



ELSEVIER

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

[www.elsevier.com/locate/jes](http://www.elsevier.com/locate/jes)

JES

JOURNAL OF  
ENVIRONMENTAL  
SCIENCES[www.jesc.ac.cn](http://www.jesc.ac.cn)

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

Saibal Ghosh<sup>1</sup>, Sandip Mondal<sup>1</sup>, Jajati Mandal<sup>2,\*</sup>, Abhishek Mukherjee<sup>1,\*</sup>, Pradip Bhattacharyya<sup>1,\*</sup>

<sup>1</sup>Agricultural and Ecological Research Unit, Indian Statistical Institute, Giridih, Jharkhand 815301, India

<sup>2</sup>School of Science, Engineering & Environment, University of Salford, Manchester M5 4WT, UK

## ARTICLE INFO

### Article history:

Received 1 August 2022

Revised 24 October 2022

Accepted 24 October 2022

Available online 2 November 2022

### Keywords:

Metal fractions

Microbial parameters

Mica waste soil

Soil enzymes

Rice

Random forest

## 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  $0.83 \pm 0.41$ ,  $0.41 \pm 0.19$ ,  $0.21 \pm 0.14$  and  $0.17 \pm 0.08$  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.

© 2023 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

\* Corresponding authors.

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

<https://doi.org/10.1016/j.jes.2022.10.038>

1001-0742/© 2023 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

## 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<sup>2</sup> 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 kg 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., 2014). They play a significant role in nutrient turnover and are influenced by several biotic and abiotic factors.  $\beta$ -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 assessed 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 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<sup>2</sup>) 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.

## 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, 1934). 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<sub>2</sub> 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<sub>3</sub> 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<sub>3</sub>)<sub>2</sub> 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 hr at room temperature for bound to carbonate fraction; with 0.08 mol/L NH<sub>2</sub>OH HCl, shaking (120 r/min) for 6 hr at 96°C temperature for bound to Fe and Mn – oxide phase metal; for organic matter fraction 1 g soil samples with 0.02 mol/L HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> shaking (120 r/min) for 2 and 3 hr at 85°C temperature and sulphides phase fraction, with 3.2 mol/L NH<sub>4</sub>OAc shaking (120 r/min) for 30 min at room temperature, and finally digestion with concentrated HNO<sub>3</sub> at 105°C for mineral matrix fraction (Page et al., 1982). 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<sub>3</sub>. Certified reference material SRM 2710 and blank extract were used for quality assurance. The reference material was then digested in ultrapure HF/HNO<sub>3</sub>/HClO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> 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<sub>3</sub>:HClO<sub>4</sub>) in a beaker placed on an electric heating plate at 190 °C for 1 hr 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<sub>2</sub> was measured when

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<sub>3</sub>) 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 hr. The activity of acid phosphatase (APS) and β-D-glucosidase (BetaD) 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 hr 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 hr. The dehydrogenase enzyme activity of the moist sample was measured as described by the method of Casida (1964). The determination of dehydrogenase (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 hr. The microbial metabolic quotient and respiratory quotient were calculated using values of microbial biomass carbon, basal soil respiration, and substrate-induced respiration using Eqs. (1) and (2) (Alef and Nannipieri, 1995; Chakraborty et al., 2022).

$$q_{CO_2} = (R_{b-CO_2})/C \quad (1)$$

$$q_r = R_b/R_s \quad (2)$$

where,  $q_{CO_2}$  is the microbial metabolic quotient,  $R_{b-CO_2}$  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  $n_{tree}=500$  and  $m_{try}=2$ . 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/100 g 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

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

Parameter	Mean	Range (Min–Max)	Median	*Q <sub>3</sub> -Q <sub>1</sub>
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 ( $\mu$ g fluorescein/(g soil.hr))	63.5	15.1–171.7	60.1	80.6–41.3
DHG ( $\mu$ g TPF/(g.hr))	16.1	3.1–45.8	14.9	21.5–9.2
APS ( $\mu$ g p-nitrophenol/g soil.hr)	419.8	44.7–1462.2	351.9	552.4–221.7
Urease ( $\mu$ g urea/·g soil/hr)	26.6	3.3–89.4	23.6	32.0–16.8
BetaD ( $\mu$ g p-nitrophenol/g soil.hr)	30.9	16.0–61.2	29.6	39.5–20.2
BSR ( $\mu$ g CO <sub>2</sub> released/g soil.hr)	14.3	9.5–21.6	14.3	15.2–13.3
SIR( $\mu$ g CO <sub>2</sub> released/g soil.hr)	81.8	39.2–118.7	88.2	101.2–56.6
qCO <sub>2</sub>	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

