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ORIGINAL ARTICLE

Assessment of Phytoextraction Potential of Fenugreek (*Trigonellafoenum-graecum* L.) to Remove Heavy Metals (Pb and Ni) from Contaminated Soil

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KEYWORDS

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ABSTRACT: The objective of the present study was to evaluate the effect of metal mobilizing agents, ethelynediaminetetraacetic acid (EDTA) and salicylic acid (SA), on the accumulation and translocation of lead (Pb) and nickel (Ni) by fenugreek (*Trigonellafoenum-graecum*L.) plants in contaminated soil. EDTA and SA were amended at 100 mM and 1.0 mM respectively. Pb and Ni content were estimated using ICP-OES. Plant samples were prepared for scanning electron microscope (SEM) analysis to investigate metals distribution in different tissues (root, stem and leaf) of plant. The results showed that EDTA increased Pb and Ni uptake as compared to SA. SEM analysis revealed that in the presence of EDTA, the deposition of Pb particles was predominantly in vascular tissues of the stem and leaf.

INTRODUCTION

Heavy metal pollution is a growing environmental problem. There are numerous sources of heavy metal pollution [1]. Sources of soil Pb contamination are mining, smelting, dumping of municipal sewage, and battery disposal [2-4] while of soil Ni contamination are mining, refining operations, incineration of municipal waste, and sewage sludge. Nickel can disrupt metabolic pathways in the body by replacing metals in metallo-enzymes.

Plant-based environmental remediation technology, or Phytoremediaton, has been extensively practiced in recent years as an in situ, cost-effective, clean and green latent approach for the cleanup of trace metals from contaminated sites [5]. Phytoremediation is the use of

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plants for the removal of pollutants from contaminated soil or water [6]. One of the mechanisms of phytoremediation in which plants are used for the extraction of heavy metals from contaminated soils is phytoextraction. There are two main approaches proposed for the phytoextraction of heavy metals: first is the use of natural metal hyperaccumulator plants (natural phytoextraction), and second is the utilization of high biomass plants with a chemically enhanced method of phytoextraction (induced phytoextraction). Ethylene diaminetetraacetic acid (EDTA) has been the most widely-studied chelating agent in an induced-phytoextraction because of its high extraction efficiency of many metals, and to date, it has been found to be the best soil amendment for Pbphytoextraction [7-8].

Salicylic acid (SA) is a phenolic natured endogenous growth regulator and it plays important role in the regulation of physiological processes in plants [9]. Besides this, SA induces an increase in the resistance of seedlings to toxic action of heavy metals [10] by activation of systematic acquired resistance [11].

Trigonellafoenumgraecum L. is a leguminous herb. It has been recently studied for its tolerance and accumulation of heavy metals [12-14].

The objective of the present study was to evaluate the effect of EDTA and SA on the accumulation of Pb and Ni by fenugreek (*T.foenum-graecum*L.) plants in contaminated soil and to determine the distribution of Pb and Ni in the different tissues (leaves, stems and roots) of fenugreek plant.

MATERIALS AND METHODS

Experimental site

Under field conditions, a pot experiment was conducted to study the comparative effect of EDTA as well as SA on the heavy metal accumulation by fenugreek plants. Experiments were conducted at the Micromodel experimental site of the Indian Institute of Technology, Delhi. It is situated at 77.09^{o}E longitude and 20.45^{o}N latitude, and 28 m altitude above sea level. The mean maximum and minimum temperature during the study period were 18-43^{o}C and 3-15^{o}C, respectively. Main physical and chemical properties of soil are summarised in Table 1.

Table 1. Main physicochemical properties of soil

Parameter	Unit	Amount
Texture	-	Sandy loam
Clay	%	16.30
Silt	%	14.23
Sandy	%	69.47
Electrical Conductivity	mS/cm	0.28
pH	-	7.5
Cation Exchange Capacity	Cmol/kg	18.4
Organic Carbon	%	0.72
Available N	kg/ha	272
Available P	kg/ha	9.0
Available K	kg/ha	200.7
Total Pb	mg/kg	0.02
Total Ni	mg/kg	4.0

Pot experiment

The seeds of *Trigonellafoenum-graecum* L. were procured from the National seeds Corporation Ltd., BeejBhawan, Pusa, and New Delhi. About 20 seeds were sown in 11x11 cm pots containing unsterilized field soil, farm yard manure (organic carbon 12.2 %, total N 0.55 %, total P 0.75 %, total K 2.30 % and pH 7.2) and sand in a 2:2:1 ratio in October 2008. In chemical treatment, Pb, Ni, EDTA and SA were added as per the designed treatment. The designed treatment for fenugreek plants was as follows:

(T1) 100 mg/l Pb, (T2) 800 mg/l Pb, (T3) 800 mg/l Ni, (T4) 800 mg/l Pb + 800 mg/l Ni, (T5) 800 mg/l Pb + 800 mg/l Ni + 100 mM EDTA, (T6) 800 mg/l Pb + 800 mg/l Ni + 1.0 mM SA, and (T0) Control or untreated plants. The concentrations of Pb and Ni were supplied as Pb $(NO_3)_2$) and Ni$(NO_3)_2$) respectively in all treatments. Dipotassium salt of EDTA was supplied in the treatment. Plants were watered daily using tap water to maintain optimal growth conditions. Seed germination started after the seventh day of sowing and plants were thinned to 3 plants per pot after 30 days of sowing. Samples of plants were taken seven days and monthly after chelant treatment. At the end of the experiment the plants were harvested and then washed accurately, the aerial part was divided from the roots and the two parts were analysed separately to determine the metal content. Scanning electron microscopic (SEM) observations were also made to determine the localization and translocation path of metals in plant tissues.

