Essential elements in *Etlingera elatior* (Jack) R. M. Sm. and *Etlingera philippinensis* (Ridl.) R. M. Sm.

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ABSTRACT

Despite the advancements contributed to botanical research, scientific attention on many Zingiberaceae plants in spite of their numerous health-promoting applications is still few. Existing reports mostly focus on the common species of Zingiberaceae specifically on the rhizomes, with less emphasis on Philippine endemic gingers such as *Etlingera philippinensis* (Ridl.) R. M. Sm. In this study, the concentration of essential elements (Fe, Cu, Zn, Ni) in *Etlingera elatior* (Jack) R. M. Sm. and *E. philippinensis* leaves and rhizomes were determined using atomic absorption spectroscopy. Among the essential elements, Cu was found highest in *E. elatior* leaves and *E. philippinensis* rhizomes. However, the identified levels of Fe, Cu, Zn, and Ni for these two zingiberaceaes indicate that all are below the permissible limit set by World Health Organization (WHO) for plants. Thus, this study ventures in the exploration of the baseline information on the essential element content of the less studied Philippine endemic *E. philippinensis*.

Keywords: leaves, metal content, rhizomes, Zingiberaceae

INTRODUCTION

Zingiberaceae plants, known as gingers, have been popular for medicinal and culinary uses since time immemorial. Zingiberaceae family with 53 genera and over 1,200 species is known as the largest family of the order Zingiberales (Mahdavi et al. 2017). *Etlingera* is a genus belonging to the Zingiberaceae family, which is native to the Indo-Pacific region. This genus consists of more than 100 species that grow from sea level to the altitude of 2,500 m (Vairappan et al. 2012).

The plant torch ginger or scientifically known as *Etlingera elatior* (Jack) R. M. Sm., abundantly grows in Southeast Asia (Krajarng et al. 2017). The inflorescences of *E. elatior*, characterized to have a unique flavor and aroma, are traditionally used for medicinal and culinary purposes such as in

traditional dishes like "Ulam" and "Asam laksa" in Malaysia. It has been further reported that the daily intake of raw inflorescence could reduce diabetes and hypertension. Furthermore, when these are taken with bitter leaves *Vernonia amygdalina* Del (Asteraceae), it is said to relieve flatulence in postpartum women (Wijekoon et al. 2011).

Etlingera elatior contains a high amount of total phenolics, flavonoids, and vitamin C contents (Rachkeeree et al. 2018; Sungthong et al. 2018). Both leaves and rhizome of *E. elatior* exhibit antibacterial, antioxidant, antiproliferative, and apoptotic activities (Juwita et al. 2018). The inflorescence of *E. elatior* contains potassium, calcium, magnesium, phosphorus, and sulfur (Wijekoon et al. 2011). *Etlingera elatior* possesses essential oils which could be potentially used as a new source of natural antioxidant and antibacterial in the pharmaceutical and food industries (Abdelwahab et al. 2010). *Etlingera elatior* is found to contain appreciable levels of total phenolic and total flavonoid contents. GC-MS studies resulted in the identification of 73 compounds in the plant. Accordingly, the most abundant components of this plant include β-pinene (24.92%) and 1-dodecene (24.31%).

A certain species of ginger which is endemic in the Philippines is *Etlingera philippinensis*. (Ridl.) R. M. Sm. with basionyms such as *Hornstedtia philippinensis* Ridl., *Amomum philippinense* (Ridl.) Merr., *Achasma philippinensis* (Ridl.) B.L. Burtt and R.M. Sm. (Newman et al. 2004). *Etlingera philippinensis* reaches a height of 2–2.5 m tall. Its rhizomes are long and creep along the soil, and have cone-shaped inflorescence (with 7-12 flowers) which either grow distantly from the main rhizome or are partially buried in the soil (Mendez et al. 2017). This plant has a dominant reddish color in its entire inflorescence and the flowers do not bloom altogether at the same time (Mendez et al. 2017). High 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities were also observed in the water extracts of *E. philippinensis*, and the plant's extract contained alkaloids, flavonoids, saponins, tannins and steroids (Barbosa et al. 2016). Its leaves contain chlorogenic acid (Barbosa et al. 2017). Furthermore, this species showed high antioxidant activity and total phenolic content (Mabini and Barbosa 2018).

