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THE IMPACT OF SOIL HEAVY METAL ACCUMULATION ON THE GROWTH OF SUNN HEMP (*Crotalaria juncea* L.)

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Abstract:

Sunn Hemp plants, specifically *Crotalaria juncea*, are effective in absorbing and reducing the accumulation of heavy metals like cadmium, zinc, lead, and copper in soil. This plant species belongs to the Fabaceae family and is not only important for its phytoremediation capabilities but also for its agricultural value as a fiber and manure crop. Overall, Sunn hemp plants are crucial in mitigating the harmful effects of heavy metal contamination. The experiment was conducted in a greenhouse using a Completed Randomized Design (CRD) with 5 treatments and 6 replications to assess the impact of varying concentrations of heavy metals on Sunn hemp growth. The heavy metals were created by combining Cadmium (Cd), Copper (Cu), Lead (Pb), and Nickel (Ni). Each treatment of heavy metal contamination using 200ml of solution: T0 is the negative control. T1 containing 0.9 (Cd) + 4.0 (Cu) + 0.18 (Pb) + 0.7 (Ni). T2 containing 11.90 (Cd) + 19.80 (Cu) + 36.00 (Pb) + 28.90 (Ni). T3 containing 23.80 (Cd) + 39.60 (Cu) + 72.00 (Pb) + 57.80 (Ni). T4 containing 35.70 (Cd) + 59.40 (Cu) + 108.0 (Pb) + 115.60 (Ni). The study analyzed the impact of heavy metal concentrations on the growth of sunn hemp. Data included soil pH, plant height, flower count, pod count, root and shoot length, fresh and dry weight. Results showed that T4 showed excellent growth performance in terms of height, number of flowers, and dry weight. Sunn hemp has the ability to store heavy metals to a greater extent in its roots and has a remarkable capacity for long-term survival.

Keywords:

Crotalaria Juncea, Heavy Metals, Phytoremediation

Introduction

Heavy metal defines as some metal that cause several damages to the soil and plants when they are highly concentrated. For example, of heavy metals are cadmium (Cd), mercury (Hg), lead

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(Pb), chromium (Cr), iron (Fe), and copper (Cu). This kind of contamination is persistent in the soil environment, widely dispersed, and both biologically harmful. There are several ways to recognize heavy metal contamination through the soil, water in rivers, air on atmosphere and many others. Additionally, there is no denying that heavy metals are hazardous and toxic. Heavy metals degrade the quality of the air, water, and soil, which negatively affects the health of plants, animals, and human beings. For soil, it had to bear the accumulated amounts of heavy metals in soil and plants negatively which will affect their physiological processes like photosynthesis, gas exchange, and nutrient uptake, which reduces plant growth and causes an overabundance of dead matter. Besides, each heavy metal has a unique chemical structure, composition, and physical characteristics. Moreover, the composition of the parent rocks, the degree of weathering, the chemical, physical, and biological characteristics of the soil, as well as the climatic conditions, are some of the factors that affect the presence and division of these metals in the earth. The main factor contributing to the environment's degradation is human activity, which is steadily growing day by day.

Sunn hemp also known as Sunnhemp, brown hemp, Madras hemp, Indian hemp or Sannhemp (Bhardwaj et al., 2005). The scientific name is known as *Crotalaria juncea* L. The word "rattle" in the genus name "Crotalaria" describes the sound made when the mature pods seeds are shaken. This genus' species are widely distributed throughout the tropical, subtropical, and to a lesser extent, temperate zones countries. Besides, Sun hemp is originated from India and be indigenous Indian crop since the beginning of agriculture. This plant species is from legume family (Fabaceae) that has high agricultural relevance since it can be fiber and manure crop. Furthermore, the reason of Sunn hemp as cover crop because it can be used to improve crop yields during a crop rotation and lessen the impact of invertebrate pests. Sun hemp residue enriches the soil with nutrients and organic matter and may improve the soil microbiota. Other than that, the majority of studies on vegetables planted after hemp have discovered higher vegetable yields. Sun hemp also kills nematodes and weeds. Allelochemical plant defense compounds found in its residue prevent or postpone the growth of seeds from weeds and the life cycle of nematodes (Cheng & Cheng, 2015).