Soil and plant analysis

The organic C, N, P and K were estimated by the methods of Walkley and Black, Micro-kjeldahl, Olsen and Flame photometer, respectively, in soil and farmyard manure as described by Rowell [15].Cation exchange capacity was carried out using the $BaCl_2$ method. Metals concentration in soil was determined using the aqua regia extraction method. Plant samples were washed with tap water and dried at 70^{o}C for 48 h. The dried material was digested with aqua regia. All determinations were performed in triplicate. Metal concentrations in solutions were analysed by ICP-OES (Varian Vista-MPX CCD Simultaneous ICP-OES, Varian Australia Pty. Ltd) with a Ni detection limit of 0.007 mg/l and a Pb detection limit of 0.01 mg/l.

Scanning Electron Microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX)

Plants were separated into roots, stems, and leaves, frozen in liquid nitrogen, and then fractured into small pieces with a blunt knife. The pieces were freeze-dried overnight at −30 °C while under a vacuum (15–25 torr) using a freeze–dry system. Samples were mounted on aluminium stubs with double-sided carbon tape. Tissue structures and abnormalities of roots and leaves of plants were observed and evaluated using a scanning electron microscope (JSM-840A) coupled to Energy dispersive X-ray spectrometer. The EDX analyses were conducted at accelerating voltages of 10–30 kV and working distance of 15 mm. Several randomly selected areas (approximately 2 mm × 3 mm) on each sample were scanned using the SEM/EDS for 20 frames (approximately 30 min).

STATISTICAL ANALYSIS

The experiment was conducted as a factorial randomized block design with each treatment replicated thrice. Statistical analysis of the data was done following analysis of variance (ANOVA) in MINITAB version 15 software; when the ANOVA was significant, the means were separated using Tukey difference at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Plant growth

Effect of treatments on seed germination and plant dry weight is shown in Table 2.

Table 2. Effect of treatments on seed germination and dry weight in*Trigonellafoenumgraecum* L.

Treatment	Seed germination (%)	Dry weight (g)
T0	100±0.5	3.8±0.31
T1	90±1.5	2.0±0.50
T2	100±0.6	2.7±0.16
T3	95±1.4	1.7±0.48
T4	90±1.9	1.2±0.19
T5	85±1.2	1.4±0.09
T6	95±1.6	2.3±0.25

Values are mean ± standard deviation (n=3)

Application of SA with heavy metal (Pb^{2+} and Ni^{2+}) treatment helped in reducing the inhibitory effects of these metals on seed germination along with increased dry weight. This finding is supported by Mishra and Choudhari [16]. Seed germination was observed 85% in the presence of EDTA (T5) and 95% in the presence of SA (T6). Seed germination (%) were significantly (P=0.028) decreased by EDTA. A potential decrease in total plant dry weight was observed in plants treated with T4. EDTA decreased significantly (P<0.05) dry weight, whereas SA stimulated dry weight compared to T4 (Pb + Ni) treatment. It was 1.4 g and 2.3 g dry weight in the presence of EDTA and SA respectively. No significant (P<0.05) negative effect on plant growth was observed in the presence of SA.

Plant metal accumulation

Table 3 shows Pb and Ni concentrations in root and shoot part of fenugreek plants.

Table 3. Accumulation of Pb and Ni in shoots and roots of *Trigonellafoenumgraecum* L

Treatment	Shoot concentration (mg/kg DW)		Root concentration (mg/kg DW)		Translocation factor (TF)	
	Pb	**Ni**	**Pb**	**Ni**	**Pb**	**Ni**
T0	23±8	47±11	68±22	124±25	0.33	0.37
T1	164±105	35±15	271±160	112±17	0.60	0.31
T2	429±218	58±13	684±250	106±20	0.62	0.54
T3	31±12	216±135	53±17	634±241	0.58	0.34
T4	17624±985	5322±576	43140±969	15291±586	0.40	0.35
T5	401±252	170±112	2888±755	1187±481	0.14	0.14
T6	512±214	230±98	1653±682	871±364	0.30	0.26

Values are mean ± standard deviation (n=3)

According to Pugh et al. [17] the normal and phytotoxic levels of Pb accumulation in plants were 0.5–10 mg/kg and 30-300 mg/kg respectively. According to Kataba-Pendias and Pendias [18] the plant toxicity limits of Ni were 10-100 mg/kg. Lead bioaccumulation in roots and shoots of plants was significantly different. Pb and Ni concentration in root organs of the plants was higher than that in shoot organs in all treatments. Pb concentration increased in both organs of the plant in T4 treatment, but it decreased with T5 and T6 treatment. In

T4 treatment, Pb concentrations are increased in root and shoot organs significantly ($P \leq 0.05$) as 43,140 mg/kg DW and 17,624 mg/kg DW, respectively. Translocation factor was evaluated which is the ratio of shoot metal concentration over root metal concentration. Translocation factor in Pb and Ni treatments were ordered as follows: T2>T1>T4>T6>T5 and T4>T3>T6>T5 respectively. Salicylic acid helped in the translocation of Pb and Ni in the aerial parts of the plants.

