

# Effectiveness of arsenic phytoremediation using *Ludwigia octovalvis* in a pilot reed bed system

[Harmin Sulistiyaning Titah, Siti Rozaimah Skeikh Abdullah, Mushrifah Idris, Nurina Anuar, Hassan Basri, Muhammad Mukhlisin ]

**Abstract**— *Ludwigia octovalvis* (Jacq.) P.H. Raven is a terrestrial tropical plant in Malaysia having the ability to uptake and accumulate arsenic in their tissue. The effectiveness of arsenic phytoremediation by *L. octovalvis* in pilot reed bed system using arsenic spiked sand was conducted to determine the feasibility using this plant in a large scale of phytoremediation. The depth of arsenic spiked sand in a reed bed system was 10 cm with another two fine and medium gravels layer below, each at 10 cm depth. All treatments were carried out without addition of fertilizer, bacteria or aeration. The higher arsenic uptake and accumulation reached  $528.5 \pm 68.3$  mg/kg in leaves of *L. octovalvis* after 42 days of exposure at arsenic concentration of 39 mg/kg. Based on the translocation factor (TF) and bioaccumulation factor (BF) values, indicating phytoextraction has role process in arsenic phytoremediation using *L. octovalvis* in a pilot reed bed system using spiked sand. The effectiveness of arsenic phytoremediation increased with time exposure. The effectiveness uptake percentages of total arsenic mass at concentrations of 5, 22 and 39 mg/kg on Day 42 were 100, 43.8 and 18.5% respectively. It was concluded that *L. octovalvis* has the potential to be used in pilot arsenic phytoremediation.

**Keywords**— arsenic, uptake, accumulation, performance, pilot reed bed, effectiveness.

## I. Introduction

Arsenic (As) is listed as the first hazardous substance according to the United State Agency for Toxic Substances and Disease Registry (ATSDR) [1]. Commonly sources of As contamination were mine waste (primarily sulfide, iron and tin), tanneries, metal smelters and geothermal activity. As also has been used in the following were embalming fluids, paint pigments, insecticides, herbicides, defoliant and metal alloys [2]. The toxicity and redistribution of arsenic in the environment make it evoke public concern [3].

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One of green technologies to remediate arsenic is phytoremediation. Phytoremediation takes advantage of the unique, selective and naturally occurring uptake capabilities of plant root systems, together with the translocation, bioaccumulation and pollutant storage/degradation abilities of the entire plant tissue [4]. Phytoremediation includes several processes namely, phytoextraction, phytodegradation, rhizofiltration, phytostabilization (phytosequestration), phytovolatilization and phytohydraulics [5]. Phytoremediation can be conducted in situ or ex situ method [6]. Based on ex situ method, the phytoremediation can be carried using a reed bed. Reed bed system is designed to simulate the microbiological, chemical and physical processes that occur naturally. A reed bed system works through cleansing power of three elements involving soil dwelling microbe, the physical and chemical properties (soil, sand or gravel) and finally the plant themselves [7]. The effectiveness of phytoremediation is, however, inconsistent and depends on several factors associated with soil and plant characteristics as well as the chemistry of arsenic in the environment [8]. Several soil amendments like lime, phosphate (P), compost are used to enhance the effectiveness of phytoremediation [9]. According to Memon [10], some systems can be used to enhance the effectiveness of phytoremediation, such as selection plant species as varieties with high potential of metal accumulation, using different agricultural method such as adjusting pH and adding chelates agent to increase the bioavailable of heavy metal, and employing biotechnological methods to increase the ability of the plants to enhance the remediation rate of the polluted areas.

*Ludwigia octovalvis* has been described as a plant that can survive on a contaminated site in Malaysia [11]. In Malaysia, it is known as “buyang samalam”, “lakom ayer” and “pujang malam”. *L. octovalvis* is a cosmopolitan plant with a mainly tropical distribution. Based on a previous study, *L. octovalvis* could uptake and accumulation of arsenic in their tissue [12].

The objective of this study was to determinate the effectiveness of arsenic phytoremediation by *L. octovalvis* using arsenic spiked sand in a pilot reed bed system.

