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# Assessment of hyperaccumulator plants in native flora grown around the solid waste open dumpsite in District Karak, Pakistan

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**Abstract**
Rapid industrialization and urbanization have caused serious threat to our environment and open dumpsites are one of the main sources polluting our environmental factor by releasing heavy metals into water, air and soil. Phytoremediation is a novel and ecofriendly approach to remediate heavy metals pollution. The current study was carried out to investigate phytoremediation potential of native plants growing around the municipal solid waste open dumpsite in district Karak. Four dominant plants species namely *Astragalus creticus Lam*, *Allium carolinianum DC*, *Arisaema jacquemontii Blume* and *A. spinosus* along with soil samples were assessed for three heavy metals Chromium (Cr), Nickel (Ni) and Iron (Fe) using atomic absorption spectrophotometer. Principle component analysis and correlation model was used to find associations between heavy metals uptake pattern and plant species. Phytoremediation potential of the studied plants were calculated through translocation factor (TF), bioaccumulation factor (BAF) and bioaccumulation coefficient (BAC). The results shows that Fe had the highest mean concentration of 1465.6±161 mg/kg$^{-1}$ in dumpsite soil followed by Cr and Ni with mean concentration and standard deviation of 1184±295 mg/kg$^{-1}$ and 273±164 mg/kg$^{-1}$, respectively. Similarly, based TF, BAC and BAF all the analyzed plant species were hyperaccumlator for Cr and only accumulator for Ni and Fe. The study concluded that *Astragalus creticus Lam Allium carolinianum DC*, *Arisaema jacquemontii Blume* and *A. spinosus* has the potential candidate to remediate Cr, Ni and Fe metals pollution.

**Keywords:** Bioaccumulation, Heavy Metals, Hyper Accumulator, Translocation, Open Dumpsite

## 1. Introduction

Uncontrolled and poorly handled waste disposal results in heaps of refuse on open dumping sites emitting toxic pollutants that have a major impact on the environment and human health (Naz *et al.*, 2021). Many disease-carrying vectors, such as rats, fleas, and mosquitoes, find landfills as an ideal breeding ground (Verona *et al.*, 2019). Open dumps have long been thought of as the ultimate tool for disposing of garbage at the lowest possible expense (Ayyub *et al.*, 2015). Owing to the lack of any form of protection for emissions control and the toxic substances leaching, old dumpsites are a source of heavy metals contamination (Khan *et al.*, 2018). Heavy metals in soils as a result of industrial activities is one of the most pressing issues of the modern world. High quantities of heavy metals have a negative impact on environmental health, food quality, plant health and production (Akhtar *et al.*, 2018) [1]. Atmospheric deposition of metals from mining and smelting operations, heavy metals from open dumpsite and the usage of pesticides and sewage sludge are the main sources contributing to metals pollution (Ali *et al.*, 2013) [2]. Metal contamination causes significant harm to the local flora, altering the community structure by causing an increase in resistant herb populations and a decrease in plant biodiversity as well as bioaccumulation in food plants with higher health risks for humans (Zhoufaz *et al.*, 2018) [33]. Previous studies have reported that all the open dumping sites in Pakistan are posing serious threat to natural resources (soil, water, or sediments) and Cu, Zn, Cd, Pb, Ni, Fe, Cr and as were commonly found in higher concentration than permissible or baseline providing a high ecological danger. Metals are categorized into essential and non-essential primarily (Zhang *et al.*, 2012) [32]. For regular plant development in to their significance to biological systems, necessary micronutrients maintain the growth cycle while non-essential hazardous metals including Ni, Pb, Cd, As, Cr, and Hg even in minute quantities have deleterious effects on plants health (Maleki *et al.*, 2017). Many researchers have brought up the connection between heavy metals pollution and health problems. People who live or work near open dumps have a greater chance of congenital birth defects, cancer, and respiratory illness (Zhou *et al.*, 2018). The evidence linking waste open dumps and incinerators to health problems such as cancer, fertility conditions have long been reported (Naveed *et al.*, 2020). To avoid these health issues and to remediate the deadly metals or to limit its health impact, phytoremediation is an emerging technology used extensively for metals pollution. Plants that grow in heavy metal-rich soils are known as Metallophytes (Li *et al.*, 2016). Metal tolerance has evolved in metallophytes as a result of prolonged exposure to an abundance of various metals in different form. Metallophytes are plants that have a unique capacity to withstand metal toxicity while surviving and reproducing in metalliferous soils (Nazir *et al.*, 2015). Metal markers are plants that can absorb heavy metals in above-ground biomass (Suresh *et al.*, 2018). Metal excluders are plants that can absorb heavy metals from the substrate in their roots and transfer into the above-ground plant biomass. Although certain plants are not suitable for metal extraction, demonstrated that they can be useful for phytostabalisation purpose (Xia *et al.*, 2018). The technique is comparatively new in the research and in implementation area. According to scientists, the Baker and Brooks norm for hyper accumulators is the most cited (with a total of 1376 citations to date) however, is extra conservative in determining plants as a hyperaccumulator. Plant species which can accumulate 100 mg/kg cadmium, 1000 mg/kg nickel, copper, and lead on a dry weight basis, and 1000 mg/kg zinc and manganese in their aerial sections in their natural environment could be considered as hyper accumulators (Brook and baker, 1989) [4]. Hyper accumulators are plants with concentrations of 100 mg/kg cadmium, selenium, and trallium; 300 mg/kg cobalt, copper, and chromium; 1000 mg/kg nickel, iron, and arsenic; 3000 mg/kg zinc; and 10000 mg/kg manganese can be grouped as hyper accumulators (Van der ent *et al.*, 2013). Hyper accumulators (without reducing yield) contain a 100-fold higher metal content in aerial parts than non-accumulator or crop plants (Chaney *et al.*, 2007; Lasat, 2002). Just a few trials have been performed to assess the effectiveness of phytoremediation in the environment, although most testing is currently limited to laboratories and other greenhouse scale experiment. Since field is something different and considered as real world where many variables are at play at the same time, results in the field can vary from those obtained in the laboratories (Ji *et al.*, 2011). The aim of this study was to evaluate the Eco toxicity effects of some heavy metals regarding the plant growth and metal accumulation in four plants plants spceies *Astragalus creticus Lam Allium carolinianum DC, Arisaema jacquemontii Blume* and *A. spinosus* to search hyper accumulator plants for heavy metals remediation.