Soil contamination has been becoming significant issues because of a number of anthropogenic activities, such as the use of heavy metals, agriculture activities, waste disposal, natural sources, mining and smelting, industrial waste and air pollution have both direct and indirect effects on the soil according to Figure 1. The heavy metals are mainly included Cadmium (Cd), Nickel (Ni), Copper (Cu), Arsenic (As), Mercury (Hg), Lead (Pb), and Zink (Zn). Since heavy metals are unable to breakdown and endure in the environment indefinitely, they are the most challenging and intractable soil contaminants for remediation. The most prevalent heavy metals found in contaminated soils are Cd and Pb. Besides, waste from construction projects and improper soil storage in manufacturing mines are additional potential sources of heavy metal soil pollution. It proved that numerous environmental issues, such as loss of vegetation, rice pollution, contaminating groundwater, and Cd or Pb toxicity in the food chain, have been attributed to their accumulation in soils. However, due to their toxic nature, poor biodegradability, perseverance in the environment, and bioaccumulation in the food chain, heavy metals have posed as serious threat to both human health and the environment (Alengebawy et al., 2021). They are responsible for a number of human disorders, including illnesses like Parkinson's, Alzheimer's, and Menken’s. In addition, they can also have an impact on the human cardiovascular systems, as well as some organs like kidneys, liver, and heart.

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Furthermore, in this research we mainly focused on observe heavy metals accumulated such as cadmium (Cd), Copper (Cu), Lead (Pb) and Nickel (Ni) in the contaminated soil.

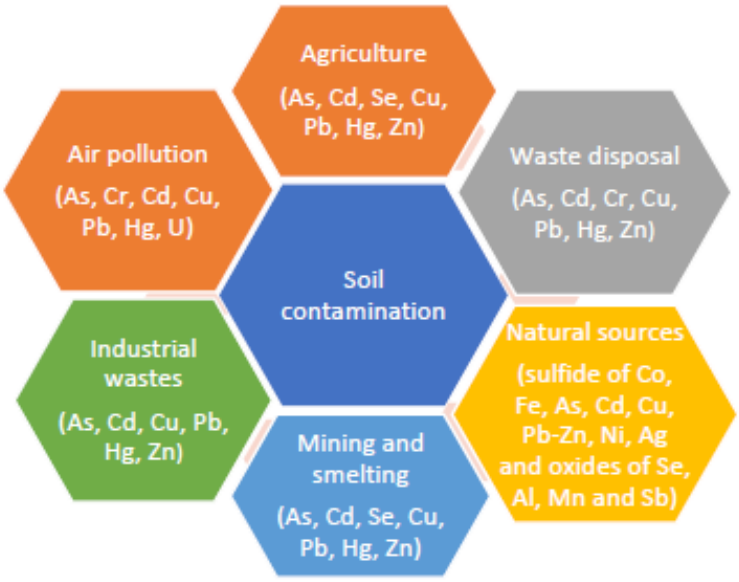

Figure 1: Source Of Heavy Metal That Leads To Soil Contamination (Ugwu, 2019)

Figure 2*: Sunn Hemp*

The quickest growing species of the genus Crotalaria, is sunn hemp (*Crotalaria juncea* L.). Besides, it also happens to be the one used most frequently as green manure in the tropics. It is additionally grown as a crop for animal feed and fibre. According to Mosjidis et al., (2013) this

