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DISPERSION OF CADMIUM-RESISTANT BACTERIA IN CADMIUM -CONTAMINATED SOILS AT MAE SOT DISTRICT, TAK PROVINCE

การกระจายตัวของแบคทีเรียต้านทานแคดเมียมในดินปนเปื้อนแคดเมียม บริเวณอำเภอแม่สอด จังหวัดตาก

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Abstract

Cadmium contamination in cultivated soils and rice grain grown in Mae Sot district, Tak Province, Thailand was brought to public attention in 2003. Cadmium is considered highly toxic to plants, animals and microbes. However, some bacteria have developed the ability to detoxify cadmium and other heavy metals there by conferring resistance on these microbes. The aims of this research were to study the quantities and dispersion of viable soil bacteria and cadmium-resistant found in cadmium contaminated soils at Mae Sot District. Cadmium-resistant bacteria were isolated and further investigated for their cadmium-resistance ability. Cadmium and zinc concentrations were found in 15 soil samples ranging from 0.20 to 1,021.75 mg/kg and 18.80 to 80,575.00 mg/kg, respectively. The maximum number of viable bacteria in soils collected in November 2007 and March 2008 were 4.3×10^8 CFU/g and 1.3×10^7CFU/g soil, respectively. The smallest number of viable soil bacteria was found in soils containing a high concentration of cadmium. The number of cadmium-resistant bacteria that grew in TSA medium amended with 10 mM $CdCl_2$ ranged from 3.2×10^4 to 1.3×10^5 CFU/g soil. 9 strains of cadmium resistant bacteria were selected for quantitative study of their level of resistance to cadmium toxicity. The results revealed that only 2 strains, TN6 and TM6, exhibited strong resistance to cadmium toxicity. 97.62% of TN6 and 84.62% of TM6 cells survived when placed in 3mM $CdCl_2$. These 2 strains of cadmium resistant bacteria, TN6 and TM6, were identified as Alcaligenes sp. and *Arthrobacter* sp.,

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respectively, through 16S rDNA sequencing.

Keywords: Cadmium-resistant bacteria, Cadmium-contaminated soil, *Alcaligenes*, *Arthrobacter*

บทคัดย่อ

การปนเปื้อนของแคดเมียมในดินบริเวณพื้นที่เพาะปลูกและเมล็ดข้าวที่เก็บเกี่ยวจากอำเภอแม่สอด จังหวัดตากของประเทศไทย ได้รับความสนใจจากประชาชนในปี พ.ศ. 2546 แคดเมียมเป็นโลหะหนักที่มีความเป็นพิษสูงต่อพืช สัตว์ รวมทั้งจุลินทรีย์ อย่างไรก็ตาม แบคทีเรียบางชนิดมีความสามารถในการต้านทานความเป็นพิษหรือกำจัดความเป็นพิษของโลหะหนักได้ ดังนั้น เป้าหมายของงานวิจัยนี้จึงทำการศึกษาจำนวนของแบคทีเรียที่มีชีวิตและแบคทีเรียที่ต้านทานแคดเมียมที่กระจายตัวอยู่ในดินที่ปนเปื้อนแคดเมียม บริเวณอำเภอแม่สอด จังหวัดตาก และนำแบคทีเรียต้านทานแคดเมียมที่คัดแยกได้มาทดสอบความสามารถในการต้านทานแคดเมียม ผลการทดลองพบว่า ตัวอย่างดินจำนวน 15 ตัวอย่าง มีปริมาณแคดเมียมและสังกะสีอยู่ในช่วง 0.20-1021.75 และ 18.80-80,575 มก./กก. ตามลำดับ ส่วนจำนวนแบคทีเรียที่มีชีวิตในดินที่เก็บในช่วงเดือนพฤศจิกายน 2550 และเดือนมีนาคม 2551 ที่พบมากที่สุดมีจำนวน 4.3 $\times 10^8$ และ 1.3 $\times 10^7$ CFU ต่อกรัมดิน ตามลำดับ และพบจำนวนแบคทีเรียในปริมาณต่ำในดินที่ปนเปื้อนแคดเมียมที่ความเข้มข้นสูง สำหรับจำนวนแบคทีเรียต้านทานแคดเมียมที่สามารถเจริญได้ในอาหารเลี้ยงเชื้อ TSA ที่เติมแคดเมียมเข้มข้น 10 มิลลิโมล่าร์ อยู่ในช่วง 3.2 $\times 10^4$ ถึง 1.3 $\times 10^5$ CFU ต่อกรัมดิน และสามารถคัดเลือกแบคทีเรียต้านทานแคดเมียมได้จำนวน 9 สายพันธุ์ เมื่อนำมาศึกษาระดับการต้านทานความเป็นพิษต่อแคดเมียม พบว่ามีแบคทีเรียเพียง 2 สายพันธุ์คือ TN6 และ TM6 ที่มีความสามารถในการต้านทานความเป็นพิษของแคดเมียมได้ในระดับสูง โดยร้อยละของการรอดชีวิตต่อความเป็นพิษของแคดเมียมที่ความเข้มข้น 3 มิลลิโมล่าร์ของแบคทีเรียสายพันธุ์ TN6 และ TM6 เท่ากับร้อยละ 97.62 และ 84.62 ตามลำดับ เมื่อทำการจำแนกจีนัสแบคทีเรียทั้งสองสายพันธุ์โดยใช้วิธีการวิเคราะห์ลำดับเบส 16S rDNA พบว่า แบคทีเรียสายพันธุ์ TN6 และ TM6 จัดอยู่ในจีนัส *Alcaligenes* sp. และ *Arthrobacter* sp. ตามลำดับ

คำสำคัญ: แบคทีเรียต้านทานแคดเมียม, ดินปนเปื้อนแคดเมียม, *Alcaligenes, Arthrobacter*

Introduction

Heavy metals are continuously released into the environment by natural means and as a result of human activity. Essential metals such as iron, copper, zinc, and manganese play important roles in biological systems. On the other hand, mercury, lead, and cadmium are non-essential elements. In contrast to these metals, which are toxic even in trace amounts, the essential metals tend to only produce toxic effects when the metal intake is in extreme excess[1]. High concentrations of heavy metals in the environment are known to be toxic to most organisms and these effects are increasingly being studied. In plants, such effects of heavy metal contamination may include growth inhibition, structural damage, and a decline of physiological and biochemical activities[2].

In Mae Sot District of northwestern Thailand, cadmium contamination of cultivated soil and rice grain was brought to public attention by a joint investigation of International Water Management Institute (IWMI) and the Department of Agriculture (DOA) in 2003[3]. Soils in this area contain high concentrations of cadmium and zinc due to deposit of zinc ore. Cadmium is one of the most toxic heavy metals to living organisms. In plants, generally speaking, and quite frequently in agriculturally important crops, Cadmium inhibits root and shoot growth, affects nutrient uptake and homeostasis, diminishes respiration activities through the inhibition of phosphatase and sulphatase, alters the microbial community structure, and reduces the level of dehydrogenase activity of and biomass produced by soil microbes [4, 5]. However, some bacteria have acquired resistance to the detrimental effects of Cadmium through heavy metal detoxification. Such microorganisms have evolved a number of heavy metal tolerance mechanisms including active efflux, extra- and intracellular complexation, extracellular precipitation, crystallization, and transformation[6].

This study focused on assessing the distribution of viable bacteria and cadmium-resistant bacteria in cadmium-contaminated soils in Mae Sot District of Tak Province. Additionally, the level of cadmium resistance in isolated cadmium-resistant bacteria was investigated. Cadmium-resistant bacteria were utilized for microbial remediation of cadmium contamination in water, sediment and soils.

