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Effect of Organic soil amendment on Hemp

Mounica Talasila

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EFFECT OF ORGANIC SOIL AMENDMENT ON HEMP

A Thesis presented to
The Faculty of Hutson School of Agriculture
Murray State University
Murray, Kentucky.

In partial fulfilment of requirements for
the degree of
Master of Science

Submitted by
Mounica Talasila
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// Acharya Devo Bhava // (Teachers are equal to God).

Abstract

The research study is about the impact of different levels of organic soil amendment on the levels of psychoactive compounds produced in industrial hemp. Inorganic or chemical pesticides or fertilizers are not as extensively used in hemp as in other crops. Apart from this, using an organic soil amendment would be an environmentally sustainable way. A randomized block design (RBD) model was used to study the impacts of the soil amendment. The soil amendment used is called “Rehab” a product of Organilock created from the remnants of animals like fish and chicken. This research design has 3 different soil amendment levels (B-14 gallons per plot, C-21 gallons per plot and D-28 gallons per plot) and a control treatment(No amendment), each replicated three times to minimize the errors. The soil and plant tissue samples were sent to the lab for analyzing the amount of THC and CBD content in plant tissue and the amount of essential nutrients in soil. The THC content was below 0.3% in all the four treatments. This means that the use of soil amendment does not have any negative impact on THC levels of the hemp crop. However, when the CBD levels are compared, all the four samples were below 10% which is on the lower end of the regular CBD yield. Samples from Treatment B (14 gallons per plot) has slightly higher dry weight, higher soil nutrients and overall CBD levels but the data is not statistically significant enough to say that this treatment performed better than the other 3 treatments.

Keywords: soil amendment, hemp, soil sample analysis, THC and CBD levels

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EFFECT OF SOIL AMENDMENTS ON HEMP PRODUCTION

Background

Before its production was regulated across the world, Hemp was a versatile crop used for a variety of products ranging from food (oilseed), paper, fuel to textile and canvas production. The earliest known paper sample in mankind's history was found in China and is made of hemp (Cherney, 2016). Over 100 different cannabinoids are identified in hemp along with CBD(CannaBiDiol). Hemp's popularity declined gradually as the same plant that is used for fiber extraction is used for marijuana and hashish production. This is due to the presence of THC's (TetraHydroCannabinol), the hallucinogenic substance in the plant. The crop was slowly banned during the 1930s in North America. The first blow was the Marijuana Tax Act of 1937 which didn't ban the crop altogether but levied additional taxes on commodities thereby indirectly putting pressure on farmers growing the crop (Malone, 2019). Slowly, by 1961 it was banned worldwide as a result of the Single Convention on Narcotic Drugs by the United Nations (Baxter, 2009). For over a century, the conflicted public policies pertaining to hemp are due to limited knowledge of the crop regardless of the political incentives.

Until a few decades ago, Hemp fields were a common sight in Kentucky when the state was once "Hemp capital of the world" before the crop slowly disappeared (Comer, 2017). Later, as the crop was regulated, the cropland under hemp shifted towards other profitable crops like tobacco, corn and soybean. At one point, Kentucky farmers produced the finest hemp seed in the world (Comer, 2017). With the gradual legalization of hemp in many states of the United States, the dynamics are changing, and hemp is coming back into the scene as one of the reliable and profitable crops. Now the winds of deregulation of hemp throughout the states are picking up due

to shift in legislative uncertainties, business frameworks, switching costs and benefits (Malone, 2019). The CBD extracted from hemp is slowly being used in treating various disorders like anxiety, depression, joint pains and also insomnia (Hilderbrand, 2018). The creation of a new code number for all Marijuana extracted chemicals by DEA (Drug Enforcement Authority) from January 3rd, 2017 has helped exploring new ways of using Hemp/Marijuana extracts in treating human or animal ailments. All these extracts are grouped under new code number (7350) for controlled substances (DEA, 2016). The agricultural processing industries of America can be interested in procuring the domestic product instead of importing it from other countries (Williams, 2015). This is an excellent opportunity for the domestic market to capitalize on the demand for the product. This gives a scope of developing the hemp processing and marketing industries as well (Comer, 2017).

Nitrogen Fixing is a natural process, but the excess use of chemical fertilizers is increasing the rate of Nitrogen fixed in soil. Fertilizer usage has been increasing in modern day agriculture and the cultivation is slowly turning towards fertilizer intensive crop production. In the US, the Midwest region contributes to more than half of ammonia emissions through fertilizer usage (EPA, National emissions inventory data & documentation, 2014). This indiscriminate use of fertilizers in agriculture will result in run off excessive nutrients like Nitrogen and Phosphorus. These nutrients will then build up in ground water, downstream ecosystems resulting in subsequent nutrient pollution (EPA, Nutrient Pollution, 2017). This nutrient pollution is not only limited to water ecosystems but also adversely affecting soil acidification and ozone depletion processes (Bashir, 2013). In order to balance any leaching or fixing loses of soil nutrients due to incognizant use of chemical fertilizers, organic amendments can be used as an alternative. These amendments can be used in moderation to regulate loses as well as replenish soil health.

Statement of the problem

The main concern about choosing hemp crop for research is that very little is known about growing hemp and there are no set agronomic practices like other major crops. Even a slight abiotic stress like flooding, drought, imbalanced nutrient supplements and heat can also cause the THC levels to spike up (George, 2019). As of now, there are no pre-designed cultivation practices to regulate the THC and CBD levels of Industrial Hemp. This initial stage of hemp research would be a best time to experiment on the hemp growth and design agronomic practices for growing as well as propagating hemp.

Purpose of the study

In order to achieve the final desired chemistry in the product, crop inputs like fertilizers and other plant protection chemicals can be used in combination with other cultural practices. The study is designed to answer the following questions based on the results:

1. What is the impact of this organic soil amendment on soil fertility and the available nutrients? Soil test is used to determine these levels.
2. How are the THC and CBD levels of hemp plants affected by this soil amendment?
3. Is it economically beneficial to use organic soil amendment as a source of soil nutrients?

