

## Research Article

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# Heavy metals uptake ability from water by the Himalayan alder growing in Riparian habitat of Sino Japanese regions in Pakistan

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### Abstract

The excess amount of heavy metal in soil pose a serious risk for the life and food chain of living beings. The recommended and feasible procedure to combat heavy metal is plantation of trees with excellent capacity of accumulation. *Alnus nitida* is a plant species grown on the banks of rivers and streams in western Himalayas and Hindu Kush region of the Sino-Japanese belt. It plays a significant role in accumulation of heavy metals from water. In current study it was explored in the west from Bajaur to Kashmir in the east. For heavy metals analysis shoot, leaves Root and Bark of *Alnus nitida* were washed thoroughly under tap water and then placed in an 81 °C oven to dry it for about 5 minutes. After acid digestion plant material were put in falcon tube to raise the level of sample to 40 ml through distilled water. The accumulation of heavy metal in Himalayan alder was assessed via atomic absorption spectroscopy. The Heavy Metals analyses were carried out to find the bioaccumulation ability of Alder by using standard formulas of bio concentration factor (BCF), Translocation Factor (TF) and Biological Accumulation Coefficient (BAC). It was observed an efficient accumulator of all the heavy metals as revealed by the values of BCF in shoots, BCF in roots and also the TF. Himalayan Alder has greatly declined through out in its habitat as a result of drought, developmental projects, deforestation and other anthropogenic activities. Current study can provide a baseline for further comprehensive studies heavy metals studies of other such species.

**Keywords:** Heavy metals; Himalayan alder; Pakistan; Sino Japanese regions

### Introduction

Over the recent past decades Heavy metals sources have been increased due to rapid industrialization and use of inorganic materials in domestic as well as at industry level. From biological point of view the metalloids and

metals can be harmful both for animals and plants at low concentration [1]. Heavy metals are of two types essential and non-essential. Non-essential metals don't play role in physiological functions i.e. Pb, Hg, Se, As and Cd. Essential elements are required for

metabolism i.e. Cu, Ni, Zn, Mo, Co and Fe [2]. Environmental pollution due to heavy metals led to an alarming situation due to disturbance of biogeochemical cycles and rapid industrialization [3]. Both anthropogenic and natural activities add heavy metals in environment. Natural sources are soil Erosion, weathering of valuable minerals and volcanos. The anthropogenic activities are Mining, smelting, use of pesticides etc [4]. Different herbs, Shrubs and Trees are used to eliminate heavy metals from the environment. All the plants show potential capability to reduce the amount to a range of least or eliminate it [5]. Tree species with phytofiltration are unable to grow in polluted area, for that reason different ecotypes of same species are grown in heavy metals rich area [6]. Plant species with mycorrhizal affiliation may reduce the risk of heavy metals on plant physiology as it resists the harmful effect of metals and enhance the growth of species. Himalayan alder is a special tree having mycorrhizal association and with excellent heavy metals accumulation capability. The alder tree absorb heavy metals and reduce the risk to its physiological or anatomical features by unique association with fungi [7-9]. Heavy metals accumulation studies on Alder species were carried out in different countries. As compare to Salix and Acer *Alnus glutinosa* was the plant species with greater survival rate planted in a highly alkaline anthropogenic sediment [10]. The *Alnus nitida* heavy metal studied were carried out first time from Pakistan in the present research work. The genus *Alnus* is distributed in various temperate regions of the world. According to [11] about 40 of its species distributed in Bhutan, China, Bangladesh, Europe, India, Korea, Japan, Nepal and America and Northern Hemisphere. *Alnus* are separated from each other by unique characters of nitrogen fixation [12].

The present research work is based on *Alnus nitida* a native plant of northern areas of Pakistan playing great role not only economically as well as medicinally for the local inhabitants of the area. Many species in different areas of the world by are under

consideration for test of accumulation of heavy metals. The present research work will provide a base for future researchers to consider different heavy metal accumulator plants to recommend species best for water purification.