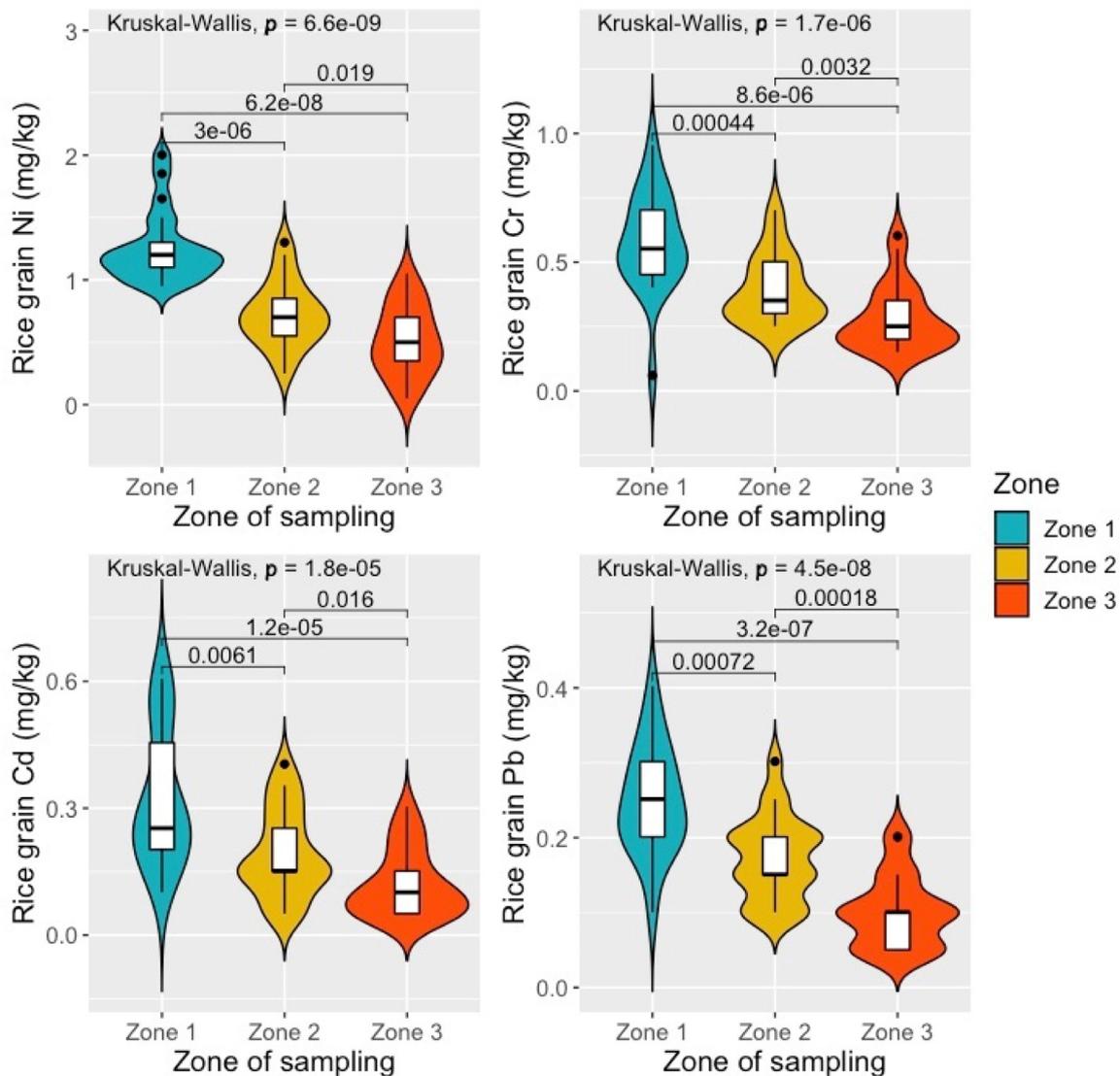
\*Q<sub>3</sub>-Q<sub>1</sub>: Represents the inter quantile range, between 3<sup>rd</sup> Quantile (Q<sub>3</sub>) and 1<sup>st</sup> Quantile (Q<sub>1</sub>).

