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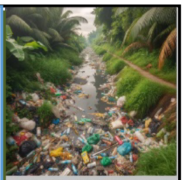

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Plastics-polluted river

Propagation and Feasibility Study of *Pennisetum purpureum* for Removal of Arsenic in Constructed Wetland

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ABSTRACT

Various species of plants have recently been identified as hyperaccumulators, capable of storing and resisting high concentrations of toxic metals. A perfect hyperaccumulator for phytoremediation must exhibit rapid growth and produce significant biomass. In the case of *P. purpureum*, roots displayed a growth rate of 1.19 cm/day, stems 4.78 cm/day, with an absolute growth rate (AGR) peaking at 4.16 ± 0.091 gm/day after 77 days. Regression analysis ($y = 0.138x$, $R^2 = 0.9904$) revealed a strong correlation between dry and wet weights. Water content in *P. purpureum* ranged from 70.59 ± 1.27% to 85.54 ± 1.54%. Typically propagated from seeds, *P. purpureum* is known for its ease of cultivation, rapid growth, and simple seed collection for subsequent generations. At 77 and 105 days, respective calorific values for the whole plant were 19,888 ± 238.66 J/g and 18,405 ± 220.86 J/g, indicating potential bioenergy use post-phytoremediation. This study comprehensively examines the physical growth, growth rate, biomass, water content, and calorific value of *P. purpureum*, revealing its robust root system and rapid growth characteristics. Thus, *P. purpureum* emerges as a promising candidate for heavy metal uptake in environmental remediation efforts.

INTRODUCTION

Arsenic (As) and arsenic-containing chemicals are listed as harmful materials with reportable quantities [1]. Polluted water utilized for drinking, food preparation as well as irrigation of food crops poses the extreme threat to public health from As [2]. Long-term exposure to As from drinking-water including food can cause cancer along with skin lesions [3]. Nowadays there are many kinds of technologies to restore polluted areas with As. One of the technologies that belong to a green technology is the remediation of soil as well as groundwater contaminated with As using plants. This technology is known as phytoremediation of As in constructed wetland. Constructed wetlands are a reliable, cost-effective natural process that have been helpful in eliminating a variety of contaminants, including arsenic [4]. Constructed wetlands (CWs) are artificial processes that have been created to utilize the natural processes incorporating the plants, soils, as well as related microbial populations of wetlands to help purify wastewater [5]. Napier grass **(***Pennisetum purpureum***)** is a monocotyledonous flowering plant belonging to the family Poaceae (the family of grasses) including the genus *Pennisetum* [6, 7]. *Pennisetum* is an immensely wide including varied genus composed of roughly 140 species with distinct basic chromosome numbers of 5, 7, 8, or 9, a wide variety of ploidy stages from diploid to octoploid, sexual as well as apomictic reproductive behaviours, along with annual, biennial, or perennial lifespans [8].

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Napier grass is a perennial C4 grass species native to Sub-Saharan Africa, from which it is thought to have spread to various tropical as well as subtropical areas throughout the globe [9]. It has been claimed that it is adaptable to thrive in a broad variety of soil types including agro-ecologies, from sea level to 2100 m, as well as that it can endure brief dry spells, while it grows most in locations with yearly precipitation ranging between 750 as well as 2500 mm [7]. Napier grass has established in regions of Central as well as South America, tropical Asia, Australia, the Middle East along with the Pacific islands because to its broad agro-ecological adaption [7]. As a result, it is now commonly cultivated in tropical as well as subtropical places across the globe. Napier grass is most typically spread through vegetative cuttings, tillers, as well as seeds [7]. It is a perennial bunchgrass with quick growth that is morphologically robust, long, as well as deeply rooted [10].

This grass is prolific, convenient to grow, drought resistant, generally adaptable to a wide range of soil including environmental situations, particularly in tropical soils [11]. It is apparently a plant that can withstand environmental stress as well as is resistant to disease, according to Negawo et al. [12]. It is among the foremost essential tropical crops and is additionally recognized as elephant grass or Uganda grass [12]. Napier grass has a number of excellent attributes., together with vigorous and deep root system with first growing characteristics in any types of soil; high production per unit area, resistance to disease, susceptibility to periodic drought, including high water usage efficacy, making it a selection for heavy metal phytoremediation [13]**.** The purpose of this research is to do a feasibility study by understanding the propagation, growing characteristics, dry weight, wet weight, growth rate, absolute growth rate, water content and correlation between dry weight and wet weight of this variety of Napier grass.