Having identified the aforementioned elements found in the *E. philippinensis* component is not that surprising since medicinal and some spice plants normally contain essential and non-essential metals (Wagesho and Chandravanshi 2015). However, having deeper understanding on the essential element contents of plants could aid in the development of mineral biofortification. Thus, this study aims to determine the levels of essential metals (copper, Cu; iron, Fe; zinc, Zn; and nickel Ni) found in the leaves and rhizomes of *E. elatior* and *E. philippinensis* and in the soil source where the plants were grown.

METHODS

Plant and Soil Materials Collection

Leaves and rhizomes of *E. elatior* (Figure 1A, B) were collected from Purok 16 of Musuan, Maramag, Bukidnon (7°46'49"N and 125°03'29"E) while leaves and rhizomes of *E. philippinensis* (Figure 1C, D) were collected from Gutapol, Kibawe, Bukidnon (7°29'15"N and 125°03'38"E).

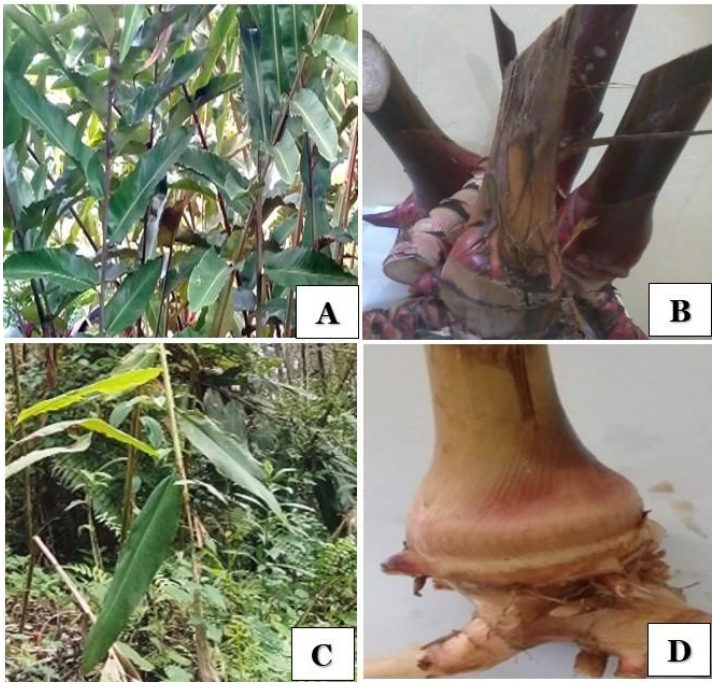

Figure 1. *Etlingera elatior* (Jack) R. M. Sm. (A) leaves and (B) rhizomes and *Etlingera philippinensis* (Ridl.) R. M. Sm. (C) leaves and (D) rhizomes.

Soil samples were collected with slight modification based on the method described by NRCS (2007). Six representative soil samples, with equal amounts of about a standard shovel (roughly ~1 kg), were collected in a zig-zag manner from the source field of the plant samples. Each sampling depth was 60.96 cm deep. Each of the collected representative soil samples was placed in a polyethylene bag, mixed, and air dried. The dried soil samples were then thoroughly mixed. Finally, the 1 kg dried soil sample was then sent for metal analysis.

The collected plant samples were also placed in separate clean plastic bags and were transported to Central Mindanao University (CMU), University Town, Musuan, Bukidnon.

Sample Preparation

The collected leaf samples were thoroughly washed with tap water, then rinsed with distilled water, and were air-dried for three to four weeks under the shade at ambient temperature. In the same manner, the rhizomes were thoroughly washed with tap water followed by distilled water, and then peeled, grated, and lastly, air-dried. Finally, the air-dried samples were separately powdered using a food processor and were securely stored in correctly labelled ziploc bags.

Meanwhile, foreign debris from the collected soil samples were removed. This was followed by the soil being pulverized and then were air-dried for three weeks by spreading thinly on a clean surface. Once dried, the soil samples were sifted in a 2 mm sieve and were stored in properly labelled ziploc bags.

Digestion/Ashing and Metal Analyses of Samples

Soil samples for analysis of Fe, Cu and Zn. Soil samples (12.50 g) were placed into a 50 ml centrifuge tube, added with 25 ml Diethylene triamine pentaacetic acid (DTPA), and then were shaken for 1 h. The mixtures were filtered and read in an atomic absorption spectrophotometer (AAS-Agilent 280 Fast Sequential) (Biddle 1997).