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<sub>2</sub>: metabolic quotient; QR: soil microbial respiration quotient.

0.72 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 (OXDCd), 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 compari-

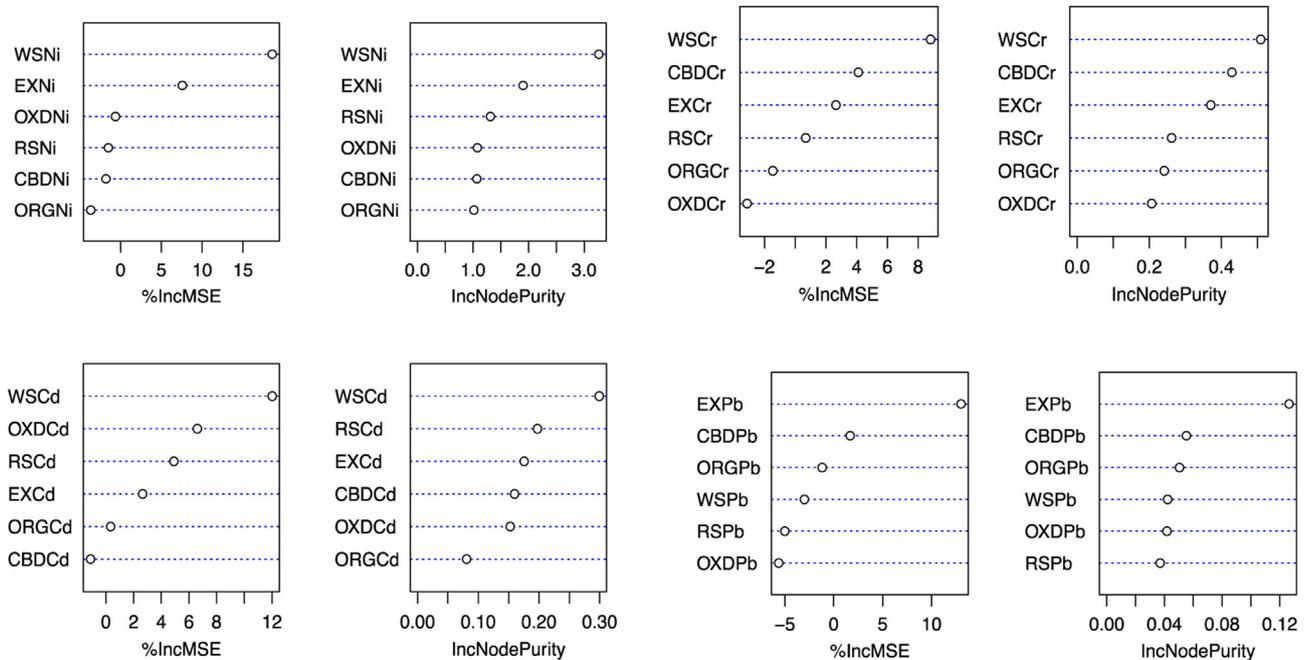


**Fig. 1** – Violin plots representing the comparison among the 3 zones in terms of heavy metal (Ni, Cr, Cd and Pb) content in rice grain.

