WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 1530-1544. Research Article ISSN 2277– 7105

AN INVESTIGATION OF GROWTH AND YIELD OF *HELIANTHUS ANNUUS* ON DIESEL CONTAMINATED SOIL AND BIOREMEDIATED DIESEL CONTAMINATED SOIL

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Article Received on
12 October 2014,

Revised on 06 Nov 2014,
Accepted on 30 Nov 2014

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ABSTRACT

In this work, we investigated the effect of diesel contaminated soil and bioremediated diesel contaminated soil on the growth and yield of *Helianthus annuus* L. The diesel contaminated soil was amended separately with different ratio of poultry droppings (0.5, 1.0, 1.5, 2kg). Results show that diesel contamination in soil significantly affected the physical and chemical properties of soil and also retarded the growth of sunflower. Poultry manure amended with diesel contaminated soil was able to remedy the effect of the diesel pollution and enhance the growth and yield of sunflower. The remediation depends on the amount of poultry droppings used and maximum growth constraints were seen in 2kg of poultry waste amended with diesel contaminated soil. This work has revealed that soil contaminated by diesel may possibly contain adverse effects on growth and yield of sunflower, but this can be remediated by addition of natural nutrients particularly poultry waste which acts as a bioremediator and enhances the soil fertility. It has been suggested that bio remediated (poultry manure) diesel contaminated soil is suitable for crop cultivation.

KEYWORDS: Bioremediation, Diesel oil, Poultry droppings amendments and Sunflower.

INTRODUCTION

Diesel is a composite combination of aromatic and alkane compounds acquired from the gas-oil fraction through petroleum separation. [1] Contamination of soil by petroleum derivatives is a widespread ecological problem. Soil pollution by oil spills is frequently monitored in

metropolitan regions due to the progression and distribution of petroleum products through continuous consumption of diesel fuel by many vehicles and generators and also by the transportation through longer distance. [2,3] The petroleum products contain volatile and non-volatile compounds in which some gaseous compounds can easily volatilize and non-volatilize compounds remain as residues in soil. These compounds will affect the physical and chemical nature of soils. [4] Pollution with petroleum derived compounds can lead to water and oxygen shortage and also deficiency in nitrogen and phosphorous. [5]

Diesel fuel is not a complete killer but it eradicates plants cells on contact. Pollution by diesel fuel can destroy the roots and prevent the plant from uptake of water and other nutrients. It can also disrupt plant and water in soil. [6] Contamination of soil with petroleum hydrocarbons will adversely reduce the soil microorganisms, fertility of soil and growth of plants. [6] They also described that the accumulation of diesel oil in soil lead to continuous reduction in organic carbon substances in soil. [5]

Diesel oil is phytotoxic to plants even at low concentrations. The soil contaminated with diesel oil can cause decrease in length of the radicals of *Arachis radical, Sorghum bicolor, Zea mays* and *Vigna unguiculata*. [7] They also reported that the soil with contamination of petroleum derivative substance will significantly hinder the phases of seed germination, reduce percentage of germination, plant height, biomass and leaf production in *V. unguiculata*. Hence, there is a need to find out remediation methods to challenge the consequences of some of the petroleum products on plants. [8]

One of the best methods to renovate contaminated soil is bioremediation process. [9] Diesel contaminated soils are modifiable to bioremediation since microorganisms are competent in degrading petroleum hydrocarbons and the microbial biomass is generally considered to be important in bioremediation. [10] Bioremediation of diesel contaminated soil can be enhanced by stimulation of the indigenous microorganisms, by providing nutrients and oxygen into the soil (bio stimulation) [11] or through inoculation of an enhanced microbial consortium into the soil (bio augmentation). [12, 13]

Helianthus annuus L. (Sunflower) is a member of the Asteraceae family. It is grown in different climatic regions of the world. It is a very fast-growing industrial oil crop with a high biomass which can be used for uptake of toxic metals (Cu, Zn, Pb, Hg, As, Cd, Ni) from soil in heavily contaminated areas. [14]

The aim of the present study is to evaluate the effect of diesel contaminated soil and bioremediated diesel contaminated soil on the growth and yield of *Helianthus annuus* L.

MATERIALS AND METHODS

Collection of Poultry Droppings and Soil

The poultry droppings (feathers free) were obtained from a poultry farm located at Sulur, Coimbatore, Tamil Nadu, India. The red soil for the study was taken from the agricultural lands near Pollachi, Coimbatore, Tamil Nadu, India.

Experimental Design

The study was carried out with three replications at Karpagam University campus, Coimbatore (11˚16'N; 76˚58'E), Tamil Nadu. The agricultural soil taken for the study was contaminated synthetically by diesel oil. Different ratios of diesel contaminated soil and poultry droppings (w/w) have been used. Diesel contaminated soil and poultry droppings were mixed and put into separate tanks (1 m depths). Six different ratio of diesel contaminated soil and poultry dropping were maintained. Treatments were allowed to compost for 40 days at normal room temperature. The treated samples were collected for further analysis. The physicochemical properties of normal soil, diesel contaminated soil and poultry droppings are shown in Table 1.

Table 1: Physicochemical properties of normal soil, contaminated soil and poultry droppings.

