

BIOCHEMICAL CHARACTERISTICS OF *THIONIC* FLUVISOL LINKED TO LAND USE TYPES IN SOUTHERN VIETNAM

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ABSTRACT

Thionic Fluvisols soil in Southern Vietnam is like typical acid sulfate soil in the tropics and is severely polluted due to human activities. Salinity intrusion and industrial wastewater contamination are the main cause of environmental degradation in soil ecosystem. This research was aimed to determine a link between biochemical soil properties and land use types to provide suitable solutions for afforestation and soil restoration. Soil sampling was conducted in five different land use types at four soil layers (O, AB, B_j and C_p). The five land use types were sugarcane crop; *Melaleuca* plantation; 2-year *Acacia* plantation; 5-year *Acacia* plantation; and control (grass-covered land). The results showed that soil in those five land use types were very acidic (pH ≤ 4) having poor-nutrient condition with range of orthophosphate content of 378 - 640 mg/kg, N-NH₄ of 586 - 999 mg/kg and N-NO₃ of 830 - 1,112 mg/kg. Concentration of toxic ions was very high with large variation among land use types and soil depths i.e. 1,799 - 12,403 mg SO₄²⁻/kg; 22 - 1,645 mg exchangeable Fe/kg and 34 - 88 mg Al³⁺/kg soil. The lowest concentration of exchangeable Fe³⁺ and SO₄²⁻ ions were found in sugarcane and *Melaleuca* plantations, respectively. Twenty-three sulfur-oxidizing bacteria and two iron-oxidizing bacteria were identified. All these bacteria were initially identified as *Thiobacillus* sp. Sugarcane and *Melaleuca* plantations exhibited the most diverse *Thiobacillus* species which linked to reduction of exchangeable Fe and SO₄²⁻ concentrations in these two land use types. This study indicated that *Thiobacillus* sp. could grow well in the *Thionic* Fluvisols. It is proposed that *Melaleuca* and sugarcane species could reduce iron and sulfur contents in *Thionic* Fluvisols in the tropics.

Keywords: *Acacia*, acid sulfate soil, *Melaleuca*, sugarcane, *Thionic* Fluvisols, *Thiobacillus* sp.

INTRODUCTION

Thionic Fluvisols are acid sulfate soils containing high SO₄²⁻ content, having very low pH (3 - 4) and containing high concentration of exchangeable Al³⁺ and Fe³⁺. High content of SO₄²⁻ accumulating in the soil mainly originates from an oxidation of potential minerals containing sulfur to form sulfuric acid (H₂SO₄) (Sokolova & Alekseeva 2008). While acid sulfate soil derives from sulfides oxidation containing minerals such as organic matter, sulfate, iron and aluminum, it could be formed in brackish water soil or other highly potential sulfate soil areas with a participation of microorganisms (Dao & Hoang 2005). Potential acid sulfate soil is a large source

of heavy metal which causes pollution when being exposed to oxygen due to natural phenomena or artificial drainage, oxidizing pyrite to create H₂SO₄ and thus, decrease soil pH (Breemen 1993; Nguyen *et al.* 2004). Generally, iron and aluminum are often considered as toxic elements in the soils and greatly affect plant growth and development because excess of these elements can be toxic to plants (Nguyen *et al.* 2004; Dao & Hoang 2005). Concentrations of exchangeable Fe (Fe²⁺ and Fe³⁺) and Al³⁺ exceeding 500 and 135 mg/kg, respectively, are toxic to rice (Le *et al.* 2000). When soil pH is less than 4.5, macro-nutrient ions (N, K, Ca, Mg, P) have relatively low concentration in acid soils. In contrast, Al and Fe are normally abundant in the soil with low pH (Brady & Well 2002). The more

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acidic the soil is, the higher concentrations of these two ions are.

According to Dao and Hoang (2005), concentrations of Fe^{2+} , Al^{3+} and SO_4^{2-} ions in acid sulfate soil are much higher than concentrations that can be tolerated by plant. Sulfur and sulfate are two main compounds involved in the formation process of acidic soil. Total sulfur in two layers of Jarosite (Bj) and Pyrite (Cp) is an index to distinguish between an acid sulfate soil and other soil types. Major forms of sulfur in the acid soil are FeS , FeS_2 , H_2S , SO_3^{2-} , and SO_4^{2-} . Concentration of SO_4^{2-} ions greatly varies among different types of acidic soils. SO_4^{2-} ions are toxic to plants and are obstacles for land reclamation. In Southern Vietnam, total sulfur in the acidic soil ranges from 1 - 4% and can reach 5 - 6% when the acidic soil experiences a long-term flood (Dao & Hoang 2005). Metabolism and transformation of sulfur forms are complex with participation of sulfur-reducing bacteria in anaerobic conditions (Lamers *et al.* 2012; Pester *et al.* 2012).

A total area of potential acid sulfate soil in Southern Vietnam was 2,415,727 ha (in 2005) (Ho *et al.* 2010). This area had active acidic soil area of 279,946 ha in 2005, which increased to 1,078,169 ha in 2011 (Pham *et al.* 2011). Concentrations of Fe and S ions increased while concentrations of K^+ , Al^{3+} , Total Organic Carbon (TOC), N and P tended to decrease between year of 1975 and 2005 (Ho *et al.* 2010). Kyuma (1976) and Le (2003) showed that TOC content in the acidic soil located in the Mekong Delta was pretty high, ranging from 1.0 to 4.8% for soil having high concentration of sulfur ions and ranging from 3.2 to 5.2% for soil having low concentration of sulfur ions. Due to high content of TOC, the acidic soil also contains high concentration of Total Nitrogen (TN) (0.15 - 0.25%). However, concentration of Total Phosphorus (TP) (0.01 - 0.05%) and Total Potassium (TK) (0.03 - 0.09%) were very low in the acidic soil.

