Nickel acquisition affected by root density of mono- and mixed-cropping peanut and choy sum

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Abstract
Nickel (Ni) and associated minerals (Cr and Mn) are naturally occurring substances in ultramafic laterites soil. It may be found in our vegetables and grains when agriculture is grown in ultramafic laterites. This study aimed to assess the contamination of Ni in edible crops affected by soil volume in mono- and mixed cropping on limonitic laterite soil. The investigation was conducted on Peanut (Arachis hypogaea L.) and Choy Sum (Brassica rapa var. parachinensis) in three different pots sizes-representing soil volume to support root growth, which was filled with 0.5 kg (small), 1.0 kg (medium), and 1.5 kg (big) of limonitic laterite soil, respectively. The limonitic soil has a 7.884 mg kg⁻¹ Ni concentration. The experiment shows that Ni concentration in peanut and Choy Sum shoots of monocropping in small, medium, and big pots achieve 20, 90, 120 mg kg⁻¹ and 51, 67, and 95 mg kg⁻¹, respectively. Meanwhile, in mixed cropping, Ni concentration in small, medium, and big pots of peanut and Choy Sum shoots are lower only by 33, 50, and 51 mg kg⁻¹ and 15, 52, and 63 mg kg⁻¹, respectively. Contamination of Ni in Peanut and Choy Sum shoots increases with the increasing soil volume, and mixed cropping is a potential strategy to reduce the acquisition of Ni.

INTRODUCTION
Nickel (Ni) has been in high demand. Its value is also predicted to keep rising due to its usage as the main component of Li-ion batteries and the big plan for mass production of hybrid and electric vehicles (HEVs) (Pirmana et al., 2023; Shahzad et al., 2018; Stampatori et al., 2020). Mining companies are already positioning themselves to build a new plant project or even join a consortium in anticipation. However, as the potential keeps growing, the risk follows behind, e.g., food contamination.

Indonesia has been one of the significant suppliers in the Ni mining industry globally. Like many mine sites scattered around the nation, Indonesia provided around 11% as the second larger global resources (Revindo & Aditya, 2020). One of Indonesia’s oldest and most well-known Ni mining areas is in Sorowako, Sulawesi, which mainly comprises laterite soil. In mining areas, agriculture activities are directly impacted by mining. While mining occupies much less of the total land area than agriculture, the negative footprint of mining on human health, ecological status, and natural biodiversity can be orders of magnitude higher than agriculture. The extraction and processing of minerals are destructive processes and changing abiotic and biotic conditions at multiple spatial scales (site, landscape and regional) (Sonter et al., 2018).

The effect of root volume restriction on plant growth is well documented and comparable with soil compaction’s effect. In general, root and shoot growth inhibition is similarly affected by root restriction stress, which is also characterized as “bonsai effect” (Ismail & Davies, 1998; Wilson, 1988). It is shown that root-derived hormonal signals mainly regulate this shoot growth inhibition, and nutritional factors are only of secondary role (Li et al., 2021; Puig et al., 2012; Rahayu et al., 2005; Wheeldon & Bennett, 2021). This root-restriction stress has practical implications for pot-grown plants in horticulture or in fertigation systems with drippers. As a consequence of an increased root length density under root volume restriction, the overlapping of individual roots with their rhizosphere and depletion zones of quickly plant-accessible
fractions of nutrients such as P or K is increasing (Feng et al., 2022; Isaac & Borden, 2019; Rubio et al., 2001). The limited research on the effects of restricted root growth on Ni uptake by food crops highlights the need for studies to better understand this phenomenon, which could have implications for food safety.

The objective of this study is to examine the influence of spatial availability or accessibility on Nickel uptake by non-hyperaccumulator plants, specifically a legume (Soybean) and non-legume (Cabbage-Choy sum). To achieve this, we implemented a root volume restriction approach using various pot sizes. The impact of root volume restriction on heavy metal uptake in non-hyperaccumulator and hyperaccumulator plants has not been previously investigated, but such knowledge could contribute to the advancement of practical phytoextraction technology. Through our study, we aim to determine the potential level of Ni contamination in food crops when grown under different conditions, namely monoculture and mixed cropping, governed by root growth in distinct soil volumes.

