INTEGRATED FARMING SYSTEM

AT

UNION DUCRAY

Final Report

Evaluation Committee

February 2003

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INTEGRATED FARMING SYSTEM AT UNION DUCRAY

EXECUTIVE SUMMARY

Integrated Farming System (IFS) is a scientifically proven concept in many countries such as China, Vietnam, Fiji, and Brazil etc. In Mauritius, the IFS was introduced by Prof. G. Chan, Consultant. The validity of the IFS project was demonstrated through a pilot project funded by MRC at Union Ducray from 1995 to 1997.

Based on their past experience in IFS, Union Ducray submitted a project proposal on IFS in Rodrigues to the Ministry of Local Government and Rodgriues. The proposal was not found to be technically and economically viable by the Ministry.

Mauritius Research Council (MRC), in collaboration with the Ministry of Local Government and Rodrigues, decided to review the project proposal submitted by Union Ducray on Integrated Farming in Rodrigues. The aim of the study was to objectively review and assess the project proposal and to make its recommendations to the Ministry. A steering committee for the same was set-up with the representatives of the relevant Ministries/ Departments.

During the steering committee meeting, the members were made aware about the two opposite views regarding tests that were conducted by the different parties including University of Mauritius. As a result, the Ministry of Health had put a ban on sale of crayfish at Union Ducray. In view of the conflicting scientific arguments leading to the ban, the committee decided that it would be improper to make recommendations on the St. Gabriel, Rodrigues proposal without a full scientific understanding of the apparent lackings of the Union Ducray project. Therefore, it was decided to undertake the evaluation of the Integrated farming System at Union Ducray by independent reviewers.

Thereafter, an Evaluation Committee was set up in April 2002 comprising of representatives from the Ministry of Health and Quality of Life, Agricultural Research and Extension Unit (AREU), Central Water Authority (CWA), Ministry of Fisheries and Ministry of Agriculture, Food Technology and Natural Resources to undertake a scientific audit of the Integrated Farming System (IFS) at Union Ducray with the following objectives -

- To evaluate the existing Integrated Farming System at the Union Ducray Farm
- To determine whether the crayfish produced in the IFS were fit for human consumption
- To recommend/ propose any improvement in the existing system.

Mauritius Standards Bureau (MSB) was selected by the Evaluation Committee as the agency to carry out physicochemical and microbiological analysis of the IFS at Union Ducray farm over a period of four weeks. The sample collection and analysis were undertaken by MSB in September 2002 and the final results of the analysis were submitted in November 2002.

The results of the microbiological investigations carried out on 80 samples of crayfish over a period of four weeks showed that they were contaminated with Coliform as well as Faecal coli. According to the norms set up by Association Française de Normalisation (AFNOR), the total Coliform count in live crayfish should be 0 CFU/g and that of Faecal coli 1CFU/g. Therefore, the crayfish was not found to be fit for human consumption.

According to the microbiological results for the water samples, it was clearly shown that water from the outlet of the oxidation pond and stream was always contaminated with Coliform and Faecal coli and that this water was continuously contaminating the pond water. This may be the reason why the crayfish was always contaminated. However, it was found that the purged crayfish was less contaminated than those, which were not purged.

The results of physicochemical analysis showed that the IFS at Union Ducray was working properly. It was clearly demonstrated that as the wastewater from the pigsty goes through the different processes, thereby resulting in acceptable level of physicochemical composition of the water in the fishponds.

The evaluation committee is of the view that the existing system needs some improvements and therefore has recommended appropriate pond management practices and aseptic precautions to be undertaken by the farm manager.

TERMS OF REFERENCE

Evaluation of the Integrated Farming System at Union Ducray

Background

Integrated Farming System (IFS) is a scientifically proven concept in many countries such as China, Vietnam, Fiji, and Brazil etc. In Mauritius, the validity of the IFS project has been demonstrated through a pilot project funded by MRC at Union Ducray.

Based on their past experience in IFS, Union Ducray submitted a project proposal on IFS in Rodrigues to the Ministry of Local Government and Rodgriues, which was not found to be technically and economically viable.

Mauritius Research Council (MRC), in collaboration with the Ministry of Local Government and Rodrigues, decided to review the project proposal submitted by Union Ducray on Integrated Farming in Rodrigues. The aim of the study was to objectively review and assess the project proposal and to make its recommendations to the Ministry. A committee for the same was set-up with the representatives of the relevant Ministries/ Departments.

During the committee meeting, the members were made aware about the two opposite views regarding tests that were conducted by the different parties including University of Mauritius. As a result, the Ministry of Health had put a ban on sale of crayfish at Union Ducray. In view of the conflicting scientific arguments leading to the ban, the committee decided that it would be improper to make recommendations on the St. Gabriel proposal without a full scientific understanding of the apparent lackings of the Union Ducray project. Therefore, it was decided to undertake the evaluation of the Integrated farming System at Union Ducray by independent reviewers.

Objectives

To undertake a scientific audit of the Union Ducray Integrated Farming project which would include consideration of an inbuilt mechanism to monitor health and safety standards

Scope of Work

1. To review existing literature on IFS, specifically on Union Ducray project
2. To liaise with all stakeholders
3. To undertake relevant tests as specified in annex A.
4. To interpret the data with respect to existing standards/ regulations and assess the environmental and health risks, if any
5. To recommend a monitoring system for an Integrated Farming System
6. To recommend any improvement to the existing system
7. To submit a report to the Council

Annex A

ENVIRONMENTAL MONITORING OF THE
INTEGRATED FARMING SYSTEM AT UNION DUCRAY FARM

1. Carry out a **microbiological monitoring** of the IFS at Union Ducray farm over a period of **four weeks** as follows:

    1. Collect and analyse water samples **twice weekly**. For a particular sampling day collect samples **in the morning (before 9.00 am) and in the afternoon (between 2.00 and 4.00 pm)** at the following locations (see Figure enclosed) and analyse for the parameters indicated.

Sampling Point	Location	Parameters
1	Water Inlet	TC, FC
4	Outlet of Sedimentation tank	TC, FC, Taenia
5	Outlet of Oxidation Basin	TC, FC
Ist Fish Pond	*First fish pond (Four samples from the four corners of the pond)*	*TC, FC*
2nd Fish Pond	*Second fish pond (Four samples from the four corners of the pond)*	*TC, FC*

    2. Carry out **microbiological testing of crayfish weekly** for the following parameters:

No. of Cray Fish Samples	Parameters
1st Sample: 5 crayfish from 1st fish pond and 5 crayfish from 2nd fish pond **2nd Sample:** 10 crayfish from purging tank	TC, FC, Staph.aureus/ gms, Clostridium perfringens/ gms, Salmonella/ gms, Vibrio/ gms and Taenia (cysticercosis).

    A representative sample should be prepared on each set of 10 crayfish to make two samples for the study.

Note: Collection, presentation and analysis of samples should be carried out according to standard WHO guidelines.

2. Carry out an **environmental monitoring** of the IFS at Union Ducray farm **over a period of four weeks** as follows:

Collect and analyse samples twice weekly. For a particular sampling day collect samples in the morning (before 9.00 am) and in the afternoon (between 2.00 and 4.00 pm) at the following locations (see Figure enclosed) and analyse for the parameters indicated.

Sampling Point	Location	Parameters
1	Water Inlet	pH, TSS, COD
2	Inlet of Digester	TSS, COD, pH, BOD
3	Outlet of Digester	COD, BOD_5, TSS, pH
4	Outlet of Sedimentation tank	COD, BOD, TSS, DO, pH, NO_3, NO_2, NH_3, PO_4, SO_4
5	Outlet of Oxidation Basin	COD, BOD, TSS, pH, DO, NO_3, NO_2, NH_3, PO_4, SO_4
6	Outlet of first fish pond	COD, BOD, TSS, pH, DO, NO_3, NO_2, NH_3, PO_4, SO_4
7	Outlet of second fish pond	COD, BOD, TSS, pH, DO, NO_3, NO_2, NH_3, PO_4, SO_4

Note: Collection, presentation and analysis of samples should be carried out according to standard WHO guidelines

LIST OF ACRONYMS

AFNOR	Association Française de Normalisation
AREU	Agricultural Research and Extension Unit
BOD	Biochemical Oxygen Demand
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
CWA	Central Water authority
DO	Dissolved Oxygen (mg/l)
EEC	Enteropathogenic E.coli
FC	Faecal Coliforms (MPN/100 ml)
IFS	Integrated Farming System
NO_3	Nitrate (mg/l)
NO_2	Nitrite (mg/l)
NH_3	Ammonia (mg/l)
MPN	Most Probable Number
MRC	Mauritius research Council
MSB	Mauritius Standards Bureau
PO_4	Phosphate (mg/l)
SGS	Société Générale de Surveillance (Mauritius) Ltd.
SO_4	Sulphate (mg/l)
TC	Total Coliforms (MPN /100 ml)
TOR	Terms of Reference
TSS	Total Suspended Solids (mg/l)
UASB	Upflow Anaerobic Sludge Blanket

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INTEGRATED FARMING SYSTEM AT UNION DUCRAY

FINAL REPORT

1. INTRODUCTION

Integrated Farming System (IFS) is a scientifically proven concept in many countries such as China, Vietnam, Fiji, and Brazil etc. In Mauritius, the IFS was introduced by Prof. G. Chan, Consultant. The validity of the IFS project was demonstrated through a pilot project funded by MRC at Union Ducray from 1995 to 1997.

IFS at Union Ducray consists of three distinct phases (*Figure 1*). The first phase is the digestion of the pig waste from the sty into an Upflow Anaerobic Sludge Blanket (UASB) digester and sedimentation tank, where 60% reduction in Biochemical Oxygen Demand (BOD) is achieved in an anaerobic condition. The second phase is the aerobic treatment in an oxidation pond, where a further 30% BOD reduction is achieved by photosynthesis. A third phase is the complete mineralisation of the organic wastes into stable nutrients in the stabilisation pond, resulting in the prolific growth of various natural and rich planktons which are then used to feed fish or crayfish. The pond systems have been designed for aerobic treatment and are more effective than most conventional wastewater treatment processes at pathogen destruction. Pond systems can remove 99.99% to 99.999% of pathogenic bacteria, viruses and intestinal parasite eggs, so that the treated effluent is biologically safe for agricultural reuse (Journey and Mc Niven, 1996).

Following various investigations conducted at the IFS in the past, conflicting scientific arguments were obtained leading to the ban on sale of crayfish by the Ministry of Health and Quality of Life. Therefore, the Mauritius Research Council (MRC) set up an evaluation committee, to undertake a scientific audit of the IFS at Union Ducray.

The Evaluation Committee consists of representatives from the Ministry of Health and Quality of Life, Agricultural Research and Extension Unit (AREU), Central Water Authority (CWA), Ministry of Fisheries and Ministry of Agriculture, Food Technology and Natural Resources (*Annex 1*).

The main objectives of the Evaluation Committee were -

1. To evaluate the existing Integrated Farming System at the Union Ducray Farm
2. To determine whether the crayfish produced in the IFS were fit for human consumption
3. To recommend/ propose any improvement in the existing system.

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2. METHODOLOGY

The Evaluation Committee was set-up in April 2002 to undertake the study as per the enclosed terms of reference (TOR). In the first meeting it was decided to invite quotations from laboratories for testing of microbiological and physicochemical parameters as stated in the TOR.