## 2. Materials and methods

### 2.1 Description of the study area

The study area is located between 32°1'50 N and 71°1'52 E at an altitude of 469 meters above from the sea level having total population of 40,852 persons (GoP, 2017). Overall, the climate of the area is semi-arid, and the average rainfall is 450 mm/year. The average temperature ranges from 45°C in summer and to 10°C in winters (Javed *et al.*, 2019). The total area of the district is 3,372 square kilometers (Tabassum *et al.*, 2012) and the total population of the district is about 707,000. Physiography of the district is rough having plains and hills with wide valleys. It is among the richest district regarding precious mineraloids e.g., salt, gypsum, oil, and gas etc. (Khan *et al.*, 2011).

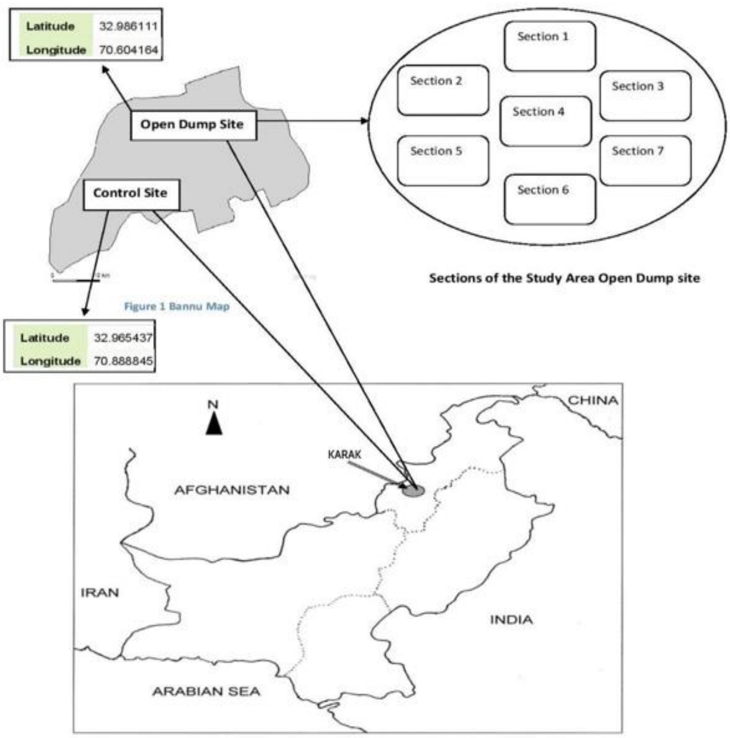


**Fig 1:** Map of the study area

### 2.2 Plants samples collections and identification

Soil and plant samples were collected from the major solid waste open dumpsite by using random sampling techniques. For plants samples, species frequency and accessibility were considered. Similarly, soil samples were collected from the root zone of each plant samples and were checked for the background concentration of the selected heavy metals. The collected samples were carried in polyethylene bags to the Central Laboratory of the University of Haripur for further analysis. Apart from it, plants species were identified with help of smart phone apps such as Plant Net and Plant snap and through experience faculty members of Forestry Department of the University of Haripur.

### 2.3 Sample preparation and chemical analysis

Plant samples were divided into roots and shoots and carefully washed with distilled water for about three minutes to remove dirt particles from the plants. Following cleaning and two weeks of air drying at room temperature, plant samples were ground into powder using a steel pistel and morter. Then soil samples were sieved using a 2-mm stainless steel sieve after being allowed to air dry for two weeks at room temperature. A mixture of 15 ml from Perchloric acid ($HClO_4$), Nitric acid ($HNO_3$) and Sulfuric acid ($H2SO_4$) with ratio of 1:5:10 was prepared. Then each plant of (0.5g) was added to the solution and left-over night. The samples were carried to digestion block and heated for 1 h at 80°C then the temperature was raised up to 120–130°C until a transparent solution was obtained. Each solution tubes were cooled, filtered through Whitman usually through (0.45 µm) and diluted to 50ml with deionized water. (Adesodun *et al.*, 2010; Padmavathiamma and Li, 2007; Zhuang *et al.*, 2007). The total metal concentration was calculated using a flame atomic adsorption spectrophotometer after the plant and soil samples were digested using the hot-block digestion process (USEPA Method 3050).

### 2.4 Phytoremediation potential of analyzed plants

Phytoremediation potential of the studied plants were calculated as followed by using the equation by Adesodun *et al.*, (2010).

$$Translocation\ factor = \quad Metal\ conc.in\ \frac{Shoots}{Root}$$

$$Bioaccumulation\ coefficient = \quad Metal\ conc.in\ \frac{Shoots}{Soil}$$

$$Bioaccumulation\ factor = \quad Metal\ conc.in\ \frac{Root}{Soil}$$

### 2.5 Statistical analysis

Principle component analysis and was used to find the behavior heavy metals in soil-plant system and heavy metals distribution in different plants parts. Kaiser Criteria which is accepted widely was considered for number of PCs which proposed eigenvalues>1 and accumulated variance ≥70 should be retained in the system. The software used were graph pad and SPSS.

