Treatment of urea manufacturing facility effluent by *Hopea odorata* and *Khaya ivorensis*

Sara Yavari, Amirhossein Malakahmad, Nasiman B. Sapari and Saba Yavari

**ABSTRACT**

Phytoremediation is an environmentally friendly and sustainable alternative for treatment of nitrogen-enriched wastewaters. In this study, Ta-khian (*Hopea odorata*) and Lagos mahogany (*Khaya ivorensis*), two tropical timber plants, were investigated for their performances in treatment of urea manufacturing factory effluent with high nitrogen (N) content. Plant seedlings received four concentrations of N (190, 240, 290 and 340 mg/L N) in laboratory-scale constructed wetlands every 4 days for a duration of 8 weeks. The solution volumes supplied to each container, amount of N recovered by plants and plant growth characteristics were measured throughout the experiment. Results showed that Ta-khian plants were highly effective at reducing N concentration and volume of water. A maximum of 63.05% N recovery was obtained by Ta-khian plants grown in 290 mg/L N, which was assimilated in the chlorophyll molecule structure and shoot biomass. Significant positive correlations have been shown between N recovery percentages and plant growth parameters. Ta-Khian plants can be applied as suitable phytoremediators for mitigating N pollution in water sources.

**Key words**: constructed wetlands, eutrophication, industrial discharge, nitrogen removal, timber plants

**INTRODUCTION**

Urea fertilizer factories produce thousands of cubic metres of effluents per day, containing considerable levels of urea and ammonia-nitrogen. Discharge of this effluent causes serious damage to ecosystems of water bodies. There are different technologies to reduce nitrogen (N) to concentrations below discharge limits set by the US Environmental Protection Agency (EPA) (Marhaini et al. 2012).

Steam strippers and water scrubbers are among the techniques to recycle and reuse N. These techniques are very efficient and can be used for treatment of effluents with ammonia concentration in the range of 100–1,300 mg/L. However, their installation and operation are costly (Negulescu 1994). Biological recovery methods, such as aerobic-nitrification and anaerobic-denitrification, are among remediation methods that use microorganisms to transform ammonia to nitrate and then nitrate to nitrogen and gaseous oxygen. High concentrations of ammonia in effluent, as toxic and lethal compounds to microorganisms, are the major drawback for this type of recovery system (Marhaini et al. 2012). Application of other methods such as ion exchange and chlorination also encounter some obstacles. Huge cost and low removal efficiency are some of those limitations (Leaković et al. 2000). Phytoremediation as a sustainable and eco-friendly alternative technique can be used for recovery of N-polluted industrial discharges (Li et al. 2007). The EPA has also reported different phytoremediation technologies to degrade, extract, contain or immobilize pollutants from soil and water (EPA 2000). In phytoremediation, suitable plants are being used for removing contaminants from water (Sehar et al. 2016). Plants are known to be nitrogen- and water-demanding. They are able to remove N at a maximum rate of approximately 200 kg/yr by assimilation into plant biomass (McKeon et al. 2005). However, plant species are different in terms of their tolerance to N concentrations and water supplied. Wetland plant species such as softstem bulrush (*Scirpus validus*), lake sedge (*Carex lacustris*) and common cattail (*Typha latifolia*) could remove about 82% N from domestic wastewater containing 56 mg/L N while reed canary grass (*Phalaris arundinacea*) showed weaker performance.

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compared to those species at reducing N (Fraser et al. 2004). Abe & Ozaki (2007) reported that Italian ryegrass (Lolium multiflorum Lam.), papyrus (Cyperus papyrus L.), or kenaf (Hibiscus cannabinus L.) removed effectively more N from domestic wastewater ponds than hanana (Brassica campestris L. var.), African marigold (Tagetes recta L.), sorghum (Sorghum vulgare Pers.) and reed (Phragmites communis Trin.). In a comparison of ornamental plants, canna (Canna generalis) showed higher removal of total N than heliconia (Heliconia psittacorum) in the constructed wetlands loaded with domestic wastewater (Konnerup et al. 2003).