Operational definitions

CBG - CannaBiGerol is the precursor of all the cannabinoids and this compound produces the primary cannabinoids like CBC, THC and CBD through biosynthetic pathways (Cherney, 2016).

CBD – Cannabidiol is the principal non-toxicant cannabinoid compound in Hemp used for therapeutic purposes. There are 2 alcohol groups in the chemical structure hence the name ‘Diol’. It is synthesized in the perigonal bracts of inflorescence (Cherney, 2016).

THC - Tetrahydrocannabinol. It’s the hallucinogenic cannabinoid compound that causes the “high” in the marijuana users (Place, 2019) It is concentrated in the resin heads of capitate stalked trichomes glands (Cherney, 2016).

CBN - CannaBiNol is produced when the THC in stored marijuana is degraded and is about 10% psychoactively potent as THC (Cherney, 2016).

ISO 17025 accreditation: It is the accreditation given to laboratories that can carry out tests, sampling and calibrations. These standards given to the laboratories are revised every 5 years (ISO, 2017).

Hyperaccumulator plant: It is a plant used for removing toxins from the soil like heavy metals by absorbing them (Rafiq, 2015).

N-nitrosamines: These are the compounds with a general structure of $N-N=O$ with 2 R groups ranging from a simple Hydrogen to complex chemical compounds and are formed when nitrites react with secondary or tertiary amines.

Assumptions

- a. The soil conditions are uniform all over the trial plot.
- b. The root growth is assumed to be equal for all plants. The soil amendment applied to each treatment plot is equally available to all the plants in the plot. These two assumptions are important as they will eliminate the calculation of each plant and each plot exclusively.

- c. There are minimum to zero leaching losses of soil amendment and all the product applied to the plot stays within it. This is important because it eliminates the calculations of leaching losses from each plot separately.

Most of these assumptions are backed up by some measures to lower the skewing they cause otherwise. For example, in order to reduce the differences caused in the result by assuming each plot has got the same amount of soil amendment, it is hand raked into the soil and the top layer of each plot has pretty much the same amount of treatment. To nearly nullify the leaching losses and the subsequent changes in the adjacent treatments, there are buffer zones between different treatments and around the entire research plot. Lastly, to minimize the error in soil testing as much as possible, soil samples can be collected from random sites on the plot. This will not only minimize the error but also gives a representative sample of the entire plot.

Limitations of the study

- a. This study is not repeated over years. So, data from only one year is made available to draw conclusions. This leaves a possibility that the other determining factors like weather, soil fertility and others might not be same in the consecutive years.
- b. Results from one soil type cannot be applied to other soil types as the available soil nutrients and OC matter are different for each soil type.
- c. There is no standard procedure to test the produce for cannabinoid percentages (THC and CBD levels) and most of the laboratories for controlled substance testing follow different procedures to test the hemp samples.

- d. Hemp requires certain soil conditions like good drainage for better growth. This makes it difficult to carry out multi location trials as the soils are not uniform all over the country and different tests are to be designed for each soil type.

Significance of the study

Given the recent changes in the agriculture scenario, Industrial Hemp can be considered as a novel crop in North America. Most of the crops have agronomical, economic, processing and/ or social issues that hinder their adaptability and in the case of hemp, it has all of these. In general, hemp has relatively higher requirements of the NPK fertilizers (Baxter, 2009). It is a row crop and hence there is going to be a risk of soil erosion and the subsequent depletion of soil nutrients from the top layer of soil. This study focuses on improving the soil fertility to suit the needs of hemp using organic means. Given the ambiguity on the phytochemicals to use on hemp along with the heavy rates of soil nutrients depletion, organic supplements seem to be an excellent substitute for the inorganic fertilizers. If the crop responds positively to this soil amendment, then it can be used to change the soils that were previously thought to be unsuitable for hemp cultivation.

Chapter 2

Literature review

The purpose of the study and the problems faced to complete the study were discussed in first chapter. There were previous research works done on soil amendments, improvement of soil fertility, regulating hemp growth and the chemistry in general. This chapter discusses the available literature on these topics discussed in the earlier chapter. This discussion helps to identify the scope for further research in the above-mentioned areas as well as provide the information of existing research work.

Importance of soil health

Soils play a key role in managing environmental changes and supply of food all over the world. This is the reason why FAO has recognized soils as a key issue for managing the deterioration of the environment (Blanco, 2019). When a soil is repeatedly used to raise crops on it, that results in draining of the nutrients from the soil without a way of giving back what we are taking from the soil. The changes in the soil might be of various physical, chemical and biological components like soil texture, pH, Ion Exchange Capacity or simply the essential nutrients being fixated in soil and being no longer available for plants. Apart from these primary losses, many secondary losses in form of soil erosion, soil degradation might occur rendering the soil completely useless for crop production any longer. The crops not only drain nutrients from the soil, they also absorb cations to the soil during ion exchange (Blanco, 2019). This results in acidification of soil. Moreover, uptake of the cation exchange can affect the Soil Organic Carbon (SOC) levels by controlling the way of biomass production and level of immobilization. This also has a secondary effect on sequestration of carbon. If the agriculture intensification continues it will result in the deterioration of arable soils. Hence, we need to adopt corrective measures to conserve soil health and fertility.

Previous research on fertilizer usage in crops

Fertilizers are a major part of the crop input. They are used for correcting deficiency of soil nutrients to the recommended amount, to make the nutrients available to plants and increased yields. There are 3 major methods of applications to add fertilizer for crop growth i.e. Broadcasting, fertilization at planting and Side-dressing. Out of the 3 methods, the most effective

would be a combination of fertilization during planting and side dressing after the plant grows (Balasubramanian, 2015)

Too much use of fertilizer on crops will increase the yield in the end but it has some permanent hindering effects on soil health as well. In the USA, particularly the Midwest region has higher percentage of ammonia emissions into the atmosphere from Crop fertilizer usage compared to the other parts (Balasubramanian, 2015). Using fertilizers more than the recommended level will build up heavy metals, Phthalate Acid Esters (PAE) apart from making the other essential elements fixed in the soil. Excessive Nitrogen fertilizer usage can cause soil acidification along with nutrient imbalance (Cherney, 2016). Also using excessive fertilization increases imbalance of nutrients in soil, lowers the nutrient use efficiency along with polluting the environment by volatilizing ammonia and denitrification processes (Cherney, 2016).