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plant has been domesticated for many centuries and grows wild on the Indo-Pakistani subcontinent, in which it is believed to have its origins. Sunn hemp is a legume that can grow in a variety of climates and soil types. It has a high yield, fixes nitrogen, and resists a number of nematodes. As a result, it can be used as a summer cover crop to enhance soil productivity, preserve and maintain water and soil resources, and minimise the transport of pollutants across the water's surface. Sunn hemp suitable to grows in tropical and subtropical regions of the world and recommend climate is warm season crop since it is intolerant of frosts. In addition, it requires a minimum of 40 cm of well distributed rainfall during the growing season. The growth rate for sunn hemp is about 60 – 90 days. Moreover, since sunn hemp is a legume that can grow in a variety of climates and soil types. Once established, it is tolerant of drought. It cannot tolerate standing water though, as plants become yellow and stunted. Moreover, Mosjidis et al., (2013) states that Sunn hemp is extremely vulnerable to hypoxia, a temporary oxygen shortage in the soil. If water remains in one place for a prolonged period of time due to poor drainage, plants grow shorter and a perfect stand may be reduced to almost nothing.

Materials and Method

Experimental Site

The experiment was conducted in greenhouse of Faculty of Plantation and Agrotechnology UiTM Arau, Perlis. The coordinate for the location is 6°27’10‘’N 100°16’15‘’E. The experiment was conducted from September 2023 until February 2024. The study location are warm with temperatures between 25.5°C and 35.7°C, which suitable for germination and growths *Crotalaria juncea* L .While, for the Agrotech laboratories, Star Complex UiTM, Kampus Arau were used for laboratory work.

Figure 3: Experimental Site In The Greenhouse

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Experimental Design

The replicates were treated with five different treatments under arrangement of complete randomized design (CRD). This experiment had been conducted inside the greenhouse and there would no interaction with outside factor such as rainfall that might affect the results. Five treatments had been used in this experimental design, followed by six replications of each treatment had been used, resulting in 30 plants. Table 1 shows the permissible range limit of heavy metal in soil that has been control by Department of Environment Malaysia in maximum and minimum used for cadmium, copper, lead and nickel.

Table 1: Permissible Range Limit Of Heavy Metal In Soil

Element	Soil	
	Min	Max
	mg/L	mg/L
Cadmium, Cd	0.9	11.90
Lead, Pb	0.18	36.00
Copper, Cu	4.0	19.8
Nickel, Ni	0.70	28.90

Source: Department of Environment Malaysia (DOE, 2009)

Table 2: Treatment Range With Interaction Of Heavy Metal Concentrations. The Units Of Measurement For This Treatment Are Milligrams Per Liter (mg/L) (DOE, 2009)

	Concentration applied (mg/L)			
Treatment	Cd	Pb	Cu	Ni
T0 (control)	0	0	0	0
T1 (min)	0.9	0.18	4.0	0.70
T2 (max)	11.90	36.00	19.80	28.90
T3	23.8	72.00	39.6	57.80
T4	35.7	108.00	59.4	115.60

The solution poured into 200 mL to each polybag. The plants was initially treated with 50mL of the heavy metal one week before to planting. Following planting, 50 mL of the element was poured each of the next three weeks.

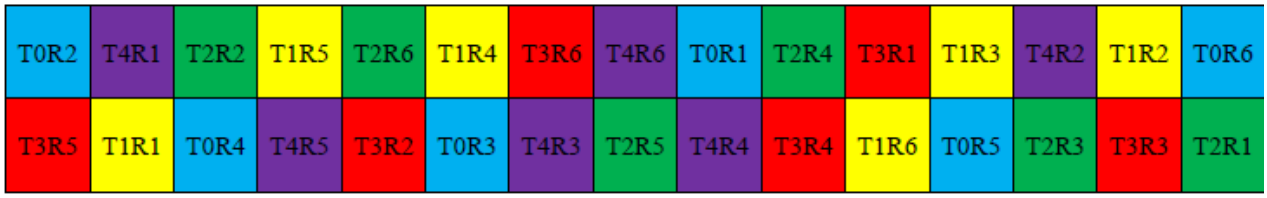

Figure 4: Experimental Layout in CRD

* T represents the type of treatment that will be applied to each arrangement
* R represents the replication of each treatment

