



Vol 20, No 3 (2022): July 2022

Table of Contents

Research Article

Analisis Hubungan Konstruksi Sumur Gali dan Sanitasi Lingkungan Terhadap Jumlah Bakteri Coliform Dalam Air Sumur Gali (Studi Kasus: Desa PAL IX, Kecamatan Sungai Kakap)

PDF
437-
444

Jtin Yeni Syafarida, Dian Rahayu Jati, Aini Sulastrri

| Language: ID | DOI: [10.14710/jil.20.3.437-444](https://doi.org/10.14710/jil.20.3.437-444)

Received: 3 Dec 2021; Revised: 6 Feb 2022; Accepted: 13 Feb 2022; Available online: 26 Feb 2022; Published: 21 Feb 2022.

Estimasi Nilai Hydraulic dan Solid Loading Rate Tipe Pengendapan Diskrit dan Flok Pada Proses Lumpur Aktif Untuk Pengolahan Limbah Cair Industri Kertas

PDF
445-
456

Fatihaturrizky Amelia, Owen Jacob Notonugroho, Satyanto Krido Saptomo, Allen Kurniawan

| Language: ID | DOI: [10.14710/jil.20.3.445-456](https://doi.org/10.14710/jil.20.3.445-456)

Received: 5 Jan 2022; Revised: 11 Feb 2022; Accepted: 21 Feb 2022; Available online: 27 Feb 2022; Published: 5 Jul 2022.

Pola Distribusi Spasial-Temporal Hotspot dan Variasi Standardized Precipitation Index pada Lahan Gambut Tropis di Kepulauan Meranti, Riau

PDF
457-
464

Maulana Dwi Putra Riyadi, Yudi Setiawan, Muh Taufik

| Language: [ID](#) | DOI: [10.14710/jil.20.3.457-464](https://doi.org/10.14710/jil.20.3.457-464)

Received: 5 Feb 2022; Revised: 5 Mar 2022; Accepted: 13 Mar 2022; Available online: 18 Mar 2022; Published: 3 Jul 2022.

Pengaruh Penambahan Gliserol Mentah Limbah Industri Biodiesel Terhadap Produksi Biogas dari Kotoran Sapi Menggunakan Anaerobic Digester Sistem Batch

[PDF](#)
465-473

Nelsy Mariza Syahyuda, Fadjar Goembira, Shinta Silvia

| Language: [ID](#) | DOI: [10.14710/jil.20.3.465-473](https://doi.org/10.14710/jil.20.3.465-473)

Received: 18 Feb 2022; Revised: 4 Mar 2022; Accepted: 10 Mar 2022; Available online: 18 Mar 2022; Published: 4 Jul 2022.

Proyeksi Konservasi Tanah dan Air Mitigasi Penurunan Jasa Lingkungan Tata Hidrologi DAS Hulu Brantas

[PDF](#)
474-483

Raushanfikr Bushron, Latief Mahir Rachman, Dwi Pujo Tejo Baskoro, Soemarno Soemarno

| Language: [ID](#) | DOI: [10.14710/jil.20.3.474-483](https://doi.org/10.14710/jil.20.3.474-483)

Received: 25 Jan 2022; Revised: 7 Mar 2022; Accepted: 10 Mar 2022; Published: 5 Jul 2022.

Dampak Penurunan Kualitas Air Laut Dari Kegiatan Operasi Floating Storage and Offloading (FSO) Challenger Lepas Pantai Blok Bawean

[PDF](#)
484-493

Ai Siti Patimah, Sigit Heru Murti, Agus Prasetya

| Language: [ID](#) | DOI: [10.14710/jil.20.3.484-493](https://doi.org/10.14710/jil.20.3.484-493)

Received: 4 Feb 2022; Revised: 4 Mar 2022; Accepted: 10 Mar 2022; Available online: 18 Mar 2022; Published: 3 Jul 2022.

Pemanfaatan Scrap Besi Menjadi Copperas dan Ekstrak Kulit Rambutan untuk Pembuatan Nanopartikel Besi yang Ramah Lingkungan

[PDF](#)
494-507

Sunardi Sunardi, Mardiyono Mardiyono, Nur Hidayati

| Language: [ID](#) | DOI: [10.14710/jil.20.3.494-507](https://doi.org/10.14710/jil.20.3.494-507)

Received: 30 Jan 2022; Revised: 6 Mar 2022; Accepted: 12 Mar 2022; Available online: 20 Mar 2022; Published: 4 Jul 2022.

Penilaian Tingkat Cemar Logam Berat Pada Lahan Pertanian di Hulu Sungai Citarum, Jawa Barat

[PDF](#)
508-516

Cicik Oktasari Handayani, Sukarjo Sukarjo, Triyani Dewi

| Language: [ID](#) | DOI: [10.14710/jil.20.3.508-516](https://doi.org/10.14710/jil.20.3.508-516)

Received: 13 Jan 2022; Revised: 5 Mar 2022; Accepted: 6 Mar 2022; Available online: 29 Mar 2022; Published: 5 Jul 2022.

[Kesuburan Tanah Di Bawah Tegakan Berbagai Jenis Bambu Pada Tanah Andosol-Regosol](#) PDF
517-523
Sutiyono Sutiyono, I Wayan Susi Dharmawan, Ujang W Darmawan

| Language: [ID](#) | DOI: [10.14710/jil.20.3.517-523](https://doi.org/10.14710/jil.20.3.517-523)
Received: 4 Feb 2022; Revised: 9 Mar 2022; Accepted: 13 Apr 2022; Available online: 20 Mar 2022; Published: 4 Jul 2022.

[Model Pengelolaan Banjir: Systematic Review dan Arahan untuk Masa Depan](#) PDF
524-545
Veybi Djoharam, Widiatmaka Widiatmaka, Marimin Marimin, Dyah R Panuju, S.D. Tarigan

| Language: [ID](#) | DOI: [10.14710/jil.20.3.524-545](https://doi.org/10.14710/jil.20.3.524-545)
Received: 2 Feb 2022; Revised: 9 Mar 2022; Accepted: 12 Mar 2022; Available online: 22 Mar 2022; Published: 4 Jul 2022.

