Immobilization and mobilization effect of ammonium molybdate on phytoremediation of toxic heavy metals in soil

Megha Tharayil, Harikumar Puthenveedu Sadasivan Pillai

Water Quality Division, Centre for Water Resources Development and Management, Kozhikode, Kerala, 673571, India, Email: meg8tee@gmail.com

ABSTRACT

Rapid industrialization and urbanization have resulted in elevated emission of toxic heavy metals to the environment. Many of the current remediation techniques available for heavy metal removal from contaminated sources are expensive, time consuming and environmentally not sound. Unlike organic compounds, metals will not degrade, and therefore requires effective cleanup techniques to reduce or remove toxicity. Phytoremediation, an emerging cleanup technology for contaminated area is both low-tech and cost effective. The objective of this study was to investigate the effect of ammonium molybdate on phytoremediation of toxic heavy metals (Cd, Pb, Cu, Ni and Zn) from soil by *Amaranthus retroflexus*. Five concentrations of ammonium molybdate solutions having Mo contents 0.05, 0.10, 0.30, 0.50, 1.00 g/L respectively were added to pots containing *Amaranthus retroflexus*. Ammonium molybdate shows immobilization and mobilization effect on phytoremediation of toxic heavy metals in soil. It was found that ammonium molybdate has the potential ability to precipitate with Pb and Zn and it decreases the biotoxicity of these metals to plant. Ammonium molybdate also has the ability to chelate and form more soluble fractions with Cd, Cu and Ni and it increases the bioavailability of these metals to plant. From the bioconcentration factor and translocation factor, it was found that application of ammonium molybdate to soil is a promising technology in phytoremediation.

Keywords: Ammonium molybdate, phytoremediation, bioconcentration factor, translocation factor.

INTRODUCTION

Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution. The primary sources of this pollution are the burning of fossil fuels, mining and smelting of metalliferous ores, metallurgical industries, municipal wastes, fertilizers, pesticides and sewage [1]. Environmental pollution by heavy metals is now a global issue that requires considerable attention. This is due to the fact that unlike many substances, metals are not biodegradable and hence accumulate in the environment. Trace amount of some heavy metals such as Cu, Zn, Fe and Co are required by living organisms, however, any excess amount of these metals can be detrimental [2]. Non-essential heavy metals include arsenic, antimony, cadmium, chromium, mercury, lead; these metals are of particular concern because they cause air, soil and water pollution [3]. Decontamination of such soils has therefore, become imperative for the safety of animals and humans. A number of techniques have been developed to remove metals from the contaminated soils. However, many sites remain contaminated because of economical problem of the available technologies. Techniques such as excavation and disposal of contaminated soils in landfills are not environment friendly and may serve as secondary pollution sources [4]. Therefore, new environmental friendly and less expensive techniques are required. With current trends moving towards greener technologies, the focus is shifting to phytoremediation, where plants are used for the uptake of metals or pollutants from the environment or transform them into harmless compounds [5]. Phytoremediation presents a cheap, noninvasive, and safe alternative to conventional cleanup techniques and can be accomplished by phytoextraction, phytodegradation, phytostabilization, phytovolatilization and rhizofiltration [6].

Green plants are not only the lungs of nature with an ability of purifying impure air by photosynthesis, but some species also have the unique ability to uptake, tolerate and even hyperaccumulate heavy metals and other toxic substances from soils and water through roots and concentrate them in roots, stem and leaves[7]. The high bioconcentration factor, which is the ability of the plant to extract metals from the soil and the efficient root to-shoot transport system endowed with enhanced metal tolerance provide hyper accumulators with a high potential detoxification capacity. The heavy metal accumulation capacity of plants belonging to the Amaranthaceae family was reported earlier. *Amaranthus retroflexus* is a good metal accumulator and it has been used for the uptake of cadmium, mercury, zinc and copper [8].

Plants that accumulate metals can extract metals from soils (phytoextraction) or, on the other hand, are used in combination with soil amendments to improve soil conditions (phytostabilization) [9]. Chelate-enhanced phytoremediation has been proposed as an effective tool for the extraction of heavy metals from soils by plants. The most frequently used solutions for extraction also have deficiencies: ethylene diamine tetraacetic acid (EDTA) is expensive and toxic, and presents a low level of biodegradability [10,11]; nitrilotriacetic acid (NTA) is also the toxicant as a class II carcinogen [12]; nitric acid (HNO3) is lethal to soil micro-flora and destructive to the physical and chemical properties of soil; hydrochloric acid (HCl) can alter soil properties [13]; citric acid is a nontoxic acid that forms relatively strong complexes. It is easily biodegradable, but it presents of lower effectiveness in the removal of metal ions [14]. EDGA enhanced metal solubility but plant uptake did not increase accordingly [15].

