

# **Impact of Aquaculture Drugs and Chemicals on Aquatic Ecology and Productivity**

**Report  
June 2013**

**Dr. Md. Khalilur Rahman  
Principal Scientific Officer  
&  
Project Director**



**Ministry of Fisheries and Livestock  
Bangladesh Fisheries Research Institute**

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on  
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## Foreword

It is a pleasure for me to note that a report on research output on Impact of Aquaculture Drugs and Chemicals on Aquatic Ecology and Productivity is going to be published under the initiative of the Impact of Aquaculture Drugs and Chemicals on Aquatic Ecology and Productivity Project of BFRI. Aquadrugs and chemicals are widely used to produce fish and shrimp throughout the country. Rate and amount of use of aquadrugs and chemicals are increasing day by day as a result of intensification and diversification of aquaculture. Aquadrugs and chemicals are applied in every steps of aquaculture from pond preparation to harvest. The project has been designed to undertake research covering all aspects of use of chemicals and drugs in aquaculture to find out the impacts of such use on aquatic biota as well as in human health. Fish species diversity and density in open waters of Bangladesh are declining day by day due to over exploitation and degradation of habitats. Therefore, aquaculture has been emerged as an alternative for meeting the increasing demand. Bangladesh has 761 rivers, 146,890 ha of ponds, 5,488 ha of oxbow lakes and 140,000 ha of brackishwater areas, which offers excellent opportunities for culture of various species of fish prawn and shrimp. In order to fulfill the increasing demand, aquaculture in Bangladesh is being intensified. Farmers are stocking their pond at a greater rate, which necessitates greater use of fertilizers and supplementary feeding. Feed manufacturers are using different feed additive and growth enhancer for increased growth. High stoking density and use of artificial feed increases the risk of diseases outbreak in culture waters leading to mass mortalities and economic losses. EUS disease in fish seriously out broke in 1988. Shrimp aquaculture in Bangladesh seriously affected by SEMBV in 1994, which has become a major problem in recent years and led to great losses to shrimp producers.

The BFRI and BAU conducted research on various aspects of use of aqua-drugs and chemicals and this report is the outcome of three years endeavor. Results and findings of eight research projects have been highlighted.

I am very much hopeful that the report will be useful for the fishers, farmers, aquadrugs treaders, sellers, users, extension workers, students, teachers, specialists, decision makers, development partners and other interested people.

I praise all who have contributed in conducting research and preparing this report, which I am sure, will be an asset to help in augmenting conservation of fishery resources by applying appropriate aquadrugs and chemicals in farms.

**Prof. Dr. Subhash Chandra Chakraborty**  
Director General

## **Acknowledgement**

I like to express my deep appreciation to Prof. Dr. Subhash Chandra Chakraborty, Director General, Bangladesh Fisheries Research Institute (BFRI) for his support in implementing the project.

My thanks also go to the MoFL, Planning Commission and IMED, Dhaka, Bangladesh for their help and cooperation during project approval, execution, extension and evaluation.

I am also thankful to Dr. Yahia Mahmud, CSO and Director (F & A), Bangladesh Fisheries Research Institute (BFRI) for his dedicated interest, works and support in formulating and developing the project initially, to strengthen and conduct research on impacts of aquadugs.

I like to give thanks Mr. Md. Alamgir, SSO and former PD of the project for his cordial help and co-operation in executing the project.

My thanks also to all the Principal Investigators, who conducted research on aquadugs and provided valuable results.

My colleague in the Institute, Mr. Md. Razibul Karim, Executive Engineer, Mr. Md. Azizul Haque, Store Officer, Mr. Md. Enamul Haque, Accountant and Mr. Rezaul Karim Reza, Computer Operator, I would like to thank for their assistance throughout this programme. I also wish to thank Mr. Md. Razab Ali, Motor Diver, BFRI for his assistance in diverse ways.

Special thanks to Dr. Jubaida Nasreen Akhter, Senior Scientific Officer, Freshwater Station, BFRI for her generous help and support during implementing the project.

Finally, I like to express my gratefulness to all the staff of the project, fishermen, farmers, chemical and drug traders, sellers, users and representatives of different Agro-Chemicals Companies, who helped us to collect information on use of chemicals and drugs in aquaculture.

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June 2013

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3	Dr. Momtaz Begum, SSO, HQ, BFRI, Mymensingh	Determination on Efficacy of Selective Chemicals and Drugs Used in Aquaculture in Bangladesh
4	Dr. Gias Uddin Ahmed, Professor, BAU, Mymensingh	Study on Impact of Aquaculture Drugs and Chemicals on Fish Health, Productivity and Biodiversity
5	Dr. Md. Nurul Absar Khan, Professor, CVU, Chittagong Dr. Md. Shaheed Reza, Associate Professor, BAU, Mymensingh	Influence of Chemicals and Drugs on Microbial Flora used Indiscriminately in Aquaculture
6	Dr. Md. Kamal, Professor, BAU, Mymensingh	Determination of Safety Soses of Aqua Drugs in Bangladesh
7	Dr. Gias Uddin Ahmed, Professor, BAU, Mymensingh	Testing of Local Herbal Products for Fish/Shrimp Disease Prevention and Control
8	Dr. Md. Shahab Uddin, Associate Professor, SAU, Sylhet Mohammed Zaher, CSO, MFTS, Coz's Bazar	Factors Causing Emerging Shrimp Diseases and Development of Their Health Management Strategies

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## **Executive Summary**

Development project on Impact of Aquaculture Drugs and Chemicals on Aquatic Ecology and Productivity has been undertaken to strengthen research activities of the Institute. The project has been designed to undertake research covering all aspects of use of chemicals and drugs in aquaculture to find out the impacts of such use on aquatic biota as well as in human health. Fish species diversity and density in open waters of Bangladesh are declining day by day due to over exploitation and degradation of habitats. Therefore, aquaculture has been emerged as an alternative for meeting the increasing demand. Bangladesh has 761 rivers, 146,890 ha of ponds, 5,488 ha of oxbow lakes and 140,000 ha of brackishwater areas, which offers excellent opportunities for culture of various species of fish prawn and shrimp. In order to fulfill the increasing demand, aquaculture in Bangladesh is being intensified. Farmers are stocking their pond at a greater rate, which necessitates greater use of fertilizers and supplementary feeding. Feed manufacturers are using different feed additive and growth enhancer for increased growth. High stocking density and use of artificial feed increases the risk of diseases outbreak in culture waters leading to mass mortalities and economic losses. EUS disease in fish seriously out broke in 1988. Shrimp aquaculture in Bangladesh seriously affected by SEMBV (Systematic Ectodermal Monodon Baculovirus) in 1994, which has become a major problem in recent years and led to great losses to shrimp producers.

In aquaculture, chemicals are used mainly in the treatment and prophylaxis of disease problems, which constitute the largest single cause of economic losses. However, the increasing use of chemicals in aquaculture has lead to widespread public concern. The concerns related to human health due to chemical use in aquaculture are repeatedly found in the published literature. They include allergic reactions in the previously sensitized persons triggered by chemical residues, and the potential impact on human health resulting from the emergence of drug-resistance bacteria caused by used of sub-therapeutic levels of antibiotic and by antibiotic residues persisting in the sediments of aquaculture environments.

Results and findings of eight research projects have been highlighted. The benefits of chemical usage are many. Chemicals increase production efficiency and reduce the waste of other resources. Currently, huge number and amount of chemicals and drugs are

in use in aquaculture. Chemicals have many uses in aquaculture, the types of chemicals used depending of the nature of the culture system and the species being cultured. Chemicals and drugs are essential components in pond and tank construction, soil and water management, enhancement of natural aquatic productivity, transportation of live organisms, feed formulation, manipulation and enhancement of reproduction, growth promotion, health management, and processing and value enhancement of the final product. It assists in increasing hatchery production and feeding efficiency, and improves survival of fry and fingerlings to marketable size. It is also used to reduce transport stress and to control pathogens, among many other applications. Farmers use different forms of limes, such as, calcium oxide (CaO), slake lime {Ca(OH)<sub>2</sub>}, agricultural lime (CaCO<sub>3</sub>) and dolomite lime {CaMg(CO<sub>3</sub>)<sub>2</sub>} and fertilizers, such as, urea, TSP and MP.

