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Effects of *Zingiber officinale* Roscoe. extracts in growth media on nitrate and phosphate removals for growths and accumulations by *Ipomoea aquatica* Forsk

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Abstract

The most effective measure for prevention and mitigation of water quality and ecological degradation from eutrophication is the minimization of NO_3-N and PO_4-P to the standards set out before discharging into waterways. Floating plants can absorb excessive NO_3-N and PO_4-P from sewage water effectively via their roots for growth. This mechanism supports ecological sustainability, as the result of their natural abundance, well-adaptation and aesthetics in water ecosystem. The experimental design was set up to achieve the objectives of comparing the growths of *I. aquatica* (water spinach) by fresh biomass experimented in 4 treatments of media growth (NO_3-N and PO_4-P concentrations at 20 mg-N/L and 1 mg-P/L) with the additions of *Z. officinale* (ginger rhizome) extracts at 0, 2,000, 4,000, and 6,000 ppm, respectively. Their efficacy of NO_3-N and PO_4-P removals (%) are analyzed. Analyses of variances (ANOVA) at a confidence level of 95% and Duncan Post Hoc Tests were performed to determine the differences among efficiency of NO_3-N and PO_4-P removals (%) and growths of *I. aquatica*. The results revealed that the application of the 4,000 ppm extract was appropriate for *I. aquatica* growth and the remediation of nutrient-rich waters. This is owing to the highest biomass of 130.08±25.33 g (sig=.015) which was mainly derived from the upper parts (leaves and stems) and the uttermost NO_3-N at 67.9±6.20%, while the second most PO_4-P at 95.55±3.98 % (95.75±3.22% in the controls) removals were found in 18 and 15 days. Moreover, the far below European Commission maximum levels for nitrate as contaminants in fresh vegetables (for fresh spinach) at 86.95± 4.40 mg-N/Kg may strengthen the safety for human and animal consumption.

Keywords: nitrate and phosphate removals, biomass growth, nitrate and phosphate accumulation, ginger, water spinach

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1. Introduction

Wastewater containing high contents of NO_3-N and PO_4-P, which are nutrients for plant growths, when discharged to inland and marine water bodies is the major concerned for eutrophication. In general, the amounts of NO_3-N found are ≤ 1 mg-N/L, while detection of phosphorus at 0.01 mg-P/L or less may cause serious effects in surface waters [1]. Utilization of floating plants in constructed wetland systems is appropriate in many respects [2 - 4], owing to co-operational working of bacteria and roots conducting to absorption of excess nutrients, NO_3-N and PO_4-P, and stored in the tissue. Plants with the greater absorption power for nutrients should be able to recover quickly when exposed to nutrient-rich waters and absorb nutrients well [5, 6]. Hence, the distinct characteristics of selected plants are included higher growth rates, biomass, and storage of nitrogen and phosphorous in the tissue [7]. In a study aimed for improving the quality of dairy manure wastewater using three floating species, *Eichhornia crassipes*, *Hydrocotyle umbellate*, and *Pistia stratiotes*, *E. crassipes* absorbed TN and TP at 91.7 and 95.8% [8].

I. aquatica, a floating plant, can grow accelerated in the water and extend up to 16 cm/day, when physical factors and concentrations of inorganic nutrients are appropriate [9]. *I. aquatica* has high in nutritional values. Hence, it has been used mainly as a green vegetable and animal feed [10]. In constructed wetland treatment systems, it often collaborates with other floating plants to absorb nutrients [11] and heavy metals from sewage water [12, 13]. In a study on the remediation of water quality caused by eutrophication (TN and TP at 2.70 and 0.57 mg/L) at Huajia pool of Zhejiang University in China, using *I. aquatica*, the efficacy for absorption at 41.5 and 75.5% was found, respectively [14].

