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Research

Metals Accumulation of Tropical Shrub *Melastoma malabathricum* L. (Melastomataceae) Populations and Their Relation To Soil Edaphic Factor

Norshahida Saberi[1], Mohd Izuan Effendi Halmi[2], Muhammad Saiful Ahmad Hamdani[1], Noor Amalina Ramle[3] and Khairil Mahmud[1,4]*

1. Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), 43400 Seri Kembangan, Selangor, Malaysia
2. Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia (UPM), 43400 Seri Kembangan, Selangor, Malaysia
3. Centre of Toxicology and Health Risk (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia (UKM), KL Campus, 50300, Kuala Lumpur, Malaysia
4. Unit of Biodiversity, Institute of Bioscience, Universiti Putra Malaysia, 43000 Serdang, Selangor, Malaysia
*Corresponding author: khairilmahmud@upm.edu.my

ABSTRAK

Melastoma malabathricum L. (Melastomataceae) is a widely distributed tropical shrub that grows in Southeast Asia. Recent research found that *M. malabathricum* has a bioremediatory potential that can accumulate high concentrations of toxic metals such as Al, Pb, As, and Cr. Thus, this study aimed to investigate the metal hyperaccumulation in *M. malabathricum* from various populations and their relation to soil edaphic factors. We collected mature leaves and soils of *M. malabathricum* from 15 different populations in Negeri Sembilan, Selangor, and Pahang in Peninsular Malaysia. These 15 populations consist of lowland forests, dump sites, riparian, oil palm and rubber plantations, paddy fields, and mining sites. We found that *M. malabathricum* accumulated high concentrations of Al (3.45±1.58 to 8.697±1.61 mg g^{-1}) followed by Fe (1.02±0.02 to 1.07±0.04 mg g^{-1}), Pb (0.013±0.001 to 0.016±0.001 mg g^{-1}), As (0.008±0.005 to 0.23±0.004 mg g^{-1}), and Cr (0.005±0.0003 to 0.02±0.01 mg g^{-1}). The highest concentration of soil Al was 85.95±5.00 mg g^{-1}, Fe with 69.960±7.47mg g^{-1}, Pb with 0.192±0.03 mg g^{-1}, As with 0.156±0.06 mg g^{-1} and Cr with 0.133±0.03 mg g^{-1}. We found no significant association between high foliar metal concentrations of the metals (Al, Pb, As & Cr) with the soil chemical properties but some soil metal elements were intercorrelated with foliar metal concentrations. Understanding the potential of *M. malabathricum* in accumulating high levels of metal elements, provides useful information for phytoremediation works. Further research is required to investigate the mechanism uptake and tolerance of heavy metals in *M. malabathricum.*

Key words: Heavy metals accumulation, *Melastoma malabathricum*, soil chemical properties, phytoremediation, environment

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INTRODUCTION

Soil contamination is one of the worldwide serious problems including in Malaysia. Soil contamination has been attributed largely to human expansion activities in the form of urbanization and industrialization (Patra *et al*., 2020). These activities have led to the continuous release of contamination and are easily transferred to natural resources (surface water, groundwater & air), and accumulate in the food chain, thus compromising human health (Patra *et al*., 2020). Besides, these contaminations have been reported to cause negative effects on agricultural productivity, and soil ecosystem, reduce soil properties, and affect human health (Shah & Daverey, 2020). Metal contamination has become an environmental concern due to its long-term persistence. Metal contamination of the soil arises through natural occurrences and anthropogenic activities. Typical metal contamination in soil is due to heavy metals and metalloids. The extensive use of agrochemicals (pesticides & fertilizer) plays an important role in agricultural soil. Heavy metals and metalloids may enrich soil through industrial sludge, mining activities, municipal waste, and transportation. Once heavy metal contaminates the soil, it will be kept in the soil for many years because of its non-biodegradable nature (Sumiahadi & Acar, 2018).

Heavy metals such as chromium (Cr), lead (Pb), cadmium (Cd), nickel (Ni), copper (Cu), and some metalloids such as aluminum (Al) and arsenic (As) are highly toxic to living things and categorized among the most hazardous substances (Shah & Daverey, 2020). High exposure to these metals has been linked to adverse health effects and is considered to be carcinogenic to humans as well (Patra *et al*., 2020). These toxic elements accumulate in the soils, and potential contamination in the food chain through uptake at the primary producer level and then through consumption at consumer levels (Shah & Daverey, 2020). In addition, the accumulation of these metals in crop plant systems has become a great concern. The metal contamination that is attached to soil particles will be absorbed by plant roots and accumulate in crop plants (Rahman & Zaim, 2015). The accumulation of metals significantly affects the physiological and biochemical processes in plants and restrains their growth. Toxicity of metals destroys membranes, damages different types of biomolecules by producing reactive oxygen species (ROS), and inhibits the early stages of germination of seeds (Patra *et al*., 2020).

