DATABASE CREATION AND MAPPING OF DISCOVERED EXTREMOPHILES IN INDONESIA

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i3L – Indonesia International Institute for Life Sciences

School of Life Sciences

In partial fulfillment of the requirements for the Bachelor of Science in

Bio Technology

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2020

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Abstract

Background – Indonesia, which possesses diverse geographies, holds a big potential for extremophile exploration. Despite all the research conducted to identify extremophiles in Indonesia, no study has compiled all of these findings yet.

Objective – This study aims to compile and map all of the discovered extremophiles in Indonesia to further address the current state of research on Indonesian extremophiles.

Methodology – A database containing information of organism's name, type of extremophile, type of environment, location of discovery, location's physicochemical condition, location's province, potential application, publication year, journal source, research title, and link source was created. Using the database, information as follows was presented visually: Total amount of extremophiles research used in this study per year in Indonesia (column chart), total amount of extremophiles per type in Indonesia (pie chart), total number of extremophiles based on Indonesian region (pie chart), and total amount of extremophiles type based on type of environment in Indonesia (pie chart). The database was then mapped by using Google My Maps and published by using WIX web application.

Result and Conclusion – The database could be accessed in http://bit.ly/extremoresearchid, and the map could be accessed in https://victorsandyawan.wixsite.com/extremoresearchid. While the extremophile research in Indonesia is currently lacking, there is an abrupt increase of interest in this topic. Most of the discovered extremophiles are thermophile which are majorly obtained from volcanic-hot spring. A collaboration with researchers taking interest in Indonesian extremophile is recommended to futher improve the data collection for this map.

Keywords: Extremophile, Indonesia, Database, Map, Research

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List of Abbreviations

EEZ	**Economic Exclusive Zone**
DD	**Decimal degrees**
DMS	**Degrees, minutes, seconds**
URL	**Uniform Resource Locator**

CHAPTER I: INTRODUCTION

1.1 Background

Indonesia is the world's largest archipelago. Each of Indonesia's region has its own unique geography, making Indonesia one of the most biodiverse country in the world. Indonesia's diverse nature includes extreme environment as it is located in Pacific Ring of Fire, a region with active tectonic plate movements and volcanic eruptions, creating naturally harsh environments where organisms must adapt in order to survive (ICAO, 2007). The organisms capable to survive in these extreme conditions are called extremophiles. This classification includes every branch of organism: Bacteria, Archaea, and Eukarya (Rampelotto, 2013).

Extremophiles are classified based on the extreme conditions where the organisms are able to survive: extreme temperature (thermophile and psychrophile), extreme pH (acidophile and alkaliphile), extreme pressure (barophile), extreme amount of metal and/ or chemical, and high ionizing radiation (radiophile). Several organisms may also be polyextremophiles, allowing them to withstand two or more extreme conditions (Rampelotto, 2013).

Extremophiles have certain unique physiology that enables these organisms to sustain its survival. This unique physiology enables extremophiles to improve the current state of biotechnology. A famous example is *Thermus aquaticus* which possesses thermostable DNA polymerase. *T. aquaticus*' DNA polymerase, named taq polymerase, is currently widely adopted for polymerase chain reaction (PCR) due to its activity in wide range of temperature (Bell, 1989).

1.2 Problem Formulation

Despite all the research conducted to identify extremophiles in Indonesia, no study has compiled all of these findings yet. A study to compile all discovered extremophiles in the form of table is necessary to ease the data finding. Visualization through mapping is also beneficial to help locating the discovered extremophiles for future research. This study would also track the progress of extremophiles' research in Indonesia by visualizing the total research papers publish per year.

To the best of the author's knowledge, this study is the first to compile, review, and map the discovered extremophiles in Indonesia.

1.3 Objectives

This study aims to compile and map all of the discovered extremophiles in Indonesia to further address the current state of research on Indonesian extremophiles.

1.4 Scope of Work

- **Data Collection**

The review is conducted to address the following research questions as foundation:

  - What species are the discovered extremophiles in Indonesia?
  - What types are the discovered extremophiles classified as?
  - Where are the extremophiles discovered in Indonesia?

In addition to these foundational questions, there are several aspects which would be documented as well:

  - What are the known functions of the discovered extremophiles?
  - What is the environment type of the sampling location?
  - What are the known physicochemical conditions of the sampling location?
  - What is the journal source of the respective research discovery?
  - When is the respective study published?

- **Data Analysis**
    - List all the collected information in the form of table
    - Create a map to facilitate the visualization of the collected information
    - Create a website as a platform to publish the map
    - Present the following summary using pie chart:
        - Total amount of extremophiles research used in this study per year in Indonesia
        - Total amount of extremophiles per type in Indonesia
        - Total number of extremophiles based on Indonesian region
        - Total amount of extremophiles type based on type of environment in Indonesia

CHAPTER II: LITERATURE REVIEW

2.1 Indonesia's Geography

Indonesia is the largest archipelagic country in the world, where 65% of its area is water. Indonesia has five largest islands: Sumatra, Java, Kalimantan (Borneo), Sulawesi, and Papua, where most of the population resides.

Indonesia's geography is very promising to conduct research on extremophiles. Indonesia has numerous locations where extremophiles discoveries are possible. In the sea, Indonesia owns 7000 m deep Java trench near Java and hydrothermal vents in Sulawesi (Bertolino et al., 2017; Masson et al., 1990). Indonesia is also located in the Ring of Fire where most tectonic activities take place; Therefore, Indonesia has numerous volcanoes. The mud volcano and volcanic hot springs have been found to harbor extremophiles (Gupta, Srivastava, Khare, & Prakash, 2014; Pikuta, Hoover, & Tang, 2007; Rampelotto, 2013).

2.2 Extremophiles

Among all known living things, there are organisms capable to survive in hostile and even lethal condition for common living creature. This group of organisms, known as extremophile, has been found to thrive in extreme temperature, pH, pressure, salinity, water availability, metal and/ or chemical concentration, and ionizing radiation. Extremophiles have been found 6.7 km deep inside the earth's crust, more than 10 km deep inside the ocean with pressures as far as 125 MPa, from extremely acidic (pH -0.06) to basic condition pH 12.5), and from intense temperature of deep sea hydrothermal vent (122°C) to frozen water (-15°C) (Merino et al., 2019; Rampelotto, 2013). Having adapted to its respective niche environment, extremophiles are able to tolerate certain extreme living condition and often require this condition to survive. To further facilitate its survival, extremophiles might also possess metabolisms towards sulfur, methane, and metals (Rampelotto, 2013).

