

Analysis of acute toxicity in some
selected pesticide on freshwater mussel,
Lamellidens marginalis



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For the degree of

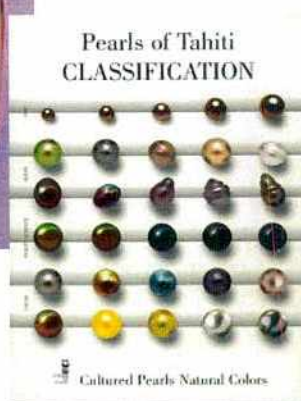
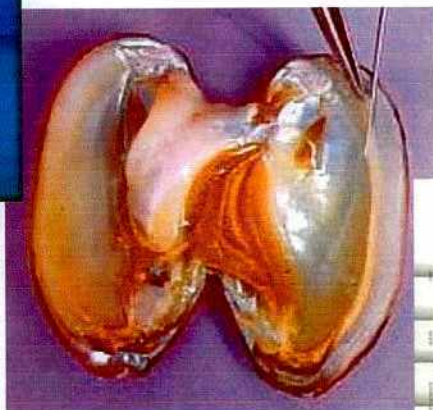
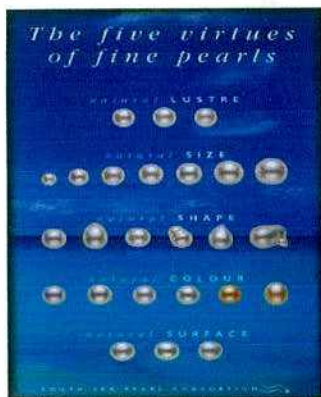
DOCTOR OF PHILOSOPHY IN ZOOLOGY

By

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March, 2006



DEDICATION

To my beloved Parents

425599

ঢাকা
বিশ্ববিদ্যালয়
গ্রন্থাগার



DECLARATION

I do hereby declare that this thesis submitted to the University of Dhaka for the degree of Doctor of Philosophy in Zoology is carried out under the supervision of Professor **Dr. Miah Mohammed Abdul Quddus** and **Dr. Jahanara Khatoon** and has not been submitted elsewhere for the award of any degree or diploma.

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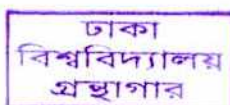
CERTIFICATE

The thesis titled "Analysis of acute toxicity in some selected pesticide on freshwater mussel, *Lamellidens marginalis*" submitted by **Md. Nazrul Islam**, Registration No. 27; Session 2002-2003 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Zoology on September 10, 2006. This thesis has been carried in the Laboratory of Pesticide Residue Monitoring and Research in Environment Institute of Food and Radiation Biology, Savar, Dhaka.

It is further certified that the work presented here is original and suitable for submission and such help or source of information has been availed of in this connection is duly acknowledged.

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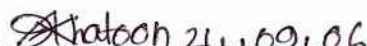
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LIST OF ABBREVIATIONS

A – Appendix

AEC – Atomic Energy Commission

AERD – Agrochemical and Environmental Research Division

AI – Active Ingredient

BBS – Bangladesh Bureau of Statistics

DAE – Department of Agricultural Extension

IPM – Integrated Pest Management

N.G.O. – Non-Government Organization

NMIDP – National Minor Irrigation and Development Project

PAB – Pesticide Association of Bangladesh

WARPO – Water Resources Planning Organization

ASTM – American Society for Testing Materials

APHA – American Public Health Association

FP – Formulated Product

GLEAMS – Groundwater Loading Effects of Agricultural Management Systems

CMC – compartmental mixing-cell

SEHD – Society for Environment and Human Development

IFRB – Institute of Food and Radiation Biology

BC – Basement Count

AERD – Agro-Chemical and Environmental Research Division

FAO/IAEA – Food and Agriculture Organization/ International Atomic Energy
Agency

USEPA – U.S. Environmental Protection Agency

PPIS – Pesticide Product Information System

SRDI – Soil Resources and Development Institute

ATIA – Assisting Transformation to Irrigated Agriculture.