Several traditional remediation methods have been used to remove or reduce the concentration of metals in soil but these techniques produce different pollutants and are expensive (Cioica *et al*., 2019). Phytoremediation is now recognized as a new appropriate method that uses selective plants to decontaminate hazardous substances and site restoration. This method involves stabilizing the metals in the rhizosphere and subsequently translocating them into aerial parts. The contaminated biomass is harvested on the maturity of the plants. Consequently, the contaminants are removed from the soil (Patra *et al*., 2020). Some plants have the ordinary ability to accumulate toxic metals without having any injuries and they are classified as “hyperaccumulators”. Hyperaccumulator plants can exceed 100 times or more than the normal concentrations of accumulated metals or metalloids in their aboveground biomass without showing signs of phytotoxicity, although in some cases they can have low productivity. According to Reeves *et al*. (2018), hyperaccumulating plants are defined based on their metal concentrations of foliar tissues in their natural habitat (mg g^{-1}), i.e., >0.1 for Cd; >0.3 for Cu and Cr; >1 for As, Al, Ni, and Pb; >3 for Zn; and >10 mg g^{-1} for Mn. The selection of efficient hyperaccumulating plants is important for phytoremediation of metal-contaminated soils. Recent reports have discovered over 700 plant species across the world classified as hyperaccumulator plants. Among the plants for metals and metalloids accumulators listed were from family Brassicaceae, Cunouniaceae, Caryophyllaceae, Asteraceae, Euphorbiaceae, Cyperaceae, Fabaceae, Lamiaceae, Violaceae, Poaceae, Melastomataceae, Theaeceae, and Rubiaceae (Rascio & Navari-Izzo, 2011).

The study species was the tropical shrub *Melastoma malabathricum* (Melastomataceae), which is a known Al accumulator plant (Khairil & Burslem, 2018). *M. malabathricum* occurs from islands in the Indian Ocean to South and Southeast Asia, China, Taiwan, Australia, and the South Pacific Ocean and is found in a range of natural vegetation types, as well as wasteland, secondary forest, and roadsides (Patek-Mohd *et al*., 2018). Several reports found that *M. malabathricum* is a potential bioremediator since it can accumulate high concentrations of Al (Khairil & Burslem, 2018), uranium and thorium (Saat *et al*., 2015), and other heavy metals such as Cu, Fe, Mn, Pb, As, Zn and Cd (Yeo & Tan, 2011; Selamat *et al*., 2014; Patek-Mohd *et al*., 2018). However, the information on the edaphic influence on the metal accumulation of *M. malabathricum* is less reported. Therefore, this study was conducted to investigate the foliar metal elements concentrations accumulated between *M. malabathricum* populations and their association with the soil edaphic factor. Support for this is that differential expression of the metal accumulation trait may be associated directly with tolerance to high metal concentrations. This paper contains two hypotheses as below:

1. The foliar metals concentrations and soil physico-chemical properties may significantly vary among *M. malabathricum* populations.
2. The variations of this metal element concentration are significantly influenced by the soil edaphic factor of the population or habitat.

MATERIALS AND METHODS

Study species

Melastoma malabathricum (Melastomataceae) is native to tropical and subtropical regions of Asia including Malaysia, Indonesia, and Singapore. *M. malabathricum* is a shrub or small tree that can reach up to 3-4 meters in height. It has opposite, elliptical to ovate leaves that are about 7-15 cm long and 4-8 cm wide (Saat *et al*., 2015). In Malaysia and other tropical countries, it grows wild and is commonly found in previously cleared land, waste places, and roadsides (Yong, 2014). Scientific findings also revealed the wide pharmacological actions of various parts of *M. malabathricum*, such as antinociceptive, anti-inflammatory, wound healing, antidiarrheal, cytotoxic, and antioxidant activities (Joffry *et al*., 2012).

Soil and leaves sampling

Soil and leaves of *M. malabathricum* a total of 15 sampling sites were selected (Table 1; Figure 1) from different habitat populations which are palm oil plantation, post-mining, rubber plantation, secondary forest, forest reserve, paddy, roadside, and riparian. The sampling sites represent almost all the natural habitats occupied by *M. malabathricum* within Peninsular Malaysia. Four undamaged mature leaves from fully exposed locations were collected and pooled per individual to form 3 samples per population. Three soil samples from 0-15 cm depth were collected using an auger within a 1-2 m distance from the *M. malabathricum* individual that had been sampled for leaves following the collection method by Khairil *et al*. (2014) and Mahmud *et al*. 2020). The leaf and soil samples were transported to the Universiti Putra Malaysia, for analysis.

Table 1. Sampling location of *M. malabathricum* populations

Plot	Population	Habitat	Latitude	Longitude
S1	Salak Tinggi	Oil Palm Plantation	2.820436	101.7261
S2	Bagan lalang	Oil Palm Plantation	2.608281	101.7012
S3	Kuala Selangor	Secondary Forest	3.197553	101.3087
S4	Bestari Jaya	Ex Tin Mining	3.42081	101.4485
S5	Bestari Jaya	Ex Tin Mining	3.405182	101.4248
S6	Serendah	Ex Tin Mining	3.363868	101.6111
S7	Bera	Riparian	3.274358	102.5447
S8	Kuala bera	Riparian	3.34684	102.5225
S9	Bera	Dumpsites	3.288658	102.5285
S10	Tasek Bera	Forest reserve	3.122463	102.6299
S11	Bera	Forest reserve	3.301747	102.6656
S12	Jempol	Rubber Plantation	2.992433	102.4692
S13	Jempol	Rubber Plantation	3.001963	102.4167
S14	Kuala Pilah	Paddy field	2.745879	102.1645
S15	Kuala Pilah	Paddy field	2.737863	102.1068