So far, the research on residue accumulation on hemp has shown the accumulation of pesticides like abamectin, chlorfenapyr, dichlorvos, metalaxyl and others in the leaf tissues. But there are also instances where the fertilizers have caused contamination in cannabis. In one of the research studies, excess Nitrogen fertilizer is given to the crop with limited potassium supplement and this resulted in crop loss due to stalk breakage (Baxter, 2009). However, some recent studies on the importance of Nitrogen alone as a fertilizer shows that the nutrient is not very effective in altering the THC levels. But these are the studies that were not replicated over a period and hence we do not have a comprehensive data yet (Place, 2019). Instances where the carcinogenic N-nitrosamines levels were high as a result of spraying liquid fertilizers were reported in *Cannabis* (McPartland and McKernan, 2017). To solve this problem, we need to resort to methods that eliminate these toxic traces and provide the essential nutrients and restore soil health i.e. organic compounds (Mehdizadeh, 2019).

Role of hemp in soil reclamation

Hemp is one of those versatile crops which can be targeted for phytoremediation. Its characteristics like shorter crop cycle (around 180 days), non-food crop (fiber hemp), and higher biomass makes it one of the best choices for Hyperaccumulator plants. The first popular instance of using Hemp as a Phyto accumulator was seen in the reclamation of Chernobyl in 1998 by the Ukraine's Institute of Bast Crops (Rafiq, 2015). The need to remove the excessive heavy metal from soil is high since the heavy metals also have certain secondary disadvantages like contaminating the food chain, interfering the crop growth (Chen, 2016). The problem with using hemp as an accumulator is that the hemp genome is not sequenced yet. Therefore, it is difficult to identify the genes involved in heavy metal tolerance and accumulation in hemp (Rafiq, 2015). Hemp especially fiber hemp can be used for bioremediation in croplands with heavy metal contamination particularly lead and cadmium contamination. But this can also be both pro and con for hemp since this heavy metal contamination is a disadvantage in CBD hemp (McPartland, 2017). The two most affected factors are pH and organic matter of the soil (Chen, 2016). Even if grown on the non-contaminated soils, about 42% of the plants' biomass returns to the soil which can be tilled into soil as the buds are the only economical part (Baxter, 2009).

Summary

From the above-mentioned sources, it is evident that the soil amendments used for fertilizing purposes not only improved the crop yield but also prevented the deteriorating effects on soil due to agriculture intensification. In the light of regulations on Hemp crop, the biological crop protection/ nutrient supplement methods appear as a better conventional option. Further,

hemp has been proven to be a soil reclamation crop in many heavy metal contaminated sites especially in cadmium contamination.

Chapter 3

Methodology

Methodology of the research contains the planning and execution of the research trial. It explains the research design used to carry out the field trial, instrumentation used to carry out the research, the validity of the instruments used, data collection and its consequent analysis.

Research Design

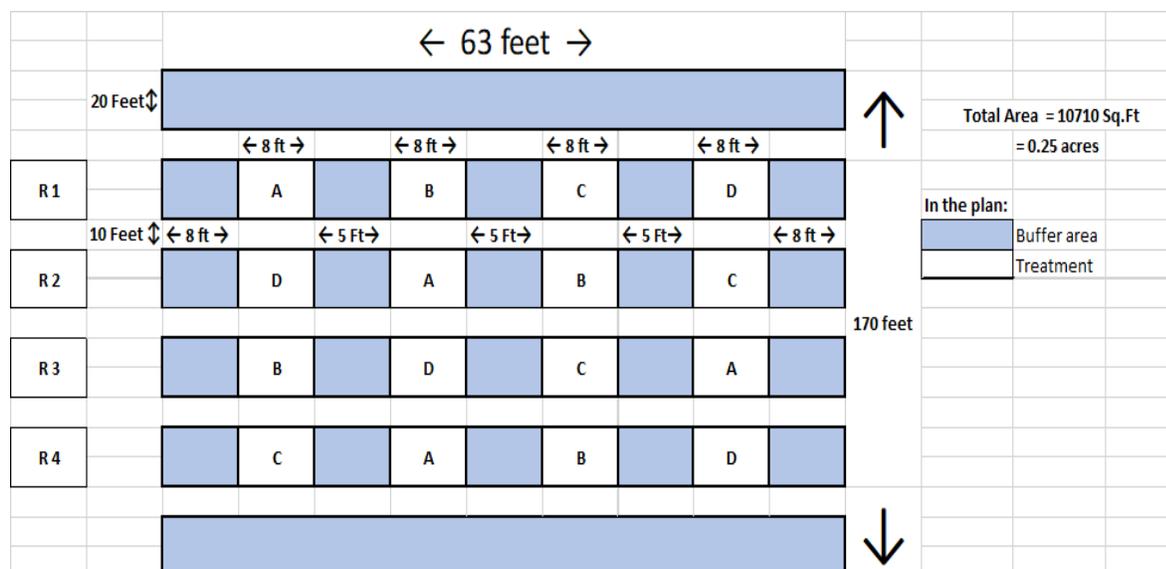
The trial plot was designed using a completely Randomized Block Design (RBD). This design helps in maintaining the randomization of treatments which is a key to get an average effect of the treatment throughout the replications (Suresh, 2011). The layout is a 4×4 plot with 4 treatments, each replicated 4 times with 16 treatments in total. The 4 treatments are a control (A) and 3 different dosages of soil amendment B, C, D at the rate of 14, 21 and 28 gallons per plot, respectively. The soil amendment can be either top dressed or incorporated into soil by light tilling. In this case, the amendment was first spread out and hand raked into the topsoil. The hemp plants were transplanted using a mechanical transplanter with a spacing of 45×45 inches.

