Original Research Article

POTENTIALITY OF WEED PLANTS FOR PHYTOREMEDIATION OF HEAVY METAL POLLUTED SOIL

ABSTRACT

Soil remediation through plants is the return of soil to a condition of ecological stability together with the establishment of plant communities it supports to conditions prior to disturbance. Phytoremediation offers to metal-contaminated sites an innovative and cost-effective option to address recalcitrant environmental contaminants. Processes include using plants that tolerate and accumulate metals at high levels and using plants that can grow under conditions that are toxic to other plants while preventing. This article addresses key research, potential benefits and the potential future needs for phytoremediation. Total concentrations of heavy metals have been estimated in soil and weed plant samples growing in an industrial area of Rajkot region.Results indicated that uptake patterns of metals by weed plant tissues were more or less related with nature of metals and species specifications. All the studies suggested that each weed plant could be used as a modern tool as a biomonitor.

Keywords: Biomonitors, Environmental contaminants**,** Heavy metals, Plant communities, Phytoremediation

1 INTRODUCTION

Phytoremediation is combined multidisciplinary approach to the cleanup ofcontaminated soils, which combines the disciplines of plant physiology, soil chemistry, andsoil microbiology. Phytoremediation has been applied to a number of contaminants in smallscalefield and laboratory studies. These contaminants include heavy metals,radionuclides, chlorinated solvents, petroleum hydrocarbons, PCBs, PAHs, organophosphateinsecticides, explosives, and surfactants [1].Certain species ofhigher plants can accumulate very high concentrations of metals in their tissues withoutshowingtoxicity [2]. Such plants can be used successfully to clean up heavymetal polluted soils if their biomass and metal content are large enough to completeremediation within a reasonable period.

Comment [N'BJ1]: Full spell as it is first time use in paragraph

Comment [N'BJ2]: Supposed to have more than 1 cited reference. The cited ref no 1 only refer to heavy metal which is arsenic.

Comment [N'BJ3]: Statement too general. What metal? How high is high concentrations to show the toxicity level?

Phytoremediation has secured popularity with government agencies and industry in thepast ten years. This popularity is based in part on the relatively low cost of phytoremediation,combined with the limited funds available for environmental cleanup. The costs associatedwith environmental remediation are staggering. Currently, $6-8 billion per year is spent forenvironmental cleanup in the United States, and $25-50 billion per year worldwide [3]. Because biological methods are eventually solar-driven,phytoremediation is on average tenfold low-cost than engineering-based remediation methods.The way that phytoremediation is normally completed in situ adds to its expense adequacy and may decrease openness of the contaminated substrate to people, natural life, and the climate.So that, government agencies like toinclude phytoremediation in their cleanup strategies to stretch available funds, corporations (e.g., electric power, oil, chemical industry) like to advertise their involvement with thisenvironment-friendly technology, and environmental consultancy companies increasinglyinclude phytoremediation in their package of offered technologies.

Comment [N'BJ4]: The cited reference used was outdated (2009) to support the statement.

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In general, either in situ or ex situ, remediation technologies accomplish one of two things: They either remove the contaminants from the substratum (site decontamination or clean-up techniques) or lessen the risk of exposure (site stabilization techniques) to the contaminants. One “gentle” plant-based site stabilization approach, suitable forheavily contaminated sites, is phytostabilization aimed to decrease soil metal bioavailabilityusing a combination of plants and soil amendments [4]. Anotherapproach directed towards real decontamination is trace element phytoextraction,representing use of plants for trace element removal from the soil by concentrating them inthe harvestable parts [5]. An opinion exists that trace element phytoextractionwill be more economically feasible if, in addition to metal removal, plants produce biomasswith an added economical value [2].

Recently, many plants have been reported to accumulate highlevel of the toxic metals in their sinks in the aquatic and terrestrialecosystems. Such plants can remove the pollutant metals from soil andwater [6,7]. This potentialapproach to clean the environment, termed phytoremediation, draws on ourcenturies of experience in cultivating crops and is emerging as a low costtreatment technology for the contaminated environment.

Comment [N'BJ7]: 'Recently' - however the cited reference was 2009 and 1997..

Indian Mustard (*Brassica juncea*L), Italian serpentine plant*(Alyssum bertolonii)* and *Thlaspicaerulescens*are some of the mostpotential terrestrial plant species which have recently been used to extractheavy metals from soil and sediments and translocate these metals to theroots, harvestable stalks and leaves ~~etc.~~ of the plants [8].Many other plants ~~eg.~~for instancespinach, cauliflower, cabbage, radish, onions, potato, wheat and *Eichhornia*~~etc~~. collected from Ni and Cr contaminated fields in the industrial vicinity ofSonepat (Haryana) have been reported to possess high sink for the metalaccumulation [9]. Therefore, it appears thatselecting a more appropriate plant species for the phytoextraction(phytoremediation), i.e. removal of heavy metals from the contaminatedsoils, sludges and sediments in the vicinity of the industrial towns havingspecific metal contaminants using a potential plant species/cultivar is yet a task ahead. For such an application, the contaminated sites with richvegetation can provide naturally evolved hyperaccumulator and the metaltolerant species and cultivars which can be used commercially for thecleaning of the contaminated sites in a systematically cultivated manner.

Comment [N'BJ8]: What type of heavy metals?

Comment [N'BJ9]: 'many' - should refer to more than 2 references.. And cited ref no 9 was not related to the statement at all.

The selection of many potential phytoremediators need an areawise/metalwise screening keeping the Indian cultivars acclimatised for thespecific agroclimatic conditions in mind. An attention to find out a localcultivar of commercially potential phytoremediator plants targeting to thecleaning of the specific sites of cities, towns and metropolitans sufferingfrom the heavy metal contamination.

Comment [N'BJ10]: Objective was too general. What is the interest heavy metal that need to study?

2 MATERIALS AND METHODS

2.1 Field survey

Comment [N'BJ11]: Provide map of study area

Rajkot is most developing city of Gujarat and epicenter of the Saurashtra region. As per industrialization, there are more than 500 foundry units in Rajkot region. Most of foundry units in Rajkot produce grey iron castings for domestic market and also export to worldwide. Industrial products include bearings, diesel engines, kitchen knives, watch part, automotive parts ~~etc.~~ there are so many forging industries, casting industries and machine tools production units.