Scanning electron microscopic observations

Scanning electron microscopy (SEM) has been one of the most convenient and useful instruments for the observation of the surfaces of the samples in a variety of application fields. Elemental analysis on the surface is also performed by an energy dispersive X-ray analysis (EDX) system attached to SEM. Cross-section of plant organs were examined using SEM attached with EDX unit for elemental analysis.

Table 4. Element accumulation (%) in plant tissues of fenugreektreated with different Pb and Ni treatments.

Treatment	Plant tissues	C	O	S	K	Ca	Fe	Ni	Au	Pb
T1	Leaf	ND	89±10	0.3±0.2	2±2	2±2	4±7	ND	2±1	0.1±0.0
	Stem	62±10	26±5	0.3±0.3	4±5	5±8	0.4±0.4	ND	1±0.2	0.06 ± 0.0
	Root	18±6	75±6	0.03±0.0	2±0.8	1±1	3±0.2	ND	1±0.4	0.02±0
T2	Leaf	70±1	29±1	0.1±0.1	0.3±0.0	1±0.2	0.2±0.4	0.11±0.0	ND	ND
	Stem	55±13	27±12	0.1±0.9	2±3	1±1	1±1	0.4±0.2	12±9	ND
	Root	51±10	37±11	0.11±0.3	1±0.6	2±1	1.05±0.89	0.07±0.2	7±1	ND
T3	Leaf	70±4	27±3	0.3±0.2	0.3±0.1	1±1	0.03±0.0	0.01±0.0	1.5±0.2	0.07±0.0
	Stem	57±14	31±9	0.13±.02	1.6±1	1.1±1	1±1.5	0.02±0.0	7±2	0.07±0.1
	Root	30±18	57±15	-0.2±0.2	3±3	2±4	1±2	0.1±0.4	6±5	0.2±0.5
T4	Leaf	66±15	28±11	0.2±0.2	0.4±0.2	4±4	-0.01±0.4	0.01±0.1	2±0.4	0.01±0.0
	Stem	65±16	26±15	0.25±0.2	1±1	3±4	0.3±0.0	0.03±0.2	5±5	0.02±0.1
	Root	71±8	24±6	0.09±0.1	1±0.4	1.3±1	0.3±0.7	0.02±0.1	2±1	0.03±0.0
T5	Leaf	78±4	17±4	0.02±0.0	0.5±0.1	0.5±0.2	0.09±0.2	0.06±0.0	3±0.3	0.1±0.0
	Stem	66±16	25±13	0.53±0.3	2±2	2±1	1±0.9	0.01±0.0	4±1	0.03±0.1
	Root	61±27	30±22	-0.24±0.7	2±3	1±1	2±2	0.04±0.0	4±2	0.01±0.0
T6	Leaf	52±21	37±19	0.11±0.2	2±0.5	1±0.6	2±2	ND	5±2	0.05±0.0
	Stem	71±4	23±5	0.11±0.0	0.6±0.1	1±0.6	0.2±0.2	ND	4±2	0.08±0.0
	Root	64±8	26±9	-0.24±0.8	3±2	1±0.6	0.5±2	ND	5±2	0.1±0.4

Values are mean ± standard deviation (n=3)
ND- Not determined

Percentage element accumulation in leaf, stem and root part of fenugreek with different treatments is shown in Table 4. Pb accumulation was highest in root, followed by stem and after then leaf at T1 and T5 treatments. Ni accumulation was found highest in root at T5 whereas Ni accumulation was highest in stem at T4 and in leaf at T6. This comparative study shows that overall percentage Ni accumulation was more in T2 treatment while percentage Pb accumulation was higher in T3 treatment. No particular relationship could be established between the concentration of the minor elements and the type of treatment, except for Ca and Fe. Accumulation of Pb and Ni affected the presence of Ca and Fe as Ca was highest in T4 treatment (Pb+Ni) and Fe was highest in T1 (100 mg/l Pb) treatment. The presence of EDTA caused decrease in Ni accumulation in stem from 0.03% to 0.01%. EDTA increased Fe uptake from 0.6% (T4) to 3% (T5). Mari et al. [19] identified a chelant (Ni-nicotianamine complex) in *Thalspicaerulescens* which helps in translocation of Ni. A study by Wycisket al. [20] showed free histidine as the most effective translocator of Ni as compared to malic acid and citric acid. Histidine and nicotianamine mediated transport

help in enhanced Ni translocation in the absence of EDTA. Whereas, translocation of Ni is limited in the presence of EDTA as EDTA-bounded Ni was expected not to be able to complex with histidine.

Analysis of leaf, stem and root samples at 500X magnification in SEM/EDX indicated some differences in the treatments which are shown in Figure 1, 2, 3, 4, 5 and 6.

