PROJECT PROPOSAL

Project name	**Evaluating the potential of Vetiver grass (*Chrysopogon zizanioides*) for the treatment of surface water in Mekong Delta for cooking and drinking purposes in Mekong Delta**
Budget	**40,000 AUD**
Duration	**24 months**
Investigators	**Principle investigator: Dr Luu Thai Danh** **Investigators: Dr Duong Minh Vien** **Dr Le Viet Dung** **Dr Tran Chi Nhan**

I. BACKGROUND

Most of people living in the Mekong Delta of South Vietnam are exposed to unsafe levels of chemical contaminants in their drinking water. The limited number of water purifying plants, lack of wastewater treatment facilities, high population density and intensive agricultural production make this problem more serious. The population of the delta is about 20 million, with a density of 427 people per km^2 (GSO, 2011) that is nearly double the national population density. Water purifying plants are only available in the urban regions, while about three quarters of its inhabitants living in the rural areas where water used for drinking and cooking purposes heavily depend on the available natural water sources including surface water (rivers and canals), ground water and rainwater. In addition, there is no wastewater treatment facilities installed at anywhere in the Mekong Delta, it means that all domestic wastewater generated by both urban and rural inhabitants is freely discharged into water systems. The last but the most important factor contributing to the degradation of water quality is intensive agricultural production in this region. The delta, with an area of about 4 million ha, is one of the most highly productive agricultural areas in the world (MRC, 2002). About 50% of the delta area is used for rice production (GSO, 2011). Depending on the area and its water regime, one to three rice crops per year are produced with double cropping being dominant (1.3 million ha) in the delta. The area with three rice crops per year covers about 0.4 million ha. The Mekong Delta is also a major production area of fruits such as mango, longan, pineapple, bananas, and others. Annual crops, including sugar cane, maize, peanut, sweet potato and cassava add up to less than 10% of the cultivated land area (GSO, 2008). About 18% of the delta is employed for aquaculture (GSO, 2011). Animal production

occupies only a small area of the delta. The main processes of agricultural production causing water pollution include runoff and tile drainage, that release suspended matter, phosphorus, nitrogen, plant protection products, metals, pathogens, salts, veterinary medicines, feed additives and hormones to freshwater systems (Casali et al. 2008; Diaz 2001; Causape et al. 2004; Ongley 1996).

Water in the Mekong Delta is general abundant and provide valuable supports for a wide range of production systems and particularly for drinking purposes, however the degradation of water quality in recent years has imposed a serious health hazard to locals. The available natural water sources include surface water (rivers and canals), ground water and rainwater. A 10 year report (1998–2008) of the Department of Natural resources and Environment of Can Tho City (DONRE Can Tho, 2009) showed a continuous decrease in surface water quality of the main canals and rivers and emphasized high microbial, organic, ammonia, and nitrite pollution. Furthermore, a recent study of Phung et al. (2015) revealed the serious contamination of surface water in which all parameters presented in Table 1, except NO_3^-, exceed the levels set by national guidelines for residential use and other purpose. The use of rainwater is limited in the rainy season due to the fact that under monsoon climate, the lower Mekong Delta receives an average annual rainfall of above 2000 mm with 80% of that amount falling during the rainy season generally from May to October (GSO 2013). Harvested rainwater is considered to be safe and free of taste, smell, color and suspended particles. However, the recent study of Chau et al. (2015) showed that rainwater is contaminated by agrochemicals. Over the past decade, groundwater has become an important source of drinking water in the Mekong delta and it is tapped wherever the high salinity is not compromising its use (i.e. below 1 g L^{-1} TDS, Total Dissolved Solids) (Buschmann et al. (2008). It is estimated that about 465,230 wells deliver a total of ca. 1.2 million m^3 day^{-1} for industrial purposes, domestic water supply, and partly for irrigation (Delta Alliance 2011). The studies of Berg et al. (2007) and Buschmann et al. (2008) demonstrated that groundwater in the Mekong Delta is highly contaminated with arsenic and heavy metals. In addition, groundwater is also polluted by agrochemicals due to leaching (Chau et al., 2015). Consequently, degradation of water quality is becoming a serious concern in the region.

