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# STUDY OF BIOLOGICAL REMEDIATION TECHNOLOGIES IN RELATION TO THE QUALITY AND QUANTITY OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) CROP ALONG WITH THE RESTORATION OF METAL CONTAMINATED SOIL

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**Abstract**

The discharge of heavy metals due to anthropogenic activities is the primary concern regarding soil pollution in the current advancement of the world. Therefore it is essential to design novel, cost-effective technologies for soil remediation. In the current paper, the restoration of Cadmium (Cd) metal-contaminated soil concerning the growth of the Sunflower (*Helianthus annuus* L.) is reported. For this purpose, two natural bio-stimulators, i) *Vesicular Arbuscular mycorrhizae* (VAM) fungi and ii) Tea waste (TW), were used as biological remediation technology to restore the Cd-contaminated soil. These stimulators are readily available and effectively check metal's movement from soil to roots; additionally, these are the natural sources and can be worked as natural fertilizers for plants growth. Ultimately, an organic crop free from pesticides, synthetic and hazardous chemicals can be obtained with equal yield in terms of quality and quantity. The experiment was conducted in natural habitat in three replicates for each remediator with control as standard plant. The soil pots were prepared, and laboratory-prepared Cd solution with different concentrations was added, followed by applying **a)** VAM and **b)** TW. The soil's physical properties, including, Soil pH, Electrical conductivity (EC) and Total Dissolved Solids (TDS) were monitored as health-associated properties of the soil. Results showed that the pH of the soil under different Cd (10ppm-50ppm) treatments increased by 13.8%, while in control plants (no Cd added), the treatment also increased the pH by 5.5%. The soil EC increased (13.3%) in Cd-contaminated soil, were found decreased in the presence of both remediators. Results revealed that the high-quality growth of Sunflower (*Helianthus annuus* L.), in the presence of both remediators, indicated the effective control of the metal movements from soil to roots. It was established that the Tea waste acts as a suitable adsorbent to adsorb the metal on its surface and to facilitate the movements of the other essential ions. Furthermore, VAM also traps the metal and increases the movement of essential ions; consequently, remarkable growth of the plants exposes the soil restoration to contamination. It was concluded that both of the remediators are readily accessible and can easily be used by the farmers as the best biofertilizers to control metal's destructive effects, followed by healthy beneficial crop.

**Key words:** Natural biostimulators, Growth, Enzymes, pH, EC.

## Introduction

The uses of biological entities like bacteria/ microorganisms or plant species to detoxify or control or remove organic and inorganic hazardous compounds from the soil is termed bioremediation. It is also called green technology to save the environment from harmful compounds. Bioremediation and phytoremediation are the most essential and appropriate ways to remove harmful matters (HMs) from the ecosystem Sunflower (*Helianthus annuus* L.) is usually considered one of the significant ornamental plants and a great source of vegetable oil. Sunflower is an excessive-yielding oil seed crop with the potential to close the gap between present edible oil use and local production. In addition, it is also a short-season crop (maximum 90-120 days) that can be cultivated twice a year. The yields of Sunflower genotypes are affected in unexpected ways in response to diverse natural surroundings. Various ecological variables must be assessed for the best-yielding, higher adaptive, and robust vegetation ranges (Lu'quez *et al*., 2002.).

Nowadays, agriculture faces a severe threat from the metallic waste discarded in the open atmosphere, which poses a challenge to crop production to feed a thick population while the amount of agricultural land relative to the increasing population is declining. Advanced and different soil protective and crop protective substances are in demand to increase the food supply for ever increasing population with a good amount of crop production. Herbicides, fungicides, or bio-engineered crops are in common practice. All these substances increase plant productivity as they are effective against weed growth, insect effects, and plant-based pathogens and diseases. The use of these substances is controversial, including claims that such substances are harmful to humans and the environment. In particular, genetically modified crops harm the entire general nourishment chain. Since metal stresses the electrical conductivity (EC) of the soil with lower pH, which puts pressure on average crop production, an improved crop-promoting substance that controls EC and restores pH is required. Plant expansion and soil concentrates should be maintained with sufficient yield for food production and industrial use.

Soil EC measures soil health and acts as an indicator to the soil's physical and chemical properties that impact plant growth. It is an important feature that the farmer can use for efficiently and inexpensively soil variability within a field efficiently and inexpensively. Soil EC and pH are essential factors of the soil related to nutrient mobility from soil to the roots of the plants; it is also a measure of the salinity of the soil and nutrients present in the soil. It also described the activity of the soil microorganism, which helps in the development of plants. Although EC does not directly measure specific ions or salt compounds, it is associated with concentrations of nitrates, phosphate, potassium, sodium, chloride, sulphate, and ammonia in non-saline soils. Determining EC can be a convenient and cost-effective way to estimate the amount of nitrogen (N) available for plant growth in certain non-saline soils. Minzan *et al.* [CN 102539921B]., (2014) described the method, which was simple, suitable, and fast with perfect accuracy of the electrical conductivity of the leaves tacked with the plants for real-time on-site to determine the physiological and biochemical index measurement for plant.

In most cases, the EC of soil measures the soil's ability to conduct electricity. The availability of nutrients in the soil is determined by EC, which is the most critical factor in fertility. The EC indicates the availability of nutrients in the soil. The higher the EC value, the more negatively charged sites (clay and organic particles) must be present in the soil, and the more cations (positively charged ions) (Sodium ($Na^+$), Ammonium ($NH_4^+$) must be retained in the soil. Salimi *et al*., 2012, discussed the effect of the electrical conductivity of irrigation water and compost on the uptake of Cadmium (Cd) and zinc (Zn) by Sunflower (*Helianthus annuus* L). The high EC values in the irrigation water negatively affected the biomass production of the plants. TDS and EC are associated with each other because EC evaluates soluble ions, but TDS is the function of soluble salts exclusively in such saline situations.

VAM Fungi are recognized for their long-term stay in the natural environment, where they are adapted (in terms of morphology, ecology, and metabolism) to the environment and are important for natural processes such as decomposition and nutrient cycling (Archana & Jaitly, 2005). Tea waste (TW), a type of agricultural waste, is produced in significant quantities yearly. TW, for example, has an insoluble cell wall made up of cellulose, lignin, tannin, and structural proteins with specific functional groups that can form physicochemical reactions with heavy metals and other contaminants, allowing it to be used to remove harmful substances from solutions and wastewaters (Yang *et al*., 2016).

However, there have been a few investigations on its application in soil remediation. Azmat and Akhter (2010) grew *Vigna radiata* under chromium stress with tea waste mixed with soil as a suitable adsorbent, which can protect plants from $Cr^{3+}$ phytotoxicity by modifying numerous metabolic processes (Azmat & Akhter, 2010).