Applications of woody species for phytoremediation have been receiving more attention recently. Woody plants are huge living organisms with thousands of leaves that have ability to transpire a large amount of water through their leaf stomata (Dimitriou & Aronsson 2014). Plants such as shisham (Dalbergia sissoo), acacia (Acacia mangium) and eucalyptus (Eucalyptus hybrid) have been reported to transpire water 7 to 13 times more than the soil matrix alone (Thawale et al. 2009). They are able to uptake 350–450 m³ of wastewater per hectare of land area per day (Thawale et al. 2009). Also, it is reported that root systems of fast-growing plants such as eucalyptus (Eucalyptus hybrid), willow (Salix spp.) and poplar (Populus spp.) can remove 95% N from municipal discharges containing an annual load of 100 kg N (Duggan; Dimitriou et al. 2009).

Ta-khian (Hopea odorata) and Lagos mahogany (Khaya ivorensis) belong to Dipterocarpaceae and Meliaceae families, respectively, and are widely distributed in rainforest areas in the world (Laongpol et al. 2005). These high-quality timber plants possess properties of fast growth, high rates of biomass production and high filtering capacity due to their extensive root systems (Laongpol et al. 2005). Despite their potential, there is lack of information on the phytoremediation capability of Ta-khian and Lagos mahogany plants. This study aims to evaluate remediation potential of Ta-khian and Lagos mahogany to remove N from real effluent of a urea fertilizer factory.

**MATERIAL AND METHODS**

**Experimental design**

A bench-scale subsurface constructed wetland was designed by fitting two polyethylene pots of different sizes. The outer pot (202 mm rim diameter, 170 mm bottom diameter, 190 mm tall) had an overflow valve 100 mm from the top, and enclosed a drained inner pot (200 mm rim diameter, 160 mm bottom diameter, 150 mm tall) which was filled with pea gravel (Figure 1).

**Plant material**

Two-foot seedlings of Ta-khian and Lagos mahogany were purchased from Mata Ayer Research Stations, Perlis, Forest Research Institute Malaysia (FRIM) (Figure 2). The seedlings were acclimatized 4 weeks before the initiation of experiments. The seedlings were then placed in the constructed wetland and fed with 40% modified Hoagland’s solution (Table 1). Hoagland’s solution provides necessary nutrients for a wide range of plant species.

**Collection and analyses of effluent**

Untreated discharged real effluent of a urea production facility in Malaysia was collected and transported to the laboratory and stored at 4°C. Then it was brought to room temperature before the experiment. The effluent was analyzed to determine total organic carbon (TOC) using a TOC-L Shimadzu-Japan apparatus, chemical oxygen demand (COD) according to Hach Method 8000, pH by a portable pH meter (model EW 53013, Hach Sension), total N (TN) using the Hach Test ‘N Tube tests, orthophosphate (PO₄³⁻) by PhosVer 3 method (Hach Method 8190) and potassium (K), iron (Fe), zinc (Zn), by atomic absorption spectroscopy, AAS (Model AA 6800 Shimadzu-Japan). The effluent composition is presented in Table 2.

![Figure 1](https://iwaponline.com/wst/article-pdf/75/7/1684/453854/wst075071684.pdf)
High concentration of total N was detected due to presence of urea and ammonia-N in the effluent. The effluent had also an alkaline pH probably due to the presence of unreacted ammonia. The other characteristics were in the acceptable range.

**Experimental procedures**

Four test solutions were prepared by adding different concentrations of N fertilizer (in the form of NH$_4$NO$_3$) into real wastewater (Table 3). T1 and L1 had the N concentration (190 mg/L) equal to average concentrations of those collected from urea manufacturing wastewater. Nitrogen concentrations were increased in other solutions (T2 to T4 and L2 to L4) in 50 mg/L intervals to investigate plants’ performances in extreme conditions. The initial pH of the nutrient solutions was adjusted in the suitable range for plants (6–6.5) with 2 N H$_2$SO$_4$ or 2 N NaOH. Initially, the constructed wetlands containing plants were emptied and rinsed with tap water (pH = 7 and electrical conductivity = 66.5 μS/cm). Then, they were filled with the test solutions until the solution started to escape from overflow valves. Aqueous solution with a predetermined concentration of nitrogen (i.e. 190 mg/L) was added to each pot every 4 days to maintain the water level at 10 cm below the gravel surface. Each time, the volume of solution supplied to each container was recorded and added to the previous ones. Therefore, the total volume was obtained from the sum of the supplied volumes during the 8-week experiment. The containers were arranged in a completely randomized...
design with four replicates. Replicates were placed under a shade in an open area exposed to natural diurnal variation in light and temperature. Air temperature and relative humidity were determined by a maximum and minimum thermometer and a hygrometer, respectively. The wind speed was recorded based on the meteorological data.

