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Research Article

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The Phytoattenuation of the Soil Metal Contamination: The Effects of Plant Growth Regulators (GA_3 and IAA) by Employing Wetland Macrophyte Vetiver and Energy Plant Sunflower

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Abstract

The phytoattenuation, a novel green remediation concept, has been successfully demonstrated while employing vetiver and biostimulator (gibberellic acid GA_3 and indol-3-acetic acid IAA) to gradually mitigate the soil [1] Cu levels. The effectiveness of stimulator GA_3 and IAA was in the descending sequence GA_3 > IAA. Biostimulator has been demonstrated plant growth enhancement and been employed for agricultural operation. The on-site tests demonstrated Cu levels were gradually decreasing during 4 months monitoring time periods. The soil metal level reduction achieved a satisfactory level which complied with local environmental standards. After more rounds of planting and harvesting, the soil metal concentration expected to be further dropped while on-site operation was executed. Green remediation concepts such as the phytoattenuation need to be taken as serious concern while the Earth has faced recent unpresdent damage Japan tsunami, Green house effect, unpredicted weather fluctuation worldwide, and serious endangered species issues.

Keywords: The phytoattenuation; Heavy metals; Vetiver (*Vetiveria zizaniodides)*; Sunflower; Biostimulator; Gibberellic acid (GA_3); Indol-3-acetic acid (IAA)

Introduction

The 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 the soil and groundwater resource quality. Regulatory agencies, academia, remediation consulting firms, on-site professional engineers all contribute the proud ten years in terms of the soil and groundwater clean-up contribution. However, some of technologies were un-environmental friendly even detrimental and damage to Taiwan precious the soil and groundwater resources. In Article one of the current Taiwan the soil and groundwater Act, it clearly stated which the soil is a precious nature resources. The 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 the soil acid washing are damage employed in almost every single local environment agency the soil clean-up project. Lot of money, effort and time has been spent during past ten years. Most of the spending is not improving the 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 the soil acid washing simply treat the soil as garbage, waste, and junk, not the soil law indicated the soil is a resource. The purpose of this paper is aimed to raise all you concerns and care toward our precious the 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 the soil damage.

A novel green remediation approach intends to convey in this paper by employing plant to gradually reduce the soil metal contamination through several rounds of planting and harvesting. Unlike phytoextraction, the phytoattenuation aims to reduce the soil metal pollution in a gradually and less aggressive approach such as chelator assisted remediation [1-3]. The initial pollution level generally is lower than most the soil contamination sites. Therefore, plant is easier to propagate to increase biomass inducing reliable metal uptake. The conceptual model is shown in figure 1.

Attenuation is borrowing from the concept "natural attenuation"

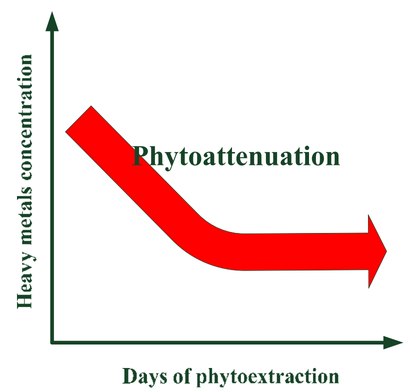

Figure 1:

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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 [4]. 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 the soil environment. The choice of plant is more flexible than phytoextraction. Plant is not necessary to be a hyper accumulator 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 the soil background concentration (Figure 2). 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 the Macrophyte such as the vetiver, the cattail, and the reed which has been demonstrated to be easily propagation and capable to reduce water and sediment metal levels [4]. The harvested plant wastes should be properly managed to prevent the secondary environmental contamination. An alternative plant is the energy macrophyte 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 the soil erosion control due to its unique morphological and physiological characteristics. Vetiver is also a high biomass plant with remarkable photosynthetic efficiency which renders it tolerant against various harsh environmental conditions. Vetiver with deep-rooted and higher water-use can