Plant analysis

Foliar concentrations analyses were conducted following Alarefee *et al*. (2021). The leaves samples were cleaned with tap water and rinsed a few times with distilled water to remove adhering materials. The leaves from each population were oven-dried at 50-55 °C for 4 days. The samples were then ground in a blender and digested using the dry-ashing method for P, K, Ca, Mg, Al, As, Pb, and Cr. Dry-ashing (oxidation) is normally performed by placing the sample in an open vessel (crucible) and destroying the organic (combustible) parts in the sample by heat in a muffle furnace at a temperature of 500 °C. In this method, 1 g of oven-dried sample was weighed into a crucible and placed in a muffle furnace to ash at an initial temperature of 300 °C for 1 hr, and then the temperature was subsequently raised to 500 °C for 4 hr. After cooling in a desiccator, the samples were then placed in a fume cupboard. A few drops of distilled water were added to the ash samples, followed by 2 mL of concentrated HCl, and then it was allowed to evaporate to dryness on a hot plate. Subsequently, 10 mL of 20% HNO_3 (200 mL HNO_3 in 1 L distilled water) was added to the samples and was then placed in a hot bath for 1 hr. The samples were then filtered using a Whatman No. 2 filter paper into a 100 mL volumetric flask and made up to volume with distilled water. The concentration of P, K, Ca, Mg, Al, As, Pb, and Cr contained in the soil were determined by Microwave Plasma Atomic Emission Spectroscopy (Agilent 4200 MP-AES). The total nitrogen content (% N) was extracted using the Kjeldahl method, followed by identification through distillation and titration with 0.01N HCl.

Soil analysis

Analyses on the soil physico-chemical properties were conducted following Alarefee *et al*. (2021). The soil samples were air-dried and then passed through a 2 mm sieve and were analyzed for selected chemical and physical properties. Soil pH was measured at a soil-to-distilled water ratio of 1:2.5, and the pH was afterward read using a pH meter. Electrical conductivity (EC) was determined at the soil-to-distilled water ratio of 1:5, and EC was subsequently measured using an electrical conductivity meter. Organic matter (OM) content was determined by the weight loss after drying at 105 °C for 6 h and then ignition at 600 °C in a muffle furnace for 6 hr. Particle size distribution was determined using the pipette method. In this method, the inorganic soil particle was separated into sand, silt, and clay fractions. The CEC was measured after successive extraction (three times) using 1 M ammonium acetate (NH4-OAc) adjusted to pH 7.0 and determined with titration of 0.01N HCl. The total nitrogen (% N) was extracted using the Kjeldahl method and identified by distillation and titration of 0.01N HCl. Total nutrient elements and some heavy metals/metalloids (P, K, Ca, Mg, Al, As, Pb & Cr) were analyzed using Microwave Plasma Atomic Emission Spectroscopy (Agilent 4200 MP-AES).

Fig. 1. Sampling locations map.

Statistical analysis

All analytical results were performed as an average of three replicates. All data were analyzed for normal distribution before further statistical analyses. The precision of the data was calculated and expressed as mean and standard error (SE). Data were subjected to an ANOVA procedure using R-studio version 2023.03.1 Build 446. Pearson correlation test was employed to define the relationship between nutrients and heavy metals/metalloids in the plant and soil. The association of nutrients and heavy metal/metalloid elements in soil and plants was identified using Principal Component Analysis (PCA). The significance level was set at a 95% confidence level (α=0.05).

RESULTS AND DISCUSSION

Foliar metal concentrations among wild populations of *M. malabathricum*

The lowest and highest mean ± SE elements concentrations in the leaves from different habitats were as follows: N ranged from 1.62±0.09% to 2.61±0.10%; P from 0.41±0.03 to 0.48±0.03 mg g^{-1}; K from 0.64±0.22 to 1.13±0.15 mg g^{-1}; Ca from 1.59±0.51 to 3.39±0.32 mg g^{-1}; Mg from 0.14±0.03 to 0.336±0.13 mg g^{-1}; Fe from 1.02±0.03 to 1.07±0.04 mg g^{-1}; Al from 3.45±1.59 to 8.69 ±1.61 mg g^{-1}; Pb from 12.98±0.73 to 16.66±0.73 mg kg^{-1}; and Cr from 4.64±0.34 to 21.91±17.67 mg kg $^{-1}$; and As from 7.87±4.56 to 23.47±4.21 mg kg^{-1} (Table 2). The foliar elements concentrations showed no significant differences among the 15 wild populations of *M. malabathricum* (p>0.05). There was no regular pattern in the heavy metal/metalloid concentrations from the 15 sampling sites. The order of macronutrients by mean concentration for all populations in the *M. malabathricum* leaves was N > Ca > P > K > Mg, while for trace and heavy metal/metalloids, it was Al > Fe > Pb > As > Cr, respectively.

The highest foliar N concentration was found at site S7 with 2.61±0.10 %. S2 contributed the highest foliar of P (0.48±0.03 mg g^{-1}), K (1.13±0.15 mg g^{-1}), Mg (0.336±0.13mg g^{-1}) and Pb (16.66±0.73mg kg^{-1}). The highest accumulation of Al was detected at S1, (8.7±1.6 mg g^{-1}), foliar Fe concentration at S8 (1.066±0.04 mg g^{-1}), foliar As at S15 (23.47±4.21 mg kg^{-1}), Pb at S2 (16.66±0.73 mg kg^{-1}) and Cr at S11 (14.24±5.56 mg kg^{-1}) (Table 2). All trace and heavy elements (Al, Fe, Pb, As & Cr) showed concentrations exceeding the normal limit as stated in Table 2.