The letters for inviting quotations were sent to 14 organizations/ laboratories as from April to May 2002 and it was re-launched in August 2002. Quotations from only two organisations were received, namely Société Générale de Surveillance (Mauritius) Ltd. (SGS) and Mauritius Standards Bureau (MSB) (*Annex 2*).

Mauritius Standards Bureau (MSB) was selected as the agency to carry out microbiological and physicochemical monitoring of the integrated farming system at Union Ducray farm over a period of four weeks as their quotation was according to the specifications and the price was reasonable (also because they are an authority on standards in Mauritius).

MSB had suggested an alternative for microbiological testing of crayfish in their quotation. The Evaluation Committee agreed to the suggestion made by MSB, that is, a composite and representative sample to be prepared on each set of 10 crayfish to make two samples instead of testing 20 individual crayfish.

Thereafter, the Evaluation Committee members and the representative of the MSB met the Director of Union Ducray Sugar Estate on 20 June 2002 to apprise him on the aim of the study and that the samples would be drawn over a period of four weeks. The Evaluation Committee members and the representative of the MSB laboratory also visited the Integrated Farming project to familiarize themselves with the project and to identify the sampling points as well as to prepare a sampling protocol. (*Copy of the sampling protocol is enclosed as Annex 3*).

The sampling points were identified and confirmed by all members. Thereafter, the sampling protocol was discussed and finalized. It was decided to collect the water samples from the seven points (*Figure 1*), namely (1) water inlet, (2) inlet of digester, (3) outlet of digester, (4) outlet of sedimentation tank, (5) outlet of oxidation basin, (6) outlet of first fish pond and (7) outlet of second fish pond, on Mondays and Thursdays, at 9.00 hours and 14.00 hours for four weeks. The crayfish samples were collected on Thursdays at 9.00 hours. The sample collection and analysis were undertaken by MSB in September 2002.

Water Samples

Water samples were collected in 1 litre and 2 litre sterile glass bottles by the workers on-site under the supervision of trained scientific personnel from MSB for microbiological and physico-chemical analysis respectively.

Based on the level of pollution, it was decided that the water samples would be collected in the following sequence - sampling points 1, 6,7,5,4,3 and 2.

Samples for microbiological tests were transported in icebox and all tests were performed upon arrival at the laboratory. Samples for chemical tests were transported in carton boxes. The transportation time from the farm to the laboratory was 45 minutes to 1 hour.

Crayfish Samples

A metallic trap was used to fish at least 10 crayfish (5 from each pond) on every Monday and Thursday. The crayfish harvested on Thursdays were transported directly to the lab for microbiological testing. Those collected on Mondays were purged in stream water, in concrete tanks next to the fishponds, for three days prior to collection and transportation to the laboratory. At all times the crayfish were picked with bare hands by the manual workers of Union Ducray Farm. On every Thursday a total of 20 Crayfish were transported in clean and dry black plastic bags from the farm to the lab. They were kept alive until homogenised in a grinder before testing.

The tests were performed immediately afterwards. No washing of the sample prior to maceration was done. The transportation time from the farm to the laboratory was 45 minutes to 1 hour.

Submission of Reports

Bacteriological results were received three weeks after collection of the first samples. It was than noticed from the results submitted that no final titration had been performed. MSB was then informed by the Committee to perform complete bacterial titration of samples during the last week and to collect and test 12 additional samples from the two fishponds.

The final results were communicated by the Microbiologist of MSB to the Executive Director of MRC on 8 November 2002 (*Annex 4*).

3. RESULTS

MICROBIOLOGICAL

Crayfish before purging

For the first three weeks of the study the crayfish was found to be contaminated with Coliform (>110 CFU/g), but no final titrations were performed. During the last week total Coliform was present at a load of 4600 CFU/g. Similarly, for the isolation of Faecal coli, >110 CFU/g were present during the first three weeks followed by 460 CFU/g during the last week.

The Clostridium perfringens count varied from 33 CFU/g to 240 CFU/g. No Salmonella, Staphylococcus aureus and Vibrio parahaemolyticus were isolated from all samples tested, *Table 1.*

Crayfish after purging

The total Coliform count was found to be >110 CFU/g during the first three weeks, when no final titration was carried out. During the fourth week a total Coliform count of 460 CFU/g was obtained.

The Faecal coliform count was >110 CFU/g during the first two weeks whereas during the last two weeks the count was reduced to 24 and 43 CFU/g respectively.

The Clostridium perfringens count varied from 38 CFU/g to 260 CFU/g. No Salmonella, Staphylococcus aureus and Vibrio parahaemolyticus were isolated from all samples tested, *Table 1.*

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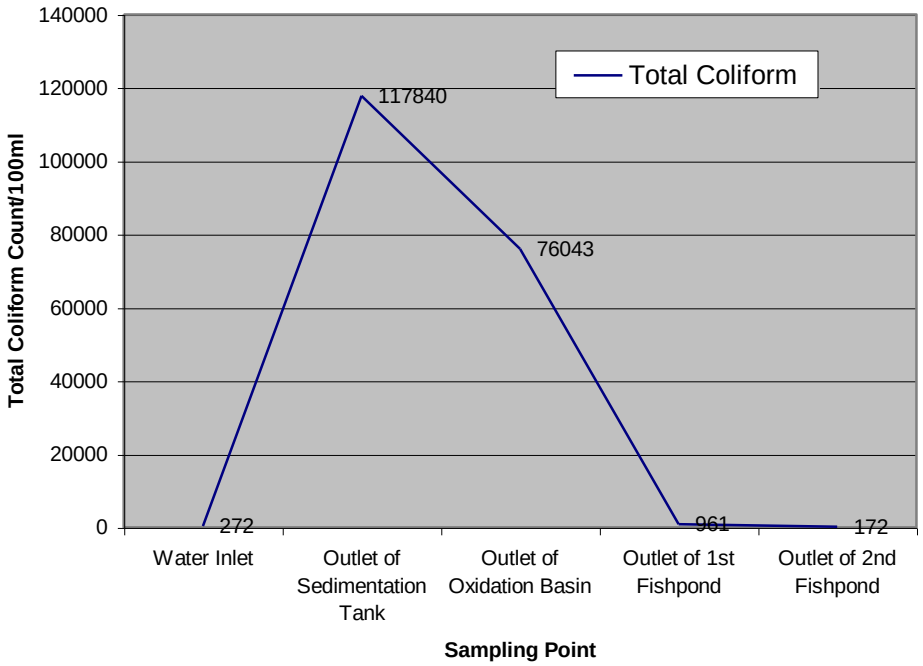
Water Samples

According to Figure 2 the total coliform count at point (1), the water inlet (stream water), there was an average count of 271 CFU/100mL. The average count was based on the results of 28 samples collected on 8 days during a period of 4 weeks. There was a marked increase in bacterial count in the (sedimentation tank) sample point (4), that is, >117, 840 CFU/100 mL. In twelve samples the bacterial count was greater than 1100 CFU/100 mL and no end point was determined. A high bacterial count is expected in this sample due to the active proliferation of micro organisms during the process of decaying as well as load of faecal Coli present in faecal wastes. In the oxidation pond there was also a high bacterial count >76,043 CFU/100 mL. In the first 12 samples the bacterial count was >1100 CFU/100 mL and no exact CFU was determined. However, in the two fishponds, that is, sample points (6) and (7) there was a marked decrease in the CFU/100 mL - 960 CFU/100 mL and 171 CFU/100 mL respectively.

Figure 2: Average Total Coliform Count/100ml from Different Sampling Points



A similar pattern was observed with the Faecal coliform *(Figure 3).* It is important to note that on one occasion, that is, on the 12th sample the total Coliform as well as the Faecal coliform count was 0 CFU/100 mL *(Table 2)* in both crayfish ponds. During the last two days of samples collection, four samples were collected in the morning and afternoon respectively at different sites from each pond. Eight water samples were collected from each pond (points 6 and 7) and out of the 16 samples collected, from point 7, one sample was free from total Coliform and five showed complete absence of Faecal coli.

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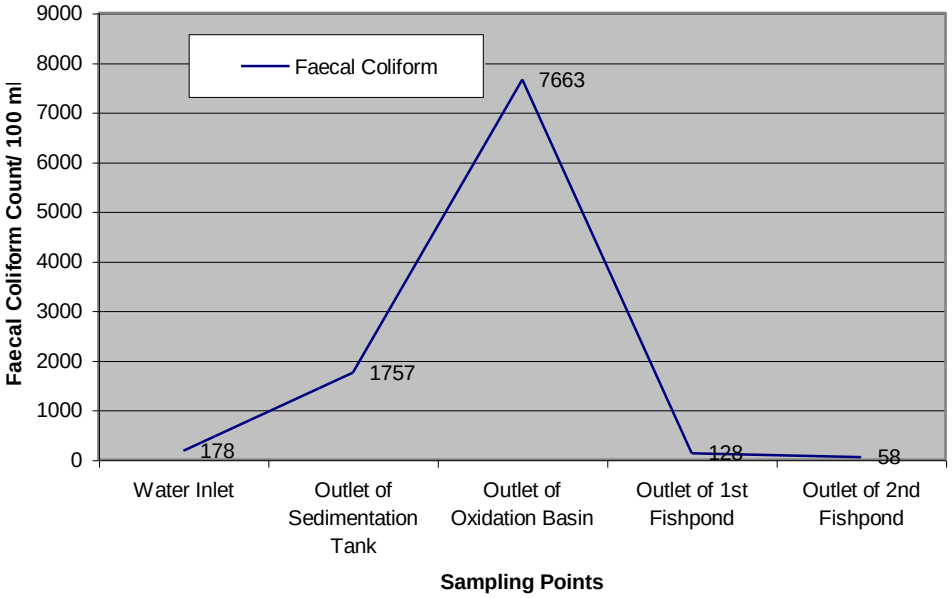
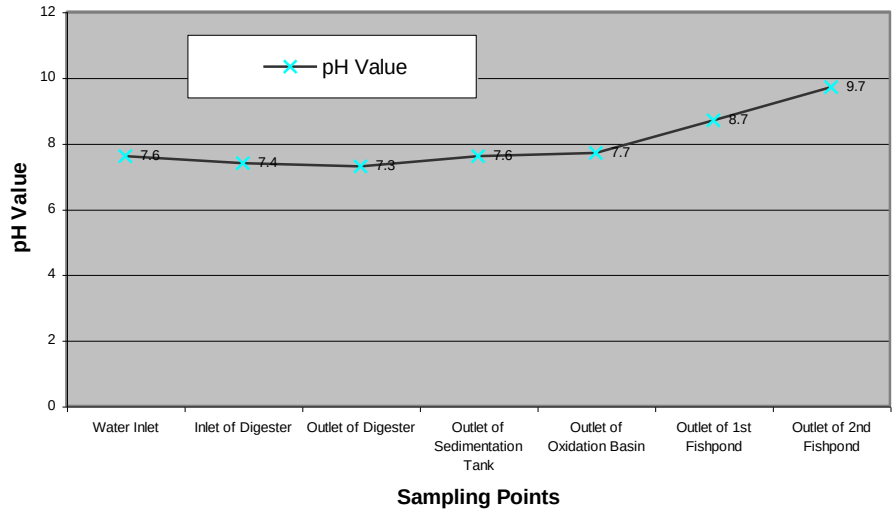
Figure 3: Average Faecal Coliform Count from Different Sampling Points



Throughout the study the pH varied from 8.4 to 10.6 (average 9.7) at point 7, whereas at point 6 the pH variation was from 7.6 to 10.0 (average 8.7) as shown in table 2 and 3 and figure 4.

