Laboratory-scaled Developments and Field-scaled Implementations of Using Vetiver Grass to Remediate Water and Soil Contaminated with Phenol and Other Hazardous Substances from Illegal Dumping at Nong-Nea Subdistrict, Phanom Sarakham District, Chachoengsao Province, Thailand

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Abstract

The community at the Nong-Nea subdistrict, Phanom Sarakham district, Chachoengsao province, Thailand, has been suffered from illegal dumping of industrial wastewater containing high concentrations of phenol (C_6H_6O). Various government agencies reported that the phenol concentration of drinking water exceeded the maximum contamination level (1 µg/L) by more than 250-fold. To protect the Nong-Nea community's health and ecology, decreasing phenol exposure and migration is mandatory.

This project aimed to use vetiver grass to degrade phenol in the accessible illegal dumping source zone, contaminated runoff, and a shallow groundwater plume within surface water (Tad-Noi creek). Two strategies for using vetiver systems include: 1) using vetiver grass on a floating platform for the treatment of phenol-contaminated wastewater; and 2) using vetiver hedgerows along Tat-Noi creek to decrease the exposure of villagers to residual phenol migrating along the creek.

Laboratory-scale experimental results suggested that phenol degradation by vetiver involves two phases: Phase I, phytopolymerization and phytooxidation assisted by root-produced H_2O_2 and peroxidase (POD), followed by Phase II, a combination of Phase I with enhanced rhizomicrobial degradation. The first 360–400 hours of phenol degradation was dominated by phytopolymerization and phytooxidation. Phenol was rapidly detoxified via transformation to phenol radicals, followed by polymerization to non-toxic polyphenols or regioselective polymerization with natural organic matters prior to being precipitated as particulate polyphenols (PPP) or particulate organic matters (POM). After this first phase, phenol decreased from 500 mg/L to around 145 mg/L, while PPP and POM increased, as indicated by the increase of particulate chemical oxygen damand (COD). Synergistically, rhizomicrobial growth was ~100-fold greater on the roots of the vetiver grass than in the wastewater and participated in the microbial degradation of phenol at this lower phenol concentration, increasing the phenol degradation rate by more than 4-fold. This combination of POD-assisted phytopolymerization, phytooxidation, and rhizomicrobial degradation completely eliminated phenol in the wastewater in less than 700 hours.

Based on these results, from August 28–29, 2014, we implemented the first field-scale vetiver system, as 0.12 million vetiver grass bare roots were cultivated to create 1.2-kilometer vetiver hedgerows along Tat Noi Creek. Similarly, on December 5, 2015, we implemented a field-scale treatment of illegally dumped wastewater in a 768-cubic meter pond using vetiver grass on 45 floating bamboo platforms. We completed one round of wastewater treatment within 56 days of treatment. To our knowledge, this is the first time that vetiver systems have been used for field-scale phenol degradation. Such environmental restoration projects can be a model for the more than 50 communities recently affected by illegal dumping.

Keywords: Phenol-contaminated Wastewater, Root-produced H_2O_2 and Peroxidase, Rhizomicrobial Degradation, Field-scale Implementation, Phytoremediation

Introduction

Over the last few years, illegal dumping of industrial waste and wastewater has become a major environmental problem in Thailand. It is estimated that in 2013 alone, around 1.8 million tons of hazardous industrial waste disappeared from the tracking system (Crime News Team, 2013), and likely ended up in illegal dumping sites in remote areas. As intensively reported on the news over the last two years, more than 50 cases of illegal industrial waste dumping were found in remote areas close to residential zones throughout the country, but most (40 cases) are in the eastern part of Thailand (Figure 1a) (Bootkote, 2013). Nevertheless, the most infamous illegal dumping case in Thailand is probably the dumping of phenol (C_6H_6O)-contaminated wastewater in a “15-rai” pond (1 rai = 1,600 m^2) in the Nong-Nea Subdistrict, Phanom Sarakham District, Chachoengsao Province (Figure 1b and c). This case has gained much public and media attention since February 2012 due to several interesting and unique aspects of the incident.

First, Nong-Nea villagers organized voluntary teams to monitor illegal dumping in their neighborhood and were able to seize trucks conveying phenol-contaminated wastewater (40 mg/L phenol) to dump into the 15-rai pond. Second, after the discovery of the illegal dumping of phenol-contaminated wastewater, several government agencies conducted field survey to assess the impact of illegal dumping on human and ecological health. They estimated that more than 800 villagers were at risk of phenol exposure via the consumption of phenol-contaminated shallow groundwater (Phenrat, 2013), which the villagers rely upon for consumption. However, as a result of illegal dumping, shallow groundwater was contaminated with as much as 225 µg/L of phenol (see Figure 2a), while the acceptable level of phenol in drinking water is only 1 µg/L (Phenrat and Teeratitayangkul, 2014). Phenol is a toxic substance causing irritation and kidney inflammation, and disinfection of phenol-contaminated water using chlorine yields carcinogenic pentachlorophenol. Third, the ecological impacts of the incident were quite pronounced. For example, the Nong-Nea neighborhood is well-known as a food production area. During the illegal dumping incident, several pig farmers reported strange miscarriages (Figure 1e), which resulted in only 4–5, as opposed to 15–20, surviving piglets (Health Impact Assessment Coordinating Unit, 2013). Phenol is known to cause miscarriages, especially if it is transformed to chlorophenols (Lee and Morris, 1962) due to disinfection via chlorination, a routine practice by pig farmers. Additionally, fish in many farms died in large numbers, and rubber trees also yielded much less milk. The villagers believed that these unpleasant decreases in agricultural product yield were a result of illegal dumping (Health Impact Assessment Coordinating Unit, 2013). Fourth, in 2013, Mr. Prajob Naowaopas (Figure S1a), a village headman and leader of Nong-Nea environmentalists, was murdered by a contract killer after his environmental campaign that brought the illegal dumping and contamination issue to the government's attention for environmental cleanup and legal punishment to the dumpers. Yet, this does not discourage the Nong-Nea villagers from fighting for the homeland. Instead, Mr. Prajob becomes a heroic icon of environmental campaign for Nong-Nea cleanup and reclamation of their quality of life.

Last but not least, even though the court ordered the waste management facility that illegally dumped wastewater into the 15-rai pond, to treat the dumped wastewater in December 2012, phenol contamination has been continuously detected in shallow groundwater wells in Nong-Nea village for almost two years after the treatment (i.e., phenol was still detected in November 2014, as shown in Figures 2b and c). The remaining phenol in the shallow groundwater system might be due to phenol leaching into the shallow aquifer before or after treatment of the 15-rai pond.In addition, the 15-rai pound might not be the only phenol source contributing to the contamination of surface water and the shallow groundwater system, as at least 5 more illegal dumping sites (Figure 1b) were discovered in Nong-Nea after the 15-rai incident. Three of the five are suspected to release phenol to the environment. Two of them are waste management facilities, while another belongs to Mr. Manus Sawasdee, a Nong-Nea resident who is a victim of illegal dumping. Two years ago, Mr. Manus's pond (Figure 1d) was illegally dumped with industrial wastewater a containing high concentration of phenol (C_6H_6O) (as high as 500 mg/L at the beginning of the incident), as well as other hazardous organic substances, such as petroleum hydrocarbons (TPHs), formaldehyde, and metals such as arsenic, chromium, copper, lead, and nickel. Like the 15-rai pond, Mr. Manus's pond is at high elevation. When it rains, the runoff from this pond might carry phenol and other hazardous substances to lower waterways, as evident by the migration of phenol along Tad-Noi creek to the lower residential areas (Figure 2).

To reduce the health risk from phenol exposure through consumption of contaminated shallow well water, a research group lead by T. Phenrat, the first and corresponding author of this present article, was assigned by the government to develop and install ozonation units to eliminate phenol from the drinking water (Figure S1b)). We were successful in equipping 40 Nong-Nea households with ozonation units capable of oxidatively degrading phenol in the drinking water. Nevertheless, this attempt does not stop phenol migration in shallow groundwater and surface water systems, as ozonation neither addresses the contamination sources nor degrades the contaminated plume. Instead, ozonation is just a passive point-of-use treatment for short-term risk reduction. As the phenol contamination appears to have expanded with time (Figures 2b and c), the number of households contaminated by phenol may increase as well. Obviously, passive, point-of-use phenol treatment alone is not a sustainable solution. Nong-Nea villagers need an effective, practical, sustainable remediation measure to reclaim their shallow groundwater and soil and to reduce the extent of the contamination. A remediation technique that allows community involvement will be of great benefit for Nong-Nea, which has an active environmentalist community.

The phytoremediation of phenol by vetiver grass is a great remedial action candidate for the Nong-Nea incident. First, phytoremediation is known for its cost effectiveness and ease of use. Thus, the Nong-Nea villagers can participate in the phytoremediation implementation. Second, phytoremediation is the technology of choice for widely spreading contamination, such as phenol-contaminated runoff from illegal dumping sources. Most importantly, a recent study found that vetiver grass could degrade phenol in the laboratory (Singh et al., 2008). Nevertheless, neither a laboratory-scale study using real wastewater nor a field-scale application of vetiver grass has been examined for phenol removal. The study pointed out that phenol removal was associated with H_2O_2 and peroxidase (POD) produced by the vetiver roots. However, no complete mechanistic understanding of phenol degradation by vetiver grass, especially in a complex system for real application, is available.

This on-going research aims to use vetiver systems to protect the Nong-Nea community by degrading phenol and other contaminants in the accessible illegal dumping source zone contaminated runoff, the shallow groundwater plume in surface water (Tad-Noi Creek), and shallow groundwater systems. Our specific research objectives include: 1) determining the phenol removal mechanisms via a vetiver system; 2) evaluating the performance of phenol degradation by vetiver grass on a floating platform and underground permeable reactive barrier made of vetiver root, 3) implementing a field-scale application of vetiver on a floating platform to treat phenol-contaminated wastewater at Mr. Manus's pond via community involvement; and 4) implementing vetiver hedgerows along Tat Noi Creek to decrease the exposure of villagers to residual phenol migrating along the creek via community involvement. Because this research is large in scope and involves various laboratory-scale and field-scale implementations, in this research article, we report the complete missions of the research (i.e., (1) and (2), and the on-going ones (i.e. (3) and (4)), and the one to be conducted in the near future (i.e. (5)).

