) was 99.14 mg/kg and ranged from 23.79 to 396.82 mg/kg. The mean FDA, DHG, APS, Urease and BetaD were 63.55 μg fluorescein/g soil/hr, 16.15 μg TPF g/hr, 419.86 μg *p*-nitrophenol/(g soil·hr), 26.66 μg urea hydrolysed/(g soil·hr) and 30.99 μg *p*-nitrophenol/(g soil·hr) respectively. The available and total N of the soil was 127.76 mg/kg and 0.24% and ranged from 50.96 to 221.20 mg/kg and 0.03% to 0.77% respectively. The mean exchangeable P, Ca, Mg and Na were 1.04, 2921.30, 307.82 and 19.63 mg/kg respectively. The mean DTPA extractable Ni, Cr, Cd and Pb were 3.27, 3.96, 0.05 and 1.31 mg/kg respectively and ranged from 0.83 to 5.67, 1.30 to 5.50, 0.007 to 0.148 and 0.19 to 6.47 mg/kg respectively. The mean rice grain content of Ni, Cr, Cd and Pb were 0.83, 0.41, 0.21, 0.17 mg/kg respectively.

2.2. *Nickel, chromium, cadmium and lead content in rice grain*

The violin plots in Fig. 1 represent the heavy metal content in the rice grain across the different sampling zones. The non-parametric Kruskal-Wallis test revealed that a significant difference ($p < 0.05$) in rice grain metal content was observed across the different zones. The abundance of heavy metals in rice grain followed the trend of zone 1 > zone 2 > zone 3. The mean Ni content in zone 1 was 1.25 mg/kg followed by 0.72

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Table 1 – Descriptive statistics of the soil parameters and heavy metal content in rice grain (*n*=63).

Parameter	Mean	Range (Min–Max)	Median	*Q_3-Q_1
pH	6.0	4.7–8.3	5.8	6.4–5.3
EC (mSm/cm)	5.0	1.6–27.0	4.1	5.7–3.0
TOC (%)	0.6	0.2–1.0	0.61	0.73–0.49
CEC (meq/100 g)	7.8	2.9–16.1	7.6	9.4–6.0
MBC (mg/kg)	99.1	23.7–396.8	88.6	120.4–60.9
FDA (μg fluorescein/g soil/h)	63.5	15.1–171.7	60.1	80.6–41.3
DHG (μg TPF/(g·h))	16.1	3.1–45.8	14.9	21.5–9.2
APS (μg *p*-nitrophenol/g soil/h)	419.8	44.7–1462.2	351.9	552.4–221.7
Urease (μg urea/g soil/h)	26.6	3.3–89.4	23.6	32.0–16.8
BetaD (μg *p*-nitrophenol/g soil/h)	30.9	16.0–61.2	29.6	39.5–20.2
BSR (μg CO_2 released/g soil/h)	14.3	9.5–21.6	14.3	15.2–13.3
SIR(μg CO_2 released/g soil/h)	81.8	39.2–118.7	88.2	101.2–56.6
qCO_2	0.05	0.009–0.136	0.045	0.06–0.03
QR	0.19	0.08–0.38	0.16	0.22–0.14
Available N (mg/kg)	127.7	50.9–221.2	127.6	144.9–108.3
Total N (%)	0.2	0.03–0.7	0.1	0.2–0.1
Exchangeable P (mg/kg)	1.0	0.1–5.2	0.4	1.0–0.3
Exchangeable Ca (mg/kg)	2921.3	796.9–6076.6	2415.7	4457.8–1606.3
Exchangeable Mg (mg/kg)	307.8	68.7–893.7	268.7	402.5–181.8
Exchangeable Na (mg/kg)	19.6	1.84–213.8	5.5	18.9–3.6
Available Ni (mg/kg)	3.2	0.8–5.6	3.1	3.6–2.8
Available Cr (mg/kg)	3.9	1.3–5.5	4.3	4.9–3.1
Available Cd (mg/kg)	0.05	0.007–0.148	0.04	0.07–0.03
Available Pb (mg/kg)	1.31	0.19–6.47	1.23	1.78–0.96
Rice Ni (mg/kg)	0.83±0.41	0.05–2.00	0.80	1.10–0.52
Rice Cr (mg/kg)	0.41±0.19	0.06–0.95	0.40	0.55–0.25
Rice Cd (mg/kg)	0.21±0.14	0.05–0.60	0.20	0.27–0.10
Rice Pb (mg/kg)	0.17±0.08	0.05–0.40	0.15	0.20–0.10

*Q_3-Q_1: Represents the inter quantile range, between 3rd Quantile (Q_3) and 1st Quantile (Q_1).
EC: electrical conductivity; TOC: total organic Carbon; CEC: cation exchange capacity; MBC: microbial biomass Carbon; FDA: Fluorescein diacetate hydrolyzing activity; DHG: dehydrogenase; APS: acid Phosphatase; BetaD: Beta D Glucosidase; BSR: basal soil respiration; SIR: substrate induced respiration; qCO_2: metabolic quotient; QR: soil microbial respiration quotient.