S. No	Properties	Normal soil	Diesel contaminated soil	Poultry droppings
1	pH	6.8 ± 0.02	5.7 ± 0.01	6.9 ± 0.01
2	Electrical conductivity (dSm^{-1})	1.40 ± 0.45	2.56 ± 0.12	1.02 ± 0.15
3	Organic carbon (%)	38.1 ± 2.15	45.23 ± 1.16	35.26 ± 1.02
4	Nitrogen (%)	1.54 ± 0.05	2.78 ± 0.05	0.65 ± 0.01
5	Phosphorous (%)	0.77 ± 0.01	0.65 ± 0.02	0.32 ± 0.01
6	Potassium (%)	1.58 ± 0.01	1.34 ± 0.01	0.43 ± 0.01
7	C:N ratio	24.70 ± 2.13	69.55 ± 1.78	54.60 ± 1.59
8	Zinc (mg/kg)	92.78 ± 0.02	32.96 ± 0.02	10.20 ± 0.01
9	Copper (mg/kg)	2.56 ± 0.01	10.50 ± 0.06	2.10 ± 0.05
10	Manganese (mg/kg)	78.90 ± 2.56	62.00 ± 2.15	1.42 ± 0.01
11	Iron (mg/kg)	3547.9 ± 1.65	5028.02 ± 2.28	160.01 ± 2.25
12	Sodium (mg/kg)	28.75 ± 2.09	28.10 ± 0.84	30.23 ± 2.59
13	Calcium (mg/kg)	2.4 ± 0.18	10.20 ± 0.19	1.20 ± 0.10
14	Lead (mg/kg)	Nil	2.06 ± 0.08	1.00 ± 0.10
15	Cadmium (mg/kg)	Nil	0.10± 0.01	0.80 ± 0.10
16	Urease ($\mu g\ g^{-1}$)	0.50 ± 0.01	0.15 ± 0.02	0.10 ± 0.02

17	Alkaline phosphatase (µg g^{-1})	0.46 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
18	Acid phosphatase (µg g^{-1})	0.38 ± 0.005	0.10 ± 0.01	0.15 ± 0.01
19	Bacteria (10^{-6} CFU)	96	42	112
20	Fungi (10^{-5} CFU)	32	18	58

Effect of diesel polluted soils amended with various concentration of poultry manure on *Helianthus annuus* growth and yield

Collection of Seeds

Helianthus annuus (Co-4) seeds were obtained from Department of Oil Seeds, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, and India.

Experimental works

A preliminary pot experiment was conducted at Karpagam University Campus, Eachanari, Coimbatore, Tamil Nadu, India, during June to September 2014. Seeds were surface sterilized with 1% sodium hypochlorite solution for 10 min and rinsed with double distilled water. Different ratios of diesel contaminated soil and poultry droppings (w/w) have been used with normal soil as control. Ten seeds were sown in earth pots (30 cm diameter and 25 cm deep) in diesel contaminated soil with different dosage of poultry droppings and treatment details are presented in Table 2. The pots were kept under natural photo radiation. There were three replication pots for each treatment and the plants were harvested on 30, 60 and 90 (Day After Sowing) DAS. The soil moisture was maintained, which ranged from 4% to 8%. The biometric, quality and yield characters of test crops were carried out by using the standard procedures. [15]

Table 2: Treatment details.

Treatment	Treatment Details
T_1	9.5 kg of diesel contaminated soil + 0.5 kg of poultry droppings
T_2	9 kg of diesel contaminated soil + 1 kg of poultry droppings
T_3	8.5 kg of diesel contaminated soil + 1.5 kg of poultry droppings
T_4	8 kg of diesel contaminated soil + 2 kg of poultry droppings
T_5	10 kg of diesel contaminated soil
T_6	10 kg of poultry droppings

Determination of biochemical, quality and yield characters of *Helianthus annuus*

The different growth and yield parameters in sunflower like shoot length, root length, fresh weight, dry weight, head diameter, seed number and total seed yield were recorded at maturity (30, 60 and 90 DAS). The plants from each treatment were carefully uprooted, washed in running tap water to flush out the soil particles. The plant height, root length, shoot

length and head diameter measured (cm) using a meter rule. Chlorophyll contents were determined by standard procedure. [16] Total carbohydrates were determined as described by Hedge and Hofreiter. [17] and reducing sugars were determined by the method of Miller. [18] Proteins were determined by Lowry's method. [19] Oil from seed was extracted with petroleum ether (boiling point 40–60^{0}C) using soxhlet assembly for 6 h. The solid material from the soxhlet assembly was extracted twice with petroleum ether and residual weight was taken at the end. Oil was then calculated on weight basis. [20]

Statistical analys

The experiment was performed twice and One-way ANOVA was used to examine the significant variations among different treatments for studied constraints. Tukey's test was carried out to identify the identical type of the treatments for their different properties. All the data were analyzed using SPS 16.0 software. Probability levels used for statistical significance were $P < 0.05$ for all tests.

RESULTS AND DISCUSSION

Effect of poultry manure amendment on diesel contaminated soil on growth profile and seed yield of *Helianthus annuus*

Growth (shoot and root), fresh and dry weight of sunflower seedlings as influenced by the application of various doses of poultry manure amendment on diesel oil contaminated soil are presented (Figure 1a, 1b and 2a, 2b). The root and shoot length were maximum in T_4 treatment when compared to other treatments. On 30, 60, 90 DAS, the root length was high in T_4 (11.87, 21.17 and 39.37 cm). Among all the treatments, T_5 treatment had very short root formation (4.30, 8.70, 14.37 cm) on 30, 60 and 90 DAS respectively. The highest shoot formation (59.50, 84.17 and 120.70 cm) was seen in T_4 and lowest shoot formation (14.50, 48.93 and 69.43 cm) in T_5 on 30, 60 and 90 DAS.

On 30, 60 and 90 DAS, T_4 treatment showed maximum increase (64.63, 129.61 and 142.79 g) in fresh weight among all other treatments. The lowest values of 25.37 g on 30 DAS, 54.27 g on 60 DAS and 66.14 g on 90 DAS was observed in control. Dry weight on 30, 60 and 90 DAS was found to be highest in T_4 (22.53, 43.58 and 55.76 g) and lowest dry weight was registered in T_5 (7.92, 15.34 and 26.68). Maximum number of petals (35.33) were seen in T_4 and less number of petal formation (15.00) in T_5 treatments. Among all the treatments, the head diameter of sunflower was maximum (5.90 cm) in T_4 and minimum head diameter was observed (1.67 cm) in sunflower grown in diesel oil contaminated soil without adding poultry

manure (T_5 treatments) and are presented (figure3). It has been reported that poultry droppings treated diesel contaminated soil could induce the germination percentage, plant height, root length, leaf length, leaf width, moisture content and leaf numbers in *Delonix regia, Bauhinia sp* and *Cassia siamea*. [21]