Nong-Nea's Risk and Risk Reduction Strategies Using Vetiver Systems

Prior to discussing laboratory approaches and field-scale implementations in detail, we discuss Nong-Nea villagers' risk and risk reduction strategies using vetiver systems to understand the logic of the three vetiver application strategies. Figure 3 illustrates a risk conceptual model from illegal dumping at Nong-Nea village. There are two types of source zones, accessible and inaccessible. The accessible source is Mr. Manus's pond, while the inaccessible sources are two waste management facilities. These two are inaccessible because the owners do not allow any stakeholders, including researchers and government agencies, to assess or remediate their properties. Thus, we had no choice but to investigate the contaminant signal in shallow groundwater and soil outside their fences using a membrane interface probe (MIP), and we found a high potential of contaminant release from their properties (Figure S2). Contaminants may be released from accessible and inaccessible source zones via overtopping the dike, infiltration or percolation, and spills. Released contaminants can be transported via runoff, shallow groundwater flow, and surface water flow (through Tad-Noi creek). Nong-Nea villagers and aquatic and terrestrial animals may be exposed to contaminants via drinking water, dermal contact, or the food chain. Inhalation of contaminated dust is also possible, but it is not likely to be a substantial risk.

Thus, risk reduction strategies include: 1) treatment of contaminants in the accessible source zone, and 2) interception and degradation of contaminants released from the inaccessible source zones to surface water and shallow groundwater systems. Vetiver systems can be applied for both strategies. First, vetiver grass on floating platforms can be used to treat contaminated wastewater in Mr. Manus's pond (Figure 4a). Second, vetiver hedgerows with an underground root barrier along Tad-Noi creek can be used to reduce contaminant migration from Tad-Noi creek to the shallow groundwater system (Figure 4b). Last, a deep underground permeable reactive barrier of vetiver root can be used to intercept contaminated shallow groundwater plume from the inaccessible sources (Figure 4c). These three risk reduction strategies can be implemented with community involvement. However, laboratory-scale studies are needed to ensure success. Next, we will discuss essential laboratory studies and report the progress of field-scale implementations.

Figure 1. (a) Forty illegal dumping sites in eastern Thailand, (b) The Nong-Nea subdistrict is surrounded by at least five illegal dumping sites, (c) the "15-rai" site during on-site treatment of illegally dumped wastewater containing high phenol concentrations, (d) Mr. Manus's pond affected by the illegal dumping of wastewater containing high concentrations of phenol and total petroleum hydrocarbons, and (e) reported strange miscarriages in pigs believed to be caused by phenol contamination of shallow groundwater.

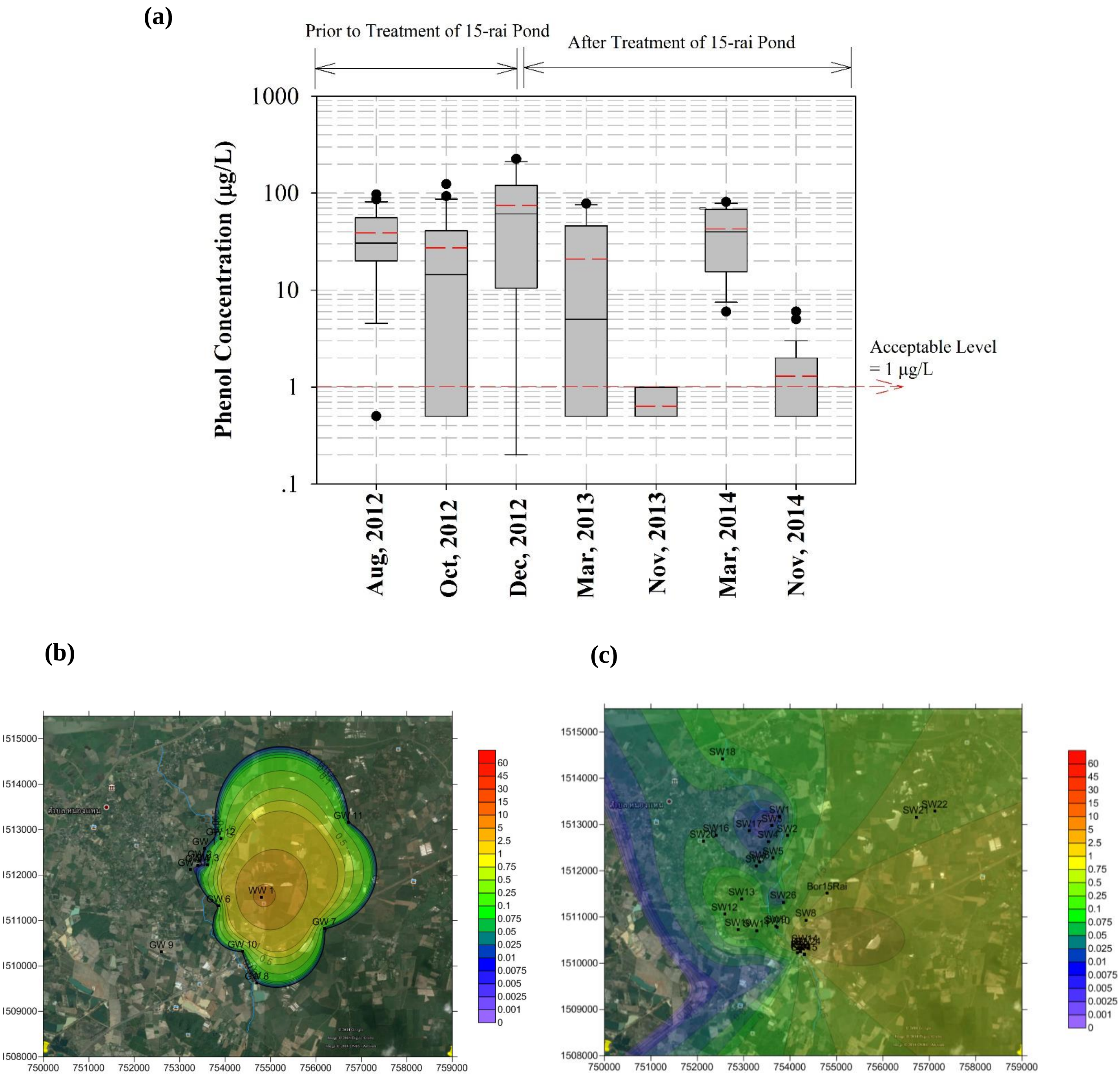
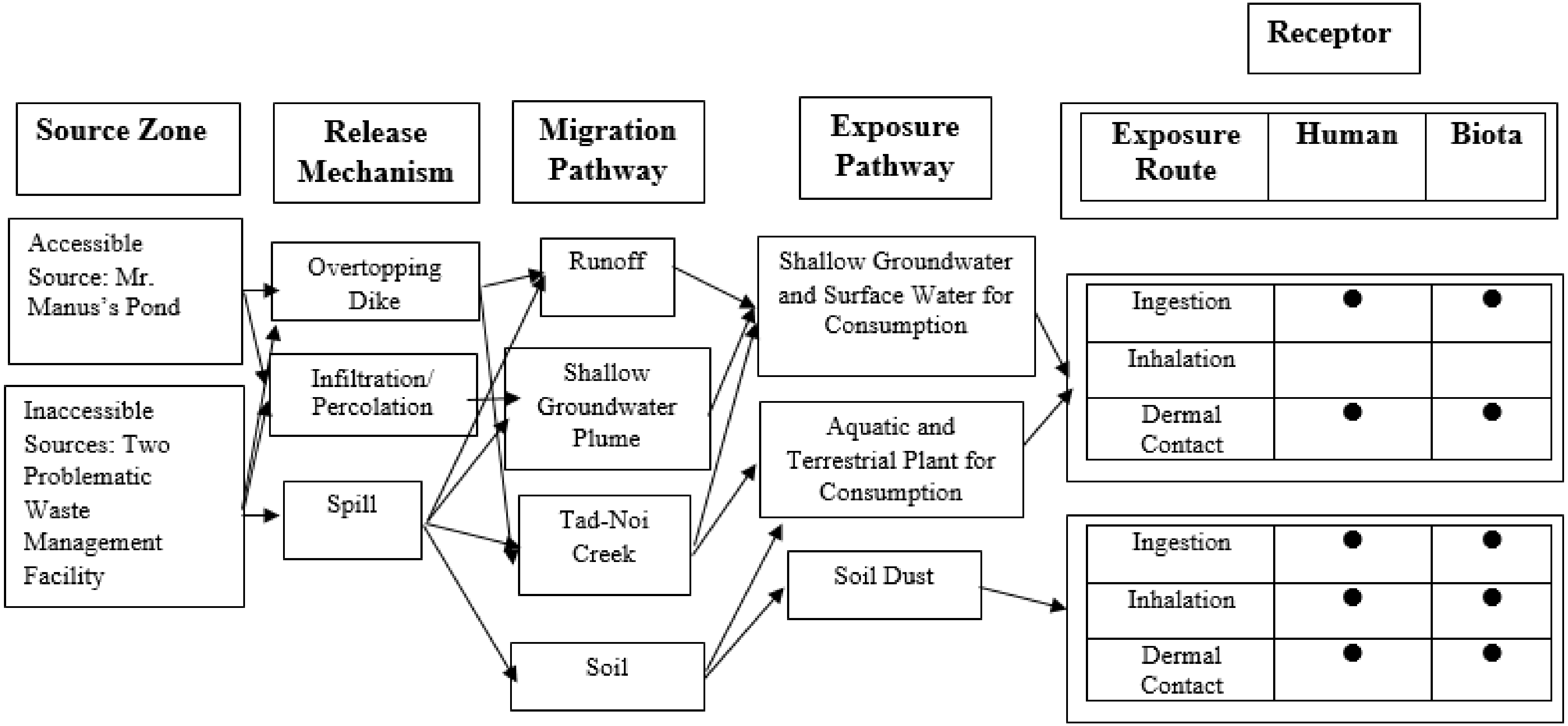


Figure 2. (a) Phenol concentration in shallow groundwater wells of Nong-Nea villagers from August (two months after the "15-rai" incident) to November, 2014, (b) Contour of phenol contamination in Nong-Nea shallow groundwater in June 2012 (the beginning of the "15-rai" incident), and (c) Contour of phenol contamination in Nong-Nea shallow groundwater in March 2014.



Exposure Route	Human	Biota
Ingestion	●	●
Inhalation		
Dermal Contact	●	●

Exposure Route	Human	Biota
Ingestion	●	●
Inhalation	●	●
Dermal Contact	●	●

Figure 3. Risk conceptual model of Nong-Nea villagers and living organisms due to hazardous substances released from illegal dumping sources.

