



## Phytomining: Effect of sulfur on the nickel yield of Magkono (*Xanthostemon verdugonianus*)

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### Abstract

This study investigated the effects of sulfur on the nickel yield of *Xanthostemon verdugonianus* seedlings over a two-month period. Two groups were analyzed: one treated with elemental sulfur (0.46 g S/kg soil) and a control group without sulfur. The nickel uptake and biomass production of the sulfur-treated plants were compared to those of the control plants. Analysis revealed that sulfur-treated plants exhibited increased nickel yield and biomass compared to the control group, with average nickel concentrations of 2.42% and 1.61%, respectively. Statistical analysis showed a significant difference in nickel yield between the control and sulfur-treated groups ( $F = 16.833$ ,  $p = 0.0093$ ), indicating a significant effect at the 5% significance level. Additionally, sulfur-treated seedlings produced more biomass (15.92 g) compared to the control (8.06 g). The linear relationships between nickel yield and dry weight were also examined. Analysis revealed a strong positive relation between the two variables ( $r = 0.753$ ).

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## Introduction

Traditional methods of mining have historically caused enormous environmental problems, necessitating the development of new processing routes and technologies to mitigate these issues (Githiria *et al.*, 2020). The conventional nickel extraction process, for instance, generates significant environmental impacts, including nickel-containing waste streams from batteries, catalysts, wastewater, and electrolyte bleed-off, which can contaminate soil, water, and air, thereby adversely affecting both the environment and human health (Haddaway *et al.*, 2022). Additionally, the environmental impacts and energy demand of nickel processing are expected to rise with the increasing demand for stainless steel and other nickel-related alloys. The removal of nickel from diverse waste sources is a global concern to maximize the use of the precious metal and to reduce environmental impact during waste disposal. Recent advancements have been made in understanding the environmental and economic implications associated with this process (Begum *et al.*, 2022).

Phytomining is a sustainable alternative to traditional nickel mining, involving the cultivation of high-biomass plants that accumulate significant metal concentrations, which can be harvested and processed using existing smelters or refineries (Ragini *et al.*, 2021). This method is particularly suited for heavily contaminated soils and low-grade ore deposits, where conventional mining would be prohibitively expensive. Nickel hyperaccumulators on the other hand, serves as 'bio-ore' that can economically justify the costs of phytoextraction development (Losfeld *et al.*, 2012, 2015). Although still an emerging technology, phytomining has garnered significant research interest since its introduction by van der Ent *et al.*, 2015, describing the agricultural process of growing hyperaccumulator plants on sub-economic ore deposits and extracting metals from their biomass. The global phytoremediation market, valued at 34–54 billion US dollars, is expanding, particularly in developed nations, showcasing the effectiveness of phytoextraction. This technology hinges on factors

such as biomass yield, metal accumulation rate, and tolerance to specific metals, with research focusing on identifying potent hyperaccumulators and understanding the molecular mechanisms of metal accumulation and tolerance. Phytomining offers a cost-effective alternative for on-site metal removal and presents opportunities for biofortification, biofuel production, and the generation of metal-rich biochar (Ali *et al.*, 2017).

Phytomining technologies have primarily focused on nickel due to the efficiency with which hyperaccumulators absorb this metal, presenting a promising alternative to conventional extraction methods. Enhancing biomass production through fertilization is crucial for improving the efficiency of phytomining and phytoextraction (Jacobs *et al.*, 2017, 2019).

Research by Sissou *et al.* (2021) demonstrated that sulfur amendments significantly increase the phytoextraction of cadmium, chromium, and nickel, with respective fold increases of 1.6, 3.3, and 12.6, highlighting the potential of sulfur in enhancing metal uptake from contaminated soils. Similarly, Rosenkranz *et al.* (2019) found that *Odontarrhena chalcidica*, a herbaceous plant, treated with sulfur and intercropped with legumes, produced higher shoot biomass and nickel yield compared to *Noccaea goesingensis*, also a herbaceous plant, with the highest yield reaching 55 kg Ni ha<sup>-1</sup> under sulfur treatment. Moreover, sulfur's role in plant growth and development underscores its importance in meeting the rising demand for high-quality crops amidst environmental stresses (Zenda *et al.*, 2021). These findings collectively emphasize the need to optimize sulfur utilization in phytomining to achieve sustainable and efficient metal extraction.