[Dampak Perluasan Lahan Tambak Terhadap Keanekaragaman Makrozoobenthos Di Ekosistem Mangrove](#) PDF
546-552
Mahmudin Mahmudin, Fajria Sari Sakaria, Veranika Veranika

| Language: [ID](#) | DOI: [10.14710/jil.20.3.546-552](https://doi.org/10.14710/jil.20.3.546-552)
Received: 30 Dec 2021; Revised: 9 Mar 2022; Accepted: 11 Mar 2022; Available online: 22 Mar 2022; Published: 5 Jul 2022.

[Valuasi Ekosistem Mangrove di Pesisir Kayong Utara, Kalimantan Barat](#) PDF
553-562
Ajeng Apriani, Aji Ali Akbar, Jumiati Jumiati

| Language: [ID](#) | DOI: [10.14710/jil.20.3.553-562](https://doi.org/10.14710/jil.20.3.553-562)
Received: 9 Feb 2022; Revised: 13 Mar 2022; Accepted: 17 Mar 2022; Available online: 26 Mar 2022; Published: 6 Jul 2022.

[Short Communication: Biochemistry Analysis and Molecular Approach to Identify the Cultured Bacterial from Ex-Tin Mining Lakes](#) PDF
563-569
Andri Kurniawan, Oedjijono Oedjijono, Tamad Tamad, Uyi Sulaeman

| Language: [EN](#) | DOI: [10.14710/jil.20.3.563-569](https://doi.org/10.14710/jil.20.3.563-569)
Received: 12 Feb 2022; Revised: 14 Mar 2022; Accepted: 16 Mar 2022; Available online: 28 Mar 2022; Published: 5 Jul 2022.

[Pemanfaatan Bionanomaterial Chitosan dari Limbah Cangkang Kulit Udang Sebagai Adsorben dalam Pengolahan Air Gambut](#) PDF
570-578
Shinta Elystia, Nur Anisyah Handayani Hasibuan, Zultiniar Zultiniar

| Language: [EN](#) | DOI: [10.14710/jil.20.3.570-578](https://doi.org/10.14710/jil.20.3.570-578)

Received: 2 Feb 2022; Revised: 13 Mar 2022; Accepted: 16 Mar 2022; Available online: 26 Mar 2022; Published: 5 Jul 2022.

[Pengaruh Kedalaman Sedimen Terhadap Emisi Gas Metana \(CH₄\) di Situ Kuru](#)

Putri Permata Utari Andini, Zahra Yunisa, Armar Riliansyah Tamala, Nurul Akhirati Hasanah, Muhammad Indra Maulana Rizki, Megga Ratnasari Pikoli, Irawan Sugoro

[PDF](#)
579-
587

| Language: [ID](#) | DOI: [10.14710/jil.20.3.579-587](https://doi.org/10.14710/jil.20.3.579-587)

Received: 1 Feb 2022; Revised: 12 Mar 2022; Accepted: 20 Mar 2022; Available online: 29 Mar 2022; Published: 5 Jul 2022.

[Evaluasi Aspek Teknis Operasional Pengelolaan Persampahan di Kecamatan Putussibau Utara Kabupaten Kapuas Hulu](#)

Meideristi Eka Suciutami, Arifin Arifin, Robby Irsan, Rizki Purnaini, Y. Fitrianiingsih

[PDF](#)
588-
596

| Language: [ID](#) | DOI: [10.14710/jil.20.3.588-596](https://doi.org/10.14710/jil.20.3.588-596)

Received: 12 Jan 2022; Revised: 16 Mar 2022; Accepted: 23 Mar 2022; Available online: 30 Mar 2022; Published: 5 Jul 2022.

[Pengembangan Wisata Pantai di Kalimantan Timur Berdasarkan Persepsi Pengunjung](#)

Ardiyanto Wahyu Nugroho

[PDF](#)
597-
608

| Language: [EN](#) | DOI: [10.14710/jil.20.3.597-608](https://doi.org/10.14710/jil.20.3.597-608)

Received: 9 Feb 2022; Revised: 13 Mar 2022; Accepted: 18 Mar 2022; Available online: 31 Mar 2022; Published: 5 Jul 2022.

[Hubungan Pengetahuan dan Perilaku Pengunjung Wisata Pantai di Bali Selatan dalam Membuang Sampah Masker](#)

Desak Putu Risky Vidika Apriyanthi, Ayu Saka Laksmi-W, Ni Putu Widayanti

[PDF](#)
609-
614

| Language: [ID](#) | DOI: [10.14710/jil.20.3.609-614](https://doi.org/10.14710/jil.20.3.609-614)

Received: 19 Nov 2021; Revised: 12 Mar 2022; Accepted: 25 Mar 2022; Available online: 5 Apr 2022; Published: 5 Jul 2022.

[Pengaruh Perubahan Tutupan Lahan Terhadap Jasa Ekosistem Pangan Di Taman Nasional Danau Sentarum](#)

Hanif Andryannur, Aji Ali Akbar, Aini Sulastri

[PDF](#)
615-
627

| Language: [ID](#) | DOI: [10.14710/jil.20.3.615-627](https://doi.org/10.14710/jil.20.3.615-627)

Received: 17 Feb 2022; Revised: 15 Mar 2022; Accepted: 27 Mar 2022; Available online: 1 Apr 2022; Published: 5 Jul 2022.

Pengaruh Paparan Pencemar Udara Terhadap Stres Oksidatif: Sistematis Review PDF
628-
636
Kusmiyati Kusmiyati, Norma Tiku Kambuno, Pius Selasa, Ferry William Frangky Waangsir

| Language: [ID](#) | DOI: [10.14710/jil.20.3.628-636](https://doi.org/10.14710/jil.20.3.628-636)
Received: 24 Feb 2022; Revised: 13 Mar 2022; Accepted: 21 Mar 2022; Available online: 2 Apr 2022; Published: 5 Jul 2022.

Analisis Kesejahteraan Masyarakat Petani Dataran Tinggi Dieng Menggunakan Pendekatan Penghidupan Berkelanjutan PDF
637-
645

Isna Rahmawati, Iwan Rudiarto

| Language: [ID](#) | DOI: [10.14710/jil.20.3.637-645](https://doi.org/10.14710/jil.20.3.637-645)
Received: 25 Feb 2022; Revised: 15 Mar 2022; Accepted: 26 Mar 2022; Available online: 7 Apr 2022; Published: 5 Jul 2022.