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mg/kg in zone 2 and a minimum of 0.51 mg/kg in zone 3. The Cr content was maximum in zone 1 (0.57 mg/kg) followed by zone 2 (0.39 mg/kg) and minimum at zone 3 (0.28 mg/kg). The highest Cd content was 0.32 mg/kg at zone 1 followed by 0.19 mg/kg (zone 2) and 0.12 mg/kg (zone 3). Zone 1 had the maximum Pb content of 0.24 mg/kg followed by 0.17 mg/kg and 0.10 mg/kg at zones 2 and 3 respectively. However irrespective of the zones of sampling the rice grain Cd content was less than the maximum tolerable concentration of 0.4 mg/kg as per the codex recommendation JEFCA (2017). The Pb content in rice grain in zone 1 was higher than the codex recommended value of 0.2 mg/kg. In zones 2 and 3 the Pb content in rice was less than the codex-recommended maximum tolerable concentration. The concentrations of water-soluble and exchangeable metal fractions were positively correlated with the total metal content of rice grain; Ws-Ni ($r = 0.57^{**}$) and Ex-Ni ($r = 0.41^{**}$), Ws-Cr ($r = 0.45^{**}$) and Ex-Cr ($r = 0.39^{**}$), Ws-Cd ($r = 0.49^{**}$) and Ex-Cd ($r = 0.24$), Ws-Pb ($r = 0.26^{**}$) and Ex-Pb ($r = 0.50^{**}$), respectively. In DTPA extractable fractions of metals Ni ($r = 0.56^{**}$), Cd ($r=0.28^{**}$) and Pb ($r = 0.55^{**}$) were found highly positive correlated with rice grain compared to other metals **(Appendix A Fig. S2)**. These findings are well corroborated with the previous findings by Zhou et al, (2014).

2.3. *Importance of the fractions of Ni, Cr, Cd and Pb*

The variable importance plot from the random forest models for each metal can be observed in (Fig. 2). The %IncMSE shows how much the model accuracy decreases if we leave out that variable. The IncNodePurity is the measure of how pure the nodes are at the end of the tree without each variable. More useful variables achieve higher increases in node purities, that is to find a split which has a high inter-node variance and a small intra-node variance. For Ni it was observed that the water-soluble (WSNi) and the exchangeable (ExNi) were the two most important variables. For Cr it was a water-soluble fraction followed by the carbonate-bound Cr (CBDCr) and exchangeable Cr. In the case of Cd the water-soluble fraction (WSCd) contributed the most followed by oxidisable Cd (OXD Cd), exchangeable Cd (EXCd) and residual Cd (RSCd). The exchangeable fraction of Pb (EXPb) and carbonate-bound Pb (CBDPb) were the only important fraction governing the uptake of Pb in rice grain. Variable Importance plot shows how worst the model performs without each variable. In our study for Ni (WSNi and EXNi), for Cr (WSCr, CBDCr and EXCr), for Cd (WSCd, OXDCd, EXCd and RSCd) and for Pb (EXPb and CBDPb) are the important variables in absence of which the model will have