The addition of chelators effectively increased the mobility of target heavy metals in soils, and significantly enhanced the accumulation of these heavy metals in aerial parts of the plants but the application of chelators had inhibitory effects on the growth of the plants [16]. The ammonium molybdate (containing nitrogen and molybdenum) is fertilizer to plants, which can produce more biomass [17]. In this study, we assessed phytoextraction potential of the *Amaranthus retroflexus* with ammonium molybdate, to evaluate the ability of the *Amaranthus retroflexus* to remediate soils contaminated with multiple heavy metals. Heavy metals selected for study include two essential (copper (Cu) and zincZn)) and three toxic (cadmium (Cd), lead (Pb) and nickel (Ni)) elements.

MATERIALS AND METHODS

Physicochemical characteristics of soil samples

Soil used for the experiment was spiked with heavy metals (Cd, Pb, Cu, Ni and Zn) solutions. The contaminated soil received the metals Cd as $CdSO_4$; Pb as $Pb(NO_3)_2$; Cu as $CuSO_4$; Ni as $NiSO_4$ and Zn as $ZnSO_4$. Physicochemical factors of soil such as pH, electrical conductivity (EC), texture, calcium, magnesium, organic carbon, inorganic phosphorous, sodium, potassium were analyzed.

Plant culture and experimental design

Each plastic pot was filled with 5Kg of soil. *Amaranthus retroflexus* seeds were germinated in pots containing soil to a depth of 1cm under normal condition. After seedlings grew for 10 days, 18 seedlings were transplanted to pots containing heavy metal contaminated soil at a rate of one seedling per pot. The experiment consists of 5 treatments (No.1, No.2, No.3, No.4 and No.5) and control with replicates for each. Experiments were exposed to natural day and night temperatures. The plants were watered daily with 300 ml of distilled water per pot. In the case of 5 treatments, 50 ml of ammonium molybdate solutions (having Mo contents 0.05, 0.10, 0.30, 0.50 and 1.00 g/L respectively) were added to pots containing *Amaranthus retroflexus*. Ammonium molybdate solution was not applied in control [17].

Analysis of heavy metals in soil and plant

After 45 days the plants were harvested and heavy metal concentrations in plants and soil were determined. The extraction of heavy metals from soil was done by using perchloric acid-nitric acid mixture. The powerful oxidizing and dehydrating properties of hot, concentrated perchloric acid were extremely effective in decomposing organic matter and sulphides. Nitric acid dissolves the majority of the metals occurring in nature, with the exception of gold and platinum. 0.2g of soil was digested with 20ml of concentrated HNO_3, 5ml distilled water and 10 ml of $HClO_4$ and heated on a hot plate for 2 hrs. The mixture was heated until the white fumes come and the soil become white. Then the solution was filtered and made up to 50 ml. The filtrate was analyzed for heavy metals using Atomic Absorption Spectrophotometer (AAS) (Thermo Series). Replicates were carried out as part of the measurement.

The plants were harvested after 45days. The lengths of the roots and shoots were measured. The roots and shoots were separated and washed with distilled water to remove soil and dust. The plant parts were dried in an oven at 70°C for 72 hr and the dry weight were recorded by electronic balance. 0.2 g of plant parts were digested at 150°C for 200 min with 10 ml mixtures of $HNO_3/HClO_4$ (4:1) [18]. After complete digestion, the volume of digested samples was adjusted to 50 ml with distilled water. Subsequently, the amount of heavy metals was determined by Atomic Absorption Spectrophotometer (AAS) (Thermo Series). In this experiment, three replicates were maintained.

Bioconcentration and translocation factor

The bioconcentration factor (BCF) was used to determine the quantity of heavy metals absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil and is calculated using the below given formula [19]. The higher BCF value the more suitable for phytoextraction (BCF Values>2 were regarded as high values) [20, 21].

$$\text{Bioconcentration factor} = \frac{\text{Metal concentration in the plant tissue}}{\text{Metal concentration in the soil}}$$

To evaluate the potential of *A. retroflexus* for phytoextraction, the translocation factor (TF) was calculated. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant [22]. Metals that are accumulated by plants and largely stored in the roots of plants are indicated by TF values<1, with values greater indicating translocation to the aerial part of the plant [21].

$$\text{Translocation factor} = \frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in roots}}$$

RESULTS AND DISCUSSION

Physicochemical characteristics and heavy metal concentration of the soil samples were given in table 1. As compared with controls, the concentration of Ni, Cd and Cu in treated soil were lower but the concentrations of Pb and Zn in treated soil were higher (Table 2).

