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Screening of *Xanthium strumarium* (IAPS) Growing on Abandoned Habitats in Khyber Pakhtunkhwa, Pakistan: Perspectives for Phytoremediation

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Featured Application: The research work has a wide range of future implications in the field of phytoremediation. It is distinct from many other studies regarding alien invasive species that are usually considered a threat to ecosystem biodiversity and conservation.

Abstract: The ecological impacts of invasive alien plant species (IAPS) are well-documented, but a dearth of studies exist concerning its economic, livelihood, biotechnological, and health risk assessment perspectives. IAPS management is currently expanding to ecological indicator and biotechnological aspects. Hence, this study aimed to investigate the phytoremediation potential, biomedical, and bio-safety applications of *X. strumarium* growing in different abandoned habitats in Khyber Pakhtunkhwa, Pakistan. In this study, 45 plants and soil samples were gathered from 15 abandoned sites and analyzed for Pb, Cd, Cu, and Zn concentrations; bioaccumulation (BA); and translocation factor (TF). The assayed Pb and Cd concentration was higher and above threshold in both soil–plant samples. BAF was found higher in roots than intact plants despite having a significant accumulation of Cd, Pb, and Zn, which shows high metals tolerance of this IAPS. PCA-ordination explained a high cumulative variance (98.9%) and separated roads and densely populated sites with comparatively high metals concentration, indicating the pseudometallophyte nature of *X. strumarium*. Soil, sand, and plant biomass were shown to be the major determinants affecting the heavy metals concentration and its phytoremediation significantly, which may be due to the soil's metalliferous nature in the study area. This IAPS exhibited strong translocation and hyperaccumulation capacity in different functional traits with comparatively high Pb, Cd, and Zn (≥1 TF) mobility and, hence, can effectively be used for Pb phytoextraction and phytostabilization of Cd, Cu, and Zn, respectively. Likewise, several other non-spontaneous IAPS growing on such abandoned habitats might be promising for developing a reasonable strategic framework for heavy metals mitigation and health risk implications in this region.

Keywords: invasive plants; *X. strumarium*; bioaccumulation and translocation; phytoremediation potential

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Citation: Ullah, R.; Khan, N.; Ali, K.; Khan, M.E.H.; Jones, D.A. Screening of *Xanthium strumarium* (IAPS) Growing on Abandoned Habitats in Khyber Pakhtunkhwa, Pakistan: Perspectives for Phytoremediation. *Appl. Sci.* **2021**, *11*, 11704. https://doi.org/10.3390/app112411704

Academic Editor: Zhujian Huang

Received: 21 November 2021
Accepted: 7 December 2021
Published: 9 December 2021

1. Introduction

The balance in the ecosystem is significantly disrupted by the infiltration of invasive alien plant species (IAPS), negatively affecting valuable native plant species, resulting in biodiversity loss [1]. The favorite hotspot for IAPS is polluted soils due to lack of competitors and also because IAPS have the potential to withstand harsh conditions, whereas native plants fail to tolerate and propagate [2]. These plants develop specific physiological and biochemical mechanisms to function normally on lands polluted with heavy metals, forming heavy metal-resistant populations [3]. Heavy metals are non-degradable pollutants, and their hyperaccumulation is a matter of great concern because of their contamination and toxicity in the natural environment [4]. Soil and air contaminated with heavy metals affect the quality of the environment and pose threats to living organisms and human beings. In soil, heavy metal pollutants leach to plant roots through absorption, thereby entering the living system, subsequently bioaccumulating and magnifying in the upper trophic levels. Its effect is more pronounced and explicitly lethal for living biota [5]. Heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), etc. are added to soil and air through different sources and are considered a problem of great concern [6]. Likewise, increasing urbanization and industrialization throughout the globe make this an urgent issue [7] due to severe threats to the environment by discharging different pollutants in effluents [8]. Vehicular emissions are considered the main source contaminating areas near to roads and many other accessible urban and rural areas [8]. The most common metals released by vehicles via different processes include Cd, Pb, zinc (Zn), nickel (Ni), Cu, and iron (Fe) [9–11].

Pb and Cd are nonessential heavy metals, as they are naturally not found within organisms, and have no metabolic function in biological systems. Zn and Cu are essential heavy metals that are required in small quantities for normal plant growth. However, excess quantities can be lethal, causing different abnormalities [12]. Physiological functions such as nutrient absorption, photosynthesis, and gaseous exchange in plants are reduced/ceased by metal toxicity. It can also reduce growth, yield, and dry matter accumulation [13]. These metals cause cancer, cardiovascular and neurological disorders, diabetes, and atherosclerosis [14]. Possible sources of these metals are fossil fuel combustion; wear and tear of automobile tires; metals in catalysts; degradation of various vehicle parts, especially paints; and many other industrial operations [15]. In phytoremediation, plants extract soil pollutants and accumulate them in various functional parts [16]. The roads and industrial zone soils are multi-element contaminated soils, and cleaning of such soils can be negatively influenced if the hyper-accumulator cannot adapt to soils with high concentrations of several toxic elements [10]. Bioaccumulation (BAF) and translocation factor (TF) have been applied for evaluating the plant's ability to absorb heavy metals from the soil and translocate to different plant parts. BAF is a ratio of heavy metal concentration in biota to that in soil. It is an appropriate measure of the capability of native plants for heavy metals uptake, e.g., [17–20]. Nevertheless, very few studies have explored the effects of invasive plant on heavy metals contamination in the soil resulting from traffic emissions in roadside and industrial farmlands, e.g., [21–25].

Xanthium strumarium L., belonging to the family Asteraceae, is one of the emerging invasive plant species currently invading croplands and non-cropped areas in Khyber Pakhtunkhwa, Pakistan [26]. This plant species originated in South or Central America, and spread gradually towards North America, where it grows near rivers and shores, dispersing through water and animals [27]. Chinese pharmacopeia has documented 60 different compounds from the fruit of this plant species that are used for treating various diseases, including rhinitis, nasal sinusitis, headache, gastric ulcer, urticarial, rheumatism, bacterial and fungal infections, and arthritis [28]. *X. strumarium* has an economic impact in pastures, where cattle, sheep, and pigs may be poisoned by eating young plants. The cotyledons contain a toxic compound, carboxyatractyloside, which is absent in older plants [29]. Previously many studies have been performed to assess the level of some heavy metals of vehicular origin along the roadside areas, e.g., [9,10]; however, little attention has been given to the phytoremediation potential of this IAPS. Therefore, this study has been performed to evaluate the phytoremediation potential of *X. strumarium*. In addition, this study also highlights the need to assess the phytoremediation potential and response of this IAPS toward common road-industry-borne heavy metals specifically Pb, Cd, Zn, and Cu accumulation—compared with reference range to elucidate its suitability for phytoremediation, medicinal, and forage purposes.

