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Spatial Distributions and Model Selections of Commercial Estuarine Fish (Sciaenidae) Populations Related to Water Quality, Chl-a, and AML in Musi River mouth, South Sumatra

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Abstract

Estuary and river mouth are essential habitats for many commercial estuarine fishes, including the Sciaenidae family. While recently, estuaries have been threatened by anthropogenic marine litter (AML) transported from nearby land and river. An important type of AML is plastic litter since it takes a long degradation time. In the South Sumatra Province, Indonesia, one of the vital estuaries is the Musi estuary. This paper aims to map the spatial distributions of two Sciaenids, including *Panna microdon* and *Otolithoides pama*, and Sciaenid's environmental covariates, including water quality, chlorophyll a, and plastic litters in Musi estuary and model the correlations of Sciaenids with their covariates. The maps were developed using GIS, and the model was validated using AIC methods. The data were collected from 3 river mouths in the west, central, and east of the Musi estuary. The data showed that the populations of both Sciaenids were higher in the east river mouth rather than in the west. Sciaenid populations were positively correlated with high salinity, DO, chlorophyll a, moderate transparency, and low temperature. A high load of AML's frequency (7.54 items/m^2) and weights (36.8 gram/m^2) has reduced both Sciaenid populations in the central river mouth of the estuary. In contrast, low AML loads in the east have correlated with high Sciaenid populations. Model selection based on AIC values shows the best model for *P.microdon* retained an effect of AML weight with AIC values of 22.591 and 28.321 for *O. pama.* This concludes that the weight of plastic litter in estuary water was the main limiting factor for Sciaenid populations in Musi.

Keywords: AIC, anthropogenic marine litter, estuary, model selection, Sciaenidae

1. Introduction

The Sciaenidae family includes 70 genus and 270 species [1] with geographical distribution, including the Atlantic, Indian, and Pacific oceans. Some sciaenid species can be considered to have a commercial value for aquaculture production, the red drum (*Sciaenops ocellatus*) [2] and the yellow croaker [3]. In Indonesia, the production of Sciaenidae accounted for 12,404 t, ranked as the second most commercial fish caught [4]. Several Sciaenids are new to aquaculture and have good aquaculture potential worldwide, e.g., *Argyrosomus japonicas* distributed in Australia, southern Africa, and Taiwan; *Sciaena umbra* found in Greece and Turkey; *S. ocellatus* inhabits China, USA, Israel, Mexico, and Taiwan; *Umbrina cirrosa* distributed in Cyprus, Spain, Greece, Italy, and Turkey; and *A. regius* inhabits Spain, Egypt, France, Italy, Morocco and Turkey [5]. Advantages of Sciaenids to be a commercial aquaculture species are due to their relatively easy broodstock management, fast growth rate, and good feed conversion ratios [5].

In Indonesia, Sciaenids have been the dominant species caught from estuaries. Sciaenids are an important stock in Malaka waters besides fishes from Scaridae, Mullidae, and Nemipteridae families. Sciaenids were dominant in waters from Panipahan in Riau Province to Tanjung Balai, Asahan in North Sumatra [6]. Among other Sciaenids, *Johnius belangerii* is the most caught and consumed species [6]. Besides this species, other Sciaenids that have commercial value is *Otolithoides pama*. The biomass of *O. pama* accounted for 0.49% of the total biomass of fish caught in the Barito estuary [7].

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O. pama was also caught from Pasuruan water [8]. Another commercial Sciaenid found in the estuary is *Panna microdon*. In the Mayangan estuary in West Java, *P. microdon* is among eight Sciaenids inhabiting this estuary [9]. O. pama and P. microdon in estuaries indicate that the estuary is an essential ecosystem for Sciaenids.

One of the important estuaries in Indonesia is the Musi estuary. This estuary located on the east coast of Sumatra Island receives three rivers from the mainland. The river delivering nutrient combined with mangrove covers in the estuary has made this estuary has a high biodiversity of estuarine species, including the commercial species. Previous research [10] in 2006 has caught and recorded 107 fish species, including shrimp species. Sciaenid was recorded as the dominant fish family in one of the rivers in the Musi estuary. Current research in 2021 [10] has confirmed a significant increase of estuarine fish annually in Musi. Even the last record shows there were up to 10 fish families in Musi.

Despite the high diversity of estuarine fish species, Musi estuary has been threatened by pressures ranging from land-use conversion and pollution nearby Musi's intact ecosystem. In an aquatic ecosystem, pollution has significant effects since it can spread poison and hazardous material that can spread rapidly following the river current. One pollution in the aquatic and marine ecosystems is the presence of anthropogenic marine litter (AML) [11, 12, 13], including plastic litters. AML is transported by rivers to the river mouth and estuary and is often eaten by estuarine fishes, concentrating toxic chemicals in fish tissues and filling their stomachs, causing fish to starve. Plastic aquatic debris is much more than a mere aesthetic problem. Despite growing research on fish diversity in the Musi estuary, research on how the plastic litters and water quality affect the Sciaenid's fish population is still limited. Then this study first aims to map the spatial distributions of 2 Sciaenid's species, including *P. microdon* and *O. pama*, water quality, chlorophyll a, and anthropogenic marine litter, in this case, is plastic litters Musi estuary. Secondly, the correlations of Sciaenids with the water quality, chlorophyll a, and plastic litters are also assessed. The results of this study will contribute to the conservation of Sciaenids in supporting the sustainability of the estuarine fishery in Musi.

2. Methodology

2.1. Study Area

The study area was an estuary ecosystem located in the Musi river mouth (Figure 1) with a longitude of 104.8^0-104.9^0 East and a latitude of 2.3^0-2.4^0 South. The studied ecosystem is adjacent to the Bangka Strait in the north and receives water from three rivers in the south, i.e., Banyuasin in the west, Musi in the central, and Upang in the east. The width of Banyuasin is 1,670 m, Musi is 712.7 m, and 926.3 m for Upang. Banyuasin has a water flow rate of 0.344 m/sec [14]. While the debit of Musi river was ranging from 1.08 m^3/sec. to 22.15 m^3/sec. [15, 16]. The depth of the Musi river was 7 m due to the sedimentation. In comparison, Banyuasin depths varied from 0.17 m in the shallowest point downstream to 9.91 m upstream. Shallow parts of Banyuasin were related to the sedimentation [17]. Musi estuary ecosystem was dominated by swamp and wetland. This ecosystem receives average daily rainfall ranging from 65.22 m to 76.81 mm [18]. The sampling locations in the study area consisted of three locations in each river mouth, giving a total of nine locations. Sampling location in Banyuasin covers 162.94 km^2, in Musi 103.6 km^2, and 48.27 km^2 in Upang. Sampling locations were located from upstream, central, to downstream. Samplings were done weekly on April 2020, giving a total of four sampling times. The samples were taken in April 2020, considering this month represented a transitional season representing fish species both from dry season ended up in March and wet season started in May.

2.2. Water quality survey

Water quality surveys include in situ chlorophyll-a, dissolved oxygen (DO), salinity, sediment, temperature, and transparency. Those variables were measured in each sampling location with three replications for each location (Table 1). The chlorophyll a was measured using YSI 6025 chlorophyll probe, DO and temperature were measured using multi-parameter (Lutron DO 5510), salinity with a refractometer (Atago), and transparency with a Secchi disk. The geographical locations of samplings were recorded using Etrex Garmin GPS handheld. Sediments were collected from the bottom of the river [19] using Ekman grab sampler and sieved using sediment sieves [20] sizing 0.075 mm [21].

2.3. Fish survey

The fish survey followed methods from previous studies [22, 23]. Fish samples were taken using gillnet with a mesh size of 2 inches [24] and identified using the fish identification guide book [25, 26]. Fish samples then were calculated for a frequency (%) of appearance using the following equation:

$$\text{frequency} = \frac{\text{number of fish species i caught}}{\text{Total number of species caught}} \text{ x } 100\%$$

2.4. Anthropogenic marine litter (AML) survey

The AML assessment was conducted in situ in the surface water of Musi estuary in designated sampling locations. In each sampling location, the survey was conducted within a 1 m x 1 m grid, and there was a 1 m^2 grid

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randomly located in each sampling location. Then the collected AML samples were weighed. The litter was defined as numbers of items/m^2 and weights of items (gr/m^2). The AML survey and collection emphasized and included all non-organic discarded materials made of plastic, e.g., bags, bottles, cups, packaging, sheets, and straws [27, 28, 29, 30, 31]. The selection of plastic as AML samples was based on the consideration that plastic has a long degradation time. Then the organic AMLs, including rubber and wood, part of the house, boat, and tree, were excluded from the samples.

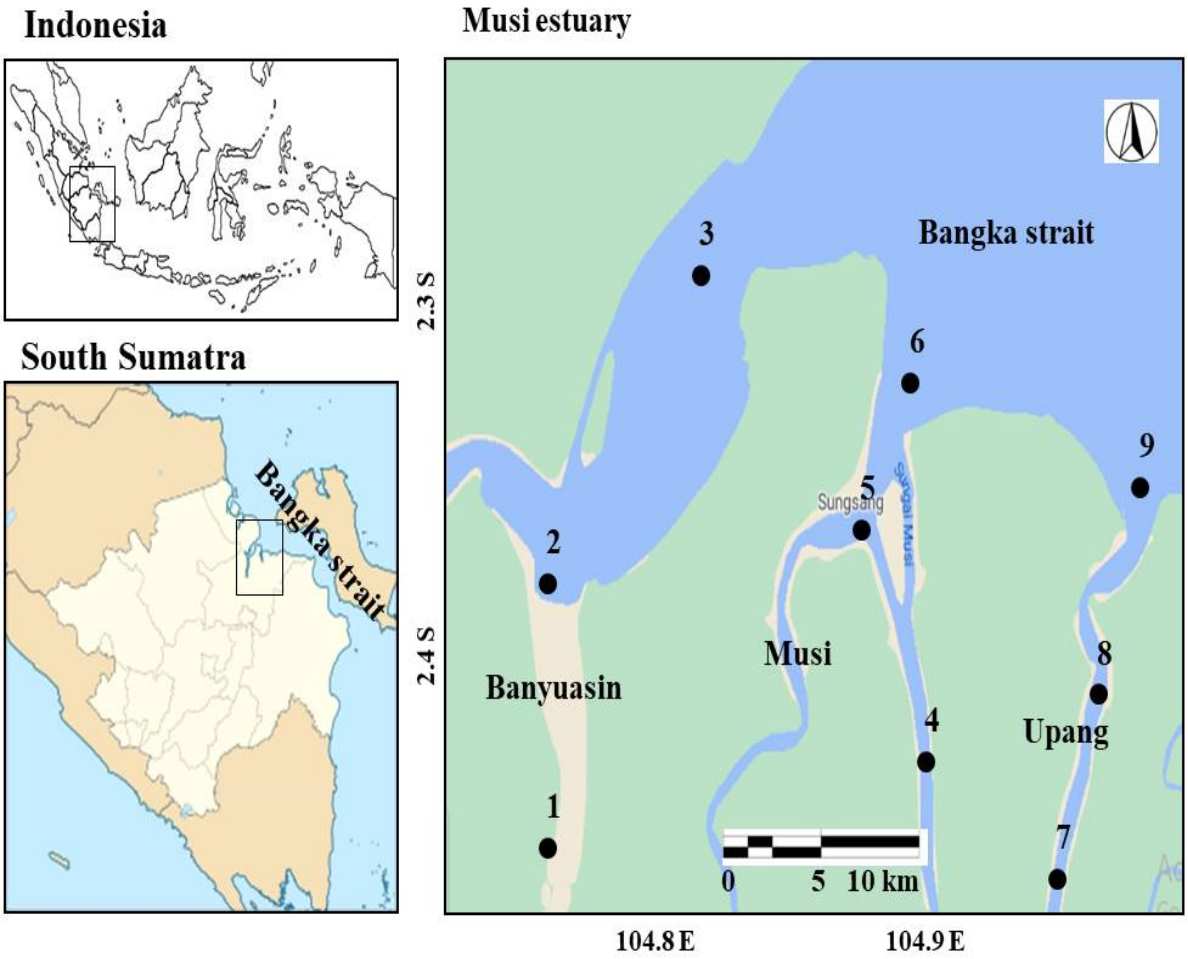

Figure 1 Nine sampling locations (1-9) in 3 river mouths (Banyuasin in west, Musi in central, Upang in east) of Musi estuary, South Sumatra Province

Table 1 Water quality variables

Variables	Unit	Measurement methods
Chlorophyll a	mg/m^3	YSI 6025 chlorophyll probe
Dissolved oxygen (DO)	mg/l	Lutron DO 5510 multi parameter
Anthropogenic Marine Litters	Item/m^2, gr/m^2	1 m x 1 m grid survey
Salinity	‰	Atago refractometer
Sediment	na	Ekman grab, sieve
Temperature	^0C	Lutron DO 5510 multi parameter
Transparency	cm	Secchi disk
Geocoordinate	Decimal degree	Garmin Etrex GPS handheld

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2.5. Mapping and spatial analysis

Mapping and spatial distribution analysis of fish, water quality, chlorophyll a, and AML consisted of developing the presence map of those variables and then interpolating those variables' patterns. First, the recorded geocoordinate of fish, water quality, chlorophyll a, and plastic litter variables in each sampling location were inserted into a table. Then, the recorded data in the table were mapped using Geographical Information System (GIS) with ArcView version 3.2 to pinpoint the geolocations of fish, water quality, chlorophyll a, and plastic litter variables in 3 sampled river mouths in Musi. To create a pattern map, the GIS tables containing fish, water quality, chlorophyll a, and plastic litter variables were interpolated [32, 33, 34].

2.6. AIC data analysis

Sciaenid population correlations with water quality and AML were analyzed using Principal Component Analysis (PCA) and modeled using Akaike Information Criterion (AIC) following Cavada [35]. The AIC was developed using linear regression. The measured parameters included in AIC, residual standard error, R-squared, P and F values, and % contribution of each covariate. To build the model using R version 4.1.1, 5 covariates correlating with Sciaenid population explanatory including salinity, temperature, transparency, AML measured in items/m^2 and gram/m^2 were included in the analysis to develop the model. The best model was selected based on the model that has the lowest AIC values.

3. Results and discussion

3.1. Sciaenidae spatial distribution

This paper assesses the spatial distribution of two Sciaenid species. Those species are *Otolithoides pama* and *Panna microdon*. In 3 river mouths in the Musi estuary (Figure 2), frequency ranges of *O. pama* (11.1-17.9%) were higher than *P.microdon* (8.1-11.6%) and indicating *O. pama* was more dominant. Among 3 river mouths in Musi, the highest *O. pama* population with a frequency up to 17.9% was observed in the eastern parts of Musi estuary or the Upang river, mainly in the river mouth areas. In the central parts of the estuary or the Musi river mouth, the *O. pama* population was lower than other populations in Banyuasin and Upang river mouths.

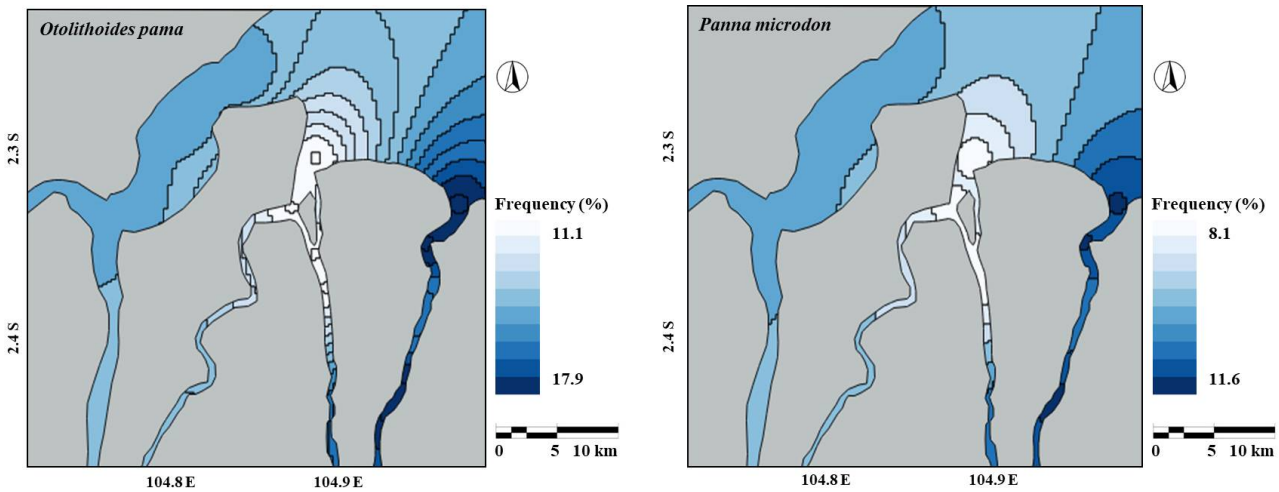

Figure 2. Frequencies (%) of *Otolithoides pama* (11-17.9%) and *Panna microdon* (8.1-11.6%) in 3 river mouths of Musi estuary, South Sumatra Province.

A similar spatial distribution was also observed for *P. microdon*. The lowest *P. microdon* frequencies were also observed in the Musi river mouth. Then the population increased in the western parts of the Banyuasin river mouth, and the highest population was observed in the Upang river mouth in the east. In this river mouth, the *P.microdon* frequencies can be high as 11.6%.

3.2. Water quality

The result of comprehensive water quality spatial distributions in the Musi estuary is presented in Figure 3. Musi estuary was receiving freshwater from 3 rivers mixed with saltwater from Bangka strait. The highest salinity (22‰) was recorded in the Banyuasin river mouth in the west, and the lowest salinity was observed in the central Musi river mouth. The water is similar to freshwater in this

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river mouth since the salinity can be as low as 4‰. A similar spatial distribution was also recorded for water temperature. The lowest temperature was recorded in the central and east parts of the estuary. In the west parts or in the Banyuasin river mouth, the water was warmer than other river mouths since the temperature can be as high as 31 ^{0}C. The water clarity of the Musi estuary was varied. Central and east river mouths of Musi had less clarity.In comparison, clear water was observed in the west part of Musi in the Banyuasin river mouth. Low clarity in the central Musi estuary was related to the land uses along the Musi river. This river was passing settlements and receiving surface runoff from anthropogenic activities that increased the amount of sediment entering the river.

The water transparency in Musi was correlated with the dissolved oxygen (DO) (Figure 3). Banyuasin river mouth has the highest DO (9.9 mg/l) since the water was also clearer. In contrast, murky water with low transparency values in the central estuary has the lowest DO ranged from 3.6 to 6.0 mg/l. Correlations of water transparency with the DO were related to the phytoplankton photosynthesis. Clearwater will allow sunlight penetrations through water and support phytoplankton photosynthesis [36]. As a result of photosynthesis, phytoplankton will release oxygen into the water and increase the DO levels [36]. This also explains high chlorophyll recorded as high as 20 mg/m^3 in Banyuasin and Upang river mouths that were characterized by high transparency and DO levels. Chlorophyll is essential to the existence of phytoplankton that can be used as an indicator organism for the health of particular estuary water.

3.3. Anthropogenic marine litter (AML)

The spatial distributions of AML in Musi estuary and river mouth (Figure 4) were varied. Plastic litter was accumulated in the central parts of the estuary near the Musi river mouth. Here the density of plastic litter can be as high as 7.54 items/m^2. The plastic litter density then decreases in west and east river mouths with density as low as 1.84 items/m^2. Similar spatial patterns were also observed for weights of plastic litters. East parts of an estuary or Upang river mouth have the lowest weight of plastic litter. At the same time, the weight of plastic litter was increased almost fourfold in the Musi river mouth or the central estuary. This central area has the highest AML considering that the central estuary received water from 2 Musi river tributaries. Besides that, the land use in upstream areas of the Musi river was dominated by settlements instead of forests that contribute to the discharge of anthropogenic litter to the Musi river [36].

3.4. Model selections for Sciaenidae

Table 2 presents the best model to describe the correlations of *O. pama* and *P.microdon* with their environmental covariates. Both species showed a model result similarity based on AIC values. Model selection for *O. pama* resulted in the best model containing the weight of AML, and other covariates have less significant effects. AIC values describing the correlation significance of *O. pama* frequency and AML measured in gram/m^2 was 28.321. Similarly, the best model for *P.microdon* retained an effect of AML weight with an AIC value of 22.591. Those AIC values indicate that plastic litter weight as measured AML is the best covariate to predict the correlations of Sciaenids with AML covariates.

Table 2. AIC model selections of *Otolithoides pama* and *Panna microdon* frequencies with covariates (water quality variables and plastic litter contents)

Variables	AIC	Residual standard error	R-squared	F	P	% contribution
Otolithoides pama (freq)						
Covariates:						
~salinity (‰)	32.357	9.14	0.055	0.117	0.56	21.54
~temperature (°C)	32.279	9.229	0.037	0.076	0.62	17.16
~transparency (cm)	32.508	9.14	0.055	0.117	0.56	14.11
~items/m^2	29.579	6.521	0.519**	2.16	0.02***	23.27****
~gram/m^2	28.321*	5.572	0.648**	3.697	0.00***	23.90****
Panna microdon (freq)						
Covariates:						
~salinity (‰)	26.552	4.467	0.055	0.117	0.56	20.16
~temperature (°C)	26.629	4.51	0.037	0.076	0.62	16.54
~transparency (cm)	26.552	4.467	0.055	0.117	0.56	14.90
~items/m^2	23.851	3.187	0.519**	2.16	0.02***	24.27****
~gram/m^2	22.593*	2.273	0.519**	3.697	0.00***	24.11****

*selected best model, **significant correlation, ***significant with P<0.05, ****high % contribution

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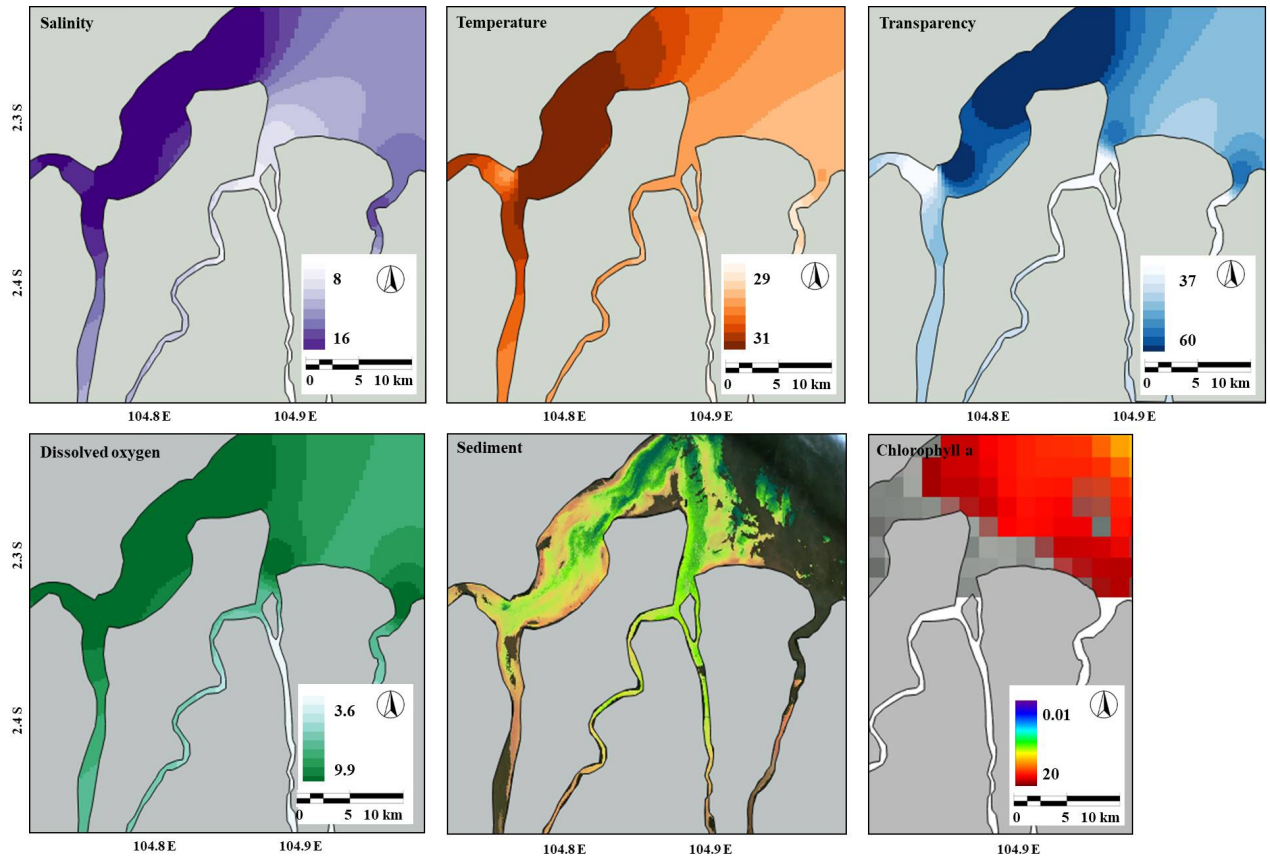

Figure 3. Spatial patterns of water quality variables including salinity (8-16 ‰), temperature (29-31 °C), transparency (37-60 cm), dissolved oxygen (3.6-9.9 mg/l), sediment (brown color), and chlorophyll a (0.01-20 mg/m^3) in 3 river mouths of Musi estuary, South Sumatra Province.