Hence, the main aim of the present work is to study how the restriction of root on the non-hyperaccumulator plant from the legume’s family (efficient in N fixation) and non-legume plant affect Ni acquisition in Ni naturally contaminated soil. This study emphasized the role of root density on Ni acquisition of mono and mixed cropping. We aimed to assess Nickel acquisition due impact of root growth in different soil volumes, and whether sharing rhizosphere between legume and non-legume will affect Ni uptake.

METHODS
Study Site, Plant Preparation, and Soil Condition
The pot experiment was conducted at the Faculty of Agriculture of Tadulako University, Palu, Indonesia. The experiment was conducted on peanuts (Arachis hypogaea) collected from local farmers and Choy sum (Brassica rapa var. parachinensis). Both plants directly sowed in subsoil (overburden) of ultramafic from Ni mining area obtained from Sorowako, Indonesia. Peanut seeds were inoculated with Rhizobium sp. (produced by PT. Centra Biotech Indonesia) prior to seeding and Choy sum was planted three weeks later.

Ultramafic soil from the Ni mining area of Sorowako region used in this experiment is overburden. There are two overburden types, namely limonitic and saprolitic laterite. The limonitic laterite, which contains 7.884 mg kg⁻¹ of Ni total concentration, was used in this experiment.

The limonitic laterite soil was sieved through a 3-4 mm filter. After measuring water holding capacity (WHC), the basal fertilizers of 100 mg N, 150 mg K, and 100 mg P kg⁻¹ soil were homogenously mixed into the soil by first diluting in 10 wt%. The WHC of the soil is determined by the amount of water held in the soil sample vs. the dry weight of the sample. Fertilized soil was then re-sieved before being filled into pots comprised of three different sizes with a double set of the medium pot. The pot was filled with soil according to its size, but all pots were kept with a similar bulk density of about 1.15 g cm⁻³.

Experimental Design
The experiment used a randomized block design. Peanuts and Choy sum were planted in three different pot sizes with specific soil volumes. The small pot (S) contained 0.5 kg, the medium pot (M) was filled with 1 kg, and the large/big pot (B) was 1.5 kg of air-dried soil respectively. They were divided into two sets of experiments, monocropping composed of single species per pot and mixed cropping composed of both species in the same pot with four replicates. Each pot's population was reduced to two plants per species. Therefore, four plants will be co-existed in one pot in the mixed-cropping group. Each set of experiments contained 16 pot units.

Plant Maintenance
Since water contributes the most to plant growth, thus the watering has to be done according to the growth rate of the plants. A gravimetric of the watering method was employed. Pots were regularly weighed, and the loss of water will be replaced. Water content was kept at 60% of water holding capacity (WHC) in the early growth period and 70% throughout the last experiment stage. WHC during the growth period was kept by weighing the pots and watering them to meet the designed water level. During the growth stages, the weight of the biomass was considered. Deionized water was utilized to prevent the effect of other substituents that might blend in tap water to affect the experiments.

Variable Measured and Data Analysis
Visual symptoms were observed. The experiment then concluded at the end of the peanuts' flowering stage (2 months after the peanuts were planted). Shoots and roots of both peanut and choy sum were harvested. Shoots were washed thoroughly with deionized water and dried at 60 °C for 48 hours, and the dry weight was recorded.
Shoot-dried samples were mashed and sieved before being sent to the BALITTAN laboratory in Bogor for Ni concentration analysis.

Root samples were dried on water-absorbed paper. After carefully washing with tap water-preventing root loss, the roots also being dried at 60°C for 48 hours for the dry weight to be recorded. The roots dry weight is measured per pot, not per plant.

This study observed dry biomass production of root and shoot and Ni shoot concentration. Statistical analysis was performed using SPSS 25. Means and standard deviations are presented for all data. One-Way ANOVA was used for mean comparisons, followed by the Duncan test, and means were marked with different letters showing significant differences (p<0.05).

RESULTS

Visual Growth and Symptoms

Peanuts in all treatments fully germinated within 5-8 days after sowing. However, differences in growth pace were observed after the second week, in which larger soil volume encouraged peanuts to grow faster and bigger and produce more leaves. However, it also encourages Ni toxicity because, after the third week, it is apparent that chlorosis and necrosis were more severe than those in smaller soil volumes.

