Chemical and Microbial Analyses of Organic Milkfish Farm in Negros Occidental, Philippines

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Abstract - *This study was undertaken to provide baseline information on the chemical and microbial profiles of a milkfish farm that practices organic aquaculture in a brackishwater system. A one-hectare pond from the total of 13-ha. farm was subjected to chemical and microbial analyses for two production cycles from August 2015 to May 2016. The owner followed the organic milkfish farming protocol practiced since 2009. The pond was fertilized with vermicast and stocked with wild-caught milkfish fingerlings of 2-3 inches body length. Soil and fertilizer profiles, water quality, heavy metal, and antibiotic residues were analyzed. Production data were taken to compare survival between dry and wet seasons. Soil and fertilizer analyses showed high levels of magnesium, iron, manganese, copper, and nickel, but nil for cadmium. Water analyses showed positive for Escherichia coli, with the highest level of 220 MPN/100 ml from the water source during the wet season. Harvested milkfish were found negative to the antibiotic's chloramphenicol and nitrofuran; but positive to cadmium at 0.24 mg/kg. Milkfish production was higher during the wet season, with 86% survival, compared to the dry season with 70%. All the parameters analyzed were within limits, except for cadmium, and bacterial contaminations observed higher in the water source during the wet season. Results suggest the potential of organic aquaculture in brackishwater systems. However, a scheme to decrease microbial and chemical contaminations, such as a bio-filtration system is recommended, especially for farms that have open water sources like brackishwater fishponds.*

Keywords: *organic aquaculture, brackishwater aquaculture systems, heavy metals, antibiotic residue, microbial load*

INTRODUCTION

Organic aquaculture has become popular with increasing production of organic products worldwide [1]-[2]. In the Philippines there were a few organic aquaculture practitioners certified as organic, with only two farms certified in 2017 [3], by Organic Certification Center of the Philippines and Negros Island Certification Services. In other countries, numerous species have been certified by various certifying bodies. Some of these include trout in Switzerland (certified by Bio Suisse), trout and salmon in Norway (Debio), carp, salmon, mussels, trout and shrimp in Germany (Naturland), salmon, crayfish and oyster in New Zealand (Biorgo), etc. [4]. However, in the Philippines, no organic milkfish farm has been certified as organic [5].

There are a lot of factors to consider to be certified organic. These include but not limited to antibiotic residue, heavy metals, and farm management practices [6]. Organic farming practice is being promoted to avoid the negative impacts of these substances both to humans and the environment [7]-[15]. Moreover, another threat for organic milkfish culture in brackishwater systems is cross contamination due to open water sources. Usually, brackishwater aquaculture farms utilize rivers or estuaries as water source.

However, pollution levels in our river ecosystems are significantly increasing due to human and environmental factors. Pollution can be caused by certain circumstances such as but not limited to soil erosion, improper discharge of domestic wastes, agricultural, and industrial practices and poor or either lack of water waste management programs. Wastewater Treatment Plants (WWTP) is also an obvious high-risk source both in terms of number and strain of pathogens. Furthermore, during periods of high rain fall or plant failure, WWTP may release large amounts of poorly treated effluents [16]. Domestic pollution also contributes in contaminating our water sources which is sometimes caused by seepage from broken septic tanks and pit latrines [17]. Agricultural and other pollutions also adds-up to pollution which emanates mainly from carried out fertilizers, pesticides, manure, refuse dumps, decaying matter, herbicide and fecal matter from irrigation water and runoff water after the rain [18]-[19]. These contaminations may occur since the Province of Negros Occidental has vast agricultural area, also tagged as "Sugar Capital of the Philippines" Aside from certain human activities, wild and domestic animals using the same drinking water can also contaminate it through direct defecation and urination [20]-[21]. Because rivers serve as basins and can catch majority of these contaminants and deliver it to the downstream, these may eventually enter brackishwater farms or be discharged in estuaries.

Organic milkfish culture is quite harder to push in Negros Occidental due to constraints of cross contamination [22]. In the Philippines, milkfish farms are mostly brackishwater, an open and exposed aquaculture system. However, milkfish being a top commodity next to seaweed [23], is potential to be explored. Moreover, the culture of milkfish without the use of inorganic chemical and synthetic feeds is currently practiced in the Province of Negros Occidental, making milkfish a new potential organic farming commodity.

