Original Research Article

A COMPARATIVE STUDY OF PHYTOREMEDIATION OF HEAVY METALS IN SOIL USING BRASSICA JUNCEA, AND GREEN GRAM

Abstract

With the increase in Industrialization and urbanization, the pollution by heavy metals such as arsenic, cadmium, chromium, etc accumulates in water and soil rapidly. As the heavy metals are non-biodegradable and toxic, they accumulate in the environment causing devastation to the living organisms thereby. Few heavy metals have got carcinogenic and mutagenic properties at lower concentrations. Phytoremediation is the process by which plants are used for remediating contaminated sites. Phytoremediation is a “Green Technology” that is a cost-effective way to mitigate measures and revegetate heavy-metal polluted soil and water. More than 400 species of plants have been found to remediate heavy metal contamination sites as they have got high tolerance to heavy metals. Brassica juncea and Green gram are found to have high heavy metals tolerating capacity. But plants that uptake heavy metals are slow growing and produce very little biomass and they uptake very little heavy metals in a short span. Silica obtained from coconut husk and paddy straw is also found to have heavy metals absorption properties. When silica is fused along with the Phyto remediating plant, the efficiency of the process increases. Silica powder is used in soil, while silica gel is made to float in hydroponics. The remediation capacity is measured using Atomic Adsorption Spectroscopy. The study is comparative to find the efficiency of plants infused with silica in up taking heavy metals. The remediated plants are further used for the production of Biofuels.

Keywords: Heavy metals, Phytoremediation, Brassica juncea, Green gram, silica

Introduction

Soil pollution has become a significant issue in many countries due to increased industrialization (Bolan N et al.,2016). Numerous studies have claimed that industrial activity such as fast urbanization, and population increase have contaminated the environment and negatively impacted the quality of the air, water, and soil. Heavy metals rank first among the most important soil pollutants coming from both natural and man-made sources because of their long-term harmful effects (Ankur et al., 2015). In regions with high industrial activity, soil metal content is typically seen to increase. These places have a few times more metal build-up than pristine sites (Wang P et al.,2006). Heavy metals, unlike some biological compounds, do not degrade over time, and while they are essential to life at some levels, they become poisonous when their levels exceed the limit values (Erik et al.,2006). It is recognized that heavy metals can be carcinogenic, teratogenic, poisonous, or lead to cardiovascular issues when they are ingested into humans at concentrations greater than the limit values recommended by the World Health Organization (WHO) (Zheng YM et al., 2005). As a result, metal pollution in places where there are agricultural operations is a serious concern(Peng RH et al., 2021). Heavy metals, unlike other organic materials, do not decompose over time, even though they are necessary for life at particular amounts (Qiao M et al.,2001). Some techniques, like soil cleaning, have a negative impact on biological activity, soil structure, and fertility, while some demand expensive engineering (Gamerdinger AP et al., 1999). Therefore, the low-tech, in-place method of phytoremediation is appealing since it provides site restoration, partial decontamination, maintenance of the biological activity and physical structure of soils, is possibly affordable, unsightly, and there is a chance for bio-recovery of metals. Phytoremediation is a new method that has great potential for remediating and recovering contaminated environments(Poon CS et al 2001). The ongoing need for contaminated site cleanup in both developed and developing countries necessitates critical, serious, and rapid study of phytoremediation as a cost-effective, promising, and novel environmental technological solution (Agrawal M et al., 2007). Phytoremediation, or the use of plants or plant products to stabilise or restore contaminated environments, involves three major metal-remediation strategies: phytoextraction rhizofiltration, and phytostabilization(Steinnes E et al.,2006).

Mining, industry, and agriculture have accelerated the release of metals into ecosystems, causing serious environmental problems and posing a threat to human and animal health (Yang L et al., 2010). Excessive metal concentrations in contaminated soils can degrade soil quality and possibly contaminate the food chain. Cadmium (Cd), Chromium (Cr), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni), and Zinc (Zn) are the most common heavy metals found at hazardous waste sites.Industrial wastes have the potential to contaminate the groundwater and soil (Zhou Q et al., 2010). Around the world, the tanning business is viewed as a potential contributor to environmental pollution (Pacyna JM, 1986). An important component of tannery waste is the poisonous heavy metal chromium (Cr), whose build-up in soil and water has raised considerable environmental concerns in India, notably in Tamil Nadu (Hanssen JE et al.,1987).

Chromium (Cr) is one of the most toxic and dangerous metals in the environment. In addition to its natural occurrence, Cr is released into the environment through a variety of industrial activities such as electroplating, tanning, polishing, painting, pigment manufacturing, and wood preservation(Avudainayagam S et al.,2018).Higher quantities of these metals can cause metabolic abnormalities and growth suppression in most plant species, both of which can result in mortality.The degree of metal absorption by plants varies greatly between species and plant regions (Juste C et al., 1994). Several studies have found that arsenic and chromium levels in roots are higher than in leaves or stems.Though phytoremediation, using bioamendments that use harvestable parts of plants to remove toxins, provides a green and environmentally acceptable way for cleaning metal-contaminated soils and rivers, there is a risk of food chain contamination if edible crops are used for the purpose. Farmyard manure, coconut husk silica powder, poultry manure, press mud compost, and biochar have all been shown to minimize hazardous pollutants in soil environments (Ahmed R et al.,2017).These bio amendments provide the opportunity to increase pH, specific surface area, porous structure, surface functional groups, and the ability to be used as an adsorbent to immobilize heavy metals in soil.Pollutants are taken from plant roots and transported to shoots or other aboveground biomass such as stems, leaves, and fruits via phytoextraction (Bolan Net al.,2016).