Slight regression in growth was visible in mixed culture after cohabiting by choy sum for a week, compared to mono-culture peanuts in their respective soil volume. The differences were not much apparent, yet it still visible, especially for those grown in medium soil volume. Peanuts begin the flowering stage around the fifth week. It also affiliates with the soil volume, encouraging peanuts in larger containers to produce more flowers earlier, followed by smaller volume. Mixed cultures followed a similar pattern to mono-cultures. Although all peanuts were able to produce flowers, most of the flowers ended up withered, along with most older leaves, as the results of symptoms getting worse. Pods were not investigated since the harvest was done at roughly the end of the vegetative growth of peanuts.

Choy sum developed similarly to peanuts in terms of growth and toxicity, but the effect of the neighbouring plant was more visible. Choy sum in mixed-cropping was smaller than their mono-cultured in respective soil volume. They are more similar in size to the mono-cultured one volume below them. Regarding toxicity, smaller volumes, and mixed cropping produce healthier plants rep-

Figure 1. Mono-crop Peanut grown in A) small B) medium C) large soil volume on (left) 4th and (right) 7th week after planting (WAP)

Figure 2. Mono-crop Choy sum grown in A) small B) medium C) large soil volume on (left) 1st and (right) 4th WAP
represented by greener and thicker leaves.

The analysis of variance for the main factors (Species, Soil volume/pot size, group (mono and mixed cropping), and the interactions are reported in Table 1. All main factors had significant effects with probability level >0.001 on dry shoot weight, Ni concentration, and root dry weight, but species significant with P>0.005 on root/shoot ratio. Interactions between pot sizes and species (AB) are significant (P>0.001) only on dry shoot weight and Ni concentration but not significant on root dry weight and root/shoot ratio at all. Interactions between pot sizes and neighbouring (AC), as well as species and neighbouring (BC), had significant effects (P>0.001) only on Ni concentration and not on dry shoot weight. The interactions between all main factors (ABC) are significant (P>0.001) on both shoot dry weight and Ni concentration as well.

Table 1. Ni Concentration of Mono- and Mix-Peanut and Choy Sum after grown in pots with different sizes under controlled growth conditions

<table>
<thead>
<tr>
<th>Pot Sizes</th>
<th>Nickel Concentration (ppm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mono-cropping</td>
</tr>
<tr>
<td></td>
<td>Peanut</td>
</tr>
<tr>
<td>Small</td>
<td>20 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medium</td>
<td>90 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Big</td>
<td>120 ± 5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Nickel concentration in shoots

Choy Sum has a higher concentration of Ni than peanuts of monoculture. In mixed cropping, their Ni concentration was significantly lower in both crops than monoculture cropping system (Table 1). However, increasing soil volume supported higher Ni uptake in both plants.

Dry Matter Yields

Peanuts were more tolerant and produced higher dry weight in root and shoot (Table 2). Despite no significant differences in the impact of soil volume on root/shoot ratio and root density for Choy sum, the results still align with their respective soil volume. Root densities of both plants reduced with increasing of soil volumes, but Ni uptake increased in large soil volumes. Peanut showed better tolerance to nickel contaminated soil, with higher dry matter accumulation and root/shoot ratio (Table 2). The presence of peanut also improved the soil condition for Choy sum growth, as indicated by the higher shoot biomass and root length.

Comparing both groups, crops in the mixed group have low shoot biomass than their respective mono-cultured crops, with choy sum in large volume. Surprisingly an exception. Mixed cropping root of choy sum dry matter is assuredly higher than root dry matter of choy sum in the mono-group; aside from large volume, they are lower than mono-cultured peanuts. The combined root/shoot ratio is lower than both mono-cultured species, and the root density order is jumbled.

However, Ni toxicity is one of many factors to be considered in this study. The results show that differences in growth and yield are apparent among different soil volumes despite being planted in similar growing media (Table 2 and 3). The growth of samples in smaller pots is inhibited when compared to those in bigger pots, visibly (Figure 1 and 2) and statistically (Table 2 and 3). Increasing the rooting volume proved to increase the dried mass significantly.