Contents of Al^{3+} , Fe^{3+} , SO_4^{2-} and other nutrients in acid sulfate soil greatly vary among soil layers and seasons (Le *et al.* 2000; Le 2003). Soil in the Mekong Delta had average organic layer depths (O) of 20 - 23 cm, transitional layer (AB) of 23 - 46 cm, Jarosite layer (Bj) or sulfur forms producing layer of 47 - 88 cm and Pyrite layer (Bp) greater than 89 cm (Duong *et al.* 2010). Total Organic Carbon (TOC) and Total Nitrogen (TN) in layer O of acidic soil are 5.47% and 0.26%,

respectively (Ngo 2010). Other studies in Southern Vietnam conducted by Nguyen *et al.* (2011) showed that soil pH, TOC and TN in topsoil layer (O) were respectively 3.38 - 3.75; 2.24 - 4.04%; and 0.15 - 0.27%. Concentrations of Al^{3+} , Fe^{3+} and SO_4^{2-} ions not only varied due to seasonal changes, but also depended on depths of soil layers (Do & Nguyen 1999; Tran *et al.* 2011). Tran *et al.* (2011) showed concentrations of exchangeable Fe ions were seasonally fluctuated in layer O, which could reach a peak of 11,780 mg/kg at the beginning of the rainy season, but dramatically decreased to 521 mg/kg at the end of the rainy season while Al^{3+} concentration greatly varied among soil layers, fluctuated from 0 to 749 mg/kg in soil layer A and 104 to 1,109 mg/kg soil in soil layer Cp. Other study measuring concentrations of Fe^{2+} , Al^{3+} and SO_4^{2-} in layer Bj of different acid sulfate soils in Southern Vietnam showed that SO_4^{2-} concentration was less than 0.15%, 0.11 - 0.25%, and 0.27 - 0.74% for acidic soils having less, medium and high activity of sulfur forms; while Fe^{2+} and Al^{3+} concentrations ranged from 29 - 166 mg/kg and 73 - 111 mg/kg, respectively (Ngo 2002).

Vegetation density and composition reflect soil biological property and fertility depending on soil location, type and depth (Dang *et al.* 2009). At soil depth of 0 - 22 cm, microbial density could reach 232×10^5 CFU/g, however, vegetation density was much lower at soil depth higher than 55 cm (Araragi & Tangcham 1979). Hitomo & Naoto (2005) identified four species of *Thiomonas* genus that could reduce sulfur from paddy rice in Sado Island of Japan, including *T. cuprina*, *T. intermedia*, *T. perometabolis* and *T. thermosulfate*. These microorganisms could transform the concentration of Thiosulfate ions from 1.51 to 1.60 mM from initial Thiosulfate concentration of 1.61 mM used for the cultural media (Hitomo & Naoto 2005). Gram negative *Thiobacillus* isolated from paddy soil in India is highly capable of reducing sulfur forms (Rajagopal & Sridar 2007). Results of other study conducted in acid sulfate soil in Binh Chanh District of Ho Chi Minh City showed that a total number of sulfur-reducing microbes at soil depths of 0 - 20 and 20 - 40 cm ranged from 1.6×10^5 to 8.9×10^5 CFU/g soil (Nguyen & Phan 1992). The study also identified *Thiobacillus* sp. which mainly contributed to oxidize sulfate ions and pointed out that the presence of *Thiobacillus* sp.

depended on land use types and cultivation time of crops.

In order to improve acidic status of sulfate soil, some researchers planted different species on sulfate soil (Le *et al.* 1999; Wathinee *et al.* 2015; Kogawara *et al.* 2006). Watanabe *et al.* (1997) conducted trials on adaptability of *Melaleuca cajuputi* and *Melastoma marabathricum* on acid sulfate soil in Thailand and observed that these species grew well. *Melaleuca cajuputi* was capable of accumulating high aluminum concentration in its root system, while *Melastoma marabathricum* accumulated Al in its foliage. Satoshi *et al.* (2006) showed that in a greenhouse condition having lack of oxygen and acidic pH of 5.8, *M. cajuputi* grew better than *E. camaldulensis*. Le *et al.* (1999) and Thai (2009) planted *Melaleuca leucadendra* and *Melaleuca cajuputi* on acid soil located in the Mekong Delta and observed that *Melaleuca leucadendra* and *Melaleuca cajuputi* could adapt to the Mekong Delta region with survival rate greater than 75%.

Pham and Pham (2009) and Pham and Vu (2014) planted *Eucalyptus* sp. and *Melaleuca* sp. on seasonally inundated soil in the Mekong Delta and observed that *Eucalyptus camaldulensis*, *Eucalyptus tereticormis*, *Melaleuca leucadendra*, *Melaleuca viridiflora* and *Melaleuca cajuputi* could grow very well on the acid sulfate soil.

Melaleuca sp. can fix Al^{3+} ions into its root system through symbiosis activities of soil microorganisms associated with the root which can release organic acids to neutralize Al^{3+} ions (Tran 2012). This capability of *Melaleuca* sp. leads to the role of *Melaleuca* sp. in reclamation of sulfate-affected soil in the Mekong Delta.

In addition to highly adaptable *Melaleuca* sp. in the Mekong delta (Nakabayashi *et al.* 2001; Duong *et al.* 2005), there are many native plant species grow on acid sulfate soil in the Mekong Delta (Dang *et al.* 2009; Pham *et al.* 2014). Nine species dominating sulfate soils are *Melaleuca cajuputi*, *Polygonum tomentosum*, *Nyssa fruticans*, *Eleocharis dulcis*, *Nymphaea pubescens*, *Sonneratia caseolaris*, *Melastoma* sp., *Cryptocoryne ciliata* and *Annona glabra*. There are several indigenous species adapting to seasonally flooded sulfate soil such as *Calophyllum inophyllum*, *Thespesia populnea* and *Gluta velutina* (Dang *et al.* 2009; Pham *et al.* 2014).

Environmental quality of acid sulfate soil in Southern Vietnam is seriously degraded and

therefore, threatens biodiversity of the ecosystem leading to the decrease in crop production. It is important to use every possible effort to rehabilitate and restore degraded acid sulfate soil quality based on soil biochemical characteristics. Biological approach is among efforts to restore the degraded acid sulfate soil.

This study was aimed to determine biochemical characteristics of acid sulfate soil in relation to different vegetation types grown on the soil and to select appropriate crops to renovate degraded acid sulfate soil.

MATERIALS AND METHODS

Several steps were conducted to achieve the research objectives i.e. 1. measuring nutrients concentrations (C, N, P, K) and toxins (exchangeable Fe^{3+} , Al^{3+} , SO_4^{2-}) of acid sulfate soil in Southern Vietnam; 2. isolating and identifying microorganisms that are capable of reducing sulfur and iron; and 3. evaluating potential relationship between biochemical characteristics and land use types.

Study Site

This study was conducted on five different land use types located in Binh Chanh protected forest, Binh Chanh District of Ho Chi Minh City. This location represented *Thionic* Fluvisols soil (acid sulfate soil) having high concentration of SO_4^{2-} . This area was highly polluted due to anthropogenic activities and industrial wastewater. The study site was surrounded by a canal system to water several species of plants growing in the area.

