Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2020.25297

187 (2020) 87–92
May

Phytoremediation efficiencies of *Spirodela polyrhiza* and *Brassica oleracea* in removing nutrients from treated sewage effluent

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Received 11 June 2019; Accepted 20 December 2019

ABSTRACT

The study investigates the capacity of phytoremediation as a post-treatment step for the nutrient-rich-treated sewage effluent from Saga City sewage treatment plant, Saga, Japan. Phytoremediation in the context of this study is the removal of nutrients such as ammoniacal nitrogen, nitrate nitrogen and phosphorus from the nutrient-rich-treated sewage effluent by plants. In this study, *Spirodela polyrhiza* (*S. polyrhiza*) and *Brassica oleracea* (*B. oleracea*) were used to phytoremediate the treated sewage effluent collected from the Saga City Sewage Treatment Plant under laboratory scale. Plants were grown in polypropylene planter box supplied with 8,000 mL treated sewage effluent under indoor environment and full water retention throughout the experimental studies. The removal efficiency and daily absorption of nutrients by phytoremediation plants were determined. It was found that the most optimal removal efficiency and average daily nutrient removal rate by *S. polyrhiza* throughout the experiment were 92.42% ± 1.29% or 15.4 mg/L/d for ammoniacal nitrogen achieved in day 1, 78.69% ± 10.31% or 2.68 mg/L/d for nitrate-nitrogen achieved in day 4, and 93.45% ± 3.26% or 0.51 mg/L/d for phosphorus in day 3 of an experiment. On the other hand, the removal efficiency and average daily nutrient removal rate by *B. oleracea* throughout the experiment gave a total of 8 d where 76.07% ± 10.38% or 1.68 mg/L/d for ammoniacal nitrogen, 78.38% ± 0.40% or 1.19 mg/L/d for nitrate-nitrogen and 67.40% ± 10.91% or 0.10 mg/L/d for phosphorus. The overall findings demonstrated that phytoremediation by *S. polyrhiza* was far more effective in removing nutrients from the nutrient-rich-treated sewage effluent compared to *B. oleracea*. The significance of the study includes reducing the possibility of eutrophication outbreak caused by the disposal of treated sewage effluent, advocating less dependency on global demand for non-renewable phosphorus resources in the agriculture sector, and solving food demand due to the increasing world population.

Keywords: Phytoremediation; Treated sewage; Effluent; *Spirodela polyrhiza*; *Brassica oleracea*

1. Introduction

Treated sewage effluent is particularly high in nitrogen and phosphorus [1]. In general, the concentration of total nitrogen is reported to be around 40–55 mg/L [2], while the presence of total phosphorus (TP) concentration in a treated sewage effluent is reported to be below 10 mg/L [3]. However, studies have shown that even at a concentration of 0.02 mg/L of TP, eutrophication can occur [4]. At the end of sewage treatment, the nutrient-rich-treated sewage effluent is released into surface water such as rivers and streams. This phenomenal is practiced throughout the world [5]. The discharged nutrient-rich-sewage can alter ecosystem

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structure in all aquatic habitats and eventually pose threats to human health.

The studied area of this study is located in the Saga city which is the capital city of Saga Prefecture, located in the Western part of Japan, Kyushu Island. There are 20 local sewage treatment plants located in the Saga Prefecture. The study focuses on the Saga City sewage treatment plant, which is the largest sewage treatment plant in the Saga Prefecture. The sewage treatment plant spans across an area of 4,299.9 ha and handles maximum sewage volume of 81,500 m^2/d. Currently, the Saga City sewage treatment plant treats an average of 53,230 m^2 sewage per d.

In Japan, adhering to the National Effluent Standard is mandatory before discharging the treated sewage effluent into the open water bodies. Thus, contaminants such as biochemical oxygen demand and heavy metals present in treated sewage effluent are within the allowable limits. Also, treated sewage effluent has no physicochemical and biological effect on the agricultural crops despite its high biochemical oxygen demand, and it also contains nutrients such as nitrogen and phosphorus which is highly demanded plant growth and development [6]. Therefore, the treated sewage effluent is an ideal medium for plant growth while reducing nutrients which may cause eutrophication along the discharging path. As the need to alleviate nutrient contents in treated sewage effluent before disposal increases, phytoremediation is an ideal approach to be taken.