The aims and objectives of the current research were the application of two natural stimulators tea waste (TW) and (VAM) to save *Helianthus annuus* L. crops irrigated with wastewater containing laboratory-prepared Cd metal. The impact of these remediators and Cd metal was monitored on soil health-associated properties like pH, EC, and TDS for the first time to understand the mechanism of the movements of the ions and metals. Bioremediation was also investigated for the growth of the plant *Helianthus annuus*.

## Materials and Methods

**Sample site and seed collection:** Initially, the hybrid seeds of sunflower (*Helianthus annuus* L.) HYSUN 39 was obtained from a local market. For convenient use, seeds were washed 3-times with normal tap water and soaked in water at room temperature overnight prior to planting. Earthen clay pots of around 5 L (22.5 x 16.5 x 18.0 $cm^3$) were prepared for seed sowing, then one-month-old plants were shifted to the prepared field area at the University of Karachi Latitude: 24.9400 Longitude: 67.1200; (Fig. 1) during the sunflower crop season in January 2021 (seed sown date: 21 January 2021). A watering nozzle was used to irrigate twice a week until the top 6 inches of soil was moist.

**Preparation Cd metal wastewater:** Laboratory-prepared wastewater containing Cadmium of different concentrations (10 to 50 ppm) was obtained from Cadmium Chloride salt AnalaR grade, which was used as toxic metal wastewater in soil.

**Collection of tea waste:** Used tea packets were collected from the local canteens of the University of Karachi, Pakistan and dried in the open air for a whole day (Fig. 2).

**Isolation of VAM fungi spores from the soil:** 100 gm of *Ixora* sp. soil was mixed with 400 mL water in a 1000 mL beaker mixed well with a spatula to form a suspension and then allowed the heavier particles to settle down. The resulting suspension was sieved at 710 µm to remove large organic matter and roots. The filtrate was further sieved through a series of 250 µm, 75 µm and 45 µm sieves. The residue on the 45 µm sieve was washed well in a 1000 mL beaker with additional water and the spores were collected. The collected spores were identified as *Archaeospora undulata* (Family, *Archaeosporacea*) using a digital light microscope OPTIKA B-290TB (Fig. 3).

**Soil snalysis:** Soil pH, Electrical conductivity (EC) and Total Dissolved Solids (TDS) were measured before and after the application of both remediators onto the soil using pH meter (ADWA AD 111) and TDS, EC meter (ADWA AD 330) respectively. Soil samples were air-dried and sieved using stainless steel 100mm mesh sieve. Initially, a 20 gm soil sample was added into 50 mL deionized water and kept for 1 day for TDS, Electrical conductivity, and pH measurements (Zdolec *et al*., 2008).

**Atomic absorption spectroscopy (AAS) of soil:** 65% Nitric Acid 1M solutions prepared from Nitric Acid AnalaR specific gravity 1.42 were used for metal analysis on AAS. Samples were prepared using 8gm soil in 100 mL 65% Nitric acid solution and kept for shaking upto 4 hours on a shaker. Using a Varian atomic absorption spectrophotometer Thermo Scientific™ iCE™ 3000 Series AAS with deuterium background correction, the extracts were filtered, and the final extractions were tested for lead, Cadmium, zinc, copper and nickel. Triplicates of each sample were made.

**Seed sowing:** At the initial stage, the soil was prepared using two biological wastes **a)** Tea waste (TW), **b)** VAM to study remediation of the harmful effects of Cadmium. Primarily, 1000gm soil was mixed in pots with 4gm air-dried tea waste (treatment #1), 30 mL VAM (treatment #2) and with a combination of Tea waste and VAM (treatment #3) respectively. All three treatments and control soil was also treated with different concentrations of Cd (30mL of each 10ppm, 20ppm, 30ppm, 40ppm & 50ppm Cd) and were added separately in all pots.

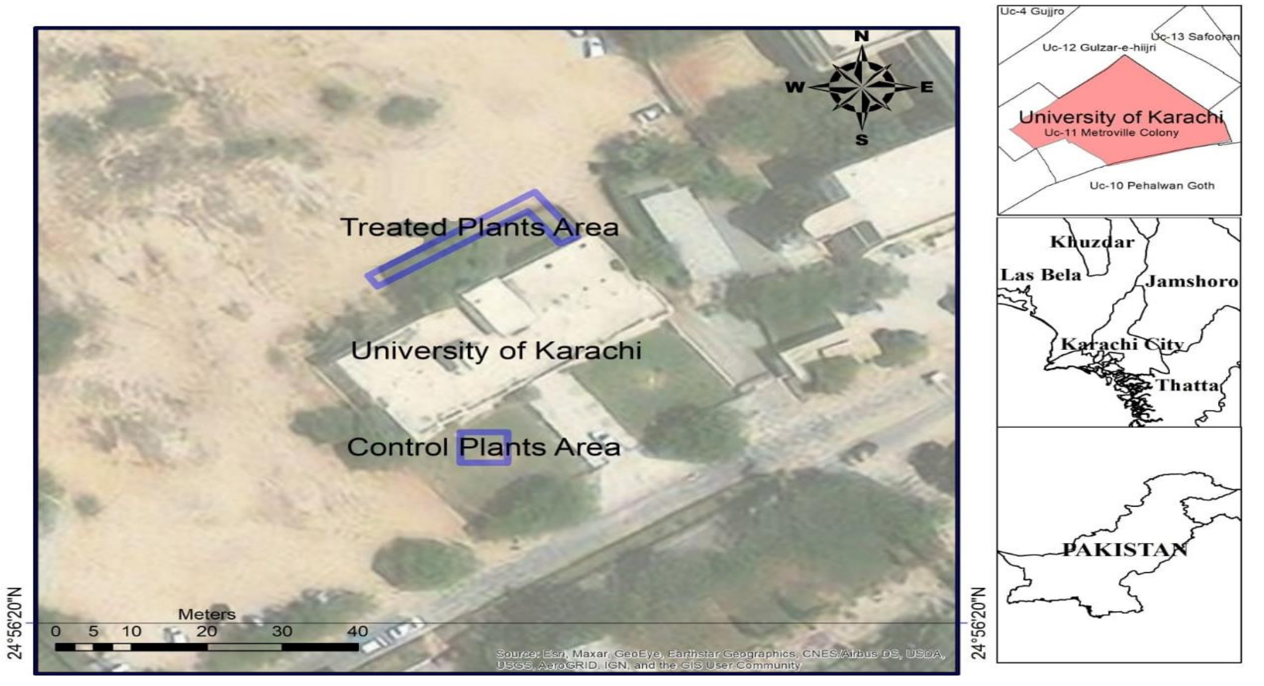



Fig. 1. GPS location for field area.