## 3. Results and discussion

### 3.1 Soil properties and metal concentrations

In the dumpsite soil, pH ranged as 5.3-16.2 with mean concentration and standard deviation of 11.8±6.3 while in the control site soil, pH ranges as 6.1-8.3 with means and standard deviation of 6.4± 1.9, respectively as shown in the Table 1. The previous studies showed that typical soil the study area has lower pH then demonstrated by the dumpsite soil. Such higher pH value could be attributed the anthropogenic organic input to the dumpsite soil. Similarly, Table 1 shows mean EC value with standard deviation in the dumpsite soil was 10.4±3.3 which is way higher than the previous studies result in the same area. Higher EC value of the dumpsite soil could be regarded to the metallic scrap in the dumpsite soil being disposed often in the form of workshop waste as the municipality had no protocol for industrial waste to impose. In the same manner organic contents in the dumpsite soil ranged as 2.5-4.8 mg/kg$^{-1}$ with mean concentration and standard deviation of 3.54±1.1 mg/kg$^{-1}$ (Table 1). The relatively higher organic contents in the dumpsite could be attributed to the organic inputs in the form of animal waste, sewage sludge, plant leaves and organic municipal waste. Previous studies reported that due to the mixed collection of food waste from households in Pakistan, MSW is diverse in character. Waste generated in low- and middle-income countries has a higher moisture and organic contents than waste produced in high-income or developed countries. Likewise, soil texture varied in different section of the dumpsite because of the different type of waste disposal into the dump site. However, the soil type was dominantly loamy sand with less clay and silt contents. Heavy metals concentration was higher in the dumpsite soil then in the control site soil. Among the analyzed metals, the highest concentration was observed in soil Cr with mean concentration and standard deviation of 1465.6±161 mg/kg$^{-1}$, followed by Ni and Fe with means values and standard deviation of 1184±295 mg/kg$^{-1}$ and 594.3±218 mg/kg$^{-1}$, respectively (Table 2). In contrary to the dumpsite soil, the analyzed metals concentration was lower in the control site soil. Since metals are more soluble in acidic media than in basic media, soil pH may to improve heavy metal bioavailability. In the current study, the lower range of pH was observed 5.3 which demonstrate quite acidic environment thus feasible for heavy metals bioavailability. The higher concentration of heavy metals in the dumpsite demonstrate that different nature of wastes is being thrown without any prior screening which pose serious threat to the environmental factors.

**Table 1:** Physiochemical parameters of dumpsite and control site soil

| S. No | Parameters | Dumpsite | | Control site | |
|---|---|---|---|---|---|
| | | Range | Mean±Std | Range | Mean±Std |
| 1 | pH | 5.3-16.2 | 11.8±6.3 | 6.1-8.3 | 6.4±1.9 |
| 2 | EC mS/cm | 5.1-18.3 | 10.4±3.3 | 7.3-9.5 | 8.6±1.4 |
| 3 | OM mg/kg | 2.5-4.8 | 3.54±1.1 | 1.06-2.5 | 1.32±0.4 |
| 5 | Soil Texture % | Loamy sand | | Loamy sand | |
| | Silt | 17 | | 11.8 | |
| | Clay | 11 | | 13 | |
| | Sand | 68.2 | | 74.2 | |

### 3.2 Heavy metals in Plants

Depending on the type of plant, metal levels might differ in that plant (Alloway *et al.*, 1990). Heavy metals are either passively absorbed by plants with the mass flow of water into the roots or actively transferred across the plasma membrane of the root epidermal cells. Under normal growing conditions, plants may be able to capture certain metal ions to a greater amount than the surrounding medium

(Kim *et al.*, 2003). Heavy metals are ingested by plants from their roots from the soil solution. Metals can be transported from roots to xylem vessels or retained in the roots system where they are deposited in vacuoles (Jabeen *et al.,* 2009; Prasad, 2004). Vacuoles are cellular organelles that have a lower metabolic function or none at all. Metals sequestration in vacuoles is one of the most effective ways to remove excess metal ions from the cytosol, and can interfere with cellular metabolic reactions. Hyperaccumulators plants, complex metals compartmentalization in vacuoles is a part of the tolerance mechanism (Bothe *et al.,* 2011).

In the dumpsite plants, Cr concentration was highest in the *A. Spinosus* roots with means concentration and standard deviation value of 419±128 mg/kg$^{-1}$ followed by *A.Carolinianum* 301.6±85 mg/kg$^{-1}$ and the lowest Cr concentration was founded in *A. jacquemontii* root with mean value of 106.3±71 mg/kg$^{-1}$, respectively. Similarly, *A. spinosus* accumulated highest Cr contents in shoots with mean value and standard deviation of 128.6±33 mg/kg$^{-1}$ followed by *A.carolinianum* 59.6±22 mg/kg$^{-1}$ and lowest value was observed in the shoot of with mean concentration and standard deviation value of 26.6±7 mg/kg$^{-1}$ The overall Cr concentration was higher in plant roots comparative to plants shoots which shows that most of the Cr is accumulated in the root and its translocation is very low towards shoots. The uptake of the Cr by plant depends upon the background concentration. Further Cr concentration showed significant variation from those plants grown in the control site. The higher bioavailability of Cr is probably due the higher solubility of Cr in water rich environment which was provided by the dumpsite leachate. The other reason may be the preferential uptake of Cr against other metals like Cd and Pb as Cr is one the micro essential nutrient in plants growth cycles up to required limits. The current study results are in line with previous study conducted by (Khan *et al*., 2010).