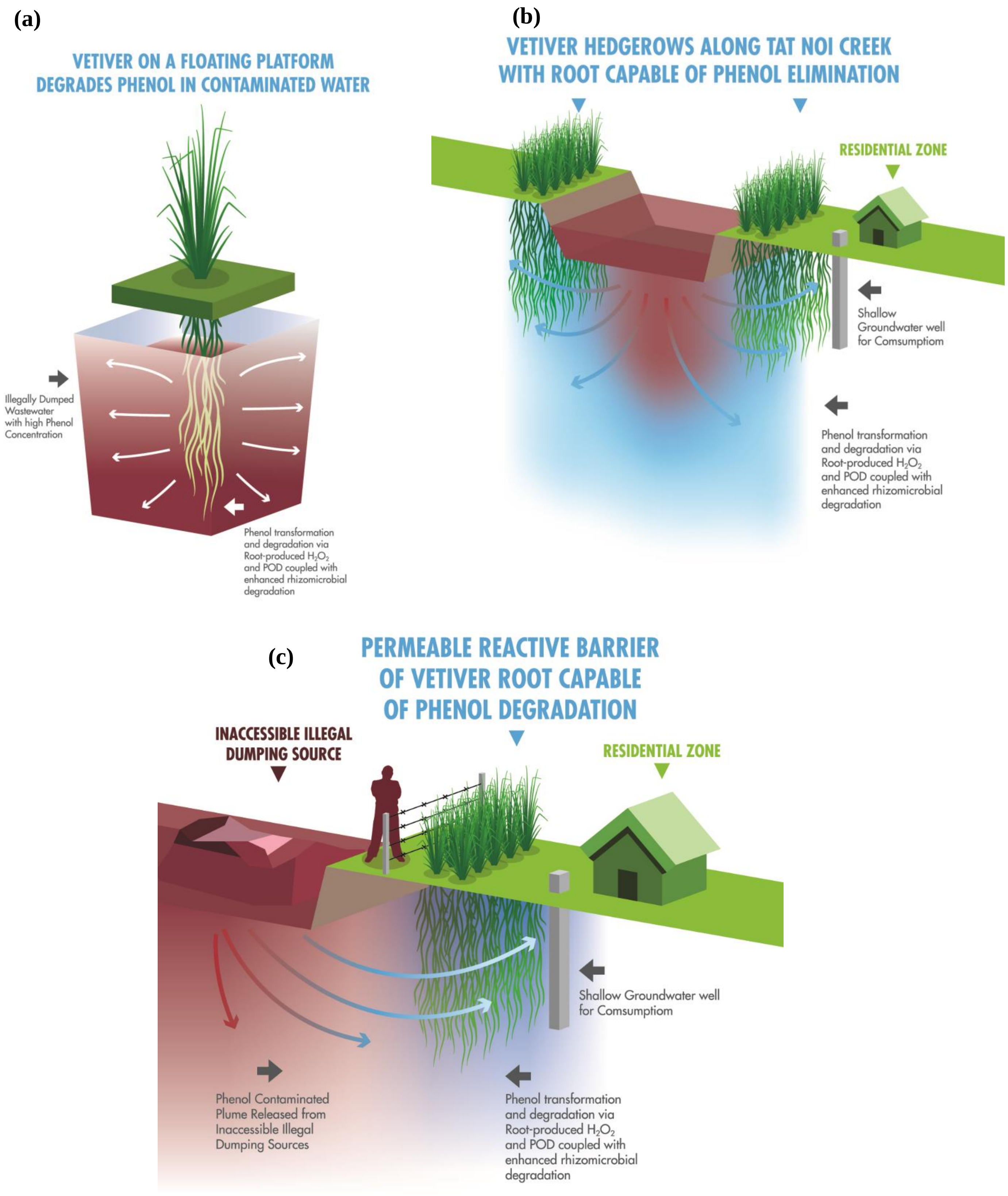


Figure 4. (a) Vetiver grass on floating platforms to treat contaminated wastewater in Mr. Manus's pond, (b) Vetiver hedgerows with an underground root barrier along Tat-Noi creek to reduce contaminant migration from Tat-Noi creek to the shallow groundwater system, and (C) deep permeable reactive barrier of vetiver root to intercept contaminated shallow groundwater plume from the inaccessible sources.

Materials and Methods

Vetiver grass, Phenol, Wastewater, and Soil

Vetiver grass (*Vetiveria zizanioides* (L.) Nash) used in this study was the (bare root) Songkla-3 ecotype obtained from Chachoengsao Development Station. Phenol was 99% purity obtained from Panreac (Barcelona, Spain). Wastewater and soil used in laboratory studies were obtained from Mr. Manus's pond and surrounding area to represent real contaminated wastewater and soil. Wastewater was spiked with phenol for experimental studies.

Chemical and Physical Analysis

Phenol in water and wastewater samples from laboratory and field-scale studies were measured using the 4-amino-antipyrine method 8047 with a Spectroquant® Move 100 mobile colorimeter (Merck KGaA, Darmstadt, Germany). Chemical oxygen demand (COD) was measured by the closed reflux method (Standard Method 5220 C), while tannin and lignin were measured by Standard Method 5550. Volatile organic acids (VOA) were quantified by Standard Method 5560. pH, oxidation-reduction potential (ORP), and conductivity were measured with appropriate probes with proper calibration. Hydrogen peroxide (H_2O_2) was measured by reacting it with dissolved titanium to form yellow pertitanic acid. The colored complex was measured by a spectrophotometer at 400 nm (Lei and Xuanzhen, 2008). Peroxidase (POD) activity was determined at 25ºC with a spectrophotometer following the formation of tetraguaiacol as described by Singh et al. (2006). One unit of POD activity (U) represents the amount of enzyme catalyzing the oxidation of 1 µmole of guaiacol in 1 min at 25ºC. Other contaminants, including metals and metalloids, total petroleum hydrocarbons (TPH), cyanide, formaldehyde, polycyclic aromatic hydrocarbons (PAHs), pesticides, and other semi volatile organic compounds (SVOCs), were measured by appropriate analytical methods by a certified analytical laboratory (ALS, Thailand).

Microbial and Rhizomicrobial Characterization

To characterize microbes and rhizomicrobes, vetiver roots were collected and surface sterilized by washing with 75% ethanol and 5% NaOCl, followed by rinsing with sterile distilled water. Surface sterile roots were ground and mixed with potassium dihydrogen phosphate buffer pH 6.8 (Dudeja et al., 2012). Root and wastewater samples were subjected to ten-fold serial dilutions. The pour plate technique on standard plate count agar was used for total viable counts, and the spread plate technique was applied to determine yeasts and molds on Sabouraud dextrose agar and *Pseudomonas spp*. on *Pseudomonas* agar. Standard plate count agar and Sabouraud dextrose agar plates were inverted and incubated for 48 h at 35ºC. Sabouraud dextrose agar plates for yeast and mold counts were upturned and incubated for 5 days at 25ºC. Colonies growing on the plates were counted and recorded as colony forming units per g (CFU/g) of each sample. Colonies were purified on standard plate count agar for bacteria and Sabouraud dextrose agar for yeasts and molds. The pure isolates were stored on the same media at 4ºC. Isolated colonies were characterized by Gram staining, cell morphology, and biochemical tests as described in Bergey's Manual of Systematic Bacteriology (Garrity et al., 2001).

Laboratory Study of Phenol Degradation by Vetiver Grass on a Floating Platform

Vetiver grass on a floating platform is a well-established approach for wastewater treatment (Danh et al., 2009; Roongtanakiat, 2009; Truong and Stone, 1996; Truong and Heart, 2001; Trong and Baker, 1998). Nevertheless, no such system has been used for phenol degradation. To design a field-scale vetiver system on a floating platform for phenol removal, we first needed to understand the phenol degradation mechanism. Thus, phenol degradation by vetiver grass on a floating platform was conducted in glass tanks (Figure 5a) filled with 35 L of real wastewater from Mr. Manus's pond, in which the initial phenol concentration was adjusted to 500 mg/L, the maximum phenol concentration measured at this pond two years ago. Five different experimental conditions (P1 to P5) using different numbers of vetiver (P1 = 20, P2 = 40, P3 = 60, P4 = 80, and P5 = 100) plants in 35 L of aerated wastewater were examined. Two control studies without vetiver grass, but with and without aeration, were also conducted. Phenol degradation kinetics were determined over 1,400 h. Similarly, the kinetics of COD removal, VOA formation, lignin and tannin removal, H_2O_2 formation, and peroxidase formation were monitored over 1400 h to verify the phenol degradation mechanism.

To understand the synergistic effects of vetiver grass and microbes, this study also isolated and characterized native microorganisms after the phenol treatment. Vetiver roots and treated wastewater samples from P1 and P5 were collected and examined for microbial content after four different treatment times. Total viable counts of yeasts, molds, and *Pseudomonas spp*. were quantified.

Laboratory Study of Phenol Degradation by a Permeable Reactive Barrier of Vetiver Root

To design field scaled vetiver hedgerows along Tad-Noi creek and permeable reactive vetiver root barrier (PRVRB) surrounding the inaccessible sources, we needed to evaluate its effectiveness in phenol removal. Thus, we conducted a permeable reactive vetiver root barrier (PRVRB) study in a flow-through tank (Figure 5b) packed with a sand:soil mixture. The soil was from Mr. Manus's area. We flowed phenol-spiked DI water (500 mg/L of phenol) through the tank at a specific discharge of 30 cm/day. The phenol-contaminated water was intercepted by a PRVRB (20 plants over a length and width of 40 and 20 cm, respectively). Vetiver grass was grown in uncontaminated soil until its root length was 20–30 cm prior to its installation as the root barrier. Concentrations of phenol, COD, and VOA were quantified as a function of time.

Figure 5. (a) Laboratory-scale vetiver grass in a floating platform and (b) Laboratory-scale PRVRB.

Field-scale Implementation of Vetiver Systems and Community Involvement

After laboratory evaluations, we presented our research results regarding the benefit of using vetiver systems to the Nong-Nea community. Additionally, we used the phenol removal rates to design three field-scale implemntations. On August 28–29, 2014, we implemented the first field-scale vetiver system, the vetiver hedgerows, along Tat Noi Creek. Devember 5, 2015, we implemented the field-scale treatment of illegally dumped wastewater at Mr. Manus's pond. A new treatment pond was prepared with an HDPE liner to prevent the infiltration of cotnamianted water. The contaminated water was pumped from the illegal dumping pond to the HDPE pond. Vetiver grass on 45 floating bamboo platforms were used to treat the wastewater in the HDPE pond. In April or May, 2015, we plan to implement the last prototype of vetiver system, a field-scald PRVRB with engineered, extended root zone to intercept plume in shallow groundwater from an inaccessible source zone.