Data Collection

The data collection of heavy metals in soil and plant which is Cd, Cu, Pb, and Ni were taken at laboratory in acid digestion method by used ICP-OES. The soil pH also was recorded before planting and after harvest. Next, the data collection for plant growth parameters such as plant height (cm), number of flowers, number of pods were carried out after the seedlings been transplanted into polybag. The first readings were recorded two weeks after transplant. While,

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for the root length, shoot length, fresh weight and dried weight, it was taken at laboratory for recording after 60 days of planting by removing the plants form the polybags. The growth duration of sunn hemp was about 60 – 90 days All of the data had been measured and recorded in ordered to obtain information about heavy metals content in soil and plant after being exposed to different concentration rate of heavy metals and sunn hemp growth performance.

Statistical Analysis

The data were analysed using SPSS (Statistical Package for Social Science (SPSS) Version 26) software using one-way ANOVA and further tested using Tukey test if the one-way ANOVA were found to be significant.

Results And Discussion

Heavy Metals Content In Soil

Heavy metals in soil had been analyse in acid digestion method by used Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to identify the heavy metals such as Cd, Cu, Pb, and Ni. The evaluation of heavy metals content in soil after being exposed to different concentration rate of heavy metals was evaluated and the results are presented in graph below for element Cu, Ni and Pb. However, Cd element had been not absorbed and detected in soil contaminated.

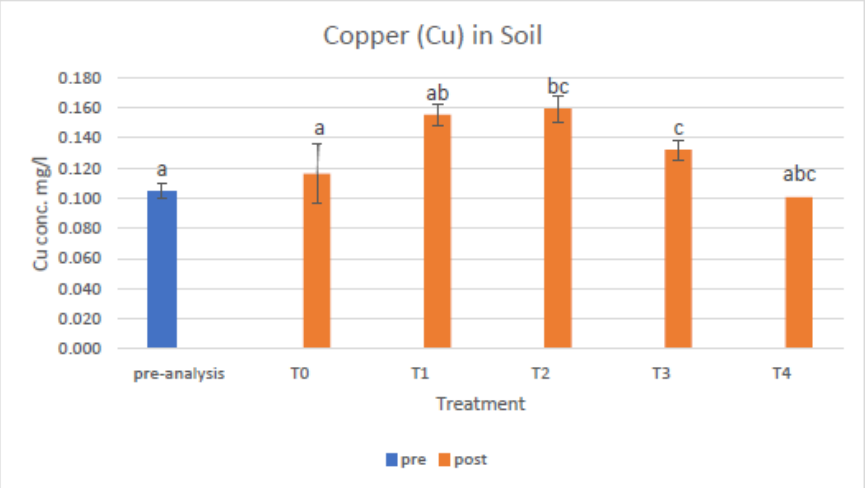

Figure 5: Copper (Cu) in Soil

Based on the Figure 5, the result show there significant different ($P<0.05$) in contaminated soil that content Cu element. From the graph, the highest concentration rate of Cu in soil is T2 which is 0.159 mg/L since the concentration rate were applied for T2 is 200ml solution of 11.90 (Cd) + 19.80 (Cu) + 36.00 (Pb) + 28.90 (Ni). Meanwhile, for the lowest concentration rate of soil can absorbed for Cu is T4 which is 0.100 mg/L it will be further discuss in Cu absorption in plants. In previous study stated that, the availability of the Cu, which is impacted by both internal which is plant-associated and external were soil-associated factors (Shweta, 2008). Therefore, this study adopted the limit proposed by (Al Saad et al., 2018) which Cu in soils were below the safe limit which range about 0.6 mg/kg which far above WHO standard.