The replications of treatments are arranged in such a way that no two adjacent treatments are the same. At the same time, all the sample sizes (treatment) are same. The key concept of having a randomized design model for the trial is to balance the size of sample across the trial along with balancing covariates (Suresh, 2011). A randomized trial layout ensures to prevent any selection bias and avoids the overestimation of the results from each treatment. This means there is equal

basis for all the treatments in the trial along with minimizing the negative effects of any confounding variables.

Figure 1

Layout of the research plot



Variables

In a plot size of 8×20 feet and 45×45 inches spacing, there were 11-12 plants in each plot. A buffer space of 5 feet is left between the two adjacent plots to prevent any seepage of soil amendment into the adjacent plot and thereby altering the data. Around the trial plot, a buffer zone of 20 feet is maintained to separate it from the adjacent trials. Randomization helps to prevent selection bias while sampling the data and minimizes any accidental bias. It ensures each treatment plot gets the same average amount of inputs. Maintaining equal number of plants in each treatment means minimizing any imbalance in the study by preventing negative impact on statistical analysis (Suresh, 2011).

The soil fertility level in each plot, the consequent effect on the levels of THC and CBD levels in plants and dry weight are going to be the variables in this research design. Through this design the effect of soil amendment on psychoactive compound (THC and CBD) content in hemp can be studied. Apart from the variables mentioned above, there are confounding variables that might affect the results. Confounding variables are the unaccountable variables in the experiment design that cannot be controlled but they influence the research results (Suresh, 2011). These variables cannot be eliminated altogether but their effect on the trial can be limited. They may be any external factors like biotic or abiotic stresses, pest and disease outbreaks etc. Measures are taken to ensure the internal validity of the experiment does not decrease. For example, by placing buffer zones around the plot and in between the treatments, we can minimize the changes caused by the seepage of soil amendment into adjacent plots. Maintaining 4 replications for each treatment means the error will be minimized and subsequently the average performance of the treatment is obtained in the end.

Sample selection

The plot area is 170×63 feet i.e. 0.25 acres. The spacing between the plants is 45×45 inches. The total plant population of the trial plot is 762 plants. Some of these plants are in the buffer zones along the trial plot and between adjacent treatments. Excluding the plants in buffer zones, the total number of plants used for the study are 192 plants. These are the total number of plants in the 16 treatments (each plot has 11-12 plants in it).

Out of the 192 plants, sampling is done twice for the analysis of plant tissue samples and once for weighing dry weight of the plants. The plant samples are collected twice during the growth period, one during the flowering of the plants and one at the end of growth period. This gives us

the changes in levels of THC and CBD in plants over a period. As for the soil samples, one pretest soil sample was collected before the application of soil amendment. After the growth period, four different soil samples were collected from 4 treatments, respectively. These samples are collected from each replication of the treatment resulting in a representative sample of the whole treatment.

Instrumentation

Randomized Block Design (RBD) is used to get the average of the effect of treatment on the plants. The plant samples are sent to the lab for plant tissue analysis and soil samples to the soil testing labs. The labs that undertake soil and plant tissue analysis are to be ISO 17025 accredited (Snyder, 2019). It is the accreditation given to laboratories that can carry out tests, sampling and calibrations. These standards given to the laboratories are revised every 5 years. Soil testing is done for the primary and secondary nutrients and for some important trace nutrients. Plant sample analysis is done for the THC and CBD levels. Dry weight of plants is measured by common scale.

Data Collection Procedure

Data collected can be divided into 2 major parts - soil samples and plant tissue samples. A representative soil sample was collected before starting the research trial and after concluding the trial. The plant tissue samples are collected after the plant starts flowering. These samples are collected from the top 20cms of the main shoot. This is the place of synthesis and storage of CBD and THC. The highest content of cannabinoids is found in the perigonal bracts. These envelop the pistils and seeds (Cherney, 2016). This is the reason we collect plant samples from the top part of shoot. Out of the 11-12 plants in each treatment, the plants at the center of the plot are selected to get the plant tissue sample.

Data Analysis

Samples are prepared before they are sent to their respective labs for analysis. In the case of soil samples, the sample is dried to remove excess moisture and placed in soil sample collection bags. It is then tested for the N, P, K, Ca, Mg and other essential microelements like Zn along with the pH, CEC levels in soil. The before and after results of soil sample analysis gives the difference caused in soil fertility by different soil amendment doses. In case of plant tissue, the sample is air dried in an oven at constant temperature (70-75-degree Celsius) for 2-3 days in loosely sealed paper bags. They are crushed after drying using mortar and pestle. All the larger stem bits are removed with sieves leaving only crushed inflorescence parts with flowers and their bracts . These samples are then tested for THC and CBD. The mean of these results from all replications of a respective treatment will give us the average effect of the treatment on the THC and CBD levels. By comparing the averages of the 4 different treatments, we can recommend the best dosage of soil amendment to regulate the CBD and THC levels with minimum residue. On the other hand, the soil test differences give us the effect of soil amendment on the soil nutrient levels.

Sampling and data collection problems

Testing CBD/THC levels throughout the growing period is important in the Hemp crop as the THC levels are to be below 0.3% all the time. For testing these plant material samples, the farmers need to send them to labs with ISO 17025 accredited labs alone (Snyder, 2019). One major constraint to consider while analyzing plant tissues is their stability. Some compounds may be lost due to volatility or degrade during the process of storage. In the case of *Cannabis sativa*, the freshly collected oil has monoterpenes, sesquiterpenes and a trace amounts of ketones and esters. There was a decrease in oil content after storing for one month and 3 months compared to freshly

extracted oil samples. There was no difference qualitatively with respect to major components of the oil (Samir A Rossi, 1996). This shows the importance of testing the samples for the oil content as soon as possible rather than storing them. This boils down to a point of availability of the ISO 17025 accredited labs and the speed with which they do the testing along with their efficiency. Soil sampling and testing is another key test in this research to assess the effect of a soil amendment on soil health. The sites of soil sampling might also affect the results i.e. if the site from where the soil is collected is not representative of the entire plot it might give accurate results. Hence proper caution must be employed while sampling the soil for testing.