The most frequently used chemicals and drugs includes Timsen, Geofersh, Geoprime, Geotox, Oxygold, Oxymax, Oxyflow, Oxymax, Oxymore, Aquamycine, Oxyentine and Renamycine. Among the drugs tested, Timsen was found best in all aspects of improving fish health and water quality. Antibiotics were found effective to control bacterial disease in fish.

Disease is one of the most critical problems of fish production both in culture system and wild condition of Bangladesh. Fishes have been suffering from many diseases such as Epizootic Ulcerative Syndrome (EUS), tail and fin rot, fungal, parasitic and bacterial infections. In most cases hemorrhages, septicemia, lesions and gill damage are the common symptoms of the diseased fish. Edwardsiellosis and bacterial haemorrhagic septicaemia disease was identified as problem and associated in cultured *Pangasianodon hypophthalmus*. *Streptococcus agalactiae* is a major problem in tilapia culture, especially in the cage culture. *Flavobacterium columnaris* caused up to 90% mortality in fry of Thai koi (*Anabas testudineus*) and Tilapia (*Oreochromis niloticus*). Currently, diseases in Shing (*Heteropnaustes fossilis*), Thai koi (*A. testudineus*) and Tilapia (*O. niloticus*) are threat to the fish growers. Pathogens, excessive high stocking density, higher input and inadequate husbandry practices accelerate diseases in the fish farm. In an interview-based questionnaire survey there were 16 relative sources of risk identified from the disease farms. Sources of risk for disease outbreak includes higher stocking density, water quality deteriorate, pond connected to other water body, incomplete pond

preparation and disease in the previous season in culture ponds were found 92%, 88%, 84%, 72% and 72%, respectively in the disease farm. Emerging diseases causing mass mortality were recorded in *H. fossilis*, *P. hypophthalmus* and *A. testudineus* in different Upazillas under Mymensingh district. In December 2012, mass mortality was observed in *Catla catla* at Mahamaya Lake in Mirsora Upazilla under Chittagong district.

Ten different categories and 50 different types of chemicals were found to use in aquaculture activities. They are antibiotics, disinfectants, gas removal, oxygen supplier, vitamins and minerals, growth promoter, insect killer, algae killer, predator killer and pH balance. Among those, 15 types are widely used by the farmers such as Renamycin, Amoxifish, Ossi-C, Timsen, Aquamysine, Aquamycine, Virex, Aquakleen, Geolite gold, Oxy Dox F, Polgard plus, Charger gel, Seaweed, Bactisal and Deletix. Productions of Pangas and Koi in Gouripur and Muktagacha Upazillas were almost double in the chemical treated ponds compared with non treated ponds.

In Upazillas like Fulpur, Muktahacha and Fulbaria EUS, dropsy, Edwardsiellosis diseases were recorded with Pangas and Tilapia. Shing had 90-100% mortalities within very short period from unknown diseases with no obvious symptoms (except swollen abdomen and spots) provided 30-100% recovery after application of drugs. In EUS affected Tilapia in Fulpur (20%) and Muktagacha (30%) and farmers used Renamycin, Polgard plus and Ossi-C with a result of 80-95% recovery. In Edwardsiellosis affected Pangas in Fulpur (80%) and Fulbariaa (50%), farmers used Renamycin, Timsen, Polgard plus and Ossi-C in Fulpur and Geolite and Timsen in Fulbaria having 80% recovery in both Upazillas. It was thus observed that aqua drugs played excellent role in recovery of fish diseases and maintenance of health. Histopathology in the control ponds, skin-muscle, liver, kidney and gill of fish had almost normal structure. However, in the chemical treated ponds, the above mentioned investigated organs of fishes had remarkable pathological changes like necrosis, hemorrhage, vacuum, melanocytes and partial loss of organs. Thus fishes of chemical treated ponds although clinically looked normal, but histopathologically they were severely affected.

Microorganisms reside in the water and sediment of aquaculture facilities, as well as in and on the cultured species. They may have positive or negative effects on the outcome of aquaculture operations. Positive microbial activities include elimination of toxic

materials such as ammonia, nitrite, and hydrogen sulfide, degradation of unused feed, and nutrition of aquatic organisms such as shrimp, fish production. Quantitative estimation of aerobic heterotrophic bacteria in pond water and in fish gill filaments and intestine of Ruhu, Catla, Shingi, Magur, Pangas, Tilapia and Thai koi after feeding of OTC medicated feed were estimated, and compared with that of pond condition. There were significant changes in the total bacterial load after feeding antibiotic medicated diets. The pattern of change in bacterial load in 5 test species was more or less similar. Antibiotic resistance was developed in bacteria which were collected from OTC treated feed for 5 days. Shelf life of ice stored Ruhu fish (that were fed antibiotic medicated diet prior to killing) showed fish were acceptable upto 16 days of storage, indicating little or no effect of antibiotics on their shelf-life and post-harvest quality.

Chemicals and drugs including antibiotics are applied in ponds to improve water quality and control diseases. Antibiotics are a group of natural or synthetic compounds that kill bacteria or inhibit their growth and heavy amounts of antibiotics are administered in fish feed for prophylactic (disease prevention) and therapeutic (disease treatment) purposes in aquaculture facilities worldwide. Despite the widespread use of antibiotics in aquaculture facilities, limited data are available on the specific types and amounts of antibiotics used. Application dose of chemicals and drugs is important as lower doses do not improve water quality or control disease while higher dose can kill fish with severe consequences on water quality. Due to health concern, EU and US FDA banned several chemicals and drugs those are not allowed to use in aquaculture. Safty doses of commonly used chemicals and drugs have been determined that will help to minimize deterioration of aquatic habitat and will help to increase fish production.

Chemotherapy has progressed internationally for treating the most diversified infectious disease of fish. However, there are problems associated with the use of such chemicals. Thus it is the demand of the time to look for alternative means of commercial synthetic drugs. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced. Herbal medicine or phytochemicals have antiviral, antibacterial, antifungal and antihelminthic properties. Medicinal plants are vital source of drugs from the ancient time. In Bangladesh, different kinds of medicinal herbs are available which grow in roadside, small jungles are fellow

lands and most of them are cultivable with very low cost. Many species of these herbs are used as directly human food or as medicine such as, *Andrographis paviculata*, *Azadirachta indica*, *Basella alba*, *Allium cepa*, *Allium sativum*, *Calotropis gigantean* and *Monordica chorantia*.

Experiments were conducted on selection of medicinal plant products, preparation of extracts (crude, semi-crude and fine), selection and collection of fish species, isolation of bacteria and use of medicinal plant extracts on disease recovery of fishes. Twelve medicinal plant products, six crude extracts, two semi-crude extracts and four fine extracts were collected and prepared to observe their effect on disease recovery of fish. Diseased sarpunti and rui were treated with neem seed oil, kalojira seed oil, neem leaf extract and mehagoni seed extract for 28 days. Neem seed oil extracts exhibited satisfactory recovery in third week with 6 ml/kg feed dose. Disease fish treated with kalojira seed oil showed the best performance in respect of disease and wound recovery. Better recovery were found in third week at a dose of 4 ml/kg feed and 6 ml/kg feed. However, fishes treated with neem leaf extract and mehagoni seed oil extract showed poor performance in respect of disease recovery of fishes. Among doses 6 ml/kg feed exhibited the best performance followed by 4 ml/kg feed. More intensive and elaborate studies are necessary to observe the effects of chemicals, drugs and others with more biochemical parameters.

