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EDTA and Plant Growth Regulators (GA_3 and IAA) by Energy Plant to Evaluate Phytoattenuation

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Abstract

The concurrent usage of chelator and biostimulator demonstrated satisfactory of pytoattenuation through several of planting and harvesting by using energy plant sunflower and vetiver. EDTA increased the soil metal mobility and further plant uptake while the stimulator GA_3 and IAA was in the descending sequence. Biostimulator has been demonstrated e plant growth enhancement and been employed for agricultural operation after. 3 cycles of planting and harvesting Cu levels had demonstrated gradually metal decreasing. The control, stimulator GA_3 and IAA, and stimulator remaining copper levers were, Soil copper contents were, for initial, 1st, 2nd, and 3rd session, respectively. The soil metal level reduction achieved an acceptable level. More rounds of planting and harvesting, the soil metal concentration expected to be more effective in on-site operation. Sunflower can be employed to mitigate soil metal contamination through concurrent use of chelator and biostimulator.

Keywords: Phytoattenuation; Heavy metals; Vetiver (*Vetiveria zizaniodides*); Sunflower; EDTA; Biostimulator; Gibberellic Acid (GA_3); Indol-3-Acetic Acid (IAA)

Introduction

Soil and groundwater remediation act has been enacted and executed since year 2000. It has been ten good years till today where lots of remediation techniques progressively employed to improve Taiwan soil and groundwater resource quality. Regulatory agencies, academia, remediation consulting firms, on-site professional engineers all contribute the proud ten years in terms of soil and groundwater clean-up contribution. However, some of technologies were un-environmental friendly even detrimental and damage to Taiwan precious soil and groundwater resources. In Article one of the current Taiwan soil and groundwater Act, it clearly stated that soil is a precious nature resources. Soil definitely is not a waste, shame on us most of current most commonly employed remediation are unlawful and merely aiming to save time and money consideration without any care to our land. Dig-and-dump and soil acid washing are damage employed in almost every single local environment agency soil clean-up project. Lot of money, effort and time has been spent during past ten years. Most of the spending is not improving soil quality.

It is really confusing regarding the lesson learned and gained while used these chemical physical, not environmental friendly treatment techniques. Two remediation approaches, namely dig-and-dump and soil acid washing simply treat soil as garbage, waste, and junk, not the soil law indicated soil is a resource. The purpose of this paper is aimed to raise all you concerns and care toward our precious soil property, toward remediation engineers and particularly those governmental authorities who have so far never taken it as deep thought of current serious situation regarding soil damage.

A novel green remediation approach intends to convey in this paper by employing plant to gradually reduce soil metal contamination through several rounds of planting and harvesting. Unlike phytoextraction, phytoattenuation aims to reduce soil metal pollution in a gradually and less aggressive approach such as chelator assisted remediation [1]. The initial pollution level generally is lower than most soil contamination sites. Therefore, plant is easier to propagate to increase biomass inducing reliable metal uptake.

Attenuation is borrowing from the concept "natural attenuation" which has been commonly proposed as a remediation approach for organic pollutants such as DNAPL (dense non-aqueous liquid) solvent TCE (tri-chloro ethylene) and PCE (tetra-chloro ethylene) or LNAPL (light non-aqueous liquid) petroleum product BTEX (benzene, toluene, ethyl benzene, and xylene. Natural attenuation mainly used natural pollution mitigation mechanism including microbial degradation, adsorption, volatilization, etc. This approach is targeted to pollutant which is not degraded in a reasonable time using conventional remediation techniques, technical imperfectability, or the cost beyond the affordable monetary amounts, economical imperfectability.

Cu is used as the fodder additives for preventing swine diarrhea and skin abrasion [2]. Cu has been reported the toxicity to phytoplankton and been employed as algaecide for serious eutrophication mitigation. The careless management of Cu wastewater from swine industries could damage the water and soil environment.

The choice of plant is more flexible than phytoextraction. Plant is not necessary to be a hyperaccumulator and biomass production is not required to be enormous. Using several sessions of agricultural planting and harvesting, the metal contamination is gradually to reduce to an acceptable soil background concentration. The only concern is the time requirement for the whole attenuation operation. If the site has the emergent health and ecological damage concern, the aggressive remediation takes into the substation list to be conducted to ensure public health and ecological protection.

Possible ideal plants include wetland water pollution mitigation macrophytes such as vetiver, cattail, and reed which has been demonstrated to be easily propagation and capable to reduce water

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and sediment metal levels [2,3]. The harvested plant wastes should be properly managed to prevent the secondary environmental contamination. An alternative plant is the energy macrophytes such as sunflower and Chinese cabbage. After harvesting, the residue plant can be reused to produce bio-fuel which is green and substitute to petroleum fuels to lesson current energy concern.

