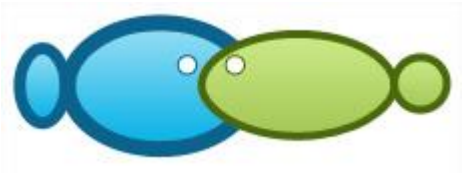

Water fungus populations as an indicator of water quality in Mooat Lake, Modayag District, Bolaang Mongondow Regency, North Sulawesi, Indonesia

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Abstract. The application of pesticides on agricultural land has a negative impact that is detrimental to the environment and human health. One of the negative dangers is that pesticide contamination can endanger the life of freshwater fish and water microorganisms, especially the Phycomycetes class. The purpose of this study is to determine the population level of water fungus species that act as an indicator of Mooat Lake water quality. This research was conducted for 1 year. Measurement of the level of the mushroom population was carried out by using stratified dilution method and the CFU value was calculated. From the macroscopic and microscopic isolation and identification results, 3 classes were obtained, namely Phycomycetes class with 2 orders, namely order Saprolegniales (*Saprolegnia* sp.) and order Chytridiales (*Rhizophydium pollinis*), Aspergillus class (*Aspergillus niger*), and Penicillium class (*Penicillium citrinum*). From this study, the highest and fastest colony growth was found in JA2 (*Saprolegnia* sp.) fungal isolates (222 colonies) with a mean number of CFU/mL spores of 0-27.6x10^{-5} and the lowest in JA3 (*Aspergillus niger*) fungal isolates with 46 colonies with the lowest mean number of CFU/mL spores were 0-1.6x10^{-5}.
Key Words: dilution, fungi, negative impact.

Introduction. Lake Mooat is one of the three lakes that has the potential to be developed in North Sulawesi Province, located at an altitude of 1080 meters above sea level in Modayag District, Bolaang Mongondow Regency. Moat Lake has great potential, namely as a tourism destination because it has quite a lot of tourist attractions, ranging from unique and distinctive natural objectives to cultural attractions, making it an appropriate place to. Its existence provides benefits in terms of water sources, electricity generation, irrigation, fisheries, tourism, etc. In 1985 the Mooat Lake area experienced changes caused by illegal cultivation, illegal grazing, forest burning, and deforestation (Wowor 1991).

One form of stagnant freshwater ecosystem (lentic) is a lake. This ecosystem occupies a relatively small area on the earth's surface compared to marine and terrestrial habitats (Thomas et al 1992; Conell & Miller 1995; Effendi 2003). Lakes can be formed from the deposition of late runoff from water flows or the flow of groundwater to the earth as a result of glaciation, earthquakes, or volcanic activity (Miller 1992; Horne & Goldman 1994). Gaseous pollutants, dissolved materials, and particulates can pollute a lake water system through air, soil, agricultural runoff, and factory and industrial waste (Edward 1993). Along with the development of time and an increase in the human population, the activity around the area has increased, and there is also an increase in waste that will pollute the lake environment. Likewise what happened to the Mooat Lake ecosystem, where around the lake there are horticultural and agricultural lands located in Modoinding District, South Minahasa Regency which is a geographical area of Lake Mooat and the activity on these agricultural lands is very high, especially in the use of pesticides to control pests and plant disease.

The physical, chemical, and biological properties of water are indicators of the quality of the lake ecosystem. Likewise, contamination of aquatic organisms such as fish

and water fungi can be used as an indicator of water quality in ecosystems in the area. The use of pesticides by farmers in the area around Lake Mooat is inevitable. One of the methods of controlling pests commonly used by farmers in North Sulawesi Province, especially in South Minahasa District is through spraying with insecticides, because this method is easy to implement and can kill pests quickly.

The results of interviews with agricultural farmers in Modoinding District in controlling carrot plant disease, they use a lot of pesticides. According to them, during cultivating carrots, the use of pesticides to control the disease was continuously carried out. A survey conducted in North Sulawesi in 1990 showed that almost all farmers used pesticides to control pests and agricultural diseases (Sembel 2010).