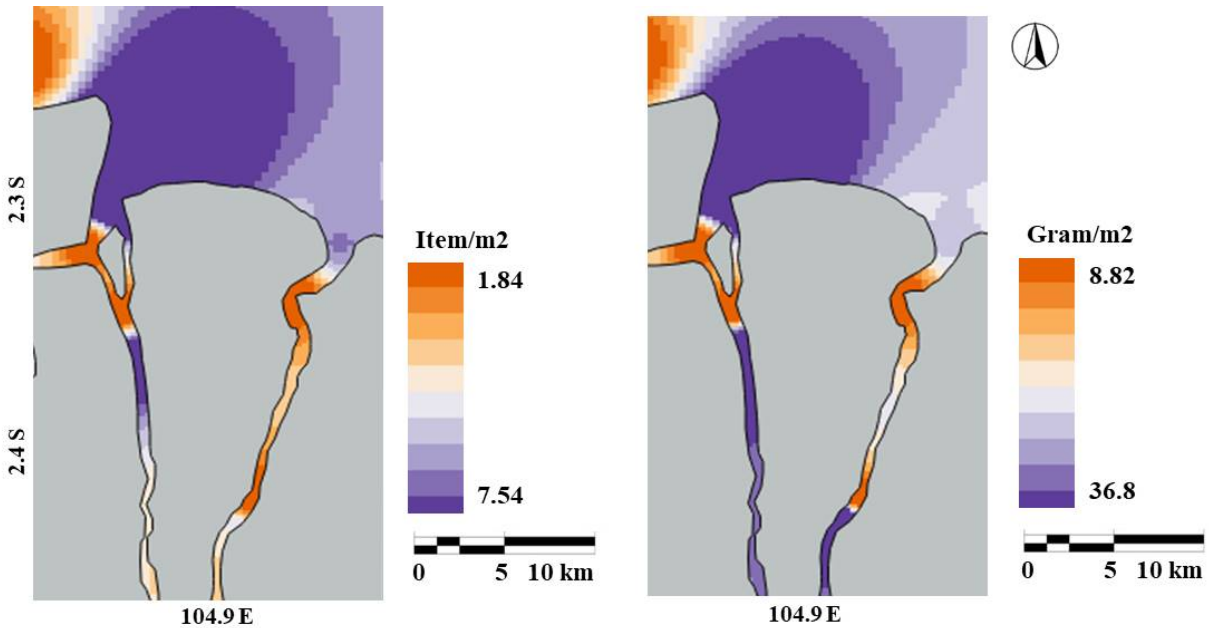

Figure 4. AML contents (1.84-7.54 items/m^2, left and 8.82-36.8 gram/m^2, right) in 3 river mouths of Musi estuary, South Sumatra Province.

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3.5. *Correlations of Sciaenids with water quality and AML*

Both Sciaenid's species populations had either positive or negative correlations with environmental factors (Figure 5, 6). Positive correlations can be seen for high salinity, DO, cholophyll a, and moderate transparency covariates. An increase in salinity was followed by an increase in Sciaenid populations. Sciaenid is a fish that is common in coastal areas. This fish prefers high salinity. Presences of Sciaenid species in the river mouth of Musi estuary are considering that Sciaenid lives inshore over sandy or muddy bottoms and use estuarine environments seasonally as nursery grounds during the juvenile stage and as feeding grounds during the young adult stage [37]. In the Musi estuary, Sciaenids were observed to prefer a river mouth in the east with moderate transparency levels rather than inhabiting clear water in the west. Sciaenid species are known to inhabit water with various transparency levels, from murky to clear water. The transparency tolerance range of Sciaenid was 2.5-60 cm [37], while in Musi, the lowest transparency level was only 20 cm. Estuary water with low transparency can protect against predators and indicate more food availability [23].

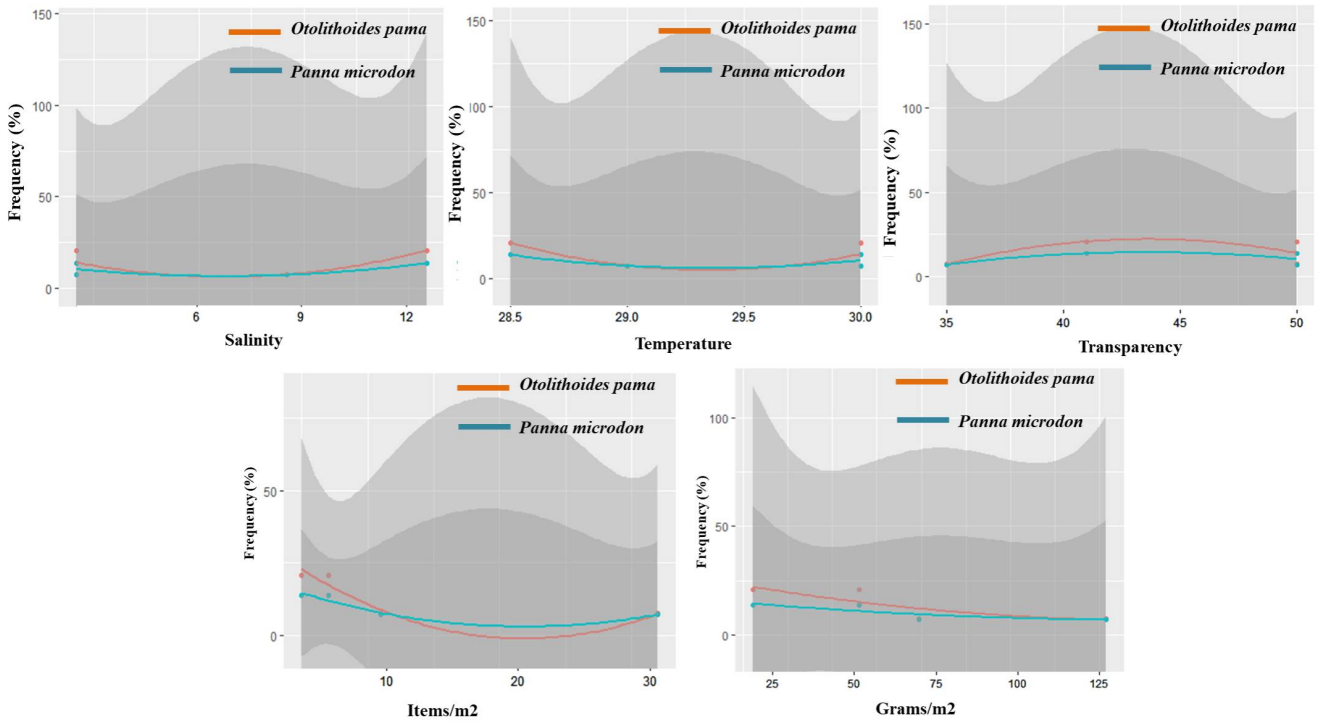

Figure 5. Trends (shaded area for 95%CI) of *Otolithoides pama* and *Panna microdon* frequencies with salinity (‰), temperature (°C), transparency (cm), and AML contents (items/m^2, gram/m^2) in 3 river mouths of Musi estuary, South Sumatra Province.

High chlorophyll a was observed in west and east river mouths, while low in the central river mouth. Similar spatial patterns were observed for *O. pama* and *P. microdon* frequencies that were also high in the east river mouth. This finding is similar to other results in coastal areas [38, 39]. On the Coast of the South China Sea [38], chlorophyll a contents of 0.06 mg/m^3 was followed by fish density as high as 2,767 individuals. In the Asian tropical sea, fish abundance was dependent on chlorophyll contents [39]. In Indonesia's sea, several fish species were correlated with chlorophyll a abundances, including sardine, fringe scale sardine, [40] and flying fish [41]. In the Musi estuary, high chlorophyll a contents were observed in coastal areas. This finding is comparable to the chlorophyll a contents that mostly dominate the Kendal coast, West Java [42]. Chlorophyll a indicates a presence and abundance of phytoplankton that was a food resource for zooplankton. While zooplankton is a diet of Sciaenids, this explains Sciaenids' correlations with chlorophyll a [42].

In this study, a negative correlation (Figure 6) of the presence of AML in the forms of plastic litters with the estuarine Sciaenid's populations in river mouth has been reported. The finding in this study is comparable to the other studies [43, 44, 45]. Marine litter is a global concern [43] since it can impact fish species at different levels of

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biological organization and habitats in many ways, namely through entanglement in, or ingestion of, litter items by individuals, resulting in death and/or death or severe suffering. Estuary and river mouth were some of the ecosystems that were vulnerable to plastic litter pollutions. Plastic litter density (Table 3) in Musi can be categorized as moderate.

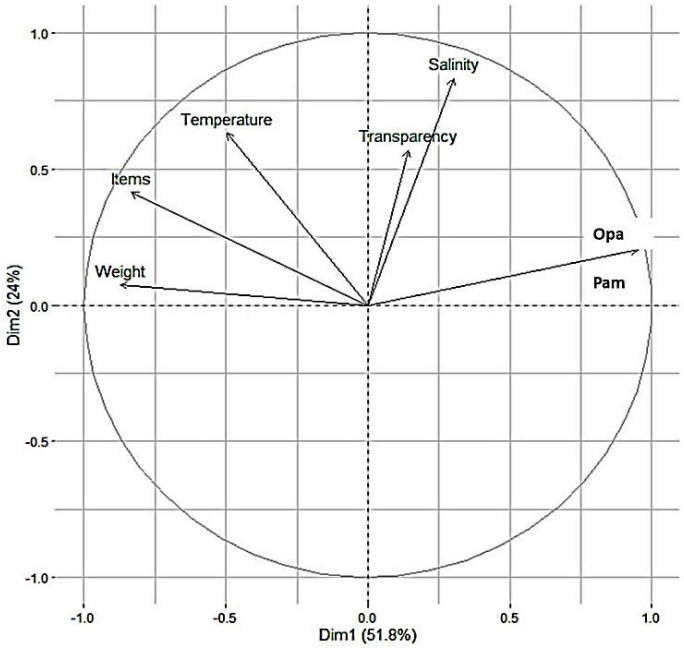

Figure 6. PCA of *Otolithoides pama* (Opa) and *Panna microdon* (Pam) frequencies with salinity (‰), temperature (°C), transparency (cm), and AML contents (items/m^2, weight in gram/m^2) in 3 river mouths of Musi estuary, South Sumatra Province.

Table 3. AML content (1.84-7.54 plastic items/m^2) comparisons with other estuary and river mouth locations

Locations	Items/m^2	Source
Musi	1.84-7.54	This study
Bandar Lampung	21.9	[46]
Padang	3.35	
Pangkal Pinang	4.18	
Manado	16.18	[47]

In comparison with other coastal ecosystems in Sumatra, plastic litter density in Musi was below the values of plastic litter density recorded in Bandar Lampung. In contrast, plastic litter density in Musi was still larger than Padang and Pangkal Pinang coasts. A high density of plastic litter in Musi might be related to the land use conditions of the Musi river. Settlements and less forested areas dominated the upstream of the Musi river. This condition leads to the increase of surface runoff, including the transport of plastic litter discharged from nearby settlements along river banks. The consequences of plastic litter in the river include accumulations of plastic in river mouths that affect the populations of estuarine. Plastic can indirectly pollute the intact river mouth and estuary ecosystems [44] also plastic litter can directly ingested by fish and reduce the fish populations [45]. The AIC model confirmed that weight (AIC: 22.593-28.321) followed by density (AIC: 23.851-29.579) of plastic litters were the primary covariates that were best describing the Sciaenid population in the river mouth of Musi estuary. Since Sciaenid fish [48] in this study has a commercial value, then plastic litters management aiming to reduce the weight of plastic litters should be implemented in the future.

4. Conclusion

Two commercial Sciaenid species populations in Musi estuary were distributed spatially in east parts of estuary in Upang river mouth. Spatial presences of these Sciaenid species were strongly correlated with AML covariates rather than with salinity, DO, chlorophyll a, transparency, and temperature covariates. Low Sciaenid species populations were observed in areas with high loads of anthropogenic

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marine litter, in this case, plastic litter. To conclude, weight followed by the density of plastic litter in estuary water was the main factor limiting Sciaenid population spatial distributions in Musi.

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Biological, Technical, and Financial Feasibilities Study of *Spirulina* sp. Biomass Production with Modified Commercial Medium in Indonesia

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Abstract

Spirulina sp. is the most common cyanobacteria commodity used in the bioindustry for functional food, source of protein, bioactive compounds, and biopigment. Production of *Spirulina* sp. still facing several problems such as high cost of culture medium with the less effective result, especially in developing countries. The medium was modified with commercial chemicals and fertilizers locally available in Indonesia to reduce the production cost. This study aimed to assess the biological, technical, and financial feasibility of *Spirulina* sp. biomass production using a modified commercial medium. Based on the biological feasibility study, the modified commercial medium (ZK1 and ZK2) gives a similar result to the standard medium, equal for growth rate and protein content. However, the result contains lower fat, carbohydrates, and biopigment. The financial feasibility analysis suggested that the system is feasible starting from 1.2-1.5 kg biomass production in a month. The best result gained on the production capacity of 5 kg biomass using ZK2 medium, with NPV of IDR 183,208,962 (US$12,769), IRR of 73%, B/C ratio of 7.8, and payback period in 7 months. It can be concluded that modified commercial medium was biologically, technically, and financially feasible to be applied in industrial biomass production of *Spirulina* sp.

Keywords: bioindustry; commercial medium; feasibility study; mass production; *Spirulina*

1. Introduction

Spirulina sp. is blue-green algae containing highly bioactive compounds introduced as the "best food for the future" at the World Food Conference since 1974. *Spirulina* sp. could be used as a source of nutritional supplements with several advantages, e.g., high potential to be cultured even in a limited space, integrable with aquaculture production systems, and accountable in needs of protein and vitamin sources for food crisis (1). In 1993, the World Health Organization (WHO) introduced *Spirulina* sp. as an "interesting food for multiple reasons, contain rich iron and proteins, also eligible to be administered to children without any risk". Since then, *Spirulina* sp. treated as a superfood, a highly beneficial healthy food, and market demand for *Spirulina* sp. product continue to increase (2). According to Meticulous Research (3) the global *Spirulina* sp. market is estimated to grow to 68,025.2 tons per year in 2025. The fastest growth will likely occur in the Asia-Pacific region, within any sectors such as the nutrition sector, the food and beverage industry, agriculture, and the cosmetics industry.

The biomass production of *Spirulina* sp. is considered less feasible in developing countries because of high production costs due to high medium costs and production technology. Research and development of alternative medium for *Spirulina* sp. cultivation has been widely done in Indonesia, including utilizing industrial wastewater. However, the alternative medium is limited to laboratory scale, and the unstandardized nutrient of culture medium could affect biomass product quality. In this study, the standard medium was modified with commercial chemicals and fertilizers locally available in Indonesia to reduce the production cost and make it feasible for industrial biomass production without decreasing the nutrient content. The

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feasibility study was carried out by analyzing the biological, technical, and financial feasibilities of modified commercial medium for the biomass production of *Spirulina* sp.

2. Materials and Method

This research was conducted from September 2019 to March 2020 in Aquatic Ecology Laboratory and Biopond Room SITH ITB. The study was conducted in three steps: the analysis of biological feasibility; analysis of technical feasibility or operational; and financial feasibility analysis, on a pilot scale (with 500 L of medium volume) outdoors.

2.1. Research Materials

Spirulina sp. culture obtained from the Brackish Water of Fisheries and Aquaculture Center (BBPBAP) Jepara, Indonesia. The used culture medium is differentiated into three formulations, i.e., the Zarrouk Medium as a standard medium for *Spirulina* sp. (ZM), Zarrouk Medium substituted with commercial chemicals and fertilizers (ZK1), and substituted medium with reduction of some commercial chemicals and fertilizers (ZK2). Formulations of each medium are shown in **Table 1**.

The phosphate source on the standard medium, KH_2PO_4, is modified and substituted with Super Phosphate in ZK1 and ZK2. The substitution was performed due to the less affordable cost of K2HPO4 and its unavailability of commercial grade. The cost of K2HPO4 amounted to IDR 9,000 per gram while the cost of Super Phosphate was IDR 4 per gram, then this substitution suppresses the medium cost significantly. Super Phosphate was also used by Raoof et al. (4) and Ranjith et al. (5) to reduce medium cost for industrial scale.

Table 1. *Spirulina* sp. medium formulations.

Zarrouk Medium (g.l^{-1})		ZK1 medium (g.l^{-1})		ZK2 medium (g.l^{-1})	
$NaHCO_3$	16.8	$NaHCO_3$*	16.8	$NaHCO_3$*	16.8
$NaNO_3$	2.5	$NaNO_3$*	2.5	$NaNO_3$*	2.5
K_2SO_4	1	K_2SO_4*	1	Super Phosphate	0.33
NaCl	1	NaCl*	1	$MgSO_4$*	0.2
K_2HPO_4	0.5	Super Phosphate	0.33	Na_2EDTA*	0.08
$MgSO_4$	0.2	$MgSO_4$*	0.2	$FeCl_3$*	0.01
Na-EDTA	0.08	Na_2EDTA*	0.08	Note :	
$CaCl_2$	0.04	$CaCl_2$*	0.04	*: commercial grade	
$FeSO_4$	0.01	$FeCl_3$*	0.01		

2.2. Biological Feasibility Study

The assessment of biological feasibility is conducted based on biomass production performance and nutrient quality of *Spirulina* sp. Measurement of growth rate was carried out by taking Optical Density data at a wavelength of 520 nm (OD_{520}) using UV-Visible spectrophotometer. Obtained data were then converted into total biomass with a standard regression curve. Biomass productivity is calculated with the following equation :

$$Px = \frac{Xm - Xi}{Tc} \qquad (6)$$

where Px=Productivity (g.l^{-1}.d^{-1}); Xm=maximum biomass concentration (g.l^{-1}); Xi= initial biomass concentration (g.l^{-1}); Tc=maximum biomass time (days).

Proximate analysis is performed to determine proteins, fat, carbohydrates, and biomass water content. Protein content was measured with Semimicro Kjeldahl method, fat analysis with Hydrolysis (*Weibull*) method, carbohydrates with Titration (i.e., Luff-Schoorl method) , and biomass moisture analysis with Gravimetric (Oven) method.

Chlorophyll and carotenoid content were analyzed by mixing the methanol solvent with 10 mL centrifuged biomass of *Spirulina* sp. culture at 6,000 rpm for 5 minutes. The supernatant was then separated from biomass to be extracted using 10 ml solved methanol for 30 minutes. Then, centrifugation was carried out to obtain pigment extract at 6,000 rpm for 15 minutes. The supernatant was observed with a spectrophotometer at a wavelength of 461 nm, 650 nm, 653 nm, and 664 nm [6]. The chlorophyll and carotenoid content were then calculated using the following equation :

$$\text{Chlorophyll (mg/L)} = 25.5 \times A650 + 4 \times A653 \qquad (7)$$
$$\text{Carotenoids (mg/L)} = (A461 + (0.046 \times A664)) \times 4 \qquad (7)$$

2.3. Technical Feasibility Study

The technical feasibility is assessed based on the biomass production scheme and operational necessities for biomass

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production. Materials, equipment, and facilities required for cultivation were accounted for technical analysis based on their specification and quantity. The production scheme was developed based on the real condition in *Spirulina* sp. production site during the study period.

2.4. Financial Feasibility Study

The financial feasibility was analyzed based on collected data from surveys and experiments. Before being analyzed for financial feasibility, all data were projected to produce 1,2-1,5 kg, 3 kg, and 5 kg dry biomass/period during five years of production. The financial feasibility is analyzed based on the investment feasibility, which is determined by the net present value (NPV), internal rate of return (IRR), benefit-cost (B/C) ratio, and payback period. The formulas are described below :

$$NPV = \frac{Net\ Value\ 1}{(1+r)} + \frac{Net\ Value\ 2}{(1+r)^2} + \cdots - Investment\ Cost \quad (8)$$

$$IRR = i_1 + \frac{NPV_1}{NPV_1 - NPV_2} \times (i_2 - i_1) \quad (8)$$

$$BC\ ratio = \frac{\sum PV\ Net\ Value}{\sum PV\ Investment} \times 100\% \quad (8)$$

$$Payback\ Period = \frac{Investment}{net\ value/\ year} \times 1\ year \quad (8)$$

where, i1 = discount rate 1; i2 = discount rate 2; NPV1 = net present value 1; NPV2=net present value 2.

2.5. Statistical Analysis

All data were subjected to one-way variance analysis (ANOVA) using IBM SPSS Statistics.

3. Results and discussion

3.1. Biological Feasibility Analysis

3.1.1. Growth Rate and Biomass Production

Biomass production profile was measured to determine *Spirulina* sp. growth on a pilot scale that will be used to determine the production flow, harvest time, and find out the amount of biomass produced. Before entering the stationary phase, the harvest time was chosen when the culture growth reached the middle of the log phase. This consideration aims to keep the culture in the log phase after harvesting to the maximum production rate. *Spirulina* sp. biomass production profile in pilot-scale and biomass productivity is shown in **Figure 1** and **Table 2**.

A single production period using ZK1 medium was carried out for 24 days with three harvesting times without medium addition. The first harvest was on the 10th culture day with the amount of biomass 1.48 gram/liter, the second harvest was on the 17th culture day with the amount of biomass 1.44 gram/liter, and the third harvest was on the 24th day with the amount of biomass 1.33 gram/liter. Whereas on ZK2 medium, a single production period was carried out for 26 days. The first harvest was done on the 10th day with the amount of biomass produced was 1.68 gram/liter, the second harvesting on the 18th day with the amount of biomass was 1.61 gram/liter, and the third harvesting on the 26th day with the amount of biomass was 1,52 gram/liter. Biomass produced from ZK2 medium was more than biomass from ZK1 medium ($p<0.05$). For 500-liter scale production, the use of ZK1 medium can produce 379 grams of dry biomass, while the ZK2 medium produces 429 grams of biomass. Therefore it is known that biomass productivity for ZK1 medium is 0.140±0,031 gram/liter/day and ZK2 medium is 0.144±0,017 gram/liter/day (**Table 2**).