Prokaryote is one of the earliest forms of organism which had occupied earth. Its simple structure and quick reproduction make prokaryote exceptionally easier to adapt with new environment, including extreme environment; Thus, most extremophiles are prokaryotes.

2.2.1 Temperature

Depending on the temperature, extremophile is grouped into thermophile and psychrophile. Thermophilic organism grows optimally above 50°C, while psychrophilic organism grows optimally at low and subzero temperature (Pikuta et al., 2007).

Thermophile is generally classified into moderate thermophile (optimum growth above 50°C), thermophile (optimum growth above 70°C), and hyperthermophile (optimum growth above 80°C). Thermophile has been found in hot springs, mud volcano, and hydrothermal vents around volcanically active area underwater (Madigan, 2000; Pikuta et al., 2007; S. et al., 2014). Thermophile's physiology has a wide diversity, each adjusted with its natural habitat; All of its cellular components, however, must be thermostable (Kelly & Adams, 1995; Ladenstein & Antranikian, 1998; Van de Vossenberg, Driessen, & Konings, 1998; Wiegel & Adams, 2003).

Pikuta, Hoover and Tang (2007) suggests several factors that increase thermal stability: Increase in secondary structure, aromatic stacking, hydrophobic interactions, metal binding capacity, and oligomerization, as well as decrease in loop length and labile residues (such as cysteine, asparagine, and glutamine) (Yano & Poulos, 2003). Fujiwara (2002) also suggests that the chromosomes of hyperthermophiles appear to be densely packed with genes required for essential functions. *Methanopyrus kandleri* strain 116 discovered from Kairei hydrothermal field grows at 122°C, which is the highest temperature for living condition ever recorded, making this organism the most hyperthermophilic organism currently known (Rampelotto, 2013).

Psychrophile is defined by organism which thrives in low and subzero temperature, and unable to grow at temperature above 15°C due to metabolic absence (Pikuta et al., 2007). Room temperature is lethal for psychrophile, as its enzymes and proteins are very sensitive and rapidly destabilize under this condition (Pikuta et al., 2007). There is no further classification of psychrophile due to the estimated limit of life seems to be around -20°C (D'Amico, Collins, Marx, Feller, & Gerday, 2006). Psychrophile inhabits all permanently cold environment from deep sea, mountains, and even polar regions (D'Amico et al., 2006).

Psychrophile adapts with this extreme condition commonly by several mechanisms. Generally, psychrophile's phospholipid membrane adapts by increasing the ratio of polyunsaturated-saturated fatty acids, conversion of trans fatty acid to cis-fatty acid, changing the composition of lipids in the membrane, and reducing the size or charge of lipid heads. Psychrophile enhances its membrane by upregulating lipopolysaccharide (LPS) biosynthetic gene, peptidoglycan biosynthesis, membrane transporter, outer membrane proteins, and exopolysaccharide (EPS) biosynthesis. Psychrophiles might produces ice nucleating protein and/ or antifreeze protein. Other common mechanisms are downregulation of flagellar motility and energy metabolism, biosynthesis of trehalose to stabilize cellular membranes, biosynthesis of carotenoid pigments to buffer membrane fluidity, and accumulation of osmotic molecules such as mannitol and glycine (Maayer, Anderson, Cary, & Cowan, 2014). *Rhodotolura glutinis* fungi found to cause frozen food spoilage is able to live at -18°C, making it the most psychrophilic organism currently known (Maayer et al., 2014).

2.2.2 pH

Extremophiles are classified into acidophile if it is able to thrive in acidic condition below pH 5 and alkaliphile if it thrives in alkalic condition above 8 (Johnson & Schippers, 2017).

Acidophile is further classified into moderate acidophile with optimal growth between pH 3-5 and extreme acidophile with optimal growth at or below 3 (Johnson & Schippers, 2017). Acidophile has been discovered in solfataric fields, sulfuric pools, geysers, acid sulfate soils, as well as metal and coal mine (Johnson & Schippers, 2017; Sharma & Kawarabayasi, 2012). Acidophile has proton impermeable membrane; This membrane properties is achieved either by creating tetraether lipids with ether linkages (instead of common bacterial ester linkage) to prevent acid hydrolysis or by creating rigid monolayers of bulky isoprenoid core (Sharma, Parashar, & Satyanarayana, 2016). Acidophile generates proton intracellularly to prevent other protons from entering the cell (Sharma et al., 2016). All acidophiles have cytoplasmic buffering molecules containing basic amino acids (lysine, histidine, arginine, etc) to capture protons and act as a buffer to prevent damage when there is a sudden increase presence of protons (Sharma et al., 2016). Acidophiles also have abundant secondary transporters and quick damage-fixing chaperones (Sharma et al., 2016). The currently known lowest pH for surviving acidophile is -0.06 which *Picrophillus oshimae* and P*icrophillus torridus* thrive in; Both of these organisms are discovered in solfataric hydrothermal area in Hokkaido, Japan (D'Amico et al., 2006; Rampelotto, 2013).

Alkaliphile grows optimally at pH above 8 (Gupta et al., 2014). This group of organisms has been found in ammonified soils, alkali soda lakes, and alkaline hydrothermal vents (Preiss, Hicks, Suzuki, Meier, & Krulwich, 2015). Alkaliphile utilizes cell walls containing acidic polymers to adsorb sodium and hydronium ions and repel hydroxide ions, or peptidoglycan with excess hexosamines and amino acid (Horikoshi, 1999). Alkaliphile has also been found to utilize sodium ions membrane transport to maintain the intracellular amount of Na^+ and

H^+ (Horikoshi, 1999). Cyanobacteria is the most alkaliphilic organism found currently; This organism is able to survive in pH between 12 to 13 (Pikuta et al., 2007).

2.2.3 Pressure

Barophile is organism with optimal growth at high pressure (above 40 MPa) (Pikuta et al., 2007). This group of organism is commonly found in deep lake and deep subsurface regions (Pikuta et al., 2007).

