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Original Research Paper

Brassica juncea and *Medicago sativa* as A Phytoremediator for removal of Chromium and Arsenic

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ABSTRACT

The aim of this work is to examine the effectiveness of phytoremediation, a process that uses *Brassica juncea* (Indian Mustard) and *Medicago sativa* (Alfalfa) plants to remediate contaminated soil with Cr and Ar. An economical and ecologically appropriate way to remove, immobilise, and degrade contaminants from soil and water is through phytoremediation. With this experiment, plants can grow in a controlled environment with different Cr and Ar concentrations in soil and addition of organic compost. This entails evaluating the plants' capacity to absorb metal, monitoring variations in the concentrations of metal in the plants' roots, stems, leaves, and seeds, and looking into how organic matter affects the efficiency of phytoremediation. The findings showed that plants accumulated large amounts of chromium and arsenic across all experimental plants, but the highest accumulation was observed in the root system, which suggested that the plants were involved in the process of rhizofiltration. The roots pick up much more of the metals than the aerial of the plant, including stems, leaves, and seeds, thereby minimizing metal translocation to the parts of the plant that can be ingested by animals and, in turn, humans. This is a fundamental criterion for phytoremediation for assurance of a safe and effective process. Overall, the present study underscores the ability of phytoremediation in the remediation of heavy metal-polluted soils, especially under the use of organic growing media. It has made me understand the usefulness of this method for the effective and efficient cleaning of the soil in comparison with traditional methods, which could benefit the environment and future cost savings. Further research should be concerned with field-scale experiences and examine the potential of phytoremediation approaches in the range of environmental conditions.

Key Words	Phytoremediation, Heavy metals, Brassica juncea, Medicago sativa, Organic compost
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INTRODUCTION

Impact of Heavy Metals: Chromium and Arsenic

Chromium and arsenic are hazardous elements that are dangerous for the sustainability of ecosystems as well as for human health. Arsenic is an element and a potent toxin, which can be of geogenic or anthropogenic origin, the former being sources including rocks and the latter being mining and industrial activities. Drinking water containing arsenic is likely to cause some diseases despite the dosage used in this analysis being relatively small, bearing in mind that arsenic-contaminated water may cause skin problems, cancer, and other development complications in a child. Arsenic was found to contaminate the groundwater, and this has led to many cases of arsenic poisoning in different parts of Asia, especially Bangladesh (Srivastava et al. 2021).

Several industries, for example, electroplating and leather tanning, produce chromium, some of which is the hexavalent chromium ion Cr (VI) that has been rated to be a severe pollutant (Kumar et al. 2018). The overall properties of this type of chromium make it dangerous to human health, especially because it is poisonous and causes cancer. Chromium can be hazardous to human health depending on the dosage; especially through intake of water containing Chromium at high concentrations, one can develop respiratory diseases, skin diseases, and have an increased tendency of suffering from cancer. Due to their endurance in the environment, arsenic and chromium are difficult to rehabilitate from water; hence, their removal is paramount to public health. The use of phytoremediation as an eco-friendly solution to this issue has been growing in popularity. In order to evaluate the impact of five different soil management techniques (cow manure, sewage sludge, leaf compost, vermicompost, and chicken manure) on the accumulation of Cd, Cu, Mo, Ni, Pb, and Zn in the leaves and pods of three different varieties of mustard (Black mustard, yellow mustard, and Mighty mustard), this study aims to investigate the efficacy of phytoremediation in agricultural settings. Anjan Nepal et al (2024). Brassica juncea was grown hydroponically to examine its morphological and physiological reactions to trichlorfon (TCF) and cadmium (Cd) stressors as well as its capacity for phytoremediation. The results demonstrated that when the concentration of Cd rose, B. juncea's biomass and root shape were strongly inhibited. Malondialdehyde and soluble protein levels, as well as superoxide dismutase activity, rose with the increased Cd concentration, while B. juncea's chlorophyll a fluorescence intensity and chlorophyll content dropped. TCF did not significantly affect these B. juncea morphological and physiological characteristics at varying concentrations. Under the co-exposure to Cd and TCF, the biomass and physiological state of B. juncea were mostly controlled by the Cd level. B. juncea may collect Cd in many ways. Chao Zhang et al (2023).

Water Quality and Its Significance

The quality of water is very crucial for the survival of all the species on planet Earth. Get deterioration of agriculture, health of people, and last but not least, the ecology of planet. Potable water is therefore very essential for man’s existence, as diseases such as cholera and dysentery spread very fast through waterborne diseases (Prasad et al. 2021). Since most countries in the world today recognize clean water as a basic human right, the same governments at all levels must ensure that they strive as much as possible to ensure that the water supply meets or exceeds international standards.

Various pollutants that are capable of reducing the quality of water include discharge from agricultural and industrial activities and poor disposal of waste (Sharma et al. 2018). Forces such as chemicals, heavy metals, pesticides, and viruses can highly contribute to compromised water safety. The problem of the presence of heavy metals in the environment has recently stepped out of the background and become one of the key problems. Some heavy metals, including arsenic and chromium, are more dangerous as they are not biodegradable and can stay in the body of an organism for many years and therefore pose an extended threat to future generations of both human and animal beings.

Characteristics of *Brassica juncea* (Indian Mustard)

Brassica juncea, commonly known as Indian mustard, belongs to the Brassicaceae family and originates from Central Asia. This annual herbaceous plant typically grows to a height of 40-150 cm. Its leaves are lobed with crenate or serrate margins; the basal leaves are stipulate and petioled, while the cauline leaves are exstipulate and sessile. Indian mustard produces small yellow flowers arranged in racemes. Its root system consists of shallow, fibrous roots, supported by lateral branching underground stolons, which help the plant adapt to various soil textures. While it prefers light, well-drained soils, it is versatile and tolerates a range of soil types, including saline and alkaline conditions. Originally grown in mild climates, Indian mustard can also withstand drought and extreme heat. The plant is valued for its seeds, which are used to produce mustard oil, and as a spice (Patel et al. 2019). Its leaves are consumed as greens or vegetables. Additionally, Indian mustard is utilized in bioremediation to remove heavy metals from soil. However, it is vulnerable to pests like aphids and cabbage worms, and diseases such as downy mildew and blackleg.

Characteristics of *Medicago sativa* (Alfalfa):

Medicago sativa, commonly known as Alfalfa, belongs to the Fabaceae (Leguminosae) family and originates from the Mediterranean and southwestern Asia. It is an herbaceous perennial legume that typically grows to a height of 30-90 cm. Its trifoliate leaves consist of three oblong leaflets, with the terminal leaflet being longer than the two side leaflets (He et al. 2018). Alfalfa produces small, purple to blue flowers on racemes and has a well-developed, deep root system, which can extend over 5 meters, allowing it to withstand dry conditions. It thrives in well-drained loamy soil with a neutral pH range of 6.5 to 7.5. This plant adapts well to the middle and southern latitudes, being both frost-tolerant and heat-resistant. Primarily grown as a fodder crop due to its high protein, vitamin, and mineral content, Alfalfa is also valuable for soil improvement through nitrogen fixation. However, it is susceptible to pests such as weevils and aphids, and fungal diseases like root rot and anthracnose.

Phytoremediation techniques:

Concepts:

The primary idea of phytoremediation is the utilization of plants to absorb, accumulate, and purify pollutants found in the industrial effluent water and contaminated soil. Phytoremediation is a cost-effective and environmentally friendly technique.

