Graduate School of Frontier Sciences, The University of Tokyo

Study on Social Environment, Department of Socio-cultural Environmental Studies

2014-2015

Master's Thesis

Status of Antibiotic Residues in Shrimp and Prawn Muscle in Bangladesh and Associated Health Risk Assessment

（バングラデシュにおけるエビ身肉中抗生物質の残留状況と健康リスク評価）

July, 2015

Advisor: Professor Jun SASAKI

Student ID: 47136822

Md. Boduruzaman

Abstract

The study was carried out to find out the sources of nitrofurans and chloramphenicol that are available in the shrimp and prawn muscle of Bangladesh and also to assess the associated health risk for the people of Bangladesh. Data on the presence of antibiotics in shrimp and prawn muscle and feed were collected from the Upazila (subdistrict) Fisheries Offices of Satkhira, Khulna and Bagerhat district, Department of Fisheries (DoF) under the Ministry of Fisheries and Livestock, Bangladesh and total 83 shrimp and prawn farms of which 49 contaminated and 34 non-contaminated with banned antibiotics, 5 feed factories and 9 hatcheries were interviewed with pretested questionnaire. On the other hand, it was investigated 10 VMD/AMD shops to justify the name, composition and company name of chemicals that were mentioned by the farmers and hatchery and feed factories representatives. However, on the basis of information mentioned by the farmers during questionnaire survey, 12 PL (Post Larvae of shrimp/prawn), 02 shrimp/prawn shell and 02 unknown chemicals samples were collected for nitrofuran and chloramphenicol test. Antibiotic tested data, questionnaire survey data and test result of collected samples revealed that nitrofuran and chloramphenicol antibiotics are coming from hatchery, feed factories and use of contaminated shrimp/prawn shell as feed ingredient at farm level. Moreover, existing concentration of SEM in prawn signifies the potential carcinogenic effect to the consumers whereas that of AHD in shrimp has no significant effect. The carcinogenic risk (CR) of SEM (4.69E-06) through consumption of prawn was higher than the USEPA threshold level 1.0E-06. So, it should encourage the hatchery and feed mill operators and farmers to use probiotics and approved antibiotics instead of nitrofuran and chloramphenicol antibiotics and to maintain the withdrawal period and also encourage the farmers to refrain from the using of contaminated shrimp/prawn shell as feed ingredient. At the same time, it should not provide contaminated shrimp/prawn to the children and also build public awareness about it.

Acknowledgement

Firstly, I would like to express my sincere and heartfelt gratitude and regards to reverend teacher and research supervisor Prof. Jun SASAKI for his kind help, suggestion, generous guidance and untiring supervision throughout the research work as well as preparing this thesis.

I would like to express my sincere gratitude and appreciation to respected co-supervisor Assoc. Prof. Hiroyasu Sato for his valuable suggestion and instructions to carry out the research work and also in writing the thesis. I also grateful to my respected teacher Assoc. Prof. Yukio Koibuchi who helped me in numerous ways during the research field selection with his keen opinion and constant inspiration.

I am greatly indebted to District Fisheries Officers (DFOs) and Upazila Fisheries Officers (UFOs) of Satkhira, Khulna and Bagerhat district for their co-operation and assistance during my questionnaire survey and data collection.

I would like to express my immense indebtedness to all members of WESTECH, Jun SASAKI laboratory especially HaeChong O, Hiroto Higa, Kusumoto Yoshimu and MAANO Desiree Eve for their inspiration and assistance in many aspects during my research period.

I would like to express my sincere gratitude to Asian Development Bank-Japan Scholarship Program authorities and University of Tokyo for their financial assistance that made my study possible.

Finally, I would like to express my appreciation to my mother and other family members together with the friends for their inspiration, never-failing support and co-operation during study period at the University of Tokyo.

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Chapter 1

Introduction

1.1 Background

In Bangladesh, shrimp (*Penaeus monodon*) and prawn (*Macrobrachium rosenbergii*) farming is one of the most important sectors from the economical point of view and the position of this sector is second in terms of foreign currency earning. During the last three decades, this sector has drawn a special attention to the people due to its high export potential. Actually Bangladesh exports almost all shrimp to EU, USA and Japan (ASCC 1995; Islam 2008; Ahmed et al. 2008). During 2010-11, Bangladesh exported 51,672 MT of prawn and shrimp, valued at US$ 470.16 million of which around 80% was shrimp by value (EPB 2011). Shrimp and prawn farming also plays a vital role in employment. Approximately 1.2 million people are directly involved in shrimp and prawn production activities and 4.8 million household members are also indirectly involved in this sector. In addition, the livelihoods of around 400,000 rural people, most of them women, are associated with shrimp and prawn fry fishing in the coastal area of Bangladesh (USAID 2006).

At present, 275232.10 ha area has been brought under shrimp and prawn farming of which 76.1 % area for shrimp and the rest 23.9 % area for prawn culture (DoF 2013). In the past, to maximise production various antibiotics and chemicals like nitrofuran, chloramphenicol, oxytetracycline, tetracycline, malachite green and crystal violet etc. were used prophylactically and therapeutically in shrimp and prawn farming to control microorganisms (Nowsad,2007; Shamsuzzaman and Biswas, 2012; Hossain et al., 2013).

Nitrofurans, especially furazolidone (AOZ), furaltadone (AMOZ), nitrofurantoin (AHD), nitrofurazone (SEM), belong to a class of broad spectrum antibiotics of which parent compounds and their metabolites are depicted in Figure 1.1(M. Vass et al., 2008).

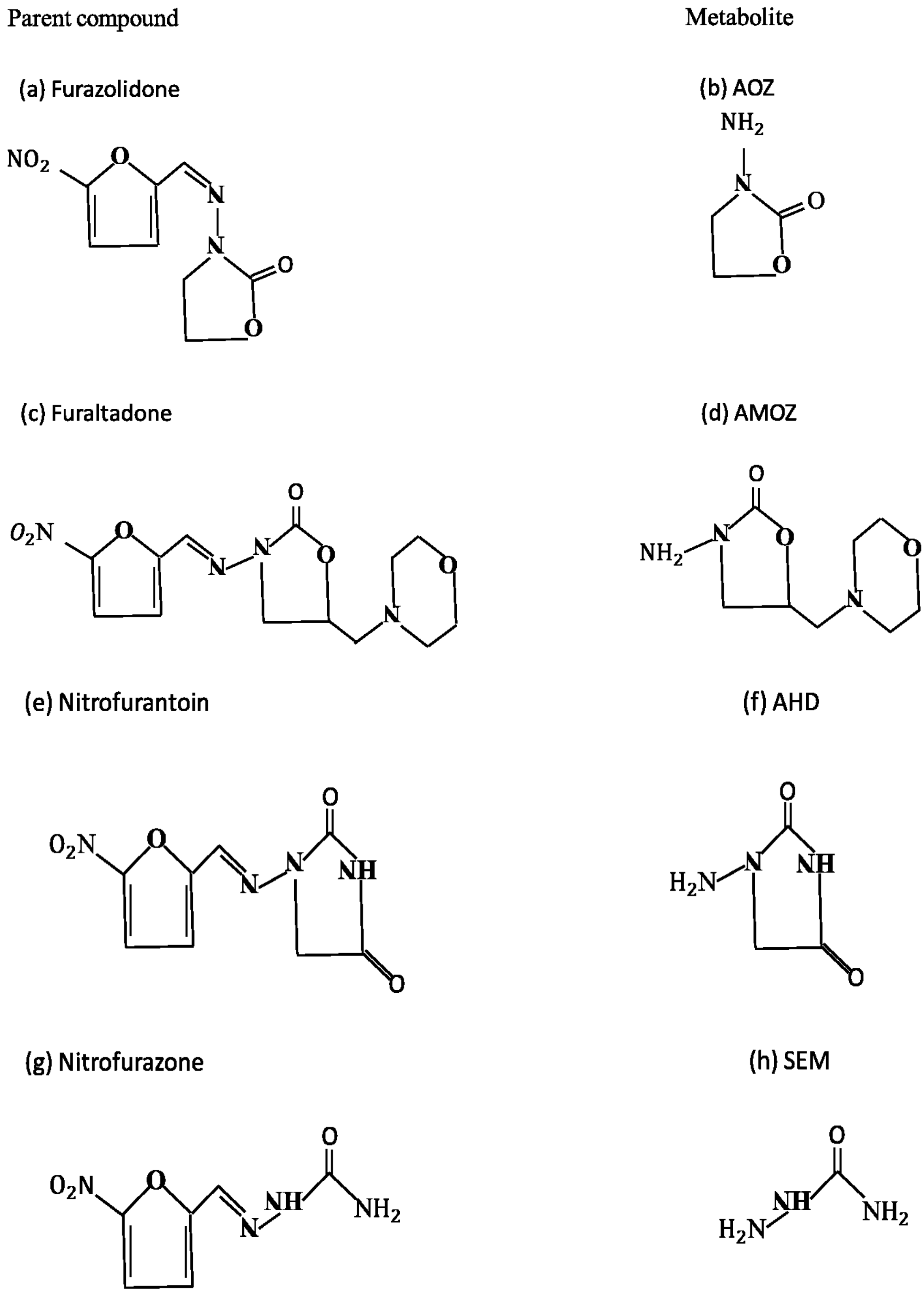


Figure 1.1 Structures of furazolidone, furaltadone, nitrofurantoin and nitrofurazone

In aquaculture, nitrofurans are commonly used as a growth promoter and in the prophylactic and therapeutic treatments of bacterial and protozoan infections such as gastrointestinal enteritis caused by *Escherichia coli* and *Salmonella* spp., fowl cholera and coccidiosis black heads (Draisci et al., 1997). In 1995, the use of nitrofurans for livestock production was completely prohibited in EU (Commission Regulation, 1995) due to concerns about the carcinogenicity of the drug residues and their potential harmful effects on human health (Van Koten-Vermeulen, 1993). After ingestion, nitrofuran parent drugs are metabolized to furazolidone (AOZ), furaltodone (AMOZ) and nitrofurantoin (AHD) rapidly and form corresponding tissue-bound metabolites (Nouws and Laurensen, 1990). The half-life of this parent drug is 7-63 min. This instability results in rapid depletion of nitrofurans in blood and tissue (Nouws and Laurensen, 1990). However, the formed metabolites (AOZ, AMOZ, AHD and SEM) bind with the protein in the body and remain for many weeks after treatment (Cooper et al., 2005).

Nitrofurans found in various food items specially poultry and aquaculture products of Thailand, China, Taiwan, India, Vietnam, Equador, Brazil and Bangladesh. In 2007, EU inspection found nitrofuran contamination in shrimp, honey and canned meat products of over nine countries, the highest incidence rate being from India (37%), China (37%), Bangladesh (10%) and Thailand (5%) (M. Vass et al., 2008). These antibiotics have carcinogenic and non-carcinogenic effects on human body though it's not possible to assess the health risk caused by furaltadone (AMOZ) due to lack of scientific information.

Chloramphenicol (CAP) is a broad spectrum antibiotic that against gram-positive and gram-negative bacteria causes aplastic anaemia (M. Vass et al., 2008). It has mentioned that chloramphenicol prohibited in USA and Japan and explicitly banned in Canada and European Union countries for its carcinogenic characteristics. However, it's used in shrimp culture of Latin America and Asia where shrimps are grown for export to USA, EU and Japan to control the diseases and also in human and veterinary medicines (GEASAMP 1997). Until now it's not possible to assess the CAP carcinogenicity due to lack of scientific information though CAP is treated as carcinogenic by IARC (International Agency for Research on Cancer) in human (Hanekamp et al., 2003).

Minimum Required Performance Limit (MRPL) of nitrofurans and chloramphenicol was established by EU for shrimp and prawn in 2003. MRPL of nitrofurans, especially furazolidone (AOZ), furaltadone (AMOZ), nitrofurantoin (AHD), nitrofurazone (SEM) was 1µg/kg whereas that of chloramphenicol was 0.3µg/kg. It indicates that designated laboratories must be able to detect nitrofurans and chloramphenicol at this concentration, and importing countries will reject or destroy shrimp and prawn consignment above mentioned concentration level (Commission Decision 2003/181/EC). In 2008 and 2009, 18 and 44 consignments of Bangladesh respectively were rejected by EU due to presence of nitrofuran and chloramphenicol in shrimp and prawn muscle (DoF 2014).

In recent years, many Southeast Asian Countries especially Bangladesh has been facing difficulties to meet with the present food safety standards of the importing countries specially Eu, USA and Japan. These countries have imposed a lot of non-tariff rules and regulations regarding food safety on the shrimp and prawn export of Bangladesh. To meet with the requirements, Government of Bangladesh (GOB) imposed a voluntary suspension on the export of prawn to EU countries for six months, executed from 20 May 2009 to 19 November 2009. To identify the sources of contaminations and prevent the entrance of banned antibiotics and chemicals in the shrimp value chain, Bangladesh Government has adopted many necessary steps. These include implementation of training programs for the officials of Department of Fisheries (DoF) and the stakeholders of shrimp and prawn value chain, strengthening of monitoring activities under National Residue Monitoring Control Plan (NRCP), testing of the presence of banned antibiotics and chemicals in shrimp and prawn meat and different brands of feed and feed ingredients sample and adopting measures to implement traceability in the shrimp and prawn value chain in toto (DoF 2009). When banned antibiotics especially nitrofuran and CAP found in a sample all the shrimp and prawn of the farm from where the sample was taken caught and sold in the local market for domestic consumption. It's a threat for the the people of Bangladesh.

1.2 Objectives

The objectives of this research are as follows:

- To find out the sources of the antibiotics that are available in the shrimp and prawn muscle of Bangladesh.
- To assess the health risks for the people of Bangladesh from antibiotics in shrimp and prawn muscle.

Chapter 2

Literature Review

2.1 Act, rules and regulations

EU (2003) amended Decision 2002/657/EC to set up Minimum Required Performance Limits (MRPLs) for certain residues in food of animal origin. In this amendment, it was mentioned that member states shall ensure the methods which used for detecting chloramphenicol, nitrofuran metabolites and medroxyprogesterone substances meet the MRPLs set out in Annex II. As per Annex II, the MRPLs of nitrofuran metabolites (SEM, AMOZ, AOZ and AHD), chloramphenicol and medroxyprogesterone are 1 µg/kg, 0.3 µg/kg and 1 µg/kg respectively.

Ioannis et al. (2005) mentioned that EU made several regulations and directives on food safety issues. Regulation EC 178/2002 states the general principles and obligations for exporting food to Europe. It has mentioned in the Article 11 of Regulation EC 178/2002 that the presence of chloramphenicol, nitroimidazoles, malachite green and nitrofurans residues excludes foods from exportation to EU. In addition to, Regulation EC 882/2004 explains the general procedures for official control, Regulation EC 852/2004 indicates the general hygienic requirements for all the food business operators and Regulation EC 853/2004 laid down additional specific requirements for food business related with foods of animal origin, including fishery products.

Legislating an act “Fisheries Hatchery Act, 2010” (GB, 2010) and a rules “Fisheries Hatchery Rules, 2011” (GB, 2011) Bangladesh Government has banned the use of chloramphenicol and nitrofuran in the production of shrimp and prawn PL (Post Larvae). Rule11 (1) explicitly states that chemicals that mentioned in Group-A and Group-B of the Schedule 3 are prohibited totally in the hatcheries. On the other hand, Bangladesh Government has also banned the use of nitrofuran and chloramphenicol in fish feed and animal feed. Section 14(1) of “Fish and Animal Feed Act,2010” (GB, 2011) states clearly that antibiotic, growth hormone, steroid and pesticide will not use in the fish and animal feed”. The list of prohibited chemicals has mentioned in Group-A and Group-B of the Schedule 7 under “Fish and Animal Feed Rules, 2011”.

The constitution of Bangladesh (GB, 1972) gives more importance to food safety. Article 15 states “it shall be a fundamental responsibility of the state to secure the provision of the basic necessities of life including food” and article 18 states “the state shall regard the raising of the level of nutrition and the improvement of public health as moving its primary duties”. Both articles indicate that the state must be ensured food safety in all levels of the state through legislation of appropriate laws.

2.2 Antibiotics, feed and chemicals

Aftab Uddin et al. (2006) found that various types of chemicals were used in Bangladesh shrimp hatcheries to control the pathogens and increase the feeding efficiency so that mortality rate could reduce. Chloramphenicol, erythromycin, prefuran and oxytetracycline were used by 40, 25, 20 and 15% of the surveyed hatcheries in the broodstock tanks to prevent bacterial infections after eye stalk ablation whereas 31, 25, 12, 13 and 19% hatcheries used chloramphenicol, erythromycin, malachite green, furazolidone and neomycin sulphate in larval rearing tank for prophylactic and metaphylactic treatment.

EU (2007) reported that 90% shrimp farm of Bangladesh follows extensive culture technique where shrimps are not fed with formulated feed at all, but most of the farmers apply home-made mixture of rice bran and wheat bran where as in the rest 10% farm shrimps are feed with formulated feed during culture period.

FAO (2005) reported that chloramphenicol is a broad-spectrum antibiotic that used for treatment of serious infections in human body. But, it has not been possible to identify the safe dose of it due to the unpredictable effects of doses on different patients. That’s why USA has prohibited its use in food-producing animals and animal food products.

Frappaolo et al. (1986) reported that antibiotics used in animal feeds to control bacteria population and maintain healthy environment. As a result, the intestinal bacteria of the animals have become resistant to antibacterial medicines and usually the resistance can be transferred to pathogenic bacteria by R-factors. It causes reducing the efficiency of antibiotic treatment for diseases caused by the resistant pathogens.

GEASAMP (1997) mentioned that chloramphenicol and nitrofuran prohibited in USA and Japan and explicitly banned in Canada and European Union countries for its carcinogenic characteristics. However, these are used in shrimp culture of Latin America and Asia where shrimps are grown for export to USA, EU and Japan. GEASAMP also mentioned that chemicals applied in aquaculture farms in order to control the diseases. So, chemicals should be sold in the market with proper labelling-withdrawal period with reference to species and temperature, potential hazards to environment and human health (including treatment in case of accidental ingestion or contact), storage requirements and expiry date and disposal methods for unused products.

K. Holmstrom et al. (2003) found that about 74% shrimp farmers used antibiotics in the management of their ponds along the Thai coast. They used the antibiotics namely tetracyclines, quinolones, sulphonamides, chloramphenicol, gentamycin, trimethoprim, tiamulin and some unidentified antibiotics for preventive management and daily purpose. The authors assumed that the use of these antibiotics could develop antibiotic resistance amid the pathogens that caused infections of the cultured animals and humans.

K. Hoenicke et al. (2004) found that SEM can occur naturally, e.g. in algae, shrimps and eggs and it also can be formed from natural substances, e.g. arginine and creatine. They also found SEM in samples treated with hypochlorite or bleaching commonly used in food processing for disinfection. SEM was found in samples at the concentration of 0.3-20 μg/kg after treatment with 1% active chlorine.

Lyle-Fritch et al. (2006) documented the use and application rates of chemicals and biological products for shrimp farming in Sinaloa, Mexico. About 106 types of chemicals and biological products were identified and on an average 41.7 products were applied in each farm. The most commonly used products were soil and water treatment compounds, fertilizers, antibiotics, feed additives and disinfectants etc. The authors also mentioned that shrimp farms of Sinaloa utilized a higher number of chemicals and biological products with compare to that of Philippines and Thailand

McCracken et al. (2011) found trace amount of SEM in meat of wild-caught prawn (*Macrobrachium rosenbergii*) in Bangladesh where as high concentration of SEM (up to 100× higher) was detected in shell. So, the authors commented that SEM might be produced in shrimps naturally.

Nowsad et al. (2009) investigated the source of nitrofuran in shrimp and prawn through questionnaire survey. The authors found that most of the hatchery operators and prawn farmers in Mymensingh district would not know about the fatal effects of nitrofuran where as those people of Cox's Bazar and Khulna would know about that of nitrofuran, but, they kept mum on this issue. In contrast, various types of antibiotics that sold in local market were found to be used in shrimp hatcheries. So, the authors concluded that both the shrimp/prawn hatchery and poultry/fish feed used in culture ponds might be the possible sources of nitrofuran in exportable shrimp/prawn products of Bangladesh.

Paul et al. (2011) found that various types of drugs were used in different prawn (*Macrobrachium rosenbergii*) farms of north and south 24 Parganas districts of West Bengal, India namely chloramphenicol, nitrofurans, oxytetracycline and formalin etc. Most of the farms used chloramphenicol (40%) followed by oxytetracycline (23%), erythromycin (20%) and nitrifurans (10%).

Pfenning et al. (2003) reported that presence of chloramphenicol in animals is a burning question globally. Due to its fatal side effects, such as bone marrow depression, aplastic anaemia and other blood disorders, use of chloramphanicol was banned in food producing animals. But, it is finding in several foodstuffs including sea foods from Asia until now.

Raffi et al. (2011) found chloramphenicol in farmed shrimp ranged from 0.02 to 0.3μg/kg and in wild shrimp ranged from 0.01 to 0.04μg/kg shrimp meat. The author assumed that it might be come from the released shrimps which were disease affected from the culture pond into the natural water body.

S. Graslund et al. (2003) documented the use of chemicals and biological products that were used in shrimp farming of Thailand. Farmers used on an average 13 types of chemicals and biological products namely soil and water treatment compounds, fertilizers, pesticides and disinfectants, antibiotics, microorganism products, immunostimulants, vitamins, feed additives and uncertain groups etc. The authors also mentioned that these products could have negative effects on the cultured shrimps, cause a risk of food safety, human and adjacent ecosystem.

Chapter 3

Materials and Methods

3.1 Study sites

The present study was carried out in Satkhira, Khulna and Bagerhat districts, the south-western coast of Bangladesh (Figure 3.1). The number and area of shrimp farm were 59,163; 21,601; 34,579 and 66,735; 36,402.82; 46,724 ha whereas that of prawn farm were 11,522; 29,574; 39,570 and 9,317; 13,027.88; 18,468 ha in Satkhira, Khulna and Bagerhat district respectively. Producing shrimp and prawn these districts play a vital role in the economy of Bangladesh. About 70% of country's shrimp and 80% of total prawn produced in these three districts, which accounted for 70% of total shrimp and prawn export in 2009-2010 (DoF 2011).