Land Use Types

Five land use types were selected for conducting sample collections i.e. sugarcane plantation, 2-year *Acacia* plantation, 5-year *Acacia* plantation, 10-year *Melaleuca* plantation and grass-covered land (as control; the grass was dominated by *Eleocharis* sp. and *Cyprus* sp.).

Statistical Analysis

This study was designed using Completely Randomized Design with six replications for each treatment. The treatments were the five land use



Figure 1 Study sites in the Mekong delta, Vietnam

types i.e. sugarcane plantation, 2-year *Acacia* plantation, 5-year *Acacia* plantation, 10-year *Melaleuca* plantation and grass-covered land (as control; the grass was dominated by *Eleocharis* sp. and *Cyprus* sp.). ANOVA of this experimental design including the Tukey's test for determining the significant differences were computed using Minitab 17 software at $p < 0.05$. Sigmaplot 12 software was used to develop charts.

Soil Samples Collection

Six different soil sampling points were randomly selected in each land use type to collect soil samples and to determine soil profiles. Soil samples were collected from four soil layers

i.e. O (organic layer), AB (transitional layer between A and B_j), B_j (Jarosite layer) and C_p (Pyrite layer) (Duong *et al.* 2010). Soil samples were air-dried for two weeks, then were sieved using 2-mm mesh size sieve. Soil samples were analyzed at the Soil Laboratory of Forest Science Institute of South Vietnam. Macronutrient elements and toxins were analyzed from soil samples (TCVN 1995).

Different batches of soil samples for isolating and identifying microbes capable of transforming or oxidizing iron and sulfur were also collected from O and AB soil layers of the same soil sampling points in each land use type (Araragi & Tangcham 1979; Nguyen & Phan 1992).

Table 1 Analytical methods used to measure soil nutrients and toxins

Criteria	Analytical method
pH	Soil: DDI water = 1 : 5
Total Nitrogen (TN)	Kjeldahl method
Total Phosphorus (TP)	Dry-ashed and 6N HCl
Total Potassium (TK)	Dry-ashed and 6N HCl
Total Organic Carbon (TOC)	Walkley – Black
NH ₄ ⁺	Soil: 2M KCl = 1 : 10
NO ₃ ⁻	Soil: 2M KCl = 1 : 10
Orthophosphate	Mehlich 3
Al ³⁺	Mehlich 3
SO ₄ ²⁻	Turbidimetric method
Fe ³⁺	Mehlich 3

Analytical methods applied for measuring macronutrients (C, N, P, K, NH_4^+ , NO_3^- , Orthophosphate) and toxic ions (Exchangeable Fe^{3+} , Al^{3+} , SO_4^{2-}) are presented in Table 1.

Isolation and Identification of Sulfur-oxidizing Microbes

Isolation of sulfur-oxidizing microbes was carried out based on methods described by Rajagopal and Sridar (2007), using the Starkey and Thiosulfate culture media. These culture media had initial pH of 8.0. The total number of sulfur-reducing microbes was determined using method described by TCVN (2005) and computed using Equation 1 developed by TCVN (2005):

$$A = N / (n_1 V f_1 + \dots + n_i V f_i)$$

where:

A = numbers of bacteria cells in 1 g soil (colony forming units or CFU/g)

N = total colony count

n_i = number of disks having colony growth at each dilution

V = amount of sample solution in each disk

f_i = dilution concentration in each disk

In order to screen microbes capable of sulfur oxidation and/or transformation, the isolated microbes were continually cultured in Thiosulfate media supplemented by three different concentrations of $\text{Na}_2\text{S}_2\text{O}_3$ i.e. 5, 10 and 15 g/L culture media.

Once screened, these microbes were tested for their biochemical characteristics such as Catalase, Voges-Proskauer (VP), Indole, Citrat, Gram, and nutritional types using methods described by Nguyen *et al.* (1978) and Nguyen (2005a; 2005b). The biochemical characteristics test were carried out prior to classifying and identifying these microbes. Total numbers of microbes where determined afterward.

Isolation and Identification of Fe-oxidizing Microbes

Iron-oxidizing microbes were isolated using culture media containing 1% of Fe^{2+} ion. This method was described by Suparna *et al.* (2014). Total number of Fe-reducing microbes was determined using methods described by TCVN (2005) and computed using Equation 1. The isolated microbes were continually cultured in culture media containing 3% and 6% of Fe^{2+} ion. These media were used to screen iron-tolerant microbes as well as microbes capable of iron oxidation. Classification and identification of the screened microbes were carried out based on growth probability test in culture media having four levels of pH i.e. 3, 5, 7, 9. Biochemical characteristics test was also carried out prior to classifying and identifying the microbes. The biochemical characteristics test included Catalase, Gram, mobilization ability of colonies and assimilation ability of organic carbon. Total numbers of microbes where determined afterward.

RESULTS AND DISCUSSION

Soil pH

Soil pH is an important indicator as it relates directly to crops development, microbial activity and biochemical reactions occurring in soils. The study results showed that soil pH of the five land use types were less than 4.0 (Table 2). Soil pH differed among land use types within each soil layer of O, AB and Bj (Table 2). Although sugarcane plantation and grass-covered land (control) had higher pH than the 2-year Acacia plantation, 5-year Acacia plantation and Melaleuca plantation, the pH difference was not significant.

Table 2 Soil pH of five land use types within each soil layer

Soil layer	Sugarcane plantation	2-year <i>Acacia</i> plantation	5-year <i>Acacia</i> plantation	<i>Melaleuca</i> plantation	Grass-covered land (control)
O	3.8 ^{ab}	3.3 ^b	3.5 ^{ab}	3.2 ^b	3.9 ^a
AB	3.6 ^{ab}	2.9 ^c	3.4 ^{bc}	3.5 ^{ab}	4.0 ^a
Bj	3.5 ^{abc}	3.0 ^c	3.2 ^{bc}	3.6 ^{ab}	4.0 ^a
Cp	3.3 ^{ns}	3.3 ^{ns}	3.3 ^{ns}	3.8 ^{ns}	3.7 ^{ns}

Note: Numbers within a row (soil layer) followed by the same letter did not differ significantly at $p < 0.05$
ns = non significantly different

Concentrations of N and P among Land Use Types

Total Nitrogen (TN) is an important criteria used to evaluate soil fertility depending on the content of soil organic matter. Nitrogen in soil is produced by microbial decomposition of soil organic matter and is a result from fixation of nitrogen gas by rhizosphere in plant root system.

Nitrogen mainly accumulated in topsoil layer (O layer) and AB layer for all land use types (Fig. 1). TN concentration in the topsoil layer was higher compared to those in deeper soil layers within each land use types. TN concentration ranged from 2.3 g/kg (in sugarcane plantation) to 5.2 g/kg (*Melaleuca* plantation) (Table 3).