son 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 in-

creased 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



**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 Accuracy, IncNodePurity: Mean Decrease Gini).**

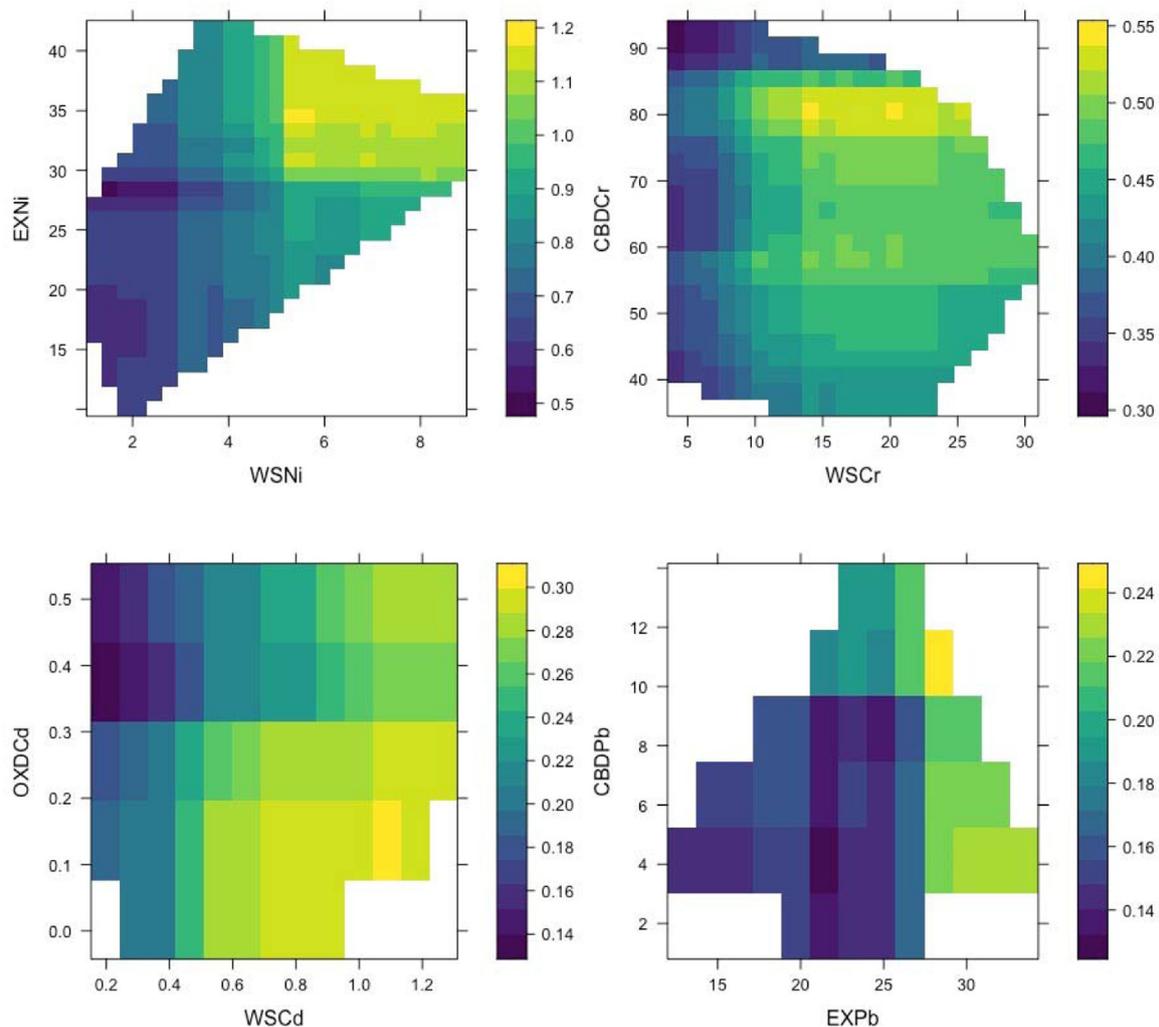
reveals the relationship between the variables in a model we're interested in and the expected outcome by marginalising the model output over the distribution of the predictor variables.

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  $\text{cmol}^+/\text{kg}$ ) as compared to zones 1 (mean = 10.51  $\text{cmol}^+/\text{kg}$ ) and 2 and (mean = 7.66  $\text{cmol}^+/\text{kg}$ ) respectively, which may be the result of a lack of inorganic colloids and low organic carbon in this mica contaminated soil (Shyamsundar et al.,



**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.**

2014; Chen et al., 2021). The concentration of organic carbon 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 carbon 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 carbon 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.

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 in-

dicates 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 (Dzionic 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).