Barophile survives by having unsaturated fatty acids-packed cell membrane to increase membrane fluidity under high pressure, upregulated chaperones-encoding genes, modified respiratory chain, different porins expression, and osmolytes production (Jebbar, Franzetti, Girard, & Oger, 2015; Merino et al., 2019; Oger & Jebbar, 2010). The most resistant barophile known is *Thermococcus piezophilus* which survives up to 125 MPa (Merino et al., 2019).

2.2.4 Salinity

Halophile defines organism which requires salt to grow (Amoozegar, Safarpour, Noghabi, Bakhtiary, & Ventosa, 2019). It is classified based on the salt concentration where the organism is suitable to live: slight halophile (1-5% NaCl), moderate halophile (5-20% NaCl), and extreme halophile (20-30%) (Amoozegar et al., 2019). Halophiles are notably found in saline lakes, salt pans, salt marshes, and saline soil across the world (Amoozegar et al., 2019).

There are two fundamental strategies that halophile utilizes: maintaining high intracellular salt concentration equivalent or higher than the external salt concentration and maintaining low intracellular salt concentration within cytoplasm by balancing osmotic pressure through the use of organic solutes and ion pumps activation (Pikuta et al., 2007). *Haloferax mediterranei* is the most halophilic organism currently known (Han et al., 2012). It thrives at 30% NaCl seawater in seawater evaporation near Alicante in Spain (Han et al., 2012).

2.2.5 Ionizing Radiation

Radiophile stands for highly resistant organism towards high level of ionizing and ultraviolet radiation (Gupta et al., 2014). Radiophile is able to survive under starvation, oxidative stress, and high amount of DNA damage (Daly, 2000). The first and currently most resistant radiophile is *Deinococcus radiodurans* which survives 30 kGy during food conservation and storage (Pikuta et al., 2007). Makarova et al. (2001) concludes that *D. radiodurans* has expanded Nudix hydrolase and plant dessication resistance-associated proteins that contribute to the resistance on extreme radiation and numerous nucleotides repeats that may contribute on a role in stress response.

2.2.6 High Chemical Concentration

While not widely accepted, extremophiles capable to thrive in high concentration of metals are known as metallophiles (Gupta et al., 2014). Metallophiles have been found in mines and contaminated environments with high concentration of copper, lead, chromium, zinc, arsenic, cadmium, cobalt, silver, and other metals (Gupta et al., 2014). Metallophile applies the use of protein-metal association and heavy metal efflux system (active transport) to reduce the intracellular metal concentration (Gupta et al., 2014). *Acidithiobacillus caldus* KU and *Sulfolobus metallicus* BC have been found to be resistant towards toxic concentration of arsenic (Pikuta et al., 2007). Several species of *Acidiphillum spp.* are also resistant towards cadmium (Pikuta et al., 2007). *Ralstonia metallidurans* is found to be resistant with high concentrations of cadmium, chromium, cobalt, copper, mercury, nickel, lead, thallium, and zinc (Mergeay, 2006).

Several species of organisms have also adapted on environment with high chemical concentration (e.g. sulfur, methane). These organisms do not have any direct extremophile classification, even though they are sometimes called according with their ability, such as sulfur reducer and methanogen. One example of this organism is sulfur oxidizer *Halothiobacillus halophilus* (Pikuta et al., 2007).

2.3 Mapping Organism

An effort to geographically map organism has been highly valued in scientific communities. Organism mapping plays a crucial role in the subject of evolutionary biology research and to answer the question of “the origin of life” (Bowker, 2000). Organism mapping has also been used to keep track the spread of organisms to investigate certain organism’s behavior pattern (e.g. bird migration) (Schleidt & Crawley, 1980). The same method has also been used to keep track on the spread of organism to the previously uninhabited areas; This type of study is important to prevent significant social, economic, and ecological impacts such as the spread of disease (Etherington, 2012). Organism mapping would also be necessary to ease the search of the already discovered organisms.

This study proposed the creation of Indonesian extremophiles database from available studies by using mapping technique to significantly ease the search on this group of organisms.

CHAPTER III: MATERIALS AND METHODS

3.1 Data Collection

The dataset of extremophiles in Indonesia was obtained through Google Scholar, utilizing search terms in English (*Indonesian extremophiles, Indonesian thermophile, Indonesian psychrophile, Indonesian acidophile, Indonesian alkaliphile, Indonesian barophile, Indonesian halophile,* and *Indonesian radiophile*) and Indonesian language (*ekstremofil Indonesia, termofil Indonesia, psikrofil Indonesia, asidofil Indonesia, alkalifil Indonesia, barofil Indonesia, halofil Indonesia,* and *radiofil Indonesia*) from all timeframe and compiled in the form of table. The studies in the first 20 pages of Google Scholar result from every search term were collected. The obtained variables were consisted of organism's name or label, type of extremophile, type of environment, location of discovery, location's physicochemical condition, location's province, potential application, publication year, journal source, research title, and Uniform Resource Locator (URL). Location coordinate and miscellaneous information were also inputted when provided.

The nearest species identity was used as organism's name when ≥ 99% phylogeny similarity had been achieved; Otherwise, the term "*sp.*" was used.

The datasets were filtered using several parameters as in **Table 1**. The basis of these criteria is explained in the discussion section.

Table 1. Parameters for datasets filtering

No	Parameter
1	The location of discovery is in Indonesia's territory including its Economic Exclusive Zone (EEZ)
2	The organism is obtained in environment with well -preserved natural condition, with exception of accidental occurrence (e.g. oil tanker spill)
3	Research with event-based timeframe is excluded (e.g. bacterial investigation during Ramadhan)
4	Research with unclear location and uncontactable corresponding author is excluded
5	Request of exclusion from respective author is conducted accordingly
6	Study using areal coordinate as the location of discovery is excluded
7	The organism conforms with the provided parameter of extremophile characteristic as in **Table 2**.

Table 2. Parameters for extremophile determination

Type of Extremophile	Parameter
Thermophile	Grow at temperature ≥ 50°C
Psychrophile	Unable to grow at temperature > 15°C
Acidophile	Grow at pH ≤ 5
Alkaliphile	Grow at pH ≥ 8
Halophile	Grow in saline condition
Barophile	Grow at pressure ≥ 40 MPa
Radiophile	Resistant toward high level of ionizing and ultraviolet radiation
Others	Other extreme condition such as the presence of chemical or metal in high concentration

3.2 Mapping and Data Analysis

Using the dataset, information as follows was presented visually: total amount of extremophiles research used in this study per year in Indonesia (column chart), total amount of extremophiles per type in Indonesia (pie chart), total number of extremophiles based on Indonesian region (pie chart), and total amount of extremophiles type based on type of environment in Indonesia (pie chart).