Results and Discussion

Laboratory-scale Development

Phenol Degradation by Vetiver Grass on a Floating Platform: A Proposed Mechanism and Proof of Concept

Figure 6 illustrates the kinetics of phenol, COD, tannin and lignin removal, as well as VOA transformation, kinetics for all five different experimental conditions (P1 to P5). The overall phenol and COD removal rates appear to qualitatively increase with the number of vetiver grasses used in the treatment, i.e., P1<P2<P3≈P4<P5. H_2O_2 and POD were detected in the wastewater and vetiver roots, respectively (Figures 7a and b) as suggested by Singh et al., 2006. Nevertheless, we were unable to apply 1st order reaction kinetics to the phenol degradation results. It turns out that 1st order kinetics, which are typically used to model phenol degradation reactions (Singh et al., 2006; Esplugas et al., 2002), could not model the entire set of kinetic data. Instead, we needed to divide the kinetic data into two phases to model each phase with 1st order kinetics (Figure 6a). This suggests that phenol degradation in wastewater by vetiver grass is likely a two-phase process. Considering all the evidence, we proposed a new mechanistic understanding of phenol degradation by vetiver grass, as depicted in Figure 8. The two phases are: Phase I, phytopolymerization and phytooxidation assisted by root-produced H_2O_2 and POD, followed by Phase II, a combination of Phase I with enhanced rhizomicrobial degradation.

To understand the proposed mechanism, let us consider the phenol degradation and by-product formation of P5 shown in Figure 7c. According to this figure, the first 360–400 h of phenol degradation was dominated by phytopolymerization and phytooxidation at a rate constant of $3.3 \times 10^{-3}\ h^{-1}$. This is 11 times faster than the phenol removal rate without vetiver grass ($3 \times 10^{-4}\ h^{-1}$), presumably due to hydrolysis or photodegradation. Microbes might not be effective in degrading phenol at this high concentration (500 mg/L), as phenol is inhibitory to microbes at this concentration (Hugo, 1978; Nweke and Okpokwasili, 2010). Instead, H_2O_2 and POD produced by vetiver roots assisted in the first phase of phenol transformation. Phenol was detoxified via POD-catalyzed transformation to phenol radicals, followed by polymerization to non-toxic polyphenols or regioselective polymerization with natural organic matters prior to being precipitated as particulate polyphenols (PPP) or particulate organic matter (POM), respectively (Figure 8). This is evident by the rapid removal of dissolved COD (because dissolved phenol contributes to dissolved COD) and the rapid increase of particulate COD (because PPP and POM contribute to particulate COD) (Figure 7c). In addition, because the total COD decreased exponentially in the first phase, phytooxidation assisted by root-produced H_2O_2 and peroxidase (POD) yielded more oxidizing VOA or CO_2 as by-products, which also occurred at the same time as the phytopolymerization of phenol and the precipitation of PPP, as suggested above.

After the first phase, phenol decreased from 500 mg/L to around 145 mg/L, while PPP and POM, which are non-toxic to microbes, increased. Synergistically, rhizomicrobes intensively grew on the roots of vetiver grass and participated in microbial degradation of phenol at this lower concentration, increasing the phenol degradation rate ($9.7x10^{-3}$ h^{-1}) by more than 4-fold in comparison to the phenol degradation rate in the first phase and by approximately 32-fold compared with the phenol removal rate without vetiver grass (Figure 7d).

The hypothesis of enhanced rhizomicrobial degradation is supported by microbial and rhizomicrobial analysis. While at 430 h, the total viable counts (TVC) of microbes in treated wastewater samples was 10^3–10^4 CFU/mL, the mean fungal population was less than 10 CFU/mL. In contast, vetiver root samples showed TVC as high as 10^5–10^6 CFU/g and the fungal count was more than10^2 CFU/g. The total microbial population obtained from the roots was more than that in the water samples because of the special relationship between an endophyte and host (Danh et al., 2009). TVC in wastewater and vetiver root increased at the rate of 0.01 to 0.02 CFU mL^{-1} h^{-1}. Six genera of bacteria (*Rhodococcus*, *Pseudomonas*, *Bacillus*, *Micrococcus*, *Enterobacter*, and *Alcaligenes*) and three genera of fungi (*Candida*, *Trichosporon*, and *Rhodotorula*) were identified (Table 1 and Figure 9). All of them were able to degrade phenol and polyphenols (Krastanov et al., 2013), which explains the rapid phenol degradation during phase II.

Table 1. Characteristics of isolated colonies from treated wastewater and vetiver roots

Genera	Morphology	Gram	Motility	Oxidase	Catalase	O/F	IMVIC
Bacteria							
Rhodococcus	Filamentous	+	–	–	+	+/–	––++
Pseudomonas	rod	–	+	+	+	+/–	–––+
Bacillus	short rod	+	+	–	+	+/+,	–++–
Micrococcus	rod	+	–	+	+	+/–	–++–
Enterobacter	spherical	–	+	–	–	+/–	––++
Alcaligenes	short rod	–	+	+	+	+/+	––++
	rod bacilli					+/+	
Fungi							
Candida	Oval	ND	ND	ND	ND	ND	ND
Trichosporon	Septate	ND	ND	ND	ND	ND	ND
Rhodotorula	hyphae	ND	ND	ND	ND	ND	ND
	Oval						

For P5, the combination of POD assisted phytopolymerization and phytooxidation, and rhizomicrobial degradation completely eliminated 500 mg/L of phenol in wastewater within 800 h, while the natural attenuation of phenol by microbes in wastewater alone will take as long as 2.36 years considering a degradation rate of $3x10^{-4}$ r^{-1}. The same reaction mechanism was also true for P1 to P4, but at different reaction rates (see Figure 7d). Nevertheless, the phenol degradation rate in this study is slower than that reported in a previous study. Singh et al. (2008) reported that after four days, vetiver grass in aseptic Murashige and Skoog liquid medium decreased phenol at an initial concentration of 500 mg/L by 76%. Conversely, in our study of the same time period vetiver grass degraded just 25% of the phenol at the same initial concentration. This is presumably because our study was conducted using real wastewater with COD, organic load (such as total petroleum hydrocarbon), and inorganic reducing species; the actual pollutant loads are not available for aseptic Murashige and Skoog liquid medium. These organic and inorganic reducing loads consume the oxidative capabilities of H_2O_2 and POD and might compete with phenol degradation. As seen in Figure 6f, the wastewater samples started with negative

ORP, which required the consumption of an oxidant such as H_2O_2 to become positive 500 h later.

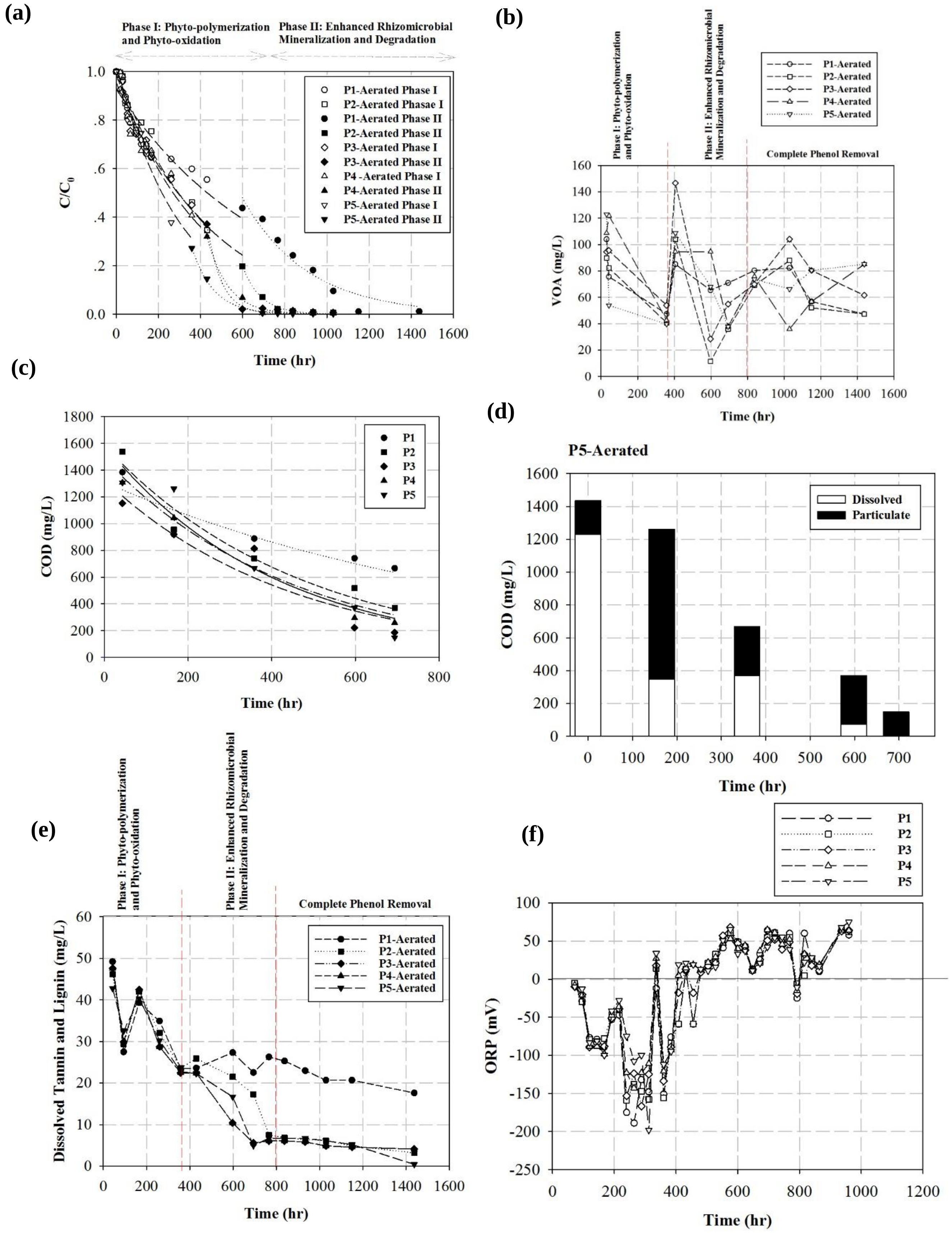


Figure 6. Experimental kinetics of (a) Phenol removal, (b) VOA transformation, (C) COD removal, (d) COD fractionation (for P5), (e) dissolved tannin and lignin removal, and (f) ORP for P1 to P5 according to the two-phase reaction hypothesis.

