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The Phosphorus and Sulphur Distribution and Culturable Bacterial in Time Chronosequence of Ex-Tin Mining Ponds

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ABSTRACT

The tin mining had caused ecological changes that can be occured to macro- and microecosystem. This article aims to study the pattern distribution of element of phosphorus (P) and sulphur (S) and also to identify culturable bacterial that were isolated form ex-tin mining ponds in time chronosequence. The elemets of P and S were detected by X-Ray Fluorescence (XRF) and the bacterials was isolated in medium agar and biochemistry identification by microbact. The concentration of element of P and S showed the average of P concentration increased in time chronosequnce of ex-tin mining ponds, whereas the average of S concentration showed dynamic pattern. In ex-tin mining pond with age < 1 year the average concentration of P was 33,725 mg.L-1 and S was 311,45 mg.L-1. In ex-tin mining pond with age 5-10 years were P (59,8 mg.L-1) and S (451,75 mg.L-1). In ex-tin mining pond with age > 15 years were P (67,44 mg.L-1) and S (386,125 mg.L-1). While, the culturable bacterials were *Kurthia* spp; *Actinobacillus equuli*; *Bacillus amyloliquefaciens*; *Bacillus* spp; *Micrococcus* sp; *Enterobacter gergoviae*; *Veillonella* sp; *Enterobacter aerogenes*; *Moraxella bovis*; *Nitrobater* spp; and *Enterobacter agglomerans*.

Keywords: *phosphorus, sulphur, tin mining, culturable bacteria*

1. Introduction

Tin mining activity had contributed to ecological problems. The soil changes by tin mining activity caused a degradation of soil composition, structure, quality, and physical or biological characteristics, and changes of macro and microorganisms in their natural habitats (Kurniawan et al., 2016). The other problem was a formation of ex-tin mining ponds and consequectly, the ponds become a reservoir of water.

The characteristics of waters showed acidic pH value and often highly acidic (pH < 4) (Kolmert and Jhonson, 2001), low dissolved oxygen (Ashraf et al., 2011), heavy metals accumulation (Daniel et al., 2014), and low cation exchange capacity, organic matter, nitrogen, phosphorus, macronutrient, and also clay content in soil texture (Oktavia et al., 2014). This condition caused the waters can not be used for primary activities.

A recovery of natural succession even takes a long time. Thefore, information about lush of the waters, especially phosphorus and sulphur were an important part to explained the thropic level and acidic value of waters in time chronosequence. It can be used to determine the eco-management of ex-tin mining ponds.

In additon, an understanding of bacterial' life in ex-tin mining ponds were also important. It can explained bacterial' response to the environmental changes caused by the tin mining chronosequence. The understanding of bacterial's life in ex-tin mining becomes an important focus to investigated adaptation capacity of them during ecological changes process or microbial succession pattern in change through time.

This studi about culturable bacterial aims to identify their characteristic of biochemistry. Further, the characteristics can indicate their capability and it can be used as a successor and bioremediator agent to recover water quality quickly.

2. Material and Methods

The study stations were located in Bangka Regency, Bangka Belitung Archipelago Province of Indonesia. The study areas were encoded as Station A (pond in age < 1 year), Station B (pond in age 5-10 years), and Station C (pond in age > 15 years).

The coordinate of Station A were 01°59' S in points 36,0"; 36,2"; 36,4"; 36,5"; 36,6" and 106°06' E in points 36,5"; 36,9"; 37,3"; 37,4"; 37,5". The coordinate of Station B were 01°59' S in points 41,3"; 41,4"; 41,5"; 42,4"; 42,5" and 106°06' E in points 39,2"; 39,5"; 41,4"; 42,7"; 43,1". The coordinate of Station C were 01°55' S in points 40,9"; 58,9"; 59,1"; 59,2"; 59,5" and 106°06' E in points 19,5"; 19,7"; 19,9"; 22,4"; 29,2".

In the each of research stations points, the water sampling was done to water samples < 4m in depth (encoded Station A.1; Station B.1; and Station C.1) and the composite sampling were done to water and sediment samples > 4m in depth (encoded Station A.2; Station B.2; and Station C.2).