*A. spinosus* was the leading plant species to uptake Ni in root with mean concentration and standard deviation value of 273±164 mg/kg$^{-1}$ while the lowest Ni concentration was founded in root of *A. creticus Lam*. with mean value 28.6±24 mg/kg$^{-1}$, respectively. Similarly, highest Ni contents in shoots with mean value and standard deviation of 99±43 mg/kg$^{-1}$ were observed in *A. spinosus* and the lowest value was observed in the shoot of *A. jacquemontii* with mean concentration and standard deviation value 22.3±13 mg/kg$^{-1}$ of the overall Ni concentration was higher in plant roots comparative to plants shoots which shows that most of the Ni is accumulated in the roots and its translocation is very low towards shoots. Such higher translocation of Ni in plant species is very rare and has very adverse toxic effects on plants growth. The effect of plants exposure to Ni for a long time can cause growth inhibition and drying of leaves. The Ni higher concentration may be because of the hospital waste being disposed into the dumpsite which may eventually ended in the plants uptake via root. The current study results are in line with the previous study conducted by (khan *et al.,* 2017) in the same context.

*A.carolinianum* concentrated highest Fe in roots with means concentration and standard deviation value of 525.6±270 mg/kg$^{-1}$ and the lowest concentration was founded in root of A. jacquemontii with mean value of 231±38 mg/kg$^{-1}$ (Table 2). Similarly, *A. spinosus* accumulated highest contents in shoots with mean value and standard deviation 251±127 mg/kg$^{-1}$ and the lowest concentration was observed in the shoot of *A. jacquemontii* with mean concentration and standard deviation value of 88±24 mg/kg$^{-1}$ as shown in the (Table 2). The overall Fe concentration was higher in plant roots comparative to plants shoots which shows that most of the Fe is accumulated in the root and its translocation is very low towards shoots. Plants usually uptake Fe contents to maintain growth and cellular activities. However, higher Fe contents may disturb the basal level of magnesium and potassium leading to nutritional disturbances (Anjum *et al.,* 2015). The higher Fe, Cr and Ni contents in the plants species represent that the dump site was seriously polluted with heavy metals.

**Table 2:** Mean concentration and standard deviation of heavy metals in mg kg$^{-1}$ in the dumpsite, control site soil and plants roots and shoots

| | | **Cr mg kg$^{-1}$** | | | **Ni mg kg$^{-1}$** | | | **Fe mg kg$^{-1}$** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plants** | | Soil | Root | Shoot | Soil | Root | Shoot | Soil | Root | Shoot |
| **A. creticus Lam.** | Ref | 45.41 | 25.23 | 14.76 | 16.34 | 13.87 | 44.23 | 76.97 | 34.34 | 9.98 |
| | Range | 156-465 | 56-187 | 29-78 | 122-265 | 20-699 | 44-704 | 1322-1643 | 245-785 | 365-113 |
| | Mean | 315.31±154 | 118.5±65 | 46.8±27 | 190.7±71 | 28.6±24 | 15.5±9 | 1465.6±161 | 525.6±270 | 251±127 |
| **A. jacquemontii** | Ref | 14.65 | 21.23 | 6.56 | 37.35 | 12.2 | 8.54 | 3.32 | 45.21 | 23.56 |
| | Range | 128-432 | 63-189 | 18.31 | 265.441 | 32.178 | 12.37 | 481.678 | 187.258 | 63.112 |
| | Mean | 268.3±153 | 106.3±71 | 26.6±7 | 365±90 | 121.3±78 | 22.3±13 | 580.3±98 | 231±38 | 88±24 |
| **A.carolinianum** | Ref | 45.54 | 23.65 | 12.43 | 11.43 | 17.65 | 6.87 | 40.6 | 43.67 | 10.65 |
| | Range | 342-651 | 218-389 | 34.78 | 123.316 | 34.211 | 23.156 | 376.784 | 234.456 | 46.221 |
| | Mean | 513.6±157 | 301.6±85 | 59.6±22 | 226±97 | 133.6±90 | 85.6±66 | 575.6±204 | 345±111 | 133.6±87 |
| **A. spinosus** | ref | 15.76 | 34.76 | 20.67 | 25.76 | 56.87 | 13.65 | 39.76 | 15.87 | 32.67 |
| | Range | 867-1451 | 345-567 | 89.165 | 347.763 | 132.453 | 56.143 | 568.987 | 123.432 | 45.213 |
| | Mean | 1184±295 | | 128.6±33 | | 273±164 | 99±43 | 780.4±209 | 293±156 | 108.3±91 |