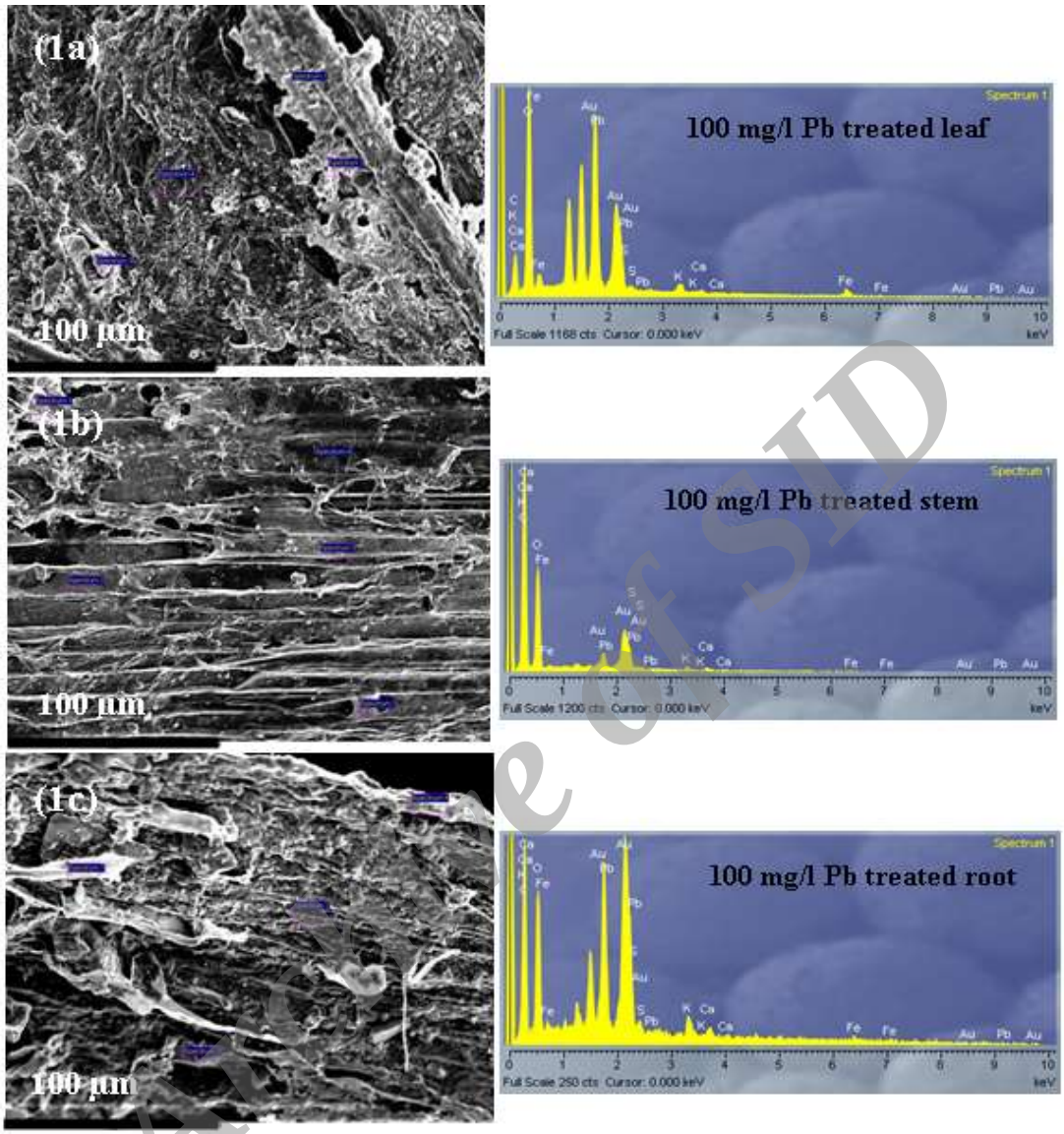

Figure1. Scanning electron micrographs and EDX spectra of Pb distribution in leaf, stem and root of fenugreek grown in the presence of 100 mg/l Pb treatment. (a) Pb treated leaf, (b) Pb treated stem, (c) Pb treated root. The EDS spectrum is taken from the area indicated by the square