OBJECTIVES OF THE STUDY

The general objective of this research was to assess the chemical and microbial contamination in an organic milkfish farm in Negros Occidental. Specifically, this study ought to: 1) determine milkfish production in the wet and dry seasons; 2) profile the nutrient contents in soil and organic fertilizers; 3) determine the microbial load in the water source and inside the pond; and 4) analyze the heavy metal and antibiotic residue in harvested milkfish.

METHODS

This study was conducted in one of the brackishwater milkfish farms in the Province of Negros Occidental who practices organic aquaculture. The farm does not feed any commercial feeds nor use synthetic chemicals throughout the operation; but has been used in intensive shrimp farming before 2009. A one-hectare pond from the total of 13-ha. farm was subjected to chemical and microbial analyses for two production cycles representing the wet (August to December 2015) and dry season (January to May 2016). The owner followed the organic milkfish farming protocol practiced since 2009. The farm uses brackishwater from a river with a distance of approximately 3 km from the river mouth. The pond was fertilized with vermicast and stocked with wild-caught milkfish fingerlings of 2-3 inches body length at 2,500 pcs fingerlings per hectare. Soil and fertilizer profiles, water quality, heavy metal, and antibiotic residues were analyzed. Production data were taken to compare survival between dry and wet seasons. The farm is non-certified organic and have not yet considered for certification.

Soil and Fertilizer Sampling

A kilogram of soil samples was collected twice, prior to pond preparation. Soil samples were collected, boring approximately 15 cm deep, 2 cm thick and 5 cm wide from 12 different sites randomly taken following an S-shaped pattern throughout the one-hectare fishpond. Also, 1 kilogram of fertilizer was randomly taken in the different bags at about 100 grams/ bag.

Soil and Fertilizer Analyses

Fishpond soil and organic fertilizer were subjected to chemical analysis. Copper, iron, cobalt, nickel, manganese, chromium, cadmium, and magnesium were analyzed at the Regional Fisheries Laboratory of the Bureau of Fisheries and Aquatic Resources VI. These chemicals were only analyzed once, prior to the beginning of the production. The following were analyzed twice, before each production cycle: pH, organic matter, phosphorus and potassium for soil samples; and pH, organic matter, nitrogen, phosphorus, and potassium for organic fertilizer. The latter were submitted at the Department of Agriculture VI, Soils Laboratory for analyses.

Water Sampling

Water samples were collected every two months for bacteriological profile. One sample was collected from the water inside pond and the other sample was collected from the river outside the farm which served as water source. The water samples were filled in a one-liter sterile bottles and preserved in a polystyrene box with ice.

Water Analyses

Water samples were analyzed at the Regional Fisheries Laboratory of the Bureau of Fisheries and

Aquatic Resources VI. Water samples were analyzed for heterotrophic plate count, total coliform, fecal coliform and *Escherichia coli*. Microbial analysis was done using the Bacteriological Analytical Manual [24].

Fish Sampling

Fish samples for antibiotic residue analysis (Chloramphenicol and Nitrofuran) were taken before and after the harvest for each cycle. Prior to stocking, 50 pcs milkfish fingerlings were sampled for analyses; 1 kilogram after harvest. On the other hand, a 1 kilogram of fish sample for heavy metals such as Mercury (Hg), Cadmium (Cd) and Lead (Pb) were only taken after harvest. Analysis of heavy metals for the second harvest failed due to lack of reagents. Fish samples were damaged due to prolonged storage and it's impossible to acquire for another sample since all milkfish were disposed by the farmer to the market right after harvest.

Fish Analyses

Fish samples were tested for presence antibiotic residues, chloramphenicol and the nitrofuran metabolites AMOZ (3-amino-5-morpholino-methyl-2-oxazolidinone) and AOZ (3-amino-2-oxazolidinone) using ELISA (Enzyme-linked Immuno-sorbent Assay) test kits. Furthermore, fish samples were analyzed for heavy metals such as mercury (Hg) using CV-AAS (Cold Vapor- Atomic Absorption Spectroscopy) and cadmium (Cd) and lead (Pb) using FAAS (Flame Atomic Absorption Spectroscopy).