Surface water is the most abundant and available for domestic use as compared to other sources. Recently, surface water contamination has been more and more serious due to the continuous and increased discharge of agrochemicals into water systems. In the context of the rapid agricultural development in the Mekong Delta since the mid of 1980s, agricultural pesticides have been used in increasingly large quantities (Chau et al., 2015). From the last decades, expenditure and application of pesticides in the Vietnamese Mekong Delta were reported at higher levels in comparison to some other Asian countries such as India, the Philippines, and Indonesia (Dung and Dung 1999). Annually, an estimation of about half a million tons of pesticides are used in the Mekong Delta (Hien, 2009). These pesticides can potentially pollute the water systems of the delta, causing adverse effects to non-target organisms in aquatic environment (Sebesvari et al. 2012).

Table 1. Range of water quality parameters at 38 sampling sites of Mekong River in Can Tho city, Mekong Delta, Vietnam (2008-2012). Source: DONRE Can Tho, 2009.

Water quality parameters	**Range**	**Median**	**National guidelines for residential use and other purpose**
pH	6.7 – 7	6.9	
BOB_5 (mg L^{-1})	8.8 – 26.2	10.9	4
COD (mg L^{-1})	11.9 – 38.7	15.5	10
DO (mg L^{-1})	2.7 – 5.3	4.1	≥ 6
SS (mg L^{-1})	31.7 – 74.8	45.1	20
Fe (mg L^{-1})	0.23 – 0.95	0.49	0.5
NH_3 (mg L^{-1})	0.24 – 2.7	0.46	0.1
NO_3^- (mg L^{-1})	0.65 – 2.3	1.05	2
NO_2^- (mg L^{-1})	0.03 – 0.2	0.06	0.01
Total coliform (MPN per100 ml)	8000 – 390000	20000	2500

Surface water samples from various sampling sites in the lower Mekong Delta of Vietnam were highly contaminated with a range of pesticides (Table 2). The Vietnamese National Technical Regulation for Drinking Water Quality (QCVN 01:2009/BYT) had no guideline values for pesticides. So the potential health threats caused by pesticides have been assessed by comparing the concentrations of pesticides with the parametric guideline value of 0.1 µg L^{-1} set for a single pesticide and 0.5 µg L^{-1} for total pesticide concentrations by the European Commission, as well as with the World Health Organization toxicity classes. Isoprothiolane, a fungicide, was the most frequently detected compound (in 97.8% of all surface water samples), followed by two inseticides fenobucarb (91.2%) and fipronil (83.4%). The median concentration of isoprothiolane was 0.55 µg L^{-1}, while fipronil and fenobucarb were quantified at median concentrations of 0.17 and 0.15 µg L^{-1}, respectively. One of the most used fungicides, propiconazole was also found in 39.2% of the analyzed samples with a median concentration of 0.5 µg L^{-1}. These four compounds are classified as WHO class II (moderately hazardous) pesticides (WHO 2010). In the rural areas of the Delta, surface water still serves as one of the main drinking water sources, especially during the dry season. Direct intake via drinking is one of the possible exposure routes to pesticides. In addition, surface water is widely used for personal hygiene and washing of food items, dishes, and clothes thus opening up another exposure pathway that potentially threatens human health (Van Toan et al., 2013). Based on "the worst case scenario", it can be roughly estimated that people living in rural areas using surface water may consume up to 50 µg of pesticides per day (Table 2) through drinking plus additional amount via other exposure pathway. This figure intends to increase with the amount of imported and used pesticides over time.

Table 2. Pesticide residues detected in surface water samples from March 2012 to January 2013. Source: Chau et al. (2015).

Compound	Frequency of samples detected with pesticide (%)	Maximum concentration (µg L^{-1})	Median concentration (µg L^{-1})	Frequency of samples exceed 0.1 µg L^{-1} (%)	Daily exposure (µg day^{-1})	WHO toxic class
Herbicides						
Butachlor	55.8	0.81	0.25	50.3	1.22	III
Pretilachlor	71.8	0.85	0.21	63	1.28	U
Fungicides						
Propiconazole	39.2	4.76	0.5	39.2	7.14	II
Tebuconazole	37	1.34	0.34	30.9	2.01	III
Hexaconazole	67.4	1.79	0.46	60.8	2.69	III
Difenoconazole	7.2	3.18	1.1	7.2	4.77	II
Isoprothiolane	97.8	8.49	0.55	91.7	12.74	II
Trifloxystrobin	16	0.56	0.16	15.5	0.84	III
Azoxystrobin	66.3	2.41	0.49	61.3	3.62	III
Insecticides						
Fenobucarb	91.2	2.32	0.15	64.1	3.48	II
Quinalphos	78.5	1.33	0.17	63	2	III
Thiamethoxam	4.4	0.95	0.63	4.4	1.43	III
Fipronil	83.4	0.41	0.17	51.4	0.62	II
Cypermethrin	0.6	0.77	0.77	0.6	1.16	II
Assumed daily intake of total pesticide concentration (µg L^{-1})					50	