TN concentration in topsoil layer (O layer) was slightly different from that in AB layer, however, it was significantly different from TN concentration in Bj and Cp layers, with exception for *Melaleuca* plantation and grass-covered land (control). Concentration of NO_3^- ranged from 830 - 1,112 mg/kg soil, while concentration of NH_4^+ ranged from 586 - 999 mg/kg soil (Table 3). Concentrations of TN, NO_3^- , NH_4^+ decreased with increasing soil depth (Fig. 1). The topsoil

layer of *Melaleuca* plantation always contained the highest concentrations of TN (5.2 g/kg), NO_3^- (1,112 mg/kg) and NH_4^+ (999 mg/kg soil) (Table 3). Sugarcane and 2-year *Acacia* plantations showed the lowest concentrations of NO_3^- and NH_4^+ (Table 3).

Concentrations of total phosphorus (TP) and orthophosphate in topsoil layer (O layer) were low for all land use types (Table 3; Fig. 2). Range of TP and orthophosphate concentrations in topsoil layer (O layer) were 785 - 958 mg/kg and 378 - 640 mg/kg, respectively (Table 3; Fig. 2). Low concentration of orthophosphate might be due to partial binding of orthophosphate with Al^{3+} and Fe^{3+} to form Al-P and Fe-P complex (Bertsch 1996; Brady & Well 2002). Under acidic condition (pH < 4.0) the increase of Al^{3+} and Fe^{3+} concentrations resulted to a reduction of orthophosphate ions through Al-P and Fe-P complexation (Brady & Well 2002). TP concentrations in topsoil layer (O layer) were not significantly different among the five land use types. The highest orthophosphate concentration was observed in the topsoil layer (O layer) of the 5-year *Acacia* plantation (Table 3; Fig. 2).

Table 3 Variations of N and P concentrations among land use types

Criteria	Soil layer	Sugarcane crop	2-year <i>Acacia</i> plantation	5-year <i>Acacia</i> plantation	<i>Melaleuca</i> plantation	Grass-covered land (control)	<i>p</i> value
TN (g/kg)	O	2.28 ^b	2.37 ^b	2.50 ^b	5.18 ^a	2.36 ^b	0.002
	AB	1.68 ^{ab}	1.96 ^a	2.14 ^a	2.00 ^a	0.98 ^b	0.012
	Bj	0.86 ^b	1.12 ^{ab}	1.19 ^{ab}	1.84 ^a	0.72 ^b	0.003
	Cp	0.82 ^{ns}	1.98 ^{ns}	0.96 ^{ns}	1.12 ^{ns}	0.50 ^{ns}	0.091
NH_4^+ (mg/kg)	O	611.7 ^{bc}	585.7 ^c	861.8 ^{ab}	998.7 ^a	775.2 ^{bc}	0.001
	AB	448.5 ^b	441.2 ^b	736.5 ^a	467.3 ^b	386.1 ^b	0.003
	Bj	133.8 ^b	171.1 ^b	504.6 ^a	526.5 ^a	266.6 ^b	< 0.001
	Cp	106.6 ^{ns}	136.9 ^{ns}	219.0 ^{ns}	152.0 ^{ns}	227.8 ^{ns}	0.078
NO_3^- (mg/kg)	O	830.0 ^{bc}	625.1 ^c	1070.1 ^{ab}	1111.8 ^a	991.4 ^{ab}	< 0.001
	AB	656.1 ^{abc}	586.1 ^{bc}	898.4 ^a	850.7 ^{ab}	436.6 ^c	0.001
	Bj	263.4 ^c	268.0 ^c	599.9 ^{ab}	706.5 ^a	447.3 ^b	< 0.001
	Cp	218.1 ^b	218.1 ^b	294.4 ^{ab}	395.1 ^a	216.2 ^b	0.012
TP (mg/kg)	O	784.6 ^{ns}	800.6 ^{ns}	957.9 ^{ns}	871.7 ^{ns}	810.9 ^{ns}	0.118
	AB	578.9 ^{bc}	737.7 ^{abc}	758.0 ^{ab}	535.4 ^c	939.8 ^a	< 0.001
	Bj	553.2 ^{bc}	687.2 ^b	671.3 ^b	457.9 ^c	905.1 ^a	< 0.001
	Cp	603.9 ^b	628.6 ^b	591.1 ^b	519.3 ^b	855.7 ^a	0.004
PO_4^{3-} (mg/kg)	O	493.7 ^{ab}	462.4 ^b	640.0 ^a	395.5 ^b	377.7 ^b	0.001
	AB	307.2 ^b	417.3 ^{ab}	461.0 ^a	297.3 ^b	303.0 ^b	0.002
	Bj	280.5 ^{ab}	362.2 ^a	349.8 ^a	274.3 ^{ab}	219.5 ^b	0.013
	Cp	337.3 ^a	289.0 ^{ab}	315.6 ^a	249.3 ^{ab}	156.5 ^b	0.012

Note: Numbers within a row (soil layer) followed by the same letter did not differ significantly at $p < 0.05$
 ns = non significantly different