Analisis Korelasi Tingkat Pemahaman Masyarakat terhadap Perilaku Pemilahan dan Pengolahan Sampah di Dusun Pade Mare Lombok Utara PDF
646-
653

Lolom Hutabarat, Agnes Mulyani

| Language: [ID](#) | DOI: [10.14710/jil.20.3.646-653](https://doi.org/10.14710/jil.20.3.646-653)
Received: 14 Feb 2022; Revised: 17 Mar 2022; Accepted: 24 Mar 2022; Available online: 3 Apr 2022; Published: 5 Jul 2022.

Pengukuran Tingkat Eko-Efisiensi Batik Cap Menggunakan Metode Life Cycle Analysis (Studi Kasus: Batik Encim pada Kampong Batik Kauman Pekalongan) PDF
654-
664

Novie Susanto, Thomas Triadi Putranto

| Language: [ID](#) | DOI: [10.14710/jil.20.3.654-664](https://doi.org/10.14710/jil.20.3.654-664)
Received: 5 Mar 2022; Revised: 31 Mar 2022; Accepted: 4 Apr 2022; Published: 5 Jul 2022.

Pengaruh Bahan Baku Kompos terhadap Pertumbuhan dan Produksi Cabai Merah Keriting (Capsicum annuum L.) PDF
665-
671

Tiwit Widowati, Nuriyanah Nuriyanah, Liseu Nurjanah, Sylvia J.R. Lekatompessy, Rumella Simarmata

| Language: [ID](#) | DOI: [10.14710/jil.20.3.665-671](https://doi.org/10.14710/jil.20.3.665-671)
Received: 2 Mar 2022; Revised: 28 Mar 2022; Accepted: 2 Apr 2022; Available online: 10 Apr 2022; Published: 4 Jul 2022.

Potensi Dampak Lingkungan Pada Industri Minyak Goreng Sawit Dengan Metode Life Cycle Assessment PDF
672-
677

Serkiyani Adyaksa Krisi, M. Abu Jami'in, Mirna Apriani

| Language: [EN](#) | DOI: [10.14710/jil.20.3.672-677](https://doi.org/10.14710/jil.20.3.672-677)

Received: 15 Feb 2022; Revised: 1 Apr 2022; Accepted: 4 Apr 2022; Available online: 11 Apr 2022; Published: 5 Jul 2022.

Komposisi Rayap Dapat Menentukan Tingkat Ketergangguan Habitat : Studi Kasus di
Kabupaten Dharmasraya Provinsi Sumatera Barat

[PDF](#)
678-
684

Sri Heriza, Damayanti Buchori, Idham Sakti Harahap, Nina Maryana

| Language: [ID](#) | DOI: [10.14710/jil.20.3.678-684](https://doi.org/10.14710/jil.20.3.678-684)

Received: 12 Jan 2022; Revised: 11 Mar 2022; Accepted: 30 Mar 2022; Available online: 12 Apr 2022; Published: 4 Jul 2022.

Penilaian Daur Hidup Proses Distribusi BBM di PT Pertamina (Persero) Fuel Terminal
Parepare

[PDF](#)
685-
695

Putri Alifa Kholil, Mochamad Arief Budihardjo, Fuad Muhammad, Ketut Karno

| Language: [ID](#) | DOI: [10.14710/jil.20.3.685-695](https://doi.org/10.14710/jil.20.3.685-695)

Received: 24 Jan 2022; Revised: 28 Mar 2022; Accepted: 12 Apr 2022; Available online: 20 Apr 2022; Published: 4 Jul 2022.

Short Communication: Biochemistry Analysis and Molecular Approach to Identify the Cultured Bacterial from Ex-Tin Mining Lakes

Andri Kurniawan^{1*}, Oedjijono², Tamad³, Uyi Sulaeman⁴

¹Department of Aquaculture, University of Bangka Belitung; email: andri_pangkal@yahoo.co.id

²Faculty of Biology, University of Jenderal Soedirman

³Faculty of Agriculture, University of Jenderal Soedirman

⁴Faculty of Mathematics and Natural Sciences, University of Jenderal Soedirman

ABSTRACT

There are two methods to identify the bacterial characteristic, namely biochemical analysis and the 16S ribosomal ribonucleic acid gene (16S rRNA) sequencing analysis. The research aimed to identify the cultured bacterial from ex-tin mining lakes by biochemistry analysis and molecular approach. Nine bacterial were cultured and isolated in nutrient agar and then biochemically characterized by microbact™ 12A and 24E (Oxoid) identification kits. In addition, molecular analysis by 16S rRNA gene was sequenced primer 1492R and primer 27F. Based on biochemistry analysis, these bacterial were identified as belonging to species of *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *Enterobacter aerogenes*; *Enterobacter agglomerans*; and *Nitrobacter* spp. The sequence analysis in gene bank of NCBI indicated that these species had similarity with *Klebsiella variicola* strain F2R9 (Accession NR_025635.1); *Enterobacter cloacae* subsp. *dissolvens* strain LMG 2683 (Accession NR_044978.1); *Serratia marcescens* strain NBRC 102204 (Accession NR_114043.1); *Bacillus marisflavi* strain TF-11 (Accession NR_118437.1); *Falsibacillus pallidus* strain CW 7 (Accession NR_116287.1); *Klebsiella pneumoniae* strain DSM 30104 (Accession NR_117683.1); and *Nitrobacter winogradskyi* strain Nb-255 (Accession NR_074324.1). However, phylogenetic tree was constructed by Neighbor-Joining Test showed the cultured bacterial were not in the same clade and also with *Salmonella enterica* subsp. *enterica* strain LT2 (Accession NR_074910.1); *Bacillus amyloliquefaciens* strain BCRC 11601; and *Escherichia coli* strain NBRC 102203 (Accession NR_114042.1) as in group species and *Micrococcus luteus* strain NCTC 2665 (Accession NR_075062.2); *Chloroflexus islandicus* strain isl-2 (Accession NR_148571.2); *Flavobacterium gondwanense* (Accession M92278.1); and *Cytophaga aurantiaca* strain JM110 (Accession MN758870.1) as their out group.