*Xanthostemon verdugonianus*, or Magkono, is a prized Philippine tree known for its exceptional density and weight, making it valuable for uses such as electrical posts, saltwater piling, tool handles, and various novelties. Despite being relatively widespread, its supply is limited. Magkono is most

notable in Surigao, Sibuyan, and Agusan but is found in other regions of the Philippines as well (Ruales *et al.*, 2023). As the hardest timber in the Philippines, *X. verdugonianus* is a potential hyperaccumulator, particularly in nickel-contaminated areas like Agusan and Surigao.

This study aims to explore sustainable alternatives to conventional nickel extraction methods through phytomining technology, specifically focusing on enhancing metal accumulation and biomass production in hyperaccumulator plants. In particular, it investigates the potential of sulfur application to enhance nickel yield and biomass production in *Xanthostemon verdugonianus* seedlings, thereby optimizing the efficiency of phytomining as a sustainable alternative to traditional nickel extraction methods.

**Materials and methods**

*Experimental set-up, harvest, and analysis*

Seedlings of *Xanthostemon verdugonianus*, grown in nurseries and approximately 8-10 months old (26 - 45 cm tall), were transplanted into larger pots with soil contaminated with Nickel (see Table 1 for soil composition). The seedlings were randomly divided into two groups: one treated with sulfur and the other serving as a control (without sulfur). This setup followed a Complete Randomized Design (CRD), ensuring equal numbers and approximately uniform initial sizes across groups (see Fig. 1).

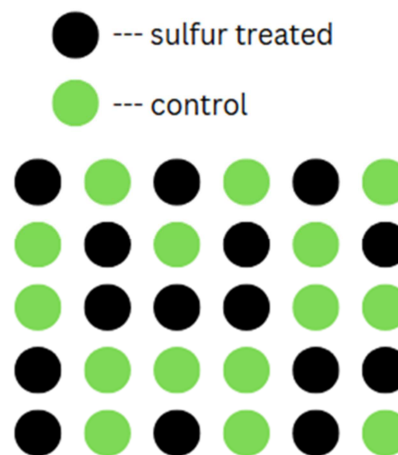
**Table 1.** Elements present in the soil with their average content by weight (mean ± SD; n = 3)

Element	(Wt. %)
Fe	28.57 ± 2.142
Ca	12.72 ± 0.772
Al	8.23 ± 4.292
S	0.15 ± 0.088
K	2.57 ± 0.284
Ni	5.20 ± 0.083
Mn	0.52 ± 0.049
Cr	0.04 ± 0.003
Ti	1.73 ± 0.175

Data obtained from XRF analysis.

Elemental sulfur (powdered form) was mixed into the soil for the treated group at a concentration of

0.46 g/kg of soil, this is the same amount applied in the study of Rosenkranz *et al.*, 2019. A fabric layer was placed atop the soil as mulch to retain moisture, reduce Ni runoff, and suppress weed growth. Seedlings were watered daily using spray bottles, and the experiment lasted for two months until the harvest phase. Throughout the experiment, plant growth, status, and size were continuously monitored. At the end, all plant tissues were carefully harvested, washed, measured for height, and dried at room temperature for 3 weeks to be weighed, pulverized, and sent for X-ray fluorescence (XRF) analysis.



**Fig. 1.** Illustration of the experimental set-up

*Statistical analysis*

To address the objective of evaluating the effect of sulfur treatment on nickel yield in *X. verdugonianus*, two statistical approaches was employed. Firstly, a one-way analysis of variance (ANOVA) was utilized to test for significant differences in nickel yield between the two treatment groups: plants grown with sulfur treatment and control (no sulfur). This analysis determined whether the addition of sulfur has a statistically significant impact on the plant's ability to accumulate nickel. Secondly, Pearson's correlation analysis was conducted to examine the strength and direction of the linear relationship between nickel yield and dry weight. This analysis provided insights into the potential relationship between plant growth and nickel accumulation. By employing these statistical tools, the study aimed to draw meaningful

conclusions about the role of sulfur in phytomining processes involving *X. verdugonianus*.

**Results**

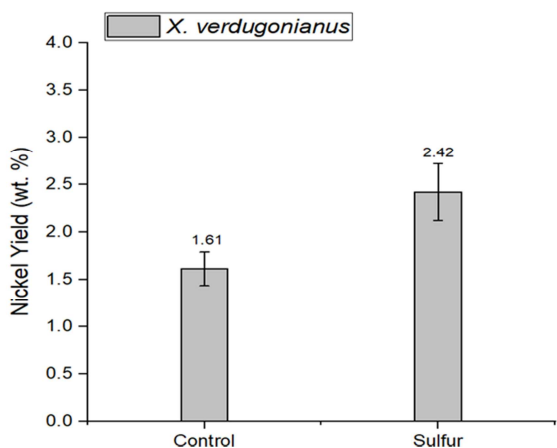
*Nickel yield*

The focus of the experiment is the increase in nickel yield of *X. verdugonianus* as a result of applying sulfur as compared to control which has no sulfur treatment after 2 months. Table 2 shows the data from XRF analysis of *X. verdugonianus*.