MATERIALS AND METHODS

Site description
All the study was conducted in a glasshouse of Ladang 15 (2°59′07"N 101°44′11"E)[14] of Universiti Putra Malaysia (UPM), Malaysia.

Duration of the experiment
The study was conducted during the period of June 2020 to September 2020.

Temperature and humidity of glasshouse
During the experiment, the temperature of the glasshouse was 30°C to 36°C and the humidity was 60% to 85%.

Propagation procedure of plants
At the primary phase, the seeds were found from *Pennisetum purpureum* initially from the crude oil polluted place in Malacca, Malaysia. The propagation of *P. purpureum* to prepare plant stock was directed utilizing the seeds of *P. purpureum* in garden soil at the glasshouse for 2nd generation. *P. purpureum* plants were grown through seeds of 2.5–4 mm in diameter. *P. purpureum* seeds were done in the germination tray using peat moss for germination. After one week of age, 15 seedlings were transferred from germination tray to a (99 x 63 x 30) cm plastic container and transplanted using garden soil 3: 2: 1 (fertile soil: organic matter: sand). A total of 720 *P. purpureum* plants were planted in the glasshouse.

Determination of physical growth of *P. purpureum*
At the time of propagation of *P. purpureum* plants, a total of three replicates were taken at a given time. Length, wet weight assessment was carried out immediately after the plants were harvested (Organization for Economic Co-operation and Development (OECD) 208 [14]). After clearing them from the soil attached to the roots, the three plants were separated into roots, stems and leaves.
Each section was weighed as wet weight (g) using weighing device. The next step was to dry the various parts of the plant at 80^{o}C for 2–3 days [15,16]. After drying, each part of the plant was placed in a Ziplock plastic bag with the aim of making the plant cool to the surrounding environment, and then weighed as dry weight (g).

Determination of macro nutrients, micronutrients and non-essential elements
According to Roose [17] and Zalesny et al. [18], nutrients are divided into 3 categories namely macro nutrients (N, P, K, Mg, Ca, S), micronutrients (Zn, B, Mn, Fe, Cu, Na, Cl) and non-essential elements (Al, Pb, Cd, Cr, Ni, As). **Table 1** shows the results of nutrient content in the soil.

Table 1. Amount of macro nutrients, micronutrients and non-essential elements (mg/kg) in garden soil.

Nutrients		Content (mg kg^{-1})
Macronutrient	N (Total)	325.19±12.36
	P	12.38±1.03
	K	9.61±0.87
	S	89.09±5.47
	Ca	96.27±6.89
	Mg	71.09±4.35
Micronutrient	Zn	2.08±0.32
	Cu	9.26±1.48
	Fe	9.96±2.04
	Mn	2.9±0.38
	Na	8.77±0.54
	Cl	6.31±0.39
Non-essential elements	Pb	-
	Cd	-
	Cr	-
	Ni	-
	Al	-
	As	-

Macro (N, P, K, S, Ca and Mg) and some micro-nutrients (Na and Cl) were analyzed using ion chromatography (IC) (Metrohm 882 Compact Plus, Switzerland). Sand sample preparation were done by extraction method for anions [19] and cations [20] using de-ionized water for analysis. A total of 5 g of sand samples was added to 50 mL of de-ionized water (1:10) and extracted utilizing a BIOSAN multi-RS-60 (Latvia) soil extraction at 60 rpm for 1 hour at room temperature.

The samples were then sliced with an incubator (Eppendorf centrifuge 5810R, USA) at 3000 rpm for 10 minutes as well as then filtered utilizing a filter paper (Whatman, Germany). Some micronutrients (Zn, Cu, Fe and Mn) and non-essential elements (Pb, Cd, Cr, Ni, Al and As) were analyzed using an inductively coupled plasma-optical emission spectrometer ICP-OES Optima 7300DV (Perkin Elmer, USA).

Calorie determination
Determination of calorific values of *P. purpureum* plants was utilized using a Bomb Calorimeter (C 5000 1.25 IKA, USA). This calorific value was determined to find out the ability of *P. purpureum* plants as an alternative for bioenergy. After the plants were dried, each part of the plant (roots, stems, leaves and fruits) was crushed using a grinder (Panasonic, Japan). A total of three replications of plant part were used to determine the calorific value of the plant.