## II. Materials and Methods

### A. Design of pilot reed bed

The pilot reed beds were constructed of fiberglass tanks, the walls of which were 5 cm in thickness and black in color with dimensions of 92 x 92 x 60 cm (Figure 1). A layer of medium gravel ( $\Phi$  in 20 mm) was placed at the bottom of the

reed bed, and another layer of fine gravel ( $\Phi$  in 10 mm) was placed at the top. The depth of both the medium and fine gravel layers were 10 cm. Based on our previous study [13], the depth of arsenic spiked sand as the top layer in the similar size of pilot reed bed was 30 cm indicating could cause toxic effect on *L. octovalvis* so that the arsenic spiked sand in this study was reduced to only 10 cm.

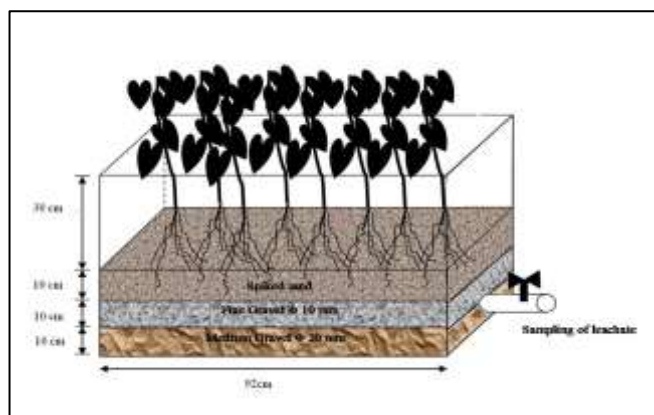


Figure 1. Design of the pilot reed bed.

## B. Propagation of the plant species

*L. octovalvis* species were propagated from seeds in a greenhouse using garden soil. The seeds were planted in plastic crates (37 x 27 x 10 cm). After 3 weeks, individual seedlings were transferred into polybags. All of the plants used in the experiment were 8 weeks old.

## C. Spiked sand preparation and sampling

The sand used for study, first analyzed to determine the content of macro nutrient, micro nutrient and trace element. Based on the analysis, the sand contained macro nutrients of 29.2 mg/kg N (nitrate) and 1.2 mg/kg K, 13.0 mg/kg  $\text{SO}_4^{2-}$ , 86.5 mg/kg Ca, 7.4 mg/kg Mg and micro nutrient of 6.4 mg/kg Cl, 5.5 mg/kg Fe, 0.04 mg/kg Zn and 1.62 mg/kg Mn. The trace elements were not detected.

The sand was spiked with an arsenic salt, sodium arsenate dibasic heptahydrate ( $\text{AsHNa}_2\text{O}_4 \cdot 7\text{H}_2\text{O}$ ) (Sigma Aldrich, USA). The following four different concentrations of arsenic were prepared: 5, 22, and 39 mg/kg including one tank acting as control. The arsenic concentrations were selected based on a range finding test as reported by Titah et al. [14]. After sand being spiked with arsenic, each reed bed was planted with 100 healthy *L. octovalvis* plants. All treatments were carried out without additional fertilizer, rhizobacteria or aeration.

The reed beds were watered every two days. The moisture content of the spiked sand was monitored using a moisture meter (Decagon, USA). Three plants from each concentration (i.e., each pilot reed bed) were harvested after 14, 28, and 42 days of exposure. The fresh and dry weights were measured for each part of the sampled plants (roots, stems, and leaves).

All of the plant parts were dried in an oven at 80 °C for two days for the dry weight measurement [15].

Sampling of the spiked sand was conducted after 0, 14, 28, and 42 days of exposure and monitored for pH, temperature and Oxidation-Reduction Potential (ORP) using a pH meter (Cyberscan pH 300, Singapore). All samplings were conducted in three replicates.

## D. Analysis of arsenic in sand and plant

The total extractable arsenic concentration in spiked sand was determined using wet digestion method [16]. All of the samples were placed in hot block tubes, and the hot blocks were placed in a block digester (AIM 600 Digestion System, Australia). Sand extraction to determine arsenic bioavailable was carried out using a method described by Quevauviller [17]. The supernatant was collected in polyethylene bottles and stored at 4 °C for further arsenic analysis.

The plant materials (roots, stems and leaves) were dried prior to the extraction procedure. In this experiment, the arsenic extraction from the plants was performed using a modified wet digestion method [18, 19, 20]. The arsenic content in sand and plant was analysed with an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), Optima 7300DV Perkin Elmer (USA).