3.2 Antibiotic residue measurement data

Data on the presence of antibiotics in shrimp and prawn muscle and feed were collected from the Upazila (subdistrict) Fisheries Offices of Satkhira, Khulna and Bagerhat districts, DoF under the Ministry of Fisheries and Livestock, Bangladesh. Actually shrimp and prawn muscle and feed samples tested in FIQC Lab that follows the method of US FDA/CFSAN (2004) and Young (2003) (DoF 2009). The reporting limit $CC\alpha$ (μg/kg) of FIQC Lab was 0.13, 0.15, 0.12, 0.23 and 0.08 μg/kg for AHD, AMOZ, AOZ, SEM and CAP respectively. The followed procedure described below:

3.2.1 Sample treatment

One kilogram sample generally collected in polyethylene bag and kept in refrigerator. Then the sample brought to the chemical laboratory and kept in air to normalize the temperature. After few minutes, it was washed with tap water and head and shell removed from the body. After air drying, 200g samples pasted using a blender (EQSOP 017) and from the paste 3.0 g taken for extraction procedure.

3.2.2 Extraction procedure

Added 6 ml ethyl acetate to 3.0 g tissue and homogenised for 1 minute (ESOP 001). Then centrifuged at 2000 rpm for 15 minutes (ESOP 004) and removed 4 ml of upper phase reduced to dryness at 70˚C under N_2 atmosphere (ESOP 019). After that, it`s dissolved in 2 ml of isooctane/chloroform (2:3), vortex for 1 minute and added 0.5 ml of diluted Tissue Extraction Buffer and vortex for 2 minutes. Then centrifuged at 2000 rpm for 15 minutes and upper phase was ready for application.

3.2.3 Test procedure for CAP

- Pipette 25 µl standards from lower to higher concentration into the wells of the microtitre plate supplied for SEM.
- Pipette 25 µl QC sample.
- Pipette 25 µl sample.
- Added 100 µl conjugate to each plate.
- Covered the microtitre plate with adhesive film.
- Gently tapped the microtitre plate from side to side for a few seconds before incubating for 1 hour at room temperature (+19 to 25˚C) in the dark.
- Inverted plate and tapped out liquid.
- Washed the plate 6 times with diluted wash buffer over a 10-15 minutes..
- Added 125 µl of one shot substrate solution into each well.
- Incubated for 20 ± 2 minutes at room temperature (+19 to 25˚C) and after that, added 100 µl of Stop Buffer to stop the enzyme reaction.
- Measured the optical density of each well at 450nm and 630 nm wavelength within 10 minutes of stopping the colour reaction.

3.2.3.1 Measurement and calculation

- Calculate the mean absorbance of the standards, controls and samples.

- Standard curve drawn plotting the mean relative absorbance (%) that obtained from each reference standard against its concentration in ng/ml on a logarithmic curve.
- Relative absorbance (%) = absorbance standard (or sample) × 100/absorbance zero standard
- Read control and sample absorbance from the standard curve.
- To convert results for muscle samples from ng/ml to ng/g it`s multiplied by 0.25.

3.2.4 Test procedure for SEM

- Pipette 25 µl standards from lower to higher concentration into the wells of the microtitre plate supplied for SEM.
- Pipette 100 µl QC sample.
- Pipette 100 µl sample.
- Added 50 µl SEM-HRP conjugate and mixed well by gently for 1 minute.
- Incubated the plate for 30 minutes at room temperature (20-25˚C/68-77˚F).
- Washed the plate 3 times with 250 µl of 1X wash solution.
- Added 100 µl of TMB substrate and mixed the solution for 1 minute.
- Incubated for 20 minutes at room temperature (20-25˚C/68-77˚F) and after that, added 100 µl of Stop Buffer to stop the enzyme reaction.
- Read the plate with 450nm wavelength immediately.

3.2.4.1 Measurement and calculation

- Calculate the mean absorbance of the standards, controls and samples.
- Standard curve drawn plotting the mean relative absorbance (%) that obtained from each reference standard against its concentration in ng/ml on a logarithmic curve.
- Relative absorbance (%) = absorbance standard (or sample) × 100/absorbance zero standard
- Read control and sample absorbance from the standard curve.
- To convert results for muscle samples from ng/ml to ng/g it`s multiplied by 2.

In the same way, AHD, AOZ and AMOZ were tested.

3.3 Questionnaire interview

To conduct the questionnaire survey, at first it was collected the list of contaminated and non-contaminated shrimp and prawn farms from the Upazila Fisheries Offices of Satkhira, Khulna and Bagerhat district and then the farms were selected randomly. A total of 83 shrimp and prawn farms (49 contaminated and 34 non-contaminated) of which 29 (14 contaminated and 15 non-contaminated) at satkhira shader, debhata, kaliganj, shymnagar, ashasuni, tala and kolarowa upazila of Satkhira, 25 (19 contaminated and 6 non-contaminated) at Khulna shader, batiaghata, terokhada, paikgacha, digholia, rupsha, dumuria and fultala of Khulna and 29 (16 contaminated and 13 non-contaminated) at bagerhat shader, rampal, mongla, fakirhat, chitalmari and kachua of Bagerhat district, from those farms samples were taken for antibiotics together with chemical tests by the Fish Inspection and Quality Control (FIQC) wing of DoF, 5 feed factories (1,2 and 2 factories in Satkhira, Khulna and Bagerhat district respectively) and 9 hatcheries (7, 1 and 1 hatchery in Satkhira, Khulna and Bagerhat district respectively) were interviewed (Figure 3.2 and Figure 3.3) through personal contact with the farmers, feed factory representatives and hatchery technicians from 03 May, 2014 to 12 May, 2014 and also from 11 September, 2014 to 03 October, 2014. On the other hand, it was investigated 10 VMD/AMD (Veterinary Medicine/Aquaculture Medicine Shop) shops (5, 2 and 3 shops in Satkhira, Khulna and Bagerhat district respectively) to justify the name, composition and company name of chemicals that were mentioned by the farmers and hatchery and feed factories representatives. The interviews were conducted with pre-tested questionnaires developed for the study.

3.4 Sample collection

The farmers mentioned a river and 11 hatchery names from where they purchased shrimp and prawn PL (Post Larvae) during questionnaire survey. So, 12 PL samples (05 prawn and 07 shrimp) of which 01 natural prawn PL on 28 April, 2015 and 01 hatchery prawn PL on 20 September, 2014 from kulia bazar, debhata, Satkhira (22˚ 38′ 36.30″ N and 88˚ 59′ 22.43″ E); 01 hatchery prawn and 04 hatchery shrimp PL from karim super market, Satkhira (22˚ 42′ 38.78″ N and 89˚ 4′ 12.58″ E) on 20 September, 2014; 01 hatchery prawn on 12 September, 2014 and 02 hatchery shrimp PL on 27 April, 2015 from chuknagar bazar, dumuria, Khulna (22˚ 50′ 24″ N and 89˚ 17′ 42″ E); and 01 hatchery prawn and 01 hatchery shrimp PL from foyla bazar, rampal, Bagerhat (22˚ 38′ 3.24″ N and 89˚ 38′ 49.74″ E) on 26 April, 2015 were collected, labelled and stored in refrigerator. One polybag was purchased for one prawn PL sample that contained 4000 PL whereas that of one shrimp PL sample contained 5500 PL. On the other hand, on the basis of information mentioned by the farmers 02 shrimp/prawn shell from Khanjahan Ali bridge market (22˚ 46′ 49″ N and 89˚ 34′ 51″ E) and 02 unknown chemicals samples from sunadanga bus stand market (22˚ 48′ 50.53″ N and 89˚ 32′ 34.18″ E) on 13 September, 2014 (Figure 3.5) were also collected and labelled. The weight of each shell and chemical sample was 1kg and 500gm respectively. All the samples were sent to the Institute of Food Science & Technology (IFST) Lab, Dhaka, Bangladesh that followed the method LC-MS-MS Q-TRAP for the analysis of nitrofuran and chloramphenicol. The reporting limit CCα (μg/kg) of IFST Lab was 0.08, 0.03, 0.04, 0.10 and 0.03 μg/kg for AHD, AMOZ, AOZ, SEM and CAP respectively. The sampling stations shown in Figure 3.4.

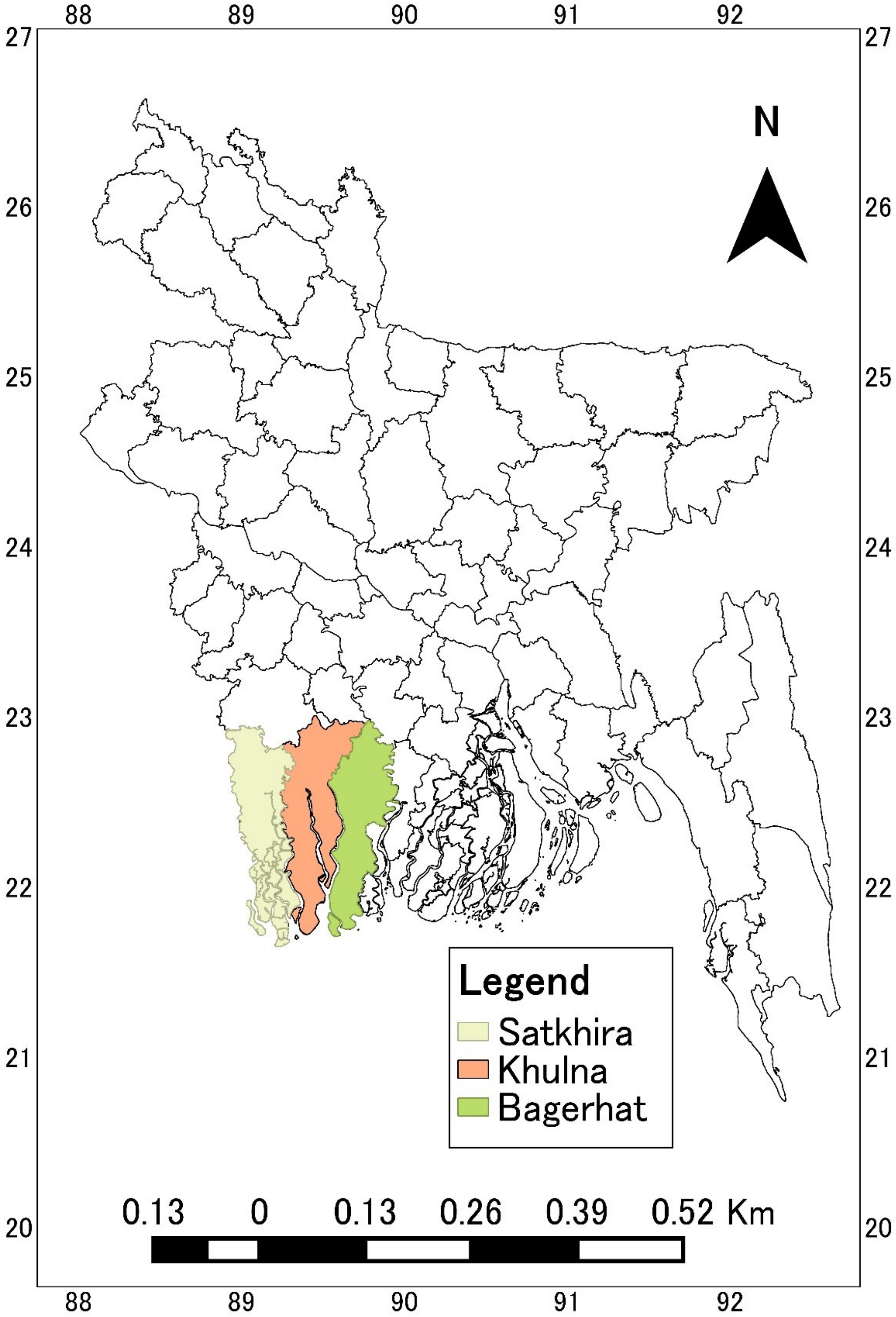


Figure 3.1 Study area of Bangladesh

Prawn farm, Dumuria, Khulna

Shrimp farm, Mongla, Bagerhat

Figure 3.2 Questionnaire survey at shrimp and prawn farm

Hatchery, Shymnagar, Satkhira

Feed mill, Satkhira

Figure 3.3 Questionnaire survey at hatchery and feed mill

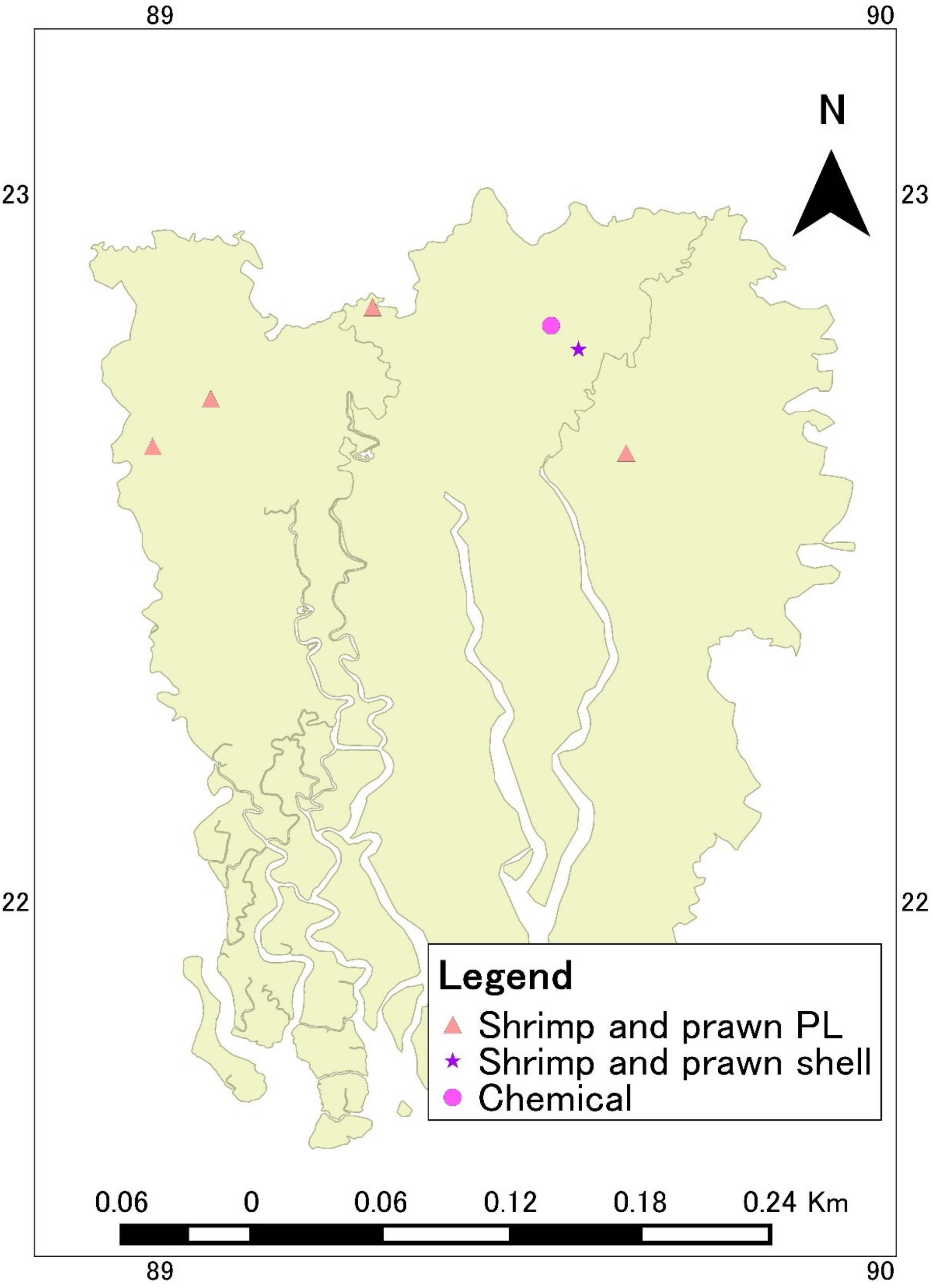
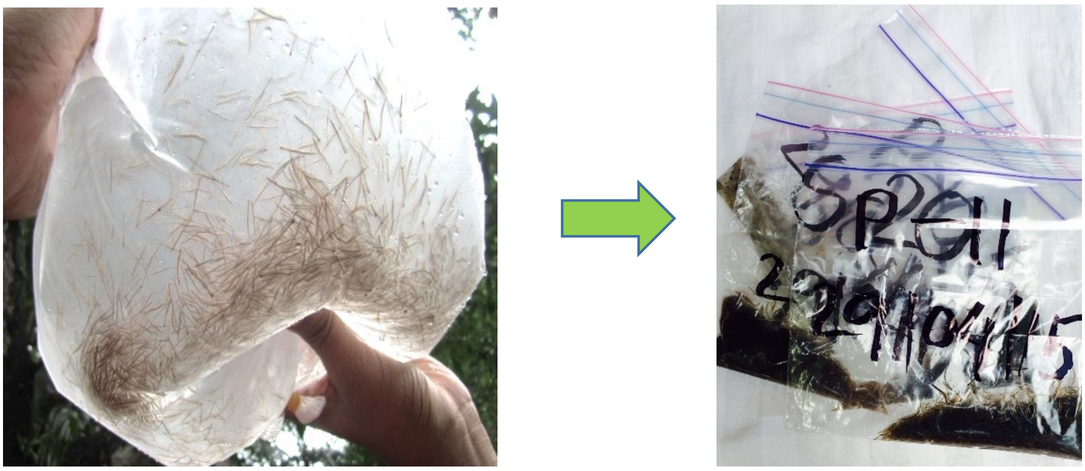


Figure 3.4 Sampling point



Shrimp and prawn PL (Post Larvae) sample

Chemical-1

Chemical-2

Figure 3.5 Collected samples

3.5 Statistical analysis

3.5.1 Analysis of variance

The data were statistically analysed in MS Excel 2013. Single factor analysis of variance was used to compare the values of nitrofuran and chloramphenicol tested data. In that case, least significant difference at $P < 0.05$ was considered to measure the significant differences among the values.

3.5.2 Correlation

Karl Pearson correlation co-efficient equation was used to evaluate the inter-element relationship among depth of pond water (m), canal water, river water, ground water, neighbour pond water (Figure 3.6), water Exchange, fertilizer, cowdung and contamination. The equation is as follows:

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[\{n\Sigma x^2 - (\Sigma x)^2\}\{n\Sigma y^2 - (\Sigma y)^2\}]}} \qquad (i)$$

(Bobko, 2001)

In this case, all parameters except water depth were inserted in MS Excel 2013 as 1 and 0 when Yes=1 (use) and No=0 (no use).

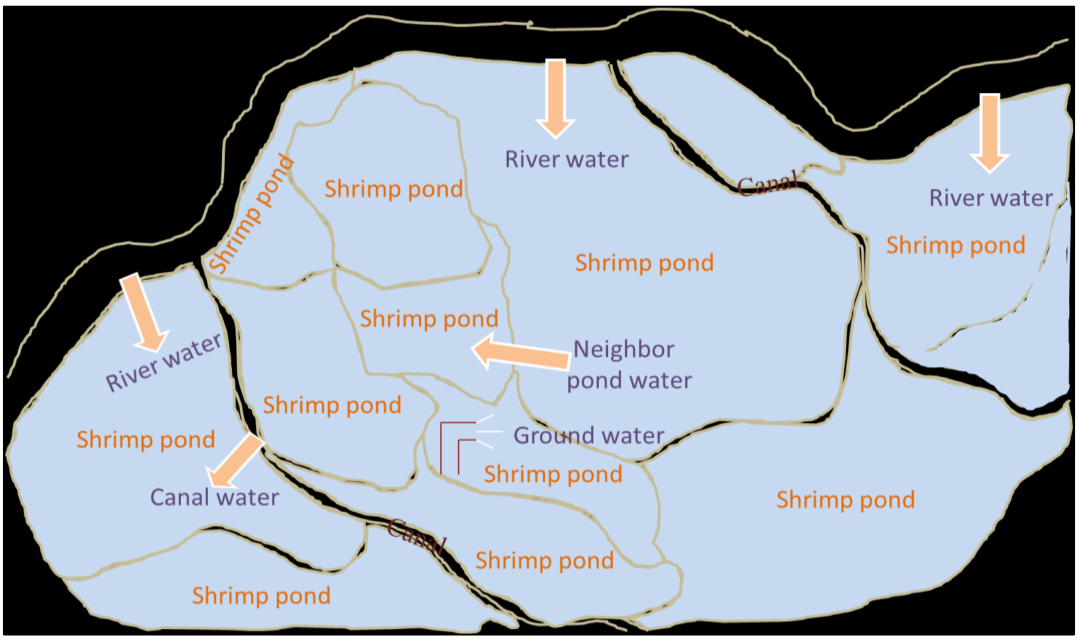


Figure 3.6 Sources of water for shrimp/prawn farming

3.5.3 Regression analysis

Multiple linear regression model was executed to measure nexus between shrimp with carp production and production oriented factors. On the other hand, the same model was used in prawn with carp production for same purpose. The model equation is as follows:

$$Y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \beta_4 x_{4i} + \beta_5 x_{5i} + \beta_6 x_{6i} + \beta_7 x_{7i} + \varepsilon_i \qquad (ii)$$ (Amin et al. 2015)

Where,

i = 1, 2; 1 means shrimp with carp and 2 means prawn with carp

Y = Production (kg/ha)

β_0 = Intercept (Constant)

X_1 = Stocking density (Thousand/ha) (Stocking density-the number of PL that released in a unit area)

X_2 = Farm area (ha)

X_3 = Feed (kg/ha)

X_4 = Depth of pond water (m)

X_5 = Fertilizer (kg/ha)

X_6 = Lime (kg/ha)

X_7 = Number of used chemicals

Ɛ = Error term

3.5.4 Normal distribution

The following equation was also used to draw normal distribution graph of body weight.

$$z = \frac{x - \mu}{\sigma} \qquad (iii)$$ (http://onlinestatbook.com/)

Where,

z = Normal standard deviate

μ = Mean

σ = Standard deviation

3.6 Health risk assessment

The widely used health risk assessment model that developed by NRC (National Research Council) and NAS (National Academy of Sciences), USA was usually used to estimate the risk caused by contaminant. A full-fledged risk assessment model consists of four steps, namely hazard identification, exposure assessment, dose response assessment and risk characterization (Lee et al., 2006).

3.6.1 Hazard identification

Nitrofuran metabolites (SEM, AOZ, AMOZ and AHD) and chloramphenicol antibiotic drugs were detected in shrimp and prawn muscle of Bangladesh. These antibiotics have carcinogenic and non-carcinogenic effect on human body (Vass et al., 2008; Hanekamp et al., 2003; Van Koten-Vermeulen, 1993; GEASAMP 1997; Pfenning et al. 2003 and EU 1995). But, until now it's not possible to assess the CAP carcinogenicity due to lack of scientific information though CAP is treated as carcinogenic by IARC (International Agency for Research on Cancer) in human (Hanekamp et al., 2003).