**Table 2.** XRF analysis of control and sulfur treated *X. verdugonianus* seedlings

Control		Sulfur treated	
Replicates	Nickel yield (Wt. %)	Replicates	Nickel yield (Wt. %)
C1	2.9635	S1	2.1158
C1	1.7066	S2	2.3063
C3	1.7257	S3	2.4315
C3	1.4086	S4	2.8216

However, diagnostics of the data revealed that one replicate in the control group is very influential (C1). That is, the residual of this observation had a large cook's distance  $D = 0.805$  which was greater than the suggested value  $4/(p - n - 1) = 4/(8 - 2 - 1) = 0.800$  (Bruce and Bruce, 2017). This indicated that the replicate was an outlier. Hence, the researcher decided to remove this case.



**Fig. 2.** Nickel yield for control and sulfur treated ( $\text{mean} \pm \text{SD}$ ;  $n = 3, n = 4$ )

Fig. 2 illustrates the average nickel content in the seedlings' tissues as measured by XRF analysis. The control group had an average nickel content of 1.61%

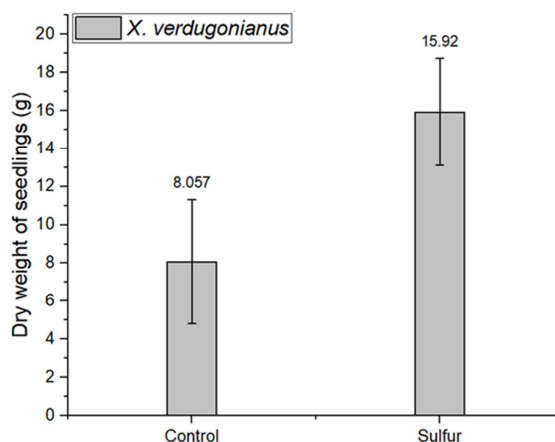
by weight, while the sulfur group exhibited a higher nickel concentration of 2.42% by weight.

ANOVA was used to determine if the observed difference in nickel yield between the two groups indicates a significant effect of sulfur on *X. verdugonianus*. The analysis revealed that the sulfur-treated group had a significantly higher average nickel content (2.42 wt. %) compared to the control group (1.61 wt. %). The result of the test confirmed this difference as statistically significant, with an F-value of 16.833 and a p-value of 0.0093 at the 5% significance level. This provides strong evidence of a positive effect of sulfur on the nickel yield of *X. verdugonianus*.

**Table 3.** Average concentrations of other elements in the tissues of *X. verdugonianus*, control and sulfur treated ( $\text{mean} \pm \text{SD}$ ;  $n = 4$ )

Element	<i>X. verdugonianus</i>	
	Control (Wt. %)	Sulfur treated (Wt. %)
Fe	52.69 $\pm$ 5.54	65.70 $\pm$ 1.96
Ca	25.58 $\pm$ 4.60	11.82 $\pm$ 1.85
Al	1.06 $\pm$ 0.53	3.95 $\pm$ 0.50
S	2.34 $\pm$ 0.43	3.53 $\pm$ 1.44
K	5.21 $\pm$ 2.24	2.33 $\pm$ 0.49
Ti	0.33 $\pm$ 0.20	0.38 $\pm$ 0.05
Mn	0.41 $\pm$ 0.20	0.45 $\pm$ 0.22
Cr	1.57 $\pm$ 0.79	1.58 $\pm$ 0.20

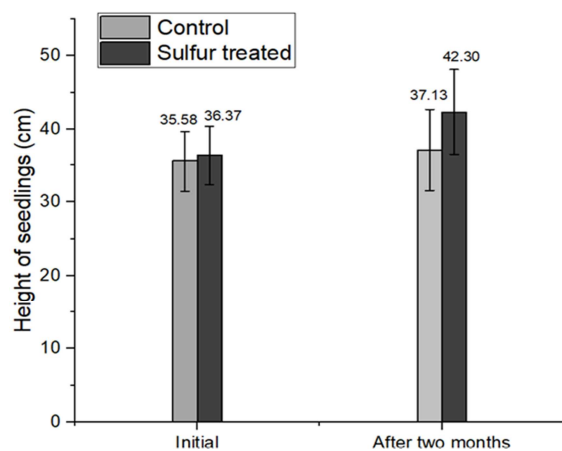
Table 3 presents the average concentrations (Wt. %) of other elements in the tissues of *X. verdugonianus*, comparing control with sulfur treated. The data were obtained using X-ray fluorescence (XRF) analysis.