**Data collection**

Volumes of solution added to each container were recorded regularly during the 8-week experiment. In the last part, samples in each container were analyzed to determine total N using the Hach Test ‘N Tube tests. The percentage of recovered N was calculated using Equation (1):

\[
R = \left( \frac{C_i - C_f}{C_i} \right) \times 100
\]

where \(C_i\) and \(C_f\) are initial and final concentrations of N, respectively, and \(R\) is recovered N percentage.

Number of leaves in each plant was recorded before harvesting. Chlorophyll content index of leaves also was determined by a SPAD-502 chlorophyll meter. The SPAD values of leaves of tropical plant species have been reported to be highly correlated to their chlorophyll content \((R^2 \approx 0.89)\) following a homographic model \((\text{Coste et al. 2010})\). At the end of the experiment, plants were harvested and sectioned into leaves, stems, and roots. All plant parts were separately oven dried (48 h at 80°C) and weighed to record oven-dry weight of samples.

**Data analysis**

Analysis of variance and correlation analyses were conducted by SAS (version 9.1, SAS Institute, Inc., Cary, NC, USA). Duncan’s multiple range tests at probability levels of \(\alpha = 0.05\) were calculated between the different treatments.

**RESULTS AND DISCUSSION**

Consumption of effluent by plants

The volume of effluent demanded by plants in each experimental set is shown in Figure 3. The demanded effluent volume increased in all experimental sets over time. Plant demand for water is not a constant quantity and it is dependent on the type of plant, plant’s growth rate and environmental factors such as temperature, humidity and wind \((\text{Lambers et al. 2008})\). In general, Ta-khian seedlings had a higher uptake ability compared to Lagos mahogany plants. The demand in set T3 (290 mg/L TN) was significantly greater than the other experimental sets. Schulze & Hall \((1982)\) reported that only a small part of water taken up by plants is involved in metabolic processes; about 97% of water transpires through leaf pores. Therefore, higher number of leaves in Ta-khian, especially in T3, was probably the main reason for higher uptake (Table 4). Table 4 shows plant growth parameters and explains the assimilation of uptaken nitrogen in plants.
Figure 3 shows water volume demanded by experimental sets at each time of feeding during the 8-week period. A sudden drop in water demand which was observed from day 40 to 48 was most likely due to increase of air relative humidity (Figure 4). The high relative humidity affects directly leaf stomata and reduces water evaporation from leaf surfaces (evapotranspiration).

High water consumption by plants can be considered as an advantage for phytoremediation systems. In this condition, plants consume more of the produced effluent and reduce it significantly. Guidi et al. (2008) showed that water consumption is positively related to plant growth enhanced by the availability of nutrients. Nutrients are necessary for stimulating new and vigorous growth of shoots. Based on Figure 3, T3 consumed more water as a result of better plant growth due to appropriate N concentration. However, the higher concentration of N in T4 had not been perfectly assimilated in plants (Table 4), which subsequently decreased the water request by plants.

Figure 5 illustrates the total amount of N supplied to each experimental set. Mass of total N supplied was calculated by multiplying total volume of test solution supplied to each pot by concentration of N. T3 was supplied with higher amount of N due to greater water demand. Although T4 received less volume of effluent, high concentration of N (340 mg/L N) caused the amount of total N supplied to be as high as that of T3. Total N supplied was significantly similar in other experimental sets despite that they were fed with different concentrations of N. The lowest total N supplied was determined in L1 (190 mg/L N).