3.6.2 Exposure assessment

The exposure assessment defines the pathways through which the contaminant enter into the human body (Lee et al., 2006). Actually, nitrofuran and chloramphenicol drugs are entering into the people`s body of Bangladesh through consumption of shrimp and prawn.

3.6.3 Dose response assessment

The EDI (Estimated Daily Intake) can be calculated quantitatively by the following numerical formula:

$$\text{EDI} = \frac{C \times Fi \times Ef \times Ed}{W \times Te} \qquad (iv)$$ (Bhatti et al., 2013)

Where,

EDI = Estimated Daily Intake (mg/kg/day)

C = Concentration of contaminant in shrimp/prawn muscle (mg/g)

Fi = Fish intake (gm/person/day)

Ef = Exposure frequency (days/year)

Ed = Exposure duration

W = Average body weight

Te = Average exposure time (Ed × 365 days)

Exposure duration is 70.7 years that is life expectancy of Bangladesh (UNDP 2014) whereas Fi × Ef = Fish intake (gm/person/year) = Total production – Total export/ Total population. In this case, total population-149.77million (Population Census, 2011), total shrimp and prawn production 57784.87MT and 45162.95MT respectively whereas total export 35677.78MT and 7059.71MT respectively (Fisheries Statistical Year Book, 2012 DoF, Bangladesh) were considered. It's noted that body weight data were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRB 2012).

3.6.4 Risk characterization

Risk characterization is quantitatively presented by carcinogenic and non-carcinogenic risk (Lee et al., 2006). Carcinogenic risk (CR) can be estimated by the following formula.

$$CR = EDI \times CSF \times ADAF \qquad (v)$$ (Bhatti et al., 2013; USEPA 2005)

Where,

CR = Cancer Risk

CSF = Cancer Slope Factor $(mg/kg\text{-}day)^{-1}$

ADAF = Age Dependent Adjustment Factor

In this calculation, 1.5E+00 that was considered as cancer slope factor of SEM noted in Integrated Risk Information System (IRIS) database (USEPA 2004) whereas 10 for 0 -< 2, 3 for 2 -< 16 and 1 for >16 years old were adopted as ADAF (USEPA 2005).

To assess the non-carcinogenic risk, the following equation was used:

$$HQ = \frac{EDI}{RfD} \qquad (vi)$$ (Bhatti et al., 2013)

Where,

HQ = Hazard Quotient

RfD = Reference Dose

In this assessment, 7.0E-02 that was considered as reference dose of AHD noted in Integrated Risk Information System (IRIS) database (USEPA 2004). If HQ value is greater than one (01), it defines a non-carcinogenic toxic risk to human health.

Chapter 4

Results

4.1 Result of antibiotic tested data

4.1.1 Antibiotics detected shrimp and prawn pond

Nitrofuran and chloramphenicol were detected in 24 prawn samples (24%) out of 100 samples and 11 shrimp samples (9.6%) out of 115 samples in 2011 (DoF, 2014). Among the detected samples, 6, 12, 4 and 2 prawn samples were SEM and CAP, only SEM, only CAP and AMOZ detected respectively while 10 and 03 shrimp samples were SEM and C AP detected (Figure 4.1).

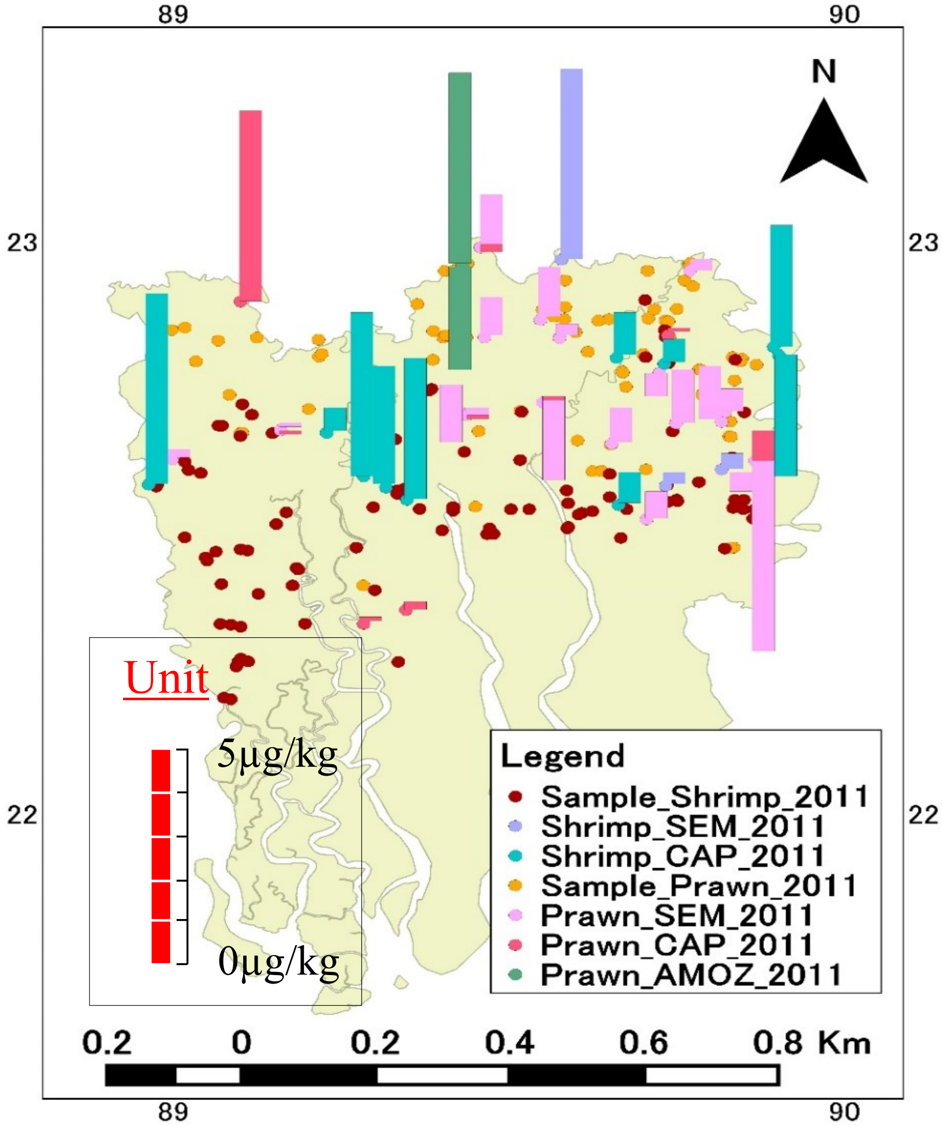


Figure 4.1 Nitrofuran and chloramphenicol detected shrimp and prawn ponds in 2011

In 2012, nitrofuran and chloramphenicol drugs were found in 13 prawn samples (10.4%) out of 114 samples and in 7 shrimp samples (5.4%) out of 130 samples (DoF,2014). Among the detected samples, 21 and 3 prawn samples were SEM and CAP detected respectively whereas 3 shrimp sa mples were CAP and another 3 shrimp samples were also AHD detected (Figure 4.2).

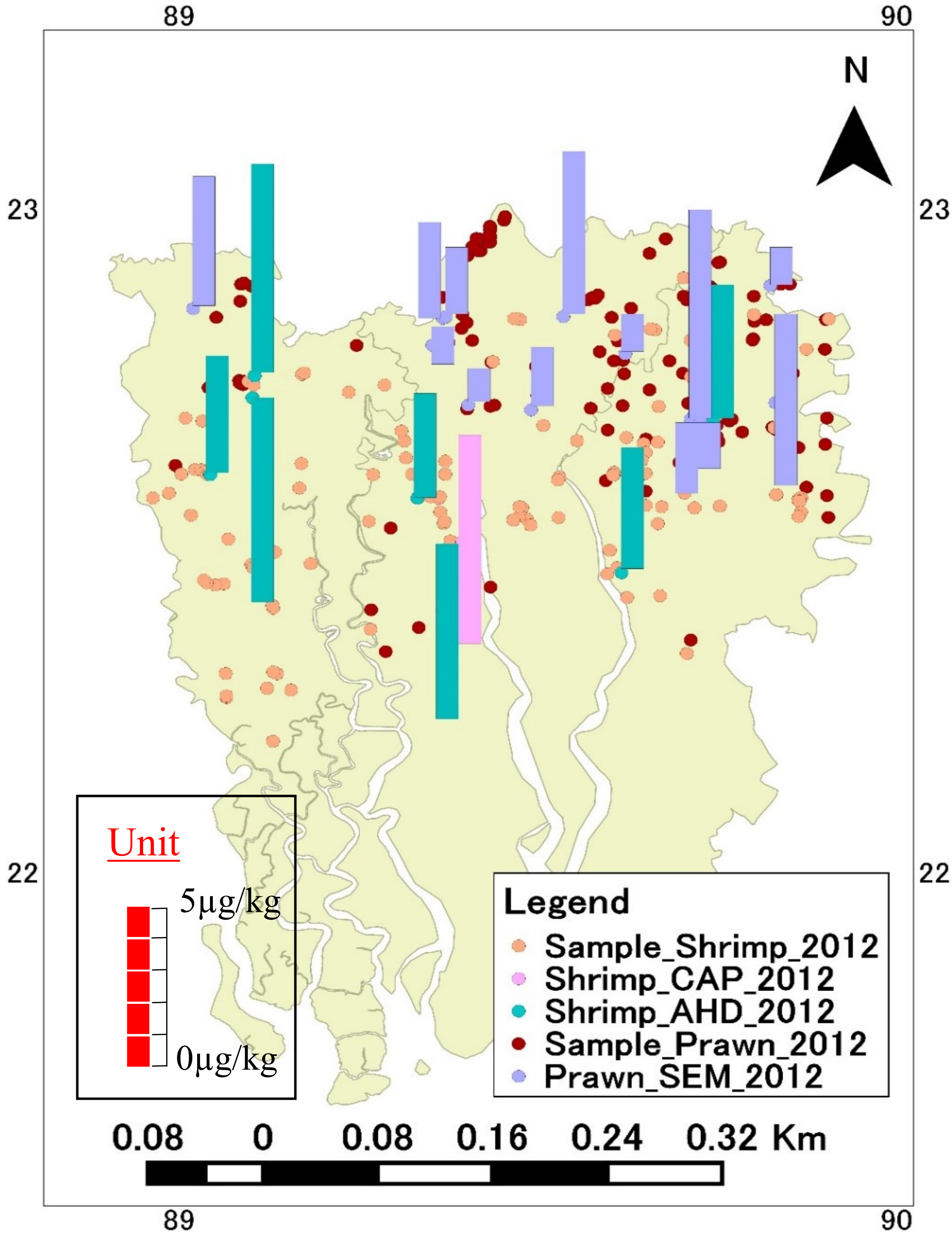


Figure 4.2 Nitrofuran and chloramphenicol detected shrimp and prawn ponds in 2012

In 2013, nitrofuran and chloramphenicol drugs were found in 24 prawn samples (21.1%) out of 114 samples and in 6 shrimp samples (5%) out of 132 samples (DoF,2014). Among the detected samples, 21 and 3 prawn samples were SEM and CAP detected respectively whereas 3 shrimp samples were CAP and another 3 shrimp samples were also AHD detected (Figure 4.3).

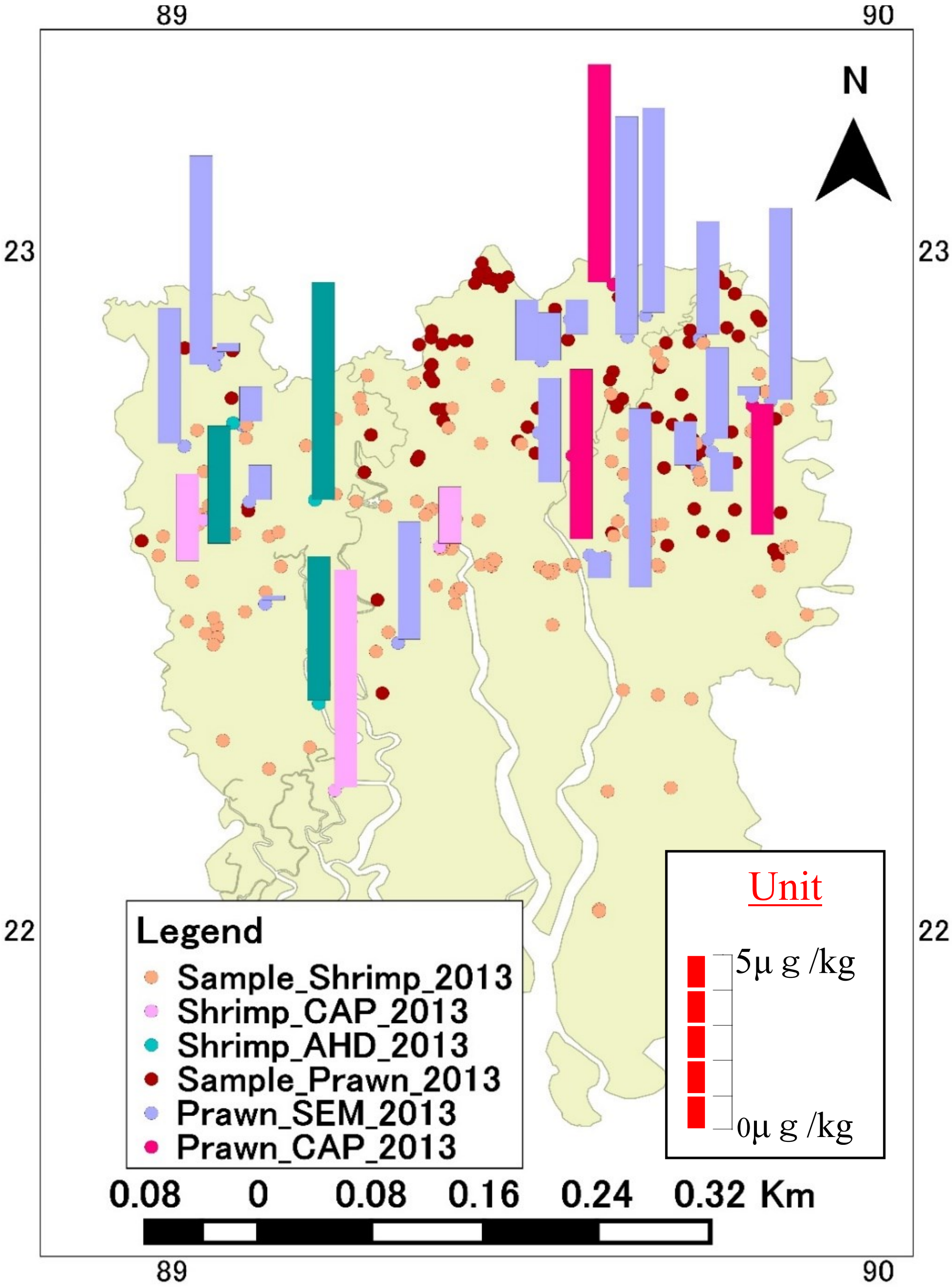


Figure 4.3 Nitrofuran and chloramphenicol detected shrimp and prawn ponds in 2013

Taking into account all the nitrofuran and chloramphenicol detected shrimp and prawn samples of 2011, 2012 and 2013, the average concentration of SEM, AMOZ and CAP was 2.44, 2.47 and 6.12μg/kg respectively ($P < 0.05$) in prawn (Figure 4.4) whereas that of SEM, AHD and CAP was 1.73, 0.42 and 1.27μg/kg respectively ($P < 0.05$) in shrimp (Figure 4.5) and their frequency distribution shown in Figure 4.6 and Figure 4.7 respectively. The Figure 4.6 and 4.7 indicate that SEM was found more in prawn whereas AHD and CAP were found more in shrimp. The Maximum Residual Limit (MRL) of nitrofuran metabolites was 1μg/kg while that of chloramphenicol (CAP) was 0.3μg/kg established by EU.

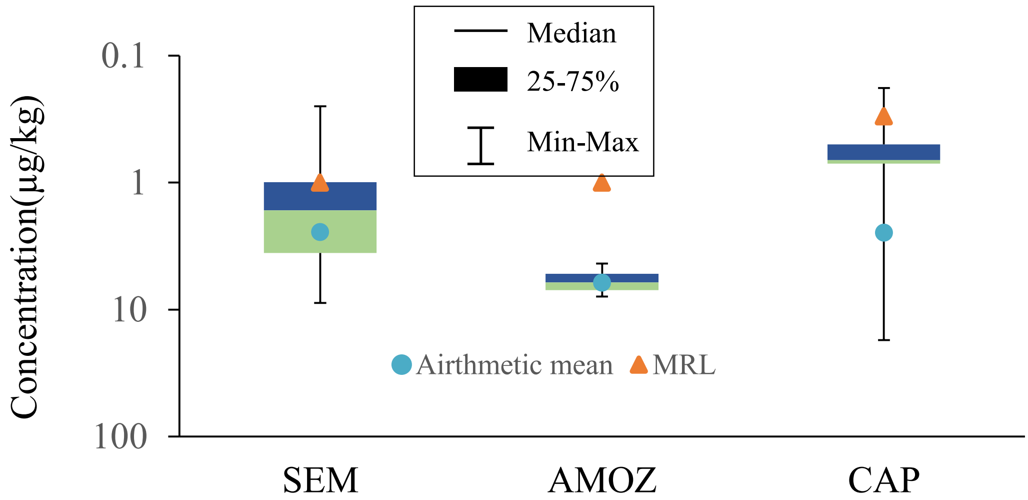


Figure 4.4 Concentration of SEM, AMOZ and CAP in prawn

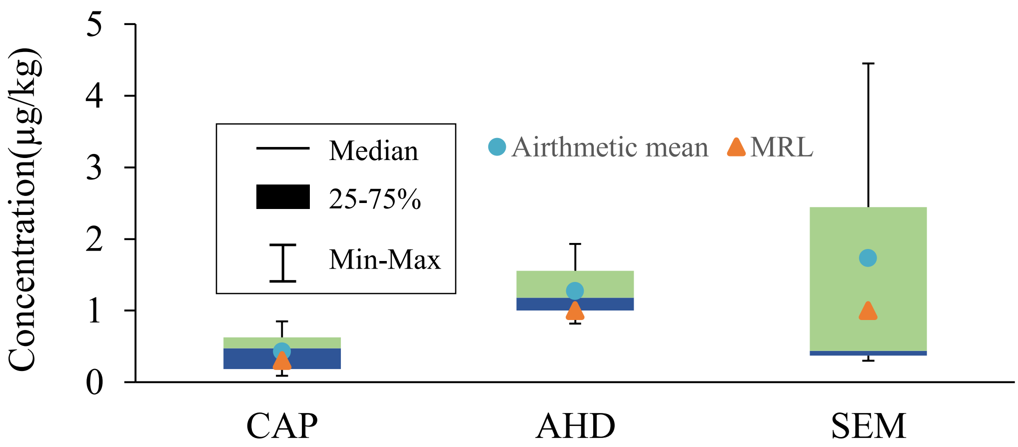
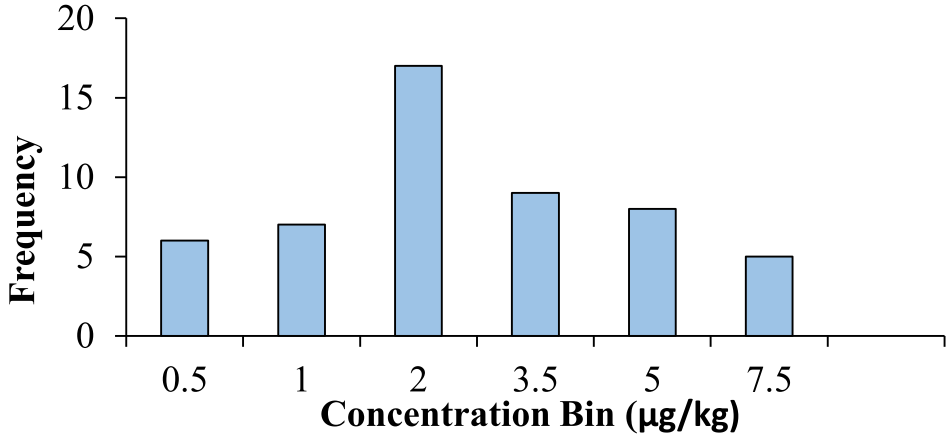
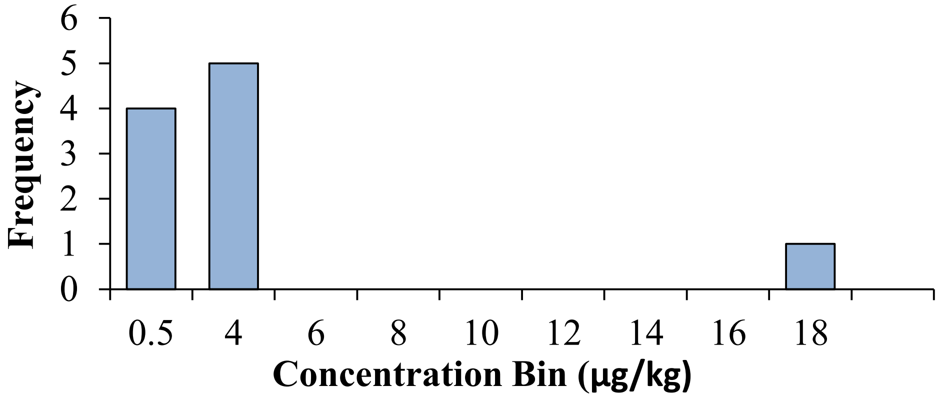


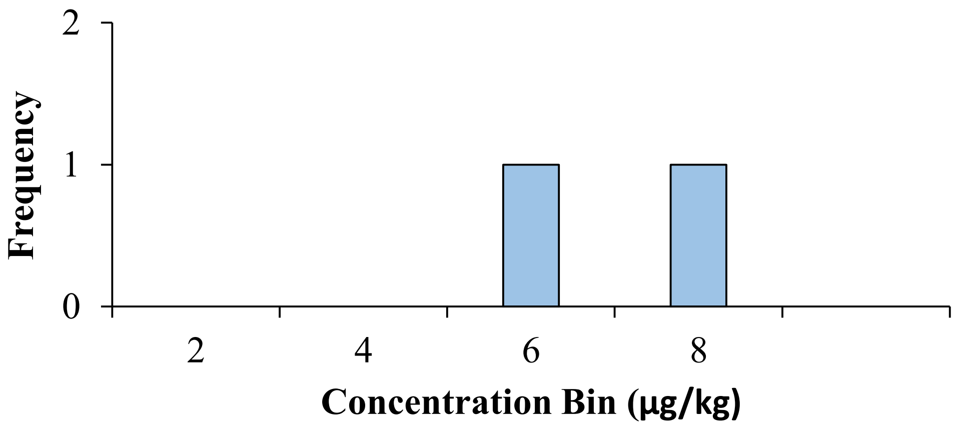
Figure 4.5 Concentration of CAP, AHD and SEM in shrimp