RESULTS AND DISCUSSION

Milkfish Production

Milkfish were harvested after five months, December 2015 for the wet season and May 2016 for the dry season. A total of 383 kgs with an average body weight of 178 g were harvested in the wet season; and a total of 314 kgs were harvested in the dry season (with an average body weight of 179 g). Survival rate in the wet season was 86%, and 70% in the dry season. As the data show, low survival rate was observed during the dry season due to occurrence of El Niño where mortality due to very high temperatures happened. However, similar results in their average body weight suggests their growth were alike between seasons

Soil and Fertilizer Profile

Table 1. shows the soil and fertilizer chemical profile as basis for soil and fertilizer efficiency; and when at elevated levels, determines its toxicity. Copper, iron, nickel, manganese, and magnesium contents in soil were 17.0, 919.3, 23.0, 173.3, and 5,398.6 mg/kg, while contents in fertilizer were 42.6, 901.5, 7.97, 241.1, and 1,268.5 mg/kg, respectively. Higher amounts of magnesium, iron and manganese were observed both in soil and fertilizer used. However, the levels for cobalt, chromium, and cadmium were nil in both soil and fertilizer. Levels recommended by Food and Agriculture Organization and World Health Organization (FAO/WHO) and DENR are presented in the 4th and 5th columns.

Pond soil is essential in providing nutrients in the water [25]. Soil quality also is necessary for the success of fish production and can be used in indicating potential aquaculture areas [26]-[27]. The levels of cupper, iron, nickel, manganese, and magnesium which serves as essential nutrients for the growth of plants are within limits. These levels may optimize the growth of natural food present in the pond, without harming the environment.

Table 1. Soil and Fertilizer Chemical Profile mg/kg).

Nutrient/ chemical	Soil	Fertilizer	FAO/ WHO	DENR
Cu	17.0	42.6	100.0	--
Fe	919.3	901.5	--	--
Co	nil	nil	50.0	--
Ni	23.0	7.97	50.0	--
Mn	173.3	241.1	--	--
Cr	nil	nil	100.0	0.1
Cd	nil	nil	0.3	0.05
Mg	5,398.6	1,268.5	--	--

Legend: -- means no available standard

The undetectable result for cobalt, chromium and cadmium is an indicator that the soil is improving its quality, from previously chemically treated shrimp farm. However, high amounts of these chemicals may pose threat to cultured species and can result to stunted growth in fishes [28]. Levels of chemicals in soil were within the limits set by FAO/WHO [29] for soils, and DENR [30] for sediments. This indicates that pond soil may have recovered from the intensive use of chemicals during intensive shrimp culture. The farm has practiced organic aquaculture since 2009, which may have aided in the soil recovery process. However, further verification studies on soil recovery after intensive use of chemicals would be necessary to understand processes on soil rejuvenation.

The soil pH, total phosphorus, total potassium and organic matter during the wet season were 6.32, 23.0 mg/kg, 388.0mg/kg, and 1.5%; while 3.41, 24.0 mg/kg, 812.0mg/kg and 2.0% on the dry season, respectively.

Table 2. Results of soil and organic fertilizer analyses on pH, Nitrogen, Phosphorus and Potassium, and Organic Matter

Production Cycle	Sample	pH	Total N (mg/kg)	Total P (mg/kg)	Total K (mg/kg)	OM (%)
First	Soil	6.32	-	23.0	388.0	1.5
Second		3.41	-	24.0	812.0	2.0
First	Fertilizer	5.70	0.55	0.02	0.10	15.66
Second		4.84	1.86	1.82	0.18	17.20

The results are indicated in Table 2 Soil pH in the wet season were nearly acidic and eventually turned acidic during the dry season with values of 6.32 and 3.41, respectively. Soil parameters were determined prior to pond preparations; therefore, it was not affected by the drying process. Moreover, the farmer believed that with him practicing organic aquaculture and not using any synthetic chemicals, the soil pH would turn to neutral (pH 7.0). This belief restrained him from using lime during pond preparation. However, fish ponds in the Philippines are mostly acidic due to acid sulphate soils. These might be attributed by high pyritic iron, active iron, active manganese, and sulfates which reduces soil ability to support natural food growth [31]. The high concentrations of manganese may explain the reduced pH levels in both soil and fertilizer used. But, the farmer's practise of frequent pond drying and water flushing is an economical way to increase pH levels as described by Singh and Poernomo [31]; but not as effective with the application lime. Fertilizer analysis on pH, total nitrogen, total phosphorus, total potassium, and organic matter in the wet season were 5.70, 0.55 mg/kg, 0.02 mg/kg, 0.10mg/kg and 15.66%, respectively while on the dry season, results were 4.84, 1.86 mg/kg, 1.82 mg/kg, 0.18mg/kg and 17.20%, respectively.

