



# Heavy Metal Accumulation and Mycorrhizal Association of the Common Agroforestry Crops in the Ultramafic Soils of Dinagat Islands, Philippines

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## ABSTRACT

Common Agroforestry crops in the ultramafic soils of Santo Niño, Cagadianao, Dinagat Islands were studied to determine their heavy metal accumulation and mycorrhizal association. Three Agroforestry farms were sampled for the crops such as coconut, sweet potato, and banana. On each farm, plant parts were collected separating the edible and the non-edible parts such as shoots and roots and were tested for accumulation of nickel and chromium via atomic absorption spectrophotometry. Soil samples within the rhizosphere of the crops were also collected for mycorrhizal studies. Results showed that all Agroforestry crops accumulated nickel and chromium on roots and shoots above the normal range at 0.05-10 mg/kg, however, only banana has nickel concentration exceeding the normal range of 5-25 mg/kg. For the edible parts, all Agroforestry crops have nickel and chromium concentration above the safety limits of 0.0028 and 0.3 mg/kg body weight per day for nickel and chromium, respectively. Only sweet potato has shoot/root quotient or translocation factor greater than one for both nickel and chromium which was 12.73 and 11.09, respectively, thus considered as hyperaccumulator species. Six genera of mycorrhiza were identified in all crops with *Glomus* as the most abundant followed by *Acaulospora*, *Scutellospora*, *Entrophospora*, *Sclerocystis*, and *Gigaspora*.

**Keywords:** Heavy metal, Agroforestry crops, ultramafic soil, mycorrhiza

## INTRODUCTION

In the Philippines, ultramafic soil accounts for about 5% of the total land area (Baker *et al.*, 1992). Ultramafic soils also known as serpentine soils are known to be naturally poor in terms of fertility and low in water holding capacity (Alban *et al.*, 2004) due to high concentration of heavy metals such as iron, cobalt, magnesium, chromium, and nickel and low concentrations of phosphorus, potassium, and calcium (Fernando *et al.*, 2008) hence, pose extreme limitations to agriculture otherwise not apt for cultivation at all.

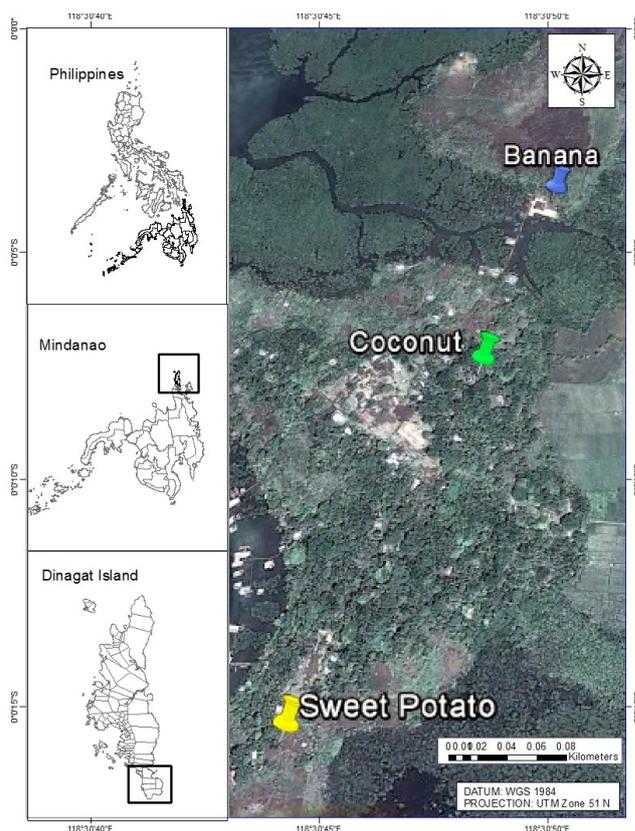
For plant species to survive and adapt in ultramafic soil amidst extreme conditions has to form symbioses with mycorrhizal fungi (Turk *et al.*, 2006). This may represent an important survival strategy and also minimize if not restrained the accumulation of heavy metals. Mycorrhizal fungi help protect the plants by storing toxic metalloids in a sheath or mantle around the roots (Turk *et al.*, 2006). It also helps in increasing nutrients and water uptake and in return, the fungi benefit from the carbon compounds provided by the plants (Ianson and Smeenk, 2014).