Materials and Methods

Study Area and Soil Sampling

The study areas included the flood plains of Mae Tao and Mae Ku Creeks in Mae Sot District, Tak Province, and neighboring areas, covering an area of approximately 110 square kilometers that encompassed the 3 sub-districts of Mae Ku, Mae Tao and Pra Taad Padaeng. The 15 soil samples were collected from different sites (Figure 1) based on the concentration of cadmium contamination in the soil. Soil samples were collected from 8 sites at Mae Tao Creek and 7 sites at Mae Ku Creek during the early of November 2007 and the late of March 2008 as the representative periods of rainy and summer seasons, respectively. Each 10g soil sample was collected with a sterilized spatula from a depth of 10 cm below the ground surface and stored in a sterilized bag for microbiological analysis. An additional 20g of each soil sample was collected at the same depth for analysis of their chemical and physical properties.

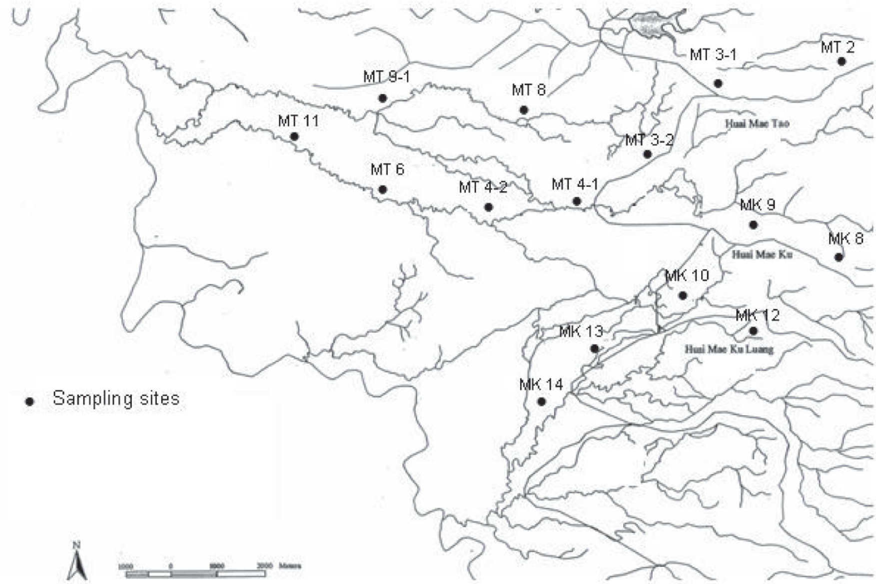

Figure 1 Map of soil sampling sites at Mae Sot District, Tak Province

Analysis of Soil Physical and Chemical Properties

Soil samples were dried in a hot air oven at 60°C for 12 hr. Subsequently, it was ground and filtered through a 2mm mesh sieve. Soil samples were tested for physical and chemical parameters, including soil texture and moisture by the hydrometer and saturation percentage methods, respectively[7, 8]. Soil pH was analyzed by glass electrode pH meter[7]. Soil organic matter was analyzed by the Walkley-Black method[9]. Soil Cation Exchange Capacity (CEC) was analyzed by the ammonium saturation method[10]. Soil samples were digested and analyzed for total concentrations of cadmium and zinc by using Flame Atomic Absorption Spectrometer (FAAS).

Enumeration of Total Viable Bacteria and Cadmium Resistant Bacteria in Soils

Soil samples were massed to 1g and suspended in 9.0mL of TSB medium. Each soil suspension was serially ten-fold diluted at 10^{-1} to 10^{-8}. Soil suspensions were collected at 10^{-4}, 10^{-6} and 10^{-8} and spread with a sterile glass rod onto TSA plate for enumeration of total viable bacteria. Soil suspensions were also collected at 10^{-3}, 10^{-5} and 10^{-7} and were spread onto TSA plate amended with 10mM $CdCl_2$ for enumeration of cadmium-resistant bacteria. All plates were incubated at

28 $^{\circ}$C in the dark for 24 hours. Colonies appearing on the agar plates were counted and calculated as CFU per gram of soil. This experiment was done in triplicates.

Isolation and Screening of Cadmium Resistant Bacteria from Soils

5g soil samples were suspended in 15mL of TSB medium amended with 3mM $CdCl_2$. Then, the soil suspension samples were shaken on incubator shaker (150rpm) at 28 $^{\circ}$C in the dark for 24 hr. Soil suspensions were transferred into 15mL of fresh TSB media amended with 5 and 10mM $CdCl_2$. Soil suspensions were spread with a sterile glass rod onto TSA plates amended with 10mM $CdCl_2$. Bacterial colonies appearing on the TSA plates amended with 10mM $CdCl_2$ were selected for further study based on the differences in colony morphology. Bacterial colonies were streaked on fresh TSA plates amended with 5mM $CdCl_2$ in order to maintain the cadmium resistant ability of isolated bacteria. A single colony of each isolate was re-streaked at least 2 times to ensure pure culture isolation. Pure culture of each isolate was sub-cultured on TSA slant and kept at 4 $^{\circ}$C.

Qualitative Analysis of Cadmium Resistance Levels in Isolated Bacteria from Soils

Cadmium resistance levels in selected bacteria from method No. 2.4 were determined by using growth inhibition zone and plate sensitivity assays with some procedural modification[11, 12]. For the inhibition zone assay, exponential phase cells (OD_{600} ~ 0.5 after 4 hours of growth) were mixed with 10mL of pre-warmed (5 $^{\circ}$C) top agar (TSA agar containing 0.7% agar) and overlaid on top of TSA agar plates (14 cm-diameter Petri dishes and poured with 50 ml of TSA agar). The agar plates were left at room temperature for 15 minutes to let the top agar solidify. A 5μL aliquot of each 0.5, 1.0, and 2.0M $CdCl_2$ was applied onto a 6-mm diameter of paper discs made from Whatman filter paper and subsequently placed on the lawn of bacterial cells. The diameters of growth inhibition zones in cadmium-resistant bacteria were measured after an overnight incubation at 28 $^{\circ}$C. Both complete (inhibition zone including the 6-mm diameter of paper disc) and partial (less dense zone) growth inhibition zones were recorded.

For plate sensitivity assay, exponential phase cells (OD_{600} ~ 0.5 after 4 hr of growth) were serially ten-fold diluted at 10^{-1} to 10^{-6}. A 10μL suspension sample at 10^{-1} to 10^{-6} was dropped onto a TSA plate (control) and TSA plates amended with 2 and 3mM $CdCl_2$. All plates were incubated at 28 $^{\circ}$C in the dark for 24 hr. Colonies appearing after incubation on the agar plates were counted and the number of surviving cells was calculated. The percentage of surviving cells is defined as the number of colony forming units (CFU) per mL recovered after treatment divided by the number of CFU prior to treatment, all multiplied by 100. The survival curves were determined by plotting the percentages of surviving cells versus the time of cultivation.

Morphological Study and Identification of Cadmium Resistant Bacteria

Each bacterial strain was streaked on a fresh TSA plate and colony characteristics were observed including distance from surface, margin and color after incubation at 28 °C for 24-48 hours. Each strain was determined to be Gram-positive or Gram-negative by Gram stain and observed cell morphology by light microscope at 1,000X. Genus of isolated bacteria was determined by 16S rDNA sequencing analysis([13]. Approximately 1500-bp of 16S rDNA was amplified in a thermocycler by using universal primers of 27f (5'-AGA GTT TGA TCC TGG CTC AG -3') and 1525r (5'-AAG GAG GTG ATC CAG CC-3'). PCR product was directly sequenced by a BigDye terminator cycle sequencing kit on an ABI 310 automated DNA sequencer. Homology of the 16s rDNA sequence of isolate was analyzed by using BLAST program from *Genbank database.*

Statistical Analysis

The physical and chemical characteristics of soil samples, growth curve of isolated bacteria, and cadmium resistance level in each bacterial strain were statistically analyzed by using mean ($\bar{X}$) and standard deviation (SD). T-test was used to compare the mean number of total viable bacteria in cadmium-contaminated soil in November 2007 versus March 2008. Statistical analysis of data was carried out with the SPSS statistical program.