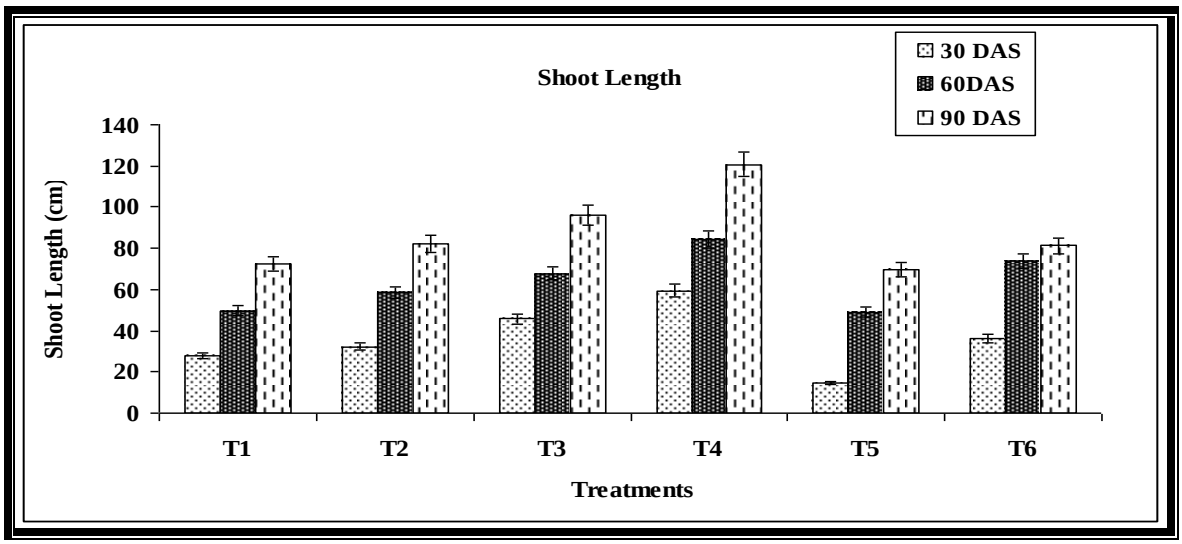

Figure 1a

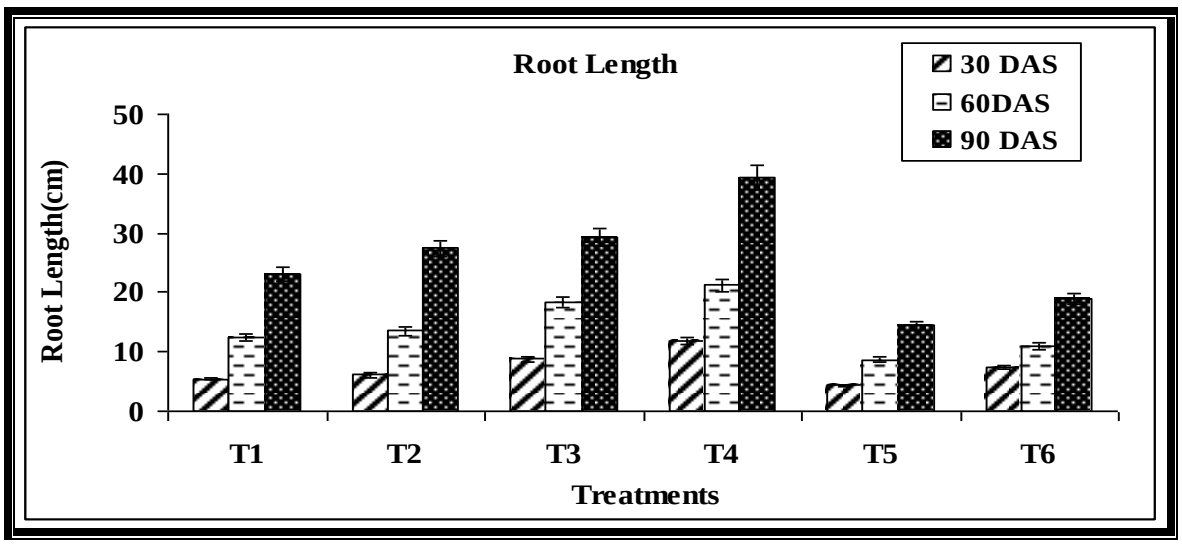

Figure 1 b

Figure 1 (a) shoot length and (b) root length in *Helianthus annuus* treated with different concentrations of poultry droppings amendment with diesel polluted soil. Data represent Mean ± SE.