Note: sampling size n = 181, daily exposure based on the "worst case scenario" using the highest detected concentration and estimating daily intake of 1.5 L surface water.

II. THE NECESSITY OF THE PROPOSED STUDY

From information mentioned above, it can be stated that surface water in the Mekong Delta is highly degraded with nutrients, heavy metals, pathogens and pesticides. The co-occurrence of a wide range of contaminants likely generates more potent toxicity effect to human and aquatic organisms than the effect of a single contaminant. The conventional treatments of surface water for domestic use, namely flocculation by aluminium sulfate and disinfection by boiling, is insufficient to remove aforementioned pesticides. As long as pesticide management remains suboptimal and water users are continuously exposed to pesticide residues, more effective water treatment practices need to be implemented at household level to reduce the likelihood of pesticide exposure (Van Toan, et al., 2013). Several methods, namely chemical oxidation (ozone, chlorine), carbon adsorption (powdered and granular activated carbon) and membrane treatments are used to remove pesticides (US EPA, 2011). These methods are quite expensive and complicated, so their application has been very limited in the rural areas of Mekong Delta. Currently, there is an urgent need for an

alternative water treatment that is simple, cheap, sustainable as well as effective in removing pesticides and other contaminants.

III. VETIVER SYSTEM

Vetiver System (VS), mainly based on vetiver grass (*Chrysopogon zizanioides*), is a good candidate for the treatment of polluted surface water in the Mekong Delta for domestic use. It is a new phyto-technology developed from research, development and application programs for numerous environmental protection purposes around the world in the last two decades. The system is now being used in over 40 countries with tropical and subtropical climates (Barbara 2004). It is due to the fact that Vetiver grass possesses nearly all the characteristics of an ideal plant for the phytoremediation of water contaminated by nutrients, heavy metals and organic pollutants.

The success of using Vetiver for phytoremediation of contaminated water depends on the interaction between its roots and contaminated bodies. Vetiver possesses a lacework root system that is abundant, complex, and extensive (Figure 1). The root system can reach 3-4 meters in the first year of planting (Hengchaovanich, 1998) and acquires a total length of 7 meters after 36 months (Lavania, 2003). Furthermore, most of Vetiver roots are very fine with an average of 0.66 mm (range from 0.2-1.7 mm) (Cheng et al., 2003). The horizontal spreading of lateral roots was in the range of 0.15-0.29 m with an average of 0.23 m (Mickovski et al., 2005). After 8 months of cultivation, Vetiver produced 0.48 kg of dry roots per plant.

Figure 1. Massive, penetrating and deep root systems.

Vetiver has a fast growing rate and high biomass production that are two important factors determining its great potential for phytoremediation. Vetiver is a C_4 plant that has high rate of photosynthesis at high light intensities and high temperatures due to the increased efficiency of photosynthetic carbon reduction cycle (Hatch, 1987). High growth rate results in high biomass production of Vetiver, about 100 tons of dry matter ha^{-1} $year^{-1}$ under tropical hot and wet conditions (Truong, 2003).

Vetiver has been demonstrated to highly tolerate and accumulate high concentrations of nitrogen (N) and phosphorous (P) that are main elements causing water pollution (Figure 2). Under hydroponic condition with a sewage effluent flow rate of 20 L min^{-1} through Vetiver roots, one square meter of Vetiver can treat 30,000 mg of N and 3,575 mg of P in eight days (Hart et al., 2003). In this application, Vetiver out-performed other crops and pasture plants, such as Rhodes grass, kikuyu grass, green panic, forage sorghum, rye grass and eucalyptus trees (Truong, 2003). Vetiver could remove up to 740 kg N ha^{-1} and 110 kg P ha^{-1} over 3 months at a nutrient-rich site and 1,020 kg N ha^{-1} and 85 kg P ha^{-1} over 10 months at a lower nutrient site (Vieritz et al., 2003). In a pot experiment (Smeal et al., 2003), Vetiver was demonstrated to have a very high recovery rate for nitrogen in shoots, but quite low for phosphorous (Table 3).