Budget and Time Schedule

Budget

This research trial required manual labor for few operations and the rest was carried out mechanically. A mechanical transplanter was used to transplant the seedlings onto the field. Weeding was done in the initial weeks after transplanting. After few weeks, the canopy fully grew, and the weed growth was almost smothered. Other operations that required manual labor are application of soil amendment by raking, irrigation and inter cultivation.

Time Schedule

The plants were transplanted onto the field as 2-week-old seedlings on July 2, 2019 and the soil amendment “Rehab” was hand raked into topsoil on July 5, 2019. The trial plot had agronomical practices like inter cultivation, weeding, irrigation and sample collection done at regular intervals. Intercultivation was started from July 16, 2019. There was plenty of rainfall at the early stages of growth hence irrigation was not provided often to let the soil dry out a little. Collection of plant samples is done two times during early flowering and seed setting stage. Soil

samples were collected two times before and at the end of research trial. Entire plants were collected from each treatment to measure the dry weight of hemp at the end of study.

Chapter 4

Results

At the end of the study, results from samples and dry weight of plants were compared to assess the impact of the organic soil amendment “Rehab” on hemp growth and soil fertility status. The soil test results give us the soil fertility levels and the dried plant sample analysis gives us the amount of CBD and THC levels in the plant with respect to the treatment. These two results were then compared to identify if the soil amendment had any significant impact on the chemistry of hemp with respect to THC, CBD levels and soil fertility with respect to the available nutrient composition. The dry weight of plants gives us the increase in plant biomass.

Soil sample analysis

Table 1

Soil test results

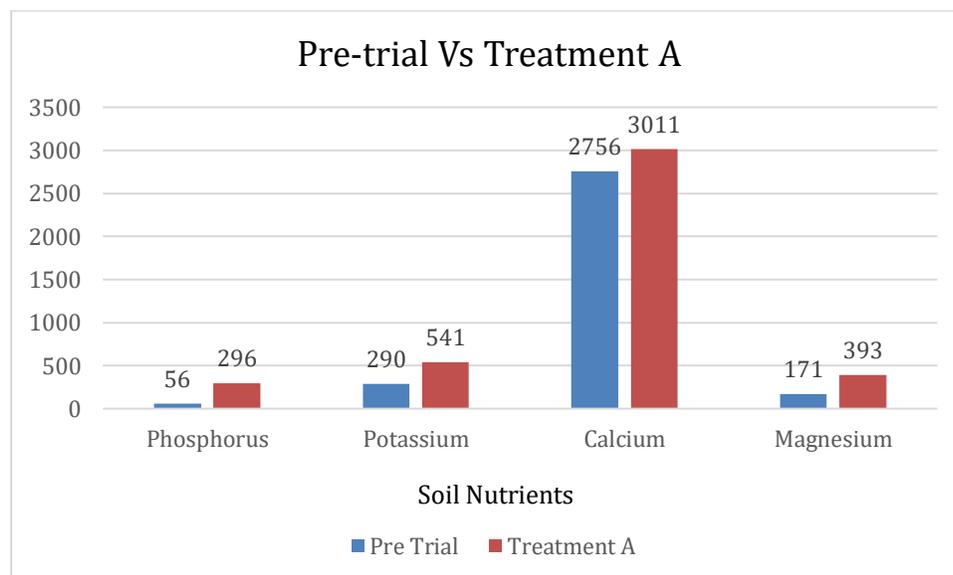
Parameter	Pre-Trial	Treatment A	Treatment B	Treatment C	Treatment D
Phosphorus	56	296	287	280	186
Potassium	290	541	651	541	411
Calcium	2756	3011	3036	3211	3731
Magnesium	171	393	403	392	333
Zinc	3.8	12	12.9	10.6	12.3
Soil pH	5.8	6.2	6.4	6.3	6.6
CEC (meq/100g)	10.8	14	15	16	15
% Base saturation					
Potassium	3.5	5	6	4	3
Magnesium	6.6	12	11	10	9
Calcium	63.9	54	51	50	61
Hydrogen	26	29	32	35	26

Note: Mehlich III was used to analyze the soil samples. Pre-trial sample was analyzed on 3/6/2019 and the samples from treatments were analyzed on 2/11/2020.

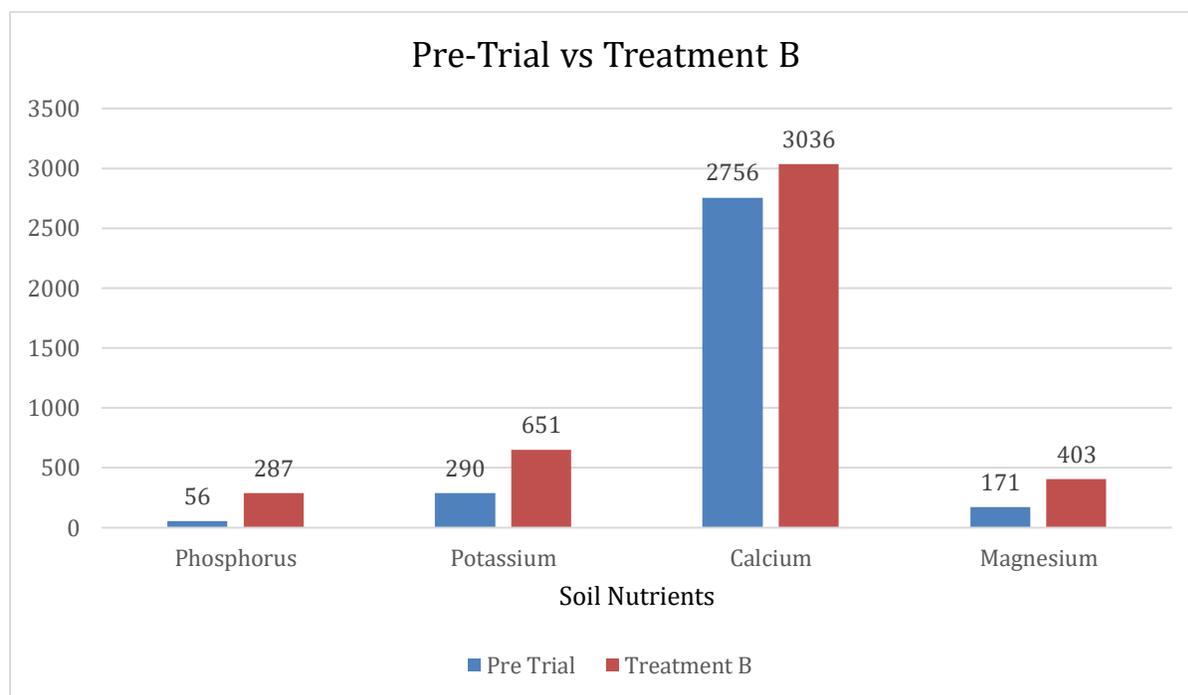
The pH level of the soil is below 7. Hence Mehlich III method is used for soil testing. The above soil sample analysis shows that there is no decrease in the amount of soil nutrient availability after the trial. Out of the three levels of Rehab, Treatment B showed slightly higher levels of Potassium and micronutrients like Magnesium and Zinc. Treatment D has slightly higher Calcium levels than the other 3 levels. When each treatment was compared individually to the pre-trial soil sample analysis, the following results were obtained.