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Currently, there have been efforts to stimulate the rates of NO_3-N and PO_4-P absorption using floating plants by growths. The introduction in plant hormone application is safer compared with synthetic hormones, some of which are mutagenic and carcinogenic in mammals [15]. *Zingiber officinale* (ginger), rhizome, consists of two groups of potentially chemical components, essential oils and oleoresin. Most of the essential oils are terpenes of which evaporated out by steam [16]. Besides the largest diversities compared to other substances produced by plants, they play important roles on the growth and development of plants in various stages [17]. However, the studies on water quality improvement using plant hormones are scarce and limited, especially the use of *Z. officinale* extract. Hence, the objectives of this study were to compare the growths of *I. aquatica* biomass in the growth media with an additional set of *Z. officinale* extracts and determine the appropriateness of *I. aquatica* biomass as a PO_4-P and NO_3-N sorbent from sewage water.

2. Materials and methods

2.1 Experimental design

The experiment was divided into four treatments, with three replicates, of which twelve plastic tanks ($40 \times 60 \times 30$ cm^3) containing 50 L of growth media, NO_3-N (KNO_3) and PO_4-P (KH_2PO_4) concentrations at 20 and 1 mg/L, were applied with the additions of *Z. officinale* extracts at 0, 2000, 4000, and 6000 ppm, respectively. *Z. officinale* which purchased from the vegetable market, Pathom Mongkol, in Nakhon Pathom Province, Thailand, was prepared and extracted as followings, soaked the sliced *Z. officinale* at approximately 1×1 cm^2 for 2 hrs in distilled water with a ratio of 1:3 wt/vol, then cooked with the high setting in Panasonic NF-M30A -Slow Cooker for 1 hr on which the lid was closed continuously to allow condensation to run down inside, and left for cooling down, strained by a muslin cloth, cooked again with a medium setting for 30 mins.

Five analogous rafts (5 rods with 2 buds for each) of *I. aquatica* with 15 cm lengths (installed by hanging with the plastic beam) were cultivated right and left alternately on the surface of media growth in the plastic tank where an air stone with a small plastic tube connecting to the Hailea-ACO-208-Air Compressor for generating aerobic conditions. On a daily basis at 5 pm, the 50L of growth media was kept consistency by the addition of tab water. The experiment period was 18 days from the February 9th-27th, 2016, carried out in the roof-top greenhouse of Science and Technology Faculty where the average highest and lowest temperature varied between 33±2-24± 3 ºC.

2.2 NO_3-N and PO_4-P removals from growth media

At 7 am, every three days, water samples were collected for the determination of NO_3-N and PO_4-P concentrations using SP-830 Plus Spectrophotometer (Metertech, Taiwan). Nitration of salicylic acid of Cataldo *et al.*[18] and phosphate using ascorbic acid of APHA, AWWA & WEF No.4500-PE [19] methods by which the absorbances at 410 and 880 nm were read, respectively. NO_3-N and PO_4-P removals (%) were determined, for example, by multiplying their concentration fractions of day1-day3 / day1 by 100.

2.3 Biomass determination

After 18 days of cultivation, *I. aquatica* rafts were harvested, washed thoroughly with deionized water and blotted dry. The fresh biomass (g) of the root (R) and the upper part (above, Ab) samples were recorded, then one day exposed in the air, and dried at 70ºC in the oven for two days. The dried root and upper part samples were weighed for the biomass, crushed by Philips-Viva chopper-HR 1396 and sieved by 40 µm mesh screen. The samples were kept dry with tightly closed lids in 30 mL glass bottles.

2.4 Water quality analysis

Water samples were collected for the determination of pH, DO, and turbidity using Senz pH (Trans Instruments, Singapore), HI 9147 Portable Galvanic Dissolved Oxygen Meter (Hanna Instruments, Rhodo Island, USA), and 2100P Portable Turbidimeter (Hach Company, Colorado, USA).

