Pak. J. Bot., 45(1): 17-27, 2013.

EFFECT OF INDUSTRIAL WASTE ON EARLY GROWTH AND PHYTOREMEDIATION POTENTIAL OF *AVICENNIA MARINA* (FORSK.) VIERH

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Abstract

Discharge of industrial waste in streams, river and coastal areas may alter the physical and chemical properties of water, which may affect the growth of mangrove. Therefore, it was anticipated that if these wastes used as organic substitute on mangrove species *Avicennia marina,* what will be the response of this species against particular industrial waste. For this purpose a greenhouse study was conducted to measure the effect of industrial waste on seed germination and growth rate of dominant mangrove species *Avicennia marina.* This study also evaluates the potential of *A.marina* for phytoremediation. Four types of industrial wastes were used to prepare seawater as treatments. The results of the final germination revealed 90% ($p<0.001$) in all treatments except ash and marble wastes. It was observed that converter slag showed overall better results while chemical industrial sludge-ash showed most deleterious affect on all growth parameters among treatments. The results of ANOVA declared significant decrease in shoot length ($F=3.54$, $p<0.05$), root length ($F=3.23$, $p<0.05$), fresh shoot weight ($F=4.61$, $p<0.01$), dry shoot weight ($F=4.05$, $p<0.01$), dry root weight ($F=3.92$, $p<0.01$) and fresh root weight ($F=3.43$, $p<0.05$) in all treatments except converter slag and sludge as compared to control. The concentrations of heavy metal varied significantly, depending upon the type of waste however, minimum values of all metals were obtained in marble waste. In contrast to marble waste Zn, Co, Mn and Pb were found maximum in prepared polluted sea water, soil, shoot and root in chemical industry sludge-ash while Fe was higher in converter slag treatment. The calculated values of Biological Accumulation Coefficient (BAC), Biological Transfer Coefficient (BTC) and Bio-concentration Factor (BCF) showed that *Avicennia marina* can efficiently act as a phytoremediation species for selected heavy metals in Pakistan mangrove ecosystem. However, there should be a limit to add chemical pollutant in this ecosystem.

Introduction

Mangroves are the woody plants that inhabit the tropical and sub-tropical coastal areas around the world. They are the major components between marine and terrestrial ecosystem and provide a variety of benefits in terms of healthy environment, biodiversity, food, fuel and fodder etc. Despite the fact that mangroves constitute an important entity and play a vital role in marine food webs, they are being badly affected due to various anthropogenic activities including pollution (Saifullah, 1997). Land reclamation activities like increasing industrialization and urban runoff leading to high risk of effluent discharges along the coastal areas have resulted in depletion of mangroves (Shete *et al*., 2007). However, there is a lack of information on assessment of heavy metal concentrations in mangroves all over the world (Lacerda, 1997).

The coast line of Pakistan extends about 1050 km where Karachi is located on the northern border of Arabian Sea. It is about 135 km long and one of the most affected area along the coast of Pakistan (Beg *et al.*, 1984 and Khan *et al*., 1999). The population of Karachi is about 20 million and it is a focal centre of industrial development and multiculturalism. It has been estimated that Karachi generates approximately 350 million gallons per day domestic and industrial waste water, which is discharged into two main rivers, via Lyari and Malir. Approximately 30% of this waste is generated from municipal sources while 70% is generated by industries (Saleem & Kazi, 1998). The heavy metals are one of the major pollutants of waste, being discharged in the city coast and create hazards for the marine ecology, particularly in the mangrove habitat (Nazim, 2011). Though, metal toxicity may affect all forms of life including microorganism, plants and animals but the degree of toxicity varies from organism to organism. Mirza *et at*., (1982) reported 250,000 ha mangrove cover which has been shrunken considerably (35%) due to extensive harvesting and industrial activities (Amjad *et al*., 2007).

It is generally considered that mangroves show ability to accumulate metals and possess a certain tolerance to relatively high levels of heavy metal pollution. Thomas & Eong (1984) and Kathiresan & Bingham, (2001) reported that mangroves are poor indicator of trace metal concentration. Generally, mangroves show less than one percent for metal concentrations between tissues and sediments (Saenger *et al.*, 1990; Rao *et al.*, 1991; Tam & Wong, 1995 and Ong-Che, 1999). They often receive inputs of heavy metals and the sediments may show significantly higher metal contamination (Mackey *et al.*, 1992; Lacerda *et al.*, 1993; Rivail *et al.*, 1996; Lacerda, 1998, Tam & Yao, 1998). It is anticipated that different mangrove species show different response to the pollution. Though, little work has been done on phytoremediation in mangroves around the world (Lacerda, 1997).

Phytoremediation is a newly emerging field, applied to clean up polluted soil, water or air (Meagher *et al*., 2000). In this process green plants can be used to remove limited amount of sequester hazardous substances especially heavy metals from a particular environment but the accumulation of heavy metals may vary from species to species. It is a cost-effective tool and long lasting aesthetic solution for remediation of contaminated sites (Ma *et al*., 2001). In recent years, it has been widely considered as an effective approach to improve metal or metalloids-contaminated soils (Chaney *et al*., 1997). However in highly polluted coastal areas of Karachi, mangroves seem to be highly affected by pollutants. Bearing these considerations in mind, present investigation was undertaken to examine the effect of various pollutants on seed germination, initial growth of seedlings and the ability of mangrove (*Avicennia marina*) seedlings to absorb and accumulate heavy metals and their effects on growth. The ultimate goal is to disclose the causal factors responsible for the damage of mangroves by accumulation of toxic substances such as heavy metals from the industrial waste.