Statistical analysis

Mathematical relationships between dry weight (g) and wet weight (g) were determined using linear least square techniques supplied through statistical toolbox of Microsoft excel 2019.

RESULTS AND DISCUSSIONS

Physical growth of *P. purpureum*

Plant sampling has been done from the plant age of 7 days to 105 days. **Fig. 1** showed the growth of *P. purpureum* roots and stems were well in the garden soil. The average root length at 7 days was 8.40 ± 0.14 cm and increased to 124.60 ± 2.12 cm at the plant age of 105 days. While the average length of the stem at 7 days was 7.22 ± 0.13 cm and at 105 days it increased to 476.00 ± 8.81 cm. Depending on the data obtained, the growth rate of the root and stem can be calculated by formula [21, 22]:

$$\text{Growth rate}\,\frac{\text{root}}{\text{stem}}\left(\frac{\text{cm}}{\text{day}}\right) = \frac{(\text{root length}/time_t - \text{root length}/\ time_0\ )}{(\text{time}_t - \text{time}_0)} \quad \text{(Eqn 1)}$$

Then,

$$\text{Root growth rate of } P.\ purpureum \ = \frac{(124.60 - 8.40)\ \text{cm}}{(105 - 7)\ \text{day}} = 1.19\ \frac{\text{cm}}{\text{day}} \quad \text{(Eqn 2)}$$

$$\text{Stem growth rate of } P.\ purpureum \ = \frac{(476.00 - 7.22)\ \text{cm}}{(105 - 7)\ \text{day}} = 4.78\ \frac{\text{cm}}{\text{day}} \quad \text{(Eqn 3)}$$

Fig. 2 showed the total number of leaves, flowers and seeds. The average leaf count at 7 days was four strands and could reach 24 leaves at 105 days. The flowers began to grow at the age of *P. purpureum* plants up to 77 days and began to bear seeds at 84 days.

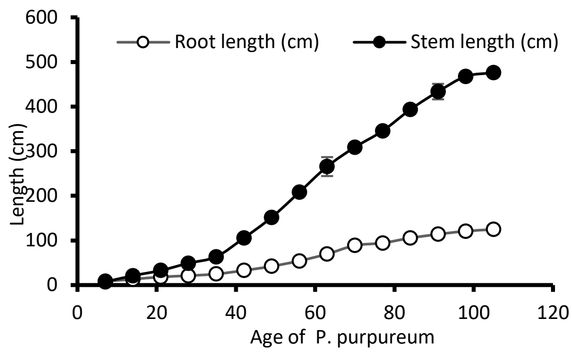

Fig. 2. Total number of leaves, flowers and fruits of *P. purpureum* throughout the propagation time. Error bars are mean ± standard deviation of triplicates.

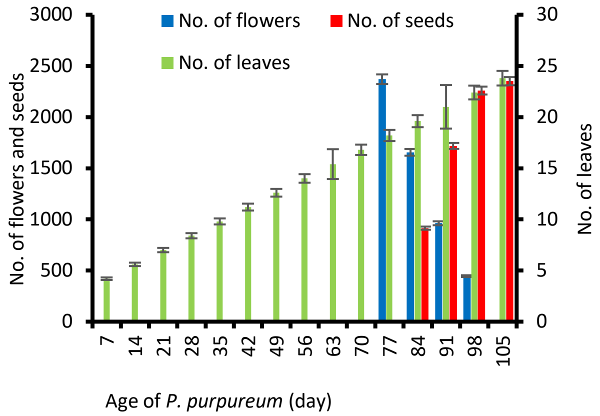

Fig. 2. Total number of leaves, flowers and fruits of *P. purpureum* throughout the propagation time. Error bars are mean ± standard deviation of triplicates.

The results presented that at the age of 105 days the seeds had matured to brown and dried. In mature spikelet there were many seeds available used for breeding. In the current study, the physical growth, total number of leaves, flowers and seeds of *P. purpureum* plants were evaluated at different days that showed an increasing trend with the rise of day. The findings of Negawo et al. [12], Morais et al., [23], Wangchuk et al. [24] and Lee et al. [25] supported these current findings who experimented on Napier grass and observed that it was a high yielding with need a least quantity of inputs as well as acreage, large biomass yield, quick growth potential and ease of propagation.