2.5 NO_3-N and PO_4-P in tissue determination

NO_3-N and PO_4-P in the root and the upper part samples were extracted from the dried plant samples of 200 ± 1.0 mg in 125 mL glass bottles with 50 ± 2.0 mL acetic acid 2%, according to Miller [20] using Fisher Scientific-Thermo Scientific MaxQ 481R HP Shaker at 180 rpm for 30 min. Three blanks were also prepared with the same extraction method. Extracted samples and blanks were filtered through highly retentive filter papers (Whatman no. 2), measured volumes (mL), and kept tightly with closed lids in 50 mL glass bottles. NO_3-N and PO_4-P contents in tissue samples were determined using Metertech-Spectrophotometer-SP-830 Plus with nitration of salicylic acid [18] and phosphate using ascorbic acid [19] methods by which the absorbances at 410 and 880 nm were read, respectively. NO_3-N and PO_4-P in the plant tissue determinations were calculated by,

$$\mathbf{AC\,(mg/kg)=(C_S-C_B)\times V_E\times df/W_S}$$

Where,

AC (mg/kg) is the NO_3-N/PO_4-P content in the dried plant sample,

C_S is the NO_3-N/ PO_4-P concentration (mg/L) in the dried plant sample,

C_B is the concentration (mg/L) of a blank,

V_E is the volume extracted (mL) of the dried plant sample,

Ws is the biomass (g) of the dried plant sample,

df is the dilution factor.

2.6 Statistics

Analyses of variances (ANOVA) at a confidence level of 95% and Duncan Post Hoc Tests were performed to determine the differences in growths, efficacy of NO_3-N and PO_4-P removals (%), and their accumulations in the tissue among *I. aquatica* raft treatments. These performances included the differences of water quality parameters, pH, DO, and turbidity among treatments.

3. Results and discussion

3.1 Effects of *Z. officinale* extracts on NO_3-N and PO_4-P removal efficiency

NO_3-N, NH_4-N and PO_4-P are the main nutrients in hydrological ecosystem where plants can absorb for their growing processes. Naturally, the degradable organic compounds, containing nitrogen and phosphorus from plant and animal debris, return these nutrients back mainly through the nitrogen and phosphorus cycles. Hence, the excess NO_3-N, NH_4-N and PO_4-P contaminated by human activities, particularly, from agricultural runoff and wastewater from households conduct to eutrophication in water bodies [21].

In this study, the growths of *I. aquatica* biomass were run parallel to PO_4-P and NO_3-N removals from the nutrient media (20 and 1 mg/L) with the additional set of *Z. officinale* extracts (0, 2,000, 4,000, and 6,000 ppm). This was to determine the extract additions in which appropriate for *I. aquatica* biomass as a PO_4-P and NO_3-N sorbent for wastewater treatment. *Z. officinale*, rhizome, comprises two groups of potentially chemical components, essential oils and oleoresin. Most of the essential oils are terpenes of which evaporated out by steam [16]. Besides, the largest diversities compared to other substances produced by plants, they play important roles on the growth and development of plants in various stages [17].