Comment [N'BJ12]: ref

The industrial effluents are pulled on in a well through the number of channels and then disposed off in drain No.8, a canal in which sewage effluents also get mixed at certain points. Water of the drain No. 8 containing industrial effluents along with the sewage waste water is lifted with the help of diesel pumps ~~etc.~~ to irrigate the crop field situated near by.

Comment [N'BJ13]: From where?

2.2 Collection of soil samples

Comment [N'BJ14]: Please state when the sample was collected

Soil samples were collected from industrial fields. After removing grasses and other vegetation from the surface, samples were taken from 0-15 cm depth from 5 to 8 different points in the same location using Auger. All the samples were mixed together and almost 5 kg taken out of it. Half of the soil samples were dried in shade and then stored in clean polythene bag. These samples were then carried to the laboratory. There is no difference in soil profile for all the samples. At each site 2-3 composite samples were taken from different horizons viz., 0-15 cm, 15-30 cm and 30-45 cm segments.Dried ~~Soil~~ soil samples were crushed in to small pieces, mixed well together and spread it to make it air dry and passed through a sieve with round holes of 2 mm diameter. One kg of soil sample was taken as representative of the original material [10]. The samples were placed in clean polythene bag with tag, which had details of location, depth of soils ~~etc.~~

Comment [N'BJ15]: Explain method to select the sampling point/station. And how the choosing sampling station can represent the whole contaminated area.

2.3 Analysis of Soil

At the outset, a comprehensive survey was conducted in order to evaluate soil from different locations of industrial areas located in Rajkot for assessment of heavy metals contamination (Cu, Cr, Mn, Pb and Cd).

2.3.1 Physico-chemical properties

Table 1. Methods for Physico-chemical properties

Properties	Methods
Soil texture	Hydrometric method [11].
Ph	Digital pH meter [12].
Electrical conductivity	Digital EC meter [12].
Bulk Density	By lab method for disturbed soil.[13]
Soil Organic Carbon	Walkley and Black method [14]
Soil Organic Matter	Van Bemmelen,[15]
Heavy Metals	Atomic Absorption Spectrophotometer (AAS), DTPA method[16]

2.3.2 Contamination factor (CF)

The CF is the ratio obtained from heavy metal concentrations in polluted and background sites [17].

CF = CmSample/ CmBackground

Where Cm represents the concentrations of metals in contaminated and background sites.

2.3.3 Pollution load index (PLI)

PLI was calculated by the following formula adopted from Muhammad et al. [18].

$$\textbf{PLI=n}\sqrt{CF1 \times CF2 \times CF3 \ldots\ldots\ldots\ldots\ldots . CFn}$$

Where n is the number of metals and CF is the contamination factor value.

2.4 Collection of weed plants

2.4.1 Survey the flora of contaminated sites

An ecological survey was done from March 2009 to March 2010. Community analysis was carried out during rainy season when majority of the plants were at the peak of their growth. In every study sites, 30 quadrats of 10 m X 10 m (100 sq m) size were randomly laid to study plant species. The herbaceous species was studied by laying 50 quadrats of lm X lm (lsq m) size randomly in each study site.

Comment [N'BJ16]: Plant samples was collected 13 years ago, however results for soil samples was present in 2020-2021. time frame of data collection of course not related to each other.

2.4.1.1 *Quantitative analysis*

The important quantitative analysis such as density and frequency of tree species, shrubs and herbs species were determined as per Curtis and McIntosh [19].

(a) Density

Density is an expression of the numerical strength of a species where the total number of individuals of each species in all the quadrats is divided by the total number of quadrats studied.

Density is calculated by the equation:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Total number of quadrats studied}}$$

(b) Frequency (%)

This term refers to the degree of dispersion of individual species in an area and usually expressed in terms of percentage occurrence. It was studied by sampling the study area at several places at random and recorded the name of the species that occurred in each sampling units.

It is calculated by the equation:

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occured}}{\text{Total number of quadrats studied}} \times 100$$

Due to the heterogeneity of the characteristics of the soil, observed in the first sampling, each subplot was divided equally, establishing 20 different vegetation sampling sites. Surveys were conducted in October-November 2021. For each survey, a 30 X 30 cm quadrat was used [20]. The quadrat was randomly placed three times within each sampling site. Plant species were listed and vegetation cover estimated. Plant species were determined and named following the keys and nomenclature proposed by Valde´s et al. [21]. Than the most frequent species were collected for metal analysis.

2.5 Analysis of Weed plants

2.5.1 Metal analysis of plants of contaminated sites.

Plant samples were collected from the same sites as the soil samples. Plant identification was confirmed by the Forest Research of India, Dehradun. At least three to five individuals of all plant species were randomly collected within the sampling areas. Fresh plant materials were washed thoroughly with the tap water, washed for at least 15 seconds with a 0.1 N HCl solution and cleaned with distilled water and then separated into leaf, root and shoot. All plant parts were oven dried at 72°C for 72 h and then ground and passed through a 500-mm stainless-steel sieve to powders. For total metal concentrations in the plant components 0.5 g of plant samples were digested. Heavy metal concentrations in plants were also determined by the method as outlined by Gupta [22]. The total metal concentrations were measured by an atomic absorption spectrophotometry (AAS A-700, Perkin-Elmer) [16]. To assess the analytical precision, three analytical replicates of each sample, an appropriate standard reference material (from Sigma-Aldrich Company) and a reagent blank were performed in each analytical batch.

2.5.2 Bio-accumulation factor (BAF)

Bio-accumulation factor is used to explain the transport of trace elements (heavy metals) from complex soil mixture to plants. The bioaccumulation factor of a metal can be calculated as the ratio between the amount of metal in the plant (dry weight) to same in the corresponding soil [23, 24]. The BAF for different heavy metals was calculated as follows:

$$BAF = C_{Plant} / C_{Soil}$$

CPlant is concentration of heavy metal in plant (dry weight) and *C Soil* is concentration of heavy metal in corresponding soil under plant. BAF =1 means plant only absorbed the heavy metal but no accumulation while BAF > 1 denotes absorption and accumulation of heavy metal by plant [25].