Phytoremediation, the use of the natural ability of plants to absorb nutrients in wastewater, has been known as a low cost, in situ and eco-friendly remediation technique [7]. It can be an alternative to conventional technologies for the remediation of nutrient-rich-treated sewage effluent. Excess amounts of nutrients in the treated sewage effluent can be incorporated to plant biomass and increase plant growth [8]. However, in a previous study, swine wastewater and coffee wastewater were reported to contain inconsistent high biochemical pollutants and may not sustain the growth of agricultural crops during phytoremediation [9]. Thus, this study used treated sewage effluent where water quality is monitored and the quality of wastewater abides by the local effluent standard. Phytoremediation in previous studies used artificial soil filter followed by phytoremediation [10]. Therefore, the results obtained include the nutrient removal efficiency of artificial soil filters. To obtain a pure removal efficiency and average daily nutrient removal rate of phytoremediation plants, the additional filters shall be eliminated from the study. Besides, most studies on phytoremediation focused on removal efficiency; hence, data on the daily nutrient removal rate is very limited. The nutrient removal rate of phytoremediation is important as the data obtained is useful enough to provide practical solutions to solve the real-life eutrophication situation. Thus, the daily nutrient removal rate must be determined in order to achieve the objectives of this study.

The experimental plants used throughout the study were *Spirodela polyrhiza* (*S. polyrhiza*) and *Brassica oleracea* (*B. oleracea*). Duckweed was suggested to be used in this experiment as it is an easy-to-grow floating aquatic plant with a fast growth rate. Due to its floating capabilities, the harvesting process is relatively simple [10]. Besides, studies have shown duckweed are capable to store a high amount of starch with a maximum of 46% of its dry mass under nutrient starvation [11]. Moreover, duckweed is resilient to a wide acceptance range of nutrient concentrations [12]. Besides, a system with free-floating plants such as duckweed may achieve higher removal of nutrients with harvesting due to multiple harvesting schedule [13]. There are five main genera of duckweed. Of the five main genera of duckweed—*Lemna*, *Spirodela*, *Wolffia*, *Wolffiella*, and *Landoltia*—*Spirodela* was chosen as it exhibited the most significant result, where nutrient removal percentage of 60% NH_3–N, 30% NO_3–N and 72% PO_4^{3-} from the synthetic wastewater were achieved in the previous studies [9]. On the other hand, to meet 2050 crop demand and production, *B. oleracea* or also known as cabbage was selected for this study due to its worldwide distribution and its high nitrogen requirement [14]. Moreover, *B. oleracea* is easy to be cultivated, grow quickly to produce large biomass and has an extensive root system [14].

With phytoremediation of treated sewage effluent, nutrients present in the treated sewage effluent are recovered by turning it into plant biomass for agricultural uses. Besides, the chances of eutrophication outbreak in the sewage discharging path will also be decreased. Therefore, this study aims to remove the nutrients in the treated sewage effluent and reduce the chances of eutrophication outbreak along the discharging path of treated sewage effluent while producing agricultural crops for other potential uses.

2. Materials and methods

2.1. Collection and preparation of samples

Treated sewage effluent sample was collected at the discharge point of the Saga City Sewage Treatment Plant, 2667 Oaza Takatoro, Nishiyoka-machi, Saga, Saga 840–0036, Japan. As referred to HACH standard, the treated sewage effluent sample was collected and stored in the polyethylene container. Fig. 1 shows the collection of the treated sewage effluent sample. Next, the collected treated sewage effluent was filtered twice with Whatman glass microfiber filters with a pore size of 1.0 and 0.45 μm respectively in the lab to remove suspended solids as shown in Fig. 2. This precaution step was taken to minimize microbial uptake of nutrients by a microbe which in turn will affect the parameter results. The standard guideline followed is following APHA 4500–P*B [15]. On the other hand, the experimental plants were prepared carefully three weeks before the experiment day. The experimental plants were *S. polyrhiza* and *B. oleracea*. The duration of the experiment was 4 d for *S. polyrhiza* and 8 d for *B. oleracea* with three replicates.