Figure 2.1

Pre-trial sample vs. Treatment A sample



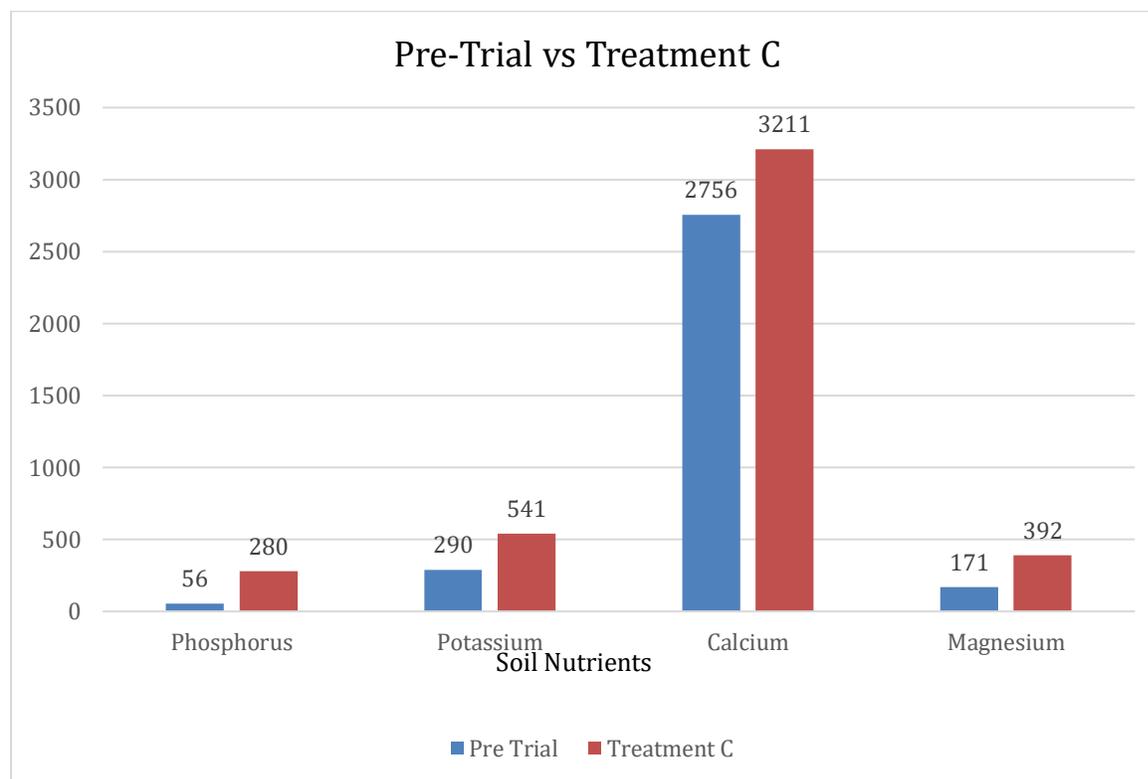
There is slight increase in all the primary (P,K) which are deemed “Very High” along with “High” levels of secondary (Ca, Mg) essential nutrients of growth.

Figure 2.2*Pre-Trial Sample Vs. Treatment B Sample*

Treatment B shows relatively higher levels of potassium levels and is the highest level of K tested in all the 4 samples. The results also show an increase in other 3 available soil nutrients (P, Ca, Mg) as well. However, the difference is not huge enough to say that it is statistically significant. The phosphorus and potassium levels are “Very High” whereas the magnesium is under “High” category.

Figure 2.3

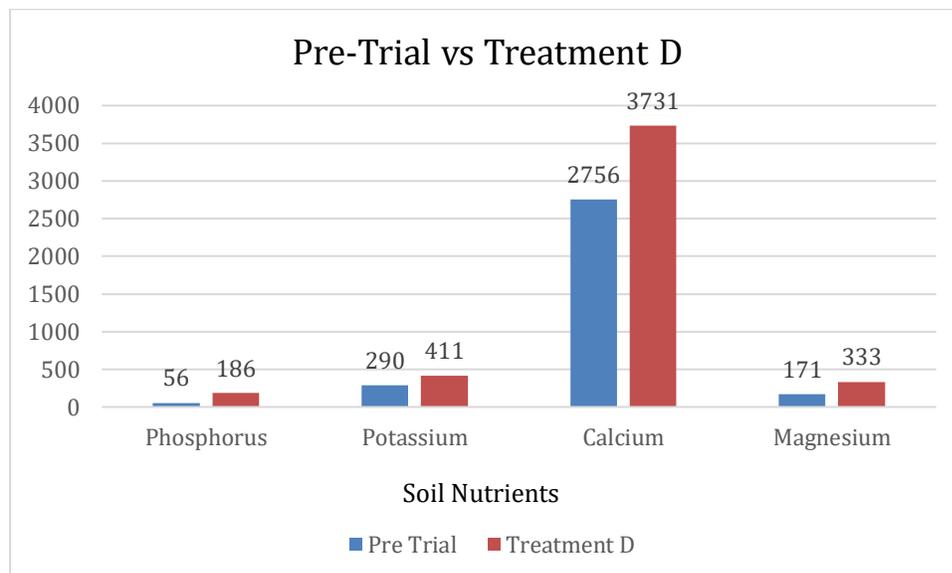
Pre-Trial Sample Vs. Treatment C Sample



The figure 2.3 shows an increase in all major soil nutrients like P,K, Ca and Mg. The phosphorus and potassium levels are “Very High” and magnesium level is “High”.