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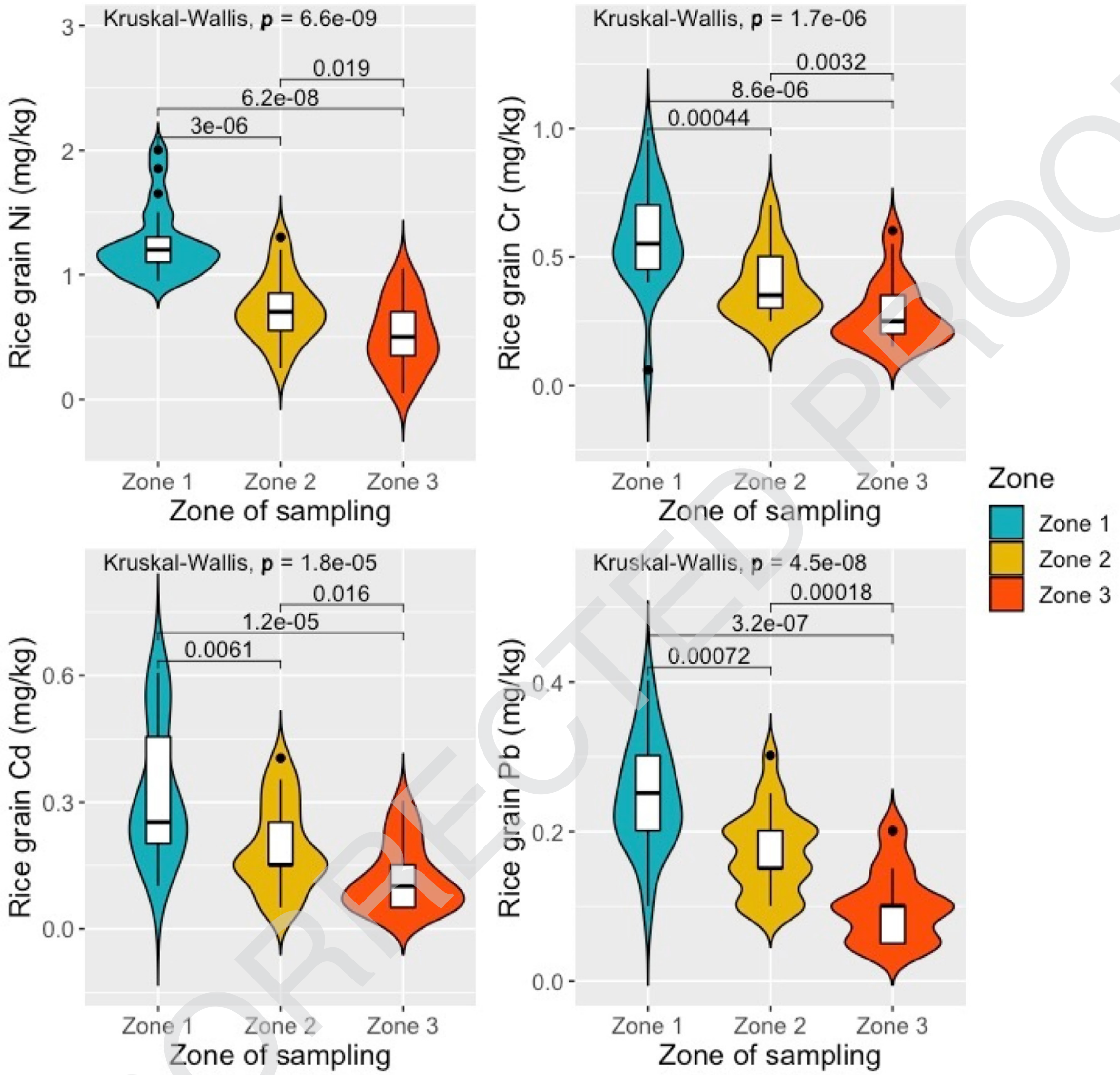

Fig. 1 – Violin Plots representing the comparison among the 3 zones in terms of heavy metal (Ni,Cr, Cd and Pb) content in rice grain.

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the worst performance irrespective of the zones. A comparison of the metal fractions with respect to the zone of sampling can be observed in **(Appendix A Fig. S3a and b)**. All metal fractions varied significantly ($p < 0.05$) higher in between the mica waste-contaminated soils of zone 1 as compared to zones 2 and 3. Significant variations existed in the metal concentrations among the mica-amended contaminated soil due to the diversity in the waste materials dumped at the mine sites over the years. Throughout soils, metals can be found in a variety of different fractions, including those that are easily leached and those that are reacting, as a result of interactions with diverse soil components.

Fig. 3 depicts the three dimension (3D) partial dependence plot of the variation of rice grain metal content for each metal with the two most important variables derived from the variable importance plots of random forest models. It can be observed that The ExNi and the WSNi both have significant contributions to grain Ni content. The grain Ni content increased when the ExNi was more than 30 mg/kg and the WSNi crossed 4 mg/kg. For Cr the CBDCr of above 70 mg/kg and WSCr above 10 mg/kg resulted in the highest uptake of Cr in rice grain. The WSCd above 0.4 mg/kg and OXDCd above 0.1 mg/kg resulted in significant uptake of Cd in rice grain. The EXPb fraction above 25 mg/kg and the CBDPb above 2 mg/kg resulted in the maximum uptake of Pb in rice grain. The partial dependence plot shows the marginal effect one or two features have on the predicted outcome of a machine learning model. The partial dependence plot depicts the marginal impact of one or two features on a machine learning model's predicted outcome (Friedman, 2001). A partial dependence plot can be used to determine whether the relationship between the target and a feature is linear, monotonic, or complex. PDPs show the link between a subset of variables (usually 1-3) and the response while accounting for the average effect of the other predictors in the model. Partial dependence reveals the relationship between the variables in a model we're interested in and the