The phytoremediation setup is described as follows. First, the planter box was filled with 8,000 mL filtered treated sewage effluent. Next, the phytoremediation plant was placed carefully into the planter box containing treated sewage effluent. The reference line was drawn on the surface of the planter box after the planter box was equipped with treated sewage effluent and the phytoremediation plant. The reference line was drawn as a guideline for replenishing the evaporation deficit. The water level in the planter box was maintained at an initial marked level before the collection of samples. Distilled water was added to the planter box if the water level dropped below the marked reference line, which was due to evaporation. 40 mL of water sample was collected at 24 h interval for 4 or 8 d depending on the plant species for the three replicates. Sterile 10 mL syringes were used aseptically to collect the water sample for physiochemical tests in the laboratory. Fig. 3 shows the schematic diagram of the phytoremediation system in this study.

Fig. 1. Collection of treated sewage effluent in the study area.

Fig. 2. Filtration of treated ewage effluent after collection.

2.2. Parameter test

The physicochemical characteristic tests of treated sewage effluent were carried out throughout the preliminary study and phytoremediation treatment. All physicochemical parameter measurements were adhering to the manufacture instruction. Table 1 shows the standard method of each parameter.

2.3. Data analysis

All data obtained from the parameter testes were recorded. The data obtained were then analyzed using statistical analysis. Software such as Microsoft Excel was used to aid the analysis. Relevant mathematical formulations were applied.

3. Results and discussion

3.1. Ammoniacal nitrogen

Fig. 4 shows the results obtained from the phytoremediation of the treated sewage effluent by *S. polyrhiza*. Within 4 d of observation, *S. polyrhiza* was able to remove 99.07% ± 0.17% of ammoniacal nitrogen. The most significant daily absorption of ammoniacal nitrogen by *S. polyrhiza* was achieved on day 2, where 15.40% ± 0.14 mg/L/d of daily absorption was obtained. In the most optimal mean, *S. polyrhiza* was able to remove 92.42% ± 1.29% or 15.40 ± 0.14 mg/L of ammoniacal nitrogen within one d. As compared to similar work from previous studies by [9], where removal of merely 60% or 12.43 mg/L/d of ammoniacal nitrogen was obtained in 2 d. As *S. polyrhiza* removed almost all of the nitrogen in the medium, the plant shall be removed from the medium. This is because significant inhibition of glycolysis during nitrogen starvation by macrophyte species was observed [16]. In other words, nitrogen content in *S. polyrhiza* will be released back into the medium if nutrient starvation takes place. Thus, the phytoremediation of nutrient-rich-treated sewage effluent by *S. polyrhiza* shall be monitored to avoid the condition of nutrient starvation after the species had absorbed all the presented nutrients.

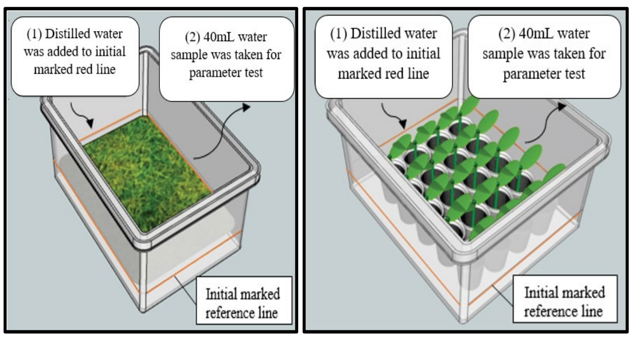

Fig. 3. Experimental set-up of phytoremediation for treated sewage effluent using *Spirodela polyrhiza* and *Brassica oleracea*, respectively.

Table 1
Standard method for each parameter

Parameter	Standard method	Method/Equipment
Ammoniacal nitrogen	HACH Method 8155	Salicylate method
Nitrate nitrogen	HACH Method 8039 HR	Cadmium reduction method
Phosphorus	HACH Method 8048	Ascorbic acid method

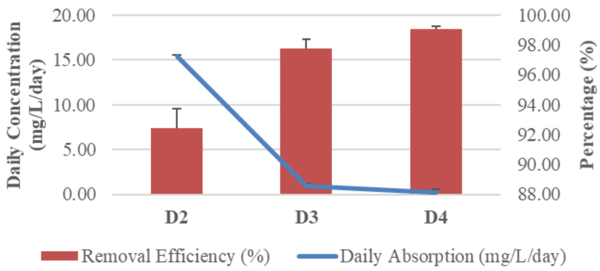

Fig. 4. Removal efficiency and daily absorption of ammoniacal nitrogen via phytoremediation by *Spirodela polyrhiza*.