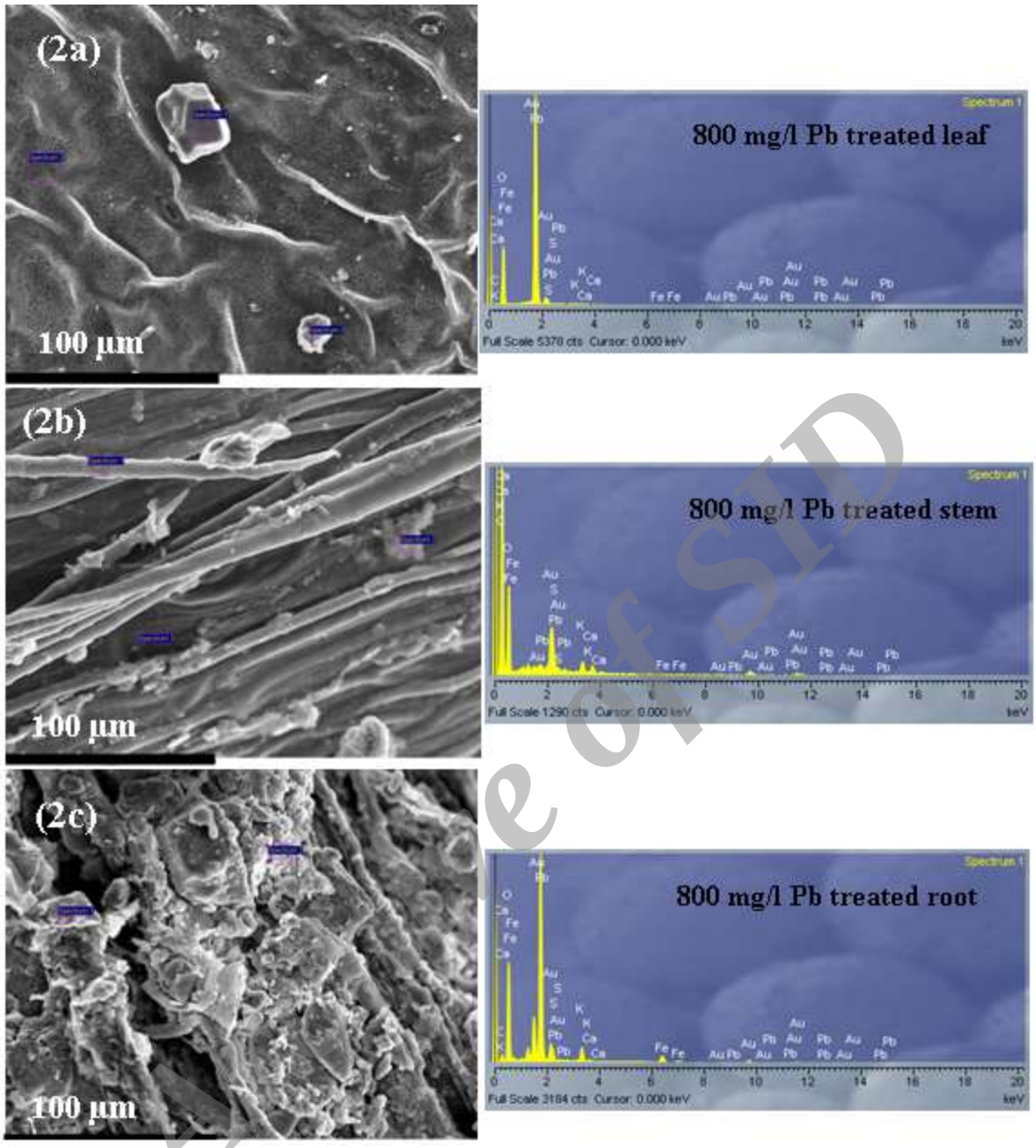

Figure 2. Scanning electron micrographs and EDX spectra of Pb distribution in leaf, stem and root of fenugreek grown in the presence of 800 mg/l Pb treatment. (a) Pb treated leaf, (b) Pb treated stem, (c) Pb treated root. The EDS spectrum is taken from the area indicated by the square