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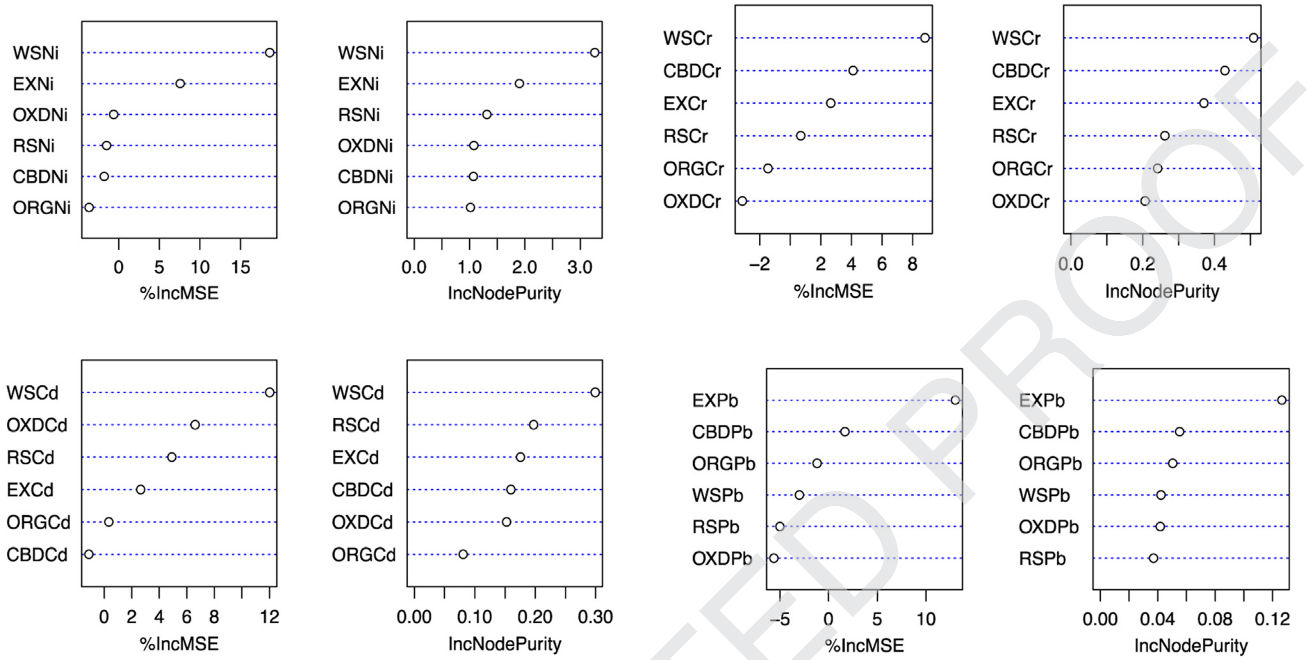

Fig. 2 – Variable Importance plot from Random Forest representing the importance of different fractions of Ni, Cr, Cd and Pb in soil influencing the uptake of metals in rice grain. (%IncMSE: Mean decrease in accuracy, IncNodePurity: Mean Decrease Gini).

expected outcome by marginalising the model output over the distribution of the predictor variables.

Principal component analysis (PCA) revealed that the first two dimensions (Dim-1, Dim-2) of PCA conserved 79.9% of total variation (Appendix A **Fig.** S4). Dim-1 and Dim-2 possess eigenvalues of 3.37 and 1.42 explaining 61.4% and 18.5% of the total explained variance respectively (Appendix A **Fig. S4a**). PCA biplot revealed the highest positive coordinate of the total, carbonate bound, exchangeable and oxide bound fractions with a contribution of 23.07%, 22.82%, 21.06% and 16.68% respectively to Dim-1. This result shows that most of the heavy metals were in carbonate-bound form following exchangeable, oxide bound and other fractions. The lowest contribution of the water-soluble form to Dim-1 indicated the lowest abundance of this fraction. The PCA in Appendix A **Fig. S4b** represents the different fractions of the metals along with the soil's biological properties. The first two dimensions explained 55.5% variation in the data (Dim-1: 45.8% and Dim-2: 9.7%). It was observed that WS-Pb, Ni and Cr and EX-Cr were found to be in positive coordination in Dim-1. Other fractions of metals were found in negative coordinates with respect to both Dim-1 and Dim-2. All the biological parameters were also found in negative coordinates with respect to both Dim-1 and Dim-2. It also implies a negative correlation between them. The abundance of both fractions of heavy metals decreases with an increase in distance from mica mines, while a reverse trend was observed in the case of microbial parameters. The metal concentration in the soil does not provide a reliable indicator for predicting the effects of metals on soil microorganisms and their enzyme activity (Bhattacharyya et al., 2008; Xu et al., 2019). The controlling metal fractions were those bound to carbonate. Using PCA is also a good way of ensuring that as much variation in the data is covered as possible (Mondal et al., 2017; Mandal et al., 2022).