Materials and Methods

A green house experiment was conducted in plastic pots on mangrove species *A. marina* under the influence of four different industrial wastes viz; (1) Chemical industry sludge and its ash (2) Boulder slag, (3) Converter slag and (4) Marble waste powder.

Sample preparation of industrial wastes: Only sludge samples were incinerated up to 1100ºC until they were converted into ash at Government Authorized Incineration Plant at Dhabeji area Sindh, Pakistan. For preparing solution all the collected waste samples were oven dried at 105^{o}C to get constant weight and 0.2g of each sample were taken into the beaker and digested in aqua regia solution (HCl: HNO_3 3:1). Samples were placed on hot plate till the solution gets dried. After drying the solution, 10ml deionized water was added and left for 24 hours so that all the metals get settled in water. The solutions were filtered with Whatman filter paper No. 41 into 100 ml volumetric flask and volume made up to 100ml using deionized water and stored for analysis. Blank sample was also prepared for the calibration. Five heavy metals Zn, Fe, Co, Mn and Pb were analyzed with Atomic Absorption Spectrophotometer (Model PG990).

Seeds of *A. marina* and seawater were collected from Sandspit. 100 g dry powder of each waste were spread in different glass containers, filled with 25 liters sea water and left for 30 days to prepare five different types of polluted sea water treatments. The ratio between waste and seawater was 1g/250ml (4g/Liter). Minimum concentrations were taken to check the individual effects of waste on germination and initial growth of *A. marina*. The experiment was carried out in plastic pots sized 8 inches in height and 5 inches in diameter filled with 500 g of loamy sand. Three replicates were taken and five seeds of *A. marina* were sown in each pot including control. All pots were irrigated by prepared polluted sea water up to 20 weeks. The growth parameters i.e. percentage of seed germination, height of seedlings and numbers of leaves were measured weekly. After twenty weeks the experiment was terminated and root length, fresh weight and dry weight of shoot and root were determined. Physical properties of prepared polluted sea water i.e. pH, salinity, total dissolved solids, conductivity and temperature were determined using multiparameter Sension Tm105.

Five heavy metals including Zn, Fe, Co, Mn and Pb in prepared water, soil and tissues were analyzed after the termination of experiment.

Sample preparation of prepared polluted water: Water samples of all treatments were filtered and diluted 90% with de-ionized distilled water and 1ml HNO_3 to stop microbial activity. Sample preparation of soil and plant tissue (shoot and root) samples were prepared following Ismail (2002).

The values of seed germination percentages were transformed using Arcsine Transformation by Zar (1999). The data were subjected to soft wares COSTAT ver.3 and SPSS ver.10 for the analysis of variance (Steel and Torrie, 1984; Duncan, 1955) and multivariate analysis respectively. The package CURVEFIT was applied to number of leaves and shoot length data. Relationship between treatment and time was obtained using following logistic curve equation.

$$Y=a/ (1+b\times\exp^{(-cx)})$$

where Y = exponential growth
X = time
a, b and c are constants

Three multivariate tests were applied on heavy metals concentration in prepared polluted sea water, soil and tissues to check the over all significant differences among treatments. P-values for two of the three tests (Wilks' lambda and Hotelling's trace) are computed exactly, and the p-values for the third (Pillai's trace) based on an F-approximation is more accurate but occasionally slightly more liberal than the default.

Phytoremediation: Phytoremediation were carried out using three biological methods, Biological Accumulation Coefficient (BAC) was described as the concentration of a particular metal accumulated in plant shoot to that of the concentration of the same metal obtained from soil.

$$BAC= [Metal]\ shoot / [Metal]\ soil$$

Biological Transfer Coefficient (BTC) was defined as the accumulated concentration of a particular metal in shoot to that in the root.

$$BTC= [Metal]\ shoot / [Metal]\ root$$

The above two equations were followed by Zu *et al*., (2005).

Bio-concentration Factor (BCF) was represented as the concentration of heavy metals accumulated in plant root divided by concentration of the same metal obtained from their respective soil (Yoon *et al*., 2006).

$$BCF= [Metal]\ root / [Metal]\ soil$$

Results

Growth parameters: Effects of various prepared polluted sea water on final seed germination percentage, shoot length, root length, number of leaves, fresh shoot weight, fresh root weight, dry shoot and root weight were recorded and summarized in Table 1.

Table 1. Analysis of variance and Duncan's Multiple Range Test for growth parameters of *Avicennia marina*, under various industrial pollutants.

Treatments	Ger (%)	NL	SL (cm)	RL (cm)	FSW (g)	FRW (g)	DSW (g)	DRW (g)
F-values	10.6***	1.79ns	3.54*	3.23*	4.61**	3.43*	4.50**	3.92**
Control	90±0a	10±0.73a	14.93±0.9 ab	15.55±0.27a	1.71±0.30ab	1.38±0.19ab	0.55±0.09abc	0.35±0.04abc
sludge	90±0a	8±0.51ab	12.25±1.44 abc	14.6±0.55 ab	1.75±0.12ab	1.8±0.11a	0.67±0.06ab	0.46±0.03ab
Ash	45±0b	5±2.23ab	7.53±2.61 c	7.30±3.28c	0.64±0.22c	0.80±0.37b	0.22±0.07c	0.19±0.08c
Boulder Slag	90±0a	5±1.45b	8.91±0.54 bc	8.95±2.85bc	1±0.47bc	0.88±0.30b	0.34±0.15bc	0.21±0.07c
Converter Slag	90±0a	9±0.65ab	17.18±1.30 a	14.78±0.56ab	2.56±0.13a	2.15±0.09a	0.87±0.05a	0.53±0.02a
Marble	60±15b	8±2.82ab	10.18±3.36 bc	8.13±2.66c	1.15±0.45bc	1.29±0.42ab	0.43±0.16bc	0.31±0.31bc
LSD	19	4.76	5.66	6.09	0.91	0.81	0.32	0.19