Numerous Brassica species have been examined as possible phytoextraction plants because they are known metal accumulators (Sathya V et al.,2022). Brassica plants have the potential to be used in phytoremediation due to their inherent resistance to heavy metals and high above-ground biomass output (Peterson E et al.,2003). It grows quickly, is resistant to heavy metals, and yields a significant amount of above-ground biomass. These traits have made this species the subject of numerous research to assess its potential for phytoremediation (von Lützow M et al., 2015). However, it has been shown that the amount of oil generated by these plants is decreased when heavy metals, such as cadmium, are present. Bioremediation of chromium and Cadmium contaminated soils is a well-known procedure that uses biological wastes to detoxify or transform toxic Cr and Cd into less hazardous forms (Jackson M (1973)). Despite its limitations, this technology retains appeal due to its low cost. We investigated the ability of specific crops (Brassica juncea and green gram) and biological wastes (Silica powder from coconut husk) to remediate contaminated soil (Le Wang L et al.,2021).

.Brassica species have demonstrated a comparatively high level of resistance to this abiotic stress, although the precise mechanism underlying this resistance is still unclear (Black IA et al.,1934) . In this study, we will assess the most current findings about the mechanisms that Brassica plants use to accumulate and tolerate heavy metal stress.A food that is high in protein is the green gram (Heimlich R et al.,2021). It has a protein content of roughly 25%, which is about three times that of grains (Ali A et al.,2021). It is often referred to as a productive green manure crop (Brooks RR et al.,2006). The goal of this study was to investigate into the phytoextraction capability of Brassica juncea and Green gram with different bioamendments such as silica powder from coconut husk to remediate Cr and Cd from polluted soil (Kimble JM et al.,1976). Furthermore, the goal of this study was to rehabilitate contaminated locations by determining suitable Phyto tolerance modifications, Cr accumulation, and translocation capacity to aerial sections of Brassica juncea and greengram (Chowdhury S et al.,2011). The goal of the current research work was to understand the toxicity of heavy metals in Vigna radiata leaf and how to treat them (Sheikh Abdullah S.R et al., 2006). Fig 1 (Graphical abstract).

Materials and methods

Description of the study area and initial Characteristics of soil

The effect of several bioamendments and plants on Cr and cd bioavailability was investigated in a pot culture experiment at the National Agro Foundation Research and Development Centre in Chennai, India (12°.98′84.01″ N, 80°.22′26.4″ E). To test metal accumulation and phytoremediation of Cr and Cd-contaminated soils, two plant species, Brassica juncea, and green gram, were planted. According to the treatment schedule, silica powder was administered and uniformly blended. A specified amount of water was given to each pot on a regular basis to compensate for moisture loss. Soil and plant samples were taken at the end of the experiment for various analyses. The physical properties such as bulk density was analysed using the wet cylinder method (Kotrba et al., 2009). And physicochemical properties such as soil reaction (pH) were analysed using potentiometry-soil water suspension of 1:2:5 ratio, Electrical conductivity (EC) was analysed using conductometry-soil water suspension of 1:2:5 ratio, and the chemical properties such as Organic carbon was analyzed using chromic acid wet digestion method, available nitrogen was analyzed using Alkaline permanganate method, Available Phosphorous was analysed using Calorimetry method (Olsen 1954), Available Phosphorous was analyzed using Neutral normal ammonium acetate method (Kotrba et al., 2009), Exchangeable Ca and Mg were analyzed using Neutral normal ammonium extract-versenate titration method, the total Cr content was analyzed by digestion with aquaregia and available Sulphur was analyzed using 0.15 percent $Cacl_2$ Turbidimetry method (Le Wang L et al.,2021).

Methods used to analyse the parameters of soil

Soil Sample Collection and Analysis

Soil samples were collected from the pot culture experiment at fortnightly intervals on the 0th, 10th, 15th, 30th, and 40th days. Soil samples were collected and dried for 2-3 days before being powdered, sieved (2 mm sieve), sealed in a polyethylene cover, and analyzed. pH was determined using a pH meter (Jackson 1973), EC using a conductivity meter (Jackson 1973), and organic carbon using a wet digestion method (Walkley and Black 1934),total Cr content digestion with aqua regia (USEPA 1979), and speciation of Cr by the sequential extraction method (Noble and Hughes 1991).

The important characteristics of silica powder are listed in Table.1. Initially 200 g of soil was taken in plastic pots and the 5g silica powder was added to the soil uniformly according to the treatment. Then 2.83 g of $k_2cr_2o_7$ was added in 1000 ml distilled water to make a Cr stock solution of 1000ppm. Similarly 2.83 g of $Cdcl_2$ was added in 1000 ml distilled water to make a Cd stock solution of 1000 ppm.

The soil in the pot was then treated with a 500-ppm aqueous solution of $K_2cr_2o_7$ and 500 ppm of an aqueous solution of $Cdcl_2$. Following that, two seedlings of local types obtained from a nearby nursery were placed into each container. Finally, watering and weeding were done as needed. The experiments were replicated three times in the order in Table 3:

Plant Collection and Analysis

On the 40th day, the plants were harvested and split into roots, shoots, and leaves for analysis. The samples were dried in a 65 °C oven to achieve constant weight, and the dry weight of each component was recorded. Finally, the dried plant samples were crushed and digested in 10 mL with a di-acid mixture (9 HNO3:4 HClO4), and the Cr and Cd concentration were measured using an atomic absorption spectrophotometer (Varian Spectra AA220). Plant height (cm), root length (cm), and shoot length (cm) were all measured.

Bioconcentration Factor (BCF) and Translocation Factor (TF)

BCF is the ratio of Cr and Cd content in plant tissue to soil concentration. The plant's propensity to acquire heavy metals is referred to as BCF. BCF>1 implies substantial metal accumulation in the shoot. The ratio of a plant's ability to extract heavy metals from the root to the shoot is expressed as TF, TF shows heavy metal build-up in the root and vice versa.

BCF=Cr in the roots (mg kg−1)/Cr in the soil (mg kg−1).

TF=Cr in stover/stalks (mg kg−1)/Cr in roots (mg kg−1) (mg kg−1).

EF=Cr in stover/stalk (mg kg−1)/Cr in soil (mg kg−1) (mg kg−1), similarly values are calculated for Cd contaminated soil.