2.6 Data analysis

Statistical analysis was done by an analysis of variance (ANOVA). Datawere considered to be significant at p % 0.05 and highly significant at p % 0.005, level of significance.

Comment [N'BJ17]: Authors not show any results of statistical study from ANOVA.

3 RESULTS AND DISCUSSION

3.1 Analysis of Soil

3.1.1 Physico-chemical properties of Soil

Texture of contaminated soil was the class sandy clay loamy which was in accord with the report of Carter and Gregorich [26]. Texture reflects the particle size distribution of the soil and thus the content of fine particles like oxides and clay. These compounds are important adsorption media for heavy metals in soils. Results showed that Shapar soil samples had sand particle between 68 to 70 % where Agricultural samples had almost 67%.Sand particles in Metoda samples were between Agricultural samples and Shapar samples. Silt particles were minimum in Agricultural samples like as 8 to 10 %. Silt particles were maximum in Metoda samples near about 12%. Silt particles in Shapar samples had also near about 10%. Clay particles were maximum (24%) in Agricultural soil samples where minimum (19%) in metoda A samples. The results obtained indicate that the dumpsite soils were highly permeable which are not suitable for waste disposal due to its high leaching capability contaminating groundwater resource in and around the waste dumpsite.

Table 2. Soil Texture

Textural composition (%)	Different soil profiles (0-15)cm soil depth			
	Metoda A	**Metoda B**	**Shapar A**	**Shapar B**
Sand (0.05– 2.0 mm)	68.75±0.85	67.84±0.85	69.27±0.85	68.9±0.85
Silt (0.002– 0.05 mm)	11.99±0.45	12.03±0.43	10.15±0.45	10.06±0.45

Clay (<0.002 mm)	19.26±0.51	20.13±0.55	20.58±0.52	21.04±0.53

Mean values ± Standard deviation of means of three replicates

pH is one of the most significant parameter which influences the availability of most of the elements in the soil to both plants and microbes. The soil when continuously amended with industrial waste alters the pH to a certain extent and thus one crucial aspect in pollution studies of soils [27]. The physico – chemical analysis of the dumpsite soil samples investigated in the present study revealed pH value was varied from 6.8 to 7.2 in summer and 6.9 to 7.3 in winter (Grapg.4.1) with a mean pH of 6.98 in summer and 7.12 in winter [28] which were in accord with the ranges reported by [29] in their earlier studies.

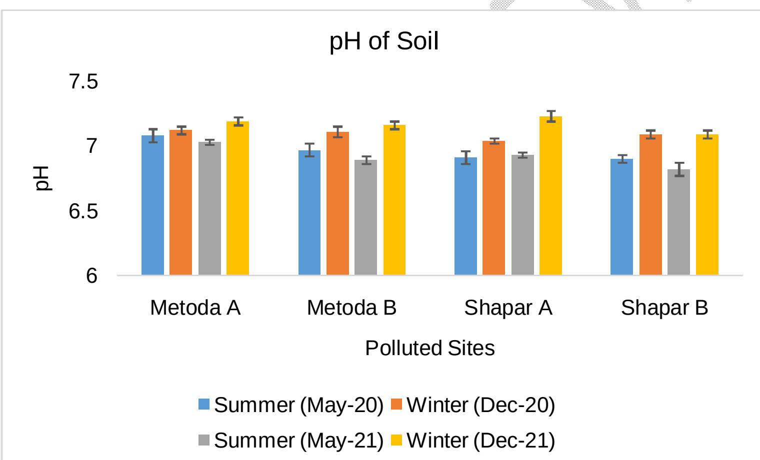

Figure 1. pH of Soil samples

Mean values ± Standard deviation of means of three replicates

The electrical conductivity recorded for the soil samples in the polluted sites ranged from 1.95 dS/m to 2.23 dS/m in summer and 1.98 dS/m to 2.3 dS/m in winter (Fig. 2**)** with a mean EC of 2.09 dS/m in summer and 2.18 dS/m in winter. The results were similar to those reported by Uba *et al.,* [30]. The mean EC recorded for the control soil samples was 0.38 dS/m in summer and 0.4 dS/m in winter. The EC recorded for the polluted site soils were slightly above the prescribed ranges for agricultural soils which might hinder the uptake of water by the plants from the soils [31].

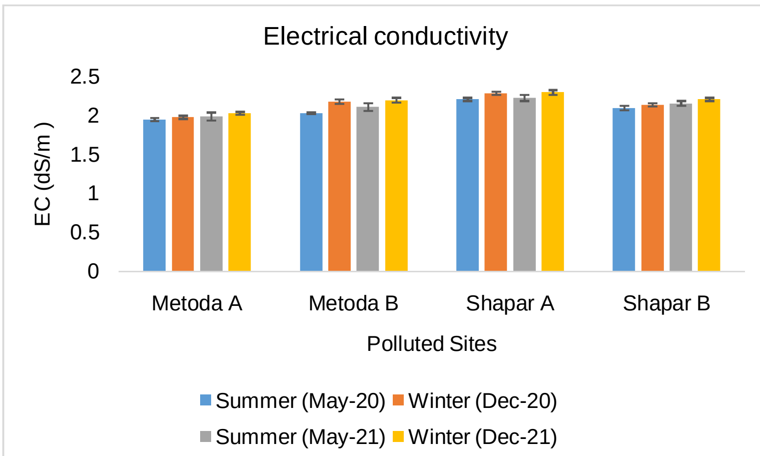

Figure 2. EC of Soil samples

Mean values ± Standard deviation of means of three replicates

Bulk density is an indicator of soil suitability for plant growth. The bulk density recorded for the soil samples in the polluted sites were ranged from 1.29 mg/m^3 to 1.32 mg/m^3 in summer and 1.25 mg/m^3 to 1.3 mg/m^3 in winterwith a mean bulk density of 1.31 mg/m^3 in summer and 1.28 mg/m^3 in winter. The mean bulk density recorded for the control soil samples was 1.38 mg/m^3 in summer and 1.37 mg/m^3 in winter.).Mathur [32] observed that bulk density of soil treated with organic manure decreased from 1.46 to 1.40 mg/m^3 which confirms the present findings. Similar results were also reported by Chaudhury [33] in a 31 years long-term field experiment for assessing soil quality under rice-based cropping system in Indo-Gangetic alluvial soils of India.