The created dataset was mapped by using Google My Maps web application. Decimal degrees (DD) format was used as the location of respective data when coordinate was provided; Otherwise, the location name was used. All the variables were inputted in the map to act as information. The created map was published digitally by using WIX web application and open-sourced to extremophile researchers.

CHAPTER IV: RESULTS

4.1 Data Analysis

From 3200 observed studies in Google Scholar, a total of 180 studies were found to conduct research on Indonesian extremophiles. Further data filtering obtained 142 published research papers which correspond with the parameters. Database could be accessed in http://bit.ly/extremoresearchid.

Based on the observation, the research on Indonesian extremophiles was first conducted in 1988 and become more popular during the second decade of 21st century (2011-2020) **(Table 3; Figure 1)**. The increase of studies might be caused by the overall improvement of molecular identification facility, the availability of research funding, as well as the availability and interest of the experts in Indonesia. Cheaper sequencing cost might also play a major role in advancing the research on this topic.

A total of 486 extremophiles were collected, with the major extremophile types are made of thermophile (256 isolates; 53%), halophile (119 isolates; 25%), thermoalkaliphile (36 isolates; 7%), alkaliphile (32 isolates; 7%), and thermoacidophile (23 isolated; 5%) **(Table 4; Figure 2)**. There was no psychrophile, barophile, and radiophile discovered in this study. An extremophile which has no clear classification is grouped as Not Applicable (N.A.).

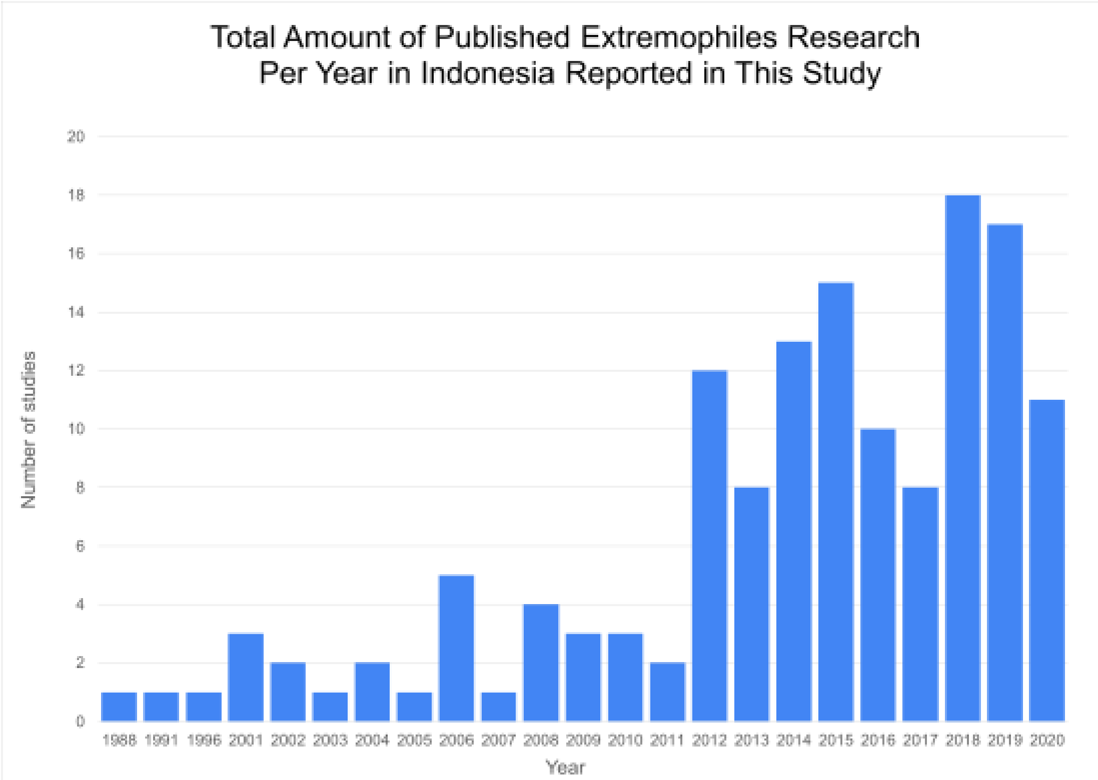

Figure 1. Total amount of published extremophiles research per year in Indonesia reported in this study

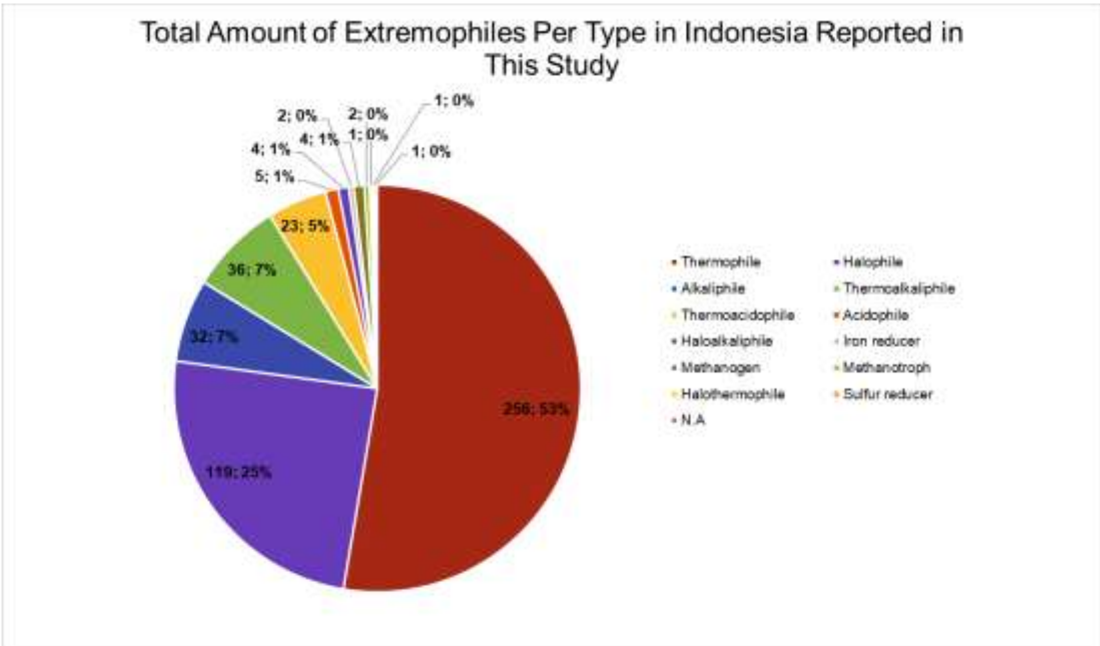

Figure 2. Total amount of extremophiles per type in Indonesia reported in this study