Fig. 2. Dried Tea waste collected from local canteens of University of Karachi.

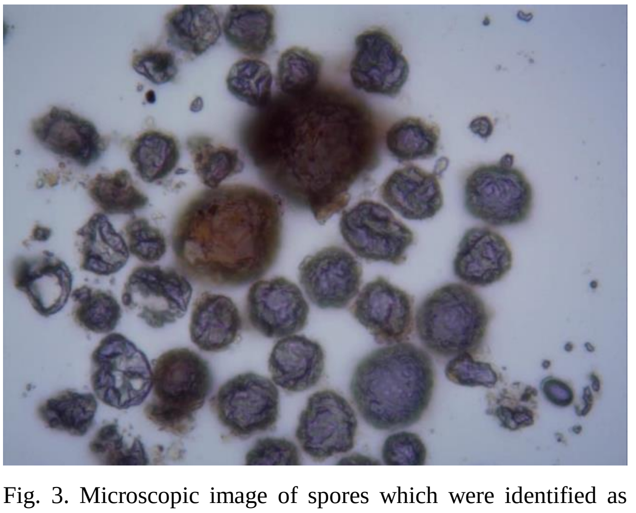


Fig. 3. Microscopic image of spores which were identified as specie *Archaeospora undulate (*Family, *Archaeosporacea)* with the help of the expert Botanist of University of Karachi by using digital light microscope OPTIKA B-290TB.

Fig. 4. Sunflower plants growth at fixed distance to each other in the field area of University of Karachi.

Pots were watered once a day. After seed germination of 20 days, plants were shifted to the field area having the same soil with plant to plant distance of 30 cm (Fig. 4). All treatments were applied in the same ratio to the field area after shifting plants on a large scale.

After almost 03 months of completion of the Sunflower crop (harvest date: 10 May 2021), analysis of field soil was repeated and several physical parameters of the plant were measured which included; stem length, stem width, capitulum width, receptacle/stamen width, ray florets size (petal), fresh capitulum weight, dry capitulum weight, seed weight and leaf size/area of three different measurements (Fig. 5).

**Statistical analysis**

Experiments were conducted in three replicates. All observations of treatments were statistically analyzed by using analysis of variance (ANOVA) with a significance value of <0.05. Further variables are studied for their Pearson correlation values. Values, higher than 0.50, are supposed to correlate with the data points (Table 1). The positive values showed a positive correlation among variables, while negative values showed a negative correlation. Values closer to 0 indicated poor negative or positive correlation. However, values nearer to 1 showed a significant correlation. All statistical analyses were performed in the Microsoft Excel program 2007.

## Results

The presence of metal and organic contents influences the soil's physical properties related to the mobility and pathways of nutrients and pollutants. pH, EC and TDS are considered soil health physical properties usually affected by metals. Therefore it is essential to assess the parameters in the presence of the metals and their remediators for sustainable agriculture. It was observed that the plants under remediation in the presence of the metal showed significant growth, which is discussed as follows.

**pH, EC and TDS of the soil under contamination and remediation:** pH is the significant parameter related to the mobility of ions in the soil and also determine the toxicity level of hazardous element in the soil, while pH variations depend upon the decomposition of the plant's carbon by a microorganism.

pH analysis showed that pH under different Cd concentrations (10ppm-50ppm) was increased 13.8% (7.2 to 7.86), showing that soil properties changed; while increased 5.5% in the soil in the absence of Cd (control soil) which was in accordance with earlier reports (Tariq *et al*., 2016), who observed pH 7.6–8.5, is reliable to exhibit higher accessibility of essential metals ions like Mg, Ca, and K, as observed in current research. Furthermore, some micronutrient metals (Fe, Zn, and Cu) are less available to the plants at these pH (Anon., 1998). Furthermore, in the presence of both remediators pH recovered from 7.2 to 7.6 showing 5.5% increases. However, it is observed from (Fig. 6) that the treatment gradually decreased the pH of the soil of the corresponding positive control plant with a % decrease of 2.6, 2.5, 2.6, 4.1 and 4.1 in 10, 20, 30, 40, and 50 ppm Cd contaminated soil respectively. This impact of pH recovery was further observed on plant growth, where healthy growth was related to soil health-associated properties. The increase in pH at higher concentrations inhibits the conversion of toxic metal into soluble ions, which is in accordance with the reported work, where it is mentioned that it decreases the availability of metals in the soil (Sintorin *et al*., 2021). Similarly in another study, the increased pH showed the defensive mechanism for the survival of the plants (Zhang *et al*., 2018).The correlation analysis of pH with other variables showed no significant association with the studied attributes.

EC, another soil health property related to ionic movement, is presented in (Fig. 7). The soil's electrical conductivity showed the soil's ionic strength, which showed a 13.3% increase, whereas EC was 357 to 412 in control and Cd-treated plants respectively, which has been remediated after Tea waste treatment and VAM treatment or the combination of both. It can be related to the presence of organic waste and VAM fungi or a combination of both. It has been stated that the increase of organic content in soil can ameliorate the immobilization of potentially toxic elements by converting them into insoluble forms (Shahkolaie *et al*., 2019). The remarkable growth of the plants also reflected the soil remediation through both stimulators on EC. The negative correlation of EC was observed with all physicomorphological properties of the plants, highest with seed and capitulum weight of plants.

The total dissolved solids (TDS) of the soil of control plants were considerably affected when Cd contamination was infused. It decreased by 72.5, 71.7, 68.5, 67.7 and 64.6% in 10, 20, 30, 40 and 50 ppm Cd-contaminated soil respectively (Fig. 8). The results indicated that TDS in TW treatment was significantly remediated compared to the other two treatment options (p-value 0.016). This may be related to the Tea waste's dissolved inorganic and organic compounds as these ions move in the direction of the water; therefore, excellent growth of the plants was observed. Only the ray floret size of a capitulum was positively correlated with the TDS of soil.