## E. Statistical analysis

All of the experimental data were subjected to an analysis of variance (ANOVA) using SPSS, version 17.0 (IBM, USA). All the experiments were performed in triplicate to compensate for experimental errors and are reported as mean  $\pm$  standard deviation (SD). Statistical significance was defined as  $p < 0.05$ .

## III. Results and Discussions

Figure 2 shows that profile of *L. octovalvis* dry weight increased till the end of experiment (Day 42) at arsenic concentration of 5 mg/kg. However, the fresh weight increased till Day 28. At arsenic concentrations of 22 and 39 mg/kg, the dry weight gradually increased, and then, it shows stable trend till the end of experiment. According to Rattanawat et al. [21], the decreasing of dry weight of biomass is one of the symptom of toxic effect of heavy metal on plant. Based on Figure 2, the increasing and stable condition of dry weight indicating that plant could grow during phytoremediation. Based on ANOVA analysis, the fresh weight shows a significant difference ( $p < 0.05$ ) for arsenic concentration of 39 mg/kg, however, the dry weight shows no significant difference ( $p > 0.05$ ) for all arsenic concentrations.

Figure 3 illustrates the arsenic uptake and accumulation of *L. octovalvis*. Among all of the concentrations, the higher arsenic uptake and bioaccumulation during the exposure was  $528.5 \pm 68.3$  mg/kg in leaves of *L. octovalvis* after 42 days of exposure in the highest arsenic concentration of 39 mg/kg. The high arsenic uptake and bioaccumulation at 5 and 22 mg/kg also occurred in leaves with  $205.6 \pm 72.4$  and  $413.4 \pm 84.6$  mg/kg respectively. Based on ANOVA analysis, the arsenic

uptake and accumulation by the whole plant of *L. octovalvis* shows a significant difference ( $p < 0.05$ ).

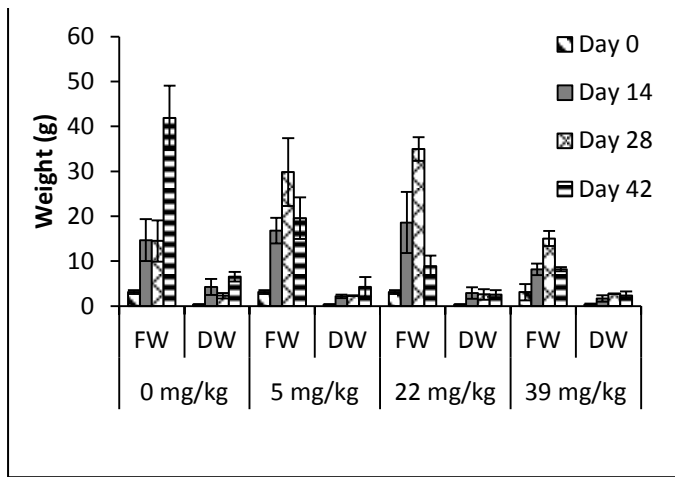


Figure 2. Fresh and dry weight of *L. octovalvis* biomass.

The accumulation of heavy metals in a plant can be quantified by two factors of translocation factor (TF) and the bioaccumulation factor (BF). The TF and BF values are respectively calculated as shown in Equations (1) and (2):

$$TF = \frac{[arsenic]_{in\ aerial\ plant}}{[arsenic]_{in\ root}} \quad (1)$$

$$BF = \frac{[arsenic]_{in\ root}}{[total\ extractable\ arsenic]_{in\ sand}} \quad (2)$$

Phytoremediation, mainly through the mechanisms of phytoextraction and phytostabilization, is a promising and practical technology to remove heavy metals from polluted environments [22]. Figure 4 shows the TF and BF value on arsenic phyoremediation in a pilot reed bed system using arsenic spiked sand. TF values are higher than 1, however, some values on 28 days of exposure are below than 1. All BF values are higher than 1. Based on the TF and BF value, it indicates that phytoextraction has the role main on arsenic phyoremediation by *L. octovalvis* in a pilot reed system. The relatively high BF and TF values of *L. octovalvis* for arsenic gave evidencethat it a potentialplant candidate for arsenic phytoextraction in sand.