According to Pattiselanno (2001), to overcome the detrimental impact caused by pests and diseases in agricultural lands, which have become serious problems, humans have since tried to erase the damage by using various methods, both traditional and traditional modern. In the initial year of the food intensification program, pest and disease problems were dealt with chemically by using pesticides. Compared with other pest and disease control techniques, the use of pesticides by most farmers is considered to be more effective, its use is more practical, and this ecosystem brings huge economic benefits (Untung 2006). Various types of pesticides have been used since this was known to be a powerful weapon to eradicate pests and plant diseases (Noya 2004).

Besides being able to help humans in their efforts to overcome pests and diseases, it turns out that the application of pesticides has a major influence on organisms and other non-target environments (Murty 1986). Excessive and continuous chemical control measures can cause negative and detrimental impacts, including environmental pollution, killing of natural enemies, resistance and resurgence of pests and the emergence of residues on agricultural commodities that are harmful to humans (Kardinan 2001).

The high use of pesticides and the slow rate of degradation of these active ingredients on agricultural land can affect water quality, especially they can cause a decrease in the number of water fungus populations in the lake, even though the pollution in lake water is still below the specified threshold. Information about the population level of the Phycomycetes class water fungus exposed to synthetic pesticides in the water body of Mooat Lake in Modayag District, Bolaang Mongondow Regency is still scant. Looking at the facts above, it is necessary to do research on the population level of water mushrooms which act as an indicator of water quality in Mooat Lake, Modayag District, Bolaang Mongondow Regency.

Material and Method

Description of the study sites. This research was conducted in Mooat Lake, Modayag District, Bolaang Mongondow Regency where in the surrounding area there is agricultural land that uses a lot of pesticides. The research took place between Febuary 2020 and November 2020. Measurement of population, isolation, and drainage levels of the class Phycomycetes was be carried out at the Advanced Laboratory, Department of Biology, FMIPA Unsrat.

Water sampling. Water samples were taken at Mooat Lake, Modayag District, Bolaang Mongondow Regency, North Sulawesi. The water samples were taken in 5 zones with different depths. Each of these zones are, among others: Zone I (water collected at a depth of 1 meter), Zone II (water collected at a depth of 1.5 meters), Zone III (water collected at a depth of 2 meters), Zone IV (water collected at a depth of 3 meters), and Zone V (water collected at a depth of 5 meters). The water of Mooat Lake in each zone was sampled 5 times which were composited together.

Testing of Phcomycetes class population levels. Population level, isolation, and identification of the types of water fungi of the class Phycomycetes were carried out by using the multilevel plate method. The dilution method used is based on the method used by Humaidi et al (1999). First, 1 ml of water suspension was taken in the first dilution

test tube (10^{-1}) and put into a test tube containing 9 ml of sterile distilled water, shaken until homogeneous to obtain a fungal suspension with a dilution of 10^{-2}. In the same way a further dilution to 10^{-7} is made. The dilutions that will be used in this study are only up to 10^{-5} dilutions.

After the dilution process, the next process is the isolation process following the Umboh method (2016), by pouring 10ml of PDA media that has been melted at 50°C from each Petri dish. To avoid bacterial contamination it is necessary to add cotrimoxazole. Next, we took 1 ml suspension of the fungus from each dilution using a syringe and put it in a Petri dish containing solidified PDA media. Then the cultures were incubated at room temperature for 7 days. Furthermore, a pure culture of different fungi was made on each Petri dish.

After a pure culture was carried out on each existing fungus, identification was then carried out. Identification was done two ways: first macroscopically by looking at the shape and color of the fungal colony, second microscopically, by looking at the structure or arrangement of hyphae, conidia, and fungal spores. The microscopic identification step is to take a small part of the fungus using an aseptic loop needle. Then placed on the slide, a solution of methylene blue is dropped. Afterwards, we covered it using a cover glass and observed it under a microscope. Hyphae, spores, and conidia were observed.