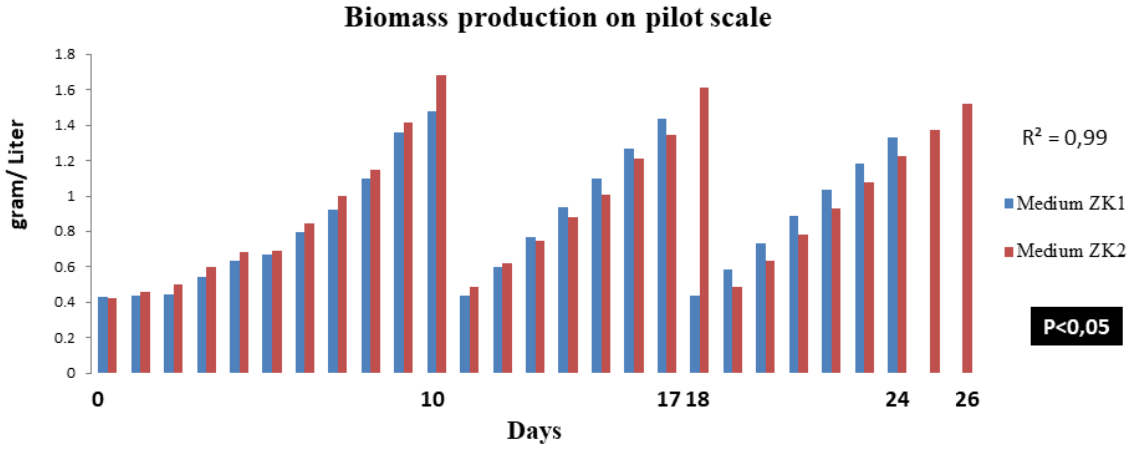

Figure 1. The biomass production profile of *Spirulina* sp. on a pilot scale.

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Table 2. Biomass production and productivity.

ZK1 medium			ZK2 medium		
Day	Biomass ($g.l^{-1}$)	Productivity ($g.l^{-1}d^{-1}$)	Day	Biomassa ($g.l^{-1}$)	Productivity ($g.l^{-1}d^{-1}$)
0	0.428		0	0.425	
10	1.48		10	1.68	
17	1.44	0.140±0.031	18	1.61	0.144±0.017
24	1.33		26	1.52	

3.1.1.Biochemical Analysis

Biochemical compounds were analyzed for protein, lipid, carbohydrates, water content, and biopigment chlorophyll and carotenoid. The results of biomass biochemical compounds from the ZK1 and ZK2 medium compared to the biomass produced from the standard medium (ZM), are shown in **Figure 2**. The biomass protein content of ZK1 medium was 52.29%, ZK2 medium was 52.25%, not significantly different.from the ZM medium, which was 52.95%. For fat content, biomass from ZK1 medium was 3.01%, ZK2 medium was 2.99%, lower than ZM medium, which was 7.16%. Also, for carbohydrates content, ZK1 medium was 6.76%, ZK2 medium was 5.97%, lower than ZM medium, which was 13.2%. Proximate content of *Spirulina* sp. from modified commercial medium (ZK1 and ZK2) generally have equivalent to the standard medium (ZM) for protein content but lower for lipid, carbohydrates, and biopigment (**Figure 2**).

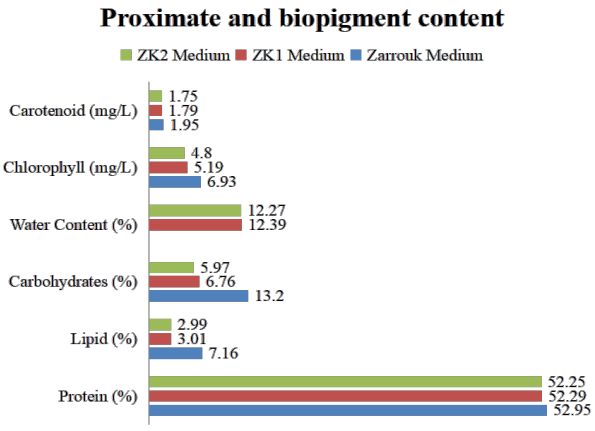

Figure 2. Biochemical analysis of *Spirulina* sp. biomass from each medium.

3.2. Technical Feasibility Analysis

3.2.1.Production Requirement

At each stage of the production flow, identification of production equipment and materials is carried out along with ongoing research. The needs for tools and materials are generally grouped into pre-production materials, production materials, and post-production materials. The need for raw materials for production is analyzed in terms of specifications and quantity that will be used for financial analysis. The primary raw materials are *Spirulina* sp. inoculum, growth medium, and freshwater. The production equipment is cultivation tanks, aeration equipment, electricity, water pumps, harvesting equipment, and drying equipment (**Table 3 – 6**).

Based on the facilities and production analysis results, the farm layout can be determined to produce *Spirulina* sp. biomass using a modified commercial medium. The sizes and specifications of the farm layout are referred to the facilities' needs of each medium and production volume (**Figure 3 and 4**).

Table 3. Production factors and specifications.

Production Factors	Specifications
Spirulina sp. inoculum	*Spirulina* sp. inoculum as a starter to have a biomass of about 1.5 $g.l^{-1}$, obtained from *Spirulina* sp. culture that has been in the maximum growth phase or stationary phase (after 10 days of cultivation).
Medium	The growth medium is made with a modified formulation. Chemicals are grounded physically before being used.
Freshwater	The freshwater has been sterilized using chlorine and Na-thiosulfate. Chlorine is added as much 1.2 $mL.L^{-1}$ and aerated for 24 hours, then add 0.06 $g.l^{-1}$ of Na-thiosulfate and aerated for 24 hours. After that, the water was sterile and ready to be used for cultivation.

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Table 4. Production materials quantity among different production capacity.

Production materials	Production with ZK1 medium			Production with ZK2 medium		
	1.5 kg	3 kg	5 kg	1.2 kg	3 kg	5 kg
Spirulina sp. starter	200 L	400 L	660 L	150 L	350 L	600 L
Medium	2000L	4000 L	6600 L	1500 L	3500 L	6000 L
Freshwater	1800 L	3600 L	5940 L	1350 L	3150 L	5400 L
Chemicals and Disinfectants	3 sets	5 sets	8 sets	2 sets	4 sets	7 sets

Table 5. Production facilities specifications.

Production Facilities	Specifications
Cultivation tank	As the main equipment for cultivation, the tank is round with 175 cm length, 100 cm width, and height of 50 cm.
Aeration Equipment	Serves to provide aeration or air supply in *Spirulina* sp. culture. It consists of an aerator, aeration hoses and air regulator, and aeration stones.
Electricity	Electricity is an energy source for lights, aerators, and water pumps.
Water pump	Function to drain water from and to the system at the beginning of cultivation and when harvesting.
Harvest Equipment	Serves to harvest biomass. It consists of filter cloth, mesh basket, and spatula to collect biomass.
Drying Equipment	Serves to dry the biomass of *Spirulina* sp. so the biomass can be stored for a long time. Drying equipment consists of an oven, drying mat, blender, and airtight container.

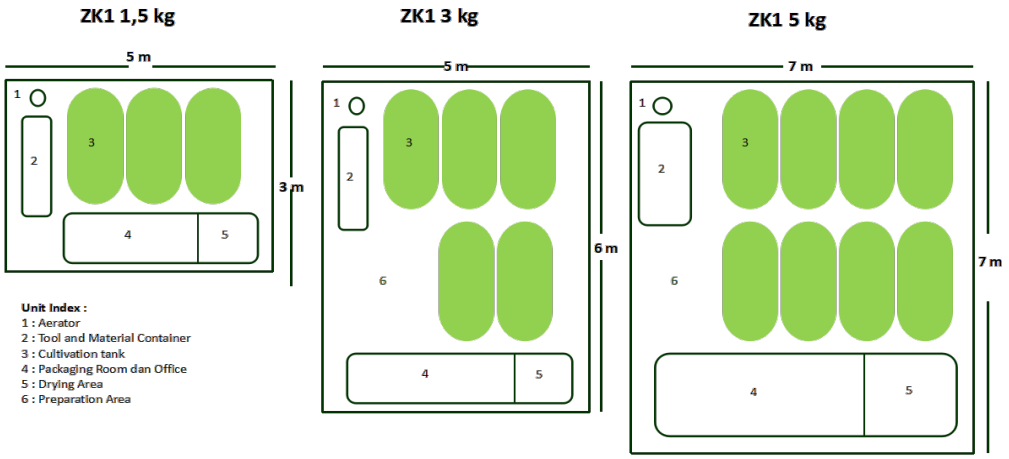

Figure 3. Farm layout of *Spirulina* sp. biomass production with ZK1 medium in different production capacities.

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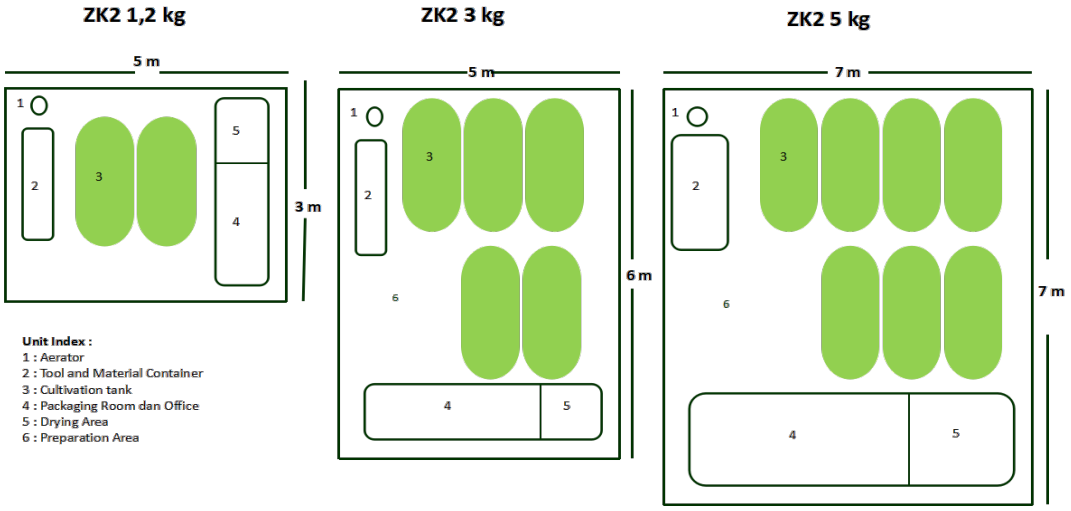

Figure 4. Farm layout of *Spirulina* sp. biomass production with ZK2 medium in different production capacities.

Table 6. Production facilities quantity among different production capacity.

Produnction facilities	Quantity (Scale)		
	1.2-1.5kg	**3 kg**	**5 kg**
Land and Building	15 m^2	30 m^2	49 m^2
Pre-Production set			
Tool and material containers	2 units	2 units	2 units
Digital scale (5kg)	1 unit	1 unit	1 unit
Beaker glass (500 mL)	1 unit	1 unit	1 unit
Drop pipette	1 pc	1 pc	1 pc
Spatula/spoon	1 pc	1 pc	1 pc
Duster	1 pc	2 pc	2 pc
Cultivation set			
Cultivation tank	3 units	5 units	8 units
Aerator	1 unit	1 unit	1 unit
Aeration hose	1 roll	1 roll	1 roll
Aeration stone	6 pc	10 pc	16 pc
Electrical terminal	1 pc	1 pc	1 pc
LED lights	3 units	5 units	8 units
Harvesting set			
Water pump	1 unit	1 unit	1 unit
PVC hose ¾ inch	6 m	6 m	6 m
Screening (100x160 cm)	1 pc	1 pc	1 pc
Harvesting containers	1 unit	1 unit	1 unit
Drying set			
Oven	1 unit	1 unit	1 unit
Alumunium pan	3 pc	3 pc	3 pc
Polyvinyl plastic (200x100cm)	1 pc	1 pc	1 pc
Blender	1 unit	1 unit	1 unit
Mortar (d. 6 cm)	1 pc	1 pc	1 pc
Spatula	1 pc	1 pc	1 pc
Biomass containers	2 units	2 units	2 units

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3.3. Financial Feasibility Analysis

The financial feasibility of *Spirulina* sp. biomass production with the modified commercial medium was analyzed based on the calculation of overall costs, such as cost of raw materials, equipment, and production facilities, the supporting costs needed during the production process, as well as the profits obtained. Furthermore, investment analysis is calculated to determine the feasibility of *Spirulina* sp. biomass production with ZK1 and ZK2 medium, each with three production scales: 1.2-1.5 kg/month, 3 kg/month, and 5 kg/month dry biomass.

The financial feasibility of *Spirulina* sp. biomass production with the modified commercial medium was analyzed with the assumptions: Single production period last about ± 30 days; products are sold per 100 grams at a price of IDR.150,000/pcs; the production costs are *Spirulina* sp. inoculum or starter cost, medium cost, electricity, freshwater, sterilization materials, and other supplies in the form of consumable goods; the investment costs are the cost of production facilities which consist of pre-production set, production set, harvesting set, and drying set, in the form of non-consumable goods; supporting cost consist of the cost of labor, packaging, and marketing; investment discount rate of 12%.

3.3.1. Financial Cost Calculation

Production costs needed for production using ZK1 medium at 1.5 kg/month production capacity is IDR. 793,300, Required cost for 3 kg/month production capacity is IDR 1,463,860, and for 5 kg/month production capacity is IDR 2,342,440. Whereas, required cost for production with ZK2 medium at 1.2 kg/month production capacity is IDR 564,830, 3 kg/month production capacity requires a cost of IDR 1,176,740, and 5 kg/month production capacity requires a cost of IDR 1,922,650.

Operational cost per production period calculated from production cost, depreciation, and supporting cost in one production period. Investment costs for production with ZK1 medium at 1.5 kg/month production capacity is IDR 12,196,400, IDR 21,434,400 for 3 kg production capacity, and IDR 36,791,400 for 5 kg production capacity. As for production with ZK2 medium at 1.2 kg, production capacity requires a cost of IDR 11,077,400, at a production capacity of 3 kg/month requires a cost of IDR 21,434,400, and at a production capacity of 5 kg of IDR 35,672,400 (**Table 7**).

3.3.2. Financial Ratio Calculation

A project is considered feasible if the NPV is positive, the IRR value is greater than the interest rate, and the value of the B/C ratio is greater than 1 (8). It is known that the ZK1 medium is feasible to be used in the production of *Spirulina* sp. with a minimum production scale of 2000 L with a production capacity of 1.5 kg of dry biomass, with a positive NPV value of IDR 35,996,141, the IRR is greater than the 12% interest rate which is 16%, the B/C ratio is greater than 1 which is 5.06, and the payback period in 11 months. The ZK2 medium is feasible to be used for the production of *Spirulina* sp. with a minimum production capacity of 1500 L with a production capacity of 1.2 kg of dry biomass, with an NPV of IDR 28,245,878, IRR of 42%, B/C ratio of 4.5, and payback period in 13 months. The best result is obtained from the production of *Spirulina* sp. with ZK2 medium with a production scale of 6000 L with a production capacity of 5 kg of dry biomass in a month (**Table 8**).

3.3.3. Cost Efficiency of Modified Commercial Medium

Modified commercial medium from the Zarrouk medium used in this study has several advantages. Those are easy to obtain because of their availability in local market suppliers, low cost, and ability to compete with the standard medium productivity. However, the disadvantage of the commercial medium is its solubility is not as good as the Zarrouk medium because the available supply form is some material in granular form, and not all are available in powder. This can be overcome by grinding the medium physically before use. Cost efficiency obtained with this modified commercial medium could reduce costs up to 96% compared to the Zarrouk medium (**Figure 5**). Comparison of medium cost and productivity compared to previous studies is shown in **Table 9**.

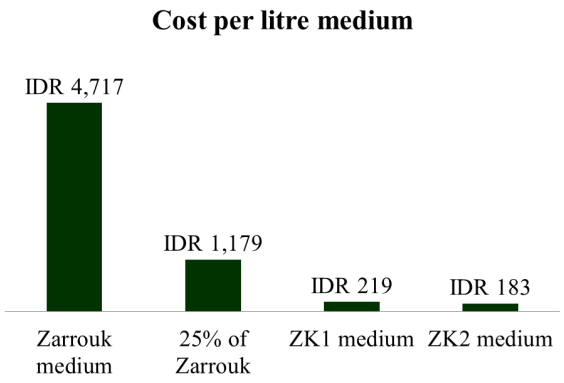

Figure 5. Modified commercial medium (ZK1 and ZK2) cost compare with Zarrouk medium.

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Table 7. Financial cost calculation among different medium and production capacities.

	ZK1 medium			ZK2 medium		
Production capacity	**1.5 kg**	**3 kg**	**5 kg**	**1.2 kg**	**3 kg**	**5 kg**
Production Cost (IDR)						
Medium	440,000	877,000	1,447,000	275,520	642,880	1,102,080
Spirulina sp. starter inoculum	200,000	400,000	660,000	150,000	350,000	600,000
Electricity	120,000	129,400	143,640	115,110	129,360	138,800
Chemicals	18,300	30,500	48,800	12,200	30,500	42,700
Freshwater	12,000	24,000	40,000	9,000	21,000	21,000
Other	3,000	3,000	3,000	3,000	3,000	3,000
Total (IDR)	**793,300**	**1,464,000**	**2,342,440**	**564,800**	**1,176,700**	**1,922,600**
Operational Cost (IDR)						
Labor (3 x 3 hours)	150,000	150,000	300,000	150,000	150,000	300,000
Packaging	50,000	50,000	50,000	50,000	50,000	50,000
Marketing	50,000	50,000	50,000	50,000	50,000	50,000
Depreciation	92,610	138,532	196,782	76,115	138,532	177,365
Total (IDR)	342,600	388,500	596,700	326,100	388,500	577,300
Total Cost/cycle	**1,136,000**	**1,852,500**	**2,940,000**	**891,000**	**1,565,000**	**2,500,000**
Capital Cost (IDR)						
Pre-production set	579,400	579,400	579,400	579,400	579,400	579,400
Cultivation set	4,312,000	6,550,000	9,907,000	3,193,000	6,550,000	8,788,000
Harvesting set	158,000	158,000	158,000	158,000	158,000	158,000
Drying set	1,147,000	1,147,000	1,147,000	1,147,000	1,147,000	1,147,000
Land and Building	6,000,000	13,000,000	25,000,000	6,000,000	13,000,000	25000000
Total (IDR)*	**12,196,400**	**21,434,400**	**36,791,400**	**11,077,400**	**2,143,4400**	**35,672,400**

*tax not included

Table 8. Financial ratio calculation among different medium and production capacities.

	ZK1 medium			ZK2 medium		
Dry Biomass	1.5 kg	3 kg	5 kg	1.2 kg	3 kg	5 kg
Revenue/cycle (IDR)	**2,250,000**	**4,500,000**	**7,500,000**	**1,800,000**	**4,500,000**	**7,500,000**
COGM (IDR)	75,727	61,750	58,784	74,245	52,175	43,500
Profit/cycle	11,14,090	2,647,477	4,560,778	909,055	2,934,737	5,059,985
NPV	35,996,141	93,088,345	160,495,608	28,245,878	105,514,441	183,208,962
IRR	16%	48,00%	71,00%	42,00%	72,00%	73,00%
B/C ratio	5.06	6.85	6.87	4.5	7.5	7.8
Payback Period	11 months	9 months	8 months	13 months	8 months	7 months

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Table 9. Medium cost and productivity among the different mediums of *Spirulina* sp. biomass production.

Medium	Medium cost/ton	Productivity ($g.l^{-1}.d^{-1}$)	Reference
RM6	16 US$	0,03	(4)
Zarrouk (Egypt)	80 US$	$5,2x10^{-5}$	(9)
Reduced Cost Medium	13 US$	$2,8x10^{-5}$	
Zarrouk (India)	7635,128 Rs	-	(5)
Modified Zarrouk	7108,128 Rs	-	
NRC	5022,46 Rs	-	
Modified NRC	5215,06 Rs	-	
NPK (P2C2)	86,62 US$	0,077	(10)
CMU02	13,14 US$	0,037	(11)
SKM	180,80 US$	0,121	(6)
MKM	5,47 US$	0,141	
Zarrouk (Indonesia)	329,86 US$	-	This study
ZK1	15,31 US$	0,140	
ZK2	12,79 US$	0,144	

4. Conclusion

Based on the biological feasibility study, the modified commercial medium (ZK1 and ZK2) gives a similar result to the standard medium (Zarrouk), equal for growth rate and protein content. However, the result contains lower fat, carbohydrates, and biopigment. The technical feasibility analysis suggested that the production period with ZK1 medium was 24 days with 3 times of harvesting (day 10, 17, and 24), and the ZK2 medium was 26 days with 3 times of harvesting (day 10, 18, and 26). The financial feasibility analysis suggested that the system is feasible starting from 1.2-1.5 kg biomass production in a month. Based on this study, it can be concluded that the modified commercial medium is feasible for Spirulina sp. biomass production at a small-scale home industry in Indonesia.

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A Review on The Production of Wine as a Post-Harvest Processing Alternative for Mango, Banana, and Purple Sweet Potato

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Abstract

West Java is one of the regions in Indonesia that produces large numbers of mango, banana, and purple sweet potato. After harvesting, these commodities will undergo physical, chemical, and physiological changes so that further post-harvest processing is needed. One of the post-harvest processing that can be done is fermentation. Fermenting mango, banana, and purple sweet potato into wine is a simple and efficient method that can increase the economic value of the product. Wine is an alcoholic beverage made from grapes; however, any fruit and tuber could be used for wine-making. The article reviews the potential of mango, banana, and purple sweet potato for wine production, the microbes involved, and pretreatments of mango, banana, and purple sweet potato.

Keywords: banana, mango, pretreatments, West Java, wine

1. Introduction

West Java is a region in Indonesia that produces a significant commodity of fruits, such as mango and banana, and tuber, such as purple sweet potato. The production of mango, banana and purple sweet potato increased every year. The latest data obtained from BPS-statistics Indonesia in 2018 West Java produced around 404,543 tons of mangos and approximately 1,13 million tons of bananas [1]. In 2015, West Java had about 456,176 tons of purple sweet potatoes [2].

Fruit and tuber commodities such as mango, banana, purple sweet potato contain a good and complete number of nutritional contents. If consumed according to the recommended portion, it can help maintain health and reduce the risk of micronutrient deficiencies. Moreover, it can also help the healing processes of several diseases, e.g., diabetes, coronary heart disease, bone, and many more [3]. Apart from their nutritional contents, fruits and tubers also have antioxidant activities originated from their pigments, flavonoids, and vitamins that they naturally contain to help protect the body from free radicals [4,5]. The physical condition of fruits and tubers is generally soft so that it is easily damaged. When harvested, they require immediate post-harvest processing. Indonesians usually consume fruits and tubers directly or make them into other products such as chips [6], flour [7], or food coloring [8].

Although the production of mango, banana, and purple sweet potato commodities is abundant, based on the BPS data, the consumption of these commodities in the Indonesian population is incomparable, and a decline occurred in the last five years. The level of public consumption is less than half the recommended level of consumption. Most Indonesians only consume 173 grams of fruit per day, lower than Recommended Dietary Allowances (RDA) of 400 grams per capita per day [3].

In 2014, Indonesia produced approximately 8,097,938 tons of fruit waste [9]. On the other hand, researchers have already predicted that these fruits and tuber production will continue to increase due to Indonesia's potentials, land availability, ecological, climate diversity, germplasm, and human resources that can still be developed more [10]. The amount of these commodities that are already overproduced is not proportional to these fruits and tuber utilization. Therefore, to not make any more waste and create a larger loss, there must be an innovation for their post-harvest processing. The purpose of post-harvest processing is to overcome the short shelf life of fresh products. One of the many post-harvest processing that can be used is fermentation. Fermentation is a simple and efficient method that can transform both fruits and tuber into various products such as pickles [11],

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kombucha [12], or other fermented beverages like cider and wine [13,14]. It is a simple and efficient procedure, and the end product can increase the quality and economic value of fruits and tubers.