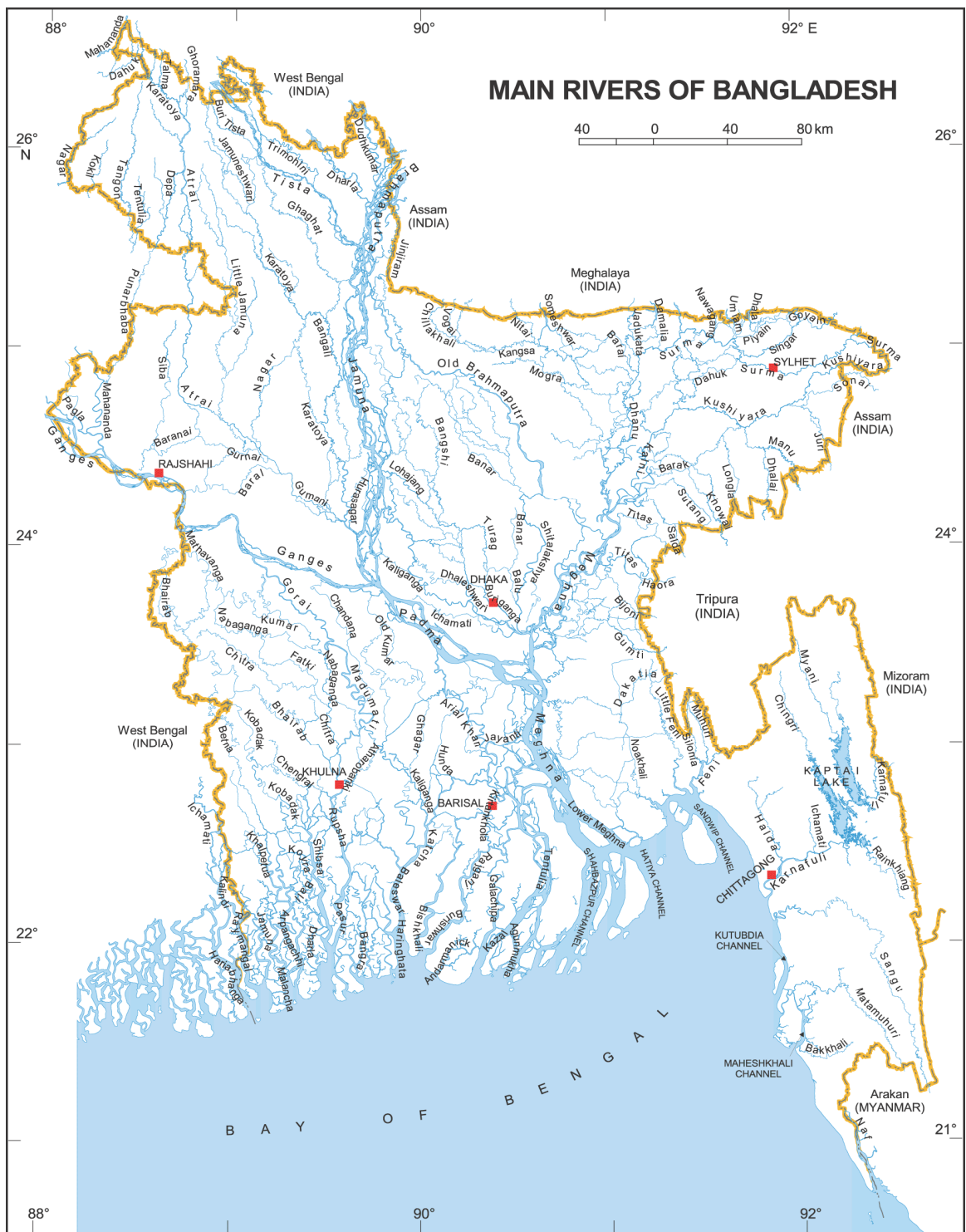
## **1. Introduction**

Worldwide pesticide usage has increased dramatically during the past two decades, coinciding with changes in farming practices and increasingly intensive agriculture. Environmental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish. Residual amounts of pesticides and their metabolites have been found in drinking water and foods, increasing concern for the possible threats to human health posed by exposure to these chemicals. Contamination of surface waters has been well documented worldwide and constitutes a major issue at local, regional, national, and

global levels (Cerejeira *et al.* 2003, Spalding *et al.* 2003). Fish exposed to insecticides exhibit a variety of physiological responses, including blood balance disturbances. In order to make an accurate assessment of the hazards that a contaminant may pose to a natural system, behavioural indices selected for monitoring must reflect the organism's behaviour in the field. Repeated opening and closing of the mouth and opercular movements are obvious indicators of a toxicant's effect in fish. Biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust 2000). Biochemical alterations are usually the first detectable and quantifiable responses to environmental change. The evaluation of haematological characteristics of fish has become an important means of understanding normal and pathological processes and toxicological impacts. Haematological alterations are usually the first detectable and quantifiable responses to environmental change (Wendelaar Bonga 1997). Information about drug residues and pharmacokinetic parameters in aquatic species is relatively sparse. In addition, it is difficult to rapidly compare data between studies due to differences in experimental conditions, such as water temperatures and salinity.

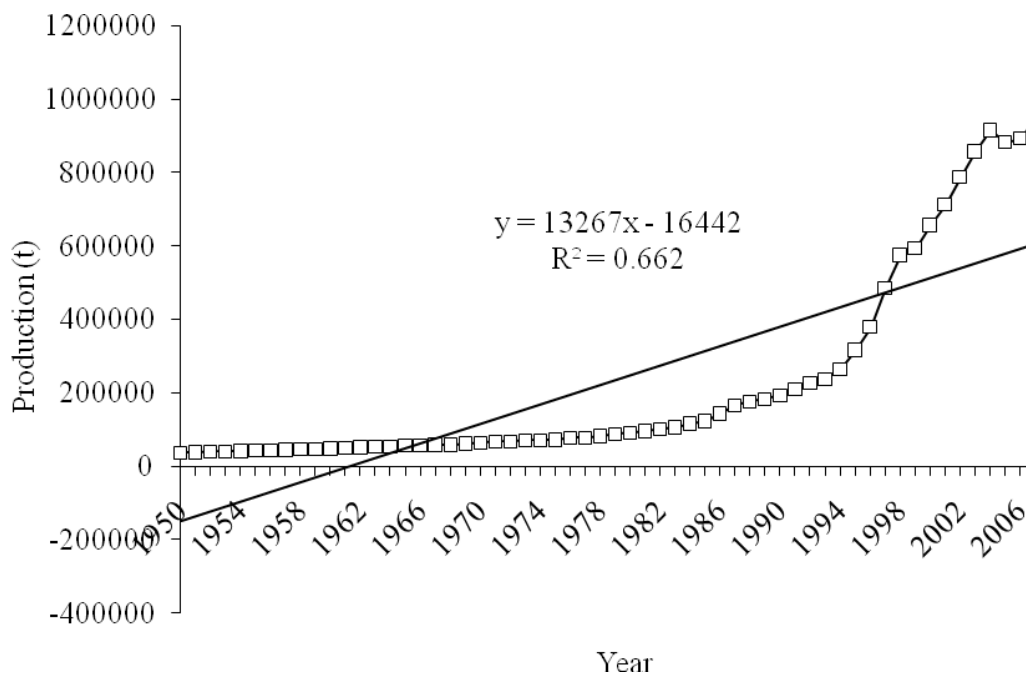
## **2. Scope of the Project**

Toxicological and environmental problems resulting from the widespread use of pesticides in agriculture have raised concerns, particularly with respect to the potential toxic effects in humans and animals. The acute exposure of rainbow trout and common carp to the pyrethroids deltamethrin, cypermethrin, and bifenthrin are associated with alterations in haematological and biochemical indices as well as in tissue enzymes, resulting in stress to the organism. Pyrethroids are therefore classified as belonging to substances strongly toxic for fish. Long-term exposure to triazines terbutryn and simazine in environmental concentrations can affect the biochemical, haematological, and biometric profiles of common carp. Biometric, blood, liver biomarkers, and histopathological responses could be used as potential biomarkers for monitoring residual pesticides present in aquatic environments and provide useful parameters for evaluating physiological effects in fish. Other classical morphologic indices (e.g. condition factor and hepatosomatic index) in fish could provide useful information for evaluating environmental stress.