Vetiver is known for its effectiveness in soil erosion control due to its unique morphological and physiological characteristics. Vetiver is also a high biomass plant with remarkable photosynthetic efficiency that renders it tolerant against various harsh environmental conditions. Vetiver with deep-rooted and higher water-use can effectively stabilize soluble metals in soils [4]. These properties enable vetiver to be an ideal candidate for phytoattenuation and have been investigated in the study.

Sunflower

An energy plant is commonly used to extract sunflower oil which can be employed as biofuel to be substitute of fossil fuel. In addition, sunflower also upgrade the landscape therefore the remediation execution can be more acceptable by general public.

Chinese cabbage

Biostimulator has been facilitated the plant growth enhancement and been employed for agricultural operation. The stimulators can be borrowed to enhance the vetiver propagation leading to expected phytoattenuation purpose. Two biostimulators, namely gibberellic acid (GA_3) and indol-3-acetic acid (IAA), were tested to evaluate vetiver metal attenuation enhancement.

In recent year, lots of researches related to phytoextraction have been conducted. The metal removal results were very optimistic the most updater researched results are shown in Table 1. Few if any study was focused on the biostimulator assisted phytoattenuation. The objectives of this study were aimed to observe the planting and harvesting attenuation cycles were required to achieve feasible soil metal levels. The effects of concurrent usage of chelator EDTA and biostimulators, GA_3 and IAA were also scrutinized to reveal the phyotattenulation effect.

Materials and Methods

Plant, biostimulators, and soil preparation

Vetiver and sunflower were collected from the University of Kaohsiung campus wetlands (22°73'N, 120°28'E) precultured for 5 days and carefully washed with distilled water. Plant samples were dried at 103°C in an oven until completely dried.

Total metal content, soil retained fractionation and plant metal uptake analysis

Harvested Plant tissue and final soil metal content analysis

Plant after last session of operation was harvested, careful washed, and air dried for metal analysis. Plant samples were dried at 103°C in an oven until completely dried. Dried plant samples were divided into root and shoot for metal accumulation assessment. These pretreated plants were digested in a solution containing 11:1 HNO_3:HCl solution via a microwave digestion apparatus (Mars 230/60, CEM Corporation) and diluted to 100 mL with deionized water. 0.2 g of dried soil adding *aqua regia* rending for microwave digestion and 2.5 g of dried for sequential extraction experiments. Metals analyses were conducted via an atomic absorption spectrophotometer (AAS, Perkin Elmer).

The fractionation of soil retained metal was investigated by a sequential extraction technique where soil samples were placed in a plastic bottle then shaking for proper mixing overnight and subjected to a five-step serial extraction procedure. The procedure of sequential chemical extraction used in this study includes a series of reagents which represented as exchangeable (1 M KNO_3), inorganically bound (0.5 M KF), organically bound (0.1 M Na P O), Fe and Mn-oxide bound (0.3 M $Na_3C_6H_5O_7$, 1 M $NaHCO_3$ and 0.5 g $Na_2S_2O_4$), and sulfide (6 M HNO_3) forms, respectively [9].

Data and statistical analysis

Data were evaluated relative to the control to understand their statistical variation. Metal concentration of plants was recorded as mg of metal per kilogram of dry biomass. A triplicate of soil and plant