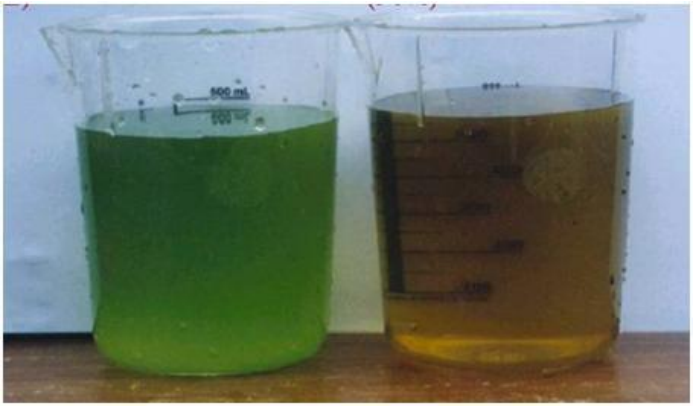

Figure 2. High N and P removal capacity of Vetiver: blue green algae infested waste water (left) with high nitrate (100 mg L^{-1}) and phosphate (10 mg L^{-1}), same effluent after 4 days of treatment with Vetiver (right) reducing N and P level to 6 and 1 mg L^{-1}, respectively. Algal infestation was eliminated from the effluent.

Table 3. Recovery rate of N and P by Vetiver.

Treatment	Recovery rate by Vetiver (%)		Recovery in soil (%)	Total
	Shoot	Root		
N (ton ha^{-1} $year^{-1}$)				
2	76.3	20.4	0.3	97
4	72.1	23.1	0.1	95.3
6	67.3	21.2	0.4	88.9
8	56.1	30.0	0.4	86.5
10	46.7	17.0	0.1	63.8
P (kg ha^{-1} $year^{-1}$)				
250	30.5	23.3	46.3	100
500	20.5	14.6	48.7	83.8
1000	16.5	14.2	40.8	71.5

One special attribute of Vetiver discovered recently has made it an excellent plant for heavy metal phytoremediation is its ability to highly tolerate and accumulate a wide range of heavy metals. Vetiver could survive and grow well on multi-heavy metal contaminated soils under glasshouse conditions with total Pb, Zn and Cu in the range of 1155 - 3281.6, 118.3 - 1583 and 68 - 1761.8 mg kg^{-1}, respectively (Danh et al., 2015). Vetiver was also demonstrated to grow well on iron ore tailings containing high concentrations of multi-heavy metals with total Fe, Zn, Mn and Cu concentrations of 63920, 190, 3220 and 190 mg kg^{-1}, respectively (Roongtanakiat et al., 2008). Under field conditions, Vetiver could grow on mine tailing soils containing total Pb, Zn, Cu and Cd of 2078 - 4164, 2472 - 4377, 35 - 174 and 7 - 32 mg kg^{-1}, respectively. Recently, Vetiver grass has been shown to accumulate high content of these metals in its roots and shoots (Table 4).

Table 4. The highest concentrations of heavy metals accumulated in the roots and shoots of Vetiver reported in the literature. Source: Danh et al. (2012).

Heavy metals	**Soil condition**		**Hydroponic condition**	
	Roots (mg kg^{-1})	**Shoots (mg kg^{-1})**	**Roots (mg kg^{-1})**	**Shoots (mg kg^{-1})**
Lead	4940	359		
Zinc	2666	642	≥ 10,000	
Chromium	1750	18	>10,000	≥ 3350
Copper	953	65		>10,000
Arsenic	268	11.2	900	
Cadmium	396 [1]	~ 44		700
Mercury			2232	
Iron	871 [3]	1197 [3]	1310 [2]	93
Manganese	552 [3]	648 [3]		
Uranium	28 [4]	164 [4]		

Note: [1] Zhang et al. (2014), [2] Lomonte et al. (2014), [3] Roongtanakiat et al. (2008), [4] Hung et al. (2012).