Figure 2.4

Pre-Trial Sample Vs. Treatment D Sample

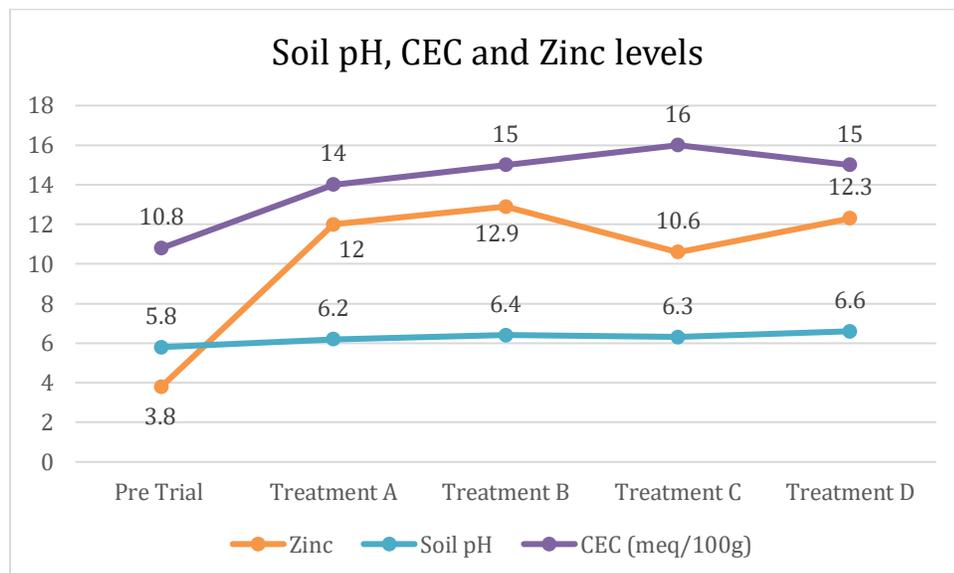


Treatment D is the highest level of soil amendment (28 gallons per plot) used. However, there is not much difference in the soil nutrient status in this treatment compared to others. The soil sample analysis shows “Very High” levels of Phosphorus and “High” levels of potassium and magnesium.

The soil sample analysis recommends a 50-150 lbs of Nitrogen for the sample tested. It is usually based on the type of crops i.e. 50 lbs for a fiber crop and 100-150 lbs for a grain crop.

Figure 3

Soil pH, Cation Exchange Capacity(CEC) and Zinc levels



The other soil attributes tested in the soil sample analysis were the pH levels of soil, cation exchange capacity and level of zinc in the soil. The pH levels and CEC (Cation Exchange Capacity) remained nearly constant across all the treatments.

The above charts show the comparison of Pre-Trial plots with respect to each treatment of soil amendment used. The nutrients levels tested include primary and secondary essential nutrients like Phosphorus, Potassium, Calcium and Magnesium along with some essential micronutrients like Zinc. As for the other major nutrients, Phosphorus is used for flower germination and thus the requirement increases steadily towards the end of the growth phase. Additionally, Phosphorus is important for efficient uptake of Nitrogen from soil. From the above results, Phosphorus and Potassium levels are “Very High*” in treatment A,B and C whereas Treatment D still has “High” levels of these 2 nutrients. The levels of Magnesium are “High*” in all four treatments.

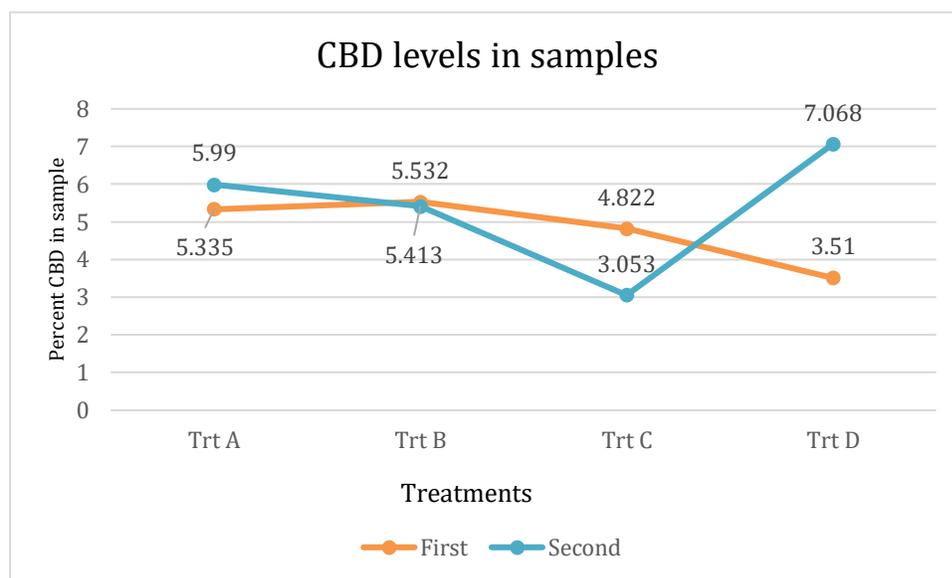
CBD and THC levels

Apart from the soil sample analysis, the dried plant samples were analyzed for CBD and THC levels in them. There are 2 samples collected during the growth period of hemp, one at end of vegetative growth phase when the flowering started (pre harvest sample) and the other at the end of seed formation stage (post-harvest sample). The CBD and THC in the samples collected are as follows