Reference	Plant species	Plant uptake concentration (mg/kg)	TF	BCF	Chelator concentration
Doumett et al. (2008) [6]	*Paulownia t.*	Root, Shoot: Cu : 570, 46 Zn : 750, 149 Pb : 750, 149	Cu: 0.08 Zn :0.2 Pb: 0.1	Cu: 0.27 Zn :0.16 Pb: 0.06	EDTA 5mmol/kg
Epelde et al. (2008) [7]	*Cynara cardunculus*	Root, Shoot: Pb: EDDS (4165, 310) EDTA (6695, 1332)	EDDS: 0.02 EDTA: 0.20	EDDS: 0.83 EDTA: 1.34	EDDS 10 mmol/kg EDTA 10 mmol/kg
Sun et al. (2009) [8]	*Sedum alfredii*	Root, Stem, Leaf, Shoot: Cu: CA (32, 10, 11, 11) EDTA (25, 12, 12, 12) Pb: CA (39, 18, 18, 18) EDTA (68, 39, 43, 40) Zn: CA (680, 2000, 1950, 1930) EDTA (380, 2030, 2000, 2030)	Cu: CA (0.03) EDTA (0.57) Zn: CA (2.88) EDTA (5.34) Pb: CA (0.45) EDTA (0.61)	Cu: CA (0.03) EDTA (0.57) Zn: CA (12.6) EDTA (10.8) Pb: CA (0.29) EDTA (0.7)	Citric acid 5 mmol/kg EDTA 5 mmol/kg
Chen et al. (2004) [4]	*Vetiveria zizanioides*	Root, Shoot: Zn: 150, 82	Zn: 0.55	Zn: 0.85	EDTA 0.8 mmol/kg
This study	*Vetiveria zizanioides*	Root, Stem, Leaf: Cu: EDDS (1818, 1459, 361) CA (926, 56, 15) EDTA (2080, 954, 86) Zn: EDDS (16388, 12412, 12036) CA (14444, 12420, 10821) EDTA (12899, 9891, 12552) Pb: EDDS (4343, 280, 197) CA (4914, 388, 103) EDTA (4632, 1878, 340)	Cu:EDDS (0.51) CA (0.04) EDTA (0.25) Zn: EDDS (0.7) CA (0.82) EDTA (0.86) Pb: EDDS (0.06) CA (0.05) EDTA (0.24)	Cu:EDDS(1.97) CA (0.88) EDTA (2.22) Zn: EDDS(1.95) CA (1.67) EDTA (1.5) Pb: EDDS(0.63) CA (0.67) EDTA (0.58)	EDDS 5 mmol/kg Citric acid 5 mmol/kg EDTA 5 mmol/kg

Table 1: Plant uptake and transportation in recent study.

samples from each treatment were recorded and used for statistical analyses. Statistical significance was assessed using mean comparison test. Differences between treatment concentration means of parameters were determined by Student's t test. A level of $p < 0.05$ considered statistically significant was used in all comparisons. Means are reported mean ± standard deviation. All statistical analyses were performed with Microsoft Office EXCEL 2003.

Results and Discussion

Background soil concentration including total metal and metal fractionation

Background soil concentration including total metal and metal fractionation is shown in Table 2.

The biostimulator vetiver propagation enhancement and Cu soil reduction

Heavy metal accumulation and translocation in vetiver of different treatment conditions in pot experiments is shown in Table 3.

Phytoattenuation evaluation

The results of the attenuation study using vetiver and two stimulators have demonstrated prominent success. After 3 cycles of planting and harvesting Cu levels had demonstrated gradually metal decreasing.

Background	(g)	mg/L	mg/kg
1	0.3059	0.1750	
		0.1730	
		0.1790	
AVG		0.1757	28.7131
2	0.3027	0.1790	
		0.1820	
		0.1750	
AVG		0.1787	29.5122
3	0.3036	0.1830	
		0.1770	
		0.1760	
AVG		0.1787	29.4247

Table 2: Soil properties and metals background metal concentrations.

	mg/kg		
1st	Control	GA_3	IAA
Root	31.0340	23.7290	26.3631
Stem	30.4890	18.8386	12.4682
Leaf	23.0339	19.2525	18.2680
Flower	18.3680	21.3030	19.9920

	mg/kg		
2nd	Control	GA_3	IAA
Root	30.9638	23.5525	27.5342
Stem	27.8275	17.9197	13.6918
Leaf	20.5684	28.6246	18.7789
Flower	17.5715	14.8193	23.0192

	mg/kg		
3rd	Control	GA_3	IAA
Root	19.0021	29.3708	21.7290
Stem	17.2471	24.9768	8.8765
Leaf	12.8762	40.5539	11.3224
Flower	10.4892	20.1269	15.0759

Table 3: Heavy metal accumulation and translocation in vetiver of different treatment conditions in pot experiments.

These results were very effective and indicted that phytoattenuation can be a green alternative to mitigate soil metal contamination with or without biostimulator assistant.

Conclusion

Vetiver and sunflower has been demonstrated as valid plant for the phytoattenuation ideal plant due to it is great biomass prorogation and metal prominent uptake. This study has demonstrated after several sessions of vetiver and sunflower planting and harvesting. The soil metal levels had been reduced. Biostimulators, GA_3 and IAA, have demonstrated effective plant propagation enhancement. Cu descending levels were statistically significant relative to the control. The soil metal level reduction achieved acceptable levels. More rounds of planting and harvesting, the soil metal concentrations expected to be much lessoned in real sites. Green remediation concepts such as the phytoattenuation and phytoextraction need to be taken as serious concern.

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