Results and Discussion

Physical and Chemical properties of soils

Of the 15 soil samples collected and analyzed for their physical and chemical properties, most had the texture of clay and the moisture percentage ranged from 3.13% to19.42% (Table 1). The mean pH of the soil samples was neutral while the percentages of organic matter contained in the samples were slightly low, ranging from 1.25%-6.72%. Cation exchange capacity of soil samples was moderately low to high (6.36-27.5 cmol/kg). Confirming the report from Al-Khashman *et al.* [14], the upper part of the soil surface was highly enriched with heavy metals (iron, nickel, zinc, lead and copper). This might be attributed to the mobility of heavy metals, which is strongly influenced by many factors such as pH, Eh and the stability of minerals.

Table 1 Physical and chemical properties of soils

Sampling site	Soil Texture	CEC (cmol/kg)	OM (%)	Moisture (%)	pH	Cadmium (mg/kg)	Zinc (mg/kg)
MT2	SCL	7.4	2.43	3.13	6.90	1021.75	65,925.00
MT3-1	L	27.5	6.72	14.40	7.02	550.25	80,575.00
MT3-2	C	24.9	3.77	11.19	6.91	2.15	695.00
MT4-1	SCL	8.1	2.90	8.38	7.83	17.08	1,085.00
MT4-2	SCL	8.1	3.12	3.62	7.73	62.75	2,582.50
MT6	C	18.4	3.47	13.67	7.58	130.30	7,747.50
MT8	SCL	17.7	2.72	8.25	6.62	17.45	742.50
MT9-1	C	9.7	2.49	4.37	7.96	45.78	1762.50
MT11	CL	17.0	3.02	10.29	6.12	5.28	296.25
MK8	C	6.36	1.60	10.41	6.36	0.40	31.25
MK9	C	7.15	1.53	16.74	7.15	0.70	94.50
MK10	C	7.84	2.12	19.42	7.84	0.80	243.50
MK12	C	7.42	1.25	14.35	7.42	0.40	18.80
MK13	C	6.79	1.25	19.26	6.79	0.20	29.55
MK14	SiCL	7.78	2.49	17.79	7.78	1.00	300.50

Remark: C = Clay; L = Loam; CL = Clay Loam; SCL = Sandy Clay Loam; SiCL = Silty Clay Loam

Cadmium and zinc concentrations in the 15 soil samples ranged widely from 0.20 to 1,021.75 mg/kg and 18.80 to 80,575.00 mg/kg, respectively. Soil sample cadmium concentrations from some sampling sites were found to be above the permissible limits of soil quality standard for agricultural use in Thailand (37 mg/kg)[15]. The highest cadmium concentration was found at sampling site MT2 at Mae Tao Creek. In terms of zinc, the highest soil concentration was found at sampling site MT3-1 at Mae Tao Creek and also exceed than the standard of zinc concentration in soil (600 μg/kg)[16]. In contrast, cadmium concentrations in soil collected from Mae Ku Creek sites (MK8-MK14) ranged from 0.2 to 1.0 mg/kg, well-below the standard.

Quantity of Total Viable Bacteria and Cadmium Resistant Bacteria in Soils

The number of total viable bacteria in 15 soil samples collected during the early of November 2007 and late-March 2008 ranged from 5.6×10^4 to 4.3×10^8CFU/g soil and 3.5×10^5 to 1.3×10^7CFU/g soil, respectively (Figure 2). The number of total viable bacteria found in highly cadmium-contaminated soil (e.g., MT2) collected in the early of November 2007 and the late of March 2008 was 5.6×10^4CFU/g soil and 3.5×10^5CFU/g soil, respectively. In addition, there was no statistically significant difference between the number of heterotrophic culturable soil bacteria in November 2007 and March 2008 as assessed by T-test at $p<0.05$. This result indicated that the season was no affecting the number of heterotrophic soil bacteria in cadmium-contaminated soils. The number of heterotrophic soil bacteria depend on some soil chemical and physical properties[6]. According Malik *et al.*[17], the total aerobic heterotrophic count was found to be 5.39×10^7CFU/g of agricultural soil, whereas it was 3.14×10^7 CFU/g of industrial soil. The number of total viable bacteria in soil contaminated with a high concentration of cadmium was low. As reported by Perkiomaki and Fritze[18], the addition of cadmium to the soil can disturb the nutrient cycling of forest ecosystems because of its potential toxic effects on microbes. Cadmium had a greater inhibiting effect on microbes than did lead[19].

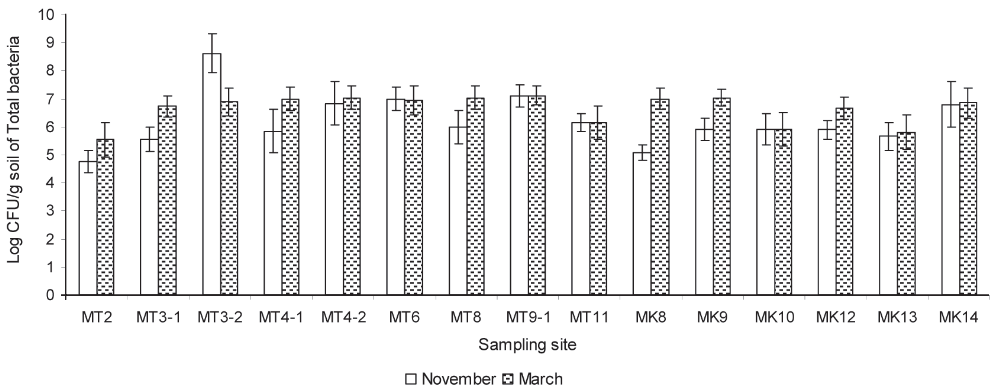

Figure 2 The number of total viable bacteria in soils collected in November 2007 and March 2008

However, a huge number of total viable soil bacteria could survive in cadmium contaminated soils even though cadmium concentration in soils was higher than standard of soil quality. Microbes apply various types of resistance mechanisms in response to heavy metals [(6)]. The number of cadmium-resistant bacteria from 15 soil samples collected during the early of November 2007 and the late of March 2008 and grown on TSA amended with 10mM $CdCl_2$ ranged from 3.2×10^4 to 7.0×10^4CFU/g soil and 3.4×10^4 to 1.3×10^5CFU/g soil, respectively (Figure 3). No cadmium-resistant bacteria were found in samples collected neither from all sampling sites in Mae Ku Creek (except sampling site MK8) nor some sampling sites in Mae Tao Creek (sampling site MT3-2, MT4-1, MT8 and MT11) due to the lower concentration of cadmium in soils. This finding reveals that soil bacteria in highly cadmium-contaminated soil had more tolerance to cadmium toxicity than soil bacteria found in soil with low cadmium-contamination. Moreover, the large number of soil bacteria found to be resistant to cadmium toxicity (10mM $CdCl_2$) was found in highly cadmium-contaminated soil (sampling site MT2) collected during both sampling periods. The study by Shuqing *et al.*[(19)] found that the number of soil microbes in cadmium concentrations of 0, 1, 5, 10, 50, 100 and 200mg/kg were 6.3×10^{10}, 8.9×10^{10}, 4.7×10^{10}, 3.4×10^{10}, 2.7×10^{10}, 2.4×10^{10}, 1.4×10^{10}CFU/g, respectively. As reported by Ryan *et al.*[(20)]), the percentage of the number of metal-tolerant strains was calculated in relation to the total viable count. In the case of each site, 79% of strains isolated from site A (metal-contaminated site) were resistant to either copper, zinc or arsenic. The level of metal resistance at site B (metal-uncontaminated site) ranged from 30%-35% in the cases of copper, zinc and arsenic. In addition, the number of heavy metal-resistant bacteria could use for an indicator of the levels of heavy metal contamination[(20)].