Various applications of Phytoremediation technique

Phytoremediation is a novel and environmentally sound approach that employs plants and their associated microorganisms to immobilize, transport, transform, and degrade contaminants from water and soil (Baltrenaite et al. 2017 & Hemani et al., 2024). To elaborate, since this method capitalizes on plants' natural air purifying qualities, it is both affordable and eco-friendly. It is comprised of several stages, like rhizofiltration, phytoextraction, phytostabilization, and phytodegradation, all of which are designed for particular pollutants.

Phytoremediation is the process of using plants to eliminate heavy metals from the soil by letting the plants' roots take in the metal and transport it to their above-ground tissues. (Vijayant Panday & Ananda Babu 2023). Phytostabilization is a technique that helps in avoiding the mobilization and bioavailability of contaminants to other organisms through fixing them onto the soils. While rhizofiltration is centered on the removal of pollutants from water, phytodegradation is more directed toward the breakdown of chemical substances (Sabreena et al. 2022). Phytoremediation deserves research because it is promising to become a great-scaled eco-technology (Panneerselvam & Shanmuga Priya 2023). It possesses the potential of restoring the degraded landscape to better optimized ecosystems in different settings, such as cities, farmland, and past mining areas. Since pollution control and environment conservation form part of the sustainable development agenda, this approach is commendable.

Though deployed mainly to increase the effectiveness of conventional phytoremediation technologies, a new approach collectively known as bio-remediation is gradually incorporating technology such as genetic engineering and nanotechnology (Wang &Delavar 2024). Such breakthroughs open new possibilities to increase the intake of toxins that can be absorbed and ingested, therefore increasing the potential of phytoremediation as a way of eradicating heavy metal pollution. (Qadir et al. 2021). Phytoremediation Techniques depicted in Fig. 1.

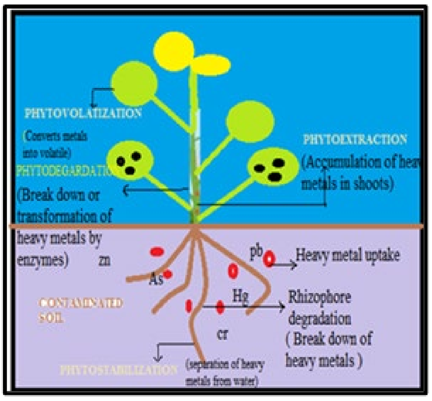

Fig. 1: Phytoremediation Techniques.

Objectives of the Study

This research work aims at identifying and evaluating the viability of using phytoremediation to tackle the pollution posed by these two pollutants, namely Chromium and Arsenic, in the water and soil. Specific objectives include:

1. To assess the efficiency of plant species, such as Brassica juncea and Medicago sativa, for the remediation activity of heavy metals chromium and arsenic and enhancement of soil and water quality under controlled experimental conditions.
2. To identify the impact of heavy metal contamination on health monitoring of plants, Morphological characteristics, plant responses with different effluent doses.

3. To analyse the heavy metal uptake with and without organic amendments and also statistical analysis.
4. To analyse impact on soil properties before planting and after harvest following phytoremediation

Current research on Phytoremediation techniques

Some empirical evidence show that phytoremediation works well in eliminating contaminants on ecosystems due to the increasing interest in the subject in the last two decades. Studies have revealed that certain species of plants have the capacity of accumulating these metals to sets of measures that are acceptable (Guerra Sierra et al. 2021). Afterwards, these plants can be collected, accumulated and perhaps disposed without any negative impact. Previous research, research and experiments have shown that Brassica juncea possesses a high potential of phytoextraction of metals including nickel, cadmium and lead.

Studies on phytoremediation have also helped in identifying the absorption and detoxifying processes that metals involve at physiological and biochemical level. Literatures on root exudates and soil microbes show that these molecules increase the solubility and bioavailability of metals thus increasing the uptake of these metals by plant. Yet, there is hope that with the application of new technologies such as plant genetic engineering, they can improve on their Toxins retention capacity. (Mohan et al. 2021).

Despite the progress made in recent years, the enhancement of the phytoremediation technologies for various pollutants and site conditions remains a difficult problem. The present study underlines the need for a better understanding of the mutual relation of soil, plants and contaminants by focused studies site-dependent. The purpose of this study is to begin building upon for future successful use of phytoremediation by conducting a review of prior research.

MATERIALS AND METHODS

Selected Industrial Wastewater

Some industries, for instance, polishing of metals, mining, and the manufacture of chemicals release wastewater containing materials that are dangerous, for instance, arsenic and chromium. These two substances therefore have significant impacts on the well-being of human beings as well as the environment. General Pasteurization: the wastewater has a pH of about 7. 2 ± 0. 3, an electrical conductivity of batch about 1200 ± 50 µS/cm, total dissolved solids concentration of batch about 800 ± 40 mg/L, Arsenic concentration is 30.5 ± 1.5 mg/l and Chromium values 50.3 ± 2.0 mg/l respectively. (Farhadkhani et al. 2018). Table 1. shows the characteristic of selected industrial waste water.

Table 1: Characteristic of selected industrial waste water

Parameter	Unit	Values
pH	-	7.2 ± 0.3
Electrical Conductivity (EC)	µS/cm	1200 ± 50
Total Dissolved Solids (TDS)	mg/L	800 ± 40

Arsenic (As) Concentration	mg/L	30.5 ± 1.5
Chromium (Cr) Concentration	mg/L	50.3 ± 2.0

Selection Criteria for Indian mustard and Alfalfa

Based on the characteristic of industrial effluent water for the investigations two plant species was selected. Brassica juncea also known as Indian mustard and Medicago sativa also known as alfalfa were chosen for phytoremediation because they have high biomass yields, are resistant to toxic levels of heavy metals and, also have the ability of bio concentrating chromium and arsenic among other metals. Because of its rather large and sprawling root system B. juncea can efficiently take up nutrients and metals from the contaminated soil and it has rather high bioconcentration factor concerning chromium (Cr) and arsenic (As). Medicago sativa promotes soil stability due to a higher density of root system and performs nitrogen fixation that helps the transportation of metals through it (Zhang et al. 2019). Both these species are highly suitable for the long-term bioremediation of polluted sites as they are capable to grow in variable type of soil, grows very fast and are also stress tolerating.

Soil and Plant Tissue Analysis

A preliminary study carried out with analysing of soil and plant tissue sample is important to reviewing and evaluating the progress of phytoremediation. Amendments and treatments can be studied by collecting soil samples at different time intervals for determining the heavy metal concentrations. In order to evaluate the effectiveness of different plant species, several samples of plant tissue are collected and contaminated pollutant concentrations are measured. Usually, in order to provide more exhaustive analysis, it is possible to take samples of soil from different layers of the soil. The samples concerning the root, stem, and leaves are gathered to analyze the increase of metals and the fact of plants' activity (Yanitch et al. 2020). By following the method, in order to determine the levels of heavy metals, the soil and plant samples are first dried, ground, and digested, and then analyzed using highly sophisticated techniques like inductively coupled plasma mass spectrometry. Table 2. shows the collection of samples, sampling timing, preparation and analysis methods was selected for the investigations.

