Evaluating the potentiality of naturally growing *Ipomoea carnea* Jacq. as an iron hyperaccumulator in Ramgram Municipality, Nawalparasi (west), Nepal

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Received: 09, August 2024 Revised: 01, September 2024 Accepted: 15, September 2024 Published: 22, November 2024

Hyperaccumulator plants, such as *Ipomoea carnea*, are known for their ability to accumulate heavy metals in their aerial parts. This study aimed to determine whether *I. carnea* is an iron hyperaccumulator. Ten sites within the Ramgram Municipality, Nawalparasi (west) district were selected randomly- five near industrial areas and five away from industries. Two plants from each site were harvested along with the soil samples nearby, following the standard protocols for collection. After sun-drying, the plant samples for four weeks and soil samples for three days, iron concentrations in roots, shoots, leaves, and soil were measured with an atomic absorption spectrophotometer using ashing and wet acid digestion. The results showed a significant correlation ($r = 0.728$, $p < 0.05$) between the root iron concentration and the total iron in the plant. The analysis of variance revealed differences in iron accumulation in the roots, stems, and leaves of *I. carnea*. In the industrial areas, the biological absorption coefficient was 0.12 as compared to 0.08 in the non-industrial areas, indicating *I. carnea* is a low accumulator. The bioconcentration factor and the translocation factor were observed to be higher in the industrial areas. Overall, *I. carnea* was not found to be an effective iron hyperaccumulator in the study area, as indicated by the biological absorption coefficient, bio-concentration factor, and translocation factor values. In addition, the soil iron concentration was within the acceptable limit in the study area.

Keywords: *Bioconcentration factor, heavy metals, iron concentration, soil pollution*

Hyperaccumulators are those species that can accumulate a particular type of metal or metalloid in their biomass which is a hundred or thousand times greater than is normal for most species (van der Ent *et al*., 2013). In accumulator plants, the concentration ratio of the metal in the plant to that in the soil is >1. These species can absorb metal from the soil in large amounts without showing a phytotoxic effect. Phytoremediation is the technique of remediation of contaminated sites using hyperaccumulator plants. It comprises phytovolatization, phytodegradation, rhizofiltration, phytostabilization, and phytoextraction (NRMRL, 2000). Rapid industrialization and urbanization have created a serious threat of environmental degradation including soil pollution. The most common heavy metals present in the soil are lead, nickel, iron, copper, zinc, mercury, arsenic, and chromium (Bakshi *et al*., 2018). There are different ways to remediate the heavy metals from soil namely, adsorption, soil leaching, electrokinetic remediation, soil removal, and isolation and replacement of contaminated soil (Nyiramigisha *et al*., 2021). When combined with forestry and bio-energy production, phytoremediation can still be economical despite its slow process (Robinson *et al*., 2003). After the Chernobyl accident in 1986, cesium and strontium were removed by using sunflowers (Ghosh *et al*., 2021). Thus, hyperaccumulator plant species can be a good alternative to remediate the soil contaminated with such heavy metals by extracting the metals in their biomass (Ghosh *et al*., 2021).

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Figure 1: *Ipomoea carnea* plants growing naturally in the study site.

Belonging to the family 'Convolvulaceae', *Ipomoea carnea* Jacq. is a shrubby plant (see Figure 1) native to tropical America and Caribbean, and it has become an invasive plant in Nepal (Shrestha *et al*., 2017). It is found growing in degraded land and waterlogged areas, particularly in tropical regions. This species usually grows around 1–3 m erect tall in open habitats whereas in shady habitats it usually likes to climb with twining stems reaching up to 5 m (Kulshrestha & Dabral, 2018). It is a fast-growing species that yields higher biomass, and has shown potential for phytoextraction of cadmium (Ghosh & Singh, 2005). Besides, it has shown the ability to accumulate metal in roots and translocate them to aerial parts in fly ash dumps (Pandey *et al*., 2016). Kulshrestha & Dabral (2018) have shown that *I. carnea* exhibited considerable metal uptake potential for Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Nickel (Ni), Lead (Pb), and Zink (Zn) in its roots as well as translocate these to above-ground parts showing high bioconcentration factor for iron.