**Fig. 1** Rivers of Bangladesh

Bangladesh is fortunate enough having extensive and huge water resources scattered all over the country (Fig. 1). The fisheries resources in the country are highly diversified. The inland capture fisheries resources consists of rivers, canals, floodplains, oxbow lakes, reservoirs, inundated paddy fields and estuaries, covering an area of 4.3 million ha. The capture fisheries include freshwater ponds (0.292 million ha) and coastal shrimp farms (0.14 million ha). There are 260 fish species inhabit rivers, haors, baors and beels of Bangladesh (Annexure 1). With these available and adequate resources, Bangladesh offers a unique opportunity to produce enough fish and shrimp for export and domestic consumption (Fig. 2). However, there are serious constraints such as the demographic pressure, industrial and agricultural developments etc. leading to serious deterioration of the aquatic ecosystems beyond ecological tolerance. Natural disasters and man-made disturbances are also considered as serious constraints in accelerating development process of the sector. In order to address these diversified issues, an appropriate and well thought research plan is essentially needed with long-term vision for a sustainable development of the fisheries sector.



**Fig. 2** Aquaculture production in Bangladesh



There are 761 rivers in Bangladesh (Fig. 1). Many rivers of Bangladesh especially passing through a town/ city are polluted due to domestic, industrial, agricultural wastes and effluents. The rivers Atrai (Natore), Balu (Tongi), Bangalee (Bogra), Bhairab (Jessore), Brahmaputra (Mymensingh), Bongshi (Gazipur), Buriganga (Dhaka), Chitra (Narail), Dhaleshwari (Munshiganj), Gomti (Comilla), Gorai (Kushtia), Haridhoa (Madhabdi), Jamuna (Jamalpur), Jamuneshwari (Joypurhat), Kaliganga (Manikganj), Kapotaksha (Jessore), Karatoa (Rangpur), Karnaphuli (Chittagong), Kheru (Bhaluka), Kirtonkhola (Barisal), Madhumati (Magura), Mahananda (Chapainawabganj), Meghna (Bhairab bazar), Mogra (Netrokona), Nabaganga (Jhenaidaha), Narashunda (Kishoreganj), Rupsha (Khulna), Shitalakshya (Narsingdi), Surma (Sylhet), Turag (Tongi), Teesta (Dinajpur) and Teetas (Brahmanbaria) are polluted due to domestic and industrial effluents. Industries along the side of the River Karnaphuli dump 50 to 60 t of wastes per day directly into the river. Moreover, 05 major canals carry domestic and municipal wastes and effluents to the River Karnaphuli. The Balu, Buriganga, Karnaphuli, Surma and Turag are the most polluted rivers in Bangladesh. Chittagong Urea Fertiliser Factory discharges untreated effluents directly into the River Karnaphuli. Water quality of rivers of Bangladesh become degraded due to leakage of oil and lubricants from millions of engine boats. In 1992, rivers of the Sundarban became highly polluted due to oil discharge/ leakage from the ships anchored at Chalna port. Rivers flowing by the side of a city/ town or have industries on the bank are most polluted as these receive municipal, domestic and industrial wastes and effluents (Table 1).

**Table 1** Major polluted rivers of Bangladesh with polluting industries, pollutants and consequences

River	Polluting industry	Discharge rate	Pollutants/ Chemicals	Remarks
Balu (Tongi)	Effluents from 268 different industries	Huge	Cd, Cr, Pb, As and Zn	No fish
Bhairab (Khulna)	Khulna News Print Mills	4 500 m <sup>3</sup> hr <sup>-1</sup>	K, Ca, Mn, Fe, Cu, Zn, As, Br, Pb, Ni, Sr, Cd, Rb and Ti	No fish
Bongshi (Gazipur)	Effluent from 187 industries	Huge	Chemical mixed effluents	No fish
Brahmaputra (Mymensingh)	Mymensingh city, industries and factories	Huge	Chemical mixed effluents	Decreased fish production

River	Polluting industry	Discharge rate	Pollutants/ Chemicals	Remarks
Buriganga (Dhaka)	Five main drains of Dhaka city	0.60-2.9 m <sup>3</sup> s <sup>-1</sup>	Untreated domestic and industrial effluents	No fish
	277 tanneries	88t waste and 22 000 litre of effluent day <sup>-1</sup>	Different chemicals	
	Passenger and merchant ships	Huge	Oil, grease and lubricants	
Chandana (Faridpur)	Faridpur Sugar Mills	Huge	Chemical mixed effluents	Decreased fish production
Dhaleshwari (Munshiganj)	Cement Factory, many other factories and industries	Huge	Chemical mixed effluents	Decreased fish production
Haridhoa, Kalagasia and Paharia (Narsingdi)	2 000 textile mills & fabric industries	Huge	Chemical mixed effluents	No fish
Ichhamoti (Pabna)	North Bengal Sugar Mills	Huge	Blackish water, Chemical mixed effluents	No fish
Jamuna (Jamalpur)	Jheel Bangla & Dewanganj Sugar Mills, Urea Fertiliser Factory	Huge	NH <sub>3</sub> , CaCl <sub>2</sub> , NaOH, H <sub>2</sub> SO <sub>4</sub> and lubricants	Decreased fish production
Jamuneshwari (Rangpur)	Shampur Sugar Mills, Rangpur Distilleries	Huge	NH <sub>3</sub> , CaCl <sub>2</sub> , NaOH, H <sub>2</sub> SO <sub>4</sub> , lubricants, hot water (over 100 °C)	No fish
Kaliganga (Manikganj)	Fabric industries	Huge	Chemical mixed effluents	No fish
Kapotaksha (Jessore)	Jessore city	Huge	Solid and liquid wastes	No fish
Karatoa (Panchagarh)	Panchagar & Satabganj Sugar Mills, Zaz Distilleries	Huge	NH <sub>3</sub> , CaCl <sub>2</sub> , NaOH, H <sub>2</sub> SO <sub>4</sub> , lubricants, hot water (over 100 °C)	No fish
Karnaphuli (Chittagong)	144 industries	10-12 million gallon day <sup>-1</sup>	Hg, Pb, Cr, Cd & As	Poisonous water, Fish diversity and density have declined alarmingly.
	297 industries	400 t waste day <sup>-1</sup>	Degradable and persistent organic and inorganic compounds	
	40-50 Oil tankers	6 000 t yr. <sup>-1</sup>	Oil, lubricants	
Khalisa danga (Natore)	North Bengal Sugar Mills	Huge	Blackish water, Chemical mixed effluents	No fish
Kirtonkhola (Barisal)	Municipal & Industries	Huge	Chemical mixed effluents & wastes	Decreased fish production

River	Polluting industry	Discharge rate	Pollutants/ Chemicals	Remarks
Kushiara (Sylhet)	Fenchuganj fertilizer factory	Huge	CO <sub>2</sub> , NH <sub>3</sub> , SO <sub>2</sub> , (CO <sub>2</sub> + N <sub>2</sub> ), oil, CaCl <sub>2</sub> , NaOH, H <sub>2</sub> SO <sub>4</sub> and lubricants	Decreased fish production
Mathabhanga (Darshana, Kushtia)	Carew & Company, Darshana Sugar Mills	Huge	Chemical mixed effluents	No fish
Meghna (Bhairab bazar)	Ashuganj Fertiliser Factory	Huge	Ammonia and other chemicals	Decreased fish production
Mongla, Pasur and Rupsha (Khulna)	Merchant ships, oil tankers and marine vessels	Huge	Oil, grease and other lubricants	Decreased fish species diversity and production
Nabaganga (Jhenaidaha)	Mobarakganj Sugar Mills	Huge	Blackish water, Chemical mixed effluents	No fish
Narashunda (Kishoreganj)	Kaliachapra Sugar Mills	Huge	Chemical mixed effluents, hot water (over 100 °C)	No fish
Narod Nad (Natore)	North Bengal Sugar Mills and Jamuna Distilleries	Huge	Chemical mixed effluents, hot water (over 100 °C)	No fish
Padma (Pakshee)	North Bengal Paper Mills	Huge	NaOH, Cl, Hg, Calcium hypochloride	Decreased fish production
Rupsha (Khulna)	Khulna city, many boats, ships & industries	10 t day <sup>-1</sup>	Solid and liquid wastes, oil, grease, lubricants	Decreased fish production
Sangu (Chittagong)	Fishermen	Huge	Fish poison (Toxic chemicals)	Fish killing
Shitalakshya (Ghorashal)	Meghna Cement Factory, Ghorasal Urea Fertiliser Factory	Huge	NH <sub>3</sub> , CaCl <sub>2</sub> , NaOH, H <sub>2</sub> SO <sub>4</sub> and lubricants	No fish
Surma and Dhanu (Sunamganj)	Chatak Paper and Pulp Mill	1 200-1 300 m <sup>3</sup> ha <sup>-1</sup>	NaOH, Cl, Hg, Calcium hypochloride	Decreased fish production
Tulshiganga (Noagoan)	Industries Noagoan Municipal area	Huge	Solid and liquid wastes and effluents	No fish
Turag (Tongi)	250 different industries	Huge	K, Ca, Mn, Fe, Cu, Zn, As, Br, Pb, Ni, Sr, Cd, Rb and Ti	No fish