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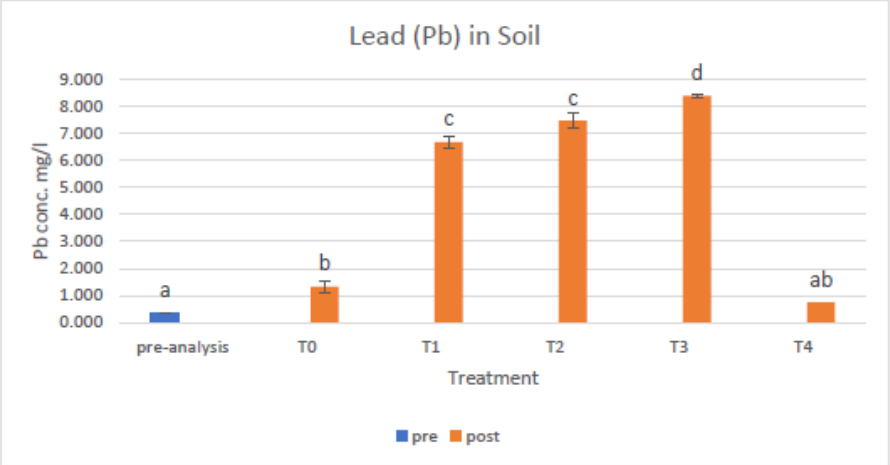

Figure 6: Lead (Pb) in Soil

Based on the figure 6, the result showed there significant different (P<0.05) in contaminated soil that content Pb element. From the graph, the highest concentration rate of Pb in soil is T3 which is 8.382 mg/L. Meanwhile, for the lowest concentration rate of soil can absorbed for Pb is T4 which is 0.753 mg/L this result will be further discuss in plant absorption results. This result shows that soil content can uptake the Pb element since the sunn hemp seeds germination both in the presence and in the absence of lead already (Silva et al., 2021). From previous study, the concentration of lead in the soil was 30.70 mg/L below which is safe limit in soil for agriculture in Malaysia.

Heavy Metals Content In Sunn Hemp Plant

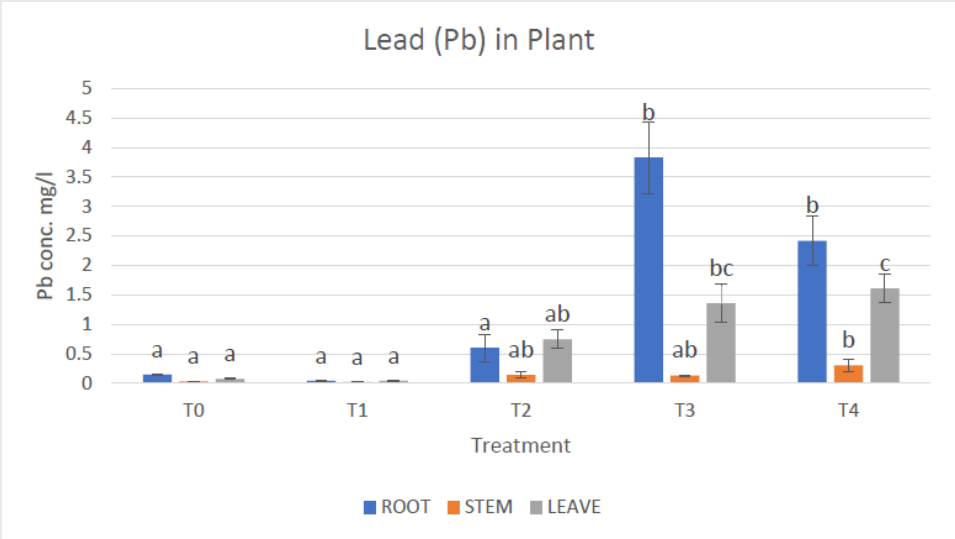

Figure 7: Lead (Pb) Content In Plant Part

Based on the Figure 7, the result showed there significant different (P<0.05) for different concentration rate of heavy metals on sunn hemp plant part which is root, stem and leaves. It was supported by (Pallavi Sharma, 2005), stated that lead is easily absorbed and accumulated element in different part of plants. For the root part, the highest concentration rate that accumulated is T3 which is 3.829 mg/l. Meanwhile, T1 is the lowest concentration rate for root to accumulate. Next, for stem part, the highest concentration rate that accumulated is T4 which is 0.308 mg/l while T1 is the lowest concentration rate which is 0.266 mg/l for stem to absorb.