According to Indonesia National Standard (SNI), wine is a fermented alcoholic beverage made from grape (*Vitis vinifera*) [15]. However, due to market demand and technological development, wine is not necessarily made from the grape. There are already a lot of wine made from non-grape fruits and tubers. To achieve a smooth fermentation process, these fruits and tubers need a pre-fermentation process called pretreatment. Fruits and tubers have different compositions; therefore, before the fermentation process starts, it is necessary to make adjustments so that the microbes used as starters can work well to metabolize properly. There are several types of pretreatments; chemical, physical, and biochemical using an enzyme. Pre-treatment is used to prepare the media condition before the microbial inoculation so that the process can run smoothly, produce optimal products, increase yield, and speed up the fermentation process [16]. This paper conducts a review to learn more about the pretreatment and fermentation process for mangoes, bananas, and purple sweet potatoes to turn into wine.

2. Fermentation of Wine

Fermentation of food from raw materials is a process found in all parts of the world and a part of some human cultures. One of the numerous fermentation products is wine. Wine is a product from a fermentation process of a grape juice or "must" [17]. The conversion of grape must into wine is a biotechnological tradition that has existed since ancient times probably one of the oldest biotechnology products that ever exists [18]. Over the centuries, many wine-making technologies and strategies have developed, resulting in a wide variety of wine products available and sold in the market today.

Wine is an alcoholic fermented beverage made with the grape as the main ingredient. In general, the first step of wine-making process is the selection of grape and crushing them until the grape must is formed. After moving the must into a tank or barrel, fermentation can start naturally or by adding the starter culture. Wine fermentations are usually carried out for roughly one to two weeks [19]. Following alcoholic fermentation, fermentation can be continued into malolactic fermentation spontaneously or purposely with lactic acid bacteria. The fermentation conditions of wine are as follows: pH range around 3.0-3.5 [20], temperature range around 20-30°C (optimum temperature with *Saccharomyces cerevisiae* inoculum is 32.3°C) [20,21], fermentation can be done by batch fermentation and liquid state fermentation [22].

3. Microbes in Wine Fermentation

Microbes do the fermentation process in wine either it is spontaneous or added. The three most common microorganisms groups involved in food fermentation are bacteria, mold, and yeast [23]. However, Saccharomyces cerevisiae as a starter culture is the most universal practice in the wine-making process [24]. In this day and age, yeast from other groups, such as *Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, or *Lactobacillus acidophilus* is also used for wine-making process. The yeast species that dominates the production of fermented alcoholic beverages in the world is *S. cerevisiae*. This strain will have a significant influence on the taste and aroma characteristics of different types of drinks. *S. cerevisiae* is a spherical or ellipsoidal yeast elongated, with a diameter of 5-10 µm. All yeasts are unicellular organisms possessing ultrastructural features. *S. cerevisiae* is eukaryotic cells [25]. In addition, this yeast has a cell wall, nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, vacuoles, microbodies, and secretory vesicles with complex extracellular and intracellular networks [26].

S. cerevisiae is the most crucial yeast species involved in the fermentation process of alcoholic beverages. This yeast was added as an inoculum because of its optimal fermentative properties. Therefore, this yeast is widely used in the fermented beverage manufacturing industry. *Saccharomyces cerevisiae* has the advantage of being a biocontrol agent through the production of toxins for spoilage microbes in making fermented drinks. This toxin will function as an antimicrobial that will inhibit the growth of spoilage microbes. This yeast can convert simple sugars into ethanol and CO_2 through the fermentation process efficiently. One of the disadvantages of this yeast is that the resulting product does not have a distinctive taste and aroma [27].

Another yeast that is often used in wine-making is *M. pulcherrima.* This yeast is known to have the ability to produce low amounts of alcohol [28]. *M. pulcherrima* has a natural antimicrobial component called pulcherrimin. Pulcherrimin has been shown to inhibit the activity of some yeasts and fungi that can interfere with the fermentation process, but *S. cerevisiae* is not affected by this antimicrobial. Therefore, the combination of *M. pulcherrima* and *S. cerevisiae* is often used to make wine [29]. When combined, both yeasts can produce aromatic components such as esters, improving the final quality of fermented beverage products. However, if used as a single inoculum, this yeast will lead to overproduction of the ethyl acetate component, which will negatively affect the product's sensory characteristics. The aroma of wine produced using a single culture of *M. pulcherrima* will smell like nail polish remover because they produce high ethyl acetate [30].

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T. delbrueckii is another non-*Saccharomyces* yeast used in wine-making [31]. This yeast is the most suitable for use in fermented beverages when combined with *S. cerevisiae* because *T. delbrueckii* has better fermentation performance than other non-*Saccharomyces* yeasts. This yeast has several roles in the fermentation process, such as producing several parameters including low acetic acid, low ethanol concentration, increasing glycerol production, releasing mannoproteins and polysaccharides, having malolactic fermentation ability, increasing some aromatic components, and decreasing the value of some aromatic components like higher alcohol which is undesirable to improve the quality of the final fermented beverage products [32].

The difference between *T. delbrueckii* and *S. cerevisiae* is the rate of CO_2 production and O_2 consumption of *T. delbrueckii* is higher than *S. cerevisiae*. Therefore, the possibility of a defect in the larger-scale production of fermented drinks with these microbes increases because their O_2 consumption differ [33]. Another disadvantage of *T. delbrueckii* is its poor growth under a strict anaerobic conditions because this yeast still needs oxygen for their growth and metabolism [34].

Furthermore, some winemakers combined yeast with lactic acid bacteria to achieve more flavorous wine products [35]. *L. acidophilus* is a rod-shaped, Gram-positive, anaerobic homofermentative bacteria that can use the glycolysis pathway or Embden-Meyerhof-Parnas pathway to ferment sugars and produce DL lactic acid. Because these bacteria can survive at pH 4-5, they are often used in the development of fermented drinks for their ability to enter malolactic fermentation [36]. Malolactic fermentation has an important role in determining the final quality of fermented beverage products. Sugars that are not used by yeast in the alcoholic fermentation process will be used by these bacteria and produce aromatic components to produce products with flavor that consumers like [37]. *S. cerevisiae*, *M. pulcherrima*, *T. delbrueckii*, and *L. acidophilus* can produce metabolites, such as alcohol and other organoleptic properties by utilizing sugar in the must through the Leloir, glycolysis pathway, and produce an alcohol content around 5.75-13.9% [38–43]

4. Wine from Mango, Banana, and Purple Sweet Potato

Although grape has been known as the primary raw material for making wine, the development of technology and market demands has allowed the same methods to be used with non-grape raw materials to create wines with a distinctive flavor and unique color. Moreover, many studies have already investigated the suitability of non-grape ingredients, such as apple [44], mango [45], banana [39], purple sweet potato [38], roselle [46], and also fruit peels [47] to make wine. Wine can be made from a large variety of fruits, tubers, and others as long as they contain enough sugar to convert into alcohol by microbes during the whole fermentation process [48]. The abundant mango, banana, and purple sweet potato require a fast and appropriate post-harvest processing to prevent great losses. One of many post-harvest processes that can be used is fermentation to make those commodities into wine. With a suitable pre-treatment, mango, banana, and purple sweet potato can be used for wine brewing using the same microbes and technique in conventional wine-making.

4.1. Pretreatment Process Before Fermentation

In making fermented drinks, pre-treatment is carried out before fermentation by adding the prepared inoculum. To have a smooth fermentation process, paying attention to what fruits and tubers contain is crucial. Sometimes microbes cannot use the complex components contained in fruits and tubers and hamper the fermentation process. For this reason, it is important to convert the complex components into simpler ones so that the microbes can use them for their nutrients. The end product of fermentation depends on this. Suppose there are no nutrients available for the inoculum. In that case, some unwanted things will happen, such as no fermentation or fermentation occurring with unwanted microbes resulting in contamination that can be dangerous. Contamination can be hazardous as it might lead to safety concerns, health problems, and foodborne diseases. The main goal of this process is to adjust and prepare the media before fermentation takes place. Several pre-treatment methods can be done by using chemical, physical, and biochemical (Figure 1).

Chemical pre-treatment is the addition of chemicals such as sulfur dioxide or sodium metabisulfite. Both sulfur dioxide and sodium metabisulfite are often used in the food and drinks industries as preservatives and antioxidants. The addition of these chemical compounds prevents the presence of microbes other than those added to grow in the fermentation medium [49]. The addition of food additives such as carbon sources, nitrogen sources, and others to support the fermentation media to create optimal conditions for the inoculum is also common. Examples of food additives are sweeteners (sucrose), preservatives (sulfites), and others.

Physical pre-treatment is by sorting, washing is a common practice to remove excess dirt, soil, pesticide residue, and lower microbes' contamination [50], cutting into a smaller size, crushing using a blender until must or juice is formed. Other physical pre-treatments that use heat are pasteurization and sterilization to kill unwanted microbes. Decomposition can occur because heat can cut the glycosidic chain in starch, converting polysaccharides into simple monomers. Heat treatment is the most commonly used technique since it

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effectively maintains product safety because it can prevent the contamination of spoilage microbes [51].

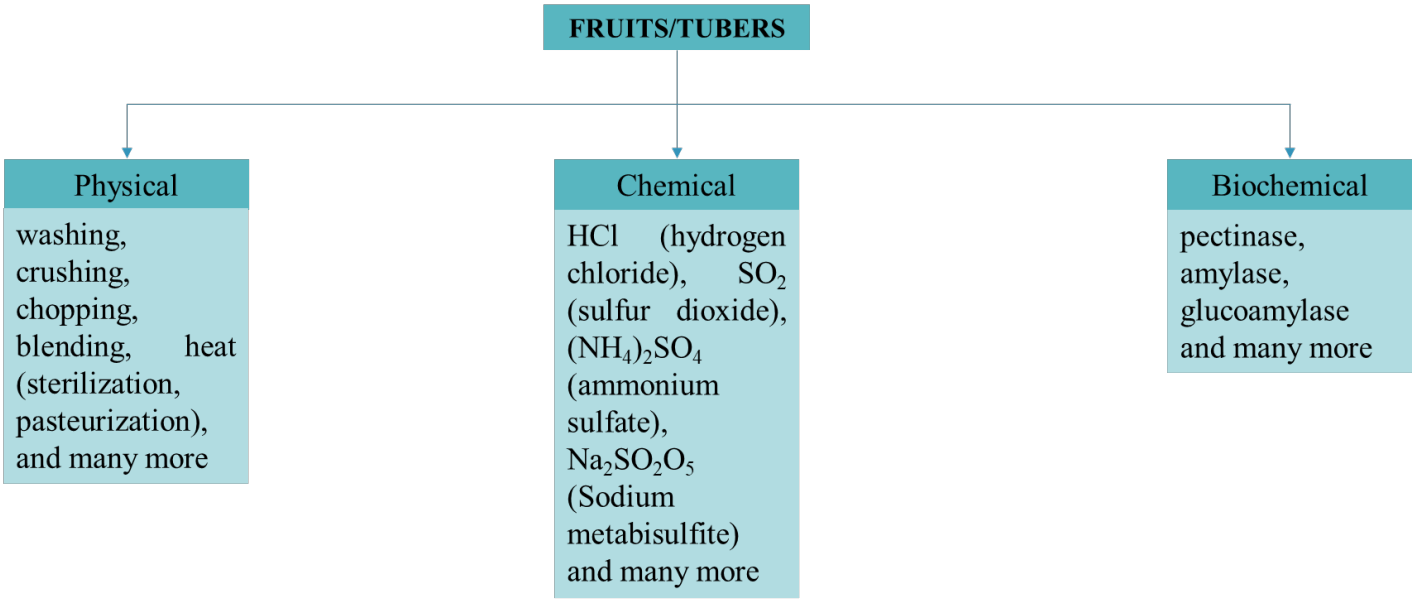

Figure 1. Three Types of Pretreatments

Biochemical pre-treatment with various additional enzymes has a function to catalyze the conversion process of complex compounds in media where the added inoculum does not have this ability. Some enzymes function to reduce various compounds that can inhibit the fermentation process [52]. The use of enzymes, e.g., pectinase, amylase, and glucoamylase, can benefit the beverage manufacturing process due to several reasons, i.e.,

a. The use of enzymes in pre-treatment will increase the efficiency of fermentation seen from the fermentation time, which becomes shorter when the complex compounds in the raw materials have been broken down into simpler compounds.
b. The use of enzymes will increase the yield; when substrate availability increases, the final product produced by the inoculum in the form of alcohol will also increase.
c. No additional filtration steps are needed because enzymes in fermentation can make the final product clear, which will improve the sensory quality.

4.2. Pre-treatments for Mango, Banana and Purple Sweet Potato

Mango is a fruit that can be easy to get in West Java. In general, mango is a fruit with a fresh sour and sweet taste and a thick to a soft texture. The pectin content influences the texture of this fruit. Mango contains approximately 0,35% of pectin. Pectin is a heteropolysaccharide component composed mainly of galacturonic acid linked by α-1,4 glycosidic bonds [53]. *Saccharomyces cerevisiae* cannot use pectin for their metabolism. That is why before fermentation they are some preparations and adjustments. Pre-treatment using pectinase is one of the easiest ways to transform pectin into simple sugar like galactose. *S. cerevisiae* can utilize galactose for the Leloir pathway and then use glucose for the glycolysis pathway [53]. The functions of the pectinase enzyme are shortening fermentation time, increasing yield and sensory quality, clarifying the final product [41].

Another fruit that can be easily accessible in West Java is the banana. Banana (*Musa sapientum*) can be found everywhere in traditional markets or supermarkets. The pectin content influences the texture of bananas. The pectin in bananas ranges from 0.65 to 1.28% [54]. The composition of bananas will change drastically as they ripen. There are a total of 9 stages in the ripening process of bananas. In stage 1, when the banana has a green skin color, the starch complex carbohydrate content is 61.7%. Entering stage 9, the last stage with yellow fruit skin with brown spots, the starch content will change to 2.6%. While the content of simple carbohydrates in the disaccharide group at stage 1 was 1.2% and increased at stage 9 to reach 53.2% [55]. The decomposition of starch in bananas occurs rapidly during the fruit ripening process. This is due to the activity of several enzymes that exist naturally in bananas [56]. By using pectinase and amylase enzyme, they can help breakdown these complex components into simple sugar that yeast can use to utilize for their metabolism.

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Not only fruits but tubers can also be used in the making of wine from non-grape. Purple sweet potato is one type of tuber widely produced in the West Java region to be found easily. Purple sweet potato is rich in starch content of around 40.1-55.1% [57], amylose content of about 15.4%, and amylopectin content of approximately 84.6% [58]. Unfortunately, the yeast used for wine fermentation, *S. cerevisiae,* cannot utilize starch for the metabolism system. It will lead to failure because the yeast does not have enough sugar for their nutrient. Therefore, starch in purple sweet potato has to be converted into simple sugar.

Two enzymes can help with the transformation, i.e., α-amylase and glucoamylase. These enzymes can break starch into glucose. The first step is liquefication using an α-amylase enzyme that works as a catalyst for hydrolysis reaction of α-1,4-D-glycosidic linkages. Then the second step is saccharification using glucoamylase. Glucoamylase enzyme functions as a catalyst for hydrolysis reaction of α-1,4-D-glycosydic linkages and α-1,6-D-glycosydic linkages [59]. When they have already transformed into simple sugar like glucose, *S. cerevisiae* can use that for the glycolysis pathway. In short, during the glycolysis pathway, the simple sugar that enzymes already prepared before fermentation can turn into ethanol or alcohol and other metabolites that can make an excellent quality of the wine.

Enzymes added to making fermented drinks will also influence the sensory attributes of the final product. The addition of enzymes in making fermented drinks has been proven to add aroma, improve color, make fermented beverages clear, help with the filtering and sedimentation process to affect the texture of fermented beverages [60].

5. Conclusion

West Java is a region in Indonesia that produces a large commodity of mango, banana, and purple sweet potato. These commodities contain a good and complete number of nutritional contents. The physical condition of fruits and tubers is generally soft so that it is easily damaged with a short shelf life. When harvested, they require immediate post-harvest processing. One of the post-harvest processing that can be used is fermentation. Mango, banana, purple sweet potato can be used as raw materials for making wine fermented drinks. Wine made from non-grape has been widely developed using the same methods, and microbes such as *Saccharomyces cerevisiae*, *Metschnokowia pulcherrima*, *Torulaspora delbrueckii*, and *Lactobacillus acidophilus* can produce wine mango, banana, purple sweet potato. Almost all fruits and tubers can be transformed into wine by paying attention to the composition so it can determine the appropriate pre-treatment. Pre-treatment is a process before fermentation to prepare and adjust the mango, banana, and purple sweet potato must so that fermentation will run smoothly because of the optimum conditions for the added microbes. Several pre-treatment methods can be done by using chemical, physical, biochemical with enzymes. Mango uses pectinase, which can decompose the pectin content. Banana uses pectinase and amylase, which can decompose pectin and starch content. Purple sweet potato uses amylase, and glucoamylase can decompose the starch content. Using appropriate pre-treatments, fermentation will run smoothly, and high quality with great organoleptic properties will be produced.

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Suitability Analysis of Kampung Pasundan Cisamaya in Mount Ciremai National Park Area as Healing Forest Site

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Abstract

Forests are ecosystems that are comfortable for human health. Spots from the forest ecosystem site suitable for healing forest must be identified. The research aims to analyze the suitability of Kampung Pasundan Cisamaya (KPC) for healing forest activities. The research method refers to the Draft Indonesian National Standard Number 9006:2021 concerning Forest tourism for health therapy (healing forest). The results showed that the KPC site is suitable for healing forest activities. Five reconnection activities with nature, namely invitation by air, invitation by vegetation, invitation by land, invitation by water, and release emotion, can be carried out well in several spots on KPC's healing forest track. Healing forest activities regularly and adequately has a positive impact on health.

Keywords: healing forest site, nature invitations activities

1. Introduction

Humans need a variety of ecosystem services provided by nature, both in the function of provision, regulatory, culture, and support [2]. Humans need to connect and affiliate with nature [7]. Forests are one of the most abundant ecosystems that provide various essential services for human lives and other living things. They may provide habitat for various animals, food, and clean water sources, or even support recreation and spirituality for the community. Forest ecosystems can create a healthy environment because the air contains abundant negative ions, phytoncides, and oxygen. Moreover, they provide acoustic sound sources, natural radiation levels, biodiversity, and create a comfortable climate within the area[8].

Healing services are provided by forests, which are ecosystems that bring numerous advantages to human health. Forest treatment has been shown in multiple studies to have a soothing impact and reduce stress levels as evaluated by psychological and physiological reactions in humans [1]. In terms of psychology, the forest environment can boost happy emotions, reduce negative emotions, improve attention concentration ability, and help people recover from attention fatigue. Several studies have indicated that walking in nature can help persons with depression and anxiety disorders enhance their cognitive performance[1]. This is due to the restorative influence of nature, which can bring relaxation to the psychological and physiological conditions of the body through flowers, trees, and water [1,3].

The forest environment has been demonstrated to lower blood pressure, pulse rate, heart rate, stress hormones, and strengthen the immune system on a physiological level. Physiological relaxation is marked by a drop in blood pressure, as demonstrated [1.3], which found a significant decrease in blood pressure in hypertensive individuals after exposure to a forest setting. Furthermore, physiological relaxation can be shown in the body's endocrine system, dramatically dropping by cortisol, adrenaline, norepinephrine, and dopamine [9]. The forest environment also affects the immune system by breathing phytoncides released by plants. Increased NK cell activity was also seen when compared to the urban environment [3]

Healing forests are forest sites possessing physical qualities that can give healing services. The biophysical attributes of the landscape and the physical elements of the environment that affect the comfort of the five human senses when they are in their ecosystem area are used to identify healing forest sites. The biophysical structure and processes of land have a significant impact on the provision of healing services; hence bio-physical forest characteristics must be examined. The first step in creating an ecosystem for this health nature therapy is suitable sites within the forest.

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2. Methodology

The research location is in Kampung Pasundan Cisamaya (KPC) Mount Ciremai National Park, Kuningan Regency, West Java Province. The research period is 4 (four) months, from December 2020 to March 2021. The KPC area is located in the National Park Utilization Zone at 6º48'36.2" South Latitude and 108º26'05" East Longitude.

The research stages are: (a) determining the suitability of the KPC site with the standard of site suitability for healing forest, and (b) determining invitation by nature activities at selected location spots. The site standard for healing forests in Indonesia refers to the Draft Indonesian National Standard Number 9006:2021 concerning Forest tourism for health therapy (healing forest). The site standard for healing forests in Indonesia refers to the Draft Indonesian National Standard Number 9006:2021 concerning Forest tourism for health therapy (healing forest). This Draft is a new SNI with the scope of establishing principles, orientation, location determination, and components as a guide in determining tourist sites in the forest and developing forest tourism programs for health therapy [6]. This forest healing program is a series of tourism activities in a forest ecosystem unit whose site and facilities and management are designed objectively and measurably to create a series of benefits in various health aspects from tourists for promotive, preventive, curative, rehabilitative, preservative and palliative. Physical environment parameters for healing forest locations consist of 6 (six) parameters: vegetation density, temperature and relative humidity, slope level, noise, wind speed, and the negative ion content of the air, as shown in Table 1.

Table 1 Healing Forest Environmental Parameters

HF Environmental Parameters	Description
Vegetation Density	Medium to dense vegetation density
Temperature & Relative Humidity	Provides a comforting effect for the body Example: The comfort level in a mountain ecosystem is at a temperature of 20 ^{C} to 26 ^{o}C and relative humidity of 40% to 80%
Slope	0% to 15% (flat to gentle)
Noise	< 50 dB
Wind Velocity	<1 m/second
Negative Air Ions (NAI)	> 1000 ion/cm^3

Source: Draft Indonesian National Standard Number 9006:2021 concerning Forest tourism for health therapy (healing forest)

This research uses drone technology and spatial mapping analysis using a geographic information system (GIS). The prospective locations candidates were obtained from the spatial analysis of the physical environment properties during the comprehensive survey phase. Aerial photo acquisition is collected from a UAV equipped with an RGB camera. The UAV was flown at 09.00-11.00 WIB with clear sky conditions through the Drone Deploy flight mission with an altitude of 250 meters and an 85% overlap. Initial aerial photos were processed using Agisoft Methashape to obtain Orthophoto and Digital Terrain Model (DTM).The physical environmental parameters (i.e., air temperature, relative humidity, light intensity, wind speed, and noise level) were measured at two times (08.00 WIB and 13.00 WIB) for five repetitions using a purposive sampling technique on spatial analysis. Measurements were made using the ET-965 IN 1 Environment Meter. Meanwhile, the recording, documentation, and recording of the GPS position during observations were carried out using the ODK Collect application.