Percentage of N recovered by the experimental plants is shown in Figure 6. Results indicate that N removal efficiencies are dependent on plant species and concentration of N load. Ta-khian plants in experimental set T3 were highly efficient in N uptake, recovering up to 63.05% of total supplied N (3,000 mg). The experimental set T1 also performed quite well in reducing N (55.92% of 1,900 mg), which was not

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**Table 4** | The leaf dry weight (g), stem dry weight (g), root dry weight (g) and number of leaves of Ta-khian (T)

<table>
<thead>
<tr>
<th>Experimental sets</th>
<th>Leaf DW</th>
<th>Stem DW</th>
<th>Root DW</th>
<th>No. of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>13.51 ± 1.32 ab*</td>
<td>25.96 ± 2.72 a</td>
<td>12.25 ± 1.23 a</td>
<td>63.50 ± 2.31 b</td>
</tr>
<tr>
<td>T2</td>
<td>11.67 ± 1.21 bc</td>
<td>20.29 ± 1.02 ab</td>
<td>8.48 ± 1.07 ab</td>
<td>63.25 ± 2.32 b</td>
</tr>
<tr>
<td>T3</td>
<td>17.47 ± 1.02 a</td>
<td>24.10 ± 2.43 ab</td>
<td>9.41 ± 1.11 ab</td>
<td>85.50 ± 1.87 a</td>
</tr>
<tr>
<td>T4</td>
<td>8.78 ± 1.07 c</td>
<td>19.21 ± 1.02 b</td>
<td>8.14 ± 0.85 b</td>
<td>46.75 ± 1.02 c</td>
</tr>
</tbody>
</table>

T1: 190 mg/L N; T2: 240 mg/L N; T3: 290 mg/L N; T4: 340 mg/L N (n = 4). *Mean (±SE) within columns followed by the same letter are not significantly different (P ≤ 0.05) based on Duncan’s multiple range tests.

DW: dry weight.

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Figure 4 | Maximum and minimum temperature, relative humidity and wind speed recorded during the experimental period (12/12/2014–10/02/2015).

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Supply and consumption of nitrogen
significantly different from T3. Results show that application of 7-month-old seedlings was able to adsorb reasonable nitrogen content from the wastewater. The growth of those seedlings used in this study to mature plants will result in further nitrogen adsorption from the wastewater. Other plants were able to decrease less than half of the supplied N. High loads of N (1,400–3,000 mg) indicate considerable efficiency of Ta-khian and Lagos mahogany, even for the least effective experimental sets, for removing N compared to herbaceous plants. Nitrogen removal efficiency by the timber plants investigated in this study was higher than those reported for common reed (*Phragmites australis*). Huett *et al.* (2005) have reported >96% N removal in nursery runoff water contaminated with 10.1 mg/L N after a 19-month period. Nitrogen removal capacity of the experimental plants was also higher than that of kenaf (*Hibiscus cannabinus*), which was reported to remove 34.65% of N from domestic wastewater polluted by 8 mg/L N (Abe & Ozaki 2007).

**Plant characteristics**

Nitrogen plays a main role in the chlorophyll molecule structure (El Gendy *et al.* 2015). Therefore, the index of foliar chlorophyll content was monitored during the experimental period. Based on results presented in Figure 7, all
experimental plants showed an increasing trend of chlorophyll index during the first month of the experiment. This indicates an increased capacity of plants to harvest energy from sunlight with increasing supply of N (Ping et al. 2014). The highest greenness content was found in T3 plants, suggesting more photosynthetic light reactions and N assimilation (Ping et al. 2014). Throughout the second month of the experiment, the index of chlorophyll remained steady in all experimental sets, despite its increase in T3. It was probably because of reduction in photosynthesis due to nutrient imbalance in plants. Urea manufacturing effluent is a rich source of N (Table 2) and poor in other essential nutrients. This nutrient imbalance can lead to overall photosynthetic disturbance in plants (dos Santos et al. 2006).