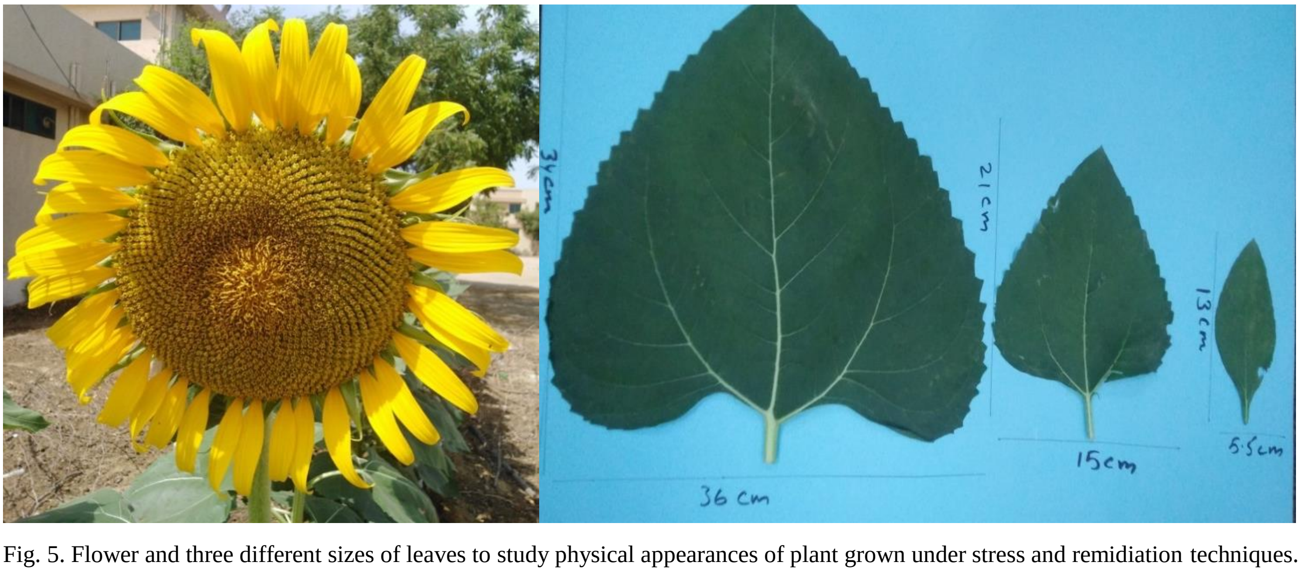



Fig. 5. Flower and three different sizes of leaves to study physical appearances of plant grown under stress and remidiation techniques.

**Table 1. Pearson correlation matrix for all variables.**

| | **Flower width in cm** | **Receptacle/stamen width in cm** | **Ray florets (Petal size) in cm** | **Fresh flower weight in g** | **Dry flower weight in g** | **Stem length in m** | **Stem width in cm** | **Seed weight in g** | **Leaf size in cm2 (Largest)** | **Leaf size in cm2 (Medium)** | **Leaf size in cm2 (Small)** | **pH** | **Electrical conductivity in mS/m** | **TDS in ppm** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Flower width in cm | 1.000 | | | | | | | | | | | | | |
| Receptacle/stamen width in cm | 0.934 | 1.000 | | | | | | | | | | | | |
| Ray florets (Petal size) in cm | 0.770 | 0.770 | 1.000 | | | | | | | | | | | |
| Fresh flower weight in g | 0.785 | 0.853 | 0.518 | 1.000 | | | | | | | | | | |
| Dry flower weight in g | 0.755 | 0.823 | 0.435 | 0.960 | 1.000 | | | | | | | | | |
| Stem length in m | 0.848 | 0.916 | 0.605 | 0.900 | 0.835 | 1.000 | | | | | | | | |
| Stem width in cm | 0.904 | 0.945 | 0.676 | 0.913 | 0.880 | 0.939 | 1.000 | | | | | | | |
| Seed weight in g | 0.801 | 0.853 | 0.482 | 0.962 | 0.961 | 0.887 | 0.910 | 1.000 | | | | | | |
| Leaf size in cm2 (Largest) | 0.813 | 0.906 | 0.665 | 0.737 | 0.671 | 0.871 | 0.882 | 0.737 | 1.000 | | | | | |
| Leaf size in cm2 (Medium) | 0.793 | 0.730 | 0.712 | 0.514 | 0.489 | 0.656 | 0.735 | 0.537 | 0.705 | 1.000 | | | | |
| Leaf size in cm2 (Small) | 0.700 | 0.789 | 0.869 | 0.578 | 0.523 | 0.663 | 0.671 | 0.540 | 0.680 | 0.662 | 1.000 | | | |
| pH | -0.180 | -0.209 | -0.185 | -0.233 | -0.147 | -0.131 | -0.029 | -0.135 | -0.013 | 0.128 | -0.339 | 1.000 | | |
| Electrical conductivity in mS/m | -0.635 | -0.663 | -0.434 | -0.837 | -0.856 | -0.605 | -0.642 | -0.812 | -0.409 | -0.254 | -0.473 | 0.476 | 1.000 | |
| TDS in ppm | 0.245 | 0.335 | 0.600 | 0.277 | 0.121 | 0.379 | 0.404 | 0.139 | 0.383 | 0.435 | 0.495 | 0.148 | 0.011 | 1.000 |

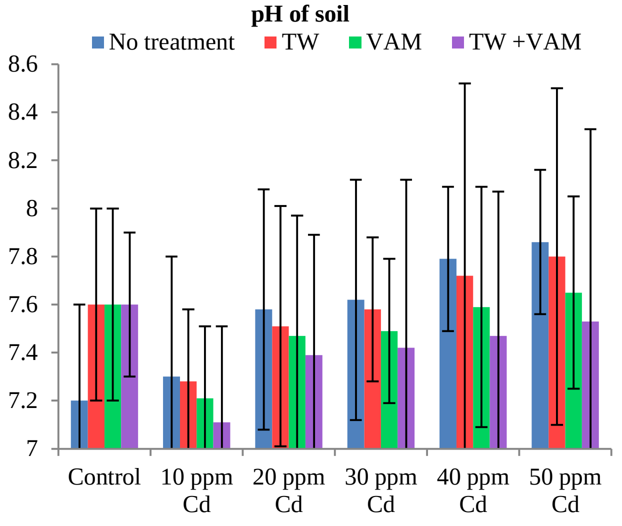



Fig. 6. pH, of soil in different treatment conditions to reduce cadmium stress. Data shown is the mean of N=3.

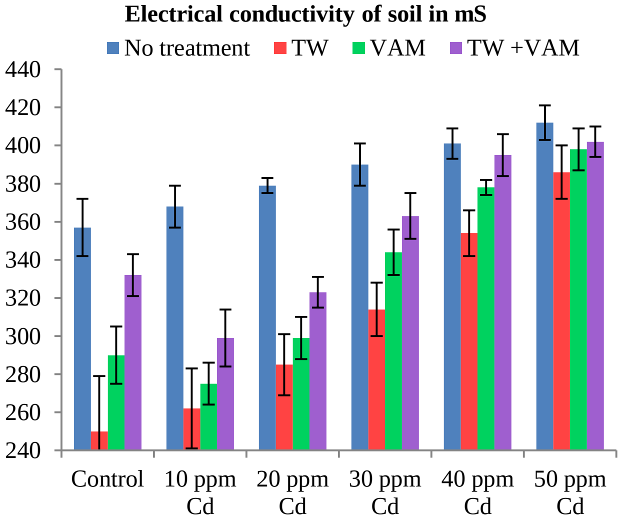



Fig. 7. Electrical conductivity of soil in different treatment conditions to reduce cadmium stress. Data shown is the mean of N=3.