Vetiver has been recently found to be highly resistant to a range of organic pollutants in growing media, including agrochemicals, antibiotics and other organic wastes (Table 5). Particularly, Vetiver was demonstrated to have ability to remove phenol, tetracycline and 2,4,6-trinitroluen (TNT) from growing media.

Table 5. The tolerance of Vetiver to the highest concentrations of organic pollutants in growing media reported in literature.

Organic pollutants		**Soil**	**Hydroponic**	**References**
Agrochemicals				
	Atrazine		20000 µg L^{-1}	1
	Diuron		2000 µg L^{-1}	2
Antibiotics				
	Tetracycline		15 mg L^{-1}	3
Others				
	Phenol		1000 mg L^{-1}	4
	2,4,6-Trinitroluene	80 mg kg^{-1}		5
			40 mg L^{-1}	6
	Benzo[A]pyrene	100 mg kg^{-1}		7
	Petroleum hydrocarbons	5%		8

Note: 1 Marcacci et al., 2006; 2 Cull et al., 2000; 3 Datta et al., 2013; 4 Singh et al., 2008; 5 Das et al., 2010; 6: Makris et al., 2007a; 7 Li et al., 2006; 8 Brandt et al., 2006.

Vetiver plantlets grown under hydroponic and aseptic conditions could remove almost all phenol from media with phenol concentration less than 200 mg L^{-1} in a period of 4 days (Singh et al., 2008). As plant investigated under aseptic conditions without the confounding effect of microorganisms, this study indicated that Vetiver was solely responsible for phenol remediation. However, the study of Phenrat et al. (2015) suggested that phenol degradation by vetiver involves two phases (Figure 6). The first phase included phyto-oxidation and phyto-polymerization of phenol assisted by root-produced H_2O_2 and peroxidase (POD). The second phase was a combination of the first phase with the enhanced rhizomicrobial degradation. Initially, phenol was rapidly detoxified to phenol radicals, followed by polymerization to non-toxic polyphenols or selective polymerization with natural organic matters, which were then precipitated as particulate polyphenols (PPP) or particulate organic matters (POM). After the first phase, the concentration of phenol significantly decreased, while that of PPP and POM greatly increased, as indicated by the increase of particulate chemical oxygen damand. Synergistically, rhizomicrobes intensively grew on the roots of vetiver grass and participated in microbial degradation of phenol at the lower concentration, increasing phenol degradation rate by more than 4-folds in comparison to phenol degradation rate in the first phase, and by approximately 32-folds compared with phenol removal rate without vetiver grass. The combined effects of root-assisted phytooxidation and phytopolymerization, and rhizomicrobial degradation resulted in the complete removal of phenol in wastewater.

Under hydroponic condition, Vetiver was demonstrated to have ability to remove 2,4,6-trinitroluen (TNT) and tetracycline (TC). Vetiver has high affinity for 2,4,6-trinitroluen (TNT) by nearly complete removal of TNT from 40 mg TNT L^{-1} solution after 8 days of treatment (Makris et al., 2007b). TNT removal kinetic of Vetiver was significantly increased by the addition of urea as a chaotropic agent (Makris et al., 2007a). No TNT was detected either in roots or shoots, but three major TNT metabolites were found in the roots, but not in the shoot, indicating TNT degraded by Vetiver roots. Similarly, Vetiver could reduce 97% of TNT in soil treated with 40 mg kg^{-1} TNT after 3 days (Das et al., 2010). Vetiver completely removed tetracycline (TC) from all treatments with three concentrations of TC (5, 10, and 15 mg L^{-1}) within 40 days, whereas no significant reduction in the TC concentrations was found in absence of Vetiver grass (Datta et al., 2013).

Vetiver can tolerate up to 20 ppm of atrazine for six weeks, even with a maximum bioavailability created by the use of a hydroponic system (Marcacci et al., 2006). It can be explained by the fact that Vetiver possesses the effective detoxifying processes involving conjugation and dealkylation of atrazine in which conjugation clearly dominates on dealkylation. The conjugated atrazine was mainly detected in leaves, while the dealkylated products were found in both roots and leaves. Furthermore, Vetiver roots were demonstrated to be able to sequester atrazine in the lipid content where Vetiver oil could concentrate atrazine. Vetiver oils in the root increase with aging thus atrazine sequestration in roots may increase with time. Because of the constant growth of the root system, some atrazine in the water could be trans-located to the shoot with the transpiration stream, where detoxification occurs. Under soil condition, the plant growth of Vetiver, measured by leaf chlorophyll activity, was not affected by the application of high atrazine concentration, equivalent to 1 mg L^{-1}. The reduction of atrazine in Vetiver treated soils was significantly greater than of the control treatment, owing to atrazine accumulation of Vetiver and microbial degradation of atrazine induced by Vetiver roots in rhizosphere (Winter, 1999). It can be concluded that the combination of these Vetiver properties make it an ideal plant for phytoremediation of atrazine and maybe extended to other agrochemicals, such as pesticides.

