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PHYTOREMEDIATION OF UREA FERTILIZER FACTORY WASTEWATER BY TEAK (*Tectona grandis*)

Urea manufacturing discharge contains a high load of nitrogen, a pollutant which needs advanced technologies to be reduced to desirable levels. However, these advanced technologies are expensive due to the complex process and cost of chemicals and maintenance. Phytoremediation has been recently considered for N removal from various wastewaters. However, the common phytoremediation plants (reeds and grasses) are not able to remove N effectively due to the recyclable nature of N through decomposition processes. Therefore, they require periodic harvestings which impose a high cost on system. In this study, the growth and phytoremediation potential of teak (*Tectona grandis*), a tropical timber plant, to treat the urea manufacturing wastewater was evaluated. Eight month old teak seedlings received 4 different concentrations of N in bench-scale constructed wetlands every 4 days for 8 weeks. The solution volumes supplied to each container and plant biomass, N recovery, and tissue nutrient concentration were measured. Teak plants showed an escalation in wastewater N uptake with increasing amount of supplied N. Total dry weight was positively correlated with total N supplied. Teak seedlings showed a considerable potential for removing nitrogen when they were supplied with up to 5 g N per pot volume (4 dm^3) during a two-month experiment.

1. INTRODUCTION

Urea fertilizer factories generate large quantities of wastewater containing high load of nitrogen (N) mainly in the form of urea and ammonium [1]. Nitrogen pollution is one of the most widely and challenging environmental problems which can cause serious ecological damage in the waters via eutrophication [2]. To efficiently remove N from urea fertilizer factory wastewater, there is the necessity of incorporating advanced technologies into existing treatment systems. However, these techniques are known to be expensive ones, due to their complexity, cost of chemicals and maintenance [3].

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As a cost-effective alternative technology, phytoremediation (defined as the use of plants to degrade, extract, or immobilize contaminants from soil and water), may be used for N removal from fertilizer production factory wastewaters. Since N is a critical component for plant growth, it is readily taken up by plants and removed from the environment [4]. However, with phytoremediation, the efficiency of the system can be exposed to changes due to the recyclable nature of N. In other words, inorganic N added to an ecosystem can stimulate the production of organic matter by plants, which is subsequently released to environment again through decomposition [5]. Therefore, the common herbaceous marsh species such as reeds and grasses, will not be able to remove N effectively if they are not harvested regularly [5]. Hence, the use of commercially important woody plants as phytoremediators can be a suitable alternative for common wetland plants, since they will compensate the cost of harvest. For example, short rotation trees, such as poplar and willow, have been successfully used for treatment of municipal wastewater and at the same time producing biomass for energy purposes [6, 7]. But few studies have examined the ability of timber plants to recover N from wastewaters. Acacia (*Acacia mangium*), neem (*Azadirachta indica*) and eucalyptus (*Eucalyptus hybrid*) are known to be suitable plants for removal of nutrient pollutants from primary treated wastewaters [8]. Similarly, shisham (*Dalbergia sissoo* L.) and African mahogany (*Khaya senegalensis*) seedlings have been applied successfully for municipal effluent phytoremediation [9, 10]. Relatively greater growth and productivity together with higher foliage mineral content caused the lowest concentrations of the nutrients in the soil associated with these species.

As a fast growing tropical timber plant, teak (*Tectona grandis*) possesses advantageous characteristics such as long-term growth period, high transpiration capacity, and industrial application; thereby it has the potential to be considered as a phytoremediator plant [11]. This high-quality timber species belongs to the *Lamiaceae* family and is widely distributed in the rainforest areas of the world [12]. Although teak has been reported to be a good species for phytoremediation of crude oil contaminated habitats [13], there is lack of information on its application for nutrient removal from wastewater. Therefore, the objective of this study is to evaluate the growth, tolerance efficiency, and phytoremediation potential of teak plants to remove N from urea fertilizer factory wastewater in a bench-scale constructed wetland.

