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Phytoremediation of Lead Polluted Soil Around Mechanic Workshop in Afor Oru Ahiazu Mbaise using Waterleaf (*Talinum Triangulare*)

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Abstract

Pollution of soil with heavy metals is a major environmental concern due to the disposal of urban and industrial waste generated by mostly anthropogenic activities. Thus, through Phytoremediation which is a plant-based approach, polluted soils are remediated using plants. In this research, the potential of Waterleaf (Talinum Triangulare) to absorb Lead from composite soil sample and consequently to remediate it,was investigated.A farmland around a Mechanic workshop located at Afor Oru Market in Ahiazu Local Government Area, Imo State was used . An uncontaminated soil was gotten from a piece of farmland in Oru Community in Ahiazu Mbaise, Imo State. This land has been lain fallow for more than five years. The Waterleaf (Talinum Triangulare) stands of 26days were collected from the nursery bed in Umuireaku Oru community in Mbaise. The contaminated Soil samples and Plant samples were analyzed for Lead concentration before and after graded periods of growth for six weeks using the Atomic Absorption spectrophotometer (AAS). The contaminated soil samples contained lead at 0 week as 438.499ppm, 2 weeks as 413.217ppm, and 4weeks as 387.935ppm and 6weeks as 362. 431ppm. The uncontaminated soil sample which served as the control sample contained 2. 310ppm.Plant samples contained 2.948ppm at 0week, 3.327ppm at 2weeks, 3.726 at 4weeks and 4.125 at 6weeks. However, after six weeks of planting the result showed reduction of Lead concentration in the soil by 17.4% and accumulation in the waterleaf (Talinum Triangulare) by 39.9%. The transfer factor for the Lead heavy metal assayed increased steadily. Thus, this demonstrated the ability of waterleaf (Talinum Triangulare) to absorb Lead heavy metal and consequently remediate the contaminated soil sample.

Keywords: Phytoremediation, Heavy metal, lead, Water leaf, Bioaccumulation, Transfer factor

INTRODUCTION

Over the years, the different components of the environment have been threatened mainly by anthropogenic activities. The soil is the most important component which is the most undervalued, abused and misused of the earth's resources (Gokul and Balamurugan, 2010). The soil forms an integral part of our agricultural resource and plays a vital role in green revolution and food security. Due to rapid urbanization and industrialization, agricultural lands have become increasingly contaminated by organic, inorganic and metallic pollutants (Sivarajasekar *et al.*, 2008, 2018b; Sivarajasekar and Baskar, 2014a, b, c). This affects the ecosystems, surface, subsurface, ground water, food quality and health (Ayoub *et al*., 2010; Sivarajasekar and Baskar 2015a, b). Heavy metals are obtained from natural and anthropogenic sources. Naturally, heavy metals are associated with the volcanic eruption, wind erosion, forest fire and fossil fuel usage in 0general. The metals from the natural sources cause less harm in the environment in general, whereas the metals from anthropogenic sources such as smelters, thermal power plants, mines, foundries pose a threat to mankind (Nagajyoti *et al*., 2010; Tangahu *et al*.,2011; Jaishankar *et al*., 2014).

In Nigeria today, there are several ways in which lead can be transmitted to plants, animals and humans.The include smelting, recycling, stripping leaded paint, and using leaded gasoline or leaded aviation fuel; and ingestion of lead-contaminated dust and water (from leaded pipes),and food.Lead exposure can also results from inhalation of lead particles generated by burning materials containing lead, for example -glazed or lead-soldered containers) (WHO, 2010). Furthermore, there is a need for technologies to remediate contaminated lands (Sivarajasekar, 2014).

Series of approaches have been implemented to reclaim soil contaminated with heavy metals through phytoremediation. Phytoremediation is a plant-based technology employed either raw or genetically modified plant species for restoring contaminated land and water sources (Parmar and Singh, 2015). It is a reliable green technology which also helps in mitigating or reversing the effects of human activities on the environment.