Table 1. Physico-chemical characteristics and heavy metal concentration of contaminated soil.

Parameters	Value
pH	6.71±0.05
EC µS/m	24.6± 0.05
Alkalinity (mg/kg)	644±0.07
Chloride (mg/kg)	320.8± 0.61
Sulphate (mg/kg)	304.8±0. 82
Inorganic phosphorous (mg/kg)	8± 1.00
Sodium (mg/kg)	250± 0.6
Potassium (mg/kg)	75±0.5
Organic carbon %	0.279± 1.22
Exchangable Calcium`(mg/kg)	1280±1.14
Exchangable Magnesium (mg/kg)	583.2±1.38
Nickel mg/kg	155.87±1.46
Cadmium mg/kg	6.98±1.5
Cupper mg/kg	86.11±2.77
Lead mg/kg	74.79±2.32
Zinc mg/kg	109.27±1.25

Table 2. Heavy metal concentration in soil after treatment.

Treatment	Ni (mg/Kg)	Cd(mg/Kg)	Cu(mg/Kg)	Pb (mg/Kg)	Zn (mg/Kg)
Control	122.95±2.47	3.73±0.36	45.7±2.18	33.11±1.54	41.81±1.80
No.1	97.37±1.25	2.87±0.73	38.53±2.33	41.09±1.58	46.15±2.12
No.2	83.93±2.89	0.87±0.25	35.52±1.24	47.93±1.82	53.28±1.70
No.3	66.83±4.06	0.075±0.07	33.53±1.32	53.51±2.94	58.45±4.31
No.4	55.79±3.62	0.05±0.02	26.32±1.22	56.02±1.00	65.82±2.23
No.5	21.56±1.74	0.025±0.02	6.48±1.39	63.59±1.75	67.61±1.64

Amaranthus retroflexus uptakes more amount of Ni, Cd and Cu than Pb and Zn in the presence of ammonium molybdate. It may be due to that Ni, Cd and Cu were chelated and form more soluble fractions with ammonium molybdate and Pb and Zn were precipitated with ammonium molybdate. The mechanism of the reaction of ammonium molybdate with toxic metals can be reported as follows [17]:

$$Zn/Pb + Mo_2O_7^{2-} \rightarrow (Zn/Pb)\ Mo_2O_7 \quad \text{(precipitation)}$$
$$Cd/Ni/Cu + NH_4^{+} \rightarrow Cd/Ni/Cu\ (NH_3)_4^{2+} \text{ (soluble fractions)}$$

The heavy metal concentrations in the root of *A. retroflexus* are indicated in Table 3. Compared to control, the concentrations of Ni, Cd and Cu in root were higher but the concentrations of Pb and

Zn were lower. A similar trend was also observed in the shoot of *A. retroflexus* (Table 4). Ammonium molybdate has the potential ability to precipitate with Pb and Zn so it stabilizes these metals in soil and decreases the bioavailability of these metals to plant. It also has the ability to chelate and form more soluble fractions with Cd, Cu and Ni, thus it increases the bio-availability of these metals to plant. Maximum reduction of heavy metal in soil was observed in treatment No.5 (with maximum concentration of ammonium molybdate). After treatment 86.16% of Ni, 99.64% of Cd and 92.47% of Cu were reduced in soil. The result indicated that, *A. retroflexus* can remove heavy metal (Cd, Cu and Ni) efficiently with ammonium molybdate. The ammonium molybdate has the potential to enhance metal mobility in soil profiles by forming complexes with toxic metals [17]. It acts as stabilization agent for Pb and Zn and as extracting agent for Cd, Cu and Ni.

Table 3. Heavy metal concentration in the root of *Amaranthus retroflexus*.

Treatment	Ni (mg/Kg)	Cd(mg/Kg)	Cu(mg/Kg)	Pb (mg/Kg)	Zn (mg/Kg)
Control	16.92±1.6	0.91±0.03	16.6±1.23	26.41±0.68	32.49±1.28
No.1	36.44±3.46	1.53±0.3	21.87±1.7	17.74±0.72	26.74±0.84
No.2	47.14±1.36	2.15±0.26	23.83±0.36	12.56±1.00	22.7±1.38
No.3	57.39±3.27	2.80±0.17	26.81±1.14	9.63±0.61	18.39±2.48
No.4	68.81±1.81	3.07±0.14	31.37±0.78	7.73±0.94	11.75±1.24
No.5	92.66±3.39	3.45±0.11	41.18±1.69	4.51±1.38	7.72±1.03

Table 4. Heavy metal concentration in the shoot of *Amaranthus retroflexus*.