Note: Ger= Germination, NL= No. of leaves, SL= Shoot length, RL= Root length, FSW=Fresh shoot weight, FRW=Fresh root weight, DSW= Dry shoot weight, DRW= Dry root weight. Means sharing same letters in a column are non-significant, * = significant level ($p<0.05$), ** = significant level ($p<0.01$), *** = ($p<0.001$), ns=Non-significant and Same letter in same column are non significant

The result of ANOVA described the significance level of these parameters among treatments. Seed germination percentage ($F=10.60$; $p<0.001$), shoot length ($F= 3.54$, $p<0.05$), root length ($F= 3.23$, $p<0.05$) were found significant, while number of leaves was found non-significant. The order of the maximum deleterious effect on these parameters among treatments was, seed germination percentage>shoot length>root length>number of leaves. The final seed germination percentage was significantly reduced in ash and marble treatments. Shoot growth ($p<0.05$) was significantly increased in converter slag treatment, while significant reduction was found in ash treatment related to control. Root growth was significantly affected in ash, converter slag and marble waste treatment as compared to control. Number of leaves were significantly ($p<0.05$) less in converter slag treatment as compared to control.

The results of ANOVA showed significant differences in fresh shoot weight ($F= 4.61$; $p<0.01$), fresh root weight ($F=3.43$; $p<0.05$), dry shoot weight ($F=4.50$; $p<0.01$) and dry root weight ($F=3.92$; $p<0.01$) among treatments. However, fresh shoot weight was significantly reduced only by ash relative to control ($p<0.01$), while fresh root weight was significantly decreased in ash and boulder slag compared to sludge and converter slag treatment ($p<0.05$). Dry shoot weight in converter slag was significantly higher than ash, boulder slag and marble ($p<0.01$), consequently dry weight of root was also differ significantly in converter slag with ash, boulder slag and marble waste treatment ($p<0.01$) Table 1.

Heavy metals analysis of prepared polluted sea water, soil and plant tissues: Table 2 showed the analysis of variance of the heavy metals in prepared polluted sea water. The F-values of ANOVA described that Zn ($F=4.02$; $p<0.05$) Mn ($F=3.20$; $p<0.05$) and Pb ($F=4.32$; $p<0.05$) were found significant, while Fe and Co were non-significant. The order of maximum toxic effect of metals discharged in water from treatments was Pb>Zn>Mn>Fe>Co. However, Zn, Mn and Pb were found significantly higher ($p<0.05$) in sludge and ash treatment respectively as compared to control, while Fe and Co were non-significant relative to control and among treatments.

Table 2. Analysis of variance and Duncan's Multiple Range Test of prepared polluted sea water from five different industries.

Treatments	Zn mg/l	Fe mg/l	Co mg/l	Mn mg/l	Pb mg/l
F–values	4.02*	1.28ns	0.32ns	3.20*	4.32*
Control	0.02 ± 0.01b	0.03 ± 0.02a	0.26 ± 0.06a	0.05 ± 0.01b	0.05 ± 0.01c
Sludge	0.40 ± 0.06 a	0.26 ± 0.13a	0.38 ± 0.04a	0.69 ± 0.14a	0.54 ± 0.19ab
Ash	0.45 ± 0.05a	0.32 ± 0.18a	0.38 ±0.15a	0.70 ± 0.20a	0.74 ± 0.21a
Boulder Slag	0.29 ± 0.09ac	0.08 ± 0.03a	0.31 ± 0.05a	0.38 ±0.24ab	0.21 ± 0.18bc
Converter Slag	0.23 ± 0.14ab	0.34 ± 0.19a	0.29 ± 0.08a	0.25 ± 0.04ab	0.08 ± 0.07c
Marble	0.08 ± 0.07bc	0.05 ± 0.01a	0.28 ± 0.08a	0.24 ± 0.06ab	0.06 ± 0.01c
LSD	0.26	0.38	0.27	0.44	0.43

Note: * = ($p<0.05$) and ns = non-significant. Means sharing same letters in a column are non-significant

Table 3 describes the results of ANOVA for heavy metals in soil after the experiment terminated. Zn ($F=7.67$; $p<0.01$), Fe ($F=23.73$; $p<0.001$) Co (3.43; $p<0.05$) and Pb ($F=4.26$; $p<0.05$) were found significant, while Mn was observed non-significant. The order of the F-values in soil for greatest contaminated effect of metals was presented as, Fe>Zn>Pb>Co>Mn. Whereas, Zn was significantly higher ($p<0.01$) in sludge, ash and boulder slag pertinent to control. Fe was significantly greater ($p<0.001$) in converter slag and ash treatment as compared to control. Co and Pb showed significant increment ($p<0.05$) in sludge and ash related to control. Non-significant differences were observed in Mn.

Table 3. Analysis of variance and Duncan's Multiple Range Tests for heavy metal contamination in soil.

