

# Role of Synthesized Soil for Minimizing Heavy Metal Penetration into the Plant's Cell in Phytoremediation Process

M. N. Naim, N. S. Abd Rasid, N. F. Abu Bakar

**Abstract:** The advantages of synthesized soil for minimizing the heavy metal penetration into the plant cells during phytoremediation process was investigated. The synthesized soil was prepared by modifying the as-received soil into fine and coarse particles via a sieving process before subjecting the prepared soil to the lotus plant for the phytoremediation process. The as-received soil was obtained from a common paddy soil and consist of major clay elements including  $\text{SiO}_2$ ,  $\text{Fe}_3\text{O}_4$  and Ca. After the phytoremediation process for 30 days, the bulk water, soil and rhizomes were sent for inductively coupled plasma (ICP) optical emission spectrometry. The fine soil absorbed more metal content than the coarse soil and as-received soil. Further analysis was conducted by downsizing the as-received soil into the aqueous solution of pH 4, 6 and 8 and followed by an ultrasonication process. A nanometer order particle of 157, 78 and 100 nm with various particles's surface charge of 22, 4.9 and -19.9 mV were obtained from pH 4, 6 and 8 respectively. The surface charge of the clay element that contained in the as-received soil adsorbs the heavy metals onto its surface and hindered the penetration of heavy metal from entering the plant cells.

**Index Terms:** heavy metal, *Nelumbo nucifera* (lotus), Phytoremediation, soil particle size, soil particle's surface charge.

## I. INTRODUCTION

Lotus plant or *Nelumbo Nucifera* (*N. Nucifera*) is a phytoremediator plant that has been proven to have the best contaminant removal species in the post-treatment of domestic waste water, wetlands and mine area [1]-[3]. [4] The plant also has ability to accumulate high amounts of zinc (Zn) and copper (Cu) in from an industrial contaminated water body in India [5], as well as removing of phosphorus content from domestic wastewater in wetland systems [1]. *N. nucifera* consists of its own gas transportation mechanism that enable it to thermodynamically drive the gases from the leaves to the buried rhizomes below the water level. This ability enriches the rhizome area with oxygen, which in turn increases the microbial activity that is required for contaminant degradation. Furthermore, *N. nucifera* is also able to absorb contaminants due to its physical structure that includes an adventitious root formation which can be elongated to 140 cm

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maximizing the absorption from the anoxic sediment surrounding the roots. A full work consisted of the laboratory-to the field scale work conducted in Malaysia also found that the plant was capable in treating the organic matter in the contaminated surface water throughout 30 days duration. The conducted study proved that the values of BOD and COD removal can be achieved up to 97.1% and 55%, respectively [6] along the phytoremediation process. A few works of phytoremediation processes using different species of aquatic plant type in wastewater including of the free-floating plant *Eichhornia crassipes* or natively known as water hyacinth which has the capabilities to absorb arsenic up to 2 mg/L of concentration. The plant survived within 15 days before wilted on day 14 onward and dead as the concentration of arsenic increased [7]. Another study by [8] also demonstrated the ability of *Eichhornia crassipes* to absorb more heavy metals than *Pistia stratiotes*. Further study on submerged aquatic plant was also investigated using *Cadomba piauhyensis*, *Egeria densa*, and *Hydrilla verticillata* [9] in gold mines wastewater effluents for arsenic (As), zinc (Zn), and aluminium accumulation. It was found that highest removal efficiency of As and Zn shown by *E. densa* were 95.2% and 93.7% respectively. Aluminium removal efficiency recorded the highest by *C. piauhyensis* which was 83.8% of removal. Despite the advantages of the plant to treat the contaminants is well known, the studies on the accumulation of unwanted heavy metals into the plant cells was also needed to avoid excess accumulation of heavy metals or unwanted metal salts from the food pyramid that can cause fatal to the animal or human. The media that connected the transportation of nutrient or metal salt between plants and contaminants in the bulk media were controlled by the soil. However, there was a limited study about the role of soil properties when the plants were used as the phytoremediator for treating contaminated water. Thus, the study between the heavy metals in plant absorption and soil adsorption processes were conducted in this work to investigate the control parameter to minimize the occurrence of unwanted contaminant penetration into the plant cell.

