Developing detoxification and purification technologies of ponds with pesticide residue for organic tiger shrimp farming development

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Abstract. The technological improvement of marginal land was conducted through examination on three treatments; land soaking and washing (A), land remediation (B) and land reclamation (C). The highest average closure rate of \textit{klekap} (66.667±5.774 \%) was gained from B, and the highest average closure rate of \textit{Chaetomorpha sp} (88.667±8.083 \%) was derived from A. The \textit{Apocyclus} discovery through the study can become preliminary information to reveal the existence of \textit{Phromima Suppa}, the indicator of tiger shrimp farming success. The land remediation (B), followed with contaminants detoxification employing plankton, is more effective in eliminating pesticide residue, depressing bacteria population and creating quality water and pond bottom soil according to the organic tiger shrimp production standard.

1. Introduction
Pathogen infection is a threat to the sustainability of the global shrimp industry. Approximately 70 percent of the total area of ponds in Indonesia has been unproductive because of pathogen infection, particularly the type of WSSV virus (\textit{white spot syndrome virus}) \cite{1} and \textit{Vibrio harveyi} \cite{2}\cite{4} have infected pond shrimp in Indonesia, Vietnam, and Malaysia. The pathogen infection is triggered by environmental degradation as an effect of improper land management standards as well as the uncontrolled use of unsustainable production inputs, especially antibiotics, pesticides, and other chemicals.

The use of thiodan type damages the environmental balance as the impact of the death of microorganisms such as Nitrosomonas and Nitrobacter bacteria that play a vital role in nutrient and food chain cycles \cite{5}. That the natural feed needed as a nutrient source is not available in the ponds inhibits shrimp growth. Pesticide residues bind nutrients and soil nutrients so they are not available as natural feed.

The international shrimp market implements food safety and product traceability for shrimp commodities exports, and Indonesian shrimp industry has received a warning from buyers of various EU (European Union) countries and the USA. In some cases, Indonesian shrimp products are indicated to contain antibiotic contaminants, pesticides, chemicals, and other substances as well as bacteria.
exceeding standard requirements, therefore, they are vulnerable to lose the markets in the EU. The EU continues to highlight Indonesian fishery products and impose "zero tolerance" towards Indonesian fishery products, especially shrimp contaminated with antibiotics, pesticides and other chemicals and substances. In fact, the global community has declared 2020 as an organic global year. The demand for organically produced food products has increased, and the organic shrimp are more expensive 1 USD than the non-organic. Purification technology development becomes the solution to the problem of productivity of land polluted by the pesticide. Developed in accordance with the criteria of organic shrimp culture, the technology aims to produce organic shrimp fulfilling the international market provisions. Tiger shrimp (Penaeus monodon), which is still an important commodity for the Indonesian fishery industry, is very susceptible to environmental stress and pathogen infection. The research was conducted to discover land purification technology that can normalize degraded ponds according to the requirements of organic shrimp farming.

2. Methods
Designed in the form of Completely Randomized Design (RAL), the research was conducted at ponds in Suppa Sub-district, Pinrang Regency, South Sulawesi Province from May to December 2016. The land normalization involved three treatments; (1) soaking and washing of the land with tide (A treatment), (2) land remediation through soil surface reversal followed by detoxification of contaminants using plankton (B treatment), and (3) land reclamation through replacement of the pond bottom soil layers with soils from outside the field (C treatment) (Table 1). Each treatment consisted of three replications. The observation parameters used to determine the success of the treatments were (1) closure of klekap and moss (lumut), (2) plankton abundance and diversity, (3) water quality, (4) quality of pond bottom soil, and (5) concentration of antibiotic contaminants, pesticides, and microbes.