According to Rengsirikul et al. [26] and Williams et al. [27], there are 2 vital groups of Napier grass cultivars depending on morphology: normal or tall (up to 4-7 m) (length of stem) varieties (for instance, 'Australiano,' 'Bana,' and 'French Cameroon,') as well as dwarf or semi-dwarf (2 m) (length of stem) varieties (for instance, 'Mott'). Normal types have been observed to yield up to double as much as dwarf variants.

Growth rate of *P. purpureum*

Plant growth rate is the rate of increase in biomass, size or number of plants [22]. Absolute growth rate (AGR) (g day^{-1}) is a simple plant growth index that shows the increase in size each time [22]. Usually, the dry weight of the whole plant is used. Based on Kubota [22] and Sheteiwy et al. [28], the equation to determine the absolute growth rate is:

$$\text{Absolute growth rate (AGR)} = \frac{dW}{dt} = \frac{(W_2\text{-}W_1)}{(t_2\text{-}t_1)} \quad \text{(Eqn 4)}$$

Where,

AGR = absolute growth rate (g day^{-1})
W_1 = The dry weight of the plant at the beginning (g),
W_2 = The dry weight of the plant at the end (g),
t_1 = The start time (d) and
t_2 = The end time (d)

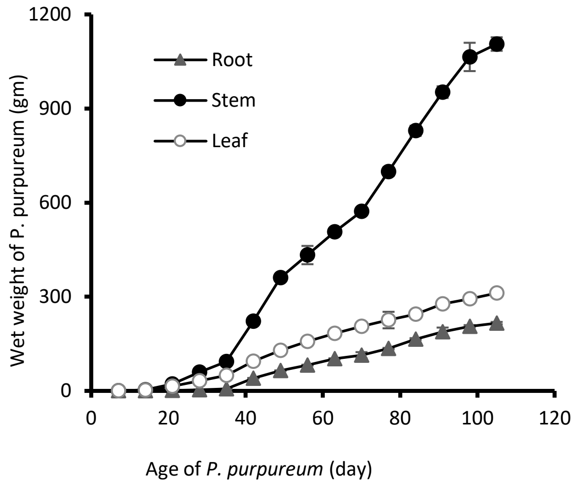

Fig. 4. Wet weight of root, stem and leaf of *P. purpureum* during propagation in garden soil. Error bars are mean ± standard deviation of triplicates.

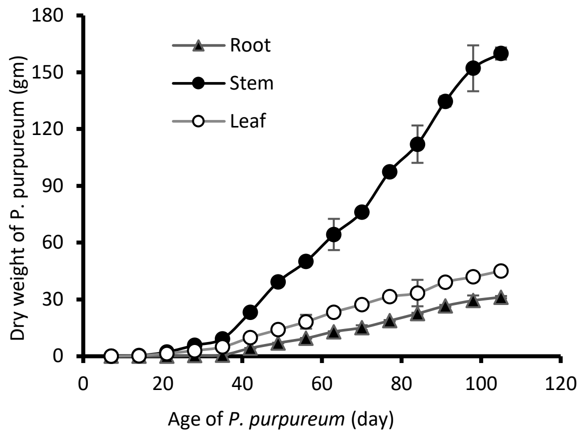

Fig. 5. Dry weight of root, stem and leaf of *P. purpureum* during propagation in garden soil. Error bars are mean ± standard deviation of triplicates.

Based on **Fig. 3** (overall data in Appendix B1), it can be seen that average *P. purpureum* plant growth has a different absolute growth rate. Absolute growth rates ranging from 7 days to 35 days showed small values (0.01 ± 0.0002 to 0.83 ± 0.018 g day^{-1}) but at age 42 days up to 105 days absolute growth rate reached a maximum value of 1.83 ± 0.04 g day^{-1}. At the age of 7 to 70 days it is expected that young *P. purpureum* plants are in a period of vegetative growth marked by the growth of new branches and new leaves. At the age of 77 days, the flower buds begin to grow, indicating that they have entered a generative growth period. These findings were parallel to the results of grass (*Sorghum halepense*) [29], switchgrass (*Panicum virgatum*) [30], maize (*Zea mays*) [31] along with sugarcane (*Saccharum officinarum*) [32].