## II. METHODOLOGY

### A. Preparation of the coarse and fine soil particles

The clay type of soil was dried at 70°C overnight in the oven. The soil was crushed using commercial blender (Waring Blender 8011S, USA) before sieving through 300 and 100 µm sieve tray to separate the fine soil. Size distribution of the sieved soil particle was measured using Malvern Mastersizer 2000. The soil was mixed with distilled water until the exact concentration were achieved. A 600 mL of distilled water was filled in a 1000 mL beaker. The pump was set up at 2000 rpm rotation speed to flush the cell from dirt. After removing the dirt, the sample was poured into the beaker until the concentration of the prepared soil was above the limit of detection.

### B. X-ray fluorescence (XRF) spectroscopy

The chemical compositions of the samples were tested by X-ray fluorescence (XRF) spectroscopy (JEOL JSX-3100R II, Japan). The samples were prepared by pouring the solid content into a mould and covered with transparent microporous film. The samples were arranged on the stage and the standard measurement was performed by referring the standard operating procedure provided by the manufacturer

### C. Thermal Gravimetric Analysis (TGA)

Analysis for soil absorption rate using degradation analysis was conducted using TGA (STA 6000 Pyris 1 TGA, USA). 12 mg soil sample was suspended in platinum pan with the tare weight 240 mg. Samples were heated to 200°C and held for 10 min before ramped at 10°C/min and the final temperature was 750°C was used. Heating rate of 10°C/min was used to provide optimal resolution in soil from temperature of 200°C until 600°C.

### D. Preparation of the cultivated phytoremediation plant; *N. nucifera*.

The adult *Nelumbo nucifera* plant with the length of 40-60 cm was collected from local pond located in Kampar, Perak, Malaysia. The plant needs to be re-cultivated for the climate adaptation purpose inside a 1×3×0.5 m (L×W×H) polymer tank in Faculty of Engineering, UPM as shown in Fig. 1.



**Fig. 1:** *N. nucifera* plant was re-cultivated inside the polymer tank in Faculty of Engineering, UPM

After two weeks of acclimatization, the plants were exposed with three different phytoremediation conditions that

consisted of fresh contaminated surface water, followed by the addition of coarse soil and fine soil in separated boxes. The plant's rhizomes were analyzed after the completion of the phytoremediation process to identify the heavy metal trace in its cells using inductively coupled plasma optical emission spectrometry ICP-OES.

### E. Induced Coupled Plasma (ICP)

The rhizome samples were pre-rinsed using deionized water and were oven-dried at 39 °C for 5 hours [10]. The sample were crushed into powder using mortar and pestle and sieved using standard set of sieves. A 0.5 g sample was weighed and transferred into a 10 mL beaker followed with addition of 5 mL of concentrated nitric acid (HNO<sub>3</sub>). The beaker was heated up on a hot plate at 65°C and was left for 1 hour for pre-digested. After an hour, a 5 mL of HNO<sub>3</sub> were dropped slowly into the solution until the solution becomes clear and dissolved. The solution was filtered out using filter paper and then a 30 mL of deionized water was added (Daran et al., 2017). The sample was analysed by the inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer OPTIMA-7300 DV, USA) for Mg, Cd, Ca, Cr, Al, As, K, Pb. The operation was conducted about 4 hours after the equipment was switched on to allow the gas circulation.

### F. Preparation of the nanometer order soil particles

Clay particles were synthesized through processes of drying and purification of the obtained as-received clay soil. A miller, fluidized bed separator, and an ultrasonic probe were used to reduce the size of the obtained clay soil into nano-meter size order. The as-received clay soil was dried by direct sunlight to facilitate the milling process. Then, the clay was milled using a Cutting mill SM200 (Retsch, Germany), in order to break the hard soil structure. The fluidization process was performed to the obtained soil for the removal of the biomass, and a suitable compressed air was chosen to provide the optimum velocity during the fluidization process. The soils were fluidized to ensure the separation of particles into different sizes, which purified particles of large sizes sank, and small size soil or impurities raised to the bed surface for removal. The milled clay is fluidized by a Swan 2 HP Air Cooled Piston Compressor SVP-202, Taiwan, with a maximum flowrate of 225 L/min. The nanometer scale clay particles were obtained by mixing the purified particles with distilled water and subjected the mixtures to the ultrasonic probe Q500 Sonicator (Qsonica, US) until the aggregated soil turned into a suspended solid. The setting of the sonication condition was set to 500 W, 20 kHz, an amplitude of 50% for 10 minutes. This ultra-sonication was a secondary process that reduce the clay particles size and increased the surface area for adsorption process. The surface charge of the suspended clay was adjusted by mixing the particles in HCl and NaOH solution for 24 hours. Different pH of 4, 6 and 8 were used to adjust the surface charge of the particles from its natural condition. Finally, the dried clay particles were prepared by drying the samples solution in the oven for an overnight.