In an agricultural country like the Philippines, farmers intend to raise crops on any parcel of land despite prevailing adverse soil conditions. In Dinagat Province, an ultramafic island, Agroforestry crops like coconut, sweet potato, and banana are widely cultivated and consumed by the locals. If these crops have developed an association with arbuscular mycorrhizal fungi (AMF) and thrive well, the possibility of metal accumulation in tissues and edible parts of the crops is still inevitable because not all heavy metals could be contained by the mycorrhiza. The risks of consuming agroforestry crops planted in ultramafic soils still remain because continued consumption, even in small amounts for a prolonged period may have serious implication to human health. Heavy metal-contaminated food can reduce some essential body nutrients causing brain retardation, gastrointestinal cancer, malnutrition, and other diseases (Dioka *et al.*, 2004). Thus, this study was conducted to investigate the heavy metal uptake of the agroforestry crops such as coconut, sweet potato, and banana in the area.

**MATERIALS AND METHODS**

**Location of the Study**

The study was carried out at Barangay Sto. Niño, Municipality of Cagdianao situated in the southern portion of the province of Dinagat Islands with geographic coordinates 09°54' N and 125°38' E (Fig. 1). The climate in the province is a Type II characterized by no dry season, but with a very pronounced maximum



**Figure 1.** Map of the Study Area

rainy period from November to February (Alban *et al.*, 2004). The whole of Dinagat Island soil type is ultramafic hence classified as mineral land based on DENR’s land classification. Despite the adverse soil conditions various portions are still cultivated and planted with agroforestry crops such as coconuts, sweet potato, and banana.

Three agroforestry farms were sampled since not all crops were found on a single agroforestry farm. All farms sampled were less than one (1) hectare. Farm one (1), two (2) and three (3) were dominated by banana, coconut, and sweet potato, respectively. These agroforestry crops were mainly cultivated for subsistence. Excess crops for consumption, especially coconut were being sold to the community and to the nearest city which is Surigao. Table 1 shows the geographic coordinates and elevation of the three agroforestry farms.

**Table 1. Geographic coordinates and elevation of the Agroforestry farms**

Agroforestry farms	Coordinates		Elevation (masl)
	Latitude	Longitude	
Banana (Farm 1)	9°54'59.51"N	125°38'53.17" E	9
Coconut (Farm 2)	9°54'52.25"N	125°38'51.25" E	19
Sweet potato (Farm 3)	9°54'39.79"N	125°38'46.58" E	12

**Sampling**

In each farm plant parts were collected separating the edible and the non-edible parts such as shoots, roots, and stem. These samples were washed using distilled water and placed in containers and were properly labeled.

**Oven-drying**

Prior to oven-drying, the samples were thinly chopped and were placed in blotters. These were then oven-dried at 60°C temperature for 3 weeks. From the oven-dried samples, a 300-gram was obtained for laboratory analyses.

**Soil sampling**

The soil samples were collected within the rhizosphere of the different crop parts. Soil samples obtained from the plot were thoroughly mixed and a composite sample of approximately 2 kg plot was collected for laboratory analyses and for mycorrhizal studies.

**Separating Mycorrhizal Spores from Soil**

a. Soil sieving and centrifugation (after Daniels and Skipper, 1982 and Tommerup, 1992).

The collected soils per plot were weighed to obtain 100 grams sample preparations at 10 proportions per plot. A substantial volume of distilled water was added to each 100-gram samples and undergone decantation process through a series of sieves after allowing heavy soil particles to settle for a few seconds. The washing and decanting process was repeated until the water is clear. A 150µm, 180µm, and 250µm core screen fractions were used to ensure soil aggregates were broken apart to free spores.

The sievings that settle at 150µm, 180µm, and 250µm core screens after decantation were collected and placed into the centrifuge tube. Centrifugation follows within 5 minutes at 2000 rpm to remove substantial amounts of floating organic debris. After centrifugation, the supernatant and floating debris were discarded. The pellet was then suspended in 50% sucrose solution and was shaken vigorously. This was followed by another centrifugation for 1 minute at 2000 rpm to separate the spores.

**Spore Count**

The supernatant from the three finest screens (*i.e.* 150µm, 180µm, and 250µm) was collected and carefully transferred to Petri-dishes. Spores were then counted via the stereoscopic microscope.