3. Results and discussion

3.1. Biophysical characteristics of the site

The results of aerial photography using a UAV that has been processed into a Digital Terrain Model (DTM) and aerial photos are used to interpret the slope of the land and the density of the area's vegetation canopy. Drone technology is utilized in spatial mapping to create clearer and more real-time photos than satellite images [13]. The reason for this is because aerial photography is not affected by weather and is not affected by cloud cover. The aerial photo reveals that the location is composed of a heterogeneous forest with a spring in the eastern part (Figure 1). Before established as a conservation area, Kampung Pasundan Cisamaya was an agroforestry area managed by Perum Perhutani. Therefore,

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tree stands in the area grew naturally or were cultivated, e.g., *Macaranga rhizinoides, Pangium edule, Gnetum gnemon, Durio zibethinus, Pinus merkusii, Ceiba pentandra, Baccaurea racemosa*, *Pterocarpus indicus,* and *Artocarpus integra.*

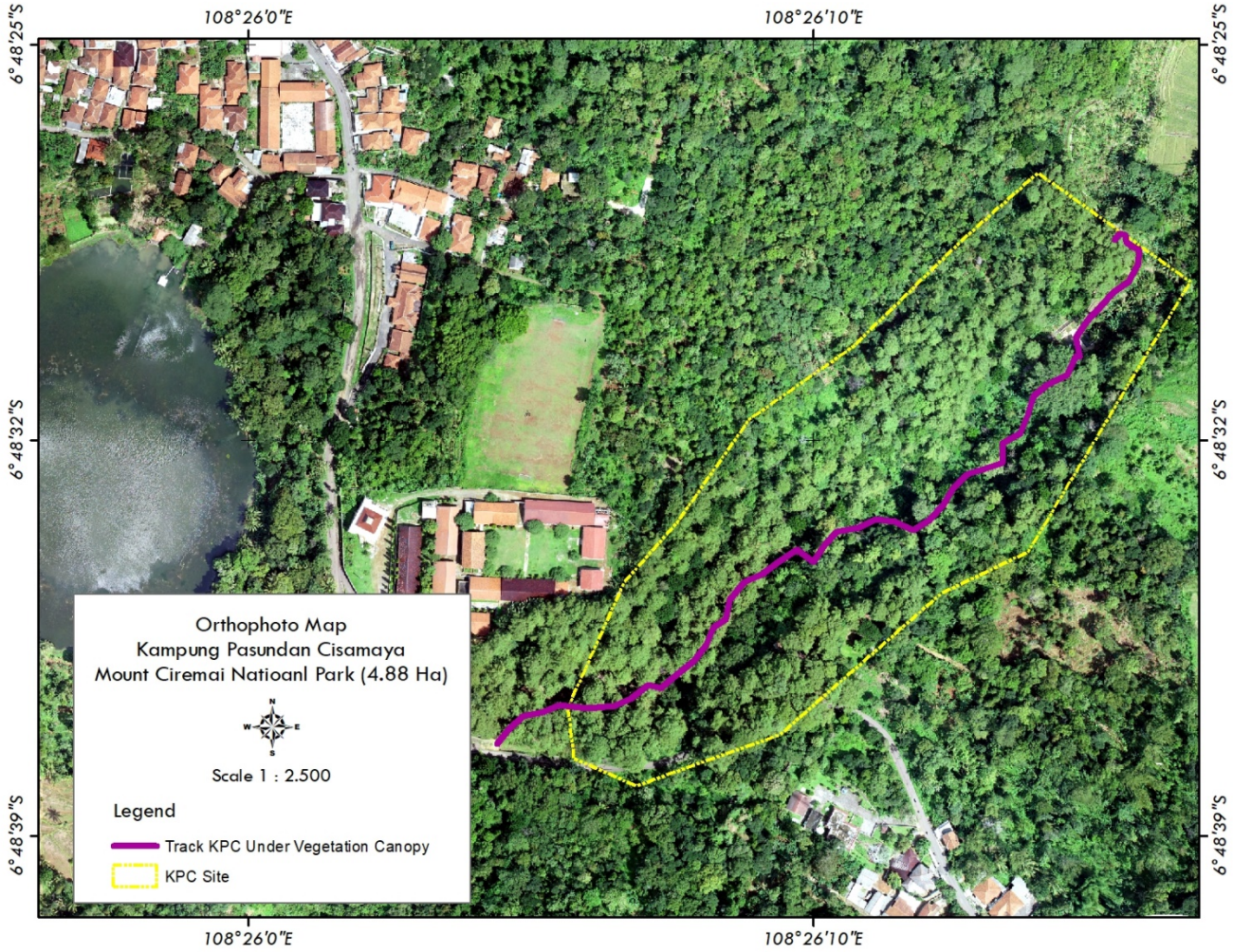

Figure 1 Orthophoto Map of KPC

The aerial photo is used to create a slope and vegetation canopy density map. Based on the land slope obtained from DTM data processing, Kampung Pasundan Cisamaya has a convex topography with a gradient ranging from 0% to 85% with an elevation ranging from 354-414 meters above sea level. The slope in the Kampung Pasundan Cisamaya area can be seen in Figure 2. Based on the classification of slope class in Minister of Public Works Regulation No.41/Prt/M/2007 concerning Guidelines for Technical Criteria for Cultivation Areas, this area is dominated by sloping, steep, to slightly steep land with the acquisition of land percentages of 24.3%, 27.6%, and 29.4% of the total land area, respectively (Figure 2). Meanwhile, land topography with very steep grades was only found in 1.7% of the total land area. An increase in elevation within a certain limit can have a positive effect on mental health, while land that is too steep will provide physiological stress [10].

Vegetation canopy density is carried out by processing the vegetation index transformation on aerial photographs using the Green-Red Vegetation Index (GRVI) method. The results obtained are vegetation index values in the range of -0.31 to 1, divided into three classes of canopy density, namely rare, medium, and dense classes (Figure 1.c). The GRVI value can discriminate between vegetation (value > 0), water bodies (value = 0), and soil (value <0), according to [15].When detecting grass, the value will be negative. The GRVI value will grow to positive as the measured vegetation density increases. The land in the Kampung Pasundan Cisamaya area is dominated by dense canopy density with a range of vegetation index values between 0.1 and 1 which reaches

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88.8% of the total area. The proportion of medium and sparse canopy density with a vegetation index value of -0.31 to 0.1 covered 10.7% and 0.5 percent of the total land area, respectively. The presence of tree stands with high canopy density has more potential to block sunlight and reduce the air temperature in the shade to be cooler than areas with sparse canopy (Sulistyana and Pratiwi, 2011).

3.2. Physical Characteristics of Healing Forestsin KPC

The characteristics of the healing forest ecosystem which are considered to have a relaxing effect on the psychological and physiological conditions of the body are the slope of the land that is not steep, covered with vegetation. It has to provide a comfortable stimulus for the body's five senses (i.e., temperature, humidity, light intensity, wind speed, and noise level) [6]. Meanwhile, the optimal conditions for the parameters of temperature, humidity, noise level, and light intensity use the recovery/treatment room condition approach as stipulated in the Regulation of the Minister of Health of the Republic of Indonesia Number 7 of 2019 concerning Hospital Environmental Health, and Indonesian National Standard Number 9006:2021 concerning Forest tourism for health therapy (healing forest) [6].

Based on the overlay analysis, three forest sites along the trekking route were thought to provide healing benefits (Figure 3). This location is further investigated by measuring microclimate and noise levels that affect healing services (Table 2). In the morning, the average air temperature is 25.8°C, and in the afternoon, it is 27.8°C. However, the air temperature measurements in the morning and afternoon show an increase and decrease in temperature that is not constant at each site. Because it is surrounded by vegetation, spot A has cooler morning temperatures than spots B and C, with more open landscape structures. At the three locations, the midday temperatures are nearly identical. The relative humidity in the morning and evening at each measurement point does not reveal a noticeable trend in the average relative humidity measurement. Spot C has a lower relative air humidity than the other sites, while spot A has a higher relative air humidity than the rest. This is because spot C is a more open area, where spot A is surrounded by vegetation. Therefore, spot C receives more sunshine, indicating a better evaporation process [12].

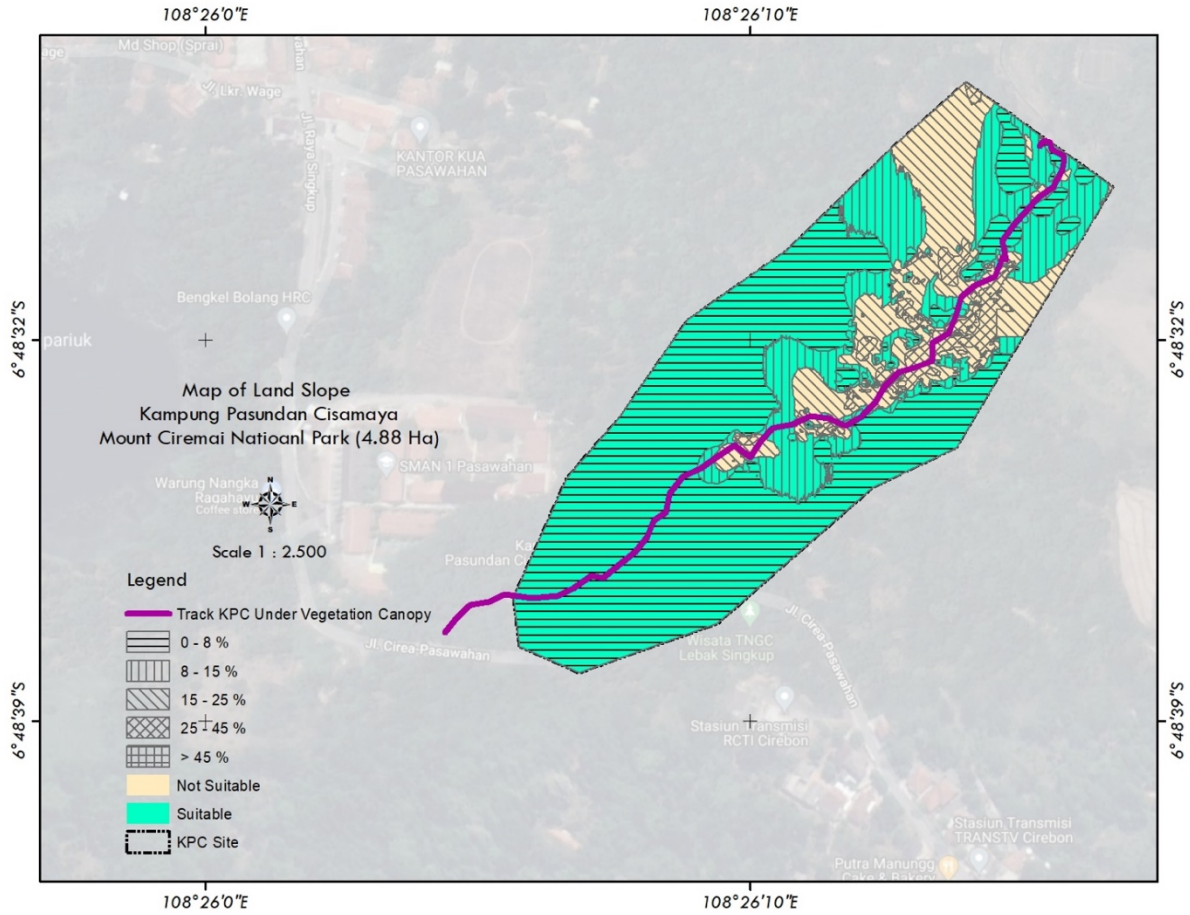

Figure 2 Map of Land Slope of KPC

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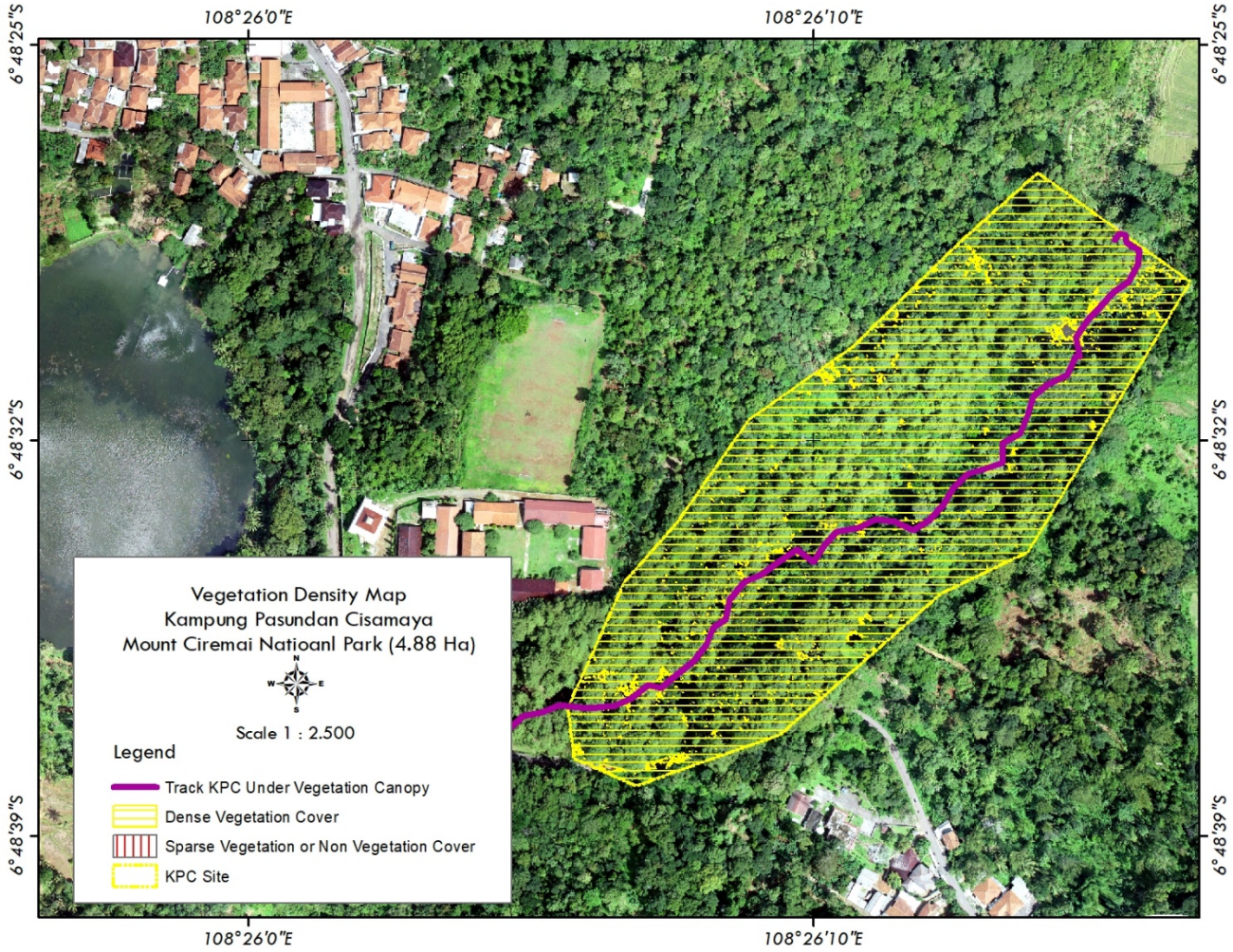

Figure 3. Vegetation Density of KPC

Table 2 Results of the sites' physical characteristics measurements

Time	Spots	Physical Characteristics				
		Air Temperature (°C)	Relative Humidity (%)	Light Intensity (Lux)	Wind Speed (m/s)	Noise Level (dB)
Morning (08.00-10.00)	A	24.61	83.41	3919.20	0.29	46.55
	B	2668	74.06	3467.00	0.20	49.60
	C	26.21	78.68	9506.80	0.74	59.15
Afternoon (12.00-15.00)	A	28.04	79.14	3960.40	0.52	48.54
	B	27.27	81.71	7588.00	0.20	41.72
	C	28.17	75.38	9893.20	0.49	57.81

The average light intensity was 5,631 Lux in the morning and 7,147 Lux in the afternoon in two time zones. Because the light intensity is direct sunshine, which can approach 10,000 lux, Spot C receives more than 9,000 lux in the morning and afternoon. The existence of vegetation is one of the variables in establishing a microclimate of air temperature, humidity, and light intensity; the denser the vegetation, the more stable the microclimate (Fitrani et al., 2016). Nonetheless, sun exposure at a particular time is critical for physical and mental wellness in people of all ages. For example, a few hours of morning exposure to 2,500 lux sunshine can boost a person's cognitive ability, attention,

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performance, and mood[4]. Spot B has a low average wind speed, which is 0.2 m/s, while spot C and A have an average wind speed that is quite felt to be more comfortable than spot B. Wind speed can also affect the concentration of negative ions in the air[15]. Low noise levels and natural sounds are important factors in health tourism and forest bathing activities [2,9.10]. Spot A and B have a pleasant noise level; however, spot C has a noise level of 59.15 dB due to its proximity to a water spring, which is considered tolerable.

3.3. Healing Activities in The KPC Site

The activities in the healing forest site are fun and mindful, relaxing activities. The activities in the healing forest site are fun and mindful, relaxing activities. In some countries, this activity was called forest bathing. People in suitable healing forests will feel comfortable and positively affect health (physical and psychological). Here are some examples [1]: (a) a peaceful mind can reduce stress. Many diseases begin with a stressful mind. The more often a person stay in the forest to heal the tension will reduce the disease symptoms; (b) the clean forest air is good for breathing, as well as inhaling natural aromatherapy from trees in the form of phytoncides which have an impact on immunity; (c) when walking leisurely under the trees (forest), the positive hormone endorphins is released; (d) digital detox against everyday technology that affects health; (e) connect with relaxing nature; (f) natural sounds as nature sound therapy; and (g) boosts a positive mood. To accelerate health effects at the healing forest site, reconnection with nature is established through nature activities. Healing activities at healing forest sites are known as invitations by nature. There are five invitations by nature, i.e., air, vegetation, land, water, and release emotion [rsni, amos]. In one track of the healing forest, there are several spots for the invitation by nature activities. Invitation by air is carried out during healing activities by breathing fresh air from forest ecosystems that are not polluted. Walking barefoot is one of the invitation by nature techniques. As for the invitation by vegetation, water, and release emotion, the site conditions must be analyzed. Figure 4 shows the invitation by nature spots at KPC. There are three spots, i.e., spots for invitation by vegetation, release emotions, and invitation by water.

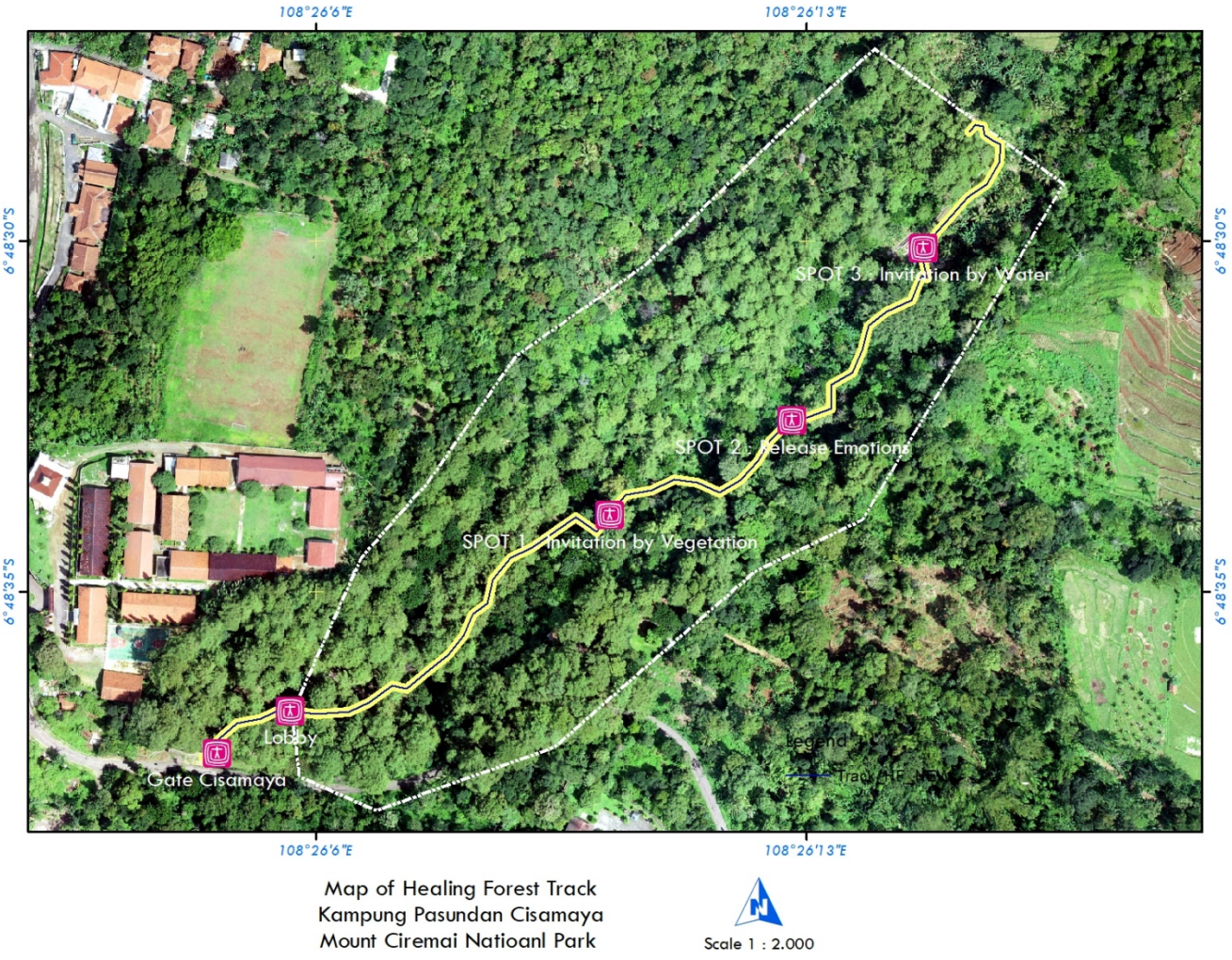

Figure 4. Map of Healing Forest Track in KPC

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Invitation by vegetation activities is connecting the body with vegetation as natural elements. Focusing the eyes on the leaves of the trees enhances the relaxation of the eyes. Being near trees that release phytochemicals in the form of inhaled phytoncides also relaxes the body. The relaxation effect is more potent when we hug the tree slowly while breathing well and having mindful thoughts. In the spot to release emotions, we can scream. The spot for emotion release is a spot that is at an elevation lower than the standing point, for example, a valley full of vegetation. The valley absorbs the sound released, so no sound bounces back to the person screaming. Invitation of water is carried out at the water flow spot. We immerse our feet into the water slowly and feel the water calm our feelings.

Invitation by nature activities in forest ecosystems relax the body and reduce stress. By reducing stress, the body will feel fitter and healthier.

4. Conclusion

Kampung Pasundan Cisamaya (KPC) located in the Utilization Zone of Mount Ciremai National Park, is suitable for a healing forest site. Five activities from invitations by nature, namely invitation by air, land, vegetation, water, and emotion release, can be carried out at the KPC site during the healing forest activity. Healing forest activities regularly and adequately have a positive impact on health.

Acknowledgements

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High-Fat Diets-Induced Metabolic Disorders to Study Molecular Mechanism of Hyperlipidemia in Rats

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Abstract

Hyperlipidemia is a lipid metabolism disorder occurring due to consumption of a high-fat diet (HFD), which contributes to atherosclerosis and cardiovascular disease development. HFD causes metabolic problems in Rodentia animals like human metabolic abnormalities, making it a popular model for studying the signaling systems involved. Hyperlipidemia is a condition in which the body's cholesterol levels elevate. In recent years, several studies have investigated the relationship between HFD feeding and hyperlipidemia and signaling pathways involved in cholesterol homeostasis. However, this signaling mechanism in lipid metabolism has not been fully explained, so additional analysis is needed. The present study aimed to investigate the mechanism that occurs from hyperlipidemia due to HFD feeding. The method used is a literature review approach following the PRISMA scheme for selecting the primary literature, including identification, screening, eligibility test, and inclusion. Eleven articles included primary literature with credibility (H-index) of 20, 33, 71, 92, 93, 162, 180, 192, and 332 (six articles from Q1 journals and five from Q2 journals). Long-term administration of HFD directly affects lipid metabolism, including an increase in the concentration of total cholesterol, triglycerides, LDL, and a decrease in HDL concentration, followed by an increase in body weight. In addition, HFD also disrupts adipose tissue and insulin resistance. The conclusion of this study is that HFD can cause hyperlipidemia either directly or indirectly by inducing insulin resistance, which contributes to lipid metabolism disorders.

Keywords: high-fat diet, hyperlipidemia, atherosclerosis, insulin resistance

1. Introduction

Metabolic syndrome (MetS) is a group of metabolic abnormalities that include hypertension, obesity, insulin resistance, and hyperlipidemia that contribute to the development of atherosclerosis and cardiovascular disease (CVD) [1]. Hyperlipidemia is a common health problem in society due to various factors such as genetics, gender, diet or diet, age, obesity, sedentary lifestyle, smoking, alcohol intake, and high blood pressure [2]. Hyperlipidemia is characterized through elevated concentrations of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-C), as properly as reduced concentrations of high-density lipoprotein-cholesterol (HDL-C). Hyperlipidemia is one of the main risk factor of coronary heart disease (CHD) [3].