Evaluation of chlorophyll content

Leaves were collected separately from the seedlings grown in control and heavy metal contaminated soil. The leaf tissues where placed in a glass beaker with 8 ml of acetone and 2 ml of ethanol for 18 hours in darkness. After 18 hours, the chlorophyll a and chlorophyll b was analysed at 665nm and 649nm respectively using UV spectrophotometer and absorbance were noted as mentioned in the formula for the estimation of chlorophyll content as,

$C_a=13.95A_{665} – 6.88A_{649}$

$C_b= 24.96A_{649} -7.32A_{665}$

Calculation of germination percentage, vigour index

The seedling which had the maximum length was taken out gently without disturbing the plants.Roots by adding water to the soil. The roots were washed using distilled water. The seedlings were kept on a paper and the root length and shoot length was measured by using 30mm scale. Germination percentage was calculated by initially counting the number of seeds sprouted after being sown.

(Number seeds sprouted / Total number of seeds sown) x 100 = Germination %.Thevigor index was also calculated by using the below formula.

Vigor index= Germination % × mean of seedling length (root + shoot)

Statistical analysis

Statistical software SPSS Version 22 was used to analyze experimental data from the experiment to identify the effect of treatments and factorial completely randomized design (pot culture experiment). The F test employed a significance level of 0.05. Panse and Sukhatme proposed and calculated the variance analysis (1967). A simple Pearson correlation analysis was also performed to examine the link between soil characteristics and crop yield when treated tannery effluent at various dilutions was combined with amendments (Blyth 1994).

Results and Discussion

Characteristics of Soil and Bioamendments

The experimental soil had a pH of 7.01 and an EC of 0.642 dS m^{-1}, and the other characteristics are listed in Table 2. After the addition of Bioamendments the pH of the soil varied between 6.03 and the EC of the soil varied between 0.668 dS m^{-1}. The organic carbon of the bioamendments added soil was found to be 6.06%. Physiochemical characteristics of bioamendments used is given in Table 4.

Changes in the pH of soil after the addition of Bioamendments

The adsorbent properties and ionization level of metal ions in aqueous solutions are affected by the pH of the solution. The pH of the soil was significantly modified as a result of the addition of bioamendments. In soil with Brassica juncea and Green gram, the pH ranged between 7.34-7.45 and 7.06-8.01. The most significant factor that affects plant heavy metal uptake is soil pH. According to this study, the pH of the soil was alkaline in the control soil and soils with lower quantities of heavy metals in all the treatments. The pH increased as the metal content increased. The pH of the soil gradually increased in the crops from 0 to the 40th day. The bioamendments had a significant effect on soil pH. Initially, in brassica juncea, the pH of the chromium-contaminated soil was 6.099, and cadmium contaminated soil the pH of the soil was 6.134. After the addition of bioamendments, the pH of the soil was found to be 6.99 and 7.21 respectively. Similarly, in soil remediated by Green gram, the initial Ph of the soil before the addition of amendment was found to be 6.099 in Cr-contaminated soil and 6.134 in Cd-contaminated soil. After the addition of amendments, the Ph value increased by 6.98 and 7.13 respectively. As the pH rises, the soil becomes alkaline, and metal ion bioavailability falls. Lower pH is good for metal availability but bad for plants (Hutchinson et al. 2007).Effect of Bioamendments on pH of Cr and Cd contaminated soil with Brassica juncea and Green gram is given in Fig 2**.**

Changes in Soil Electrical Conductivity (EC) After Bioamendments Addition

EC ranged from 0.311 to 0.301 in the soil during the experiment with Brassica juncea and in the experiment with Green gram, the EC ranged between 0.311 to 0.309. EC value increased with the presence of heavy metals, but in the trials after being remediated by plant and soil, the EC value showed a gradual decrease in the soil, enunciating the effective remediating capacity of the plant and the Bioamendments.Effect of Bioamendments on EC of Cr and Cd contaminated soil with Brassica juncea and Green gram is given in Figure 3.

Estimation of Organic Carbon in the soil after the addition of Bioamendments

Soil Organic Carbon (SOC) has been demonstrated to have a significant influence on SOC in soil bioamendments. Initially, the SOC was found to be highest (0.26%) in the silica-amended soil and lowest (0.13%) in the control soil (T1). Organic carbon bioamendments are injected into the soil and interact with microorganisms to supplement and make available other nutrients to plants (Barajas-Aceves 2005). However, in the Brassica juncea, the maximum SOC (0.71%) was recorded in the soil amended with silica powder (T2) and the smallest (0.46%) in the control soil (T1). Similarly in Green gram the maximum SOC (0.75%) was recorded in the soil amended with silica powder and in control the smallest (0.49) was recorded. As a result, silica powder is more likely to act as a soil conditioner and nutrient transformation catalyst than as a primary source of nutrients (Lehmann et al. 2003). It is consistent with the findings of Wiesmeier et al. (2015), who observed a significant decrease in SOC of the the organic manure-amended soils due to the C mineralization and its respective losses.

Changes in Water-Soluble Chromium and Cadmium: Brassica Juncea

Chromium is water soluble, and the use of bio-additions resulted in significant variations in the concentration of water-soluble Cr and Cd. Water soluble Cr ranged from 500 ppm in control and 260 nm in the soil remediation with brassica juncea along with silica powder. And in Cd-contaminated soil, the initial value of water-soluble Cd in control was found to be 500 ppm and, in the soil, remediated by brassica juncea along with silica powder the value of Cd was found to be 280 ppm. The concentration of H_2O-soluble Cr and Cd steadily reduced from the 0th to the 40th day.

Changes in Water-Soluble Chromium and Cadmium: Green gram

The use of bioamendments resulted in significant variations in the concentration of water-soluble Cr and Cd. Water soluble Cr ranged from 500 ppm in control and 280 nm in the soil remediation with green gram along with silica powder. And in Cd-contaminated soil, the initial value of water-soluble Cd in control was found to be 500 ppm and, in the soil, remediated by Green gram along with silica powder the value of Cd was found to be 290 ppm. The concentration of H2O-soluble Cr and Cd steadily reduced from the 0th to the 40th day.