Figure 4: Average pH Value at Different Sampling Points



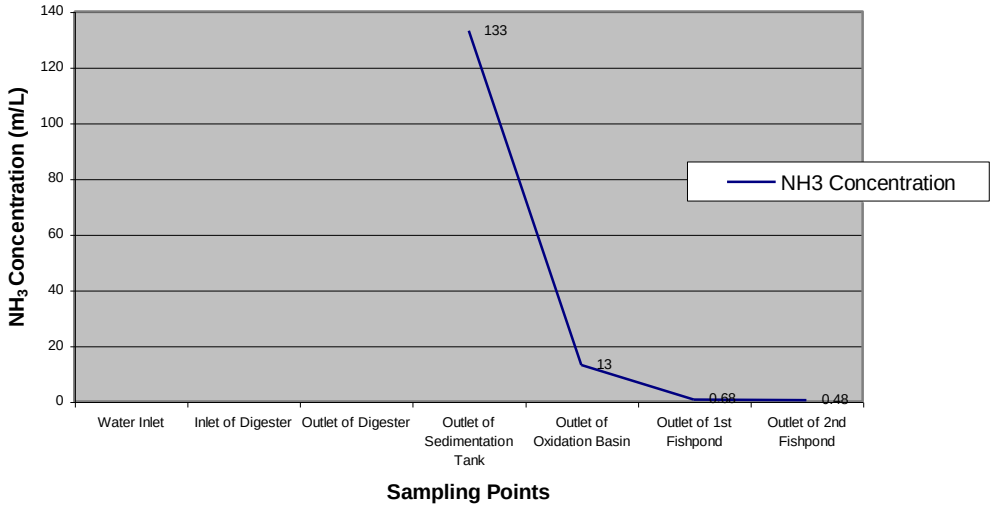
PHYSICOCHEMICAL ANALYSIS OF WATER SAMPLES

The Results of analyses shows the following trends for different parameters.

Ammonia

The average level of ammonia at the outlet of the sedimentation tank was found to be 133 mg/L, which is channeled to the oxidation pond. In the oxidation pond there was a tenfold decrease in the level of ammonia, 13.35 mg/L, which was further reduced in the fish pond. The values of ammonia in the water leaving the first and second fishponds were 0.68 and 0.48 mg/L respectively. *(Refer to Figure 5.)*

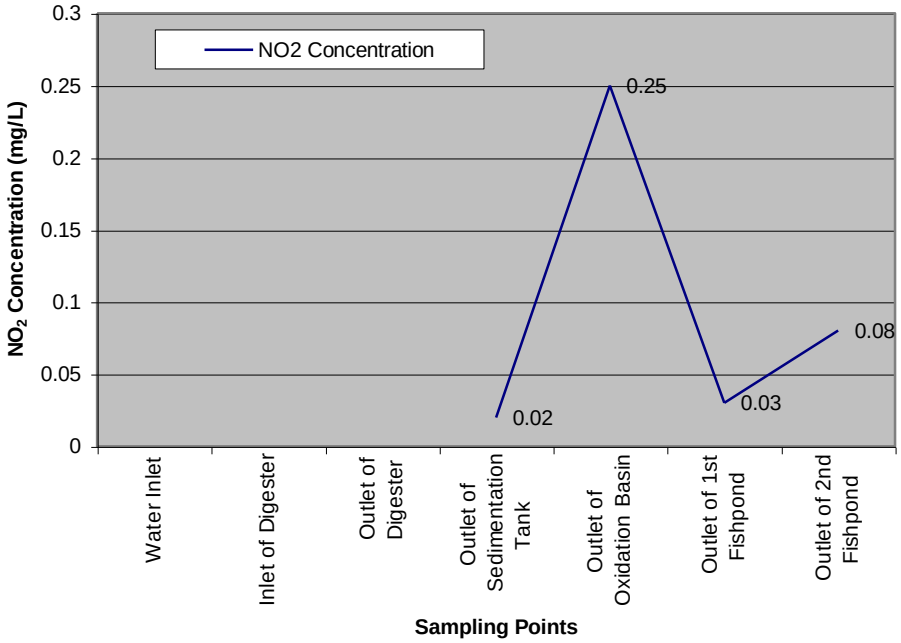
Figure 5: Average Ammonia (NH_3) Concentration from Different Sampling Points



Nitrite

At the outlet of the sedimentation tank the average value of nitrite was found to be 0.024 mg/l. It was observed that the value of nitrite increased to 0.25 mg/L in the oxidation pond, it was then further reduced in both fishponds. The average values of nitrite in the water leaving the first and second fishponds were 0.025 and 0.08 mg/L respectively. *(Refer to Figure 6.)*

Figure 6: Average Nitrite (NO_2) Concentration at Different Sampling Points

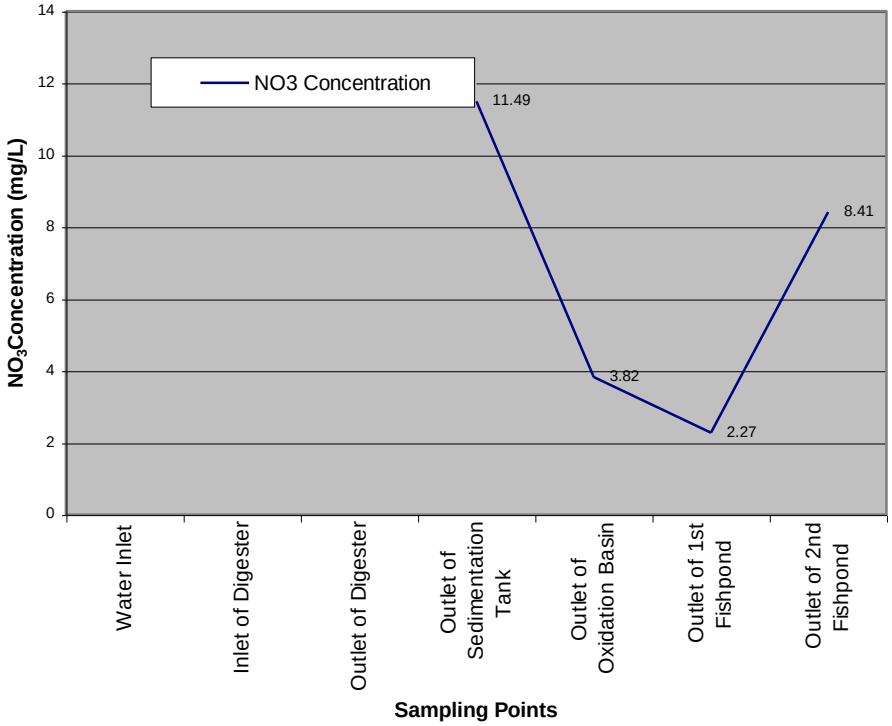


Nitrate

The average value for nitrate emanating from the sedimentation tank was found to be 11.48 mg/L. Within the oxidation pond it was observed that there was an average nitrate loss of 66.7 %.

Only an average value of 3.82 mg/L of nitrate was found to reach the fish ponds. *(Refer to Figure 7)*

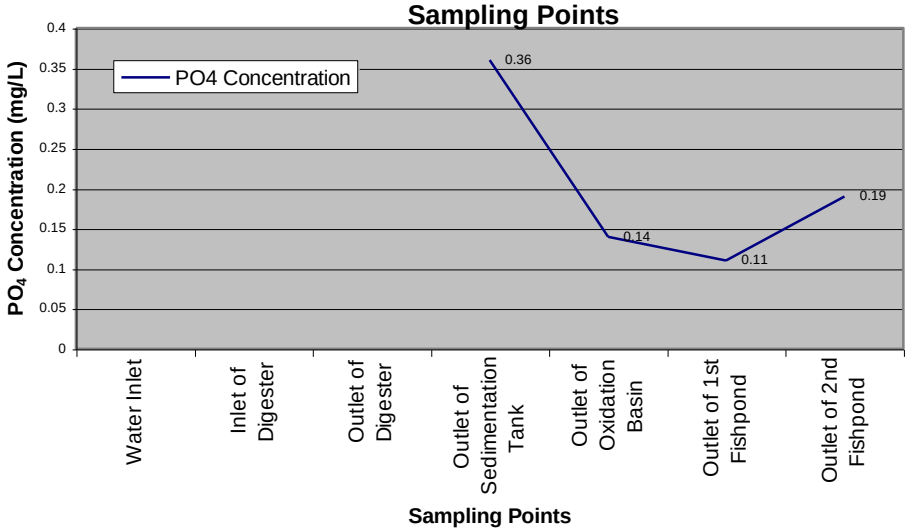
Figure 7: Average Nitrate (NO_3) Concentration at Different Sampling Points



Phosphate

The average amount of phosphate leaving the sedimentation tank was 0.36 mg/L. In the oxidation pond it decreased to 0.14 mg/L. The level of phosphate in the fishponds varied from 0.11 to 0.19 mg/L. *(Refer to Fig 8)*

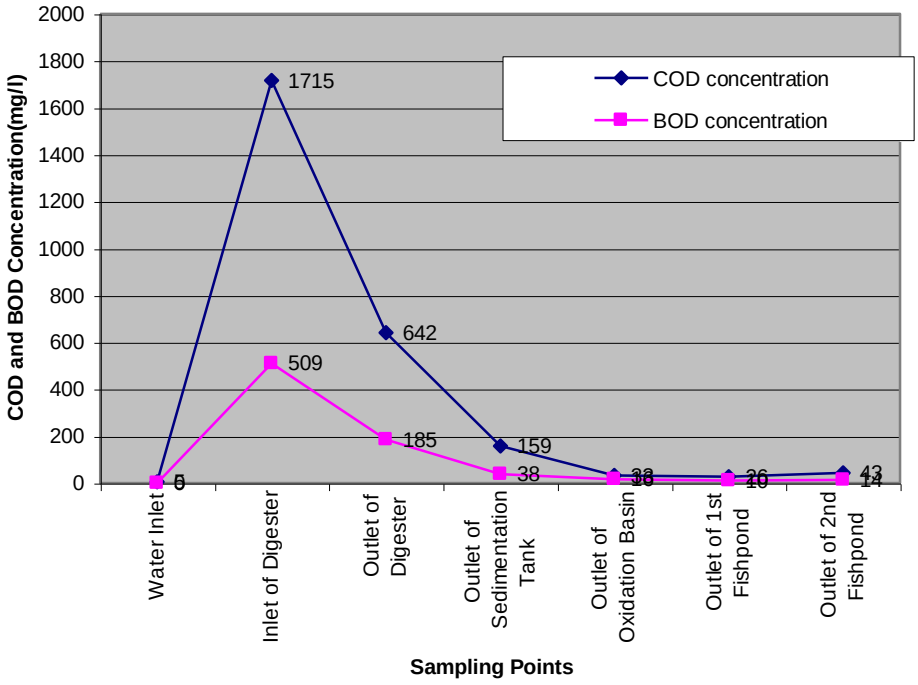
Figure 8: Average Phosphate (PO_4) Concentration at Different Sampling Points



Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)