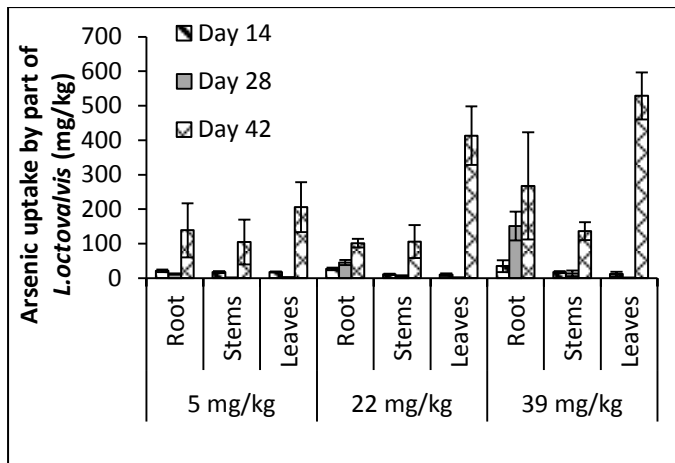


Figure 3. Arsenic uptake and accumulation in part of *L. octovalvis*.

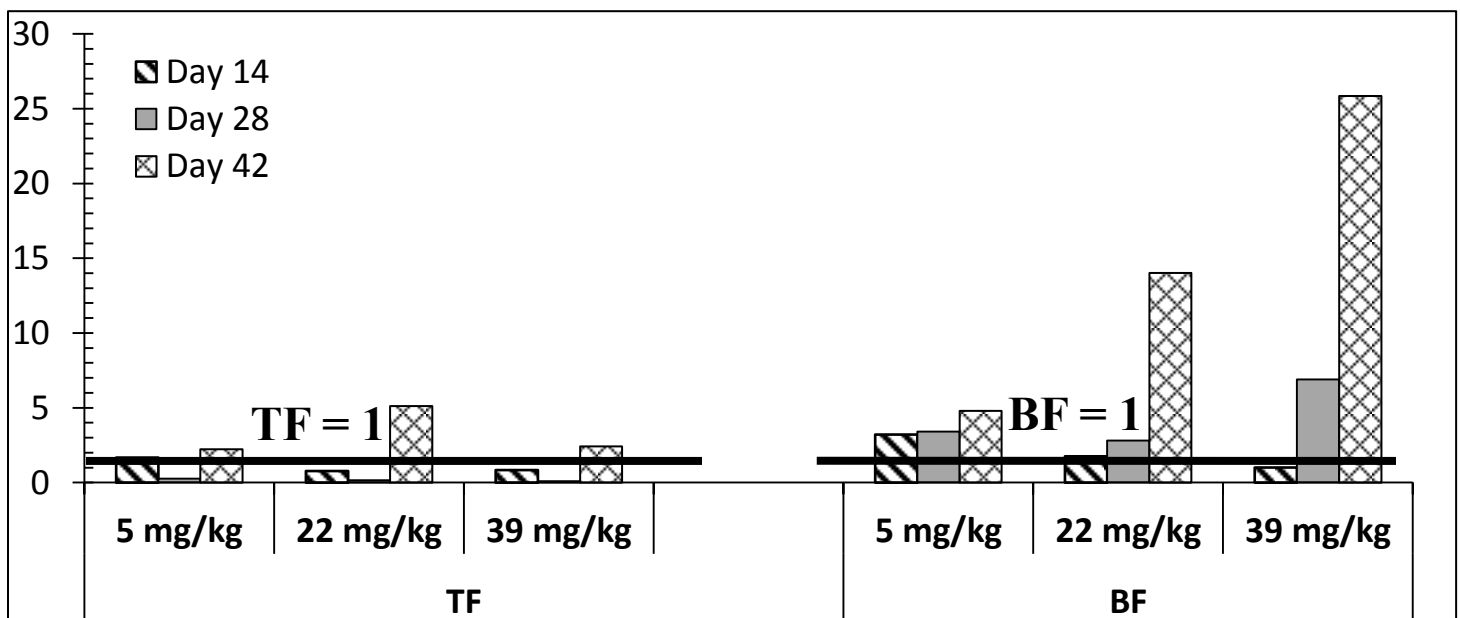


Figure 4. TF and BF value during arsenic phytoextraction.