From a total of 34 provinces, research on Indonesian extremophiles have only been conducted in 20 provinces, including Aceh, North Sumatra, West Sumatra, Jambi, South Sumatra, Lampung, Greater Jakarta, West Java, Central Java, East Java, Bali, West Nusa Tenggara, East Nusa Tenggara, East Kalimantan, North Sulawesi, Central Sulawesi, South Sulawesi, Southeast Sulawesi, Maluku, and Papua **(Table 5; Figure 3)**. The majority of Indonesian extremophiles are discovered from volcanic environments such as hot spring (266 isolates; 55%), crater (40 isolates; 8%), mud volcano (27 isolates; 6%), volcanic river (12 isolates; 2%), hydrothermal vent (4 isolates; 1%), and geyser (3 isolates; 1%) **(Table 6; Figure 4)**. The remaining environments only take up 27% of the total extremophiles: Sea (polluted, unpolluted, and desalinated: 68 isolates; 14%), beach (31 isolates; 6%), lake (7 isolates; 1%), and lime soil (28 isolates; 6%).

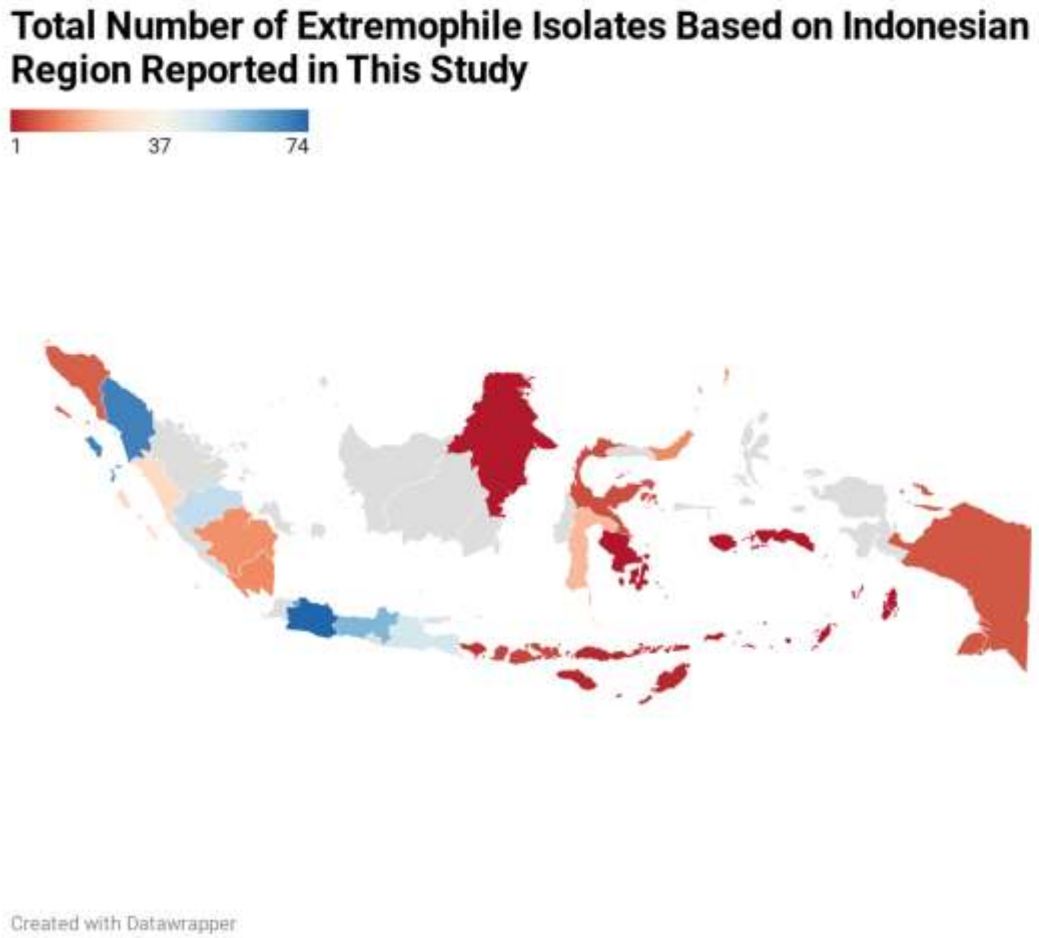

Figure 3. Total number of extremophile isolates based on Indonesian region reported in this study