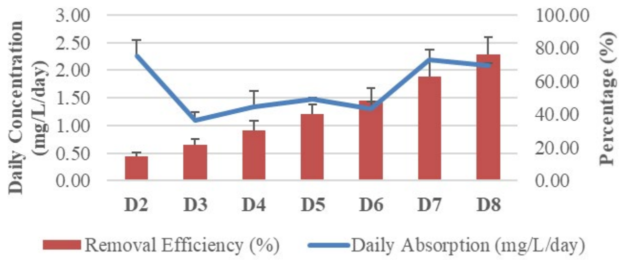

Fig. 5. Removal efficiency and daily absorption of ammoniacal nitrogen via phytoremediation by *Brassica oleracea*.

Fig. 5 shows the results obtained from the phytoremediation of treated sewage effluent by *B. oleracea* in the experiment with a duration of 8 d. *B. oleracea* was able to remove 76.07% ± 10.38% or an average of 1.68 mg/L/d of ammoniacal nitrogen in 8 d. The maximum daily absorption achieved was in 2 d, where daily absorption of 2.26 ± 0.29 mg/L/d of ammoniacal nitrogen with phytoremediation by *B. oleracea* was obtained. The removal efficiency and daily absorption of ammoniacal nitrogen by *B. oleracea* were fluctuated throughout the experiment, whereas the achieved removal efficiency increased with days. In previous studies, *B. oleracea* was reported to contain a total of four growing stages [17]. During the third stage, whereby cabbage head formation took place, the *B. oleracea* will favor nitrogen over other nutrients such as phosphorus and potassium. As the *B. oleracea* used in the study is still in its juvenile stage, the uptake of nitrogen is expected to gradually increase until the beginning of the fourth growing stage of *B. oleracea*.

3.2. Nitrate nitrogen

Fig. 6 shows the results obtained from the phytoremediation of treated sewage effluent by *S. polyrhiza*. The removal efficiency and daily absorption of nitrate-nitrogen with phytoremediation by *S. polyrhiza* were peaked in day 4 of the experiment, whereby 78.69% ± 10.31% of removal efficiency and 3.91 ± 0.20 mg/L/d of daily absorption were obtained. Generally, 78.69% ± 10.31% or an average daily absorption of 2.68 mg/L/d of nitrate-nitrogen was removed by *S. polyrhiza* within 4 d. The data can be compared with results from previous similar studies, where 30% or an average daily absorption of 0.44 mg/L/d nitrate nitrogen was removed in 12 d by the same species [9]. There is a huge difference between the current study and the previous results obtained. These may be caused by environmental factors such as the surrounding temperature and the presence of sunlight, which greatly affects the nutrient transformation of nitrate-nitrogen throughout the experiment [18]. The nutrient transformation was accelerated by higher temperatures with more presence of sunlight [18]. Since the studies were conducted during the summer in Japan, the higher temperature and longer exposure time to natural sunlight were experienced compared to the studies conducted in Malaysia by previous studies.

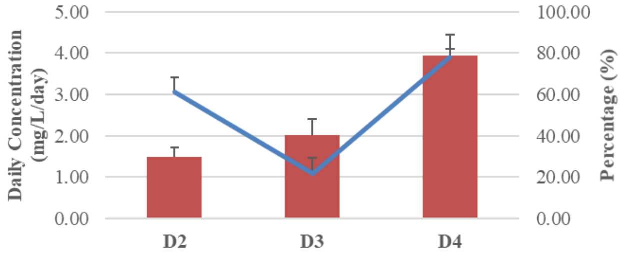

Fig. 6. Removal efficiency and daily absorption of nitrate-nitrogen via phytoremediation by *Spirodela polyrhiza*.