Based on our previous findings [23], in order to give a true assessment of phytoremediation performance, with the uptake concentration by *L. octovalvis* was converted to the total arsenic uptake to indicate the effectiveness of the phytoremediation through the following equation:

$$\text{Total arsenic uptake (\%)} = \frac{C_{\text{As in plant}} \times DW_{\text{plant}} \times N_{\text{plant}}}{C_{\text{bioavailable As in sand}} \times W_{\text{sand}}} \times 100 \quad (3)$$

Where,

$C_{\text{As in plant}}$  = concentration of arsenic uptake per plant (mg/kg) at the end of exposure

$DW_{\text{plant}}$  = dry weight of plant (kg) at the end of exposure

$N_{\text{plant}}$  = number of plants

$C_{\text{bioavailable As in sand}}$  = concentration of arsenic bioavailable in sand (mg/kg) at the initial exposure

$W_{\text{sand}}$  = total weight of sand (kg) used

Based on Figure 5, the effectiveness of arsenic phytoremediation increased with time. The effectiveness at arsenic concentration of 5 mg/kg on Day 42 is higher (100%) compare that effectiveness at 22 and 39 mg/kg. The effectiveness at arsenic concentrations of 22 and 39 mg/kg on Day 42 were only 43.8 and 18.5% respectively. The study was conducted using arsenic spiked sand without additional fertilizer. However, based on our study [23], the effectiveness of arsenic phytoremediation using *L. octovalvis* at arsenic concentration of 39 mg/kg in spiked sand could increase to 49.6% after applying NPK fertilizer on Day 42.

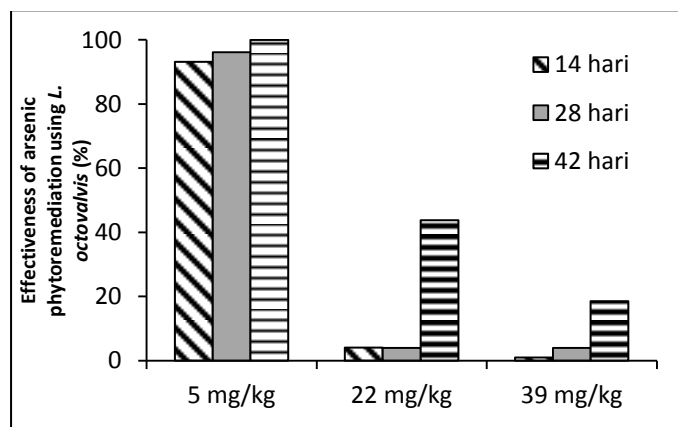


Figure 5. Effectiveness of arsenic phytoremediation.

#### iv. Conclusions

The dry weight of *L. octovalvis* increased and then stable, indicating that plant could grow during phytoremediation. The higher arsenic uptake and accumulation reached  $528.5 \pm 68.3$  mg/kg in leaves of *L. octovalvis* after 42 days of exposure at the arsenic concentration of 39 mg/kg. Based on the TF and

BF values, the phytoremediation mechanism through phytoextraction has played the main role in the arsenic phytoremediation by *L. octovalvis* in a pilot reed bed system, giving evidence that *L. octovalvis* has the potential as a good bioaccumulator for arsenic. The higher effectiveness of arsenic phytoremediation occurred at arsenic concentration at 5 mg/kg. The effectiveness of arsenic phytoremediation increased with time at all arsenic concentrations (5, 22 and 39 mg/kg).