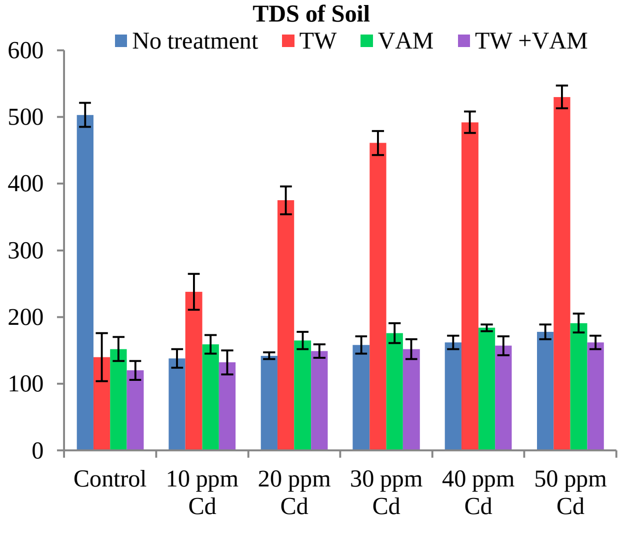



Fig. 8. TDS of soil in different treatment conditions to reduce cadmium stress. Data shown is the mean of N=3.

**Physicomorphological properties of the plants under contamination and remediation of Cd:** The soil properties like pH, EC and TDS are directly linked with the plant's development; if the pH of the soil or nutrient solution is too low or too high, the growth of the plants will be under stress. Therefore, physicomorphological properties of the plants, including length of the stem, leaves and flower; both fresh and dry weight of capitulum; and weight of the seed were also studied and correlated with soil attributes.

Initially, fresh and dry capitulum weight, capitulum and stamen width and ray floret size were measured. Sunflower plants treated to various levels of Cd stress showed reduced growth of upto 95 and 85% in both fresh and dry capitulum weights respectively (Fig. 9). After treatments, a remarkable increase was observed in capitulum weight; however, with the increased Cd concentration, this increase was gradually reduced. When the amount of Cd was low, for instance, in 10, 20 and 30 ppm cases, the two treatments, TW and VAM, showed similar behavior. On the other hand, in the case of 40 and 50 ppm of Cd concentrations, capitulum weight was significantly higher (p-value 1.67E-09) with TW treatment when compared with VAM treatment.

Conversely, parameters of the size of the capitulum, stamen and ray floret were significantly different (p-value 1.71E-10, 4.19E-08, and 3.04E-06, respectively) from those without treatment under various level of toxicity. However, the three treatment effects were not significant under various level of toxicity. The difference in weight but not in size was probably due to the weight of the seeds in the capitulum.

Furthermore, Cd stress reduces the stem length and width of sunflower plants (Fig. 10). However, remediation was observed in all three treated plants, where tremendous growth was observed with TW treatment, linked with the nutrients in Tea waste and Cd metal adsorption on the Tea waste. Similarly, the leaf area of sunflowers without treatment showed the same pattern of reduced growth as was observed in the flower and stem (Fig. 11). However, the treatment did not show any regular increase or decrease in the size of the leaves. Nevertheless, the TW treatment had significantly affected (p-value 6.03E-06) the leaves' size compared with the other two treatments, which were VAM and TW+VAM.

The significant factor in oil crops is the weight of the seed. The seed weight was affected under different doses of the Cd stress (10ppm to 50ppm) 24% decreased (Fig. 12). After applying the remediation technique of TW in control plants (without Cd), a significant (p-value 8.28E-11) increase in seed weight was observed (24.5%), whereas this was gradually decreased as the concentration of Cd increased (upto 31.4%). The same pattern of inverse relation was observed in the other two treatments, VAM and TW+VAM. However, the TW treatment is the best among all three treatments regarding seed weight.

Pearson correlation of physicomorphological properties resulted in a positive association of these properties with TDS while negative with pH and electrical conductivity (Table 1). Only EC gave significant associations among these, while pH and TDS resulted in non-significant associations with the remaining attributes.