Table 2: Sample and Analysis Methods for the investigations

Sample Type	Sampling Time Points	Preparation Method	Analysis Method
Soil	Pre-treatment, Mid-treatment, post-treatment	Air-drying, Grinding, Sieving	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
Plant Tissue (Roots)	Pre-treatment, Mid-treatment, post-treatment	Washing, Drying, Grinding	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Plant Tissue (Leaves)	Pre-treatment, Mid-treatment, post-treatment	Washing, Drying, Grinding	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)
Plant Tissue (Stems)	Pre-treatment, Mid-treatment, post-treatment	Washing, Drying, Grinding	Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Seed Morphology Characteristics

Brassica juncea (Indian Mustard):

The seeds of Brassica juncea are round or slightly oval-shaped, small in size, typically ranging from 1.5 mm in diameter. Their colour varies from dark brown to reddish-brown or black. The seed surface has a glossy texture, enhancing their appearance despite their small size. Fig. 2 shows the collected Brassica juncea seeds for the investigation purposes.

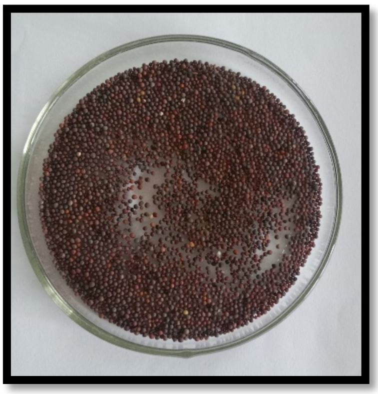

Fig. 2: *Brassica juncea* seeds.

Medicago sativa (Alfalfa):

The seeds of Medicago sativa are small, round, and have a kidney-like or reniform appearance. They typically measure between 1.5 mm to 1.8 mm in length. The seed colour can range from light brown to yellowish-brown, and they have a smooth surface with a high-gloss luster. Fig. 3 shows the collected Alfalfa seeds for the investigation purposes.

Fig. 3: Medicago sativa seeds.

Treatments and Amendments

Phytoremediation treatments often include organic and inorganic amendments applied in order to improve the properties of the soil and make available for plant uptake heavy metals like arsenic and chromium (Patel et al. 2021). Typically, compost are the most common organic additives that increase the microbial growth and the cation exchange capacity, thus increasing the metal plant uptake. To improve the dissolution of metals and increase the chances of plants absorbing the metals and addition of compost it increases the rate of plant growth and constitutes an efficient way of increasing the uptake of chromium and arsenic by hyperaccumulators such as Medicago sativa and Brassica juncea. (Rather et al. 2022).

Characteristics of Composting

Composting characteristics are mainly classified into physical, biological and chemical properties. The plants are typically grown within physical characteristics of composting are shown in the Table 3. Physical parameter is the main supporting for plants growth of both *Brassica* and *Medicago*. Temperature and humidity are a key parameter determining the success of composting operations in the experiment can accurately assess metal uptake and growth impacts in *Brassica juncea* and *Medicago sativa*, ensuring repeatable and reliable results. Apart from the physical characteristics environmental condition also should be monitored during the investigation. For phytoremediation techniques compost can be main beneficial for suppling the nutrients for plant growth and establishment and reduces the solubility of heavy metals from the industrial effluent.

Table 3: Physical characteristics of compost

S.No	Compost physical characteristics	Range
1	pH	
2	Temperature	22–28°C
3	Humidity	45-60%
4	Moisture content	40-60%
5	Organic content	35-55%

Statistical Analysis for the Phyto -Techniques

In order to identify statistically significant differences among values, content of plant tissues (taproot, lateral root, stem, and leaf), were analysed using one-way ANOVA and post-hoc Tukey's test (p\0.05). ANOVA was used to analyse the relationship between plant height and growth. The use of statistics helps the researcher to quantify the parameters of relations between variables, which include the association between plant species and heavy metal concentration in the soil. It is used in deciding whether or not some observed patterns were really high or really low purely by chance. It becomes relevant here to quantify such results to justify the feasibility of phytoremediation approaches and the extent of metal adsorption by Medicago sativa and Brassica juncea.

A multiple comparison test is carried out to find out which of the treatments offered in the experiment are significantly different after a significant result is obtained from the ANOVA. It is always used to compare all pairs of treatments while at the same time controlling for Type I errors; it's called the Tukey's Honestly Significant Difference (HSD) test. In this regard, following the analysis of variance by ANOVA to determine the variation in metal uptake across treatments, Tukey's HSD test would be used in comparing the chromium and arsenic accumulation in the roots, stems, leaves, and seeds of Brassica juncea and Medicago sativa across the various treatment codes. This procedure assists in determining which of the amendments or conditions enhances or reduces the efficiency of phytoremediation. Fig. 4 shows the statistical analysis on the phyto-techniques results.

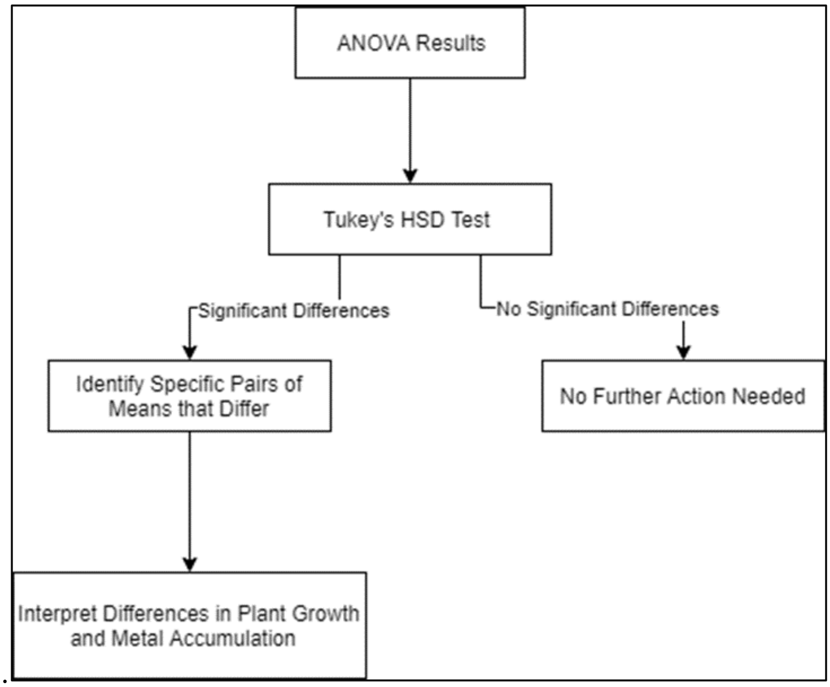

Fig.4: Statistical analysis on the phyto - techniques results.

Phytoremediation Experiment Details

Experimental Design and Setup

The experimental design involved planting seeds of Indian mustard (*Brassica juncea*) and alfalfa (*Medicago sativa*) in pots treated with industrial wastewater collected from a tanning industry in India. Additionally, composted organic matter was added to the soil to improve its efficiency in filtering various metals of interest. Each pot was then treated with solutions containing different concentrations of chromium and arsenic.

Quality and Morphological Analysis of Plant Samples

Throughout the experiment, the physical characteristics of the crops, including biomass, height, and leaf count, were recorded (Atabaki et al. 2020). Every two weeks, the chlorophyll content was measured using the SPAD meter; weekly measurements were made of the number of leaves, shoot length, and root growth. It also helps assess how well plants are doing in terms of growth and health at different pollution levels. (Kumar et al. 2022)

Plant Sample Analysis

Samples of the plants were taken. The meticulous handling did not do any damage to the root system. To get rid of dirt particles, deionised water was used to clean the plants. After that, the samples were divided into four groups: seeds, stems, leaves, and tree roots. To get a consistent weight, the samples were cleaned, let to air dry, and then baked at 70 °C. To ensure there was no contamination, these dried samples were subsequently crushed into a fine powder. The powdered samples were set aside for additional analysis and put in sealed vials.