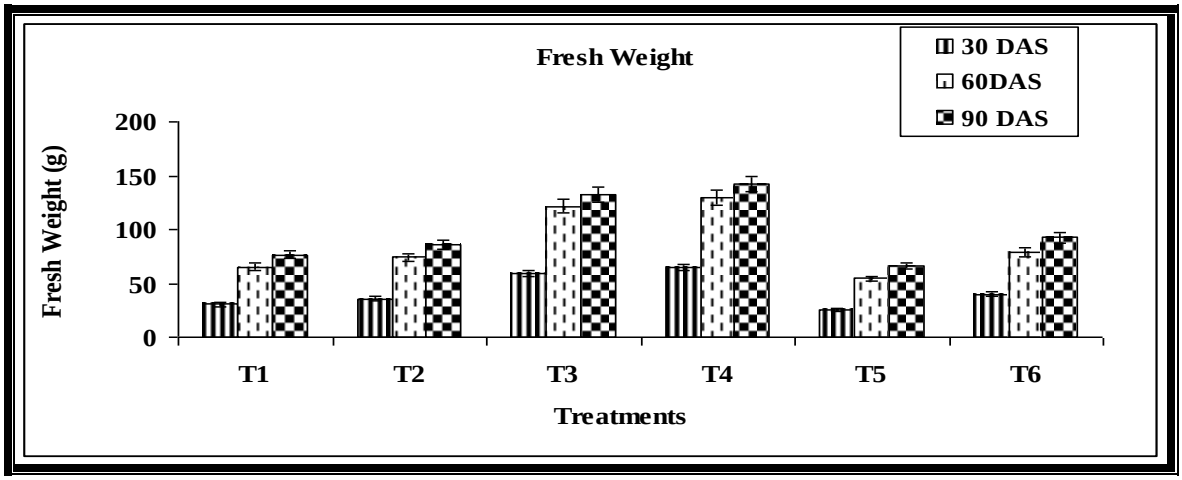

Figure 2 a

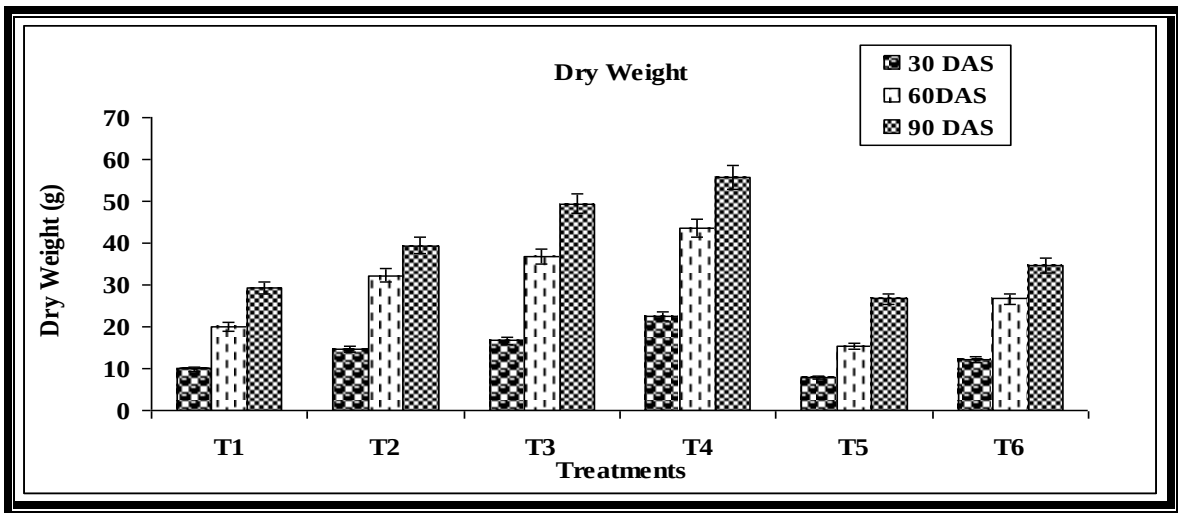

Figure 2 b

Figure 2 (a) fresh weight and (b) dry weight in *Helianthus annuus* treated with different concentrations of poultry droppings amendment with diesel polluted soil. Data represent Mean ± SE.

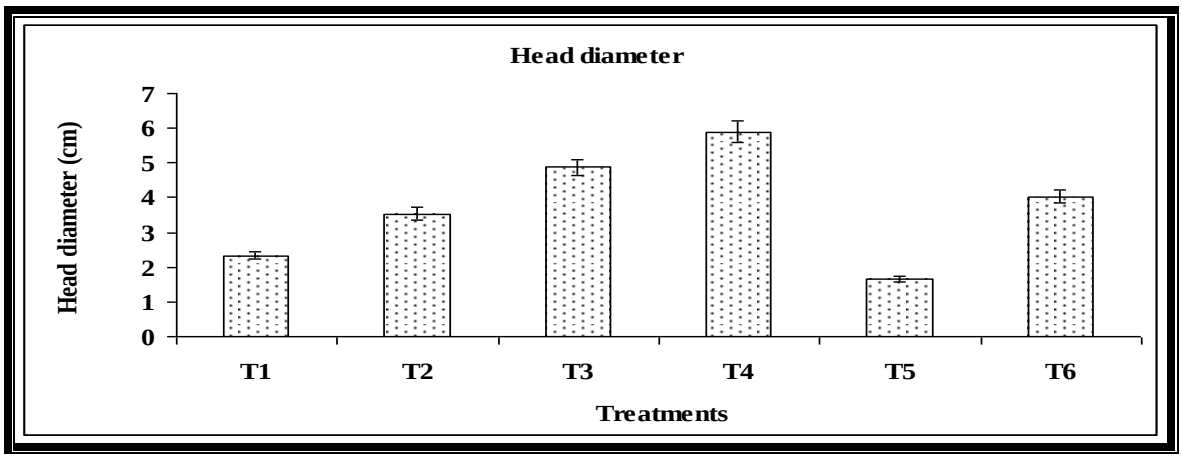

Figure 3 Head diameter in *Helianthus annuus* treated with different concentrations of poultry droppings amendment with diesel polluted soil. Data represent Mean ± SE.

Quality parameters for *Helianthus annuus*

Biochemical studies: The results on the effect of various doses of poultry manure under various diesel oil contaminated treatments on photosynthetic pigment (chlorophyll a, b and total chlorophyll) contents of sunflower are reported (Figure 4a, 4b and 4c). On 30, 60 and 60 DAS, the chlorophyll 'a' (0.20-0.27 mg g^{-1}), chlorophyll 'b'(0.15-0.21 mg g^{-1}), total chlorophyll content (0.39-0.46 mg g^{-1}) level were high in T_4 treatment. The lowest chlorophyll 'a' (0.07-0.13 mg g^{-1}), chlorophyll 'b' (0.05-0.11 mg g^{-1}), total chlorophyll (0.12-0.20 mg g^{-1}) levels were recorded in sunflower grown without amendment of poultry droppings in diesel contaminated soil. (T_5 treatment). It has been reported that diesel contaminated soil amendment with poultry manure could induce progressive increase in chlorophyll and protein contents in *Solanum melongena*. [22] They also reported that amendment of cow dung in crude oil contaminated soils enhances the growth, dry weight, chlorophyll content, leaf area and pod production of the *Glycine max*. [23] Increase in total chlorophyll contents leads to an increase in the total photosynthate produced. [24] The researcher also reported that chicken manure can improve photosynthesis and greatly improve the growth of Lettuce (*Lactuca sativa* L.) at a suitable concentration. [25] The highest leaf protein content on 30, 60 and 90 DAS was found to be in T_4 (4.87, 8.01 and 9.03 mg/g^{-1}) treatment among all the treatments and lowest protein level was obtained in T_5 (0.95, 1.51 and 2.23 mg/g^{-1}). On 30, 60 and 90 DAS, the total carbohydrate content was significantly high in T_4 (13.23, 15.26 and 17.92 mg/g^{-1}) and low level of carbohydrates was seen in T_5 (5.77, 8.48 and 10.36 mg/g^{-1}). The maximum level of reducing sugar was recorded in T_4 (9.5, 10.16 and 11.5mg/g^{-1}) and the lowest content of reducing sugar was observed in control (T_5 -5.95, 6.8 and 7.7 mg/g^{-1}) on 30, 45 and 90 DAS (Figure 5a,5b and 5c).