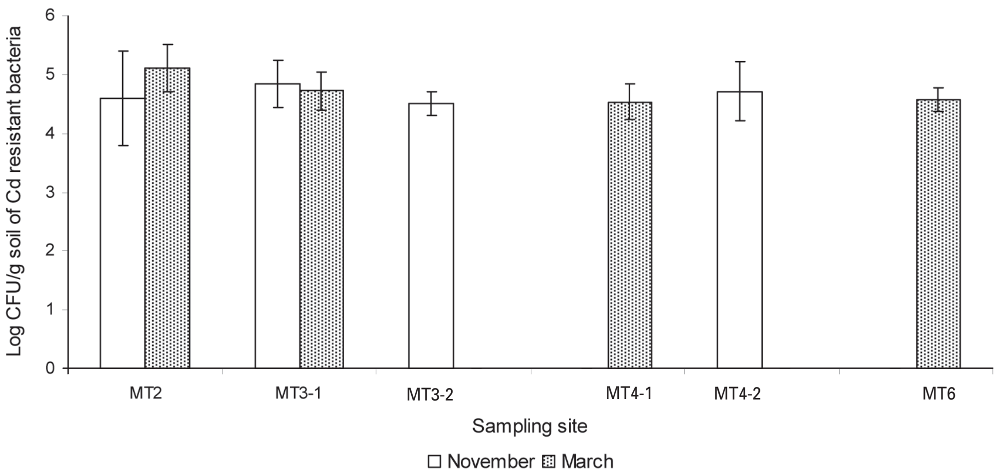

Figure 3 The number of cadmium resistant bacteria grown on TSA plate supplemented with 10 mM $CdCl_2$ in cadmium contaminated soils

Isolation of Cadmium Resistance Bacteria and their Resistance Levels against Cadmium Toxicity

The finding that cadmium resistant bacteria could survive in the soils that contaminated with high concentration of cadmium prompted us to isolate and screen the potent cadmium resistant bacteria from cadmium contaminated soils. A total of 9 bacterial strains, 3 of them isolated from soil samples collected during the early of November 2007 (sampling site MT4-2, MT6 and MT9-1) and 5 of them isolated from soils collected during the late of March 2008 (sampling site MT2, MT3-1, MT3-2 and MK8), were selected and assessed their resistance levels against cadmium toxicity by growth inhibition and plate sensitivity assays. Growth inhibition and plate sensitivity assays showed that most of the selected bacterial strains were isolated from highly cadmium-contaminated soil. Two bacterial strains, TN6 and TM6, exhibited higher resistance to $CdCl_2$ than other bacterial strains. Both TN6 and TM6 bacterial strains were isolated from cadmium contaminated soil (sampling site MT6) at Mae Tao Creek collected during the early of November 2007 and the late of March 2008. Soli sample in site MT6 contained high concentration of cadmium (130.30 mg/kg soil). For the same chemical, a bacterial strain with a larger clear zone correlated with higher sensitivity to the chemical as compared to strains with smaller clear zone diameter[21]. The inhibition zone diameters around each of the 6-mm discs containing 10 μL of 2M $CdCl_2$ of bacterial strains TN6 and TM6 were 13.5 mm and 11.5 mm, respectively (Figure 4). As reported by Chandy[22], the relative sensitivity of marine bacteria isolated from the Al-Jubail coast to different metals, in terms of the growth inhibition zone, was studied. At 10 μg cadmium, a diameter of growth inhibition zone was about 10 -15 mm. This finding indicates that some cadmium-resistant bacteria increase resistance under higher levels of cadmium toxicity because cadmium could induce the alteration of bacterial response or resistance mechanisms. However, cadmium-resistant bacteria could not alter or less ability to alter their resistant potential against very high concentration of cadmium (sampling site MT2 and MT3-1) due to its toxic effect of cadmium. Heavy metal-contaminated site was the preferable environment for gene transfer of multiple metal resistant determinants in metal-resistant bacteria isolated to other bacterial isolates[20].

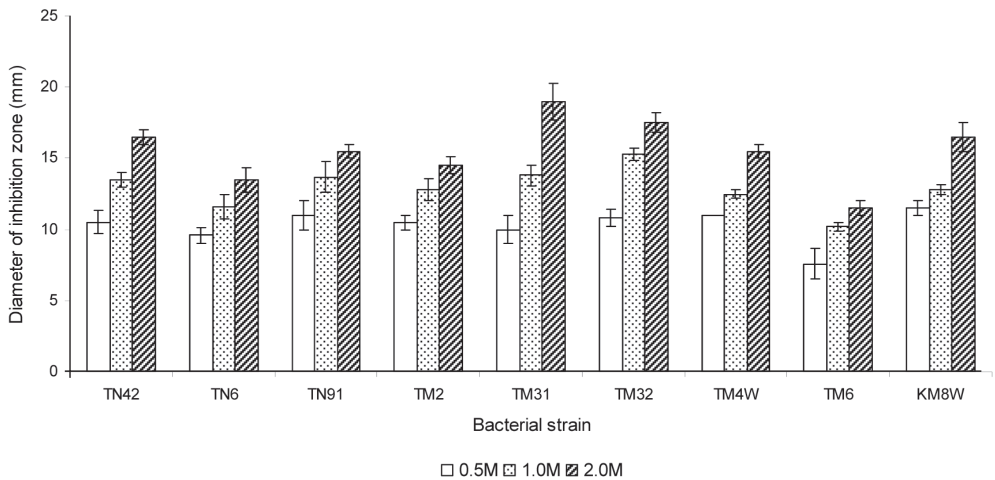

Figure 4 Diameter of growth inhibition zone against various concentrations of $CdCl_2$ in cadmium resistant bacteria

In addition, experiments were then performed to find out the percentages of survival cells of the potent cadmium resistant bacteria against cadmium toxicity. The percentage of TN6 and TM6 cells surviving after cultivation on TSA plate amended with 3mM $CdCl_2$ was 97.62% and 84.62%, respectively. This result indicated that bacterial strains TN6 and TM6 had the highest ability to resistant against cadmium toxicity. Both bacterial strains might use either specific or non-specific resistance mechanism against cadmium toxicity.

Genus Identification of the Potent Cadmium Resistant Bacteria

The genus of TN6 and TM6, two highly cadmium-resistant bacterial strains, were identified by 16S rDNA sequencing analysis as *Alcaligenes* sp. and *Arthrobacter* sp., respectively. The study by Trajanovska et al. reported that resistance to a range of heavy metal ions was determined for lead-resistant and other bacteria that had been isolated from a battery-manufacturing site contaminated with high concentrations of lead[23]. Several isolates of Gram-positive (belonging to the genera *Arthrobacter* and *Corynebacterium*) and Gram-negative (*Alcaligenes* spp.) bacteria were resistant to lead, mercury, cadmium, cobalt, zinc and copper, although the levels of resistance to the different metal ions were specific to each isolate.