Table 2

CBD values

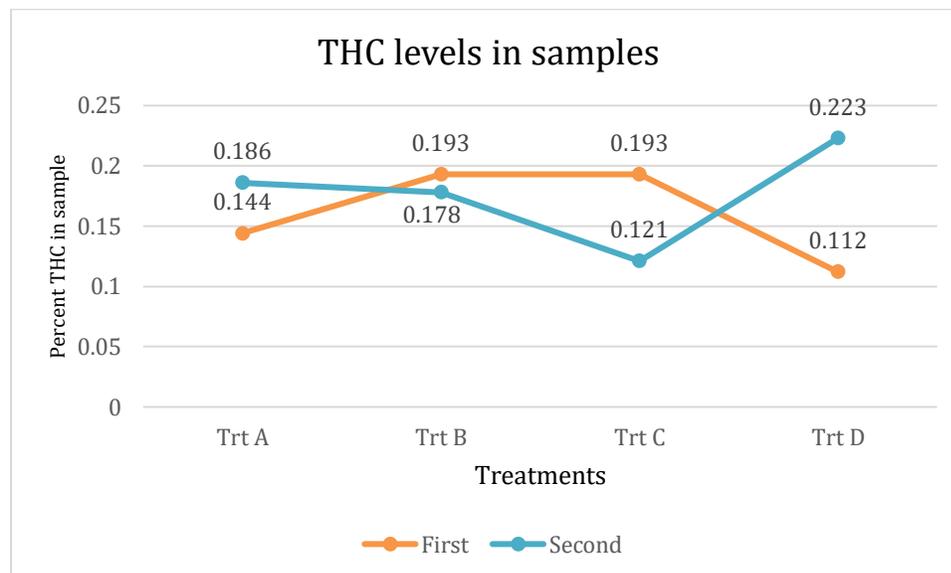
Sample collection Date	A	B	C	D
9/26/2019	5.335	5.532	4.822	3.51
12/12/2019	5.99	5.413	3.053	7.068

Figure 4*Levels of CBD in the samples*

The crop in the trial plot is a CBD variety and the table 2 has the individual CBD levels of each treatment. All the 4 treatments have CBD levels less than the commercially profitable levels (>10%). Treatments A and D saw an increase in CBD levels from first to second sample whereas treatments B and C saw a decrease in CBD levels after seed setting. However there seems to be no significant difference in the CBD levels among the treatments.

Table 3*THC values*

Sample collection Date	A	B	C	D
9/26/2019	0.144	0.193	0.193	0.112
12/12/2019	0.186	0.178	0.121	0.223

Figure 5*Levels of THC in the samples*

The pre harvest samples must have a <math><0.399\%</math> delta THC in order to be allowed into the market, only then the plants will be deemed as “Compliant variety”. The above data shows us that the THC levels are below the acceptable limits. The same trend of increase as in CBD levels is seen in treatments A and D i.e. there was an increase in THC levels from first to second sample. Whereas in treatments B and C, there was a decrease in THC levels from first to second sample.

Table 4*Plant Dry weight*

Replication	A	B	C	D
1	1.54	2.64	1.98	1.76
2	1.32	2.42	1.54	1.98
3	1.98	1.76	1.76	1.32
4	2.42	1.98	1.1	0.88
Average(lbs)	1.815	2.2	1.595	1.485

The average dry weight of each replication of treatment are as recorded in table 3. The averages of all treatments do not show much difference. Among the 4, Treatment B shows slightly higher dry weight closely followed by treatment A.

Chapter 5

Conclusions and Recommendations

The organic soil amendment “Rehab” was prepared using the remnants of animals like fish, chicken, and was designed to improve the soil nutrient content. There was a report of study conducted on sweet potatoes at Murray State University using Rehab in troughs. However, there was no field level study where several deciding factors act on establishing a healthy crop stand. Three different rates of “Rehab” were used against a control in this study to determine if any one of them would prove helpful regulating the levels of CBD and THC in hemp along with being beneficial to soil health and fertility. The conclusions from the study, results of plant tissue and soil sample analysis, and future recommendations for both researchers and growers are discussed in this chapter.

Conclusions

Soil Fertility and nutrient availability

For this study, two soil samples were collected. One was before the research plot was laid out which is the representative sample for the entire plot. The second sample was collected from each treatment in the research plot. The pH of the soil is slightly acidic or neutral in all the samples. This indicates that the addition of soil amendment did not turn the soils to alkaline pH. A pH of 6 - 7.5 is considered ideal for most of the commercial crops which means that use of this soil amendment helps in maintaining optimum soil pH for the following crops on the same piece of

land. This slightly acidic pH is important to prevent Zinc deficiency as high pH sometimes causes deficiency of essential nutrients like zinc. The available soil nutrients did not seem to be improved with addition of Rehab as there was no significant difference among the treatments.

THC and CBD levels

All the treatments had THC levels below permissible levels i.e. 0.3% (Agriculture, 2019). The CBD levels recorded are on the lower end of commercial CBD crop yield. While this might be due to one of the several constraints, Rehab did not improve the CBD levels in the hemp plants. Among the 4 pre harvest samples from the treatments, treatment B showed slightly higher CBD levels but when the samples collected at almost seed setting stage, treatment D recorded higher CBD.

Plant Dry Weight

All the treatments recorded nearly equal dry weight and when individual treatments were considered, treatment B had slightly higher dry weight compared to other 3 treatments. Since the plants are CBD variety, dry weight is not much emphasized. But in case of fiber hemp, this dry weight plays a crucial role in determining the yield from crop.

Future research

The role of hemp in soil reclamation by taking up heavy metals has a huge scope for future research. The amount of heavy metals absorbed by the hemp is to be established and the threshold levels of heavy metals in CBD Hemp should be identified. This gives us an opportunity to reclaim the arable soils contaminated with heavy metals while rotating regular crops with hemp on same land. Apart from the testing for CBD and THC levels, hemp needs to be tested for presence of microbial contaminants and heavy metals that might result in Eutrophication. This data is from a

single cropping season and cannot be considered as a representative performance of variety or the soil amendment. The study can be repeated over a period to determine if there is any soil nutrient buildup due to organic soil amendment added to soil.

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