### G. Zeta potential measurement of the clay particles

The behavior of the suspended nanoclay particles was analyzed under the different solutions of pH 4, 6 and 8. The zeta-potential is measured using the Particulate Systems zeta/nano particle analyzer Nanoplus (Micrometrics, USA). Two types of flow-cell were used to measure the particles size and zeta-potential value of each pH solution. To minimize the error, the clay particles were allowed to sediment and only the supernatant was injected into the flow-cell for zeta-potential for measurement purposes. All samples were subjected to centrifugal force for 180 minutes under a speed of 5000 rpm using a 5804R Centrifuge (Eppendorf, Malaysia) to ensure the sampling were taken from only the supernatant layer.

### H. Adsorption of NO<sub>3</sub><sup>-</sup> onto the prepared nanometer order soil

Seven polystyrene boxes with a dimension of 37×25×26 cm were set up to hold the lotus plants under a controlled environment. All boxes were consisted of lotus plants and immersed with 3L of contaminated water. Two of the boxes were consisted of control and as-received soil, two of the boxes were immersed with coarse and fine soil and the rest were consisted of 200 ml of nanoclay particles of three different pH values of 4,6, and 8. During the treatment, water samples were collected from the phytoremediation boxes for 5-day interval and sent to the environmental laboratory for the water quality analysis. pH and nitrate concentration were measured in-situ by using LAQUAtwin nitrate meter (HORIBA scientific, Japan). Water sample was dropped into the sensor and measurement were taken after a constant reading was achieved.

## III. RESULTS AND DISCUSSION

### A. The role of soil in phytoremediation

The obtained as-received soil were separated into fine and coarse particle size using the sieving method. As the size of the soil reduces, large surface area was obtained from the soil, thus make the soils tends to hold more nutrients. Clay particles generally have the negative surface charge [11] because of its mineralogical compositions that consisted of SiO<sub>2</sub>, Al, Fe and Ca. When the particle were immersed in the aqueous, the surface charge density increased and large surface area provided numerous nutrient binding site which creates the fine size soils surface to adsorb high amount of salts or nutrients on its surface.

This mechanism was adopted by the *N. Nucifera* plants as its nutrient storage during the phytoremediation process as the plant are naturally grow with the clay type of soil. An XRF analysis in Fig. 2 proves that the major composition of the paddy soil consists of clay particles. From the analysis of the clay particles, it was found that three major components were SiO<sub>2</sub> = 42 %, Fe<sub>3</sub>O<sub>4</sub> = 37% and Ca = 16%. From the analysis we hypothesized that the adsorption sites were influenced by Fe and Ca due to their affinity to combine with the contradict surface charge when immersed in the aqueous solution.

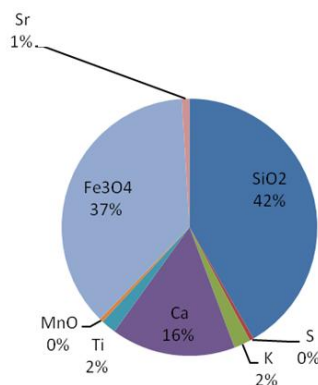


Fig. 2: Element of the clay composition that consist in the paddy soil analyzed by XRF.