The purpose of this study was to find out whether the naturally growing *I. carnea* within the Ramgram Municipality, Nawalparasi (west) district meets the criteria of iron hyperaccumulator and to assess its iron phytoremediation capability. The study area possesses different industries like alcohol distillery, steel plants, paper processing plants, brick kilns, and other small wood-based industries. Industrial activities are considered major contributors of heavy metals to environmental contamination (He *et al.*, *2005;* Mpewo *et al*., 2023). The study also aimed to investigate if these industries are causing iron contamination in the area within 200 m of their surroundings.

Materials & methods

Study site

The study was conducted within the Ramgram Municipality of Nawalparasi (west) district, western Nepal (see Figure 2) in 2023. The municipality is situated between 27°28'0"–27°35'0" N latitudes and between 83°35'0"–83°43'0" E longitudes. Encompassing over an area of 128.32 sq. km, it is bordered by Sunwal Municipality on the north, Devdaha Municipality, Omsatiya and Rohini rural municipalities on the west, Sarawal Rural Municipality on the east, and Palhi Nandan Rural Municipality on the south. It lies nine km. south of Mahendra Highway from Sunwal. The Ramgram area includes various industries such as steel plants, paper mills, alcohol distilleries, brick kilns, and other small-scale wood industries. The area within a 100 m radius around these industries was considered as contaminated with heavy metals.

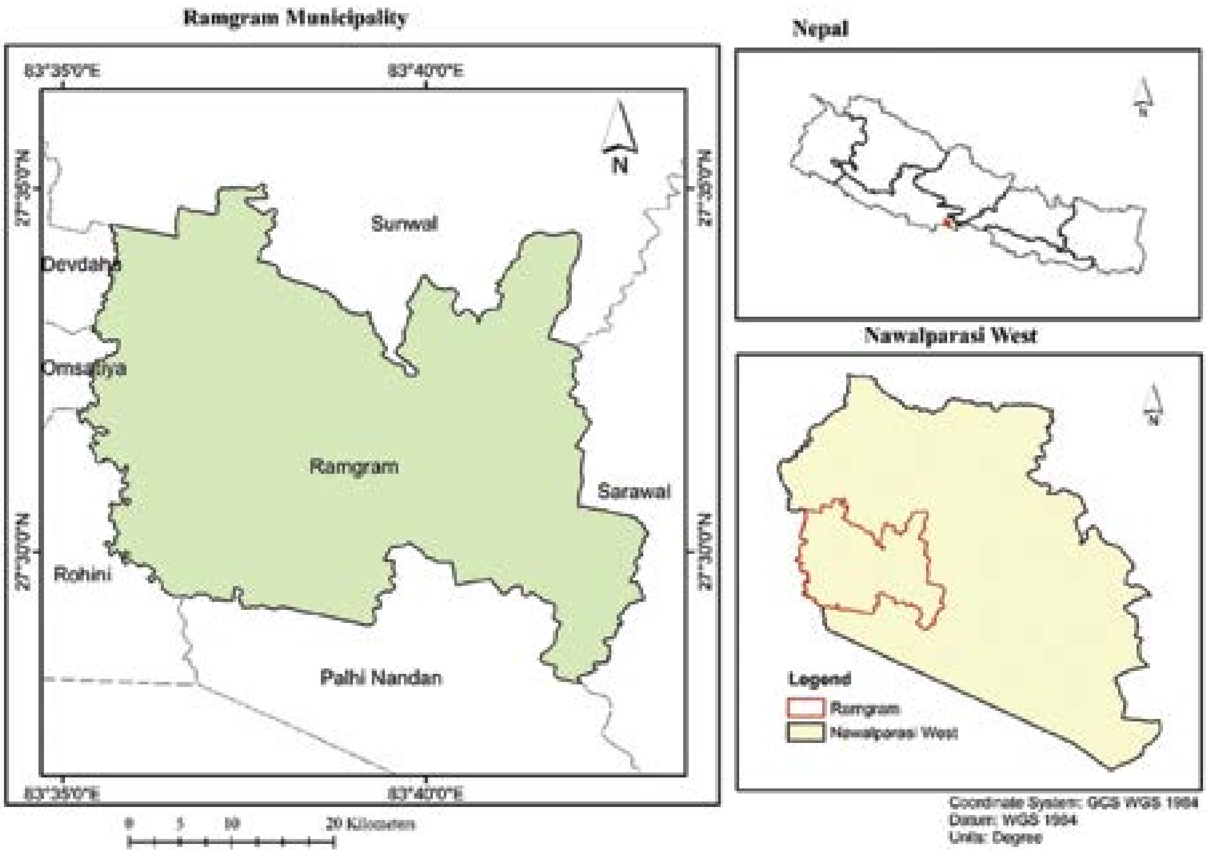

Figure 2: Location of the study area (Ramgram Municipality), Nawalparasi (west) district in the map of Nepal.