Results

Isolation and identification of Phycomycetes class fungi. Results of identification by making observations were matched with the books Compendium of Soil Mushrooms (Domsch et al 1980), Introduction to Common Tropical Fungi by Gandjar et al (1999), Introduction to Food-Borne Mushrooms (Samson et al 1981), and the identification book Pictorial Atlas of Soil and Seed Fungi (Watanabe 2002). Water fungus isolates according to macroscopic and microscopic observations classified as: 3 classes, namely class Phycomycetes with 2 orders, namely order Saprolegniales (*Saprolegnia* sp.) and order Chytridiales (*Rhizophydium pollinis*), class Aspergillus (*Aspergillus niger*), and class Penicillium (*Penicillium citrinum*). Macroscopic and microscopic identification of all soil fungi obtained can be seen in Figure 1, Figure 2, Figure 3 and Figure 4.

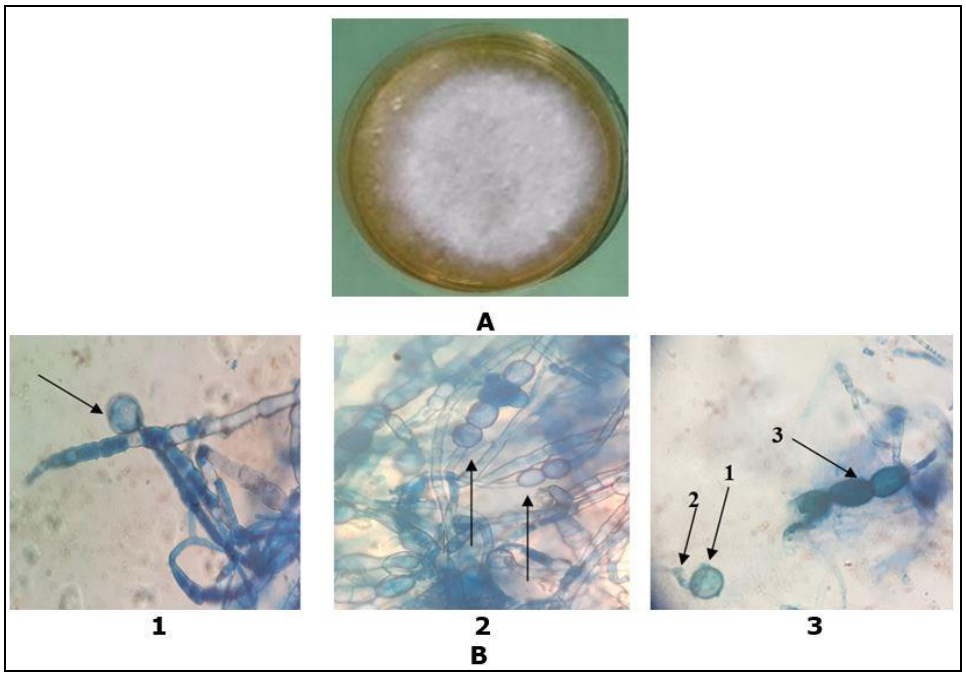

Figure 1. *Rhizophydium* sp. (A: Form of macroscopic morphology; B: Microscopic morphological form (400X magnification microscopic structure); 1: Zoosporangia, arrow; 2: Zoosporangium supported by branched protruding stalks; 3: Young zoosporangia, arrow 1, with basal rhizoid, arrow 2, Zoosporangium, arrow 3).