The result of this study found that adding *Z. officinale* extract at 4,000 ppm was suitable for removals of NO_3-N and PO_4-P concentrations at 20 mg/L and 1 mg/L (**Figure 1 and 2**). Firstly, NO_3-N removal occurred slowly and increased continuously with time which was in accordance with increase of treatment efficiency in constructed wetland wastewater treatment, when the hydraulic loading rate minimized and retention time increased [22]. In addition, the gradual increase of NO_3-N removal at the beginning may attribute to the acquirement of PO_4-P than NO_3-N where the plants take up PO_4-P for newly root and shoot generation [22, 23]. On day 18, the removal efficiency at greater than 50% found at which 67.9 ± 6.20%. This may derive from greater contents of NO_3-N requirements compared with at the beginning. Plants take up more nitrogen for branch and leaf building up activities, nitrogen is not only the main component of the protein in protoplasm of cells, but also of chlorophyll that is essential for photosynthesis [22, 23]. While the NO_3-N removal was reluctant at the beginning, PO_4-P removal on day 3 was raised distinctively where removal of more than 92% found in all treatments (100% found at 6000 ppm of *Z. officinale* extract addition). This was attributed to; 1) PO_4-P promoting early root formation and growth along bilateral nodes resulting in newly leave occurrences on those nodes [23]. 2) The application of relatively low PO_4-P concentration of 1 mg/L for preventing the stress of *I. aquatica* rafts in media growth; however, this given concentration was closed to the total phosphorus from effluents of freshwater aquaculture [24]. The negative removal percentage on day 6 and 9 derived from some parts of the initial stems forming the raft structure being blemish and swelling, resulting in dis-integration, decay by microbes, and the return of PO_4-P to the growth media. From day 12 to 15, the percent removal was improved subsequently and reached the maximum in the control and the 4000 ppm extract addition treatments (95.75±3.22 and 95.55±3.98 %), in contrast to the recurrence of decrease in percent removal on day 18. This may explain as mentioned earlier. In this study, the biomass of *I. aquatica* was found at higher efficiency for NO_3-N and PO_4-P removal than that of Hu, Ao, Yang, & Li [14] in which the water from Huajia pool, Zhejiang University in China where eutrophication was prevail, NO_3-N and TP of 0.32 mg/L and 0.57 mg/L eliminated by 41.5 and 75.5%, respectively. In addition, removal of the present study was surpassed that of Awuah *et al.* [25] in which revealed the efficiency of TP removal among three floating plants, *Pistia stratiotes* (water lettuce), *Spirodela polyrhiza* (duckweed) and algae at 33% and 9%, whereas the

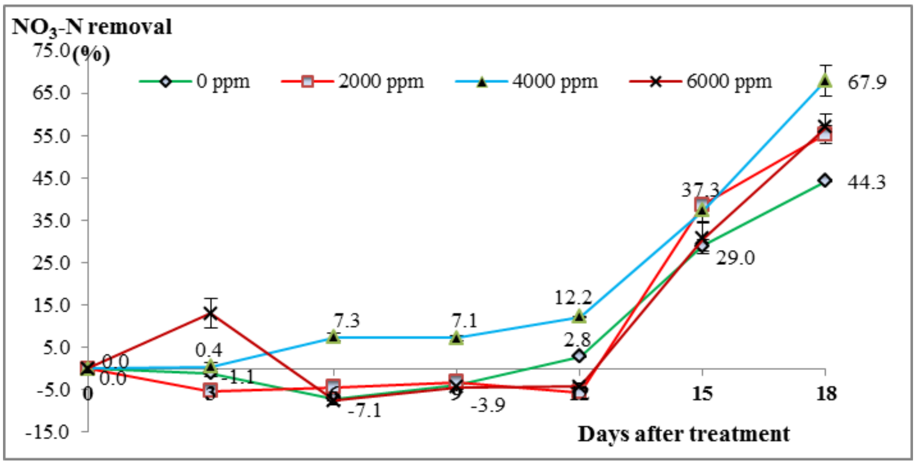

Figure 1 Effects of *Z. officinale* extracts on NO_3-N removals from growth media by *I. aquatica*. Values represent the means±SE of three replicates.