(a)

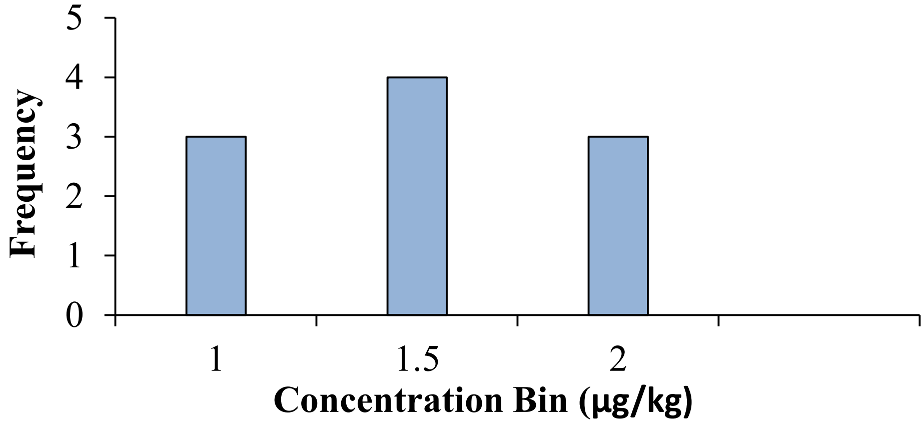


(b)

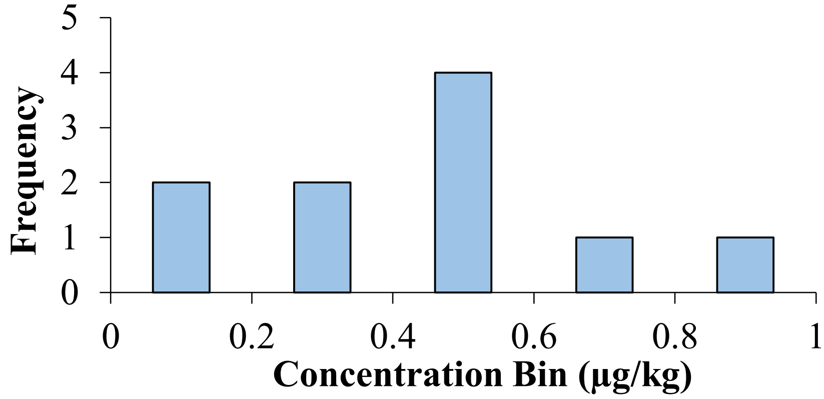


(c)

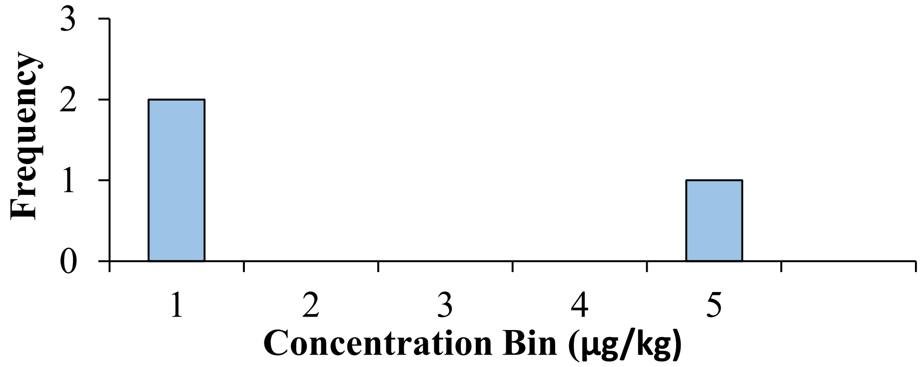
Figure 4.6 Frequency distribution of (a) SEM, (b) CAP and (c) AMOZ detected prawn samples



(a)



(b)



(c)

Figure 4.7 Frequency distribution of (a) AHD, (b) CAP and (c) SEM detected shrimp samples

4.1.2 Farm contamination rate

Nitrofuran and chloramphenicol drugs were detected in 10 prawn samples (20.4%) out of 49 samples, 27 prawn samples (18.5%) out of 146 samples and 24 prawn samples (16.7%) out of 144 samples in Satkhira, Khulna and Bagerhat district repectively (Figure 4.8).

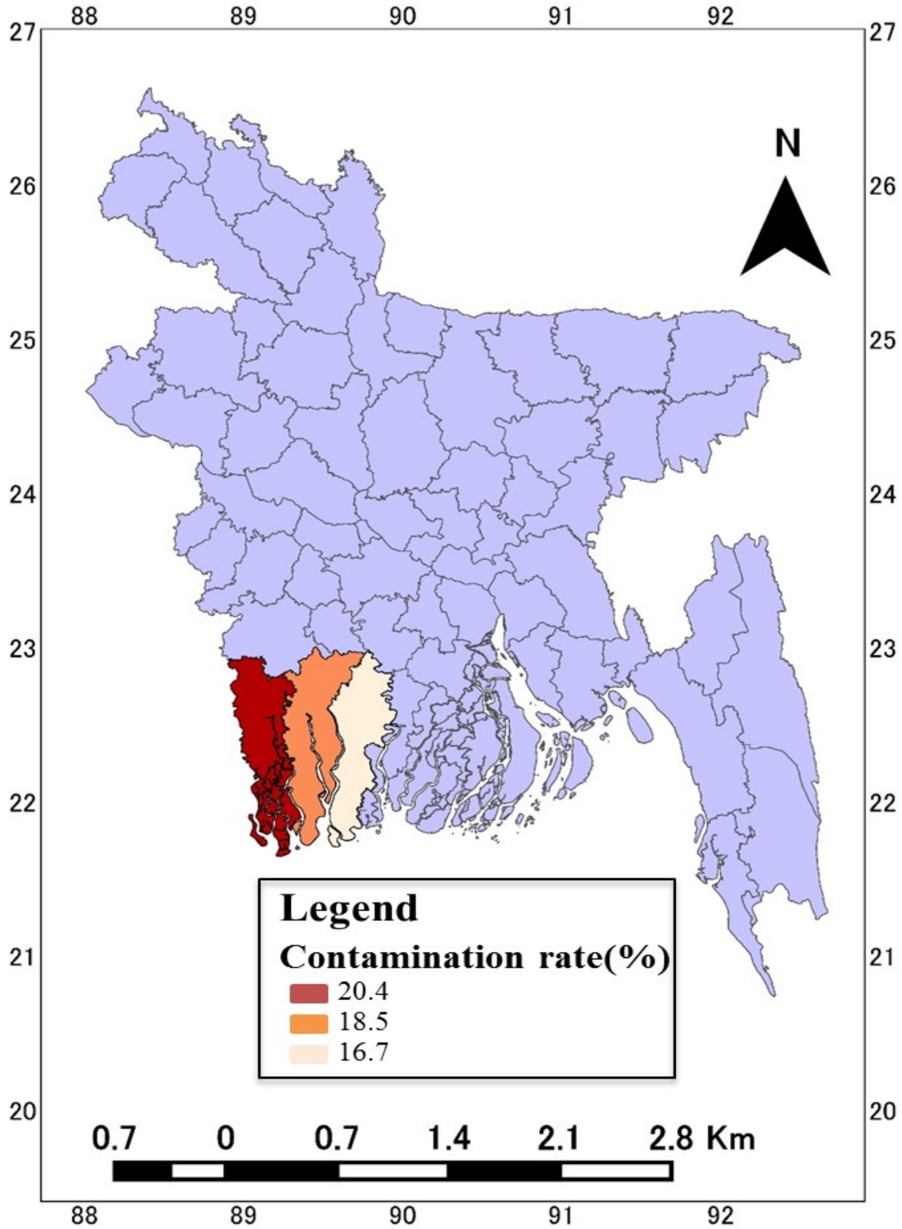


Figure 4.8 Prawn farm contamination rate in Satkhira, Khulna and Bagerhat district

Nitrofuran and chloramphenicol antibiotics were found in 10 shrimp samples (7.8%) out of 128 samples, 6 shrimp samples (4.8%) out of 126 samples and 8 shrimp samples (6.5%) out of 123 samples in Satkhira, Khulna and Bagerhat district respectively (Figure 4.9).

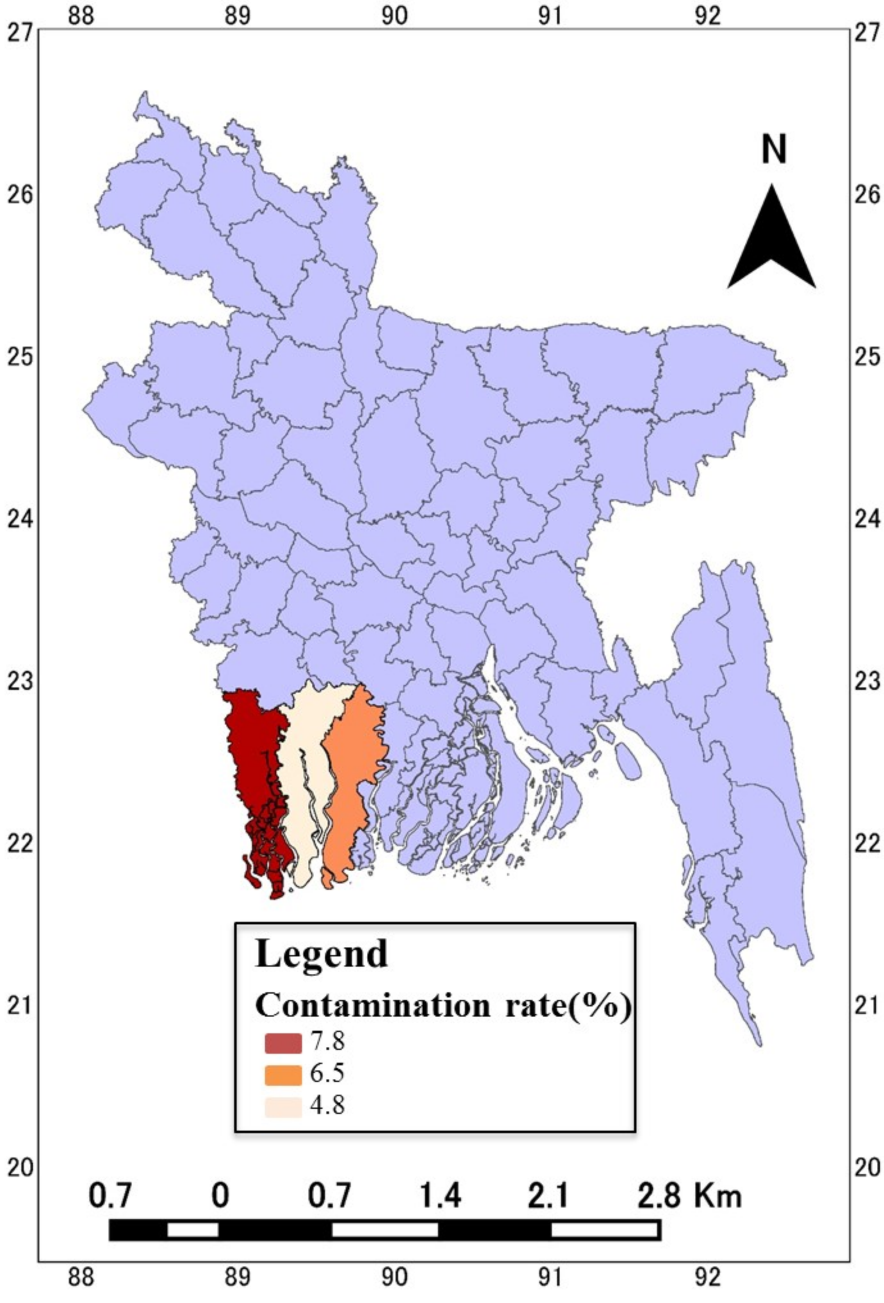


Figure 4.9 Shrimp farm contamination rate in Satkhira, Khulna and Bagerhat district

4.1.3 Concentration of antibiotics in feed for shrimp and prawn

In 2011, 50 feed samples of different companies were tested. Tetracycline, oxytetracycline and chlortetracycline antibiotics were detected in 17 samples in which 15 samples were above the Maximum Residual Limit (MRL) in terms of antibiotic concentration. Among the detected samples, 5, 6, 1 and 5 samples were tetracycline, tetracycline and oxytetracycline, tetracycline and chlortetracycline, and tetracycline, oxytetracycline, chlortetracycline detected samples respectively. The MRL of tetracycline, oxytetracycline and chlortetracycline was 100µg/kg established by EU. The average concentration of tetracycline, oxytetracycline and chlortetracycline was 652.28, 90.78 and 203.31µg/kg respectively (Figure 4.10).

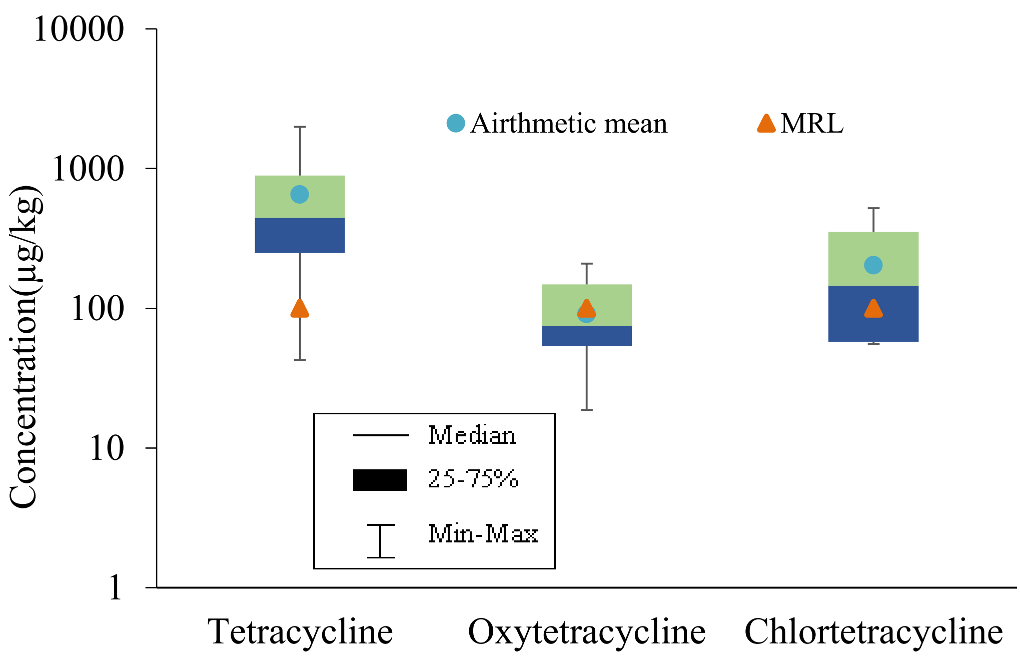


Figure 4.10 Concentration of tetracycline, oxytetracycline and chlortetracycline in feed for shrimp and prawn in 2011

Tetracycline, oxytetracycline and chlortetracycline were detected in 11 samples out of 50 samples in 2012 in which 1 sample was above the MRL. Among the detected samples, 1, 7, 2 and 1 samples were tetracycline, oxytetracycline, tetracycline and chlortetracycline and tetracycline and oxytetracycline detected samples respectively. The average concentration of tetracycline, oxytetracycline and chlortetracycline was 11.28, 30.48 and 8.5µg/kg respectively (Figure 4.11).

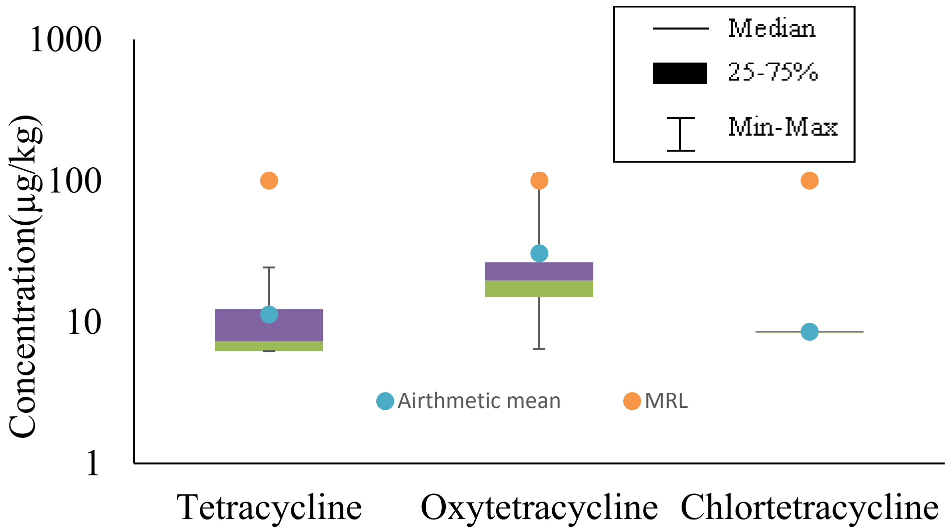


Figure 4.11 Concentration of tetracycline, oxytetracycline and chlortetracycline in feed for shrimp and prawn in 2012

In 2013, 30 feed samples of different companies were tested. Tetracycline, oxytetracycline and chlortetracycline antibiotics were detected in 10 samples and AHD was found in 2 samples. Among the detected samples, 5, 1, 1 and 3 samples were oxytetracycline, tetracycline and oxytetracycline, tetracycline, oxytetracycline and chlortetracycline and tetracycline and chlortetracycline detected samples respectively. The average concentration of tetracycline, oxytetracycline and chlortetracycline was 287.91, 135.58 and 81.21µg/kg respectively (Figure 4.12) whereas that of AHD was 18.65 (Figure 4.13).

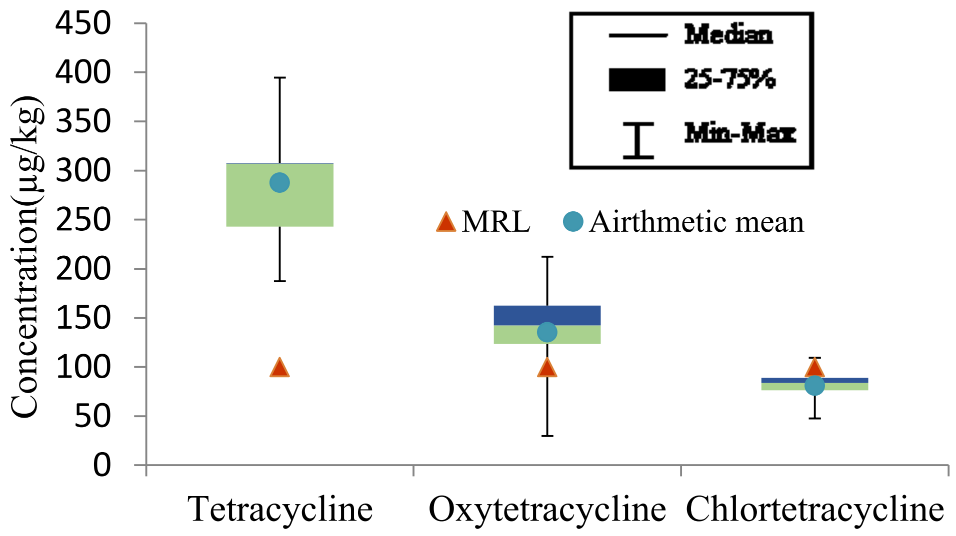


Figure 4.12 Concentration of tetracycline, oxytetracycline and chlortetracycline in feed for shrimp and prawn in 2013

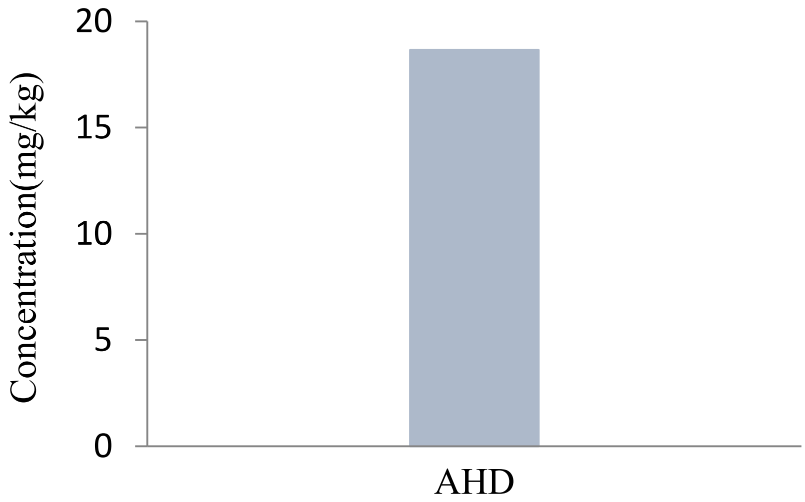


Figure 4.13 Concentration of AHD in feed for shrimp and prawn in 2013

4.1.4 Antibiotics in feed ingredient, PL and unknown chemicals

Depending on the information mentioned by the farmers during survey 5 prawn PL, 7 shrimp PL, 2 shrimp and prawn shell and 2 unknown chemicals were collected and sent to the Institute of Food Science & Technology (IFST) Lab, Dhaka, Bangladesh for nitrofuran and chloramphenicol test. The reporting limit CCα (µg/kg) of IFST Lab was 0.08, 0.03, 0.04, 0.10 and 0.03 µg/kg for AHD, AMOZ, AOZ, SEM and CAP respectively. As per test report, AOZ was found in 1 prawn PL and 1 shrimp PL whereas CAP and SEM were found in 1 shrimp PL and 1 shrimp and prawn shell sample respectively. On the contrary, SEM and AOZ were also found in chemical 1 and chemical 2 respectively. The concentration of AOZ was 0.6 and 1.6µg/kg respectively in prawn PL and shrimp PL samples and 36.0µg/kg in chemical 2, the concentration of SEM was 0.80 and 27.00µg/kg in shrimp and prawn shell and chemical 1 respectively while that of CAP was 0.45µg/kg in shrimp PL (Table 4.1).