Sampling

A total of ten locations were chosen within the study area (see Figure 3) on the basis of the objectives of the study. The sampling sites, both industrial and non-industrial, were selected randomly. Sampling locations S1 to S3 were positioned around the periphery of the alcohol distillery, steel plant, paper plant, and plastic recycling plant, respectively, within a 50–100 meter range. Meanwhile, S4 and S5 were located at a waste disposal site. Therefore, samples S1 to S5 were classified as industrial samples (polluted areas). The remaining locations (S6 to S10) were situated along roadsides and agricultural fields, more than 1 km away from industrial sites. However, S6 was only 600 meters far from the waste disposal site (S5), and S10 was 500 meters away from the steel plant. Two whole plants were collected from each sampling site by carefully digging them out, and they were labeled according to their respective sampling locations. Soil samples were collected from the corresponding sites within 15 cm following the techniques used in Naveen & Madhukar (2022). The whole plant samples were sun-dried for four weeks and soil samples were dried for three days. The root, stem, and leaves were separately stored in a zip-lock plastic bag and labeled.

Figure 3: Sampling locations within the study area.

Soil and plant analysis

Both the plant and soil samples were analyzed for iron content using an Atomic Absorption Spectrophotometer (AAS) (Analytik Jena: novAA 350) in accordance with the standard techniques established by the AOAC (1990). The dried plant and soil samples were placed in a muffle furnace for ashing at 500 °C for 20 minutes. Following this, one gram of the ash sample was taken and digested in 6M HCl, placed in a hot bath for about two hours. Once digestion was complete, the resulting sample was analyzed for iron content using the AAS.

Determination of Bio-concentration Factor, Biological Absorption coefficient, and Translocation Factor

Bio-concentration factor (BCF) is the ratio of the metal accumulated in the root and the metal present in the soil (Lorestani *et al*., 2011). It is calculated as:

$$BCF = \frac{Metal\ concentration\ (root)}{Metal\ concentration\ (soil)}$$

The biological absorption coefficient (BAC) is the ratio of the metal present in the plant and the metal present in the soil (Cui *et al*., 2007; Li *et al*., 2007). It is calculated as:

$$BAC = \frac{Metal\ concentration\ (plant)}{Metal\ concentration\ (soil)}$$

The translocation factor (TF) is the ratio of metal concentration in the leaves & stems and the metal concentration in the root (Mellem *et al*., 2009). It is calculated as:

$$TF = \frac{Metal\ concentration\ (leaf + stem)}{Metal\ concentration\ (root)}$$

Statistical Analysis

The data were statistically analyzed for analysis of variance (ANOVA) and Pearson Correlation using the data analysis tool pack in Microsoft Excel. Statistical significance was established at a 5% level of significance.

Results

Quantification of iron in soil and plant samples

The concentrations of total iron in various parts of the *I. carnea* plant (root, stem, leaf, and total plant) as well as the iron concentration in the soil for ten distinct samples are presented in Table 1.

Table 1: Iron concentration in soil and plant samples (mg/kg)

Sites	Sample No.	Root	Stem	Leaf	Soil	Total plant	Remarks
1.	S1	469.47	299.50	1493.0	23445.6	2261.50	Polluted (Industrial area)
2.	S2	930.74	274.60	424.6	14903.4	1629.90	
3.	S3	1796.20	161.70	544.3	10436.6	2502.20	
4.	S4	471.44	93.40	373.8	18874.6	938.67	
5.	S5	1263.30	92.48	552.3	15859.0	1908.00	
6.	S6	597.05	161.10	156.8	13409.0	914.95	Non-polluted (Non-industrial area)
7.	S7	811.05	89.82	284.6	21988.8	1185.50	
8.	S8	1427.60	343.70	415.0	22239.4	2186.30	
9.	S9	383.62	170.50	357.9	10313.9	911.98	
10.	S10	1214.40	199.50	372.4	23668.3	1786.30	

The term "total plant" refers to the overall iron concentration in the plant, calculated by adding the iron concentration values from the leaf, stem, and root. The highest total iron concentration in the whole plant was found at S3 (plastic recycling plant) and the lowest was found at S9 (agricultural field). On the other hand, the highest soil iron concentration was found at S10 (the side nearby a less busy road) and the lowest was found at S9 (agricultural field). In the whole plant, the order of total iron concentration was:

S3 (2502.2 mg/kg) > S1 (2261.5mg/kg) > S8 (2186.3mg/kg) > S5 (1908 mg/kg) > S10 (1786.3 mg/kg) > S2 (1629.9 mg/kg) > S7 (1185.5 mg/kg) > S4 (938.67 mg/kg) > S6 (914.95 mg/kg) > S9 (911.98 mg/kg).