The parameters of research include element concentration of phosphorus (P) and sulphur (S) in waters and also characteristic of biochemistry from culturable bacterial. The elements concentration of samples were measured by X-Ray Fluorescence (XRF) that calibrated by three light spreader metals of copper (Cu), molybdenum (Mo), and aluminum (Al). While, the isolation of culturable bacterial was done in medium agar and analysis gram, motility, catalase, oxidase, glucose, ornithine, indole, citrate, and voges-proskauer (VP) were identificated by microbact™ 12A and 24E (Oxoid, UK) Identification Kits (Septiama et al., 2008; Osuntokun et al., 2018).

3. Results and Discussion

2.1 The concentration of phosphorus and sulphur

The concentration of element of P and S in time chronosequence of ex-tin mining ponds showed the average of P concentration increased from Station A to Station B and Station C, whereas the average of S concentration increased from Station A to Station B and then decreased in Station B. The average of P concentration were 33,725 mg.L-1 (Station A), 59,8 mg.L-1 (Station B), and 67,44 mg.L-1 (Station C). While, the average of S concentration were 311,45 mg.L-1 (Station A), 451,75 mg.L-1 (Station B), 386,125 mg.L-1 (Station C) (**Table 1**).

The understanding of both element of P and S in ex-mining activity was important. The element of P can indicated thropic level, whereas element of S can produced acide mine drainage by oxidation sulfide minerals.

The ex-mining ecosistems were deficient in nutrients, include P (Huang et al., 2011). However, the chronosequence ranging in time of ex-tin mining ponds contributed to the ecological change. The age of ecosystem had correspondences with ecological succession that were followed by changes of nutrient cycling and physico-chemical characteristics (Moreno-de las Heras et al., 2008). The element of P concentration in Table 1 showed increasing pattern that can indicated the change of ecosystem in eutrophication level because the presence of P in a ecosystem caused eutrophication (Sibrell et al., 2009; Abdel-Raouf et al., 2012).

Table 1. Concentration of P and S in time chronosequence of ex-tin mining ponds

No.	Elements	Density (g/cm^3)	Concentration (mg.L-1)					
			Station A		Stasion B		Stasion C	
			A.1	A.2	B.1	B.2	C.1	C.2
1.	Phosphorus (P)	1,823	12,2	55,25	25,7	93,9	88,3	46,58
2.	Sulphur (S)	1,96	60,8	562,1	163	740,5	471	301,25

Notes: *Station A (pond in age < 1 year), Station B (pond in age 5-10 years), and Station C (pond in age > 15 years). The station code (A.1, B.1, and C.1) showed water samples < 4m in depth and the station code (A.2, B.2, and C.2) showed composite samples (water and sediment > 4m in depth).*

The eutrophication can indicated positive changes of ex-tin mining ponds ecosystem. The presence of phosphate becomes one of indicator for eutrophication and a parameter to predicted biomass abundance. The mobilization of phosphate in water-sediment interphase contributed to physic-chemical factors of waters (Maher et al., 2002; Quirós, 2003; Mahadevaiah et al., 2007; Topcu and Pulatsu, 2014; Lei et al., 2017). Indirectly, the physic-chemical changes can impacted to organism' life, especially microorganisms as first life in the ecosystem. The microorganism' life can implicated to succession and water quality change there.

The element of S can produced acid condition in mine ecosystem (RoyChowdhury et al., 2015). The mining wastewaters typically contain metal sulfide minerals, particularly the pyrite (FeS_2) was oxidized in contact with oxygen and water become an acid mine drainage (Chun-bo et al., 2007). The overall, pyrite oxidation by reaction respectively (Descotes et al., 2002):

$$FeS_2 + 7/2\ O_2 + H_2O \rightarrow Fe^{2+} + 2\ SO_2 + 2\ H^+$$
$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O \rightarrow 15\ Fe^{2+} + 2\ SO_2 + 16\ H^+$$

The element of P concentration in Table 1 showed increasing from Station A to Station B and decreasing in Station C. This pattern can indicated oxidation process in Station B was higer than Station C and then this process implicated on pH value change. The element of S in form SO_4^{2-} was a significant factor in acid mine drainage, besaide metals such as Al, As, Fe, K, Mg, Mn, Na, and Zn (Campaner et al., 2014). The consequence of pH value change contributed to dissolved organic and anorganic materials (Akan et al., 2013; Kuriata-potasznik et al., 2016), water solubility from nitrogen and amonia (Luo et al., 2015), oxygen depletion (Hou et al., 2013), and other parameters of water that impacted to biology activity.