2. Materials and Methods

2.1. Experimental Materials

Soil bulk samples and *X. strumarium* plants at the reproductive stage were collected from fifteen different abandoned habitats in Khyber Pakhtunkhwa, Pakistan (34°26′59.42″ N and 72°0′45.65″ E; Figure 1) from July to September 2019. Pollutants in the experimental sites discharged from automobiles, and its workshops derivatives or industrial effluent predominately include plastics residues, pesticides, and marble factories beside domestic sewages in residential areas. The geological features of the study area revealed 13 land-cover classes extracted from 37 sub-categories. The relatively high mean annual temperature (34.96 ± 1.36 °C to 0.67 ± 0.97 °C) and low precipitation (384 mm to 639 mm) contributed to heavy metals contamination in addition, the road, urban, and industrial sources. At each site, five cores of soils were sampled at a depth of 0–20 cm (at rooting zone) by stainless steel trowel to avoid contamination and were mixed thoroughly to achieve composite samples [30]. Soil samples were air-dried and then passed through a 2 mm sieve, oven-dried, and was placed in sealed polythene bags in the laboratory. Forty-five plants (3 plants/site) healthy with sound structural characteristics were randomly harvested using a destructive method for robust results and to minimize the risks of invasive species spread and extinction of rare and threatened species within the natural plant communities [31]. The harvested plants were washed immediately with fresh water and subsequently oven-dried at 80 °C for 24 h. The plant's functional parts were separated (i.e., roots, stems, leaves, and fruits), converted into a fine powder using a commercial grinder, and sieved through a 1.5 mm mechanical shaker to remove ample residues [32]. The powdered samples were stored in precisely labeled polythene bags for acid-digestion and metal analysis.

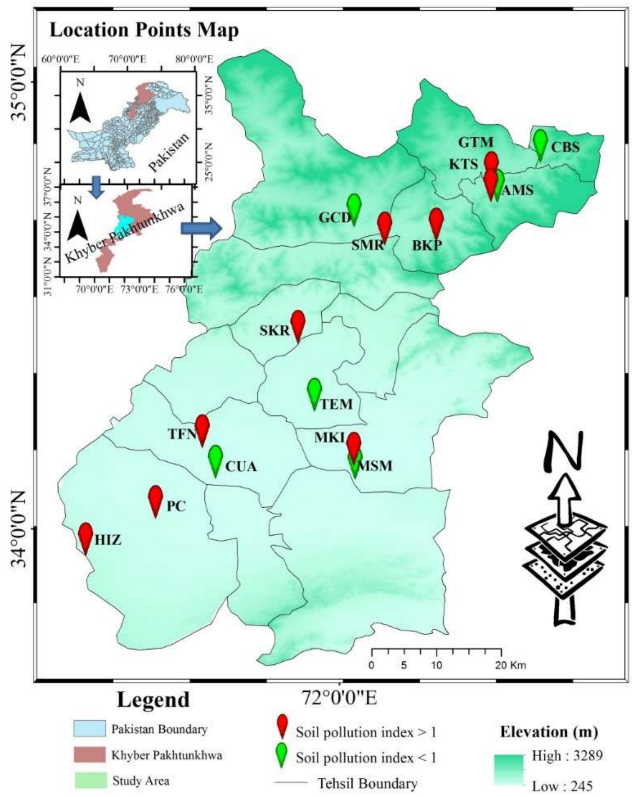

Figure 1. Study area map along with sampling point for collection of plant and soil samples in abandoned sites of Khyber Pakhtunkhwa, Pakistan. Note: KTS (Kanju Town Junction), GTM (GT Road Mingora), SKR (Skhakot Road), SMR (Shamozu Road), PC (Peshawar), HIZ (Hayatabad Industrial Zone), MKI (Mardan Kid Industry), BKP (Barikot Crush Plant), CUA (Charsadda Urban), MSM (Mardan Shiekh Malton), TFN (Tarnab Form), TBM (Takht Bhai), GCD (Gulabad College Degree), AMS (Amankot), CBS (Charbagh street).

2.2. Soil–Plant Samples Preparation and Heavy Metal Analysis

For acid-digestion, a 0.25 gm oven-dried sample at 105 °C was taken and subsequently, ashed at 450 °C was added in nitric acid following the procedure outlined by Yafa et al., (2004) [33]. The digested samples were dried on hot plates for evaporating all the fumes and then added to a 25 mL volumetric flask with 2% *v/v* diluted nitric acid. Samples were filtered with ashless Whatman 45 filter paper in a polypropylene centrifuge tube and stored at 4 °C. Parallel, blank reagents were processed with experimental samples in the analysis (<0.5%) and considered as reference water samples to determine metals concentration accuracy following the standard protocol described by Yafa et al., (2004) [33]. Consequently, acid-digested samples were analyzed for heavy metals concentration using atomic absorption spectrophotometer (VARIAN model-AA 2407, USA) in the Department of Chemistry, Kohat University of Science and Technology (KUST).

2.3. Determination Phytoremediation Potential

Prior analysis we calculated soil pollution index (SPI) following Wu et al., (2014) [34] by computing the equation: PI = ([Cd]soil/3 + [Cu] soil/100 + [Pb] soil/100 + [Zn] soil/300)/4 to find out the contamination level in the abandoned habitats. Phytoremediation potential was assessed by computing bioaccumulation (BAF) or bioconcentration (BCF), and translocation factor (TF)/mobility index (MI) of mean metal concentrations. BAF was determined by the formula; BAF = metal concentration in living parts/metal concentration in soil [35]. Here we determined two types of BAF: roots and intact-plants bioaccumulation to know the phytoaccumulation potential and biomedical safety if subsequently used for medicinal purposes. Higher values of transfer factors indicated higher mobility or availability of a particular metal to the plants. Hence high BAF values may put forth the potential health risks to the consumers [36]. Here we determined transfer or mobility factors in root–stem (R-S), stem–leaves (S-L), and leaves–fruits (L-F) to distinguish the potentially hyperaccumulating part of *X. strumarium*. The analysis was computed with the formula; TF or MI = metal concentration in receiving part (mg/kg)/metal concentration in source (mg/kg). In addition, various soil parameters, i.e., soil texture, pH, organic matter, and electrical conductivity were determined. The factors that significantly influence metals concentration, bioaccumulation, and translocation were included in the results. In soil parameters, soil texture was determined using the Bouyoucos hydrometer [37], while the organic matter percentage was determined by following [38].

2.4. Statistical Analysis

Pb, Zn, Cu, and Cd mean concentrations were calculated and compared site-wise to evaluate soil–plant samples with a higher concentration of heavy metals. One-way ANOVA analyzed specific site effects and metal concentrations differences in different plant functional parts, indicating the importance of habitat types. BAF and TF were computed based on mean values using paired t-test, and ANOVA followed by post hoc Tukey HSD tests for determining the pair-wise significant differences between root and intact plant phytoremediation potential besides variation in the translocation factor from R-S, S-L, and L-F, respectively. PCA-ordination was applied to explore relationships between metal concentrations in plant functional parts and soil textural properties spatially structured using PC-ord software (ver. 6). Descriptive statistics were analyzed in MS-Excel, whereas univariate analysis and graphics were carried out in Graphpad statistical package (ver. 8).