Treatments	Zn mg/kg	Fe mg/kg	Co mg/kg	Mn mg/kg	Pb mg/kg
F–values	7.67**	23.73***	3.43*	0.78ns	4.26*
Control	0.006 ± 0.002c	0.17 ± 0.03b	0.05 ± 0.01c	1.52 ± 0.25a	0.79 ± 0.01b
Sludge	0.75 ± 0.17a	0.26 ± 0.09b	0.87 ± 0.27ab	2.47 ± 0.58a	1.1 ± 0.13a
Ash	0.78 ± 0.13a	1.64 ± 0.25a	0.98 ± 0.25a	2.51 ± 0.54a	1.12 ± 0.08a
Boulder Slag	0.45 ± 0.21ab	0.25 ± 0.06b	0.67 ± 0.19abc	2.43 ± 0.36a	0.91 ± 0.01ab
Converter Slag	0.16 ± 0.04bc	1.69 ± 0.23a	0.66 ± 0.20abc	2.42 ± 0.37a	0.83 ± 0.02b
Marble	0.05 ± 0.01bc	0.18 ± 0.05b	0.21 ± 0.11bc	2.27 ± 0.33a	0.82 ± 0.06b
LSD	0.38	0.47	0.60	1.30	0.21

Note: * = (p<0.05), ** = (p<0.01), *** = (p<0.001) and ns = non-significant. Means sharing same letters in a column are non-significant

The result of ANOVA for heavy metals accumulation in shoot dry biomass was described in Table 4. The maximum F-values of the heavy metals were described in that order, Fe>Zn>Mn>Pb>Co. Only Zn (F=7.23, p>0.01) and Fe (F=83.44; p<0.001) were recorded significant whereas, Co, Mn and Pb were non-significant. Zn was significantly higher (p<0.01) in sludge, ash and marble treatment compared to control. The significantly higher accumulation of Fe (p<0.001) was recorded in sludge, ash and converter slag treatment related to control. Co and Pb were non-significant among treatments. The amount of Mn (p<0.05) was significantly accumulated in sludge and ash treatment.

Table 4. Analysis of variance and Duncan's Multiple Range Tests of heavy metal accumulation in shoot.

Treatments	Zn mg/kg	Fe mg/kg	Co mg/kg	Mn mg/kg	Pb mg/kg
F–values	7.23**	83.44***	0.096ns	2.36ns	0.39ns
Control	0.01 ± 0b	1.24 ± 0.26c	0.32 ± 0.16a	0.41 ± 0.18b	0.61 ± 0.28a
Sludge	1.35 ± 0.30a	2.58 ± 0.36b	0.41 ± 0.20a	1.13 ± 0.20a	0.84 ± 0.14a
Ash	1.41 ± 0.23a	6.99 ± 0.39a	0.48 ± 0.24a	1.14 ± 0.13a	0.88 ± 0.07a
Boulder Slag	0.34 ± 0.32b	1.35 ± 0.18c	0.39 ± 0.17a	0.87 ± 0.24ab	0.81 ± 0.12a
Converter Slag	0.31 ± 0.12b	7.14 ± 0.35a	0.37 ± 0.18a	0.72 ± 0.23ab	0.80 ± 0.08a
Marble	1.25 ± 0.22a	1.32 ± 0.26c	0.33 ± 0.15a	0.49 ± 0.19b	0.68 ± 0.14a
LSD	0.71	0.96	0.58	0.62	0.49

Note: ** = (p<0.01), *** = (p<0.001) and ns = non-significant. Means sharing same letters in a column are non-significant

The heavy metal analysis of root discussed in Table 5. The F-values of ANOVA showed that only Fe (F=152.02; p<0.001) and Mn (F=3.49; p<0.05) were found significant, while Zn, Co and Pb were non-significant. The order of the maximum accumulation of these heavy metals in root dry biomass were presented as Fe>Mn>Zn>Co>Pb. However, the mean values of Zn (p<0.05) were significant in sludge and ash treatment compare to control. Fe (p<0.001) was significantly accumulated in roots in all treatments except marble compared to control. The accumulation of Mn (p<0.05) was comparatively significant with control in sludge and ash treatment, while Co and Pb were non-significant among treatments.

Table 5. Analysis of variance and Duncan's Multiple Range Tests of heavy metal accumulation in root.

Treatments	Zn mg/kg	Fe mg/kg	Co mg/kg	Mn mg/kg	Pb mg/kg
F–values	2.57ns	152.02***	1.32ns	3.49*	0.82ns
Control	0.001 ± 0b	1.26 ± 0.22d	0.23 ± 0.08a	0.65 ± 0.18b	0.71 ± 0.12a
sludge	0.90 ± 0.40a	4.48 ± 0.42c	0.41 ± 0.23a	1.40 ± 0.26a	0.93 ± 0.05a
Ash	0.91 ± 0.34a	9.04 ± 0.32b	0.81 ± 0.10a	1.46 ± 0.15a	1.01 ± 0.17a
Boulder Slag	0.63 ± 0.21ab	4.16 ± 0.22c	0.39 ± 0.24a	1.23 ± 0.19ab	0.86 ± 0.08a
Converter Slag	0.32 ± 0.27ab	11.92 ± 0.47a	0.37 ± 0.17a	0.83 ± 0.14ab	0.76 ± 0.13a
Marble	0.01 ± 0b	1.64 ± 0.31d	0.34 ± 0.12a	0.71 ± 0.19b	0.73 ± 0.17a
LSD	0.79	1.05	0.53	0.59	0.40

Note: * = (p<0.05), *** = (p<0.001) and ns = non-significant. Means sharing same letters in a column are non-significant

Multivariate analysis for heavy metal in water, soil, shoot and root: Table 6a describes summary of multivariate analysis of heavy metals in sea water, soil, shoot and root with their mean vectors. Pilli's Trace for intercepts and treatments were found significant ($p<0.001$) in all samples.

Table 6a. F-values of multivariate test (Pillai's Trace) for heavy metal in water, soil, shoot and root.