Fig. 7 shows the results obtained from the phytoremediation of treated sewage effluent by *B. oleracea*. 78.38% ± 0.40% of removal efficiency was obtained at the end of the experiment. Besides, the daily absorption of nitrate-nitrogen with phytoremediation by *B. oleracea* was peaked in day 5, whereby 0.41 ± 0.10 mg/L/d of daily absorption was obtained. Throughout the eight; s experiment, *B. oleracea* was able to remove 78.38% ± 0.40% or an average daily removal of 1.19 mg/L/d of nitrate-nitrogen. The yielded result is poor as compared to the removal efficiency by *S. polyrhiza*. However, the removal efficiency of nitrate-nitrogen achieved in the current study is better compared to the phytoremediation result obtained with *S. polyrhiza* in the previous similar study [9]. These may mainly due to external factors, where nitrate-nitrogen was able to undergo nitrate-ammonification [19] and denitrification [20]. The nutrient transformation causes nitrate nitrogen to undergo oxidation, followed by

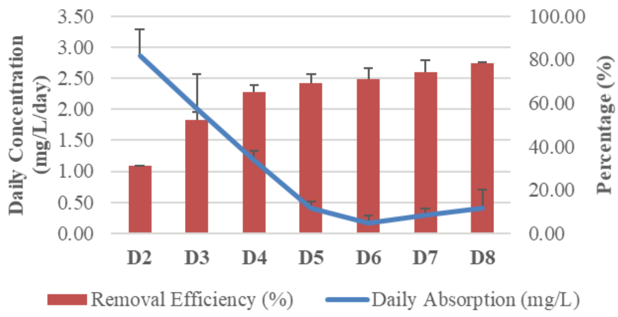

Fig. 7. Removal efficiency and daily absorption of nitrate-nitrogen via phytoremediation by *Brassica oleracea*.

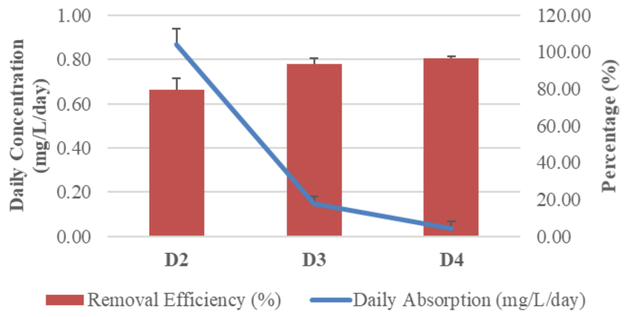

Fig. 8. Removal efficiency and daily absorption of phosphorus via phytoremediation by *Spirodela polyrhiza*.

concentration decrement of nitrate nitrogen and production of nitrous oxide, nitric oxide and nitrogen gas.

3.3. Phosphorus

Fig. 8 shows the results obtained from the phytoremediation of the treated sewage effluent by *S. polyrhiza*. *S. polyrhiza* was able to achieve removal efficiency of 96.90% ± 0.57% in 4 d. The daily absorption of phosphorus with phytoremediation by *S. polyrhiza* was peaked in day 2 of the experiment, whereby 0.87 ± 0.07 mg/L/d of daily absorption were obtained. Overall, *S. polyrhiza* was able to remove 93.45% ± 3.26% or an average of 0.51 mg/L/d daily absorption of phosphorus within 3 d. The phosphorus removal result obtained by *S. polyrhiza* in this experiment is better compared to the previous similar study where 72% or 0.47 mg/L/d of phosphorus removal is attained at day 10 [9]. The daily removal rate obtained from both studies were quite similar. This is because phosphorus is relatively stable [21].

Fig. 9 shows the results obtained from the phytoremediation of treated sewage effluent by *B. oleracea*. The removal efficiency of phosphorus with phytoremediation by *B. oleracea* were peaked at the end of the experiment, whereby 67.40% ± 10.91% of removal efficiency was obtained. Besides, the daily absorption of phosphorus with phytoremediation by *B. oleracea* was peaked in day 4 of the experiment, whereby 0.17 ± 0.02 mg/L/d of daily absorption was obtained. Generally, the removal efficiency and absorbed concentration of nitrate-nitrogen by *B. oleracea* fluctuated throughout the experiment. Throughout the 8 d experiment, *B. oleracea* was able to remove 67.40% ± 10.91% or 0.10 mg/L/d of phosphorus. The yielded result is poor as compared to the removal efficiency by *S. polyrhiza*. However,