Diet plays a vital position in regulating the concentration of lipids and lipoproteins in the blood. At the same time, a lifestyle with high-energy consumption, lack of physical activity, stress, and anxiety with a relatively frequent frequency are factors that cause the development of obesity, atherosclerosis, and CVD [3,4]. A high-fat diet (HFD) can trigger rodent metabolic disorders like in humans [5]. Metabolism disorders due to HFD feeding are considered more relevant than monogenic animal models (germline defect leptin production/signaling) because in humans, it rarely occurs. A widely used animal model for studying physiology, behavior, metabolic disorders, toxicology, and cardiovascular disease is the rat, especially the white rat (*Rattus norvegicus*) (6,7). Rats are an animal model of human disease that is more convenient and beneficial than other organisms (8). The advantages are small size, simplicity, low maintenance cost, and short life cycle. Rats are excellent model animals for biomedical research (7).

HFD is widely used to study signaling mechanisms of metabolic disorders and CVD. There is a relationship between high saturated fats or saturated acids intake with atherosclerosis and CHD [6]. Hyperlipidemia due to long-

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term feeding of HFD is a metabolic disorder that leads to an increase in LDL deposits in the circulatory system, leading to the potential to be atherogenic and contribute to the development of CVD (10,11). Previous studies showed that long-term HFD feeding could significantly increase the concentrations of TC, TG, LDL-C, and VLDL-C compared to the control group, while HDL-C decreased (12,13). In addition, HFD also causes weight gain (14), and 50-60% of overweight people tend to suffer from hyperlipidemia (10,15).

Based on the description above, this literature review study has three goals: (1) determine how the cellular mechanism of HFD increases the concentration of TC, TG, LDL-C, and VLDL-C, and decreases the concentration of HDL-C, (2) determine the effect of HFD on the functional physiology of the body, and (3) determine the time required to induce hyperlipidemia using HFD.

2. Research Method

This study belongs to the category of literature review with the format of semi-systematic. This research used to be carried out by evaluating and analyzing qualitative and quantitative facts from the primary literature that had been formerly selected. Data analysis used to be carried out by quantitative and descriptive methods.

In a literature review-based study, the primary literature becomes a main essential component as a source of data and discussion analysis. Therefore, proper determination of the primary literature is crucial to produce a good literature review-based study. The primary literature was searched through three article search sites, i.e., Google Scholar, National Center for Biotechnology Information (NCBI), and Elsevier's repository with the keywords "*Rattus norvegicus*", and "High Fat Diet", as well as implementing filers "year >2010" and "availability open access". The search results obtained are then sorted according to relevance to the topics discussed, have high credibility, the necessary data, and parameters.

The sorting technique of primary literature candidates used the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) method by identification, screening, eligibility, and inclusion (16,17). The process of searching and sorting the primary literature is presented in the flow chart in **Figure 1**. The primary literature obtained is 11 articles with credibility (H Index) 20, 33, 71, 92, 93, 162, 180, 192, and 332, which are from the journal Q1 has six articles, and Q2 journals have five articles. Eleven primary pieces of literatures can be seen in **Table 1**, and the variety of variables in the primary literature is shown in **Table 2.**

3. Results and discussion

3.1. High-Fat Diet-Induced Metabolism Disorder

Diet is one of the factors that affect the concentration of cholesterol in the body. Other factors include genetics, little physical activity, stress, and anxiety with relatively frequent frequency. Especially high-fat diet (HFD) greatly contributes to the development of atherosclerosis, obesity, and coronary heart disease (CHD) (3,18).

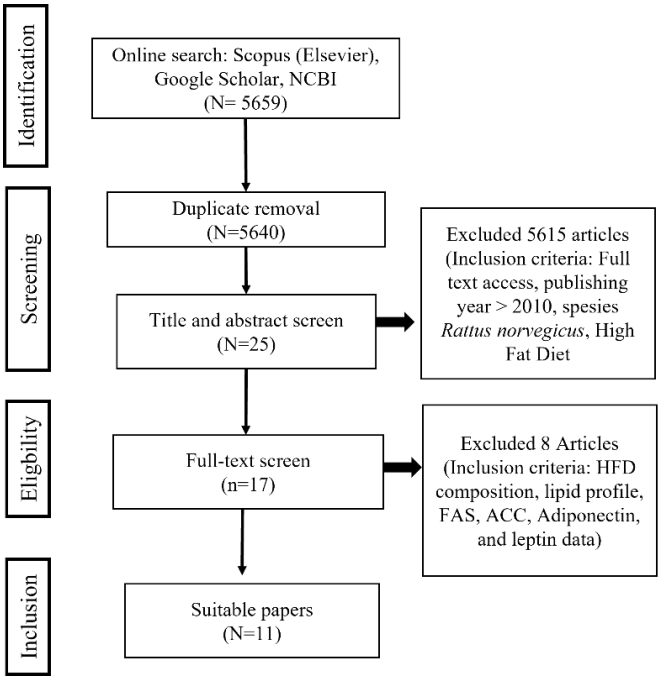

Figure 1. Flow charts of literature review PRISMA

HFD is metabolized in the liver and can accelerate *De Novo* lipogenesis and lipoprotein levels. HFD can also cause oxidative stress by increasing reactive oxygen species (ROS) and reducing antioxidant enzymes (19,20). In addition, HFD can induce obesity, hypertriglyceridemia, hyperlipidemia, hypertrophy, hepatic steatosis, insulin resistance, and beta-cell dysfunction in muscle and liver (9). Furthermore, the energy produced by HFD is higher than regular feed, thus contributing to strongly obesity (obesogenic is an external environmental factor that causes weight gain) (9,15,21).

According to Wang et al.(21), at the beginning of HFD administration, lipid profile parameters did not show any significant changes. This finding demonstrated that cholesterol has a regulatory feedback mechanism, indicating that extra fat in the body has no effect. Feedback regulation of cholesterol is a physiological process in humans and animals that adapts to changes in cholesterol concentrations from the diet to maintain cholesterol homeostasis in blood and peripheral tissues (22). The relationship between cholesterol absorption and biosynthesis is key in maintaining cholesterol homeostasis, which is a negative feedback mechanism. If there is an increase in cholesterol synthesis, the absorption of cholesterol decreases. Contrary, if there is an increase in cholesterol absorption, cholesterol synthesis will decrease (23). However, due to the long-term

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administration of HFD, the feedback regulation ability slowly decrease and is harmful to the body, especially to the heart and liver (24). Long-term HFD feeding can have a direct or indirect impact on lipid metabolism (9,25). HFD can also lead to insulin resistance, which disturbs glucose homeostasis and indirectly affects lipid metabolism disorders (26,27).

Table 1. Primary literature list

No.	Year	Publication	Article Title	Pages	Authors	Journal
1	2011	BioMed Central Ltd.	Comparison of Dietary Control and Atorvastatin on High Fat Diet Induced Hepatic Steatosis and Hyperlipidemia in Rats	Vol. 10, Issues 23	Ji, G., Zhao, X., Leng, L., Liu, P., & Jiang, Z.	Lipids in Health and Disease ISSN 1476511X **H-INDEX 71** **Q2**
2.	2021	Elsevier	Impact of protocatechuic acid on high fat diet-induced metabolic syndrome sequelae in rats	Vol. 907: 174257	Nour, O.A., Ghoniem, H.A., Nader, M.A., Suddek, G.M.	European Journal of Pharmacology ISSN 00142999 **H-INDEX 180** **Q1**
3.	2021	Elsevier Ireland Ltd	Effective amelioration of hepatic inflammation and insulin response in high fat diet-fed rats via regulating AKT/mTOR signaling: Role of Lepidium sativum seed extracts.	Vol. 266:113439	Abdulmalek, S.A., Fessal, M., El-Sayed, M.	Journal of Ethnopharmacology ISSN 03788741 **H-INDEX 192** **Q2**
4	2013	Elsevier BV	Metabolic features of rats resistant to a high-fat diet.	Volume 7, issue 4: e243-e250	Akieda-Asai, S., Koda, S., Sugiyama, M., Hasegawa, K., Furuya, M., Miyazato, M., Date, Y.	Obesity Research and Clinical Practice ISSN 1871403X **H-INDEX 33** **Q2**
5	2021	Elsevier Ireland Ltd	Effects of Danhong injection on dyslipidemia and cholesterol metabolism in high-fat diets fed rats.	Vol. 274: 114058	Du, H., Li, C., Wang, Z., He, Y., Wang, Y., Zhou, H., Wan, H., Yang, J.,	Journal of Ethnopharmacology ISSN 03788741 **H-INDEX 192** **Q2**
6	2020	Elsevier BV	Potential mechanisms of improvement in body weight, metabolic profile, and liver metabolism by honey in rats on a high fat diet	Vol. 14: 100227	Gohar, A., Shakeel, M., Atkinson, R.L., Haleem, D.J.	PharmaNutrition ISSN 22134344 **H-INDEX 20** **Q2**
7	2021	Elsevier Masson	Supplementation of cumin seed powder prevents oxidative stress, hyperlipidemia and non-alcoholic fatty liver in high fat diet fed rats.	Vol. 141: 111908	Miah, P. Mohona, S.B.S., Rahman, Md.M., Subhan, N., Khan, F., Hossain, H., Sharker, S.Md. Alam, Md.A.	Biomedicine & Pharmacotherapy ISSN 07533322 **H-INDEX 92** **Q1**
8	2019	Hindawi Publishing Corporation	Antiobesity, Regulation of Lipid Metabolism, and Attenuation of Liver Oxidative Stress Effects of Hydroxy-α-sanshool Isolated from anthoxylum bungeanum on High-Fat Diet-Induced Hyperlipidemic Rats.	Vol. 2019: 5852494	Wang, L., Fan, W., Zhang, M., Zhang, Q., Li, L., Wang, J., Zhu, L., Wei, D., Peng, W., & Wu, C. [2019]., 2019, 5852494	Oxidative Medicine and Cellular Longevity ISSN 19420994 **H-INDEX 93** **Q1**
9	2017	Public Library of Science .	Resveratrol and caloric restriction prevent hepatic steatosis by regulating SIRT1-autophagy pathway and alleviating endoplasmic reticulum stress in high-fat diet-fed rats	Vol. 12, issue 8: e0183541	Ding, S., Jiang, J., Zhang, G., Bu, Y., Zhang, G., & Zhao, X.	PLoS ONE ISSN 19326203 **H-INDEX 332** **Q1**
10	2018	MDPI Multidisciplinary Digital Publishing Institute	Regulatory Efficacy of the Polyunsaturated Fatty Acids from Microalgae Spirulina platensis on Lipid Metabolism and Gut Microbiota in High-Fat Diet Rats.	Vol. 19, issue 10: 3075	Li, T. T., Liu, Y. Y., Wan, X. Z., Huang, Z. R., Liu, B., & Zhao, C	International journal of molecular sciences **H-INDEX 162** **Q1**
11	2018	MDPI Multidisciplinary Digital Publishing Institute	Regulatory Efficacy of Spirulina platensis Protease Hydrolyzate on Lipid Metabolism and Gut Microbiota in High-Fat Diet-Fed Rats.	Vol. 19, issue 12: 4023	Hua, P., Yu, Z., Xiong, Y., Liu, B., & Zhao, L.	International journal of molecular sciences **H-INDEX 162** **Q1**

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Table 2. Primary literature variable variation

Reference	HFD containing	Energy food	*Rattus norvegicus* strains	Initial Weight rats [g]	Condition	Method	Blood collected
Ji et al., 2011	Casein (25.8 %), Cystine (0.3 %), Lodex (16%), Sucrose (9%), Solka Floc (6%), Lard (31%), Soybean oil (3 %), mineral (6%), vitamin (0.3 %). (Fat 60%, carbohydrate 20%, and protein 20%)	5.21 Kcal/g	Male Sprague-Dawley	180 – 220	12 h light-dark cycle at 22–26 °C	free access to water and feed	Abdominal aorta
Nour et al., 2021	Normal Pellet Diet (365 G/Kg), Casein (300 G/ Kg), Beef Tallow (310 G/Kg) And Vitamins and Minerals Mix (60 G/Kg). (Fat 58%, Carbohydrate 17%, Protein 25%).	-	Male Sprague–Dawley	150 –200	12 h light-dark cycle at 22 °C	free access to water and feed	Retro-orbital venous plexus
Abdul-malek et al., 2021	Butter (310 g/kg), casein (253 g/kg), cholesterol (10 g/kg), vitamins (60 g/kg) and minerals, yeast powder (1.0 g/kg) and sodium chloride (1.0 g/kg). (58% fat, 17% carbohydrate, 25% protein).	-	Male Sprague-Dawley	105 ± 15	12 h light-dark cycle at 22°C ± 2°C	free access to water and feed	retro-orbital venous plexus
Akieda-Asai,et al., 2013	Casein (25.8 %], Cystine [0.3 %], Lodex (16%), Sucrose (9%), Solka Floc (6%), Lard (31%), Soybean oil (3 %), mineral (6%), vitamin (0.3 %). (Fat 60%, carbohydrate 20%, and protein 20%).	5.2 kcal/g	Male Sprague-Dawley & Wistar	150 -180	-	free access to water and feed	-
Du et al., 2021	Basic Feed (67.5%), Egg Yolk Powder (10%), Pig Fat (10%), Sucrose (10%), Cholesterol (2%), And Sodium Cholate 0.5%.		Male Sprague-Dawley	150 ± 20	12 h light-dark cycle at 22°C ± 2°C	free access to water and feed	Retro-orbital venous plexus
Gohar et al., 2020	Casein (25.8 %), Cystine (0.3 %), Lodex 16%, Sucrose (9%), Solka Floc (6%), Lard (31%), Soybean oil (3 %), mineral (6%), vitamin (0.3 %). (Fat 60%, carbohydrate 20%, & protein 20%).	5.2 kcal/g	Male Wistar	120 –140	12 h light-dark cycle at ± 23°C	free access to water and feed	Heart
Miah et al., 2021	Beef fat. The source of fat was local beef market	-	Male Wistar	185 –200	12 h light-dark cycle at 22°C ± 3°C	free access to water and feed	Abdominal aorta
L. Wang et al., 2019	Basic Feed (73%), Cholesterol (1.5%), Pig Fat (10%), Egg Yolk Powder (5%), Sucrose (10%), And Bile Salt (0.5%)	-	Male Wistar	220 ± 20	12 h light-dark cycle at 22°C	free access to water and feed	Abdominal aorta
Ding et al., 2017	Standard Chow (60%), Custard Powder (8%), Lard (12%), Sugar (12%), Peanut Podwer [6%], and Milk (1%). (Fat 41.26 %, Carbohydrates 39%, Protein 19.13%].	4.59 kcal/g	Male Wistar	190 - 270	12 h light-dark cycle at 22°C - 23°C	free access to water and feed	Heart
T.T. Li et al., 2018	Normal Diet (67%), Sugar (20%), Lard (10%), And Cholesterol (3%).	-	Male Wistar	± 200	-	free access to water and feed	Heart
Hua et al., 2018	Normal Diet (67%), Sugar (20%), Lard [10%], and Cholesterol (3%).	-	Male Wistar	223 -227	-	free access to water and feed	Heart

3.2. High Fat Diet Induces Lipid Metabolism Disorders

Hyperlipidemia is a lipid metabolism disorder that causes an increase in the concentration of total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), and a decrease in the concentration of high-density lipoprotein-cholesterol (HDL-C). Hyperlipidemia is the cause of CHD and other cardiovascular diseases (CVD) (3). As reported previously, it was shown that long-term or chronic administration of HFD in rats could induce a significant increase in TC, TG, LDL-C, and VLDL-C compared to the control group, while HDL-C decreased (13,28,29). Based on **Table 3**, lipid profile parameters showed no significant changes at the beginning of HFD treatment. This finding indicates that cholesterol has a regulatory feedback mechanism (21), which in the 2nd-week lipid profile and body weight did not change significantly, and the atherogenic index was low. However, from the fourth week to the eighth week, lipid profile parameters, namely TC, TG, and LDL increased, and HDL decreased compared

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to controls in each study. HFD feeding showed a significant increase in lipid profile from the fourth week, suggesting that the rats had hyperlipidemia.

However, from the tenth week to the seventeenth week, the lipid profile tends to decrease, but bodyweight increases, which means the rat is still obese. This is possible because the lipid profile data were taken from different studies, so the use of feed composition, test reagents, and the formula for calculating the lipid profile is different, resulting in different results (13,30). However, in many studies, the lipid profile tends to increase over time with HFD administration. The highest lipid profile parameters in rats induced by hyperlipidemia using HFD occurred at the eighteenth week, indicated by very high TC, TG, and low HDL. The highest atherogenic index was 20.43, which indicates a very high atherogenic index. It is a powerful marker for predicting the risk of atherosclerosis, coronary artery disease, and cardiovascular disease (30,31). In addition, according to Kammar-Garcia et al. (32) it was reported that a high atherogenic index was correlated with a high prevalence of obesity and abdominal adiposity.

According to Han et al. (24), HFD feeding on rats caused a decrease in the amount of food consumption but still increased body weight. Energy from fat contributes to weight more than non-fat energy. Thermogenesis is the energy to digest, absorb, and store nutrients (33). Thermogenesis for fat is only 2-3%, while protein is 25-30%, and carbohydrates are 6-8%. Therefore, fat has a much higher energy efficiency of 97-98%, while protein is only 70-75%, and carbohydrates are 92-94%. This is what causes HFD to stimulate obesity by increasing energy uptake (18,34). **Table 3.** shows that feeding HFD can increase rat body weight. Individuals who are overweight have a 50-60% risk of developing hyperlipidemia (10).

3.3. HFD Induces Increase Free Fatty Acids (FFAs)

One of the causes of lipid metabolism disorders is increased Free Fatty Acids (FFAs) in the blood (24,28,35). **Figure 2.** shows the increased levels of FFAs due to HFD feeding. FFAs are an energy substrate for the body by the oxidation of FFAs, especially for heart contraction. However, excessive levels of FFAs are one of the risk factors for CVD by increased levels of heart-type fatty acid-binding protein (H-FABP), causing changes in the morphology of the myocardium layer (24). Cardiomyocytes generate up to 70% of their energy requirements by the oxidation of FFAs. Nevertheless, in patients with coronary heart disease, levels of FFAs may lead to the severity of heart failure. FFAs have amphiphilic and detergent-like qualities, a persistent increase in their concentration can cause cardiac dysfunction, such as ion channel abnormalities, membrane integrity, and heart contraction interference (24,36).

Long-term feeding of HFD, which has a high content of saturated fatty acids (SFAs) may lead to a decrease in the levels of polyunsaturated fatty acids (PUFAs) in hepatocyte cells (37). PUFAs maintain cell membrane fluidity, inhibit inflammatory processes, reduce the secretion of proinflammatory cytokines by macrophages, maintain cardiac ventricular rhythm, improve vascular endothelial cell function, and reduce triglyceride synthesis in the liver (1,38). However, long-term HFD feeding can reduce PUFAs, especially in hepatocytes, causing oxidative stress and inflammatory conditions (37,39). These conditions make sterol regulatory element-binding protein (SREBP-1c) activation (9). This is a transcription factor that regulates genes that play a function withinside the synthesis and increases the absorption of FFAs, cholesterol and TG by stimulating the expression of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) proteins (40). It can be concluded that the administration of HFD can increase the expression of FAS and ACC proteins, as shown in **Figure 3.** ACC protein plays a role in inhibiting fatty acid transport to the mitochondria, and thereby fatty acids will be esterified into a large proportion of TG and stimulate *De Novo* lipogenesis (27).

Furthermore, TG is converted into VLDL. VLDL will then become LDL, resulting in an increase in LDL concentration followed by a decrease in HDL concentration (36). The hypothesis of the HFD mechanism that induces lipid metabolism disorders can be seen in **Figure 4.**

Lipolysis occurs in adipocyte cells in response to oxidative stress and inflammation, allowing more FFAs to be transported to the circulatory system (41). Increased levels of FFAs in the circulatory system, then FFAs will be sent to various tissues, especially to the heart and liver. In the liver, fatty acids will be esterified into TG under normal conditions and oxidized in the mitochondria (36). However, suppose fatty acid ranges are chronically high. In that case, the maximum of the fatty acids could be esterified into TG, which might also cause disrupting lipid metabolism characterized by the aid of using growth in LDL and a lower HDL (36,42).

3.4. High-Fat Diet-Induced Endoplasmic Reticulum (ER) Stress

The content SFAs of HFD also causes endoplasmic reticulum (ER) stress (43). ER stress is due to the accumulation of protein misfolding because of SFAs. This may be detected with the aid of using the presence of protein kinase R-like endoplasmic reticulum kinase (PERK), endoribonuclease inositol-requiring kinase-1 (IRE1), and activation of transcription factor-6 (ATF-6) (27). In general, ER stress causes a decrease in the mRNA translation process, resulting in a reduction of protein synthesis. However, the

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transcription process usually continues, specifically in genes concerned with protein folding and protein degradation. ER stress causes inflammation through activation of IκB kinase-mediated by-activated IRE1 and c-JunN-terminal kinase (JNK) (44). Cell dysfunction due to ER stress by SFAs is found in various cell types and has different cellular mechanisms, such as macrophages and hepatocyte cells. In hepatocytes, ER stress stimulates the activation of X-Box Binding Proteins (XBP1s), thereby increasing *De Novo* lipogenesis in the liver, **Figure 4**. This causes an increase in the concentration of TG, VLDL, LDL, and a reduction in HDL (27). However, in macrophages, ER stress causes increased inflammation, mitochondrial dysfunction, and production of ROS, which interfere with insulin signaling in hepatocytes and skeletal muscle (44,45).

Table 3.Lipid Profile Concentration and Body Weight

Reference	TC [mg/dL]	TG [mg/dL]	HDL [mg/dL]	LDL [mg/dL]	VLDL [mg/dL]	AI	Initial body weight [g]	Final body weight [g]	Duration of diet [weeks]
L. Wang et al., 2019	96.67	97.43	30.94	19.33	19.49	2.1	220	240	2
T.T. Li et al., 2018	174.01	70.86	15.47	23.20	14.17	10.3	210	410	4
Hua et al., 2018	154.68	115.15	11.60	23.20	23.03	12.3	224.28	418.53	4
L. Wang et al., 2019	108.28	162.98	35.96	37.90	32.60	2.0	220	280	6
Du et al., 2021	146.95	124.00	19.33	29.00	24.8	6.6	150	450	6
Gohar et al., 2020	225	250	40	200	50	4.625	140	400	8
Miah et al., 2021	300	225	25	250	45	11	71.71	377.65	8
Akieda-Asai et al., 2013	50	60	20	18	12	1.5	180	580	10
Adyab et al., 2019	62.6	38.97	46.79	7.73	7.79	0.3	237.17	476.5	10
Nour et al., 2021	90.7	103.4	42.8	25.3	20.68	1.12	179.2	436.4	14
Adyab et al., 2019	85.07	73.52	51.04	25.91	14.70	0.7	237.17	565.83	17
Ding et al.,2017	81.21	115.15	29.00	25.14	23.03	1.8	190	620	18
Abdulmalek et al., 2021	300	215	14	190	43	20.43	98	402	18
Ji et al., 2011	168.60	80.60	32.10	147.72	16.12	4.25	220	542.14	20

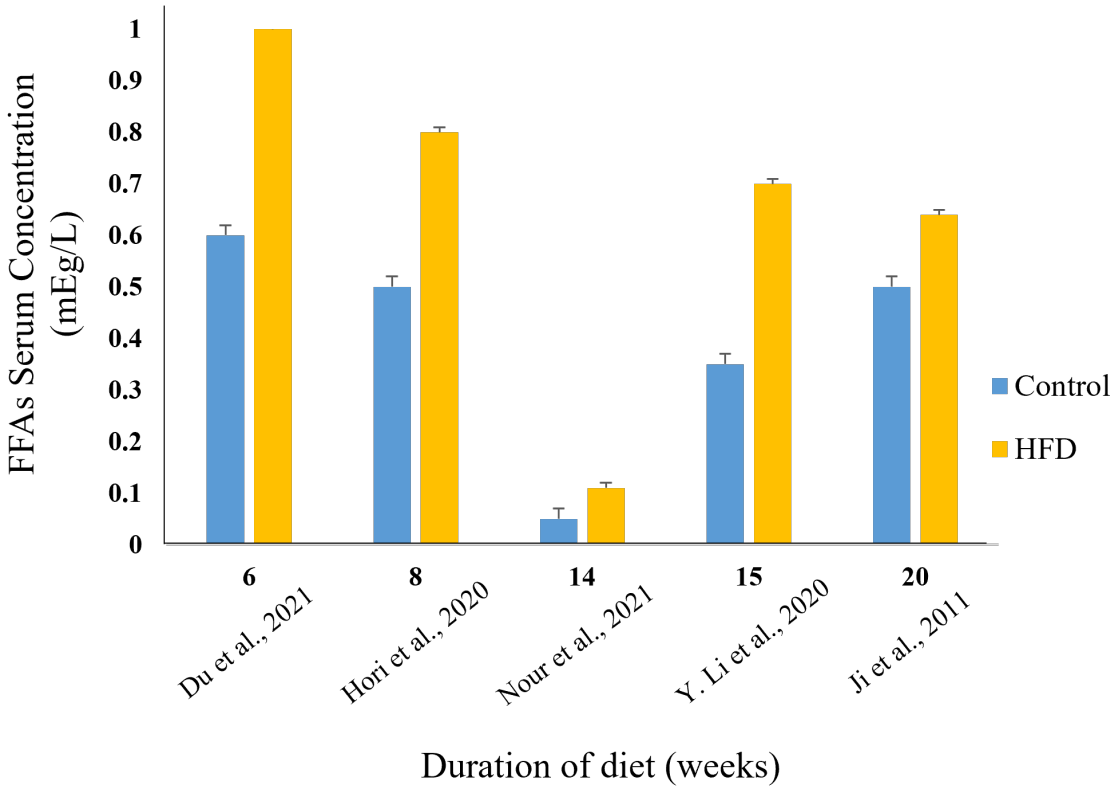

Figure 2. Free Fatty Acids Serum Concentration.