The soil-plant transfer of metals and nutrients is natural and a part of the nutrient cycle. Metals are taken up in different concentrations by plants, most often through soil solutions, and higher metal accumulation indicates higher metal contents in soil. The highest metal concentrations found in *Melastoma malabathricum* in this study were Al followed by Fe, N, K, P, Ca, Mg, Pb, As, and Cr. Although Al is commonly associated with toxicity and impaired growth in most plants, a beneficial effect of this element has been noted in several plants, especially in Al hyperaccumulators such as *M. malabathricum*. The presence of Al can stimulate growth efficiency and improved uptake of nutrients as in *Camellia sinesis* (Amirah *et al*., 2023), *Medicago sativa* (Wang *et al*., 2016), *Melastoma malabathricum* (Bojórquez-Quintal *et al*., 2017; Khairil & Burslem, 2018), *and Fagopyrum esculentum* (Horbowicz *et al*., 2011). *M. malabathricum* can accumulate high concentrations of Al and can reach up to >10 mg g^{-1} Al (Watanabe & Osaki, 2006), and 15.5 mg g^{-1} Al (Khairil & Burslem, 2018). We found foliar Fe concentrations were significantly higher compared to other mature plant tissues as suggested by Ekin (2022) which are in the ranges of 0.05-0.25 mg kg^{-1}. Previous research by (Watanabe *et al*., 2006) showed the ability of *M. malabathricum* to accumulate Fe is higher than 1 mg g^{-1} and 10 mg g^{-1} in the shoot and root respectively. The higher absorption of Fe in the plant due to the special characteristic of metal elements function as a stressing agent and low substrate of Fe fraction (Rusmanta *et al*., 2019).

Pb, As, and Cr are non-essential metals for plants, and their accumulation in plants is due to their relative immobility. These metals are less immobile or transported within the plant and therefore tend to accumulate in the tissues. The accumulation of non-essential metals in plants can cause toxicity and potentially harm the plant's growth and development (Rascio & Navari-Izzo, 2011). The normal concentration of foliar Pb in mature leaves is around 1.1-1.7 mg kg^{-1}, but our study showed an average concentration of 14.5±0.38 mg kg^{-1} in *M. malabathricum* leaves which is above the normal limit for plants (Ekin, 2022). *M. malabathricum* can accumulate up to 2,200 mg kg^{-1} Pb in the leaves and higher in the root (13,000 mg kg^{-1}) in the Pb-treated soil (Ashraf *et al*., 2011; Selamat *et al*., 2014). Moreover, *M. malabathricum* can survive in soils with As concentrations of up to 40 mg kg $^{-1.}$ It exhibits a high translocation factor, suggesting its efficiency in transporting As to the upper parts of the *M. malabathricum*, particularly reaching 570 mg kg^{-1} in the stem and leaves (Selamat *et al*., 2014). Cr can cause toxicity in plants when concentrations exceed 2 mg kg^{-1} (Aloud *et al*., 2022). Cr in *M. malabathricum* from all habitats exceeded the safe limit and could be harmful to the local public. No clear study to show the ability to accumulate Cr in the *M. malabathricum* tissues. However, similar to the previous study by a native plant of *H. acutigluma* which can accumulate Cr up to 10.2 mg kg^{-1} on their shoot (Chu *et al*., 2019).

Most plants have gradually formed an avoidance mechanism and tolerance mechanism in the heavy metal stress environment. Avoidance mechanisms include plants affecting the mobility of heavy metals and microbial activity through root exudates, fine isolation, and regionalization of cell walls, cell membranes, and vacuoles. Avoidance mechanisms are to reduce the amount of heavy metals in the plants. The tolerance mechanism is that the plant itself has mechanisms to reduce the toxicity of heavy metals in the body, including chelation, osmotic adjustment, and antioxidant systems (Yu *et al*., 2019).

Table 2. Mean (±SE) of foliar metal concentrations of 15 *M. malabathricum* populations