Vetiver is highly adaptable to extreme weather conditions. It can thrive and survive under the prolonged drought and flood. The extensive and long root of Vetiver, mentioned above, can utilize deep soil moisture supporting the survival of Vetiver grass up to 6 months under drought condition (Figure 2). Moreover, Vetiver grass is considered as a hydrophyte (wetland plant) due to its well-developed sclerenchyma (air cell) network. Consequently, Vetiver can thrive under hydroponics conditions. Vetiver was demonstrated to be tolerant to the complete submergence for more than 120 days (Xia et al. 2003). Similarly, Vetiver can survive more than 3 months under muddy water in a trial conducted in 2007 to stabilise the Mekong river bank in Cambodia. Under partial submergence, it can stand up to 8 months in a trial in Venezuela (Figure 3).

Figure 3. Vetiver survival under prolong drought (left) in Australia (note: all native plants were brown off) and submergence of 25 cm for 8 months (right) in Venezuela. Source: www.vetiver.org.

From special characteristics of Vetiver mentioned above together with the successful field studies of applying Vetiver around the world for wastewater treatment (Danh et al., 2015), it can be suggested that Vetiver grass is a right choice for phytoremediation of polluted surface water. Particularly, VST is considered as a non-expensive, easily implemented and environmentally friendly approach that the local population in the Mekong Delta can afford with minimum cost and effort.

IV. OBJECTIVE

The objective of this study is to demonstrate the ability of Vetiver grass grown in constructed wetland and under hydroponic condition for purifying surface water contaminated with nutrients, pathogens, heavy metals and pesticides in the Mekong delta for domestic use. Furthermore, the outcomes of this study will be used to design a simple, non-expensive Vetiver treating unit for surface water purification.

V. MATERIALS AND METHODS

5.1. Experiemental design

Vetiver growth chamber units will be constructed with rectangular shape, each covering a surface area of 1.2 m^2. River sand will be used as growth medium. Each unit has a dimension of 2 m x 0.6 m x 1 m (length x width x height), and a medium depth of 0.6 m. The experimental set-up of vetiver for surface water treatment includes a unit planted with vetiver grass on river sand (constructed wetland treatment), another unit filled with river sand without vetiver (control treatment), last unit planted with vetiver grass on surface water only (floating platform treatment) (Figure 4). Vetiver will be planted at a density of 10 plants per m^2. Vetiver will be cultivated in the growth chamber for 3 months before experiment starts.

River or canal water will be pumped into a storage tank, and stay still for 24 hours to settle down suspended particles. Water will be delivered to each unit to reach a depth of 0.6 m and retained for 24, 48, 72 and 96 hours, then water will be discharged from the systems. Water from treatment 1 and 2 will be passed through a gravel filled section before discharge. One treating cycle will include three stages: water refill of storage tank and particle settlement, water refill of treating units and retention, and water discharge. Each retention time will be tested for 40 cycles, the quality of inlet and outlet water will be assessed every fifth cycle during the treating period. All samples will be collected and stored at 4°C until analysis.