Table 3. Bulk Density of Soil

Bulk Density(mg/m^3)

Site	Metoda A	Metoda B	Shapar A	Shapar B
Summer (May-20)	1.31±0.03	1.3±0.02	1.29±0.01	1.29±0.01
Winter (Dec-20)	1.29±0.02	1.28±0.02	1.26±0.01	1.25±0.02
Summer (May-21)	1.32±0.01	1.32±0.01	1.31±0.02	1.3±0.01
Winter (Dec-21)	1.29±0.02	1.3±0.01	1.3±0.02	1.29±0.02

Mean values ± Standard deviation of means of three replicates

The soil organic carbon in the polluted site soils ranged from 0.78% to 1.02% in summer and 0.8% to 0.96% in winter with a mean of 0.9% in summer and 0.88% in winter. The mean soil organic carbon recorded for the control soil samples analyzed was 0.57% in summer and 0.58% in winter. The high soil organic carbon of the dumpsite soils is due to the high organic and compostable matter degradation [34]and also silt content in the soils which might have contributed to the excessive accumulation of SOC [35].

Table 4. Organic Carbon of Soil

Organic Carbon %				
Site	**Metoda A**	**Metoda B**	**Shapar A**	**Shapar B**
Summer (May-20)	0.9±0.02	0.96±0.02	0.78±0.02	0.81±0.02
Winter (Dec-20)	0.89±0.03	0.92±0.02	0.81±0.02	0.8±0.03
Summer (May-21)	0.98±0.02	1.02±0.03	0.87±0.02	0.9±0.02

Winter (Dec-21)	0.91±0.03	0.96±0.03	0.81±0.03	0.86±0.02

Mean values ± Standard deviation of means of three replicates

The SOM (Soil Organic Matter) of soil samples in the polluted site soil samples were ranged from 1.34% to 1.76% in summer and 1.38% to 1.66% in post - monsoon with a mean of 1.55% in summer and 1.52% in winter. The mean soil organic matter recorded for the control soil samples analyzed was 0.96% in summer and 1.00% in winter.However among all the soil samples analyzed control (Agricultural) soil samples recorded lower SOM percentages compared to the polluted site soils which was in accord with the previous studies of [36]. Soil organic matter is thus one crucial characteristics impacting soil physical properties.

Table 5. Soil Organic Carbon

Soil Organic Matter %				
	Metoda A	**Metoda B**	**Shapar A**	**Shapar B**
Summer (May-20)	1.55±0.05	1.66±0.06	1.34±0.07	1.40±0.06
Winter (Dec-20)	1.53±0.05	1.59±0.06	1.40±0.07	1.38±0.05
Summer (May-21)	1.69±0.06	1.76±0.05	1.50±0.07	1.56±0.07
Winter (Dec-21)	1.57±0.05	1.66±0.04	1.40±0.06	1.48±0.06

Mean values ± Standard deviation of means of three replicates

Copper content in the soils of the polluted site soil samples were ranged from 3.9 mg/kg to 6.83 mg/kg in summer and 6.38 mg/kg to 9.01 mg/kg in winter with a mean of 5.37 mg/kg in summer and 7.7 mg/kg in winter. The results recorded for polluted site soils might be due to the introduction of the industrial wastes which might increase the soil copper levels [37]. The mean copper content of the control soil samples recorded was 1.46 mg/kg in summer and 1.98 mg/kg in winter. Among all the samples analyzed polluted site soils recorded lower copper levels beyond the International Standards for Agricultural soils.

The amount of manganese varies greatly and is generally present in the form of manganese associated with organic matter, exchangeable manganese or manganese oxides. The mean Mn content in the soils of the polluted site ranged from 23.5 mg/kg to 25.6 mg/kg in summer and 24.4 mg/kg to 29.41 mg/kg in winter with a mean of 24.55 mg/kg in summer and 28.71 mg/kg in winter. The results recorded for the polluted site soil samples were lower than the ranges reported in a similar study by Udeme [38]. The mean Mn content of the control soil samples recorded 13.51 mg/kg in summer and 16.33 mg/kg in winter. Among all the soil samples analyzed polluted site soils recorded higher Mn content than the control soil samples due to the solubilizing effect of organic matter on Mn. The high Mn levels in polluted site may be due to the discarded battery materials and other metallic discards [39].

The mean Cr content in the soils of the polluted site samples were ranged from 0.21 mg/kg to 0.4 mg/kg in summer and 0.21 mg/kg to 1.02 mg/kg in winter with a mean of 0.31 mg/kg in summer and 0.62 mg/kg in winter. Similar study by Nwajei [40] also reported lower levels of Cr in the surface soils of a waste polluted site. The mean Cr content in the control sample recorded was 0.24 mg/kg in summer and 0.22 mg/kg in winter.

The mean Pb content in the soil samples from the polluted site ranged from 2.68 mg/kg to 19.21 mg/kg in summer and 24.6 mg/kg to 41.02 mg/kg in winter with a mean of 10.95mg/kg in summer and 32.81 mg/kg in winter presented. The mean Pb content in control soil sample analyzed was recorded 0.2 mg/kg in summer and 0.21 mg/ kg in winter. Among all the soil samples analyzed polluted site soils recorded higher Pb content than the control soil samples which was similar to the previous findings of Ogunyemi[41]. The high Pb levels in polluted site soils may be due to the discarded plastics, glasses, paints, ceramics, batteries etc; The overall mean Pb levels recorded for the dumpsite soils samples in both the seasons were beyond the WHO standards for soil and below the International Standards for Agricultural soils.

The mean Cd content in the soils of the polluted site samples were ranged between 0.18 mg/kg to 0.25 mg/kg in summer and 0.23 mg/kg to 0.38 mg/kg in winter with a mean of 0.22 mg/kg in summer and 0.31 mg/kg in winter. The mean Cd content in control soil samples was recorded 0.02 mg/kg in summer and 0.03 mg/kg in winter. Among all the samples analyzed polluted site soils recorded higher Cd levels than the control soil samples. The overall mean Cd levels in polluted site soils were within the permissible ranges of the International Standards for Agricultural soils and WHO. The results obtained were contrary to a similar study of Awokunmi[42] who reported very high levels of Cd levels in the surface soils of the polluted site. Many industrial processes required cadmium content raw materials and many industrial operations required cadmium as a catalyst; hence these applications are responsible for increase the lead content [43].