2. MATERIALS AND METHODS

Experimental system design. Since a continuously flooded condition is not ideal for growing and thriving of the experimental plant, a bench-scale subsurface constructed wetland was modeled after Polomski et al. [5]. The system was prepared by fitting two polyethylene pots of different sizes. The smaller inner pot (3.2 dm^3) with drainage holes was filled with pea gravel and placed into the outer aquatic pot (8 dm^3). An overflow valve was placed in 100 mm of top of aquatic pot to give a water depth of 90 mm below the gravel surface in gravel pots (Fig. 1).

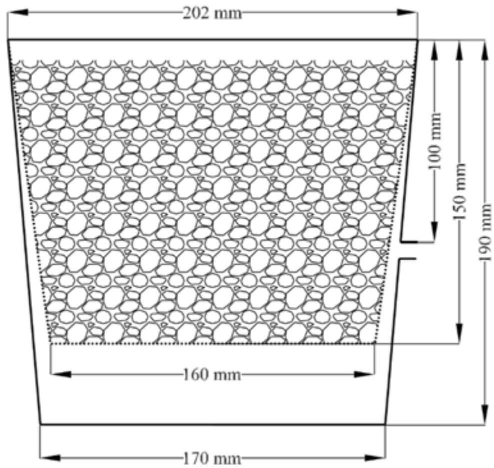

Fig. 1. The schematic of the experimental bench-scale wetland consisting of a gravel-filled pot inserted into an aquatic pot

Plant material. Eight month old teak seedlings were purchased from the Mata Ayer Research Stations, Perlis Forest Research Institute Malaysia (FRIM). Four weeks before the initiation of the experiment, 16 teak seedlings were transplanted into the experimental systems for acclimatization. Additionally, four containers without plants were inserted between the experimental plants to determine the evapotranspiration rate by plants and to estimate the N recovery ability of plants in the experimental constructed wetlands. During the acclimatization, all containers were fertilized weekly with a 40% modified Hoagland's solution.

Wastewater analyses. Influent from a urea production plant in Malaysia was collected in polyethylene gallons and transferred to the laboratory and stored at 4 °C before starting the experiment. Subsamples of the wastewater were analyzed to determine total organic carbon (TOC) using a TOC-L Shimadzu apparatus, chemical oxygen demand (COD) according to HACH, Method 8000, pH using a portable pH meter (EW 53013, HACHSension), total N with HACH Test'N Tube tests, orthophosphate by the PhosVer 3 method (HACH, Method 8190) and potassium (K), iron (Fe), zinc (Zn) by the atomic absorption spectroscopy (AAS), AA 6800 Shimadzu. The wastewater is characterized by high concentration of total N (Table 1), which mainly originated from urea and ammonium compounds. It has also an alkaline reaction, due to the presence of unreacted ammonia in the wastewater remaining from the urea synthesis. The other characteristics are in the normal range [14].

Table 1

Composition of wastewater effluent collected from a urea plant in Malaysia

Parameter	pH	TOC	COD	TN	[PO_4^{3-}]	[K]	[Fe]	[Zn]
Concentration, mg·dm^{-3}	9.3	46.8	20.7	190	1.3	1.8	0.4	<0.1

Experimental procedures. Four test solutions were prepared using collected wastewater and adding various amounts of N in the form of NH_4NO_3 (mg N·dm^{-3}): T1 – 190, T2 – 240, T3 – 290, and T4 – 340. The pots without plants (T0) received the same solution with T4. The initial pH of the nutrient solutions was adjusted to 6.3 with 1 M H_2SO_4 or 0.02 M NaOH. In June 2014, the small pots containing the plants were lifted from the bigger pots and flushed with tap water (pH = 7, and electrical conductivity = 66.5 µS·cm^{-1}). They were then returned to the big pots, which had been emptied and rinsed with tap water. The test solutions were poured into the pots with plants until it began to flow from the overflow valve. Thereafter, the solutions were added every four days to maintain the water level at the 10 cm below the gravel surface. The five treatments were arranged in a completely randomized design with 4 replications. The pots were placed under a shade house with the average temperature of 36.8 °C and relative air humidity 85%.