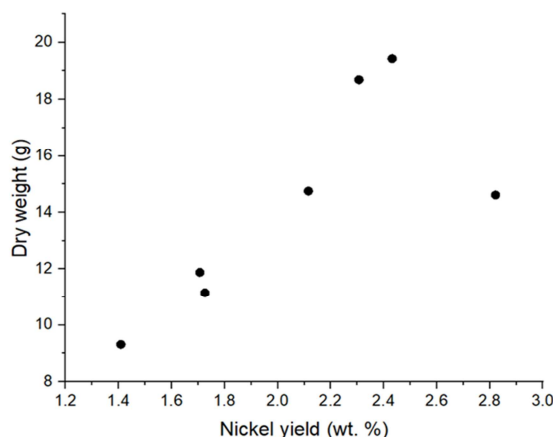
**Fig. 3.** Dry weight of control and sulfur treated *X. verdugonianus* ( $\text{mean} \pm \text{SD}$ ;  $n = 14$ ). Significant difference at  $p < 0.05$

*Biomass production*

Fig. 3 presents a comparative analysis of the dry weight of *X. verdugonianus* between two groups: the control group and the sulfur-treated group. In the control group, the average dry weight is recorded at 8.06 g, whereas in the sulfur-treated group, the average dry weight notably increases to 15.92 g. This significant difference suggests that the sulfur treatment nearly doubles the dry weight of *X. verdugonianus* compared to the control group, highlighting the substantial impact of sulfur treatment on the growth of the plant.



**Fig. 4.** Height comparison between initial and two months after for control and sulfur-treated *X. verdugonianus* (mean ± SD; n = 14), significant difference at  $p < 0.05$



**Fig. 5.** Linear relationship between nickel yield (Wt. %) and dry weight (g)

Fig. 4 presents the average height of *X. verdugonianus*. Initially, the control group was 35.58

cm and the sulfur group was 36.37 cm. After two months, the control group grew by 4.3% to 37.12 cm, while the sulfur group increased by 16.3% to 42.30 cm, showing a significant difference.

Fig. 5 shows the linear relationship between nickel yield and dry weight of the plant using Pearson correlation.

Analysis revealed a strong positive correlation between nickel yield and dry weight ( $r = 0.753$ ), indicating that nickel content in plants increases as biomass increases. This supported the study of Jacobs *et al.*, (2017, 2019) that improving plant growth through fertilization improves yield in phytomining. In the case of this study, elemental sulfur serves as the fertilization agent.

**Discussion**

These results seem to agree with numerous previous researches indicating that sulfur plays a significant role in improving plant health. This is supported by several factors. Firstly, sulfur is a vital macronutrient for plants, ranking fourth in importance after nitrogen, phosphorus, and potassium. It contributes to the primary structure of proteins and the functionality of enzymes, being a key component of amino acids like cysteine and methionine. Additionally, sulfur aids in various physiological processes of plants and offers protection against environmental stresses such as heavy metal contamination and pests, thanks to its antioxidative properties (Zenda *et al.*, 2021). Furthermore, sulfur enhances nutrient accumulation, partially increases biomass, and improves ionic equilibrium even in nickel-exposed environment (Matraszek *et al.*, 2016). It also bolsters growth, photosynthetic activity, yield, and quality, and studies have shown that sulfur fertilization can alleviate the negative impacts of abiotic stress on plants, thereby improving the productivity of important agricultural crops (Shah *et al.*, 2021). Sulfur not only aids in nickel (Ni) exposure but also improves the accumulation of other heavy metals such as arsenic (As) by stimulating thiol production and forming complexes with arsenite-

phytochelatins (Chen *et al.*, 2017), and lead (Pb) by enhancing photosynthetic activity and transpiration rates (Saifullah *et al.*, 2016).

### Conclusion

The study highlights the essential role of sulfur in enhancing the nickel yield and biomass production of *X. verdugonianus*, showing that sulfur supplementation significantly increases nickel yield and biomass compared to plants without sulfur. At 5% significance level, ANOVA revealed a significant difference in nickel yield ( $p = 0.0093$ ). A strong positive linear relation were observed between nickel yield and dry weight of plants ( $r = 0.753$ ) indicating that nickel yield increases as biomass increases.

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