Conclusion

This research focused on the quantity of viable soil bacteria and cadmium-resistant bacteria found in cadmium-contaminated soils at Mae Sot District, Tak Province. The resistance capabilities of selected soil bacteria against cadmium toxicity were also investigated. As compared to soil with a lower cadmium concentration a lower number of viable bacteria were found in soils containing high levels of cadmium (MT2) in the early of November 2007 (5.6×10^4 CFU/g soil) and the late of March 2008 (3.5×10^5 CFU/g soil) was found. The number of cadmium-resistant bacteria in high cadmium-contaminated soils (sampling site MT2) collected during November 2007 and March 2008 was 3.9×10^4 and 1.3×10^5 CFU/g soil, respectively. The results indicate that a high number of cadmium-resistant bacteria may be found in highly cadmium- contaminated soils. Most bacteria isolated from such soil had a greater ability to resist cadmium toxicity than soil bacteria isolated from soil with low cadmium contamination. The bacterial strain TN6 and TM6 showed the strongest resistance ability against cadmium toxicity and they were identified as *Alcaligenes* sp. and *Arthrobacter* sp., respectively. This finding suggests that selected cadmium-resistant bacteria might be useful for microbial remediation of cadmium in contaminated soils.

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WATER MANAGEMENT FOR INTEGRATED FARMING; REUSE OF WATER FROM *Macrobrachium rosenbergii* FARMING FOR OFF-SEASON RICE PRODUCTION

การจัดการน้ำเพื่อการเกษตรเชิงบูรณาการ; การใช้น้ำจากบ่อเลี้ยงกุ้งก้ามกรามเพื่อการทำนาปรัง

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Abstract

Water management for integrated farming, reusing of water from shrimp (Macrobrachium rosenbergii) farm for off-season rice production was studied at Ban Na Vee, Khoawong District, Kalasin Province. The experiment was conducted in 2008 during dry season, from February to June. The integrated rice cultivation was using left-over water from shrimp rearing pond compare with that using water directly from the reservoir - for field preparation and rice growing throughout 4 months period. Factors on quality of water and sediment in the rice fields were monitored monthly. Additionally, indexes on rice growth and production were also determined monthly. Later, the partial cost-benefit was used to interpret the benefit of each treatment.

The results indicated that water quality factors of the rice field using water from shrimp rearing pond, i.e. pH, alkalinity, COD, TKN and phosphate were significantly higher than those of the field using water directly from the reservoir. Soil quality factors of rice field using water from shrimp rearing pond, for example pH, ammonia-N, TKN and TP were higher than those of rice field using water directly from the reservoir. Indexes on rice growth - height and number of shoot of rice field using water from shrimp rearing pond were higher than those of the rice field using water directly from the reservoir. Furthermore, the yield from the rice field using water

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from shrimp rearing pond was about 5% higher than that of the field using water directly from the reservoir. These could be concluded that left-over water from the shrimp rearing pond can be reused for off-season rice growing. Consequently, the farmers get higher rice production and additional benefit from shrimp production from the integrated farming.

Keywords: off-season rice, Macrobrachium rosenbergii, water quality.

บทคัดย่อ

การจัดการน้ำเพื่อการเกษตรเชิงบูรณาการนี้เป็นการศึกษาการทำนาปรัง ณ พื้นที่บ้านนาวี ตำบลสงเปลือย อำเภอเขาวง จังหวัดกาฬสินธุ์ โดยเปรียบเทียบการทำนาโดยใช้น้ำจากบ่อเลี้ยงกุ้งก้ามกราม (NP1) กับการทำนาโดยใช้น้ำจากอ่างเก็บน้ำโดยตรง (NP2) โดยเริ่มศึกษาตั้งแต่เดือนกุมภาพันธ์ 2551 และสิ้นสุดในเดือนมิถุนายน 2551 ประกอบด้วยการเก็บข้อมูลคุณภาพน้ำ ตะกอนดิน ตรวจวัดการเจริญเติบโต วัดผลผลิตข้าว จากนั้นนำมาเปรียบเทียบการใช้น้ำ ต้นทุน และผลตอบแทนที่ได้ของทั้งสองระบบ

จากการศึกษาพบว่า นาข้าวที่ใช้น้ำจากบ่อเลี้ยงกุ้งมีค่าองค์ประกอบคุณภาพน้ำเป็นดังนี้ คือ อุณหภูมิ (NP1=NP2), pH (NP1>NP2), alkalinity (NP1>NP2), COD (NP1>NP2), BOD (NP1>NP2), TOC (NP1>NP2), TKN (NP1>NP2) และ total phosphorus (NP1>NP2) และมีองค์ประกอบส่วนตะกอนดิน ได้แก่ แอมโมเนีย ไนโตรเจนทั้งหมด และฟอสฟอรัส สูงกว่านาข้าวที่ใช้น้ำจากอ่างเก็บน้ำอย่างมีนัยสำคัญ ($P<0.05$) เนื่องจากน้ำจากบ่อเลี้ยงกุ้งก้ามกรามได้ผ่านการจัดการน้ำเพื่อการเลี้ยง การให้อาหาร การเติมปูนมาร์ล ซึ่งองค์ประกอบที่ต่างกันนี้ส่งผลให้การเจริญเติบโตของข้าวที่ได้รับน้ำจากบ่อเลี้ยงกุ้ง เช่น ความสูง และการแตกกอในแต่ละช่วงอายุข้าวสูงกว่าข้าวที่ใช้น้ำจากอ่างเก็บน้ำอย่างมีนัยสำคัญ ($P<0.05$) และให้ผลผลิตข้าวในปริมาณน้ำที่ใช้เท่ากันสูงกว่าประมาณ 5 เปอร์เซ็นต์ เมื่อเปรียบเทียบต้นทุนการผลิตบางส่วนต่อผลตอบแทน พบว่าการปลูกข้าวมีต้นทุนการผลิตในแปลงนาเท่ากันคือ 925 บาท ผลตอบแทนของนาข้าวที่ใช้น้ำจากอ่างเก็บน้ำคิดเป็นเงินสุทธิ 4,582 บาทต่อไร่ ส่วนนาข้าวที่ใช้น้ำจากบ่อเลี้ยงกุ้งคิดเป็นเงินสุทธิ 4,863 บาทต่อไร่ สรุปได้ว่าจากการใช้น้ำมวลเดียวกันนาข้าวที่ใช้น้ำจากบ่อเลี้ยงกุ้งก้ามกรามให้ผลผลิต และผลตอบแทนสูงกว่าแปลงข้าวนาปรังที่ใช้น้ำจากอ่างเก็บน้ำ

คำสำคัญ: นาปรัง, กุ้งก้ามกราม, คุณภาพน้ำ

Introduction

Water resource management plays a key role in success of farming. Currently, majority of the agricultural practices in Thailand rely on rainfall or natural stream as the main source of water. Due to lacking of the water resource management, yields of cultivation closely depend on the quantity of water. In abundant year, the farm could obtain good yield whereas in the drought year, the farmer would face the loss[1].

Based on the problem, an integrated farming combining with water resource management practice is proposed to make the most out of available water resource. The study was conducted at the upstream area of Phaya Young watershed in Khoawong District, Kalasin Province. In the area, there was a small reservoir able to supply limited amount of water during dry season which was not sufficient for the off-season rice cultivation in large scale. An integrated farming, combining shrimp farming and rice cultivation, was one of the schemes that give promising perspective. Shrimp farming takes 8 months per round of cultivation. If the farmer started the cultivation during rainy season, the time frame will overlap with the dry season. At that time, the water from the shrimp pond will be valuable. The water together with the nutrients from shrimp wastes or from left-over feed could provide sensible off-season rice cultivation. This scheme makes a good use of water that might be left as waste and also provides excellent water treatment, thus, helps improve the surrounding environment, collaterally. This efficient uses of water resource could also give a ground for sustainable agricultural practice which is highly advantageous[2].