Samples	Intercept	Treatments
Water	16.34***	1.64***
Soil	1301.41***	2.68***
Shoot	189.23***	2.53***
Root	773.97***	1.91***

Note: *** = (p<0.001)

The test between treatments for prepared polluted sea water was presented in Table 6b. Zn, Mn and Pb were found significant ($p<0.05$) while Fe and Co were non-significant among treatments. The highly significant results between treatments for soil were recorded. The F-values of Zn was found significant ($p<0.01$), Fe ($p<0.001$), Co and Pb ($p<0.05$) while Mn was non-significant among treatments. The test between subjects in treatments for shoot showed F-values of Zn ($F=7.23$; $P<0.01$) was significant and Fe ($F=83.44$, $p<0.001$) while Co, Mn and Pb were non-significant. In the case of root, the tests between treatments are presented in Table 6B. The F-values of Zn, Co and Pb were non-significant whereas Fe ($F=152.02$) and Mn ($F=3.49$) were significant ($p<0.001$ and $p<0.05$) respectively.

Table 6b. Multivariate tests for heavy metal between-treatments effects of prepared polluted sea water, soil, shoot and root.

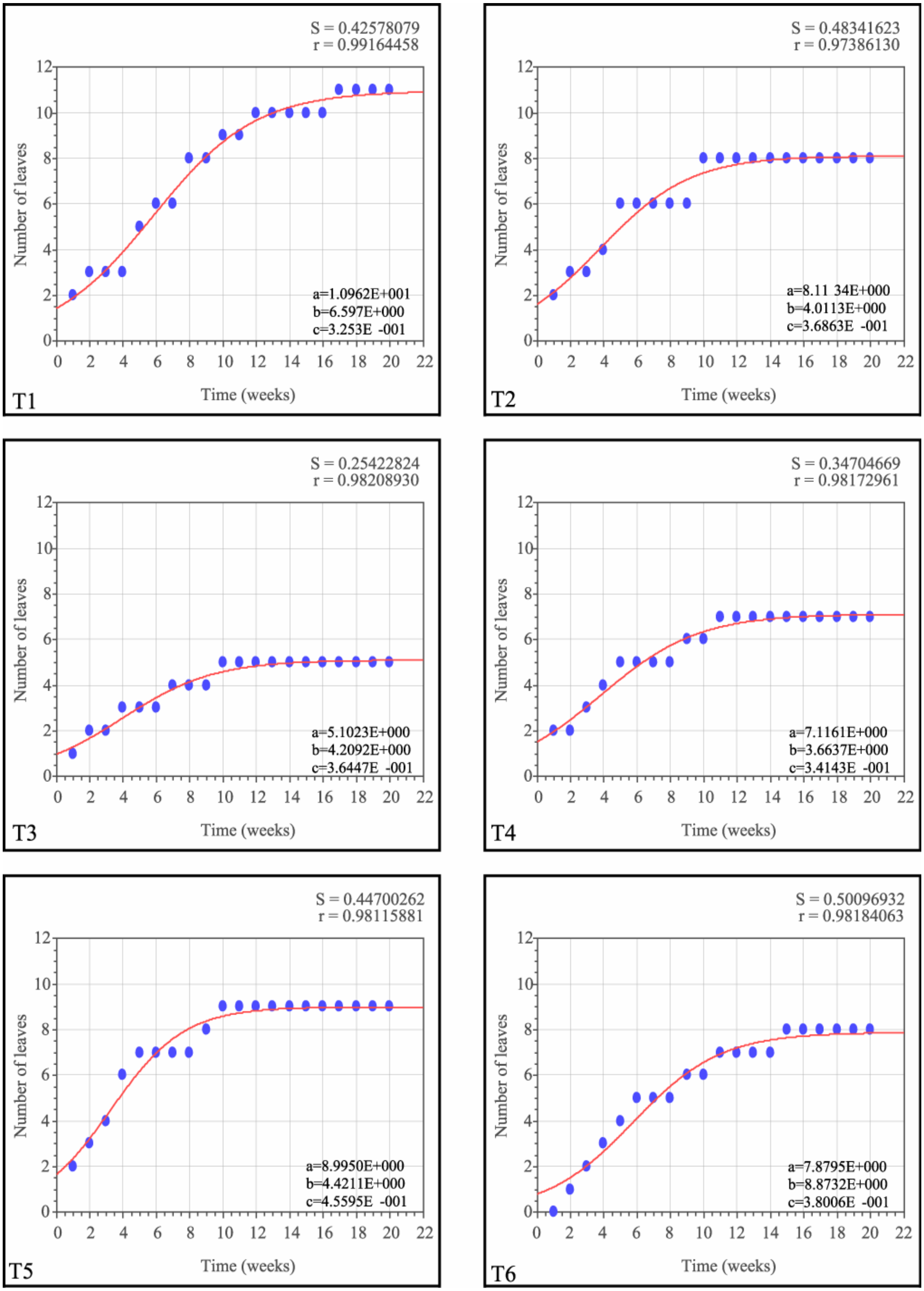
S and DV	A			B			C			D		
Treatments	**M S**	**FV**	**P<**	**M S**	**FV**	**P<**	**M S**	**FV**	**P<**	**M S**	**FV**	**P<**
Zn	0.08	4.02	p<0.05	0.36	7.67	p<0.01	5.85	7.23	p<0.01	2.60	2.57	ns
Fe	0.05	1.27	ns	1.69	23.73	p<0.001	122.18	83.44	p<0.001	268.28	152.02	p<0.001
Co	0.007	0.32	ns	0.40	3.43	p<0.05	0.05	0.09	ns	0.59	1.32	ns
Mn	0.20	3.20	p<0.05	0.42	0.79	ns	1.45	2.36	ns	1.96	3.49	p<0.05
Pb	0.25	4.32	p<0.05	0.06	4.26	p<0.05	0.15	0.39	ns	0.21	0.82	ns
Error												
Zn	0.02			0.04			1.94			2.42		
Fe	0.04			0.07			3.51			4.23		
Co	0.02			0.11			1.29			1.07		
Mn	0.06			0.54			1.47			1.34		
Pb	0.05			0.01			0.92			0.62		