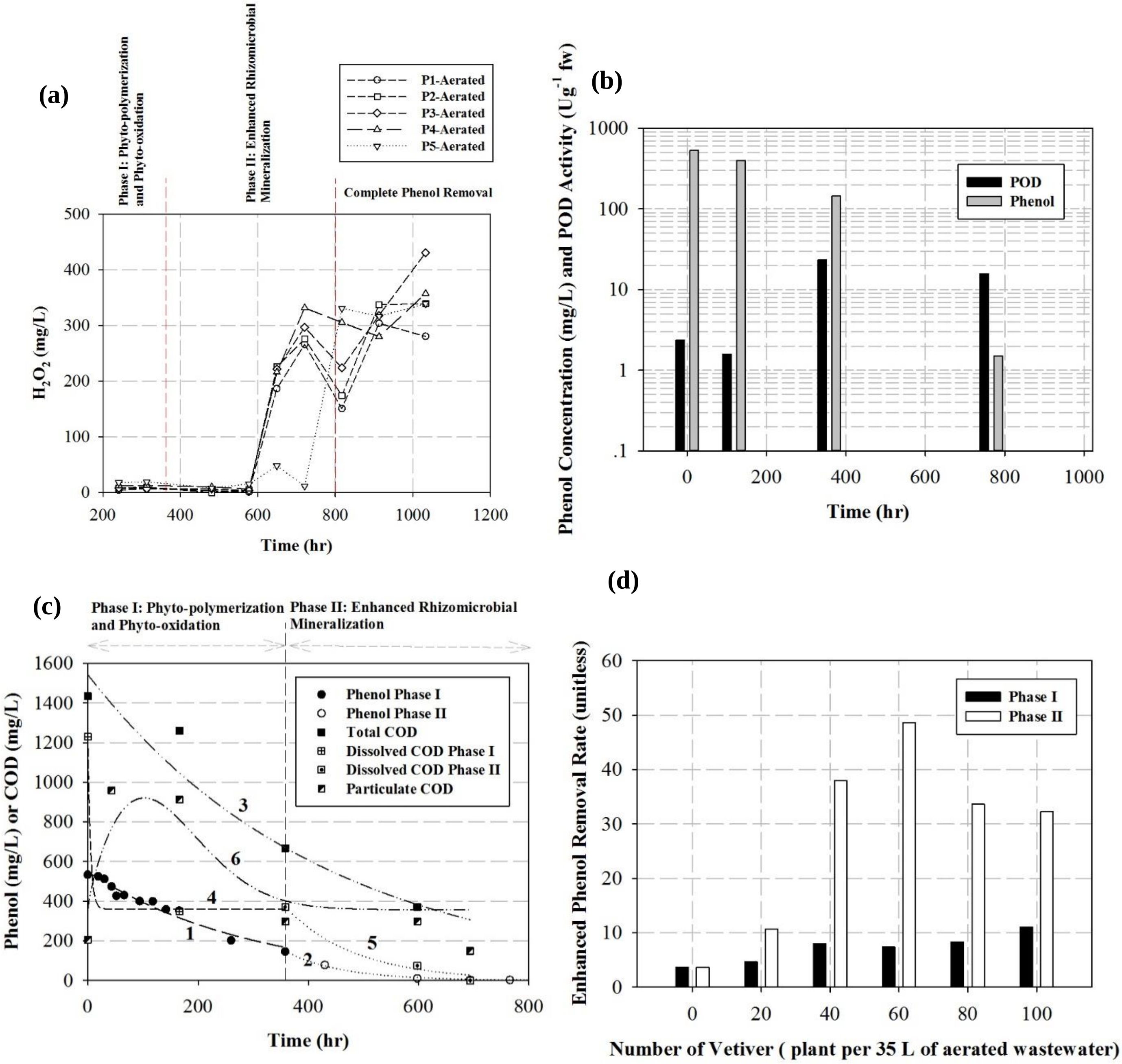


Figure 7. Experimental kinetics of (a) H_2O_2 formation, (b) POD activity and phenol concentration, (C) phenol concentration together with total COD, dissolved COD, and particulate COD for P5, and (d) Summary of the enhanced phenol removal rate as a function of the number of vetiver grasses in a floating platform. Typically, one would redefine all abbreviations in a figure legend.

To our knowledge, this is the second study of phenol degradation by vetiver grass, but it is the first that systematically reveals the complete phenol degradation mechanism by vetiver grass in real wastewater. Our proposed mechanism is different from that reported by Singh et al. (2008), who conducted there experiment in aseptic Murashige and Skoog liquid medium, in which rhizomicrobes might not play substantial role in phenol degradation. In addition, Singh et al. (2008) did not conduct their phenol degradation study until phenol was completely degraded to meet the discharge standard. Thus, they might not have noticed phase II of the degradation mechanism in which vetiver rhizomicrobes substantially increase the rate of phenol detoxification.

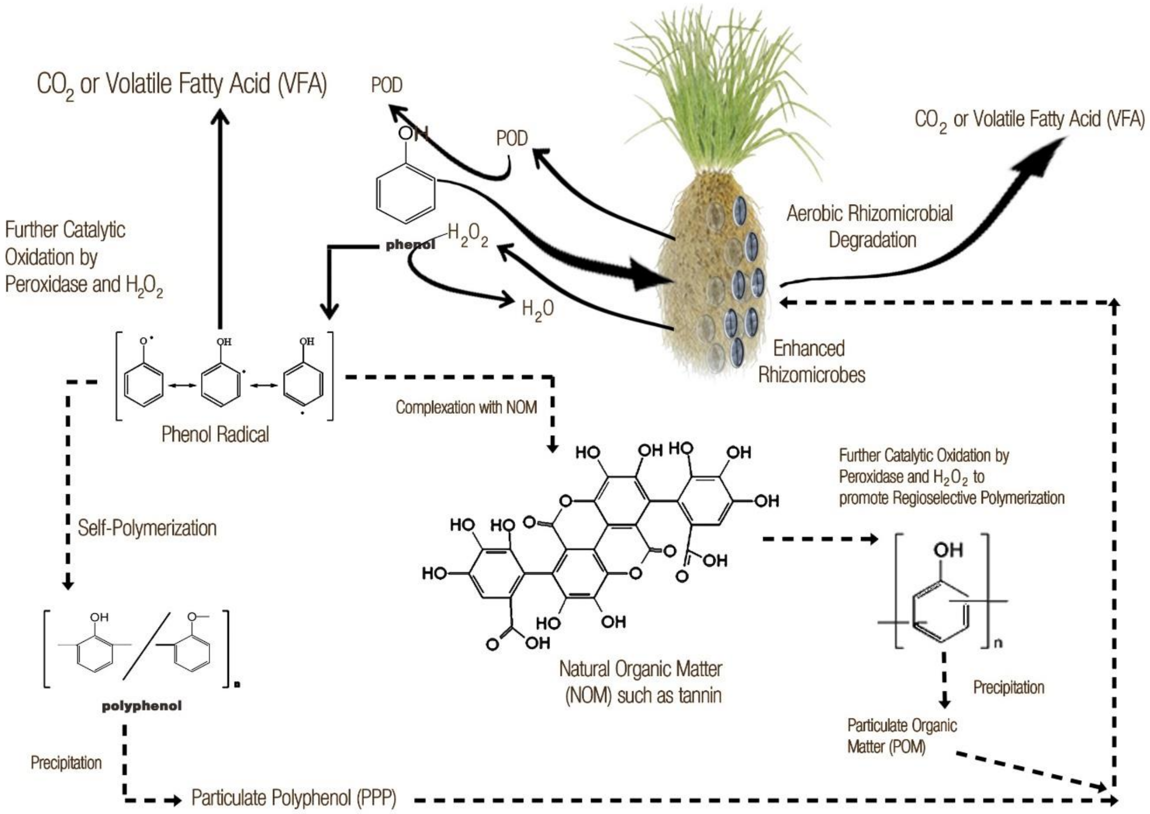


Figure 8. Conceptual mode of two-phase phenol degradation by vetiver grass in wastewater. The two phases are: Phase I, phytopolymerization and phytooxidation assisted by root-produced H_2O_2 and POD, followed by Phase II, a combination of Phase I with enhanced rhizomicrobial degradation. Again, it is usually best to redefine all abrreviations.

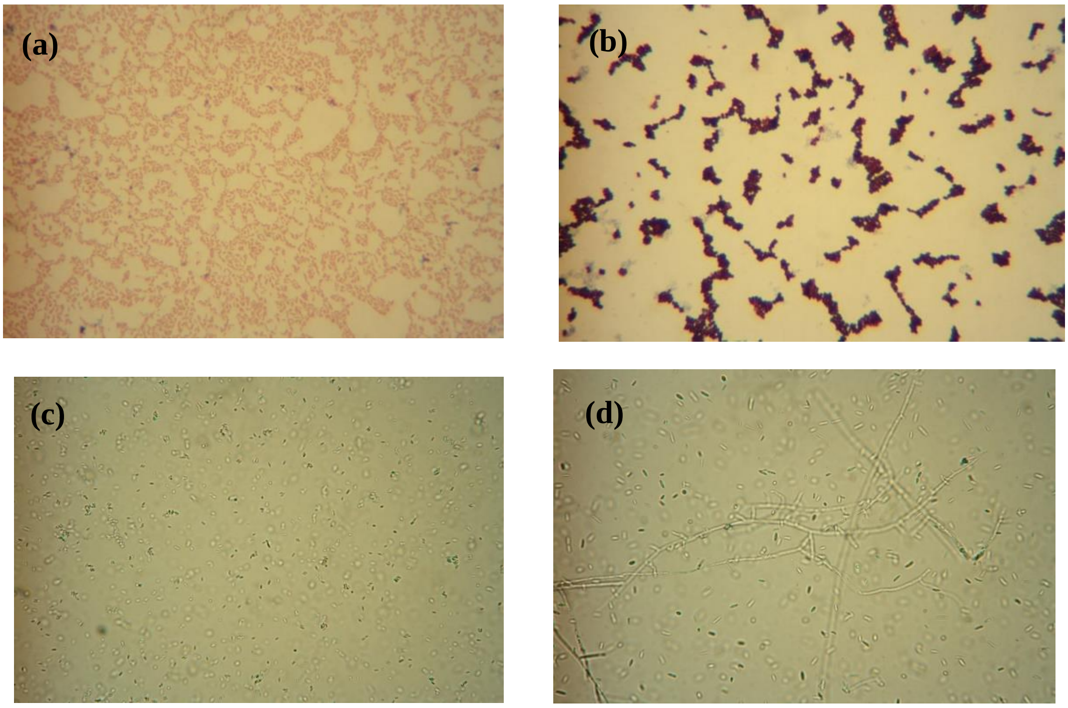


Figure 9. Isolated colonies from treated wastewater and vetiver roots, including (a) *Pseudomonas spp*., (b) *Micrococcus spp*. 1,000X magnification, (c) *Candida spp*. 400X, and (d) *Trichosporon spp*. 400X.