Research Methods

Site Selection

The study was conducted during February -June 2008, in an area within the Nam Young watershed, a small 3 rais farm at Ban Navee of Khaowong District, Kalasin Province. The shrimp rearing pond (PR) was 40 m. width by 60 m. long, made up of 2,400 m^2 meters space. The pond contained 1.2 m. depth of water, made up of 2,880 m^2. At the time of need, the water from the shrimp pond at the 20 cm. height from the floor will be transferred via a pipe to the designated rice field in adjacent area. The 30 m. by 40 m. or 1,200 m^2 rice field (NP1) was designed to rely on the water from the shrimp pond (Figure 1). As a comparable study, another rice field (NP2) with the same size was assigned to rely solely on the water from the reservoir, the Phaya Young (Figure 2). Both rice fields were planted with Chai Nath 1 rice variety. Sampling points for water and sediments were assigned as the plus sign in a circle symbols as in Figure 3, four spots for each pond. During the study, the samples were collected four times - on day 0, 30, 70 and 100, taking from the planting date.

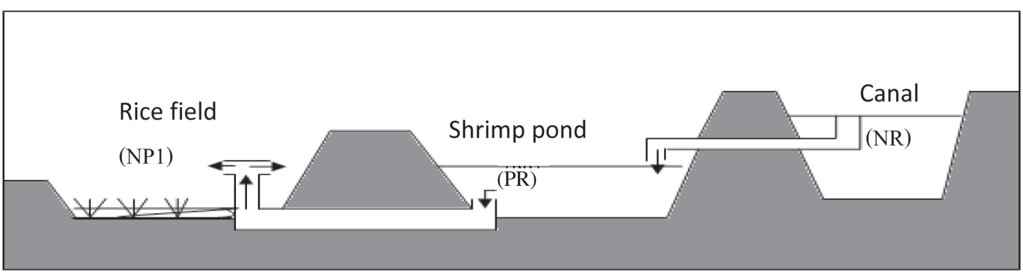


Figure 1 Diagram presents the water allocation design of the integrated use; the water was transferred from the Canal (NR) to the shrimp pond (PR). Then, when need, the water from the shrimp pond was piped in to the designated rice field (NP1).

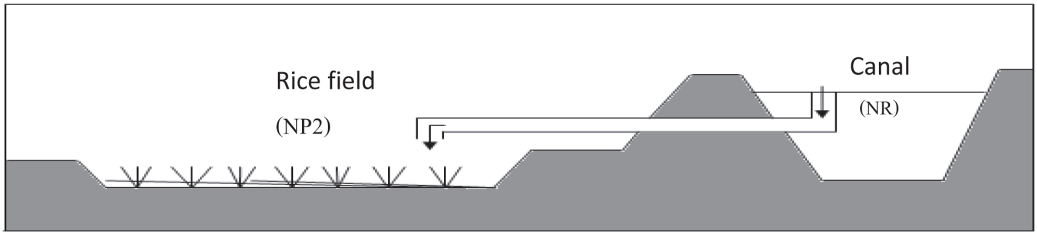


Figure 2 Diagram represents the water allocation design of rice field (NP2) to rely on water from the reservoir via a canal (NR).

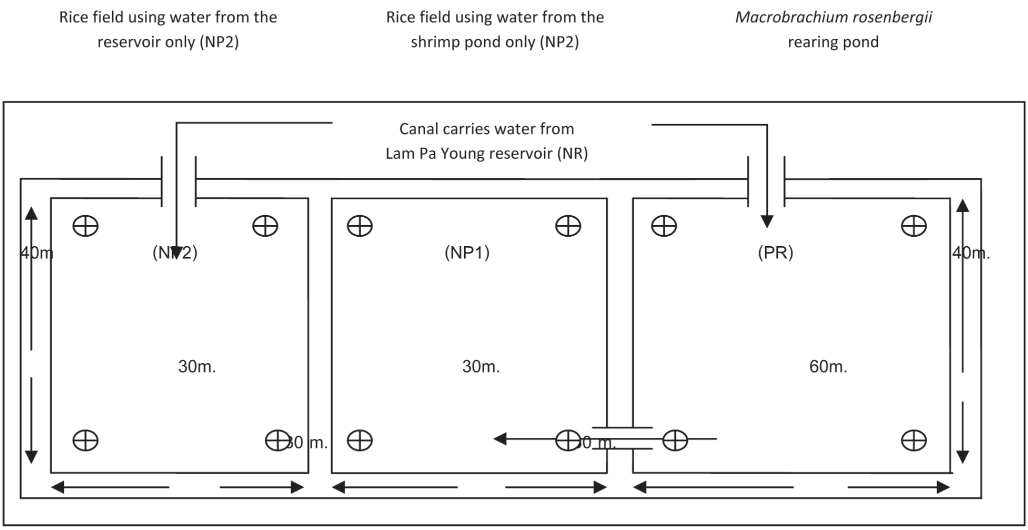


Figure 3 Diagram representing the scheme of the study.

Field Preparation and Treatment

Both rice fields were prepared by releasing the water from each source; water from reservoir to the NP2 and water from the shrimp pond to the NP1, by keeping equal height. Then, they were plowed and left standing for a week, later, furrowed into level. The seeds were soaked for 24 hr and covered for 48 hr before planting, at 20 kg/rai. After day 15-20, the water was released and controlled to be at about 5-10 cm. in both fields. The water level was maintained throughout the period until day 105 which the water was discharged out of both fields. At that stage, the rice was ready to be harvested.

Water, Sediment Sampling and Rice Growth Index

Water samples were collected at each point indicated in the diagram (Figure 1). Some factors i.e. temperature, pH were measured on the spot. On the other hand, the other factors were analyzed at the specific laboratory of the Environmental Research Institute of Chulalongkorn University.

The sediments were also collected in the same manner.

The growth indexes of the rice were height and number of stems. Those were recorded on day 0, 30, 70 and 100, respectively. The rice was harvested at day 120. The harvest was by sampling from 1 m^2 area, four samplings from each rice field.

Laboratory Test Method

Factors of water quality; temperature, pH, Alkalinity, COD, DO, TOC, TKN, total phosphorus were determined according to Strickland & Parson,[(4)]. Also, factors of the sediments: pH and nutrients (nitrogen-ammonia and nitrate; and, available phosphorus) were verified according to Strickland & Parson,[(4)].

Data Analysis

Analysis of variance; ANOVA method was used to compare factors of water quality, sediments from each rice field and factors on growth of rice; height, number of stems and rice yield.

Results

Water Usage

The profile of water usage for the 2,400 m^2 shrimp-pond is as following: at initial stage, 1,920 m^3 of water was filled at 80 centimeters deep; then, at the initial of 1^{st} month, 960 m^3 of water was added to the 120 centimeters deep; during the 2^{nd} and 3^{rd} month, the water was re-leased out depends on the environment; in the 4^{th} month, the water was being drained to be used in the rice field (NP1).

In both rice fields, in each 1,200 m^2, initially, 240 m^3 of water was released into the field. During, days 15-20, 120 m^3 of water was added and kept at the level of 10 centimeters from the ground. During day 45-50 and day 80-85, twice, the water was added to the rice fields to keep the level of water at 20 cm. from the ground. The water usage was 180 m^3 each. In total, the water for rice cultivation in each field was 720 m^3. While, 5,040 m^3 of water was used for shrimp rearing. Among those number, 2,160 m^3 was taken out of the pond.

Table 1 Water Use Profile of Shrimp rearing and off season rice cultivation

Water uses	Type of water uses (m^3)		
	PR	NP1	NP2
1 Shrimp pond preparation; start shrimp rearing	1,920	-	-
2 at initial of the 1st month	960	-	-
3 in the 2nd month	720	-	-
4 in the 3rd month	720	-	-
5 Preparation of the rice fields; start rice cultivation	240	240	240
6 during day 15-20	120	120	120
7 during day 45-50	180	180	180
8 during day 80-85	180	180	180
Total water uses	5,040	720	720

Note: PR = Shrimp rearing pond

NP1 = Rice field using water from the shrimp rearing pond

NP2 = Rice field using water from the reservior

Water Quality

Factors on water quality - pH, alkalinity, COD, TOC, total nitrogen or total phosphorus of each location were displayed in Figure 4.