**Phytoremediation potential of plants**

The BCF, which is defined as the ratio of the metal concentration in the roots to that in the soil, may be used to measure a plant's capacity to acquire metals from soils. The TF, which is defined as the ratio of metal concentration in the shoots to the roots, is used to assess a plant's capacity to move metals from the roots to the shoots (Naz *et al.,* 2019). Similarly, BAF refers to the heavy metal's concentration in roots to that in soil which BAC and TF shows the metals ratio in soil to shoot and root to shoot respectively. Based on the mentioned parameters we grouped plants species into excluders, accumulator and hyperaccumulator. Plants with BAF, BAC and TF values >1 are considered as excluder. Similarly, plants having BAF values between 1-10 and BAC and TF value >1 are considered as accumulator while plant species with BAF>10 along with BAC and TF values >1 are perfect hyper accumulator specie for any metal. However, all the mentioned criteria merely obey most of the time and most of the author prefer to categories plants based on TF and BAC only. So, in case Cr, Ni and Fe, all the analyzed plants namely Astragalus *creticus Lam, Arisaema jacquemontii Blume, Allium carolinianum DC* and *A. spinosus* showed as a potential hyperaccumulator plant as shown in the Fig (2, 3, 4). Since as per the followed criteria, BAC and TF values were >1 hence demonstrated good potential to remediate all the analyzed plants.