Biotransformation and Bioavailability of Cr in Contaminated Soil

Chemical bioavailability is a current notion to reduce chemical availability even when in touch with soil. According to Favas et al. (2011), availability is affected by soil origin, mineralogy, characteristics (physical and chemical), and edaphic variables. Metal speciation describes the several forms in which an element can be found. As a result, understanding metal speciation in soils is critical for better understanding its transit and interaction. Diverse water sources and amendments influence Cr and Cd speciation in the current study via variations in soil chemical properties and metal adsorption, complexation, and chelation. Choppala et al. (2016) observed surface complexation or adsorption onto soil particles to change the water-soluble fraction into fractions with poorer solubility.

Effect of Cr and Cd on Brassica juncea and Green gram height and root length

Heavy metal phytotoxicity and severe sterility of contaminated soils were the principal limiting factors for plant development (Rotkittikhun et al. 2007; Norwood et al. 2013). Brassica juncea has a plant height of 5 to 8 cm. The highest plant height, 8 cm, was reported at T1 (Uncontaminated soil), followed by T3 (Cd contaminated soil), while the lowest plant height, 5 cm, was recorded in T2 (Cr contaminated soil). In Green gram, the plant height ranges from 18.5 cm to 29.3 cm. The highest plant height, 29.3 cm, was reported at T1 (Uncontaminated soil), followed by T3 (Cd contaminated soil), while the lowest plant height, 18.5 cm, was recorded at T2 (Cr contaminated soil). Plant growth is affected by the uptake of heavy metals. heavy metals hinder the uptake of essential nutrients from the soil such as sulfur, boron, phosphorous, etc decrease the height of the plants. The no. of seedlings was found higher in T1 in both plants and low in T2 in both plants. This signifies the uptake of Cr to be higher compared to Cd by the plants. Vigour index of Brassica juncea is given in Table 5 and Vigour index of Green gram is given in Table 6. Pictorial representation of the calculation of Vigour index of Brassica juncea is given in Fig 4 and that of Green gram is given in Fig 5

Estimation of Chlorophyll content

The chlorophyll contents were removed from the leaves which turned the acetone ethanol mixture green in color, the chlorophyll content decreased in T2 as the metal uptake by plants hinders the supply of nutrients to the plants, thus affecting the chlorophyll content. In Brassica juncea, chromium-contaminated soil the chlorophyll content ranged between 3.1862 and 2.1743 in Green gram the chlorophyll content ranged between 3.2034 and 2.1976. The chlorophyll content of plants is given in Table 7

Estimation of Sulphur content

The following results were obtained for the sulphur content in the soil with heavy metals using UV spectrophotometer for green gram and Brassica juncea and is given in Table 8.

There is not much decrease in the concentration of sulphur in both the plants. There are no significant changes in the concentration of sulphur content in the soil. The plants uptake the heavy metals and trace amount of sulphur are taken up by the plants. Sulphur is the important macro nutrient required for the plant’s growth. But due to the presence of heavy metals in plants it inhibits the uptake of organic matters which lead to the poor growth. The concentration of sulphur has reduced from 24.7% in T1 to 23.1% in T2 in brassica juncea, while the concentration decreases from 24.5 in T1 to 24.0 in T2 in green gram.

Estimation of Phosphorus available in soil

The following results were obtained for the phosphorus content in the soil with heavy metals using UV spectrophotometer for the green gram and Brassica juncea and the Phosphorous content in the soil is given in Table 9.There is no significant change in the concentrations of phosphorous in the soil. The heavy metals hinder the uptake of phosphorous by the plants. Phosphorus is essential in plants for absorbing, storing, and converting solar energy into biomolecules such as adenosine triphosphate (ATP) that drive biological activities from germination to maturity. In Brassica juncea the Phosphorous uptake by plants ranges from 2.09 in T1 to 2.0 in T3, while in Green gram the Phosphorous content ranged from 2.09-2.06, demonstrating no significant change in its uptake by the plants.

Estimation of Boron available in the soil

The following results were obtained for the boron content in the soil with heavy metals using UV spectrophotometer for green gram and Brassica juncea. The boron content in soil is given in Table 10.Boron content in the soil decreases by 10%. Boron is the essential nutrient required by the plants in their healthy growth. But since plant uptakes a lot of heavy metal it hinders the uptake of boron by the plants.

Estimation of Calcium, Magnesium, Sodium and Potassium

The following results were obtained for the Calcium, Magnesium, Sodium, and Potassium content in the soil with heavy metals using atomic absorption spectrophotometer for the green gram and Brassica juncea. The calcium, sodium and potassium content in the soil grown with green gram is given in Table 11 and calcium, sodium and potassium content in the soil grown with Brassica juncea is given in Table 12.

There are no significant changes in the concentration of Calcium, Magnesium, Sodium, and potassium in the soil for both plants. Due to heavy metal uptake by the plants and silica, it resists the adsorption of these micro elements that is required for the healthy growth of the plant. Hence the growth of plants is affected.

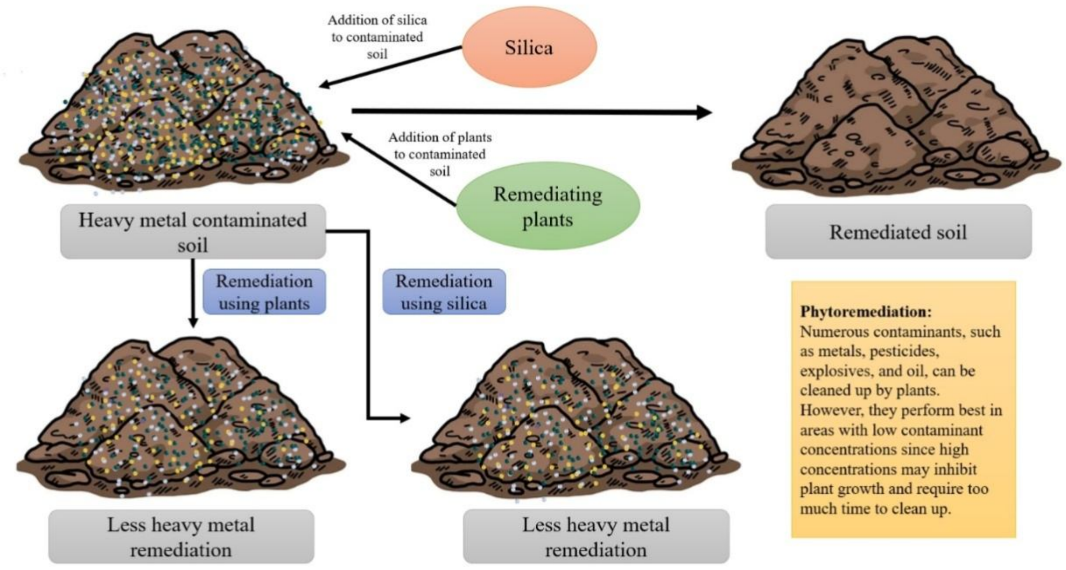
Heavy metal content in Brassica juncea and green gram