Measurement of Chromium and Arsenic Concentration in Plant Organs

Thus, the levels of arsenic (As) and chromium (Cr) in plant tissues were measured using inductively coupled mass spectrometry (ICP-MS) (Heitland et al. 2017). The components were broken down under carefully controlled settings in a microwave digestion facility using a 3:1 nitric oxide to hydrogen peroxide ratio. ICP-MS was used to analyse the digested solutions and determine the concentration of Cr and Ar in the roots, stems, leaves, and seeds of each plant.

Data Interpretation

The results obtained at the end of the experiment show that the process of phytoremediation helped to decrease the level of heavy metals in the soil, especially chromium and arsenic. The means obtained for the metals are significantly higher in the root system than in stems, leaves, and seeds, which indicates that plants have used rhizofiltration, whereby metals are taken up and stored mainly in the root system without translocation to the aerial parts. This is considered advantageous in phytoremediation because it prevents the likelihood of metals being ingested by other creatures and making their way through the food chain or affecting wildlife by consuming tissues of the plants.

Specifically, the use of organic compounds significantly increased the efficiency of metal absorption. Chromium and arsenic levels were higher in plant tissues grown on soils supplemented with compost, suggesting that these substances increased the solubility and availability of heavy metals. By improving soil structure conformation and enhancing plant metal tolerance while preventing phytotoxicity symptoms, the amendments help plants grow.

RESULTS AND DISCUSSION

Monitoring of Plants Health

To maximize absorption of metals and plant health, it is necessary to monitor condition and development of Medicago sativa and Brassica juncea during phytoremediation. Biotic parameters which include plant growth, chlorophyll content in leaves, biomass produced and root elongation are recorded at different intervals of time. Bi-weekly evaluation is done in terms of height and biomass of plants that maybe hampered by heavy metal stresses (Jan et al. 2024). Further, chlorophyll index is checked on every two weeks by SPAD (Soil Plant Analysis Development) meter to measure photosynthesis activity. Some of the typical test performed include chromium and arsenic content from soil and plant tissue samples respectively. Thus, to improve the outcomes of remediation and to support well-being of plants, it is necessary to control such factors and make necessary changes to the types of added soil and watering frequency. Table 4. shows the parameter and frequency of measurement and measurement method

Table 4: Parameter and Frequency of Measurement and Measurement Method

Parameter	Frequency of Measurement	Measurement Method
Plant Height	Weekly	Measuring Tape
Leaf Chlorophyll Content	Bi-weekly	SPAD Meter
Biomass Production	Weekly	Dry Weight Analysis
Root Development	Monthly	Root Excavation and Imaging
Metal Concentration (Cr, As)	Monthly	ICPMS

Morphological Characteristics

We evaluated seed morphology by measuring the length and diameter of the seeds. A digital caliper was used to measure the length of each seed. The diameter of Indian mustard seeds is 1.5 mm, while the length of alfalfa seeds ranges from 1.5 mm to 1.8 mm

Plant Growth and Response to Varying Effluent Doses

The impact of arsenic and chromium on plants was observed based on the changes in development in various concentrations of the both metals in the soil (Saud et al. 2022). Fifty to 100 percent concentrations affected the plants negatively by decreasing their overall height, the number of leaves, and dry matter accumulation. But with the with the addition of the organic matter such as compost, some of the adverse consequences were offset since this improved plant growth, particularly at low levels of contaminants. Table 5. shows the effect of contaminant concentration on plant growth.

Table 5: Effect of Contaminant Concentration on Plant Growth

Plant species	Dose	Average Plant Height (cm)	Biomass (g)
Brassica juncea	B1 (Control)	120 ± 5	95 ± 4
	B2 (50%)	105 ± 4.5	82 ± 3
	B3 (100%)	90 ± 3.8	73 ± 2.5
Medicago sativa	M1 (Control)	15 ± 3.2	60 ± 3
	M2 (50%)	12 ± 2.5	45 ± 2
	M3 (100%)	10 ± 2.5	38 ± 2

Accumulation of Chromium and Arsenic - Indian Mustard and Alfalfa

Consistent with previous findings, this study also showed that level of metal accumulation was higher in root > stem > leaves > seed parts. Table 6 indicates the Accumulation of Cr and Arsenic in various parts of brassica juncea (mg/kg dry weight) and depicted in Fig. 5. And the statistical analysis using one-way ANOVA for Accumulation of Cr and As in various parts of Medicago sativa of concentration of 50% of average value is 30.9%, standard deviation value is 33.5 and the F-ratio value is 6.77544. The p-value is .035272. And also the statistical analysis using one-way ANOVA for Accumulation of Cr and As in various parts of Medicago sativa of concentration of 100% of average value is 35.8%, standard deviation value is 40.9 and the F-ratio value is 6.1133 The p-value is .042676.

It was established that the roots took in far higher levels of chromium and arsenic than any other parts of the plant that grew above the ground. The organic amendments in the soil improved metal accumulation in the plants, most notably on their roots. Table 7 indicates the accumulation of Cr and arsenic in various parts of Medicago sativa (mg/kg dry weight) and depicted in Fig. 6. And the statistical analysis using one-way ANOVA for Accumulation of Cr and As in various parts of Medicago sativa of concentration of 50% of average value is 38.759%, standard deviation value is 45.7 and the F-ratio value is 5.75488. The p-value is .047541. And also, the statistical analysis using one-way ANOVA for Accumulation of Cr and As in various parts of Medicago sativa of concentration of 100% of average value is 45.7%, standard deviation value is 52.8 and the F-ratio value is 5.73639 The p-value is .047811.

Table 6: Accumulation of Cr and Arsenic in various parts of brassica juncea (mg/kg dry weight)

Treatment Code	Cr in Roots (mg/kg)	Cr in Stems (mg/kg)	Cr in Leaves (mg/kg)	Cr in Seeds (mg/kg)	Ar in Roots (mg/kg)	Ar in Stems (mg/kg)	Ar in Leaves (mg/kg)	Ar in Seeds (mg/kg)	Average	SD

B1(Control)	0	0	0	0	0	0	0	0	0	0
B2(50%)	144	55	38	18	29	13	7	6	38.75	45.7
B3(100%)	165	70	40	20	34	12	9	8	44.75	52.8

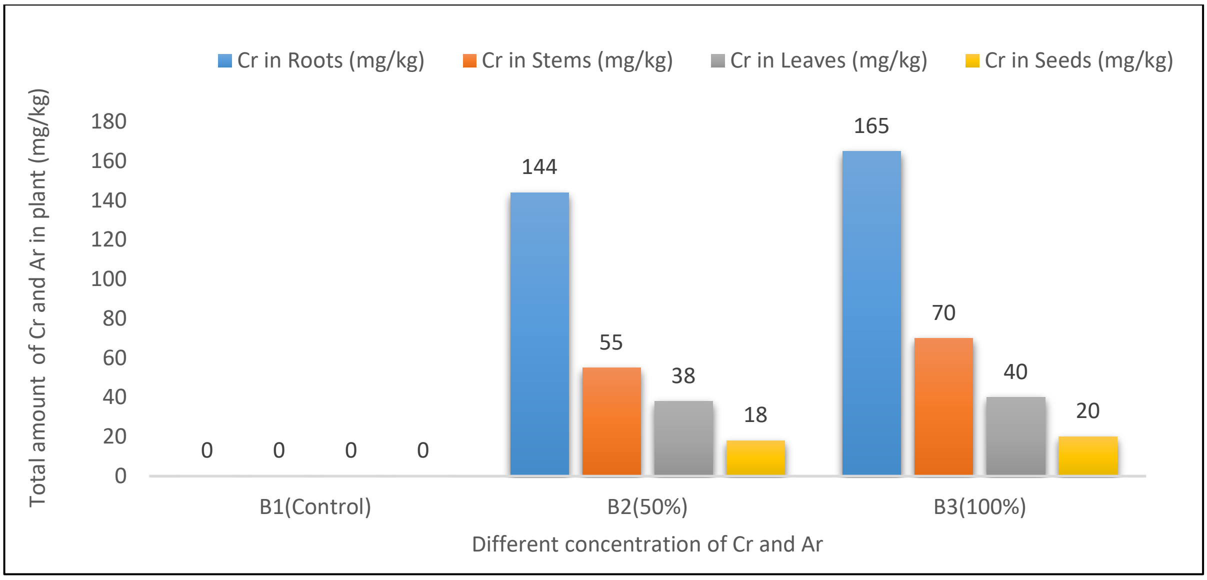

Fig. 5 : Accumulation of Chromium and Arsenic in all part of Brassica juncea.