**Spore Identification**

Spores suspended in the supernatant were collected using a syringe. This was then placed on the glass slide and was covered with a cover-slip and was identified under a compound microscope. Photographs of spores were taken and were compared using Brundrett *et al.*, (1996), Morton and Benny (1990), and Elmore (2006) for identification.

**Shoot/Root Quotient or Translocation Factor**

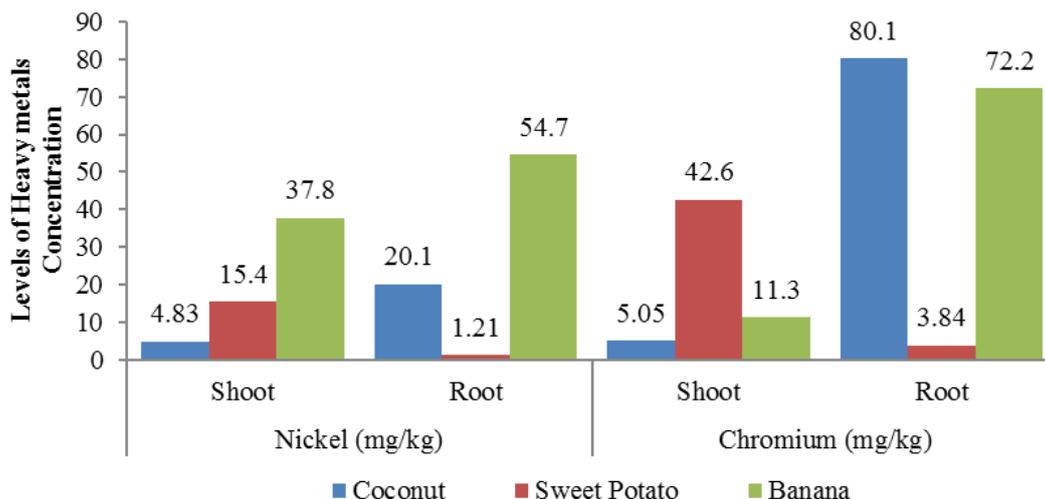
To evaluate the ability of the plant to accumulate or translocates heavy metal in their tissues, shoot/root quotient (SRQ) or translocation factor (TF) was used. The species that has SQR or TF greater than one (1) is considered as hyperaccumulator otherwise, characterized as heavy metal excluders (<1) (Rotkittikhun *et al.*, 2006).

$$SRQ \text{ or } TF = \frac{\text{Heavy metal concentration in the shoot}}{\text{Heavy metal concentration in the root}}$$

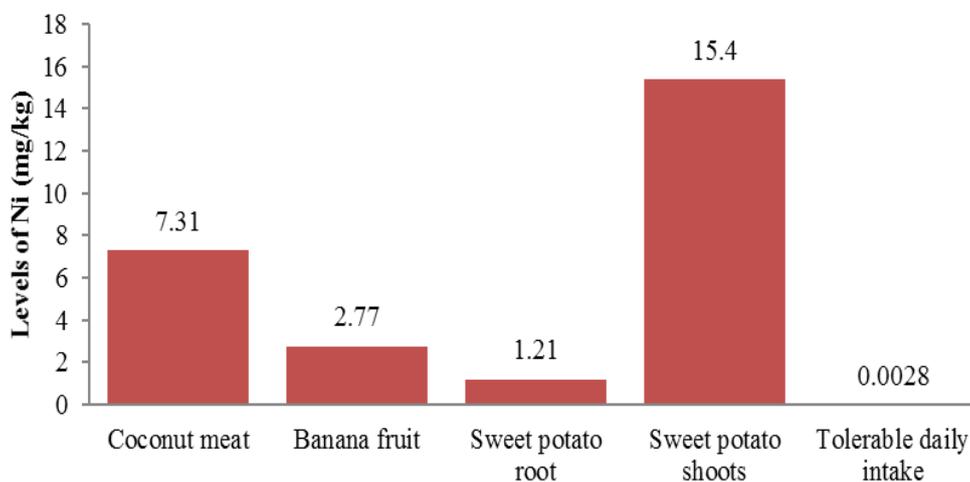
**RESULTS AND DISCUSSIONS****Heavy Metal Accumulation**