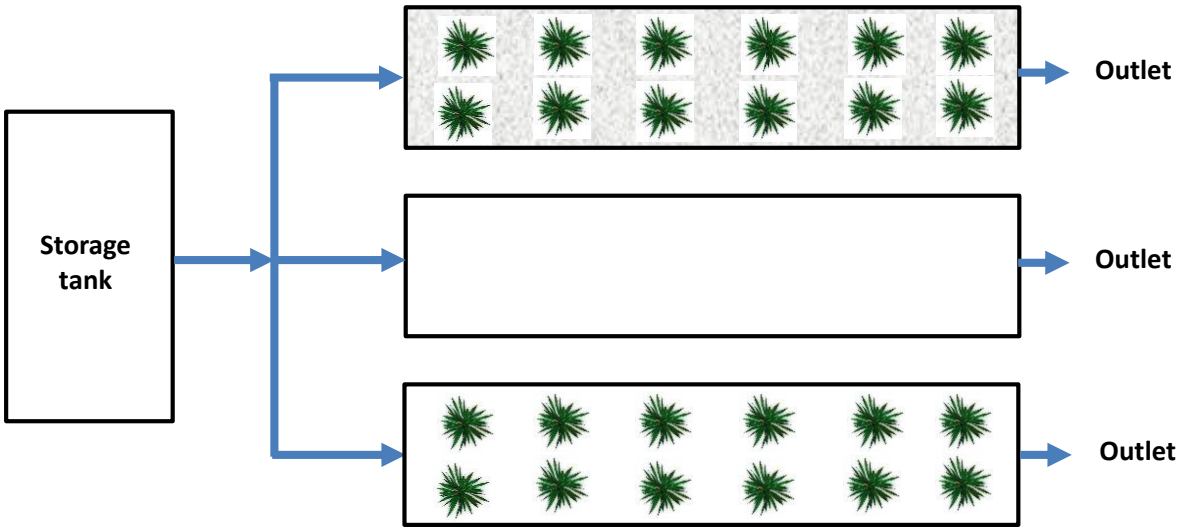

Figure 4. Top view of treating units: bottom (vetiver and sandy medium), middle (sandy medium) and top (vetiver floating platform).

5.2. Analysis

5.2.1. General water quality analysis

Suspended solids (SS), biological oxygen demand (BOD), chemical oxygen demand (COD), NH_4^+, NO_2^-, NO_3^-, total nitrogen (TN), PO_4^{3-}, total phosphorous (TP), Fe, pH, pathogen from sampling water will be measured by using the related analytical methods in The Standard Methods for the Examination of Water and Wastewater, APHA (1998).

5.2.2. Pesticide analysis

The selection of target pesticides for assessing the potential risk of local population with respect to pesticide pollution will be based the selection and results of studies by Van Toan et al. (2013) and Chau et al. (2015). The selecting criteria in these studies include i) pesticide use (frequency and amount), ii) expected fate (occurrence and persistence) in aquatic

ecosystem based on physio-chemical properties, such as solubility in water, hydrolysis half-life, octanol-water partition coefficient, soil sorption and soil degradation half-life, iii) potential risk to the aquatic life and human health, and iv) the availability of analytical method. Ten pesticides, namely butachlor, pretilachlor, propanil (herbicides); buprofezin, cypermethrin, endosulfan, fipronil (insecticides); isoprothiolane, propiconazole (fungicides), were detected in water samples (Van Toan et al. 2013). In the study of Chau et al. (2015) fourteen pesticides were detected, including butachlor, pretilachlor (herbicides); fenobucarb, quinalphos, thiamethoxam, fipronil, cypermethrin (insectidies); propiconazole, tebuconazole, hexaconazole, difenoconazole, isoprothiolane, trifloxystrobin, azoxystrobin (fungicides). Consequently, there are 18 pesticides selected in this study (Table 6).

A multi-residue pesticide analysis will be performed according to the method of Chau et al. (2015). Water sample (500 ml) will be adjusted to pH 4, followed by addition of 10 g NaCl, then filtered through glass fiber filter (pore size 1 μm). One microgram δ-HCH will be spiked right after as surrogate standard. Water sample will then extracted through Strata C18-E cartridge which will be preconditioned by sequential eluting of 6 mL n-hexane, 6 mL ethyl acetate, 2 mL methanol, and 2 mL HPLC water. Nitrogen gas flow will be used to dry the C18-E cartridge. Target pesticides adsorbed on the solid phase of the cartridge will be eluted by 9 mL ethyl acetate followed by 9 mL n-hexane. The eluate will be concentrated to ca. 500 μL by rotary evaporation with toluene as keeper and then transferred to amber vials, filled up to ca. 1 mL by toluene and stored at −20 °C until analysis. The extracted compounds will be analyzed with a gas chromatograph (GC) equipped with mass spectrometry detector using electron impact (EI) mode (GCMSQP2010 plus, Shimadzu, Japan), installed with a DB-1 fused silica capillary column (length 30 m; inner diameter 0.25 mm; film thickness 0.25 μm). The GC oven temperature will be programmed as followed: the initial temperature will be set at 80°C for 2 min, increased at a rate of 10°C/min to 150 °C, held for 5 min, then increased at a rate of 5 °C/min to 230 °C, kept on increasing at a rate of 2 °C/min to 250 °C/min, and finally increased to 280 at 20 °C/min, held for 10 min. A post temperature of 300 °C will be applied for 10 min.