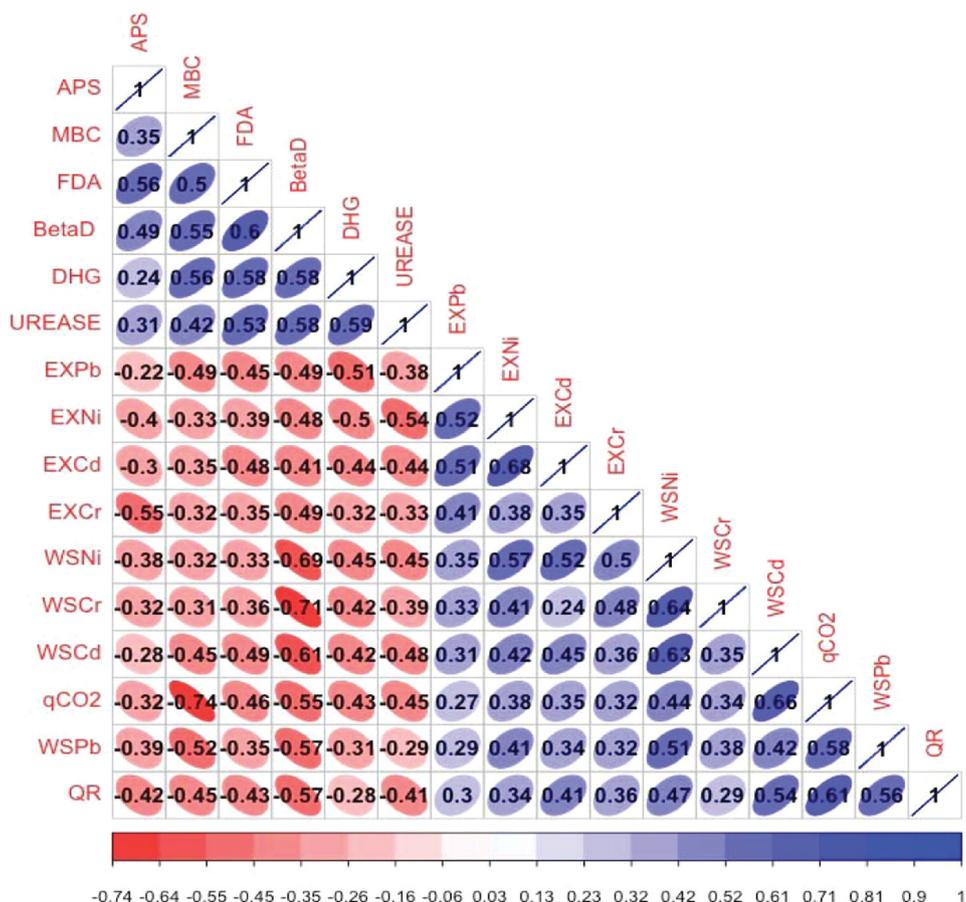


Fig. 4 – Interactions between metal bioavailability and microbial activity are based on spearman correlation ( $p < 0.05$ ).

### 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, BetaD, 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. 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 responsible 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 occu-