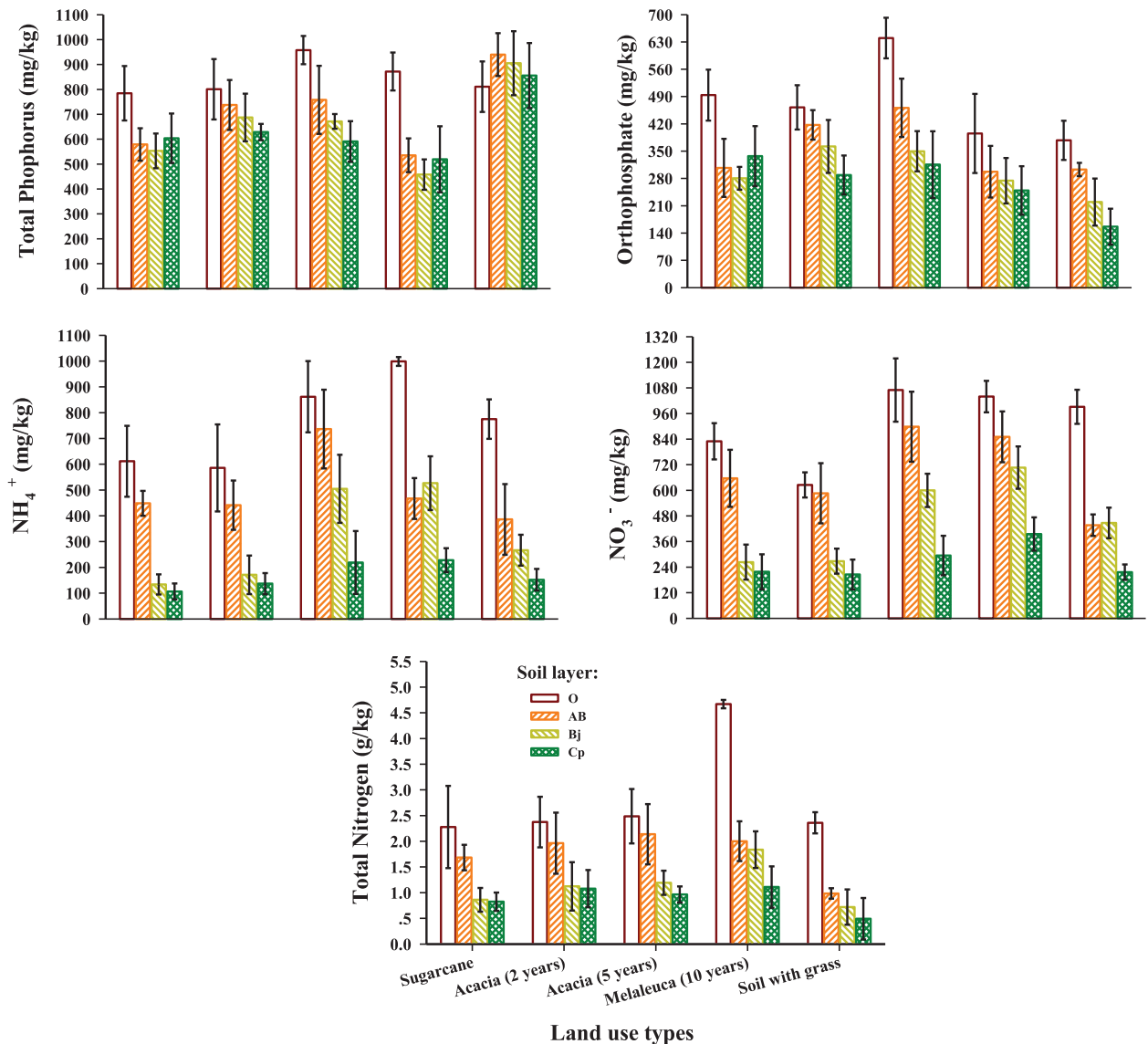


Figure 2 Concentrations of total and bioavailable N and P in five land use types

Variation of TOC and TK among Land Use Types

TOC concentration within each land use type decreased with increasing soil depth (Table 4). In each of land use types, TOC concentration in the topsoil layer was higher than those in deeper layers. Within topsoil layer, the lowest TOC concentration (2.98%) was observed in sugarcane plantation, whereas the highest TOC concentration (5.29%) was observed in *Melaleuca* plantation. Differences of TOC concentration were significantly different (Table 4). TOC concentrations in all land use types observed in this study were slightly lower than the average TOC concentration observed in previous study conducted in the Mekong Delta i.e. 5.5% (Ngo 2010).

The lowest TK concentration was observed in *Melaleuca* plantation ranging from 0.494 to 0.564%. Other land use types showed similar TK concentrations, ranging from 0.641 to 0.769%, which was higher than TK concentration in *Melaleuca* plantation. Differences in TK concentration, however, were not significant. TK concentrations showed in this study were within the results of previous study conducted in the Mekong Delta (Ngo 2002). Although five land use types did not show a significant difference of TK concentrations in their topsoil layer, they were significantly different among vegetation types within taking into account of each other lower layers (AB, Bj, Cp) (Table 4).

Concentrations of TN, TP, TOC and TK were significantly lower in topsoil layer (O layer) than

Table 4 Variations of total organic carbon (TOC) and total potassium (TK) among land use types

Soil layer	Sugarcane crop	2-year <i>Acacia</i> plantation	5-year <i>Acacia</i> plantation	<i>Melaleuca</i> plantation	Grass-covered land (control)	<i>p</i> value
TOC (%)						
O	2.98 ^b	3.08 ^b	3.44 ^b	5.29 ^a	3.21 ^b	< 0.001
AB	2.38 ^{bc}	2.96 ^{ab}	3.66 ^a	3.57 ^{ab}	1.39 ^c	< 0.001
Bj	1.29 ^b	1.88 ^b	1.72 ^b	3.08 ^a	1.71 ^b	0.001
Cp	1.83 ^{ns}	2.50 ^{ns}	2.06 ^{ns}	1.89 ^{ns}	2.22 ^{ns}	0.903
TK (%)						
O	0.662 ^a	0.650 ^a	0.641 ^a	0.494 ^b	0.660 ^a	0.006
AB	0.691 ^a	0.659 ^a	0.652 ^a	0.511 ^b	0.751 ^a	< 0.001
Bj	0.769 ^a	0.702 ^a	0.696 ^a	0.514 ^b	0.696 ^a	< 0.001
Cp	0.694 ^{ab}	0.675 ^b	0.759 ^a	0.564 ^c	0.654 ^b	< 0.000

Note: Numbers within a row (soil layer) followed by the same letter did not differ significantly at $p < 0.05$
 ns = non significantly different

those in deeper soil layers (AB, Bj, Cp) within each land use type (Table 3 & 4).

Bioavailability of Fe, Al and S Elements in Soil

Concentration of exchangeable Fe ions was particularly very low topsoil layer of sugarcane plantation (20 mg/kg soil), 2-year *Acacia* plantation (48 mg/kg soil) and 5-year *Acacia* plantation (37 mg/kg soil). Concentration of exchangeable Fe ions was highly accumulated in Cp soil layer of all land use types ranging from 684 mg/kg (in sugarcane plantation) to 2,645 mg/kg (in grass-covered land). The highest concentration of exchangeable Fe ions was observed in the AB, Bj and Cp soil layers of

Melaleuca plantation. The lowest concentrations of Al^{3+} and SO_4^{2-} ions were observed in the grass-covered land (control) and *Melaleuca* plantation (Table 5).

Concentration of SO_4^{2-} ion in all soil layers of *Melaleuca* plantation was not significantly different, ranging from 3 to 4 g/kg soil. The lowest concentration of SO_4^{2-} ion was observed in the AB, Bj and Cp soil layers of *Melaleuca* plantation. The highest concentration of SO_4^{2-} ion was observed in the 2-year *Acacia* plantation and 5-year *Acacia* plantation for all soil layers, ranging from 9.2 to 12.4 g/kg. The concentration differences within the same soil layer were not significant.