Data collection. The solution volumes supplied to each container were recorded during the eight-week experiment. At the end of the experiment, August 2014, wastewater samples from the aquatic containers were collected and analyzed for total N using the HACH Test'N Tube tests. The percentage of recovered N was determined by:

$$R = \frac{C_i - C_f}{C_i} \times 100\% \tag{1}$$

where C_i and C_f are the initial and final concentrations of N, and R is the percentage of recovered N.

The effect of N treatments on plant growth was evaluated by weekly monitoring of plant height from the gravel surface to the tallest plant part. A non-linear logistic growth model was used to determine the dynamics of plant growth by modeling the changes in the plant height. Data on plant height were regressed against days using the following equation:

$$y = \frac{A}{1 + b\mathrm{e}^{-cx}} \tag{2}$$

where y is growth parameter, x is day, and A, b and c are regression constants. The A and $A/(1 + b)$ are the asymptotic level and initial value of each parameter, respectively.

As an index of the chlorophyll content, the greenness of new fully expanded leaves was estimated using a SPAD-502 chlorophyll meter. The SPAD values are highly correlated to chlorophyll content ($R^2 \approx 0.9$) and follow exponential or second-order polynomial functions [15].

After termination, plants were harvested and separated into leaves, stems, and roots. All plant parts were separately oven dried for approximately 48 h at 80 °C, weighed and ground in Mortar grinder (Rocklabs, NZ) to pass through a 40-mesh (0.425 mm) screen. Dry ashing was used for leaf tissue digestion [16]. Determination of K, Ca, Mg, Fe, Zn, Mn, and Cu was carried out using AAS (model Z-5000 HITACHI). Phosphorous was analyzed spectrophotometrically [17]. Nitrogen concentration was evaluated by the Kjeldahl method [18]. The N content (g) was calculated using the following equation to normalize the differences in N concentrations ($g \cdot g^{-1}$) as a result of growth differences among treatments [5].

$$\text{N content} = \text{plant dry weight} \times \text{nitrogen concentration} \tag{3}$$

Statistical analysis. Regression analyses were performed using Excel for description of changes in biomass and N recovery relative to N supplied. Analysis of variance (ANOVA) was performed by SAS (version 9.1, SAS Institute, Inc., Cary NC USA) to determine significance of treatment effects on wastewater composition and plant characteristics. The treatment means were compared using Duncan's multiple range tests ($\alpha = 0.05$).

3. RESULTS AND DISCUSSION

The effect of total N supplied was plotted against total dry biomass of each container (Fig. 2). Dry weight accumulation rates were highly correlated with increasing levels of N over the 8-week period (p-value < 0.001).

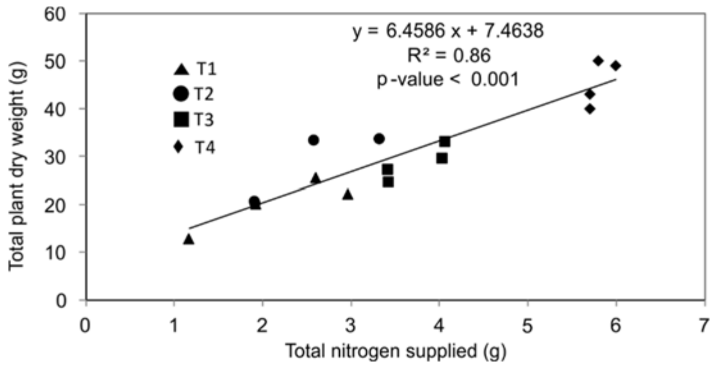

Fig. 2. The effect of supplied nitrogen in wastewater effluent from a urea factory on total dry weight of teak plant during the 8-week of experiment

This indicates that as the amount of N increases, the plants assimilate greater amount of N and produce more dry weight. It is probably a plant growth response induced by added N, because of an inherent limitation of N in plants [19]. Also, there was a linear relationship between N content of whole plant tissues and the amount of N supplied to each container (p-value < 0.001) (Fig. 3). These findings were in accordance with studies that showed dynamics of N uptake and dry matter accumulation in plants [20, 21].