2.4. *Soil chemical and biological properties across 3 zones*

The non-parametric Kruskal-Wallis test as can be observed from the violin plots (showing the distribution of the data) in Appendix A **Fig. S5** revealed that all the chemical properties of soil, pH, EC, TOC, CEC, total and available N, exchangeable P, Ca, Mg and Na were statistically non-significant ($p > 0.05$) in terms of zones of sampling from the source of contamination. The tested soils were found natural to slightly acidic in nature zones 3 and 2 based on pH values. The range of pH values between the three zones were (6.32 to 8.3), (5.5 to 6.31), and (4.78 to 5.44) for zones 1, 2, and 3 respectively. The lowest pH value was found in zone 3 due to the production of organic acids by potassium solubilizing bacteria (KSB); it has previously been reported that KSB bacteria can change soil pH by producing organic acids (acidolysis) (Meena et al., 2015; Saha et al., 2016). The results of these studies are found to be similar, to the earlier findings (Ciarkowska et al., 2017). pH and CEC values were found to vary significantly ($P < 0.05$) across all three sample sites (zones 1, 2, and 3) (Appendix A **Fig. S5**). There was a lower CEC capacity in zone 3 (mean = 4.86 cmol$^+$/kg) as compared to zones 1 (mean = 10.51 cmol$^+$/kg) and 2 and (mean = 7.66 cmol$^+$/kg) respectively, which may be the result of a lack of inorganic colloids and low organic carbon in this mica contaminated soil (Shyamsundar et al., 2014; Chen et al., 2021). The concentration of organic C was significantly ($p <$ 0.05) higher in zone 3 (mean = 0.81%) as compared to zone 2 (mean = 0.62) and zone 1 (mean = 0.40) respectively. In available N, zone 3 (mean = 163.00 mg/kg) was found significantly

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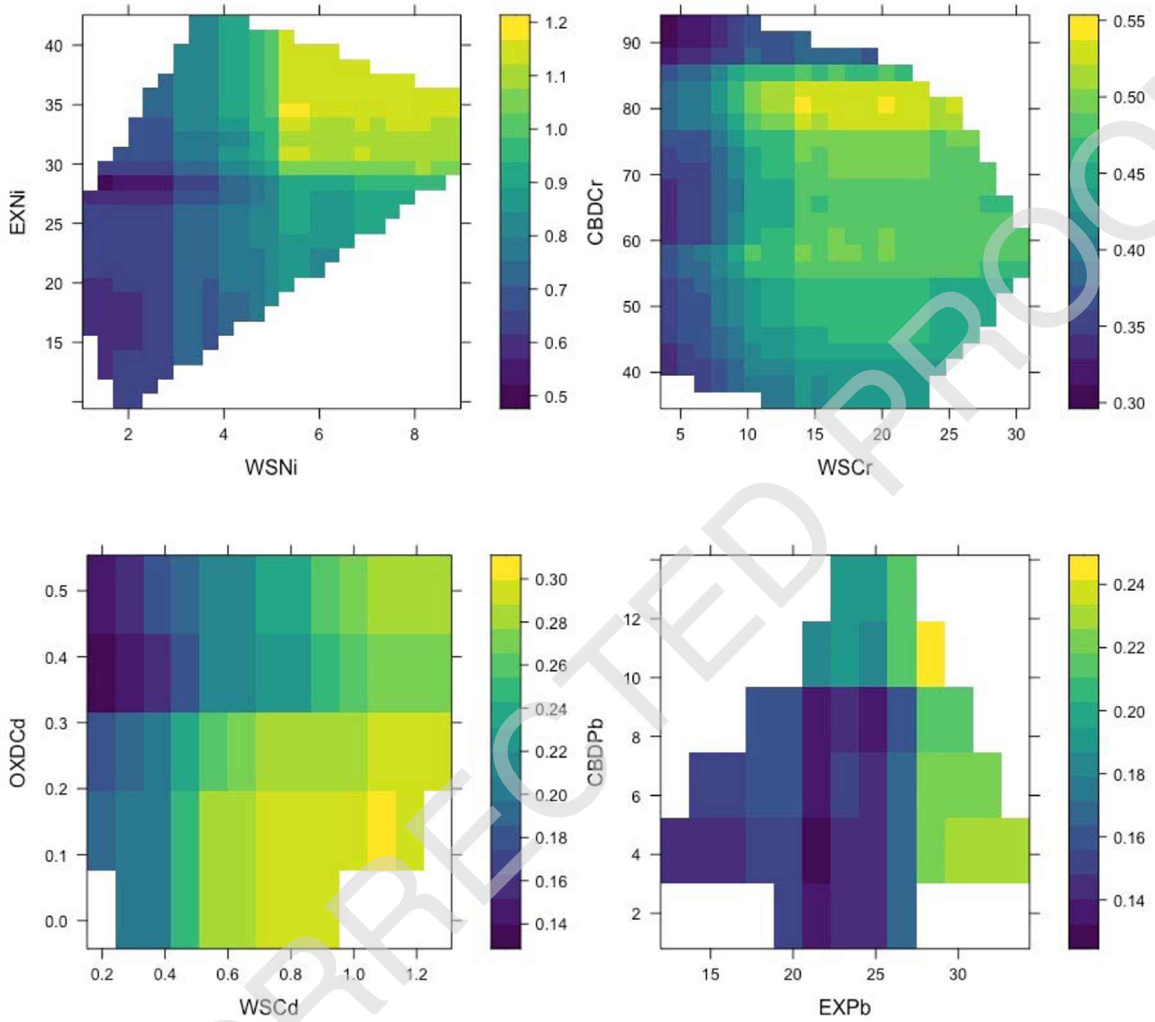

Fig. 3 – Partial dependence plot from random forest model showing the marginal effect of two most important fractions of soil Ni, Cr, Cd and Pb affecting the content of the metals in rice grain (represented by values and colour intensity at right side of each plot). All the parameters have the unit of mg/kg.