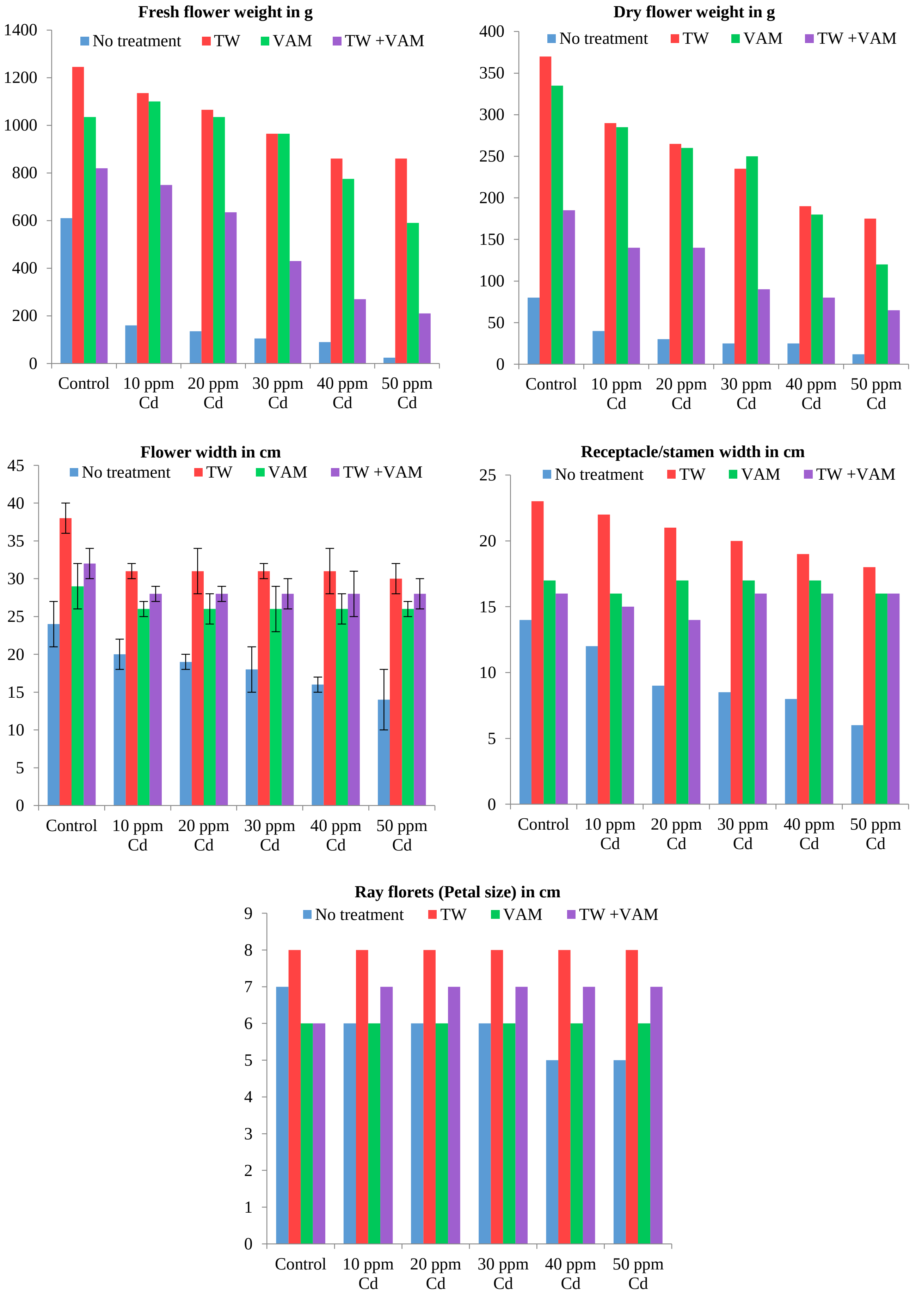
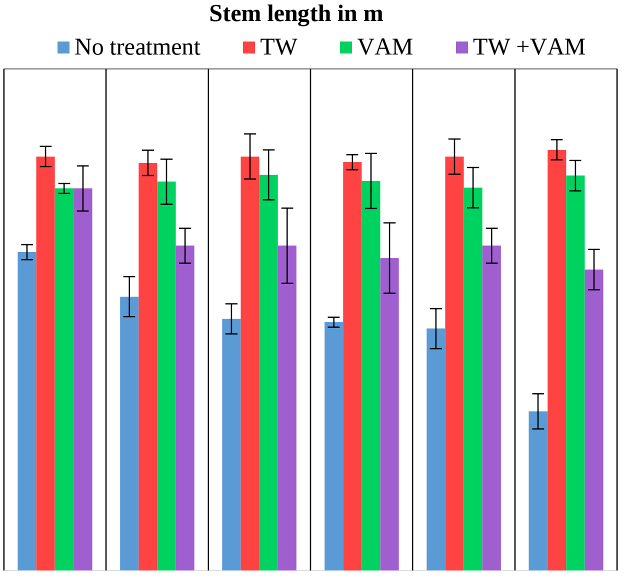



Fig. 9. Physical parameters of flower of sunflower in different treatment conditions to reduce cadmium stress. Data shown is the mean of N=3.



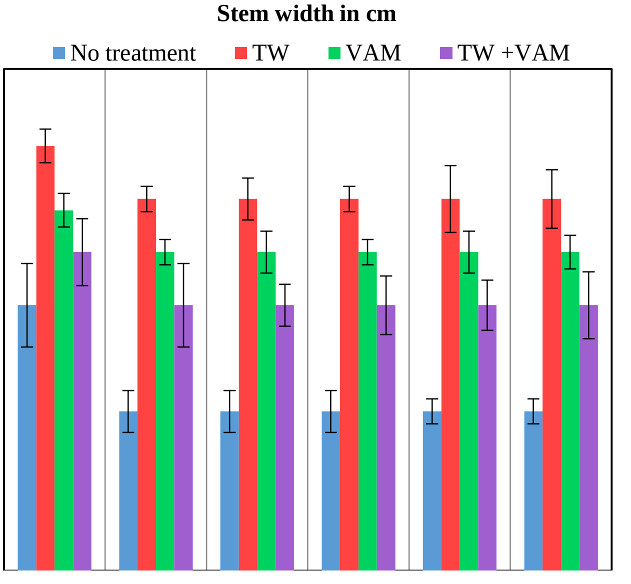
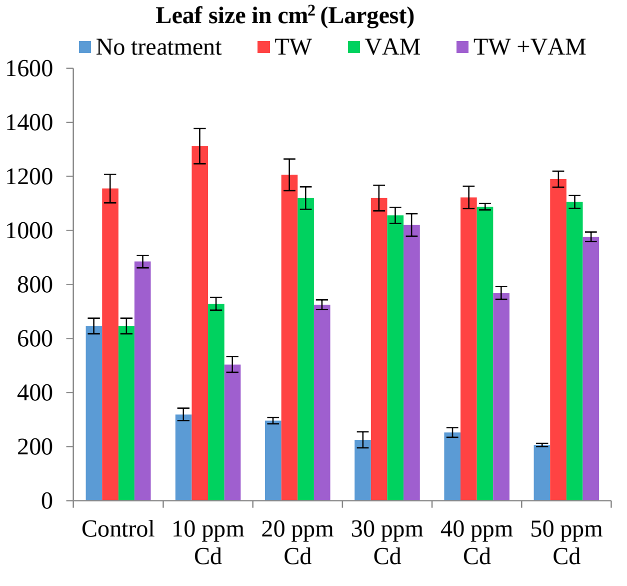
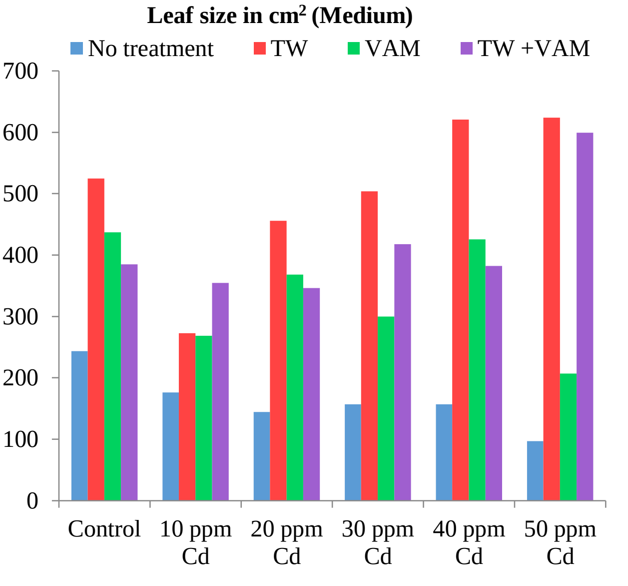




Fig. 10. Physical parameters of stem of sunflower in different treatment conditions to reduce cadmium stress. Data shown is the mean of N=3.