There are many phytoremediation techniques, this includes Rhizofiltreration, phytostabilisation, phytoextraction, phytovolatilization and phytodegradation. Phytoextraction is the technique used in this study. Phytoextraction is also known as phytoaccumulation. It is the process where plants absorb the contaminants from contaminated sites along with other nutrients and water required for their growth. The absorbed contaminant is not destroyed but get accumulated in the shoots, leaves and other plant parts (Rashid *et al*., 2014). The pollutant is sequestered in the cell of the plant until it is harvested. This technique is promising as it helps to mitigate the effects of anthropogenic activities on the soil.

The major objectives of this study are: Preparing soil and plant samples from the selected study area, Determining the Transfer Factor which is the transfer of the heavy metal from the soil to the plant body, Determining the metal uptake rate that is the rate at which the plant sample remediated or Bio accumulated the lead pollutant, soil pH before and after analysis, Comparing the levels of concentration of lead in the soil and plant samples after experiment analysis and Suggesting possible measures to manage the contamination to ensure safety to human, animal and the soil environment.

MATERIALS AND METHODS

Study Site

The study area was chosen in the south-east geopolitical region of the Federal Republic of Nigeria. This study area is located at Oru community in Ahiazu Mbaise Local Government Area of Imo State, Nigeria. It has its headquarters at Afor Oru. Oru community is located on a latitude of 5°32’27.96” North and Longitude 7°16’6. 42” East.The study area is the artisanal mechanic workshop located in the vicinity of the Afor Oru Market in Oru community in Ahiazu Mbaise. The area was selected based on the multi- activities involving vehicle and motorcycle repair and maintenance that leads to the production of toxic waste such as carbide, emission of leaded gasoline, engine oil, grease and crude oil.

Population and Activities

Oru community in Ahiazu local Government Area has an area of 111km2 (43sq mi) with its headquarters at Afor Oru. The population growth according to the last population census in 2006 was 170,902. Oru community in Ahiazu local government is blessed with the biggest market in Mbaise. This market is known as Afor Oru Market. It deals with all sorts of business activities. It deals with the selling and buying of assorted goods. It is known as the biggest market in Mbaise because many people from different parts of the country come there to buy and sell their goods. It also deals on Timber sales. In terms of services rendered in Afor Oru market place is the artisanal mechanic workshop. This is of utmost importance because of the use and disposal of toxic waste like leaded gasoline, engine oil and crude oil by the artisanal mechanics into the soil environment and emissions of leaded gasoline into the environment by moving vehicles and motorcycles.