Table 7: Accumulation of Cr and Arsenic in various parts of Medicago sativa (mg/kg dry weight)

Treatment Code	**Cr in Roots**	**Cr in Stems**	**Cr in Leaves (mg/kg)**	**Cr in Seeds**	**Ar in Roots**	**Ar in Stems**	**Ar in Leaves**	**Ar in Seeds**	**Average**	**SD**
M1 (Control)	0	0	0	0	0	0	0	0	0	0
M2 (50%)	107	41	35	20	25	8	6	5	30.9	33.5
M3 (100%)	130	50	29	25	30	9	7	6	35.8	40.9

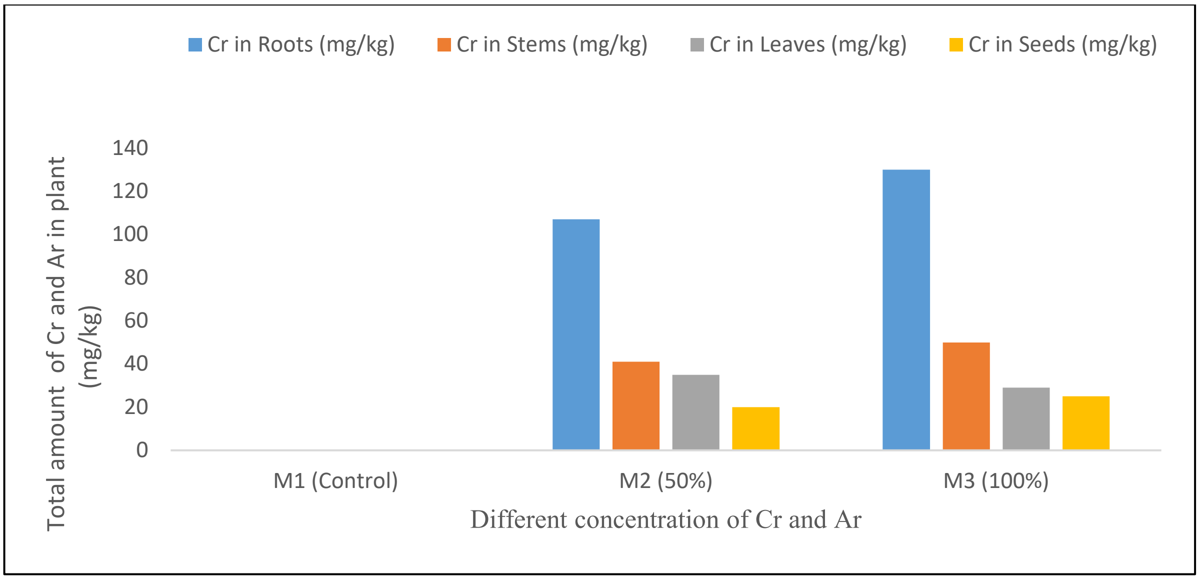

Fig.6: Accumulation of Chromium and arsenic in all parts of Medicago sativa.

Comparison of statistical values Medicago sativa and Brassica juncea

To assess statistically significant variations in values, one -way ANOVA was used to analyze all of the data. Measurements of tissues (leaf, stem and seeds) were examined. one-way ANOVA $p<0.05$. A one -way ANOVA with repeated measures was utilized to examine how plant height and growth in the both plant species comparison of statistical values Medicago sativa and Brassica juncea. For plant species 1 Brassica juncea concentration of 50% of average value is 38.75%, standard deviation value is 45.7 and the F-ratio value is 5.75488. The p-value is. 047541 and concentration of 100% of average value is 44.7%, standard deviation value is 52.8 and the F-ratio value is 5.73639 The p-value is .047811 and plant species 2 Medicago sativa of concentration of 50% of average value is 38.9%, standard deviation value is 33.5 and the F-ratio value is 6.77544. The p-value is .035272 and concentration of 100% of average value is 35.8%, standard deviation value is 40.9 and the F-ratio value is 6.1133 The p-value is .042676. Brassica juncea takes a higher growth accumulation of Ar and Cr.

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Comparison of Root vs. Aboveground Accumulation

Chromium and arsenic mobility were higher in the root tissues as compared to the aboveground tissues of the plant. Such differences are usually observed in phytoremediation work where roots are known to be the main location of metal accumulation and first depot (Ullah et al. 2021). In this experiment, the roots had much higher levels of both metals, which can be attributed to the fact that they are in direct contact with the tainted soil and are responsible for taking in water and nutrients where dissolved metals usually follow.

Nevertheless, the concentration of metals in the stems, leaves, and seeds was found to be comparatively lesser than that in the roots. Little transport of the heavy metals from the root to the above-ground portion was observed, meaning while the plant is good at uptake of the contaminants, most of the metal is tied to the roots. Indeed, this implies that the role of the plant in phytoremediation is mainly in rhizofiltration (the absorption of contaminants through the roots without transport to other parts of the plant). Table 8. indicates the Chromium and Arsenic Concentration in Roots vs. Aboveground Parts (mg/kg dry weight) and depicted in Fig. 7.

Table 8: Chromium and Arsenic Concentration in Roots vs. Aboveground Parts (mg/kg dry weight)

Plant species	Concentrations	Plant Part	Chromium (Cr) (mg/kg)	Arsenic (As) (mg/kg)
Brassica juncea	Control	Below ground	0	0
		Above ground	0	0
	50%	Below ground	144±5	29±1
		Above ground	111±3	26±3
	100%	Below ground	165± 6	34±1.2
		Above ground	130±3	29±4
Medicago sativa	Control	Below ground	0	0
		Above ground	0	0
	50%	Below ground	107 ± 4	25 ± 0.8
		Above ground	96 ± 3.2	19 ± 0.9
	100%	Below ground	130 ± 5	30 ± 0.9
		Above ground	104 ± 4	22 ± 0.7