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

### 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.

### Appendix A Supplementary data

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

### REFERENCES

- Alef, K., Nannipieri, P., 1995. *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, London, UK, pp. 214–217.
- Amoakwah, E., Arthur, E., Frimpong, K.A., Lorenz, N., Rahman, M.A., Nziguheba, G., et al., 2022. Biochar amendment impacts on microbial community structures and biological and enzyme activities in a weathered tropical sandy loam. *Appl. Soil Ecol.* 172, 104364.
- Atakpa, E.O., Zhou, H., Jiang, L., Ma, Y., Liang, Y., Li, Y., et al., 2022. Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria. *Chemosphere* 290, 133337.
- Basak, B.B., Biswas, D.R., 2009. Influence of potassium solubilizing microorganism (*Bacillus mucilaginosus*) and waste mica on potassium uptake dynamics by sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. *Plant Soil* 317, 235–255.
- Bascomb, C.L., 1964. Rapid method for the determination of cation-exchange capacity of calcareous and non-calcareous soils. *J. Sci. Food Agric.* 15 (12), 821–823.
- Beckett, P.H.T., 1989. The use of extractants in studies on trace metals in soils, sewage sludges, and sludge-treated soils. *Adv. Soil Sci.* 9, 143–176.
- Bhattacharyya, P., Chakraborty, A., Chakrabarti, K., Tripathy, S., Powell, M.A., 2005. Chromium uptake by rice and accumulation in soil amended with municipal solid waste compost. *Chemosphere* 60, 1481–1486.
- Bhattacharyya, P., Tripathy, S., Chakrabarti, K., Chakraborty, A., Banik, P., 2008. Fractionation and bioavailability of metals and their impacts on microbial properties in sewage irrigated soil. *Chemosphere* 72, 543–550.
- Breiman, L., 2001. Random forests. *Mach. Learn.* 45, 5–32.
- Brookes, P.C., 1995. The use of microbial parameters in monitoring soil pollution by heavy metals. *Biol. Fertil. Soils* 19, 269–279.
- Candeias, C., Melo, R., Ávila F., P., da Silva F., E., Salgueiro R., A., Teixeira P., J., 2014. Heavy metal pollution in mine–soil–plant system in S. Francisco de Assis – Panasqueira mine (Portugal). *Appl. Geochem.* 44, 12–26.
- Casida, Jr L.E., Klein, D.A., Santoro, T., 1964. Soil dehydrogenase activity. *Soil Sci.* 98, 371–376.
- Chakraborty, P., Sarkar, S., Mondal, S., Agarwal, B.K., Kumar, A., Bhattacharya, S., et al., 2022. *Eisenia fetida* mediated vermi-transformation of tannery waste sludge into value added eco-friendly product: An insight on microbial diversity, enzyme activation, and metal detoxification. *J. Clean. Prod.* 348, 131368.
- Chander, K., Dyckmans, J., Joergensen, R., Meyer, B., Raubuch, M., 2001. Different sources of heavy metals and their long-term effects on soil microbial properties. *Biol. Fertil. Soils* 34, 241–247.
- Chen, N., Fu, Q., Wu, T., Cui, P., Fang, G., Liu, C., et al., 2021. Active iron phases regulate the abiotic transformation of organic carbon during redox fluctuation cycles of paddy. *Soil Environ. Sci. Technol.* 55, 14281–14293.
- de Aguiar Santiago, F.L., Silva, A.O., Carneiro, M.A.C., Gastauer, M., Ramos, S.J., Caldeira, C.F., et al., 2022. Revegetation of mined areas influences the physiological profile of bacterial communities and improves the biochemical functions of soil. *Pedobiologia* 90, 150793.
- Dewis, J., Freitas, F. (Eds.), 1984. *Physical and Chemical Methods of Analysis of Soils and Waters*. Food and Agriculture Organization, Soils Bulletin (10), Rome.
- Dick, R.P., Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., 1997. Soil enzyme activities as integrative indicators of soil health. In: *Biological Indicators of Soil Health*. CAB International, New York, pp. 121–156.
- Dilly, O., Buscot, F., Varma, A., 2005. Microbial energetics in soil. *Microorganisms in Soils: Roles in Genesis and Functions*. Soil Biology, 3 Eds.. Springer-Verlag, Berlin, Heidelberg.
- Dinesh, R., Chaudhuri, S.G., 2013. Soil biochemical/microbial indices as ecological indicators of land use change in mangrove forests. *Ecol. Ind.* 32, 253–258.
- Doran, J.W., Parkin, T.B., Doran, J.W., Jones, A.J., 2015. Quantitative indicators of soil quality: a minimum data set. In: *Methods for Assessing Soil Quality*, 49. Soil Science Society of America, Madison, USA, pp. 25–37.
- Dzionek, A., Dzik, J., Wojcieszynska, D., Guzik, U., 2018. Fluorescein diacetate hydrolysis using the whole biofilm as a sensitive tool to evaluate the physiological state of immobilized bacterial cells. *Catalysts* 8, 434.
- Friedman, Jerome H., 2001. Greedy function approximation: a gradient boosting machine. *Ann. Stat.* 1189–1232.
- Ghosh, A.K., Bhattacharyya, P., Pal, R., 2004. Effect of arsenic contamination on microbial biomass and its activities in arsenic contaminated soils of Gangetic West Bengal. *India. Environ. Int.* 30, 491–499.
- Groditskaya, I.D., Karpenko, L.V., Pashkeeva, O.E., Goncharova, N.N., Startsev, V.V., Baturina, O.A., et al., 2022. Impact of forest fires on the microbiological properties of oligotrophic peat soils and gleyed peat podzols of bogs in the northern part of the Sym-Dubches Interfluvium, Krasnoyarsk Region. *Eur. J. Soil Sci.* 55, 460–473.
- Huang, H., Ji, X.B., Cheng, L.Y., Zhao, F.J., Wang, P., 2021. Free radicals produced from the oxidation of ferrous sulfides promote the remobilization of cadmium in paddy soils during drainage. *Environ. Sci. Technol.* 55 (14), 9845–9853.
- Huang, J., Li, Z., Zeng, G., Zhang, J., Li, J., Nie, X., et al., 2013. Microbial responses to simulated water erosion in relation to organic carbon dynamics on a hilly cropland in subtropical China. *Ecol. Eng.* 60, 67–75.
- James, G., Witten, D., Hastie, T., Tibshirani, R., 2013. *An Introduction to Statistical Learning with Applications in R*. Springer, New York.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2017. Report of the Eleventh Session of the Codex Committee on Contaminants in Foods. Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission: Rio de Janeiro, Brazil. pp.8.