However, in the soil, the order was:

S10 (23668.3 mg/kg) > S1 (23445.6 mg/kg) > S8 (22239.4 mg/kg) > S7 (21988.8 mg/kg) > S4 (18874.6 mg/kg) > S5 (15859 mg/kg) > S2 (14903.4 mg/kg) > S6 (13409 mg/kg) > S3 (10436.6 mg/kg) > S9 (10313.9 mg/kg).

The results also showed that the roots and leaves accumulated more iron than the stem in the industrial areas (see Figure 4). However, the average iron concentration in the stem from the non-industrial areas was found to be slightly higher.

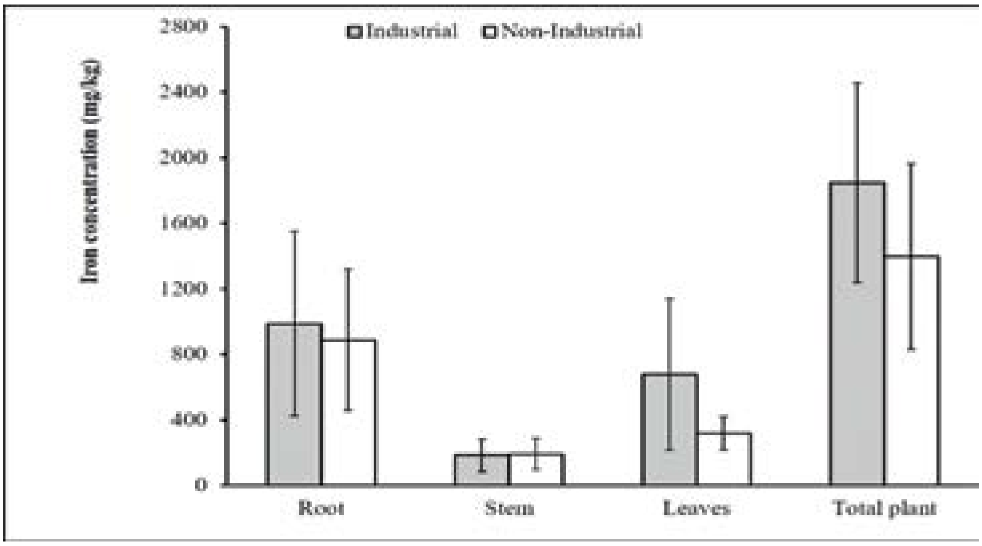

Figure 4: Mean iron concentration in different plant parts in industrial and non-industrial areas of Ramgram Municipality.

***Translocation of iron in different parts of* I. carnea**

The TFs between the stem and root, leaf and stem, leaf and root, and shoot (leaf + stem) and root were calculated separately (Table 2). The BCF was determined to assess the ratio of the metal accumulated in the roots and the metal present in the soil. In addition, the BAC was also calculated to find out how much iron was accumulated from the soil by the plant. The mean values of the BAC, BCF, and TF were found to be higher in the industrial area than in the non-industrial area (see Figure 5 & Table 2).