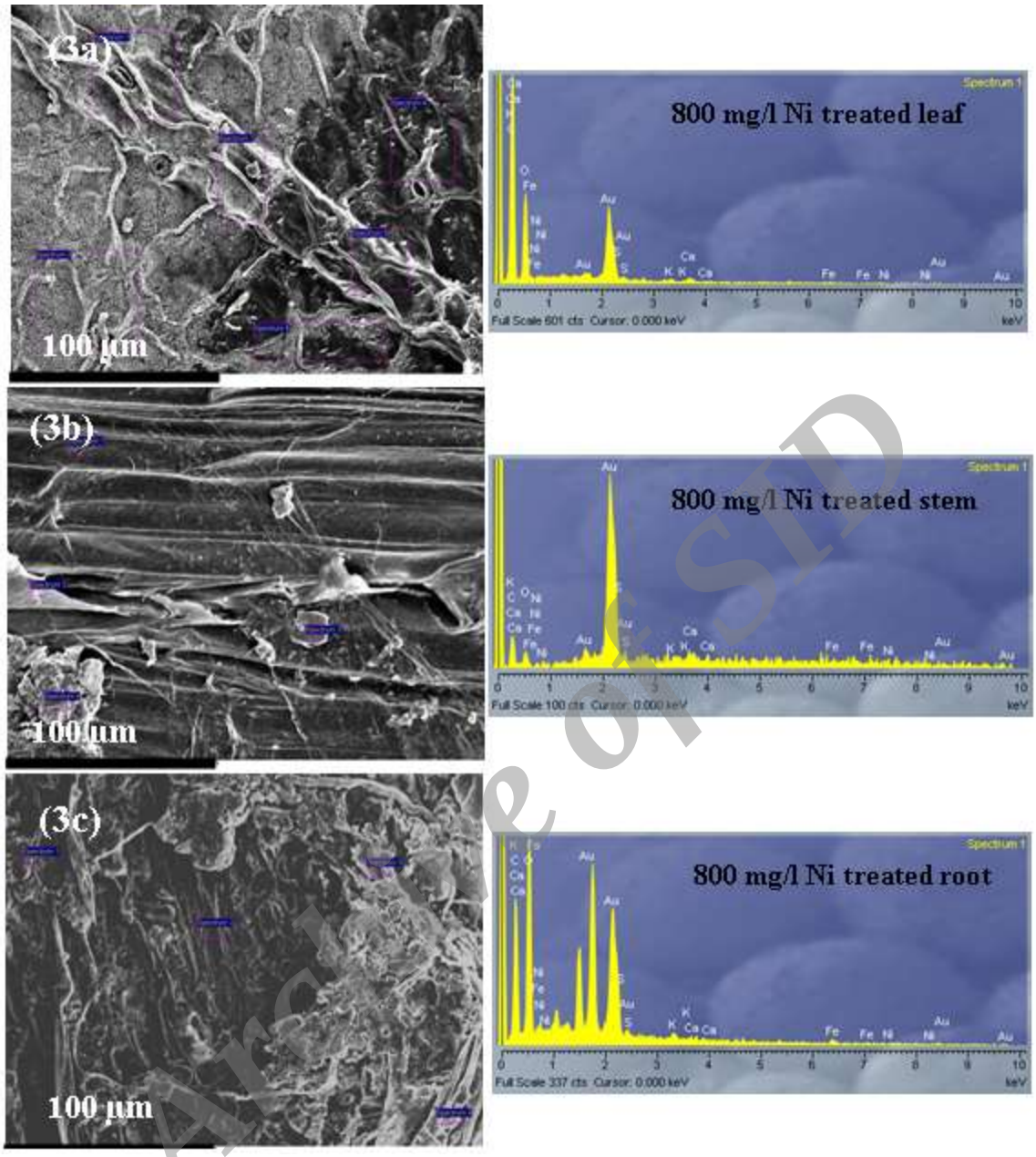

Figure 3. Scanning electron micrographs and EDX spectra of Ni distribution in leaf, stem and root of fenugreek grown in the presence of 800 mg/l Ni treatment. (A) Ni treated leaf, (B) Ni treated stem, (C) Ni treated root. The EDS spectrum is taken from the area indicated by the square

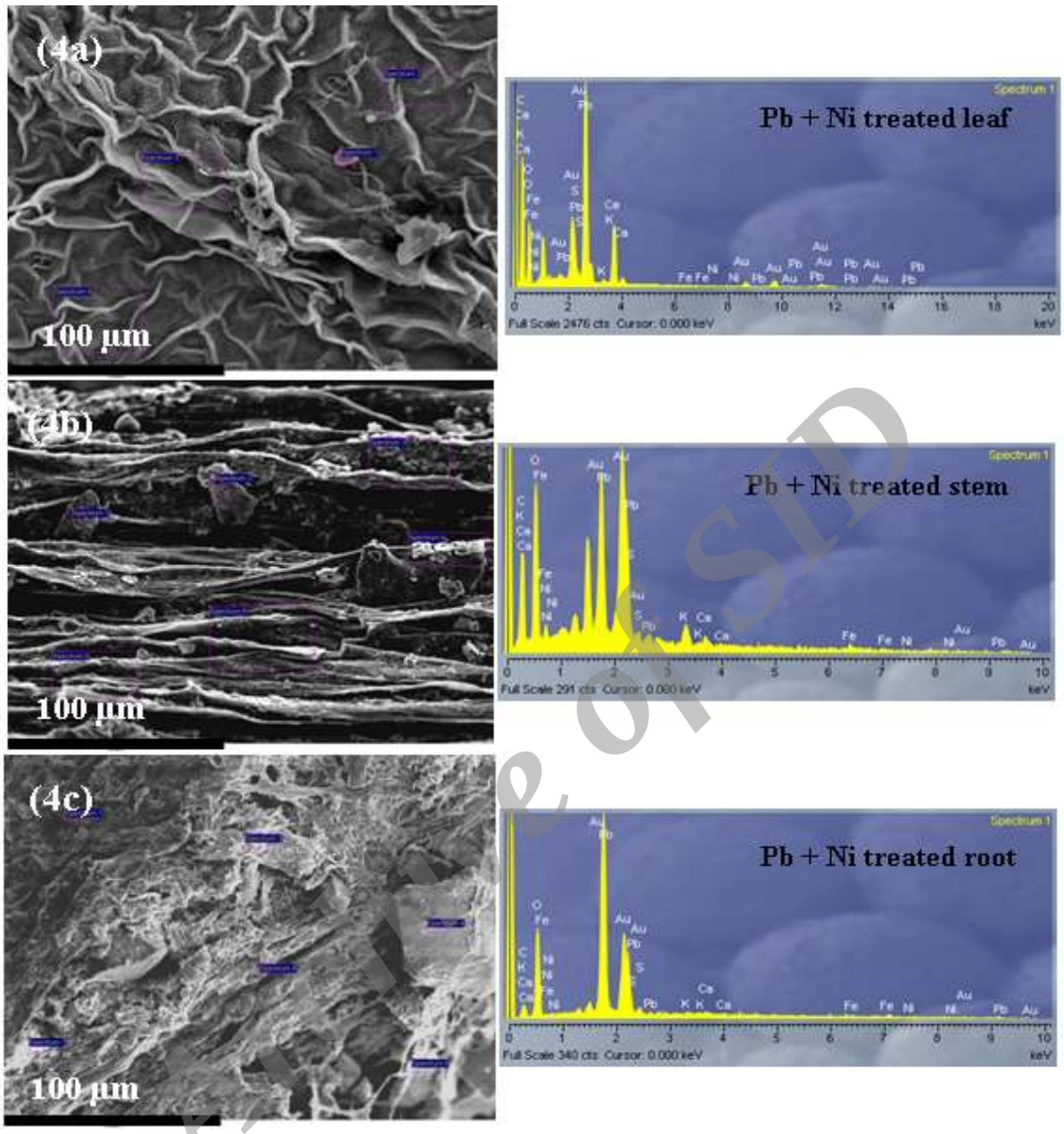

Figure4. Scanning electron micrographs and EDX spectra of Pb and Ni distribution in leaf stem and root of fenugreek grown in the presence of 800 mg/l Pb + 800 mg/l Ni treatment. (A) Pb + Ni treated leaf, (B) Pb + Ni treated stem, (C) Pb + Ni treated root. The EDS spectrum is taken from the area indicated by the square