3. Results and Discussion

3.1. Soil–Plant Metal Concentration and Its Biomedical Safety

The bio-concentration of Pb, Zn, Cu, and Cd with correction for dilution factors in soil and *X. strumarium* plant samples is presented in Figure 2A–D. To validate the concentration level at the spatial domain, we analyzed the samples at 15 abandoned habitats. Pb and Zn concentrations above the permissible level, i.e., Pb = 5 mg/kg and Zn 60 mg/kg for soil, Pb = 8.15 mg/kg, and Zn = 44.19 mg/kg for plants in eight locations. Conversely, Cd and

Cu concentration were above threshold (SPI ≥ 1) i.e., Cd = 0.1 mg/kg and Cu 10 mg/kg for soil, Cd = 0.31 mg/kg and Cu = 8.39 mg/kg for plants in all the abandoned sites except TBM, GCD, AMS, and CBS sites (Figure 1) indicating metals contamination at a broad spatial area covering parts of the Malakand, Hazara, and Peshawar Divisions in Khyber Pakhtunkhwa (formerly Northwest Frontier Province) of Pakistan (Figure 1). The substantially higher magnitudes of Pb and Cd in plant tissues except those in TFN, TBM, GCD, AMS, and CBS sites revealed that these plants are not an ideal choice for medicinal, phytochemical, or veterinary (forage) purposes, but can efficiently be utilized as phytoremediator. Cu and Zn concentrations were below the threshold in plant tissues in all the abandoned habitats and hence secured if subsequently used for biotechnological aspects. Concerning Pb, alkyl-lead compounds as anti-knock agents in fuel are the primary source of elevated levels in soils [39], or its pollution might be caused by vehicle wheels imbalance weights [40] The high Cd concentrations in soil and *X. strumarium* might originate from the wear and tear of old tires due to surface friction [15,41]. Likewise, oil spills, exhaust fumes, corrosion of various vehicle components, wearing of engine parts, and brake lines enhanced surplus Zn quantity along roads [42]. These abandoned habitats may have one or more sources adding heavy metals to the soil and translocating successively into plant tissues.

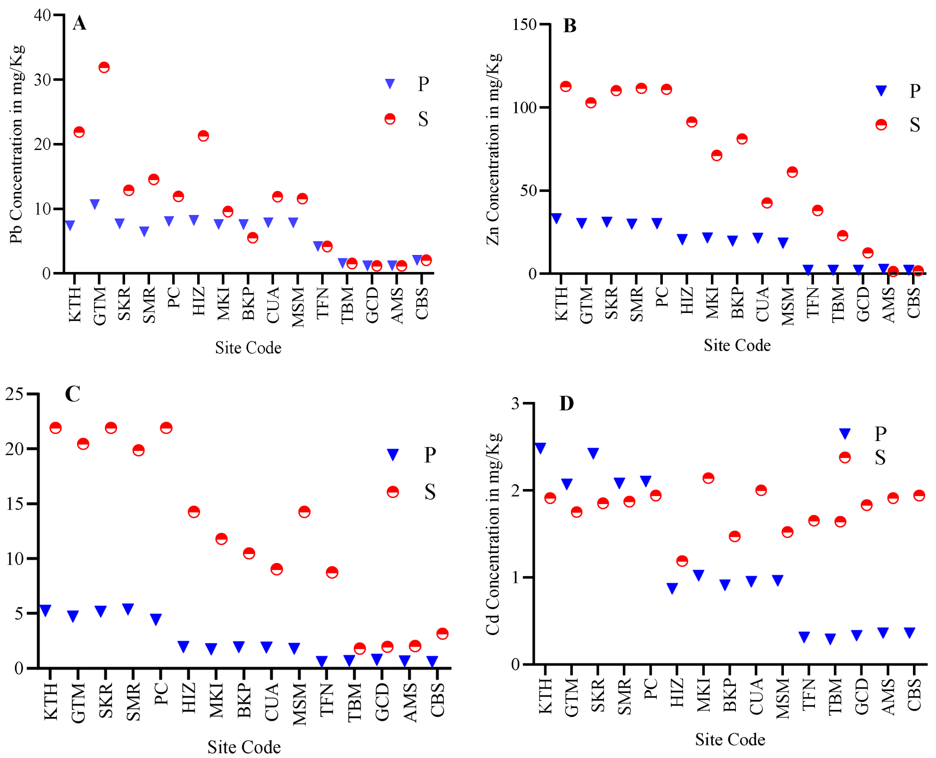

Figure 2. Bio-concentration of Pb, Zn, Cu, and Cd in plants (mg/kg) total dry biomass and its concentration in soil collected from different abandoned habitats in Khyber Pakhtunkhwa, Pakistan. Note: P (Plant), S (Soil), Threshold levels: Pb = 5 mg/kg [43], Zn = 60 mg/kg: Cu = 10 mg/kg [44], Cd = 0.1 mg/kg [45] in plants while for soil while Pb = 8.15 mg/kg, Zn = 44.19 mg/kg, Cu = 8.39 mg/kg [46] and for Cd = 0.31 mg/kg [47]. (**A**–**D**) (Sub-Figure for Pb, Zn, Cu and Cd, respectively).

3.2. Bioaccumulation Factor

Bioaccumulation factors for Pb, Zn, Cu, and Cd were assessed for phytoremediation potential of root, stem, leaves, and intact plant tissues, besides determining associated health risk if consumed for medicinal or forage purposes. Highest accumulation of Cd (1.22 ± 0.66) was recorded in root and intact-plant compared to Pb, Cu, and Zn. Our results depicted BAF >1 for Cd in some abandoned sites (i.e., KTH, GTM, SKR, SMR, and PC), indicating plant potential as hyper-accumulator with higher organic matter contents (0.83 ± 0.17), despite the high accumulation of Pb in the root (BAF > 1) in sites like SMR, PC, BKP, TBM, GCD, AMS, and CBS respectively (Table 1). Zn was significantly accumulated in the root and intact plants harvested at AMS and CBS sites, whilst Cu hyperaccumulation was not found in any abandoned site. Additionally, we observed a significant accumulation difference ($p < 0.001$) between root and intact-plant for all the sites, except Pb accumulation at the GTM site ($p < 0.45$). In contrast, a low accumulation of Zn was recorded at all the abandoned sites except AMS and CBS (BAF > 1). In contrast, the bioavailability factor for Cu was <1 at all the sites indicating the poor ability of *X. strumarium* to accumulate this metal. Generally, BAF >1 is related to the high mobility of metals in soils, possibly favored by soil physicochemical characteristics, specific plant species metabolism, and metal pollutant characteristics or derivatives, thus making its readily available for absorption by the plants [48–52]. Our aforementioned results link well with the *X. strumarium* plant screened with BAF > 1 for Cd and Pb at roadside areas in Faisalabad-Sargodha [26], while the same opposite low sign pattern for Zn accumulation and its mobility is also clearly depicted. Soil texture plays a crucial role in heavy metal availability for absorption in plant species [48]. In our study, we observed the soil textural pattern as sand (40%) > silt (33%) > clay (26%), advocating highly mobilized heavy metals, thereby enhancing its availability for absorption and accumulation in plants as reported by Andersson (1979) [53]. This behavior of soil texture with heavy metal absorption seems to be typical as Qian et al., (1996) [54] reported higher availability of Pb and Cu in fine-sand soils and [55] in sandy soils. The same relationship between higher soil sand contents and mobility strength of Cd was also noted by [56]. In other words, increased soil sand contents are correlated with the mobility of heavy metals. Hence, the inland sandy soil texture enhanced these metals' mobility and accumulation extent in *X. strumarium* in this region. The Khyber Pakhtunkhwa province lies under the most substantial sandy soil influence of the Indus Basin System (IBS), often facing heavy floods in semiarid regions. Hence, even a slight increase in flood frequency particularly in monsoon conditions may enhance sand particles, perhaps increasing mobility and accumulation of heavy metals both in native and exotic plants. Given the importance of low soil organic matter (SOM) for metals availability, the negative relationship between heavy metals availability and mobility in plants is not surprising. Consequently, the availability of Pb and Cd was negatively affected by organic matter contents (Table 1), previously endorsed by [57,58] in different plant species. In contrast, we found a positive correlation between organic matter and Zn availability, which agrees with the findings of [59]. The slight differences in this study can be attributed to other soil characteristics, of which pH and electrical conductivity play a vital role in the bioavailability of heavy metals [60].