## Materials and methods

### Site description

Research area is located in Sino-Japanese belt occurring in Himalayan-Hidukush territory of Pakistan. Survey was extended for heavy metals analysis thought out the area of Bajaur, Dir, Swat, Buner, Haripur and Kashmir. In the above mentioned selected zones few areas were analyzed for heavy metals analysis including Muzaffarabad (Kashmir), Nokhara, Pandh, Afreen tang, Rahat kot, Kokarai (Swat) Gokand (Buner) Ghor, Chianrkot, Manyal (Dir) (Fig.1)

### Sample collection

At each sampling area *Alnus nitida* vigorous and healthy tree was selected to collect samples for analysis. All the plant samples were collected in samples, taking selected portion of root, leaf and bark and packed it in plastic bags in sampling area and brought to the laboratory as soon as possible to analyze it. The field visit and collection of samples was carried out in September 2017.

### Heavy metals analysis

The shoot, leaves Root and Bark of *Alnus nitida* were washed thoroughly under tap water (roots were scrubbed with a brush carefully) and then placed in an 81 °C oven (company Walls) to be dried for about 5 minutes.

Plant metal uptake was determined by wet acid digestion [13]. The 0-.5 g dried plant material was grinded manually. Then 10 ml Nitric-Perchloric acid (HNO<sub>3</sub>-HClO<sub>4</sub>) mixture (3:1 ratio) was supplemented to plant material in conical flask, and left it for overnight. After the initial digestion, the flasks having the material were then transferred to fume hood, and temperature was raised to 70 OC for 60 minutes. Temperature was steadily raised until the brown fumes turned into white fumes. Mixture was then permitted to cool for a few minutes, and diluted carefully with distilled water. Extract was filtered via filter paper (Whatmann No. 42) and 50 ml volume was raised using

distilled water. Filtered samples were then used to determine the concentrations of desired metal i.e. Ni, Cu, Mn, Co, Zn, Cd, Cr, Pb, Mg, Ca, Na, K by flame atomic absorption spectrometry (Varian FAAS-240, Triad Scientific, New Jersey, USA) [14].

#### Formulae used for the analysis

#### Calculation of bioaccumulation coefficients (BAC), bio concentration factor (BCF) and translocation factor (TF)

The bioaccumulation factor and bioconcentration factor provide an index of the

ability of the shoots and roots to accumulate the metal with respect to the metal concentration in the soil. (Malik *et al.* 2010). Biological Concentration Factor (BCF) was calculated as metal concentration ratio of plant roots to soil given in equation 2 [15]. Translocation Factor (TF) was described as ratio of heavy metals in plant shoot to that in plant root given in equation 2 [16]. Biological Accumulation Coefficient (BAC) was calculated as ratio of heavy metal in shoots to that in soil given in equation 1 [17].

$$\text{BAC} = \frac{\text{Metal Concentration in shoot (mg/kg)}}{\text{Metal Concentration in soil (mg/kg)}} \quad (1)$$

$$\text{BCF} = \frac{\text{Metal concentration in roots (mg/kg)}}{\text{Metal concentration in soil (mg/kg)}} \quad (2)$$

The translocation factor was calculated according Ahmad *et al.* (2015)

$$\text{TF} = \frac{\text{Metal concentration in shoot (mg/kg)}}{\text{Metal concentration in roots (mg/kg)}} \quad (3)$$