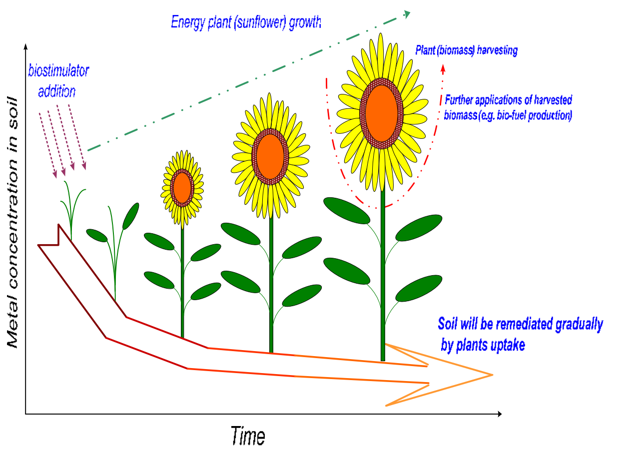

Figure 2: Schematic diagram of pot experiment.

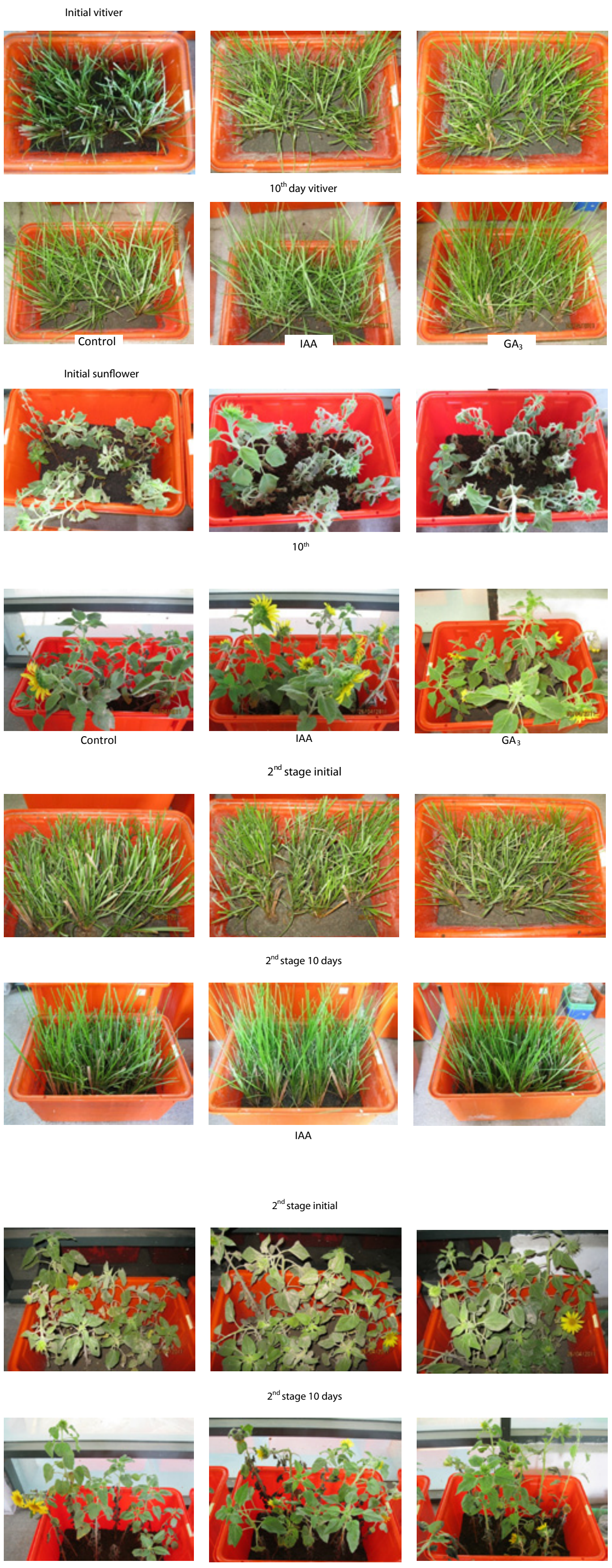

Figure 3:

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Reference	Plant species	Plant uptake concentration (mg/kg)	TF (transfer factor)	BCF (biological concentration factor)	Chelator concentration
Doumett et al. [9]	*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 5 mmol/kg
Epelde et al. [10]	*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. [11]	*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
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: Previous research results.

effectively stabilize soluble metals in the soils [5,6]. These properties enable vetiver to be an ideal candidate for the phytoattenuation and have been investigated in the study. Sunflower (*Helianthus annuus*) is a fast-growing crop which has been commonly used for phytoextraction of metal contaminated the soils. Sunflower has the potential as biofuel to become the substitute of fossil fuels, especially the increasing oil prize in recent years. The higher biomass production of sunflower, contribute them being the candidates of phytoextraction contaminant and then harvested as potential fuel substitution.

The biostimulator has been facilitated the plant growth enhancement and been employed for agricultural operation [7,8]. The stimulators can be borrowed to enhance the vetiver propagation leading to expect the phytoattenuation purpose. Two biostimlators, 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 the phytoextraction have been conducted. The metal removal results were very optimistic. The most updater researched results are shown in table 1. [9-11]. Few if any study was focused on the biostimulator assisted the phytoattenuation. The objectives of this study were aimed to observe the planting and harvesting attenuation cycles were required to achieve feasible the soil metal levels. The effects of the biostimulator, GA_3 and IAA were also scrutinized to reveal the stimulator effect.

Materials and Methods

Plant, the biostimulator, and the 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. The soil used in this study was also collected from the campus wetlands and mixed well before use. Figure 2 presents the schematic diagram of pot experiment. The biostimulators, GA3 and IAA were added to the pots to enhance the removal of Cu in the soil.

Total metal content, the soil retained fractionation and plant metal uptake 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 the deionized water. 0.2 g of dried the 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 Spectrophotometry (AAS, Perkin Elmer).

The fractionation of the soil retained metal was investigated by a sequential extraction technique where the 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 $Na_4P_2O_7$), 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 [12].

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 the soil and plant 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 the soil concentration including total metal and metal fractionation

Table 2 shows the analysis results of the field soil copper concentration. As can be seen, the copper concentrations in the soil ranged from 13.7 to approximately 21 mg/kg. Different state of copper concentration is presented in Table 3.

IAA and GA3 both performed satisfactory vetiver growth enhancement relative to control. GA3 generally possessed better propagation upgrade than IAA. Both biostimulator could employ for the vetiver growth increase which is the merit for further plant uptake. Biostimulators commonly employed in agricultural amendment to enhance produce propagations (Table 2). Appling in contaminated site mitigation was unprecedented and the results were optimistic. The price the addition the biostimulator needs to further evaluate.

The phytoattenuation evaluation

The results of the attenuation study using the vetiver and two stimulators have demonstrated prominent success (Table 3). After 4 cycles of planting and harvesting Cu levels had demonstrated gradually metal decreasing. The control, the stimulator, and stimulator remaining copper levels were progressing descended. Figure 3 shows the observation of the plant growth. The results show that the plants grew well in the copper contaminated soil. This indicates that the plants are feasible to be applied for the phytoattenuation of the copper contaminated soil. Figure 4 show the Cu concentration in different parts of vetiver and sunflower. The results demonstrate that copper in the soil could be adsorbed by the plants, and then distributed to the roots, stems, and leaves of the plant. Table 4 presents the BCF, TF and PEF of vetiver and sunflower. These results were very effective and indicted which the phytoattenuation can be a green alternative to mitigate the soil metal contamination with or without the biostimulator assistant.

Field the soil copper concentration	Initial
A	20.95 ± 4.30
B	22.09 ± 0.41
C	20.69 ± 0.76
D	15.26 ± 0.24
E	18.45 ± 0.56
F	18.87 ± 0.31
G	16.78 ± 0.39
H	14.19 ± 0.29
I	13.85 ± 0.13
J	13.66 ± 0.21

Table 2: Field the soil of copper concentration (mg/kg).