Dry weights of leaves, stem and roots and number of leaves of Ta-khian and Lagos mahogany are presented in Tables 4 and 5, respectively. Evidently, Ta-khian seedlings grown in T4 (340 mg/L N) showed the least amount of growth compared to T1, T2 and T3. Higher concentration of N in T4 compared to T3 could not increase growth of Ta-khian plants. Lack of other nutrients (such as phosphorus and potassium) in high concentration of N affects the plant growth in sampled solutions. Phosphorus improves the photosynthesis process and potassium acts as catalyst to activate various enzymes in plants (Maathuis 2009; Malvi 2011). Similar results were observed in Lagos mahogany seedlings grown in L4 (Table 5). In the other experimental sets, shoot growth has been stimulated by increasing N rates, suggesting acceptable N concentrations for the experimental plants. As for root dry weight, high concentrations of N in T4 and L4 had least effects on below-ground biomass. Reduced root growth is a result of limited carbon allocation to roots (Ericsson et al. 1996). Carbon deficiency in roots was probably caused by excess amount of NH₄⁺ taken up by plants, which increases the competition between root growth and NH₄⁺ as sinks for carbon skeletons (Ericsson et al. 1996).

Table 5 | The leaf dry weight (g), stem dry weight (g), root dry weight (g) and number of leaves of Lagos mahogany (L)

<table>
<thead>
<tr>
<th>Experimental sets</th>
<th>Leaf DW</th>
<th>Stem DW</th>
<th>Root DW</th>
<th>No. of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>6.45 ± 1.98 ab*</td>
<td>19.52 ± 1.89 a</td>
<td>17.47 ± 1.72 ab</td>
<td>37.25 ± 0.98 ab</td>
</tr>
<tr>
<td>L2</td>
<td>7.57 ± 1.23 a</td>
<td>14.89 ± 1.21 b</td>
<td>12.82 ± 1.37 b</td>
<td>44.00 ± 0.76 a</td>
</tr>
<tr>
<td>L3</td>
<td>5.77 ± 1.43 ab</td>
<td>11.51 ± 1.78 c</td>
<td>21.24 ± 2.31 a</td>
<td>33.50 ± 0.79 ab</td>
</tr>
<tr>
<td>L4</td>
<td>4.86 ± 1.02 b</td>
<td>10.90 ± 2.06 c</td>
<td>12.75 ± 0.98 b</td>
<td>28.00 ± 0.86 b</td>
</tr>
</tbody>
</table>

L1: 190 mg/L N; L2: 240 mg/L N; L3: 290 mg/L N; L4: 340 mg/L N.
DW: dry weight.
*There is no significant difference with the same letter based on Duncan’s multiple range tests (α = 0.05).
Correlation analysis between N recovery percentages and plant growth characteristics are shown in Table 6. The data reveal that there were significant positive correlations between percentage N recoveries and leaf dry weight, number of leaves and chlorophyll content index. Positive correlation between amount of N and chlorophyll content is well documented for a number of plant species (El Gendy et al. 2015). There was no significant difference between the percentage of recovered N and the root dry weight. Roots are not probably the initial sink for N assimilation (Ericsson et al. 1996). It seems that a small quantity of N is enough to meet the root growth requirement (Shangguan et al. 2004). A negative correlation between root growth and increasing supply of N has been reported by other researchers (Shangguan et al. 2004; Xu et al. 2015).

### CONCLUSION

The seedlings showed considerable potential for removing N, as high as 63.05%, when they were supplied with as much as 3 g N in solution. Besides N removal, plants decreased a considerable volume of effluent via transpiration. Nitrogen taken up by plants was mostly assimilated in above-ground parts of plants, involved in chlorophyll molecule synthesis and increasing photosynthesized carbon compounds. High ability of tropical timber plants for N uptake suggests potential application of phytoremediators for mitigating N pollution from polluted effluent generated from domestic or industrial sources. Additional research needs to be done to find optimum balances of nutrients for the timber plants to maximize their efficiency in phytoremediation.

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### REFERENCES


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### Table 6

<table>
<thead>
<tr>
<th>Characters</th>
<th>Leaf dry weight</th>
<th>Stem dry weight</th>
<th>Root dry weight</th>
<th>Number of leaves</th>
<th>Chlorophyll content index</th>
</tr>
</thead>
<tbody>
<tr>
<td>% N recovery</td>
<td>0.9142**</td>
<td>0.7547*</td>
<td>−0.4432</td>
<td>0.8944**</td>
<td>0.8930**</td>
</tr>
</tbody>
</table>

Correlation coefficient shows significance at 5% (*) and 1% (**)