Table 6. Heavy metals of Soil (in mg/kg)

	Elements	Cadmium	Lead	Chromium	Manganese	Copper
Metoda A	Summer (May-20)	0.2±0.01	2.68±0.08	0.4±0.01	25.5±0.62	6.68±0.32
	Winter (Dec-20)	0.23±0.06	30.01±0.42	0.41±0.05	26.01±0.63	6.92±0.52
	Summer (May-21)	0.2±0.01	18.4±0.51	0.4±0.01	25.6±0.52	6.83±0.41
	Winter (Dec-21)	0.31±0.01	36.31±1.02	1.02±0.04	29.41±0.87	8.34±0.69
Metoda B	Summer (May-20)	0.21±0.01	2.69±0.12	0.31±0.02	25.00±0.28	6.05±0.5
	Winter (Dec-20)	0.27±0.01	24.6±0.78	0.38±0.04	25.87±0.81	6.38±0.25
	Summer (May-21)	0.2±0.05	14.51±0.58	0.35±0.03	25.21±0.42	6.12±0.21
	Winter (Dec-21)	0.32±0.01	28.06±1.25	0.98±0.01	28.06±0.85	7.59±0.22
Shapar A	Summer (May-20)	0.18±0.01	2.68±0.05	0.32±0.01	24.00±0.96	3.9±0.05
	Winter (Dec-20)	0.32±0.02	32.78±1.04	0.21±0.01	24.4±0.84	6.57±0.52
	Summer (May-21)	0.25±0.02	19.21±1.02	0.21±0.02	23.9±0.74	5.28±0.5
	Winter (Dec-21)	0.37±0.02	41.02±0.21	0.29±0.02	26.54±1.05	7.91±0.5
Shapar B	Summer (May-20)	0.21±0.01	2.68±0.05	0.40±0.01	23.5±0.88	4.09±0.05
	Winter (Dec-20)	0.38±0.02	30.14±0.85	0.32±0.01	25.93±1.04	7.82±0.14

	Summer (May-21)	0.23±0.02	15.67±0.52	0.30±0.01	23.53±0.99	5.42±0.5
	Winter (Dec-21)	0.3±0.01	39.10±0.75	0.40±0.01	26.88±1.05	9.01±0.6

Mean values ± Standard deviation of means of three replicates

3.1.2 Contamination factor and Pollution load index

Heavy metal concentrations in soil of Rajkot industrial zones are mentioned in table 1 & 2. With the help of background value (agricultural soil) of the heavy metals the contamination factor and pollution load index (PLI) was calculated which reflects the pollution of metals in soil. Contamination factor of metals in Metoda soil sample was Cu (1.98), Mn (1.58), Cr (1.58), Cd (7.67) and Pb (166.72). The PLI for this site was 397.51. Contamination factor of Shapar soil sample was Cu (2.24), Mn (1.58), Cr (1.23), Cd (12.67) and Pb (167.44). The PLI value for this site was 480.49. The Shapar site was highly contaminated when compared to the Metoda sites. These high values of PLI in the study area could be due to open dumping of industrial waste that may cause a potential health risk to the local community as well to grazing animals. In this study, the PLI values were greater than those reported by Muhammad et al. [18].

Comment [N'BJ18]: Table 1 & 2 not show the concentration of heavy metal

Accumulation of lead in soil was significantly high compare to other metals found in their respective presence in soil during the study period. The intensity of adverse effects of several heavy metals depends upon the form and percentage distribution in soil. The soil parameters such as soil texture, organic matter content, pH, redox potential will affect the mobility of metal and its translocation [44]. Most of the trace metals are found in crystalline state and are immobile. Oxides of iron and manganese are generally coated on organic matted present in soil and fine particles of clay along with other colloidal material which are generally active provide mobility platform to trace metal. Several human interventions in environment geochemical cycle of trace metals, resulting in soil and water contamination which finally enters in food chain [44].

The CF values at the Metoda site were observed to be in the order of Pb>Cd>Cu>Mn≥Cr. According to the Muller classification, Pb and Cd were present at very highly polluted levels; others were highly polluted. The CF values at the Shapar site were observed to be in the order of Pb>Cd>Cu>Mn>Cr. The concentrations of Pb, Cd and Cu were present at very highly polluted levels; others were highly polluted level, as suggested by Muller. These results revealed that the Metoda and Shapar sites showed higher CF values for Pb, Cd, Cr, Mn, and Cu. The Shapar site showed higher CF values for Cd and Cu.

3.2 Survey the flora of contaminated sites

In the surveys of the polluted sites, more than 20 vascular plant species, representing 16 genera and 11 families were found. Most of these plants were annual or biannual and perennials were less represented. Tableshows the species that were present in more than 55% frequency and density of the sampling sites. Moreover, under a Mediterranean condition, greatest development of annuals occurs in spring, coinciding with the March and June surveys. In general, species richness, vegetation cover and biomass production were significantly higher in the industrial zones in each survey. This pointed to a positive effect of the metal concentration in enhancing plant colonization and plant development in spite of the chemical heterogeneity revealed by soil analyses. The results obtained from these areas clearly indicate the adverse conditions (very high metal contamination) of the polluted soil. The positive effects of the pollution might be related to an increase in some species density. There were strong positive correlations between soil metal concentration and the number of species, vegetation cover and biomass production. The second thing was the increase in soil pH reduced trace element solubility and thus potential toxicity to plants and microorganisms. Therefore the increase in soil pH seems to be the most important heavy metals effect in reducing trace element solubility. Moreover, the nutrients added through the pollution could also contribute to improving soil fertility in this soil.

Comment [N'BJ19]: ?

Comment [N'BJ20]: Authors not show any correlation study.