Vetiver	Initial	1th	2th
Control	30.03 ± 0.73	28.05 ± 1.57	26.71 ± 1.33
IAA	34.07 ± 1.54	32.64 ± 0.73	31.03 ± 0.43
GA_3	34.15 ± 0.47	29.74 ± 1.47	27.62 ± 0.48
Sunflower	Initial	1th	2th
Control	31.39 ± 3.2	24.85 ± 0.26	24.90 ± 0.37
IAA	32.50 ± 2.29	29.20 ± 0.51	27.59 ± 0.56
GA_3	30.09 ± 0.43	29.55 ± 0.82	28.25 ± 1.26

Table 3: Different state of copper concentration (mg/kg).

Factors	Plant	Treatment	1st	2nd	3rd
BCF	Vetiver	Control	1.91 ± 0.14	1.18 ± 0.23	0.68 ± 0.06
		IAA	1.31 ± 0.24	0.94 ± 0.07	0.77 ± 0.10
		GA_3	1.74 ± 0.18	1.17 ± 0.09	0.84 ± 0.15
	Sunflower	Control	1.00 ± 0.34	0.35 ± 0.05	0.33 ± 0.01
		IAA	0.55 ± 0.10	0.66 ± 0.32	0.48 ± 0.22
		GA_3	0.70 ± 0.10	0.57 ± 0.09	0.62 ± 0.13
TF	Vetiver	Control	0.34 ± 0.05	0.61 ± 0.14	0.79 ± 0.27
		IAA	0.33 ± 0.05	0.61 ± 0.14	0.68 ± 0.13
		GA_3	0.22 ± 0.05	0.58 ± 0.17	0.63 ± 0.24
	Sunflower	Control	0.77 ± 0.14	0.64 ± 0.12	0.71 ± 0.07
		IAA	0.94 ± 0.30	0.72 ± 0.27	1.10 ± 0.24
		GA_3	0.77 ± 0.24	0.72 ± 0.46	0.82 ± 0.26
PEF	Vetiver	Control	0.10 ± 0.01	0.11 ± 0.01	0.08 ± 0.02
		IAA	0.07 ± 0.02	0.09 ± 0.02	0.08 ± 0.01
		GA_3	0.06 ± 0.01	0.10 ± 0.02	0.08 ± 0.02
	Sunflower	Control	0.24 ± 0.11	0.07 ± 0.02	0.07 ± 0.01
		IAA	0.16 ± 0.05	0.13 ± 0.03	0.16 ± 0.08
		GA_3	0.17 ± 0.07	0.13 ± 0.09	0.14 ± 0.01

Table 4: BCF, TF and PEF of vetiver and sunflower.

wavelenght	functional group	plant
3300~3500	N-H in amines and amides	Vetiver Sunflower
3400~3200	-OH stretch from –COOH and –COH	Vetiver Sunflower
1600~1650	Aromatic –C=C vibration, -C=O stretch from H-binded conjugated ketones, and –COO- asymmetric stretch1,2	Vetiver Sunflower
1000~1200	C-O stretch of polysaccharides	Vetiver Sunflower
1080	Si-O bending	The soil

Table 5: The FTIR analysis results of the soil and the root of sunflower and vetiver.

Figure 5 shows the FTIR diagram of the soil and the root of sunflower and vetiver, and the results are presented in Table 5. The results show that functional groups including N-H in amines and amides, OH stretch from –COOH and –COH, Aromatic –C=C vibration, -C=O stretch, and C-O stretch of polysaccharides were presented in the roots of vetiver and sunflower. The functional groups could help the roots adsorb copper, resulting in the remove of copper from soil.

Three stage of the phytoattenuation observation demonstrated positive medium to low contaminated level the soil mitigation which can be used for further similar site application. The phytoattenuation though is not effective for high level metal contamination while it is environmental friendly without using dig-and –dump rather EDTA the chelation enhancing expected to be well received worldwide.

Conclusion

Vetiver 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 planting and harvesting. 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.