Table 2. Dry matter production and root/shoot ratio of Mono- Peanut and Choy sum plants after being grown in pots with different sizes under controlled growth conditions

<table>
<thead>
<tr>
<th>Pot Sizes</th>
<th>Dry Matter (g pot&lt;sup&gt;-1&lt;/sup&gt; D. M)</th>
<th>Root Density (w/w)</th>
<th>Root/Shoot Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>Peanut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>1.64±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medium</td>
<td>1.87±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.28±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.28±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Big</td>
<td>2.09±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.73±0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.82±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Choy sum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>0.46±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.92±0.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medium</td>
<td>1.14±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.31±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.31±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Big</td>
<td>1.27±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.78±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.19±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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There are no significant differences between values in the same column that are followed by the same letter (Duncan test at the 5% level).
DISCUSSION

Several theories can be considered regarding which factors induced differences in growth among different soil volumes. All centred around the root as the occupant of underground space. First is to consider roots as the key to the nutrients and water supply needed for plant metabolism throughout their lifetime. This represents the external factors more as it emphasizes the circumstances of the rhizosphere. That is saying to restrict plant roots also meant limiting the supply flow to the plant itself. It could affect the plants’ system and morphology in the short or long term. Physical root volume restrictions may have caused secondary water or nutrient limitations, obscuring the direct physical limiting effect. Spatial area may primarily have effects through micro-climate and the rate at which roots encounter nutrients (Kharkina et al., 1999; Yu et al., 2015). They also concluded that doubling the medium volume while holding nutrient concentration constant doubles the optimal number of roots, thus doubling the plant’s total harvest and net harvest (Brown et al., 1987; Kharkina et al., 1999; Yu et al., 2015). Moreover, Oxygen (O₂) also appears to be a limiting factor that affects root respiration in regards to limited spatial available for restricted root, which can be explained as the reduction of total pore space by increasing the root mass in the container (Kharkina et al., 1999). The possibility is even higher because of the nature of serpentines soil that has high day and silt and naturally low in major essentials nutrients (Chiarucci & Baker, 2007).

Another possibility related to roots as internal factors is the ability of roots to produce root-synthesized growth substances which can cause imbalances in plant hormones as the main reasons that effectively alter plant morphologies. Around 5 to 25% of net fixed carbon is released by plants into the rhizosphere in the form of compounds ranging from simple organic anion to complex polymer mucilage (Canarini et al., 2019; Kawasaki et al., 2016). The number of root exudates being released under drought in which photosynthesis is being constrained is reported to equal that under a control situation, thus indicating the vital role of these compounds for plant survival under extreme conditions (Williams & de Vries, 2020). Root exudates are believed to significantly form a direct communication pathway between plants and Rhizosphere microbes. Rhizodeposits, including root exudates, are the primary carbon sources for the rhizosphere microorganisms, and the compounds present in the root exudates can induce behavioural changes in the bacteria by altering their gene expression and determining the compositions of the root colonizing microbes (Canarini et al., 2019; Kawasaki et al., 2016). In turn, rhizosphere microorganisms can benefit plant growth by increasing nutrient supply, suppressing pathogens, and other less-studied cases (Kawasaki et al., 2016).

However, Herz and Kawasaki reported the exudation behaviour is mainly driven by local environmental conditions surrounding roots rather than species identity, species-specific traits, or neighbouring plants (Herz et al., 2018; Kawasaki et al., 2016). Furthermore, it is also believed that the suppression of roots would inevitably affect root exudation (Cai et al., 2007). Therefore, it is more reasonable that the root’s substances are instead the product of external factors, as in the first conjecture.

Substantively, the effect of soil volume on root growth could change from negative to positive depending on how nutrients were supplied (Murphy et al., 2013). Differences in root space and soil amounts do not actively affect the concentration but can give access to a diverse pool of the total amount of nutrients available.

Regardless, it is not limited to essential nutrients but applies to all substances available in growing media, including heavy metals. Considering the high concentrations of Ni, explain how samples in small pots are not as much affected by toxicity symptoms as samples in bigger pots. The argument is then proved in Tables 2 and 3 that crops in smaller pots indeed
acquire less nickel than those in bigger pots. Moreover, life quality is also noticeably better in visual observation, proven by root-shoot ratio and root density.