Keywords: bacterial, biochemistry, ex-tin mining lakes; molecular; 16S rRNA gene

ABSTRAK

Terdapat dua metode untuk mengidentifikasi karakteristik bakteri, yaitu analisis biokimia dan analisis sekuensing gen 16S ribosomal ribonucleic acid (16S rRNA). Karakterisasi bakteri telah dilakukan melalui analisis morfologi dan biokimia dan dikonfirmasi melalui pendekatan molekuler menggunakan sekuensing gen 16S ribosomal ribonucleic acid (16S rRNA). Penelitian ini bertujuan untuk mengidentifikasi bakteri yang dapat dikultur dari danau pascatambang timah melalui analisis biokimia dan pendekatan molekuler. Sembilan bakteri berhasil dikultur dan diisolasi di media nutrient agar dan kemudian secara biokimia dikarakterisasi menggunakan microbact™ 12A and 24E (Oxoid) identification kits. Lebih lanjut, analisis molekuler menggunakan gen 16S rRNA dilakukan sekuensing dengan primer 1492R dan primer 27F. Berdasarkan analisis biokimia, bakteri-bakteri tersebut termasuk ke dalam spesies *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *Enterobacter aerogenes*; *Enterobacter agglomerans*; dan *Nitrobacter* spp. Analisis blasting pada *gene bank* di NCBI mengindikasikan bahwa spesies-spesies tersebut memiliki kemiripan atau similaritas dengan *Klebsiella variicola* strain F2R9 (Accession NR_025635.1); *Enterobacter cloacae* subsp. *dissolvens* strain LMG 2683 (Accession NR_044978.1); *Serratia marcescens* strain NBRC 102204 (Accession NR_114043.1); *Bacillus marisflavi* strain TF-11 (Accession NR_118437.1); *Falsibacillus pallidus* strain CW 7 (Accession NR_116287.1); *Klebsiella pneumoniae* strain DSM 30104 (Accession NR_117683.1); dan *Nitrobacter winogradskyi* strain Nb-255 (Accession NR_074324.1). Namun, pohon filogenetik yang dikonstruksikan dengan Neighbor-Joining Test menunjukkan bahwa bakteri yang dikultur tersebut tidak berada pada *clade* dan juga dengan *Salmonella enterica* subsp. *enterica* strain LT2 (Accession NR_074910.1); *Bacillus amyloliquefaciens* strain BCRC 11601; dan *Escherichia coli* strain NBRC 102203 (Accession NR_114042.1) yang digunakan sebagai spesies *in group species* maupun *Micrococcus luteus* strain NCTC 2665 (Accession NR_075062.2); *Chloroflexus islandicus* strain isl-2 (Accession NR_148571.2); *Flavobacterium gondwanense* (Accession M92278.1); dan *Cytophaga aurantiaca* strain JM110 (Accession MN758870.1) sebagai *out group*nya.

Kata kunci: bakteri, biokimia, danau pascatambang timah, molekuler, gen 16S rRNA

Citation: Kurniawan, A., Oedjijono, Tamad, Sulaeman, U. (2022). Short Communication: Biochemistry Analysis and Molecular Approach to Identify the Cultured Bacterial from Ex-Tin Mining Lakes. Jurnal Ilmu Lingkungan, 20(3), 563-569, doi:10.14710/jil.20.3.563-569

* Penulis Korespondensi: andri_pangkal@yahoo.co.id

1. Introduction

Tin mining activity in Bangka Belitung Archipelago Province produces a water in a mined land looked like a lake, called kolong. The waters have poor characteristics such as low dissolved oxygen (DO) (Ashraf et al. 2011), cation exchange capacity (CEC), poor nutrient and organic component (Oktavia et al. 2014), acidic pH, and also heavy metals contamination (Kurniawan et al. 2019). Tin mining activities can also cause damage to the ecology, include distraction and alteration of microorganisms' ecology and functional stability of microbial community (Kurniawan et al. 2018; Kurniawan, 2016; Li et al. 2014). The ecological damage due to mining activities causes ecosystem imbalance and also changes in the diversity of microorganisms (Lad & Samant, 2015; Giri et al. 2014; Singh et al. 2013; Ashraf et al. 2010; Vyas and Pancholi, 2009; Fan et al. 2002).

The microorganisms' capability to responds the ecosystem changes quickly can be utilized as an indicator to understanding the changes of water quality (Lau & Lennon, 2012; Moscatelli et al., 2005; Niemi & McDonald, 2004; Paerl et al., 2003). This is used to link the relationship between environmental changes to the microbial diversity because they have role in biogeochemical and biotransformation cycles in the biosphere (Gadd, 2010; Prosser et al., 2007). It is important for understanding the microbial community diversity, structures, dynamics, and functional. The existence and biochemical characteristics of microorganisms in an environment can be known by growing them in synthetic medium and identification by 16S rRNA gene. This gene sequence is a biological marker that is widely studied to explain the existence of microbe in an environment, molecular evolution for

taxonomic classification, and microbial phylogenetic analysis. The 16S rRNA gene has a hypervariable region so that it can be used to identify microbes (Yang et al. 2016; Lozupone and Knight, 2008).

This research aimed to identify the diversity of microbes, especially the cultured bacterial from ex-tin mining lakes in Bangka Regency, Indonesia. Identification of bacterial by molecular approach showed name of species based on the gene, besides biochemical characteristics. This research showed the potential of bacterial as a bioindicator and their role in the ecosystem with their characteristics of biochemistry. Further, their capability as bioremediator can be elaborated to remediate and recover the waters quality of abandoned tin mining lakes.

2. Method

2.1. Study area

The research stations were located in Bangka Regency, Bangka Belitung Archipelago Province, Indonesia. The research areas were encoded as Station A (lake < 1 year), Station B (lake 5-10 years), and Station C (lake > 15 years). The coordinates 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 coordinates 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 coordinates 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" (Figure 1) (Kurniawan et al. 2018).