Table 7. Dominant plant species of polluted sites

NO	Plant Name	Family
1	*Alternanthera caracasana*Kunth.	Amaranthaceae
2	*Amaranthus spinosus*L.	Amaranthaceae
3	*Calotropis gigantea* L.	Apocynaceae
4	*Cyprus haspan*L.	Cyperaceae

5	*Datura stramonium* L.	Solanaceae
6	*Ipomoea aquatica* Forssk.	Convolvullaceae
7	*Phyllanthus nirudi*L.	Phyllanthaceae
8	*Withaniasomnifera*L. (Dunal)	Solanaceae

3.3 Metal analysis of plants of contaminated sites.

In general, plants growing in the contaminated soils had higher concentrations of micronutrients and trace elements than control plants.

Cadmium

The highest Cd concentration was measured 2.60 mg/kg in *C. gigantea* at shapar which is higher than the permissible limit set by WHO [45]. The result of Cd concentration of the plants species from both polluted sites were grouped together with Cd concentration of the Soil from both sites and correlated to find the correlation coefficient between them.

Lead

The highest Pb concentration was measured 36.34 mg/kg in *A. spinosus*at shapar which is higher than the permissible limit set by WHO[45]and the lowest of 0.0 mg/kg in *I. aquatica* at shapar as shown in table. The result of Pb concentration of the plants species from both polluted sites were grouped together with Pb concentration of the Soil from both sites and correlated to find the correlation coefficient between them.

Chromium

The highest Cr concentration was measured 64.30 mg/kg in *A. spinosus*at shapar which is higher than the permissible limit set by WHO [45]. The result of Cr concentration of the plants species from both polluted sites were grouped together with Cr concentration of the Soil from both sites and correlated to find the correlation coefficient between them.

Manganese

The highest Mn concentration was measured 28.08 mg/kg in *W. somnifera*at metoda which is higher than the permissible limit set by WHO [45]. The result of Mn concentration of the plants species from both polluted sites were grouped together with Mn concentration of the Soil from both sites and correlated to find the correlation coefficient between them.

Copper

The highest Cu concentration was measured 43.68 mg/kg in *I. aquatica* at metoda which is higher than the permissible limit set by WHO[45]. The result of Cu concentration of the plants species from both polluted sites were grouped together with Cu concentration of the Soil from both sites and correlated to find the correlation coefficient between them.

Table 8. Metal analysis of weed plants of Metoda site (in mg/kg)

Plant Name	Cd	Pb	Cr	Mn	Cu
*Alternantheracaracasana*Kunth.	1.48±0.05	11.23±0.5	4.09±0.04	18.04±0.05	2.03±0.05
*Amaranthusspinosus*L.	0.59±0.02	21.32±0.11	36.40±0.13	12.03±0.8	3.09±0.04
*Calotropisgigantea*L.	1.87±0.06	3.22±0.12	2.35±0.12	8.90±0.11	9.19±0.12
*Cyprus haspan*L.	0.98±0.01	1.79±0.01	4.36±0.11	9.65±0.11	11.64±0.11
*Daturastramonium*L.	0.12±0.01	0.43±0.01	2.70±0.05	5.96±0.05	1.22±0.04
Ipomoea aquatic Forssk.	0.26±0.01	0.07±0.01	6.09±0.05	18.93±0.22	43.68±0.37
*Phyllanthusnirudi*L.	0.12±0.02	1.09±0.05	3.06±0.05	12.37±0.05	11.71±0.12
*Withaniasomnifera*L. (Dunal)	2.08±0.05	4.75±0.07	4.09±0.05	28.08±0.43	12.44±0.17

Mean values ± Standard deviation of means of three replicates

Table 9. Metal analysis of weed plant of Shapar site (in mg/kg)

Plant Name	Cd	Pb	Cr	Mn	Cu
*Alternantheracaracasana*Kunth.	1.02±0.05	17.38±0.25	5.78±0.15	12.21±0.14	0.10±0.04
*Amaranthusspinosus*L.	0.45±0.05	36.34±0.2	64.30±0.50	3.06±0.12	2.01±0.05

*Calotropisgigantea*L.	2.60±0.05	10.50±0.12	4.30±0.10	7.74±0.11	18.20±0.24
*Cyprus haspan*L.	1.09±0.1	2.38±0.11	3.09±0.10	5.97±0.21	22.36±0.51
*Daturastramonium*L.	0.05±0.01	0.18±0.01	0.30±0.01	1.10±0.05	0.70±0.01
*Ipomoea aquatica*Forssk.	0.10±0.01	0.00±0.00	4.55±0.09	16.74±0.24	32.41±0.65
*Phyllanthusnirudi*L.	0.09±0.01	0.93±0.08	2.13±0.05	7.27±0.21	9.91±0.20
*Withaniasomnifera*L. (Dunal)	1.69±0.08	5.34±0.11	3.69±0.10	25.66±0.50	16.03±0.50

Mean values ± Standard deviation of means of three replicates

3.4 Bioaccumulation factor (BAF)

Ability of a plant to accumulate metals from contaminated soils was evaluated by the BAF, according to studies of Fayiga[46] and Yoon [47]. This study assumed that plants with BAF values > 1 are accumulators, while plants with BAF values < 1 are excluders [48].Metoda and Shapar both polluted sites results showed that most of weed plant species had BAF values > 1 which indicating that they had the potential for use as accumulators of heavy metals. The success of the phytoremediation process depends on heavy metal removal by the plants [49]. Conversely, the accumulation of heavy metals from soil to plant parts were an extremely multifaceted process which affected by several factors, which exert different influences on the process by means of various mechanisms. There wereso many influencing factors include the chemical forms of the heavy metals, pH of the soil, conductivity of the soil, organic carbon,organicmatter content, plant species, climatic conditions, and irrigation with polluted water [50].