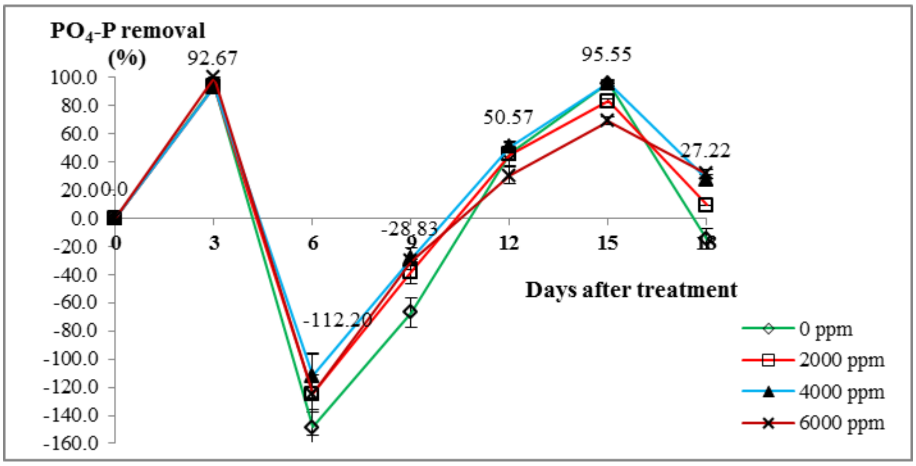

Figure 2 Effects of *Z. officinale* extracts on PO_4-P removals from growth media by *I. aquatica*. Values represent the means±SE of three replicates.

19% of TP increased in the algae pond. Consequently, the appropriate concentration of *Z. officinale* extract added in the growth media may influence on the *I. aquatica* growth acceleration, following with the increase of those improving removal.

3.2 Effects of *Z. officinale* extracts on biomass of *I. aquatica*

In this research, analysis of variance revealed significant differences among the growth of biomass in treatments of which the addition at 4,000 ppm of *Z.officinale* extract conducted to the maximal biomass of 130.08 ± 25.33 g (*sig* = *.015*), while there were no significantly different means of biomass between other treatments, including the control (**Figure 3 and 4**). Furthermore, nearly all biomass was derived from the stem and leave parts. Consequently, the concentration of *Z. officinale* extract at 4,000 ppm, added in 50 l of growth media, containing NO_3-N and PO_4-P of 20 and 1 mg/L was helpful for accelerating the growth of *I. aquatica.* This is in agreement with *Z. officinale* volatile oils containing in the extraction, of those mostly are terpenes, the most plentiful chemical compound group that plays a fundamental role in relation to the growth and development of plants in various stages [17]. This compound has properties similarly to a plant hormone that can stimulate the growth of roots and shoots. Additionally, in a study to increase the numbers and sizes of *Z. officinale* rhizomes, it was reported that chlormequat (2-chloroethyl)-trimethylammonium chloride of the concentrations, 180 and 200 ppm when sprayed onto the leaves of *Z. officinale* in the active growth phase may have effects on restraining GAS synthesis which produced naturally in small amounts, resulting in

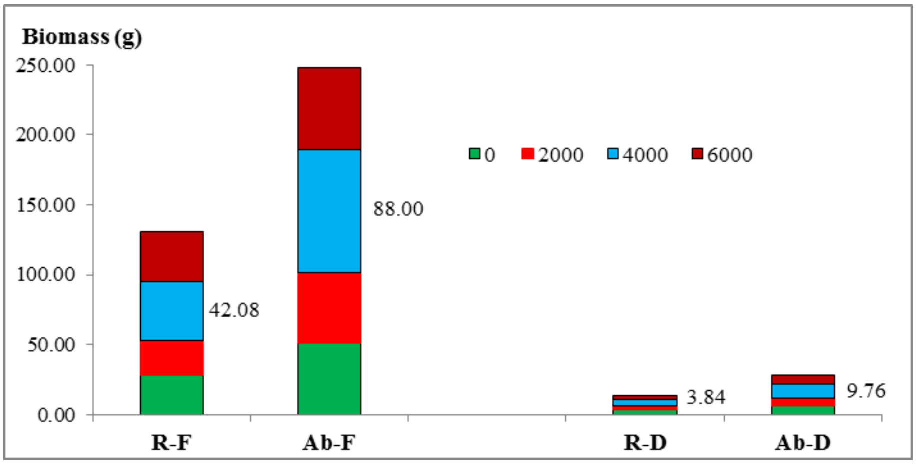

Figure 3 Biomass of *I. aquatica* after treatments. R/Ab-F and R/Ab-D represent the means of three replicates of the fresh (left) and dry (right) biomass of roots (R) and leaves-stems (Ab), respectively.