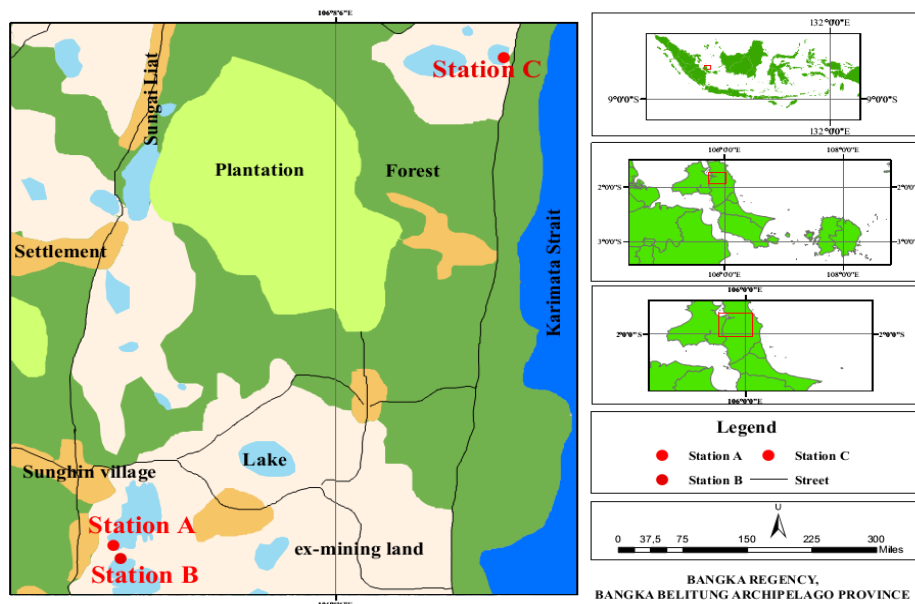


Figure 1 Research location along with the research stations in Bangka Regency, Bangka Belitung Province Archipelago. Station (A) was ex-tin mining lake in < 1 year; Station (B) 5-10 years; Station (C) > 15 years

2.2. Identification of the cultured bacterial

The bacterial of ex-tin mining lakes were isolated by nutrient agar (NA) and showed nine bacterial isolates (Table 1) that were prepared for biochemistry analysis. The biochemical characteristics of cultured bacterial isolates were identified by microbact™ 12A and 24E (Oxoid) identification kits (Osuntokun et al. 2018). While, molecular analysis was done by 16S rRNA gene analysis with primer 1492R (5'GGTTACCTTGTTACGACTT3') as reverse primer and primer 27F (5'GAGTTTGATCAT GGCTCAG3') as forward primer for Polymerase Chain Reaction (PCR). The DNA template was prepared from an individual colony of each species of the cultured bacterial and then the amplification of the 16S rRNA gene was carried out by PCR. The denaturation process in PCR was occurred at 94 °C (2 min), annealing process at 94 °C (1 min) with 35 cycles, and a final extension at 72 °C (3 min) (Senthilraj et al. 2016). The product quality of PCR was visualized by 0.80% agarose gel with amount of DNA ladder loaded per lane 0.1 µg, 1 kb DNA ladder (bp), and volume of sample loaded per lane was 1 µL.

2.3. Sequence analysis

Sequence analysis was carried out by First Base Agent. Sequence alignments were analyzed by Program BioEdit and then were compared with bacterial genes in National Center for Biotechnology Information (NCBI) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The phylogenetic tree was constructed with Neighbor-Joining Test in Program Mega 6.06. The phylogenetic tree for sequences of samples was constructed and compared with *Klebsiella variicola* strain F2R9 (Accession NR_025635.1); *Enterobacter cloacae subsp. dissolvens* strain LMG 2683 (Accession NR_044978.1); *Serratia marcescens* strain NBRC 102204 (Accession NR_114043.1); *Bacillus marisflavi* strain TF-11 (Accession NR_118437.1); *Falsibacillus pallidus* strain CW 7 (Accession NR_116287.1); *Klebsiella pneumoniae* strain DSM 30104 (Accession NR_117683.1); *Nitrobacter winogradskyi* strain Nb-255 (Accession NR_074324.1); *Salmonella enterica* subsp. enterica strain LT2 (Accession NR_074910.1); *Bacillus amyloliquefaciens* strain BCRC 11601 (Accession NR_116022.1); and *Escherichia coli* strain NBRC 102203 (Accession NR_114042.1) as in group species of Phylum Proteobacteria and Firmicutes of Kingdom Bacteria. While, out group species for the phylogenetic tree were *Micrococcus luteus* strain NCTC 2665 (Accession NR_075062.2) from Phylum Actinobacteria; *Chloroflexus islandicus* strain isl-2 (Accession NR_148571.2) from Phylum Chloroflexi;

Flavobacterium gondwanense (Accession M92278.1) from Phylum Bacteroidetes; and *Cytophaga aurantiaca* strain JM110 (Accession MN758870.1) from Phylum Cytophagia.

3. Result

Mustikasari The biochemistry analysis showed some characteristics of nine cultured bacterial (bac 1, bac 2, and bac 3 from Station A; bac 4, bac 5, and bac 6 from Station B; and bac 7, bac 8, and bac 9 from Station C). Kurniawan et al. (2018) have reported some biochemical characteristics of these cultured bacterial such as gram, oxidase, motility, ornithin, glucosa, indole, Voges-Proskauer (V-P), citrate, and catalase. The other properties of biochemistry was investigated (Table 1) and these characteristics indicated bacterial of *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *E. aerogenes*; *E. agglomerans*; and *Nitrobacter* spp.

These cultured bacterial was isolated and identified their DNA with PCR. The product of PCR showed the DNA quality and estimation of base pair (bp) of the 16S rRNA gene were about 1,400-1,500 bp (Figure 2).

The sequence analysis produced profile of sequences and then they were blasted in NCBI. The sequence analysis of 16S rRNA gene showed that the name of bacterial species did not represent the results of biochemistry analysis, there were differences species of these bacterial, although the blast of NCBI website showed that the cultured bacterial had high (90-100 %) similarity with strains were used as in group species. The research evidence revealed species name which analyzed by biochemistry approach to the cultured bacterial were different with blasting investigation in gene bank of NCBI.