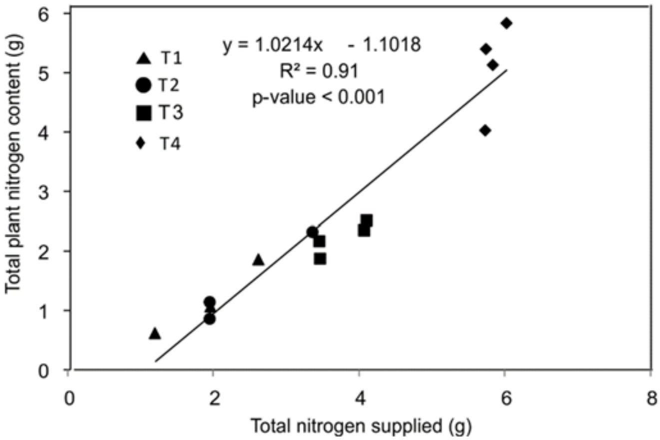

Fig. 3. The effect of supplied nitrogen in wastewater effluent from a urea factory on total plant nitrogen content during the 8-week of the experiment

A large portion of changes in dry biomass of the plants is proportional to the changes in plant height during the experiment. The weekly monitoring of the plants height indicated that the pattern of growth was quite similar in the all experimental sets (Fig. 4).

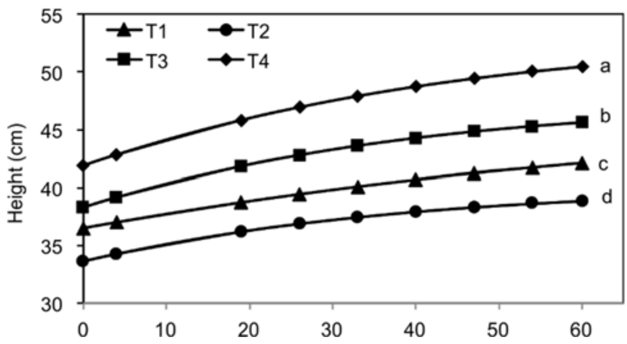

Fig. 4. Height of teak plants grown in wastewater effluent from a urea factory during 60 days in the form of $y = A/(1 + be^{-cx})$; ad a) there is no significant difference with the same letter based on Duncan's multiple range tests ($\alpha = 0.05$)

There was an increasing growth tendency throughout the experimental period in the all investigated plants. T4 showed the greatest growth compared to T1, T2 and T3 which indicates a growth response to added N fertilizer.

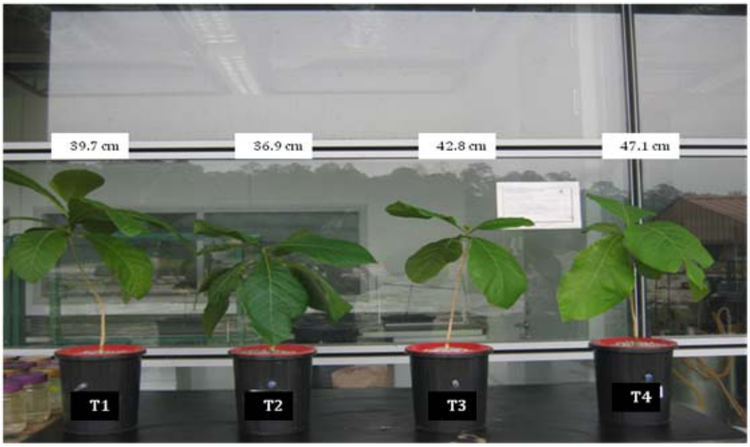

Fig. 5. The height of teak seedlings of different experimental sets after 60 days: T1 – 190 mg N·dm^{-3}, T2 – 240 mg N·dm^{-3}, T3 – 290 mg N·dm^{-3}, T4 – 340 mg N·dm^{-3}