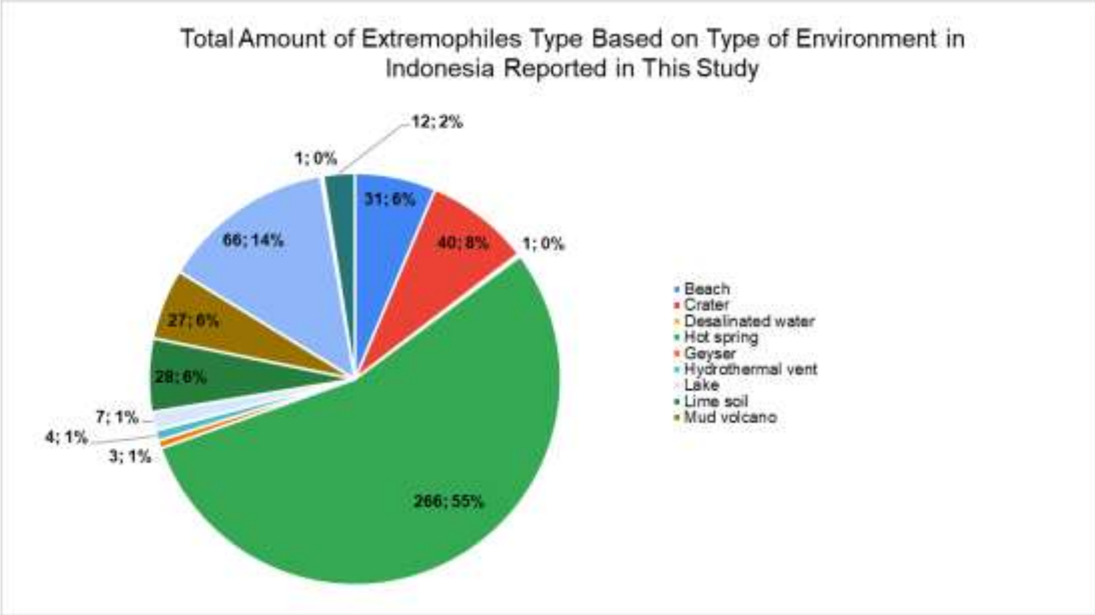

Figure 4. Total amount of extremophiles type based on type of environment in Indonesia reported in this study

4.2 Mapping and Web Page

The map is consisted of markers positioned to the respective extremophile's sampling location. Markers are colored depending on its type of extremophile. Each marker contains information as follow: name, type of extemophile, type of environment, location's condition, location, location province, potential application, publication year, journal source, journal title, and URL. Coordinate and additional note is also provided if it is available **(Figure 5; Figure 6)**.

The map application provides searchbox and direction icons to ease the search of certain isolate and give the direction to the sampling location respectively.

Figure 5. Mapping result (initial display)

The map is published in https://victorsandyawan.wixsite.com/extremoresearchid. The website is consisted of 1 webpage containing 5 strips: title, map, project information, set parameters, and contact information **(Figure 7)**.

CHAPTER V: DISCUSSION

5.1 Study Overview

This study created a map with intent to facilitate any individual on seeking digitally available studies of Indonesian extremophiles. The map would also serve as a platform for scientists to exhange research discoveries in this field.

Google My Maps web application is chosen due to its user-friendly interface when inputting new research data. Being directly integrated with Google Map, Google My Maps also allows a more approachable user-experience.

A website is created as a publishing platform for the map. The website is consisted of 1 web page containing 5 strips of title, map, project information, set parameter, and contact information. The 2nd strip is chosen for a more immediate access to the map. Project information describes the purpose of the website creation, while set parameter describes the characteristics required for respective extremophiles classification in the map. Researchers who are interested to contribute on this map are able to contact directly using the attached contact information.

5.2 Search Term and Set Parameter Selection

Having numerous classifications, only 8 general types of extremophiles are used as the search term: thermophile, psychrophile, acidophile, alkaliphile, barophile, halophile, and radiophile. For every search term, this study selected only the first 200 results. Using both Indonesian and English language for all search terms, a total of 3200 search results were obtained for selection. The amount is considered enough to represent the majority of research in this topic. This is proven from the total of only 180 research studies which conduct research on extremophile discovery after selection.

The selected studies further underwent data selection using the set parameters **(Table 1)**. The first criterion discusses about Economic Exclusive Zone (EEZ). EEZ is a territory stretching 200 nautical miles from a country's coast where a country is given special rights for exploration and use of marine resources, making any sampling activity within this area a legal conduct (United Nation, 1980). The second criterion mentions the need of a well preserved sampling location to maintain the authenticity of the environmental condition; A special exception is given for accidental occurrence as it is unintended (e.g. oil spill). The third parameter refers to studies with aim to compare extremophile composition on a certain location during and after a specific event (e.g. National holiday); These research are excluded with consideration of dynamic extremophile composition in the location of study during those events. The fifth parameter excludes any research using areal coordinate (e.g. 07°00'00" S to 07°50'00" S, 112°00'00"E to 112°50'00"E), as sampling location might be unclear. The sixth parameter describes that request for data removal, during and after this study, would be conducted accordingly to respect the authors' rights. Finally, the seventh criterion refers to the extremophile classification for this study.

The extremophiles possessing characteristic within the parameter are categorized as one of the stated extremophile classification: thermophile, psychrophile, acidophile, alkaliphile, halophile, barophile, radiophile, or others **(Table 2)**. *Others* classification includes any organism capable to survive in extreme environment not mentioned in the provided classifications; Extremophiles categorized in this group usually involve the ability to survive in high concentration of chemical in nature (e.g. sulphur and iron). Data which does not conform with the set parameters was removed. A total of 142 research studies were obtained after final selection.

A more accurate extremophile classification, such as moderate thermophile or hyperthermophile, are not used with purpose to maintain the minimum amount of location marker identity (color) and ease the view of the map.

Having no established universal classification rules for all types of extremophile, the set parameters selected for this study are chosen based on the clarity of the description provided by other studies. The set parameters of thermophile, psychrophile, and barophile are based on a study by Pikuta, Hoover and Tang (2007); Acidophile and alkaliphile are based on Johnson and Schippers (2017); Halophile is based on Amoozegar *et al.* (2019), and radiophile is based on Gupta *et al.* (2014). There seems to be no set value which informs the minimum concentration of chemical and/or radiation to be categorized as extremophile; Therefore, the statement of *high concentration* in the observed studies is used to identify radiophile as well as other extremophiles living in chemical-rich environment unsustainable for life.

5.3 Attached Information in the Map

Several information such as organism name, type of extremophiles, type of environments, location of discovery, location coordinate, location's province, and journal title are provided as general information. Only organisms with phylogeny similarity of ≥ 99% are stated with its species name to maintain the accuracy of naming. The accuracy of sampling location is maintained as well by using location coordinate when provided. The coordinate inputted for mapping location uses decimal degrees (DD) instead of degrees, minutes, seconds (DMS) format due to the incapability of Google My Maps on recognizing the later format; Therefore, a new variable, location in Google Map, is added as the location data which will be automatically processed in the map. Despite the usefulness of this approach, Google My Maps, however, is still incapable to accurately position the marker according to the inputted coordinate; Therefore, manual positioning was done to adjust the marker location.

The information showing potential application is provided to accomodate users who are interested in utilizing a certain capability of the available extremophile. Sampling location's condition could be used to decide the condition which the extremophiles could be cultivated in. Publication year and journal source are provided for user to decide the reliability of the finding. Link is also available to view the published research.