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($p < 0.05$) higher concentration of N as compared to zone 2 (mean = 127.79 mg/kg) and zone 1 (mean = 92.47 mg/kg) respectively. The higher organic C content of zone 3 (>100 m) soil than zone 2 (50–100 m) and zone 1 (<50 m) was due to the presence of a high amount of organic materials present in rice agricultural fields. It was previously reported that there was a higher concentration of organic C present in soils containing a greater amount of organic matter (Tripathy et al., 2014). The low level of nitrogen available in the samples is related to the low organic matter contribution (due to the samples' acidity, resulting in a low positive charge). These studies found similar results to earlier ones (Park et al., 2011; Shyamsundar et al., 2014). The available P was higher in sample zone 3 (mean = 5.95 mg/kg) compared to zone 1 (mean = 4.68 mg/kg) and zone 1 (mean = 3.79 mg/kg) respectively (Appendix A **Fig. S5**). Phosphorus availability is influenced by organic matter, and when organic matter is present, phosphorus availability increases. In addition, organic molecules will compete with phosphate adsorbing on soil surfaces, thereby reducing phosphorus retention, thereby increasing phosphorus availability.

2.5. *Soil microbial biomass carbon and metabolic quotient*

Microbial biomass is an integral part of soil organic matter and a labile pool for plant nutrients (Tang et al., 2019; Bhattacharyya et al., 2008). Therefore, the suppression of the soil microbial biomass could often lead to a decrease in the rate of nutrient recycling and the volume of the labile nutrient pool. The MBC value in the mica waste soils was markedly higher in zone 3 (mean = 140.80 mg/kg) than in zone 2 (mean = 91.64 mg/kg) and zone 1 (mean = 64.95 mg/kg) and varied significantly ($p < 0.05$) at each of the three sites (Appendix A **Fig. S6**). In nearby mines side soils (zone 1), the concentration of MBC was lower due to a low organic matter content. The ranged of MBC was zone 3 (74.83 to 396.81 mg/kg) medium zone (53.24 to 195.20 mg/kg), and lower in zone 1 (23.78 to 142.17 mg/kg) respectively. According to Tripathy et al. (2014), the presence of higher organic matter in soil is correlated with higher MBC. The variation in MBC in the landfill soils is related to the interplay of physicochemical properties as well as bioavailable metals in these soils.

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The microbial activity in mica waste amended soils was assessed using three parameters, like basal respiration (BR), and FDA hydrolyzing activities. qCO_2, or microbial metabolic quotient, is an indicator of microbial response to disturbance, based on the ratio of basal respiration to microbial biomass (Dinesh and Chaudhuri, 2013; Tripathy et al., 2014). qCO_2 measures the efficiency of the soil MBC in utilizing C resources, with qCO_2 value higher for zone 1 (mean = 0.07) than zone 2 (mean = 0.17) and zone 3 (mean = 0.03) respectively (Appendix A **Fig. S6**). Increasing qCO_2 showed a shifting of energy by microbial biomass from growth to maintenance, pointing to metal-induced stress on the soil as a consequence of microorganisms spending more energy to survive in mica waste-contaminated soils, resulting in faster respiration and less efficient incorporation of the fresh substrate into new microbial biomass (Zhang et al., 2010; Xiao et al., 2017). Chander et al. (2001) stated that metals disturb the biological division of energy between growth and maintenance in soil microorganisms, which then require greater quantities of C for maintenance, reducing the amount of C incorporated into the biomass. In addition, the high qCO_2 value indicates that microbial respiration occurred faster than their biomass growth, which indicates that most of the biomass energy was used for survival rather than growth (Chakraborty et al., 2022). These results clearly indicate that zone 3 (agricultural field) was the more favourable site for microbial proliferation compared to zones 1 and 2.

2.6. *Soil microbial respiration quotient (QR)*

A microbial respiration quotient is a ratio of basal soil respiration (BSR) to substrate-induced respiration (SIR), which indicates how microbial communities respond to environmental perturbations. BSR is metabolically inherent, whereas SIR pertains to metabolically active populations (Tripathy et al., 2014; Amoakwah et al., 2022). BSR and SIR are dependent on two groups of microorganisms in soil: the K-strategists, which are autochthonous microbial populations, and the R-strategists, which are zymogenous microorganisms (Dilly, 2005). It was found that the QR value in zone 1 soil (mean = 0.26) was considerably higher than in zone 2 (mean = 0.16), and zone 3 (mean = 0.15) (Appendix A **Fig. S6**). It reflects that dormant populations of metabolically active organisms are suppressed. Hence, the suppressive effects of soil acidity seem specific to metabolically active zymogenous populations, indicating that biomass synthesis is less efficient under heavy metal loadings in tailings, and the reduction of biomass in heavy metal-contaminated soils is mainly due to inefficient biomass synthesis. Higher concentrations of QR suggest a shift from energy-based enhancement to ecosystem maintenance (Grodnitskaya et al., 2022). An extended QR ratio also indicates a shift from an increase in energy to the maintenance of the ecosystem (Brookes, 1995; Bhattacharyya et al., 2008).