Sampling location	N (%)	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	Al (mg g^{-1})	Fe (mg g^{-1})	Pb (mg kg^{-1})	As (mg kg^{-1})	Cr (mg kg^{-1})
S1	2.38±0.29	0.41±0.02	0.64±0.22	1.59±0.51	0.17±0.07	3.45±1.58	1.04±0.07	14.27±3.58	11.20±5.16	9.95±2.81
S2	2.20±0.18	0.48±0.03	1.13±0.15	2.49±0.54	0.33±0.13	7.22±1.80	1.03±0.02	16.66±0.73	12.10±7.26	8.50±1.0
S3	1.87±0.05	0.45±0.01	0.83±0.08	2.79±0.39	0.32±0.03	6.26±0.51	1.02±0.25	16.61±1.82	10.28±7.31	5.27±0.59
S4	2.44±0.18	0.45±0.01	0.93±0.04	2.44±0.18	0.23±0.03	5.94±0.48	1.04±0.10	13.45±1.35	14.32±7.73	4.64±0.34
S5	1.62±0.09	0.43±0.03	0.97±0.22	2.22±0.37	0.14±0.03	6.02±1.29	1.04±0.01	15.34±1.67	17.73±6.09	6.69±2.01
S6	2.31±0.12	0.44±0.01	0.74±0.14	3.39±0.32	0.28±0.04	4.93±0.77	1.04±0.04	14.46±0.97	21.53±14.63	21.91±17.67
S7	2.63±0.12	0.44±0.02	1.02±0.03	2.31±0.05	0.26±0.01	6.27±0.43	1.03±0.03	13.06±0.79	9.68±3.35	15.51±6.79
S8	1.97±0.65	0.45±0.01	0.80±0.03	2.91±0.16	0.22±0.02	8.15±0.45	1.07±0.44	13.04±1.09	12.47±2.14	5.13±0.78
S9	1.90±0.10	0.47±0.04	0.84±0.09	2.79±0.08	0.33±0.06	8.63±0.64	1.04±0.08	14.18±1.73	10.38±3.51	12.28±3.38
S10	2.14±0.12	0.44±0.02	0.92±0.11	2.57±0.49	0.21±0.01	8.69±1.61	1.05±0.02	14.09±0.77	24.40±2.19	10.34±1.41
S11	1.92±0.16	0.43±0.02	0.96±0.10	2.19±0.27	0.28±0.01	6.65±0.57	1.05±0.04	13.02±1.03	9.56±5.18	14.24±5.56
S12	1.90±0.29	0.41±0.03	0.74±0.17	2.36±0.61	0.18±0.05	7.29±2.08	1.06±0.05	12.98±0.73	7.87±4.56	8.68±1.54
S13	1.73±0.03	0.43±0.02	0.85±0.03	2.71±0.16	0.14±0.01	6.49±1.05	1.05±0.04	14.47±0.55	23.256±9.58	8.45±3.07
S14	2.37±0.23	0.45±0.05	0.70±0.18	2.16±0.54	0.30±0.12	5.65±2.26	1.04±0.10	15.25±2.45	12.39±5.20	4.67±0.44
S15	2.21±0.15	0.44±0.02	0.86±0.03	2.54±0.41	0.23±0.01	6.21±0.58	1.05±0.03	16.59±0.46	23.47±4.21	10.02±4.11
F-VALUE	1.50	0.97	0.10	1.16	1.26	1.22	0.72	0.74	0.62	0.76
P-VALUE	0.17	0.51	0.48	0.35	0.27	0.31	0.74	0.72	0.83	0.70
*Normal concentration	1.5-4	2-5	0.1-5	3-30	0.1-1	0.015-0.1	0.05-0.25	1-1.7	5-10	0.1-0.5

p<0.05*, *p*<0.005**, *p*<0.001***

*Average concentrations of trace and heavy elements in mature leaf tissue for various plant species (Ekin, 2022).

Correlation elements among foliar metal concentrations of *M. malabathricum*

There were several significant foliar metal concentrations among *M. malabathricum* populations. Al shows a positive significant correlation with most nutrient elements (P, K, Ca & Mg) ($p<0.05$) (Table 3). There was also a significant correlation between foliar Mg-Pb and As-Cr in *M. malabathricum* ($p<0.05$). The metal foliar concentrations distribution and association were explained by Principal Component Analysis (PCA) (Figure 2). The first axis of a PCA of the foliar data explained 28.02% of the variation and the first six PC axes cumulatively explained 93.61% of the variance. The first PC axis was positively associated ($P<0.05$) with variation in As and Fe (Figure 2). The second PC axis was positively associated with variation in concentrations of Mg, Pb, and N and other elements were negative ($P<0.05$). PC1 explained 28.02% of the data variation and P, Mg, and Fe as the highly weighted factors. PC2, which contributed to 19.85% of the studied variances, displayed the main loading factor for N, Ca, and Al.

Several reports mentioned that Al may benefit Al hyperaccumulators like *M. malabathricum* in stimulating other nutrients such as Ca and Mg (Mahmud & Burslem, 2020). Other Al hyperaccumulators such as *Symplocos*, were also reported to have a significant correlation with Ca concentration (Schmitt *et al*., 2016). This association both at the leaf level and the entire plant level had provided evidence for a genetic disposition of the mechanisms underlying the uptake of Ca in the presence of Al. The genus *Symplocos* is known to have calcium oxalate crystals stored in its leaf tissue. This oxalate can bind to both Ca and Al and could therefore act as a regulating molecule for the accumulation of both elements. Moreover, the formation of crystals with metal inclusion and subsequent storage in the vacuole is also hypothesized to be a possible mechanism to reduce the toxic effect of Al and other metals (Schmitt *et al*., 2016).