Quality assurance and quality control

Analytical grade purified water will regularly processed together with each batch of samples. The recovery in the range from 70 to 130% of a surrogate standard (δ-HCH) that was added to the samples prior to extraction will be accepted in order to monitor the extraction process. Extracted samples with the standard recovery out of this range will be not considered. The detected concentrations will not be adjusted to the recovery rate of the standard.

Method detection limit (MDL) of each pesticides will be determined via analysis of a series of water samples (n=9) spiked with pesticide concentrations close to the expected detection limit (in this study is limit of quantitation (LOQ)) (Ripp, 1996). Analytical results below the calculated MDL will be not reported.

5.2.3. Statistical analysis

Data were tested for normal distribution via Kolmogorov–Smirnov test at p=0.05 level. In case of normal distribution and equal variance, depending on the number of groups, either a t-test, a Welch-test or a one-way ANOVA was applied. In case of non-normal distribution, depending on the number of groups, either a MannWhitney Rank Sum test, a Mann Whitney U test, a Wilcoxon Signed Rank test, or a Kruskal–Wallis ANOVA on Ranks test was applied (Systat, 2008; Toutenburg, 2002).

Table 6. Analysed pesticides, their physiochemical properties and WHO toxicity class.

Pesticides	**Solubility at 20°C (mg L^{-1})**	**Octanol-water partition coefficient, Log (Kow)**	**Soil sorption, Koc, ml g^{-1}**	**Hydrolysis half-life, $DT_{50,\ water}$ (av, days)**	**Half-life in soil, $DT_{50,\ soil}$ (av, ays)**	**WHO toxicitiy classes**
Herbicides						
Butachlor	20	4.5	700	–	56	III
Pretilachlor	50	4.1	–	Stable	30	U
Propanil	225	2.29	400	364	0.4	II
Fungicides						
Propiconazole	150	3.7	1086	53.5	214	II
Tebuconazole	36	3.7	1023	Stable	63	III
Hexaconazole	18	3.9	1040	Stable	122	III
Difenoconazole	15	4.4	–	Stable	130	II
Isoprothiolane	54	3.3	–	–	–	II
Trifloxystrobin	0,61	4.5	1642 – 3745	40	7	III
Azoxystrobin	6.7	2.5	589	Stable	78	III
Insecticides						
Fenobucarb	420	2.8	1068	20	18.5	II
Quinalphos	17.8	4.4	1465	39	21	III
Thiamethoxam	4100	−0.13	56.2	Stable	50	III
Fipronil	3,78	3.75	427–1248	Stable	142	II
Cypermethrin	0.009	5.3	156,250	179	60	II
Buprofezin	0.46	4.8	10,624	stable	46.2	III
Endosulfan	0.32	3.13	11500	20	86	II
Profenofos	28	1.7	2016	Stable	7	II

VI. EXPECTED OUTCOMES

+ To determine which retention time and treatment will give the best quality of treated water.

+ The general quality of treated water will be complied with the Vietnamese guidelines for residential use and other purposes.

+ The concentration of single pesticide and total pesticide residues in treated water will be complied with the parametric guideline value of 0.1 and 0.5 µg L^{-1} set by European Commission, respectively.

+ Construction and display of a simple, non-expensive Vetiver treating unit for surface water purification.

VII. BUDGETS

Description	Year 1	Year 2	Total	Contributor
Direct cost				
Personnel	$5000	$5000	$10000	Can Tho University
Equipment				
Vetiver growth chambers and storage tank	$3000	0	$3000	
Analysis				
Gases	$2000	$2000	$4000	
Solvents	$3000	$3000	$6000	
Chemicals	$3000	$6000	$9000	
General lab consumables	$2000	$2000	$4000	
Other				
Travel expenses	$2000	$2000	$4000	
TOTAL	**$20000**	**$20000**	**$40000**	

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