Permeable Reactive Vetiver Root Barrier (PRVRB): Laboratory Proof of Concept

The PRVRB effectively degraded phenol in a laboratory flow-through study. Figure 10a illustrates two sampling points: 1) Pre-PRVRB representing the phenol concentration prior to entering the PRVRB, and 2) Post-PRVRB representing the phenol concentration after passing through the PRVRB. One PV is around 56 h at flow rate of 30 cm/day. All the breakthrough curves gradually increase from time zero and reach a steady state after 56 h (Figure 10b). By comparing the breakthrough curve prior to entering and after passing through the PRVRB at the steady state, the 40-cm PRVRN achieved 28% and 38% removal of phenol and COD, respectively, at a 32-h retention time. With this finding, we estimated that the 1st order phenol and COD removal rate constants were 0.010 and 0.012 h^{-1}, respectively. The phenol and COD removal rates by the PRVRB were greater than that of vetiver on a floating platform at the initial state (Phase I at 78 h), but similar to that of Phase II (kphenol = 0.0097 h^{-1}), as the presence of soil presumably stimulates rhizomicrobial growth at a faster rate than in wastewater, thereby moving the system to Phase II faster. This hypothesis needs further verification.

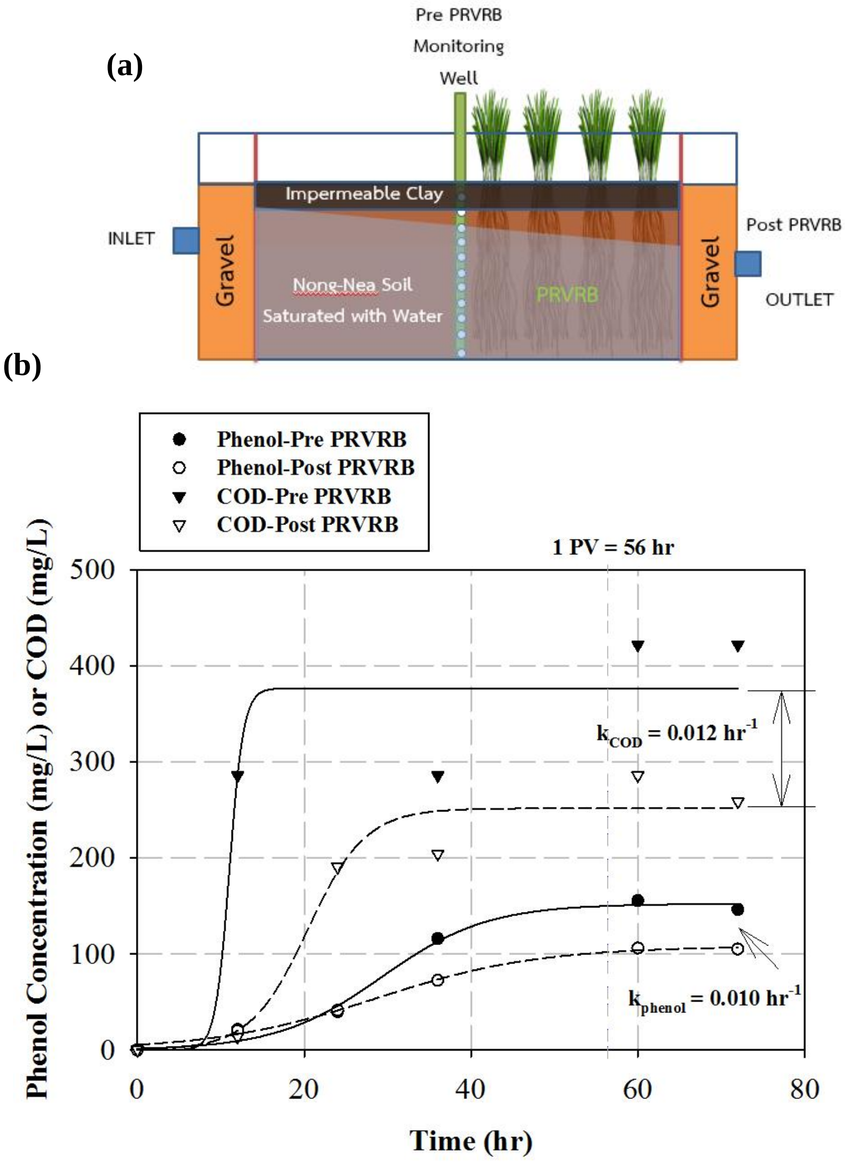


Figure 10. (a) Sampling points (Pre-PRVRB and Post-PRVRB) of laboratory-scaled PRVRB experiment and (b) Experimental breakthrough curves of phenol and COD sampled at Pre- and Post-PRVRB together with the predicted trends using reactive transport model.

Field-scaled Implementations and Community Involvement

During August 28–29, 2014, (the month of the Queen's birthday celebration in Thailand), more than 100 volunteers, including Nong-Nae villagers, undergraduate students from Naresuan University, primary school students from the Nong-Nea community, representatives of the Office of the Royal Development Projects Boards, local government agencies, and news reporters and actors and actresses from Channel 3 TV Thailand (see Figure 11) were present at the site to cultivate 0.12 million vetiver grasses (bare roots) to create a 1.2-km vetiver fence along Tat Noi creek, the major route of transporting residual phenol to the shallow wells of the villagers. Three to five rows of vetiver hedgerows were planted, covering around 1–1.5 m of the creek banks. Vetiver hedgerows are widely accepted as an effective measure to prevent erosion of creek banks (Greenfield, 1990; Truong, 2014). However, here at Nong-Nea, the volunteers aimed to use vetiver hedgerows to transform phenol to harmless polyphenols through the action of H_2O_2 and peroxidase produced from the roots of vetiver grasses, followed by the rhizomicrobial degradation of phenol to harmless by-products, as shown in the aforementioned laboratory study.

According the previous section, the phenol degradation rate by the PRVRB was 0.01 r^{-1}. With this information, our theoretical calculation suggests that the 1.5-km vetiver fences with a width of 1.5 m should decrease phenol transport through the PRVRB along the 1.5-mk creek banks at a removal efficiency of 40% (at a surface water flow velocity of 50 cm/min, which is quite a conservative estimate for a small creek like Tat-Noi creek) and should decrease phenol migration through soil (perpendicular to the creek) to the shallow wells at a removal efficiency of around 70% (at a seepage velocity of 30 cm per day). We collected water samples from Tat-Noi creek and the roots of vetiver hedgerows four months after cultivation. We found that the roots were as long as 50 cm. In addition, some phenol residual was detected in the creek, while the roots processed POD to protect Nong-Nea villagers (Figure 12a). To our knowledge, this is the first time that a vetiver fence has been used for phenol degradation at a field scale. This voluntary activity was reported in 18 news clips on Channel 3 from 27 to 29 August, 2014. Please see some new clips in the Supporting Materials.

Similarly, December 5, 2014 (the King Bhumibol's birthday), we launched the second field-scale vetiver system for the Nong-Nea community, i.e., the treatment of illegally dumped wastewater in Mr. Manus's pond using vetiver grass on floating platforms. Two treatment ponds (HDPE ponds 1 and 2) were prepared with an HDPE liner to prevent the infiltration of contaminated water or sediment. The wastewater consists of two separable constituents, contaminated water and contaminated sediment. The sediment that settled at the bottom of the pond was much more contaminated than the water. The sediment contained very high concentration of hazardous total petroleum hydrocarbons (TPH) (27.5 mg/kg), while the water contained 296 mg/L of COD (the allowable COD is 120 mg/L), and 775 µg/L of hazardous total petroleum hydrocarbons (TPH) (the allowable TPH concentration is 300 µg/L). A very low phenol concentration (<0.025 mg/L) was detected in the water, as heavy rain over the last two years gradually overflowed phenol out of the pond to Tat-Noi creek or enabled phenol to infiltrate into the shallow groundwater system.

The contaminated water was first pumped from the illegal dumping pond to HDPE pond 1. We treated the contaminated water first to remove all liquid wastewater from the illegal dumping pond so that we could move the contamianted sediment at the bottom of the illegal dumping pond to HDPE pond 2 for subsequent treatment using vetiver grass.

A total of 21,600 vetivers were used to crate 45 floating bamboo platforms for the treatment of the contaminated water in HDPE pond 1. The vetivers in the floating bamboo platforms were a result of a voluntary collaborative effort between our field researchers and Nong-Nea villagers (Figure 13). Some of them even participated in collecting samples and analyzing COD in the field study, as we planned to train them to be community researchers for the Nong-Nea community and also for other communities affected by illegal dumping or other kinds of wastewater-related environmental problems.

Vetiver grass can also be used to treat COD and TPH in the contaminated water as suggested in literature (Brandt et al., 2006; Cook and Hesterberg, 2013). After, 30 and 56 days of treatment, COD decreased to 120 mg/L and 52.5 mg/L, respectively, while TPC became 407 and 336 µg/L after 30 and 56 days of treatment, respectively. This suggested that 45 floating vetiver platforms (21,600 plants) can treat COD and TPH in the 12 m x 23m x 2 m HDPE pond 1 (the total volume of 768 m^3) in around 2 months. The treated water will be used for agricultural irrigation. We are going to start the last round of treatment of contaminated water followed by the treatment of the contaminated sediment in HDPE pond 2.