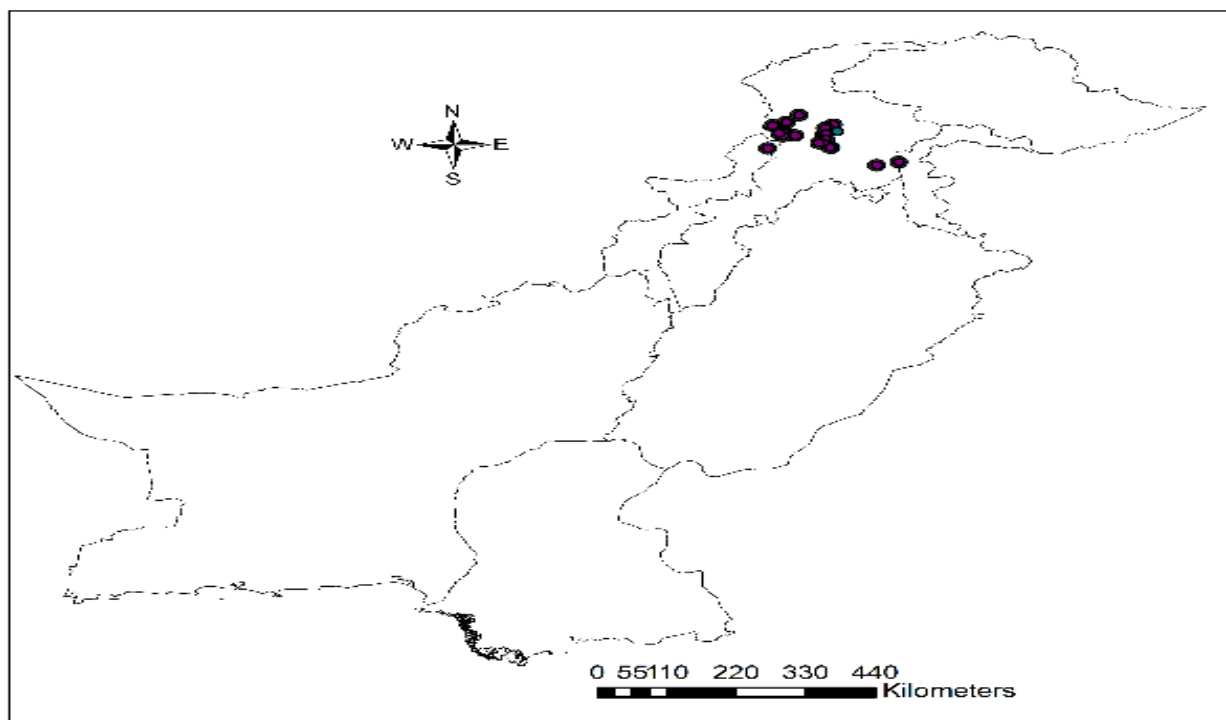


Fig. 1. Localities in Pakistan from which *Alnus nitida* specimen were collected

## Results and discussion

The Heavy metals uptake was examined in various parts of Himalayan Alder. The accumulation of metals in different part were previously observed by other scientists as [18-20]. The [21] recorded excellent accumulation of metals in roots of the *Alnus nepalensis*. In present study Himalayan alder was collected from different region of western Himalayas to find the accumulation of metals. The localities were started from Kashmir in the east to Bajaur in the west. Six localities were targeted to find the accumulation of 12 heavy metals. The main reason for the presence of heavy metals in the area were industries, hotels, Domestic effluents and Construction work. The local people are facing many health issues while acquiring orally from them regarding the water quality. The disease of kidneys, cancer and digestive problems are common in the area. Different heavy metals absorbing plant especially planation of *Alnus nitida* will help to reduce the pollutants from the environment. After collection and analysis of samples it was noticed that Ni is present in all of them however its amount varies in water, soil and plant parts and also according to the site of collection (Table 1). In water samples highest Ni contamination is present in sample collected from Chinarkot (404.5 $\mu$ /L) followed by Ghor (401.5 $\mu$ /L). Highest Ni contaminated soil was observed in Muzaffarabad (323.4 $\mu$ /g) while lowest contaminated is in Gokand (164.65  $\mu$ /g). Average values of Ni contents in various samples revealed that plant showed efficient uptake of Ni and translocate it in different plant tissues. Highest Ni accumulation was observed in shoot (241.27) followed by root (202.77) (Fig. 2). The order of Ni accumulation in plant parts was in order as followed shoot>root>bark>leaves. Cu is present in all of them however its amount varies in soil and plant parts and also according to the site of collection (Table 1). In water samples highest Cu contamination is present in sample collected from Rahat Kot (105.2  $\mu$ /L) followed by Afreen tang (27.45  $\mu$ /L). (Fig. 3) Highest Cu contaminated soil was observed in Rahat Kot (184.9  $\mu$ /g) while