Table 3. Pearson's correlation coefficient (*r*) between metal foliar concentrations of *M. malabathricum* populations

	N	P	K	Ca	Mg	Al	Fe	Pb	As
P	0.24								
K	0.19	0.59**							
Ca	0.02	0.52**	0.43**						
Mg	0.28	0.79**	0.49**	0.56**					
Al	-0.09	0.57**	0.63**	0.66**	0.52**				
Fe	0.07	-0.12	-0.13	-0.28	-0.21	-0.03			
Pb	0.14	0.49**	0.55**	0.25	0.41**	0.25	-0.06		
As	-0.03	0.09	0.09	0.40**	0.08	0.22	-0.08	0.08	
Cr	0.1	-0.14	-0.20	0.09	-0.02	-0.15	-0.01	-0.19	0.36*

$p<0.05$*, $p<0.01$**, $p<0.001$***

Soil mineral and heavy metals/metalloids concentration

The mean concentration of different essential and heavy metal/metalloids showed that Al was present in the highest amount followed by Fe > N > Mg> Ca > K > P > Pb > As > Cr (Table 4). The highest Fe was recorded at S13 (69.960±7.48 mg g^{-1}), Al in S5 (85.954±5.01 mg g^{-1}), Pb of 0.192±0.070.34 mg g^{-1} in S5, whereas As and Cr were 0.192±0.121 mg g^{-1} and 0.133±0.03 mg g^{-1} in the S13 and S9 populations. Based on the ANOVA, there were significant differences observed in the soil chemical properties ($p<0.05$) among the 15 *M. malabathricum populations* except for Ca ($p>0.05$). The soil physico-chemical distribution and association were displayed in the PCA. The PC axes of the soil data explained 23.42% of the variation and the first six PC axes cumulatively explained 85.42% of the variance (Figure 3). The first PC axis was positively associated ($P<0.05$) with variation in concentration of Ca, Cr, Mg, K, Fe, As, Pb, and Al (Figure 3). The second PC axis was positively associated with variation in soil parameters of silt, clay, OM, and P and other elements were negative ($P<0.05$).

Excessive accumulation of metals in soil can contribute to metal pollution and contamination, which can have adverse effects on the soil, plants, and the surrounding ecosystem. The accumulation of metals in the soil can be a result of human activities such as mining, industrial processes, the use of certain pesticides and fertilizers, and waste disposal activities. The presence of excessive metals in the soil can make it difficult for plants to grow and can also be toxic to animals and humans that consume them. In addition, the metals can leach into groundwater, further spreading the contamination (Edelstein & Ben-Hur, 2018). Besides, the availability of metals such as O, Si, Al, Fe, K, Na, Ca, P, and Ti in soil also may be contributed by natural processes.

In general, the concentration of soil metal elements in our study decreased in the order of Al>Fe

> Pb > As > Cr. Activities such as agriculture, mining, and other industrial activities may increase the levels of heavy metals/metalloid concentrations in the soil. Additionally, land application of sewage sludge, organic waste manure, industrial byproducts, and irrigation with wastewater are major sources of heavy metals in agricultural systems (Srivastava *et al*., 2017). According to Manan *et al*. (2019) significant amount of heavy metal (Cu, Zn, Pb & Ni) was found in oil palm plantations due to the continuous application of chemical fertilizers. Mining sites such as gold mines, tin mines, and iron ore mines were found to increase higher concentrations of heavy metals/metalloids including As, and Pb and they are less fertile compared to other soils (Rajoo *et al*., 2017). Several bauxite mining areas such as Johor, Pahang, and Sarawak bauxite mining contribute to the higher concentrations of Cr, Pb, Ni, Al, and Fe due to the acid mine drainage when mined materials are exposed to air and water (Ismail *et al*., 2018; Kusin *et al*., 2018).

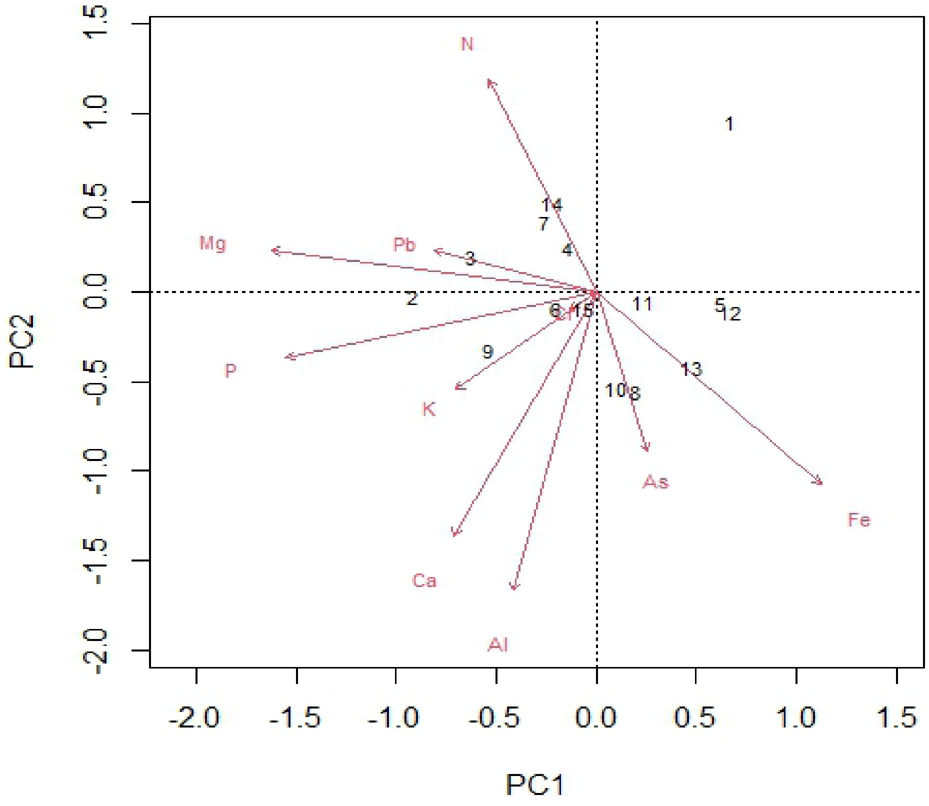

Fig. 2. Biplot of scores for principal component axes (PC) 1 and 2 from Principal Component Analysis of concentrations of 10 elements in leaves derived from 15 *M. malabathricum* populations. PC1 and PC2 accounted for 28.02% and 19.85% of the total variation, respectively. The arrows show the loadings of each element on the first two PC axes.