The phylogenetic tree (Figure 3) was constructed by involving bacterial of in group species and out group species. All of the cultured bacterial were not in the same clade with in group species such as *K. variicola* strain F2R9; *E. cloacae subsp. dissolvens* strain LMG 2683; *S. marcescens* strain NBRC 102204; *B. marisflavi* strain TF-11; *F. pallidus* strain CW 7; *K. pneumoniae* strain DSM 30104; *N. winogradskyi* strain Nb-255; *S. enterica* subsp. enterica strain LT2; *B. amyloliquefaciens* strain BCRC 11601; and *E. coli* strain NBRC 102203. Further, the phylogenetic tree showed sequences of the cultured bacterial were also different form their outgroup species such as *M. luteus* strain NCTC 2665 from Phylum Actinobacteria; *C. islandicus* strain isl-2 from Phylum Chloroflexi; *F. gondwanense* from Phylum Bacteroidetes; and *C. aurantiaca* strain JM110 from Phylum Cytophagia.

Table 1. Biochemistry Characteristics of Bacterial by Microbact™ 12A and 24E

Biochemistry Characteristics	Research Stations								
	Station A			Station B			Station C		
	bac 1	bac 2	bac 3	bac 4	bac 5	bac 6	bac 7	bac 8	bac 9
Gram*	+	+	-	-	-	-	-	-	-
Spore	+	+	-	-	-	-	-	-	-
Oxidase*	+	+	-	+	-	+	+	+	-
Motility*	-	-	+	-	+	-	-	-	+
Nitrate	+	+	+	+	+	+	+	+	+
Lysin	-	-	+	-	+	-	-	-	+
Ornithin*	-	-	+	-	+	-	-	-	-
H ₂ S	-	-	+	-	-	-	-	-	-
Glucosa*	-	-	+	+	+	+	+	+	+
Mannitol	-	-	+	-	+	-	-	-	+
Xylose	-	-	+	+	+	+	+	+	+
ONPG	+	+	+	-	+	-	-	-	+
Indole*	-	-	-	-	-	-	-	-	+
Urease	-	-	+	-	-	-	-	-	+
V-P*	+	+	+	+	+	+	+	+	+
Citrate*	-	-	+	-	+	-	-	-	+
TDA	-	-	-	-	-	-	-	-	-
Gelatine	-	-	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-	-	-
Inositol	+	+	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-	-	-
Catalase*	+	+	-	-	-	-	-	-	-
Coagulase	-	-	-	-	-	-	-	-	-
Haemolysis	Beta	Beta	Alpha	Alpha	Alpha	Alpha	Alpha	Alpha	Alpha
Sensitive novobiosin	No	No	No	No	No	No	No	No	No
Starch hydrolysis	-	-	-	-	-	-	-	-	-
Casein hydrolysis	+	+	-	-	-	-	-	-	-

Legend: Station A (lake < 1 year), Station B (lake 5-10 years), and Station C (lake > 15 years). The asterisk (*) and biochemistry analysis indicated species *Bacillus amyloliquefaciens* (bac 1); *Bacillus amyloliquefaciens* (bac 2); *Enterobacter gergoviae* (bac 3); *Nitrobacter* spp. (bac 4); *Enterobacter aerogenes* (bac 5); *Nitrobater* spp. (bac 6); *Nitrobater* spp. (bac 7); *Nitrobater* spp. (bac 8); and *Enterobacter agglomerans* (bac 9) (Kurniawan *et al.*, 2018).

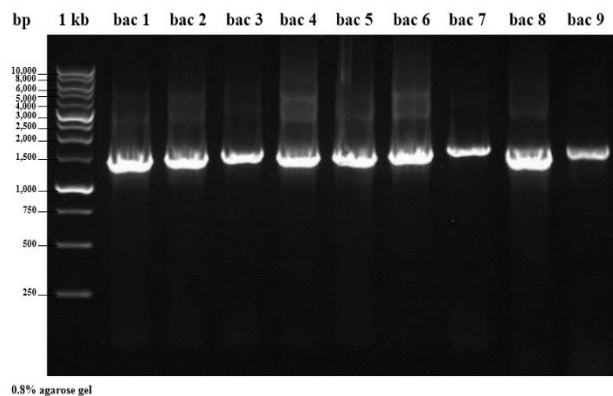


Figure 2 The PCR quality of nine cultured bacterial species for sequencing process

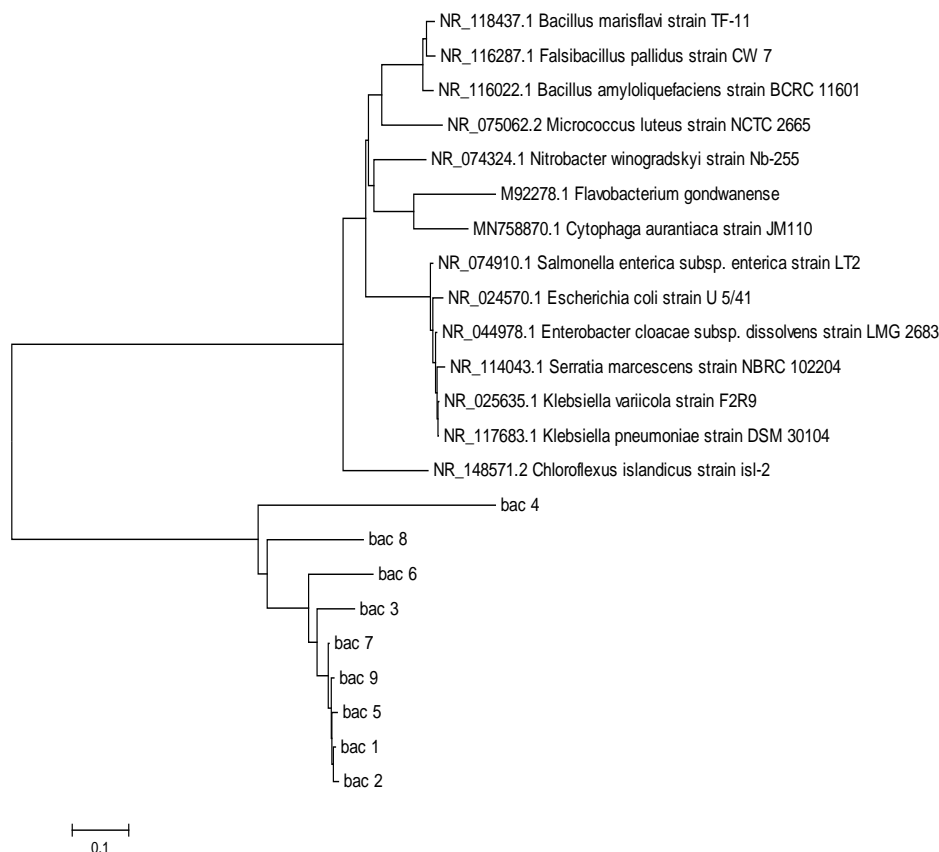


Figure 3 The phylogenetic tree showed that cultured bacterial were different from some species sequences database of NCBI. The biochemistry analysis indicated species *Bacillus amyloliquefaciens* (bac 1); *Bacillus amyloliquefaciens* (bac 2); *Enterobacter gergoviae* (bac 3); *Nitrobacter* spp. (bac 4); *Enterobacter aerogenes* (bac 5); *Nitrobater* spp. (bac 6); *Nitrobater* spp. (bac 7); *Nitrobater* spp. (bac 8); and *Enterobacter agglomerans* (bac 9)