The remediation efficiency of Brassica juncea and Brassica juncea with silica is given in Table 12 and the remediation efficiency of Green gram and Green gram with silica is given in Table 13**.**Graphical representation of the remediaiating capacity of Brassica juncea and green gram along with silica is given in Figure 6.

The heavy metal uptake by brassica juncea is more efficient than green gram. The concentration of heavy metals has decreased by 30% by Brassica juncea and 50% by Brassica juncea and silica. The chromium content in soil is greater than Cadmium and Nickel. Green gram reduces the heavy metal by 20% and 40% with silica.Pictorial representation of the overall process of Remediation by Brassica juncea and Green gram along with silica is given in Figure 7.

CONCLUSIONS

Heavy metal pollution in soil, water, and air is a serious concern these days and requires immediate action against its remediation. As phytoremediation is a cost-effective and natural method, steps are taken in using plants as a remediating agent. Many plants such as vetiver, green gram, asola, sunflower etc have got more tolerant capacity towards heavy metal Effective remediation strategies are required because soil contamination is a major global issue. Phytoremediation is a solar-powered, environmentally friendly technology that is well-liked by the locals. In the near future, it is anticipated that heavy metal phytoextraction will be a financially viable method for agromining. Improved knowledge of how heavy metals are absorbed by plants from soil will also help to advance agromining, which can be utilized to extract metals even from biomass from harvestable plants. Comparing the remediation study between Brassica juncea and green gram, Brassica juncea proves to have better-remediating capacity than green gram. In this study, Cr was found to be in greater concentration in the soil sample, followed by Cd. These three metals were present in greater concentration. Silica proved to have more remediating capacity than the plants. More than 50% of the metals were remediated by the plants with silica. One of the most crucial processes for the tolerance of Brassica species appears to be the chelating of heavy metals. An effect of metal toxicity that is frequently mentioned is an increase in citrate levels, and this appears to be a key factor in these plants' ability to tolerate heavy metals. This is because this organic acid may be involved in chelating the metals, which facilitates their translocation and accumulation in the shoots and lessens their toxicity. This conclusion is supported by the evidence that exogenous application of citrate to a contaminated medium enhances both plant tolerance and heavy metal uptake. Many people believe that phytochelatins have a significant role in certain Brassica species' ability to resist the toxicity of heavy metals.



Phytoremediation: Numerous contaminants, such as metals, pesticides, explosives, and oil, can be cleaned up by plants. However, they perform best in areas with low contaminant concentrations since high concentrations may inhibit plant growth and require too much time to clean up.

Fig 1 Graphical abstract of the phytoremediation of Contaminated soil using plants and Bioamendments