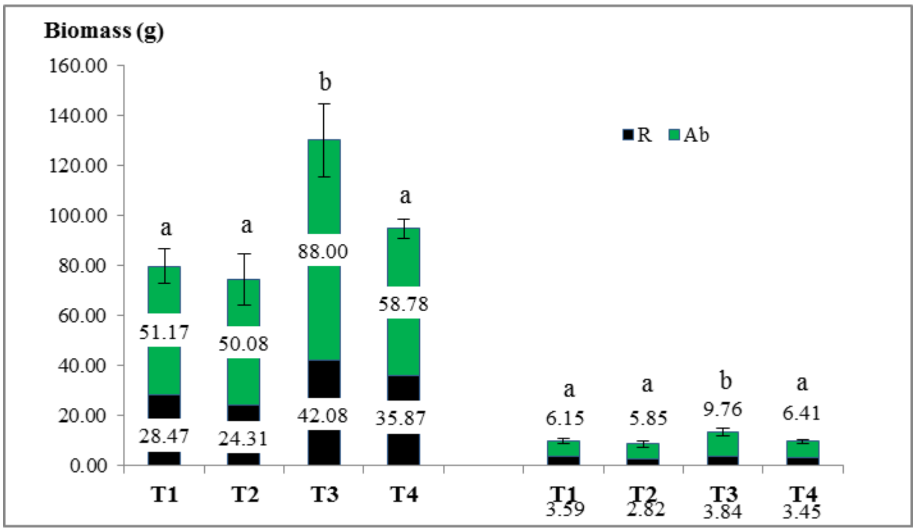

Figure 4 Biomass of *I. aquatica* after treatments. R and Ab represent the means of three replicates of the fresh (left) and dry (right) biomass of roots (R) and leaves-stems (Ab), respectively. Different letters above the columns show significant differences between treatments.

stimulation of auxin and cytokinin hormone syntheses, following with the numbers and sizes of rhizome increments [26].

3.3 Effects of *Z. officinale* extracts and *I. aquatica* growth on water quality

The water quality parameters of pH (6.2-8.4) and DO (6.7-7.5) among 4 treatments, 0, 2000, 4000, and 6000 ppm were in agreement with the *optimum* ranges for *aquaculture*, pH 6-9 and DO 5 mg/L-saturation [27]. The pH on day 9 sharply dropped to 6.9 in all treatments derived from the addition of 10% HCl for suitability of plant growth. In addition, the turbidity at 5.0-39.3 NTU complied with of those in general aquaculture ponds at ≤50 NTU [27]. Furthermore, the significant difference of the maximum turbidity in *Z.officinale* extract at 6,000 ppm treatment among the others was in accord with the minimum efficiency of PO_4-P removal (**Figure 5**).

3.4 Effects of *Z. officinale* extracts on NO_3-N and PO_4-P accumulations in *I. aquatica*

For NO_3-N and PO_4-P accumulations in tissues (**Figure 6**), roots (R) and upper parts (Ab), the greater found significantly in the roots (*sig = .00*). There was no significant difference of NO_3-N between 63.03 ± 6.12- 90.34 ± 21.0 6 mg-N/Kg and PO_4-P between 131.50±47.54 - 347.80±97.09 mg-P/Kg in the upper parts among the treatments. However, the present levels were much less compared with a previous study