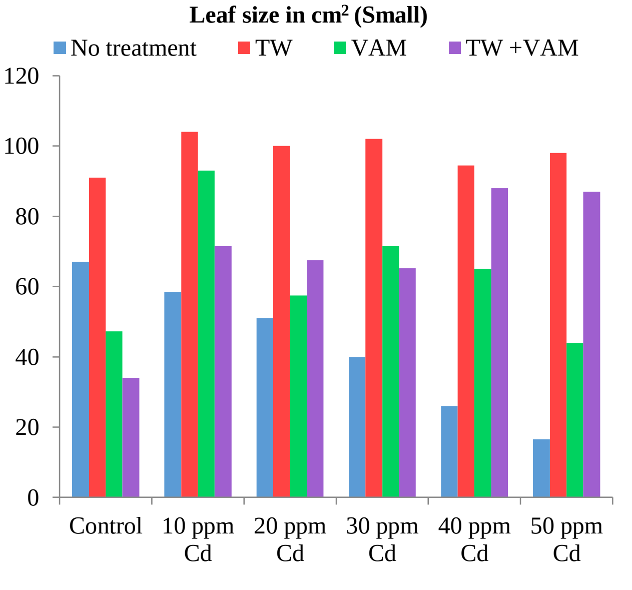




Fig. 11. Area of three different levels of leaves of sunflower in different treatment conditions to reduce cadmium stress. Data shown is the mean of N=3.

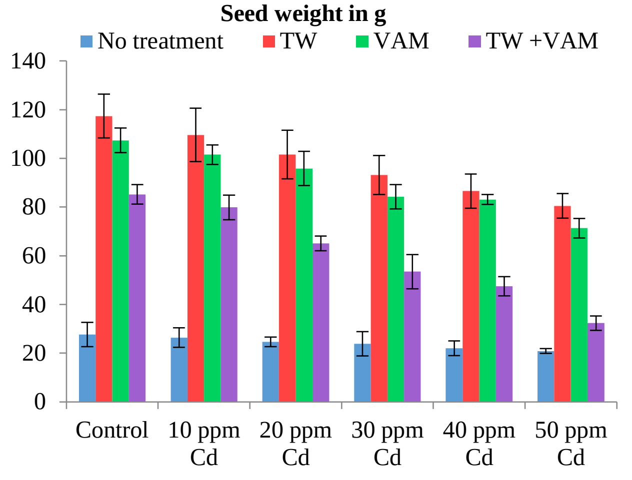



Fig. 12. Sunflower seed weight in different treatment conditions to reduce cadmium stress. Data shown is the mean of N=3.

**Soil restoration under remediation:** The concentration of essential metals like Fe, Cu, Ni and Co in fresh soil and control plants' soil was very much similar to the soil stressed with 50ppm Cd addition (Fig. 13). Similarly, these essential metals were unchanged even after providing treatments. However, Pb was detected in the soil and the control plants' soil, probably due to trash burning near plant growth areas or any other source of soil contamination. Surprisingly, Pb was not found when treatments (TW and VAM) were given to soil on Cd-contaminated plants, which might also be blocked by these stimulators. Interestingly, Cd, was not detected initially in fresh soil alongside the soil of control plants. It was detected when Cd 50ppm Conc. was given to soil. This stress of 50ppm Cd was found to be effectively remediated with TW treatment more than the VAM treatment.

## Discussion

The current soil enrichment formulation using Tea waste and VAM was intended to increase nutrient mobility from soil to plants under Cd metal contamination. That may act as natural pesticide resistance, increase crop productivity, bind to heavy metals and express the remediation of Cd metal that has no biological function in plant growth and is toxic for their growth.

The soil analysis, including pH, EC and TDS, was conducted before seed sowing and after the full maturity of plants in three different sets; and restoration of soil health-associated properties was investigated on the plant's growth. It was observed that the Cd stress reduced the overall quality and quantity of sunflower crops. It was previously reported that Cd stress disturbed the behavior of major metabolic enzymes and resulted in leaf necrosis, leading to growth reduction (Amaya-Carpio *et al*., 2009.) Several growth increment functions, including membrane potential at the proton pump, were altered under Cd stress, resulting in decreased cell division and elongation rates (Karcz & Kurtyka, 2007). Similarly, in another study, Cd was responsible for reduced biomass accumulation in sunflowers (Al-Dhaibani *et al*., 2013). Morphological traits of plants were also severely affected by Cd stress as it diminished hydraulic conductivity and disturbed cell wall extensibility (Ehlert *et al*., 2009).

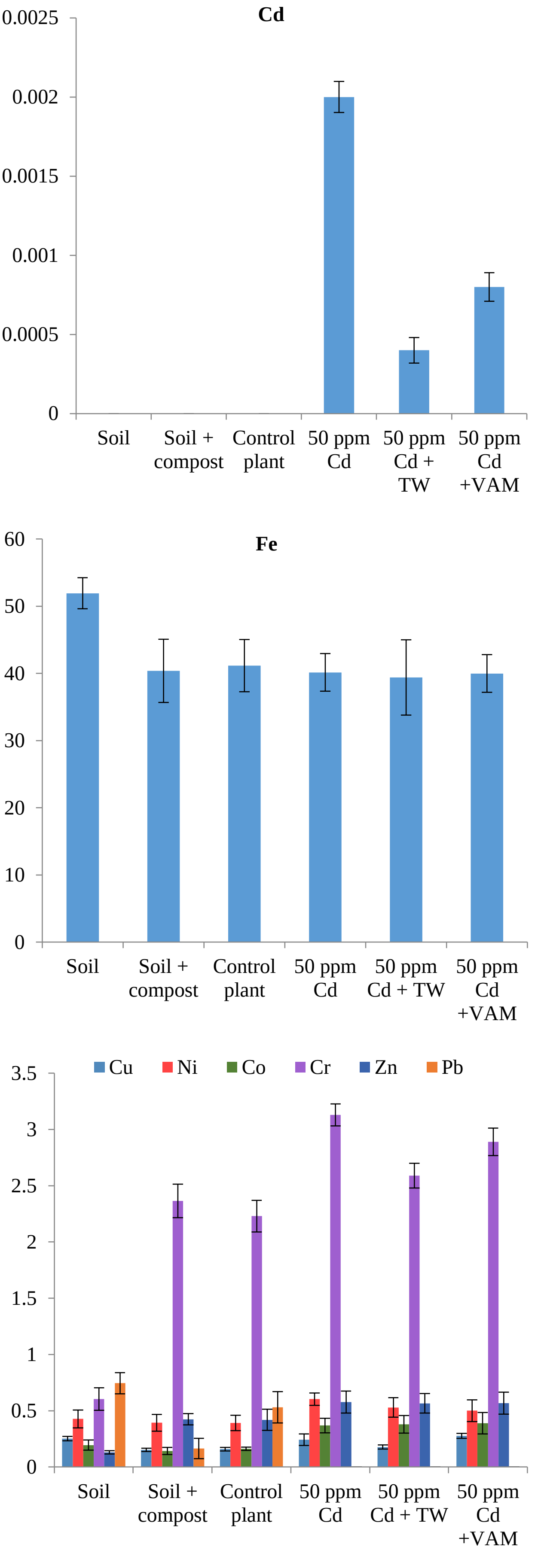



Fig. 13. Concentration of various metals in soil in different treatment conditions. Data shown is the mean of N=3.