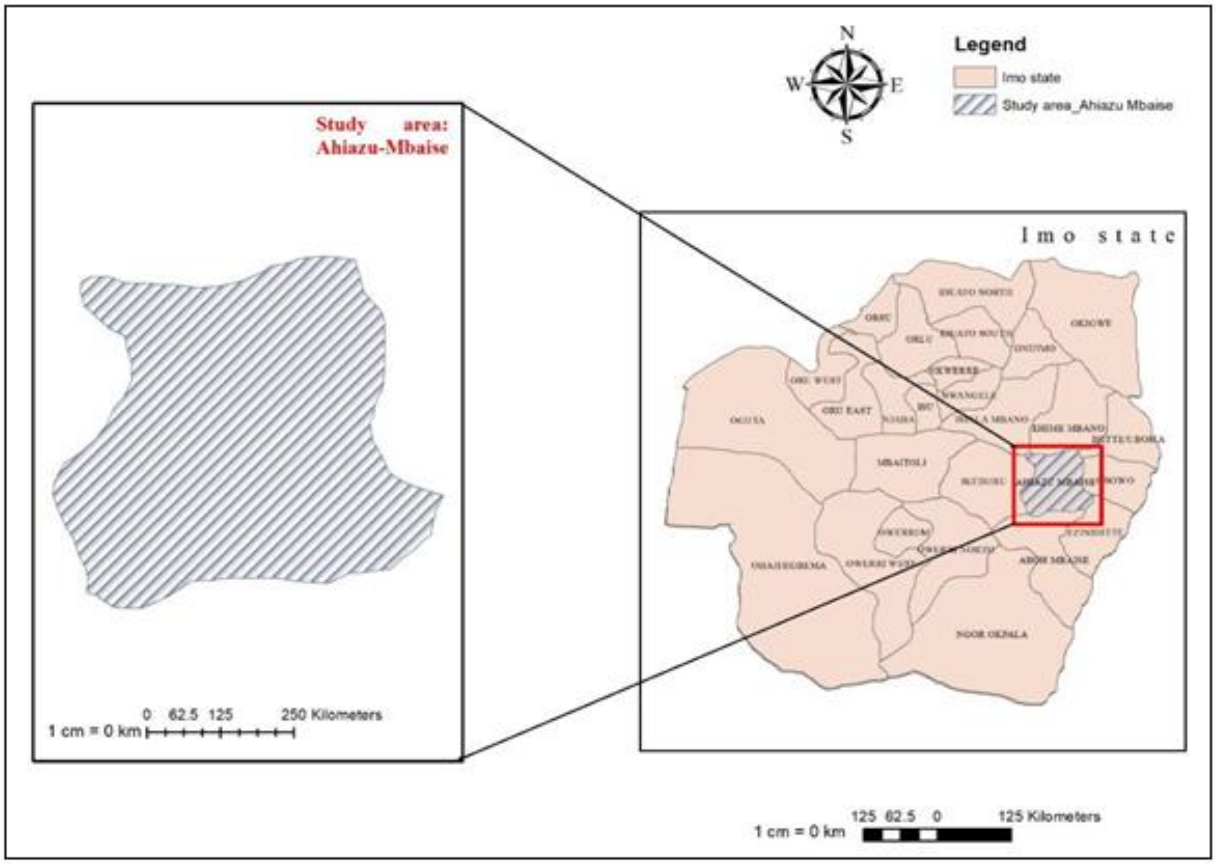


Fig: 1. Map of Study Area

Plant Sample

The plant sample used in this research is water leaf (*Talinum Triangulare*). This is selected based the fact that it has the ability to tolerate and accumulate lead (Aiyesanmi *et al*., 2012).

Soil Sample

The soil samples were collected from the farmland around the mechanic workshop at Afor Oru Ahiara, Ahiazu Mbaise (Imo State, Nigeria).

Sampling Equipment

The following materials and equipment were used. They include polyethylene bags, buckets, hand auger, cutlass, hand gloves and Shovel.

Plant and Soil Sampling

Water leaf (*Talinum Triangulare*) was used in this research and was selected based on the fact that it is a fast-growing plant, good accumulator and tolerate lead (Aiyesanmi *et al.*, 2012). The Waterleaf (*Talinum Triangulare*) stands of about 26days or more were collected from the nursery bed in Umuireaku Oru community in Mbaise. This place is not common with anthropogenic activities. In this study, two sets of soil samples were collected. Soil sample A (Contaminated soil) was collected from the farm around the mechanic workshop at Afor Oru market in Ahiazu Mbaise (Imo state, Nigeria). This place is well known for its anthropogenic activities. The farm was first cleared of debris and then the topsoil was taken at depths of 0.5cm to 5cm using the hand auger. The samples from this site were homogenized to obtain an individual composite Soil sample. Four kg of the composite soil sample was then filled into the polyethylene bag.

Soil sample B (uncontaminated soil) was collected from a piece of land at Umuireaku village in Oru community of Ahiazu Mbaise (Imo state, Nigeria). This farmland has been led fallowed for more than five years. The farm was as well firstly cleared of debris and then the topsoil was collected at the deaths 0.5cm to 5cm using the hand auger. The samples were then homogenized to obtain a composite sample. Twenty kilogramme of the soil sample was then filled into the polyethylene bag. The soil sample B (uncontaminated soil) would then act as a control sample.

Experimental Design

Four Kilogramme of the composite soil samples was weighed into each of the three plastic buckets, that’s to say that four kilogramme of composite soil sample was weighed into three plastic buckets and then also four kg of uncontaminated composite sample was also weighed into the one plastic buckets. The buckets will be labelled as (bucket+2wks), (bucket + 4wks) and (bucket + 6wks) and the uncontaminated soil as (control bucket). the were then taken into the greenhouse. Five stands of plant samples were planted in each of the three plastic buckets and watered with fifty ml of water for eight hours according to (Yee *et al.,* 2013). At an interval of every two weeks, ten gramms of the soil samples and five plants stands from each of the groups were harvested, pretreated and analyzed for Lead content concentrations. The last was harvested at week 6.