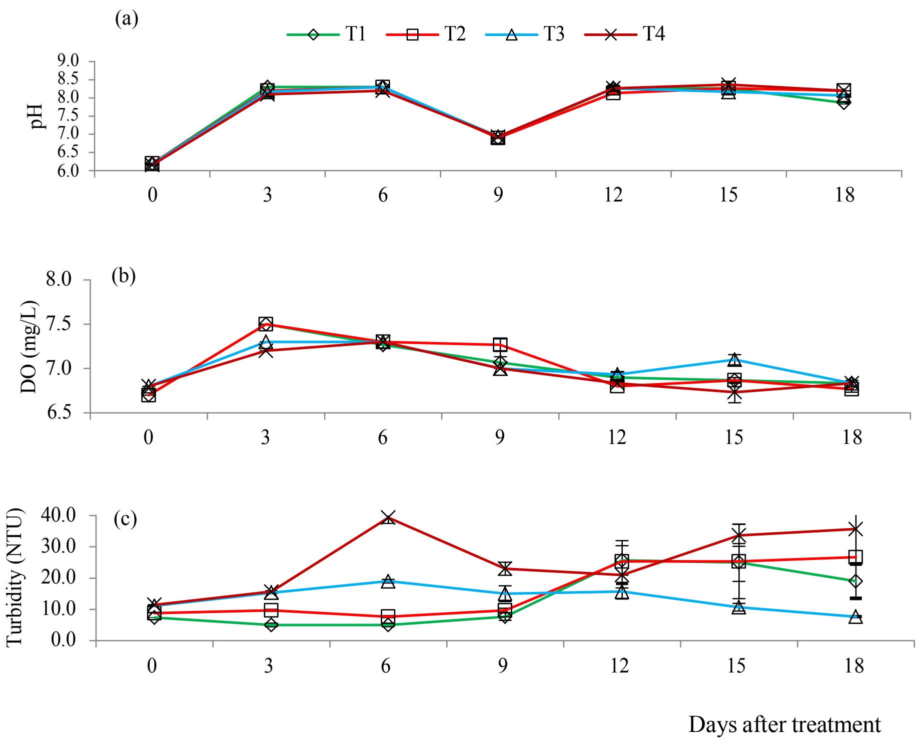

Figure 5 Effects of *Z. officinale* extracts and *I. aquatica* growth on the water quality of growth media (a) pH, (b) DO, and (c) Turbidity. T1-T4 are the additions of *Z. officinale* extracts at 0 (control), 2,000, 4,000, and 6,000 ppm, respectively. Values represent the means±SE of three replicates.

on NO_3-N and PO_4-P accumulations in *I. aquatica* samples of 3 stations from the Tha Chin River, a distributary of the Chao Phraya River, Thailand, where the levels in tissues were < 5000 mg-N/kg and < 2900 mg-P/kg, respectively [28]. It is important to note that all NO_3-N levels accumulated in tissues of this study were much less than the maximum levels specified by EC [29] of ≤ 2,500 mg/kg as contaminants in fresh vegetables (for fresh spinach). Therefore, this confirms the suitability and safety as a green vegetable for culinary usage, and for animal feed. Besides, larger amounts of NO_3-N accumulations in the roots (R) compared with those in the upper parts (Ab) of the current study is indicated that the proper concentration of *Z. officinale extract* applied to NO_3-N and PO_4-P rich water may escalate the efficacy of *I. aquatica* biomass uptake and removal of these nutrients, promoting growth augmentation, and improvement of water quality.

4. Conclusions

NO_3-N and PO_4-P in wastewater reductions prior to discharging into natural waterbodies, e.g. rivers, canals are the major keys for accomplishment in recovery, improvement, and protection of water quality from eutrophication. Floating plant adoptions for nutrient removals in constructed wetland wastewater treatments provide many useful benefits, according to low budget investment and apparent availability. Furthermore, successful minimization of the treatment system is not only dependent on high efficacy of floating plant removals, but also physical and biological properties of wastewater. In this study, the result revealed that the application of *Z. officinale* extract of 4000 ppm added in growth media (NO_3-N and PO_4-P of 20 and 1 mg/L) was capable of stimulating *I. aquatica growth, conducting* to the maximal biomass at 130.08 ± 25.33 g (analysis of variance, ANOVA; *sig = .015*), that mainly obtained from the upper parts, leaves and stems (Ab). Besides, the topmost NO_3-N of 67.9±6.20% and the second most PO_4-P of 95.55±3.98 % (95.75±3.22% in the controls, though) removals had occurred on day18 and 15, the much below from EC maximal levels for nitrate as contaminants in fresh vegetables (for fresh spinach) at 86.95± 4.40 mg-N/Kg may confirm the safety for human and animal consumption.