lowest contaminated is in Manyal (18.45  $\mu$ /g). Average values of Cu contents in various samples revealed that plant showed efficient uptake of Cu and translocate it in different plant tissues. (Fig. 4) Highest Cu accumulation was observed in leaves (78.9) followed by (72.1). The order of Cu accumulation in plant parts was in order as followed Soil>root>water>leaves (Fig. 4) Mn is present in all of them however its amount varies in soil and plant parts and also according to the site of collection (Table 1 & Fig. 4). In root samples highest Mn contamination is present in sample collected from Afreen tang (269.1  $\mu$ /L) followed by Gokand (216.15  $\mu$ /L). Highest Mn contaminated soil was observed in Muzaffarabad (536.8  $\mu$ /g) while lowest contaminated is in Muzaffarabad (8.7  $\mu$ /g). Average values of Mn contents in various samples revealed that plant showed efficient uptake of Mn and translocate it in different plant tissues. Highest Ni accumulation was observed in stem (321.27) followed by root (18 $\mu$ /g). The order of Mn accumulation in plant parts was in order as followed soil>Roots> stem >leaves. (Fig. 5) Zn is present in all of them however its amount varies in water, soil and plant parts and also according to the site of collection (Table 1) (Fig. 6). In root samples highest Zn contamination is present in sample collected from Afreen tang (324.45  $\mu$ /L) followed by Gokand (319.15  $\mu$ /L). Highest Zn contaminated soil was observed in Afreen tang (345.6  $\mu$ /g) while lowest contaminated is in Chinarkoy (40 $\mu$ /g). Cr is present in all of them however its amount varies in water, soil and plant parts and also according to the site of collection (Table 1) (Fig. 7). In root samples highest Cr contamination is present in sample collected from Pandh (2194.1  $\mu$ /L) followed by Nokhara (77.65 $\mu$ /L). Pb is present in all of them however its amount varies in water, soil and plant parts and also according to the site of collection (Table 1 & Fig. 7). In root samples highest Pb contamination is present in sample collected from Rahat kot (321.9  $\mu$ /L) followed by Gokand (131.85 $\mu$ /L). Mg is present in all of

them however its amount varies in water, soil and plant parts and also according to the site of collection (Table 1 & Fig. 8). In root samples highest Mg contamination is present in sample collected from Rahat kot (841 $\mu$ /L) followed by Manyal (142.2  $\mu$ /L). Ca is present in all of them however its amount varies in water, soil and plant parts and also according to the site of collection (Table 1 & Fig. 9). In root samples highest Ca contamination is present in sample collected from Nokhara (2363.18  $\mu$ /L) followed by Pandh (2100  $\mu$ /L). (Fig. 10) Na is present in all of them however its amount varies in water, soil and plant parts and also according to the site of collection (Table 1 & Fig. 11). In root samples highest Na contamination is present in sample collected from Rahat kot (6614.1  $\mu$ /L) followed by Muzzafarabad (3786.6  $\mu$ /L). K is present in all

of them however its amount varies in water, soil and plant parts and also according to the site of collection (Table 1 & Fig. 12). In root samples highest K contamination is present in sample collected from Rahat kot (163.075  $\mu$ /L) followed by Muzzafarabad (187.75  $\mu$ /L). Plant was observed an efficient accumulator of Ni as revealed by the values (>1) of BCF in shoots, BCF in roots and also the TF (Fig. 2 & Fig. 13). From current sampling and research it is revealed that plants growing at localities Alnus showed highest Phyto-extraction of heavy metals. Reason behind is the presence of increased heavy metals contents in soil sample as compared to others [21, 22]. Plant was observed an efficient accumulator as revealed by the values (>1) of BCF in Stem, BCF in roots and also the TF.