Plate1: Plant growth for the different growth periods

However, the initial Lead concentration in plant sample and contaminated soil sample were first analyzed for, that is ten gm of the soil sample and five stands of water leaf were collected and stored in sample bags and labelled (0weeks soil) and (0weeks plant). The initial Lead concentration of the samples were analyzed for.

Laboratory Analysis

Apparatus for Soil and Plant Analysis

The following apparatus were used in the laboratory analysis: Beaker, Burette, Conical Flask, Erlenmeyer flask, electronic weighing balance, filter paper, funnel, water bath, measuring cylinder, Mortar and pestle, PH meter, Sieve and extraction cupboard.

Spectrophotometer methods were applied using the Atomic Absorption Spectrophotometer (Agilent Technologies, 200 Series AA) to the metal, using the wavelength of 520nm.

Reagents

The reagents used were hydrochloric acid and Nitric acid (aqua regia method).

Pretreatment

Preparation of Plant Sample

Five stands of Water leaf (*Talinum Triangulare*) from each of the groups in the buckets (Contaminated soil) were washed under tap water and then into two changes of distilled water. The samples were air dried for twenty-four hour and then oven dried in an air circulatory oven at (80°c) for five hrs. The samples were crushed with mortar and pestle and then sieved using a screen of 0.25mm openings (60 mesh size).The sieved samples were stored in plastic bags, labelled according to their week of harvesting and then digested. They analytes were filled in vial bottles and then stored in the refrigerator for the Lead analysis.

Preparation of Soil Sample

Ten grammes of the composite samples were air-dried for 24hr and then oven dried in an air circulatory oven at (80°c) for 5hrs.The soil samples were then ground with mortar and pestle. The powder was passed through 0.25mm openings (60 mesh size). The sieved samples were then stored in plastic bags and labelled accordingly and then digested. The analytes were filled into vial bottles and then stored in the refrigerator until due for the Lead content analysis.

Digestion of Samples

The Powdered Samples (Soil and plant) were digested (Kudirat *et al.*, 2011). Five grammes of each of the powdered samples was weighed in an analytical weighing balance. They were weighed into 250ml digestion flask. A 20ml of 10:1 digestion mixture (hydrochloric acid and nitric acid) was added. The mixture was heated in a water bath at approximately 95^{O}C until the whole sample dissolved and white fumes were observed. The solution was then allowed to cool to room temperature, filtered using a What man No. 42 filter paper into beaker and made up to 50ml mark with deionized water.

Lead Analysis

The concentrations in ppm of the metal in the filtered samples were determined by means of an Atomic Absorption Spectrophotometer (Agilent Technologies, 200 Series AA). Specific metal standards in the linear range of the metal were used to calibrate the equipment. The metals were analyzed by direct air-acetylene flame method (APHA 3111-C). The concentrated and digested samples were then aspirated and the actual concentrations were obtained by referring to the calibration graph and necessary calculations.

Analysis for PH of Soil Sample

Contaminated Soil sample of twenty gramme was weighed and then added to 20 ml of distilled water in the ratio of 1:1. This was left for 30 minutes but was stirred intermittently. The electrode pH was rinsed with distilled water and dipped into the sample solution and a stable reading was recorded. The procedures include

a) Dry the electrode by gently wiping with soft tissue.
b) Calibrate the pH meter as follows:
c) Immerse the electrode in freshly prepared buffer pH 4 for solution (if required adjust the pH meter reading to s pH 4)
d) Remove the electrode from the buffer
e) Rinse thoroughly with distilled, water and dry with soft tissue.
f) Repeat same for buffer pH 7 and buffer pH 9 solutions, adjusting if required
g) Confirm the calibration by immersing the electrode again in buffer pH 4, pH 7 and pH 9 solutions Mix the sample thoroughly to provide homogeneity
h) Weigh 20g of the soil samples into a beaker
i) Add 20ml of distilled water, stir and allow to stand for 30mins
j) Immerse the electrode into the beaker containing the sample and read the pH on the pH meter.
k) Note the temperature and record.
l) Rinse the electrode with distilled water and immerse in a beaker containing distilled water Until the Next Measurement.