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#### References

- [1] Glick, B.R., "Research review paper: using soil bacteria to facilitate phytoremediation," *Biotechnology advanced*, 2010, vol. 28, pp. 367-374.
- [2] Charlson, J., "Phytoremediation of arsenic", Iowa State, 2009, Pp. 1-20. <http://home.eng.iastate.edu/~tge/ce421-521/jcharlie-pres.pdf>
- [3] M.A. Rahman, H. Hasegawa, "Aquatic arsenic: Phytoremediation using floating macrophytes. *Chemosphere*," 2011, vol.83(5), pp.633-646.
- [4] S.N Hosamane, "Removal of Arsenic by Phytoremediation - A Study of Two Plant Spices. *International Journal of Scientific Engineering and Technology*," 2012, vol.1(5), pp.218-224.
- [5] ITRC, "Phytotechnology Technical and Regulatory Guidance and Decision Trees, Revised," 2009, <http://www.itrcweb.org/Guidance/GetDocument?documentID=64>
- [6] USEPA, "Arsenic treatment technology for soil, waste dan water," 2002. [www.epa.gov/tioclu-in.org/arsenic](http://www.epa.gov/tioclu-in.org/arsenic)
- [7] P. Pinkson-Burke, "Sludge can be an asset from watershed to well. Community water and waste management solutions," 2005, A publication of RCAP Solutions, Inc. [www.rcapsolutions.org](http://www.rcapsolutions.org)
- [8] R.D. Tripathi, S. Srivastava, S. Mishra, N Singh, R. Tuli, D.K Gupta, and F.J.M. Maathuis, "Arsenic hazards: strategies for tolerance and remediation by plants," *TRENDS in Biotechnology*, 2007, vol.25, pp.158-165.
- [9] M. Santiago and N. S. Bolan, "Phytoremediation of arsenic contaminated soil and water," in 19th World Congress of Soil Science, Soil Solutions for a Changing World 1 – 6 August 2010, Brisbane, Australia, pp. 189-192.
- [10] A.R. Memon, D. Aktoprakligil, A. Ozdemir, and A. Vertii, "Heavy metal accumulation and detoxification mechanisms in plants," *Turkish Journal of Botany*, 2001, vol.25, pp. 111-121.
- [11] A. Rahman, A. Amelia, A. Nazri, I. Mushrifah, N.S. Ahmad, and J.A. Soffian, "Screening of Plants Grown in Petrosludge - A Preliminary Study towards Toxicity Testing in Phytoremediation," *Colloquium on UKM-PRSB Phytoremediation*, Malaysia, August, 2009.
- [12] H.S. Titah, S.R.S. Abdullah, M. Idris, N. Anuar, H. Basri, and M. Mukhlisin, "Arsenic toxicity on *Ludwigia octovalvis* in spiked sand," *Bulletin of Environmental Contamination and Toxicology*, 2013a, vol. 90(6), pp.714-719.
- [13] H.S. Titah, S.R.S. Abdullah, M. Idris, N. Anuar, H. Basri, and M. Mukhlisin, "Phytotoxicity and uptake of arsenic by *Ludwigia octovalvis* in pilot reed bed system," *Environmental Engineering Science*, 2014, vol.31(2), pp.71-79.
- [14] H.S. Titah, S.R.S. Abdullah, M. Idris, N. Anuar, H. Basri, and M. Mukhlisin, "Arsenic range finding phytotoxicity test against *Ludwigia octovalvis* as first step in phytoremediation," *Research Journal of Environmental Toxicology*, 2012, vol.6(4), pp.151-159.



- [15] T.T. Huynh, W.S. Laidlaw, B. Singh, D. Gregory, and A.J.M Baker, "Effects of phytoextraction on heavy metal concentrations and pH of pore-water of biosolids determined using an in situ sampling technique. *Environmental Pollution* 2008, vol.156, pp.874–882.
- [16] USEPA, "SW 846: Method 3050B – Acid Digestion of Sediments, Sludge and Soils, Rev. 2," 1996, <http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3050b.pdf>
- [17] P.h. Quevauviller, " Methodologies in soil and sediment fractionation studies, single and sequential extraction procedures. Royal Society of Chemistry, U.K, 1998.
- [18] Plank, C.O, "Plant Analysis Procedures for the Southern Region of the United States, Southern Cooperative Series Bulletin #368," 1992, <http://www.cropsoil.uga.edu/~oplank/sera368.pdf>
- [19] Kalra, Y.P, "Handbook of references method for plant analysis," 1998, CRC Press, Boca Rotan, Florida, USA.
- [20] E.E.J.M. Temminghoff, and V.J.G Houba, "Plant analysis procedures," Second edition, 2004, Kluwer, the Netherlands.
- [21] C. Rattanawat, S. Rujira, P. Narupot, K. Maleeya, and P. Prayad, "Effect of soil amendments on growth and metal uptake by *Ocimum gratissimum* grown in Cd/Zn-contaminated soil. *Water Air Soil Pollution*, 2011, vol.214, pp. 383–392.
- [22] C. Grabisu, J. Hernandez-Allica, O. Barrutia, I. Alkorta, and J.M. Becerril, "Phytoremediation: a technology using green plants to remove contaminants from polluted areas. *Reviews on Environmental Health*, 2002, vol.17, pp.75-90.
- [23] H.S. Titah, S.R.S. Abdullah, M. Idris, N. Anuar, H. Basri, and M. Mukhlisin, " Effect of applying rhizobacteria and fertilizer on the growth of *Ludwigia octovalvis* for arsenic uptake and accumulation in phytoremediation," *Ecological Engineering*, 2013b, vol.58, pp.303-313.