Table 4. Mean (±SE) of soil nutrient and heavy metal/metalloid concentration from *M. malabathricum* populations

Sampling location	N (%)	P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	Al (mg g^{-1})	Fe (mg g^{-1})	Pb (mg kg^{-1})	As (mg kg^{-1})	Cr (mg kg^{-1})
S1	0.26±0.06	0.298±0.05	0.52±0.22	0.28±0.07	1.68±0.32	31.47±10.1	19.19±4.94	73.68±5.52	22.84±4.54	105.43±39.88
S2	0.14±0.04	0.20±0.01	0.32±0.07	0.16±0.02	1.25±0.02	38.13±7.56	33.85±7.72	49.67±10.84	35.59±14.72	112.81±28.12
S3	0.09±0.03	0.16±0.003	0.36±0.02	0.37±0.17	1.34±0.07	20.57±3.49	22.27±1.82	41.44±3.64	55.33±17.19	49.03±3.39
S4	0.21±0.05	0.20±0.002	0.20±0.02	0.32±0.06	1.28±0.01	21.75±2.76	14.00±0.99	47.21±6.10	44.25±8.10	49.07±12.72
S5	0.13±0.01	0.21±0.01	0.22±0.03	0.19±0.02	1.26±0.08	85.95±5.01	30.05±3.39	192.03±70.34	60.18±16.36	72.17±14.05
S6	0.19±0.02	0.20±0.005	0.44±0.02	0.25±0.03	1.62±0.13	14.25±3.84	13.89±0.39	56.80±11.74	43.03±9.49	41.92±5.82
S7	0.13±0.02	0.20±0.003	0.91±0.19	0.35±0.04	2.00±0.19	83.04±3.92	48.18±12.9	150.50±95.62	50.54±9.83	111.29±10.51
S8	0.25±0.004	0.18±0.01	0.13±0.03	0.22±0.03	1.40±0.07	29.72±2.03	25.31±1.93	82.79±8.92	32.96±16.37	54.54±9.99
S9	0.10±0.03	0.19±0.01	0.04±0.01	0.30±0.07	1.10±0.01	19.79±2.80	54.12±7.61	24.29±2.39	15.68±4.09	133.98±28.30
S10	0.13±0.03	0.18±0.001	0.07±0.02	0.26±0.03	1.14±0.03	11.99±3.62	24.84±1.78	21.28±0.87	32.97±13.74	40.99±5.541
S11	0.13±0.01	0.19±0.01	0.11±0.02	0.19±0.01	1.29±0.05	19.53±5.04	16.71±4.44	23.34±3.35	44.10±6.867	49.22±14.48
S12	0.15±0.02	0.20±0.01	0.13±0.03	0.18±0.01	1.12±0.02	13.24±2.57	15.03±0.55	31.24±2.20	37.00±7.69	17.07±2.36
S13	0.16±0.01	0.21±0.01	0.19±0.05	0.33±0.08	1.43±0.17	29.99±10.3	69.96±7.47	57.31±16.53	156.68±60.33	109.60±19.62
S14	0.13±0.02	0.23±0.01	0.16±0.02	0.32±0.04	1.23±0.03	69.26±13.3	12.71±2.83	180.63±32.46	42.02±9.76	27.11±6.16
S15	0.12±0.01	0.18±0.01	0.12±0.02	0.21±0.01	1.11±0.01	26.91±6.07	7.18±0.14	90.23±2.65	18.15±5.55	7.04±0.54
MEAN	0.15±0.01	0.20±0.01	0.26±0.04	0.26±0.02	1.35±0.04	34.37±3.83	27.15±2.81	74.83±10.71	46.09±6.29	65.42±6.8
F-VALUE	3.005	4.273	7.834	1.256	4.542	15.257	11.197	2.976	3.046	5.258
P-VALUE	0.006**	0***	0***	0.29	0***	0***	0***	0.006**	0.005**	0***
*Natural concentration in soil	NA	NA	0.0014	NA	0.141	42567	12.140	10.37	15.6	6

p<0.01*; *p*<0.05**; *p*<0.001***, NA=Not Applicable
*Typical range of naturally occurring metals concentrations (Department of Environment Malaysia, 2009)