To the author's knowledge, this is the first time that vetiver on floating platform is used for TPH and COD removal in field scale, especially for community prevention from hazardous constitutes from illegal dumping. This voluntary activity was reported in a news clip on Channel 3. Please see the new clips in the Supporting Materials

Last but not least, we are planning to implement a field deep PRVRB to intercept contaminated shallow groundwater plume from the inaccessible sources. For this mission, we need an engineering root development stimulation strategy in order to grow the root barrier as deep as 5 m (Negri et al., 2013). We are conducting laboratory studies to understand factors affecting root development and how to engineer them to achieve the goal (Figure 12b). We should have enough understanding to launch the last field scaled vetiver system in either April or May of 2015.

Conclusion: Nong-Nea Model

This study proposed a two-phase mechanism of phenol degradation in wastewater by vetiver grass. Phase I is phytopolymerization and phytooxidation assisted by root-produced H_2O_2 and POD, while Phase II is a combination of Phase I with enhanced rhizomicrobial degradation. This study also evaluated the feasibility of phenol degradation on a laboratory scale using vetiver grass in a floating platform and PRVRB. Phenol was degraded at rate constants of 9.7×10^{-3} $h^{-1}$for vetiver grass in a floating platform (100 plants per 35 L of wastewater) and 10×10^{-3} $h^{-1}$for the PRVRB (20 plants over a length and width of 40 and 20 cm, respectively). Although these phenol degradation rates are around 10 times slower than those achieved with advanced engineering techniques, such as ultrasound (phenol degradation rate =111 x 10^{-3} h^{-1}), the phytoremediation of phenol and other hazardous substances at Nong-Nea village by vetiver grass is much more suitable when considering the practicality of the technique and the widespread nature of the contamination. Advanced engineering techniques are suitable for source zone treatment, but are mostly economically unpractical for widespread contaminants.

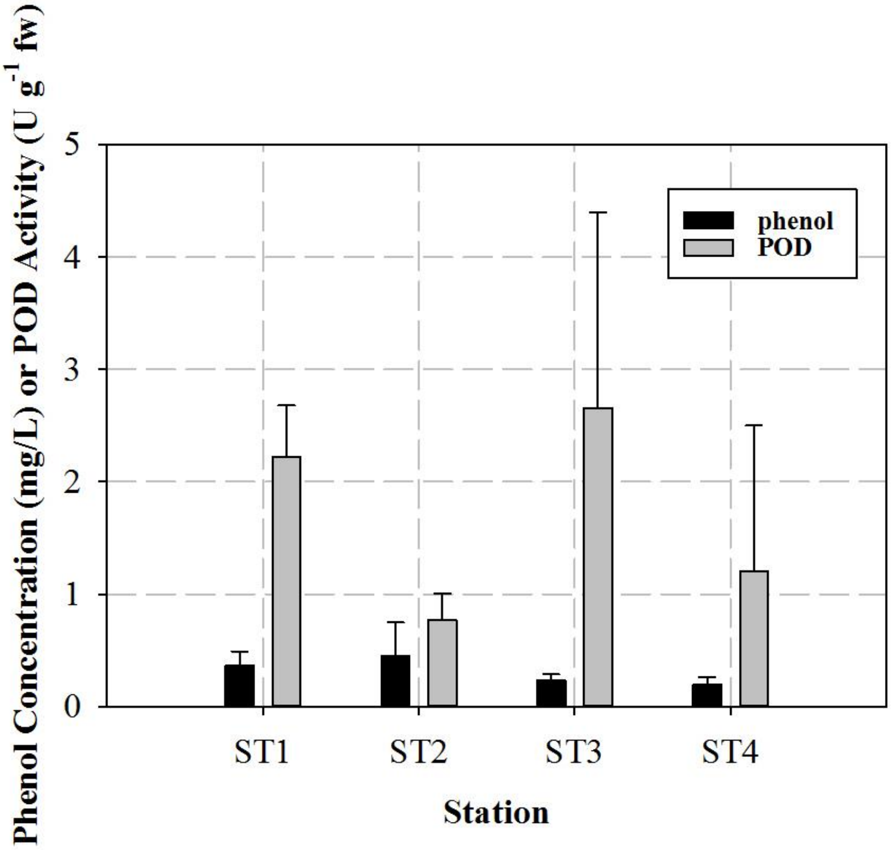
The on-going field implementations of vetiver systems are very successful in terms of treatment performance, community participation, and public communication. The successful uses of vetiver systems at Nong-Nea village will be a model of community-based remediation for the communities affected by 50 cases of illegal dumping in residential areas throughout the country. For example, waiting for government agencies to remove contamination due to illegal dumping in a community might take years (if it happens at all), while an affected community can use vetiver systems to treat illegally dumped wastewater in the meantime. This will reduce the concentration of contaminants in the source zone, which decreases contaminant migration and exposure of villagers. In such an environmental crisis, the successful use of vetiver systems at Nong-Nea can provide an affected community hope and a sense of empowerment.

In addition, government agencies can also benefit from this study. For example, most responsible government agencies do not expect illegal dumping; thus, they have not secured a budget to conduct an appropriate cleanup of contaminated area. As a result, they might have to wait for the next fiscal year prior to obtaining a budget for the cleanup. Vetiver systems can be a very effective short-term solution to degrade contaminants while waiting for the money needed to implement more aggressive remediation techniques for source zone treatment.

Last but not least, vetiver systems allow the public to get involved in voluntary efforts to ease the local community's suffering from illegal dumping. Following the Nong-Nea model, tri-party collaborative actions (government agencies, the public, and the local community) to install vetiver systems might happen in every community affected by illegal dumping sites.

Figure 11. Selected photos illustrating more than 100 volunteers, including Nong-Nea villagers, undergraduate students from Naresuan University, primary school students from the Nong-Nea community, representatives of the Office of the Royal Development Projects Boards (RDPB) (including the RDPB director, Suwanna Pasiri), local government agencies, and news reporters and actors and actress from Channel 3 TV Thailand voluntarily cultivated 0.12 million vetiver grasses (bare roots) to create a 1.2-km vetiver fence along Tat-Noi Creek from August 28–29, 2014. The activities involved education on how to cultivate vetiver grass and field action. This voluntary activity was reported in 18 news clips on Channel 3 from August 27–29, 2014. Again, define all abbreviations.

(a)



(b)

Figure 12. (a) Field phenol concentration and POD from different sampling stations along the Tat-Noi creek after four months of vetiver hedgerow cultivation and (b) Our current laboratory studies to understand factors affecting root development and how to accelerate the growth of vetiver roots for deep PRVRB application.

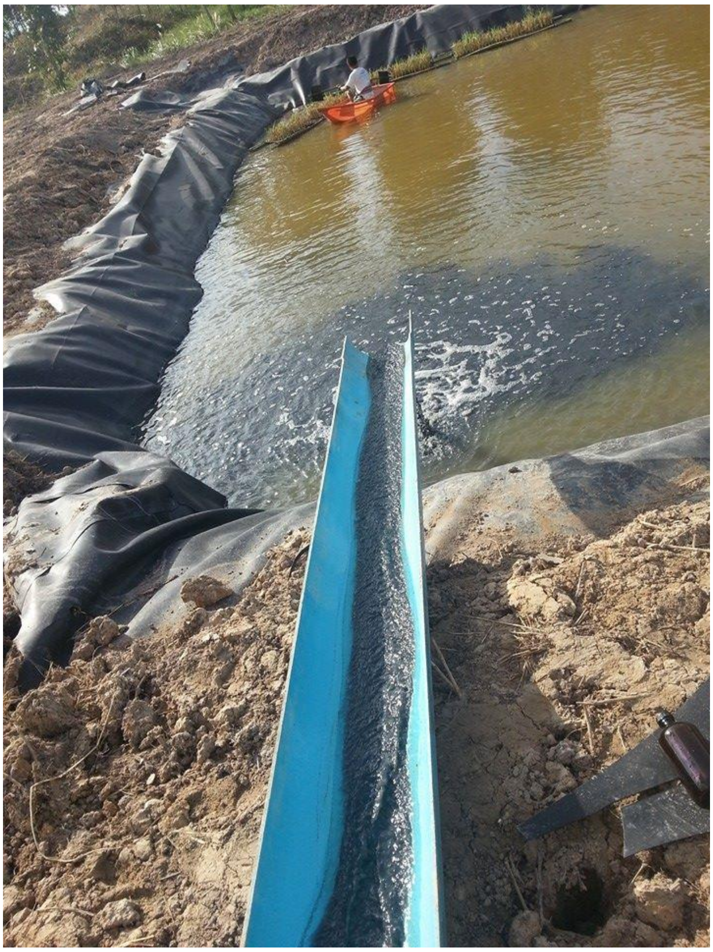


Figure 13. Selected photos illustrating our research team and 30 volunteers, including Nong-Nea villagers, primary school students from the Nong-Nea community, and news reporters from Channel 3 TV Thailand launched treatment of illegally dumped wastewater in Mr. Manus's pond using vetiver grass on floating platforms on December 5, 2014. This voluntary activity was reported in a news clip on Channel 3

Acknowledgements

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References

Brandt, R., Merkl, N., Schultze-Kraft, R., Infante, C. and Broll, G. 2006. Potential of vetiver (*Vetiveria zizanioides* (L.) Nash) for phytoremediation of petroleum hydrocarbon-contaminated soils in Venezuela. Int. J. Phytoremediation. Vol. 8 (4), pp. 273–284.

Bootkote, C. 2013. Root of hazardous industrial waste problems; PCD does not have authority while DIW cannot identify the culprit. TCIJ (http://tcijthai.com/tcijthainews/view.php?ids=2420)

Cook, R. L. and Hesterberg, D. 2013. Comparison of trees and grasses for rhizoremediation of petroleum hydrocarbons, Int. J. Phytoremediation. Vol.15 (9), pp. 844–860

Crime News Team. 2013. The Department of Industrial Works and the Department of Special Investigations opened the discussion to find a solution for hazardous industrial waste. In Manager Online (http://www.manager.co.th/Crime/ViewNews.aspx?NewsID=9560000048836)

Danh, L.T., Truong, P., Mammucari, R., Tran, T. and Foster, N. 2009.Vetiver grass, *Vetiveria zizanioides*: a choice plant for phytoremediation of heavy metals and organic wastes. Int. J. Phytoremediation. Vol. 11 (8), pp. 664–91.

Dudeja, S.S., Giri, R. Saini, R., Suneja-Madan, P. and Kothe, E. 2012. Interaction of endophytic microbes with legumes. J. Basic Microb. Vol. 52, pp. 248–260.

Greenfield, J.C. 1990. Vetiver Grass: The Hedge Against Erosion. World Bank; 3 Sub edition, pp. 88.