In Bangladesh, the aquaculture industry has started to expand from mid 80's. As a consequence of expansion, usage of chemicals and drugs has been increased as a part of management. It is reported that in commercial farming in the country the farmers exercise high stocking density, which subsequently necessitates larger use of fertilizers and supplementary feed. Thus, water quality and aquatic environment of the farms are deteriorated, which make the fish/shrimp more susceptible to diseases. The disease outbreak in aquaculture occasionally leads to mass mortalities and economic loses. Therefore, the farmers/entrepreneurs indiscriminately use chemotherapeutics, feed additives, growth enhancers etc. in aquaculture in order to increase fish/shrimp production and to combat diseases. The following are some of the commercial chemical products and drugs presently used in aquaculture: Lime, Urea, NPK, Heptachlor, Diazinon, Sumithion, Malathion, Dipterex, Diazinon, Endrin, Di-eldrin, DDT, Mirex, Malachite Green, Dicofol, Iodine, Chlorine, Zeolite, Benzalkonium chloride (BKC), Dipterex (Chlorofos, Dylox, Foschlor, Masoten, Neguvon, Trichlorofon), Formalin, Acriflavin, Antibiotics (Tetracycline, Teramycene, Nitrofurantoin, Chloramphenicol, etc), Potassium Permanganate ( $KMnO_4$ ), Disodium Ethylene Diamine Tetraacetate (EDTA), Aquasafe, Intersafe, Copper A, Povidine, Aquadine 10 and Hormone. However, the list is by no means exhaustive.

In Bangladesh, as in many other developing countries, chemicals and drugs are widely used for the treatment of bacterial diseases of fish and shrimp in commercial aquaculture. They are also used to improve water quality, killing unwanted fish and to enhance production. Particularly, after mid 80's when the aquaculture industry has started to expand in the country, usage of toxic chemicals and drugs has also been increased. Thus, the toxic chemicals and drugs subsequently get access in aquatic biota, particularly in fish and shrimp, and in turn, human health through food chain, which is very much detrimental for the consumers. This toxic deposition in human body may remain year after year, and may cause serious damage to the next generation. Moreover, the toxic chemicals may hamper water quality and pond productivity in a long run. It can also damage the microbial community of the water body. The above cumulative impact ultimately hampers the total fish production in the country, which is the serious concern.

The purposes of usage of some of the widely used chemicals/drugs in aquaculture are briefly described below-

Lime is a major chemical used in Bangladesh for soil and water treatment in aquaculture. It is used to correct pond bottom and stabilize water pH. It is also reported to ensure a healthy plankton bloom. The farmer must understand the reactions of the various types of lime to be able to use them for the right purpose and at the proper dose. Otherwise, it can result negative impact on aquatic environment.

Fertilization is a basic part of pond preparation. Plankton as food for herbivorous fish must be in adequate quantities before stocking fish. Poultry manure and cow dung are commonly used for the purpose. Inorganic fertilizers such as urea and NPK mixture are widely used in pond for phytoplankton production. If the desired phytoplankton bloom is not achieved, more fertilizer is added, which may cause water pollution.

Zeolite is applied to ponds to remove hydrogen sulphide, carbon dioxide and ammonia, as it has a strong capacity to absorb molecules. Shrimp farmers use zeolite to clean pond bottoms. Zeolite is available in the market under various brand names, and it is supplied as fine grains in bags. The effectiveness of zeolite is still questionable.

Dipterex is widely used to treat for crustacean, monogenean, and protozoan parasites in pond cultured fish. Recently, Dipterex has been applied to water storage canals and reservoirs to eliminate wild crustacean vectors of shrimp viruses that enter ponds through the water supply. These vectors were found to carry systemic ectodermal and mesodermal baculovirus (SEMBV) and yellowhead disease (YHD). To completely eliminate these crustaceans, farmers use Dipterex at 0.3-0.5 ppm or higher concentrations than those used for fish.

Diazinon, Sumithion, Malathion, Phostoxin, Endrin, Di-eldrin and DDT are widely used in pond aquaculture as insecticides and for killing predators/unwanted fish during pond preparation for fingerling stocking. It was also reported that annually 11,000 tons of 242 types of pesticides/insecticides are used in rice field in Bangladesh (Mazid 2002). The most commonly used pesticides/insecticides are Heptachlore, Diazinon, Ripchord, Sumisidin, Furan, Basudin, Eldrin and Dieldrin. The pesticides/insecticides used in rice field are being washed out into water. It has resulted in eutrophication and

deterioration of water quality, affecting the biotope and thus their fishery, and in turn, human health. Study is needed along the line for the safety of human health.

Chlorine is used routinely to disinfect water supplies in fish and shrimp hatcheries in Bangladesh and elsewhere in the world. It is also used to eliminate harmful organisms entering the pond with water. The most commonly used form of chlorine is powdered calcium hypochlorite (65% active chlorine content).

Potassium Permanganate ( $\text{KMnO}_4$ ) is one of the first chemicals to be used as a chemotherapeutant in aquaculture, and has been applied since the early part of the century. In Thailand, it is used to treat against external parasites such as monogeneans, particularly in the aquarium fish industry. When applied at the rate of about 5 ppm, it is also a good treatment for external bacterial infections such as Columnaris disease. To treat against *Aeromonas hydrophila*, it can be used at 4 ppm in excess of potassium permanganate demand. For aquarium fish, it can be used at up to 500 ppm as a dip treatment for 5 minutes. EDTA is a chemical used to improve water quality by reducing heavy metal concentrations. In shrimp larval rearing, it is applied prior to stocking of nauplii. Many hatcheries use EDTA as a treatment for ectocommusal fouling to stimulate juvenile molting. EDTA is normally applied to remove organic substances in the water. Malachite Green appeared to be very effective to treat "Ich" in both aquarium and food fish. It is also used to control *Lagenidium* in shrimp hatcheries.

BKC is one of the broad spectrum disinfectants used in aquaculture. Thai shrimp farmers use it to reduce the concentration of plankton and dinoflagellates in closed pond systems. If it is used in a very small amount (0.1-0.5 ppm) and applied only in one corner, it will not kill plankton. However, if applied in large amounts, the resulting decomposition of organic matter will have an effect on animal health.

Iodine is widely used as a disinfectant in hatcheries and ponds. Granular iodine is thoroughly dissolved in water before spraying over the pond bottom to eliminate aquatic bacteria and other pathogens. Copper compound is one of the oldest and most widely used chemicals in fish culture. It is used as a parasiticide against external protozoan infestation. If copper compounds are used continuously in shrimp ponds, copper may accumulate in the pond bottom, which is dangerous to shrimp.