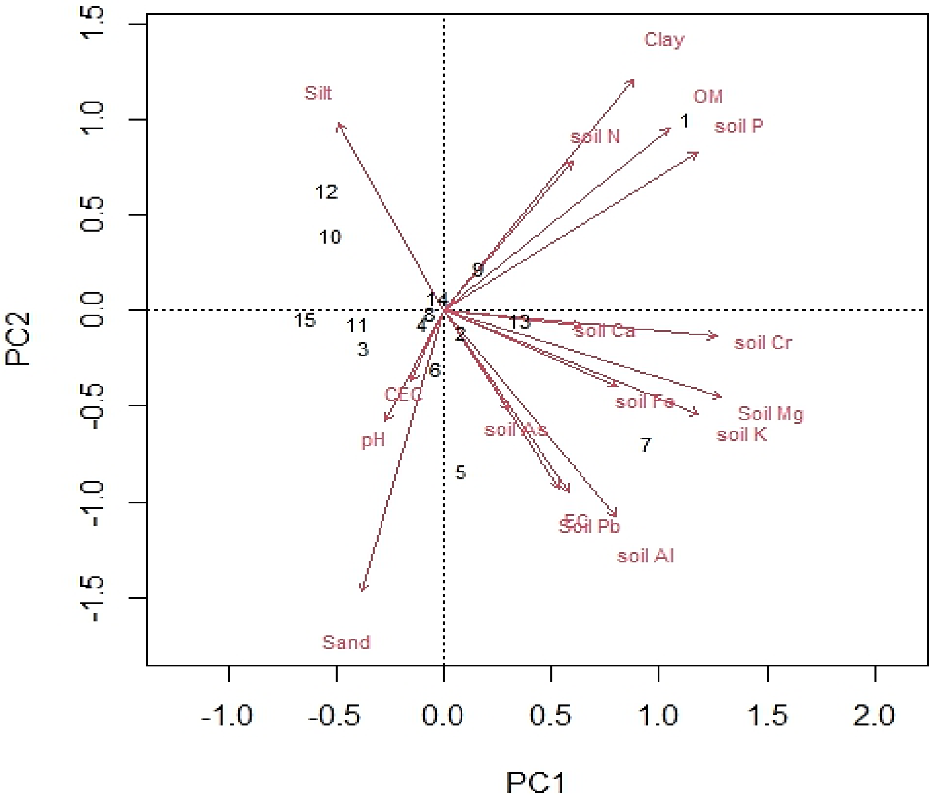

Fig. 3. Biplot of scores for principal component axes (PC) 1 and 2 from Principal Component Analysis of concentrations of 17 variables of soil derived from 15 *M. malabathricum* populations. PC1 and PC2 accounted for 23.42% and 19.13% of the total variation, respectively. The arrows show the loadings of each element on the first two PC axes.

The association of metal foliar concentrations with soil edaphic

We found foliar Al, Fe, Pb, As, and Cr concentrations of *M. malabathricum* were uncoupled to high similar metal concentrations in the soil (*p*>0.05) (Table 5). This accumulation may be influenced by other several factors such as genotype and other environmental factors which were not accounted for in this study and may have influenced the metal accumulation in *M. malabathricum*. Genetic factors can play an important role in metal accumulation in plants because certain plant species have evolved to have specific adaptations that allow them to tolerate and/or accumulate certain metals. These adaptations may include specialized transport mechanisms, increased root surface area for metal uptake, or the ability to detoxify metals through sequestration or chemical modification (Rascio & Navari-Izzo, 2011). According to (Metali *et al*., 2012; Khairil & Burslem, 2018), metal hyperaccumulation is a phylogenetic trait in metal hyperaccumulators. Besides, soil properties, microbes, and climate conditions also may play a role in determining the mobility and bioavailability of heavy metals in the soil. This requires further investigation since tropical soil has a complex microbiome compared to other regions.

CONCLUSION

We found *M. malabathricum* accumulates high concentrations of Al and Fe. Plants vary greatly in their ability to accumulate heavy metals and this accumulation appears to be a species-specific characteristic. The accumulation of metals in the leaves of *M. malabathricum* shows Al as the highest metal accumulated by this species followed by Fe, Pb, As, and Cr. However, no significant difference in variation in foliar concentration among the populations. The heavy metal/metalloid foliar concentrations of *M. malabathricum* however uncoupled with the physico-chemical or heavy metal/metalloids of the soil and this supported the hypothesis that metal accumulation is a phylogenetic trait of a metal hyperaccumulator. Further research is required to investigate the potential of *M. malabathricum* in accumulating higher concentrations of metal elements (Al, As, Pb & Cr) when grown ex-situ and under a controlled environment. Results from this study could be a guideline for future phytoremediation activities by using *M. malabathricum*.

Table 5. Pearson Correlation coefficient *(r)* of foliar metal Al, Fe, Pb, As, and Cr concentrations with the soil chemical properties

	Foliar Al	Foliar Fe	Foliar Pb	Foliar As	Foliar Cr
Soil N	-0.34	0.40	-0.41	-0.03	-0.03
Soil P	-0.67**	0.04	-0.10	-0.13	-0.03
Soil K	-0.51*	-0.49	-0.06	-0.23	0.39
Soil Ca	-0.20	-0.55*	-0.03	-0.02	-0.08
Soil Mg	-0.52*	-0.25	-0.29	-0.14	0.44
Soil Al	-0.25	-0.31	0.11	-0.13	-0.15
Soil Fe	0.32	-0.13	-0.10	0.05	0.07
Soil Pb	-0.36	-0.19	0.17	0.01	-0.19
Soil As	-0.08	0.01	-0.01	0.33	-0.11
Soil Cr	0.04	-0.33	-0.05	-0.22	0.14
Soil PC Axes					
PC1	-0.24	0.64**	-0.47	0.15	-0.08
PC2	-0.80**	-0.52*	0.11	-0.43	-0.05

*$p<0.05$; **$p<0.01$, ***$p<0.001$

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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