Soil samples for analysis of Ni. One gram sample for digestion was placed in a 250 ml flask. The sample was then heated to 95°C with 10 ml of 50% HNO_3 without boiling, cooled, and was refluxed with repeated additions of 65% HNO_3 until no brown fumes were given off. The solution evaporated until the volume was reduced to 5 ml, cooled, and was gradually added with 10 ml of 30% H_2O_2. The mixture was refluxed with 10 ml of 37% HCl at 95°C for 15 min. The digestate obtained was filtered through a 0.45 µm membrane paper. The filtrate diluted to 100 ml with deionized water and was stored at 4°C for analyses (USEPA 1996). The same procedure was conducted to the other soil samples.

Plant samples for analysis of Fe, Cu and Zn. One gram of sample was placed in a crucible and heated in a furnace at 500°C for 5 h, cooled, was added with 5 ml of 6N HCl and left to stand for 1 h. The mixture was filtered and diluted to 50 ml of distilled water. The same procedure was conducted to the other plant samples.

Metal analyses for the plant samples were done by using 1 ml aliquot of plant digest solutions into 50 ml centrifuge tubes (Nalgene type) using 1 ml volumetric pipette, adding deionized water to dilute to a final volume of 50 ml and reading the mixture in an AAS (Agilent 280 FS) (Biddle 1997).

Plant samples for analysis of Ni. One gram of sample was placed in a beaker and treated with a 10 ml of concentrated HNO_3. The mixture was then heated for 45 min at temperature of 90°C. The temperature was then gradually increased to 150°C and the sample was boiled for a minimum of 8 h to obtain a clear solution. Following this step, 5 ml of concentrated HNO_3 was added into the sample at least three times and plant digestion was continued until the volume was reduced to about 1 ml. Afterwards, distilled water was used to wash down the inner walls of the tube to keep it clean and the tube was swirled throughout the digestion process to prevent loss of sample. The sample placed inside the digestion tube was allowed to cool down, then was additionally treated with 5 ml of 1% HNO_3, and then was filtered through using Whatman filter paper No. 42 and Millipore filter paper (<0.45 µm). The solution was then transferred quantitatively to a 100 ml volumetric flask by adding distilled water (Zarcinas et al. 1987; Zheljazkov and Nielson 1996). Finally, the metal analyses were done by using Agilent 4200 Microwave Plasma Atomic Emission Spectrophotometer (MP-AES). The same steps were followed for each of the plant samples.

Statistical Analysis

An Analysis of variance (ANOVA) using SPSS version 24 was done to determine the differences in the metal content of the plants' leaves and rhizome and in the soil where the plants grew. Also, a post hoc analysis was done using Tukey's Test.

RESULTS

Variable amounts of essential trace metals (Fe, Zn, Cu and Ni) were observed among the plant and soil samples. For *E. elatior* that the concentration values in plant parts and soil ranged from 0.297 to 20.849 mg kg^{-1} for Fe, 1.687 to 8.317 mg kg^{-1} for Zn, below detection limit to 9.682 mg kg^{-1} for Cu, and below detection limit to 4.607 mg kg^{-1} for Ni (Table 1).

Zinc level was highest in *E. elatior* soil (8.317 mg kg^{-1}) followed by *E. elatior* rhizome (6.493 mg kg^{-1}) and *E. elatior* leaves (1.687 mg kg^{-1}). Cu content in *E. elatior* was significantly higher in leaves (9.862 mg kg^{-1}) than in soil (7.193 mg kg^{-1}). Copper content in *E. elatior* rhizomes was below in instrument detection limit. The rhizomes (4.607 mg kg^{-1}) of *E. elatior* contained higher level of Ni than the leaves (1.726 mg kg^{-1}).

Table 1. Metal concentrations in the leaves and rhizomes of *Etlingera elatior* and the soil where the plant was grown as determined using atomic absorption spectrophotometer. The superscript indicators of the same letters within a column implies that the sample results are not significantly different from each other; BDL indicates below detection limit.

Sample	Concentration, mg kg^{-1}			
	Fe	Zn	Cu	Ni
E. elatior leaves	1.001 ^{b}	1.687 ^{c}	9.682 ^{a}	1.726 ^{b}
E. elatior rhizome	0.297 ^{c}	6.493 ^{b}	BDL	4.607 ^{a}
E. elatior Soil	20.849 ^{a}	8.317 ^{a}	7.193 ^{b}	BDL

Analysis of variance (ANOVA) and subsequent Tukey's test revealed that *E. elatior* leaves contain significantly higher Fe content (1.001 mg kg^{-1}) than its rhizomes (0.297 mg kg^{-1}). Fe content in soil (20.849 mg kg^{-1}) was significantly higher than that in the *E. elatior* leaves and rhizomes.