Table 1. Transfer factor (bio-accumulation) from a soil–root–intact plant collected from different abandoned habitats in Khyber Pakhtunkhwa, Pakistan.

M Type	Pb		Zn		Cu		Cd			OM%
St Code	BAF (S/R)	BAF (S/P)	BAF (S/R)	BAF (S/P)	BAF (S/R)	BAF (S/P)	BAF (S/R)	BAF (S/P)	STC	
KTH	0.71 [a]	0.33 [b]	0.45 [a]	0.30 [b]	0.38 [a]	0.24 [b]	2.13 [a]	1.30 [b]	SL	0.67
GTM	0.36 [a]	0.33 [a]	0.56 [a]	0.29 [b]	0.47 [a]	0.23 [b]	2.79 [a]	1.18 [b]	SL	0.82
SKR	1.38 [a]	0.62 [b]	0.48 [a]	0.28 [b]	0.39 [a]	0.24 [b]	2.11 [a]	1.31 [b]	SL	1.45
SMR	1.531 [a]	0.794 [b]	0.50 [a]	0.27 [b]	0.51 [a]	0.27 [b]	2.58 [a]	1.11 [b]	SiL	0.38
PC	1.528 [a]	0.622 [b]	0.52 [a]	0.27 [b]	0.39 [a]	0.20 [b]	2.54 [a]	1.08 [b]	SL	0.89
HIZ	0.294 [a]	0.154 [b]	0.42 [a]	0.22 [b]	0.27 [a]	0.14 [b]	1.05 [a]	0.63 [b]	SiL	1.21
MKI	0.83 [a]	0.39 [b]	0.57 [a]	0.30 [b]	0.32 [a]	0.15 [b]	1.13 [a]	0.48 [b]	CL	0.45
BKP	1.33 [a]	0.69 [b]	0.49 [a]	0.24 [b]	0.36 [a]	0.18 [b]	0.15 [a]	0.16 [b]	SL	1.38
CUA	0.62 [a]	0.31 [b]	0.97 [a]	0.50 [b]	0.45 [a]	0.21 [b]	0.97 [a]	0.48 [b]	SiL	1.32
MSM	0.73 [a]	0.33 [b]	0.65 [a]	0.30 [b]	0.27 [a]	0.13 [b]	1.44 [a]	0.63 [b]	SiL	1.92
TFN	0.52 [a]	0.34 [b]	0.11 [a]	0.05 [b]	0.14 [a]	0.07 [b]	0.32 [a]	0.18 [b]	CL	1.21

Table 1. *Cont.*

M Type	Pb		Zn		Cu		Cd			
St Code	BAF (S/R)	BAF (S/P)	BAF (S/R)	BAF (S/P)	BAF (S/R)	BAF (S/P)	BAF (S/R)	BAF (S/P)	STC	OM%
TBM	2.10 [a]	1.06 [b]	0.20 [a]	0.09 [b]	0.80 [a]	0.38 [b]	0.33 [a]	0.18 [b]	SL	0.89
GCD	2.20 [a]	0.90 [b]	0.41 [a]	0.17 [b]	0.92 [a]	0.40 [b]	0.33 [a]	0.18 [b]	SL	0.73
AMS	1.86 [a]	0.85 [b]	4.94 [a]	2.07 [b]	0.37 [a]	0.18 [b]	0.39 [a]	0.19 [b]	SL	0.73
CBS	1.47 [a]	0.65 [b]	2.90 [a]	1.33 [b]	0.39 [a]	0.19 [b]	0.34 [a]	0.18 [b]	SL	0.41

Note: [b]: (Difference between accumulation in paired *t*-test is significant), [a]: (difference of paired *t*-test is non-significant), BAF S/R (bioaccumulation factor from soil to root), BAF S/P (bioaccumulation factor from soil to plants), Bold value (BAF >1), STC (soil textural class), SL (sandy loam), SiL (silty laom), CL (clay loam), OM% (organic matter percentage).

3.3. Translocation Factor

Translocation factors of Pb, Zn, Cu, and Cd from root to stem, stem to leaf, and leaf to fruit showed significant variations (Table 2). The resulting variations in all the plant functional parts indicate high mobilities of metals inside the plant tissues. Here, we recorded Pb-TF >1 from leaf-fruit (L-F) at six abandoned sites, four for Cd (2 each for S-L and L-F), and two sites for Cu (L-F), respectively. However, Zn-TF > 1 was not recorded in any functional part of X. *strumarium*; hence its mobility remained inactive at abandoned sites. The higher mobility of other metals indicates an ecologically inexpensive phytoremediation potential of this IAPS. As mentioned earlier, almost all the heavy metals except Zn moved to fruits in higher quantity in the majority of abandoned sites, possibly originating new resistant populations capable of tolerating heavy metal stress in time-space. TF factor or mobility index (MI) is considered one of the important factors in assessing hyperaccumulator plants (TF > 1) reveals phytoremediation potential, particularly for heavy metals [61]. Mobilization inside plants increases the level of metals in aboveground parts which may be attributed to aerial deposition and absorption of metals through surfaces of the leaves [62] as depicted by our results at few abandoned sites (e.g., TF > 1 for L-F for Pb in PC, MKI, MSM, TFN, TBM, Cu in HIZ and BKP, and Cd in BKP and CUA sites and S-L for Cd in KTH and SKR sites). Concerning phytoextraction and phytostabilization strategies often utilized in assessing phytoremediation [63], *X. strumarium* has efficient phytoextraction ability in terms of bioaccumulation and translocation of the aforementioned heavy metals into various below and aboveground parts. However, in phytostabilization, the plant changes pollutants nature in the rhizosphere, thus making it harmless or tending to make it harmless for plant growth development [64], but this phenomenon is not yet known for this IASP.

Table 2. Translocation factor of Pb, Zn, Cu, and Cd in *X. strumarium* growing on different abandoned habitats in Khyber Pakhtunkhwa, Pakistan.