| Stages of Treatment | Treatment A | Treatment B | Treatment C |
|---------------------|-------------|-------------|-------------|
| 1                   | Removal of the mud layer | Removal of the mud layer | Soil disposal of pond surface as high as 15 cm (adjusted to the basic soil structure) |
| 2                   | Soaking and washing of pond bottom soil for 4 periods of tidal with 10 days of the soaking period each | Reversal of surface soil | Replacement of surface soil with soil from outside the pond that meets the requirements for organic tiger shrimp farming |
| 3                   | Drying pond and liming it with dolomite as much as 2 ton / ha | Calcification with dolomite of 2 tons / ha | Calcification with dolomite of 2 tons / ha |
| 4                   | Provision of organic fertilizer as much as 500 kg / ha (adjusted for the level of land degradation) | Provision of organic fertilizer as much as 500 kg / ha and probiotics with a dose of 2 ppm to grow plankton | Provision of organic fertilizer as much as 500 kg / ha (adjusted for soil fertility replacement) |

3. Results and discussion

3.1. Soil quality
The results of the observation on soil quality on the reservoir pond, experimental pond and surface replacement soils are presented in Table 2.
Table 2. Results analysis of soil quality at reservoir pond, experiment pond and surface replacement soil

| No | Factors Monitored | Reservoir | Surface Layer of Experiment Pond | Bottom Layer of Experimental Pond | Surface Soil Substitute |
|----|-------------------|-----------|-----------------------------------|-----------------------------------|-------------------------|
| 1  | C-organic (%)     | 1.59      | 1.59                              | 1.69                              | 1.91                    |
| 2  | N- total (%)      | 0.62      | 0.62                              | 0.29                              | 0.45                    |
| 3  | P-available (ppm) | 66.25     | 66.25                             | 67.25                             | 55.86                   |
| 4  | SO₄²⁻(ppm)       | 3.23      | 3.23                              | 4.17                              | 3.79                    |
| 5  | Pyrite (ppm)      | 1.026     | 1.279                             | 1.156                             | 0.179                   |
| 6  | pH                | 6.52      | 6.52                              | 6.59                              | 7.20                    |

Potential pyrite (Fe₂S₂) was found in all the soil samples with a range of 0.179-1.279 ppm. The lowest concentration was found in the substitute soil sample, and the highest was found in the surface layer of the experimental pond. The pyrite contents of the reservoirs and the bottom layer of the experimental pond respectively are 1.026 and 1.156 ppm. The management of pyrite content is an important factor in choosing the right reclamation technique.

3.2. Land detoxification

3.2.1. Closure of Klekap and Moss
The effect of land normalization on klekap and moss closure is presented in Table 3. The width of klekap and moss cover gives a statistically different mean (P<0.05). B treatment produced the highest average rate of klekap (66.667±5.774%) and the lowest moss closure (10.667±2.082%). On the contrary, A treatment produced the lowest average rate of klekap (11.333±8.080%) and the highest moss closure (88.667±8.083%). The only type of moss found was Chaetomorpha sp which is the main natural food for milkfish (Chanos chanos).

Table 3. Average of klekap and moss closure

| No | Treatment | Klekap          | Moss             |
|----|-----------|-----------------|------------------|
| 1  | A         | 11.333±8.080⁴   | 88.667±8.083⁴    |
| 2  | B         | 66.667±5.774⁵   | 10.667±2.082⁵    |
| 3  | C         | 48.333±12.583⁶  | 31.667±23.629⁶   |

3.2.2. Development of plankton
The potential acidity of the experimental soil and the relatively high temperature and salinity range did not have a significant effect on the availability of plankton. The plankton density, diversity and uniformity, and dominance of each treatment did not give statistical significance (P>0.05). The number of types (genera) of plankton obtained during the study were 11 genera.

The dominant genera of zooplankton were obtained from the Apocyclopas, Brachionus Acartia, Temora, Tortanus, Schmackeria and Crustacean classes dominated by Copepoda and Oithona. The discovery of Apocyclopas through the study can be preliminary information to reveal the "mystery" of organisms from the Malacostraca subclass known as Phronima suppa (Phronima sp) which has been used as an indicator of the success of shrimp farming by the local farmers [6]. The survival rate of black tiger prawns receiving Phronima Suppa feed in the areas infected with WSSV and V. harvey is about 70 percent, while that in areas without Phronima Suppa is only about 10 percent [7].
The land remediation (B), followed by contaminants detoxification employing plankton, is more effective in eliminating pesticide residue, depressing bacteria population and creating quality water and pond bottom soil according to the organic tiger shrimp production standard.