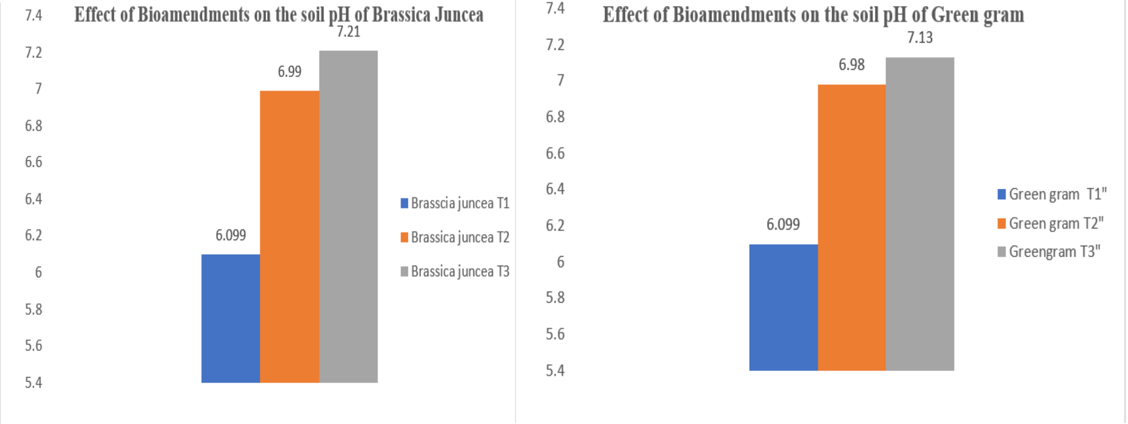


Fig 2 Effects of Bioamendments on the pH of Brassica juncea and Green gram

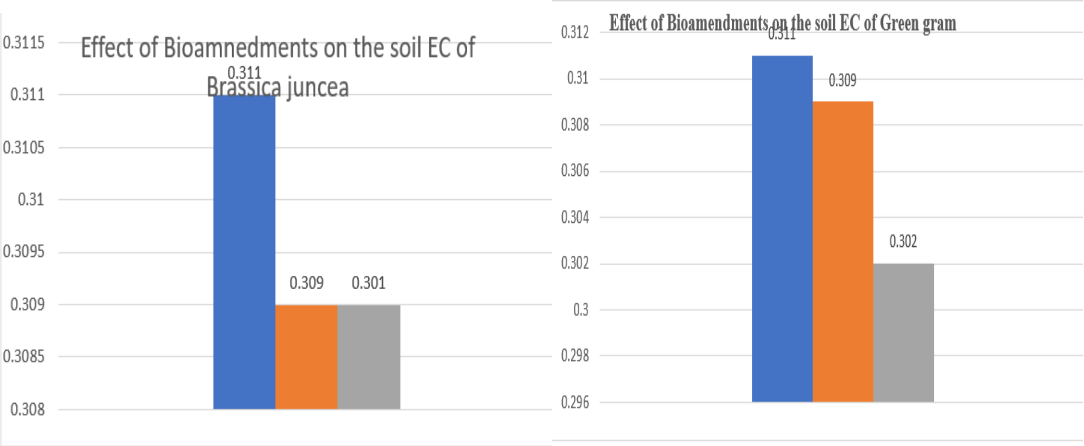


Fig 3 Effects of Bioamendments on the EC of Brassica juncea and Green gram

Fig 4 Calculating vigour index of Brassica juncea

Fig 5 Calculating vigour index of Green gram

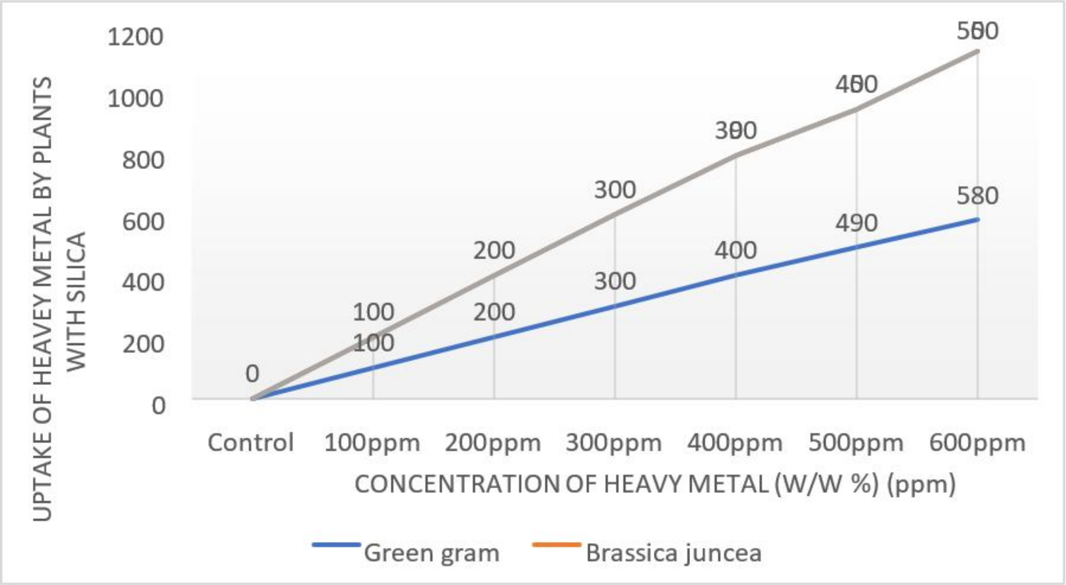


Fig 6 Graphical representation of the remediaiating capacity of Brassica juncea and green gram along with silica

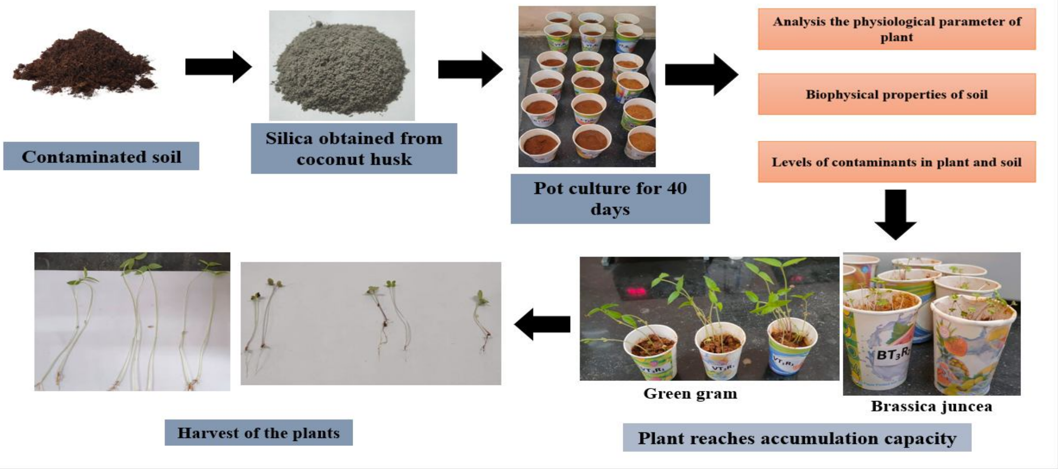


Fig 7 Pictorial representation of the overall process of Remediation by Brassica juncea and Green gram along with silica

Table 1: Methods used to analyse the parameters of the soil

Parameters	Method
Total N	Diacid extract (5:2- H2SO4 : HClO4)- semi automatic kjeldahl distillation method
Total P	Triacid extract (9:2:1- HNO3: H2SO4 : HClO4) Vanadomolybdate yellow colour method
Total K, Na	Triacid extract- neutralized with ammonia- Flame photometer
Total Cr	Atomic Absorption spectrophotometer (triple acid extract)
Total S	Diacid extract (9:4- HNO3: HClO4)- Turbidimetric method

Table 2 Characteristics of initial soil

Parameters	Unit	Value
Soil type	-	Clay loam
Bulk density	mg m^{-3}	1.05
pH	-	7.0±0.15
Electrical conductivity	dSm^{-1}	0.540±0.01
Organic matter	%	0.94±0.02
Nitrate Nitrogen	mg kg^{-1}	64.6±1.06
Available phosphorous	mg kg^{-1}	22.63±0.20
Exchangeable potassium	mg kg^{-1}	425±4.90
Exchangeable calcium	mg kg^{-1}	2605±5.42
Exchangeable magnesium	mg kg^{-1}	820±4.66
Available sulfur	mg kg^{-1}	44.5±0.89