M Type	Pb			Zn			Cu			Cd		
St Code	TF (R-S)	TF (S-L)	TF (L-F)	TF (R-S)	TF (S-L)	TF (L-F)	TF (R-S)	TF (S-L)	TF (L-F)	TF (R-S)	TF (S-L)	TF (L-F)
KTH	0.43 [a]	0.53 [b]	0.88 [b]	0.67 [a]	0.83 [b]	0.76 [c]	0.38 [a]	0.63 [b]	0.64 [b]	0.59 [a]	1.00 [b]	0.45 [c]
GTM	0.47 [a]	0.72 [b]	0.70 [b]	0.55 [a]	0.59 [b]	0.77 [c]	0.47 [a]	0.45 [a]	0.74 [b]	0.44 [a]	0.39 [b]	0.43 [a]
SKR	0.42 [a]	0.52 [a]	0.73 [b]	0.64 [a]	0.67 [a]	0.68 [a]	0.39 [a]	0.61 [b]	0.66 [c]	0.61 [a]	1.02 [b]	0.40 [c]
SMR	0.55 [a]	0.64 [b]	0.50 [c]	0.54 [a]	0.60 [b]	0.75 [c]	0.51 a	0.53 [a]	0.64 [b]	0.46 [a]	0.40 [b]	0.39 [b]
PC	0.35 [a]	0.57 [b]	1.27 [c]	0.54 [a]	0.60 [b]	0.77 [c]	0.39 [a]	0.57 [b]	0.60 [b]	0.44 [a]	0.43 [a]	0.39 [b]
HIZ	0.51 [a]	0.62 [b]	0.86 [c]	0.47 [a]	0.80 [b]	0.70 [c]	0.27 [a]	0.43 [b]	1.02 [c]	0.42 [a]	0.56 [b]	0.48 [c]
MKI	0.51 [a]	0.33 [b]	1.12 [c]	0.50 [a]	0.71 [b]	0.71 [b]	0.32 [a]	0.44 [b]	0.20 [c]	0.39 [a]	0.49 [b]	0.54 [c]
BKP	0.56 [a]	0.54 [a]	0.70 [b]	0.46 [a]	0.66 [b]	0.69 [b]	0.36 [a]	0.44 [b]	1.15 [c]	0.42 [a]	0.51 [b]	1.06 [c]
CUA	0.58 [a]	0.35 [b]	0.95 [c]	0.49 [a]	0.72 [b]	0.65 [c]	0.45 [a]	0.42 [a]	0.35 [b]	0.42 [a]	0.59 [b]	1.22 [c]
MSM	0.44 [a]	0.40 [b]	1.00 [c]	0.42 [a]	0.68 [a]	0.55 [a]	0.27 [a]	0.43 [b]	0.46 [b]	0.41 [a]	0.51 [b]	0.59 [c]
TFN	0.60 [a]	0.80 [b]	1.16 [c]	0.48 [a]	0.52 [a]	0.75 [b]	0.14 [a]	0.54 [b]	0.51 [b]	0.61 [a]	0.73 [b]	0.61 [a]

Table 2. *Cont.*

M Type		Pb			Zn			Cu			Cd	
TBM	0.63 ^{a}	0.67 ^{a}	1.17 ^{b}	0.60 ^{a}	0.40 ^{b}	0.38 ^{b}	0.8 ^{a}	0.51 ^{b}	0.46 ^{c}	0.59 ^{a}	0.48 ^{b}	0.98 ^{c}
GCD	0.62 ^{a}	0.65 ^{a}	0.53 ^{b}	0.48 ^{a}	0.33 ^{b}	0.52 ^{c}	0.92 ^{a}	0.43 ^{b}	0.49 ^{c}	0.54 ^{a}	0.81 ^{b}	0.49 ^{c}
AMS	0.70 ^{a}	0.57 ^{b}	0.68 ^{c}	0.42 ^{a}	0.43 ^{a}	0.39 ^{a}	0.37 ^{a}	0.44 ^{b}	0.56 ^{c}	0.45 ^{a}	0.70 ^{b}	0.56 ^{c}
CBS	0.48 ^{a}	0.78 ^{b}	1.20 ^{c}	0.46 ^{a}	0.54 ^{b}	0.50 ^{c}	0.39 ^{a}	0.52 ^{b}	0.52 ^{b}	0.55 ^{a}	0.71 ^{b}	0.58 ^{a}

Note: ^{a}: (Represent difference of translocation factors tested by ANOVA), St (stand), R-S (root to stem); ^{b}: S-L (stem to leaves); ^{c}: L-F (leaves to fruit), bold value (TF $\geq$ 1).

3.4. Soil–Plant Heavy-Metals Relationships

PCA-biplot reveals relationships between soil–plant heavy metal bioconcentration, BAF, TF, and physiochemical properties at spatial-scale (Table 3) (Figure 3). This multivariate approach is one of the most widely used statistical technique for identifying the pattern and sources of heavy metal pollutants [65].

Table 3. PCA-ordination explained 98.97% of the cumulative variance between metal bioconcentration, accumulation, and translocation factors of metals with soil spatial and textural properties at different abandoned habitats in Khyber Pakhtunkhwa, Pakistan.

Axis	Eigenvalues	% Variance	% Cum Var.	Broken-Stick Eigenvalues
1	9.14	38.1	38.10	3.776
2	4.97	20.74	58.84	2.776
3	2.58	10.78	69.62	2.276
4	2.15	8.96	78.59	1.943
5	1.57	6.56	85.15	1.693
6	1.29	5.37	90.53	1.493
7	0.84	3.51	94.05	1.326
8	0.53	2.23	96.28	1.183
9	0.44	1.86	98.15	1.058
10	0.19	0.82	98.97	0.947

Here, we distinguished four major clusters in the PCA-biplot ordination, showing the abandoned sites' obvious clockwise pattern. This pattern was linked with the strong gradient of textural properties particularly sand, and plant-biomass (PB) that significantly affects the heavy metals concentration and its phytoremediation (BAF and TF) by *X. strumarium*. This interesting effect may reflect the metalliferous soil nature often represents sandy, nutrient-poor, and dry substrate where plant coverage is low and patchy [66]. Several authors reported these factors since it plays a key role in the distribution and bioavailability of heavy soil metals as well as their accumulation and translocation to the living tissues, e.g., [48,67–70]. Cui et al., (2007) [71] reported PCA-ordination of heavy metal accumulation in different smelters in northeast China. Our results are somehow in compliance with the results reported by [61], since their results revealed that metals concentration in the soil and soil pH play an important role in metal BAF. The slight difference may be attributed to plant species differences and soil characteristics at a spatial scale. In our study, PCA-ordination explained 98.97% of the total cumulative variance, of which the first three axes contributed 69.62%. The pronounced 38.1% variance explained by the first axis related to the high load with corresponding high eigenvalues of Pb (λ = 0.88), Zn (λ = 0.95), Cu (λ = 0.89), and Cd (λ = 0.91) respectively. The striking pattern and high variance explained by Pb, Zn, Cu, and Cd accumulation is very similar to the findings (variance explained the first three-axis 99%; 67%) of Cui et al. (2007) and Yang et al. (2014) [71,72] while studying the agricultural land contamination by heavy metal accumulations.