Concentration of Al^{3+} ion was similar within the same soil layer among five land use types.

 Table 5 Variations of Al^{3+} , Fe^{3+} and SO_4^{2-} concentrations among land use types

Criteria	Soil layer	Sugarcane crop	2-year <i>Acacia</i> plantation	5-year <i>Acacia</i> plantation	<i>Melaleuca</i> plantation	Grass-covered land (control)	<i>p</i> value
Al^{3+} (mg/kg)	O	37.9 ^{ns}	49.7 ^{ns}	38.8 ^{ns}	40.7 ^{ns}	34.3 ^{ns}	0.101
	AB	49.9 ^{ns}	56.0 ^{ns}	61.8 ^{ns}	59.0 ^{ns}	43.8 ^{ns}	0.105
	Bj	63.4 ^{ab}	61.2 ^{ab}	74.0 ^a	52.8 ^{bc}	37.5 ^c	0.001
	Cp	84.9 ^a	63.2 ^{ab}	68.5 ^{ab}	70.8 ^a	46.2 ^b	0.003
Exchangeable Fe (mg/kg)	O	22.4 ^c	44.6 ^c	38.7 ^c	223.6 ^b	455.4 ^a	< 0.001
	AB	16.0 ^c	51.0 ^c	35.6 ^c	1462.6 ^a	958.0 ^b	< 0.001
	Bj	62.1 ^d	1052.8 ^b	489.4 ^c	1269.3 ^{ab}	1365.4 ^a	< 0.001
	Cp	684.6 ^b	1721.0 ^a	1692.4 ^a	1612.5 ^a	2144.9 ^a	< 0.001
SO_4^{2-} (g/kg)	O	1.80 ^c	10.04 ^a	7.65 ^b	2.38 ^c	3.86 ^c	< 0.001
	AB	3.24 ^b	9.57 ^a	9.00 ^a	3.33 ^b	3.45 ^b	< 0.001
	Bj	4.50 ^b	12.62 ^a	4.99 ^b	2.70 ^b	3.37 ^b	< 0.001
	Cp	10.92 ^a	10.93 ^a	8.11 ^b	3.94 ^c	8.66 ^{ab}	< 0.001

Note: Numbers within a row (soil layer) followed by the same letter did not differ significantly at $p < 0.05$
 ns = non significantly different

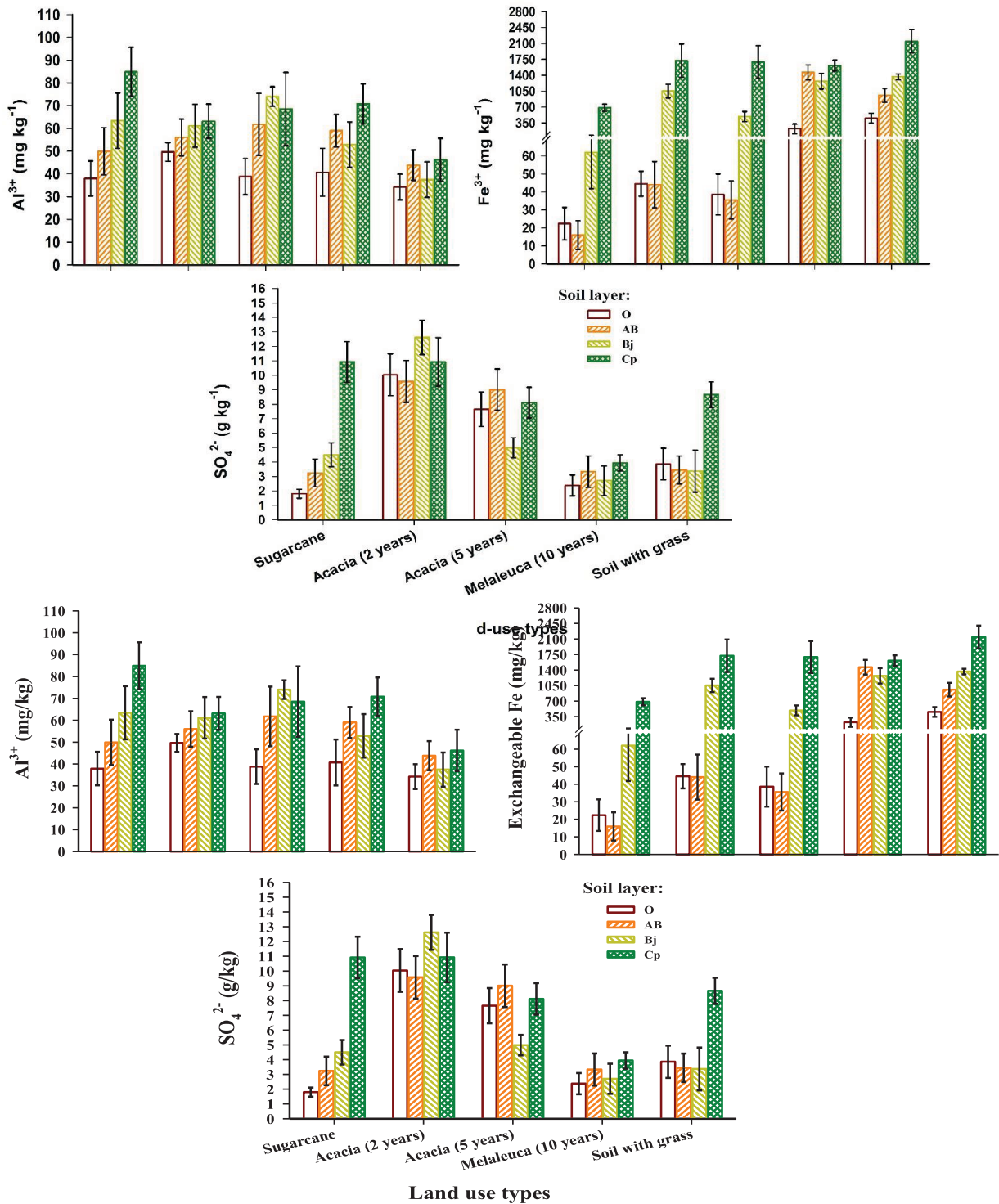


Figure 3 Concentrations of soil exchangeable Fe ions, Al^{3+} ion and SO_4^{2-} ion among land use types

Concentration of Al^{3+} ion increased with increasing soil depth. Range of Al^{3+} ion concentrations was 38 - 85 mg/kg for all soil layers among the five land use types. Concentrations of Al^{3+} ion were significantly different between O soil layer and Cj soil layer within each land use type (Table 5; Fig. 3).