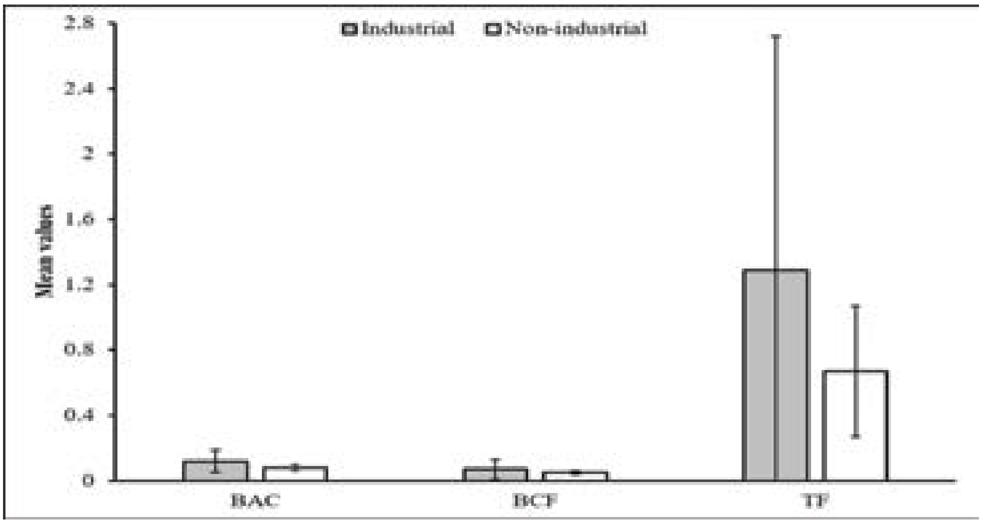

Figure 5: Mean values of BAC, BCF, and TF from industrial and non-industrial area.

Table 2: Translocation factor of iron between different plant parts

Sites	Sample no.	Stem/Root	Leaf/ Stem	Leaf/Root	Shoot/ Root	BAC	BCF
1.	S1	0.64	4.98	3.18	3.82	0.10	0.02
2.	S2	0.30	1.55	0.46	0.75	0.11	0.06
3.	S3	0.09	3.37	0.30	0.39	0.24	0.17
4.	S4	0.20	4.00	0.79	0.99	0.05	0.02
5.	S5	0.07	5.97	0.44	0.51	0.12	0.08
6.	S6	0.27	0.97	0.26	0.53	0.07	0.04
7.	S7	0.11	3.17	0.35	0.46	0.05	0.04
8.	S8	0.24	1.21	0.29	0.53	0.10	0.06
9.	S9	0.44	2.10	0.93	1.38	0.09	0.04
10.	S10	0.16	1.87	0.31	0.47	0.08	0.05

Relation between total iron concentration in soil and plant accumulation

The Pearson Correlation was conducted for different variables- root, stem, leaf, total plant (total iron concentration in the plant), and total iron concentration in the soil. The results indicated a significant correlation between the iron concentration in the roots and that in the whole plant ($p \leq 0.05$). This revealed that an increase in iron concentration in the roots was associated with a rise in iron concentration in the total plant. In addition, the iron concentration between the root-to-stem and leaf-to-stem was positive but root-to-leaf was negative although the relation was statistically insignificant in all cases.

Comparison of iron concentration in different plant parts

Single-factor ANOVA was conducted to assess whether there were significant differences in iron concentration among the leaf, stem, and root of *I. carnea*. The *p*-value (0.002478964, Table 3) was found to be less than the alpha level (0.05), indicating a statistically significant difference in iron concentration among at least two of these groups (leaf, stem, and root).

Table 3: Single factor ANOVA for comparing mean iron concentration in root, stem, and leaf

Source of variation	SS	df	MS	F	*p*-value	F crit.
Between groups	3174030.189	5	634806.0377	5.114	0.002478964	2.621
Within groups	2979222.830	24	124134.2846			
Total	**6153253.019**	**29**				

Note: SS = Sum of Squares; df = degrees of freedom; MS = Mean Square; and F crit. = F critical value.

Discussion

The iron concentration in the root of *Ipomoea carnea* was found to be the highest in the Sample S3 (1796.2 mg/kg) which is located near the plastic recycling plant followed by the Sample S8 (1427.6 mg/kg) located within a non-polluted area. Similarly, the iron concentration in the stem and leaf was found to be the highest in the Samples S8 (343.7 mg/kg) and S1 (1493 mg/kg), respectively. We observed that, with the exception of the Samples S1 and S9, the concentration of iron in the above ground parts (leaves and stems) was slightly low as compared to the underground part (roots). This differs from the findings of Pandey *et al*. (2016), who reported a higher iron concentration in the aboveground portion of *I. carnea* in Jharkhand, India. According to Alloway (1990) and Reeves (2002), the safe critical limit of iron, above which toxicity occurs in plants, is >1000 mg/kg. Except for the Samples S4, S6, and S9, the remaining seven samples in this study surpassed the threshold for iron concentration in the entire plant. Nevertheless, there were no observable indications of iron toxicity such as chlorosis and necrosis (Zahra *et al*., 2021), indicating the plant's tolerance to elevated iron levels. Despite variations in different parts, the iron concentration in the whole plant was found to be higher in the polluted area in our study. This suggests that various industries in the polluted sites may leach heavy metals into the soil, which ultimately reaches the plant body during nutritional uptake.