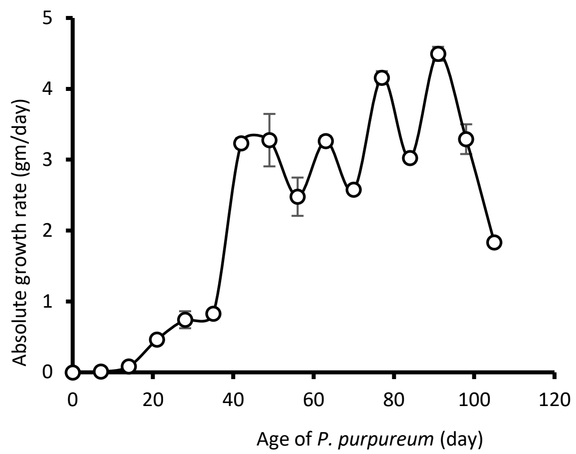

Fig. 3. Absolute growth rate (AGR) of *P. purpureum* plants. Error bars are mean ± standard deviation of triplicates.

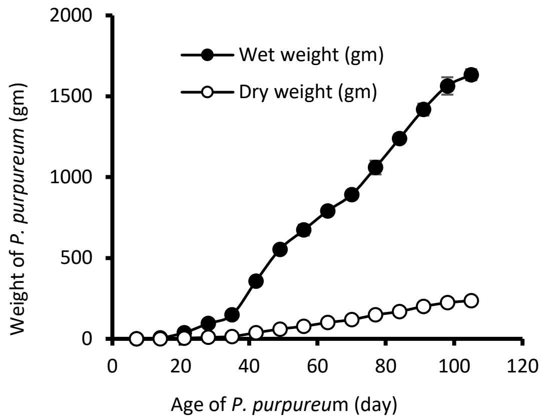

Fig. 6. Wet and dry weight of *P. purpureum* during propagation in garden soil. Error bars are mean ± standard deviation of triplicates.

Determination of the biomass of *P. purpureum* by wet and dry weight method

The goal of biomass study is to determine how much plant material is present in a given area [33]. Biomass is the total number of living things in a habitat, population or sample. Special calculations on biomass are usually expressed in dry weight (sample weight after loss of overall water content) per unit area of soil or unit volume of water [34]. During the cultivation process using the garden soil, 15 *P. purpureum* plants were able to grow well for 105 days in a (99 x 63 x 30) cm plastic container. Each of these plants contained the area:

$$\frac{\text{Area of container}}{\text{Total number of plants}} = \frac{99 \times 63 \text{ cm}^2}{15} = \frac{6237 \text{ cm}^2}{15} = 415.8 \text{ cm}^2/\text{plant} \quad \text{(Eqn 5)}$$

After the area requirement for one plant is determined, the biomass of *P. purpureum* plants at the age of 105 days during propagation can also be determined. Based on the data in **Fig. 6** it is known that the dry weight of *P. purpureum* at 105 days is 236.16 ± 4.58 g that means its biomass is 236.16 ± 4.58 g dry weight / 415.8 cm^2. **Fig. 4** and **Fig. 5** showed average wet weight and dry weight of root, stem including leaves of different ages of *P. purpureum* plants respectively. **Fig. 6** showed average wet weight and dry weight calculated at different ages of *P. purpureum* plants.

Both weights showed an increase with increasing age. *P. purpureum* is a plant that grown all year round (perennial) so it is expected that its growth will continue to reach 2-4 m. Agroecology, climate conditions, management techniques, as well as other edaphic variables have a substantial effect on the efficacy and production of Napier grass [26, 35]. Relwani, Nakat and Kandale [36], reported that biomass production of Napier grass was superior to other tropical grasses as well as Guinea grass (*Megathysus maximus*) along with Rhodes grass (*Chloris gayana*).

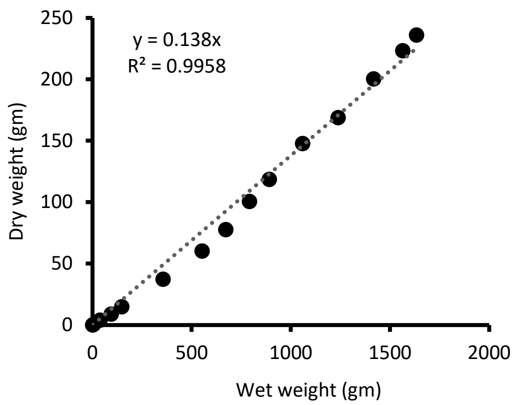

Fig. 7. Linear regression analysis of dry weight and wet weight of *P. purpureum* throughout the propagation using the garden soil.