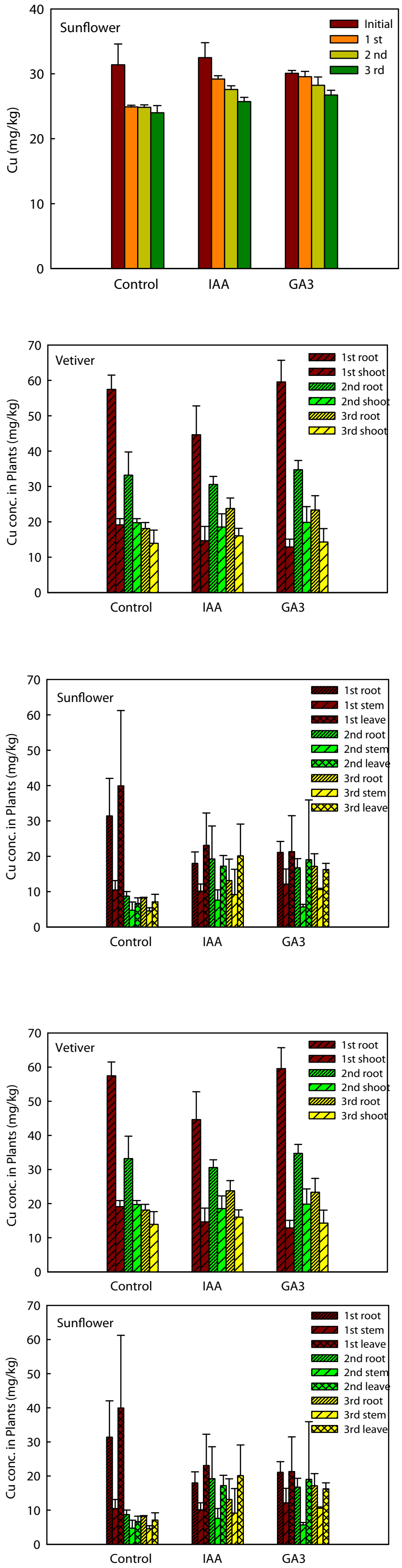

Figure 4: Cu concentration (mg/kg) in different parts of vetiver and sunflower.

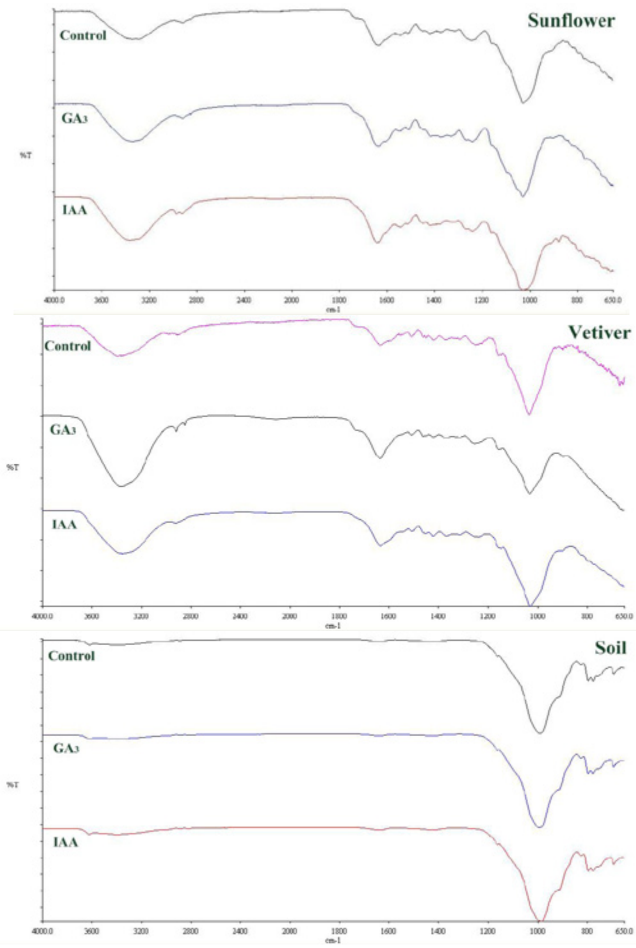

Figure 5: The FTIR diagram of the soil and the root of sunflower and vetiver.

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