Antibiotics are commonly used in aquaculture nowadays. Infections due to bacteria are major disease problems in both freshwater and brackishwater aquaculture. *Aeromonas* bacteria causes major problems in the culture of freshwater fish and other aquatic animals. Various species of *Vibrio* are involved in diseases of brackishwater species including shrimp. To treat diseased fish, antibiotics are generally applied orally by mixing with feed. Injection is applied to large fish. Antibiotics are most likely to be effective when administered at the early stage of a disease. At later stages sick fish normally refuse to eat. Antibiotics are added to fish feed as growth promoters. The use of antibiotics for aquatic animals may not only initiate environmental pollution problems but also can affect human health due to drug residues.

Among the antibiotics, Tetracyclines (oxytetracycline) were identified as the most commonly used drugs in fish culture practices. The occasional uses of chloramphenicol, oxolinic acid, nitrofurans, formalin etc. were also reported. However, the use of hazardous antibiotics in shrimp aquaculture in Bangladesh has led to widespread consumer health concern, particularly to the foreign consumers of USA and EU. Therefore, it is urgently needed to determine the residual effect of toxic antibiotics in pond sediment water and fish/shrimp health. In freshwater fish, it is used effectively against *Aeromonas hydrophila*. Oxolinic Acid is approved by the US FDA for use in aquaculture and is now widely used in shrimp for treatment of vibriosis. For shrimp, the rate of use is 2 gm/kg feed/d for 5d, and the withdrawal period is about 15 d at temperatures greater than 22 °C.

Feed Additives are used now a day in aquaculture. Artificial feeds are often advertised to contain not only protein, carbohydrate and fat, but also additives such as vitamins, minerals, carotenoid pigments, phospholipids and many others, to enhance growth and survival of cultured animals. Hormones such as corticosteroids, anabolic steroids and other steroids have been incorporated in feed in shrimp hatcheries to make the larvae look healthy and uniform in size. Vitamins (Vitamin C) are widely used in shrimp diets. In extensive culture systems, natural food may be abundant enough to provide essential vitamins, as aquatic organisms require only minute amounts of these substances for normal growth, metabolism and reproduction. However, in intensive aquaculture

systems, natural food is limited, so that the addition of vitamins to the diet is recommended.

Heavy metals (e.g. Cu, Cd, Co, Cr, Mn, Ni, Pb, Fe, Se and Zn) are persistent and non-biodegradable chemicals those can be bio-accumulated through the biological chains (Stanciu *et al.*, 2005). These metals are essential to life and are not harmful when consumed in small amounts but they can have serious side effects if consumed in large doses. The mobilization of heavy metals into the biosphere by human activity has become an important process in the geochemical cycling of these metals. Anthropogenic impacts including, industrial discharges, domestic sewage, non-point source runoff and atmospheric precipitation are the main sources of toxic heavy metals that enter aquatic systems.

Heavy metals available in soil and water can enter into fish other organisms and then in human body. So, heavy metals are ingested and accumulated in biological bodies or transport to a higher trophic level through food webs (Cui *et al.* 2011, Suci *et al.* 2010, Fu *et al.* 2011). Exposure to arsenic can cause various cancers, kidney damage, and ultimately death in human. Exposure to high levels of mercury, gold, and lead has also been associated with the development of autoimmunity, in which the immune system starts to attack its own cells, mistaking them for foreign invaders (Cai *et al.* 2010, Coeurdassier *et al.* 2010, Volpe *et al.* 2009).

Heavy metals can also seriously affect physiology and immune system of fish. As for example, heavy metals can displace the calcium of the aragonite or calcite crystalline structures by isomorphic substitution. Exposure to water-borne pollutants, a crustacean capable of osmoregulation usually results in a decrease of its  $\text{Na}^+$  and  $\text{Cl}^-$  regulation and / or of its osmoregulatory capacity. Heavy metal might nonspecifically increase the permeability of the plasma membrane to mono and divalent cations.  $\text{H}^{2+}$  increases membrane permeability.  $\text{Mg}^{2+}$  binds at the surface of the prawn oocyte plasma membrane to a receptor, which presents most of the characteristics of a divalent receptor.  $\text{Hg}^{2+}$  inhibits N/K-ATPase by binding to a cysteine located in the first transmembrane segment. A reversible blockade of the  $\text{Ca}^{2+}$  activated nonselective channel in brown fat cells by mercury and the mercurial compound thimerosal. As mercury is a strong sulphhydryl reagent; its main biological mechanism of action is to bind to exposed cysteine



residues on proteins, which leads to an impairment of channel functioning by altering channel permeation and selectivity (Gobas 1993).

Cadmium uptake in fish is inhibited by high aqueous Ca concentrations. Conversely, Cd has been shown to have an inhibitory effect on Ca uptake in fish. The inhibition of Cd uptake by Ca channel blockers has been reported in excised gills of the bivalves *Anodonta anatina*. Increasing aqueous Ca concentrations reduces Cd accumulation in both the shore crab *Carcinus maenas*.

Antibiotics are the group chemicals that have the capacity to kill or inhibit the growth of micro organisms. Like heavy metals are persistence in nature. Antibiotics (e.g. nitrofurans and oxytetracycline) are widely used in fish farms to control bacterial infectious diseases and to limit fish mortality. In fish farming, the widespread use of antibiotics (e.g. nitrofurans and oxytetracycline, tetracycline) for treating bacterial diseases has been associated with the development of antibiotic resistance in *Aeromonas hydrophila*, *A. salmonicida*, *Edwardsiella tarda*, *E. ictaluri*, *Vibrio anguillarum*, *V. salmonicida*, *Pasteurella piscida*, and *Yersinia ruckeri* (Hernandez 2005). Antibiotics are also used in pelleted feedstuffs. These substances are released to the aquatic environment from urine, faeces and also uneaten feed (Hektoen *et al.* 1995).

Nitrofurans, particularly furazolidone (FZD), furaltadone (FTD), nitrofurantoin (NFT) and nitrofurazone (NFZ), belong to a class of synthetic broad spectrum antibiotics which all contain a characteristic 5-nitrofuranyl ring. Nitrofurans were commonly used in aquaculture as feed additive (Draisci *et al.* 1997). In 1995, EU banned use of antibiotic due to concerns about the carcinogenicity of the drug residues and their potential harmful effects on human health (Van Koten-Vermeulen 1993). Since 1993, the use of nitrofurans in agriculture, livestock and aquaculture has also been prohibited in many countries like Australia, USA, Philippines, Thailand and Brazil because of a possible increased cancer-risk through long-term consumption (Khong *et al.* 2004). Like nitrofurans, oxytetracycline is generally considered a very stable substance in the environment, with half-life of 101 to 364 days (Zuccato *et al.* 2001). Oxytetracycline is used to control infectious diseases especially in carp polyculture.

Use of antibiotic, in aquaculture is one of the major concerns because there is evidence that their use may cause bacterial resistance. Moreover, overindulgence use of antibiotic

can cause serious damage of ecosystem and human being. It is estimated that over 75% of the majority of antibiotics disseminated to water at fish rearing facilities spread to the external environment (Halling-Sorenson *et al.* 1998). These drugs can accumulate in ecosystem and enter into the food chain.

### 3. Literature Review

Synthetic analogues of the pyrethrins, extracts from the ornamental *Chrysanthemum cinerariaefolium*, have been developed to circumvent the rapid photodegradation problem encountered with the natural insecticidal pyrethrins. The widespread use of these insecticides leads to the exposure of manufacturing workers, field applicators, the ecosystem, and the public to their possible toxic effects (Solomon *et al.* 2001). During investigations to modify the chemical structure of natural pyrethrins, a number of synthetic pyrethroids were produced with improved physical (involatility, lipophilicity) properties and greater insecticidal activity (knockdown). Several of the earlier synthetic pyrethroids have been successfully adapted for commercial use, mainly for the control of household insects. Other more recently developed pyrethroids have been introduced as agricultural insecticides because of their effectiveness against a wide range of insect pests and their nonpersistence in the environment. Synthetic pyrethroids are fairly rapidly degraded in soil and in plants. Ester hydrolysis and oxidation at various sites on the molecule are the major degradation processes. Pyrethroids are strongly adsorbed on soil and sediments, and minimally eluted with water. There is little tendency for bioaccumulation in organisms (Haya 1989). More than 1,000 pyrethroids have been synthesized since 1973. Their toxicity for non-target organisms is in the parts per billion (Bradbury & Coast 1989). Synthetic pyrethroids are non-systemic insecticides. Type I pyrethroids (e.g. bifenthrin, permethrin) block sodium channels in nerve filaments and cause the 'T-syndrome' in mammals. Type II pyrethroids (e.g. cypermethrin, deltamethrin) act by blocking sodium channels and affecting the function of GABA-receptors in nerve filaments. In mammals, type II pyrethroids trigger clinical symptoms known as the 'CS-syndrome' (Roberts & Hudson 1999).