The essential elements concentration values in *E. philippinensis* parts and soil ranged from 1.136 to 17.421 mg kg^{-1} for Fe, 0.727 to 6.067 mg kg^{-1} for Zn, 1.217 to 9.659 mg kg^{-1} for Cu, and below detection limit to 4.758 mg kg^{-1} for Ni (Table 2).

Table 2. Metal centrations found in the leaves, the rhizomes of *Etlingera philipinensis,* and the soil where the plant was grown as determined using an atomic absorption spectrophotometer. Superscript indicators of the same letters within a column implies that the results are not significantly different with each other. Those indicated with BDL means concentrations are below detection limit.

Sample	Concentration, mg kg^{-1}			
	Fe	Zn	Cu	Ni
E. philippinensis leaves	1.136 ^{b}	0.727 ^{b}	2.614 ^{b}	4.686 ^{a}
E. philippinensis rhizome	1.326 ^{b}	6.067 ^{a}	9.659 ^{a}	4.758 ^{a}
E. philippinensis Soil	17.421 ^{a}	0.840 ^{b}	1.217 ^{c}	BDL

In *E. philippinensis*, Zn level results show that it is significantly higher in rhizomes (6.067 mg kg^{-1}) than in the leaves (0.727 mg kg^{-1}) and that of the soil (0.840 mg kg^{-1}) (Tables 2). Moreover, Cu content is significantly highest in the rhizomes (9.659 mg kg^{-1}) followed by those in the leaves (2.614 mg kg^{-1}) and lastly in the soil (1.217 mg kg^{-1}). Meanwhile, the leaves and rhizomes of *E. philippinensis* contain similar levels of Ni.

The ANOVA results revealed that *E. philippinensis'* Fe content is statistically comparable with the rhizomes (1.326 mg kg^{-1}) and the leaves (1.136 mg kg^{-1}) but is significantly lower than in the soil (17.421 mg kg^{-1}). Fe content has a lower value in both leaves and rhizome samples of *E. philippinensis*. However, there is high Fe presence in the soil where the *E. philippinensis* samples were grown.

DISCUSSION

Rhizomes of selected ginger species such as *Alpinia officinarum*, *Alpinia galangal*, *Alpinia zerumbet*, *Alpinias calcarata*, and *Kaempferia galangal* are reported to contain quite low Cu content (0.48–2.16 ppm), moderate Zn content (1.66-14.5 ppm), Ni content (0.130–21.02 ppm), and high Fe content (17.23–85.50 ppm) (Indrayan et al. 2009). On the other hand, the Cu for the *E. elatior* and *E. phillippinensis* were found to be relatively higher than the reported range, but the Zn and Fe contents were relatively low within the range of the other ginger species. Factors influencing the concentrations of these metals include the plant species, the microclimate conditions, environmental pollution, and other factors affecting the plant growth (Mishra et al. 2006; Broadly et al. 2007).

For this study, the Fe levels in plant samples were all below the permissible limit of 20 mg kg^{-1} set by the World Health Organization (WHO) (Shah et al. 2013). Different results, however, were observed by Rachkeeree et al. (2018) wherein Fe was found below the detection limit in the *E. elatior* flowers. Juwita et al. (2018) reported that the inflorescence of *E. elatior* contained high levels of major minerals like potassium, calcium, magnesium and phosphorus. Scientifically speaking, Fe serves to carry oxygen from the lungs to the body tissues, in operational immune system maintenance, and to support metabolic energy production (Gupta et al. 2014). It plays an important role in physiological and biochemical processes. For instance, it is involved in various enzymatic activities such as the electron transport chain in cytochromes, in the synthesis of chlorophyll, and maintenance of chloroplast structure and function (Rout and Sahoo 2015). Iron toxicity from foods are rare since the body regulates iron absorption by only absorbing less iron, if the iron stored in the body are adequate. However, ingestion of elemental iron (e.g. supplement) with more than 60 mg kg^{-1} can results in severe toxicity such as gastrointestinal mucosa which can cause nausea, vomiting, abdominal pain, and diarrhea leading to a severe morbidity and mortality (Yuen and Becker 2021).

Meanwhile, the results also show that the rhizome of *E. elatior* is a good accumulator of Zn, suggesting that it is a good candidate for phytoextraction or phytoremediation in heavily contaminated soils. Hence, it

is recommended that further investigations must be conducted to determine the ability of *E. elatior* and *E. philippinensis* to accumulate heavy metals.