The highest diversity index values (1.414±0.253) and uniformity index (0.728±0.089) were produced in C treatment. Overall, plankton diversity and uniformity were moderate and relatively uniform or stable (Table 4). This condition supports the success of shrimp farming activities through the provision of natural food in a sustainable manner.

### 3.3. Purification

Compared to other treatments, the treatment of land remediation through surface soil reversal (B) followed by detoxification of contaminants using plankton is more effective in eliminating pesticide residues, suppressing bacterial populations and improving quality of water and pond bottom soil in accordance with organic tiger shrimp production standards.

#### Table 4. Concentrations of pesticides and heavy metals at experimental ponds

| No | Treatment | Pestisida (Thiodan) | Concentration Heavy metal (ppb)* | Cd | Hg | Pb |
|----|-----------|---------------------|---------------------------------|----|----|----|
| 1  | A         | not detected        | not detected                    |    | 1.621x10^{-4} | 7.85x10^{-4} |
| 2  | B         | not detected        |                                |    |                |                |
| 3  | C         | not detected        |                                |    |                |                |

* = Done composite

Thiodan type pesticide residue was not detected on all the test plots. The land processing by farmers during the research was quite effective in eliminating thiodan residues, and the land has become a good place for the development of organic shrimp farming. Cd element was undetectable in the experimental ponds. The concentrations of Hg and Pb are respectively 1.621x10^{-4} and 7.85x10^{-4} ppb.

#### 3.4. Development of organic shrimp

The concentrations of antibiotics in the experimental ponds are presented in Table 5. Antibiotics in pond waters are undetectable. The type of antibiotic analyzed was chloramphenicol.

#### Table 5. Concentrations of antibiotics in pond water experiments

| No | Treatment | Concentration |
|----|-----------|---------------|
| 1  | A         | not detected  |
| 2  | B         | not detected  |
| 3  | C         | not detected  |

Bacterial populations on weeks 5 and 9 each gave statistically different meanings (P <0.05). The highest bacterial population during the study was found in A treatment. The bacterial population tended to decrease at the end of the study except for C treatment (Table 6). On week 9, the lowest average bacterial population (400 ± 100 cfu/ml) was found in B treatment. The increasing bacterial population on C treatment was influenced by the decomposition and mineralization processes occurring as a result of the use of soil embankment. The bacterial population in the study did not exceed 500,000 units per gram, meeting the quality requirement of shrimp production. The presence of Phronima suppa that is highly sensitive to chemical contamination is an indicator of detoxification success on marginal pond [8]. Land reclamation and plankton growth have succeeded in reducing pesticide, heavy metal and antibiotic contaminants (Table 5 and 6).

The results of pond water analysis indicate that residual chloramphenicol is undetectable. This is due to the low concentration of chloramphenicol in water derived from fry, feed, domestic waste, soil bacteria, and river stream. The spread of tiger shrimp disease caused by bacteria and viruses has
encouraged most farmers around the study site to switch to milkfish cultivation, indirectly eliminating the occurrence of chloramphenicol residues through fry and feed. The layouts of ponds and irrigation channels which are not directly related to human settlements have led to a lack of domestic waste carrying chloramphenicol residues. The process or method of introducing water into experimental ponds using reservoir and filter systems contributes to the reduction of chloramphenicol residues.