- Joergensen, R.G., Wu, J., Brookes, P.C., 2011. Measuring soil microbial biomass using an automated procedure. *Soil Biol. Biochem.* 43, 873–876.
- Juma, N.G., Tabatabai, M.A., 1977. Effects of trace elements on phosphatase activity in soils. *Soil Sci. Soc. Am. J.* 41, 343–346.
- Kamaludeen, S.P.B., Megharaj, M., Juhasz, A.L., Sethunathan, N., Naidu, R., 2003. Chromium-microorganism interactions in soils: remediation implications. *Rev. Environ. Contam. Toxicol.* 178, 93–164.
- Knudsen, D., Peterson, G.A., Pratt, P.F., 1983. Lithium, sodium, and potassium. In: *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties (2nd Ed.)*. Soil Science Society of America, Madison, USA, 9, 225–246.
- Kumar, A., Maiti, S., 2015. Assessment of potentially toxic heavy metal contamination in agricultural fields, sediment, and water from an abandoned chromite-asbestos mine waste of Roro hill, Chaibasa, India. *Environ. Earth Sci.* 74, 2617–2633.
- Li, T., Song, Y., Yuan, X., Li, J., Ji, J., Fu, X., et al., 2018. Incorporating bioaccessibility into human health risk assessment of heavy metals in rice (*Oryza sativa* L.): a probabilistic-based analysis. *J. Agric. Food Chem.* 66, 5683–5690.
- Lindsay, W.L., Norvell, W.A., 1978. Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Am. J.* 42, 421–428.
- Ma, S.C., Zhang, H.B., Ma, S.T., Wang, R., Wang, G.X., Shao, Y., et al., 2015. Effects of mine wastewater irrigation on activities of soil enzymes and physiological properties, heavy metal uptake and grain yield in winter wheat. *Ecotoxicol. Environ. Saf.* 113, 483–490.
- Mandal, J., Bakare, W.A., Rahman, M.M., Rahman, M.A., Siddique, A.B., Oku, E., et al., 2022. Varietal differences influence arsenic and lead contamination of rice grown in mining impacted agricultural fields of Zamfara State, Nigeria. *Chemosphere* 305, 135339.
- Mandal, J., Sengupta, S., Sarkar, S., Mukherjee, A., Wood, M.D., Hutchinson, S.M., et al., 2021. Meta-analysis enables prediction of the maximum permissible arsenic concentration in asian paddy soil. *Front. Environ. Sci.* 9, 760125.
- Meena, V.S., Maurya, B.R., Verma, J.P., Aeron, A., Kumar, A., Kim, K., et al., 2015. Potassium solubilizing rhizobacteria (KSR): isolation, identification, and K-release dynamics from waste mica. *Ecol. Eng.* 81, 340–347.
- Mondal, A., Das, S., Sah, R.K., Bhattacharyya, P., Bhattacharya, S.S., 2017. Environmental footprints of brick kiln bottom ashes: Geostatistical approach for assessment of metal toxicity. *Sci. Total Environ.* 609, 215–224.
- Monterroso, C., Rodríguez, F., Chaves, R., Diez, J., Becerra-Castro, C., Kidd, P.S., et al., 2014. Heavy metal distribution in mine-soils and plants growing in a Pb/Zn-mining area in NW Spain. *Appl. Geochem.* 44, 3–11.
- Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. *Eur. J. Soil Sci.* 54, 655–670.
- Niemeyer, J.C., Lolata, G.B., Carvalho, G.M., de, Da, Silva, E.M., Sousa, J.P., Nogueira, M.A., 2012. Microbial indicators of soil health as tools for ecological risk assessment of a metal contaminated site in Brazil. *Appl. Soil Ecol.* 59, 96–105.
- Nishanth, D., Biswas, D.R., 2008. Kinetics of phosphorus and potassium release from rock phosphate and waste mica enriched compost and their effect on yield and nutrient uptake by wheat (*Triticum aestivum*). *Biores. Technol.* 99, 3342–3353.
- Olsen, S.R., Sommers, L.E., Page, A.L., 1982. Phosphorus. In: Page A.L., (Ed.), *Methods of Soil analysis. Part 2. Chemical and Microbiological Properties*. American Society of Agronomy, Soil Science Society of America, Madison, USA, pp. 403–430.