The results showed that only the TF surpassed a value of one in both industrial and non-industrial areas. While Kulshrestha & Dabral (2018) found a BCF for iron in *I. carnea* greater than one, this study did not detect any BCF values above one. This may be explained by the fact that the total soil iron concentration was assessed instead of the bioavailable iron. The BAC, BCF, and TF are the parameters used to describe the potential of plants to accumulate and translocate the metal. On the basis of BAC, plants can be classified into four categories: (i) high accumulator plants, with 1.0–10 BAC; (ii) moderate accumulator plants, with 0.1–1.0 BAC, (iii) low accumulator plants, with 0.01–0.1 BAC; and (iv) non-accumulator plants, with BAC < 0.01 (Aziz *et al*., 2015). As per this criteria, *I. carnea* was found to be a moderate accumulator of iron in the industrial areas and a low accumulator in the non-industrial areas. The concentration of 2500 mg/kg in leaves is considered the threshold for iron hyperaccumulators (Reeves & Baker, 2000). None of the plants sampled in our study reached this concentration. With the exception of the Sample S1, all the samples showed higher iron accumulation in the roots, similar to the findings of a study in an iron mine in Nawalparasi (east) district, where nearly all species accumulated more iron in the roots (Parajuli & Chettri, 2020). Higher concentrations of iron were observed in the plant roots, followed by the leaves, with the lowest levels detected in the stems. This indicates effective translocation of iron from the stem to the leaves. However, the stem iron concentrations in the non-industrial areas were slightly higher than those in the industrial areas. Plants growing near steel plants have been shown to accumulate high levels of heavy metals (Ogunkunle *et al*., 2017). In our study, we also found the accumulation of iron by plants greater in the industrial areas than in the non-industrial areas.

A study carried out by Bose & Bhattacharyya (2008) showed a strong positive correlation between the iron concentration in the roots with the iron concentration in the soil, indicating the increase in the root iron concentration as per the increase in the soil-available iron concentration. However, no correlation was found between the root iron concentration and the total plant iron concentration in this study, which could be because the total iron was assessed in the soil. Instead, this study found a strong correlation between the root iron concentration and total plant iron concentration, suggesting that as root iron concentration increases the overall plant iron concentration increases.

The threshold concentrations for iron in soil is 50,000 mg/kg (Mng'ong'o *et al*., 2021). This threshold concentration was not found to have exceeded even in the soils of the industrial area in our study. The highest iron concentration in the soil was found in the Sample S10 (near a less busy road, 500 m away from the steel plant). It is noteworthy to highlight that the highest concentration was not discovered in the soils of the industrial areas. This suggests that the industrial periphery is not contaminated with heavy metals, such as iron. However, it is important to note that the sample size was small and the samples were collected from the periphery of the industries. Thus, drawing a firm conclusion that industries are not polluting the soil with iron might not be reasonable based on the available evidence. On the other hand, higher iron concentration in the cultivated field might be due to the use of chemical fertilizers while on less busy roads, it could result from exhaust emissions and vehicle leakage

(Masindi *et al*., 2021). According to Cornell & Schwertmann (2003), iron is relatively abundant in cultivated soils, typically ranging from 20,000 mg/kg to 40,000 mg/kg. The concentration of iron at our study sites (7, 8, and 10), located next to the cultivated land, falls within this range.

Conclusion

According to our study, *I. carnea* does not fulfill the requirements to be considered as an iron hyperaccumulator, making it ineffective for iron phytoremediation. Based on the results, it was found to be a low accumulator of iron within the non-industrial areas and a moderate accumulator within the industrial areas. Our results, however, indicated significant iron accumulation in the roots and efficient translocation from the stem to the leaves. The concentration of iron in the soil samples was within the threshold values. Nevertheless, it is recommended to take a large sample size around an industrial area to detect a possible hotspot of pollution using a Geographic Information System.

Authors' contribution

Both the authors were equally involved in the preparation of this manuscript. However, the first author was involved in the preparation of the conceptual framework of the study, data collection, data analysis, and draft manuscript preparation whereas the second author was, moreover, involved in the preparation of the conceptual framework together with the editing & reviewing of the manuscript.

Conflict of interest

The authors declare no conflict of interest in preparing this manuscript.

Data availability

The data used in this study are accessible upon request to the corresponding author.

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