Treatment	Ni (mg/Kg)	Cd(mg/Kg)	Cu(mg/Kg)	Pb (mg/Kg)	Zn (mg/Kg)
Control	7.41±1.28	0.78±0.06	7.86±0.91	11.46±0.80	21.23±0.49
No.1	18.11±2.58	0.89±0.07	8.02±0.09	9.59±0.53	19.73±0.96
No.2	23.58±5.49	1.43±0.08	9.66±0.60	8.73±0.35	15.8±0.65
No.3	24.34±4.39	2.24±0.25	10.58±0.68	6.15±0.28	13.8±0.42
No.4	23.20±3.89	2.28±0.31	11.82±0.34	4.91±0.28	8.6±0.83
No.5	26.91±3.98	2.44±0.12	17.49±2.00	2.06±0.57	5.99±0.87

Accumulation of nickel in the plant parts increased with increasing ammonium molybdate concentration. Concentrations of Ni accumulated ranged from 16.92 mg/Kg to 92.66 mg/Kg with highest concentration being stored in the roots. Much higher Ni levels were found in the roots rather than in the shoots or leaves. Bioaccumulation of cadmium in the roots (0.91 to 3.45 mg/kg) was higher than the shoot (0.78 to 2.44 mg/kg) and leaf (0 to 0.98 mg/kg). The cadmium content in the leaf of control plants was below detectable levels. A steady increase was noticed in the accumulation of the cadmium in the plant parts with increasing ammonium molybdate. The bioaccumulation of copper by *A. retroflexus* exhibited variation among plant parts and bioaccumulation of copper in plant increases with the application ammonium molybdate. Among the plant parts, the roots accumulated more copper than the shoot and leaf.

The control plant species accumulated more amounts of lead and zinc than the treated plant species. With increase in the concentration of ammonium molybdate, the bioaccumulation of Pb and Zn decreases. The metals like Pb and Zn in soil can precipitate with $Mo_2O_7^{2-}$ and the toxicities of these metals to plant will be decreased [23]. The content of toxic metals in the root were higher than shoots, which may be related to plant uptake of toxic metals and xylem translocation from roots to shoots. Restriction of upward movement from roots into shoots can be considered as one of the tolerance mechanism [24]. The NH_4^+ ion present in ammonium molybdate chelate with toxic metals (such as Cd, Ni and Cu) and form soluble chelating complexes [25]. Ammonium molybdate increases the bioavailability of Ni, Cd and Cu in soils and the *A. retroflexus* uptakes more amount of these toxic metals with ammonium molybdate. It also increases the toxicities of these metals to plant.

After 45 days, length of shoot and root of *A. retroflexus* were measured and shoot lengths were shown in figure 1. *A. retroflexus* plant had exhibited high biomass production with the application of ammonium molybdate. The results show that ammonium molybdate could promote *A. retroflexus* plants to produce more biomass, because nitrogen and molybdenum are fertilizer, which can promote *A. retroflexus* plant to improve tillering and biomass gain. Biomass can express the tolerance of plants to toxic metals indirectly [26]. The average shoot lengths of treatment plants were longer than the control plants, but the shoot lengths of No.4 and No.5 were shorter than controls. It may be due to the reason that with addition of ammonium molybdate into soils, Cd, Ni and Cu formed more soluble fractions and the toxicities of Cd, Ni and Cu have reduced the chlorophyll content [27]. Figure 2 shows the phytotoxicity symptoms exhibited by *A. retroflexus.* Wilting and leaf necrosis have been described as typical visible symptoms of Ni^{2+} toxicity [28].

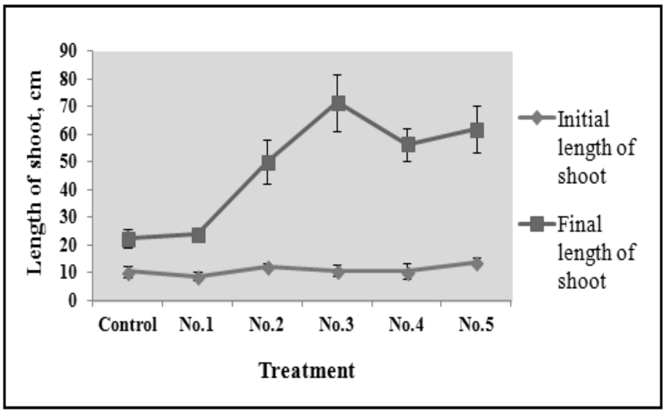

Figure 1. Shoot lengths of *A. retroflexus*.