Bioavailability of exchangeable Fe ions and Al^{3+} ion fluctuated with soil pH differences. Grass-covered land having higher soil pH exhibited lower concentration of Al^{3+} ion and higher concentration of exchangeable Fe ions (Dao & Hoang 2005).

Isolation of Sulfur- and Iron-oxidizing Microbes

Soil samples taken from O and AB soil layers were used to isolate microbes capable of reducing sulfur and iron content in the soil. Total numbers of microbes were determined afterward.

Total number of sulfur-oxidizing microbes varied from 0.75×10^5 to 172×10^5 CFU/g soil (Table 6). Total numbers of sulfur-oxidizing microbes isolated from the O soil layer were not significantly different among the five land use types. Total numbers of sulfur-oxidizing microbes isolated from the AB soil layer were significantly different among the five land use types. The highest number of sulfur-oxidizing microbes was recorded in soil samples taken from the AB soil layer of the *Melaleuca* plantation (172×10^5 CFU/g), followed by the 2-year *Acacia* plantation (134×10^5 CFU/g). These results indicated that species richness and species density of sulfur-oxidizing microbes were quite high among the five land use types.

Total number of iron-oxidizing microbes (range of 1.0×10^4 - 4.1×10^4 CFU/g for all land use types) was much lower than those of sulfur-oxidizing microbes (Table 6). Italicized numbers within brackets in Table 6 indicated the numbers of sulfur- or iron-oxidizing microbial species isolated from soil samples. These microbes survived in the sulfur- or iron-enriched culture media.

There were 23 different microbial species capable of growing well and metabolizing sulfur in sulfur-enriched culture media. Most of these microbial species were isolated from O layer. The highest numbers of sulfur-oxidizing microbes were found in the O layer of sugarcane plantation

(8 species), followed by those found in the O layer of *Melaleuca* plantation (6 species). Other species were found in the O layer of 2-year *Acacia* plantation (3 species), in the O layer of 5-year *Acacia* plantation (3 species), in the AB layer of sugarcane plantation (1 species), in the AB layer of 5-year *Acacia* plantation (1 species) and in the AB layer of grass-covered land/control (1 species). In the Starkey's sulfur-enriched culture media, colonies of these microbes had yellow, orange or dark pink color with smooth surface, which are similar to the results of studies conducted by Hitomo and Naoto (2005) and Rajagopal and Sridar (2007).

Two microbial species capable of metabolizing iron in iron-enriched culture media were found in the AB layer of sugarcane plantation (1 species) and in the AB layer of 5-year *Acacia* plantation (1 species). Diameter of these iron-oxidizing microbes colonies ranged from 2 to 4 mm. The colonies grew closely to the surface of the culture media and had golden-white color.

Identification of Sulfur-oxidizing Microbial Genera

Ten of the 23 sulfur-oxidizing microbial species performed high capacity of sulfur oxidation and thus, reduced pH of the Starkey culture media and Thiosulfate from 8.0 to 5.0 (Table 7). pH 8.0 is the initial pH of the Starkey and Thiosulfate media.

All of these ten microbial species was able to transform more than 50% of $\text{Na}_2\text{S}_2\text{O}_3$ content in culture media supplemented with 5 mg $\text{Na}_2\text{S}_2\text{O}_3/\text{L}$ (Table 7).

Table 6 Total numbers of sulfur- and iron-oxidizing microbes isolated from soil samples

Soil layer	Sugarcane plantation	2-year <i>Acacia</i> plantation	5-year <i>Acacia</i> plantation	<i>Melaleuca</i> plantation	Grass-covered land (control)
Total sulfur-oxidizing microbes (CFU/g soil)					
O	11.8×10^5 (8)	16.8×10^5 (3)	3.9×10^5 (3)	0.75×10^5 (6)	2.1×10^5 (0)
AB	31.3×10^5 (1)	134×10^5 (0)	12.5×10^5 (1)	172×10^5 (0)	11.4×10^5 (1)
Total iron-oxidizing microbes (CFU/g soil)					
O	1.9×10^4	1.4×10^4	2.4×10^4	1.4×10^4	1.3×10^4
AB	1.7×10^4 (1)	1.5×10^4	4.1×10^4 (1)	1.0×10^4	1.1×10^4

Note: Italic numbers in the brackets are the twenty-three different species of sulfur-reducing microbes and two different species of iron-reducing microbes isolated from soil samples

Table 7 Results of biochemical analyses of the ten sulfur-oxidizing microbes

Genera isolated from sulfur-enriched culture media	pH in culture media after microbes isolation (original pH = 8)	Reactions										Concentration of Na ₂ S ₂ O ₃ supplemented into the Thiosulfate culture media								
		Starkey					Thiosulfate					5 g/L			10 g/L			15 g/L		
		Starkey	Thiosulfate	Catalase	VP	Indol	Citrat	Gram	Nutritional type	GC	S-Tr (%)	GC	S-Tr (%)	GC	S-Tr (%)	GC	S-Tr (%)			
Sug-A-Gen1	5	8	pos	neg	neg	pos	neg	autotrophic	++	61.1	-	0	-	0	-	0				
Sug-A-Gen2	5	5	pos	neg	neg	pos	neg	heterotrophic	++	52.9	-	0	-	0	-	0				
Sug-A-Gen3	4.5	5	pos	neg	neg	pos	neg	heterotrophic	++	53.3	-	0	-	0	-	0				
Sug-A-Gen4	4.5	8	pos	neg	neg	pos	neg	autotrophic	+++	98.0	++	93.5	-	0	-	0				
Sug-A-Gen5	5	8	pos	neg	neg	pos	neg	heterotrophic	++	64.5	-	0	-	0	-	0				
Sug-A-Gen6	4.5	5	pos	neg	neg	pos	neg	autotrophic	++	70.6	-	0	-	0	-	0				
Mel-A-Gen7	5	5	pos	neg	neg	pos	neg	heterotrophic	+++	84.8	-	0	-	0	-	0				
Mel-A-Gen8	5	6	pos	neg	neg	pos	neg	heterotrophic	++	73.7	-	0	-	0	-	0				
Mel-A-Gen9	4.5	8	pos	neg	neg	pos	neg	heterotrophic	+++	97.1	+	33.3	-	0	-	0				
Gra-AB-Gen10	4.5	8	pos	neg	neg	pos	neg	autotrophic	+++	96.1	++	95.9	-	0	-	0				