Table 4.1 Concentration of antibiotics in feed ingredient, PL and unknown chemicals

Sl. No.	Type of sample	No. of sample	No. of contaminated sample	Type of contaminant	Contaminant concentration (µg/kg)
01	Prawn PL	05	01	AOZ	0.60
02	Shrimp PL	07	02	CAP, AOZ	0.45, 1.6
03	Shrimp and prawn shell	02	01	SEM	0.80
04	Chemical-1	01	01	SEM	27.00
05	Chemical-2	01	01	AOZ	36.00

4.2 Result of questionnaire survey data

4.2.1 Use of PL (Post Larvae)

In the present investigation, it was found that 45.8%, 13.3% and 40.9% farmers (n=83) used both hatchery and natural, natural and hatchery PL (Post Larvae) respectively (Figure 4.14). On the other side, 46.9%, 12.3% and 40.8% farmers amid the contaminated farms (n=49) and 23.5%, 35.3% and 41.2% non-contaminated farm holders (n=34) used both hatchery and natural, natural and hatchery PL respectively (Figure 4.14). The Figure 4.14 shows that most of contaminated farms used both natural and hatchery produced PL while most of the non-contaminated farms used natural PL.

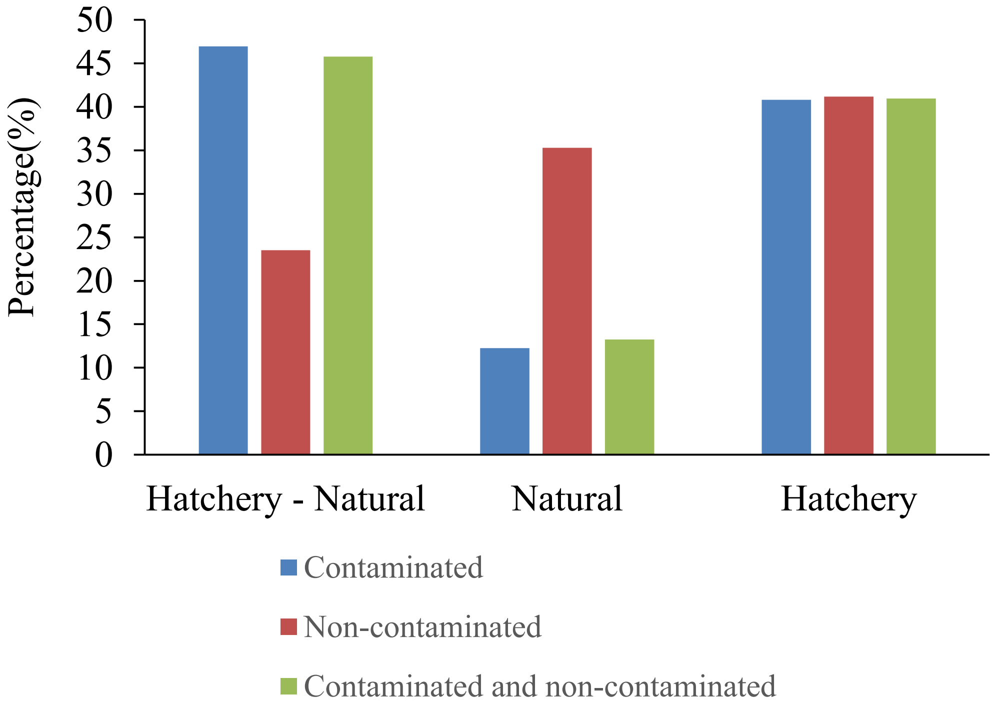


Figure 4.14 Use of PL in shrimp and prawn farm

4.2.2 Use of feed

In the present study, it was found that 62.7% farmers used factory produced feed and 25.3% farmers used handmade feed. It was also found that both feed was used in 28% (n=25) shrimp farm, 70.4% (n=27) prawn farm and 77.4% (n=31) shrimp-prawn farm (Figure 4.15) while it was used in 85.7% (n=49) contaminated and 47.1% (n=34) non-contaminated farm (Figure 4.16). On the other hand, 85.7%, 84.2% and 81.3% contaminated farm owners of Satkhira, Khulna and Bagerhat district respectively used feed for shrimp and prawn production (Figure 4.17).

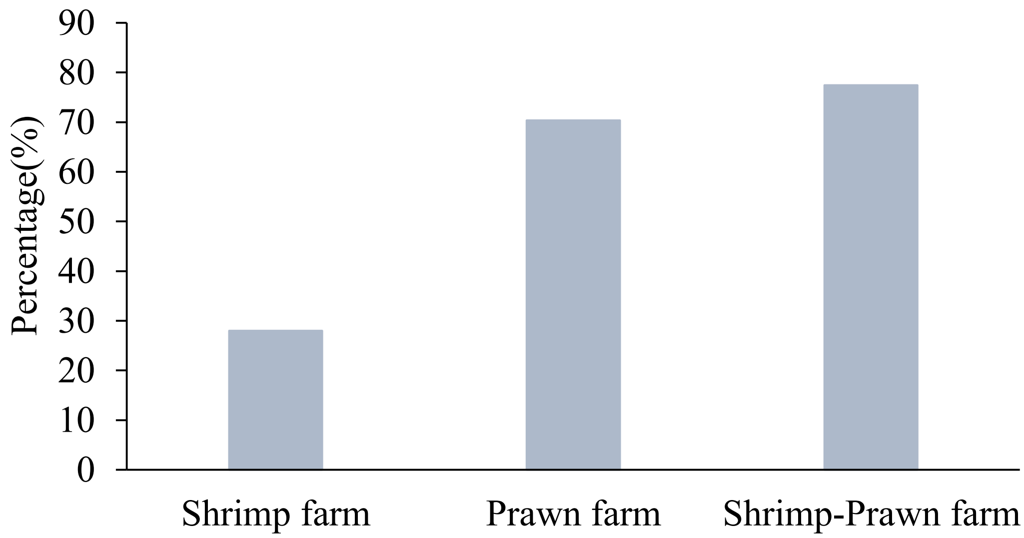


Figure 4.15 Use of feed in shrimp and prawn farm

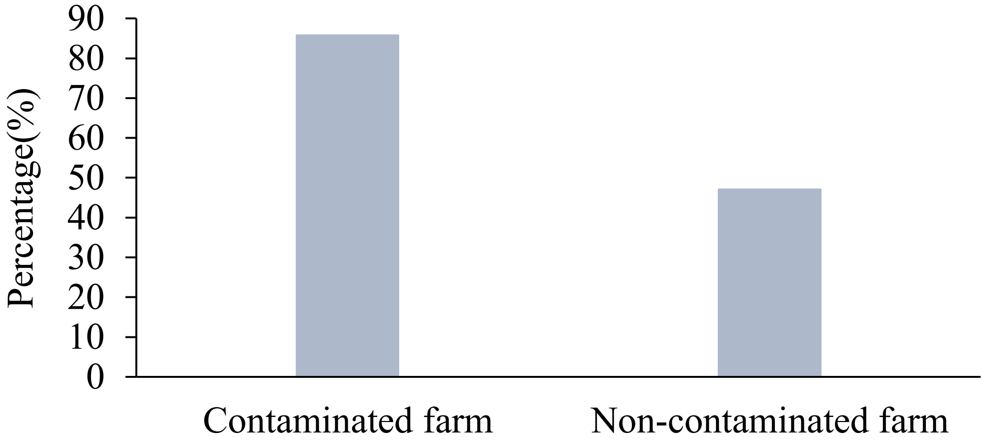


Figure 4.16 Use of feed in contaminated and non-contaminated farm

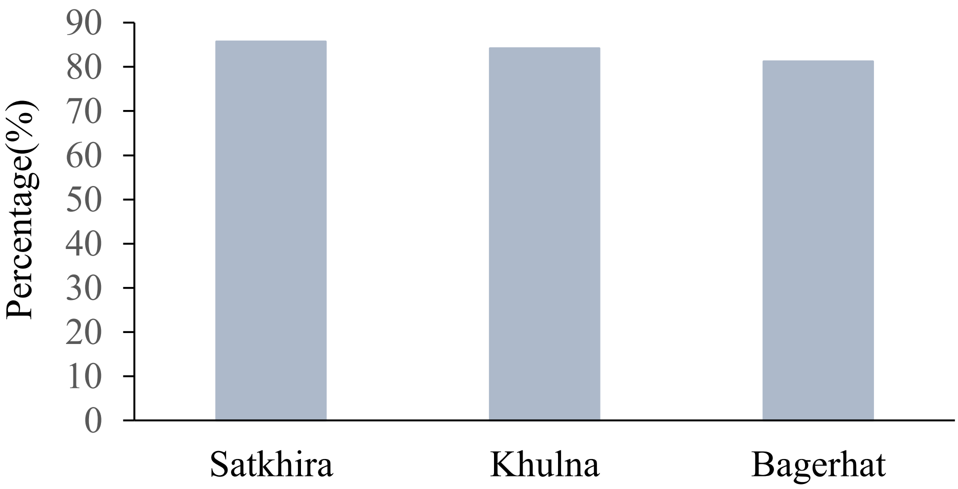


Figure 4.17 Use of feed in contaminated farm of satkhira, Khulna and Bagerhat district

4.2.3 Use of chemical and biological products

In the present study, it was identified 43 different types of chemical and biological products or substances in which 13 chemical products for pond and water quality management (Table 4.2), 5 chemicals for disinfectant (Table 4.3), 4 chemicals for oxygenation (Table 4.4), 6 antibiotics for disease treatment (Table 4.5), 5 probiotics for disease treatment and water purification (Table 4.6) and 10 chemicals were used for pest and insects control (Table 4.7).

The chemicals that listed in the Table 4.2 used in the shrimp and prawn pond to enrich the micro and macro nutrients in the soil and water so that phytoplankton and zooplankton can grow easily. Actually, phytoplankton and zooplankton are the best natural food for shrimp and prawn. In contrast, when any micro-organism appeared in the pond especially protozoa the farmers use the chemicals that mentioned in the Table 4.3 to save the shrimp and prawn from any disease that causes mortality. If disease appeared, the farmers apply antibiotics and probiotics that mentioned in Table 4.5 and Table 4.6. Not only disease, but also oxygen deficiency causes fish mortality. Generally, oxygen deficiency occurs at some circumstances especially heavy rain, drought, high stocking density and heavy use of feed etc. throughout the culture period. When it occurs the farmers use the chemicals that listed in the Table 4.4 to provide oxygen quickly. During questionnaire survey, the farmers mentioned that they grow aquatic weeds and make canal in their shrimp pond to save shrimp from high temperature at summer when the water depth remains less than 0.5m except canal. They also mentioned that a few unknown insects cut the aquatic weeds. When those insects appear they use the chemicals that listed in the Table 4.7. The Table 4.7 also indicates that a few number of farmers used thiodin and hildon that are totally banned throughout the world.

Table 4.2 Chemicals used for pond and water quality management

Trade Name	Active Ingredients	Dose (kg/ha)		Frequency	Manufacturer
		Average	Std.		
Urea	NH_2-CO-NH_2	42.89 (75.0-20.58)	11.04	76	Chemical seller
TSP	$Ca(H_2PO_4)_2.H_2O$	73.97 (150.0-11.0)	18.36	75	Chemical seller
DAP	$(NH_4)_2HPO_4$	47.5 (100.0-15.0)	21.92	14	Chemical seller
Zipsam	$CaSO_4.2H_2O$	85.45 (112.5-41.16)	16.81	19	Chemical seller
Lime	CaO, $Ca(OH)_2$	11.06 (240.0-60.0)	33.17	80	Chemical seller
Zeolite	SIO_2,Al_2O_3, Fe_2O_3,CaO,MgO, Na_2O	39.42 (52.5-21.47	6.43	46	National Agricare Imp.Exp.Ltd.
Zeocare	SIO_2,Al_2O_3, Fe_2O_3,CaO,MgO, Na_2O	0.617 (0.74-0.49)	0.12	3	Nature Care
Zeotox	SIO_2,Al_2O_3, Fe_2O_3,CaO,MgO, Na_2O	31.21 (60.0-10.0)	20.86	5	Novartis Pharmaceutical Ltd
Dolomite	$CaCO_3.MgCO_3$	49.46 (75.0-37.5)	13.87	14	Chemical seller
Aqua-Nourish P	CaO, $K_2SO_4.Al_2(SO_4)_3.24H_2O$	14.25 (15.0-11.25)	1.67	5	Aqua Chemical Co. Ltd.
Polgard Plus	3-Methyl,4-Alkyl Two Chain Brominated Compound-30%	0.75	-	2	Fishtech (BD) Ltd.
Aqua-zet	SIO_2,Al_2O_3, Fe_2O_3,CaO,MgO, Na_2O	4.87(L/ha) (7.5-3.75)	1.49	5	Lion Overseas Trading Company
Bengal Aqua	Formalin, $NaHCO_3$, $K_2SO_4.Al_2(SO_4)_3.24H_2O$	18.0	-	2	Aqua Marketing Company

Table 4.3 Chemicals used for disinfectant

Trade Name	Active Ingredients	Dose (kg/ha)		Frequency	Manufacturer
		Average	Std.		
Timsen	n-Alkyl dimethyl benzyl ammonium chloride + stabilized urea	0.36 (0.75-0.14)	0.20	15	Eon Animal Health products Ltd.
Potash	KMnO4	5.18 ppm (6-5)	0.40	11	Chemical seller
Fitkari	$K_2SO_4.Al_2(SO_4)_3.24\ H_2O$	3.20 (30-0.75)	8.05	13	Chemical seller
Bleaching	Ca(OCl)Cl	1.78 (2-1.5)	0.20	13	Chemical seller
Aquakleen	Tetradesile trimethyle ammonium bromide, amononitrogen	21.37(L/ha) (22.5-21.0)	0.75	4	Square Pharmaceutical Ltd.

Table 4.4 Chemicals used for oxygenation

Trade Name	Active Ingredients	Dose (kg/ha)		Frequency	Manufacturer
		Average	Std.		
Oxyflow	H_2O_2 10%	1.35 (1.87-0.61)	0.54	6	Novartis Pharmaceutical Ltd
Oxy gold	Sodium percarbonate 90% ($2Na_2CO_3.3H_2O_2$)	0.99 (1.87-0.49)	0.64	5	Fishtech (BD) Ltd.
Oxy Plus	O_2 promoter (H_2O_2/ Ca_2O_2)	1.17 (1.23-1.11)	0.08	2	Navana Animal Health
Oxygen Plus	O_2 promoter (H_2O_2/ Ca_2O_2)	0.86 (0.98-0.74)	0.16	2	Avon Animal Health

Table 4.5 Antibiotics used for disease treatment

Trade Name	Active Ingredients	Dose (gm/kg feed) Average	Std.	Frequency	Manufacturer
Ranamox	Amoxicillin trihydrate	9.33 (10.0-8.0)	1.15	3	Renata Pharmaceutical Ltd.
Renamycin	Oxytetracycline	4.16 (5.0-3.5)	0.76	3	Renata Pharmaceutical Ltd.
Bactitab	Oxytetracycline 20%	6 (7.0-5.0)	1.41	2	ACI Animal Health
Fish Cure	Chlortetracycline HCl	4.8 (5.0-4.0)	0.44	5	Rals Agro Ltd.
Sulphatrim	Sulphadiazine and trimethoprim	5.5 (6.0-5.0)	0.70	2	Square Pharmaceutical Ltd.
Exorich	Vitamins and minerals	5.0	-	2	Fishtech (BD) Ltd.

Table 4.6 Probiotics used for disease treatment and water purification

Trade Name	Active Bacteria	Dose (kg/ha) Average	Std.	Frequency	Manufacturer
Bioprob	*Bacillus subtilis, B. licheniformis, B. amyloliquefaciens*	0.35 (0.75-0.24)	0.22	5	Unibiocare
Profs	*Bacillus sp. and Pediococcus sp.*	0.62 (0.75-0.24)	0.25	4	Eon Animal Health
Bio-profs	*Bacillus subtilis*	0.60 (0.61-0.55)	0.02	6	Novartis Pharmaceutical Ltd
BactoGro	Aerobic and facultative anaerobic bacteria	0.63 (0.75-0.19)	0.24	5	Fishtech (BD) Ltd.
Aqua Gold	*Rhodopseudomonas Sp.*	13.17(ml/ha) (14.82-12.35)	1.42	3	Organic Pharmaceutical Ltd

Table 4.7 Chemicals used for pest and insects control

Trade Name	Active Ingredients	Dose (kg/ha)		Frequency	Manufacturer
		Average	Std.		
Virtako	Thiamethoxam + Chlorantraniliprole	0.078 (0.09-0.075)	0.006	5	Syngenta
Thiodin	Aldrin	3.75	-	1	Chemical seller
Malathion	Malathion	0.731 (0.75-0.67)	0.034	8	Century Agro Ltd.
Sumithon	Fanitrothion	0.649 (0.98-0.036)	0.421	4	Chemical seller
Rotenon	Nicouline	8.85 (9.88-8.64)	0.504	6	Chemical seller
Tobaco dust	0.5% nicotine v	7.86 (8.23-7.5)	0.516	2	Chemical seller
Hildon	Aldrin	0.4	-	1	Chemical seller
Virex	Potasium peroximono sulphate 50%	2.75 (3.0-2.5)	0.353	2	ACI animal health products
Cartop	Thiocarbamate ($C_7H_{16}ClN_3O_2S_2$)	0.245(L/ha) (0.25-0.24)	0.007	2	Syngenta
Asatop	Thiocarbamate ($C_7H_{16}ClN_3O_2S_2$)	0.475(L/ha) (0.5-0.45)	0.035	2	Corbel International, Dhaka

Farmers in the present study used on an average 6 chemical and biological products in the shrimp and prawn farm for better management (Figure 4.18). A farm used on an average 7, 7 and 6 chemical and biological products in Satkhira, Khulna and Bagerhat district respectively (Figure 4.18). On the other hand, a contaminated farm used on an average 7 chemical and biological products whereas a non-contaminated farm used an average 5 chemical and biological products (Figure 4.19).

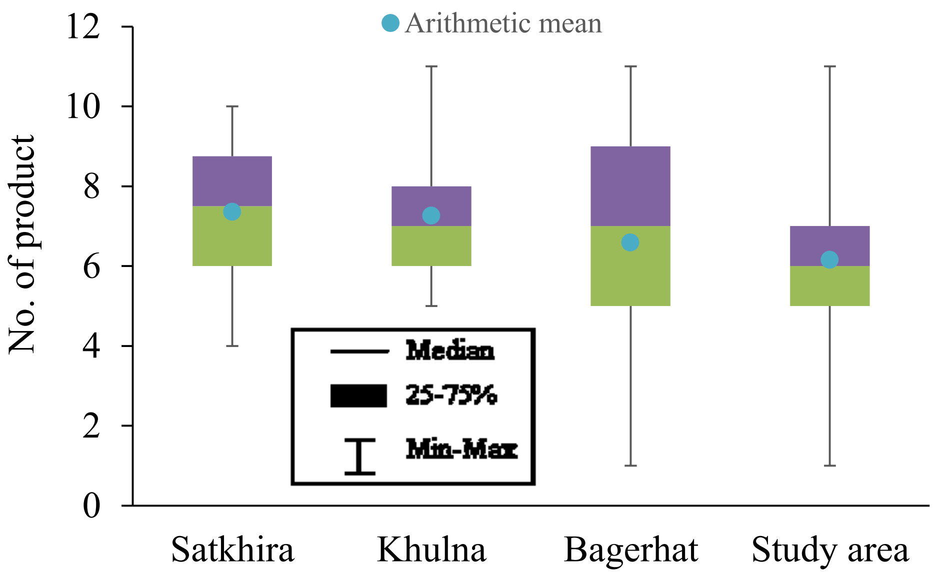


Figure 4.18 Number of chemical and biological products used per farm in study area

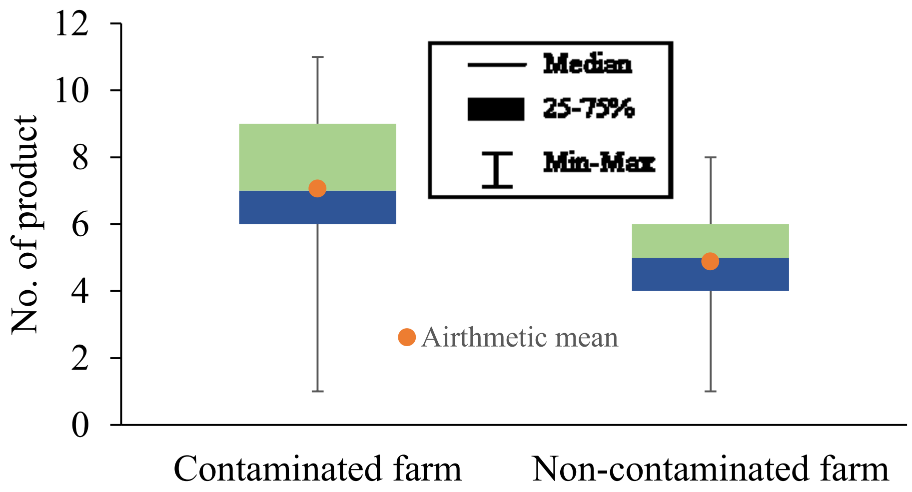


Figure 4.19 Number of chemical and biological products used per contaminated and non-contaminated farm in study area

4.2.4 Co-relation between contamination and expected parameters

Karl Pearson correlation co-efficient equation was used to draw the inter-element relationship among depth (m), canal water, river water, ground water, neighbour Pond water, water Exchange, fertilizer, cowdung and contamination. It was found positive co-relation between water exchange and canal water and contamination and neighbour pond water respectively whereas negative co-relation was found between neighbour pond water and canal water, water exchange and neighbour pond water and contamination and water exchange respectively (Table 4.8).

Table 4.8 Co-relation among expected parameters of shrimp and prawn farm

Parameters	*Depth(m)*	*Canal water*	*River water*	*Ground Water*	*Neighbour Pond water*	*Water Exchange*	*Fertilizer*	*Cowdung*
Depth(m)	1.00							
Canal water	0.03	1.00						
River water	0.05	-0.23	1.00					
Ground Water	0.38	-0.19	-0.09	1.00				
Neighbour Pond water	-0.26	-0.70	-0.33	-0.28	1.00			
Water Exchange	0.15	0.75	0.32	-0.13	-0.82	1.00		
Fertilizer	0.05	-0.14	-0.16	0.05	0.20	-0.24	1.00	
Cowdung	-0.19	-0.09	0.01	-0.16	0.16	-0.11	0.11	1.00
Contamination	-0.20	-0.42	-0.14	-0.05	0.50	-0.57	0.10	0.24

4.2.5 Use of feed in hatchery

In the present study, it was found that 88.9% hatchery operator used artemia, commercial feed and custard whereas 11.1% hatchery operator (n = 9) used artemia and commercial feed at different life stages of shrimp and prawn PL (Figure 4.20). Actually, using yolk sac (Egg), corn flour, milk powder, lecithin, chingri brood, chord liver oil and agar powder the hatchery operators formulated custard for shrimp and prawn PL.