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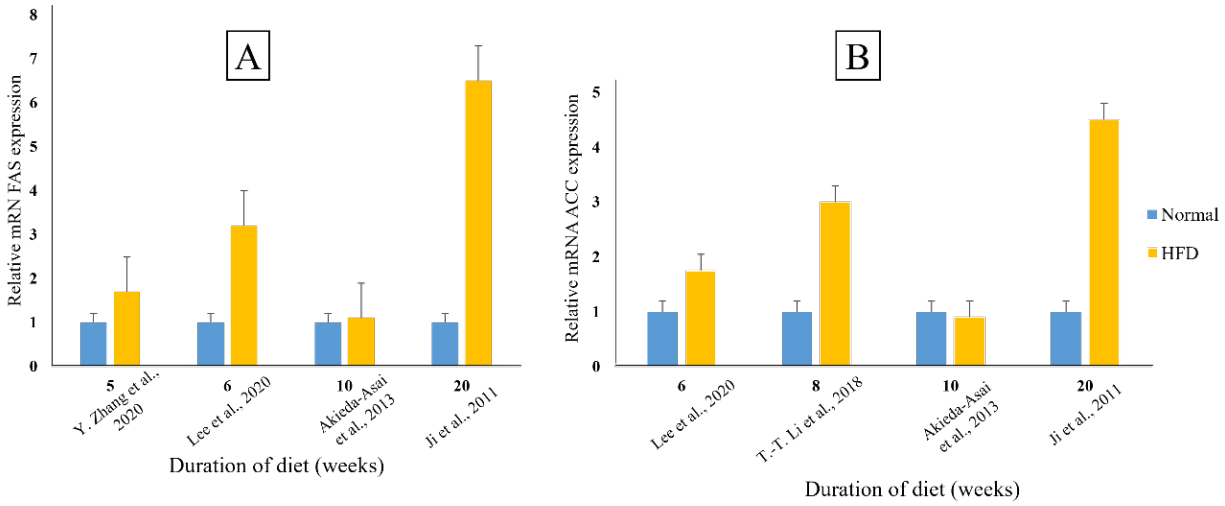

Figure 3. Relative mRNA (A) FAS and (B) ACC expression.

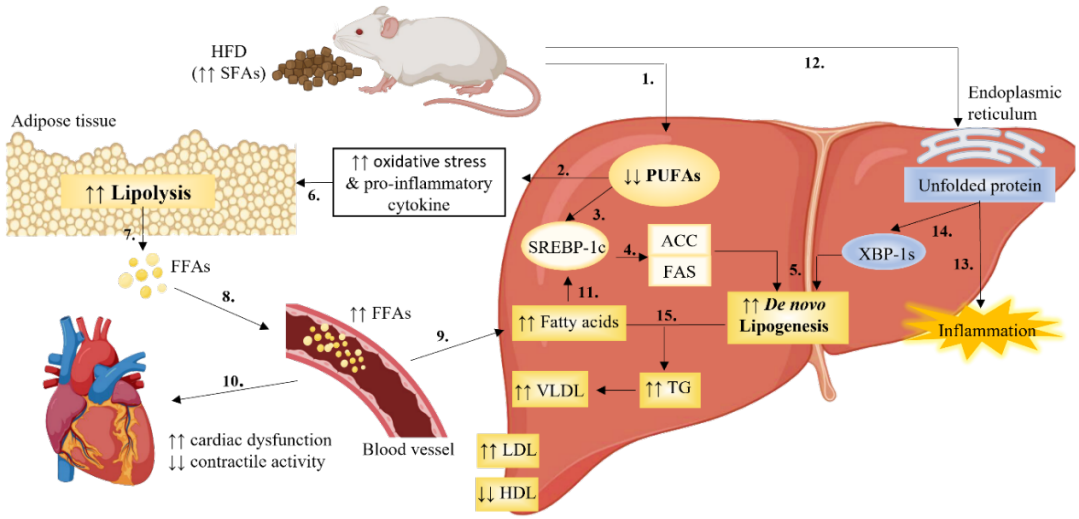

Figure 4. The hypothesized mechanism of HFD induces lipid metabolism disorders. HFD has a high content of SFAs (1), reducing PUFAs in hepatocytes, causing oxidative stress, proinflammatory conditions, and mitochondrial dysfunction in the liver (2). In addition, it activates the transcription factor SREBP-1 (3). SREBP-s stimulus proteins ACC and FAS (4). This induces an increase in *De Novo* lipogenesis (5). Oxidative stress conditions and pro-inflammatory cytokines induce increased lipolysis in adipocytes (6), leading to the secretion of FFAs (7) and more being transferred to the blood vessels (8). FFAs will be transferred to various tissues, especially to the liver (9) and heart (10). HFD causes stress on the ER (12), thereby inducing inflammation (13) and activation of XBP-1s (14). These factors increase the concentrations of TG, VLDL, LDL, and decrease HDL (15).

In macrophages, SFAs can directly induce inflammation extracellularly with the aid of activating the signaling of toll-like receptor-1/6 (TLR-1/6) and TLR-2, while intracellularly inflammation through the products of the metabolism of SFAs (44). Extracellularly, SFAs activate TLR-2 and TLR-1/6 signaling. This is the activation of IKK and NFkB, which lead to the production of pro-inflammatory cytokines (9,46). Intracellularly, metabolic products of SFAs contribute to the development of inflammation. SFAs are precursors of ceramides and sphingolipids, which are ceramides that contribute to inflammation and insulin resistance. Ceramide biosynthesis can be stimulated through TLR-4 signaling and

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may lead to insulin resistance by activating protein phosphatase-2A and PKC that inhibit Akt signaling (44). Ceramide can also activate inflammasomes to stimulate IL-1β secretion by macrophages causes inhibition of insulin signaling (47).

3.5. *High-Fat Diet Induces Adipose Tissue Disorders*

HFD causes hypoxia in adipose tissue, thereby increasing adipocyte cell death (48). This stimulated the migration of M1 macrophages. M1 macrophages infiltrate adipose tissue and secrete many pro-inflammatory cytokines, including IL-6, IL-12, and tumor necrosis factor-α (TNF-α) (2,35,49). In addition to increasing the activity of M1 macrophages, HFD also decreases the activity of regulatory T cells and macrophages M2, both of which are anti-inflammatory cells (9). TNF-α is a pro-inflammatory cytokine usually produced via way of means of macrophages and stimulates the secretion of different pro-inflammatory cytokines. IL-6 may contribute to the development of vascular disease by increasing the ability of macrophages to degrade LDLox, whereas IL-12 contributes to pro-inflammatory cell differentiation and atherosclerotic stimulation (2,50). Pro-inflammatory cytokines result in insulin resistance by way of increasing the production of suppressors of cytokine signaling proteins (SOCS), which inhibit the signaling procedure via blocking off phosphorylation of tyrosine residues from the IRS so growing proteasomal degradation of the insulin receptor substrate (IRS) (9). The hypothesis of the HFD mechanism causing adipose tissue disorders is shown in **Figure 5.**

In addition, HFD also increases the adiposity properties of adipocyte cells (51). This causes dysfunctional adipose tissue, which results in a lower in adiponectin synthesis and an increase in leptin production. (13). **Figure 6 A.** shows that HFD can reduce adiponectin production. Adiponectin is a compound secreted by adipocytes cells that plays a vital position in regulating glycolipid metabolism and energy homeostasis. High adiponectin levels inhibit inflammation by reducing pro-inflammatory cytokines and increasing the expression of the anti-inflammatory cytokine IL-10 (2). Therefore, decreased adiponectin levels cause inflammatory conditions and lead to the severity of insulin resistance (2,10).

Adiponectin can regulate fatty acid oxidation by activating the AMP-activated protein kinase (AMPK) signaling pathway. Adiponectin can protect liver tissue from lipid accumulation (52). Decreased adiponectin production causes an increase in cardiomyocyte cell apoptosis and interstitial fibrosis, thereby eliminating the contractile activity of the heart muscle leading to dysfunction and heart failure (53). In addition to affecting adiponectin, HFD also affects the concentration of leptin. HFD can growth the attention of leptin is shown in **Figure 6 B**. Leptin features as a law of power expenditure and appetite, will increase fatty acid oxidation, and decreases frame fats, and inhibits macrophages from secreting TNF-α, IL-6, and IL-12, thereby excessive leptin ranges motive multiplied subcutaneous fats deposits and obesity (54,55).

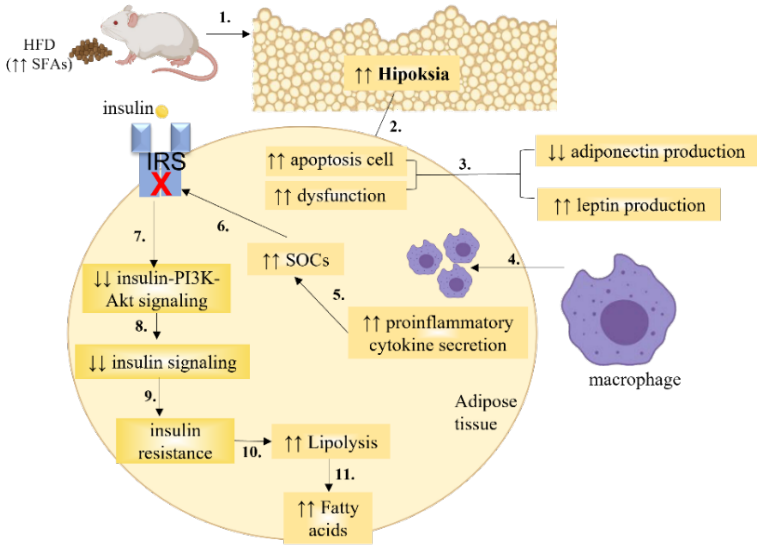

Figure 5. The hypothesized mechanism of HFD induces adipose tissue disorders. HFD can cause hypoxia (1.). Hypoxia stimulates apoptosis and dysfunction of adipocyte cells (2), thereby reducing adiponectin production followed by increased leptin production (3). This stimulates the migration of macrophages to adipose tissue (4). Macrophages secrete pro-inflammatory cytokines resulting in activation of SOCs (5). SOCs block IRS tyrosine residue phosphorylation (6), leading to

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decreased insulin-PI3K-Akt (7) and insulin signaling (8), resulting in insulin resistance (9). Insulin resistance results in increased lipolysis (10), and fatty acids (11).

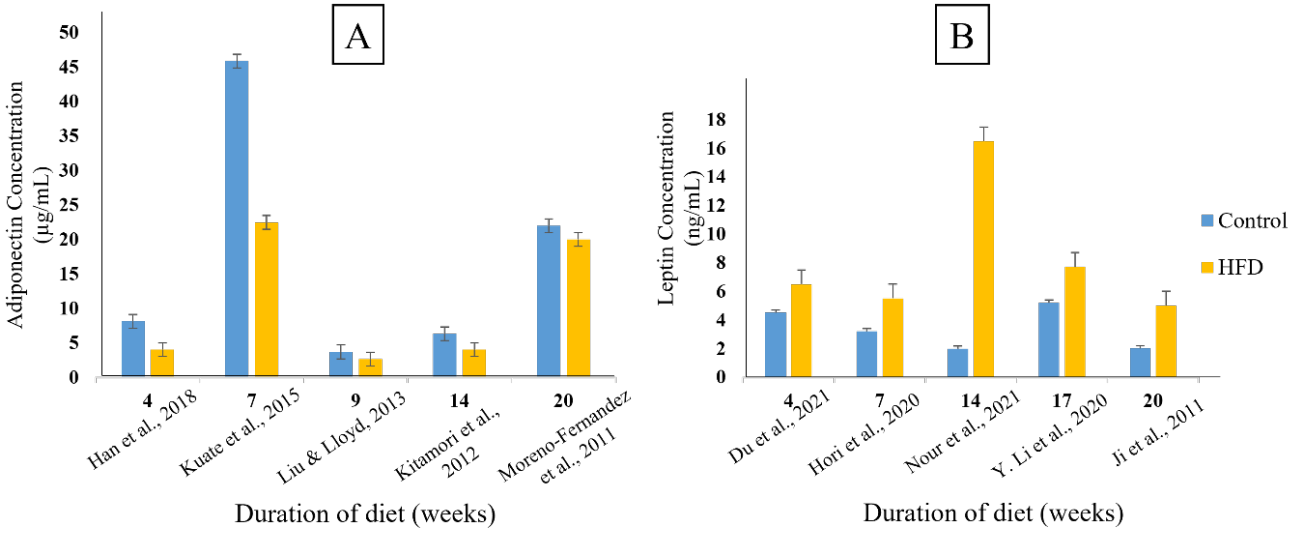

Figure 6. (A) Adiponectin and (B) Leptin Serum Concentration.

Adipose tissue is responsible for the development of insulin resistance (13). Insulin sensitive to adipose tissue will respond by storing TG through the differentiation of preadipocytes into adipocytes cells, thereby increasing uptake of fatty acids from the circulatory system and limiting the occurrence of lipolysis (27). However, in obese individuals, the amount of circulating lipids in the blood exceeds the capacity of adipose tissue for TG storage; thereby, fatty acids will accumulate in other tissues with limited lipid storage capacity, especially in the liver and skeletal muscle, and circulatory system (13). In adipose tissue, insulin plays a role in suppressing the lipolysis of TG to FFAs. However, due to the presence of insulin resistance, the process of inhibiting lipolysis did not occur, increasing the levels of FFAs and lipid deposits (27). Furthermore, there was an increase in the influx of FFAs to hepatocytes, as shown in **Figure 4.** This stimulates the synthesis and secretion of VLDL by the liver and in adipose tissue, which may lead to an increase in TG, VLDL, LDL, and a decrease in HDL (13).

3.6. High-Fat Diet Induces Insulin Resistance

Insulin is a hormone that plays a role in regulating glucose homeostasis in the body (56). Insulin binds to the insulin receptor (IR), resulting in phosphorylation of tyrosine residues on IRS1 and IRS2. This phosphorylation causes downstream phosphatidylinositol 3-kinase (PI3K) activity and the synthesis of triphosphorylated inositol (PIP3) in cell membranes. This stimulates activation of Akt (serine/threonine kinase) signaling and phosphoinositide-dependent kinase (PDK). Activation of Akt signaling leads to activation of the insulin signaling pathway and translocation of glucose transporter type 4 (GLUT4) to cell membranes for glucose uptake. In addition, the transcription factor SREBP-1c was activated for *De Novo* lipogenesis, S6K for protein synthesis, GSK3b for glycogen synthesis and inhibited phosphorylation of Forkhead Box O1 (FOXO1), thereby inhibiting gluconeogenesis (27,57).

Insulin resistance is a metabolic disease characterized via way of means of the lack of ability of insulin to stimulate the presence of glucose in goal tissues, which include the liver, adipose tissue, and skeletal muscle, resulting in systemic hyperglycemia (27). Hyperinsulinemia causes interference with gluconeogenesis, lipolysis in the liver and adipose tissue. Hyperinsulinemia is considered a cause of hypertension, dyslipidemia, and obesity. In general, insulin resistance occurs due to disturbances in insulin signaling pathways, namely insulin receptor substrate 1 (IRS-1), phosphatidylinositol 3-kinase (PI3K), and Protein Kinase B (Akt) pathways (13).

HFD feed for Rodentia animals is used to find out about insulin resistance at the molecular level and insulin signaling mechanisms. Insulin receptors are extensively dispensed in mammalian cells. However, the important sites of insulin action are hepatocytes, adipocytes, skeletal muscle, and neuronal cells. In the liver, insulin has the characteristic to inhibit gluconeogenesis and stimulate glycogen synthesis (56). In skeletal muscle cells, insulin signaling is associated with protein synthesis and glucose uptake. In contrast, in adipose tissue, insulin can inhibit lipolysis, induce glucose, and inhibit fatty acid uptake with the aid of GLUT4. In neuron cells, insulin activates satiety signals and locomotor (27).

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Long-term HFD feeding will increase the production of sn-1,2-diacylglycerol (DAGs) in the liver and muscle (48). The abundance of DAGs in liver tissue activates the calcium-independent "novel" isoform of the protein kinase C (PKC) family. In the liver, DAGs prompt PKC-ε, whereas in the muscle, it is PKC-θ. PKC activation causes phosphorylation of serine residues in the IRS, which inhibits phosphorylation of tyrosine residues by insulin stimulation, thereby decreasing insulin-PI3K-Akt signaling and inhibiting insulin signaling resulting in insulin resistance (9). In skeletal muscle cells, decreased insulin-PI3K-Akt signaling causes an impairing glucose absorption and reduction in GLUT-4 (58). The hypothesis of the HFD mechanism that induces insulin resistance can be seen in **Figure 7**.

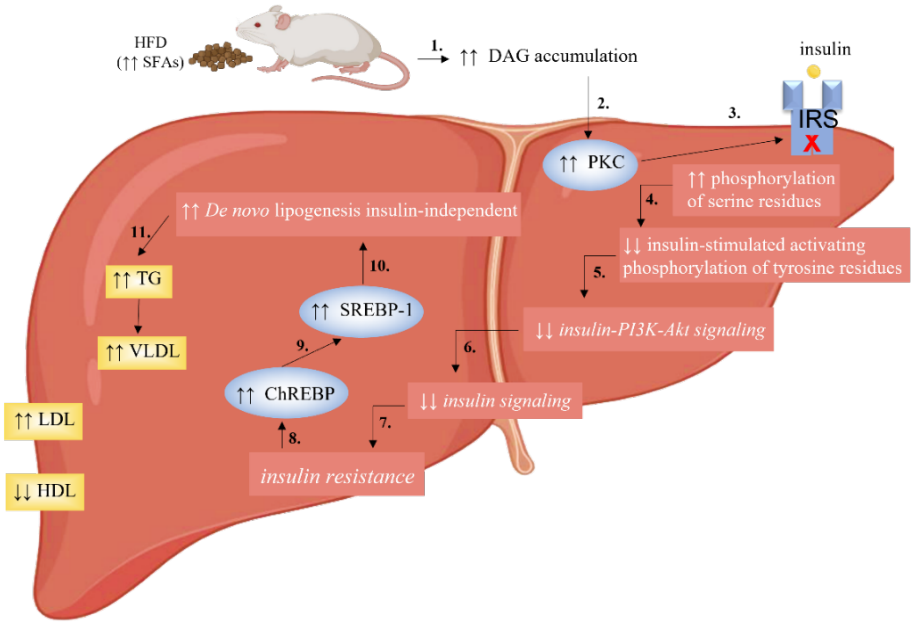

Figure 7. The HFD hypothesis induces insulin resistance. HFD induces the accumulation of DAG (1) in PKC-activated hepatocytes (2). PKC inhibits insulin-induced IRS via phosphorylation of serine residues (3), thereby inhibiting phosphorylation of tyrosine residues by insulin (4). This inhibits insulin-PI3K-Akt signaling (5) and decreases insulin signaling (6), resulting in insulin resistance (7). ChREBP activation (8) induces SREBP-1 (9), and *De Novo* insulin-independent lipogenesis occurs (10), thereby increasing the concentrations of TG, VLDL, LDL, and lowering HDL (11).

Under normal conditions, insulin stimulates lipogenesis in the liver (27). When insulin resistance occurs, there is a decrease in lipogenesis. However, this reduction in lipogenesis only occurred in animal models with genetic defects in hepatic insulin resistance (hepatic insulin receptor ablation) (59). If genetically normal, insulin resistance contributes to increased hepatic lipogenesis and hepatic steatosis in the liver (60,61). It occurs when chronic nutrient overload, especially carbohydrates (glucose and fructose), causes activation of carbohydrate response element-binding protein (ChREBP) and SREBP-1C, thereby activating signaling pathways of insulin-independent lipogenesis, and cells can produce TG (27,62).

4. Conclusion

HFD can cause hyperlipidemia either directly or indirectly. HFD induces hyperlipidemia directly by increasing the production of TG in the liver, thereby increasing TC and LDL-C and lowering HDL-C. HFD causes adipose tissue disorders and insulin resistance, thereby indirectly impacting lipid metabolism disorders. In addition, HFD also causes obesity, where individuals with obesity tend to experience hyperlipidemia. HFD can induce hyperlipidemia after administration of HFD for four weeks.

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Land Cover Change and Land Surface Temperature (LST) in North Bandung Area (NBA) from 2010-2018

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Abstract

North Bandung Area (NBA) was designated as a protected area to regulate the water system around Bandung City. Land conversion from vegetated land into built-up areas can decrease groundwater, increase the risk of floods, landslides, and Land Surface Temperature (LST). This study was conducted to describe LST distribution based on land cover types in specific years of 2010, 2014, and 2018. Landsat 5 and 8 Surface Reflectance (SR) Tier 1 imagery data, West Java land cover maps established by BAPPEDA West Java, and RBI administration maps at a scale of 1: 25,000 were used to generate a map of land cover and LST in this research. There are four land cover classes in NBA, i.e., vegetation, water bodies, open areas, and constructed areas. Within eight years observation (2010 to 2018), bare land decreased from 67.6% (2010) to 57.5% (2018). However, coverage of constructed areas increased within eight years of observation from 22.8% to 27.7 %. In addition, due to the reforestation program, vegetation coverage has slightly increased from 9.6% to 14.7%. LST can be classified into three classes, i.e., low, medium, and high temperature. The area with low and medium-class temperatures decreased from 19% to 16% and 61.3% to 51.7%, respectively. However, high LST increased in NBA 18.7% to 30.3%. The enhancement of 5% vegetation area did not significantly reduce land surface temperature in NBA due to forest conversion to constructed area. Therefore, vegetation coverage must be escalated by reforestation program around NBA to reduce land surface temperature.