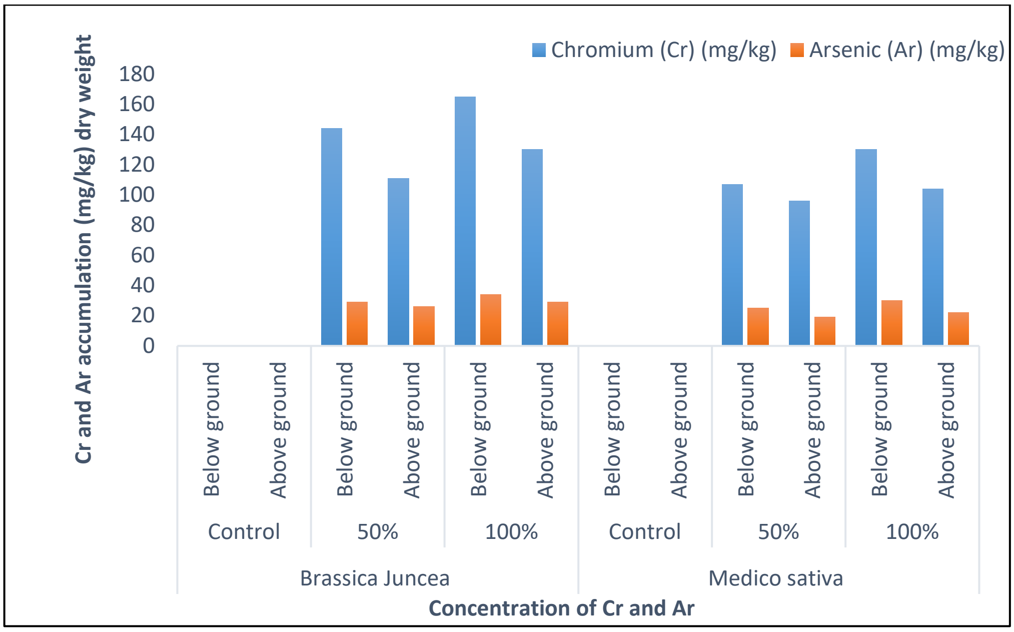

Fig. 7: Chromium and Arsenic Concentration in below ground vs. Above ground Parts.

This comparison shows that plant roots can accumulate metals much more efficiently and is, therefore, useful in phytoremediation of soils in which it is essential to minimize the movement of metals to other parts of the plant that are edible or can be harvested.

Biological and Anatomical Factors Influencing Metal Uptake

Several factors related to biological and anatomical aspects of the plant have a direct bearing on its ability to uptake and store heavy metals. This is made possible by the root morphology, which has a highly developed surface area and also penetrates deep into the soil to ensure maximum contact with the contaminated soil, hence increasing the absorption of the metals (Akcin et al. 2018). Through root surface area and root exudates that are substances secreted by the root tip, the absorption efficiency of the plant is enhanced by increasing the root or absorbing area and modifying the chemistry of the nutrient-avoiding layer about the root tips to increase the solubility and mobility of the metals, thus making them more absorbable.

Another factor is the ability of the plant to transport proteins that are located within root cells. These proteins assist in the transport and localization of heavy metals from the root cells' or membrane. Some of the metals are stored in the cell walls after they have been taken in by the plant, and this is effective in avoiding their transportation to other parts of the plant. Also, the cell walls of roots are much thicker as compared to stems, and this increases the availability of binding sites in the root system, hence increasing the uptake of heavy metals.

Additionally, the plant's ability to tolerate metals, including the synthesis of phytochelatins, small molecules that chelate to heavy metals and allow the sequestration of these metals into vacuoles, thus rendering them less toxic and permitting the plant to exist within metal-contaminated soils. These biological processes explain the plant's efficiency in growing in contaminated soil further accumulating metals and not getting metal stress.

Soil Organic Matter Estimation

Sample Collection and Preparation

To measure changes in soil organic matter, soil samples were taken from experimental pots containing plants at the beginning, middle, and end of the trial. Using a soil auger, soil samples were taken at depths of 0 to 15 cm. To eliminate debris and standardise the samples, the soil was air-dried, crushed, and sieved to a size of 2 mm after collection. Following processing, these samples were placed in airtight receptacles to facilitate additional analysis of their organic matter composition.

Walkley-Black Methodology

The Walkley-Black method, one of the most widely used techniques for estimating soil organic carbon, was employed to evaluate the organic matter content of the soil samples. This technique involves oxidising the organic carbon in the soil sample by adding a standard solution of potassium dichromate and sulphuric acid. To determine the amount of organic carbon, ferrous sulphate is used to treat the excess dichromate. Since the estimated organic matter content of soil is around 58% carbon, a factor of 1. 724 is used to calculate the percentage of organic matter.

Data Interpretation and Analysis

This made it possible to compare the amount of organic matter in the soil throughout the phytoremediation experiment with the Walkley-Black method's percentage of organic matter. To ascertain how Medicago sativa and Brassica juncea affected the amounts of soil organic matter, the data was analysed. To determine if the plants had an impact on the pace of degradation or had added biomass that contributed to the buildup of SOM, comparisons were done at the beginning, middle, and conclusion of the study. Variance analysis was one of the results examined in this field to ascertain variations in organic matter across different treatment settings. This contributed to the understanding of the general outcome of phytoremediation on the environment in enhancing the quality of the soil.

Impact of Organic Amendments on Metal Uptake Efficiency

The incorporation of organic matter such as compost significantly influenced the efficiency of uptake of the metals by the plants (Zulfiqar et al. 2023). Organic amendments enhanced the physical as well as the chemical associated with the soil fertility, including the organic matter fraction and the cation exchange capacity (CEC). By enhancing this property, better penetration of the metals in the root of the plant was made possible since they were made more bioavailable in the soil.

Amendments such as compost also bring in a population of organisms that catalyze the nutrient changes in the soil concerning heavy metals, making them bioavailable. On the other hand, given its high surface area and pore volume, where metals are adsorbed in forms that are more bioavailable to the plant roots. This enhanced the accumulation of metals, especially in the roots of the plants, without a high level of metal stress that would reduce the growth of the plant.

Table 9: Metal Uptake with and without Organic Amendments (mg/kg dry weight)

Treatment	Chromium (Cr) (mg/kg)	Arsenic (As) (mg/kg)
Without Amendments (100%) (Brassica juncea)	165 ± 6	34 ± 1.2
With Compost (Brassica juncea)	175 ± 7	38 ± 1.8
Without Amendments (100%) (*Medicago sativa*)	130 ± 5	30 ± 0.9
With Compost (100%) (*Medicago sativa*)	140 ± 5	35 ± 1.8

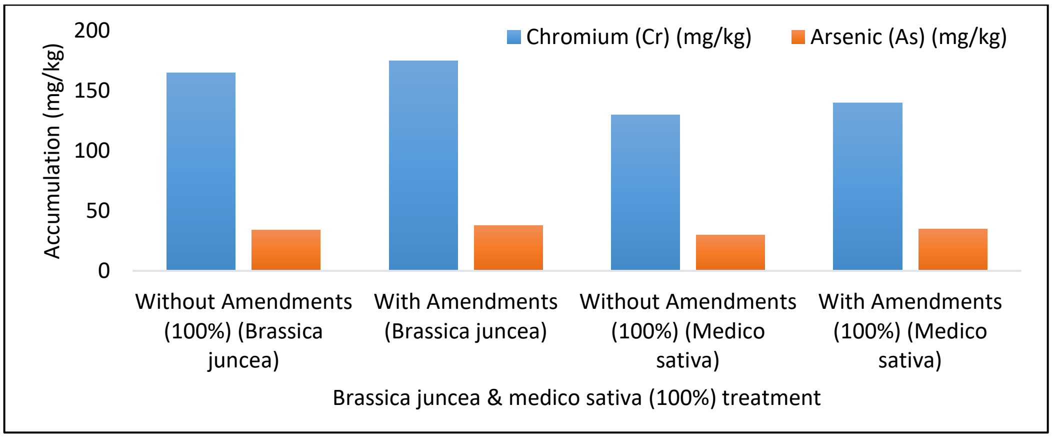

Fig. 8: Treatment of Cr and Ar Uptake with and without Organic Amendments (mg/kg dry weight)

From the data presented in the Table 9. it is evident that with the application of organic amendment, the overall absorption and accumulation of chromium and arsenic in the plant tissues was boosted. It was also evident from results that better metal uptake efficiency resulted in improved growth response and health of the plants, further stressing the role of organic amendments in enhancing phytoremediation processes. Fig. 8 shows the treatment of Cr and Ar uptake with and without Organic Amendments.