In this study, both peanut and choy sum produce a higher root-shoot ratio than in other studies, including their control (Shridhar Rao et al., 2012) and the ratio increase as the soil volume increase. The increase in root-shoot ratio could indicate a healthier plant, provided the increase came from greater root size and not from shoot deterioration (Agboola et al., 2014; Goldberg & Fleetwood, 1987). However, observing the root density that negatively correlates with the root-shoot ratio testified that it is most likely not the case for peanuts. Instead, it is evident that a larger soil volume provides access to a bigger pool of available resources and encourages faster growth. However, in the case of polluted soil, it could also worsen the toxicity symptoms. In the meantime, while different soil volumes indeed affect the biomass, it has less effect on both the root-shoot ratio and root density of choy sum, which accumulates higher concentrations of Ni in their tissue. It could imply that choy sum has a higher tolerance towards Ni than a peanut. It also suggests that the growth reduction of choy sum tends to result from root restriction rather than Ni toxicity symptoms. Unfortunately, due to the low biomass, choy sum contained less Ni than a peanut.

Both species’ different reactions may be related to the theory of root synthesized substances production. While the local environment of roots influenced the exudation behaviour, each plant secretes different compounds with different traits and attracts different microbes depending on the nature of the plant. Exudates of Brassicaceae are specifically reported to be autotoxic and possess inhibitory. Brassicaceae also reported attracting Ectomycorrhizal fungi that improve the absorption of mineral nutrition and increase the tolerance of the host toward stress environment (Zeng et al., 2003).

Similar patterns but less in value are to be expected when it comes to the mixed-cropping group. Interspecies competition is supposed to involve the work of root exudates more than in mono-cropping. Naturally, suppression in the growth of choy sum allegedly will result from peanut domination -aside from Ni toxicity- that has settled prior to intercropping. It has been proved to be the case in small and medium soil volumes in which choy sum is clearly struggling in growth, represented by the drastic reduction in biomass. In medium soil volume, the competition drove peanuts to strive for similar growth as their pair in mono-cropping despite differences in population density between mixed- and mono-cropping. However, large soil volume gives way to different development. The growth of mixed-cropping choy sum in large soil volume is surprisingly on par with mono-cropping choy sum.

Furthermore, it also seems to enjoy better condition as it visibly suffers less of the symptoms of greener and thicker leaves. The possible explanation could be either thanks to the presence of neighbour crops to share the burden of toxic metals or due to legumes specifically, as they are reported to be able to support up to 38% N uptake for their company from their root turnover and exudates (Jiao et al., 2017; Sierra & Desfontaines, 2009).

Nickel, as heavy metal, is naturally toxic. Even so, it is listed as one of the essential micro-nutrient’s plants need. Ni’s role is closely related to Nitrogen; thus, it can inhibit or stimulate N fixation depending on its availability in growing media (Saad et al., 2016; Wallace & Romney, 1980; Zaidi & Khan, 2005). Moreover, Ni effects on plant traits that are related to photosynthetic process has been proved (Batool, 2018; Someya et al., 2007), thus at length affected yield result and can even cause genotoxic effect t in form of oxidative stress (Gopal, 2014) or DNA damage (Doreswamy et al., 2004). Therefore, necrotic and chlorosis are to be expected as Ni toxicity symptoms that lead to growth inhibition. Interestingly, the last point worth noting is that even when both concentration and biomass of mixed-cropping samples are generally lower, if the Ni content rounded up per pot, then Ni-content in mixed-pot will be higher than mono-choy sum and equalled mono-peanut. These showed that by carefully maintaining proper space, intercropping could help the survival of crops on contaminated lands while optimizing the acquisition of heavy metal.

CONCLUSIONS
Less soil volume reduced biomass production but increased the root/shoot ratio, impacting higher Nickel uptake. Mixed cropping between legume (Soybean) and non-legume was reported here to reduce Nickel uptake. This mixed cropping method can employ Ni-contaminated land to produce lower Nickel concentration in the shoot.

ACKNOWLEDGMENT
The authors thank PT. Vale Indonesia Tbk for providing the overburden soil (limonitic laterite). We thank the reviewers for their careful reading of our manuscript and their many insightful comments and suggestions.
AUTHOR CONTRIBUTIONS

Conceptualization: Aiyen Tjoa, Zainuddin Basri, Henry Barus. Laboratory and experiment: Aiyen Tjoa, Leisa Reclina Christi, Henry Barus. Data curation: Aiyen Tjoa, Leisa Reclina Christi, Henry Barus. Writing original draft: Aiyen Tjoa. Review and editing: Aiyen Tjoa and Nur Edy. Funding acquisition: Aiyen Tjoa. All authors contributed to the article.

CONFLICTS OF INTEREST

The authors declare there is no conflict of interest related to financial funding and authorship order for this article.

REFERENCES