The results of heavy metal accumulation in roots and shoots of the three agroforestry crops were shown in Figure 2. Based on the result, among the three crops, banana obtained the highest concentration of nickel both in shoots and roots. Sweet potato has the lowest concentration in the roots while coconut obtained the lowest accumulation of nickel in the shoots. Bhalerao *et al.* (2015) reported that majority of plant species contain nickel at very low concentration for only 0.05-10 mg/kg dry weight and for normal functioning plants accumulation of nickel should not exceed 10 mg/kg for their metabolic needs (Lasat, 2000). Above that range, plants would show nickel toxicity symptoms like inhibition of growth, photosynthesis, seed germination, sugar transport and induction of chlorosis, necrosis, and wilt (Bhalerao *et al.*, 2015). But in plants growing in metalliferous soils, the normal range of this metal in the shoots is 5-25 mg/kg (Reeves and Baker, 2000) due to high concentration of nickel in ultramafic soils (Brooks, 1987) that can be readily accumulated since it is often mobile in plants (Welch and Cary, 1975). In this case, only banana has above the normal range for plants growing in metalliferous soils.

For Cr accumulation, the sweet potato has the highest concentrations in shoots but has the lowest in rats.



**Figure 2.** Quantified nickel and chromium in plant tissues



**Figure 3.** Nickel concentrations (mg/kg) in the edible part of Agroforestry crops

Banana ranked second both in roots and shoots. Coconut has the lowest Cr accumulation shoots but has the highest Cr in roots (Fig. 2). Chromium is often less than 1 mg/kg and rarely exceeds 5 mg/kg in normal soils and the concentration of more than 50 mg/kg are so uncommon on serpentine soils that this may be used as an indicator of soil contamination (Reeves, 2015). In vegetative organs, most of the plant's chromium toxicity starts from 10-15 mg/kg (Verbruggen *et al.*, 2009). In this study, all of the Agroforestry crops exceed the normal range of plants growing in serpentine soils. Coconut and banana even have chromium above 50 mg/kg.

#### **Edible parts**

Results through AAS showed that the shoots of sweet potato obtained the highest concentration of nickel in the edible parts, followed by coconut meat and the

lowest was sweet potato roots (Fig. 3). In general, all the agroforestry crops evaluated have accumulated higher nickel concentration above the general range of edible parts which normally ranges from 0.1 to 0.5 mg/kg (Clarkson, 1988; Andersen *et al.*, 1990; Aitio *et al.*, 2012; Fawell *et al.*, 2007; Leblanc *et al.*, 2005; Cempel and Nickel, 2006; DudaChodak and Blaszczyk, 2008; Rose *et al.*, 2010; Becker and Kumpulainen, 2011; Noël *et al.*, 2012).

A tolerable daily intake of nickel is 0.0028 mg/kg body weight per day (Benford *et al.*, 2015) and recommended a daily intake of 0.4 mg (Iyengar, 1985) suggest that frequent consumption of sweet potato roots, banana fruits, coconut meat, and especially sweet potato shoots will pose health problems. Aitio *et al.* (2012) classified nickel and nickel compounds as human carcinogens causing cancers of the lung, nasal cavity and paranasal

sinuses after inhalation. Chronic oral exposures to nickel also cause reproductive and developmental toxicity.

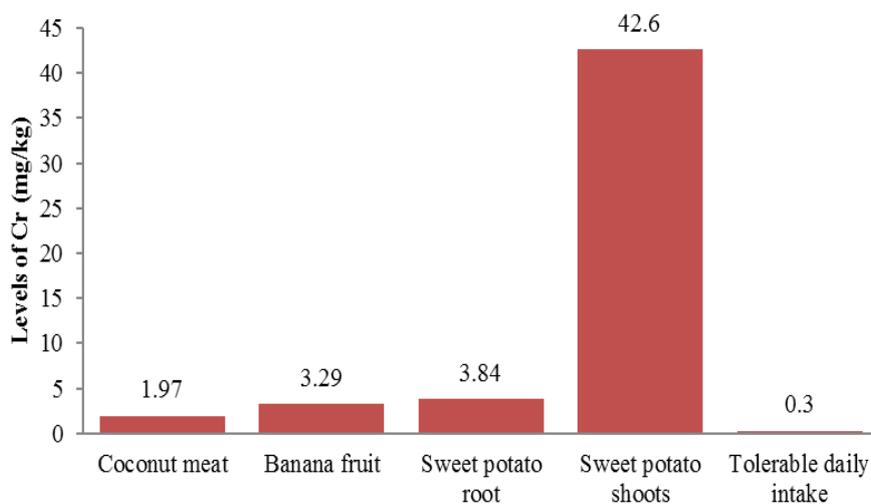
In terms of chromium concentration in the edible parts, sweet potato shoots obtained the highest followed by sweet potato roots. The lowest was obtained in coconut meat (Fig. 4). All of the crops have chromium concentration above the safety limits. The most type of chromium present in a variety of food was Cr (III) and exposure to it was mainly via diet/food rather than inhalation (Benford *et al.*, 2015). Adequate intake of chromium ranging from 0.0002-0.0055 mg/day for infants to 0.035 mg/day for males between 19 and 50 years old; 0.029-0.030 mg/day during pregnancy; and 0.044-0.045 mg/day during lactation were determined by Rusell *et al.* (2001). Havel *et al.* (1989) also established a range of 0.05-0.2 mg/day as safe and adequate daily intake for adults and adolescents. And Benford *et al.* (2014) derived a tolerable daily intake of 0.3 mg/kg body weight per day.