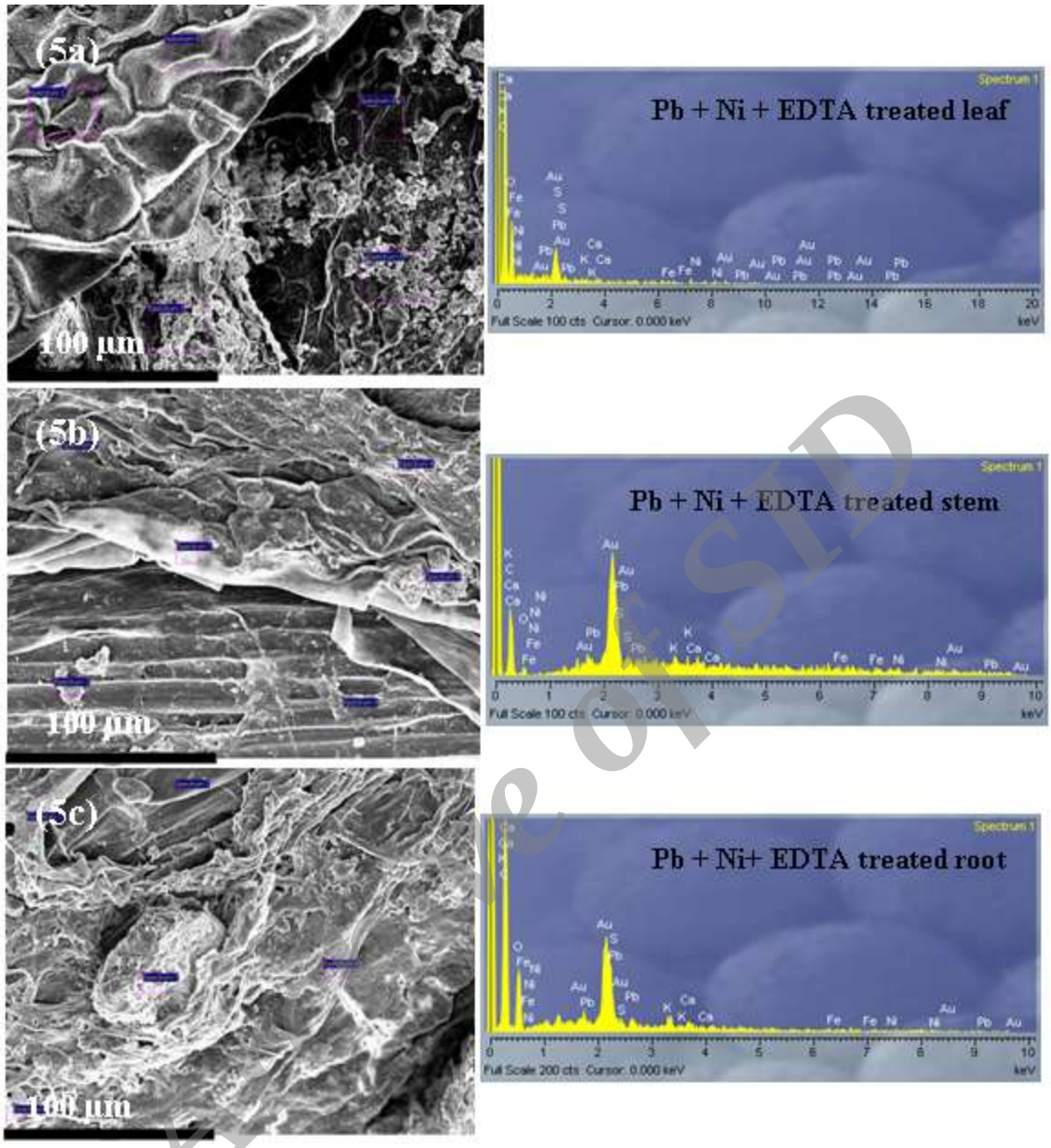

Figure 5. Scanning electron micrographs and EDX spectra of Pb and Ni distribution in leaf, stem and root of fenugreek grown in the presence of 800 mg/l Pb + 800 mg/l Ni + EDTA treatment. (a) Pb + Ni + EDTA treated leaf, (b) Pb + Ni + EDTA treated stem, (c) Pb + Ni + EDTA treated root. The EDS spectrum is taken from the area indicated by the square