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Furthermore, for leaves part the highest concentration rate is T4 which is 1.610 mg/l while T1 is the lowest concentration rate which is 0.045 mg/l to absorb in leaves part.

Overall, it showed that the root part is the highest concentration rate of lead between different parts of sunn hemp plants (Silva et al., 2021). Besides that, the potential metal accumulation by the aerial and subsurface sections of sunn hemp plants was observed because of their high lead absorption rate. In most cases, the plant was capable to hold the metal in both its aerial sections and roots, which had the largest concentration.

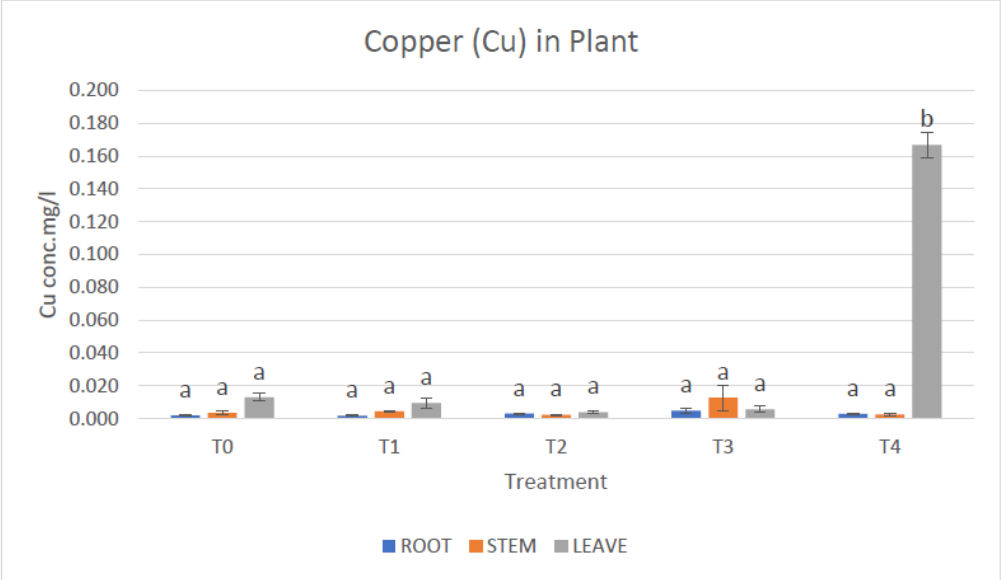

Figure 8: Copper (Cu) Content In Plant Part

Based on the Figure 8, the result showed there no significant different (P>0.05) for different concentration rate of heavy metals on sunn hemp plant part for root and stem. However, there was significant different (P<0.05) for different concentration rate of heavy metals on sunn hemp plant on part of leaves. For the root part, the highest concentration rate that accumulated is T3 which is 0.0018 mg/l while T1 showed the lowest concentration rate for root. Next, for the stem part the highest concentration rate that accumulated is T3 which is 0.013 mg/l while T2 showed the lowest concentration rate which is 0.002 mg/l. Furthermore, for leaves part the highest concentration rate is T4 which is 0.09 mg/l while T2 is the lowest concentration rate which is 0.002 mg/l to absorb in leaves part.

Overall, it showed that the leaves part is the highest concentration rate of copper between different parts of *C. juncea* plants. Additionally, too much Cu limits on leaves part effect to the uptake of other elements, resulting in nutritional imbalances in plants parts to uptake (Quagliata et al., 2021).