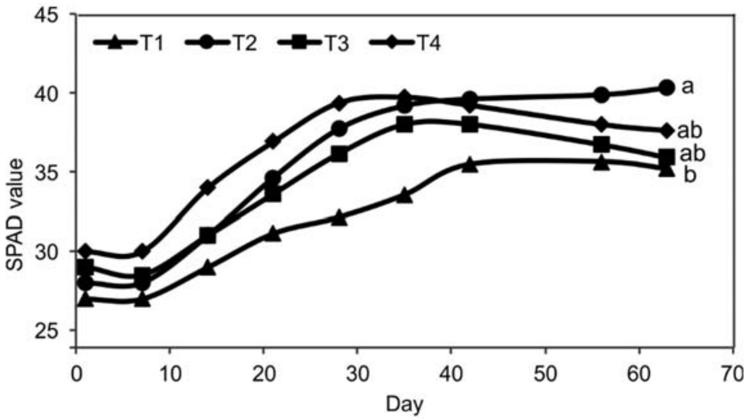

Fig. 6. SPAD value of new matured leaves of teak seedlings during the 60-day period; there is no significant difference with the same letter based on Duncan's multiple range tests ($\alpha = 0.05$)

The chlorophyll content index of the teak plant leaves throughout the 8-week duration is shown in Fig. 6. The greenness or chlorophyll content of leaves is closely related to the leaf N content since the majority of leaf N is involved in chlorophyll molecules [22]. The data for chlorophyll content index of leaves did not show similar pattern with height changes and dry biomass accumulation. Although the greenness of all the experimental plants increased initially, this trend started to decrease in T4 and T3 in the last month of experiment. The loss of greenness was also visually observed in new fully expanded leaves as symptoms of chlorosis.

Table 2

Results of nutrient analysis of fully expanded leaves of teak plants grown in urea wastewater effluent collected from a urea plant in Malaysia[1]

Treatment	Macronutrients [$mg \cdot g^{-1}$]					Micronutrients [$mg \cdot g^{-1}$]			
	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
T1	25.26a	4.62ab	7.07a	13.55a	1.23a	0.23a	0.04a	0.20a	0.01a
T2	25.40a	4.44b	7.39a	15.51a	1.19ab	0.23a	0.03a	0.17a	0.01ab
T3	29.81a	5.81a	7.60a	7.78b	1.21ab	0.23a	0.02a	0.17a	0.01b
T4	30.15a	4.79ab	7.18a	11.12ab	1.14b	0.22a	0.02a	0.23a	0.01b

[1]Based on Duncan's multiple range tests, there is no significant difference in the results denoted with the same letter ($\alpha = 0.05$).

Based on these results, although there are high N concentrations in the leaves of T4 and T3 plants (Table 2), they showed the symptom of N deficiency. It may be considered as a result of interactive effect of N and P in the photosynthesis process. Phosphorous is necessary for the formation of nitrate reductase [23]. Therefore, protein synthesis will be interrupted in P deficient condition. The urea factory wastewater has greater supply of N (Table 1) which can lead to strong P shortage, imbalance of elements and inefficient photosynthetic capacity [24]. As shown in Table 2, the foliar levels of P in the teak trees seem to be not meeting the requirements for ideal photosynthesis process. The adequate level of N and P for sufficient chlorophyll formation in teak trees has been reported to be 18.2–18.9 $mg \cdot g^{-1}$ and 10.1–12.3 $mg \cdot g^{-1}$ leaf dry weight, respectively [25]. The loss of greenness might be due to low concentrations of micronutrients in the plants' leaves (Table 2). In addition, the foliar concentration of micronutrients was lower than the critical levels in all experimental plants (<1.25 $mg \cdot g^{-1}$ for Mn, <2.5 $mg \cdot g^{-1}$ for Fe, <0.1 $mg \cdot g^{-1}$ for Cu, <0.45 $mg \cdot g^{-1}$ for Zn) which may lead to leaf chlorosis [25, 26]. While Fe has an important role in chlorophyll formation, Mn contributes to metabolism and assimilation of N [26]. Zinc is involved in protein synthesis by the accumulation of soluble N compounds and Cu functions in photosynthesis and plays role in protein and carbohydrate metabolism. Zinc and copper have been also reported to increase the P uptake by plants [27]. The amount of K in the leave tissue could not be a limiting growth factor since the average nutrient ranges in plant tissues have been reported to be around 7:3:1 (N:P:K) [28]. Teak plants have also been shown to have sufficient concentrations of Ca and Mg (Table 2). The deficiency symptoms of Ca and Mg were observed in teak plants when the foliar concentrations of Ca and Mg were lower than 5.5 and 1.0 $mg \cdot g^{-1}$, respectively [25]. The results suggest that inadequate supply of P and micronutrients in urea plant wastewater may hinder the photosynthesis activities induced by higher N concentrations. There is no significant difference with the same letter based on Duncan's multiple range tests ($\alpha = 0.05$).