Keywords: land cover; Land Surface Temperature (LST); North Bandung Area (NBA); protected area

1. Introduction

North Bandung Area (NBA) is well-known for its natural resources. Its mountain view and cool air make NBA as tourist destination [1,2]. Besides, NBA is also famous for its potential in agriculture. This area has soil and climate that support the development of agricultural business. Some districts in the NBA, such as Lembang, Parongpong, and Cisarua, produce vegetables, fruits, and flowers [3].

Furthermore, NBA is also a protected area based on the Regulation of the Province of West Java Number 2 of 2016 about Guidelines for Controlling the North Bandung Area as a Strategic Area for West Java Province [4]. Adharani and Nurzaman [5] state that NBA has a considerable influence on water management in the area below. Based on the Directorate of Geology and Environmental Management data, 60% of 108,000,000 m^3 groundwater flows into the Bandung Basin from the NBA [6]. In addition, NBA is a basin-shaped highland [7]. This area also has several tributaries that flow into the Citarum River [8]. This landscape makes NBA vulnerable to various natural disasters such as floods, landslides, and erosion [7]. Thus, land utilization in the NBA must be carried out in a controlled manner to maintain the function of water absorption and natural disasters prevention in the NBA and the area below it.

Since 1982, the Government of West Java Province has issued several regulations about the designation of NBA as a protected area. Regulation of West Java Province Number 2 of 2016 stated about Guidelines for Controlling the North Bandung Area as a Strategic Area for West Java Province [4]. These regulations are made in response to the rapid development of settlements and tourism facilities that decrease the function of water system protection and preservation in NBA [2,5,9–11]. However, development permit violations still occur in NBA [12].

The most prominent change in land cover in NBA is the conversion of vegetation into built-up areas, resulting in a decrease in water absorption in this area. Furthermore, it causes various environmental problems in Bandung Basin area, such as disturbances in groundwater reserves and

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supplies in Bandung City, Cimahi City, Bandung Regency, and West Bandung Regency. As an impact of this environmental problem is drought in dry season [5,13,14]. Moreover, land cover change in NBA can increase runoff that lead land erosion, river sedimentation, flood, and landslide [15–17].

Another environmental problem as a result from escalation of built-up areas in the NBA is increasing Land Surface Temperature (LST) which is defined as temperature of land surface area measured radiometrically, including various land surface components such as vegetation and soil [18]. Man-made structures like buildings and roads usually have low albedo and high heat storage that contribute to rise LST [19]. The negative impact of this phenomenon is reducing human health and comfort [19–21]. Therefore, this study was conducted to describe LST distribution based on land cover types in specific years of 2010, 2014, and 2018.

2. Materials and Method

2.1. Study Area

North Bandung Area (NBA) is about ±485 km^2 with altitude varies from 454-2,206 m. This area covers four cities and regencies: Bandung City, Cimahi City, Bandung Regency, and West Bandung Regency. In the northern part of NBA is adjacent with Purwakarta and Subang Regency, in the eastern part with Sumedang Regency, in western part with West Bandung Regency and Cimahi City, and in southern part with Bandung City and Bandung Regency (**Figure 1).**

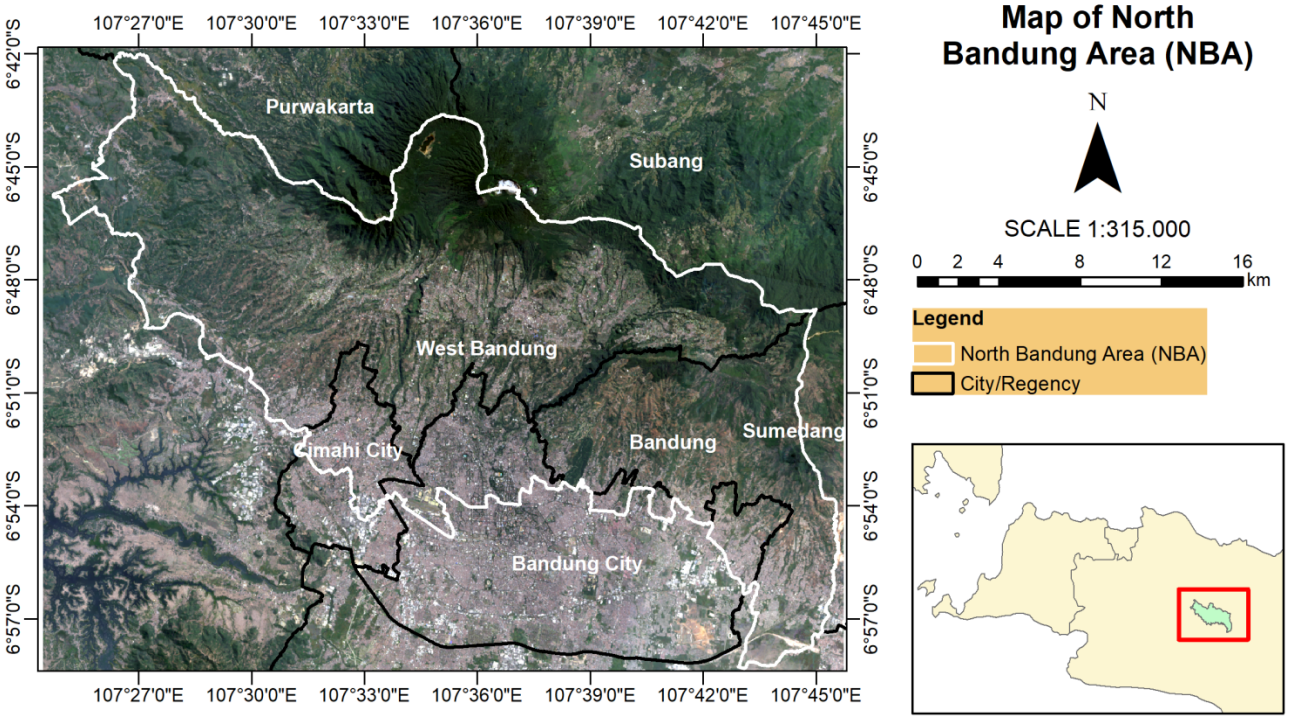

Figure 1. Map of North Bandung Area (NBA)

2.1. Data

Information of this study was obtained from Landsat 5 and 8 Surface Reflectance (SR) Tier 1 imagery data, *Peta Rupa Bumi Indonesia* or Indonesian Topography RBI administration maps at a scale of 1:25,000, and West Java land cover maps in 2010, 2014, and 2018 at a scale of 1:250,000 developed by Badan Perencanaan Pembangunan Daerah or Development Planning Agency at Sub-National Level (BAPPEDA) in West Java. Map of NBA was extracted from RBI administration maps, then overlaid with West Java land cover maps from ArcGIS Service Directory BAPPEDA of West Java Province. The acquisition time for Landsat imagery data was based on Phan et al. [22]. Information about Landsat imagery data acquisition time is shown in **Table 1**.

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Table 1. Time and Numbers of Acquired Landsat Imagery Data.

Type of Landsat Imagery Data	Acquisition Time	Data Number	Use of Data
Landsat 5 SR *Tier* 1	1 June-30 September 2009 and 2010	15	LST mapping in 2010
Landsat 8 SR *Tier* 1	1 June-30 September 2013 and 2014	24	LST mapping in 2014
	1 June-30 September 2017 and 2018	17	LST mapping in 2018

2.2. Methods

2.2.1.Classification of Land Cover Classes

The NBA map is classified using ArcMap 10.4.1 into four classes, i.e., built-up areas, vegetation, water bodies, and open areas [23] (**Table 2**). According to Saha et al. [23], these four land cover classes were based on the sensitivity of an object on the earth's surface to certain wavelengths of electromagnetic radiation that were detected by a specific band on the satellite sensor.

2.2.2.Land Surface Temperature (LST) Calculation

Method from Sultana and Satyanarayana [24] is used to calculate LST. The calculation was carried out by using Google Earth Engine. First, the cloud covers in the obtained Landsat image collection were masked using cloud masking. The Landsat image collection was then merged into one Landsat median composite image. Later, this image was clipped by RBI administration maps of NBA to obtain Landsat median composite image. Then, LST was calculated from Landsat median composite image.

Four variables are needed to calculate LST. The first variable is the Normalized Difference Vegetation Index (NDVI). This variable was calculated by using Equation 1.

$$NDVI = \frac{NIR - R}{NIR + R} \quad (1)$$

NIR in Equation 1 is a value of surface reflectance of the NIR band, and R is a value of surface reflectance of the red band. In Landsat 5, NIR and red bands are found in bands 4 and 3, respectively. In Landsat 8, NIR and red bands are found in band 5 and 4, respectively.

The second variable is fractional vegetation (PV). PV value was calculated by using Equation 2.

$$P_V = \left[\frac{NDVI - NDVI_{\min}}{NDVI_{\max} - NDVI_{\min}} \right]^2 \quad (2)$$

NDVImax is a maximum value of NDVI, while NDVImin is a minimum value of NDVI. After the PV value was obtained, the value of variable ε (emissivity) can be calculated by using Equation 3.

$$\varepsilon = 0.004 \times P_V + 0.986 \quad (3)$$

The value of the brightness temperature (TB) is then calculated. In Landsat 5 dan 8 SR Tier 1, the value of brightness temperature is found in the thermal band. Hence, the TB value can be calculated by using Equation 4.

$$T_B = Thermal \times 0.1 \quad (4)$$

The thermal band in Landsat 5 is found in band 6, and the thermal band in Landsat 8 is found in bands 10 dan 11. Due to the stray light issue in band 11 [25], this study only used band 10 in Landsat 8. In Equation 4, the value of 0.1 is a scale factor of the thermal band. TB value in Equation 4 is obtained in units of Kelvin.

After the values of the four variables are obtained, LST can be calculated. LST is calculated by using Equation 5.

$$LST = \frac{T_B}{(1 + w\frac{T_B}{p}\ln[\varepsilon])} - 273.15 \quad (5)$$

In Equation 5, w is the wavelength of emitted radiance. The w value for Landsat 5 band 6 is 11.5 μm, while the w value for Landsat 8 band 10 is 10.8 μm [26]. The p constant has a value of 1.438 X 10-2m · K. This value is obtained from Equation 6.

$$p = h \times \frac{c}{\sigma} \quad (6)$$

In Equation 6, h is Planck constant with a value of 6.626 X 10-34 J · s. Variable c is the velocity of light with a value of 2.988 X 108 m/s. Variable σ is Boltzmann constant with a value of 1.38 X 10-23 J/K.

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Table 2. New Land Cover Classes that Included Old Land Cover Classes

New Land Cover Classes (Saha et. al., 2020)	Old Land Cover Classes from BAPPEDA West Java
Built-up areas	Built-up areas (including settlements, industries, and buildings)
Vegetation	Forest
Water bodies	River/Lake/Reservoir
Open areas	Garden/Plantation, Field, Rice Field, Shrubs

2.2.3.Data Analysis

LST range classes in 2010, 2014, and 2018 was classified from NBA map, then mean LST in each year in different land cover class were analysed to clarify the significance of mean LST of NBA and the mean LST of each land cover class in the three observed years. Classification of LST range classes was carried out by using equal intervals method [27] in ArcMap 10.4.1. LST maps of NBA were classified into five classes based on Sun et al. [28] as shown in **Table 3**.

Table 3. Classification of LST in NBA

LST Class Type	Temperature Range (°C)
Very Low	0-15.7
Low	15.7-20.3
Medium	20.3-24.8
High	24.8-29.4
Very High	29.4-33.9

Kolmogorov-Smirnov normality test was used to observe LST data distribution both in NBA and in each land cover class. In addition, Kruskal-Wallis and Post Hoc Kruskal-Wallis test was used to observe whether there are significance differences among mean LST of NBA and mean LST of each land cover class in the three observed years.

3. Results and discussion

3.1. Change in Mean LST of NBA and Mean LST of Land Cover Classes

Land surface Temperature in three observed years show significant difference from 2010 (22.53±2.72°C[a]) to 2014 (23.64±2.87°C[b]) and 2018 (23.40±3.06°C[b]), however mean LST from 2014 to 2018 has slightly change as shown in **Table 4.**

Table 4. Mean LST of NBA in the Three Observed Years

NBA Mean LST±Standard Deviation (°C)	Year		
	2010	2014	2018
	22.53±2.72 (a)	23.64±2.87 (b)	23.40±3.06 (b)

*Mean LST with the same letter are not significantly different

Mean LST of each land cover class in 2010, 2014, and 2018 can be seen in **Table 5**. Built-up areas show the highest mean LST (24.98±1.93 in 2010; 26.46±2.09 in 2014, and 26.30±1.96 in 2018) and area with vegetation has the lowest LST (18.29±2.10 in 2010; 19.78±1.87 in 2014, and 19.61±2.15 in 2018). This trend is similar to study conducted by Sun et al. [28], Peng et al. [29] in China, and Saha et al. [23] in India related to calculation of LST in different land cover types.

Table 5. Mean LST of Land Cover Classes in the Three Observed Years

Land Cover Class	NBA Mean LST±Standard Deviation (°C)		
	Year 2010	Year 2014	Year 2018
Built-up Areas	24.98±1.93 (a)	26.46±2.09(b)	26.30±1.96 (b)
Water Bodies	22.22±1.97 (a)	23.70±3.26 (b)	23.14±2.73 (ab)
Open Areas	22.30±2.12 (a)	23.16±2.28 (a)	22.97±2.35 (a)
Vegetation	18.29±2.10 (b)	19.78±1.87 (a)	19.61±2.15 (a)

*Mean LST with the same letter in the same row are not significantly different

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3.2. *Distribution of Land Cover Area and Temperature Class in NBA*

The largest land cover area in NBA is open areas (garden, agricultural land, plantation, field, rice field, and shrubs) which are commonly found in West Bandung Regency and Bandung Regency in the three observed years (**Figure 2**). Based on the Government of West Bandung and Bandung Regency data, agricultural lands are spread over several districts such as Lembang, Parongpong, Cisarua, Cimenyan, and Cilengkrang. These areas have suitable environmental conditions (cool temperature and volcanic soil) to support agriculture [30]. These areas are classified as medium LST class. The percentage of open areas in NBA is about 67.56%, then 27.73% of built-up areas and 14.70% for vegetation (**Figure 3**).

Built-up areas which are covered by settlements, industries, and buildings show high and very high LST classes (**Figure 2**). This land cover class is mostly found in Bandung and Cimahi City. In addition, some built-up areas are also found in Cileunyi, Ngamprah, Cisarua, Parongpong, and Lembang District (**Figure 2**). The percentage of the built-up regions increased from 2010 (22.84%) to 2018 (27.73%) (**Figure 3**) due to various factors, such as the nice natural view [2], development of tourism activities [1,31], availability of good infrastructure (road) [6], urban expansion of Bandung City [9,10], and the development of industry and urban areas in West Bandung Regency [3].

Built-up areas are related to high temperatures due to several factors such as waterproof surfaces that decrease evaporation and materials with high thermal diffusivity; thus these physical properties will increase heat storage during the day and slow heat release at night. [23,32]. The components of built-up areas generally have high surface albedo that increases LST in this land cover class [19].

The term vegetation in this study refers to forests found in the northern part of the West Bandung Regency (**Figure 2**). Forest cover in some areas such as in Cimenyan and Cilengkrang District shows a prominent increase from 2014 (10.29%) to 2018 (14.70%) due to the reforestation program led by Perhutani [33,34]. The result from land cover change data in NBA by Samodoro et al. (2020) also shows similar results; forest cover in this area increased from 2015 (4,400.51 ha) to 2018 (6,669.84 ha) [35]. Forested areas show low and very low LST classes. Vegetation decreases LST by its shading effect, therefore reducing solar energy to the soil surface. These maps cannot determine water bodies because the area is too small (**Figure 2**).

The percentage area with a medium and low class of LST decreased from 2010 (61.29%; 19.02%) to 2018 (51.68%; 15.99%). Meanwhile, there is a significant change of high-class LST from 2010 (18.67%) to 2018 (30.29%) (**Figure 4**). Even though the forested area increased due to the reforestation program, a high percentage of the built-up regions negatively impact land surface temperature. Therefore, reforestation or rehabilitation programs should be expanded in open areas to lowering surface temperature. This result aligns with the study conducted by Lin et al. (2018) in Hangzhou, China, from 1990-2010 [36]. This data shows that the increase in heated areas was directly proportional to the rise in urban areas.

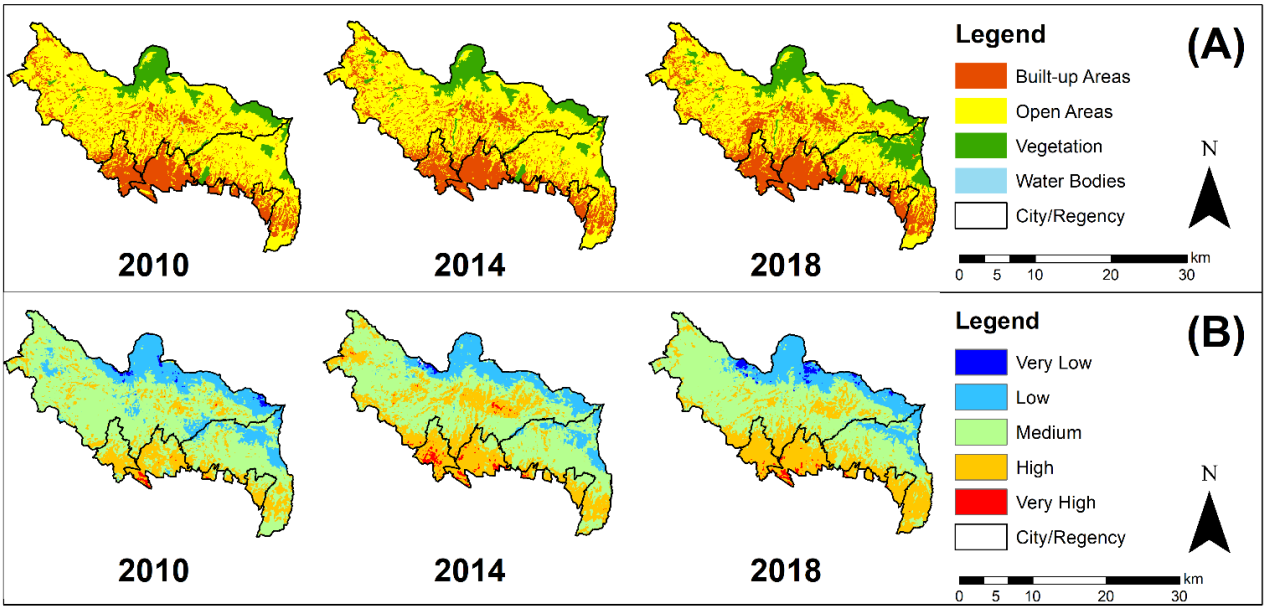

Figure 2. Maps of the Distribution of the Land Cover Class (A) and the LST Class (B) in NBA in the Three Observed Years

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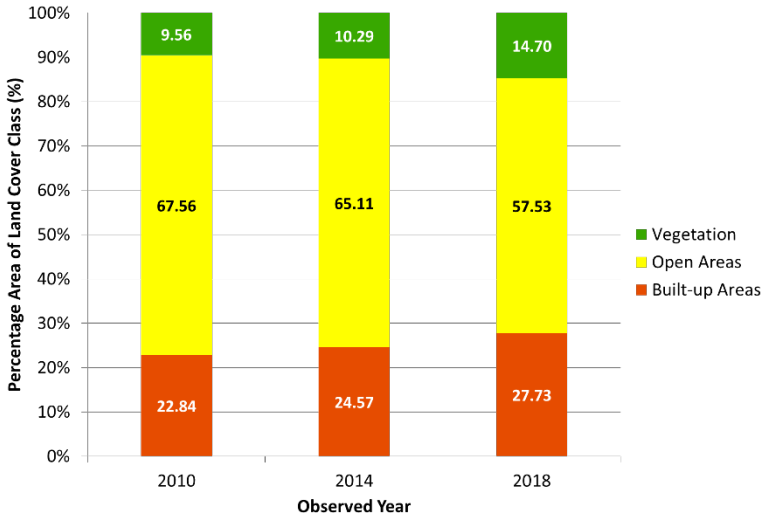

Figure 3. Percentage Area of Land Cover Class in NBA in the Three Observed Years

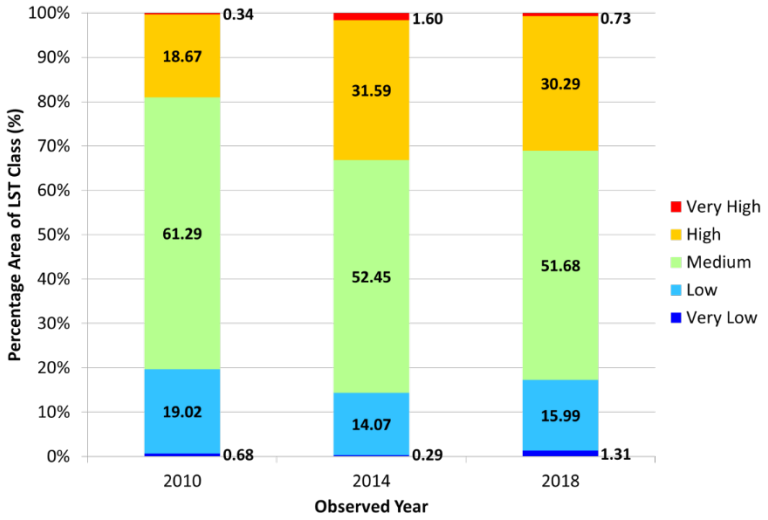

Figure 4. Percentage Area of LST Class in NBA in the Three Observed Years

Land surface temperature is affected by factors, e.g., weather, physical properties of a surface, type of surface material, material structure, surface color, and surface roughness. These material characteristics will affect thermal diffusivity, which indicates how easily heat can penetrate a material [32]. In addition, the water content will affect LST in terms of the cooling process through evapotranspiration. Especially in urban areas, the geometry of the street and Sky View Factor (SVF) also affected LST [37].

3.3. The Implication of Findings

There is a significant change of land cover area in NBA from 2010-2018. Studies related to the land cover change in NBA showed terrible impacts on the environment. Expansion of the built-up regions in the NBA can decrease the water catchment area, reducing the water reserves and supplies in Bandung City, Cimahi City, Bandung Regency, and West Bandung Regency [5,14]. In addition, NBA has a quite high level of landslide vulnerability. The existence of fields and settlements in this area will exacerbate the level of landslide vulnerability [38]. NBA also has several tributaries that flow into Citarum River [8]. The decrease of vegetation cover in this area will cause an increase of runoff and erosion. Erosion will cause river sedimentation that makes the capacity of the river to decrease. This impact will result in a flood in the upstream area of the river and the area below it [17].

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Also, the increase of LST in the NBA negatively impacts human health and comfort [19–21]. According to Arifwidodo et al. [20], negative impacts of high LST on human comfort include sleep deprivation, decrease in daily travel time, and increase in sedentary behavior, which is behaviors with less physical activities. The increasing LST will result in heat stress and other heat-related diseases in humans. Besides, several studies found that an increase in LST will encourage the use of air conditioning in households which causes electrical energy consumption to increase [20]. This impact will increase greenhouse gas emissions that result in global warming [39].

To prevent the negative impacts of land cover change in NBA, several actions should be taken, including limitation of constructed building development, providing a proportional green open space, maintaining forest cover, and expanding vegetated cover as stated in Regulation of the Province of West Java Number 2 of 2016 [4].

4. Conclusion

Land cover change in NBA from 2010 to 2018 caused an increase in LST. The percentage of open areas in the NBA decreased from 67.6% to 57.5%. Meanwhile, vegetation and the built-up regions increased from 9.6% to 14.7% and 22.8% to 27.7%, respectively. Medium class of LST in NBA shifts from 61.3% to 51.7%; low LST class decrease from 19% to 16%; and high LST class increases from 18.7% to 30.3%. Built-up areas are found in high and very high LST because they have material characteristics that decrease evaporation and increase thermal diffusivity. Therefore these physical properties can store heat during the day and slow heat release at night.

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