The soil adsorption capacity of coarse and fine soils was evaluated using the TG analysis. Both types of soils were exposed to the contaminated water to allow the contaminant adsorbed to the soil and then subjected to TG analysis after treatment. From the analysis in Fig. 3, the size difference among the soil varies the ability of the soil to adsorb and retain the nutrients. By assuming major components of the soil were consist of a clay particle, it was noticed that the surface area act as a buffer medium or resistance for the contaminants to be transported from bulk (contaminated water) to the plant cells through the root system. From the comparison of Figs. 3 (a) and (b), it was noticed that fine soil retained slightly higher amount of water than coarse soil which were about 0.67 and 0.65 mg<sub>water</sub>/ mg<sub>soil</sub> after the completion of the treatment. Water adsorption in the soil was removed when the temperature reached at 100 °C. Above the 100 °C, the degradation was caused from the binding of the contaminants or organic compound that retained onto the surface of the soil.

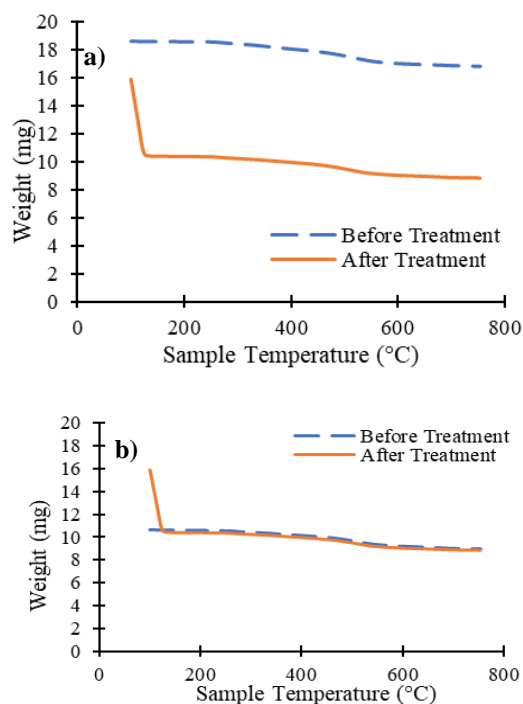


Fig. 3: TG analysis of soil composition before and after the treatment using phytoremediation method for a) coarse soil and b) fine soil.

Both soils showed a similar capacity of degradable compound degradation after 450 °C. A similar result was also obtained by [12], whereby they stated that higher concentration of heavy metals was accumulated in the finer soil. The total void and spaces in the fine soil were large, thus it provides high porosity value to the soil and retains more water and contaminant instead of directly absorb by plants.

Fig. 4 shows the ICP analysis of metal accumulation in bulk water and as-received soil at the initial condition. Several types of elements such as Pb, As, Mg, Cd, Ca, Cr, Al and K were identified from the results. Most of the concentration of the detected metals that accumulate in the water and soil were above the acceptable limit and aluminium, Al, was the highest amongst all samples (National Water Quality Standards for Malaysia, n.d.) [13]. After 30 days of the treatment process, both rhizome that were prepared in the coarse soil and fine soil were sent for ICP analysis. Arsenic (As) can be easily adsorbed due to the high content on Fe<sub>3</sub>O<sub>4</sub> in the clay [14], [15]. Fig. 4 shows that the accumulation of metal content that were absorbed in the rhizome when the treatment was conducted in coarse and fine soil. It was noticed that the fine soil adsorbed more metal content than the coarse soil.

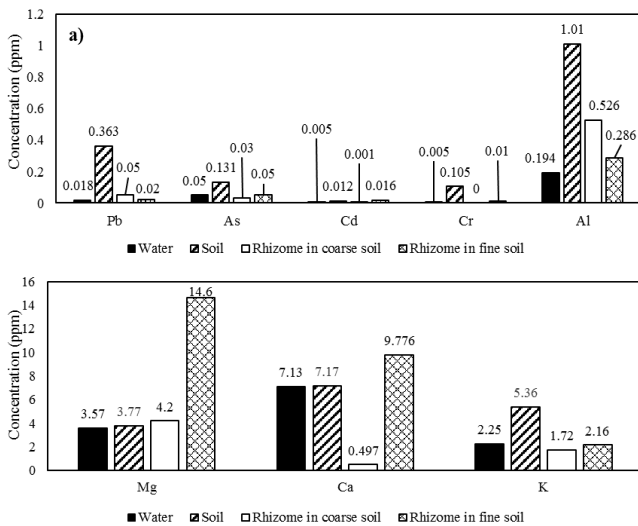


Fig. 4: Accumulation of metal trace in the bulk water, as-received (soil), and rhizomes in coarse and fine soil.