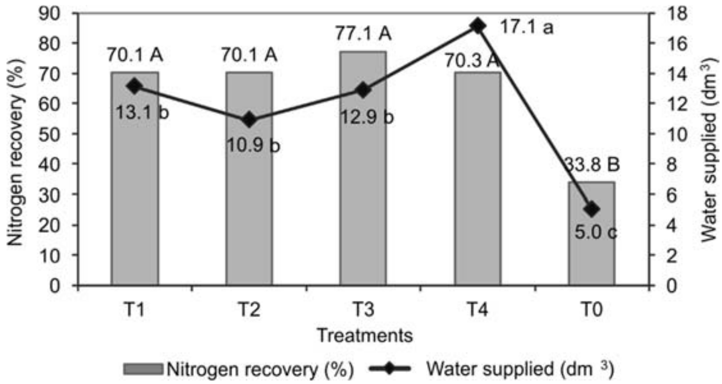

Fig. 7. The total volume of wastewater effluent supplied to each treatment and the percentage of recovered N by teak seedlings at various concentrations of N. Bars sharing the same letter and case are not significantly different based on Duncan's multiple range tests ($\alpha = 0.05$); (T1 – 190 mg $N\cdot dm^{-3}$, T2 – 240 mg $N\cdot dm^{-3}$, T3 – 290 mg $N\cdot dm^{-3}$, T4 – 340 mg $N\cdot dm^{-3}$)

As a result of evapotranspiration and N assimilation rates, T4 were supplied with greater amounts of wastewater and consequently larger amounts of N than the rest of treatments (Fig. 7). Gravel-only pots used only 5 dm^3 of solution, representing the amount of evaporation from the gravel surface. Although T1, T2, and T3 received less solution than T4, they presented similar N recovery as T4. Since the photosynthetic rate of leaves and subsequently the demand for wastewater and N in T4 and then in T3 started to decrease in the second half of the experimental period (Fig. 6), it certainly affected the final concentration of N in remaining solutions. Therefore, a following decline of the recovered N in T3 should occur as the experiment progresses.

The N recovery percentage of gravel-only pots was very low, about the half of those for treatments with plants (Fig. 7). It shows the ability of 40 cm teak seedlings to remove N during a two-month experiment.

4. CONCLUSION

Teak trees are known as fast-growing timber plants which needs 10–15 years to produce a good quality of wood. They thrive well in tropical rainforests and propagate easily by seeds and establish rapidly. Hence, they have potential as an alternative plant for phytoremediators grasses, which not only compensate the cost of harvest but also their timber is industrially important.

Over a two-month period teak seedlings fed with urea manufacturing wastewater in gravel-based, bench-sized subsurface constructed wetlands showed a considerable potential for removing N as high as 77% when they were supplied with up to 5 g N per pot volume (4 dm^3). This efficiency of teak plants for N uptake indicates that phytoremediation may be used for mitigating N pollution when wastewater effluent is applied to the land. However, this efficiency was obtained in condition under which plant growth was possibly limited by micronutrients and P deficiency. Therefore N uptake capacity likely can be enhanced by providing a more proper balance of elements required by the plant. Future research should investigate the relationship between N, P, and micronutrients, in order to minimize the nutrient imbalances and possibly increase of N concentrations in wastewater effluent.

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