2.7. *Fluorescein diacetate hydrolyzing activity (FDA)*

Among three sample zones, FDA value was found significantly ($p < 0.05$) higher in zone 3 (mean = 89.53) as compared to zone 2 (mean = 59.69) and zone 1 (mean = 41.42) respectively (Appendix A **Fig. S6**). FDA concentrations in zone 1 were lower due to heavy metal contamination in mica tailing soils, decreased uptake by active microbes, and a reduction in microbial biomass carbon availability under heavy metal stress, and inefficient biosynthesis contributes to the reduction of biomass in heavy metal contaminated soils (Atakpa et al., 2022; de Aguiar Santiago et al., 2022). FDA activity has long been regarded as a potential biological indicator to determine soil microbial activity. It is a non-fluorescent, non-polar derivative that is easily transported to the cell, where it has been hydrolysed into polar fluorescein by several enzymes viz. lipase, esterase, protease, that are involved in organic matter decomposition (Tripathy et al., 2014; Nannipieri et al., 2003). Active cells are capable of converting the FDA into fluorescein. When the cells are unable to hold the fluorescein, it eventually gets out of the cell and is measured spectrophotometrically (Dzionek et al., 2018). The finding results indicated that the presence of higher concentrations of heavy metals may be inhibited the production of the enzymes in the mine's side soils (zone 1).

2.8. *Soil enzyme activities*

The assay of enzyme activities is important for estimating the effects of metal pollution on the soil environment (Dick, 1997). Soil enzyme activity is a sensitive indicator of the effect of environmental factors on microbial functions. Dehydrogenase, urease, phosphatase, and beta-D glucosidase activities of the mica waste-contaminated soils varied significantly ($p < 0.05$) between all three sample zones (zones 1, 2, and 3) **(Appendix A Fig. S6)**. The values of DHG, urease, phosphatase, and beta-D glucosidase activities were higher in zone 3 (mean = 23.01, 678.38, 38.51, and 44.57 mg/kg, respectively) as compared to zone 2 (mean = 15.24, 341.34, 25.24, and 29.46 mg/kg, respectively) and zone 3 (mean = 10.19, 239.88, 16.22, and 18.94 mg/kg, respectively). Soils of zone 3 (>100 m) have higher enzyme activity levels, due to presence of organic matter. The enzyme activity levels were also stabilized by the enzyme-organic matter complex (Tripathy et al., 2014; Dick, 1997). Inhibition of DHG activity might be due to the reduced microbial biomass and the activity in the soils because the activity is derived from intracellular enzymes (Dick, 1997). The microbial enzyme activities in soil are used to estimate the adverse effects of pollutants on soil quality (Warman and Munroe, 2010; Niemeyer et al., 2012; Tripathy et al., 2014). Microbes are the chief controlling agent of enzymatic regulations; thus, they have the potential to respond to any environmental changes and provide detailed information on enzyme catalytic reactions associated with the biological process. They indicate soil health quality (Dick, 1997; Taylor et al., 2002).

2.9. *Metal removal interactions and microbial attributes: a correlation-based analysis*

The heat plot (Fig. 4) represents the interaction (spearman correlation) of the different fractions of metals (Ni, Cd, Cr and Pb) with the soil's biological properties. The soil biological properties APS, MBC, FDA, Beta D, Urease and DHG were positively correlated with each other at ($p < 0.05$). All the bioavailable fractions of Ni, Cr, Cd and Pb were negatively correlated with