### Table 6. The average population of bacteria in pond water

| No | Week | Treatment | Bacterial Population ALT (cfu/ml) |
|----|------|-----------|-----------------------------------|
| 1  | 5    | A         | 10,666.67±577.35^a                |
| 2  | B    | 5,400±100^b                                    |
| 3  | C    | 40±10^c                                         |
| 4  | 9    | A         | 4,200±100^a                       |
| 5  | B    | 400±100^b                                      |
| 6  | C    | 133,33±57.74^c                                 |

The bacterial population on weeks 5 and 9 gave statistically different meanings (P <0.05). The highest bacterial population during the study was found in A treatment. The bacterial population tended to decrease at the end of the study except for C treatment.

On week 9, the lowest average bacterial population (400±100 cfu/ml) was found in B treatment. The increase of bacterial population in C treatment is influenced by the decomposition and mineralization processes occurring as a result of the use of soil embankment. The bacterial population in this study did not exceed 500,000 units per gram as recommended by [9]. Soil and pond water management systems succeeded in reducing bacterial development [7]. This is reinforced by the absence of *E. coli* bacteria which are usually used as indicators for other pathogenic bacteria such as *Salmonella* sp., *Vibrio* sp. and *Staphylococcus* sp.

Water quality during the research is presented in Table 7. In general, pond water quality meets the requirement of growth and survival rate of tiger shrimp.

### Table 7. The range of pond water quality indicative scores during the study and value range of indicators eligible for the lives and growth of tiger shrimp according to references

| Water quality monitored | Water quality indicator value range during research | The value range of pond water quality indicators according to the reference |
|-------------------------|-----------------------------------------------------|--------------------------------------------------------------------------|
| Temperature (°C)        | 28.0 – 35.0                                         | 26.0 – 32.0 [10]                                                         |
| Salinity (ppt)          | 25.0 – 27.0                                         | 10.0 – 25.0 [6]                                                          |
| Dissolved oxygen (ppm)  | 4.1 – 6.5                                           | ≥ 3.0 [7]                                                                |
| BOD (ppm)               | 2.3 – 5.3                                           | ≤ 10.0 [11]                                                             |
| Acidity (pH)            | 7.8                                                 | 7.0 - 8.5 [10]                                                          |
| Nitrate (ppm)           | not detected - 0.04323                              | ≤ 8.0 [10]                                                              |
| Phosphate (ppm)         | not detected - 0.1684                               | ≤ 5.0 [10]                                                              |

In general, pond water quality in the research has been in accordance with the requirement of growth and survival of tiger shrimp except for temperature and salinity. The temperature range (28.0-35.0 °C) and salinity (25.0-27.0 °C) have exceeded the upper threshold of the recommended range, hence, it potentially inhibits growth and negatively affects the survival rate of tiger shrimp. Seasonal factor consideration becomes an important element in determining the appropriate spreading season in accordance with the physiological condition of tiger shrimp, water quality and pond soil management.
4. Conclusions
The content of organic matter at the reservoir and experiment ponds is relatively high so it potentially supports the provision of klekap and plankton required in the production of organic shrimp. The pyrite potential of the experimental pond is relatively high, requiring effective treatment and land reclamation, especially the A and B treatment.

The plankton density, diversity, uniformity and dominance are moderate and stable with phytoplankton composite components consisting of three classes; Chlorophyceae, Cyanophyceae, and Bacillariophyceae. The zooplankton composite component consists of two phyla (Mollusca and Chaetognatha) and five classes (Crustacea, Polychaeta, Chilopoda, Dinoflagellate and Rhizopoda). Apocyclopas discovery has become the initial information on producing natural feed from Phronima suppa which has been used as an indicator of the success of shrimp farming by local farmers. Bacterial populations are relatively low and tend to decrease, and the contents of pesticides, Cd heavy metals and antibiotics are not detected in all the treatments.

It takes improvement of soil and pond water management to handle the potential pyrite and heavy metal content Hg and Pb in shrimp ponds. The treatment of pond remediation through the reversal of surface soil layer followed by detoxification of contaminants using plankton (B treatment) should be chosen for the purification technology and normalization of marginal and degraded pond soil. The production of organic tiger shrimp is increased by controlling the development of pathogens and corpses through the application of a biological control system (biosecurity).

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