- Page, A.L., Miller, R.H., Keeney, D.R., 1982. *Methods of Soil Analysis*. American Society of Agronomy, Madison, USA.
- Park, J.H., Lamb, D., Paneerselvam, P., Choppala, G., Bolan, N., Chung, J.-W., 2011. Role of organic amendments on enhanced bioremediation of heavy metal (loid) contaminated soils. *J. Hazard. Mater.* 185, 549–574.
- Saha, M., Maurya, B.R., Meena, V.S., Bahadur, I., Kumar, A., 2016. Identification and characterization of potassium solubilizing bacteria (KSB) from Indo-Gangetic Plains of India. *Biocatal. Agric. Biotechnol.* 7, 202–209.
- Schnürer, J., Rosswall, T., 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl. Environ. Microbiol.* 43, 1256–1261.
- Sengupta, S., Bhattacharyya, K., Mandal, J., Bhattacharya, P., Halder, S., Pari, A., 2021. Deficit irrigation and organic amendments can reduce dietary arsenic risk from rice: Introducing machine learning-based prediction models from field data. *Agric. Ecosyst. Environ.* 319, 107516.
- Shyamsundar, P.C., Das, M., Maiti, S.K., 2014. Phyto stabilization of Mosaboni copper mine tailings: a green step towards waste management. *Appl. Ecol. Environ. Res.* 12, 25–35.
- Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angel, J.S., Bottomley, P.S. (Eds.), *Methods of Soil Analysis. Microbiological and Biochemical Properties*. Soil Science Society of America, Madison, WI, pp. 775–833.
- Tang, J., Zhang, J., Ren, L., Zhou, Y., Gao, J., Luo, L., et al., 2019. Diagnosis of soil contamination using microbiological indices: a review on heavy metal pollution. *J. Environ. Manag.* 242, 121–130.
- Taylor, J.P., Wilson, B., Mills, M.S., Burns, R.G., 2002. Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. *Soil Biol. Biochem.* 34, 387–401.
- Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal. Chem.* 51, 844–851.
- Tripathy, S., Bhattacharyya, P., Mohapatra, R., Som, A., Chowdhury, D., 2014. Influence of different fractions of heavy metals on microbial ecophysiological indicators and enzyme activities in century old municipal solid waste amended soil. *Ecol. Eng.* 70, 25–34.
- Walkley, A., Black, C.A., 1934. An examination of wet acid method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* 37, 29–38.
- Warman, P.R., Munroe, M.D., 2010. The effects of high metal concentrations in soil-compost mixtures on soil enzymes. *J. Environ. Sci. Health B* 45, 633–638.
- Xiao, L., Guan, D., Peart, M.R., Chen, Y., Li, Q., Dai, J., 2017. The influence of bioavailable heavy metals and microbial parameters of soil on the metal accumulation in rice grain. *Chemosphere* 185, 868–878.
- Xu, Y., Seshadri, B., Bolan, N., Sarkar, B., Ok, Y.S., Zhang, W., et al., 2019. Microbial functional diversity and carbon use feedback in soils as affected by heavy metals. *Environ. Int.* 125, 478–488.
- Zhang, F.P., Li, C.F., Tong, L.G., Yue, L.X., Li, P., Ciren, Y.J., et al., 2010. Response of microbial characteristics to heavy metal pollution of mining soils in central Tibet, China. *Appl. Soil Ecol.* 45, 144–151.
- Zhang, J., Yang, S., Yang, H., Huang, Y., Zheng, L., Yuan, J., et al., 2018. Comparative study on effects of four energy plants growth on chemical fractions of heavy metals and activity of soil enzymes in copper mine tailings. *Int. J. Phytoremed.* 20, 616–623.
- Zhao, F.J., Wang, P., 2020. Arsenic and cadmium accumulation in rice and mitigation strategies. *Plant Soil* 446, 1–21.
- Zhou, H., Zhou, X., Zeng, M., Liao, B.H., Liu, L., Yang, W.T., et al., 2014. Effects of combined amendments on heavy metal accumulation in rice (*Oryza sativa* L.) planted on contaminated paddy soil. *Ecotoxicol. Environ. Saf.* 101, 226–232.