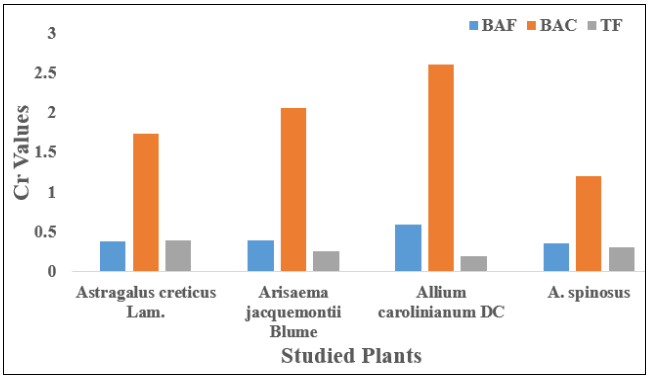


**Fig 2:** Phytoremediation potential factors of the studied plants grown in the dumpsite and control site based on Cr

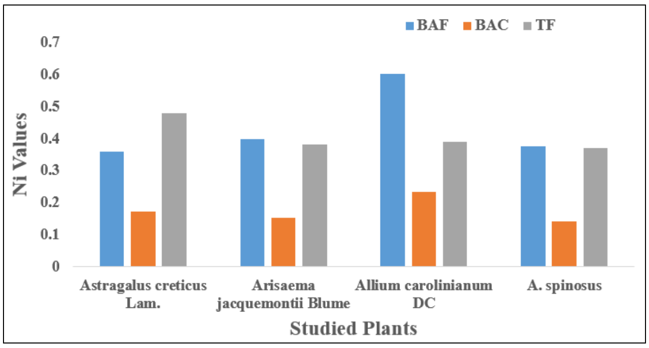


**Fig 3:** Phytoremediation potential factors of the studied plants grown in the dumpsite and control site based on Ni

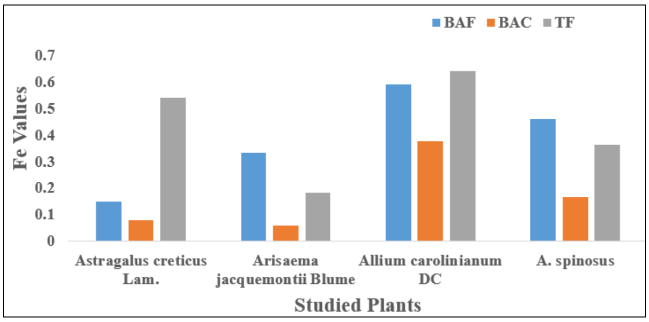


**Fig 4:** Phytoremediation potential factors of the studied plants grown in the dumpsite and control site based on Fe

### Principle component Analysis

Results of the PCA analysis revealed that in the dumpsite soil, PC1 contributed 76.3 percent variation and PC2 showed 23.6 percent of variance while the collective variance was 99.97 as shown in the (Fig 5). Soil Fe showed highest loading value on PC1 while Ni and Fe have almost same loading on PC1. In the same manner, Soil Cr and Ni had showed strong loading on PC2 while Fe has less association with PC2 (Fig 5). Since PC1 explained maximum variance of 76.3 and Fe close association demonstrate that Fe was the most dominant metal in the dumpsite soil and the Ni and Cr were secondary in term of overall concentration. Moreover, biplot vectors direction is important to understand which shows correlation among the metals. In the (Fig 5), we can apparently conclude that there is no correlation among the metals.

In the same way PCA was applied on the plant data set to find similarities, dissimilarities and associations. In the plant data set PC1 explained maximum variance of 83.7 followed by PC2 with variance of 11.7, respectively. As shown in the (Fig 5), Fe shoot showed strongest association with PC1 followed by Fe root consolidating the Fe results in the dumpsite soil as shown in the fig 5, *Astragalus creticus Lam* showed close association with PC1 followed by *Allium carolinianum DC* and *A. spinosus*, respectively. While *Arisaema jacquemontii Blume*, showed weakest association with PC1 and was rather strongly associated with PC2 which demonstrate its lower influence in term of metals uptake among the plants. As shown in the (Fig 5) root Fe and shoot Fe were strongly correlated while all the other had moderate to no correlation or very less minimum correlation.