5.4 Extremophile Research in Indonesia

Indonesia's research in extremophile field is lacking. From a total of observed 3200 research studies, only 180 studies are found to work on this topic. While there has been an increasing number of studies during the second decade of 21^{st} century, no steady rate of increase is found. Many studies do not explore the studied extremophiles in depth as well; several of these studies only conducted amylase-catalase tests and genetic identification. This is unfortunate considering that Indonesia has a diverse geographic composition and mineral resources (Devi & Prayogo, 2013; Sigit, 1965). Furthermore, extremophile research potentially plays a major role in bioremediation, bioprocessing, and bioproduction (Arora & Panosyan, 2019).

Currently, extremophile is not evenly researched across all Indonesian regions; Especially, research has yet to be conducted in Riau, Riau Islands, Bengkulu, Bangka Belitung, Banten, Yogyakarta, North Kalimantan, West Kalimantan, Central Kalimantan, South Kalimantan, Gorontalo, West Sulawesi, North Maluku, and West Papua. While extremophile research have been conducted in the regions of Kalimantan (East Kalimantan), Lesser Sunda Islands (Bali, West Nusa Tenggara, and East Nusa Tenggara), Sulawesi (Central Sulawesi), Maluku Islands (Maluku), and Western New Guinea (Papua), the research is extremely low in number.

Thermophile is the major discovered extremophiles in Indonesia. This is caused by Indonesia's geographical location which is inside the Ring of Fire, creating numerous volcanicrelated environments such as crater, hot spring, geyser, hydrothermal vent, mud volcano, and volcanic river. This same geographical reason also supports the living condition for acidophilic organisms. Volcanic environment supports mineral formation which favors the viability of alkaliphile and certain chemical-resistance extremophile (e.g. iron reducer, methanogen, methanotroph, etc). Having a large ocean area, numerous halophiles have also been discovered in Indonesia. In contrast, psychrophile, barophile, and radiophile have yet been discovered. The lack of suitable geographic conditions (e.g. tropical climate, low radiation level) might play a role in this occurrence. Psychrophile and barophile, however, might potentially be found deep in Indonesian sea, such as in deep trench which has low temperature and high pressure (Yasuhara & Danovaro, 2016).

5.5 Limitation

Different organisms by the same study are commonly obtained from the same location. In the created map, however, the markers occupying the same location overlap each other; Therefore, the access of the overlapped marker could not be reached through the map interface. The data could only be accessed through the search box and independent search from the provided list.

CHAPTER VI: CONCLUSION AND RECOMMENDATION

A collection of extremophile research in Indonesia have been successfully mapped using Google My Maps and published by using WIX web application. The created database could be accessed in http://bit.ly/extremoresearchid, and the map could be accessed in https://victorsandyawan.wixsite.com/extremoresearchid. The database and map are open-sourced for public, especially for scientific community.

While the extremophile research in Indonesia is currently lacking, there is an abrupt increase of interest in this topic. Most of the discovered extremophiles are thermophile which are majorly obtained from volcanic-hot spring.

A collaboration with researchers taking interest in Indonesian extremophile is recommended to futher improve the data collection for this map, especially in the location where the study count is really low, such as in Kalimantan, Lesser Sunda Islands, Sulawesi, Maluku, and Papua. A determined parameter value is also required to create a more precise radiophile classification.

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APPENDICES

Table 3. Total amount of published extremophiles research per year in Indonesia reported in this study

Year	Research Study
1988	1
1991	1
1996	1
2001	3
2002	2
2003	1
2004	2
2005	1
2006	5
2007	1
2008	4
2009	3
2010	3
2011	2
2012	12
2013	8
2014	13
2015	15
2016	10
2017	8
2018	18
2019	17
2020	11
Total	**142**

Table 4. Total number of extremophiles per type in Indonesia reported in this study

Type of Extremophile	Total Extremophiles
Thermophile	256
Halophile	119
Alkaliphile	32
Thermoalkaliphile	36
Thermoacidophile	23
Acidophile	5
Haloalkaliphile	4
Iron reducer	2
Methanogen	4
Methanotroph	2
Halothermophile	1
Sulfur reducer	1
N.A	1
Total	**486**

Table 5. Total number of extremophile isolates based on Indonesian region reported in this study

Region	Total Extremophiles
Aceh	8
North Sumatra	69
West Sumatra	25
Jambi	51
South Sumatra	14
Lampung	13
Greater Jakarta	66
West Java	74
Central Java	59
East Java	48
Bali	3
West Nusa Tenggara	5
East Nusa Tenggara	2
East Kalimantan	1
North Sulawesi	13
Central Sulawesi	6
South Sulawesi	20
Southeast Sulawesi	1
Maluku	1
Papua	7
Total	**486**

Table 6. Total number of extremophiles type based on type of environment in Indonesia reported in this study

Type of Environment	Total Extremophiles
Beach	31
Crater	40
Desalinated water	1
Hot spring	266
Geyser	3
Hydrothermal vent	4
Lake	7
Lime soil	28
Mud volcano	27
Polluted sea	66
Sea	1
Volcanic river	12
Total	**486**