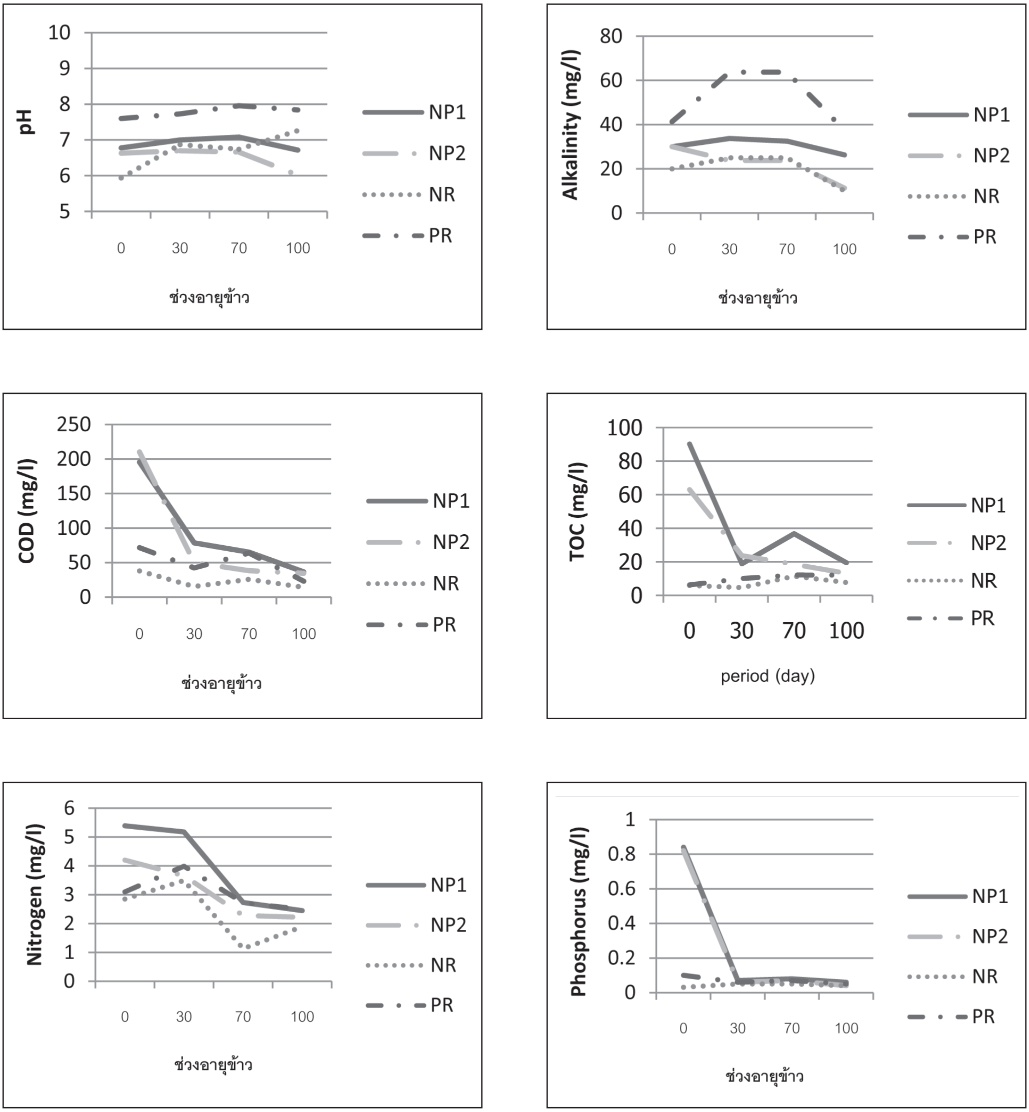


Figure 4 Factors on water quality - pH, Alkalinity, COD, TOC, Total notrogen and Total phosphorus of each source: canal (NR), shrimp rearing pond (PR), rice field which rely on water from the shrimp rearing pond (NP1) and rice field which rely on water from the canal (NP2)

Temperature

There was no significant difference in temperature of the rice field which rely on water from the shrimp rearing pond (NP1) and rice field which rely on water from the canal (NP2). Moreover, those were within the threshold of the safety standard for aquatic creatures[5].

Salinity

The salinities of the water in both rice fields were quite low, at 0-0.1 ppt. Those could be an effect from dissolving of minerals from the soil which considered benefit for rice cultivation.

Dissolved Oxygen (DO)

DO of those 4 sources was not significantly difference, and was within the standard for agricultural uses.

pH

pH of the water samples from the rice field using water from the shrimp rearing pond was higher than the others, resulting from the water treatment by adding lime during shrimp rearing.

Alkalinity

Alkalinity of the water samples from the rice field which was using water from the shrimp rearing pond was higher than the others, resulting from the water treatment by adding lime during shrimp rearing.

Chemically Oxygen Demand (COD)

On day 0, the COD of both tested fields was rather high and about equal. However, on day 30, 70 and 100, COD of the rice field using water from the shrimp rearing pond was slightly higher. On overall the COD were decreasing overtime.

Total Organic Carbon (TOC)

The TOC of each rice field gave no significantly difference. The number of each was decreasing over time, taken out via rice growing process.

Total Kjeldahl Nitrogen (TKN)

The numbers of TKN of the rice field using water from the shrimp rearing pond were higher than that using the water from the reservoir. These should be the results of the left over shrimp feed and the organic waste from the shrimps. Overtime, those numbers were lower, most likely; the nitrogen was taken by the rice growing process.

Total Phosphorus

The total phosphorus in the rice field using water from the shrimp rearing pond were slightly higher than that using the water from the reservoir. Overtime, those numbers were lower, most likely; the phosphorus was taken by the rice growing process.

Soil Quality

Factors on soil quality - pH, ammonia, nitrate or total phosphorus of each location were displayed in Figure 5

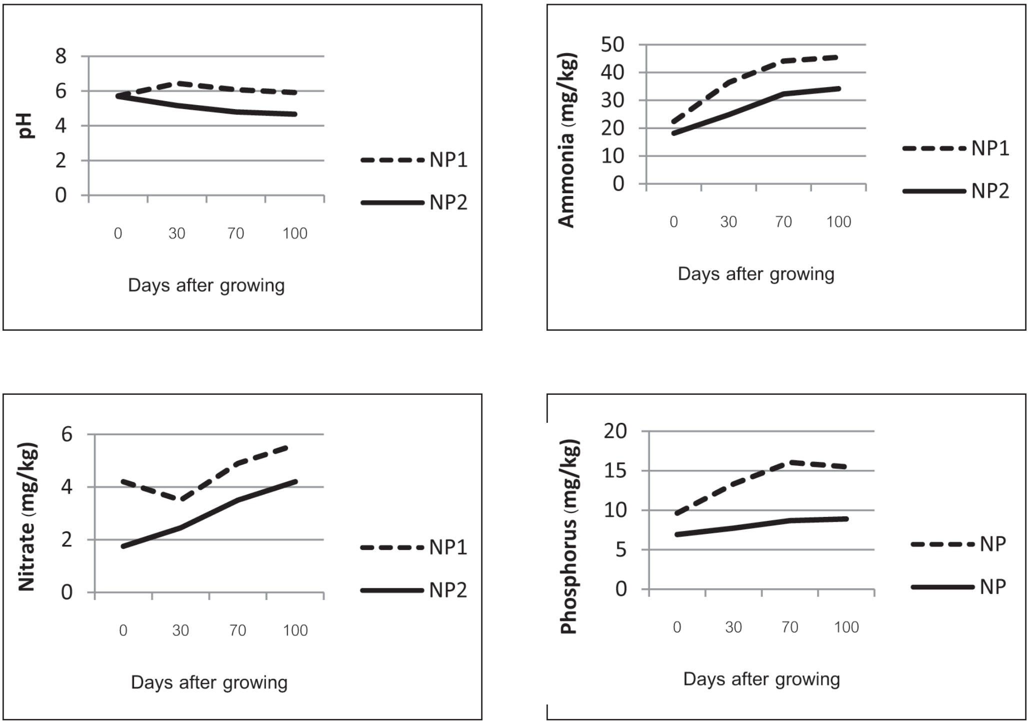


Figure 5 Factors of soil quality; pH, ammonia, nitrate and phosphorus of the rice field using water from shrimp rearing pond (NP1) compare with the one from the rice field using water from Phaya Young reservoir (NP2)