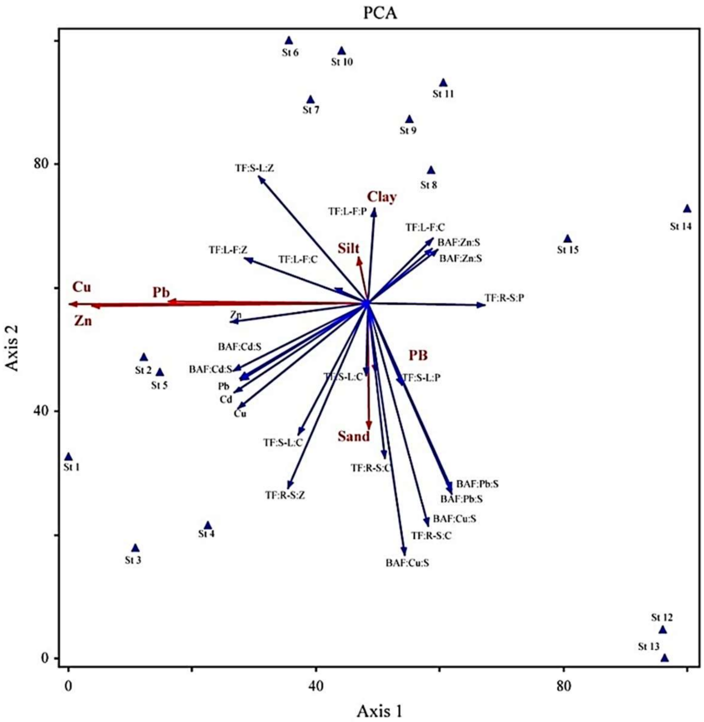

Figure 3. PCA-biplot depicting relationships between metal bio-concentration, accumulation and translocation factors of metals with soil spatial and textural properties at different abandoned habitats in Khyber Pakhtunkhwa, Pakistan. Note: St (Stand), St.1 (KTS (Kanju Town Junction)), St.2 (GTM (GT Road Mingora)), St.3 (SKR (Skhakot Road)), St. 4 (SMR (Shamozu Road)), St. 5 (PC (Peshawar)), St. 6 (HIZ (Hayatabad Industrial Zone)), St. 7 (MKI (Mardan Kid Industry)), St. 8 (BKP (Barikot Crush Plant)), St. 9 (CUA (Charsadda Urban)), St. 10 (MSM (Mardan Shiekh Malton)), St. 11 (TFN (Tarnab Form)), St. 12 (TBM (Takht Bhai)), St. 13 (GCD (Gulabad College Degree)), St. 14 (AMS (Amankot)), St. 15 (CBS (Charbagh street)).

4. Conclusions

Determining the significant players in any environmental situation is difficult due to complex nexuses among various abiotic and biotic factors. Our study found soil textural properties and plant-biomass to be the significant drivers, considerably affecting the heavy metals concentration and its phytoremediation by *X. strumarium*. Here, we derived an unequivocal conclusion that this effect might be due to the metalliferous nature of the soil at all the abandoned sites. Based on our studies, we concluded that: (1) this plant species falls under the category of facultative metallophytes (pseudometallophyte) having the ability to grow on both polluted and non-polluted soils; (2) this plant species accumulates ample quantities of bioavailable Pb, Zn, Cu, and Cd in roots and efficiently translocate to aboveground parts (i.e., stems, leaves, and fruits), hence this IAPS growing on such abandoned habitats can be used for Pb phytoextraction and phytostabilization of Cd, Cu, and Zn; and (3) concentration of Pb, Cu, and Cd were found above the threshold indicating the stress tolerance of *X. strumarium* to grow on metals contaminated soils, making this plant species unsuitable for forage, medicinal, and phytochemical purposes. As demonstrated in this research, the essential ecosystem services provided by these IAPS are of great environmental significance. They need to be explored in the future using well-established and sophisticated biotechnological approaches.

Author Contributions: The study was designed and supervised by N.K.; The field and laboratory work, data analysis, and manuscript writing was carried out by R.U. and N.K.; K.A. also contributed to manuscript writing; M.E.H.K. contributed a lot to statistical analysis and data refining; and D.A.J. provide key background information in the phytoremediation potentials and edit the manuscript in a better way for language editing; M.E.H.K. also contributed to data analysis and tabulation. All authors have read and agreed to the published version of the manuscript.

Funding: This research APC was funded by College of North Atlantic-Qatar (CNA-Q) under Seed Grant 2020, grant number SEED2020_11. An IOAP discount of 10% was applied due to membership with the Qatar National Library.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the data analyzed/generated are included and available in the manuscript.

Acknowledgments: We express our gratitude to the Master's students enrolled in the Department of Botany and the people of Malakand Division who helped with the fieldwork for this project. We are also grateful to the Chairman Department of Chemistry, KUST, for analyzing heavy metals' soil and plant samples. We also extend our gratitude to Muhammad Sadiq, Department of the Pharmacy University of Malakand, for his untiring efforts in laboratory work. We extend our valuable words of appreciation to the unknown reviewers and editorial members of Applied Sciences Journal for their helpful comments, suggestions, and language editing.

Conflicts of Interest: I hereby declare that all the authors have participated in this study and the development of the manuscript. All the authors have read the final version and given their consent for the article to be published in the Applied Sciences.

References

1. Kumari, A.; Lal, B.; Rai, U.N. Assessment of native plant species for phytoremediation of heavy metals growing in the vicinity of NTPC sites, Kahalgaon, India. *Int. J. Phytoremediation* **2016**, *18*, 592–597. [CrossRef]
2. Conesa, H.M.; Faz, Á.; Arnaldos, R. Heavy metal accumulation and tolerance in plants from mine tailings of the semiarid Cartagena–La Unión mining district (SE Spain). *Sci. Total Environ.* **2006**, *366*, 1–11. [CrossRef]
3. Shu, W.S.; Ye, Z.H.; Lan, C.Y.; Zhang, Z.Q.; Wong, M.H. Lead, zinc and copper accumulation and tolerance in populations of Paspalum distichum and Cynodon dactylon. *Environ. Pollut.* **2002**, *120*, 445–453. [CrossRef]
4. Khan, T.A.; Sharma, S.; Ali, I. Adsorption of Rhodamine B dye from aqueous solution onto acid activated mango (Magnifera indica) leaf powder: Equilibrium, kinetic and thermodynamic studies. *Toxicol. Environ. Health Sci.* **2011**, *3*, 286–297.
5. Chary, N.S.; Kamala, C.T.; Raj, D.S.S. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicol. Environ. Saf.* **2008**, *69*, 513–524. [CrossRef]
6. Khan, S.; Cao, Q.; Zheng, Y.M.; Huang, Y.Z.; Zhu, Y.G. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ. Pollut.* **2008**, *152*, 686–692. [CrossRef] [PubMed]
7. Noman, A.; Aqeel, M.; Javed, M.T.; Zafar, S.; Ali, Q.; Islam, W.; Rshad, M.K.; Buriro, M.; Kanwal, H.; Khalid, N.; et al. Histological changes in Hibiscus rosa-sinensis endorse acclimation and phytoremediation of industrially polluted sites. *J. Anim. Plant. Sci.* **2017**, *27*, 1637–1648.
8. Ali, I.; Aboul-Enein, H.Y.; Gupta, V.K. *Nano Chromatography and Capillary Electrophoresis: Pharmaceutical and Environmental Analyses*; Wiley & Sons: Hoboken, NJ, USA, 2009.
9. Ali, I.; Jain, C.K. Advances in arsenic speciation techniques. *Int. J. Env. Anal. Chem.* **2004**, *84*, 947–964. [CrossRef]
10. Zhang, Z.; Wang, J.J.; Ali, A.; DeLaune, R.D. Heavy metals and metalloid contamination in Louisiana Lake Pontchartrain Estuary along I-10 Bridge. *Transp. Res. D Transp. Environ.* **2016**, *44*, 66–77. [CrossRef]
11. Khalid, N.; Hussain, M.; Young, H.S.; Ashraf, M.; Hameed, M.; Ahmad, R. Lead concentrations in soils and some wild plant species along two busy roads in Pakistan. *Bull. Environ. Contam. Toxicol.* **2018**, *100*, 250–258. [CrossRef]
12. Noman, A.; Ali, Q.; Maqsood, J.; Iqbal, N.; Javed, M.T.; Rasool, N.; Naseem, J. Deciphering physio-biochemical, yield, and nutritional quality attributes of water-stressed radish (*Raphanus sativus* L.) plants grown from Zn-Lys primed seeds. *Chemosphere* **2018**, *195*, 175–189. [CrossRef]
13. Chen, X.; Xia, X.; Zhao, Y.; Zhang, P. Heavy metal concentrations in roadside soils and correlation with urban traffic in Beijing, China. *J. Hazard. Mater.* **2010**, *181*, 640–646. [CrossRef] [PubMed]
14. Jomova, K.; Valko, M. Advances in metal-induced oxidative stress and human disease. *Toxicology* **2011**, *283*, 65–87. [CrossRef]
15. Ozaki, H.; Watanabe, I.; Kuno, K. As, Sb and Hg distribution and pollution sources in the roadside soil and dust around Kamikochi, Chubu Sangaku National Park, Japan. *Geochem. J.* **2004**, *38*, 473–484. [CrossRef]
16. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals—concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [CrossRef] [PubMed]
17. Yoon, J.; Cao, X.; Zhou, Q.; Ma, L.Q. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* **2006**, *368*, 456–464. [CrossRef]