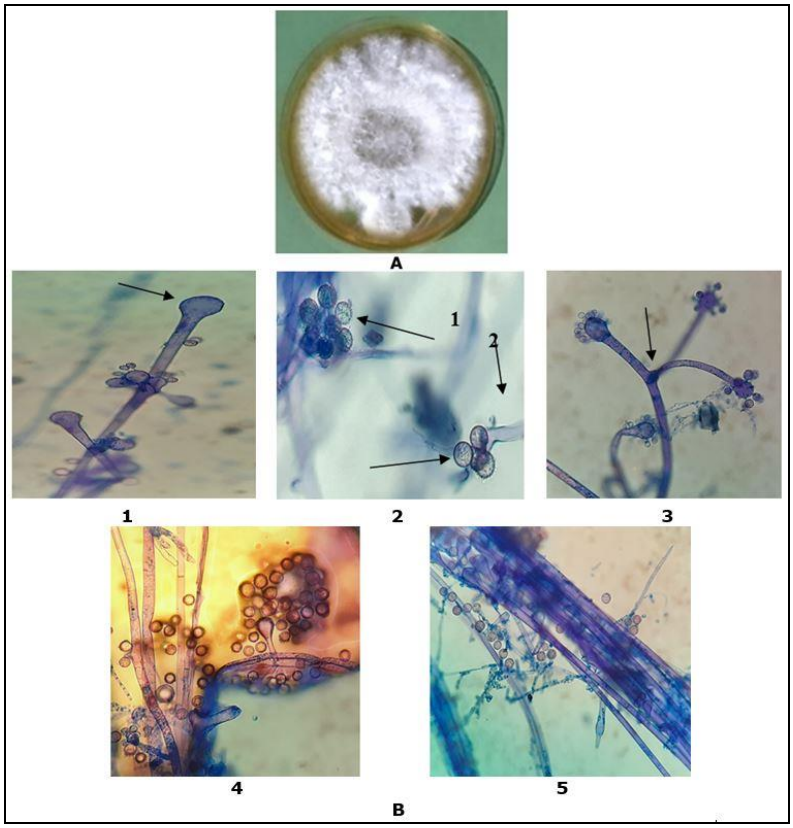

Figure 2. Fungal species *Saprolegnia* sp. (A: Macroscopic morphological form; B: Microscopic morphological form (400X magnification microscopic structure); 1: Oogonia, arrow; 2: Some oogonia, arrow 1, Antheridial hypha, arrow 2; 3: Branched conidiospores, arrow; 4: Oospores detached and scattered surrounds oogonium; 5: Oospores are scattered along conidiospores).

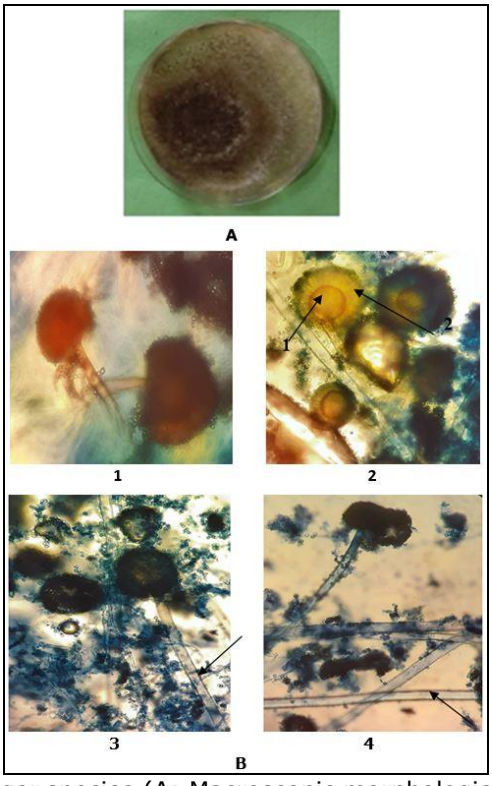

Figure 3. *Aspergillus niger* species (A: Macroscopic morphological form; B: Microscopic morphological form (400X magnification microscopic structure); 1: Intact *A. niger*; 2: Some *A. niger* which has been released from its conidia, Phialid, arrow 1, metula, arrow 2; 3: Some *A. niger* with long conidiospores, arrow; 4: septate hyphae, arrow).