Table 3. Results of Microbial Analyses of Water from the Pond and Water Source

Production Cycle	Sampling	Source	Heterotrophic Plate Count (cfu/ml)	Total Coliform (MPN/100ml)	Fecal Coliform (MPN/100ml)	Escherichia coli (MPN/100ml)
First	Initial	Pond	100	540	80	80
		WS	210	540	84	59
	Final	Pond	1,700	17	4	4
		WS	770	920	220	220
In Between	Middle	Pond	140	490	490	<1.8
		WS	620	1,300	1,300	170
Second	Initial	Pond	31,000	3,500	<1.8	<1.8
		WS	28	330	330	27
	Final	Pond	200	2,300	<1.8	<1.8
		WS	197	3,300	3,300	200

These nutrient levels showed lower amounts which may not support the growth of natural foods inside the pond. The minimal nutrient available in the pond have affected the growth of lablab and lumot which also resulted in slow growth of milkfish. It is one of the reasons why the culture period reached 4 months from stocking (5 months including pond preparation); therefore, average biomass obtained were low. In any aquaculture operation, fertilization is essential for sustainable production, thus, the use of better organic fertilizers with enough essential nutrients is very important.

Microbial Load

Water quality and sanitation are important in all aquaculture facility to promote product safety. One of the indicators in determining sanitary procedures in a farm are microbial contaminations. Here, water quality was assessed in terms of microbial load as heterotrophic plate count, total coliform, fecal coliform and *E. coli*. Based on the results of microbial analyses Table 3., the pond water and water source were found contaminated with fecal coliform and *E. coli*, with the higher numbers in the water source, the brackishwater river. Higher levels of *E. coli* were also observed during the wet season, with higher number in the water source but almost undetectable inside the pond.

The presence of coliforms particularly *E. coli* is an indicator of fecal contamination. Not far from the farm, there are piggeries where fecal materials are eroded on to the river. Also, the farm has an existing small poultry house on its other side which may have contributed to the fecal contaminations. Based on the Department of Environment and Natural Resources, Department Administrative Order 1990-34, the Philippines recommends maximum level of 70MPN/100ml for total coliforms and nil for fecal coliforms [30]. The elevated levels of fecal coliforms may posts threat to the consumers, thus, the farm management practices should adhere to good aquaculture practices.

Antibiotic and Heavy Metals

Antibiotic residue and heavy metals are among the contaminants which could greatly affect especially humans. Studies showed that consumers of products with antibiotic residues may acquire bone marrow suppression [11], and aplastic anemia and leukemia [7]-[9], [14]. Although heavy metals may not affect the fish, bioaccumulation tales place which will later harm humans after consumption [32].

Results showed negative on antibiotic nitrofuran and chloramphenicol in fish sample. However, harvested milkfish was positive to cadmium at 0.24 mg/kg. Cadmium level has exceeded the minimum amount of 0.01mg/kg [6], therefore action should be taken since the product is directly distributed to the public for consumption. Cadmium contamination and milkfish and other fishery product was also recorded in some parts of the country. These include reports on cadmium contamination in marketed milkfish in Manila Philippines [33] and oysters in Lingayen Gulf [34], which was possibly due to anthropogenic activities.

The aspect of determining the exact source of heavy metal contamination were not distinguished in this research. The negative result of cadmium in soil, but positive in harvested milkfish indicate that the source of contamination may be from the water, the food they ate, or due to handling during harvest. Nevertheless, cadmium is highly toxic [35], making this concern an urgency for the safety of the consumers.

CONCLUSION AND RECOMMENDATION

Organic aquaculture has various requirements to comply with before it will be certified as organic. Most of the parameters tested such as antibiotic residue and heavy metals except cadmium were within limits. Thus, this study shows the potential of organic aquaculture in a brackishwater system. However, the results on high level of microbial load and cadmium suggests that the farm practices be revisited and find out where the contamination begins. Microbial loads were higher in the water source and lower on the pond which suggest the contamination have begun from the water source. However, cadmium was not detected in soils therefore a thorough investigation on the elevated amounts of cadmium must be conducted to determine the source and avoid contamination. Moreover, the farm is surrounded by conventional farms which adds-up to the threats of cross contamination.

Therefore, a mechanism is necessary to push organic aquaculture in brackwater farms such as biofiltration systems, and compliance to the good aquaculture practices. All chemical tested in soil were within limits suggesting that soil is recovering from intensive use of chemicals. Understanding the processes in soil recovery after excessive use of chemicals in ponds is also essential to develop technologies to hasten the process of soil rejuvenation.

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