The average values for BOD and COD of the inlet water were found to be 0 mg/L and 4 mg/L respectively. There was a sudden increase in the level of BOD and COD at the inlet of the digester (BOD 509 mg/L and COD 1715 mg/L) due to the wastewater emanating from the pigsty. In the digester the wastewater was treated biologically and the values for BOD and COD were reduced by 64% and 63% respectively. The partly treated wastewater from the digester was chanelled to the sedimentation tank where further biological treatment occurred. This reduced the BOD and COD by a further 28.5% and 28% respectively. Further reduction in the level of BOD and COD occurred in the oxidation pond and the treated waste water leaving the fish pond had an average BOD and COD load of 12 mg/L and 33.3 mg/L respectively. (*Refer Figure 9)*

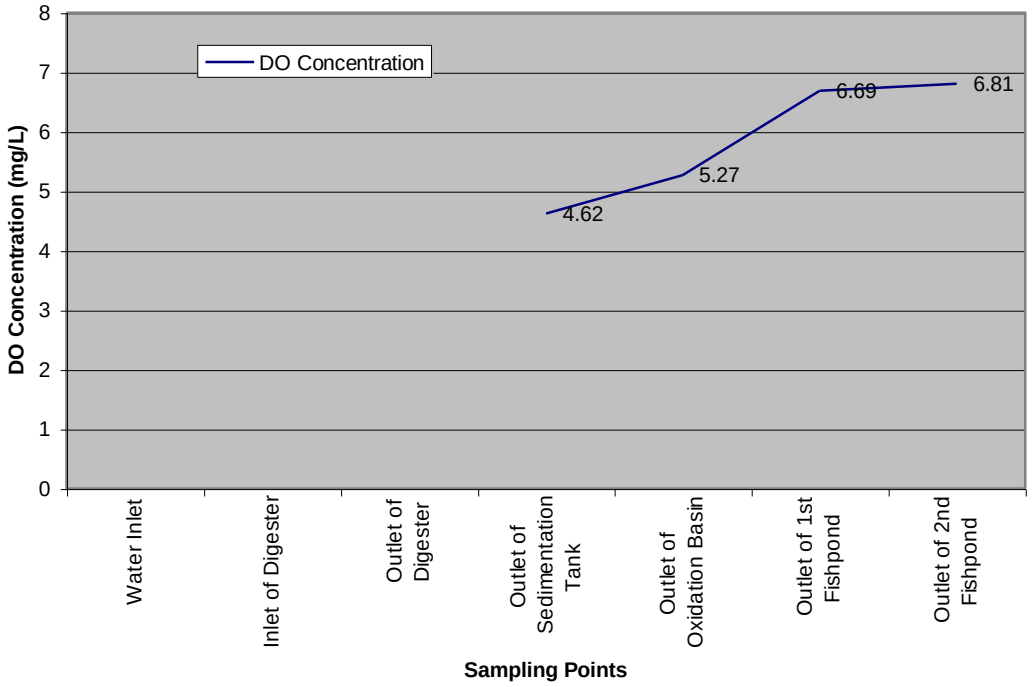
Figure 9: Average COD and BOD Concentration from Different Sampling Points



Dissolved Oxygen (DO)

As per the results of analyses it was observed that there was an increase in the DO level from the sedimentation tank to the fishponds of 4.6 to 6.8 mg/L respectively. This was in accordance with the observed reverse trend in the BOD and COD load. *(Refer Figure 10)*

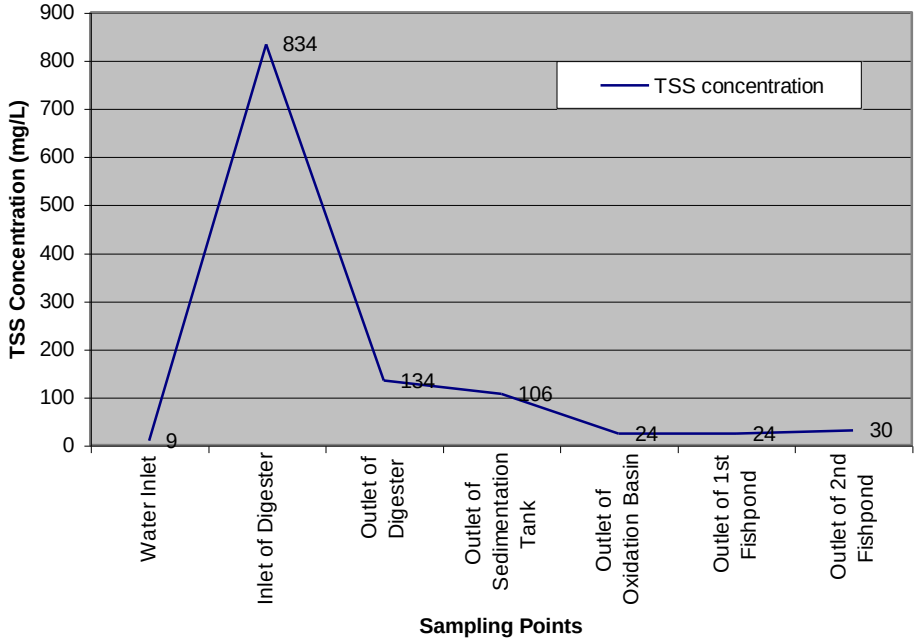
Figure 10: Average Dissolved Oxygen Concentration from Different Sampling Point



Total Suspended Solids (TSS)

The average TSS value of the stream water inlet was found to be 8.7 mg/L, compared to 834 mg/L at the inlet of the digester. At the outlet of the digester the TSS value was 134 mg/L followed by 106 mg/L at the outlet of sedimentation tank. The TSS value of the water decreased gradually from 106 mg/L to 23.5 mg/L at the outlet of the oxidation pond. At the outlet of the first and second fishponds the average TSS value was 24.2 mg/L and 29.7 mg/L respectively. *(Refer Figure 11).*

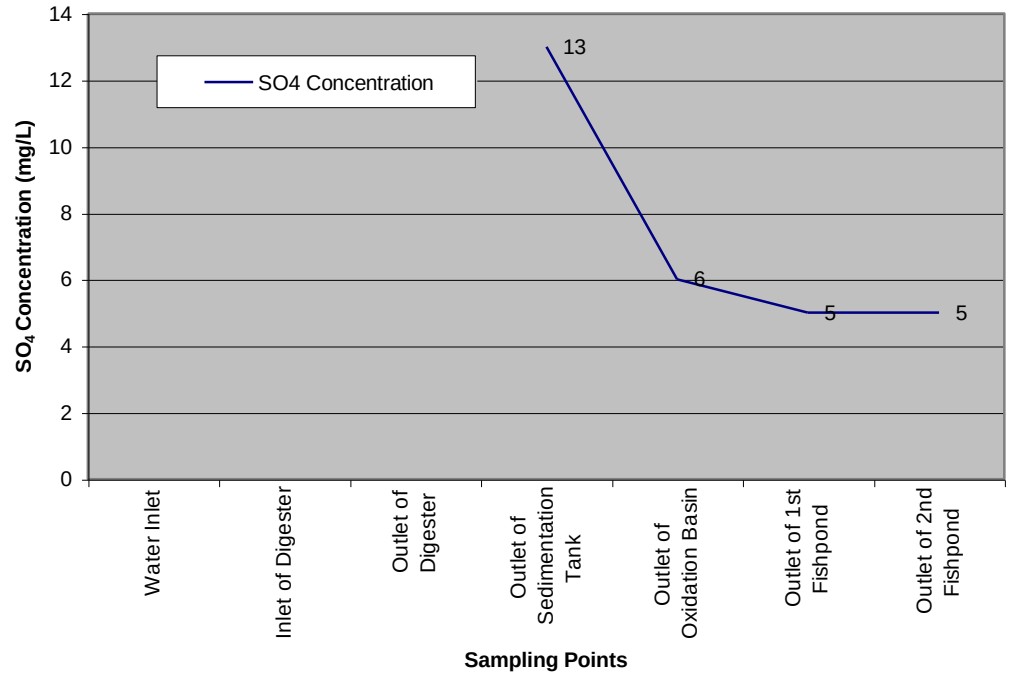
Figure 11: Average TSS Concentration at Different Sampling Points



Sulphate

It was observed that the average level of sulphate in the outlet of the sedimentation tank dropped from 12.93 mg/L to 4.56 mg/L and 5.25 mg/L in the two fishponds respectively. *(Refer Figure 12)*

Figure 12: Average Sulphate (SO_4) Concentration at Different Sampling Points

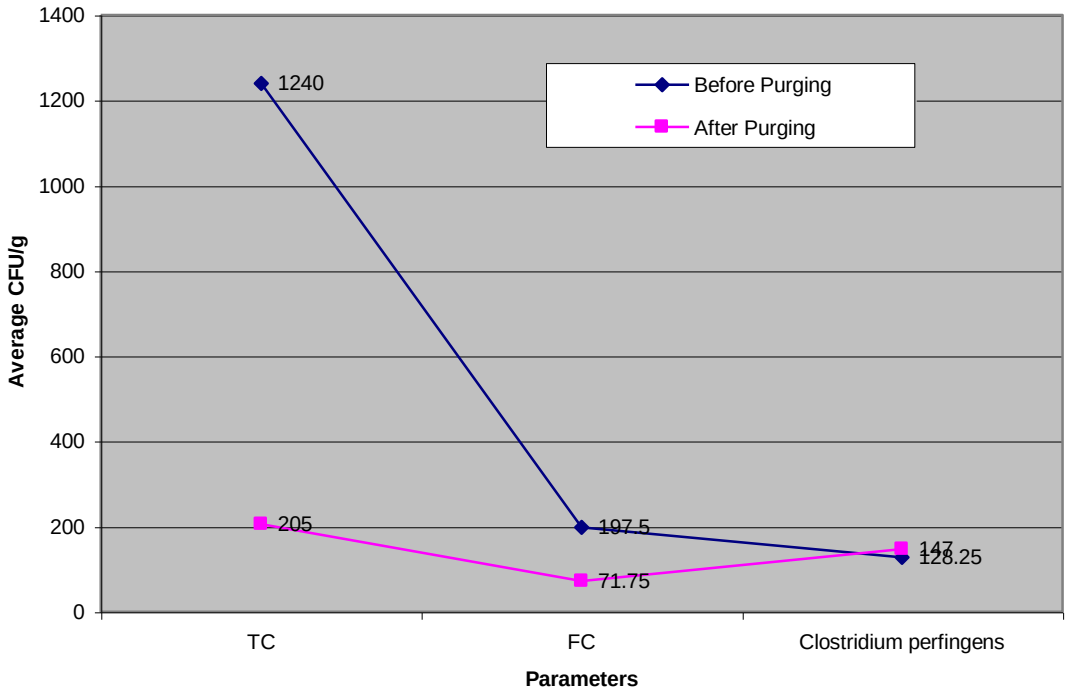


4. DISCUSSION

The microbiological investigations carried out on 80 samples of crayfish showed that they were contaminated with Coliform as well as Faecal coli. According to the norms set up by Association Française de Normalisation (AFNOR), the total Coliform count in live crayfish should be 0 CFU/g and that of Faecal coli 1CFU/g.