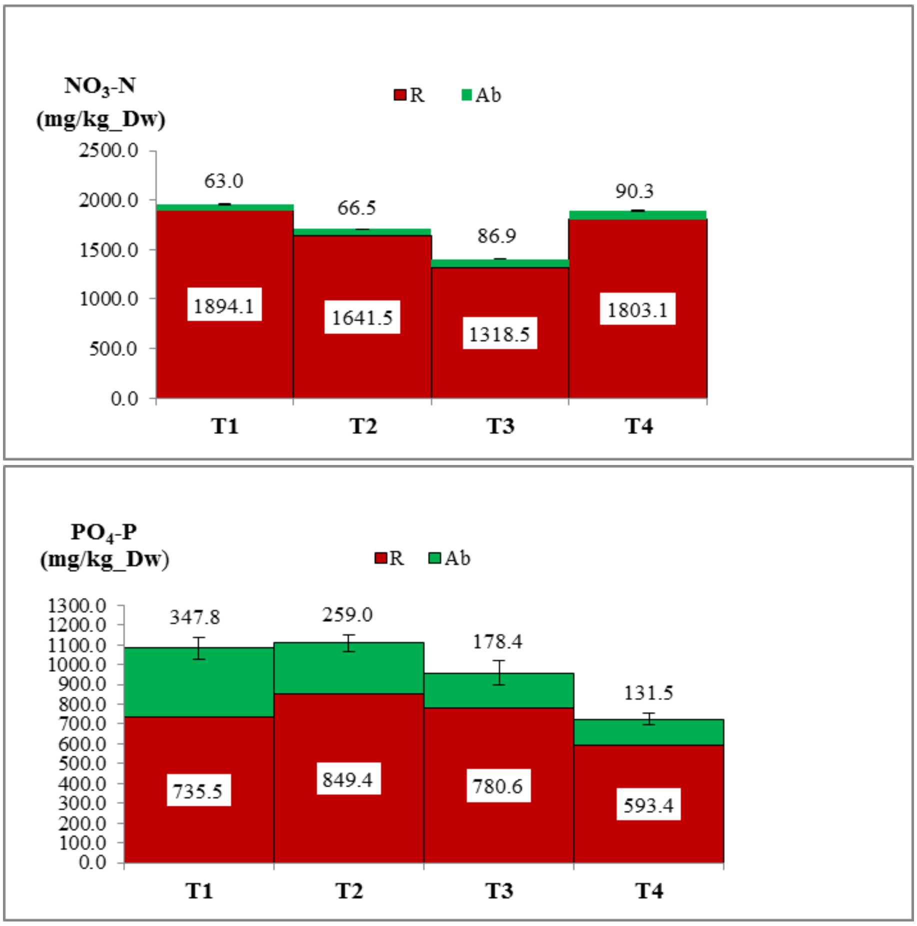

Figure 6 Effect of *Z. officinale* extracts on NO_3-N and PO_4-P accumulations in *I. aquatica*. T1-T4 values represent the means of roots (R, bottom) and of leaves-stems (Ab, top)±SE of leaves-stems (Ab) at three replicates.

For further study, the efficacy of *I. aquatica* with the addition of *Z. officinale* extract at 4,000 ppm on a consecutive range of NO_3-N and PO_4-P concentrations is recommended. Even though this research aimed to alleviate NO_3-N and PO_4-P nutrient rich water using *Z. officinale* extract for promoting *I. aquatica* growth and their efficacy of removals. In addition, using more varieties of floating plants with the greater root systems (e.g., *Typha angustifolia*, *Thalia geniculata, Limnocharis flava*) may gain more advantage on the efficacy of removals. Future studies of other pollutants, including heavy metals and organic substances in polluted waste water removals using floating plants and their pollutant metabolisms should take into consideration.

Acknowledgements

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