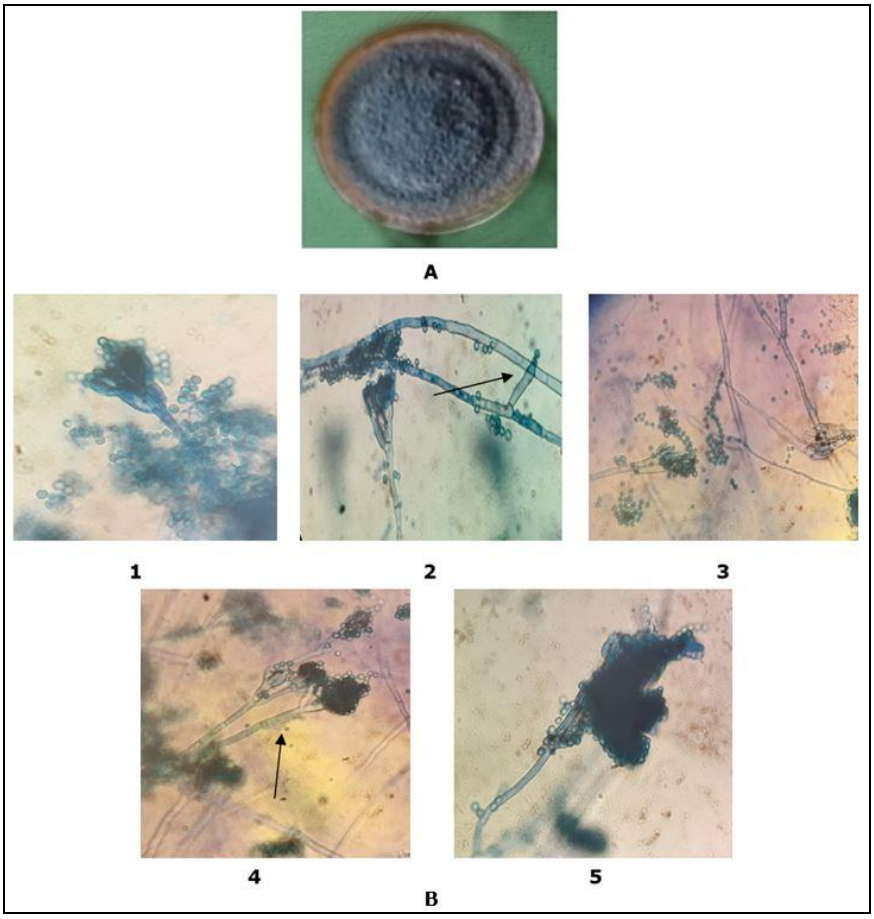

Figure 4. Species of *Penicillium citrinum* fungus (A: Macroscopic morphological form; B: Microscopic morphological form (400X magnification microscopic structure); 1: Intact *P. citrinum*; 2: Phialid form that grows on septate hyphae with conidia at the end, arrow 1; 3: Some *P. citrinum*; 4: long conidiospore, arrow; 5: conidia assemblage).

Water mushrooms that are not included in the Phycomycetes class are *Aspergillus niger* and *Penicillium citrinum*. Based on the macroscopic identification results, the characteristics of *Aspergillus niger* were obtained, namely black colonies with white borders (Figure 3A) and microscopical characteristics, namely transparent conidiosphere, round vesicles, black-brown conidia, and transparent hyphae (Figure 3B). Meanwhile, the *Penicillium citrinum* fungus is characterized by macroscopic colonies that are round green on the reverse side, yellowish-white and shaped like a fan and have a dense texture (Figure 4A) , and conidiophores are hyaline (Figure 4B).

Fungi are eukaryotic organisms, grow as hyphae or yeast cells, have cell walls that contain chitin, are heterotrophic, absorb nutrients through their cell walls and excrete extracellular enzymes into the environment and produce spores (Subandi 2010). Water fungi usually attack freshwater fish such as carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*), gouramy (*Osphronemus goramy*), Clarias catfish (*Clarias batrachus*) or Asian swamp eels (*Monopterus albus*) (Kusdarwati et al 2014). Infection in fish is usually caused by changes in the environment or season and lack of attention to water quality (Fadaeifard et al 2011).