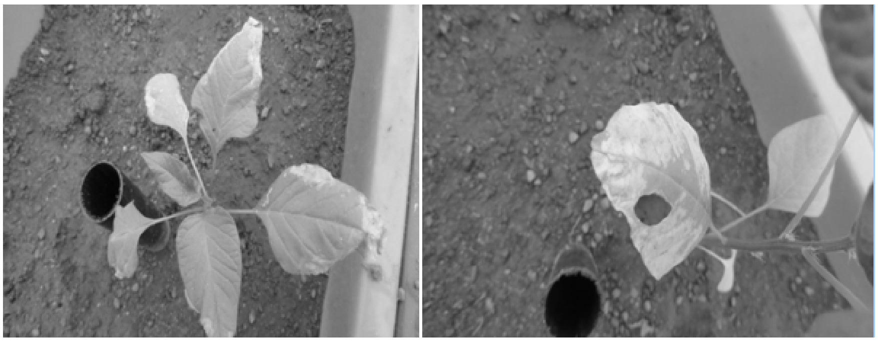

Figure 2. Toxicity symptoms exhibited by *A. retroflexus*.

BCF is the ratio of the metal concentration found within the tissues over the metal concentration found in the soil. The greater is the coefficient, the greater will be the uptake of heavy metal. It was observed that for Cd, Ni and Cu the average BCF values (Table 5) of *A. retroflexus* plant treated with ammonium molybdate were higher than control but for Pb and Zn it was lower than control.

Table 5. The average bioconcnetration factor and translocation factor value of *Amaranthus retroflexus*.

Bioconcentration factor of shoot					
Treatment	Ni	Cd	Cu	Pb	Zn
Control	0.06	0.21	0.17	0.35	0.51
No.1	0.19	0.31	0.21	0.23	0.43
No.2	0.28	1.64	0.27	0.18	0.3
No.3	0.36	29.87	0.32	0.11	0.24
No.4	0.42	45.6	0.45	0.09	0.13
No.5	1.25	97.6	2.7	0.03	0.09
Bioconcentration factor of root					
Control	0.14	0.24	0.36	0.8	0.78
No.1	0.37	0.53	0.57	0.43	0.58
No.2	0.56	2.47	0.67	0.26	0.43
No.3	0.86	37.33	0.8	0.18	0.31
No.4	1.23	61.4	1.19	0.14	0.18
No.5	4.3	138	6.35	0.07	0.11
Translocation factor					
Control	0.44	0.86	0.47	0.43	0.65
No.1	0.5	0.58	0.37	0.54	0.74
No.2	0.5	0.67	0.41	0.7	0.7
No.3	0.42	0.80	0.39	0.64	0.75
No.4	0.34	0.74	0.38	0.64	0.73
No.5	0.29	0.71	0.42	0.46	0.78

Maximum BCF value of *A. retroflexus* was found for Cd in treatment No.5. It was 97.6 and 138 for shoots and root respectively. The maximum BCF value of Ni and Cu was also found in treatment No.5. Low BCF value of *A. retroflexus* for Pb and Zn indicates that low amount of metal uptake. This indicates that *A. retroflexus* plant had higher ability to uptake Cd, Ni and Cu with ammonium molybdate. This may be due to that complexation of ammonium molybdate increased the mobility of these metals [29]. TF is a measure of the ability of plants to transfer accumulated metals from the roots to the shoots. It is given by the ratio of concentration of metal in the shoot to that in the roots [30, 31]. A TF value greater than 1 is indicative of metal accumulation and transport into the different plant parts, and less than 1 is suggestive of storage of metal in roots. In this experiment, TF values of *A. retroflexus* were lower than 1, which indicates that maximum amount of heavy metals were stored in roots.

This study therefore has proved the possibility of using ammonium molybdate with phytoremediation for phytostabilization of Pb and Zn and for phytoextraction of Cd, Ni, and Cu by *A. retroflexus*. Ammonium molybdate shows immobilization and mobilization effect on phytoremediation of the toxic heavy metals in soil. Ni, Cd and Cu were chelated and form more soluble fractions with ammonium molybdate and Pb and Zn were precipitated with ammonium molybdate. Mobilization effect of ammonium molybdate on Ni, Cd and Cu increases the bioavailability of these metals to plant. Immobilization effect of ammonium molybdate on Pb and Zn decreases the biotoxicity of these metals to plant by decreasing the bioavailability of these metals to plant. It was found that application of ammonium molybdate to soil is a promising technology in phytoremediation; it acts as a stabilization agent and as an extracting agent.

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