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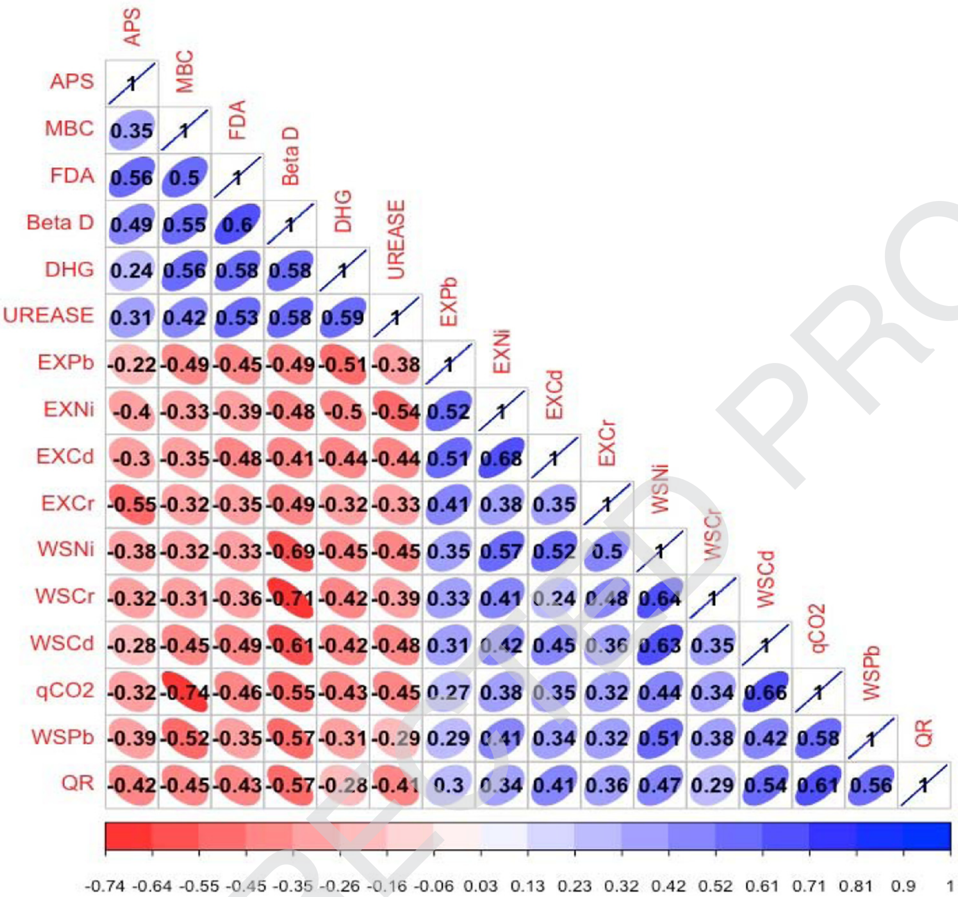

Fig. 4 – Interactions between metal bioavailability and microbial activity are based on spearman correlation ($p<0.05$).

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the soil biological properties at $p < 0.05$. On the other hand, a significant positive correlation was observed between the different fractions of metals (Ni, Cd, Cr and Pb). However, it is difficult to determine whether cadmium, chromium, lead and nickel strongly affected the soil microbial properties individually because there were highly positive correlations ($p < 0.05$) among all the metals studied and the changes in the concentration of the metals in the field were similar to each other (Fig. 4). It is suggested that the metals affected microbial properties by behaving synergistically or additively with each other (Tripathy et al., 2014). The water-soluble and exchangeable metal fractions are usually the most toxic than other fractions because they can be easily released into the water as metal ions. That can easily and direct affect soil microbes (Ghosh et al., 2004; Bhattacharyya et al., 2005). Metal ions such as Cr, Cd, Ni and Pb are thought to react with sulfhydryl groups to inactive enzymes, a reaction similar to the formation of metallic sulfide. In enzymes, the sulfhydryl group acts as an integral part of the catalyst activating site or as an involved group to maintain the proper structural relationship of the enzyme protein (Juma and Tabatabai, 1977; Kamaludeen et al., 2003). Metal can also reduce microbial enzyme activity by interacting with enzyme-substrate complexes, using denaturing the enzyme proteins, or interacting with protein-active groups (Dick, 1997; Tripathy et al., 2014). Thus, we expect that the higher the level of water-soluble and exchangeable metals, the lower activity of soil microorganisms.

3. Conclusions

Ni, Cd, Cr and Pb content in rice grain was highest in zone 1 followed by zones 2 and 3. Chromium, Ni, and Pb were found to be the most abundant heavy metals in the mica-contaminated paddy soil samples. From the fractionation study followed by modelling with RF it was observed from variable importance plots that the water-soluble and exchangeable fractions of all four metals are particularly concerning for rice grain metal uptake. According to the results, metal contamination is most critical in zone 1 soils since it contains significantly more bioavailable metals compared to zone 2 and zone 3. The results indicate that the water-soluble and exchangeable metal fractions inhibit the biological properties of soil, despite occupying only a small portion of the total metal concentration in the soil. The MBC, QR and FDA were observed to be highest in zone 3 followed by zone 2 and least in zone 1. A similar trend was observed in terms of soil enzymatic activities for DHG, urease, phosphatase, and beta-D glucosidase. It can be concluded that unplanned and inadvertently dumped mica mine wastes will contaminate the food chain and affect the soil

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quality and ecological systems of the region in the long run hence appropriate control measures should be undertaken.

Q2

Uncited References

Chen et al., 2021, Page, Miller and Keeney, 1982, Walkley and Black, 1934

Appendix A Supplementary data

Supplementary data associated with this article can be found in the online version at

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.10.038.

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