**Table 1. BAC, BCF and TF values of *Alnus nitida* in different stations**

Cu		GK-Buner	SK-Hazara	GR-Dir	MN-Dir	KK-Swat	AT-Swat	PD-Swat	NK-Swat	MZ-Kashmir
	BAC	0.071102	9.55	0.113302	2.148649	2.442049	0.996078	0.813688	1.419009	15.15517
	BCF	0.54986	0.686695	0.097167	1.621622	0.9941	7.035294	0.711027	0.698795	15.15517
	TF	1.896071	1.029181	1.697674	1.326304	1.080956	1.68107	0.976165	1.103953	1
Mg	BAC	0.531401	1.079341	0.35957	0.888889	0.829312	1.31746	1.230337	0.646259	0.549451
	BCF	0.769928	0.848802	0.117376	2.943089	1	5.190476	0.893258	1.14966	0.549451
	TF	0.690196	1.271605	3.063399	0.302026	0.829312	0.253823	1.377358	0.56213	1
Mn	BAC	0.071102	9.2103	0.113302	2.148649	2.442049	0.996078	0.813688	1.419009	15.15517
	BCF	0.54986	0.686695	0.097167	1.621622	0.978437	7.035294	0.711027	0.698795	15.15517
	TF	0.129308	13.4125	1.166052	1.325	2.495868	0.141583	1.144385	2.030651	1
Zn	BAC	0.173032	0.470022	0.386948	0.138697	0.08895	0.25307	0.018147	25.68236	2.015042
	BCF	0.132697	0.013323	0.171359	1.081747	0.99863	3.763598	0.45642	4.073446	2.015042
	TF	1.303965	35.27778	2.258112	0.128215	0.089072	0.067241	0.039759	6.304824	1
Cd	BAC	1.40625	0.757174	0.986152	0.991736	1.030367	1.061053	1.011379	1.108494	1.027778
	BCF	1.221774	1.002208	0.887755	1.010331	1.012712	1.031579	0.967871	1.017131	1.027778
	TF	1.15099	0.755507	1.110837	0.981595	1.017434	1.028571	1.044952	1.089825	1
Cr	BAC	0.18886	6.256173	4.066667	1.281834	1.246442	1.043284	1.282967	0.876334	1.086691
	BCF	0.692611	0.601852	27	0.794567	0.985157	2.897015	1.288462	0.888889	0.818681
	TF	0.272678	10.39487	0.150617	1.613248	1.265222	0.360124	0.995736	0.985876	1.327368
Pb	BAC	1.698113	1.096774	1.714286	1.371841	0.81203	1.194444	0.965278	1.019608	1.515152
	BCF	0.849057	1.225806	1	1.299639	1.150376	2.633333	0.833333	0.921569	0.681818
	TF	2	0.894737	1.714286	1.055556	0.705882	0.453586	1.158333	1.106383	2.222222
Ca	BAC	8.940901	2.509368	0.849654	5.712463	0.410692	0.371798	1.203061	0.210161	0.215341

	BCF	8.496972	3.335102	0.57031	1.734884	0.999811	0.074926	0.49326	0.78763	2.76882
	TF	1.052246	0.752411	1.489812	3.292706	0.41077	4.962209	2.438997	0.266827	1.2
Na	BAC	5.834167	1.881074	0.482173	3.302683	0.603242	1.18058	1.114004	0.256454	0.780767
	BCF	4.944279	2.945158	0.783043	2.233437	1.009646	0.677456	0.608898	0.585664	0.882769
	TF	1.179983	0.6387	0.615768	1.478745	0.597479	1.742667	1.829542	0.437885	1
K	BAC	5.834167	1.881074	0.482173	3.302683	0.603242	1.18058	1.114004	0.256454	0.780767
	BCF	4.944279	2.945158	0.783043	2.233437	1.009646	0.677456	0.608898	0.585664	0.882769
	TF	1.179983	0.6387	0.615768	1.478745	0.597479	1.742667	1.829542	0.437885	1