18. Zhao, F.J.; Lombi, E.; McGrath, S.P. Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator Thlaspi caerulescens. *Plant Soil* **2003**, *249*, 37–43. [CrossRef]
19. Balabanova, B.; Stafilov, T.; Bačeva, K. Bioavailability and bioaccumulation characterisation of essential and heavy metals contents in *R. acetosa*, *S. oleracea* and *U. dioica* from copper polluted and referent areas. *J. Environ. Health Sci. Eng.* **2015**, *13*, 1–13.
20. Zhuang, P.; Yang, Q.W.; Wang, H.B.; Shu, W.S. Phytoextraction of heavy metals by eight plant species in the field. *Water Air Soil Pollut.* **2007**, *184*, 235–242. [CrossRef]
21. Khalid, N.; Noman, A.; Aqeel, M.; Masood, A.; Tufail, A. Phytoremediation potential of Xanthium strumarium for heavy metals contaminated soils at roadsides. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 2091–2100. [CrossRef]
22. Chinmayee, M.D.; Mahesh, B.; Pradesh, S.; Mini, I.; Swapna, T.S. The assessment of phytoremediation potential of invasive weed Amaranthus spinosus L. *Appl. Biochem. Biotechnol.* **2012**, *167*, 1550–1559. [CrossRef]
23. Pandey, V.C. Invasive species based efficient green technology for phytoremediation of fly ash deposits. *J. Geochem. Explor.* **2012**, *123*, 13–18. [CrossRef]
24. Trueman, R.J.; Erber, L. Invasive species may offer advanced phytoremediation of endocrine disrupting chemicals in aquatic ecosystems. *Emir. J. Food. Agric.* **2013**, *2013*, 648–656.
25. Prabakaran, K.; Li, J.; Anandkumar, A.; Leng, Z.; Zou, C.B.; Du, D. Managing environmental contamination through phytoremediation by invasive plants: A review. *Ecol. Eng.* **2019**, *138*, 28–37. [CrossRef]
26. Marwat, K.B.; Hashim, S.; Ali, H. Weed management: A case study from north-west Pakistan. *Pak. J. Bot.* **2010**, *42*, 341–353.
27. Löve, D.; Dansereau, P. Biosystematic studies on Xanthium: Taxonomic appraisal and ecological status. *Can. J. Bot.* **1959**, *37*, 173–208. [CrossRef]
28. Kamboj, A.; Saluja, A.K. Phytopharmacological review of *Xanthium strumarium* L. (Cocklebur). *Int. J. Green Pharm.* **2010**, *4*, 129–140. [CrossRef]
29. Martin, R.B. Optimal control drug scheduling of cancer chemotherapy. *Automatica* **1992**, *28*, 1113–1123. [CrossRef]
30. Khan, N.; Ali, K.; Saeed, S. How environmental variables can determine the Chir pine (*Pinus roxburghii* sarg.) Distribution in swat Hindukush range of Pakistan: Current and future prospective of the species. *Appl. Ecol. Environ. Res.* **2021**, *19*, 2405–2424. [CrossRef]
31. Khan, W.; Ali, K.; Hussain, M.; Ali, M.; Nisar, M. Distribution and phenotypic variation in Juglans regia L. growing in Hindu Kush ranges of Pakistan. *Acta Ecol. Sin.* **2020**, *40*, 363–372. [CrossRef]
32. Rahman, I.U.; Khan, N.; Ali, K.; Ahmad, S. Vegetation–environment relationship in *Pinus wallichiana* forests of the Swat Hindukush range of Pakistan. *J. For. Res.* **2018**, *31*, 185–195. [CrossRef]
33. Yafa, C.; Farmer, J.G.; Graham, M.C.; Bacon, J.R.; Barbante, C.; Cairns, W.R.L.; Bindler, R.; Renberg, I.; Cheburkin, A.; Kylander, M.; et al. Development of an ombrotrophic peat bog (low ash) reference material for the determination of elemental concentrations. *Environ. Monit. Assess.* **2004**, *6*, 493–501. [CrossRef]
34. Wu, J.; Teng, Y.; Lu, S.; Wang, Y.; Jiao, X. Evaluation of soil contamination indices in a mining area of Jiangxi, China. *PLoS ONE* **2014**, *9*, e112917. [CrossRef]
35. Kachenko, A.G.; Singh, B. Heavy metals contamination in vegetables grew in urban and metal smelter contaminated sites in Australia. *Water Air Soil Pollut.* **2006**, *169*, 101–123. [CrossRef]
36. Rehman, Z.U.; Sardar, K.H.A.N.; Shah, M.T.; Brusseau, M.L.; Khan, S.A.; Mainhagu, J. Transfer of heavy metals from soils to vegetables and associated human health risks at selected sites in Pakistan. *Pedosphere* **2018**, *28*, 666–679. [CrossRef]
37. Day, P.R. *Particle Fractionation and Particle-Size Analysis*; No. methods of soil analysis; American Society of Agronomy Madison, Soil Science Society of America: Madison, WI, USA, 1965; pp. 545–567.
38. Nelson, D.W.; Sommers, L.E. Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis. Part 3. Chemical Method*; Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Summer, M.E., Eds.; Soil Science Society of America and American Society of Agronomy: Madison, WI, USA, 1996; pp. 961–1010.
39. Atayese, M.O.; Eigbadon, A.I.; Adesodun, J.K. Heavy metal contamination of *Amaranthus* grown along major highways in Lagos, Nigeria. *Afr. Crop. Sci. J.* **2008**, *16*, 225–235. [CrossRef]
40. Suzuki, K.; Yabuki, T.; Ono, Y. Roadside Rhododendron pulchrum leaves as bioindicators of heavy metal pollution in traffic areas of Okayama, Japan. *Environ. Monit. Assess.* **2009**, *149*, 133–141. [CrossRef]
41. Weckwerth, G. Verification of traffic emitted aerosol components in the ambient air of Cologne (Germany). *Atmos. Environ.* **2011**, *35*, 5525–5536. [CrossRef]
42. Malinowska, E.; Jankowski, K.; Wiśniewska-Kadżajan, B.; Sosnowski, J.; Kolczarek, R.; Jankowska, J.; Ciepiela, G.A. Content of zinc and copper in selected plants growing along a motorway. *Bull. Environ. Contam. Toxicol.* **2015**, *95*, 638–643. [CrossRef]
43. Kabata-Pendias, A. Behavioural properties of trace metals in soils. *J. Appl. Geochem.* **1993**, *8*, 3–9. [CrossRef]
44. Ul Islam, E.; Yang, X.E.; He, Z.L.; Mahmood, Q. Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. *J. Zhejiang Univ. Sci.* **2007**, *8*, 1–13. [CrossRef] [PubMed]
45. Macnicol, R.D.; Beckett, P.H.T. Critical tissue concentrations of potentially toxic elements. *Plant. Soil* **1985**, *85*, 107–129. [CrossRef]
46. Singh, A.; Sharma, R.K.; Agrawal, M.; Marshall, F.M. Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India. *Food Chem. Toxicol.* **2010**, *48*, 611–619. [CrossRef]
47. Alloway, B.J. Cadmium. In *Heavy Metals in Soils*; Blackie and Son, Ltd.: Glasgow, UK, 1990.