The amount of Mg and Ca of the rhizome in fine soil were higher compared to the others (14.6 ppm and 9.776 ppm, respectively). Fortunately, Mg plays a crucial part in photosynthesis thus its existence in the plant cell was considered necessary for plant respiration process. Mg was also essential for the synthesis of proteins in the plant [16] while Ca are responsible as the trace element for the plant growth.

The good finding was the accumulation of hazardous metal such as Pb, As, Cd, Cr and Al, inside the rhizomes remained low after the 30<sup>th</sup> day of treatment process although some of the elements exceeded the safety limit was detected in the as-received soil. Thus, we hypothesized that the synthesized soils that were used in this work hindered the penetration of unwanted metals in the plant cells. Further investigation was conducted by reducing the soil sizes into nanometer order and varying the surface charge of the soil with pH 4, pH 6 and pH 8.

**B. Nanometer order soil with various surface charge**

After the purification of the as-received soil and followed by sonication process, the sizes of the synthesized soils were further reduced to 157, 78 and 100 nm when they were prepared in pH 4, 6 and 8 respectively. The samples were named with pH 4 Nanoclay, pH 6 Nanoclay and pH 8 Nanoclay. Under these pH conditions, the zeta potential values for the soil particles were also adjusted to 22, 4.9 and -19.9 mV respectively. Thus, very stable suspensions were noticed in pH 4 and 8 could be due to the higher zeta potential than the as-received, coarse and fine particles. When the samples were exposed to NO<sub>3</sub><sup>-</sup> concentration of 1.2 mg/L a series of adsorption phenomenon occurs and presented in Fig. 5. To study the adsorption process, the NO<sub>3</sub><sup>-</sup> ion was preferred in the investigation compared to metal ion due to the tendency of the metal ions to react in the solution before adsorbed onto the clay surface.

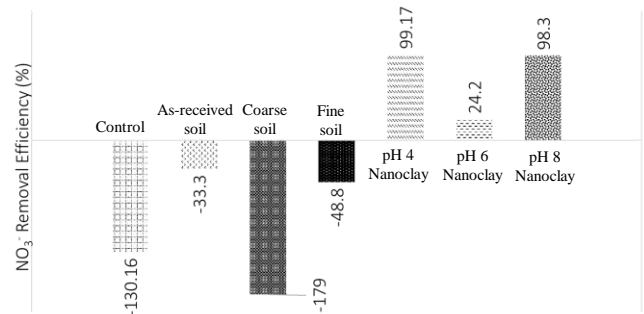


Fig. 5: Adsorption of NO<sub>3</sub><sup>-</sup> by soil types after 30 days of treatment. The negative values represent the increment of NO<sub>3</sub><sup>-</sup> concentration in the bulk solution.

From Fig.5, the adsorption of contaminants increases when the charge of the clay component in the soil composition was increased in term of zeta potential value. The highest zeta potential value of pH 4 and pH 8 removed or adsorbed NO<sub>3</sub><sup>-</sup> about 99.17 and 98.3 % respectively. The condition indicates that the adsorption site was contributes by the clay particles in the soil that were able to completely remove the NO<sub>3</sub><sup>-</sup> content that available in the bulk compared to its initial condition that represents by as-received soil, coarse and fine soil with -33.3, -179 and -48.8 % removal respectively. The plant rhizome absorbed the accumulated amount of nitrate at the rhizosphere of the lotus plant slowly. The enzyme, nitrate reductase, then becomes active and converts nitrate into nitrite [3]. The activity of nitrogen reductase is significant for the production of protein in this plant as Lotus' rhizomes contains 1.7% protein, 0.1% fat, 9.7% carbohydrate, and 1.1 % ash [17]. The amount of nitrate absorbed by the soil and later transported into the plant depends on the size of the soil particles. Soil with smaller particle size are able to efficiently transport the excess nitrate amounts to the plant as it is more porous, and it also has a higher capability in retaining the nitrate content [18] as coarse will allow the nitrate to leach into water faster after certain time of period.

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