Available manganese	mg kg^{-1}	9.31±0.20
Available copper	mg kg^{-1}	2.33±0.04
Available boron	mg kg^{-1}	0.5±0.005
Total chromium	mg kg^{-1}	BDL
Total cadmium	mg kg^{-1}	BDL
Cation exchange capacity	Cmol (p+) kg−1	21.40±0.33

BDL below detectable limit (below 0.1 ppm)

Table 3 Arrangement of the plants in series for pot experiment

Brassica juncea	T1(Control)- uncontaminated soil	T2 (500 ppm of Cr + 5 Kg of Silica powder)	T3 (500 ppm of Cd + 5 Kg of Silica powder)
Green gram	T1(Control)- uncontaminated soil	T2 (500 ppm of Cr + 5 Kg of Silica powder)	T3 (500 ppm of Cd + 5 Kg of Silica powder)

Table 4. Physiochemical characteristics of bioamendments used

Parameter	**Unit**	**Silica powder**
Ph	-	7.09±0.15
EC	dS m^{-1}	3.25±0.08
Total Nitrogen	%	1.08±0.03
Total Phosphorous	%	9.35±0.21
Total Potassium	%	0.1±0.009
Nickel	mg kg^{-1}	16.89±0.10
Lead	mg kg^{-1}	26±0.53
Chromium	mg kg^{-1}	BDL
Cadmium	mg kg^{-1}	4.46±0.04

BDL below detectable levels (below 0.01 ppm)

Table 5 Vigour index of Brassica juncea

Plant	G%	Vigour index	Seed index	Root length (cm)	Shoot length (cm)	Total plant height (cm)
BT1R1	30%	150	3	2.3	5.9	8
BT2R1	50%	325	4	3.2	3.3	6.5
BT3R1	100%	800	10	2	3	5

Table 6. Vigour index of green gram

Plant	G%	Vigour index	Seed Index	Root length (cm)	Shoot length (cm)	Total plant height (cm)
GT1R1	80%	2000	8	3.3	26	29.3
GT2R1	60%	1758	6	2.9	16	18.9
GT3R1	50%	925	5	4	21	25

Table.7 Chlorophyll content in Plants

	Concentration of heavy metals					
	T1		**T2**		**T3**	
	Chlorophyll a	**Chlorophyll b**	**Chlorophyll a**	**Chlorophyll b**	**Chlorophyll a**	**Chlorophyll b**
Green gram	4.2321	2.2678	3.2034	2.1976	4.1983	3.1956
Brassica juncea	4.1863	2.2798	3.1862	2.1743	4.9121	,’3.6815

Table.8 Sulphur content in soil

Sulphur content in soil (mg/L)

	Brassica Juncea	**Green gram**
T1	24.5	24.5
T2	24.0	24.2
T3	23.1	24.0

Table 9 Phosphorous content in soil

Phosphorous content in soil (mg/L)		
	Brassica Juncea	**Green gram**
T1	2.09	2.09
T2	2.08	2.09
T3	2.0	2.06

Table 10: Tabulation of boron content in soil

Boron content in soil (mg/L)		
	Brassica Juncea	**Green gram**
T1	1.08	1.08
T2	0.58	0.59
T3	0.56	0.55

Table 11: Calcium, Magnesium, Sodium and Potassium content in the soil grown with green gram

	Calcium	**Magnesium**	**Sodium**	**Potassium**

T1	1353.0	426.1	195.3	147.3
T2	1349.2	426.0	195.2	143.6
T3	1378.9	399.2	187.4	139.2

Table 12 Calcium, Magnesium, Sodium and Potassium content in the soil grown with Brassica juncea

	Calcium	**Magnesium**	**Sodium**	**Potassium**
T1	1380.6	426.9	148.8	157.4
T2	1367.1	419.7	148.1	156.8
T3	1388.7	398.5	146.3	147.8

Table 13 Remediation of brassica juncea and Brassica juncea with silica

Trials	**Cr (ppm)**	**Cd (ppm)**
BT1R1	600	400
BT2R1	530	367
BT3R1	450	320

Table 14 Remediation of Green gram and green gram with silica

Trials	**Cr (ppm)**	**Cd (ppm)**
VT1R1	600	400

VT2R2	560	388
VT3R3	470	450

Reference

Gupta, Ankur, and ChandrajitBalomajumder. "Simultaneous adsorption of Cr (VI) and phenol onto tea waste biomass from binary mixture: Multicomponent adsorption, thermodynamic and kinetic study." *Journal of environmental chemical engineering* 3.2 (2015): 785-796.

Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q, Li R, Zhang Z. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicology and environmental safety. 2016 Apr 1;126:111-21.

Wang Y, Qiao M, Liu Y, Zhu Y. Health risk assessment of heavy metals in soils and vegetables from wastewater irrigated area, Beijing-Tianjin city cluster, China. Journal of Environmental Sciences. 2012 Apr 1;24(4):690-8.

Meers, Erik, et al. "Potential use of the plant antioxidant network for environmentalexposure assessment of heavy metals in soils." *Environmental monitoring and assessment* 120.1 (2006): 243-267.

Li X, Poon CS, Liu PS. Heavy metal contamination of urban soils and street dusts in Hong Kong. Applied geochemistry. 2001 Aug 1;16(11-12):1361-8.

Chen TB, Zheng YM, Lei M, Huang ZC, Wu HT, Chen H, Fan KK, Yu K, Wu X, Tian QZ. Assessment of heavy metal pollution in surface soils of urban parks in Beijing, China. Chemosphere. 2005 Jul 1;60(4):542-51.

Knox AS, Gamerdinger AP, Adriano DC, Kolka RK, Kaplan DI. Sources and practices contributing to soil contamination. Bioremediation of contaminated soils. 1999 Jan 1;37:53-

Sharma RK, Agrawal M, Marshall F. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicology and environmental safety. 2007 Feb 1;66(2):258-66.