Impact on Soil Properties

In phytoremediation, it is important that the treatment process is monitored so that can assess the overall impact of phytoremediation besides the growth of plants and the removal of toxins from the soil. This is because during the process of phytoremediation, information on changes in levels of heavy metal concentrations, organic matter content, pH, and soil quality as a whole is monitored. Some of the plants include Medicago sativa and Brassica juncea, which alter the pH of the soil besides having an influence on the health of the soil and the availability of metals (Zhang et al. 2022). The toxicity of hexavalent chromium to soil microbial processes concerning soil properties and aging time. Also, these plants contribute to the content of the organic matter in the soil due to the release of the root exudates and the decomposition of biomass. To know the remediation process in the best possible way, the changes regarding these characteristics should be tracked. This would allow the evaluation of metal removal effectiveness as well as enhancement of the soil quality. Table 10. shows the impact on soil properties.

Table 10: Impact on Soil Properties

Parameter	Initial Value	Final Value (Post-Experiment)
Soil pH	6.8 ± 0.2	6.5 ± 0.3
Organic Matter Content	3.5 ± 0.2 %	4.0 ± 0.2 %
Moisture Content (Soil)	25± 2%	29 ± 2%
Cation Exchange Capacity	15 ± 1 cmol/kg	16 ± 1 cmol/kg

Data Collection and Analysis

Evaluating the success of established phytoremediation programs requires information accrued related to the program. An indication on the accumulation and intake of heavy metals, especially chromium (Cr) and arsenic (Ar), in tissues of the root, stem, leaf, and seed of plants is indicated from the concentration levels of the tissue (Ansari et al. 2021). The information from gathered samples is useful for the purpose of comparing various treatments concentrating on increasing the yield of valuable metals. Medicago sativa and Brassica juncea have been exposed to various treatments in order to investigate the effect of the treatments on the bioconcentration of metals in the tissues of plants. This information entails a significant contribution to the optimization of plants to use in the process to operate for the decontamination of soil and to find out other treatments that would make the process of phytoremediation work more effectively.

Soil pH Determination

Collection and Preparation of Soil Samples

Three separate times were used to gather soil samples from the experimental pots: before planting, during the trial, and after harvest. The results were extremely exact because the samples were taken in a grid pattern at depths of up to 15 cm. After that, stones and other undesirable particles were removed from the soil by air drying it, crushing it with a mortar and pestle, and sieving it through a 2 mm screen. For pH testing, the previously prepared soil samples were put in different, marked containers.

pH Measurement Techniques

A 1:2.5 soil-to-water suspension method was used in the lab to assess the pH. A clean beaker was filled with 10 grammes of dirt and 25 millilitres of distilled water. After thoroughly mixing the two solutions to incorporate all of the chemicals, the mixture was let to stand for half an hour. A calibrated digital pH meter was used to measure the mixture's pH after the solution had been added to bring it down to 10. Every sample was measured three times, and the mean value was calculated, in order to increase measurement reliability. This is a good method for estimating soil pH, which determines the status of metals in relation to their bioavailability and plant growth. Soil pH before planting and after harvest data indicated in Table 11. and depicted in Fig. 9.

Table 11: Soil pH before and after planting and harvest

Plant species	Treatment Code	Soil pH Before Planting	Soil pH After Harvest
Brassica juncea	B1(Control)	6.5	6.7
	B2 (50 %)	6.3	6.5
	B3 (100%)	6.4	6.6
Medicago sativa	M1 (Control)	6.5	6.7
	M2 (50%)	6.3	6.8
	M3 (100%)	6.4	6.6

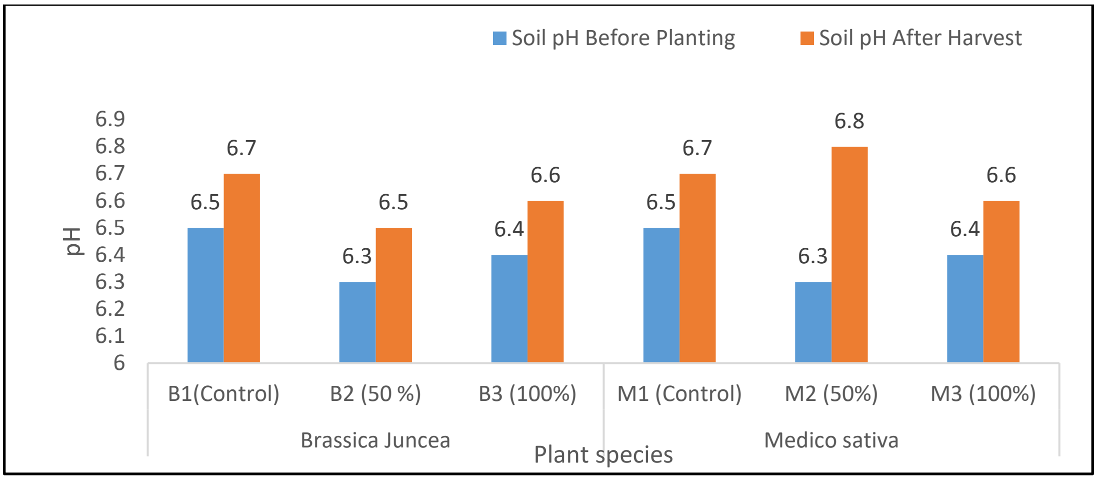

Fig. 9: Soil pH before planting and after harvests.

Comparison of Results with Other Phytoremediation Studies

The findings in this study are in concordance with studies done on the efficiency of metal uptake in hyperaccumulator plants. Several studies have supported this by showing the effectiveness of the plant in absorbing and accumulating heavy metal, especially in the root system (Sajad et al. 2020). These results affirmed the enhanced capabilities supported by organic amendments, which have been demonstrated by other research on the effects of organic amendments in the efficiency of bioremediation.

Discussion of Findings and Implications

The result of this study suggests that phytoremediation can be a viable technique for cleaning up contaminated soils with heavy metals like chromium and arsenic. The trends presented in the above results indicate that the plants, especially when combined with organic matter such as compost have the potential to uptake some of these metals, hence lowering the total contamination level in the soil.

This study has the most important implication of bolstering the position that organic amendments can actually improve the efficiency with which these metals are taken up by plants. The increased CEC and SOM in the soils enabled the plant to absorb a higher amount of metals without any inhibition of growth rate. The present study's result also supports earlier studies, which demonstrates the importance of organic amendments in enhancing phytoremediation results. The root structure and the transport proteins found in the plants found the biological and anatomical ability of plants to accumulate heavy metals in roots to be important. (Ullah et al. 2021). This makes the selected plant species very suitable to be used for remediating contaminated soils, especially where it is necessary that the metal does not translocate to the above-ground tissues, hence preventing polluting the food chain.