Esplugas, S., Gimenez, J., Contreras, S., Pascual, E., and Rodrı´guez, M. 2002. Comparison of different advanced oxidation processes for phenol degradation. Water Res., Vol. 36, pp.1034–1042

Garrity, G., Boone, D. R., Castenholz, R. W. (Eds.). 2001.Bergey's Manual of Systematic Bacteriology. 2nd ed., Springer, 722 p

Health Impact Assessment Coordinating Unit. 2013. Future of Nong-Nea Remediation. The National Health Commission Office (http://www.thia.in.th/uploads/file/Febuary%202013/Media%20Print/Nongnae_Feb%2013_1%20st%20print.pdf)

Hugo, W.B. 1978. Phenols: a review of their history and development as antimicrobial agents. Microbios. Vol. 23(92), pp. 83–5.

Krastanov, A., Alexieva, Z. and Yemendzhiev, H. 2013. Microbial degradation of phenol and phenolic derivatives. Eng. Life Sci. Issue Engineering in Life Sciences. Vol. 13, Issue 1, pp.76–87.

Lee, G. F. and Morris, J. C.1962. Kinetics of chlorination of phenol-chlorophenolic tastes and odors. Int. J. Air Wat. Poll. Pergamon Press 1962. Vol. 6, pp. 419–431.

Lei, W. and Xuanzhen, J. 2008. Unusual catalytic effects of iron salts on phenol degradation by glow discharge plasma in aqueous solution. Journal of Hazard. Mater. Vol. 161, pp. 926–932.

Negri, M. C., Gatliff, E. G., Quinn, J. J. and Hinchman, R. R. 2003. Root Development and Rooting at Depths, in Phytoremediation: Transformation and Control of Contaminants (eds. S. C. McCutcheon and J. L. Schnoor), John Wiley & Sons, Inc., Hoboken, NJ, USA. doi: 10.1002/047127304X.ch7

Nweke, C.O. and Okpokwasili, G. C. 2010. Influence of exposure time on phenol toxicity to refinery wastewater bacteria. J. Environ. Chem. Ecotoxicol. Vol. 2(2) pp. 20–27.

Phenrat, T. 2013. Restoration of Contamination at Nong-Nea Subdistrict, , Panomsarakarm District, Chachoengsao Province, Thailand. 2012 Thailand Research Expo (http://www.thia.in.th/uploads/file/August%202013/poster_research-expo/5.Board%20Research-NongNae-s.pdf)

Phenrat, T. and Teeratitayangkul, P. 2014. Vetiver on a floating platform degrades phenol in contaminated water. Vetiverim. No. 68. ISSN 0859–8878.

Roongtanakiat, N. 2009. Vetiver Phytoremediation for Heavy Metal Decontamination. Pacific Rim Vetiver Network Technical Bulletin No. 2009/1. Office of the Royal Development Projects Board, Bangkok, Thailand.

Singh, S., Melo, J.S., Eapen, S, and D'Souza, S. F.2006. Phenol removal using *Brassica juncea* hairy roots: role of inherent peroxidase and H_2O_2. J. Biotechnol. Vol. 3, 123(1), pp. 43–9.

Singh, S., Melo, J.S., Eapen, S., and D'Souza, S. F. 2008. Potential of vetiver (*Vetiveria zizanioides* L. Nash) for phytoremediation of phenol. Ecotoxicol. Environ. Saf. Vol. 71(3), pp. 671–6.

Truong, P. and Stone, R. 1996. Vetiver grass for landfill rehabilitation: Erosion and leachate control. Report to DNR and Redland Shire Council.

Truong, P.N. and Baker, D. 1998. Vetiver Grass System for Environmental Protection. Technical Bulletin N0. 1998/1. Pacific Rim Vetiver Network. Office of the Royal Development Projects Board, Bangkok, Thailand.

Truong, P. and Hart, B. 2001. Vetiver System for Wastewater Treatment. Pacific Rim Vetiver Network Technical Bulletin No. 2001/2.Office of the Royal Development Projects Board, Bangkok, Thailand.

Truong, P. 2014. Extreme Slope Stabilisation Using Vetiver System. Pacific Rim Vetiver Network Technical Bulletin No. 2014/1.Office of the Royal Development Projects Board, Bangkok, Thailand.

Supporting Materials

Figure S1 (a) Mr. Prajob Naowaopas, a village headman and leader of Nong-Nea environmentalists, was campaigning for environmental cleanup and legal punishment to the dumpers and (b) Ozonation units to degrade phenol in shallow groundwater used as drinking water were installed at the 40 most risky households and schools of the Nong-Nea community.

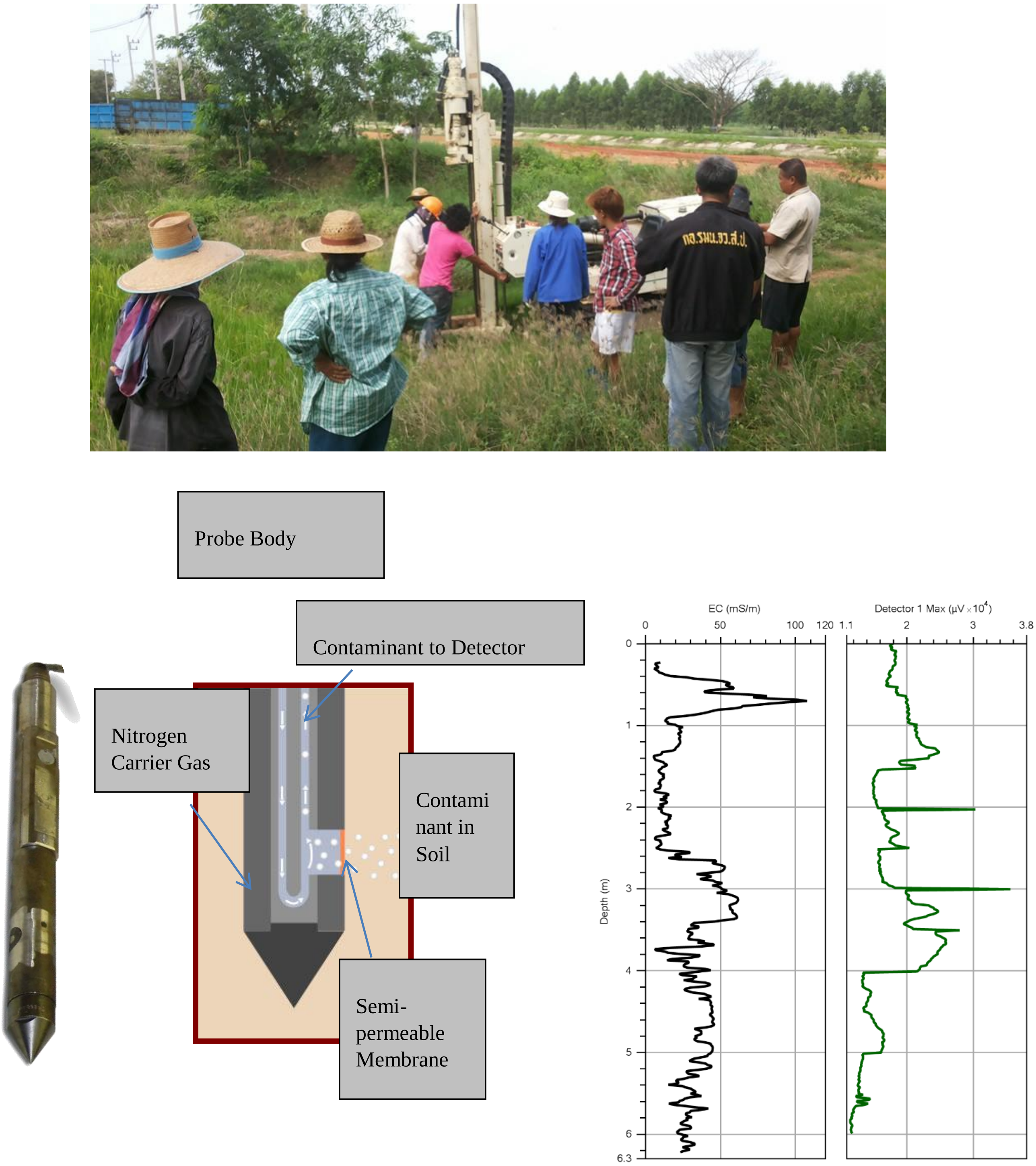

Figure S2 Membrane interface probe (MIP) for investigation of contaminant signals in shallow groundwater and soil from inaccessible contamination sources (two waste management facilities).

News Clip of Field-scale Vetiver Research and Implementation to Restore Nong-Nea Soil and Water and to Prevent Community from Exposure to Hazardous Substances

These are seven examples out of a total of 20 news clips on Channel 3.

1) http://www.krobkruakao.com/%E0%B8%A3%E0%B8%B2%E0%B8%A2%E0%B8%81%E0%B8%B2%E0%B8%A3%E0%B8%82%E0%B9%88%E0%B8%B2%E0%B8%A7%E0%B8%A2%E0%B9%89%E0%B8%AD%E0%B8%99%E0%B8%AB%E0%B8%A5%E0%B8%B1%E0%B8%87-%E0%B8%95%E0%B8%AD%E0%B8%99/104/35287/08/2014/Top-News-Variety--%E0%B8%AB%E0%B8%8D%E0%B9%89%E0%B8%B2%E0%B9%81%E0%B8%9D%E0%B8%81%E0%B8%9A%E0%B8%B3%E0%B8%9A%E0%B8%B1%E0%B8%94%E0%B8%99%E0%B9%89%E0%B8%B3%E0%B9%80%E0%B8%AA%E0%B8%B5%E0%B8%A2.html
2) https://www.youtube.com/watch?v=ZwlLbbKVTH8
3) https://www.youtube.com/watch?v=sHsuQDiWliU&list=LLCvC8LoA2S0geCOPF09EMVQ&index=5
4) https://www.youtube.com/watch?v=4tuPTz-QRi8&index=1&list=LLCvC8LoA2S0geCOPF09EMVQ
5) https://www.youtube.com/watch?v=s8dzjZeDniQ&list=LLCvC8LoA2S0geCOPF09EMVQ&index=2
6) https://www.youtube.com/watch?v=pl-71fFvNcE&index=3&list=LLCvC8LoA2S0geCOPF09EMVQ
7) https://www.youtube.com/watch?v=Un2RiVyT8fo&list=LLCvC8LoA2S0geCOPF09EMVQ&index=4