Note: Sug-A-Gen1 = Genus 1 of *Thiobacillus* sp. isolated from the A layer of Sugarcane plantation having highly transformed sulfur; Sug-A-Gen 2 = Genus 2 of *Thiobacillus* sp. isolated from the A layer of Sugarcane plantation having highly transformed sulfur; Sug-A-Gen 3 = Genus 3 of *Thiobacillus* sp. isolated from the A layer of Sugarcane plantation having highly transformed sulfur; Sug-A-Gen 4 = Genus 4 of *Thiobacillus* sp. isolated from the A layer of Sugarcane plantation having highly transformed sulfur; Sug-A-Gen 5 = Genus 5 of *Thiobacillus* sp. isolated from the A layer of Sugarcane plantation having highly transformed sulfur; Sug-A-Gen 6 = Genus 6 of *Thiobacillus* sp. isolated from the A layer of Sugarcane plantation having highly transformed sulfur; Mel-A-Gen7 = Genus 7 of *Thiobacillus* sp. isolated from the A layer of *Melaleuca* plantation having highly transformed sulfur; Mel-A-Gen 8 = Genus 8 of *Thiobacillus* sp. isolated from the A layer of *Melaleuca* plantation having highly transformed sulfur; Mel-A-Gen 9 = Genus 9 of *Thiobacillus* sp. isolated from the A layer of *Melaleuca* plantation having highly transformed sulfur; Gra-AB-Gen10 = Genus 10 of *Thiobacillus* sp. isolated the AB layer of Grass-covered land/control having highly transformed sulfur; pos = positive reaction; neg = negative reaction; GC = growth capacity; S-Tr (%) = percentage of transformed sulfur; - = no growth; + = normal growth; ++ = good growth; +++ = very good growth

Three species were able to transform more than 96% of $\text{Na}_2\text{S}_2\text{O}_3$ in Thiosulfate culture media supplemented with 5 mg $\text{Na}_2\text{S}_2\text{O}_3/\text{L}$ i.e. Sug-A-Gen4, Mel-A-Gen9, and Gra-AB-Gen10 (Table 7). These three species were able to decrease pH of the Starkey media from 8.0 to 4.5, however, these species were not able to decrease pH of Thiosulfate media (Table 7). These three species were the only species survived and grew well in Thiosulfate culture media supplemented with 10 mg $\text{Na}_2\text{S}_2\text{O}_3/\text{L}$, however, they did not survive in Thiosulfate culture media supplemented with 15 mg $\text{Na}_2\text{S}_2\text{O}_3/\text{L}$ (Table 7). These three microbial species were found in the O and AB layers of sugarcane plantation, *Melaleuca* plantation and grass-covered land/control, and thus, would be able to oxidize sulfur in the soil of these three land use types (Fig. 3).

Results of biochemical testing for the ten sulfur-oxidizing microbial species are presented in Table 7. All of these species had negative reaction toward Gram, VP and Indol tests, however, they had positive reaction toward Catalase and Citrat tests. Nutritional test showed that the ten sulfur-oxidizing microbial species consisted of three autotrophic species from sugarcane plantation, three heterotrophic species from sugarcane plantation, one autotrophic species from grass-covered land/control and three heterotrophic species from *Melaleuca* plantation (Table 7).

Autotrophic microbes are microbes that can synthesize inorganic carbon sources for their growth without the presence of organic carbon compounds. Heterotrophic microbes are microbes that can only grow well in media containing high organic carbon source and cannot

grow without organic carbon compounds (Brady & Well 2002).

Identification of these ten sulfur-oxidizing microbial species was conducted based on the results of biochemical characteristics analyses, results of studies conducted by Kantachote and Innuwat (2004) and handbook of bacterial identification written by Kelly and Harrison (1989). The identification indicated that the ten sulfur-oxidizing microbial species belong to genus *Thiobacillus*.

Identification of Iron-oxidizing Microbial Genera

Results of biochemical testing for the two iron-oxidizing microbial species are presented in Table 8. Two iron-oxidizing microbial species were isolated from the five land use types. These two microbial species showed negative reaction toward Gram test, however, showed positive reaction toward Catalase test.

These two iron-oxidizing microbial species were initially isolated in culture media containing 1% Fe^{2+} . The two iron-oxidizing microbial species grew well in iron-enriched culture media having 3 and 6% Fe^{2+} concentrations with culture media $\text{pH} \leq 5.0$.

These two microbial species showed negative reaction toward glucose, sucrose and maltose tests, indicating that these two microbial species were autotrophic.

Identification based on biochemical characteristics analyses, results of studies conducted by Kantachote and Innuwat (2004) and handbook of bacterial identification written by Kelly and Harrison (1989) indicated that these two iron-oxidizing microbial species belong to genus *Thiobacillus*.

Table 8 Results of biochemical analyses of two highly iron-oxidizing performed microbes

Genera isolated from iron-enriched culture media	Reactions		Ability to colony mobilization	Growth with iron supplement having concentration of		Growth with supplement of organic carbon sources			Growth in culture media having different pH			
	Catalase	Gram		Ability to colony production	3%	6%	Glucose	Sucrose	Maltose	3	5	7
Sug-AB-Gen1	pos	neg	-	+	+	-	-	-	+	+	-	-
Aca-AB-Gen2	pos	neg	-	+	+	-	-	-	+	+	-	-

Note: Sug-A-Gen1 = Genus 1 of *Thiobacillus* sp. isolated from the AB layer of Sugarcane plantation having highly transformed iron; Aca-AB-Gen2 = Genus 2 of *Thiobacillus* sp. isolated from the AB layer of 5-year *Acacia* plantation highly transformed iron; pos = positive reaction; neg = negative reaction; + = normal growth; - = no growth

CONCLUSIONS

The five land use types had very acidic soil with soil pH of ≤ 4.0 , low concentrations of phosphorus and potassium. Total organic carbon (TOC) in O layers in all five land use types was higher than that in deeper soil layers. *Melaleuca* plantation had the highest concentration of TOC in the O layer. Concentrations of exchangeable Fe^{3+} , Al^{3+} , SO_4^{2-} ions varied among the five land use types. The lowest concentration of SO_4^{2-} was observed in the grass-covered land (control) and *Melaleuca* plantation.

Ten sulfur-oxidizing microbial species were isolated from sugarcane plantation, *Melaleuca* plantation and grass-covered land/control and were identified as belong to genus *Thiobacillus*.

Two iron-oxidizing microbial species were isolated from sugarcane plantation and 5-year *Acacia* plantation and were also identified as belong to genus *Thiobacillus*.

It is proposed that there is a strong relationship between *Thiobacillus* sp. and sugarcane plantation which indicates that *Thiobacillus* sp. isolated from sugarcane plantation can oxidize sulfur and iron in the polluted acid sulfate soil.

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