Table 10. BAF value in weed plants of Metoda site

Plant name	Metoda soil sample (BAF value)				
	Cd	**Pb**	**Cr**	**Mn**	**Cu**
***Alternanthera caracasana*Kunth.**	7.40	4.19	10.23	0.71	0.30
***Amaranthus spinosus*L.**	2.96	7.96	91.00	0.47	0.46

***Calotropis gigantea* L.**	9.35	1.20	5.88	0.35	1.38
***Cyprus haspan*L.**	4.90	0.67	10.90	0.38	1.74
***Datura stramonium* L.**	0.60	0.16	6.75	0.23	0.18
***Ipomoea aquatica* Forssk.**	1.30	0.03	15.23	0.74	6.54
***Phyllanthus nirudi*L.**	0.62	0.41	7.65	0.49	1.75
***Withaniasomnifera*L. (Dunal)**	10.40	1.77	10.23	1.10	1.86

Table 11. BAF value in weed plants of Shapar site

Plant name	**Shapar soil sample**				
	Cd	**Pb**	**Cr**	**Mn**	**Cu**
***Alternanthera caracasana*Kunth.**	4.87	6.49	14.45	0.52	0.02
***Amaranthus spinosus*L.**	2.12	13.56	160.75	0.13	0.49
***Calotropis gigantea* L.**	12.38	3.92	10.75	0.33	4.45
***Cyprus haspan*L.**	5.19	0.89	7.73	0.25	5.47
***Datura stramonium* L.**	0.24	0.07	0.75	0.05	0.17
***Ipomoea aquatica* Forssk.**	0.47	0.00	11.38	0.71	7.92
***Phyllanthus nirudi*L.**	0.44	0.35	5.33	0.31	2.42
***Withaniasomnifera*L. (Dunal)**	8.05	1.99	9.23	1.09	3.92

4 CONCLUSION

Phytoremediation is most suitable for removal of heavy metals from the polluted sites. But the potential of phytoremediation steps, using hyper accumulators, has raised some concerns related to invasiveness and disruption of indigenous ecosystems, as the introduction of alien plants may alter ecosystem function. Therefore, the best option is to find native hyper accumulator plants from nearby regions to use them for soil remediation. The results showed that concentrations of Cd, Pb, Cr, Mn, and Cu metal in the studied native plant species were higher than the normal plant, and thereby indicating that soil was polluted and required phytoremediation.

Comment [N'BJ21]: Too general, which plant is the best to remediate the heavy metal?

Comment [N'BJ22]: What does it mean by alien plant?

Comment [N'BJ23]: Which is native/normal plant? Authors not mentioned.

6REFERENCES

[1] Rahman, M. A., & Hasegawa, H. Aquatic arsenic: phytoremediation using floating macrophytes. *Chemosphere*,(2011). *83*(5), 633-646.

[2] Wei, G., Fan, L., Zhu, W., Fu, Y., Yu, J., & Tang, M. Isolation and characterization of the heavy metal resistant bacteria CCNWRS33-2 isolated from root nodule of Lespedeza cuneata in gold mine tailings in China. *Journal of hazardous materials*,(2009). *162*(1), 50-56.

[3] Pilon-Smits, E. A., Quinn, C. F., Tapken, W., Malagoli, M., & Schiavon, M. Physiological functions of beneficial elements. *Current opinion in plant biology*,(2009). *12*(3), 267-274.

[4] Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., &Mench, M. Phytoremediation of contaminated soils and groundwater: lessons from the field. *Environmental Science and Pollution Research*, (2009). *16*, 765-794.

[5] Xu, J., Grumbine, R. E., Shrestha, A., Eriksson, M., Yang, X., Wang, Y. U. N., & Wilkes, A. The melting Himalayas: cascading effects of climate change on water, biodiversity, and livelihoods. *Conservation Biology*, (2009). *23*(3), 520-530.

[6] Ensley, B. D., Raskin, I., & Salt, D. E. Phytoremediation applications for removing heavy metal contamination from soil and water. *Biotechnology in the sustainable environment*,(1997). 59-64.

[7] Cunningham, S. D., & Lee, C. R. Phytoremediation: Plant-based remediation of contaminated soils and sediments. *Bioremediation: Science and applications*,(1995). *43*, 145-156.

[8] Schickler H, Caspi H. Response of antioxidative enzymes to nickel and cadmium stress in hyperaccumulator plants of the genus Alyssum.Physiologia plantarum. 1999;105(1):39-44.

[9] Prasad, M. N. V., Hagemeyer, J., Dietz, K. J., Baier, M., &Krämer, U. Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. *Heavy metal stress in plants: from molecules to ecosystems*,(1999): 73-97.

[10] Campos-M, M., & Campos-C, R. Applications of quartering method in soils and foods. *International Journal of Engineering Research and Application*,(2017): *7*(1), 35-39.

[11] Black, C.A. Methods of Soil Analysis, Part II, Chemical and microbiological properties. American Society of Agronomy, Madison, Wisconsin, USA,(1965): 771-72.

[12] Jackson, M.L. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi (1973).

[13] Cresswell, H. P. and G. J. Hamilton. Bulk Density and Pore Space Relations. In: Soil Physical Measurement and Interpretation For Land Evaluation. CSIRO Publishing: Collingwood, Victoria.(2002): 35 – 58.

[14] Walkley, A., & Black, I. A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil science*,(1934): *37*(1), 29-38.

[15] Van Bemmelen JM. Ueber die Bestimmungen des Wassers, des Humus, des Schwefels, der in den ColloidalenSilikatengebundenKieselsaeuren, des mangans, u.s.w.imAckerboden. *LandwirtschaftlicheVersuch Station,* (1891): 37:279-290.

[16] Kumar, V., Sharma, A., Kaur, P., Sidhu, G. P. S., Bali, A. S., Bhardwaj, R. & Cerda, A. Pollution assessment of heavy metals in soils of India and ecological risk assessment: A state-of-the-art. *Chemosphere*, (2019):216, 449-462.

[17] Riaz, A., Khan, S., Muhammad, S., Liu, C., Shah, M. T., & Tariq, M. Mercury contamination in selected foodstuffs and potential health risk assessment along the artisanal gold mining, Gilgit-Baltistan, Pakistan. *Environmental geochemistry and health*,(2018): *40*, 625-635.

[18] Muhammad, S., Shah, M. T., & Khan, S. (2011). Heavy metal concentrations in soil and wild plants growing around Pb–Zn sulfide terrain in the Kohistan region, northern Pakistan. *Microchemical Journal*, (2011): *99*(1), 67-75.

[19] Curtis, J. T., & Mcintosh, R. P. The interrelations of certain analytic and synthetic phytosociological characters. *Ecology*,(1950): *31*(3), 434-455.

[20] Cox, G. Population and community structure: Quadrat sampling techniques. *Laboratory Manual of General Ecology*, (1990): 69-73.