There was no clear evidence of genotoxic and carcinogenic effects of Cr (III) even at higher dose levels than the suggested dietary intakes (Santonen, 2009a). And an intake ranging between 1-2 mg/day shows no adverse effect (COMA, 1991). Still, frequent consumption of sweet potato shoots, sweet potato roots, banana fruits, and coconut meat could cause health risks since it is above the safety limits. Gastrointestinal effects, epigastric pain, irritation, and ulceration are the reported effects of inhalation and ingestion of high doses of chromium (Wilbur *et al.*, 2012).

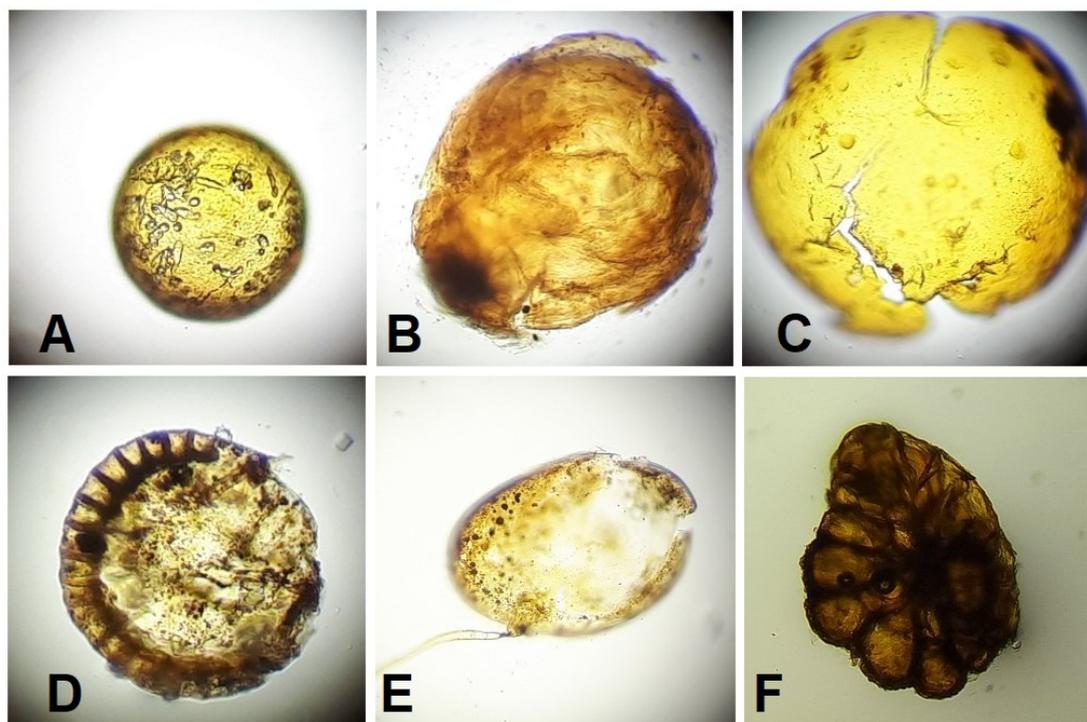
### **Mycorrhizal association**

Soil samples from the rhizospheres of banana, sweet potato and coconut showed different types of spores. The spores examined consisted of six genera, namely: *Glomus* (Glomaceae), *Acaulospora* (Acaulosporaceae), *Gigaspora* (Gigasporaceae), *Entrophospora* (Acaulosporaceae), *Scutellospora* (Gigasporaceae), and *Sclerocystis* (Glomaceae) (Fig. 5).