Soil pH

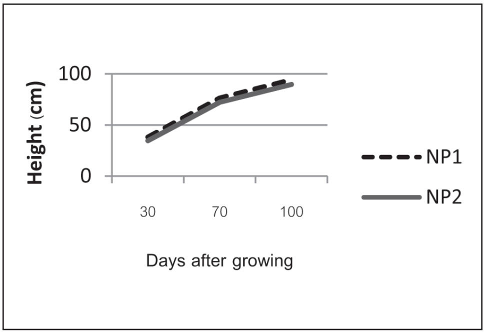
The pH values of the soil in the rice field using water from the reservoir were significantly lower than the one using water from the shrimp rearing pond (Figure 5). Samples of day 70 and day 100, gave the pH of 4.80 and 4.67, respectively. Those were considered rather acidic[9]. However, those of the samples from the rice field using water from the shrimp rearing pond were average 5.72-6.45, which are suitable for rice farming given those were around 5.0-6.5 [3].

Nutrients

The laboratory test on soil samples shows that soil from the rice field using water from the shrimp rearing pond at every sampling periods contained more ammonia, nitrate and phosphorus than the one from the rice field using water directly from the reservoir. This indicated that the left over feed and shrimp waste had provided nutrients to the field.

Rice Growth Monitoring

Rice growth was monitored based on its height and number of shoots. Those indexes were recorded on day 30, 70 and 100. Average height and average number of shoots of each case were presented as Figure 6.



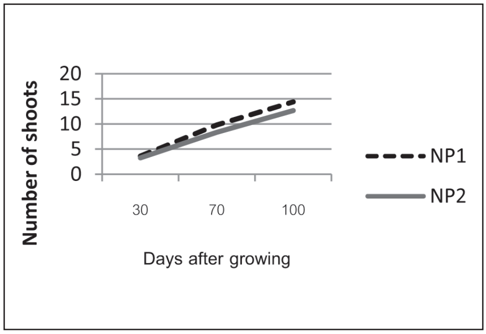


Figure 6 Rice growth pattern indicated by its height and number of shoots at each stage of the rice; those of rice field relying on water from shrimp rearing pond (NP1) compared with those of rice field relying on water directly from the reservoir (NP2)

From the study, at each stage of the growth, the average height and average number of shoots were higher in rice using water from the shrimp rearing pond than the ones using water directly from the reservoir. These together with the data on water quality and soil quality indicated that the water from the shrimp rearing pond could provide suitable environment and nutrients for rice farming.

Rice Yield

Data in table 2 points out that the rice cultivation by using water from the shrimp rearing pond showed superior yield, either considering the number of rice grain, average rice weight, number of spike or average rice weight. The equivalent yield was 423 g/m^2 or 677 kg/rai, whereas the other was at 404 g/m^2 or 647 kg/rai. From the table, it is clearly shown that yield from the rice cultivation using water from shrimp rearing pond (NP1) is higher by 5% compared with the one using water directly from the reservoir (NP2).

Table 2 Rice yield of rice cultivation using water from shrimp rearing pond (NP1) compared with the one using water directly from the reservoir (NP2).

Rice yield indicators	Yield	
	NP1	NP2
1. Number of rice grain/spike	121.30±10.14	118.45±8.54
2. Average rice weight (gram/spike)	3.34±0.09	3.29±0.07
3. Number of spike/ square meter	126.75±3.77	122.75±2.75
4. Average rice weight (gram/square meter)	423.35	403.85

Cost and Benefit

The cost of rice cultivation using water from the shrimp rearing pond, considering the whole components, was as following: construction of the shrimp rearing pond 19,000 baht, young shrimp 4,500 baht, materials for shrimp rearing pond 3,600 baht, shrimp feed 6,000 baht, rice seeds 255 baht, fuel cost 300 baht, and rice harvesting cost 400 baht-or, 34,025 baht in total. On the other hand, the cost of rice cultivation using water directly from the Phaya Young reservoir was as following: rice seeds 225 baht, fuel cost 300 baht, and harvesting cost 400 baht-or 925 baht in total. From the data, it could be seen that the investment cost for the total components of rice cultivation using water from shrimp rearing pond was much higher than that of the one using water directly from the canal of the reservoir. However, in the next round of cultivation, there will be no cost from the construction of the pond. Thus, the investment cost of the integrated system should be lower, whereas, the yield and income still the same-more profit received.

Table 3 Cost comparison of rice cultivation: A-an integrated farming using water from shrimp rearing pond, and B-using water directly from the reservoir

Item	Cost (baht)	
	A	B
1 construction of the shrimp rearing pond	19,000	-
2 fuel	300	300
3 rice seeds	225	225
4 young shrimps	4,500	-
5 rice harvesting	400	400
6 materials for shrimp rearing pond	3,600	-
7 materials for rice field	-	-
8 shrimp feed	6,000	-
9 labor cost	-	-
10 other cost	-	-
Total	34,025	925

The benefits per rai from each model were as following. Rice cultivation as an integrated farming using water from shrimp rearing pond had 6,096 baht of income and the net benefit was 4,863 baht, after adjusted the cost. Additionally, the giant freshwater shrimp gave the income of 26,527 baht or 4,460 bath of net profit. On the other hand, rice cultivation using water directly from the canal of the reservoir gave an income of 5,815 baht or net profit of 4,582 baht. However, in the integrated farming case, if the cost on construction of the pond was not considered i.e. during next round of cultivation, the net benefit per rai would be 17,127 baht rather than 4,460 baht.

Table 4 Benefit and net profit comparison of rice cultivation, the cost of construction of the shrimp rearing pond was considered: A-an integrated farming using water from shrimp rearing pond, and B-using water directly from the reservoir

Item	Benefit (baht/rai)		Net profit (baht/rai)	
	A	B	A	B
rice	6,096	5,815	4,863	4,582
** giant freshwater shrimp	26,527	-	4,460	-
total	32,623	5,815	9,323	4,582

Table 5 Benefit and net profit comparison of rice cultivation, the cost of pond construction was not considered: A-an integrated farming using water from shrimp rearing pond, and B-using water directly from the reservoir

Item	Benefit (baht/rai)		Net profit (baht/rai)	
	A	B	A	B
rice	6,096	5,815	4,863	4,582
** giant freshwater shrimp	26,527	-	17,127	-
total	32,623	5,815	21,990	4,582

Note to Tables 4-5 As of October, 2008, price of the rice was at 9,000 baht/ton and the giant freshwater prawn was at 150 baht/kg. After June, 2008, there leftover shrimp in the pond could also give some yield.

Conclusion and Discussion

The study clearly indicates that integrated farming by using leftover water from the shrimp rearing pond for rice farming gave benefit from better rice growth and better yield. Rice cultivation could benefit to the farmer 4,582 baht/rai. Typically, farmers with 1-5 rais could make use of integrated farming; fish or shrimp rearing together with rice cultivation or with other crops. The scheme supports the idea of sustainable agriculture which desirable among the farmers by enlarge.

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