Note: S and DV= Source and dependent variables, A= Treatment effects on prepared polluted water, B= Treatment effects on soil, C= Treatment effects on shoot, D= Treatment effects on root, MS= Mean Square, FV= F-value, p<=Significant level and ns= Non-significant

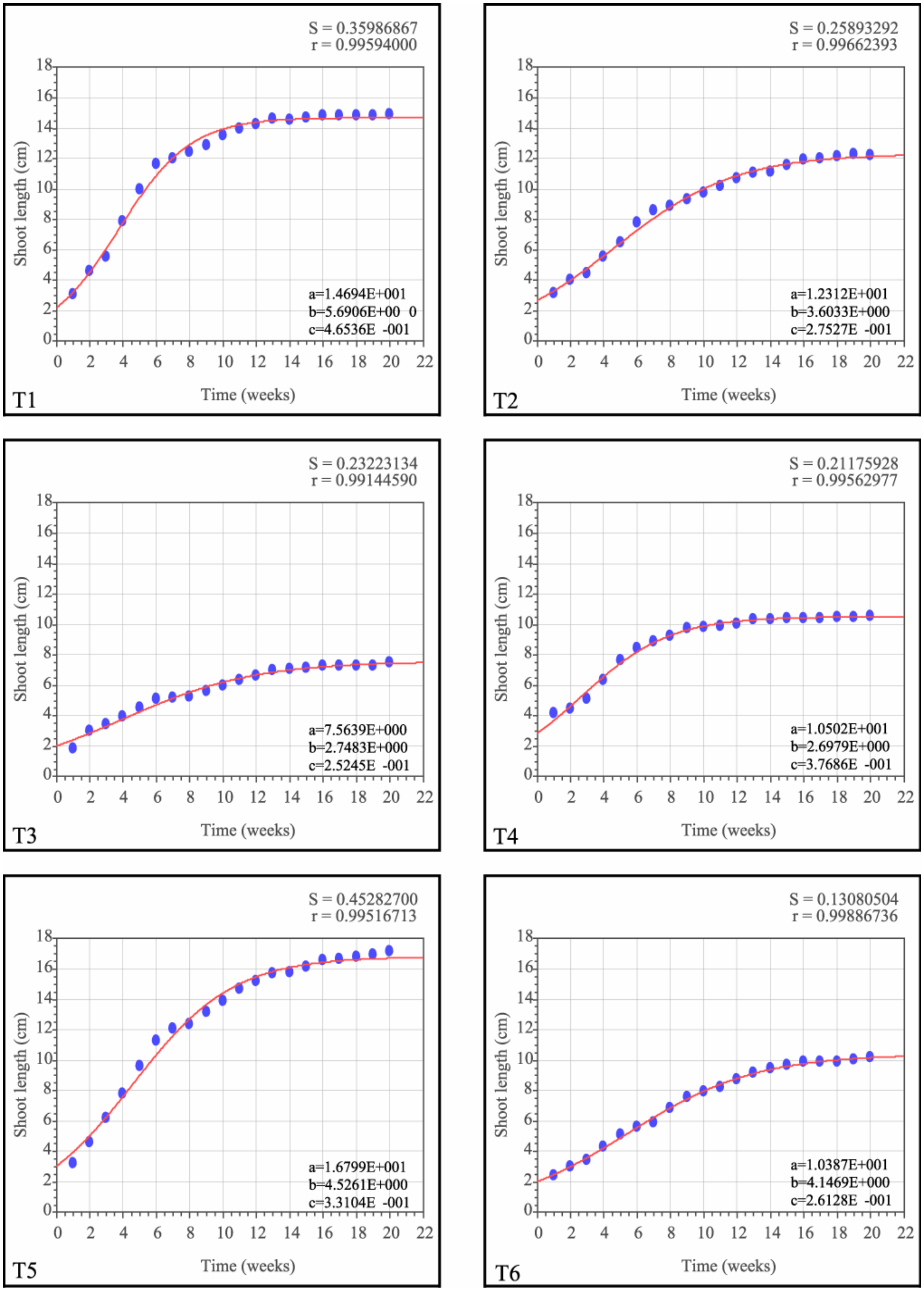
The curves obtained by plotting growth and time are typical sigmoid or S- shaped curves. Number of leaves in terms of sigmoid curve is described by various phases. The logistic curve has an inflection point which separates the curve into two equal parts of opposite concavity. The curve shows that during the initial stage (log phase) the number of leaves was lower in ash treatment compared to controls. Number of leaves increased in all treatments with time. After exponential phase the number of leaves attained stationary phase, the lowest stationary phase ($a=5.10$) was found in ash treatment and number of leaves became constant at 10 to 20 weeks of growth. The maximum stationary phase ($a=8.99$) was estimated in converter slag compared to other treatments (Fig. 1).

The characteristic concave shape in the graph of a shoot length logistic function shows that initial exponential growth is followed by a period in which growth slows down and then levels off, approaching (but never attaining) a maximum upper limit (Fig. 2). The result showed that shoot length increased in all treatments with time. During the initial stage (log phase) the increasing rate of shoot length was very low in ash treatment compared to controls. After exponential phase the length of shoot achieved stationary phase, the lowest stationary phase ($a=7.56$) was found in ash treatment and shoot length became stationary with time. The maximum increase in shoot length ($a=1.679$) was estimated in converter slag compared to controls.