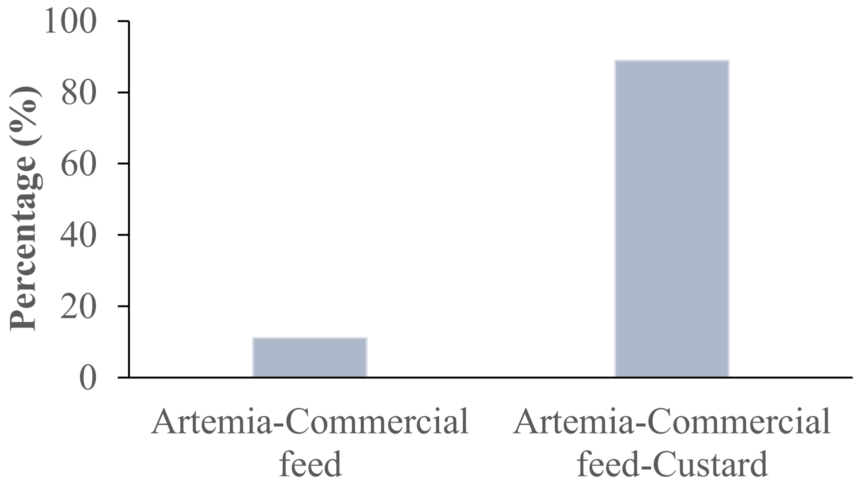


Figure 4.20 Use of feed in shrimp and prawn hatchery

4.2.6 Use of chemical in hatchery

At the time of survey, it was found 19 different types of chemical and biological products for hatchery management in which 8 chemicals for disinfectant (Table 4.9), 4 antibiotics for disease treatment (Table 4.10), 7 probiotics for disease treatment and water purification (Table 4.11).

Table 4.9 Chemicals used for disinfectant

Trade Name	Active Bacteria	Dose (ppm)		Frequency
		Average	Std.	
Bleaching	Chlorine	14.55 (20.00-12.00)	2.50	9
EDTA	Sodium thiosulphate	5.33 (6.00-5.00)	0.51	6
Sodium-bicarbonate	Sodium-bicarbonate	11.62 (12.5-10.00)	1.10	4
Potash	KMnO4	1.83 (2.00-1.50)	0.28	3
Formalin	40% HCHO	6.20 (8.00-5.00)	1.09	5
Iodine	Iodine	1.50	-	1
Dolomite	$CaCO_3.MgCO_3$	3.33 (8.00-1.00)	4.04	3
Calcium-bicarbonate	Calcium-bicarbonate	8.00	-	1

Table 4.10 Antibiotics used for disease treatment

Trade Name	Active Ingredients	Dose (ppm)		Frequency
		Average	Std.	
OTC	Oxytetracycline	1.35 (1.50-1.20)	0.16	6
Azithromycin	Azithromycin	1.35 (1.50-1.20)	0.21	2
FZ-ciprofloxacin	1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid	1.38 (1.50-1.20)	0.16	5
Erythromycin	Erythromycin	2.5		1

Table 4.11 Probiotics used for disease treatment and water purification

Trade Name	Active Bacteria	Dose (ppm)		Frequency
		Average	Std.	
ABS	Enzyme producing beneficial micro-organisms	1.5 (2.00-1.00)	0.26	8
Pro-100	*Bacillus sp.*	1.1 (1.20-1.00)	0.11	4
Epicin 3W	Natural microbes and enzymes	1.35 (1.50-1.20)	0.21	2
Epicin Hatchery	Natural microbes and enzymes	2 (2.50-1.50)	0.7	2
Aqua Pro	*Bacillus sp.*	1	-	1
VC-7	*Bacillus subtilis*	1.75 (2.00-1.50)	0.35	2
Aqua Photo	*Rhodopseudomonas Sp.* *Bacillus subtilis*	1.25 (1.50-1.00)	0.35	2

4.2.7 Use of feed ingredient and additives in feed factory

During questionnaire survey, it was found that the feed mill operators used 10 types of feed ingredients, namely fish meal, bone meal, meat meal, blood meal, rice bran, wheat bran, maize, mustard oil cake, soya bean oil cake and flour. They also mentioned that these feed ingredients were imported from India though they purchased 5-10% rice bran of their requirement from the local market. On the contrary, it was also found 14 types of additives that were used in feed formulation, namely salt, toxin binder, mould guard, liposorb, pellet binder, molasses, vitamin, chord liver oil, allzyme, sunny binder, molt nill, pump oline, biogene and biopremix. The feed factory operators mentioned that they imported all these additives from India.

4.3 Result of regression analysis

Multiple linear regression model was executed to measure nexus between shrimp and prawn production with carp and production oriented factors. In both cases, stocking density (Thousand/ha), farm area (ha), feed (kg/ha), water depth (m), fertilizer (kg/ha), lime (kg/ha) and number of chemicals were considered as production oriented factors. In case of shrimp with carp, stocking density (Thousand/ha), water depth (m), lime (kg/ha) and number of chemicals had no significant effect on production while others factors except stocking density (Thousand/ha), farm area (ha), water depth (m), lime (kg/ha) and number of chemicals had significant effect on prawn with carp production. The Table 4.12 demonstrated that shrimp with carp production was negatively influenced by farm area (ha) at 1% significant level and positively influenced by feed (kg/ha) and fertilizer (kg/ha) at 1% and 5% significant level respectively. In contrast, prawn with carp production was negatively influenced by fertilizer and positively influenced by feed (kg/ha) at 1% significant level (Table 4.13).

Table 4.12 The regression result for shrimp with carp (n=57)

Independent Variables	Coefficients	Standard Error	t Stat	P-value	Adjusted R Square	Significance F
Intercept	544.72	78.39	6.95	5.45E-09		
Farm Area(ha)	-33.83	11.41	-2.96	0.0045***	0.54	1.39E-09
Feed(kg/ha)	0.26	0.04	6.67	1.54E-08***		
Fertilizer(kg/ha)	1.26	0.55	2.27	0.027**		

** and *** represent 5% and 1% level of significance respectively.

Table 4.13 The regression result for prawn with carp (n=26)

Independent Variables	Coefficients	Standard Error	t Stat	P-value	Adjusted R Square	Significance F
Intercept	288.64	61.08	4.73	9.22E-05		
Feed(kg/ha)	0.64	0.04	18.26	3.47E-15***	0.93	2.00E-14
Fertilizer(kg/ha)	-1.75	0.42	-4.13	0.0004***		

*** represent 1% level of significance.

4.4 Risk assessment

4.4.1 Dose response assessment

4.4.1.1 SEM in prawn and AHD in shrimp muscle

The maximum and minimum concentration of SEM in prawn muscle was 8.85µg/kg and 0.25µg/kg respectively whereas the mean concentration was 2.44µg/kg (Figure 4.21). On the other hand, maximum and minimum AHD concentration was 1.93µg/kg and 0.82µg/kg respectively while mean concentration was 1.27µg/kg (Figure 4.21).

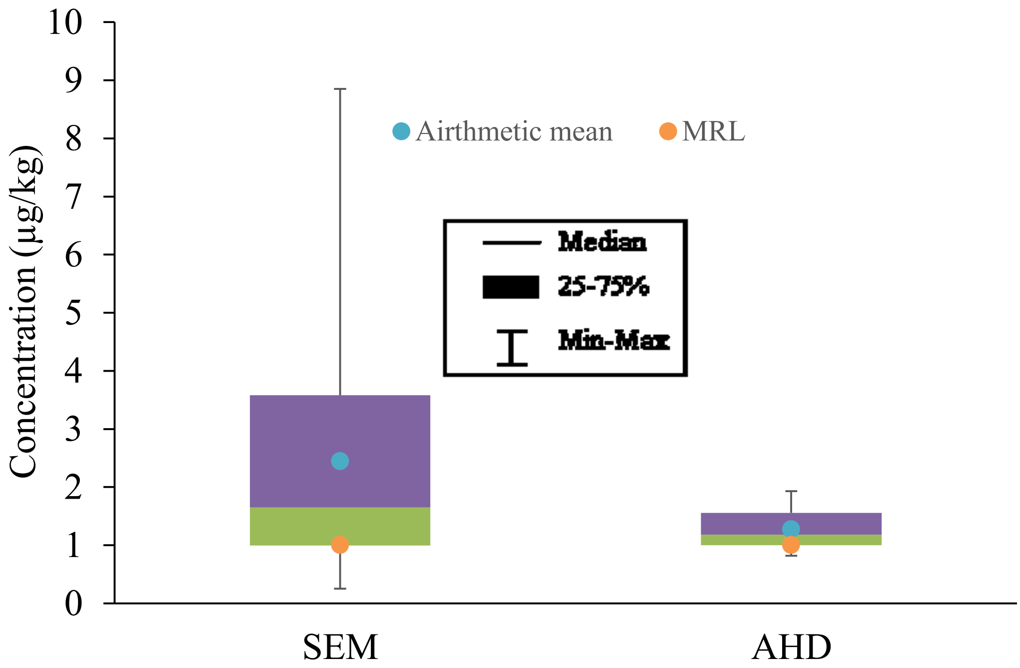
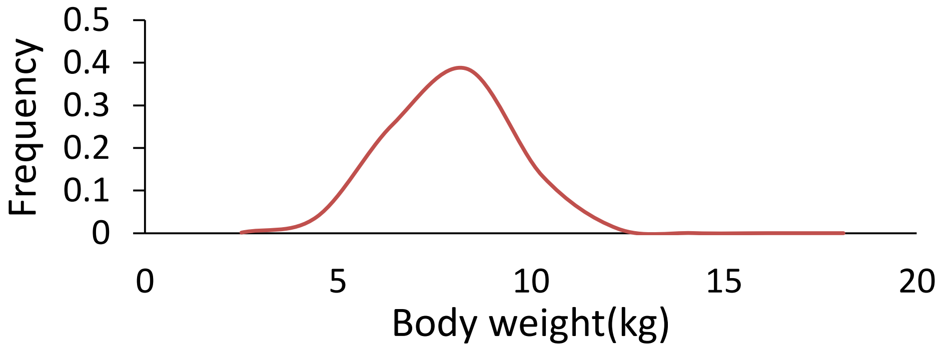


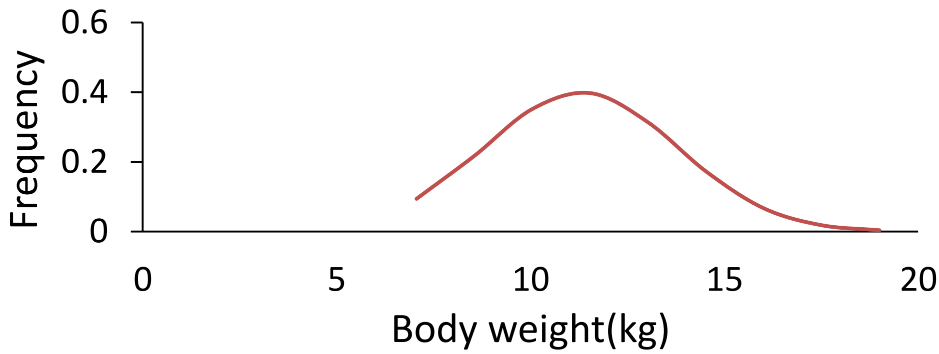
Figure 4.21 Concentration of SEM in prawn and AHD in shrimp muscle

4.4.1.2 Body weight distribution

The maximum body weight of Bangladeshi people for 0-<2, 2-<3, 3-<6, 6-<16,16-<70.7 age bins was 18.10, 19.00, 23.00, 80.51 and 98.00 kg while minimum body weight was 2.5, 7.06, 7.7, 23.7 and 23 kg for those age intervals respectively. The average body weight was 7.93, 10.85, 14.01, 36.75 and 53.5 kg respectively whereas standard deviation was 1.60, 2.49, 3.12, 14.7 and 10.68 for 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 age bins respectively. The body weight distribution for 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 age bins are shown in Figure 4.22 and Figure 4.23 (ICDDRB, 2012).

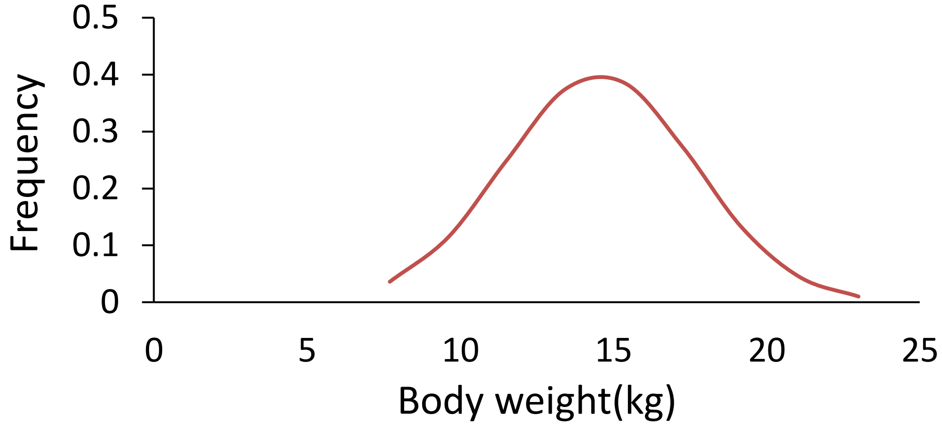


(a)

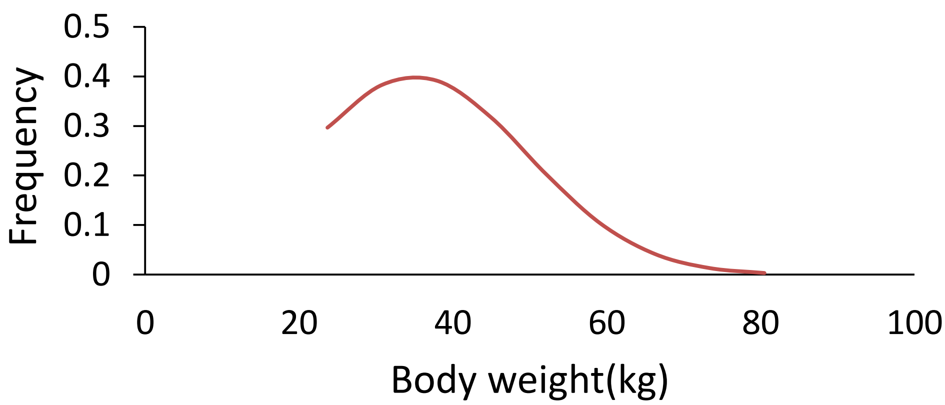


(b)

Figure 4.22 Normal body weight distribution of (a) 0-<2 and (b) 2-<3 age bins



(a)



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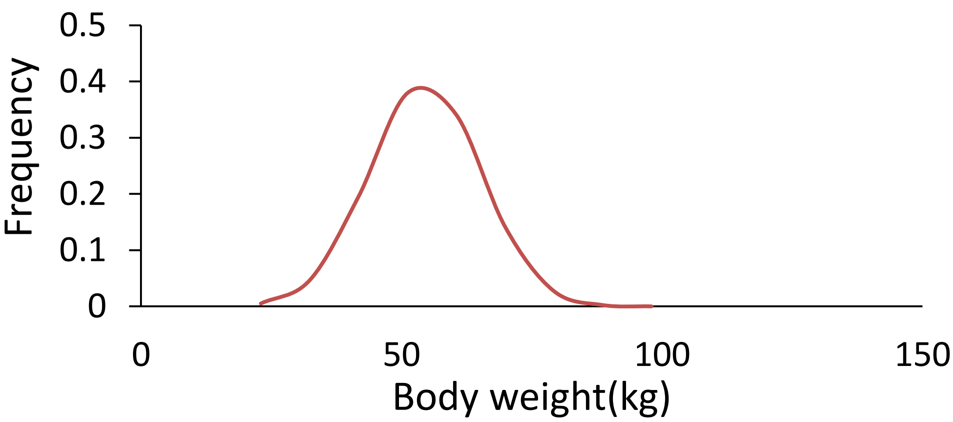


(c)

Figure 4.23 Normal body weight distribution of (a) 3-<6, (b) 6-<16 and (c)16-<70.7 age bins

4.4.1.3 EDI estimation

The estimated daily intake (EDI) that obtained from the intake of SEM through consumption of prawn was estimated for different age intervals. The EDI values of 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative 0-<70.7 age bins were 2.14E-07, 1.50E-07, 1.17E-07, 4.86E-08, 3.12E-08 and 5.62E-07 mg/kg/day respectively whereas that values of AHD intaking through consumption of shrimp were 6.19E-08, 5.07E-08, 3.94E-08, 1.64E-08, 1.05E-08 and 1.79E-07 mg/kg/day for 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative 0-<70.7 age bins respectively (Figure 4.24).

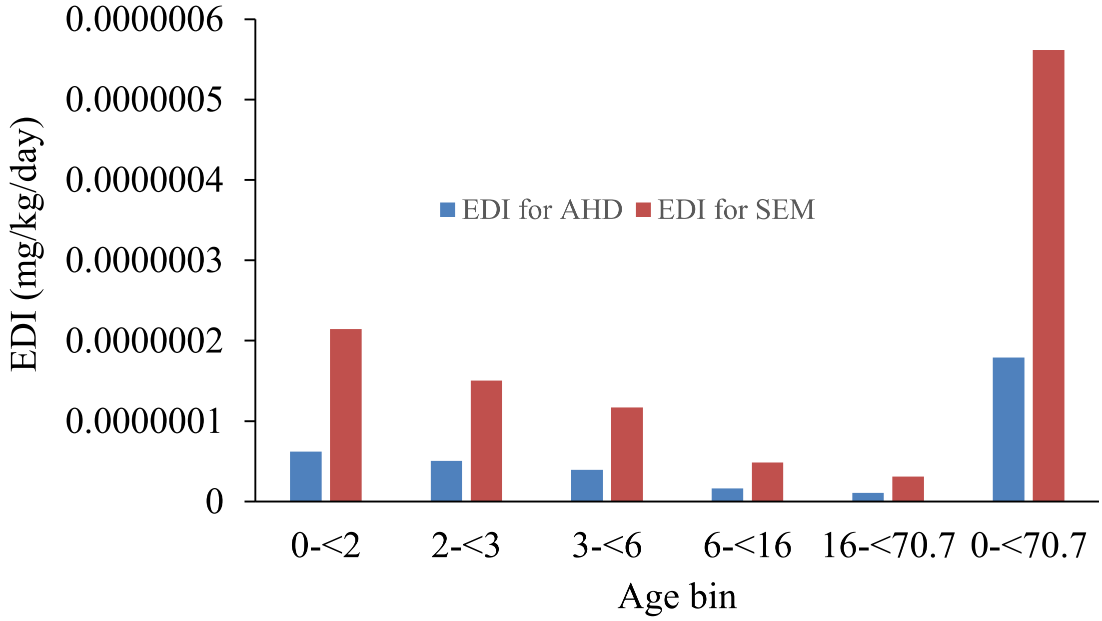


Figure 4.24 EDI values for SEM and AHD at different age bins

4.4.2 Carcinogenic and non-carcinogenic risk characterization

4.4.2.1 Carcinogenic risk caused by SEM

The carcinogenic risk (CR) that obtained from the intake of SEM through consumption of prawn was estimated for different age intervals. The CR values of 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative 0-<70.7 age bins were 3.22E-06, 6.77E-07, 5.26E-07, 2.19E-07, 4.68E-08 and 4.69E-06 respectively whereas threshold level was 1.0E-06 for causing cancer (USEPA 2004). The CR values for 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 age bins are shown in Figure 4.25 and Figure 4.26 while the average CR values for 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative value for 0-<70.7 age bins are shown in Figure 4.27.