Phycomycetes class water mushrooms population level. To determine the level of the water mushroom population in Lake Mooat, the dilution plate method was used. From this study, the highest and fastest colony growth was found in JA2 (*Saprolegnia* sp.) fungal isolates (222 colonies) with a mean number of CFU/mL spores of 0-27.6x10^{-5} and the lowest in JA3 (*Aspergillus niger*) fungal isolates with 46 colonies with the lowest mean number of CFU/mL spores of 0-1.6x10^{-5}. Water fungus colonies that grew in three

series of dilutions totaled 455 colonies. The growth of water fungus in PDA media with three dilution series is shown in Table 1.

Table 1

Number of water fungus colonies that grow in five water samples with three dilution series

No	*Sample and dilution*	*Mushroom isolates*				*Total*
		JA1	*JA2*	*JA3*	*JA4*	
1	A1P1	7	25	3	14	49
2	A1P2	6	13	2	7	28
3	A1P3	3	37	2	7	49
4	A2P1	8	3	1	4	16
5	A2P2	14	18	1	8	41
6	A2P3	2	10	0	1	13
7	A3P1	19	17	2	11	49
8	A3P2	6	12	1	3	22
9	A3P3	5	10	13	4	32
10	A4P1	9	16	2	4	31
11	A4P2	2	4	16	2	24
12	A4P3	6	9	2	1	18
13	A5P1	6	15	1	5	27
14	A5P2	8	19	0	2	29
15	A5P3	4	14	0	9	27
	Quantity	105	222	46	82	455

The results showed that the highest mean number of CFU/mL spores was found in JA2 (*Saprolegnia* sp.) fungi, namely 0-27.6x10^{-5}, and the lowest average number of CFU/mL spores was found in JA3 (*Aspergillus niger*) fungi of 0-1.6x10^{-5} (Table 2). This shows that the population of water microorganisms is influenced by factors such as organic matter, climatic conditions, types of vegetation and available humidity.

Table 2

Average number of water fungus spores growing on PDA with three dilution series

Mushroom	*Treatment*	*Average amount of spores CFU/mL*			*Range quantity of spores CFU/mL*
		1(10^{-3})	*2(10^{-4})*	*3(10^{-5})*	
JA1	A1	5.0x10^{-5}	3.0x10^{-5}	3x10^{-5}	0-5.0x10^{-5}
	A2	6.3x10^{-5}	4.6x10^{-5}	2.3x10^{-5}	0-6.3x10^{-5}
	A3	15.3x10^{-5}	2.0x10^{-5}	3.0x10^{-5}	0-15.3x10^{-5}
	A4	2x10^{-5}	2.0x10^{-5}	3.0x10^{-5}	0-3.0x10^{-5}
	A5	6.0x10^{-5}	7.0x10^{-5}	7.0x10^{-5}	0-7.0x10^{-5}
JA2	A1	21.3x10^{-5}	11x10^{-5}	27.6x10^{-5}	0-27.6x10^{-5}
	A2	3.0x10^{-5}	15.3x10^{-5}	6.6x10^{-5}	0-15.3x10^{-5}
	A3	14.3x10^{-5}	8.6x10^{-5}	8.6x10^{-5}	0-14.3x10^{-5}
	A4	3.3x10^{-5}	4.6x10^{-5}	7.3x10^{-5}	0-7.3x10^{-5}
	A5	11x10^{-5}	11x10^{-5}	1.01x10^{-5}	0-11.0x10^{-5}
JA3	A1	1.3x10^{-5}	1x10^{-5}	1.3x10^{-5}	0-1.3x10^{-5}
	A2	1.6x10^{-5}	0.3x10^{-5}	0.0x10^{-5}	0-1.6x10^{-5}
	A3	1.3x10^{-5}	0.3x10^{-5}	7.0x10^{-5}	0-7.0x10^{-5}
	A4	0.6x10^{-5}	5.3x10^{-5}	1.0x10^{-5}	0-5.3x10^{-5}
	A5	3.0x10^{-5}	0.0x10^{-5}	0.3x10^{-5}	0-3.0x10^{-5}
JA4	A1	8.3x10^{-5}	3.6x10^{-5}	3.0x10^{-5}	0-8.3x10^{-5}
	A2	2.3x10^{-5}	6.0x10^{-5}	0.6x10^{-5}	0--2.3x10^{-5}