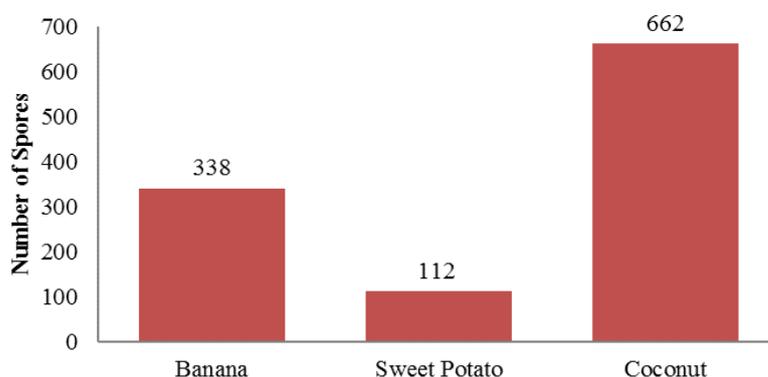
The result showed that 39 species belonging to the genus *Glomus* were recorded and considered the highest. It was followed by *Acaulospora* with 14 species and *Scutellospora* with only 4 species. Only one species were found in the genera of *Gigaspora*, *Entrophospora*, and *Sclerocystis*, respectively. This finding conforms to that of Aribal *et al.* (2017) in ultramafic soils of Mt. Kiamo where the genus *Glomus* was the most abundant and widely distributed. The dominance of *Glomus* in ultramafic soils was due to its higher metal tolerance (Martina and Vosatka, 2005; Schwartz *et al.*, 2006; Chen *et al.*, 2007; Zaefarian *et al.*, 2010; Carrasco *et al.*, 2011). Morton (1988) also considered *Glomus* species as the common species all over the world comprising more than 75 percent of the total isolates followed by *Acaulospora*. Bouamri *et al.* (2006) also mentioned that many studies revealed that different species of AM fungi were obtained depending on plant species and geographic location, but amongst AM fungal species, *Glomus* species were consistently observed while others species belonging to the genera *Acaulospora*, *Gigaspora* and *Scutellospora* were either absent or found in fewer numbers showing the same result of this study.



**Figure 4.** Chromium concentrations (mg/kg) in the edible part of Agroforestry crops



**Figure 5.** A, *Glomus sp. 6*; B, *Acaulospora sp.1*; C, *Gigaspora sp.*; D, *Entrophopora sp.*; E, *Scutellospora sp.1*; and F, *Sclerocystis sinuasa*



**Figure 6.** Total number of spores of the Agroforestry crop

On the other hand, the number of spores within the rhizospheres of the 3 agroforestry crops varied with a total of 1,112 spores counted (Fig. 6). The highest spore count was observed in the rhizospheres of coconut with 662 spores followed by banana with 338 spores while the lowest spore number was in sweet potato with 112 spores. Spore production by mycorrhizal fungi is influenced by many factors, including the host plant (Brundrett and Kendrick, 1988) through the difference in the effects on the hyphal growth and sporulation (Eom *et al.*, 2000).

*Glomus sp. 5* obtained the highest total spore count with 295 spores in all, obtained mycorrhizal spores and was

followed by *Glomus sp. 6* with 278 spores (Table 2). *Glomus intraradices* was also observed which, according to Regvar *et al.* (2003) this type of mycorrhiza was common and has adapted to heavy metal contaminated sites. In general, spore numbers were dominated by *Glomus* species followed by *Acaulospora*, *Scutellospora*, *Entrophospora*, *Sclerocystis* and *Gigaspora* species as having the lowest spore count. A small number of Gigasporaceae (*Gigaspora* and *Scutellospora*) was due to its large size, which requires long periods of their development than smaller-sized spore species (Hepper, 1984) and since they were usually found in sandy dunes (Lee and Koske, 1994).