The change of P and S in waters can indicated ecological composition and structure quality and also physical characteristics. Indirectly, they contributed to biological changes, either macro- and micro ecosystems change (Vyas and Pancholi, 2009; Giri et al., 2014; Lad et al., 2015). The biological changes include organisms and microbial communities structure (Grant et al., 2007).

2.2 The culturable bacterial

The bacterial was isolated in medium agar had an oppurtunity to studied characteristics of biochemistry. There were eight genus of bacteria that Actinobacillus, Bacillus, Enterobacter, Kurthia, Micrococcus, Moraxella, Nitrobater, and Veillonella (**Table 2**).

The change of ex-tin mining waters can impacted microorganisms diversity like methane-oxidizing bacteria (MOB), ammonia-oxidizing bacteria (AOB), and arsenic-resistant bacteria (ARB) (Jareonmit et al., 2010; Valverde et al., 2011; Sow et al., 2014a; Sow et al., 2014b) or other microorganisms like archea that were called acidophile (Navarro et al., 2013). They had a capability to respond alterations quickly in an ecosystem and the potential property can be used to predict and detect environment changes (Paerl et al., 2003; Niemi and McDonald, 2004; Moscatelli et al., 2005; Lau and Lennon, 2012). There was a significant correspondence between microorganism communities and ecological factors (Vishnivetskaya et al., 2011). The changes of ecological along time chronosequence can drived size, activity, diversity, structure, composition, and capability of microbial (Liao and Xie, 2007; Wang et al., 2007; Chodak et al., 2009; Banning et al., 2011; Bier et al., 2015).

Further, their resistance and resilience in acid mine drainage, include a metals contamination, indirectly can contributed to the ecological changes (Xie et al., 2011; Shade et al., 2012). The acidophile showed an ability to survive in acid condition like ex-tin mining. They can used residues of mining as nutition, carbon, and nitrogen source with mixotrophy as chemoheterotroph and photoautotroph (Hao et al., 2010).

Some of them were *Alcaligenes* spp., *Arthrobacter* spp., *Bacillus* spp., *Corynebacterium* spp., *Azotobacter* spp., *Pseudomonas* spp. *Rhodococcus* spp., *Aspergilus niger*, *Flavobacterium* spp., *Mycobacterium* spp., *Methanogens*, *Nocardia* spp., *Methosinus* sp., *Pleurotus ostreatus*, *Rhizopus arrhizus*, *Stereum hirsutum*, *Phormidium valderium*, and *Ganoderma applantus* (Girma, 2015).

In this research, *Actinobacillus* sp, *Bacillus* sp, *Enterobacter* sp, *Kurthia* sp, *Micrococcus* sp, *Moraxella* sp, *Nitrobater* sp, and *Veillonella* sp were isolated form the ecosystem and they were culturabled in medium agar. However, they were not representation of all microorganisme in ex-tin mining ponds ecosystem. The culturable bacterials were only 1-10% can be isolated in the laboratory (Lutton et al., 2013). Therefore, bacterial identification by the sequencing confirmation of 16S rRNA genes were needed to identify the culturable bacterials.