Phytoremediation Determination

In other to evaluate the potential Phytoextraction capabilities of plant species, two indicators are commonly checked for. They include Transfer factor and metal uptake rate

Determination of Transfer Factor (TF)

Heavy metal transfer factor (TF), also called accumulation Factor (AF) (Balkhair *et al*., 2016) or bio-concentration factor (BCF) (Sakizadeh *et al.*, 2016) describes the transfer of the metal from soil to the plant body. This was calculated by dividing the concentration of the heavy metal in the plant (Cp) by the corresponding concentration in the soil (Cs) i.e., Tf = $\frac{\text{Cp}}{\text{Cs}}$ (Rashid *et al.*, 2016).

Determination and Comparison of Rate of Remediation and Metal Uptake Rate

The rate at which the plant sample remediated or abstracted/accumulated heavy metal from the polluted soil was calculated as: R = Amount of metal lost by the soil or gained by the plant expressed in percentage of initial amount. i.e.

$$\text{R (\%)} = \frac{C^{\text{nth week}} - C^{\text{0th week}}}{C^{\text{oth week}}} \times \frac{100}{1}$$

Where, C^{nth} are concentrations of the heavy metals in the soil or plant sample at time

(Weeks) = “n” and “0” respectively.

Determining Transfer Factor

This was calculated by dividing the concentration of the heavy metal in the plant (Cp) by the corresponding concentration in the soil (Cs) i.e., Tf = $\frac{Cp}{Cs}$ (Rashid *et al*., 2016).

Determination and Comparison of the Rate of Remediation and Accumulation

The rate at which the plant sample remediated or abstracted/accumulated heavy metal from the polluted soil was calculated as: R = Amount of metal lost by the soil or gained by the plant expressed in percentage of initial amount. i.e.

$$R\ (\%) = \frac{C^{\text{nth week}} - C^{\text{0th week}}}{C^{\text{oth week}}} \times \frac{100}{1}$$

Where, C^{nth} are concentrations of the heavy metals in the soil or plant sample at time

(Weeks) = “n” and “0” respectively. This was calculated both for the rate of metal uptake rate from the soil and for the rate of accumulation of the metal in the plant sample at different growth periods and then compared. This was actually done to know the percentage increase in the plant and percentage decrease in the soil at a particular time interval (week). This would actually help to quantify the potentiality of the plant (waterleaf).

RESULTS AND DISCUSSION

From the laboratory analysis conducted, the Lead heavy metal was found to be reducing in the contaminated soil sample and increasing in the plant. This is represented in table 1.

Absorbance and Concentration of the Soil Sample

The results from (Table 1) shows that there is steady decrease in the absorbance of the soil and also steady decrease in the concentration (quantity) of the soil with respect to the growth periods. This means that the less the concentration in the soil, the less its absorbance through the growth periods. Thus, indicating reduction of Lead heavy metal in the soil sample with Time.

Absorbance and Concentration of the Plant Sample

The results from (Table1) also shows that there is steady increase in the absorbance of the plant sample and also steady increase in the concentration (quantity) of the plant with respect to the growth periods. This means that the more the concentration in the plant, the more in its absorbance. Thus, indicating accumulation of the Lead heavy metal in the plant biomass with Time.

Analysis of Soil Samples at Different Growth Periods

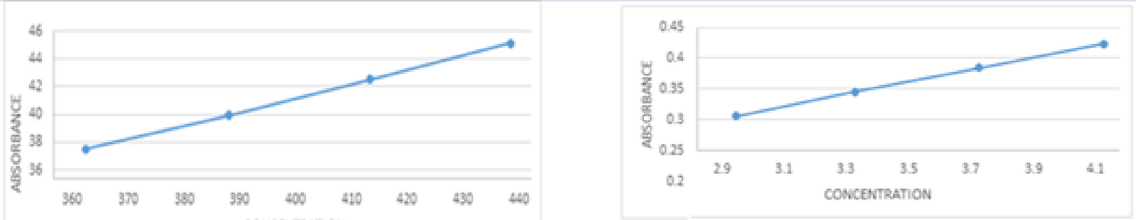
The results from (figure 2a) shows that there is steady decrease in the level of heavy metals in the contaminated soil at different growth periods. The first analysis on the soil showed that Lead was at level of 438.499(ppm). By comparison with the control which was 2.310 (ppm), the level of Lead in the contaminated soil was so high. However further analysis on the soil showed a steady decrease in the level of Lead. According to Cunningham *et al.,* (1996), heavy metals are not degradable but are bio-accumulative. This shows that some of the Lead compound in the soil are accumulating into the plant biomass through the plant root (Salt *et al*., 1995).