**Table 2.** Mycorrhiza spore count per Agroforestry crop

Mycorrhizal Species	Agroforestry Crop			Total
	Banana	Sweet Potato	Coconut	
<i>Glomus sp. 5</i>	11	36	248	295
<i>Glomus sp. 6</i>	236	6	36	278
<i>Glomus sp. 3</i>	9	23	111	143
<i>Glomus intraradices</i>	-	-	90	90
<i>Glomus sp. 1</i>	15	23	4	42
<i>Glomus sp. 24</i>	-	-	42	42
<i>Glomus sp. 10</i>	36	-	4	40
<i>Glomus sp. 8</i>	1	3	24	28
<i>Glomus sp. 34</i>	-	-	14	14
<i>Acaulospora sp. 1</i>	5	4	5	14
<i>Glomus fasciculatum</i>	-	-	11	11
<i>Glomus sp. 33</i>	-	-	8	8
<i>Entrophospora sp.</i>	6	-	1	7
<i>Glomus sp. 7</i>	1	2	3	6
<i>Glomus sp. 9</i>	3	-	3	6
<i>Glomus macrocarpum</i>	-	-	6	6
<i>Acaulospora sp. 2</i>	1	1	3	5
<i>Glomus sp. 2</i>	4	-	-	4
<i>Glomus sp. 15</i>	1	-	3	4
<i>Glomus sp. 12</i>	1	-	2	3
<i>Glomus sp. 14</i>	2	-	1	3
<i>Glomus sp. 25</i>	-	-	3	3
<i>Glomus sp. 27</i>	-	-	3	3
<i>Sclerocystis sp.</i>	-	-	3	3
<i>Glomus sp. 4</i>	2	-	-	2
<i>Glomus sp. 13</i>	1	-	1	2
<i>Glomus sp. 17</i>	-	1	1	2
<i>Glomus sp. 22</i>	-	2	-	2
<i>Glomus sp. 23</i>	-	-	2	2
<i>Glomus sp. 35</i>	-	-	2	2
<i>Acaulospora sp. 5</i>	-	2	-	2
<i>Acaulospora sp. 9</i>	-	-	2	2
<i>Scutellospora sp. 2</i>	-	-	2	2
<i>Scutellospora sp. 3</i>	-	-	2	2
<i>Glomus sp. 11</i>	1	-	-	1
<i>Glomus sp. 16</i>	-	1	-	1
<i>Glomus sp. 18</i>	-	1	-	1
<i>Glomus sp. 19</i>	-	1	-	1
<i>Glomus sp. 20</i>	-	1	-	1
<i>Glomus sp. 21</i>	-	1	-	1
<i>Glomus sp. 26</i>	-	-	1	1
<i>Glomus sp. 28</i>	-	-	1	1
<i>Glomus sp. 29</i>	-	-	1	1

Table 2. Continued....

Mycorrhizal Species	Agroforestry Crop			Total
	Banana	Sweet Potato	Coconut	
<i>Glomus sp. 30</i>	-	-	1	1
<i>Glomus sp. 31</i>	-	-	1	1
<i>Glomus sp. 32</i>	-	-	1	1
<i>Acaulospora sp. 3</i>	1	-	-	1
<i>Acaulospora sp. 4</i>	1	-	-	1
<i>Acaulospora sp. 6</i>	-	1	-	1
<i>Acaulospora sp. 7</i>	-	1	-	1
<i>Acaulospora sp. 8</i>	-	-	1	1
<i>Acaulospora sp. 10</i>	-	-	1	1
<i>Acaulospora sp. 11</i>	-	-	1	1
<i>Acaulospora sp. 12</i>	-	-	1	1
<i>Acaulospora sp. 13</i>	-	-	1	1
<i>Acaulospora sp. 14</i>	-	-	1	1
<i>Gigaspora sp.</i>	-	1	-	1
<i>Scutellospora sp. 1</i>	-	1	-	1
<i>Scutellospora sp. 4</i>	-	-	1	1
<b>TOTAL</b>	<b>338</b>	<b>112</b>	<b>662</b>	<b>1,112</b>

*Glomus* and *Acaulospora* species were observed in all crops. *Gigaspora* species were obtained only in sweet potato. *Sclerocystis sp.* was observed only in coconut. And *Scutellospora sp.* was noticed in coconut and sweet potato. Presence of *Glomus* in every Agroforestry crop can lead to assumptions that *Glomus* might have developed a good adaptive symbiotic with different host plants (Heijden *et al.*, 1998). Symbioses with mycorrhizal fungi help in increasing nutrients and water uptake of plants (Ianson and Smeenk, 2014).