[21] Valdes, L. J., Mislankar, S. G., & Paul, A. G. Coleus barbatus (C. forskohlii)(Lamiaceae) and the potential new drug forskolin (Coleonol). *Economic botany*, (1987): *41*, 474-483.

[22] Gupta, D. K., Corpas, F. J., & Palma, J. M. (Eds.). *Heavy metal stress in plants* (p. 240). Berlin: Springer(2013).

[23] Ghosh, M., & Singh, S. P. A comparative study of cadmium phytoextraction by accumulator and weed species. *Environmental pollution*, (2005): *133*(2), 365-371.

[24] Zhuang, P., Zhi-An, L. I., Bi, Z. O. U., Han-Ping, X. I. A., & Gang, W. A. N. G. Heavy metal contamination in soil and soybean near the Dabaoshan Mine, South China. *Pedosphere*, (2013): *23*(3), 298-304.

[25] Singh, R., Gautam, N., Mishra, A., & Gupta, R. Heavy metals and living systems: An overview. *Indian journal of pharmacology*, (2011): *43*(3), 246.

[26] Carter MR, Gregorich EG. Carbon and nitrogen storage by deep-rooted tall fescue (Lolium arundinaceum) in the surface and subsurface soil of a fine sandy loam in eastern Canada. Agriculture, ecosystems & environment. 2010;136(1-2):125-32.

[27] Aragaw, T. A., Wondimnew, A., &Asmare, A. M. Quantification, characterization and recycling potential of solid waste: case study Bahir Dar institute of technology. *Int J Sci Res*,(2016): *5*, 2415-2420.

[28] Sparks, D. L. Environmental soil chemistry: An overview. *Environmental soil chemistry*, (2003): *2*, 1-42.

[29] Alloway, B., & Ayres, D. C. *Chemical principles of environmental pollution*. CRC press, (1997).

[30] Uba, S., Uzairu, A., Harrison, G. F. S., Balarabe, M. L., & Okunola, O. J. Assessment of heavy metals bioavailability in dumpsites of Zaria Metropolis, Nigeria. *African Journal of Biotechnology*, (2008): *7*(2).

[31] Goswami, U., & Sarma, H. P. Study of the impact of municipal solid waste dumping on soil quality in Guwahati city. *Pollution research*,(2008): *27*(2), 327-330.

[32] Mathur, G. M. Effect of long-term application of fertilizers and manures on soil properties and yield under cotton-wheat rotation in north-west Rajasthan. *Journal of the Indian Society of soil Science*, (1997):*45*(2), 288-292.

[33] Chaudhury, J., Mandal, U. K., Sharma, K. L., Ghosh, H., & Mandal, B. Assessing soil quality under long-term rice-based cropping system. *Communications in Soil Science and Plant Analysis*, (2005):*36*(9-10), 1141-1161.

[34] Munoz, V., & Serrano, L. Elucidating the folding problem of helical peptides using empirical parameters. *Nature structural biology*,(1994): *1*(6), 399-409.

[35] Hassink, J., & Whitmore, A. P. A model of the physical protection of organic matter in soils. *Soil Science Society of America Journal*, (1997): *61*(1), 131-139.

[36] Oyedele, D. J., Gasu, M. B., &Awotoye, O. O. Changes in soil properties and plant uptake of heavy metals on selected municipal solid waste dump sites in Ile-Ife, Nigeria. *African Journal of Environmental Science and Technology*, (2008): *2*(5), 107-115.

[37] Shende, A., Juwarkar, A. S., & Dara, S. S. Phytotoxic effects of heavy metals as influenced by soil properties. *Pollution Research*,(1993): *12*(2), 75-83.

[38] Udeme, A. E. Solid waste analysis and characterization and Department of Chemistry. *University of Uyo*, (2001).

[39] Jumbe, A. S., & Nandini, N. Heavy metals analysis and sediment quality values in urban lakes. *American Journal of Environmental Sciences*,(2009): *5*(6), 678.

[40] Nwajei, G. E., Iwegbue, C. M. A., & Okafor, M. I. Heavy metals in surface soils under waste dumps from Onitsha, Nigeria. *J Biol Sci*, (2007): *7*(2), 405-408.

[41] Ogunyemi, S., Bamgbose, O. O., &Awodoyin, R. O. Heavy metal contamination of some leafy vegetables growing within Ibadan metropolis, South-western Nigeria. *Tropical Agricultural Research and Extension*,(2003): *6*, 71-76.

[42] Awokunmi, E. E., Asaolu, S. S., &Ipinmoroti, K. O. Effect of leaching on heavy metals concentration of soil in some dumpsites. *African Journal of Environmental Science and Technology*, (2010): *4*(8), 495-499.

[43] Fumikazu, S., Shusuke, S., Atsunori, M., Takashi, K., Shun, I., Jun, A., & Keiko, I. Thin-rim enhancement in Gd-DTPA-enhanced magnetic resonance images of tuberculoma. *Journal of Thoracic Imaging*, (1992): *7*(3), 64-69.

[44] Nriagu, J. O., & Pacyna, J. M. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *nature*, (1988): *333*(6169), 134-139.

[45] Sharma, R. K., Agrawal, M., & Marshall, F. M. Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. *Food and chemical toxicology*,(2009): *47*(3), 583-591.

[46] Fayiga, A. O., Ma, L. Q., Cao, X., &Rathinasabapathi, B. Effects of heavy metals on growth and arsenic accumulation in the arsenic hyperaccumulator Pteris vittata L. *Environmental pollution*, (2004): *132*(2), 289-296.

[47] Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Science of the total environment*, (2006):*368*(2-3), 456-464.

[48] Baker, A. J. Accumulators and excluders-strategies in the response of plants to heavy metals. *Journal of plant nutrition*,(1981): *3*(1-4), 643-654.

[49] Usman, A. R. A., & Mohamed, H. M. Effect of microbial inoculation and EDTA on the uptake and translocation of heavy metal by corn and sunflower. *Chemosphere*, (2009): *76*(7), 893-899.

[50] Neilson, S., &Rajakaruna, N. Phytoremediation of agricultural soils: using plants to clean metal-contaminated arable land. *Phytoremediation: Management of Environmental Contaminants,*(2015):1, 159-168.