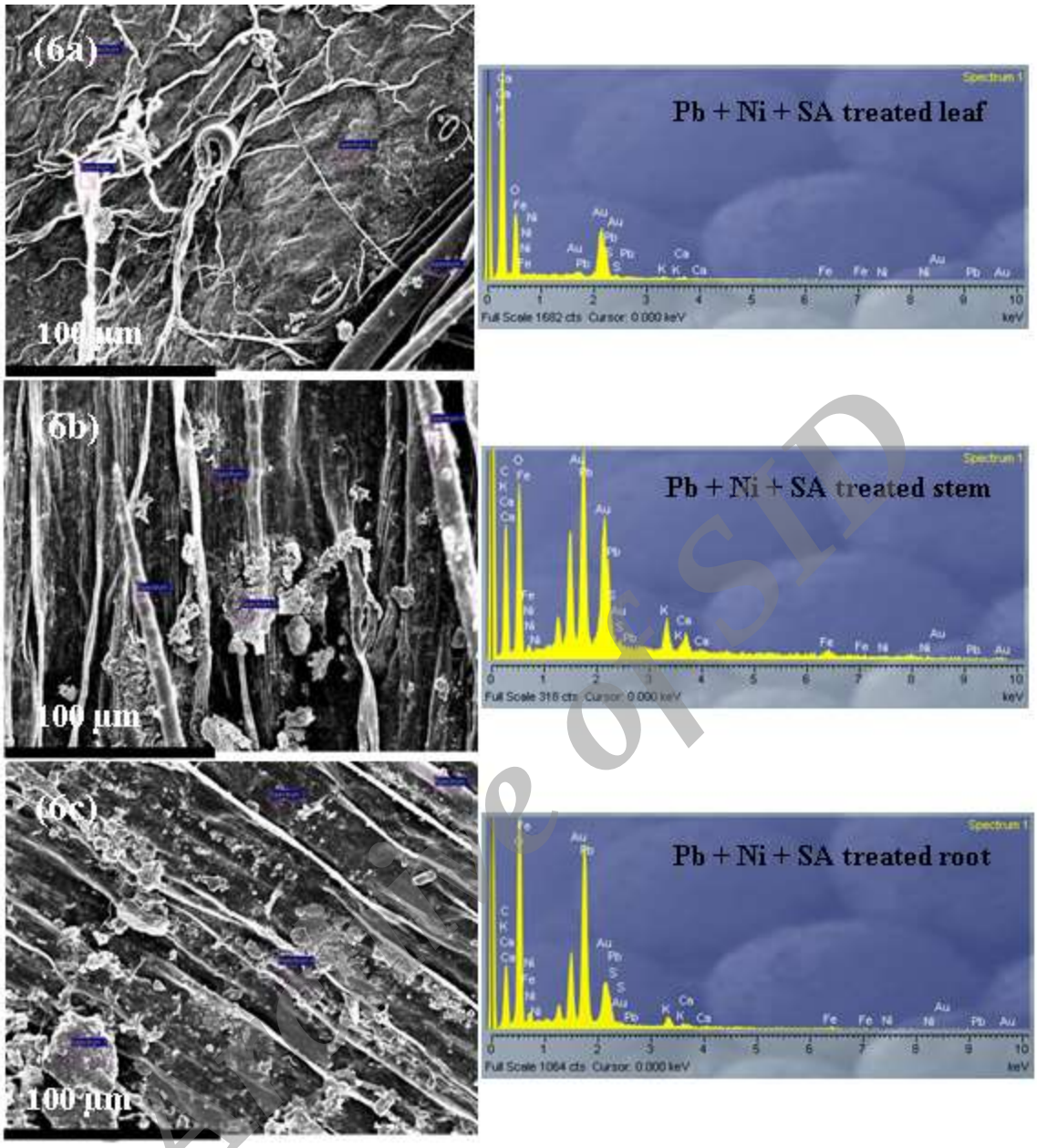

Figure 6. Scanning electron micrographs and EDX spectra of Pb and Ni distribution in leaf, stem and root of fenugreek grown in the presence of 800 mg/l Pb + 800 mg/l Ni + SA treatment. (a) Pb + Ni + SA treated leaf, (b) Pb + Ni + SA treated stem, (c) Pb + Ni + SA treated root. The EDS spectrum is taken from the area indicated by the square

SEM micrographs display various structural effects such as a reduced number of stomata on the surface of the leaf at 800 mg/l of lead treatment.

Ni was found to accumulate preferentially in the upper leaf epidermal cells. Analysis on stem cross-sections of T6 treatment revealed that nickel seems to be excluded from the stem. The examination of leaf cross-sections

pointed out the upper epidermis as the main site for nickel accumulation (Figure 2b and Figure 5b).

Some angiosperms possess exodermalcasparian strips, selectively limiting ion uptake to the symplasm, which may account for high epidermal Pb levels [21]. Relative Pb concentrations decreased rapidly through the cortex and endodermis, reaching very low levels in the stele, and thus minimal Pb was available for translocation to the shoot. The endodermis seemed to confer a degree of exclusion to Pb. Jentschkeet al. [22] has also found the endodermis to act as a barrier to Pb radial transport. Higher cell wall Pb concentrations also suggest apoplastic transport was the dominant translocation pathway. However, other studies have shown a variety of cytoplasmic responses such as active pumping of Pb complexes into vacuoles and dictyosomal vesicles, complexing by organic acids and possibly by specific metal binding proteins [23]. SEM micrographs of roots revealed accumulation of Pb and Ni mainly in cell walls, suggesting transport via the apoplastic pathway. The epidermis provided a major barrier to the transport of Pb only. The endodermis provided a barrier to movement of Pb and Ni into the stele, presumably by limiting symplastic transport. Both metals were being distributed in higher concentrations in cell walls than in the cytoplasm. A concentration gradient existed, with higher metal levels in the vascular tissue, and lower concentrations in mesophyll and water tissue, respectively. Since we found different Pb and Ni accumulation in different part of the plant this could suggest that different mechanisms may operate for uptake and accumulation of different metals.

CONCLUSIONS

The remediation of lead and nickel by fenugreek can be enhanced by the addition of EDTA. In the presence of EDTA, plant growth was significantly inhibited, whereas in the presence of SA, plant growth was as good as the control. Scanning electron microscopy confirmed the presence of lead and nickel inside tissues of the plant. SEM analysis revealed that in the presence of EDTA, the deposition of Pb particles was predominantly in vascular tissues of the stem and leaf. In roots, deposition of Pb was in the stele region. However, in the presence of SA, Pb particles were more concentrated in the cortical region. Further experiments are needed to determine the actual mechanisms involved in Pb and Ni uptake by fenugreek.

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