This study compares the effect of different treatment options for plant morphological growth and soil remediation under metal stress. Remarkably, among the three treatments, TW was found to be effective in every aspect of morphological improvement of the plant as well as soil remediation, specifically in terms of metal concentration. The enhanced growth due to TW concurs with the previous findings of Azmat *et al*., (2010) for *Vigna radiata* for chromium stress. They observed that TW addition to the soil reduced the detrimental effects of metal toxicity by increasing the concentrations of chlorophyll, carbohydrates, protein and amino acids. This indicated that Tea waste can be used to remediate damaged soil.

The current results showed that the Cd concentrations were decreased by TW addition to greater degrees when compared to VAM and their combination. The ability of fungal hyphae to bind heavy metals outside and inside the roots and confine their uptake to the higher sections may be responsible for the reduction in Cd (Hua *et al.*, 2010.). Through its effect on biomass dilution, decreasing Cd uptake to upper plant parts results in maintained growth and metabolism (Janouskova *et al*., 2007).

The metal-contaminated soil had a negative impact on plant metabolism, which was visible in the growth and morphological appearance of the plants. It was first linked to the production of reactive oxygen species, which generates an oxidative burst followed by changes in the plant's regular metabolic route. As a result of the oxidative stress, the plant's growth rate is slowed, as seen by reduced photosynthesis and transpiration rates, as well as a reduction in the leaf's surface area. An increased growth rate was reported when plants were treated with Tea leaves waste in the presence of Cd metal. A rapid growth rate and large surface indicates the absence of the Cd metal and soil remediation (Azmat, 2011).

$H_2O_2$ is a key component in plant metabolism, releasing oxygen through stomata. However, in the presence of heavy metals, reduced leaf size and stomata restrict oxygen liberation, resulting in oxidative damage in plants. The Cd-treated plant (*Helianthus annuus* L.) with tea waste had a larger surface area and a faster growth rate, indicating that Tea waste effectively controlled Cd and ROS such as $H_2O_2$, which is a vital compound and a signaling molecule, and followed the regular metabolic pathway for the plant's many metabolic processes such as translocation, photosynthesis, respiration, and transpiration. As a result, these sequences improved agricultural yield. $H_2O_2$ is currently a signaling molecule in signal transduction pathways, critical for abiotic stress acclimation and defense (Zentgraf *et al.*, 2012). When waste tea is mixed with polluted soil, normal seed germination, photosynthetic rate, amino acid, protein creation, carbohydrate formation and inhibiting automatic cell death caused by Cd are achieved followed by flowering and root system development. $H_2O_2$ is a highly stable and long-lasting molecule produced in plant cells during respiration and photosynthesis. These findings showed that metal pollution caused $H_2O_2$ to be produced in cellular transduction signaling pathways and dissociated into the *OH radical, a strong oxidizing species.

Natural materials are a good option instead of chemical fertilizers since they increase soil quality while causing no further harm to the soil, resulting in huge and high-quality and quantity crops. These natural remediates help to prevent the movement of non-essential heavy metals from the soil to the roots of plants. They act as the most cost-effective technology for replacing any standard chemical fertilizer. All that is required is transportation to add these materials for soil restoration to achieve healthy plant growth.

**Mechanism of remediation:** Soil bio-remediation is a time in current era to control the industrial wastes discharged in soil and water that involves a several methods intended to become rid of toxins like heavy metals, pesticides, hydrocarbons (petroleum and fuel residues), cyanides, volatiles or semi-volatiles from soil. It is fact that pollution regarding heavy metals require attention to control their impact in soil and water as they could not be destroyed and degrade. Therefore, they controlled by using Tea waste and VAM fungi in this research. Soil remediation by the application of these two stimulators is required to control the pollution in soil, water for the benefit to commercial cultivation or for wild flora and fauna. AM fungi are abundant in soil habitation and form valuable symbiosis with the roots of angiosperms and other plants (Verma *et al*., 2021).

It was established after observing the tremendous growth of the plants under contamination through the application of organic composite that both remediators successfully controlled the mobility of the metal in contaminated soil. It was referred to as the metal biosorption or bioremediation in the soil via wastes connected to absorption or bond formation that involved complex formation with the specific group of the compound present. Ion exchange, physical connection or adsorption, chelation, and interactions among structural polysaccharides are possibilities (Kumar *et al*., 2017). Metal biosorption on tea waste leaves based on a group of bioactive molecules called phenols and hydroxyl groups found in polysaccharides, carboxylates and oxyl groups. These chemicals are responsible for ion exchange or chelation with metal, followed by developing a complex that prevents the metal from moving and accumulating in roots (Hussain *et al*., 2018).

It's important to note that heavy metals are naturally existing soil components that aren't hazardous to the soil or humans when disposed of through anthropogenic activity. Recently, a few approaches based on readily available natural materials have been developed, demonstrating excellent metal management in the soil. These metals can't be broken down or destroyed, but they can be controlled by forming complexes or changing their oxidation states. The metal can either: **(i)** become more water-soluble and removed by leaching, **(ii)** inherently less toxic, **(iii)** less water-soluble and precipitate, becoming less bioavailable or removed from the contaminated site or **(iv)** volatilized and removed from the contaminated site as a result of its oxidation state change (Kumar *et al*., 2017).

## Conclusion

Plant growth inhibition, lower biomass production and heavy metal accumulation result in the reduction of grain yield and cause severe economic losses on account of plants growing in polluted agricultural soils. Moreover, high human health risks have been observed due to heavy metal exposure to agricultural soils. Therefore, heavy metal pollution of agricultural soils should be effectively controlled and ameliorated. This study explains the effectiveness of TEA WASTE and VAM, which can be used as a remediator of soil to control metal mobility within the contaminated soil effectively. The soil's EC, pH and TDS, considered health-associated properties within the soil quality and plant growth, were observed to be affected favorably by the metal remediation inside through remediators. It is recommended here that the Tea waste and other natural waste can be used as fertilizers to replace synthetic and hazardous chemicals to have a better yield and production of crops. However, future research is required for common farmers who can employ these features to generate healthy soil before and after harvesting crops. Tea waste composts will be manufactured with proper chemical constituents and should be available for farmers in the market.

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