Phytoremediation: Table 7 evaluates the results of BAC (Biological Accumulation Coefficient), BTC (Biological Transfer Coefficient) and BCF **(**Bio-concentration Factor) of heavy metals concentration in mangrove soil, root and shoot. The maximum values of BAC (22.05), BTC (125) and BCF (2.0) for Zn were recorded in ash treatment as compared to control. However, the lowest values of all three parameters were found in marble related to other treatments. For Fe the highest BAC (9.88), BTC (162.63) and BCF (17.14) were recorded in converter slag treatment as compared to control, while, the lowest value were found in marble waste among treatments. The highest values of BAC (6.28), BTC (1.4) and BCF (4.48) for Co, greatest accumulation of Mn BAC (0.46), BTC (0.87) and BCF (0.58) and all three parameters of Pb BAC (0.96), BTC (1.05) and BCF (0.94) were also recorded in ash treatment compared to control. The lowest accumulation of Co, Mn and Pb were estimated in marble as compared to other treatments.



Note: T 1= control, T 2= chemical Sludge, T3= chemical ash, T4= boulder slag, T5= converter slag and T6= marble waste
Fig 1. Relationship between time and number of leaves in different treatments.



Note: T 1= control, T 2= chemical sludge, T3= chemical ash, T4= boulder slag, T5= converter slag and T6= marble waste
Fig 2. Relationship between time and shoot length in different treatments.

Table 7. BAC, BTC and BCF of mangrove species *Avicennia marina* under six types of treatments.

Metals	Accumulation factors	Control	Industrial Sludge	Ash	Boulder Slag	Converter Slag	Marble
	BAC	0.74	1.8	22.05	1.79	1.95	1.57
Zn	BTC	0.53	1.53	125	1.50	10	0.97
	BCF	0.15	1.19	2	1.17	1.38	1.16
	BAC	4.21	7.07	7.26	5.36	9.88	4.24
Fe	BTC	17.27	49.44	125.6	45.91	162.63	22.77
	BCF	5.49	8.77	16.49	7.42	17.14	7.04
	BAC	0.47	1.15	6.28	0.56	0.59	0.49
Co	BTC	0.59	1	1.4	1	1	0.97
	BCF	0.47	1.56	4.48	0.58	0.82	0.55
	BAC	0.21	0.45	0.46	0.29	0.35	0.26
Mn	BTC	0.62	0.80	0.87	0.70	0.77	0.69
	BCF	0.31	0.56	0.58	0.42	0.50	0.34
	BAC	0.76	0.89	0.96	0.78	0.83	0.78
Pb	BTC	0.86	0.94	1.05	0.90	0.93	0.87
	BCF	0.84	0.91	0.94	0.90	0.89	0.89

Note: BAC= Biological Accumulation Coefficient, BTC=Biological Transfer Coefficient, BCF= Bio-concentration Factor

Discussion

In the present study the high values of pH, conductivity, TDS and temperature in converter slag indicated the high alkaline nature of this treatment in prepared polluted water because of dissolved salts and metallic ions. Conductivity, TDS and pH are temperature dependent parameters, as the temperature of water increases the velocity of ions in term of conductivity increases. The physical properties of water and soil are important to determine the appropriate use of the substrate while tissue analysis is a powerful tool for identifying the plant nutrient status because it is related to plant growth (Brady & Well, 1999). High concentrations of heavy metals were recorded in soil compared with ash treatment. Elliott & Dempsey, (1990) reported that the addition of sludge-ash significantly alters the soil chemistry.

In *Avicennia marina,* the germination started in four to five days and completed within 12 days; (Nazim *et al.,* 2010). This species showed 90% germination in all treatments except in ash and marble waste possibly due to high concentration of Zn, Co, Pb and Mn in ash treatment while in case of marble waste this may be due to unavailability of nutrients which are important for the growth of mangrove. Lovelock e*t al.,* (2007) stated that nutrient enrichment has been proposed to be an important factor, leading to increase in mangrove cover, growth and its structure. In almost all plants, nutrient availability is an important driving variable influencing growth parameter Grime, (1979); Chapin, (1980) & Tilman, (1987) and this is also the case for mangrove forests (Onuf *et al*., 1977; Boto & Wellington, 1983). Significantly higher mean height of seedlings in converter slag is most probably due to excessively high concentration of iron. Alongi, (2010) reported that mangroves growing from seedling to sapling stage have a strong nutritional requirement for iron. Nazim (2011) also described the importance of iron for the growth of *A. marina*. The thick and heavy growth of mangroves near the conveyer belt at Port Qasim also explains the importance of this element for the growth of mangroves (Personal observation). The inhibition of shoot height by chemical industry sludge-ash may be attributed to high amounts of Zn, Co, Mn and Pb which competitively interfere with other ion uptake and translocation. It also alters the activity of several enzymes, and result in variation in the levels of protein synthesis and photosynthetic activity in plant system and its growth (Lee *et al*., 1976). It was recorded that the total concentrations of heavy metals in the tissues (shoot and root) of *A. marina* were higher than in water and soil. The greater amount of lead was accumulated in root and shoot of *A. marina* in ash treatment with a significant difference compared to other treatments. The ash treatment gave the adverse effect on shoot length because this treatment possessed low salinity values with high concentration of heavy metals. Subramanian, (1982) stated that salinity seemed to be a dominant factor in controlling the uptake and accumulation of the elements in mangrove plants. The concentrations of lead were higher in roots than in shoots. Studies on lead uptake (Lane & Martin, 1977) have demonstrated that roots have ability to uptake significant quantity of Pb, simultaneously greatly restricting its translocation to above ground parts. The roots of mangroves take up the pollutant from the soil but it depends upon the biologically available metals in the soil (Ong-Che, 1999). In this study root showed overall high concentrations of all metals among treatments. According to Tam & Wong, (1995) mangrove roots appear to be barriers; they prevent metals to move from seedlings into more sensitive parts of the plant. Accumulation of pollutants especially heavy metals occur in the roots of mangroves plant but restrict its transport to aerial portions of the plant (Silva *et al*., 1990), which causes inhibition in overall growth, resulting in decreased fresh and dry biomass of plant.