4. Discussion

The biochemistry analysis served as preliminary characterization of bacterial and this identification test gives some information about morphology, physiology, chemistry, and what these microorganisms were able to do with their specific biochemical functions. While, molecular methods are always useful to identify microbes to the species or strain (Franco-Duarte et al. 2019; Bochner, 2009). The biochemical properties of nine cultured bacterial were included as species of *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *E. aerogenes*; *E. agglomerans*; and *Nitrobacter* spp. However, they can't be justified enough as those species because the molecular analysis by 16S rRNA gene did not indicate them. Approximately, more than 1400 bp in length of the 16S rRNA genes of nine cultured bacterial were sequenced. Analysis of these sequences confirmed that species were most similar to biochemistry identification species. For examples bac 1 and bac 2 namely species of *Bacillus amyloliquefaciens* by biochemistry analysis showed the different clade with *B. amyloliquefaciens* strain BCRC 11601 (Accession NR_116022.1) in gene bank of NCBI.

The results of all 16S rRNA gene sequencing presented different group with the blasted species.

They were not in the same group or clade, although they had similarity blasting percentage > 90-100% with species of Phylum Proteobacteria and Firmicutes and also so different with the other bacterial from out group species of Phylum Actinobacteria, Chloroflexi, Bacteroidetes, and Cytophagia. The 16S rRNA gene sequence has about 1,550 bp in length and this gene has differentiation at the genus level of bacterial. This gene usually related to more than one individual which the similar sequences (Clarridge, 2004).

The 16S rRNA gene as genetic marker play an important role in identification process of bacterial, the discovery of novel species, taxonomy, and also to construct the bacterial phylogeny (Al Kaabi & Al Yassari, 2019; Manjul & Shirkot, 2018; Woo et al., 2008). Whatever the explanation of this discordance, the discrepancy between these two methods gave important information. Their biochemical characteristics can be explored and elaborated to be used as biological profile of cultured bacterial from ex-tin mining lake. They can be used for various purposes such as bioremediation of ex-tin mining waters ecosystem. It due to their capacity as bioremeditors of heavy metals, wastewater, and organic pollution in the environment (Li et al. 2019; Badiefar et al. 2015; Sonia

et al. 2015; Cardak & Altug, 2014; Raja et al. 2014; Amin et al. 2013; Ogot et al. 2013; Naggat et al. 2010). In spite of their biochemical characteristics indicated their roles, however the further researches are needed to verify them. Based on the analysis of 16S rRNA gene has proven the similarity and relationship of cultured bacterial gene with the other bacterial from gene bank. The results of this study have convinced that the cultured bacterial were different with blasting results. Nevertheless, the advanced researches are also needed to prove them as novel species of bacterial.

5. Conclusion

Thirty-three of biochemical properties from the cultured bacterial were used for identification of them include carbohydrate, amino acid, and lipid utilization or degradation, gram characteristic, motility, sulphuric activity, etc. In this study, species of bacterial from ex-tin mining lakes in Bangka Regency were isolated in NA and identified with microbact test kits. There were nine cultured species from ex-tin mining waters in this study. The biochemistry analysis showed bacterial were identified as belonging to species of *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *E. aerogenes*; *E. agglomerans*; and *Nitrobacter* spp. However, phylogenetic tree was constructed by Neighbor-Joining Test showed the cultured bacterial were not in the same clade with the blasted species from gene bank of NCBI. Those bacterial were not similar with some bacterial of gene bank such as species from Genus *Bacillus*, *Enterobacter*, *Nitrobacter*, *Klebsiella*, *Serratia*, *Falsibacillus*, *Salmonella*, and *Escherichia*. They indicated the different clade with them and also with species of Genus *Micrococcus*, *Chloroflexus*, *Flavobacterium*, and *Cytophaga*. In this study, we indicate a new species bacterial were found, although this claim must be proven by further research.

Acknowledgment

I would like to thank University of Bangka Belitung for supporting in this research and publication.

Conflict of Interest

The author declares that there is no conflict of interest in this publication.

References

Al Kaabi, H., Al-Yassari, A. 2019. 16SrRNA sequencing as tool for identification of *Salmonella* spp isolated from human diarrhea cases. Journal of Physics: Conference Series 1294(6): 062041. IOP Publishing
 Amin, A., Naik, A., Azhar, M., Nayak H. 2013. Bioremediation of different waste waters-a review. Continental J. Fisheries and Aquatic Science 7(2): 7-17