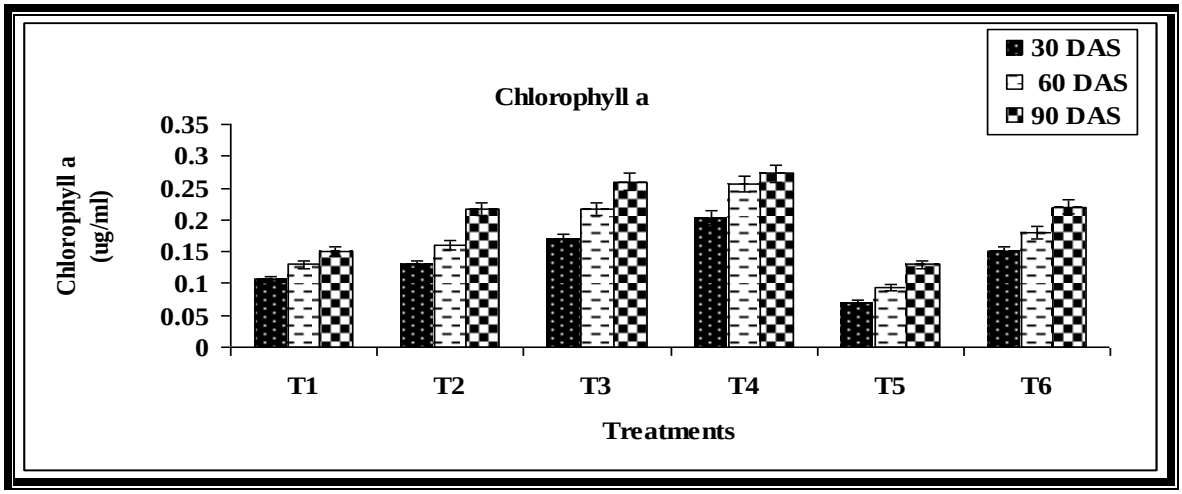

Figure 4a

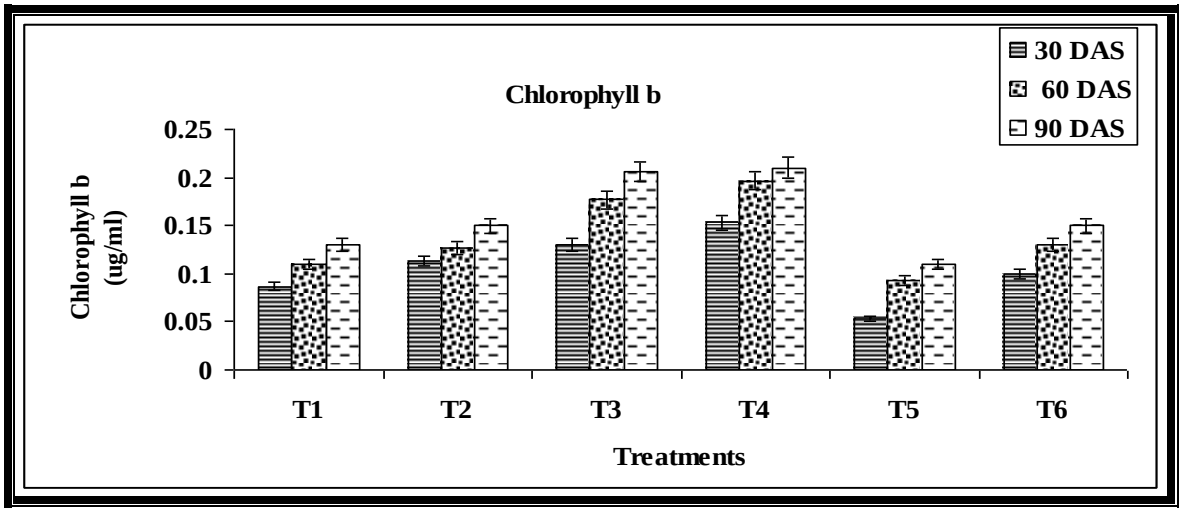

Figure 4b

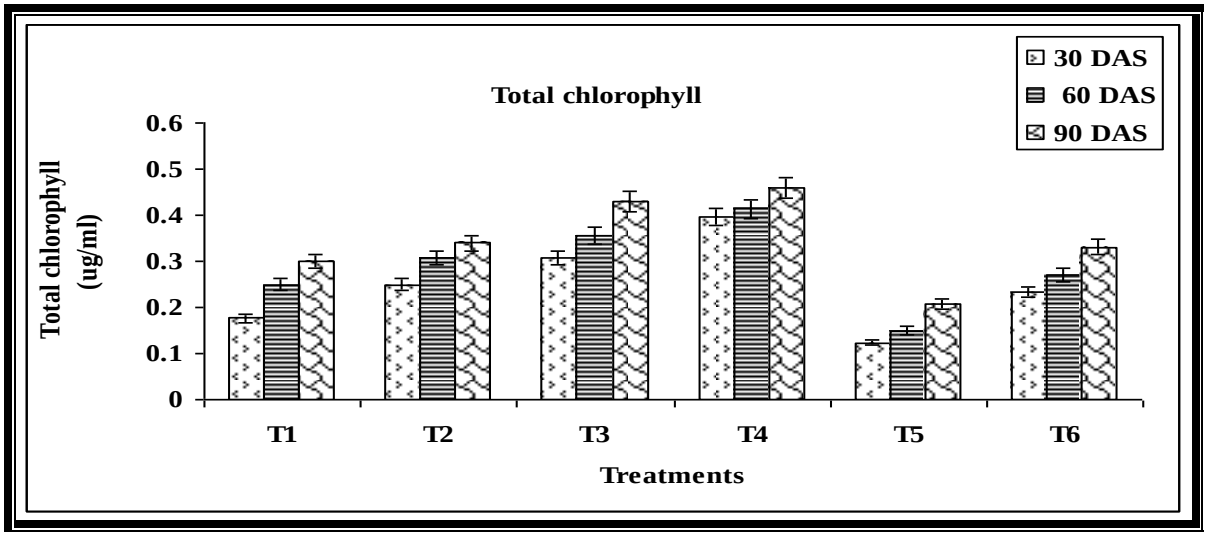

Figure 4c

Figure 4 (a) Chlorophyll a and (b) Chlorophyll b (c) Total Chlorophyll in *Helianthus annuus* treated with different concentrations of poultry droppings amendment with diesel polluted soil. Data represent Mean ± SE.