Table: 1 Analysis Result for Both the Soil (Contaminated and Uncontaminated) and Plant Sample

Sample	Absorbance	Concentration (PPM)
Initial Waterleaf	0.306	2.948
Initial soil (Contaminated Soil)	45.125	438.499
Uncontaminated Soil (Control)	0.248	2.310
Two Weeks Waterleaf	0.345	3.327
Two Weeks Soil (Contaminated Soil)	42.523	413.217
Four Weeks Waterleaf	0.384	3.726
Four Weeks Soil (Contaminated Soil)	39.42	387.935
Six Weeks Waterleaf	0.423	4.125
Six Weeks Soil (Contaminated Soil)	37.531	362.431

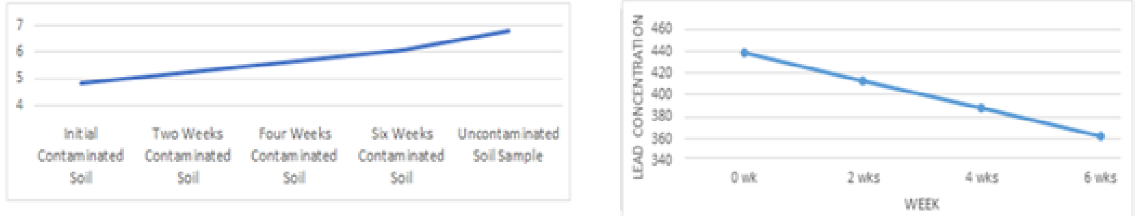
The Soil PH of the Contaminated Soil Sample

From the (figure 2c,d), the pH of the contaminated soil in the first analysis was 4.85. This indicates acidity, which can be attributed to the presence of contaminants in the soil sample such as engine oil, petrol oil, and acid from car batteries and other by-products from automobiles. The result indicated that the PH of the soil was increasing (from acidic to neutral). Increased soil PH is not good for metal solubilization, because metals are more soluble at low PH (Huang *et al.*, 1996). The increased PH indicates effective phytoremediation (Cunningham *et al.*, 1996).



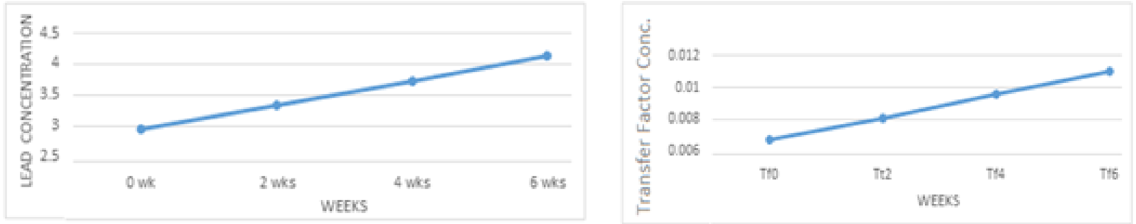
a) Contaminated Soil Sample

b) Plant sample



c)PH of the Contaminated Soil at temperature of 25°C

d) Analysis of Contaminated Soil Sample



e) Analysis of Plant Sample

f) Transfer Factor for Different Growth Periods

Figure 2: Soil and plant sample analyses.

Analysis of Plant Samples at Different Growth Periods

From (figure 2b), the level of Lead in the Waterleaf (*Talinum Triangulare*) increased steadily at different growth periods. The level of Lead in the first analysis (initial concentration) was 2.948(ppm) and then at end of growth period (sixth week) was 4.125(ppm). However, it can be deducted that the level of Lead in the plant biomass increased steadily throughout the period of growth.