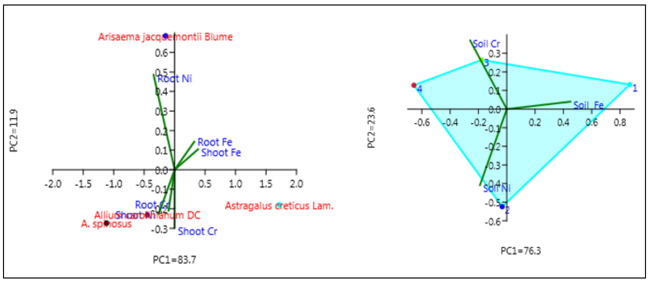


**Fig 5:** The PCA bio plot shows heavy metals distribution in different parts of plants species and in dumpsite soil

## 4. Conclusion

It was observed that overall heavy metals concentration was higher in plants roots as compare to plants shoots in dumpsite. Based on TF and BAC values for Cr, *Lam Allium carolinianum DC* was the most promising specie with highest BAC followed by *Arisaema jacquemontii Blume*, *Astragalus creticus*, and *A. spinosus*, respectively. Likewise, in case of Ni, *Lam Allium carolinianum DC* was again the most efficient specie based on TF and BAC followed by *Astragalus creticus*, *Arisaema jacquemontii Blume* and *A. spinosus*, respectively. In case of Fe, *Lam Allium carolinianum DC* proved as a potential candidate while *Arisaema jacquemontii Blume* was the least efficient specie in Fe sequestration. Overall, all the four plants demonstrated good potential for metals remediation and could be used as a hyperaccumulator. The overall, over-all heavy metals concentration was higher in the dumpsite soil posing threats to environmental factors.

**Fig 6:** View of the solid waste open dumpsite

## 5. Recommendations

1. Based on the current study findings, *Lam Allium carolinianum DC, Arisaema jacquemontii Blume, Astragalus creticus,* and *A. spinosus* should be checked in control environment for better understanding of the plants species.
2. Similarly, these plants should be study in cellular level to comprehend the mechanism and physiological changes occurs when exposed to heavy metals.
3. Native plants need to be explored for different heavy metals as they can survive in range of environment.
4. Open dumps sites should be immediately turned into engineered landfill site to avoid its deleterious impacts to biota.

## 6. Acknowledgement

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