#### Shoot/Root Quotient or Translocation Factor

To assess the potential of coconut, sweet potato and banana as nickel or chromium hyperaccumulator, the shoot/root quotient (SRQ) or translocation factor (TF) was determined (Table 5). SRQ or TF was used to evaluate the ability of the plant to accumulate or translocates heavy metal in their tissues. The species that SQR or TF greater than one (1) is considered as hyperaccumulator or has the ability to translocate heavy metals effectively from roots to shoots and can

effectively remediate heavy metal contaminated sites, otherwise characterized as heavy metal excluders or has the ability to tolerate high amounts of heavy metals. (Rotkittikhun *et al.*, 2006; Baker and Brooks, 1989).

The result showed that only sweet potato has an SRQ or TF greater than one, both in nickel and chromium, thus, considered as hyperaccumulator species and could be used to remediate nickel and chromium contaminated sites (Table 3). Coconut and banana only contain an SQR or TF of less than one both in nickel and chromium thus, classified as an excluder. Excluder species accumulate metals mostly in their roots than in shoots and show inhibited root growth than shoots (Seregin and Ivanov, 2001; Samantaray *et al.*, 1997).

Moreover, it was observed that most of the heavy metals were absorbed greater by the roots than in shoots. According to Shanker *et al.* (2005), accumulating more heavy metals in roots is a natural reaction of plants to reduce the toxic effect of it.

Table 3. Shoot/root quotient or translocation factor of Agroforestry crops

Agroforestry crop	Nickel SRQ/TF	Chromium SRQ/TF	Classification
Coconut	0.24	0.06	Ni and Cr excluder
Sweet Potato	12.73	11.09	Ni and Cr hyperaccumulator
Banana	0.69	0.16	Ni and Cr excluder

**Table 4.** Levels of total nitrogen, extractable phosphorus, and exchangeable potassium

Agroforestry crops	Total Nitrogen (%)	Extractable Phosphorus (ppm)	Exchangeable Potassium (ppm)
Banana	0.1474	0.245	42
Coconut	0.1985	0.389	39
Sweet Potato	0.0422	0.751	18

### Soil Properties

All of the soils collected from the rhizosphere of three agroforestry crops have lower levels of total nitrogen, extractable phosphorus, and exchangeable potassium (Table 4). The usual range of results has been observed since, it has been known that these micronutrients were normally at lower concentrations in ultramafic soils (Brooks, 1987). Total nitrogen concentration ranges from 0.0422 to 0.1985% were classified as very low (< 0.1%) to low (0.1-0.2%) for optimum plant growth. The moderate concentration of nitrogen ranges from 0.2-0.5% and may reach more than 1 percent (Allan, 2009). Low levels of nitrogen would result in stunted growth, and chlorosis; in severe cases, flowering and yield are greatly reduced (Silva and Uchida, 2000). Concentration of extractable phosphorus was also very low (< 5ppm) which ranges from 0.245 to 0.751ppm. The medium range was normally 13–25ppm (Ward, 1993). The lower concentration of phosphorus may cause delayed maturity and poor seed and fruit development (Silva and Uchida, 2000). Exchangeable potassium ranging from 18 to 42 ppm was classified as very low (< 40ppm) and low (41-80ppm) (Ward, 1993). A deficiency of potassium would result to chlorosis, slow and stunted growth, weak stems, and low quantity of seeds and fruits (Silva and Uchida, 2000). Generally, the all the soils collected were nutrient deficient making it infertile for plant growth. Plants that have survived have adapted to the adverse soil condition through evolution and have ultramafic soil tolerance (Proctor, 1999).

### CONCLUSION

Based on the results of the study the following conclusions are drawn:

1. All of the agroforestry crops accumulated chromium above the normal range for plants growing in metalliferous soils while only the bananas accumulated nickel. But all crops exceed the range of chromium and nickel concentration for normal soils.

2. Nickel and chromium concentration in edible parts exceed the safe limits and may pose health risks.

3. The agroforestry crops have an association with mycorrhizal fungi with the genus *Glomus* as the most abundant.

4. The sweet potato was both nickel and chromium hyperaccumulator while banana and coconut were metal excluders.

**Conflicts of interest:** The authors stated that no conflicts of interest.

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