	A3	$6.3x10^{-5}$	$1.6x10^{-5}$	$2.6x10^{-5}$	$0-6.3x10^{-5}$
	A4	$3.0x10^{-5}$	$1.0x10^{-5}$	$0.6x10^{-5}$	$0-3.0x10^{-5}$
	A5	$3.0x10^{-5}$	$1.6x10^{-5}$	$4.3x10^{-5}$	$0--4.3x10^{-5}$

Discussion. Water quality affects the growth of fungi regarding parameters such as pH, organic matter concentration, organic matter content and temperature (Willoughby 1994 and Alabi 1971). The entry of pesticides into the waters of Lake Mooat is thought to have taken place several ways, including direct use to eradicate pests and diseases of crops in the Modoinding District plantations, household waste disposal, runoff from rice fields, washing through soil, accumulation of aerosols and particulates, bulk rain and absorption from the vapor phase between air-water phases.

Usually fungi from the Phycomycetes class live in water generally as parasites or saprophytes in aquatic animals or plants, but some live on land. Fungi that are included in the Phycomycetes class in a zygospore state will be resistant to changes in environmental conditions (Anonymous 2013). *Rhizophydium pollinis* fungi species in the order Chytridiales in the class Phycomycetes, live as saprophytes or parasites in plants and aquatic animals. This species has the characteristic of being around bodies in water, releasing a haustorium to take its food from pine tree dust that falls in the water.

Pesticides will affect changes in the water conditions of Lake Mooat, especially affecting the life of water fungi. The results of research conducted by Manuaba (2006) showed residues of chlor-organic class of pesticides such as dichlorodiphenyltrichloroethane (DDT) and chlorothalonil in the water and sediments of Lake Buyan. These results indicate that the chlorothalonil type pesticide in the chlor-organic group can be bound to acidic organic matter in lake water (Caroline 1997). The nature of the pesticide DDT is very persistent with a very long half-life. Besides their hydrophobic nature, these pesticide compounds in the chlor-organic class can also spread through uptake and translocation by plants so that they are still found in the environment with a certain half-life (PIP 1995; Lee et al 2003).

From this study found very few types of water fungi, especially from the class Phycomycetes which has 6 orders (Myxochytridiales, Chytridiales, Blastocladiales, Monoblepharidales, Oomycetales, and Zygomycetales). This is due to the bioaccumulation of pesticide residues in the water bodies of Lake Mooat. The things that play a role in the occurrence of bioaccumulation are the uptake routes of contaminants into the organism and within the organism itself. Uptake can take three routes, namely: lipid, liquid, and enditotic (Newman & Unger 2003).

For phosphate-organic pesticides: dimetoat, chlorpyrifos, prefonophos, and carbamates (carbofuran and methomyl), which have a denser molecular density and high partition coefficient, make uptake more difficult than those of the chlor-organic pesticides (DDT and chlorothalonil).

Conclusions. Based on the research results, the following conclusions are drawn: from the results of isolation and identification, both macroscopically and microscopically, 3 classes were obtained, namely class Phycomycetes with 2 orders, namely Saprolegniales (*Saprolegnia* sp.) and order Chytridiales (*Rhizophydium pollinis*), class Aspergillus (*Aspergillus niger*), and class Penicillium (*Penicillium citrinum*). From this study, the highest and fastest colony growth was found in JA2 (*Saprolegnia* sp.) Fungal isolates (222 colonies) with a mean number of CFU/mL spores of $0-27.6x10^{-5}$ and the lowest in JA3 (*Aspergillus niger*) fungal isolates with 46 colonies with the lowest mean number of CFU/mL spores were $0-1.6x10^{-5}$.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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