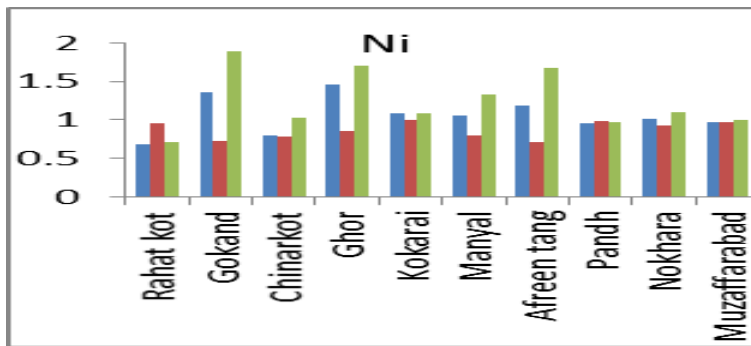


Fig. 2. Graphical representation of Ni Accumulation

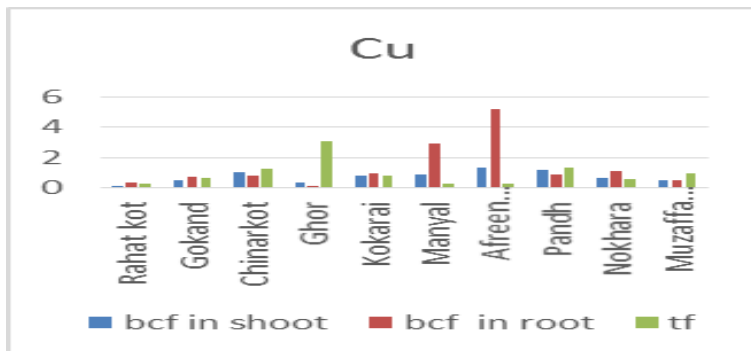


Fig. 3. Graphical representation of Cu Accumulation

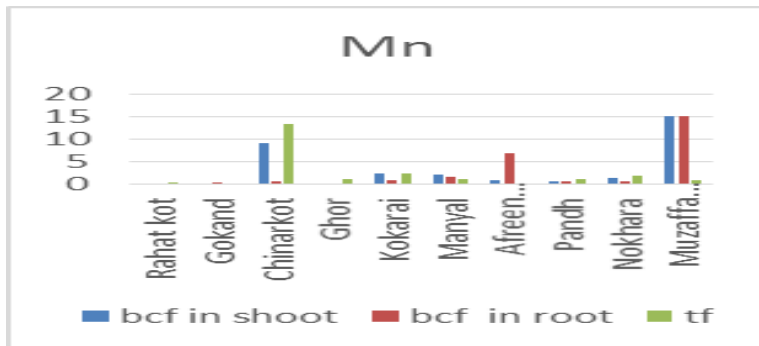


Fig. 4. Graphical representation of Mn Accumulation

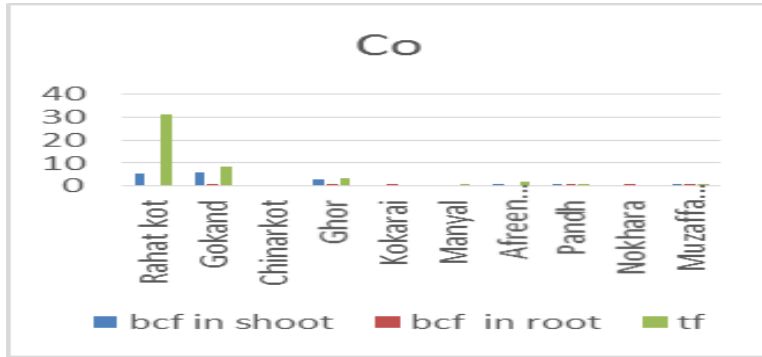


Fig. 5. Graphical representation of Co Accumulation

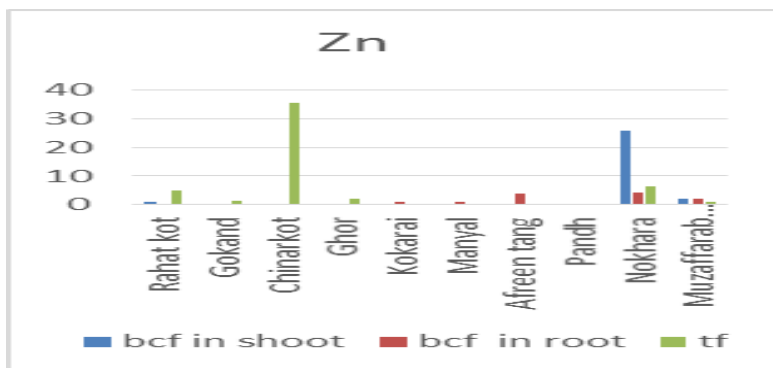


Fig. 6. Graphical representation of Zn Accumulation

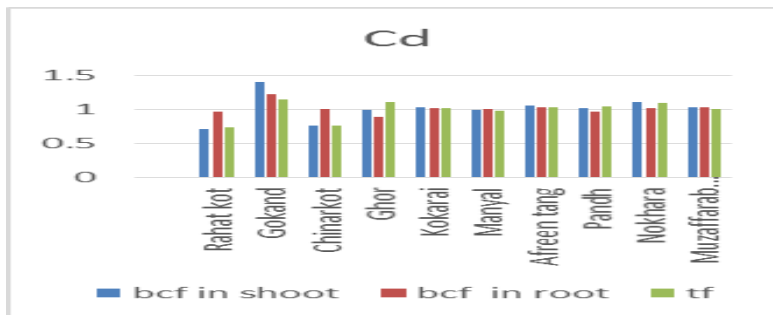


Fig. 7. Graphical representation of Cd Accumulation

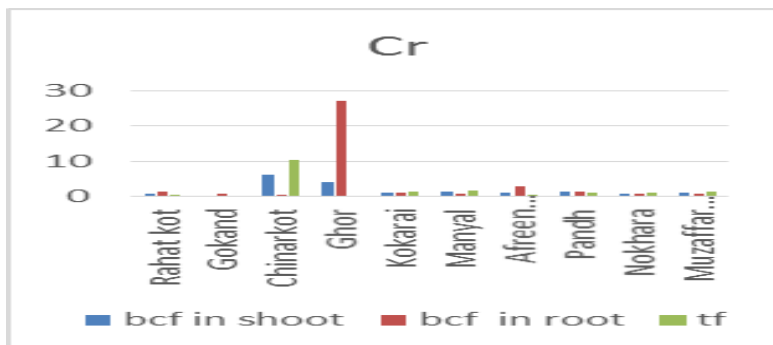


Fig. 8. Graphical representation of Cr Accumulation



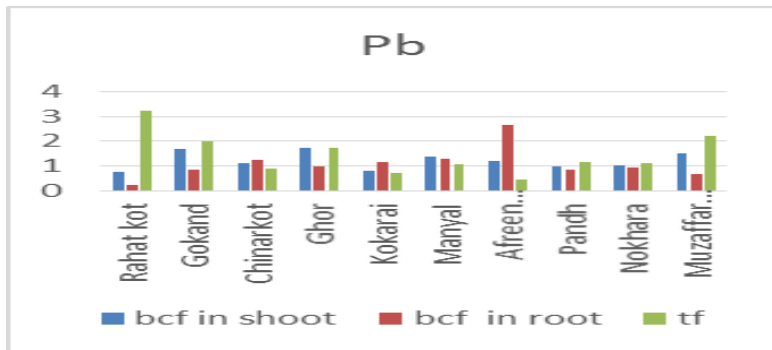