48. Kashem, M.A.; Singh, B.R.; Kawai, S. Mobility and distribution of cadmium, nickel and zinc in contaminated soil profiles from Bangladesh. *Nutr. Cycling Agroecosyst.* **2007**, *77*, 187–198. [CrossRef]
49. Tyokumbur, E.T.; Okorie, T. Bioconcentration of trace metals in the tissues of two leafy vegetables widely consumed in South West Nigeria. *Biol. Trace Elem. Res.* **2011**, *140*, 215–224. [CrossRef]
50. Khan, Z.I.; Ahmad, K.; Ashraf, M.; Shoaib, N.; Parveen, R.; Bibi, Z.; Cazzato, E. Assessment of toxicological health risk of trace metals in vegetables mostly consumed in Punjab, Pakistan. *Environ. Earth Sci.* **2016**, *75*, 433. [CrossRef]
51. Noman, A.; Ali, Q.; Hameed, M.; Mehmood, T.; Iftikhar, T. Comparison of leaf anatomical characteristics of Hibiscus rosa-sinensis grown in Faisalabad region. *Pak. J. Bot.* **2014**, *46*, 199–206.
52. Noman, A.; Aqeel, M. miRNA-based heavy metal homeostasis and plant growth. *Environ. Sci. Pollut. Res.* **2017**, *24*, 10068–10082. [CrossRef] [PubMed]
53. Andersson, A. On the distribution of heavy metals as compared to some other elements between grain size fractions in soils. *Swed. J. Agric. Res.* **1979**, *9*, 7–13.
54. Qian, J.I.N.; Shan, X.Q.; Wang, Z.J.; Tu, Q. Distribution and plant availability of heavy metals in different particle-size fractions of soil. *Sci. Total Environ.* **1996**, *187*, 131–141. [CrossRef]
55. Scokart, P.O.; Meeus-Verdinne, K.; De Borger, R. Mobility of heavy metals in polluted soils near zinc smelters. *Water Air Soil Pollut.* **1983**, *20*, 451–463. [CrossRef]
56. Eriksson, J.E. The influence of pH, soil type and time on adsorbtion and uptake by plants of Cd added to the soil. *Water Air Soil Pollut.* **1989**, *48*, 317–335. [CrossRef]
57. MacLean, A.J. Cadmium in different plant species and its availability in soils as influenced by organic matter and additions of lime, P, Cd and Zn. *Can. J. Soil Sci.* **1976**, *56*, 129–138. [CrossRef]
58. Street, J.J.; Lindsay, W.L.; Sabey, B.R. Solubility and plant uptake of cadmium in soils amended with cadmium and sewage sludge. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. *J. Environ. Qual.* **1977**, *6*, 72–77. [CrossRef]
59. Martin, M.H.; Duncan, E.M.; Coughtrey, P.J. The distribution of heavy metals in a contaminated woodland ecosystem. *Environ. Pollut. Ser. B Chem. Phys.* **1982**, *3*, 147–157. [CrossRef]
60. Brallier, S.; Harrison, R.B.; Henry, C.L.; Dongsen, X. Liming effects on availability of Cd, Cu, Ni and Zn in a soil amended with sewage sludge 16 years previously. *Water Air Soil Pollut.* **1996**, *86*, 195–206. [CrossRef]
61. Ma, J.F.; Goto, S.; Tamai, K.; Ichii, M. Role of root hairs and lateral roots in silicon uptake by rice. *Plant. Physiol.* **2001**, *127*, 1773–1780. [CrossRef] [PubMed]
62. Kachenko, A.; Singh, B. Heavy metals contamination of home grown vegetables near metal smelters in NSW. In Proceedings of the 3rd Australian New Zealand Soils Conference, Sydney, Australia, 5–9 December 2004.
63. Salt, D.E.; Smith, R.D.; Raskin, I. Phytoremediation. *Annu. Rev. Plant. Biol.* **1998**, *49*, 643–668. [CrossRef]
64. Eapen, S.; D'souza, S.F. Prospects of genetic engineering of plants for phytoremediation of toxic metals. *Biotechnol. Adv.* **2005**, *23*, 97–114. [CrossRef]
65. Hani, A.; Pazira, E. Heavy metals assessment and identification of their sources in agricultural soils of Southern Tehran, Iran. *Environ. Monit. Assess.* **2011**, *176*, 677–691. [CrossRef]
66. Borymski, S.; Cycoń, M.; Beckmann, M.; Mur, L.A.; Piotrowska-Seget, Z. Plant species and heavy metals affect biodiversity of microbial communities associated with metal-tolerant plants in metalliferous soils. *Front. Microbiol.* **2018**, *9*, 1425. [CrossRef] [PubMed]
67. Ireland, M.P. Effect of the earthworm Dendrobaena rubida on the solubility of lead, zinc, and calcium in heavy metal contaminated soil in Wales. *J. Soil Sci.* **1975**, *26*, 313–318. [CrossRef]
68. Gee, C. Mineralogy and weathering processes in historical smelting slags and their effect on the mobilisation of lead. *J. Geochem. Explor.* **1979**, *58*, 249–257. [CrossRef]
69. Alloway, B.J.; Thornton, I.; Smart, G.A.; Sherlock, J.C.; Quinn, M.J. Metal availability. *Sci. Total Environ.* **1988**, *75*, 41–69. [CrossRef]
70. Banin, A.; Navrot, J.; Perl, A. Thin-horizon sampling reveals highly localised concentrations of atmophile heavy metals in a forest soil. *Sci. Total Environ.* **1987**, *61*, 145–152. [CrossRef]
71. Cui, S.; Zhou, Q.; Chao, L. Potential hyperaccumulation of Pb, Zn, Cu and Cd in endurant plants distributed in an old smeltery, northeast China. *Environ. Geol.* **2007**, *51*, 1043–1048. [CrossRef]
72. Yang, P.; Yang, M.; Mao, R.; Shao, H. Multivariate-statistical assessment of heavy metals for agricultural soils in northern China. *Sci. World J.* **2014**, *2014*, 517020. [CrossRef]