Deltamethrin [(S)-a-cyano-3-phenoxybenzyl (1R, 3R)-3-(2, 2-dibromovinyl)-2, 2-dimethylcyclo propan-carboxylate], a widely used pesticide, is among the most effective

pyrethroid preparations (Bradbury & Coast 1989). Deltamethrin was synthesised in 1974 and first marketed in 1977. It works by paralysing the insect nervous system, giving a quick knockdown effect following surface contact or ingestion. It is commonly used to control caterpillars on apples, pears, and hops, and for the control of aphids, mealy bugs, scale insects, and whiteflies on greenhouse cucumbers, tomatoes, potted plants, and ornamentals.

Deltamethrin is the active ingredient in Butoflin, Butoss, Butox, Cislin, Crackdown, Cresus, Decis, Decis-Prime, K-Othrin, and K-Otek. It is the first potent and photostable insecticide belonging to the type II pyrethroid group. In the summers of 1991 and 1995, the pesticide caused massive eel (*Anguilla anguilla*) kills in Lake Balaton, Hungary following application for mosquito control. In 1995, the presence of deltamethrin was demonstrated in several other fish species and in sediment samples taken from the lake (Balint *et al.* 1995). The mechanism of its toxicity in fish is the same as that of other pyrethroids containing -cyano-3-phenoxybenzyl groups. They block the sodium channels of nerve filaments, lengthening the depolarisation phase. They also affect the GABA receptors in the nerve filaments (Eshleman & Murray 1991).

Cypermethrin [(RS)- $\alpha$ -cyano-3-phenoxybenzyl (1RS)-cis, trans-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane-carboxylate], another widely used pyrethroid pesticide, is among the most effective pyrethroid preparations (Bradbury & Coats 1989). Cypermethrin is the active ingredient in Ammo, Arrivo, Barricade, Basathrin, Cymbush, Cymperator, Cynoff, Cypercopal, Cyperguard, Cyperhard, Cyperkill, Cypermar, Demon, Flectron, Fligene, Kafil, Polytrin, Siperin, and Super. The mechanism of its toxicity in fish is the same as that of other type II pyrethroids (Hayes 1994). Cypermethrin is a synthetic pyrethroid used for the control of ectoparasites infesting cattle, sheep, poultry, and some companion animals. Recently, the compound has been used for the control of ectoparasite infestations (*Lepeophtheirus salmonis* and *Caligus elongatus*) in marine cage culture of Atlantic salmon, *Salmo salar* (Treasurer & Wadsworth 2004).

Bifenthrin [2-methylbiphenyl-3-ylmethyl (Z)-(1RS, 3RS)-3-(2-chloro-3,3, 3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane-carboxylate], a newer member of the synthetic pyrethroid family, is a contact insecticide and acaricide used on a variety of crops, on stored grain, and as a preconstruction termite barrier. Bifenthrin is the active

ingredient in Talstar, Bifenthrin, Brigade, Capture, Torant, and Zipak. It is a type I pyrethroid (Shan *et al.* 1997), and has some structural similarities to cypermethrin, tetramethrin, and permethrin but is characterised by greater photostability and insecticidal activity than earlier pyrethroids (Yadav *et al.* 2003). It is effective as a gut or contact insecticide that affects the nervous system of vertebrates and invertebrates. Bifenthrin acts on sodium channels at the nerve cell endings to depolarize the presynaptic terminals. It also affects cellular ATPase production (Roberts & Hutson 1999).

Triazines (a six-membered ring containing three carbon and three nitrogen atoms) are some of the oldest herbicides, with research initiated on weed control properties during the early 1950s. Triazine herbicides are categorized into two groups, the asymmetrical triazines, such as metribuzin, and the symmetrical triazines. The major commercially used symmetrical triazines are simazine, atrazine, propazine, cyanazine, ametryn, prometryn, prometon, and terbutryn. As a chemical family, the triazines are a group of pesticides with a wide range of uses. Most are used in selective weed control programs, others, such as prometon, have no selective properties, which makes them suitable for use on industrial sites (Fan *et al.* 2007). A unique member of this family is cyromazine, which is an insect growth regulator useful in livestock, vegetable, and ornamental plant applications through interference with insect moulting and pupation. As herbicides, the triazines may be used alone or in combination with other herbicide active ingredients to increase the weed control spectrum (Solomon *et al.* 1996).

Triazine's herbicidal activity is mediated through the inhibition of photosynthesis (Das *et al.* 2000) by blocking electron transport during the Hill reaction of photosystem-II (DeLorenzo *et al.* 2001). It binds to a plastoquinone-binding niche on D1, a 32-kD protein encoded by the *psbA* gene of the photosystem-II reaction complex (Das *et al.* 2000). In plants it is metabolized by oxidation to 2-hydroxy derivatives and by side-chain de-alkylation (Roberts *et al.* 1998).

Terbutryn [*N*2-*tert*-butyl-*N*4-ethyl-6-methylthio-1, 3, 5-triazine-2, 4-diamine] is used as a selective pre- and early post- emergence control agent of most grasses and many annual broadleaf weeds on a variety of crops, such as cereals, legumes, and tree fruits. It is also used as an herbicide for control of submerged and free-floating weeds and algae in water courses, reservoirs, and fish ponds (Tomlin 2003). Terbutryn is the active ingredient in

Prebane, Igran, Shortstop, Clarosan, GS 14260, Plantonit, Gesaprim Combi (with Atrazine 1:1), Senate (with trietazine), and Igrater 50WP (with metobromuron 1:1). Terbutryn is moderately toxic to fish (Meister 1992). Kidd & James (1991) reported the mean lethal toxicity of terbutryn (96 h LC50) 4 mg/L for common carp (*Cyprinus carpio*) and 3 mg/L for rainbow trout (*Onchorynchus mykiss*). Large quantities of terbutryn have been used since the mid-1980s (Larsen *et al.* 2000). Terbutryn degrades slowly, with a half-life of 240 and 180 days in pond and river sediments, respectively (Muir *et al.* 1980). Its tendency to move from treated soils into water compartments through runoff and leaching has been demonstrated, and residual amounts of terbutryn and its metabolites have been found in drinking water and industrial food products long after application (Konstantinov *et al.* 2006). The application of terbutryn has been banned in many countries because it has the potential to bioaccumulate in organisms, but is still present in waters (Rioboo *et al.* 2007). Simazine (6-chloro-*N,N'*-diethyl-1, 3, 5-triazine-2, 4-diamine) is a member of the triazine family of compounds. It is a selective herbicide used for control of annual broadleaf and grass weeds in raspberries, loganberries, highbush blueberries, apples, asparagus, and ornamentals.

Non-crop uses include total weed control in industrial areas, at airports, along shelterbelts and rights-of-way, and for aquatic weed control in ditches, farm ponds, fish hatcheries, aquaria, and fountains (Arufe *et al.* 2004). Simazine is the active ingredient in Princep Caliber 90, Princep Liquid, Caliber, Cekusan, Cekusima, Framed, Gesatop, Simadex, Simanex, Simtrol, Tanzine, Totazine, and other trade name herbicides as well as in the algicide Aquazine. Simazine is slightly toxic to fish. Hashimoto and Nishiuchi (1981) give a value of 40 mg/L for 48hLC50 for *Cyprinus carpio* and goldfish (*Carassius auratus*). Simazine degrades slowly, with an aerobic soil half-life of 91 days and an anaerobic aquatic half-life of 664 days. The depuration half-life in fish is < 7 days if the organism is transferred to uncontaminated water following exposure, indicating that simazine is rapidly excreted or metabolized (Niimi 1987).