CoŞKun M, Steinnes E, Frontasyeva MV, Sjobakk TE, Demkina S. Heavy metal pollution of surface soil in the Thrace region, Turkey. Environmental monitoring and assessment. 2006 Aug;119(1):545-56.

Wei B, Yang L. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. Microchemical journal. 2010 Mar 1;94(2):99-107.

Sun Y, Zhou Q, Xie X, Liu R. Spatial, sources and risk assessment of heavy metal contamination of urban soils in typical regions of Shenyang, China. Journal of hazardous materials. 2010 Feb 15;174(1-3):455-62.

Pacyna JM. Atmospheric trace elements from natural and anthropogenic sources. Toxic metals in the atmosphere. 1986:33-52.

Amundsen CE, Hanssen JE, Rambæk JP, Semb A, Steinnes E. Long-range transport of trace elements to Southern Norway studied by INAA of air filters. Journal Radioanalytical and Nuclear Chemistry. 1987 Aug;114(1):5-12.

Saur E, Juste C. Enrichment of trace elements from long-range aerosol transport in sandy podzolic soils of southwest France. Water, Air, and Soil Pollution. 1994 Jan;73(1):235-46

Choppala G, Bolan N, Kunhikrishnan A, Bush R (2016) Differential effect of biochar upon reduction-induced mobility and bioavailability of arsenate and chromate. Chemosphere 144:374–381. https:// doi. org/ 10. 1016/j. chemo sphere. 2015. 08. 043

Jackson M (1973) Estimation of phosphorus content: soil chemical analysis. Printer Hall, New Delhi

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37(1):29–38

Wiesmeier M, von Lützow M, Spörlein P, Geuß U, HangenE RA, Kögel-Knabner I (2015) Land use effects on organic carbon storage in soils of Bavaria: the importance of soil types. Soil Tillage Res 146:296–302. https:// doi. org/ 10. 1016/j. still. 2014. 10. 003

Sinduja M, Avudainayagam S, Davamani V, Suganthi R (2018) Uptake of mercury by marigold and amaranthus on spiked soil. Madras Agric J 105(7–9):346–351

Sinduja M, Sathya V, Maheswari M, Dhevagi P, Kalpana P, Dinesh GK, Prasad S (2022) Evaluation and speciation of heavy metals in the soil of the Sub Urban Region of Southern India. Soil Sediment Contam. https:// doi. org/ 10. 1080/ 15320 383. 2022. 20302 98

Ghosh and Singh, 2005; Kotrba et al., 2009; Garbisu and Alkorta, 2003; Halim et al.,2003; Yang et al., 2005; McIntyre, 2003)

Rono JK, Le Wang L, Wu XC, Cao HW, Zhao YN, Khan IU, Yang ZM. Identification of a new function of metallothionein-like gene OsMT1e for cadmium detoxification and potential phytoremediation. Chemosphere. 2021 Feb 1;265:129136.

Jeyasundar PG, Ali A, Azeem M, Li Y, Guo D, Sikdar A, Abdelrahman H, Kwon E, Antoniadis V, Mani VM, Shaheen SM. Green remediation of toxic metals contaminated mining soil using bacterial consortium and Brassica juncea. Environmental Pollution. 2021 May 15;277:116789.

Searchinger T, Heimlich R, Houghton RA, Dong F, Elobeid A, Fabiosa J, Tokgoz S, Hayes D, Yu TH. Use of US croplands for biofuels increases greenhouse gases through emissions from land-use change. Science. 2008 Feb 29;319(5867):1238-40.

Gao JJ, Peng RH, Zhu B, Tian YS, Xu J, Wang B, Fu XY, Han HJ, Wang LJ, Zhang FJ, Zhang WH. Enhanced phytoremediation of TNT and cobalt co-contaminated soil by AfSSB transformed plant. Ecotoxicology and Environmental Safety. 2021 Sep 1;220:112407.

Reeves RD, Brooks RR. Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. Environmental pollution series A, Ecological and Biological. 28)Cho-Ruk K., Kurukote J., Supprung P., Vetayasuporn S. Perennial plants in the phytoremediation of lead-contaminated soils. *Biotechnology*. 2006;5:1–4. doi: 10.3923/biotech.2006.1.4.

Tangahu B.V., Sheikh Abdullah S.R., Basri H., Idris M., Anuar N., Mukhlisin M. aybishenko B, Hazen TC, Long PE, Brodie EL, Conrad ME, Hubbard SS, Christensen JN, Joyner D, Borglin SE, Chakraborty R, Williams KH. In situ long-term reductive bioimmobilization of Cr (VI) in groundwater using hydrogen release compound. Environmental Science & Technology. 2008 Nov 15;42(22):8478-85. Lukaski HC. Chromium as a supplement.

Choppala G, Bolan N, Kunhikrishnan A, Bush R. Differential effect of biochar upon reduction-induced mobility and bioavailability of arsenate and chromate. Chemosphere. 2016 Feb 1;144:374-81.

Bartlett RJ, Kimble JM. Behavior of chromium in soils. II. Hexavalent forms. J. Environ. Qual.;(United States). 1976 Jan 1;5(4).

Ahmed N, Ahmed R, Ahmed S, Ahmed S, Ahmed T, Ahn C, Ahn HJ, Ahn M, Ahuja NK, Ajayi T, Akbar IB. Al Hadidi, Samer, 857 Al Hanayneh, Muhannad, 1471, 1847, 1848, 1893 Al Homssi, Amer, 2099 Al Juburi, Amar, 210, 2586 Al Kateb, Mohamad, 2433. Am J Gastroenterol. 2017;112:S1595-638.

Quievryn G, Peterson E, Messer J, Zhitkovich A. Genotoxicity and mutagenicity of chromium (VI)/ascorbate-generated DNA adducts in human and bacterial cells. Biochemistry. 2003 Feb 4;42(4):1062-70.

Chakraborty S, Chowdhury S, Saha PD. Adsorption of crystal violet from aqueous solution onto NaOH-modified rice husk. Carbohydrate Polymers. 2011 Oct 15;86(4):1533-