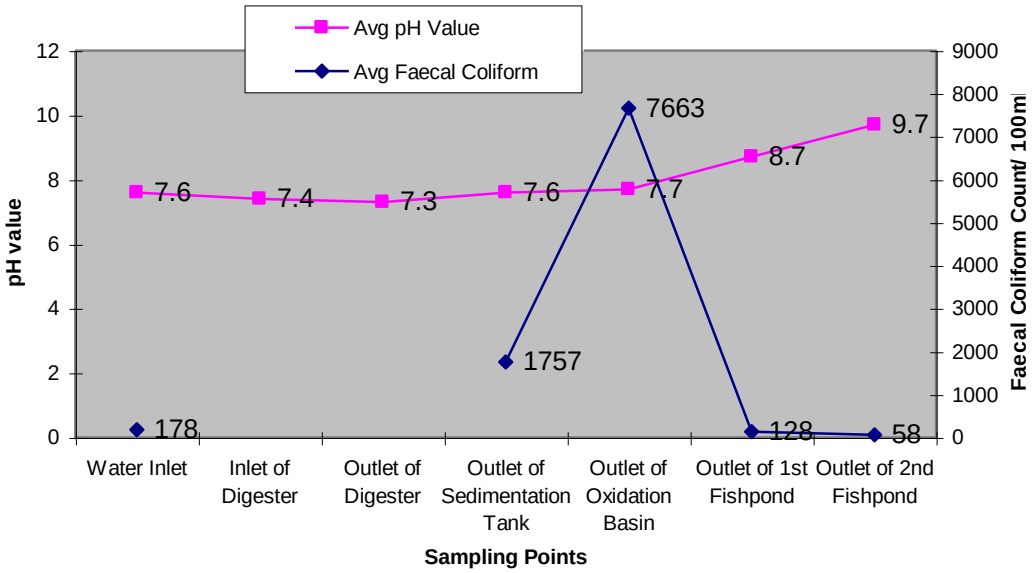
From the microbiological results, it was clearly shown that the stream water was always contaminated with Coliform and Faecal coli and that this water was continuously contaminating the pond water. This may be the reason why the crayfish was always contaminated. However, it was found that the purged crayfish was less contaminated than those, which were not purged *(Table 1, Figure 13)*. During the collection of the crayfish from the fishponds no aseptic precautions were observed. The workers working in the pigsty emptied the fish trap on the ground next to the ponds and then picked them up with bare hands. In future any samples of crayfish, which are expected to undergo bacteriological testing, should be collected and transported under the guidance of the sanitary officers working for the Ministry of Health and Quality of Life.

Figure 13: Microbiological Analysis of Crayfish



The physicochemical analysis showed that the IFS at Union Ducray was working properly. It was clearly demonstrated that as the wastewater from the pigsty goes through the different processes, thereby resulting in acceptable level of physicochemical composition of the water in the fishponds. At the same time the pH increased to more that 9.0. The high pH destroys most of the enteropathogens or food poisoning bacteria but may also hamper the growth of the crayfish. It is known that although all the Coliform genera are present in fresh faeces and in fresh pollution from faecal sources, they may not persist in water for the same length of time (WHO, European Series, No. 93). The results clearly showed that there was a marked reduction in bacterial count as the water reaches the ponds where the pH was greater than 9.0 (*Figure 14*). Therefore viability of Coliform and Faecal coli in this high pH will be strictly dependent on the time of contact at this pH. According to John Garbutt (1997), food products need to be stored at maximum pH range for a period of up to 72 hours before retailing to allow death of organisms to occur.

Figure 14: Average Faecal Coliform Count and pH value at Different Sampling Points



Out of the 28 water samples collected from the first fish pond, the average bacterial count was low, 960 CFU/100ml for total Coliform and 127 CFU/100ml for Faecal coli compared to the water leaving the oxidation basin, which was greater than 10^3 CFU/100 ml (*Table 3*). Although this pond water was rich in nutrients, the high pH destroyed the bacteria. It was observed that out of the 16 water samples collected from the second pond during the last week, 5 (31%) were free from Faecal coli. This clearly shows that complete removal of the Faecal coli, which is excreted only by warm blooded animals, is possible if appropriate pond management practices and aseptic precautions are observed.

Referring to the report of the NIOT team (*Annex 5*), it is appropriate to reduce the Coliform entry into the fishpond instead of controlling them using the concept of pH increase. It is against the concept of IFS to invest in separate chlorination system in order to prevent Coliform entry into the fishponds. However, it will be cost effective to keep the actual pH of the fishponds as such, and then to maintain the water pH above 9.0 for 72 hours prior to crayfish harvest.

*****

From the study it can be concluded that sufficient amount of nutrients were produced in the system which are meant for the prolific growth of phytoplanktons and other aquatic plants in the fish ponds. But unfortunately there is a significant loss of the nutrients within the oxidation basin due to intensive growth of aquatic floating plants.

Furthermore, the floating aquatic plants were preventing the penetration of sunlight into the oxidation pond whereby inhibiting the growth of submerged flora which is responsible for the production of dissolved oxygen in the system. It is to be noted that the dissolved oxygen plays a key role in the reduction of the BOD and COD loads and for the survival of aquatic life in the fishpond. Therefore, the wastewater treatment processes in the IFS were working satisfactorily and may prove to be more efficient for fish farming than compared to conventional farming in ponds or lakes, where the food pellets may be a source of relatively large amounts of suspended solids, that is, uneaten food pellets. Estimates of dry pellets lost from trout and salmon tank and pond culture are commonly in the range of 5-20% (Water and Health in Europe, WHO Regional Publications, European Series No. 93, pp. 66-67).

5. CONCLUSIONS AND RECOMMENDATIONS

1. At the time the microbiological investigations were carried out, the crayfish produced at Union Ducray Farm was found to be contaminated with Faecal coli, which is an indicator showing that pathogenic bacteria of faecal origin maybe present. The level of Faecal coli present in the crayfish exceeded the recommended norms, based on the Association Française de Normalisation (AFNOR) standards for live crayfish. Therefore, the crayfish was not found to be fit for human consumption.

2. However, from the results of microbiological analysis it was found that the following food poisoning pathogens - Salmonella, Staphylococcus aureus and Vibrio parahaemolyticus – as well as Taenia (cysticercosis) were absent in both the water and crayfish samples.

3. It has been clearly demonstrated that, as the wastewater from the pigsty goes through the different processes of fermentation, sedimentation and oxidation, the pH of the water in the crayfish ponds increases up to around 9, thus reducing considerably the bacterial count. Furthermore, the water coming out from the IFS conforms to the established guidelines for the purpose of discharge into the environment. This indicates that the Integrated Farming System was working in the right direction.

4. It has also been observed that stream water, which has been found to be contaminated with Faecal coli during the study period, was continuously flowing into the crayfish ponds. This was most probably the source of contamination of the pond water and crayfish with coliforms of faecal origin. Therefore, the existing system needs some improvements.

5. The Integrated Farming System was working satisfactorily during the study period as far as the wastewater treatment processes are concerned.

6. The Ministry of Health and Quality of Life should establish a set of norms and appropriate labelling for the fresh water aquaculture industry including Integrated Farming System.

Recommendations

I Recommended Improvement in the Existing System

1. The pH value of the water in the fishponds should be monitored regularly by the promoter to maintain it around 9 to ensure that the IFS is functioning properly.
2. Water should always flow in a closed circuit from digester to the fishpond.
3. At no time should the stream water be added directly to the sedimentation tank and/ or oxidation tank.
4. The inflow of water into the crayfish ponds should be controlled in such a way that only the water lost by evaporation should be replaced.
5. The oxidation pond should be free from floating aquatic plants and cleaned regularly to allow maximum sunlight to penetrate the water body for maximum oxidation processes, which are beneficial for the reduction of the BOD and COD loads.
6. The sedimentation tank should be cleaned regularly to remove the excess accumulation of sludge.
7. A further study is required to monitor the viability of the Coliform and Faecal coli in the fishponds at different time interval without adding the contaminated water from the oxidation pond and the stream.

II Recommended Monitoring System for the IFS

1. Regular monitoring of the integrated farming system should be carried out by the promoter twice yearly for –
   a. Physico-chemical analysis of water and wastewater samples
   b. Microbiological analysis of crayfish
2. A proper IFS circuit should be observed prior to collection of crayfish sample for microbiological analysis.
3. Strict aseptic precautions should be followed during the whole procedure for collection and transportation of crayfish samples to the laboratory for microbiological analysis.

III Recommended IFS Management Practices

1. No farm or pet animals should have access to the vicinity of the fishponds.
2. The workers working in the pigsty should observe appropriate sanitary measures prior to access to the fishponds at the time of crayfish harvest.
3. The product of the farm should be labelled indicating the origin of the product, e.g., “Produce of Integrated Farming (nutrients from pig farm)”, in order to allow consumers to choose the product according to their respective religious faiths and beliefs.
4. Prophylactic treatment against Taeniasis (Cysticercosis) should be given to the pigs on a regular basis.

REFERENCES

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3. Garbutt, John. (1997). Essentials of Food Microbiology. Editor Arnold, 1st Edition. Pp 76-77.

4. Journey, W. K., McNiven, S. (1996) Anaerobic Enhanced Treatment of Wastewater and Options for Further Treatment. Washington, DC.

5. WHO Regional Publications, European Series, No. 93, Water and Health in Europe, European Environment Agency, pp. 66-67.

ANNEXURE

ANNEX 1

LIST OF EVALUATION COMMITTEE MEMBERS

1. Dr. N Pyndiah, Virology, Ministry of Health and Quality of Life (Coordinator)
2. Mr. A K Gopaul, Central Water Authority (Member)
3. Mr A K Matabudul, Animal Health Laboratory, Ministry of Agriculture, Food Technology and Natural Resources (Member)
4. Mr A A Boodoo, PRS, Agricultural Research and Extension Unit (Member)
5. Mr S Jeetah, STO, Aquaculture Division, Ministry of Fisheries (Member)
6. Mrs P V Ramjeawon, Research Officer, Mauritius Research Council (Secretary)

Annex 2

EVALUATION OF THE INTEGRATED FARMING SYSTEM AT UNION DUCRAY

ENVIRONMENTAL AND MICROBIOLOGICAL MONITORING
EVALUATION COMMITTEE MEETING – 21 AUGUST 2002, 14:30 HOURS

SELECTION REPORT

1. SCOPE OF WORK

The scope of work entails carrying out environmental and microbiological monitoring of the Integrated Farming System at Union Ducray farm over a period of four weeks.

2. INVITATION FOR QUOTATIONS

The letters for inviting quotations were sent to 14 organizations/ laboratories as from April to May 2002. The invitation of quotations was re-launched in August 2002.

3. QUOTATIONS RECEIVED

At the re-launch, quotations were received from two organisations, namely -

1. Société Générale de Surveillance (Mauritius) Ltd. (SGS) – **Rs 445,640**
2. Mauritius Standards Bureau (MSB) – **Rs 285,000**

Arithmetical checks were made on the two quotations. The quotations for the two agencies mentioned above were tabulated for analysis and discussion.

4. SELECTION OF THE ORGANISATION FOR THE STUDY

Mauritius Standards Bureau (MSB) was selected by the Committee as the agency to conduct the relevant tests as they had quoted the cheapest price for the services and also because they are an authority on standards in Mauritius.

5. DISCOUNT OFFERED BY MSB

A discount of 25% is applicable on the quotation submitted by MSB

6. PRICING OF THE WORKS

The cost of the environmental and microbiological monitoring will therefore be **Rs. 285,000 (including the 25% discount).**

7. SUMMARY & CONCLUSION

Only two quotations were received. Mauritius Standards Bureau (MSB) was selected by the Committee as the agency to conduct the relevant tests as they had quoted the cheapest price for the services and also because they are an authority on standards in Mauritius. Therefore, it is recommended that the contract be awarded to Mauritius Standards Bureau.