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Growth Development

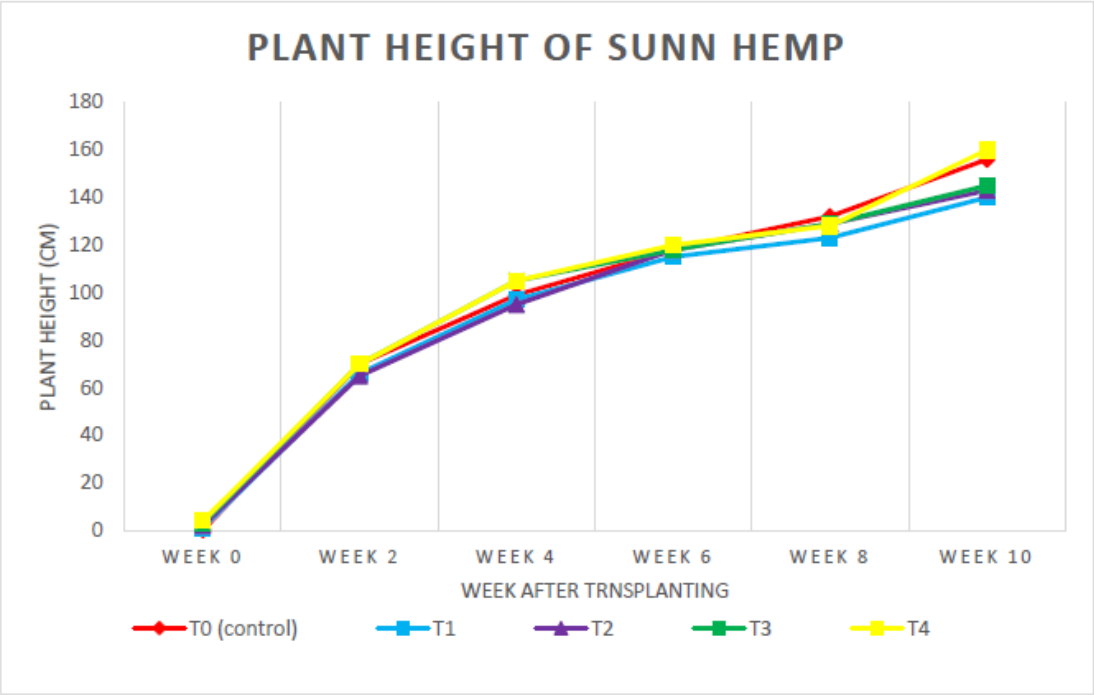

Figure 9: Effect Of Different Concentration Rate Of Heavy Metals On Height Of Sunn Hemp

The height of sunn hemp could differ based on its age, growth environment, and particular species. In ten weeks, plant height was measured every two weeks. Figure 9 shows the effects of different concentration rate of heavy metals on sunn hemp plant height. The results of the analysis of variance showed that the plant height of sunn hemp was significantly ($P<0.05$) impacted by the different concentration rate of heavy metals. Based on the results, T4 shown the highest value of plant height which is 135 cm. Meanwhile, T1 shown the lowest value of plant height after 10 weeks after transplanting (WAT) which was 129 cm. In addition, sunn hemp plant had the survival rate of 100% since no plants died throughout 56 days of experiment.

According to (Fall et al., 2020), the growth of sunn hemp can also be influenced by factors such as soil fertility, water availability, sunlight exposure, and even can grow on droughty soil with low fertility. Moreover, sunn hemp is fast growing species in its genus (UC Agriculture, 2021). From that we can see from weeks 0 sunn hemp started growth rapidly until week ten for 56 days.

Recommendation

Determination of heavy metals content in soil and sunn hemp plant after being exposed to different concentration rate of heavy metals is important in order to know limit of the plants could uptake the heavy metals in soil. This study also would like to suggest that the experiment should be conducted in the best surrounding by avoiding labs areas that are conducting other unrelated experiment. Next, prepare a conducive workplace to minimize the potential of exposure to contaminants while conducting experiments. Future research needs to be done on the growth performance. There is still lots of room for this study to be improved and updated

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on a regular basis. Further research on sunn hemp plant or focusing on other potential plant that can absorb heavy metal as plant phytoremediation.

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