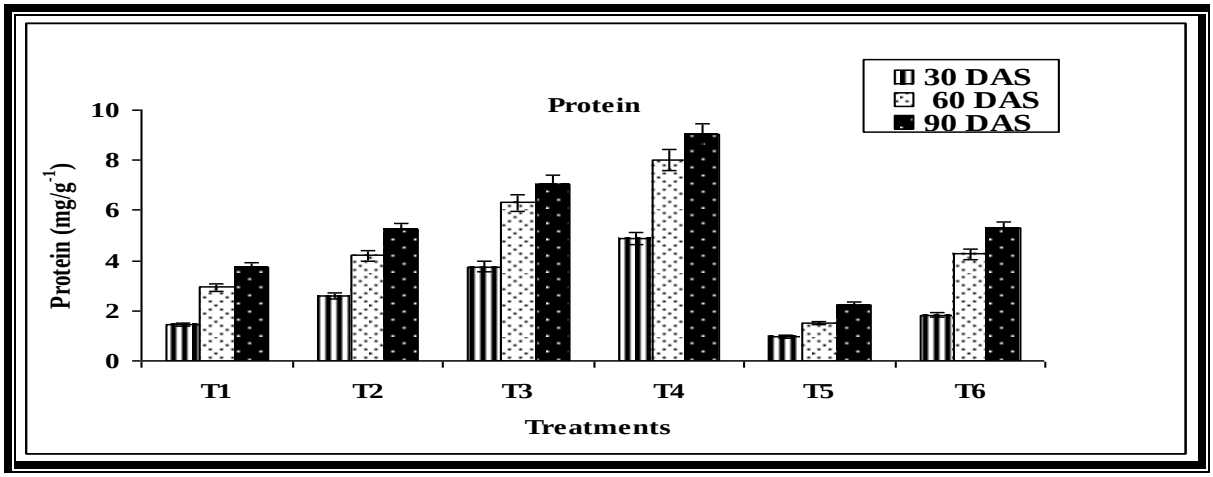

Figure 5a

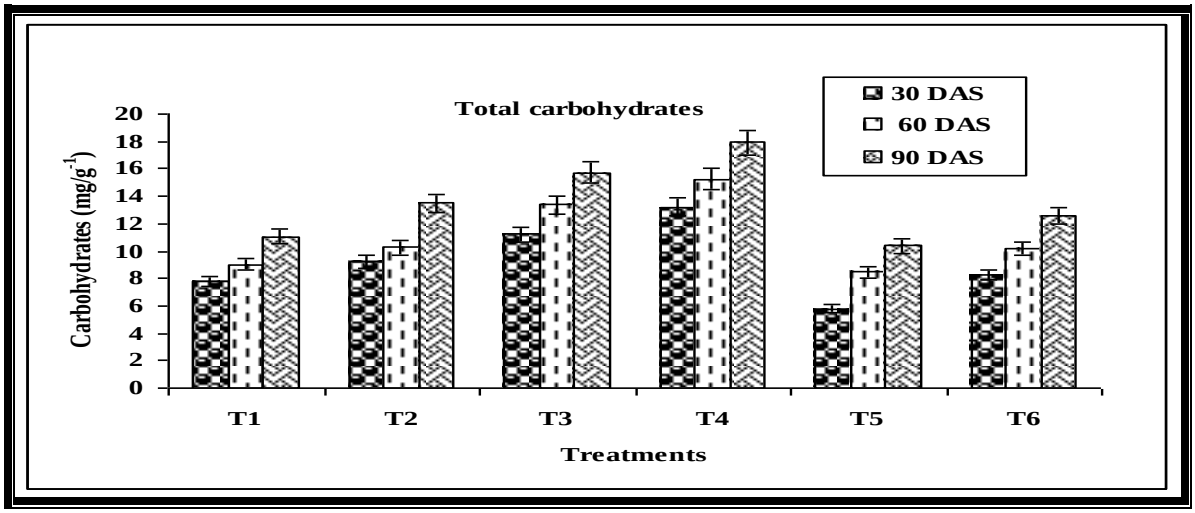

Figure 5b

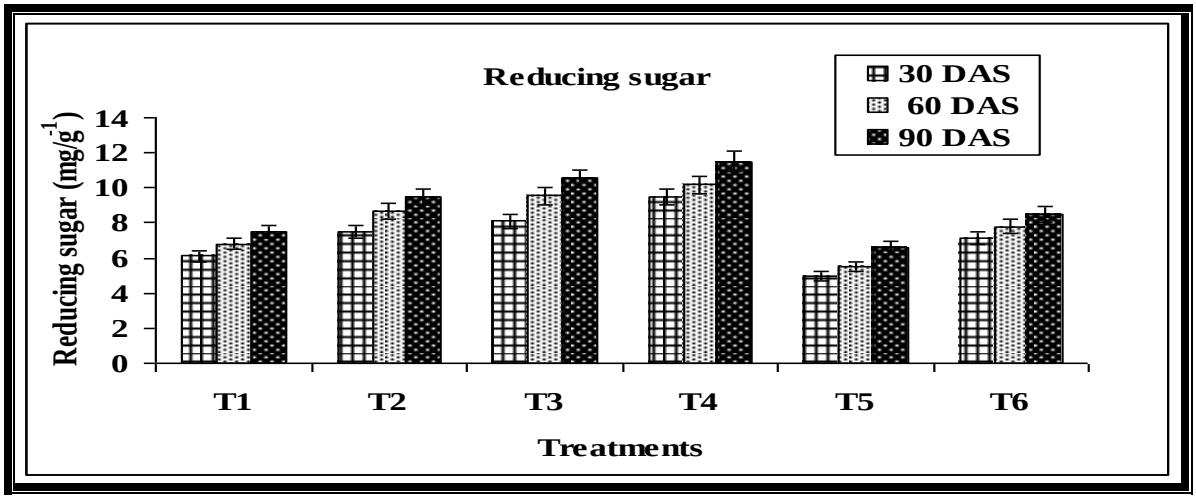

Figure 5c

Figure 5 (a) Protein and (b) Total Carbohydrate (c) Reducing Sugar in *Helianthus annuus* treated with different concentrations of poultry droppings amendment with diesel polluted soil. Data represent Mean ± SE.

Yield constituents for *Helianthus annuus*

It was revealed that diesel oil contaminated soil amendment with poultry manure significantly increased all yield parameters such as number of seeds per head, weight of seeds per head and weight of per seed per plant (Figure 6). All poultry manure treated sunflower significantly increased all yield parameters compared with control plants. T_4 treatment showed maximum (176) number of seeds per head among all other treatments. Control plants showed minimum (78) number of seeds per head. The weight of a seed was