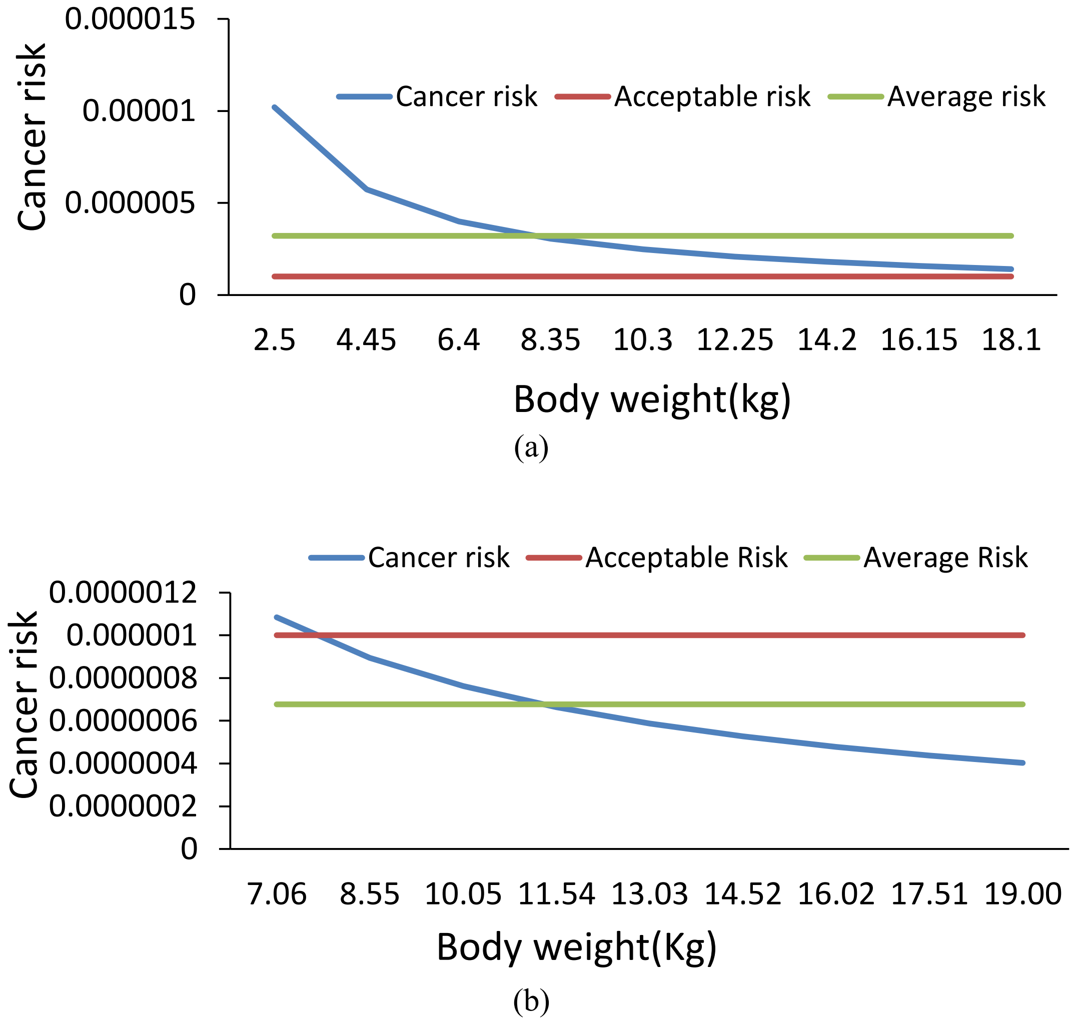
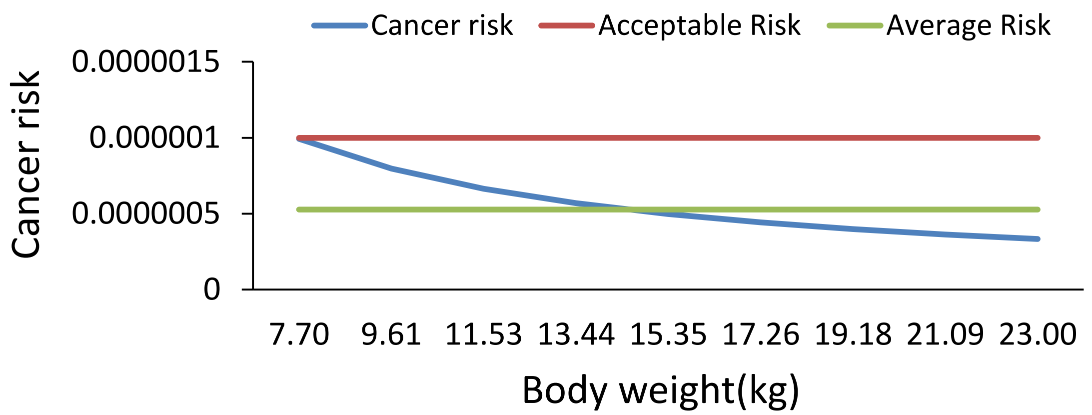
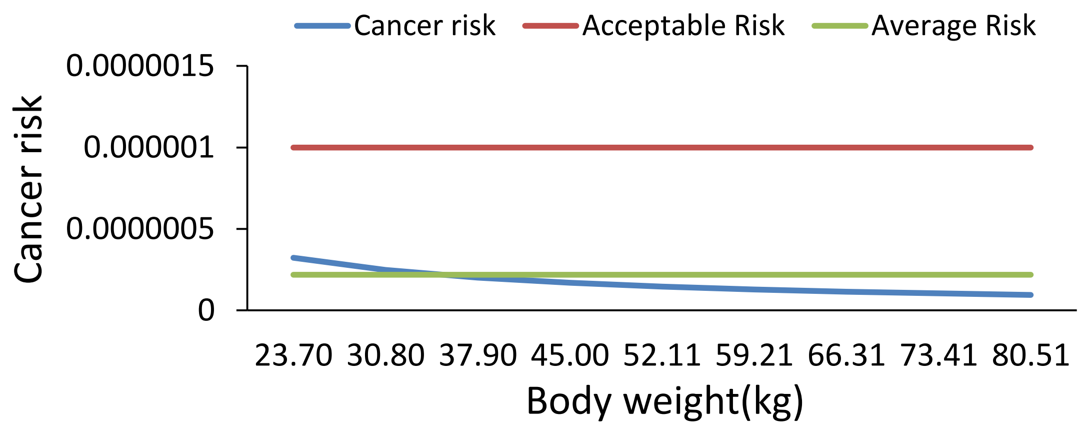


Figure 4.25 CR values of (a) 0-<2 and (b) 2-<3 age bins



(a)



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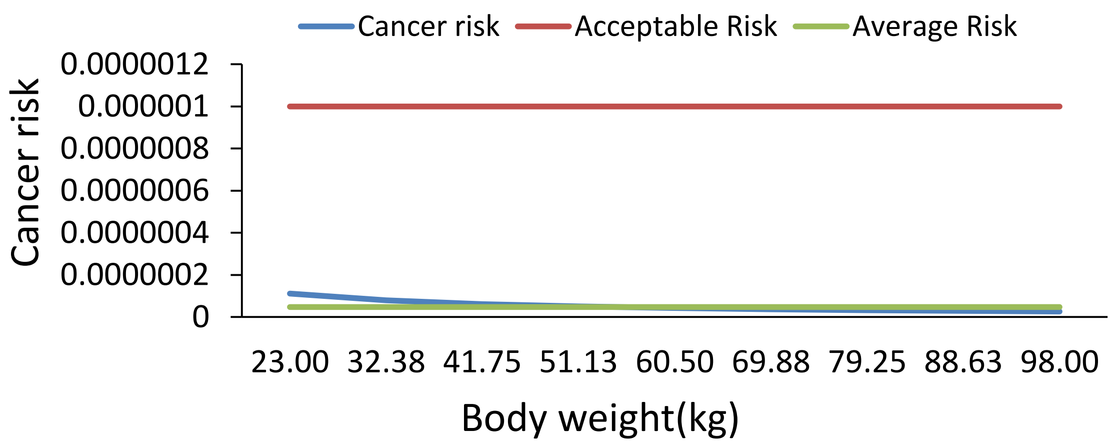


(c)

Figure 4.26 CR values of (a) 3-<6, (b) 6-<16 and (c) 16-<70.7 age bins

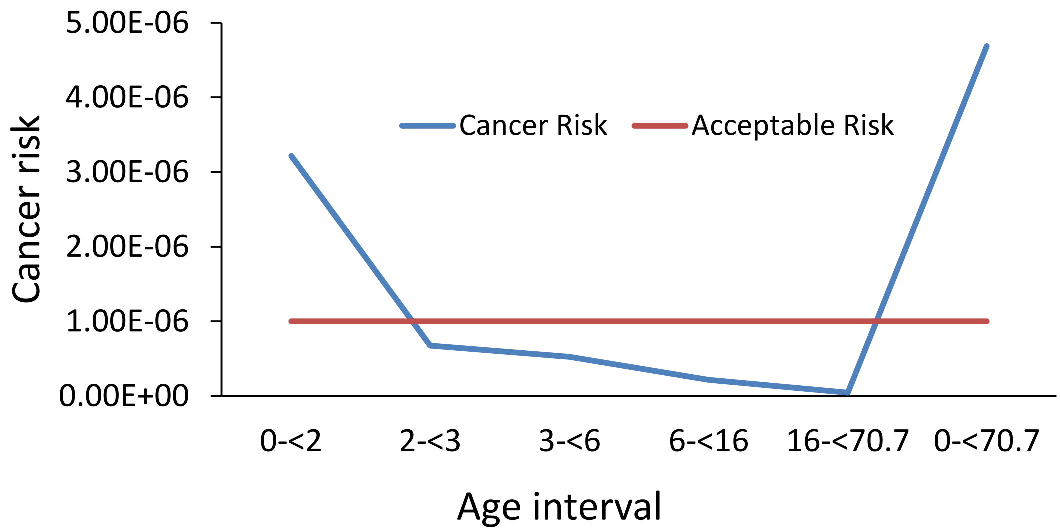
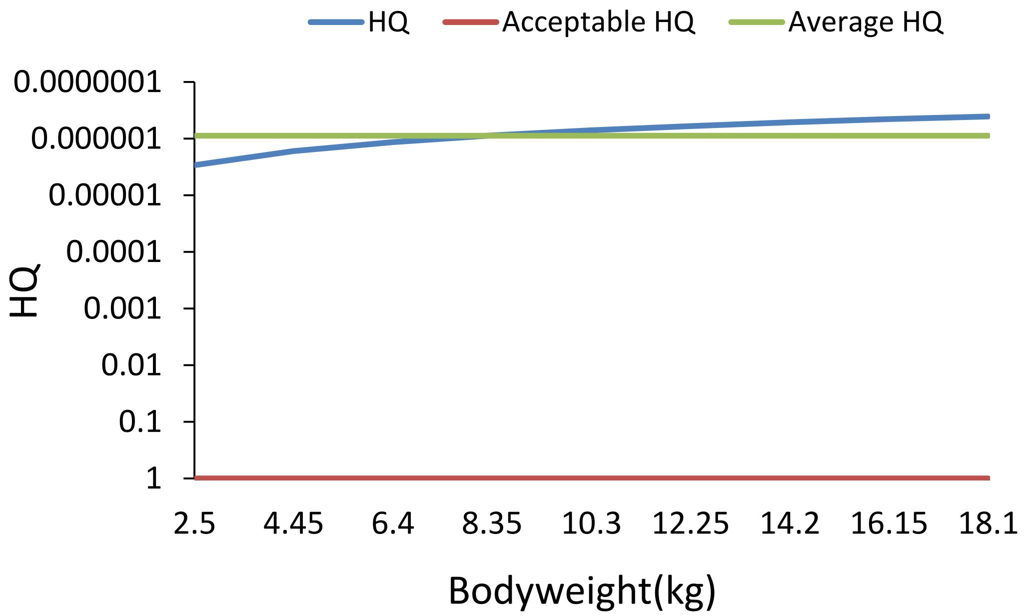


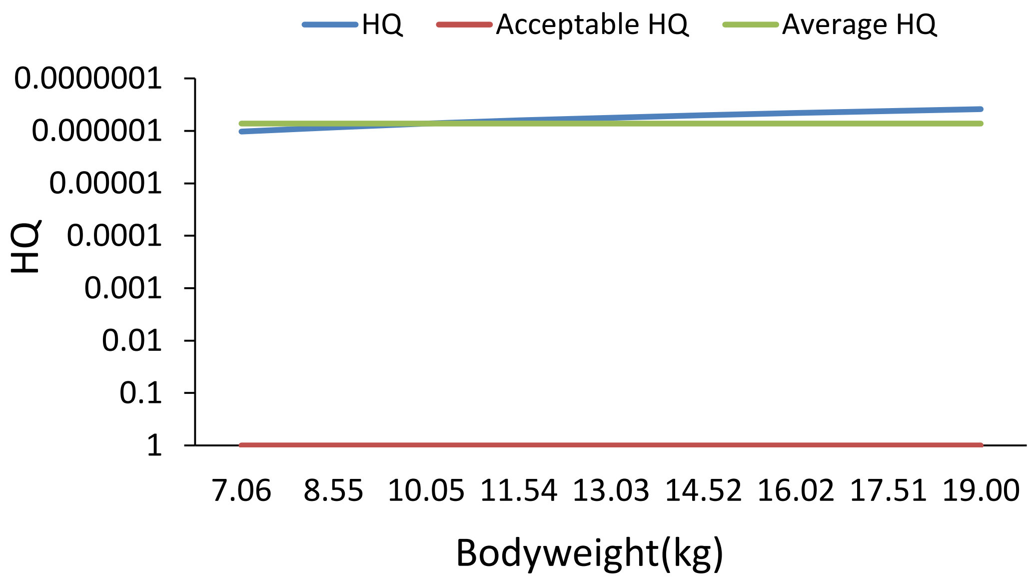
Figure 4.27 Average CR values of 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative 0-<70.7 age bins

4.4.2.2 Non-carcinogenic risk caused by AHD

The non-carcinogenic risk (HQ) that obtained from the intake of AHD through consumption of shrimp was estimated for different age intervals. The HQ values of 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative 0-<70.7 age bins were 8.85E-07, 7.24E-07, 5.63E-07, 2.34E-07, 1.5E-07 and 2.56E-06 respectively whereas threshold level was 1 (USEPA 2004). The HQ values for 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 age bins are shown in Figure 4.28 and Figure 4.29 while the average HQ values for 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative value for 0-<70.7 age bins are shown in Figure 4.30.

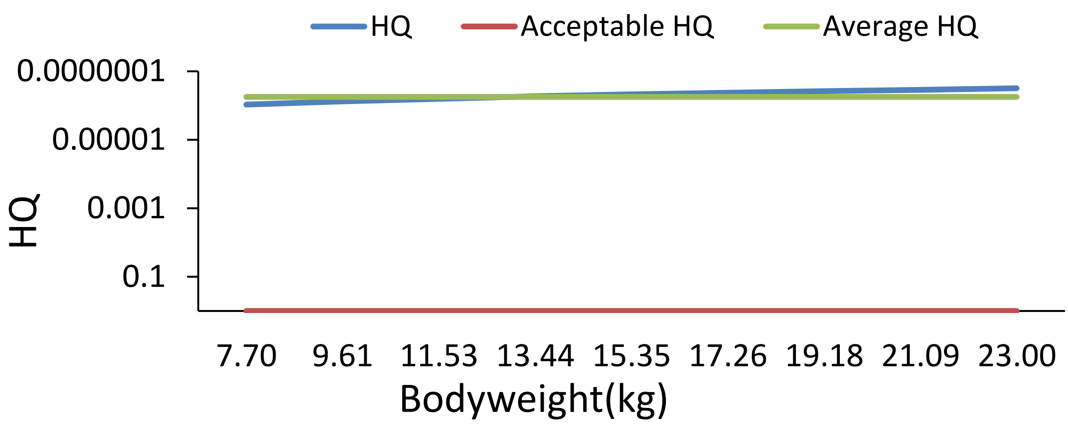


(a)

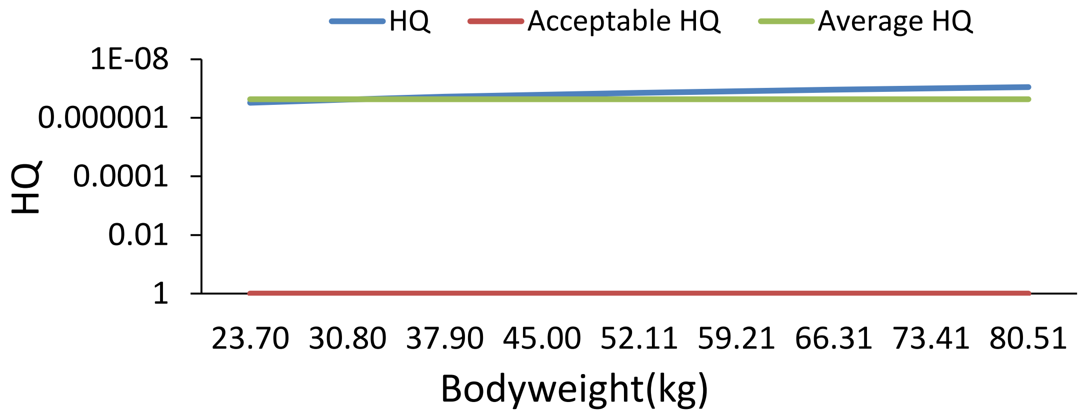


(b)

Figure 4.28 HQ values of (a) 0-<2 and (b) 2-<3 age bins



(a)



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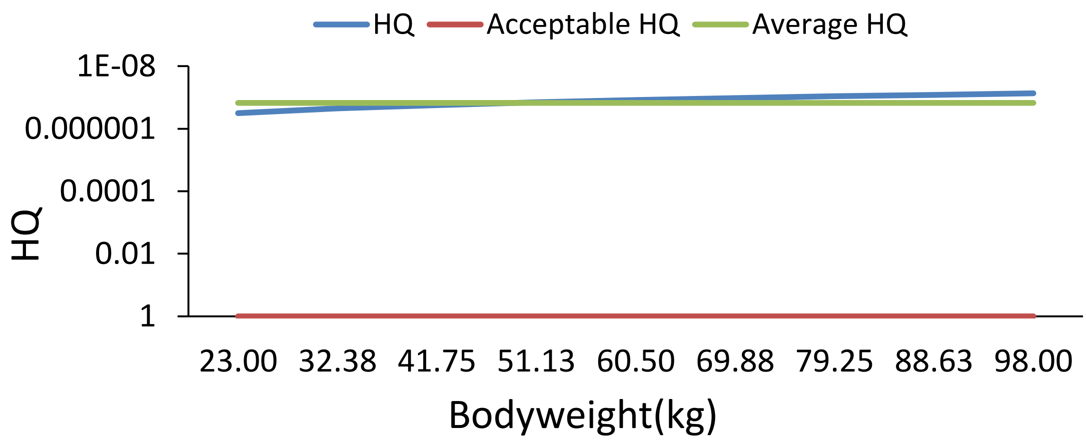

(c)

Figure 4.29 HQ values of (a) 3-<6, (b) 6-<16 and (c) 16-<70.7 age bins

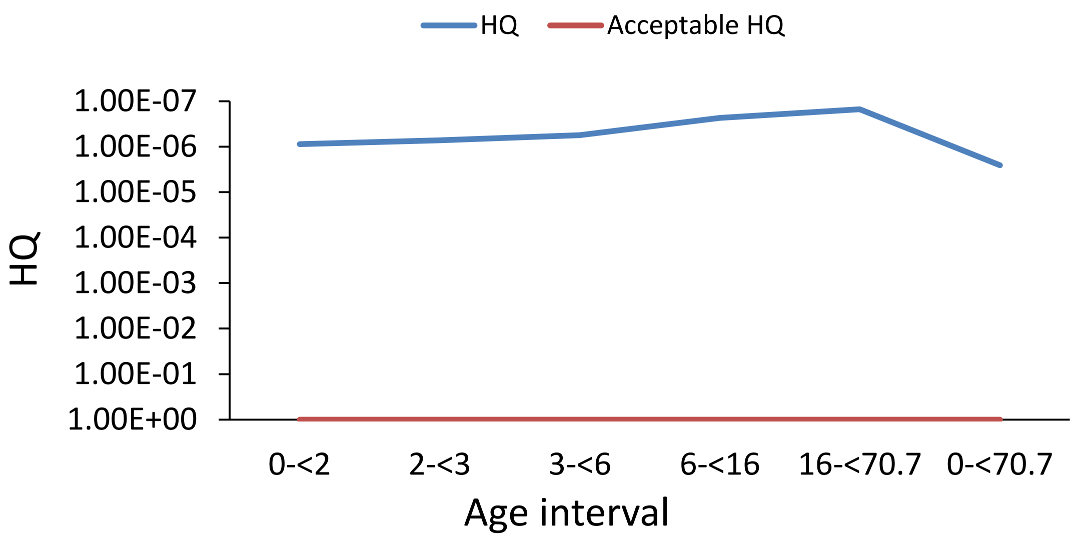

Figure 4.30 Average HQ values of 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative 0-<70.7 age bins

Chapter 5

Discussion

5.1 Source identification

Antibiotic tested data of 2011, 2012 and 2013, questionnaire survey data and test result of collected samples revealed that (i) prawn was more contaminated with nitrofuran and chloramphenicol than shrimp; (ii) contamination rate towards decreasing in case of both shrimp and prawn in 2013 with compare to that of 2011; (iii) Satkhira district was more contaminated with compare to Khulna and Bagerhat districts; (iv) SEM was found more in prawn whereas AHD and CAP were found more in shrimp; (v) it was found SEM in shrimp and prawn shell used as feed ingredient, AHD in commercial feed for shrimp and prawn, CAP in shrimp PL and AOZ in shrimp and prawn PL whereas SEM and AOZ were found in chemical-1 and chemical-2 respectively.

Questionnaire survey revealed that feed was used in 28% (n=25) shrimp-carp farm, 70.4% (n=27) prawn-carp farm and 77.4% (n=31) shrimp-prawn-carp farm (Figure 4.15) while it was used in 81.6% contaminated and 16% non-contaminated farm (Figure 4.16). On the other hand, 85.7%, 84.2% and 81.3% contaminated farm owners of Satkhira, Khulna and Bagerhat district respectively used feed for shrimp and prawn production (Figure 4.17). Questionnaire survey also indicated that 45.8%, 13.3% and 40.9% farmers (n=83) used both hatchery and natural, natural and hatchery PL (Post Larvae) respectively (Figure 4.14). On the contrary, 46.9%, 12.3% and 40.8% farmers amid the contaminated farms used both hatchery and natural, natural and hatchery PL respectively (Figure 4.14). On the other hand, Farmers in the present study used on an average 6 chemical products in the shrimp and prawn farm for better management (Figure 4.18) whereas farmers used on an average 13 types of chemicals and biological products in Thailand (S. Graslund et al. 2003). A farm used on an average 7, 7 and 6 chemical and biological products in Satkhira, Khulna and Bagerhat district respectively (Figure 4.18). On the other hand, a contaminated farm used on an average 7 chemical and biological products whereas a non-contaminated farm used an average 5 chemical and biological products (Figure 4.19).

Above all, more feed, hatchery PL and chemical were used in contaminated farm than non-contaminated farm and also used in prawn pond than shrimp pond. In contrast, it was found AHD in commercial feed for shrimp and prawn (Figure 4.13), SEM in shrimp and prawn shell used as feed ingredient, CAP in shrimp PL and AOZ in shrimp and prawn PL whereas SEM and AOZ were found in chemical-1 and chemical-2 respectively (Table 4.1). It was also found oxytetracycline, tetracycline and chlortetracycline in feed. But, these antibiotics were not found in shrimp and prawn. It means that all samples were collected after the withdrawal period of these antibiotics. So, it`s clear that nitrofuran and chloramphenicol are coming from feed and hatchery where nitrofuran and chloramphenicol drugs are using illegally. This finding has got similarity to the findings of many researchers who in Asian countries found that nitrofuran and chloramphenicol used in hatchery and feed for microbial control (Aftab Uddin et al. 2006; Frappaolo et al. 1986; K. Holmstrom et al. 2003; Nowsad et al. 2009; Paul et al. 2009). Actually, all hatchery technicians get only commission on the basis of PL production number. So, it`s assumed that they use nitrofuran and chloramphenicol drugs in order to reduce the mortality rate so that they can maximise their commission.

In 2011, Bangladesh government made a plan namely "National Residue Control Plan"(NRCP) to monitor fish and fishery products at different levels of production in regard to residues of undesirable substances especially nitrofuran and chloramphenicol residues in shrimp and prawn muscle. Under NRCP programme, the aim of the control on fish and fishery products is to assess compliance with the tolerance limits (for contaminants), maximum residue limits (MRLs for permitted substances), to reveal the illegal use of banned or unauthorized substances as well as to determine the origin of contaminant residue. In a word, the overall objective of this programme is to make the Bangladeshi fish and fishery products safe for consumers. At present, NRCP is being executed by the Department of Fisheries (DoF) under the Ministry of Fisheries and Livestock, Bangladesh. As per NRCP plan, DoF prepare sample list of shrimp/prawn and feed and send to the concerning ministry where it is approved by 31 March of every year. As stated in the approved sample list, DoF collects samples and tests in its own laboratory for monitoring purpose (DoF 2012). When banned antibiotics especially nitrofuran and CAP found in a sample all the shrimp and prawn of the farm from where the sample was taken caught and sold in the local market for domestic consumption. On the other hand, warning an official letter sent to the authority of the concerning feed factory when these antibiotics found in any feed sample. But, hatchery is not included in this plan though the present study reveals that nitrofuran and chloramphenicol are using in hatchery. So, hatchery should include in NRCP plan in order to reduce such contamination.