Transfer Factor at Different Growth Periods

From (figure 2f), it can be deducted that the transfer factor to the plant biomass increased steadily with the growth periods. This however shows effective accumulation rate in the plant biomass.

Table 2: Determination for the Rate of Remediation from the Soil and Metal Accumulation in plant

	Growth Period	*2weeks*	*4weeks*	*6weeks*
Rate of Remediation from the Soil	Rate of Remediation/ Reduction (%)	-5.77	-11.5	-17.4
Rate of Metal Accumulation in the Plant	Rate of Accumulation/ Increase (%)	12.9	26.4	39.9

Determination and Comparison of the Rate of Remediation (Reduction in the Soil) and Accumulation (Increase in the Plant)

The results from (figure 3), shows that the rate of reduction at two weeks(2weeks) was 5.77% and the last week(6weeks) was 17.4%. This means that there was steady increase in reduction rate in the concentration of Lead in the soil as a result of the growth of the plant (Waterleaf) on the soil samples for the six weeks. However, it can be deducted that the remediation using Waterleaf (Talinum Triangulare) is effective because of the steady reduction of the concentration of Lead in the soil.

Also, the results from (figure 3), shows that the rate of a metal-uptake for the two weeks (2weeks) was 12.9% and the last week (6weeks) was 39.9%. This means that there is steady increase of the concentration of Lead in the plant biomass which is because of the continuous increase in the plant biomass because of its continuous growth throughout the six weeks growth periods.

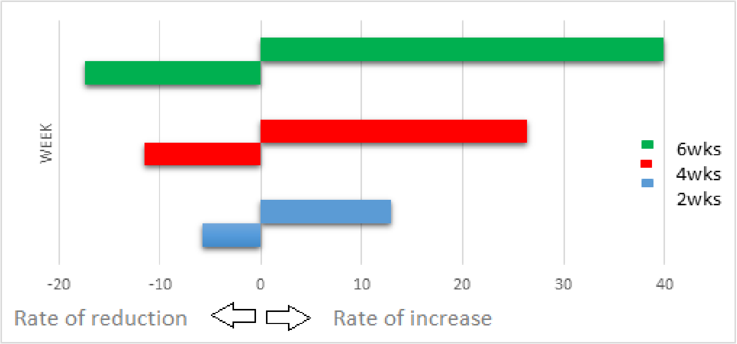


Figure: 3. Visual comparison of the Rate of Metal Remediation from the Soil to the Rate of Metal Accumulation the Plant

However, the pictorial comparison of the Lead remediation/abstraction (From Left) and the corresponding metal-uptake/accumulation (From the right) by the Waterleaf *(Talinum Triangulare*) sample (Plate 1) was obtained as the amount of metal lost by the soil or gained by the plant respectively, expressed as the percentage of initial amount showed the same pattern but differed in magnitude.

Considering Lead by the 6th week, 17.4% was lost from the soil while the same time 39.9% was gained by the plant sample. This indicates that metal-uptake rate by plants is not a function of metal concentration in the material medium only. It is also affected by other factors like pH and the distribution of metal in different soil fractions and also the nutrient management and climatic conditions which indirectly affect the transfer processes through their control of major variables that affect plant growth such as temperature, water regime and humidity. These incidental factors could be controlled to optimize the transfer processes enabling effective application of Waterleaf (*Talinum Triangulare*) in phytoextraction of the assayed heavy metals from pollute soil.

CONCLUSION

Based on the data in this study, it can be concluded that plant-based technologies for removing and detoxifying toxic trace elements from contaminated soil is effective. From the results of this research, Waterleaf (*Talinum Triangulare*) an annual vegetable is a good hyper-accumulator and can be used in phytoremediation. The use of phytoremediation is fast developing all over the world.

Based on the findings from this study, it is recommended that: Consumers of this leafy vegetable from such farmland with contaminated soil should be screened before any incident of heavy metal contamination in the food chain, More researches on phytoremediation should be carried out to upgrade existing methods, Both government and environmental agencies should encourage environmental programs on risks and dangers associated with Lead pollution and there should be preventive measures and contingency plans for an effective control of waste that are been generated.

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