Based on the results, Zn levels were all below the World Health Organizations permissible limit of Zn in plants which is 50 mg kg^{-1} (Shah et al. 2013). Zn is an immune response stimulant and membrane stabilizer, and its deficiency leads to impaired growth and malnutrition (Indrayan et al. 2009). Zinc (Zn) is a cofactor involved in many catalytic activities and structural proteins in plants. It has key structural functions in the protein domains, known as "Zn finger" domain, which interact with other molecules. The Zn finger proteins mediate DNA binding of transcription factors and protein–protein interactions (Cabot et al. 2019). Also, Zn is essential for many enzymes which are needed for nitrogen metabolism, and energy transfer (Haffez et al. 2013). Intake of naturally occurring zinc in food has no evidence of adverse effects. However, excessive intake of supplemental zinc (50-150 mg day^{-1}) is associated with the suppression of immune response, decrease in high-density lipoprotein (HDL) cholesterol, reduced copper status, and gastrointestinal distress (Trombu et al. 2001)

The Cu content in the plants obtained from this study indicates that they were below the permissible limit of Cu in plants (10 mg kg^{-1}) set by WHO (Hasan et al. 2012). Copper is a component of cytochrome oxidase, lysyl oxidase, and ceruloplasmin (Mills 1981). Copper, is an essential cofactor of numerous proteins. It is known to participate in photosynthetic electron transport, cell wall metabolism, mitochondrial respiration, hormone signaling and oxidative stress responses. Furthermore, Cu plays a significant role in many enzymatic activities such as Cu/Zn superoxide dismutase (SOD), cytochrome c oxidase, and polyphenol oxidase (Yruela 2005; Printz et al. 2016). However, consuming plants containing high amount of Cu could result to great concern to health due its toxicity to humans and animals (Kabata-Pendias and Mukherjee 2007). A high intake of Cu has been related to liver damage (Korfali et al. 2013).

On another instance, the Ni levels for this study indicates that the content were all below the permissible limit (10 mg kg^{-1}) of Ni in plants set by WHO (Nazir et al. 2015). The Ni level in soil where the plant samples were collected from was found to be below detection limit. Nickel, an essential element, acts as an activator of many enzymes such as ureases which hydrolyzes urea in plant tissue (Fabiano et al. 2015). It helps some plants to protect themselves against pathogens and herbivorous insects. At higher concentrations, however, this element is toxic to plants and other living organisms (Harasim and Filipek 2015). Human exposure to high amount of nickel is known to cause a variety of human health problems including allergy, cardiovascular and kidney diseases, lung fibrosis, lung and nasal cancer (Chen et al. 2017).

The high level of Ni in the rhizome and leaves despite its non-detection in the soil could be due to the ability of the plants to accumulate this element. Most of the Ni, however, is transported to seeds during the senescence of leaves as reported for *Mimulus guttatus* (Tilstone and Mac Nair 1997) and for soybean (Cataldo et al. 1978; Sengar et al. 2008). This may explain the results of this current study where Ni was detected in the leaves and rhizomes of *E. elatior* and *E. philippinensis* despite their non-detection in the soil.

Generally speaking, anthropogenic activities including the burning of fossil fuels for power generation, mining, smelting, emissions from vehicles, disposal of household, municipal and industrial waste, steel manufacturing, agricultural fertilizers, pesticides and cement industry accelerates the release of heavy metals (Ni, Zn, Cu, Co, and Mo) into the environment. Subsequently, there will be plant uptake through roots from the soil and by leaf adsorption from air (Mishra et al. 2017).

Certainly, at a lower level, the heavy metal micronutrients are essential elements for growth and development in both animals and plants (Tchounwou et al. 2012). Exposure of plants to high levels of heavy metal micronutrients usually results to several phytotoxic symptoms, such as leaf chlorosis, growth retardation, nutrient imbalance or low nutrient uptake, decreased stomatal conductance, inhibition of chlorophyll synthesis, reactive oxygen species (ROS) production that causes membrane, DNA and protein damage, and distortion of photosynthetic machineries (Arif et al. 2016; de Macedo et al. 2016). In humans, prolonged ingestion or exposure to a high concentration of heavy metals result to the production of free radicals that attack the DNA which cause cellular damage and mutation leading to an array of diseases (Jaishankar et al. 2014). Nevertheless, based on the results of this study the levels of Cu, Ni, Zn, and Fe in both rhizomes and leaves of *E. elatior* and *E. philippinensis* are below the permissible limit in plants as set by WHO, which means that their presence is not harmful for consumption but instead a good source of heavy metal micronutrients.

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