The metals in the chemical industry sludge were also found in great amount, but due to acidic nature of the waste, the metals were unable to be mobile in soil (Elliott and Dempsey, 1990) and give a profound effect on the growth of *A. marina* that might be due to the organic nature of sludge compared to its ash.

It is suggested that the converter slag also had overall better results with respect to fresh and dry weight of shoot and root because of its liming nature and the presence of iron. Iron is a very reactive metal especially when present extensively over a large scale on a mineral surface such as sediments. The iron concentration in waste is commonly regulated because it readily oxidized, causing rust stains, normally it does not represent a toxicity problem since it is usually present in the form of stable iron complexes (Wang and Mu, 1992). The concentration of heavy metals may be regulated because of the toxicity of these metals or their compound. The toxicity can vary with particular metal or compound and with the form in which the metal exist (Wang and Mu, 1992). Fe oxyhydroxides have received a large degree of attention in aquatic metal chemistry. This is due to their omnipresence and copious property, as well as their proven geochemical reactivity (Honey-man and Santschi, 1988). Further, it is common that Fe oxyhydroxides form surface coatings on other types of mineral substrates such as clays and carbonates Whitney, (1975); Helios-Rybicka and Forstner, (1986); Warren and Zimmer-man, (1994); Coston *et al*., (1995) and thus, while their relative mass in a suspended sediment pool may be relatively small, they often dominate the geochemical reactivity of that sediment compartment and therefore, can influence on chemical behavior of the environment. It was observed and also repeated by other workers that at coastal areas of Sindh, the mangrove plants do not survive at many places even if all other environmental factors are favorable. Therefore, on the basis of other workers our results may be suggested that besides other factors the suppression of growth (or stunted growth) and high seedling mortality (personal observation) was possibly due to the accumulation of high concentrations of heavy metals in sea-water and sediments. According to Stigliani, (1995) generally mangrove sediments have a high capacity for absorbing and holding trace metals, the disturbance or erosion may cause mangrove soil to loose their metals binding capacity, resulting in mobilization of the metals. The mangal then shifts from a heavy metals sink to heavy metals source, often these disruptions are associated with human activates (Lacerda, 1998). Zheng *et al*., (1997) suggested that mangrove plantation should be avoided from Zinc polluted sites because seedling secretes certain types of organic acids that may increase the solubility of the metals resulting in high rate of mortality due to Zn toxicity. The results presented here seem to agree the findings of Zheng *et al.,* (1997).

The study on heavy metal accumulation in mangrove shoot and root provided the understanding about the high level of risk to mangroves with the high rate of pollution. Phytoremediation of this particular species was chosen because this species grows near or on the edge of the coastal areas and seems to be highly affected by the discharge of wastes. Secondly, this species is considered as 'bio-chemical filter' but with the passage of time the area covered by this species is gradually decreasing. Mangrove plants are known as the hyper accumulating plants because they accumulate high concentration of contaminants, most commonly toxic heavy metals such as Zn and Pb (Mac-Farlane *et al*., 2007). Mangrove participates in bio-chemical remediation extended from municipal waste to industrial pollutants of both organic and inorganic (Mac-Farlane *et al*., 2007). BAC, BTC and BCF can be used to estimate a potential for phytoremediation purposes. It appeared that Zinc concentration in different samples varies to a great extent from treatment to treatment. To compare the influence of the contamination of heavy metal uptake, BAC, BTC and BCF were the best tools. BAC values for Zn and Fe for most treatments were greater than 1 which shows a special ability of the test plant to absorb heavy metals from soil transport and store them in their above ground parts (Baker and Books, 1989; Baker *et al*., 1994; Brown *et al*., 1994 and Wei *et al*., 2002). However, the value of BAC lower than 1 could be primarily considered as potential hyper-accumulators. However, the ability of this species to tolerate and accumulate heavy metals may be useful for phytostabilization. Considering the BTC values, Zn, Fe, Co Pb contained values greater than 1 in most of the treatments. It was evident that this species has great potential to accumulate Zn, Fe, Co and Pb whereas the BTC values for Mn possessed lower than 1. The overall results suggested that this species is hyper-accumulator for Fe, Zn and Co in the following order Fe> Zn> Co whereas limited ability to accumulate Pb while hypo-accumulator for Mn. This study demonstrated that *Avicenna marina* has high potential to accumulate heavy metals; however, the greater concentrations of metals inhibit the overall growth from the initial stages. Effect of heavy metals on early seedling growth will help to elucidate the causes of reduction in mangrove forest. To identify the particular industrial pollutant, this show significant inhibitory effect on germination and early growth of *A. marina* along the coast of Pakistan. Therefore, it is suggested that early or young population of mangrove should keep away from heavy metal pollution.

Conclusion

This study has showed that *A.marina* possess the high capacity to accumulate Zn, Fe, Co, Mn and Pb via its roots and accumulating in certain parts of mangrove plant. This suggests the potential of *A. marina* as a phytoremediation species for Pakistan mangrove ecosystem. As such, further study is recommended to determine the true potential of this species as a phytoremediation species. However, the degree of toxicity varies with the type and nature of the waste material that shows both positive and negative effects on various growth parameters of the plant. It is concluded that all types of industrial waste must be discharged after treatment and assortment rather than discharged directly into the sea.

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(Received for publication 10 August 2011)