Table 2. Characteristic of culturable bacteri in time chronosequence of ex-tin mining ponds

Bio-chemistry Ident.	Biochemistry characteristics and species bacteria																				
				Station A								Stasion B							Stasion C		
		A.1				A.2			B.1				B.2				C.1			C.2	
Code*	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u
Gram	+	-	+	+	+	+	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-
Motility	-	-	-	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	-	+
Catalase	+	+	+	+	+	+	-	+	-	-	+	+	+	-	-	+	+	-	+	-	-
Oxidase	-	+	+	+	-	+	-	-	-	-	+	-	-	+	+	-	+	+	+	+	-
Glucose	-	+	-	-	-	-	+	-	+	+	-	+	+	+	+	-	-	+	-	+	+
Ornithine	+	-	-	+	-	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Citrate	+	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+
VP	-	-	+	-	-	+	+	-	+	+	-	-	+	+	+	-	-	+	-	+	+

Notes: *Station A (pond in age < 1 year), Station B (pond in age 5-10 years), and Station C (pond in age > 15 years). The station code (A.1, B.1, and C.1) showed water samples < 4m in depth and the station code (A.2, B.2, and C.2) showed composite samples (water and sediment > 4m in depth).*
*) (a) *Kurthia* spp; (b) *Actinobacillus equuli*; (c) *Bacillus amyloliquefaciens*; (d) *Bacillus* spp; (e) *Micrococcus* sp; (f) *Bacillus amyloliquefaciens*; (g) *Enterobacter gergoviae*; (h) *Veillonella* sp; (i) *Enterobacter aerogenes*; (j) *Enterobacter aerogenes*; (k) *Moraxella bovis*; (l) *Bacillus* spp; (m) *Bacillus* spp; (n) *Nitrobater* spp; (o) *Nitrobater* spp; (p) *Kurthia* spp; (q) *Moraxella bovis*; (r) *Nitrobacter* spp; (s) *Moraxella bovis*; (t) *Nitrobacter* spp; (u) *Enterobacter agglomerans*

Furthermore, the metagenoms analysis become method to explore information about potential bacterial that were needed. The metagenomic analysis was a *culture-independent genomic analysis of microorganism'* communities. The analysis by polymerase chain reaction (PCR) amplification of 16S rRNA genes can be used to identify unculturable microorganisms and represented more than 99% of the microorganism in an environments (Schloss and Handelsman, 2003). By metagenoms analysis, diversity of potential bacterials will be known.

2.3 The relationship of bacteria with P and S

Phosphorus and sulphur in an environment was closely related to microorganism' life cycle because they were needed for cell activity and growth. On the other side, microorganism' activity can contributed to dynamic of P and S. Therefore, there was an interaction both of them in an environment.

In ex-tin mining ponds, the concentration of element of P and S showed concentration increasing in time chronosequnce of ex-tin mining ponds, whereas the average of S concentration showed dynamic pattern. These conditions contributed to genus of bacteria there, where gram positive bacteria were more found in ponds with age < 1 year, whereas gram negative were more found in ponds with age > 15 years.

Sulphur was among the most abundant elements on the environment and mainly present as pyrite (FeS_2) as a result of sulphide oxidation (Tan et al., 2007; Muyzer and Stam, 2008). This condition contributed to acidic pH value as acid mine drainage (AMD). The increasing of P in the ponds may caused by ecological changes as sedimentation, cation and anion exchange, pH value changes, reduction-oxidation, composting, etc along chronosequence in time.

The element P was used by the microorganism for an adaptation. In fact, in time chronosequence' effect impacted to gram negative bacterials were more than gram positive bacterials. Further, they activity caused concentration of S was decreased. This interaction indicated that gram negative bacterials had a potential activity as a sulphur reducer to redused sulphur from the environment. The sulphate-reducing bacteria (SRB) can converted sulphate ions (SO_4^{-2}) into sulphide (S^{-2}) (Sakamoto et al., 2012) and they used sulphate as a terminal electron acceptor in cell activities such as the degradation of organic compounds and also an important role in both the sulphur and hydrocarbon cycles in waste and some environment contaminants (Dar et al., 2007; Plugge et al., 2011; Hussain et al., 2016).

4. Conclussion

Tin mining caused macroecosystem changes and indirectly influenced microecosystem. The phosphorus and sulphur value and also microorganism' life in ex-tin mining ponds can be indicators that indicated the ecological changes. Distribution of P and S element had a relationship with diversity of microorganism, specially bacteria. Furthermore, in time chronosequence of ex tin mining ponds also contributed to ecological changes.

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