The dry weight and wet weight data o *P. purpureum* plants were plotted on a regression analysis. **Fig. 7** displayed the linear regression analysis obtained for y = 0.138x with R^2 = 0.9904 which showed that there was a strong relationship between the dry weight and the wet weight of *P. purpureum* plants grown using the soil.

Water content in *P. purpureum*

Water is an important factor for plant growth because all cell activities can occur in the presence of water. Availability of water is an important factor for cultivated plants in agriculture [37]. Sufficient water will support the increase in leaf area so that it is closely correlated to the production of agricultural products [28, 38].

The low amount of water can also cause limited root development, thus interfering with nutrient absorption by plant roots [28, 39]. **Fig. 8** showed the average water content in the *P. purpureum* plant during propagation was high with a range of 70.59 ± 1.27% to 85.54 ± 1.54%. This can explain that the water consumption by plants was good.

According to Barber [37], the inflation of water into plant roots for plant growth in a controlled environment varies in the range of 0.2–1.0 x 10^{-6} cm^3 / cm^2 /second. The findings of the study by Llamas et al., 2008 stated that the water content in the roots of rice grown in nutrient-rich media on day 0 reached 82.5 ± 1.7%, while that in the stem reached 87.0 ± 0.5%. In the next 10 days, the water content in the roots reached 89.6 ± 0.5% and the stems as much as 87.4 ± 0.2%. Napier grass is a C4 grass species that apparently has the potential to lower shoot dry matter including enhance carbon absorption under periods of water stress, making it a suitable crop in places susceptible to periodic droughts [40]. When exposed to water stress situations, Napier grass experiences morphological alterations such as leaf rolling, decreased stomatal conductance, as well as increased water usage efficacy [41]. Several categories of plant have been utilized in phytoremediation experiments to remediate heavy metals through absorption along with accumulation method, like sunflower (*Helianthus annuus*), alpine pennycress (*Thlaspi caerulescens*), willow (*Salix* spp) including Indian mustard (*Brassica juncea*) [42]. In Malaysia, *Scirpus grossus* [43-45] along with *Ludwigia octovalvis* [46], also correspondingly recognized as bulrush as well as long-fruited primrose-willow, are among the plants that can survive harmful impact at the polluted place.

Various types of plants have recently been identified with the ability to store as well as resist large concentrations of metals that called hyperaccumulators. The characteristic of a perfect hyperaccumulator for phytoremediation must be able to develop quickly as well as generate a substantial volume of biomass [47]. Similarly, in this experiment, physical growth, growth rate, biomass and water content determination of *P. purpureum* plant is studied, and it is seen that, *P. purpureum* has a vigorous root system and fast-growing characteristics. Those plants that contains above characteristics, can uptake heavy metals from environments. So, it can be said that *P. purpureum* may uptake heavy metals from environments.

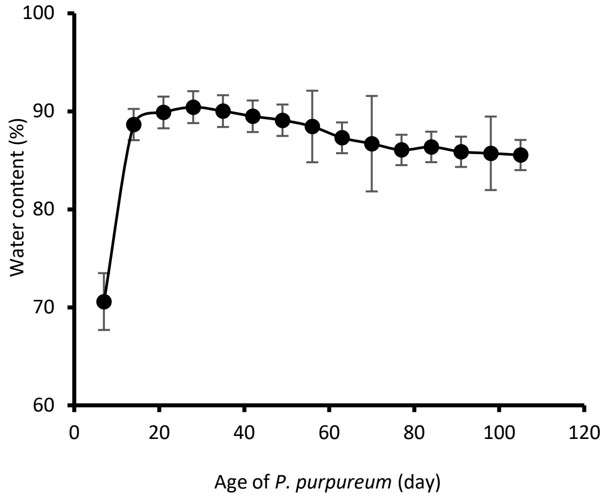

Fig. 8. Water content in *P. purpureum* plants. Error bars are mean ± standard deviation of triplicates.

Calorie determination of *P. purpureum*

Based on **Fig. 9** the calorific value of leaves was greater than the calorific value of other *P. purpureum* plant parts. While **Fig. 10** showed the average calories of the whole *P. purpureum* plant during propagation with a plant age of 77 days (19,888 ±238.66 J g^{-1}) and 105 days (18,405 ± 220.86 J g^{-1}).