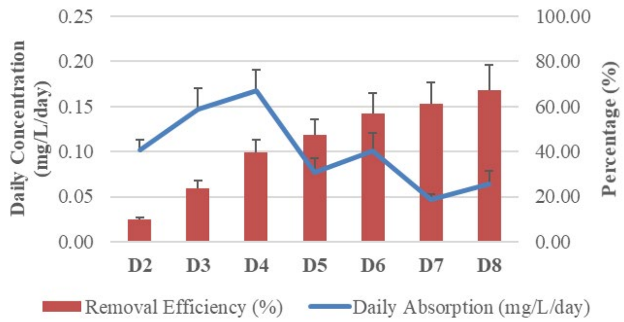

Fig. 9. Removal efficiency and daily absorption of phosphorus via phytoremediation by *Brassica oleracea*.

as *B. oleracea* is an agricultural crop, it experiences a longer life cycle than macrophyte species such as *S. polyrhiza*. The nutrient removal efficiency of *B. oleracea* is expected to be better as it approaches maturation when more outer leaf expand in later phase [17].

Table 2 summarized the signification results of removal efficiency and daily absorption and ammoniacal nitrogen, nitrate nitrogen and phosphorus obtained from the nutrient-rich-treated sewage effluent via phytoremediation by *S. polyrhiza* and *B. oleracea* throughout the experiment. Overall, *S. polyrhiza* showed a better phytoremediation efficiency as compared to *B. oleracea*. They took longer days to the desired removal efficiency of nutrients as compared to *S. polyrhiza* of the equivalent nutrients. The same trend is exhibited in the average daily removal concentration by the two species in the study. However, nutrients phytoremediation result by *B. oleracea* is expected to improve as more time is allowed for it to reach the peak of its growing curve

Table 2
Removal efficiency and average daily removal concentration of nutrients with its respective achieved days throughout phytoremediation by *Spirodela polyrhiza* and *Brassica oleracea*

	Spirodela polyrhiza	*Brassica oleracea*
Ammoniacal nitrogen	92.42% ± 1.29% or 15.4 mg/L/d in 1 d	76.07% ± 10.38% or 1.68 mg/L/d in 8 d
Nitrate nitrogen	78.69% ± 10.31% or 2.68 mg/L/d in 4 d	78.38% ± 0.40% or 1.19 mg/L/d in 8 d
Phosphorus	93.45% ± 3.26% or 0.51 mg/L/d in 3 d	67.40% ± 10.91% or 0.10 mg/L/d in 8 d

[22–30]. The comparison of the obtained results between *S. polyrhiza* and *B. oleracea* is highly dependent on the growing stage of the plant species.

4. Conclusion

Generally, *S. polyrhiza* portraits better phytoremediation efficiency and average daily removal as compared to *B. oleracea*. The removal efficiencies and average removal rate of major nutrients from treated sewage effluent was 92.42% ± 1.29% or 15.4 mg/L/d of ammoniacal nitrogen, 78.69% ± 10.31% or 2.68 mg/L/d of nitrate nitrogen and 93.45% ± 3.26% or 0.51 mg/L/d of phosphorus by *S. polyrhiza*; 76.07% ± 10.38% or 1.68 mg/L/d of ammoniacal nitrogen, 78.38% ± 0.40% or 1.19 mg/L/d of nitrate nitrogen and 67.40% ± 10.91% or 0.10 mg/L/d of phosphorus within 8 d by *B. oleracea*.

Throughout the experiment, it can be concluded that all nutrients, including ammoniacal nitrogen, nitrate nitrogen and phosphorus were absorbed and assimilated, and transmitted to organic nitrogen and phosphorus in plant body by vegetable culture. It is expected that the average daily absorption and removal efficiency of the phytoremediation plant will be improved until it reached the peak of its growing curve.

It can be concluded that *S. polyrhiza* portraits better efficiency in removing nutrients such as ammoniacal nitrogen, nitrate nitrogen and phosphorus from the current study. Cultivating *S. polyrhiza* in manageable quantity to remove nutrients in nutrients rich treated sewage effluent is suggested. *B. oleracea* could be an alternative to phytoremediate the nutrient-rich-treated sewage effluent while producing agricultural crops in addressing the food shortage to meet 2,050 crop demand.

Acknowledgments

The authors would like to thank the Institute of Lowland and Marine Research – Saga University, for technical support and the facilities provided. We are also thankful to the Ministry of Education Malaysia for giving financial aid through FRGS vot K090 and Universiti Tun Hussein Onn Malaysia via contract grant H377 and GPPS U947.

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