K. Hoenicke et al. (2004) found SEM in samples treated with hypochlorite or bleaching commonly used in food processing for disinfection. SEM was found in samples at the concentration of 0.3-20 μg/kg after treatment with 1% active chlorine. In contrast, questionnaire survey revealed that 100% hatchery technician (9 hatcheries out of 9) and 15.6% farmer (13 farmers out of 83) used bleaching powder for disinfection (Table 4.3 and Table 4.11). So, SEM might be come from the use of bleaching powder in shrimp and prawn PL. On the other hand, questionnaire survey also revealed that 88.9% hatchery operator used artemia, commercial feed and custard whereas 11.1% hatchery operator (n = 9) used artemia and commercial feed at different life stages of shrimp and prawn PL (Figure 4.20). Actually, using yolk sac (Egg), corn flour, milk powder, lecithin, chingri brood, chord liver oil and agar powder the hatchery operators formulated custard for shrimp and prawn PL. At the same time, J.C. Hanekamp et al. 2003 reported that CAP was found in milk powder which is the main component of custard. So, milk powder might be a possible source of CAP. It was also found that most of the contaminated farms do not exchange water year after year. Basically, throughout the culture period shrimps and prawns exclude their shell and also release faeces sequentially that cause recontamination. So, no water exchange may transmit nitrofuran and chloramphenicol into the shrimp and prawn muscle.

5.2 Risk caused by SEM and AHD

The carcinogenic risk (CR) that to the people of Bangladesh obtained from the intake of SEM through consumption of prawn was estimated for different age intervals. The CR values of 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative 0-<70.7 age bins were 3.22E-06, 6.77E-07, 5.26E-07, 2.19E-07, 4.68E-08 and 4.69E-06 respectively (Figure 4.27) whereas threshold level was 1.0E-06 for causing cancer (USEPA 2004). So, it`s clear that existing concentration of SEM in prawn is a threat as carcinogenic agent for the people of Bangladesh especially for the children. On the other hand, carcinogenic risk of As to the rural people of Bangladesh was 2.2E-03 and 7.59E-04 from water ingestion and cooked rice respectively (Khan et al. 2012) whereas that risk of As and Pb to the urban people was 1.9E-04 and 2.3E-05 through consumption of vegetables were higher than the USEPA threshold level 1.0E-06 (Islam et al. 2014).

The non-carcinogenic risk (HQ) that obtained from the intake of AHD through consumption of shrimp was estimated for different age intervals. The HQ values of 0-<2, 2-<3, 3-<6, 6-<16, 16-<70.7 and cumulative 0-<70.7 age bins were 8.85E-07, 7.24E-07, 5.63E-07, 2.34E-07, 1.5E-07 and 2.56E-06 respectively (Figure 4.30) that were below the threshold level 1 (USEPA 2004). It means that existing concentration of AHD in shrimp has no adverse effect on the people of Bangladesh.

Chapter 6

Conclusions and Recommendations

Antibiotic tested data, questionnaire survey data and test result of collected samples revealed that no farmer deliberately used nitrofuran and chloramphenicol drugs in the shrimp and prawn farms. But these banned antibiotics were detected in shrimp and prawn meat, shell, PL, commercial feed and two unknown chemicals. So, the present study indicates that nitrofuran and chloramphenicol antibiotics are coming from hatchery, feed factories and use of contaminated shrimp/prawn shell as feed ingredient at farm level in shrimp and prawn.

The lifetime carcinogenic risk (CR) of SEM was 4.69E-06 through consumption of prawn whereas threshold level was 1.0E-06. So, existing concentration of SEM in prawn is a threat as carcinogenic agent for the people of Bangladesh especially for the children. On the contrary, lifetime non-carcinogenic risk (HQ) of AHD was 2.56E-06 that was below the threshold level 1 through consumption of shrimp It means that existing concentration of AHD in shrimp has no adverse effect on the people of Bangladesh.

So, the following strategies can adopt to reduce the contamination:

- It should encourage the hatchery, feed mill operators and farmers to use probiotics and approved antibiotics instead of nitrofuran and chloramphenicol and to maintain withdrawal period.

- It should aware the farmers to avoid shrimp and prawn shell as feed ingredient.

- It should bring all the shrimp and prawn hatcheries under government monitoring process though hatcheries are not included in National Residue Control Plan (NRCP). So, it should include shrimp and prawn hatcheries in NRCP.

- It should not provide contaminated prawn to the children and also build public awareness about it

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Appendix A

Antibiotic detected prawn ponds in 2011

Chloramphenicol(µg/kg)	AHD(µg/kg)	AOZ(µg/kg)	AMOZ(µg/kg)	SEM(µg/kg)
ND	ND	ND	ND	0.59
ND	ND	ND	ND	1.39
3.0	ND	ND	ND	8.85
ND	ND	ND	ND	1.11
ND	ND	ND	ND	1.02
0.18	ND	ND	ND	1.66
0.5	ND	ND	ND	ND
ND	ND	ND	ND	2.59
ND	ND	ND	ND	2.61
ND	ND	ND	ND	1.17
ND	ND	ND	4.34	ND
ND	ND	ND	7.9	ND
0.5	ND	ND	ND	3.73
ND	ND	ND	ND	2.46
ND	ND	ND	ND	0.63

Antibiotic detected prawn ponds in 2011(Rest part)

Chloramphenicol(μg/kg)	AHD(μg/kg)	AOZ(μg/kg)	AMOZ(μg/kg)	SEM(μg/kg)
0.66	0.25	ND	ND	0.36
0.7	ND	ND	ND	2.4
0.71	ND	ND	ND	ND
ND	ND	ND	ND	1.79
0.45	ND	ND	ND	ND
0.67	ND	ND	ND	0.33
ND	ND	ND	ND	0.5
17.37	ND	ND	ND	ND

Antibiotic detected prawn ponds in 2012

Chloramphenicol(μg/kg)	AHD(μg/kg)	AOZ(μg/kg)	AMOZ(μg/kg)	SEM(μg/kg)
ND	ND	ND	ND	0.88
ND	ND	ND	ND	3.81
ND	ND	ND	ND	4.63
ND	ND	ND	ND	1.07
ND	ND	ND	ND	1.64
ND	ND	ND	ND	2.21
ND	ND	ND	ND	1.52
ND	ND	ND	ND	0.88
ND	ND	ND	ND	1.32
ND	ND	ND	ND	3.67
ND	ND	ND	ND	0.89
ND	ND	ND	ND	0.75
ND	ND	ND	ND	2.91

Antibiotic detected prawn ponds in 2013

Chloramphenicol(µg/kg)	AHD(µg/kg)	AOZ(µg/kg)	AMOZ(µg/kg)	SEM(µg/kg)
ND	ND	ND	ND	3.1
ND	ND	ND	ND	3.82
ND	ND	ND	ND	1.36
ND	ND	ND	ND	1.5
ND	ND	ND	ND	0.91
ND	ND	ND	ND	0.36
ND	ND	ND	ND	6.02
ND	ND	ND	ND	6.41
ND	ND	ND	ND	2.1
ND	ND	ND	ND	3.55
ND	ND	ND	ND	7.28

Antibiotic detected prawn ponds in 2013 (Rest part)

Chloramphenicol(µg/kg)	AHD(µg/kg)	AOZ(µg/kg)	AMOZ(µg/kg)	SEM(µg/kg)
ND	ND	ND	ND	1.19
ND	ND	ND	ND	6.98
ND	ND	ND	ND	3.97
ND	ND	ND	ND	1.72
ND	ND	ND	ND	0.37
ND	ND	ND	ND	1.2
ND	ND	ND	ND	1.2
ND	ND	ND	ND	4.65
ND	ND	ND	ND	7.11
ND	ND	ND	ND	0.25

Antibiotic detected shrimp ponds in 2011

Chloramphenicol(µg/kg)	AHD(µg/kg)	AOZ(µg/kg)	AMOZ(µg/kg)	SEM(µg/kg)
ND	ND	ND	ND	0.44
ND	ND	ND	ND	0.3
0.09	ND	ND	ND	ND
0.48	ND	ND	ND	ND
0.12	ND	ND	ND	ND
0.48	ND	ND	ND	ND
0.56	ND	ND	ND	ND
0.47	ND	ND	ND	ND
0.65	ND	ND	ND	ND
0.17	ND	ND	ND	ND
0.09	ND	ND	ND	ND
ND	ND	ND	ND	4.45
0.75	ND	ND	ND	ND

Antibiotic detected shrimp ponds in 2012

Chloramphenicol(µg/kg)	AHD(µg/kg)	AOZ(µg/kg)	AMOZ(µg/kg)	SEM(µg/kg)
ND	0.98	ND	ND	ND
ND	1.07	ND	ND	ND
0.7	1.39	ND	ND	ND
ND	0.82	ND	ND	ND
ND	1.61	ND	ND	ND
ND	0.94	ND	ND	ND
ND	1.64	ND	ND	ND

Antibiotic detected shrimp ponds in 2013

Chloramphenicol(µg/kg)	AHD(µg/kg)	AOZ(µg/kg)	AMOZ(µg/kg)	SEM(µg/kg)
0.23	ND	ND	ND	ND
0.34	ND	ND	ND	ND
ND	1.28	ND	ND	ND
ND	1.93	ND	ND	ND
ND	1.08	ND	ND	ND
0.85	ND	ND	ND	ND

Appendix B

SHRIMP FARMER SURVEY QUESTIONNAIRE

PART 1: IDENTITY OF RESPONDENT

1.1 Name of respondent: ____

1.2 Sex of respondent: ☐ Male ☐ Female

1.3 Educational qualification: ☐ MA/MSc ☐ BA/BSc ☐ HSC ☐ SSC ☐ <SSC

PART 2: FAMILY INFORMATION

2.1 Family members: ☐ 3 ☐ 4 ☐ 5 ☐ >5

2.2 How many children go to school, college and university? ☐ School ☐ College ☐ University

2.3 Monthly income (Tk.): ☐ 10,000-15,000 ☐ 15,000-20,000 ☐ 20,000-25,000 ☐ 25,000-30,000 ☐ >35,000

2.4 Monthly expenditure (Tk.): ☐ 10,000-15,000 ☐ 15,000-20,000 ☐ 20,000-25,000

PART 3: LOCATION OF FARM

3.1 District: ____

3.2 Upazila: ____

3.3 Village: ____

3.4 Geographical information: Longitude __________________________ Latitude __________________________

PART 4: FARM INFORMATION

4.1 Farm area (Hectare): ☐ Own land ☐ Leased land

4.2 What is the lease value (Tk. / Hectare)?

4.3 How many ponds do you have? [ ]

4.4 Average water depth (m): ____________________________

4.5 Have your farm inlet/outlet? [ ] Only inlet [ ] Inlet and outlet

4.6 Have you any aerator? [ ] Yes [ ] No

4.7 Have you any test kit box for water parameters measurement? [ ] Yes [ ] No

4.8 If yes, what is the name of test kit box?

4.9 What was your culture technique? [ ] Traditional/Extensive

[ ] Semi-intensive [ ] Intensive

4.10 What was your culture type? [ ] Mono culture [ ] Poly culture

4.11 If mono culture, which species did you culture? [ ] Shrimp [ ] Prawn

4.12 If poly culture, which species did you culture?

1. Shrimp 2. Prawn 3. Tilapia 4. Carp 5. Others

4.13 Did any animals like cow, goat and ram etc. enter your farm during culture period?

[ ] Yes [ ] No

4.14 If yes, which animal entered into your farm? [ ] Cow [ ] Goat [ ] Ram

PART 5: POND PREPARATION

5.1 Did you dry your pond completely last year? [ ] Yes [ ] No

5.2 If yes, when?

5.3 If no, what types of chemicals did you apply to eradicate predatory fish and others predatory animals?

Sl.No.	Name of Chemical	Dose	Price(Tk.)
1			
2			
3			
4			

5.4 What kind of fertilizers did you use in your farm?

Sl.No.	Name of Fertilizer	Dose	Price(Tk.)
1	Lime		
2	Urea		
3	TSP		
4	DAP		
5	Zipsam		
6	Cowdung		

5.5 What was the source of water? ☐ Canal ☐ River ☐ Ground Water

☐ Neighbour pond

5.6 Did the water source normally supply enough water to permit you to practise?
☐ Yes ☐ No

5.67 If no, indicate the months when water supply was usually insufficient:____

PART 6: PL INFORMATION

6.1 What was the source of PL? ☐ Natural ☐ Hatchery

6.2 If natural, from where did you collect PL?

6.3 If hatchery, from which hatchery did you purchase PL?

6.4 What was your stocking density?

Sl.No	Name of Species	Stocking Density(Fry/Hectare
1	Shrimp	
2	Prawn	
3	Tilapia	
4	Carp	
5	Others	

PART 7: FEED INFORMATION

7.1 Did you apply any feed? ☐ Yes ☐ No

7.2 If yes, what types of feed did you apply? ☐ Handmade ☐ Factory produced

7.3 If handmade, what kinds of ingredient did you use?

Sl.No.	Name of Ingredient	Price (Tk./Kg)	%
1			
2			
3			
4			

7.4 If factory produced, what types of feed did you apply?

Sl.No.	Name of Feed	Name of Feed Company	Price (Tk./Kg)	Dose
1				
2				
3				
4				

7.5 What is the feeding frequency? ☐ Times/Day ☐ Times/Week

☐ Times/Month

PART 8: WATER MANAGEMENT

8.1 Did you apply any chemical in your pond during culture period for water treatment?
☐ Yes ☐ No

8.2 If yes, what types of chemicals did you apply?

Sl.No.	Name of Chemical	Name of Company	Dose	Price(Tk.)

8.3 Did you exchange your pond water? ☐ Yes ☐ No

8.4 If yes, how many times did you do that?

PART 9: DISEASE MANAGEMENT

9.1 Did you find any disease in your pond? ☐ Yes ☐ No

9.2 If yes, what types of medicines did you apply?

Sl.No.	Name of Disease	Name of Medicine	Name of Company	Dose	Price(Tk.)

PART 10: MANPOWER INFORMATION

10.1 How many workers did you deploy in your farm last year?

Types of Manpower	Permanent		Temporary(8Hours=1 Labour)	
	Number	Salary/Year	Number	Wage/Day (1 Day=8Hours)
Male				
Female				

10.2 Did you give extra salary/wage to your employee for their over time? ☐ Yes ☐ No

10.3 If yes, how much did you give (Tk. / hour)? ☐ Male ☐ Female

PART 11: PADDY CULTURE

11.1 Did you grow paddy in your pond? ☐ Yes ☐ No

11.2 If yes, what types of chemicals did you apply?

Sl.No.	Name of Chemical	Name of Company	Dose	Price(Tk.)

PART 12: PRODUCTION

Name of Species	Production (Kg)	Grade	Total amount(Tk.)
Shrimp			
Prawn			
Tilapia			
Carp			
Others			

PART 13: OTHERS

13.1 What types of problems did you face in the last year?

1.

2.

3.

4.

5.

Date:…………………………………..

____
Signature of the Interviewer

Appendix C

SHRIMP/PRAWN HATCHERY SURVEY QUESTIONNAIRE

PART 1: IDENTITY OF RESPONDENT

1.1 Name of respondent: ______________________________

1.2 Designation______________________

1.3 Sex of respondent: ☐ Male ☐ Female

1.4 Educational qualification: ☐ MA/MSc ☐ BA/BSc ☐ HSC ☐ SSC

☐ < SSC

PART 2: LOCATION OF HATCHERY

2.1 Name of the hatchery: ______________________________

2.2 District: ______________________________

2.3 Upazila: ______________________________

2.4 Geographical information: Longitude ______________________ Latitude ______________________

PART 3: HATCHERY FACILITIES

3.1 How many tanks do you have? ☐ Maturation tank ☐ Spawning tank

☐ Larval rearing tank ☐ Live food culture tank

☐ Water storage tank

3.2 What is the size of the tanks? ☐ Maturation tank ☐ Spawning tank

☐ Larval rearing tank ☐ Live food culture tank

☐ Water storage tank

3.3 What types of aerator do you use?

3.4 Do you use either test kit box or digital equipments for water parameters measurement?
☐ Test kit ☐ Digital equipment

3.5 If test kit, what is the name of test kit box?

3.6 If digital equipment, what types of equipments do you use?

Sl.No.	Name of Parameter	Name of Equipment	Price(Tk.)
1	DO		
2	CO_2		
3	Salinity		
4	NH_3		
5	p^H		
6	Temperature		

PART 4: BROOD INFORMATION

4.1 What is the name of species? ☐ Shrimp ☐ Prawn

4.2 What was the price of the brood?

Sl.No.	Name of Species	Types of Species	Price(Tk.)/ Piece
1			
2			
3			
4			

4.3 How many broods did you use last year? ☐ Male ☐ Female

4.4 What was the source of your brood? ☐ Bay of Bengal ☐ River ☐ Pond

PART 5: PL INFORMATION

5.1 Which PL did you sell? ☐ PL_{5-10} ☐ PL_{10-15} ☐ PL_{15-20} ☐ PL_{20-25}

5.2 Length of one cycle? ☐ 10-15 Days ☐ 15-20 Days ☐ 20-25 Days ☐ 25-30 Days

PART 6: WATER MANAGEMENT

6.1 What is the source of fresh water? ☐ River ☐ Canal ☐ Pond ☐ Ground water

6.2 What is the source of saline water? ☐ Brine ☐ River ☐ Canal

6.3 What are the desired limits of water parameters?

Sl.No.	Name of Parameter	Desired Limit
1	DO	
2	CO_2	
3	Salinity	
4	NH_3	
5	p^H	
6	H_2S	
7	Temperature	
8	Hardness	

6.4 What kind of chemicals do you use for the sterilization of water?

Sl.No.	Name of Chemical	Name of Company	Dose	Price(Tk.)

6.5 How many times did you exchange water in a production cycle?

PART 7: FEED INFORMATION

7.1 What types of feeds did you apply? ☐ Artemia ☐ Rotifer ☐ Custard

7.2 What was the price of artemia/rotifer cyst (Tk. /Kg)?☐ Artemia ☐ Rotifer

7.3 If custard, what kinds of ingredients did you use?

Sl.No.	Name of Ingredient	Price (Tk.)	%
1			
2			
3			
4			

7.4 Do you use any probiotic? ☐ Yes ☐ No

7.5 If yes, what is the name and dose of the probiotic?

PART8: HEALTH MANAGEMENT

8.1 Did you find any health hazard/disease last year? ☐ Yes ☐ No

8.2 If yes, what types of medicines did you apply?

Sl.No.	Name of Disease	Name of Medicine	Name of Company	Dose	Price(Tk.)

8.3 Do you use any growth promoter? ☐ Yes ☐ NO

8.4 If yes, what is the name and dose of the growth promoter?

8.5 Do you use any antibiotic? ☐ Yes ☐ No

8.6 If yes, what is the name and dose of the antibiotic?

PART 9: MANPOWER INFORMATION

9.1 How many workers did you deploy in your hatchery last year?

Types of Manpower	Permanent		Temporary(8Hours=1 Labour)	
	Number	Salary/Year	Number	Wage/Day (1 Day=8Hours)
Male				
Female				

9.2 Did you give extra salary/wage to your employee for their over time? ☐ Yes

☐ No

9.3 If yes, how much did you give (Tk. / hour)? ☐ Male ☐ Female

PART 10: PRODUCTION

Name of Species	Capacity (Million)	PL Production(Million)	Price(Tk./Million)	Mortality (Million)
Shrimp				
Prawn				

PART11: OTHERS

11.1 What types of problem do you face to execute this hatchery?

1.

2.

3.

4.

5.

Date:…………………………………..

____

Signature of the Interviewer

Appendix D

FEED FACTORY SURVEY QUESTIONNAIRE

PART 1: IDENTITY OF RESPONDENT

1.3 Name of respondent: ______________________________

1.4 Designation______________________

1.3 Sex of respondent: ☐ Male ☐ Female

1.4 Educational qualification: ☐ MA/MSc ☐ BA/BSc ☐ HSC ☐ SSC ☐ < SSC

PART 2: LOCATION OF FACTORY

2.1 Name of the factory: ______________________________

2.2 District: ______________________________

2.3 Upazila: ______________________________

2.4 Geographical information: Longitude ______________________ Latitude ______________________

PART 3: INGREDIENT INFORMATION

3.1 What types of ingredient did you use last year? ☐ Rice bran ☐ Wheat bran ☐ Maize

☐ Flour ☐ Soybean oil cake

☐ Mustard oil cake ☐ Fish meal

☐ Bone meal ☐ Blood meal

3.2 What were the sources of ingredient?

Sl.No.	Name of Ingredient	Local/Imported	Country	Quantity/Year	Price(Tk./MT)
1					
2					
3					
4					
5					
6					
7					
8					
9					

3.3 Are you using the same Ingredients in this year? ☐ Yes ☐ No

3.4 If no, mention the new Ingredients.

Sl.No.	Name of Ingredient	Local/Imported	Country	Quantity/Year	Price(Tk./MT)
1					
2					
3					
4					
5					
6					
7					
8					
9					

PART 4: ADDITIVES INFORMATION

4.1 What types of additives did you use last year?

Sl.No.	Name of Additives	Local/Imported	Country	Quantity/Year	Price(Tk./MT)
1					
2					
3					
4					
5					

4.3 Are you using the same additives in this year? ☐ Yes ☐ No

4.4 If no, mention the new additives.

Sl.No.	Name of Additives	Local/Imported	Country	Quantity/Year	Price(Tk./MT)
1					
2					
3					
4					
5					

PART 5: FEED PRODUCTION

5.1 What types of feeds did you produce last year?

Sl.No.	Name of Feed	Capacity (MT)	Production (MT)	Price(Tk./MT)
1	Fish feed			
2	Poultry feed			

5.2 What types of feed are you producing in this year?

Sl.No.	Name of Feed	Capacity (MT)	Production (MT)	Price(Tk./MT)
1	Fish feed			
2	Poultry feed			

5.3 What is the feed composition? ☐ Protein ☐ Fat ☐ Carbohydrate

PART 6: MANPOWER INFORMATION

6.1 How many workers do you deploy in your factory?

Types of Manpower	Permanent		Temporary(8Hours=1 Labour)	
	Number	Salary/Year	Number	Wage/Day (1 Day=8Hours)
Male				
Female				

6.2 Do you give extra salary/wage to your employee for their over time? ☐ Yes

☐ No

6.3 If yes, how much did you give (Tk. / hour)? ☐ Male ☐ Female

PART 7: OTHERS

7.1 What types of problem do you face to execute this factory?

1.

2.

3.

4.

5.

Date:…………………………………..

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Signature of the Interviewer