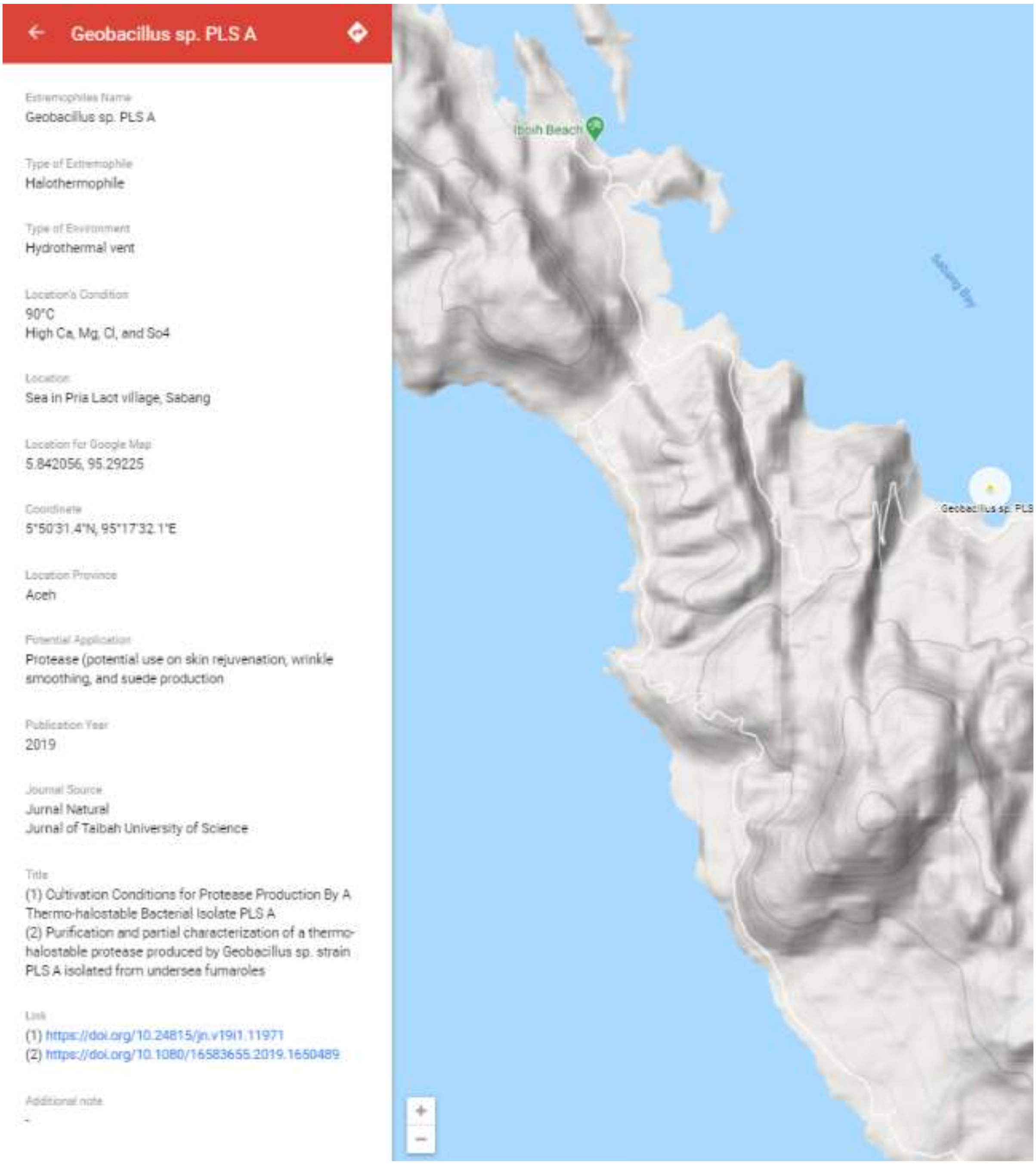

Figure 6. Mapping result (information display)

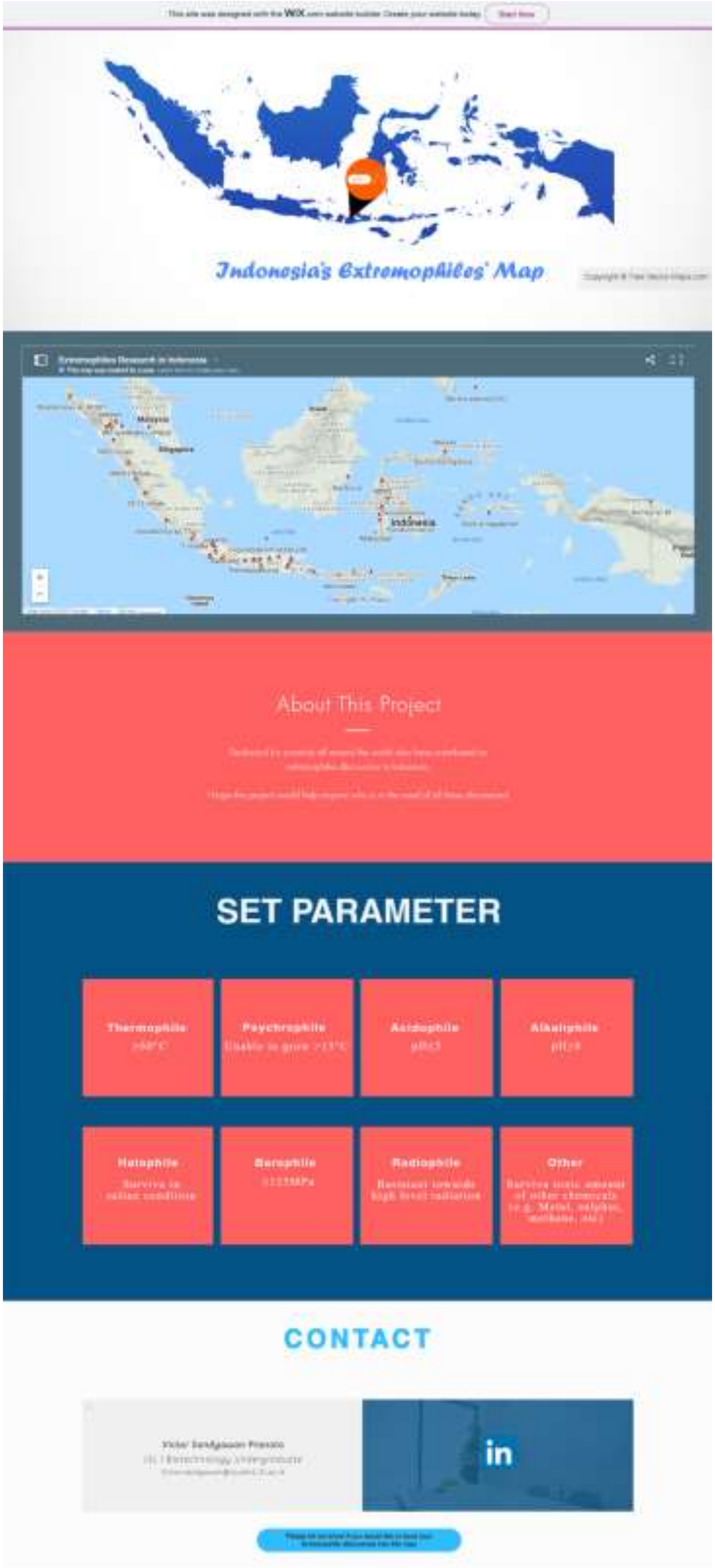

Figure 7. Extremoresearchid web page display