Evaluation Committee

Dr. R Pyndiah, Coordinator, Evaluation Committee

Mr. A K Gopaul, Member, Evaluation Committee

Mr B Hulman, Member, Evaluation Committee*

Mr D Gangapersad, MRC, Technical Secretarial for the Report

Mrs. P V Ramjeawon, MRC, Technical Secretarial for the Report

* Mr B Hulman was replaced by Mr A A Boddhoo in the Evaluation Committee in August 2002.

Annex 3

ENVIRONMENTAL MONITORING OF THE INTEGRATED FARMING SYSTEM AT UNION DUCRAY FARM

SAMPLING PROTOCOL

1. **Microbiological analysis** of water of the IFS at Union Ducray farm over a period of **four weeks** as follows:

Sampling Date	Sampling Day	Sampling Sessions
2 September 2002	Monday	0900 hours and 1400 hours
5 September 2002	Thursday	0900 hours and 1400 hours
9 September 2002	Monday	0900 hours and 1400 hours
12 September 2002	Thursday	0900 hours and 1400 hours
16 September 2002	Monday	0900 hours and 1400 hours
19 September 2002	Thursday	0900 hours and 1400 hours
23 September 2002	Monday	0900 hours and 1400 hours
26 September 2002	Thursday	0900 hours and 1400 hours

(a) Collect and analyse water samples **twice weekly**. For a particular sampling day collect samples **in the morning and in the afternoon** at the following locations (see Figure enclosed) and analyse for the parameters indicated.

Sampling Point	Location	Parameters
1	Water Inlet	TC, FC
4	Outlet of Sedimentation tank	TC, FC
5	Outlet of Oxidation Basin	TC, FC
6	Outlet of first fish pond	TC,FC, Staph.aureus/ml, Clostridium perfringens/ml, Salmonella/ml, Vibrio/ml and Taenia(cysticercosis)
7	Outlet of second fish pond	TC, FC Staph.aureus/ml, Clostridium perfringens/ml, Salmonella/ml, Vibrio/ml and Taenia(cysticercosis)

(b) Carry out **microbiological testing of crayfish weekly** as follows:

Sampling Date	Sampling Day	Sampling Sessions
5 September 2002	Thursday	0900 hours
12 September 2002	Thursday	0900 hours
19 September 2002	Thursday	0900 hours
26 September 2002	Thursday	0900 hours

Collect and analyse the following crayfish samples **weekly** as mentioned above. For a particular sampling session collect samples at the following locations and analyse for the parameters indicated for the following parameters:

No. of Cray Fish Samples	Parameters
1st Sample: 5 crayfish from 1st fish pond and 5 crayfish from 2nd fish pond **2nd Sample:** 10 crayfish from purging tank*	TC, FC, Staph.aureus/ gms, Clostridium perfringens/ gms, Salmonella/ gms, Vibrio/ gms and Taenia (cysticercosis).

***Crayfish to be purged by Union Ducray officials three days before the analyses. At least 5 crayfish should be purged from each fishpond.** The purging schedule will be as follows –

Purging Date	Purging Day	Time
2 September 2002	Monday	0900 hours
9 September 2002	Monday	0900 hours
16 September 2002	Monday	0900 hours
23 September 2002	Monday	0900 hours

A representative sample should be prepared on each set of 10 crayfish to make two samples for the study.

Note: Collection, presentation and analysis of samples should be carried out according to standard WHO guidelines.

2. Carry out an **environmental monitoring** of the IFS at Union Ducray farm **over a period of four weeks** as follows:

Sampling Date	Sampling Day	Sampling Sessions
2 September 2002	Monday	0900 hours and 1400 hours
5 September 2002	Thursday	0900 hours and 1400 hours
9 September 2002	Monday	0900 hours and 1400 hours
12 September 2002	Thursday	0900 hours and 1400 hours
16 September 2002	Monday	0900 hours and 1400 hours
19 September 2002	Thursday	0900 hours and 1400 hours
23 September 2002	Monday	0900 hours and 1400 hours
26 September 2002	Thursday	0900 hours and 1400 hours

Collect and analyse samples twice weekly. For a particular sampling day collect samples in the morning and in the afternoon at the following locations (see Figure enclosed) and analyse for the parameters indicated.

Sampling Point	Location	Parameters
1	Water Inlet	pH, TSS, COD
2	Inlet of Digester	TSS, COD, pH, BOD_5
3	Outlet of Digester	COD, BOD_5, TSS, pH
4	Outlet of Sedimentation tank	COD, BOD_5, TSS, DO, pH, NO_3, NO_2, NH_3, PO_4, SO_4
5	Outlet of Oxidation Basin	COD, BOD_5, TSS, pH, DO, NO_3, NO_2, NH_3, PO_4, SO_4
6	Outlet of first fish pond	COD, BOD_5, TSS, pH, DO, NO_3, NO_2, NH_3, PO_4, SO_4
7	Outlet of second fish pond	COD, BOD_5, TSS, pH, DO, NO_3, NO_2, NH_3, PO_4, SO_4

Note: Collection, presentation and analysis of samples should be carried out according to standard WHO guidelines.

Annex-6

MICROBIOLOGICAL STANDARDS & GUIDELINES

FOR FISH AND FISHERY PRODUCTS

WEFTA 1999

Issued By: *Mary Seaver*
Issue No.: *6*
Issue Date: *24 September 1999*

CONTENTS

MICROBIOLOGICAL CRITERIA

Microbiological Standards	Mandatory criteria that are included in legislation or regulations, failure to comply with which can result in prosecution
Microbiological Guidelines	Non-mandatory criteria usually intended to guide the manufacturer and help to ensure good hygienic practice

SEAFOOD CATEGORIES

A) Molluscs, including fresh and frozen mussels, clams, oysters in shell or shucked.

B) Fish raw materials, fresh and frozen fish and crustaceans. Usually eaten after cooking.

C) Lightly preserved fish products (i.e. NaCl <6 (w/w) in water phase, pH >5.0). This group includes salted, marinated, cold smoked and graved fish. Eaten without cooking.

D) Heat-processed (pasteurised, cooked, hot smoked) fish products and crustaceans (including pre-cooked, breaded fillets). Some products eaten with no additional cooking.

E) Heat-processed (sterilised, packed in sealed containers). Often eaten with no additional cooking.

F) Semi-preserved fish (i.e. NaCl >6 (w/w) in water phase, or pH <5.0, preservatives (sorbate, benzoate, NO_2 may be added). This group includes salted and/or marinated fish and caviar. Eaten without cooking.

G) Dried, dry-salted and smoke-dried fish. Usually eaten after cooking.

SAMPLING PLANS

TWO-CLASS PLAN

A two-class plan is concerned primarily with presence or absence of an organism in a defined amount of sample, where in a given number of samples, *n*, a certain number will show the unacceptable presence of the test organism.

THREE-CLASS PLAN

Where a rigid 'two-class' plan is not essential, use can be made of a 'three-class' plan that accept a proportion of sample units whose test results fall between unequivocal acceptability and rejection. In devising a plan for a particular food it is necessary to set values for *n, c, m,* and *M* where:

n is the number of samples units comprising the sample.

m is the threshold value for the number of bacteria; the result is considered satisfactory if the number of bacteria in all sample units does not exceed this value.

M is the maximum value for the number of bacteria; the result is considered unsatisfactory if the number of bacteria in one or more sample units is equal to or greater than this value.

c is the number of sample units where the bacterial count may be between *m* and *M*. The sample is considered acceptable if the bacterial counts of the other sample units are equal to or less than the value of *m*.

For practical purposes, *n* is frequently given a value of five, and *c* one or two.

REGULATIONS/STANDARDS FOR MICROBIOLOGICAL CRITERIA

APPLICABLE TO FISH AND FISHERY PRODUCTS

1. France
2. Norway
3. Spain
4. Australia & New Zealand
5. EU

Standards - France

Category	*Parameter*	*Specification*
A: ***Live bivalve shellfish and sea urchins***	Faecal Coliforms/g	3.0×10^2 (4)
	Faecal streptococci/g	2.5×10^3 (1)
	Salmonella /25g	Absent
B: ***Breaded or unbreaded sliced fish, chilled fresh fillets of fish***	Aerobic plate count 30ºC/g	10^5
	Faecal Coliforms/g	10
	Staphylococcus aureus/g	10^2
	Anaerobic sulphite reducers/g	10
	Salmonella / 25g	Absent
B: ***Breaded or unbreaded sliced fish, frozen or deep-frozen fillets of fish***	Aerobic plate count 30ºC/g	5×10^4
	Faecal Coliforms/g	10
	Staphylococcus aureus/g	10^2
	Anaerobic sulphite reducers/g	2
	Salmonella / 25g	Absent
B: ***Ground, raw preparations based on fish meat.***	Aerobic plate count 30ºC/g	5×10^5
	Faecal Coliforms/g	10^2
	Staphylococcus aureus/g	10^2
	Anaerobic sulphite reducers/g	10
	Salmonella / 25g	Absent
B: ***Fresh, frozen or deep frozen frogs legs***	Aerobic plate count 30ºC/g	5×10^5
	Faecal Coliforms/g	10^2
	Staphylococcus aureus */ g*	10^2 (2)
	Salmonella / 25g	Absent
B/D: ***All crustaceans including whole, cooked or raw shrimps, frozen or deep-frozen***	Aerobic plate count 30ºC/g	10^3
	Faecal Coliforms/g	1
	Anaerobic sulphite reducers/g	2
	Salmonella / 25g	Absent
D: ***Chilled, shelled cooked shrimp, and frozen or deep-frozen shrimp***	Aerobic plate count 30ºC/g	10^5
	Faecal Coliforms /g	10
	S. aureus /g	10^2
	Anaerobic sulphite reducers/g	10
	Salmonella / 25g	Absent
D: ***Pre-cooked scallops and mussels***	Aerobic plate count 30ºC/g	10^6
	Faecal Coliforms/g	10
	Staphylococcus aureus/g	10^2
	Anaerobic sulphite reducers/g	30
	Salmonella / 25g	Absent
D: ***Chilled cooked crustaceans, other than shrimps***	Aerobic plate count 30ºC/g	10^5
	Faecal Coliforms/g	1
	Anaerobic sulphite reducers/g	2
	Salmonella / 25g	Absent
D: ***Deep frozen or frozen shelled snails***	Anaerobic sulphite reducers/g	10^3 (2)
	Salmonella **/g**	Absent **(3)**

(1) This test is done in specific suspicious cases, according to the "commemoratives" in 100 ml of flesh-intervalve liquid mixture.
(2) Only tolerances of analytical origin are accepted (2 class plan)
(3) Provisional standard /1g
(4) For 100 ml
(5) These criteria also apply to frozen or deep-frozen frogs legs treated by ionising radiation.