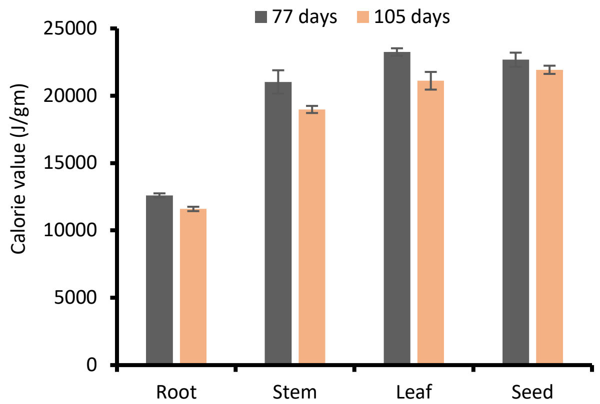

Fig. 9. Calorific value of root, stem, leaves and seeds of *P. purpureum* plant during propagation. Error bars are mean ± standard deviation of triplicates.

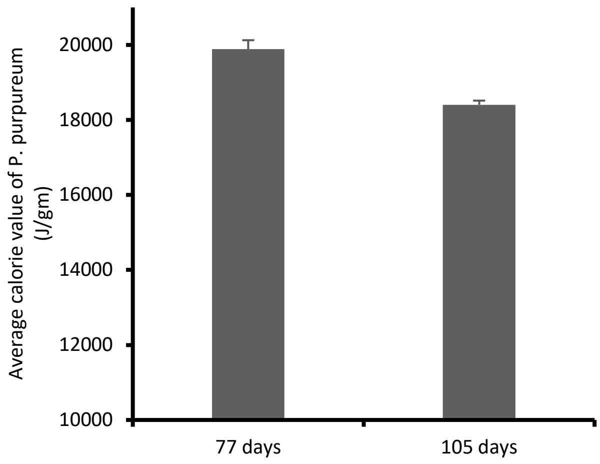

Fig. 10. Average calorific value of *P. purpureum* during propagation. Error bars are mean ± standard deviation of triplicates.

The mean calorific value at 77 days for the leaves was 23,246 ± 278.96 J g^{-1}, while the calorific values of seeds, stems and roots were 22,677 ± 272.13; 21,026 ± 252.31 and 12,602 ± 151.23 J g^{-1} respectively. The mean calorific value at 105 days for the leaves were 21,113 ± 293.47 J g^{-1}, while the calorific values of seeds, stems and roots were 21,928 ± 304.80, 18,983 ± 263.86 and 11,596 ± 161.19 J g^{-1}, respectively. Based on Kurniawan and Santoso [48], dried sugarcane plant leaves have a caloric value of up to 14,656 kJ kg^{-1} (14,656 J g^{-1}) and have the potential to produce 1,029,630 MWH of energy from 1.39 million tons. When equated, it means that one ton of dry sugarcane leaves can potentially produce 0.741 MWH (741 kWh) of energy. The calorific value of dry *P. purpureum* leaves when compared to dry sugarcane plant leaves was higher by 6.9-13.6%, indicating that *P. purpureum* has potential as a source of bioenergy.

CONCLUSION

Based on the findings of this study, it can be concluded that the growth rate of *P. purpureum* roots was 1.19 cm day^{-1} and the stem growth rate was 4.78 cm day^{-1}, while the absolute growth rate (AGR) at 77 days reached a maximum of 4.16 ± 0.091 gm day^{-1}. The dry weight and wet weight data of *P. purpureum* plants were plotted on a regression analysis. The linear regression analysis obtained for $y = 0.138x$ with $R^2 = 0.9904$ which presented that there was a strong relationship between the dry weight and the wet weight of *P. purpureum* plants grown using the soil. The range of water content in *P. purpureum* plants were 70.59 ± 1.27% to 85.54 ± 1.54%. Generally, *P. purpureum* plant is a type of plant that is grown using seeds, it is easy to grow, has fast growth and is easy to collect for mature seeds that can be used for propagation of next generation. The respective calorific values for dry leaves, dry seeds, dry stems and dry roots from *P. purpureum* plants at 105 days were 21,113± 293.47 J g^{-1}; 21,928 ± 304.80; 18,983 ± 263.86 and 11,596 ± 161.19 J g^{-1} with the average calorific values for whole plant at 77 days and 105 days were 19,888 ± 238.66 J g^{-1} and 18,405 ± 220.86 J g^{-1} respectively, suggesting that *P. purpureum* may be used as a source of bioenergy after phytoremediation of As in constructed wetland.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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