Ashraf, M., Maah, M., Yusoff, I. 2010. Study of water quality and heavy metals in soil & water of ex-mining area Bestari Jaya, Peninsular Malaysia. International Journal of Basic & Applied Sciences 10(3): 7-12
 Ashraf, M., Maah, M., Yusoff, I. 2011. Analysis of physio-chemical parameters and distribution of heavy metals in soil and water of ex-mining area of Bestari Jaya, Peninsular Malaysia. Asian Journal of Chemistry 23(8): 3493-3499
 Badiefar, L., Yakhchali, B., Rodriguez-Couto, S., Veloso, A., Garcia-Arenzana, J., Matsumura, Y., Khodabandeh M. 2015. Biodegradation of bisphenol a by the newly-isolated *Enterobacter gergoviae* strain BYK-7 enhanced using genetic manipulation. RSC Advance 5(37): 29563-29572
 Bochner, B. 2009. Global phenotypic characterization of bacteria. FEMS Microbiology Reviews 33(1): 191-205.
 Çardak, M., Altug, G. 2014. Species distribution and heavy metal resistance of *Enterobacteriaceae* members isolated from Istanbul Strait. Fresenius Environmental Bulletin 23(10a): 2620-2626
 Clarridge, J. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinical Microbiology Reviews 17(4): 840-862.
 Fan, Y., Lu, Z., Chen, J., Zhou, Z., Wu, G. 2002. Major ecological and environmental problems and the ecological reconstruction technologies of the coal mining areas in China. Acta Ecologica Sinica 23(10): 2144-2152
 Franco-Duarte, R., Cernakova, L., Kadam, S., Kaushik, K., Salehi, B., Bevilacqua, A., ... & Rodrigues, C. 2019. Advances in chemical and biological methods to identify microorganisms-from past to present. Microorganisms 7(5): 1-32
 Gadd, G. 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156(3): 609-643
 Giri, K., Mishra, G., Pandey, S., Verma, P., Kumar, R., Bisht N. 2014. Ecological degradation in northeastern coal fields: Margherita Assam. International Journal of Science, Environment and Technology 3(3): 881-884
 Kurniawan, A. (2016). Microorganism communities response of ecological changes in post tin mining ponds. Research & Reviews: A Journal of Microbiology and Virology 6(1): 17-26
 Kurniawan, A., Oedjijono., Tamad., Sulaeman, U. 2018. The phosphorus and sulphur distribution and culturable bacterial in time chronosequence of ex-tin mining ponds. Omni-Akuatika 14(3): 26-33
 Kurniawan, A., Oedjijono., Tamad., Sulaeman, U. 2019. The pattern of heavy metals distribution in time chronosequence of ex-tin mining ponds in Bangka Regency, Indonesia. Indonesian Journal of Chemistry 19(1): 254-261.
 Lad, R., Samant, J. 2015. Impact of bauxite mining on soil: a case study of bauxite mines at Ud giri, Dist-Kolhapur, Maharashtra State, India. International Research Journal of Environment Sciences 4(2): 77-83
 Lau J., Lennon J. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences 109(35): 14058-14062
 Li, Y., Chi, M., Ge, X. 2019. Identification of a novel hydrolase encoded by *hy-1* from *Bacillus amyloliquefaciens* for bioremediation of carbendazim contaminated soil and

- food. *International Journal of Agricultural and Biological Engineering* 12(2): 218-224.
- Li, Y., Wen, H., Chen, L., Yin, T. 2014. Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. *PLoS ONE* 9(12): e115024
- Lozupone, C., Knight, R. 2008. Species divergence and the measurement of microbial diversity. *FEMS Microbiology Reviews* 32(4): 557-578.
- Manjul, A., Shirkot, P. 2018. 16S rRNA gene sequencing for bacterial identification of pullulanase synthesizing thermophilic bacteria contributing to big data. *International Journal of Chemical Studies* 6(2): 2769-2773
- Moscatelli, M., Lagomarsino, A., Marinari, S., De Angelis, P., Grego, S. 2005. Soil microbial indices as bioindicators of environmental changes in a poplar plantation. *Ecological Indicators* 5(3): 171-179.
- Naggar, A., Kamel, M., Aladly, A., Ismail, N. 2010. Bioremediation of paraffinic and polynuclear aromatic hydrocarbons using laser irradiated *Bacillus amyloliquefaciens*. *Journal of American Science* 6(10): 661-670
- Niemi, G., McDonald, M. 2004. Application of ecological indicators. *Annual Review of Ecology, Evolution, and Systematics* 35(2004): 89-111
- Ogot, H., Boga, H., Budambula, N., Tsanuo, M., Andika, D., Ogola H. 2013. Isolation, characterization and identification of roundup degrading bacteria from the soil and gut of *Macrotermes michaelseni*. *International Journal of Microbiology and Mycology* 1(1): 31-38
- Oktavia, D., Setiadi, Y., Hilwan, I. 2014. Sifat fisika dan kimia tanah di hutan kerangas dan lahan pasca tambang timah Kabupaten Belitung Timur. *Jurnal Silvikultur Tropika* 5(3): 149-154
- Osuntokun, O., Jemilaiye, T., Thonda, A. 2018. Phenotypic identification and antibiogram profile of citrobacter species. *Journal of Clinical Research and Pharmacy* 1(1): 1-6
- Paerl, H. W., Dyble, J., Moisaner, P., Noble, R., Piehler, M., Pinckney, J., ..., aldes, L. 2003. Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. *FEMS Microbiology Ecology* 46(3): 233-246
- Prosser, J., Bohannon, B., Curtis, T., Ellis, R., Firestone, M., Freckleton, R., ..., Young, J. 2007. The role of ecological theory in microbial ecology. *Nature Reviews Microbiology* 5(5): 384-392
- Raja, S., Dinesh, K., Kesavan, K., Kodungallur, T., Thrissur, K. 2014. Bioremediation by using of microbes and algae with special reference to coastline environment. *International Journal of Biosciences and Nanosciences* 1(6): 130-140
- Senthilraj R., Prasad G., Janakiraman K. 2016. Sequence-based identification of microbial contaminants in non-parenteral products. *Brazilian Journal of Pharmaceutical Sciences* 52(2): 329-336
- Singh, P. K., Afzal, I., Ravi, S., Dhanesh, S., Shivi, S. 2013. A Study about ecological imbalance in Surguja (India) coalfield area due to mining. *International Research Journal of Environment Sciences* 2(4): 10-14
- Sonia, V., Rajagopalsamy, C., Ahilan, B., Francis, T. 2015. Microbial bioremediation: a potential tool for sustainable aquaculture. *Journal of Industrial Pollution Control* 31(1): 105-113
- Vyas, A., Pancholi, A. 2009. Environmental degradation due to mining in South Rajasthan: a case study of Nimbahera, Chittorgarh (India). *Journal of Environmental Research and Development* 4(2): 405-412
- Woo, P., Lau, S., Teng, J., Tse, H., Yuen, K. 2008. Then and now: use of 16s rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clinical Microbiology and Infection* 14(10): 908-934
- Yang, B., Wang, Y., Qian, P. 2016. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics* 17(1): 135-143