Simazine is the second most commonly detected pesticide in surface and ground waters in the US, Europe and Australia, presumably due to relatively high persistence (Inoue *et al.* 2006). Its degradation products are detected less frequently than atrazine and other triazine pesticides in the aquatic environment. The highest concentration reported in

surface water in the Czech Republic is 0.06 µg/L (Velisek *et al.* 2009a). In Europe simazine levels reach values up to 5 µg/L (Belmonte *et al.* 2005). Simazine was the most frequently detected pesticide in 20 counties in California, USA with concentrations ranging from 0.02 to 49.2 µg/L [US Environmental Protection Agency (US EPA)], 1994).

Ecotoxicological risks associated with pesticides depend on several factors such as type, concentration and frequency of application. Pyrethroids and triazines affect fish physiology. Major negative impact of pyrethroids is their high toxicity to fish (e.g. fish mortality in Balaton in 1991 and 1995) combined with their use for control of some parasitic diseases in fish, e.g. *Lepeophtheirus salmonis* in salmon farming. Triazine residues accumulate in fish tissue. Clinical symptoms of acute toxicity of pyrethroids in rainbow trout and common carp juveniles includes increased respiration, loss of coordination, and fish lying on their flank and moving in this orientation. Subsequent short excitation stages with convulsions, jumping above the water surface, and moving in circles alternated with resting. Necropsy performed after the acute toxicity test revealed increased watery mucus on body surfaces. The body cavity contained excess fluid and showed congestion of visceral vessels (Velisek *et al.* 2011).

Acute exposure to deltamethrin in rainbow trout is associated with a significantly ( $P < 0.05$ ) lower concentration of GLU, ALT, and significantly ( $P < 0.05$ ) greater of TP, ALB, NH<sub>3</sub>, AST, and Ca<sup>2+</sup> compared to controls. The common carp exposed to deltamethrin exhibits significantly higher ( $P < 0.05$ ) value of NH<sub>3</sub>, AST, and ALT compared to controls (Velisek *et al.* 2011).

Acute exposure to cypermethrin results in a significantly ( $P < 0.01$ ) lower concentration of ALP and significantly ( $P < 0.01$ ) higher concentration of NH<sub>3</sub>, AST, LDH, CK, and LACT in rainbow trout compared to controls fish. In common carp cypermethrin results in a significant ( $P < 0.01$ ) lower in TP, ALB, GLOB, NH<sub>3</sub>, LDH, and ALP, and a significant ( $P < 0.01$ ) higher in GLU, LACT, and CK levels compared to controls (Velisek *et al.* 2011). Acute exposure to bifenthrin results in significantly ( $P < 0.01$ ) lower NH<sub>3</sub> and significantly ( $P < 0.01$ ) higher concentrations of GLU, LDH, ALP, and CK in rainbow trout compared to control trout. Common carp exposed to bifenthrin

shows significantly ( $P < 0.01$ ) higher levels of GLU, NH<sub>3</sub>, AST and CK compared to controls (Velisek *et al.* 2011).

Acute exposure of rainbow trout to deltamethrin is associated with significantly higher ( $P < 0.05$ ) erythrocyte count, haemoglobin content, and haematocrit than in the control group. On the other hand, deltamethrin exposure in common carp led to significantly lower values ( $P < 0.01$ ) of RBC, Hb and PCV compared to controls (Velisek *et al.* 2011). Rainbow trout acute exposed to cypermethrin exhibits significantly lower ( $P < 0.05$ ) numbers of developmental forms of myeloid sequence and segmented neutrophilic granulocytes than did untreated fish. Moreover, cypermethrin exposure in common carp results in significantly ( $P < 0.01$ ) higher values of RBC, MCV, MCH, and lymphocyte count ( $P < 0.01$ ) compared to controls (Velisek *et al.* 2011). Acute exposure of rainbow trout to bifenthrin caused significantly higher ( $P < 0.01$ ) values of MCV, MCH, and neutrophil granulocyte count compared to controls. In common carp bifenthrin is associated only with significantly higher ( $P < 0.01$ ) of monocyte counts compared to control fish (Velisek *et al.* 2011).

Acute toxicity exposure (96 h) of cypermethrin causes severe teleangioectasia in the secondary lamellae of gills with the rupture of pillar cells and degeneration of hepatocytes, especially in the periportal zones in rainbow trout. Affected hepatocytes show pycnotic nuclei and many small vacuoles or one large vacuole in the cytoplasm (Velisek *et al.* 2011). In common carp, acute exposure to cypermethrin results in hyperaemia and perivascular lymphocyte infiltration in skin, mild hyperplasia of respiratory epithelium chloride cell activation in the gills and vacuolisation of pancreas exocrine cells (Velisek *et al.* 2011). Acute exposure to bifenthrin in rainbow trout and common carp is associated with degeneration of hepatocytes, especially in the periportal zones. Affected hepatocytes show pycnotic nuclei and many small vacuoles or one large vacuole in the cytoplasm. Moreover bifenthrin in common carp causes severe teleangioectasia in the secondary lamellae of gills, with the rupture of pillar cells (Velisek *et al.* 2011).

Common carp exposed to terbutryn at concentrations of 0.2 and 2 µg/L, significant ( $P < 0.01$ ) decreases in the level of CREA and Mg and a significant ( $P < 0.01$ ) increase in GLU, AST, LDH, and LACT levels in plasma were observed compared with controls

(Velisek *et al.* 2011). Biochemical profiles of carp exposed to simazine at the concentration of 2 µg/L show significantly higher activity of ALP ( $P < 0.01$ ) and ALT ( $P < 0.05$ ) than controls carp. In carp, simazine at a concentration of 4 µg/L causes a significant increase in TP ( $P < 0.05$ ), ALB ( $P < 0.05$ ), ALP ( $P < 0.01$ ) and ALT activity ( $P < 0.05$ ) compared to controls (Velisek *et al.* 2011). Common carp exposed of terbutryn at concentrations of 0.2 and 2 µg/L, RBC, lymphocyte counts, and mean corpuscular haemoglobin concentrations increases significantly ( $P < 0.01$ ), and Leuko, neutrophil granulocyte bands, and MCV decreases significantly ( $P < 0.01$ ) relative to controls. Simazine at concentrations of 0.06 µg/L, 2 µg/L, and 4 µg/L led to significant ( $P < 0.01$ ) decrease in Leuko relative to controls after 90 days exposure (Velisek *et al.* 2011).

Long-term exposure to simazine at concentrations of 0.06 µg/L, 2 µg/L, and 4 µg/L causes severe hyaline degeneration of the epithelial cells of renal tubules of the caudal kidney of common carp; while, in the control fish, the caudal kidney parenchyma was intact. The altered tubular epithelium was atrophic in tubules, with and without casts. Some tubules appear expanded, but, if they did not contain casts, were small with a thickened basement membrane (Velisek *et al.* 2011). Pyrethroid pesticides i.e. deltamethrin, cypermethrin, and bifenthrin are strongly toxic to fish. Bifenthrin is more toxic at cooler temperatures, and thus more toxic to cold water fish than to warm water species, but the toxicity of pyrethroids is little affected by pH or water hardness (Mauck *et al.* 1976). Pyrethroids are more toxic to smaller fish than larger ones (Baser *et al.* 2003). Bradbury and Coats (1989) report mean lethal toxicity of cypermethrin to various fish species in laboratory conditions to be below 10 µg/L.

Clinical symptoms (e.g. accelerated respiration, loss of movement and coordination, fish lying at the tank bottom and moving in one spot, subsequent short excitation periods with convulsions and movement in circles) observed during acute exposure of rainbow trout and common carp to pyrethroids (deltamethrin, cypermethrin and bifenthrin) (Dobsikova *et al.* 2006, Velisek *et al.* 2006a). Bradbury & Coats (1989) reported signs of fenvalerate poisoning in fish, that included loss of schooling behaviour, swimming near the water surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, increased cough