maximum (0.09 g) in T_4 treatment. T_5 treatment had only minimum (0.03 g) weight of a seed. The maximum weight of seeds per head was recorded in T4 treatment (9.47 g) and the lowest (4.20g) was

recorded in T_5 treatment (control) (Figure 7a and 7b). Treatment of *Zea mays L* seedlings with poultry manure brought about a significant increase in the number of grains per row, grain yield per row and number of rows per cob which also translated into an increase in average grain yield per plant. [26]Agyenim Boateng et al. [27] reported that grain yield of maize (*Zea mays*) was increased when treated with poultry manure. Chicken manure treatments showed highest yield and quality of lettuce. [25]

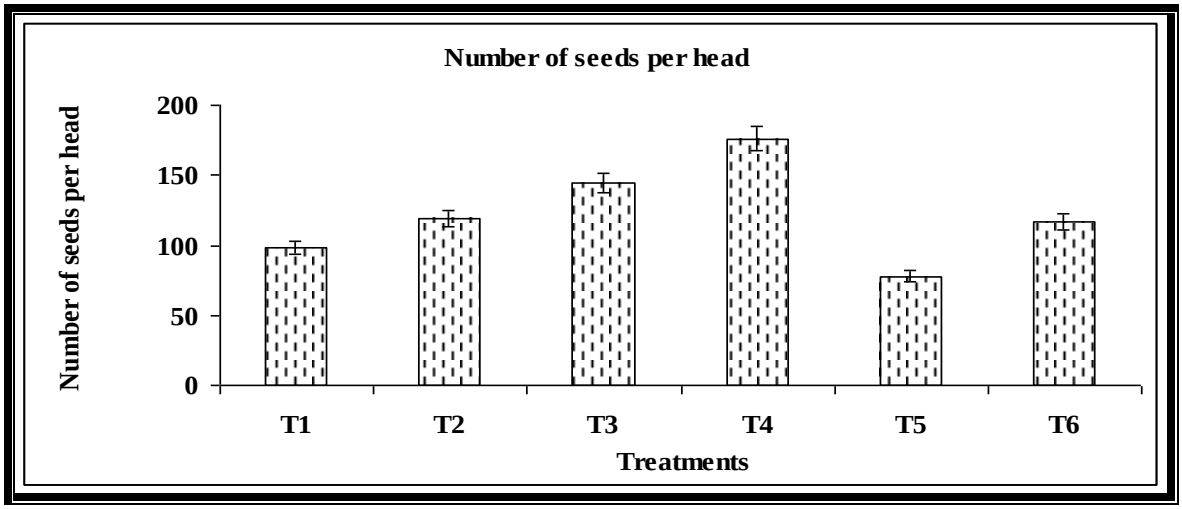

Figure 6

Figure 6 Number of seeds per head in *Helianthus annuus* treated with different concentrations of poultry droppings amendment with diesel oil polluted soil. Data represent Mean ± SE.