Fig. 9. Graphical representation of Pb Accumulation

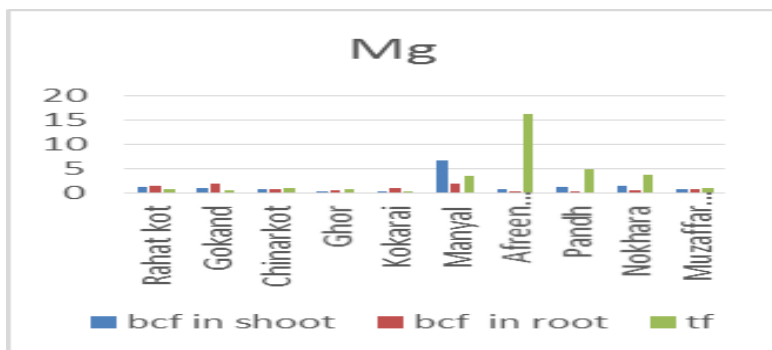


Fig. 10. Graphical representation of Mg Accumulation

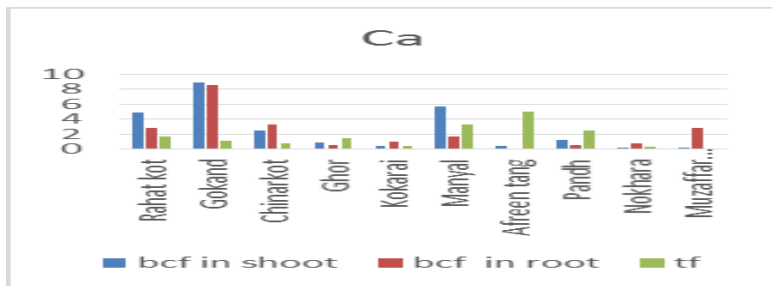


Fig. 11. Graphical representation of Ca Accumulation

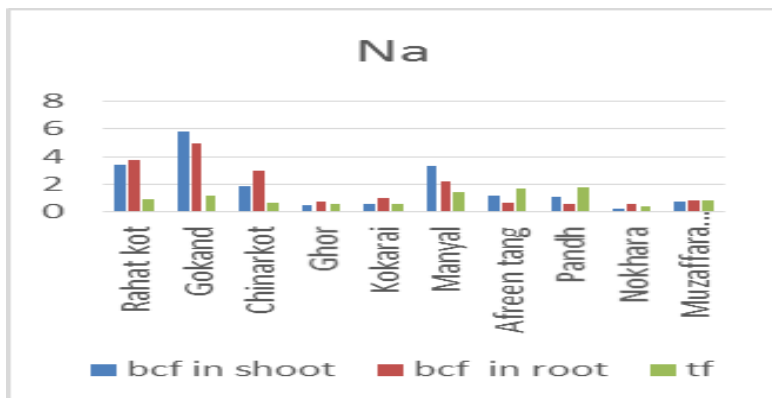
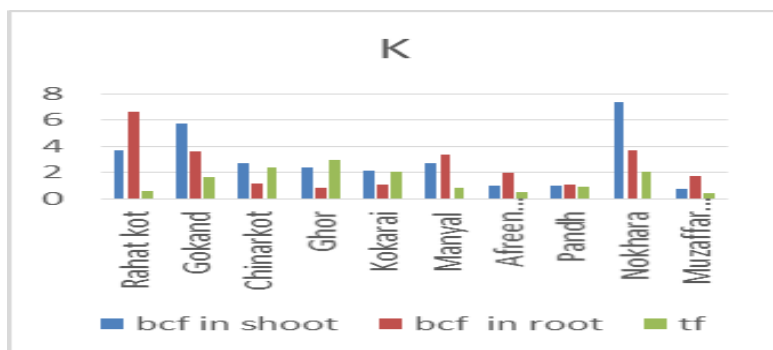


Fig. 12. Graphical representation of Na Accumulation





**Fig. 13. Graphical representation of K Accumulation**

### Conclusion

*Alnus nitida* is economically and ecologically important Holarctic plant present in Western Himalayas of Pakistan. The results of the present research work shows that Himalayan Alder is a multipurpose plant providing ecosystem services to the community. The plant is having best capability of bio-accumulation for heavy metal in hilly areas, which will help in filtering of drinkable water from heavy metals and will enhance the quality of water. Himalayan Alder conservation and afforestation is necessary for future generation otherwise the recent speed of deforestation will led it to extinction from western Himalayan regions of Pakistan.

### Author's contributions

Manuscript written: ZU Haq & SM Khan, Data Collection: Abdullah, Lab work: A Ullah, References arrangement: A Razzaq & F Manan, Data analysis: S Kamran, Revised paper: Z Ahmad, Paper format arrangement: S Rasheed.

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