Ref: See reference at end of document

Standards Norway

Category	*Parameter*	*Specification*				
		Footnotes	*n*	*c*	*m*	*M*
A/B: ***Raw crustaceans and molluscs that shall be given heat treatment***	*Thermotolerant Coliforms /g* **TABLE II**		5	3	0	10
	Salmonella spp. /25g **TABLE II**		5	0	Absent	
B: ***Raw crustaceans and molluscs to be eaten without prior heat treatment***	Thermotolerant Coliforms /g **TABLE I**		5	1	0	10
	Salmonella spp. /25g **TABLE I**		5	0	Absent	
B: ***Raw fish whole-fillet finfish – usually eaten after cooking***	TVC @ 20º/30ºC /g (a) (b) (c)	(a) (b) (c)	5	3	10^5 $5x10^6$ $5x10^5$	10^6 $5x10^7$ $5x10^6$
	Thermotolerant Coliforms /g	*(a) (c)	5	2	0 0	10 50
B: ***Raw fish minced***	TVC @30ºC/g (a)	(a)			10^6	10^7
	Thermotolerant Coliforms /g	(a)			10	50
B: ***Raw fish – Crustacea***	TVC @20º/30ºC /g	(c)	5	3	10^5	10^6
	S. aureus (Coag +ve)/g	(c)	5	2	10^2	10^3
	Salmonella spp. /25g		5	0	Absent	
C: ***Lightly preserved fish products***	TVC @20º/30ºC /g***	(a) (b)			10^4 10^6	$5x10^5$ 10^7
	Total Coliforms /g	(a)			10^2	10^3
	S. aureus (Coag +ve) /g	(a) (b)			10^2 10^2	10^3 10^3
	Listeria monocytogenes / 25g (a) + (b)		5	0	0	0
D: ***Heat processed pasteurised shellfish***	TVC @ Mesophilic aerobes /g	(i) + (iii) (ii)	5	3	10^5 10^5	10^6 10^6
	S. aureus (Coag +ve) /g **TABLE II**	(i) (ii) + (iii)	5	2	10^2	10^3
	Salmonella spp./ 25g **TABLE II**	(i)+ (iii) (ii)	5	0	Absent Absent	
	Thermotolerant Coliforms /g		5	1	0	10
F: ***Semi-preserved fish****	TVC @20º/30ºC / g	(a) (c)	5	2	10^5 10^6	10^6 $5x10^7$
	Thermotolerant Coliforms /g	(a)			0	10
	S. aureus (Coag +ve) /g	(a)	5	2	10^2	10^3
	Salmonella spp. /25g		5	0	Absent	
G: ***Dried, dry-salted, and smoke-dried fish***	*S. aureus* (Coag +ve) /g	(a) + (b)			10^2	10^3
	L. monocytogenes /g	(a) + (b)			0	0

Raw Roe
****Prawns peeled in brine***
*****Hot/Cold smoked fish***
(i) Prawns boiled frozen
(ii) Boiled bivalves
(iii) Cooked frozen crab

(a) Day of Production
(b) Last day of shelf-life
(c) Unspecified time of analysis

TABLE I

1. In cases where the threshold values given in table I are exceeded, the establishment is obliged to inform the inspection authority of the results of the examination and of any measures carried out in relation to batches that fail to meet the requirements.
2. The establishment is further obliged to review the methods used for monitoring and control of critical points to identify the source of infection and to carry out more frequent analyses.
3. If repeated controls show the establishment has not carried out effective measures to bring the production in line with the microbiological requirements, the inspection authority may adopt the measures referred to in section 23-4 (4) of the reference article.

TABLE II

1. If a batch fails to satisfy the bacteriological requirements given in table II, the batch is not permitted sold for human consumption. The inspection authority may release the batch if new documentation after reprocessing establishes that the requirements laid down in table II are satisfied.
2. In cases where threshold values in table II are not satisfied, the establishment is obliged to inform the inspection authority of the results of the examination and of the measures adopted in relation to batches that fail to meet the requirements.
3. The establishment is further obliged to to review the methods used for monitoring and control of critical points to identify the source of infection, to carry out more frequent analyses and to refrain from marketing for human consumption batches that fail to meet the requirements.
4. If the establishment fails to carry out effective measures to prevent the marketing of products in violation of the provisions of these Regulations, the inspection authority may:
   A. impose a temporary halt in production of the product group concerned.
   B. withdraw the approval for the form of production concerned.

Ref: Ministry of Fisheries 14 June 1996 laying down Quality Regulations relating to Fish and Fishery products

Standards - Spain

Category	*Microbiological parameter*	*Specification*
B: ***Fresh, refrigerated and frozen fish products***	TVC 30ºC/g	10^6
	Enterobacteriaceae/g	10^3
	Salmonella-Shigella/25g	Absent
D: ***Cooked fish products***	TVC 30ºC/g	10^5
	Enterobacteriaceae/g	10^3
	Salmonella-Shigella/25g	Absent
	S. aureus (Coag +ve)/g	10^2
E: ***Canned fish products***	TVC 30ºC/g	Absence of colonies after pre-incubation for 30 days @ 31ºC and 10 days @ 44ºC
	Sporeformers	10 spores of thermoduric "Bacilliaceae", non-pathogenic, non-toxic and unable to spoil the product.
	Botulinum toxin	Absence in the whole packing
F: ***Semi-preserved fish products in vinegar***	TVC 30ºC/g	10^3
	Enterobacteriaceae/g	10^2
	Salmonella-Shigella/25g	Absent
G(I): ***Salted and dried fish products***	TVC 30ºC/g	10^5
	Enterobacteriaceae/g	10^2
	Salmonella-Shigella/25g	Absent
G(II): ***Smoked fish products***	TVC 30ºC/g	n=5 C=3 m=10^5 M=10^6
	Enterobacteriaceae/g	n=5 c=3 m=10^2 M=10^3
	Salmonella-Shigella /25g	n=5 c=0 m=absent M=0
	Staphylococcus aureus/g	n=5 c=2 m=10^1 M=2x10^1

Ref: Order of 2 August 1991 by the Ministry of Health and Consumption laying down Microbiological Standards etc. for Fishery and Aquaculture Products.

Standards - Australia & New Zealand

Category	***Parameter***	***Specification***
B: ***Prawns & shrimps - raw, frozen***	Aerobic plate count (35ºC)/g	n=5 c=2 m=$5x10^5$ M=$5x10^6$
	S. aureus (Coag +ve) /g	n=5 c=2 m=10^2 M=10^3
	Faecal Coliforms /g	n=5 m=10^2 c=2 M=10^3
	Salmonella in 25g	n=5 m=0 c=0
	Vibrio cholerae /g	n=5 m=0 c=0
B: ***Prawns & shrimps – cooked, frozen***	APC /g	n=5 c=2 m=10^5 M=10^6
	S. aureus (Coag +ve)/g	n=5 c=2 m=10^2 M=10^3
	Faecal coliform/g	n=5 c=2 m=10 M=10^2
	Listeria monocytogenes/25g	n=5 m=0 c=0
	Salmonella in 25g	n=5 m=0 c=0
	Vibrio cholerae /g	n=5 m=0 c=0
	Vibrio parahaemolyticus /g	n=5 c=2 m=10^2 M=10^3
B: ***Fish - raw, breaded, frozen***	Aerobic plate count (35ºC) /g	n=5 c=2 m=10^5 M=10^6
	S. aureus (Coag +ve) /g	n=5 c=2 m=10^2 M=10^3
	Faecal Coliforms /g	n=5 c=2 m=10^2 M=10^3
	Salmonella in 25g	n=5 m=0 c=0

C/D: ***Shellfish – processed, requiring no further cooking***	APC 35ºC /g	n=5 c=2 m=10^4 M=10^5
	S. aureus (Coag +ve) /g	n=5 c=2 m=10^2 M=10^3
	Faecal coliform/g	n=5 c=2 m=10 M=10^2
	Listeria monocytogenes/g	n=5 m=0 c=0
	Salmonella in 25g	n=5 m=0 c=0
	Vibrio parahaemolyticus/g	n=5 c=2 m=10^2 M=10^3
D: ***Rock lobster/crayfish (cooked)***	Aerobic plate count (35ºC) /g	n=5 c=2 m=10^4 M=10^5
	S. aureus (Coag +ve)/g	n=5 c=2 m=10^2 M=10^3
	Faecal Coliforms /g	n=5 c=2 m=10 M=10^2
	Salmonella in 25g	n=5 m=0 c=0
B/F: ***Shellfish – processed, requiring cooking***	APC 35ºC /g	n=5 c=2 m=10^5 M=10^6
	S. aureus (Coag +ve) /g	n=5 c=2 m=10^2 M=10^3
	Faecal coliform/g	n=5 c=2 m=10^2 M=10^3
	Salmonella in 25g	n=5 m=0 c=0

Ref: Microbiological Standards Information Summary Proposal P178 24 June 1998. Development of Joint Australia New Zealand Food Standards

EU Guidelines

Product type	*Parameter*	*Specification /g*
D: ***Cooked crustaceans and molluscan shellfish (93/51/EEC)***	***Pathogens*** - *Salmonella*	Absent **in 25g** n=5 c=0
	Organisms indicating poor hygiene (Shelled or shucked products) - *Staphylococcus aureus* - Either: Thermotolerant coliform (44ºC on solid medium) - Or: *Escherichia coli* (on solid medium)	 n=5 m=100 c=2 M=1000 n=5 m=10 c=2 M=100 n=5 m=10 c=1 M=100
	Indicator organisms (guidelines) Mesophilic aerobic bacteria (30ºC) a) whole products b) shelled or shucked products with the exception of crabmeat c) Crabmeat	 n=5 m=10^4 c=2 M=10^5 n=5 m=$5x10^4$ c=2 M=$5x10^5$ n=5 m=10^5 c=2 M=10^6
Live Bivalve Molluscs – Ready-to-eat (91/492/EEC)	*E*. coli	230/**100g**
	Faecal Coliforms	300/**100g**
	Salmonella	Absent/**25g**

Ref: Official Journal of the European Communities. No. L 13/13. 21/1/93 (93/51/EEC)
Official Journal of the European Communities. No. L268/1. 15/7/91 (91/492/EEC)

GUIDELINES FOR MICROBIOLOGICAL CRITERIA

APPLICABLE TO FISH AND FISHERY PRODUCTS

1. ***Denmark***
2. ***Ireland***
3. ***Portugal***
4. ***UK***
5. ***ICSMF***
6. ***Belgium***

Guidelines - Denmark

Category	*Parameter*	*Specification*
B: ***Raw fish –Crustacea***	TVC @ 20ºC /g Iron agar	n=5 M=10^5 c=0
	Faecal Coliforms /g	n=5 m=10 c=2 M=10^2
	Faecal Streptococci/Enterococci /g	n=5 M=10^3 c=0
	Vibrio parahaemolyticus /25g *Vibrio vulnificus* /25g *Vibrio cholerae* /25g (Only tropical products)	Absent n=5 Absent n=5 Absent n=5
B: ***Raw fish – Whole/fillet finfish***	TVC @ 20ºC / g Iron agar	n=5 m=5x10^5 c=2 M= 1x10^7
	Faecal Coliforms /g	n=5 m=10 c=2 M=100
	Faecal Streptococci/Enterococci /g	n=5 M=10^3 c=0
C: ***Lightly preserved fish products****	TVC @ 20ºC / g Iron agar	** n=5 m=2x10^5 c=2 M=5x10^5
	S. aureus (Coag +ve)/g	n=5 m=10^3 c=0
	Faecal Coliforms /g	n=5 m=10 c=0
	Faecal Streptococci/Enterococci /g	n=5 M=10^3 c=0
	Listeria monocytogenes/25g	n=5 m=50 c=2 M=10^2
D: ***Heat-processed fish products and crustaceans. Some products eaten with no additional cooking***	TVC @20ºC Iron agar /g	n=5 m=10^4 c=2 M=5x10^5
	Faecal Coliforms /g	n=5 m=10 c=2 M=10^2
	E. coli /g /g	n=5 m=10 c=1 M=10^2
	Faecal Streptococci/Enterococci /g	n=5 m=10^2 c=2 M=10^3
	S. aureus /g	n=5 m=10 c=2 M=10^2
	Salmonella spp. /25g	n=5 m=0 (all neg) c=0
	Listeria monocytogenes/25g	n=5 m=0 (all neg) c=2
	Vibrio cholerae /g (only tropical products)	n=5 m=0 (all neg) c=2