Discussion on Statistical analysis for Growth performance of Brassica juncea and Medicago sativa

In conclusion of statistical analysis, the current paper reveals the fact that dangerous heavy metals like arsenic and chromium that are found in water are very hazardous to human health. However, using indigenous plant species in phytoremediation comes out as a very viable solution. The purpose of this research is to identify the efficiency of different plants in other to perform a better study on how optimum phytoremediation can be achieved. Improving knowledge on these processes as well as the practices that can support their application can help to restore water and soil, or in other words, it contributes to the protection of the environment using statistical analysis tool like one way ANOVA the comparison of growth performance of three different concentration samples are selected and analysed. Observed values are analysed with static tool and identify the growth performance of Brassica juncea and Medicago sativa are detailed below:

All of the data were analyzed using a one-way ANOVA to see whether any differences in values were statistically significant. Measurements of tissues (leaf, stem and seeds) were studied. $p<0.05$ for one-way ANOVA. Plant height and growth were compared between the two plant species, Brassica juncea and Medicago sativa, using a one-way ANOVA with repeated measurements. For plant species 1, the F-ratio is 5.75488, the standard deviation is 45.7, and the 50% average concentration of Brassica juncea is 38.75%. The F-ratio is 5.73639, the standard deviation is 52.8, the p-value is 047541, and the concentration of 100% of the average value is 44.7%. There is a p-value of.047811. Medicago sativa plant species 2 had an average concentration of 38.9% at 50%, a standard deviation of 33.5, and an F-ratio of 6.77544. The F-ratio is 6.1133, the standard deviation is 40.9, the concentration of 100% of the average value is 35.8%, and the p-value is.035272. A p-value of.042676 is found. Brassica juncea accumulates more Ar and Cr throughout time.

Large-scale applications and potential ecological impacts - *Brassica juncea* and *Medicago sativa*

Brassica juncea and Medicago sativa plant species is cheaper, friendly to the environment, and easy to manage metal pollution in industrial areas when used with organic soil amendments. The methods hitherto employed for undertaking soil remediation include physical removal by digging or use of chemicals, both of which are expensive and environmentally unsustainable. On this side of the argument, the process of phytoremediation appears to be an environmentally friendly technique with prospects of further application.

Environmental management

The results obtained in the course of this study have a number of implications for the implementation of phytoremediation and other related environmental initiatives. This study establishes how Medicago sativa and Brassica juncea have the potential to uptake heavy metals such as lead, cadmium, and zinc from contaminated earth and, in the process, present an economic and efficient method of soil remediation.

Practical Applications:

Utilization in Contaminated Sites: The results clearly reveal the prospect of these plant species for utilization in contaminated agricultural land, industrial waste, and polluted city areas (Sharma et al. 2018). As such, the process does not disrupt the ecosystem as much as when people employ excavation and various chemical treatments. Environmental managers can, therefore, incorporate the use of such plants whenever the level of contamination is average to draw down the costs of cleaning the soil.

Advancement in Green Technologies: This study indicates that phytoremediation with Medicago sativa and Brassica juncea catalyzes with the current trends for green approaches. Because they can bind metals without synthetic chemicals, they can now be marketed as environmentally friendly technologies for clean-up; thus, they are suitable for future green projects.

Potential for Reforestation and Land Reclamation: These species can be put into the reforestation programs, especially in the post-mining or post-industrial sites with high concentrations of heavy metals. This effectively means that the plant can be useful in the restoration of damaged soil that will in turn be useful for reforestation projects since it can spur the growth of vegetation and push for a complete rehabilitation of affected ecosystems.

Incorporation into Environmental Policies: Based on the study's results, regulatory bodies and environmental agencies should formality include the process of phytoremediation as a strategy of managing the environment. This could culminate in the setting of rules and policies concerning the utilization of plant-based remediation, hence encouraging industries to undertake environmentally friendly remedial methods.

Enhancement of Remediation Programs: Therefore, phytoremediation can effectively provide an affordable solution for dealing with other remediation programs, especially in scenarios whereby other repair strategies may be economically unfeasible and destructive to the environment.

Sustainable Development Goals (SDGs): The two plants used in phytoremediation, that is, Medicago sativa and Brassica juncea, affect more than one of the SDGs that include sustainable management of water resources and life on land.

It is recommended that large-scale field trials and research on phytoremediation involving *Medicago sativa* and *Brassica juncea* be conducted, focusing on exploring the long-term viability of using these plant species in large-scale applications, as well as assessing their potential ecological impacts. Such trials are essential for testing the effectiveness of various phytoremediation techniques, allowing researchers to gather valuable data on the suitability of these methods across different soil types and climatic conditions. This research could significantly enhance understanding and improve the practical application of phytoremediation strategies in diverse environmental contexts.

Thus, based on the practical findings of the current study, it is appropriate to state that the plant is efficacious to remediate a huge amount of material and contaminated soils and water bodies, particularly the industrial stretch (Prasad et al. 2021). This is because the plant is able to accumulate an amount of metal in its root, which is rather useful in remediation of soil contaminated with chromium and arsenic. Further, it is evident that the practical application of the organic amendments such as compost, is very advantageous for the process of phytoremediation due to the outcome of the current study. Other than improving the quantity of organic matter and adjusting the pH of the soil, they aid plant growth and give the plant a chance to absorb more of the metal.

Phytoremediation on industrial areas

There are significantly more green belt regions at the specific industry site. Depending on the water's features and industrial effluents, the plant species should be chosen, and phytoremediation techniques can be used in those specific locations. Through the careful selection of plant species that are appropriate for certain pollutants and the integration of many remediation processes, phytoremediation provides a sustainable approach to contamination management. Different pollution levels can be effectively treated with this approach. Incorporating community stakeholders into the process also increases support and improves the overall efficacy of remediation initiatives. In the end, this method turns polluted areas into healthy ecosystems by fostering environmental resilience and regeneration.

CONCLUSIONS

This study was useful to prove that the selected plant species is very efficient on the phytoremediation, which was supported by the capacity to accumulate and deposit a respectable amount of chromium and arsenic within the root structures. As mentioned in the experiment, it is clearly evident that the plant has the ability to adapt to the contaminated environment: the plant has good growth and biomass production in a lower concentration of contaminants. The study identified that the major source of heavy metals is the root system, where the authors discovered high levels of chromium and arsenic, especially in the root as compared to the stems, leaves, and seeds. Furthermore, the incorporation of organic matters like compost enhanced the efficiency of total metal uptake. About these amendments, it was possible to state that they were helpful in improving the properties of the soil for growing crops and increasing the content of organic matter and slightly decreasing the pH of the soil in order to increase the availability of heavy metals. As such, the results of this study showed that plants that have been treated with organic amendments have higher accumulations of chromium and arsenic in tissues than plants that were not treated with organic amendments. The results to up the effectiveness of phytoremediation and health and growth responses of plants under contaminated environments, implication for applied phytotechnology: A multiple successional species approach to the use of organic amendment has been recommended, noting that this increases and boosts the overall health of the plant and growth performance.

Recommendations for Future Research Directions

Subsequent research should, however, focus on increasing the utilization of this plant species in various areas. It is essential there should be further studies of phytoremediation made in the field instead of the laboratory, as this study will help to realize the efficiency of the plant in natural ways where several factors, such as climate and types of soil, may influence the plant. Additional studies should also be conducted to identify how the efficiency of the plant in uptake of the metals can be enhanced through genetic manipulation. Thus, phytoremediation could be even more effective and more used if only it had higher tolerance to higher contaminant levels and better accumulation ability.

Recommendations for policymakers and environmental practitioners

For policymakers and environmental practitioners, several steps can be taken to advance the application of phytoremediation:

When these recommendations are applied, it will be possible for policymakers and practitioners to contribute significantly to enhancing the application of phytoremediation as a typical environmental bioremediation process for ecological restoration and improvement of human health.

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