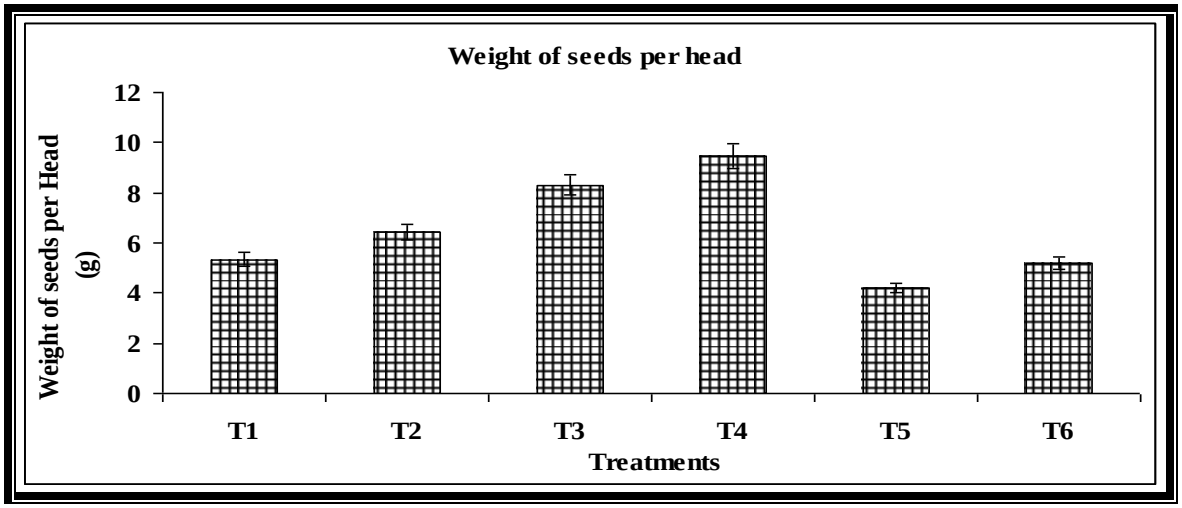

Figure 7a

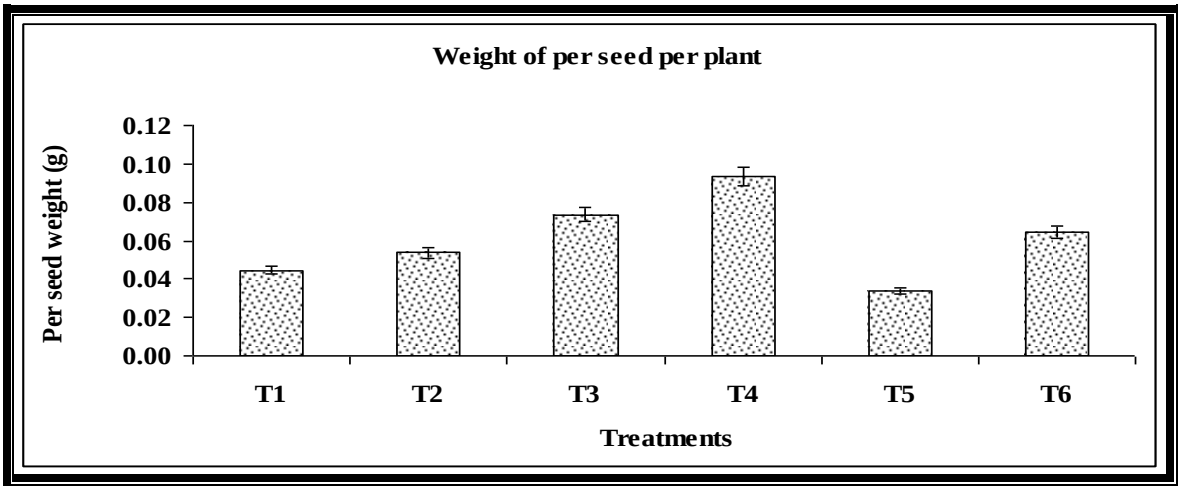

Figure 7b

Figure 7 (a) weight of seeds per head and (b) weight of per seed per plant in *Helianthus annuus* treated with different concentrations of poultry droppings amendment with diesel polluted soil. Data represent Mean ± SE.

Analysis of seeds

The diesel contaminated soil amended with poultry manure has effect on oil content of sunflower seeds are presented (Figure 8). Data indicate that diesel oil contaminated soil amendment with poultry manure treated sunflower have increased oil content. The oil content was high (36.66 %) in T_4 treatment. T_5 treatment had only low (18.67 %) amount of oil content. Treatment of *Delonix regia*, *Bauhinia sp* and *Cassia siamea* seedlings with 100g, 200g, 300g and 400g of poultry manure successfully improved their growth, leading to an increase in the net productivity in terms of seed and oil yield. [21]

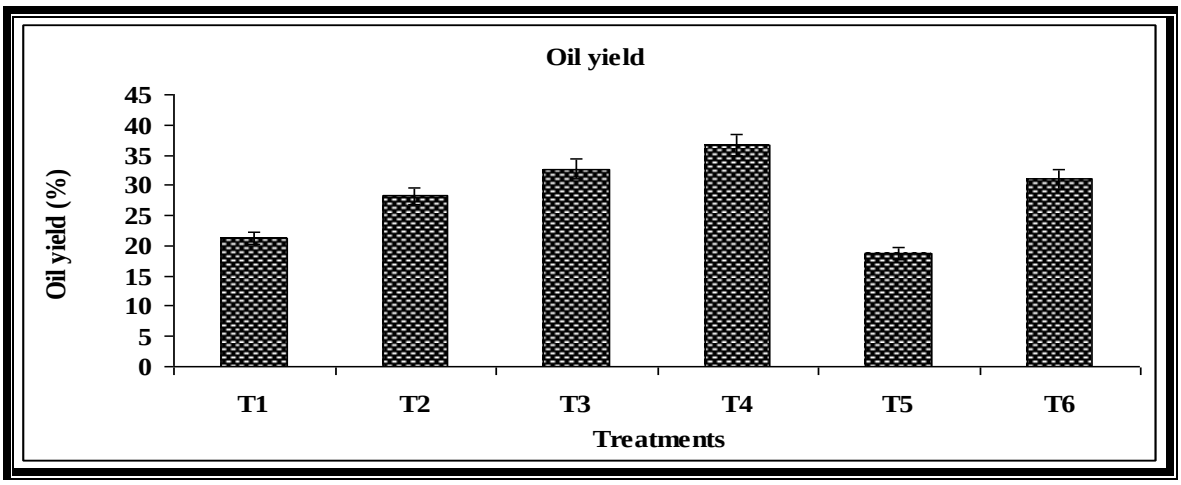

Figure 8

Figure 8 Oil yield in *Helianthus annuus* treated with different concentrations of poultry droppings amendment with diesel polluted soil. Data represent Mean ± SE.

SUMMARY AND CONCLUSION

This work was intended to conclude the possible benefits of applying poultry droppings on diesel polluted soil and assess its physiochemical properties. According to the results, it has been concluded that the poultry droppings on diesel polluted soil at 2Kg (T_4) concentration increased the growth attributes, quality parameters and yield components of *Helianthus annuus* than the control (T_5). When the concentration of poultry manure increased the growth and yield components of *Helianthus annuus* were increased. Thus, this experiment emphasized that the poultry droppings are effective bioremediation resources for diesel contaminated soil and at the same instance renovated the fertility of soil and also it enhanced the growth and yield of sunflower.

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