*Cold smoked product
**1,000,000 acceptable if the microflora is Lactic Acid Bacteria

Ref: Danish Ministry of Fisheries 28 June 1993. Danish Veterinary and Food Administration J.nr.08kt.10-533-0/98, January 1998

Guidelines Ireland*

Category	*Parameter*	*Specification*
A: ***Raw molluscan shellfish:*** ***1) Scallops, cockles***	*Salmonella* / 25g	Absent
	S. aureus /g	1/g
	SPC @22ºC/72 hours/g	2.0×10^5
	Faecal Coliforms	1
2) Oysters, mussels	*Salmonella* / 25g	Absent
	S. aureus /g	1
	SPC @22ºC/72 hours/g	3.0×10^5
	Faecal Coliforms/g	1
B: ***Fresh gutted fish; frozen fish; fish blocks***	*Salmonella* / 25g	Absent
	S. aureus /g	1
	SPC @22ºC/72 hours/g	1.0×10^5
	Faecal Coliforms	1
B: ***Fresh water fish***	*Salmonella* / 25g	Absent
	S. aureus /g	1
	SPC @22ºC/72 hours/g	1.0×10^5
	Faecal Coliforms	1
B: ***Frozen raw shrimp, prawn, lobster***	*Salmonella* / 25g	Absent
	S. aureus /g	1.0×10^2
	SPC @22ºC/72 hours/g	5.0×10^5
	Faecal Coliforms	1.0×10^1
B: ***Frozen raw breaded shrimp, prawn***	*Salmonella* / 25g	Absent
	S. aureus /g	1.0×10^1
	SPC @22ºC/72 hours/g	5.0×10^5
	Faecal Coliforms/g	1.0×10^1
B/C: ***Smoked fish***	*Salmonella* / 25g	Absent
	S. aureus /g	1.0×10^1
	SPC @22ºC/72 hours/g	1.0×10^5
	Faecal Coliforms	1
B/D: ***Breaded pre-cooked fish***	*Salmonella* / 25g	Absent
	S. aureus /g	1.0×10^1
	SPC @ 30ºC/72 hours/g	3.0×10^5
	Faecal Coliforms	1.0×10^1
D: ***Frozen cooked shrimp, prawn, lobster***	*Salmonella* / 25g	Absent
	S. aureus /g	1/g
	SPC @ 30ºC/72 hours	1.0×10^5
	Faecal Coliforms	1.0×10^1
D: ***Cooked picked crab meat***	*Salmonella* / 25g	Absent
	S. aureus /g	1
	SPC @ 30ºC/72 hours/g	5.0×10^5
	Faecal Coliforms/g	1

*All analyses to be based on 2 x 25g composite samples or subsamples from each unit or pack

Ref: Department of Health, 1992. Microbiological Guidelines for Fish.

Guidelines – Portugal

Category	*Parameter*	*Specification*
B: ***Fresh SeafoodProducts***	Aerobic Plate Count	<100,000/g
	Yeasts and Moulds	<1,000/g
	Staphylococci (Coag +ve)	<100/g
	Salmonella	Absent/25g
	Sulphite reducing *Clostridia*	<100/g
	Total Coliforms	<100/g
	E. coli	<10/g
B: ***Frozen Seafood Products***	Aerobic Plate Count	<10,000/g
	Yeasts and Moulds	<1,000/g
	Staphylococci (Coag +ve)	<10/g
	Salmonella	Absent/25g
	Sulphite reducing *Clostridia*	<10/g
	Total Coliforms	<100/g
	E. coli	<10/g
F: ***Semi-preserved products***	Yeasts and Moulds	<100/g
	Staphylococci (Coag +ve)	<2/g
	Salmonella	Absent/25g
	Sulphite reducing *Clostridia*	<10/g
	Enterococci	<10/g
	Total Coliforms	<1/g
	Total Halophilic bacteria	<1,000/g
F: ***Salted fish***	Yeasts and Moulds	<10,000/g
	Staphylococci (Coag +ve)	<2/g
	Salmonella	Absent/25g
	Sulphite reducing *Clostridia*	<100/g
	Wallemia sebi	<10,000/g
	Total halophilic bacteria/g	<10,000/g
	Bacteria responsible for “pink”/10kg	<10,000/g

(x) = Fresh
(y) = Frozen

Ref: Portuguese Veterinary Society of Food Hygiene 1971

Guidelines UK

Product type	***Parameter***	***Satisfactory***	***Fairly satisfactory***	***Un-satisfactory***	***Unacceptable - potentially hazardous***
C: Herring/roll mop and other pickled fish	Aerobic plate counts 30ºC;48± 2 h	$<10^3$	10^3 - $<10^4$	$\geq 10^4$	N/A*
D: Other fish (cooked) crustacea and seafood meals	Aerobic plate counts 30ºC;48± 2 h	$<10^5$	10^5-10^6	$\geq 10^6$	N/A *
B/C/D: Smoked fish , taramasalata and cooked shellfish	Aerobic plate counts 30ºC;48± 2 h	$<10^6$	10^6 - $<10^7$	$\geq 10^7$	N/A*
A/B/C/D/E/F/G: All seafood	*E. coli* /g *(total)*	<20	20-<100	100 - $<10^4$	$\geq 10^4$
	Listeria spp./g	ND in 25g	Present in 25g <200/g	200 - $<10^4$	$\geq 10^4$
	Salmonella spp./25g	ND in 25g			Present in 25g
	E. coli 0157 and other *VTEC*/g	ND in 25g			Present in 25g
	L. monocytogenes/g	ND in 25g	Present in 25g <200/g	200 - $<10^3$	$\geq 10^3$
	S. aureus /g	<20	20 - <100	100 - $<10^4$	$\geq 10^4$
	C. perfringens/g	<10	10 - <100	100 - $<10^4$	$\geq 10^4$
	B. cereus and *B. subtilis group***/g	$<10^3$	10^3 - $<10^4$	10^4- $<10^5$	$\geq 10^5$
	Vibrio parahaemolyticus	ND in 25 g			Present in 25g

* Prosecution based solely on high aerobic plate counts in the absence of other criteria of unacceptability is unlikely to be successful.

** *B. subtilis, B licheniformis* and *B. pumilus* when present in large numbers are also unsatisfactory or potentially hazardous. If the *Bacillus* counts, other than *B. cereus* exceed 10^4 per gram, the organism should be identified.

Ref: Adapted from – Public Health Laboratory Service 1996. Microbiological guidelines for some Ready-to-eat foods sampled at the point of sale. PHLS Microbiology Digest, 13, 41-43

Guidelines - ICMSF

Category	*Parameter*	*Specification*
A: ***Fresh and frozen bivalve molluscs***	APC/g	n=5 c=0 m=$5x10^5$
	E. coli /g	n=5 c=0 m=16
B: ***Frozen raw crustaceans***	APC/g	n=5 m=10^6 c=3 M=10^7
	E. coli /g	n=5 m=11 c=3 M=500
B/C: ***Fresh and frozen fish and cold-smoked***	APC/g	n=5 m=$5x10^5$ c=3 M=10^7
	E. coli /g	n=5 m=11 c=3 M=500
B/D: ***Pre-cooked breaded fish***	APC/g	n=5 m=$5x10^5$ c=2 M=10^7
	E. coli /g	n=5 m=11 c=2 M=500
D: ***Frozen cooked crustaceans***	APC/g	n=5 m=$5x10^5$ c=2 M=10^7
	E. coli /g	n=5 m=11 c=2 M=500
	S. aureus /g	n=5 c=0 m=10^3
D: ***Cooked, chilled and frozen crabmeat***	APC/g	n=5 m=10^5 c=2 M=10^6
	E. coli /g	n=5 m=11 c=1 M=500
	S. aureus /g	n=5 c=0 m=10^3

Ref: Sampling for microbiological analysis: Principles and specific applications, 2nd edition. The International Commission on Microbiological Specifications for Foods (ICMSF) 1978, of the International Union of Microbiological Societies.

Guidelines - Belgium

Category	*Parameter*	*Specification*	
		Target	*Rejection*
B: ***Fresh fish and fish fillets***	Total aerobic mesophilic count (30ºC)/g	$<5x10^4$	$>10^6$
	Thermotolerant Coliforms (44ºC)/g	<10	>30
	S. aureus /g	<100	>300
	Salmonella / 25g	Absent	Present
	Yeasts and moulds/g	<100	$>10^3$
C: ***Cold smoked fish***	Total aerobic mesophilic count (30ºC)/g	$<5x10^4$	$>5x10^5$
	Thermotolerant Coliforms (44ºC)/g	<10	>30
	S. aureus /g	<100	>300
	Faecal Streptococci/g	<100	>300
	Salmonella / 25g	Absent	Present
	Yeasts and moulds/g	<100	>1000
D: ***Hot smoked fish***	Total aerobic mesophilic count (30ºC)/g	<1000	>50,000
	Thermotolerant Coliforms (44ºC)/g	Absent/g	>30
	S. aureus /g	Absent	>30
	Salmonella / 25g	Absent	Present
	Yeasts and moulds/g	<10/	>1000
D: ***Cooked and peeled shrimps and crab flesh***	Total aerobic mesophilic count (30ºC)/g	m $=10^5$ 3m=$3x10^5$ M $=10^6$	
	Thermotolerant Coliforms (44ºC)/g	m =10 3m=30 M $=10^2$	
	S. aureus /g	m $=10^2$ 3m=$3x10^2$ M $=10^4$	
	Salmonella /25g	m =Absent 3m=Absent M =Absent	
B/E: ***Prepared meals based on cooked fish, shrimps, crab and scallop meat etc. and heated prior to consumption***	Total aerobic mesophilic count (30ºC)/g	$<5x10^4$	$>5x10^5$
	Thermotolerant Coliforms (44ºC)/g	<10	>100
	S. aureus /g	<100	>300
	Salmonella / 25g	Absent	Present
D/E: ***Prepared meals based on cooked fish products and eaten cold***	Total aerobic mesophilic count (30ºC)/g	$<5x10^3$	$>5x10^4$
	Thermotolerant Coliforms (44ºC)/g	<10	>30
	S. aureus /g	<10	>30
	Salmonella / 25g	Absent	Present
	Yeasts and moulds/g	<10	>1000

For raw shrimps, the total counts/g are multiplied by 10. The other microbiological limits remain the same**.**

Ref: Centre de Recherche Agronomique – Gand. Department de Peche Maritime.

References

1. Standards

France: Order of 21 December 1979
Order of 2 June 1988
Order of 13 March 1989

Norway: Ministry of Fisheries 14 June 1996 laying down Quality Regulations relating to Fish and Fishery products

Spain: Order of 2 August 1991 by the Ministry of Health and Consumption laying down Microbiological Standards etc. for Fishery and Aquaculture Products.

Australia & Zealand: Microbiological Standards Information Summary Proposal P178 24 June New 1998. Development of Joint Australia New Zealand Food Standards

EU: Official Journal of the European Communities.
No. L 13/13. 21/1/93 (93/51/EEC)
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2. Guidelines

Denmark: Danish Ministry of Fisheries 28 June 1993. Danish Veterinary and Food Administration (J.nr.08kt.10-533-0/98, January 1998)

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Belgium: Bacteriological specifications used at the Fisheries Research Station, Oostende, Belgium