G.I.A. – Gemological Institute of America

DOF – Directorate of Fisheries

NTP – National Toxicology Program

MPO – Master Plan Organization

LPI – Leaching Potential Index

HYV – High Yielding Varieties

RT – Retention Times

LDH— Lactate Dehydrogenase

SDH – Succinic Dehydrogenase

MP – Methyl Parathion

UV – Ultra Violet

^{14}C – Carbon 14

Cd – Cadmium

Fig – Figure

h – hour

ha – hectare

ppm – parts per million

a.i. – active ingredient

CPM – Count Per Minute

g – gram

Kg – Kilogram

mg – miligram

ml – mililiter

ng – nanogram

µg – microgram

MT – Matric Ton

LC₅₀ – Lethal Concentration 50

LD₅₀ – Lethal Dose 50

K_{oc} – Partition Coefficient

t_{1/2} – half life

EC – Emulcifiable Concentrate

ECD – Electron Capture Detector

GC – Gas Chromatograph

HPLC – High Performance Liquid Chromatograph

ND – Not Detected

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ABSTRACT

The aim of the study was to test the acute lethality of carbosulfan (carbamate) and malathion to *Lamellidens marginalis*. The pesticide residue analysis was also evaluated. The physico-chemical characteristics of exposure media were monitored during different trial with carbosulfan and malathion insecticide. The pH value showed a concentration dependent changes ranging from 5.9 to 8.1, ammonia increased with the increase in carbamate concentration which varied from 2.8 to 3 mg/l. Alkalinity showed a decrease level, which varied from 152 to 206 ml/l. The results show that no mortality caused to mussel exposed up to the exposure concentration of 12.87 ppm. Carbosulfan (carbamate) insecticide during the preliminary experimental period, mussel treated with 14.85 ppm carbosulfan insecticide induced 20% mortality within 6 hrs. of exposure and a complete (100%) mortality was obtained by 24 hrs. Mussel exposed to 15.84 ppm carbosulfan (carbamate) insecticide caused a complete (100%) mortality within 6 hrs. of treatment. But 14.85 ppm malathion insecticide induced mussel mortality 20% by 72h of exposure and 35% mortality occurred in the same treatment group by 96h of exposure. A complete mortality 100% was observed in mussel at 24.76 ppm malathion insecticide within 72h exposure. Acute lethality tests were conducted with a view to determine the LC_{50} values for carbosulfan and malathion in separate trials to *L. marginalis* at different exposure time. Actual trial was performed twice with each chemical. The mortality of mussel was recorded at 6h, 12h, 24h, 48h, 72h and 96h of exposure. The mortality datas were used to calculate the LC_{50} value for each of the chemicals for different exposure times. During Trial IA with carbosulfan LC_{50} values at 12h, 24h, 48h, 72h and 96h were 15.02 ppm, 13.60 ppm, 13.47 ppm, 13.33 ppm and 13.20 ppm (95% CL 14.05-15.99, 12.83-14.37, 12.70-14.24, 12.56-14.10, 12.43-13.97). During Trial IIA, the LC_{50} values were 14.26 ppm (95% CL 13.97-14.55) for 24h, 13.57 ppm (CI 12.60-14.54) for 72h, 13.35 ppm (CL 12.38-14.32) for 96h of carbosulfan respectively. Similarly, LC_{50} values for malathion at 24h, 48h, 72h and 96h of exposure times were 21.20 ppm (95% CL 19.14-23.26), 17.82 ppm (CL 12.97-22.67), 15.30 ppm (CL 10.45-20.15) 13.75 ppm (CL 9.90-17.60) respectively. During Trial IIB, with the same chemical the values obtained were 19.36 ppm (95% CL 17.42-21.30), 17.49 ppm (95% CL 15.55-19.43), 14.52 ppm (95% CL 12.58-16.46) 13.86 ppm (95% CL 11.92-15.80) at 24h, 48h, 72h and 96h exposure times respectively. Orgnochlorine residues were present in different organs of mussel samples collected from different locations, except Sample 4 (Sibganj thana), Sample 5 (Bogra sadar), Sample 6 (Sherpur thana), Sample 9 (Dhunat thana) and Sample 10 (Sonatala thana, during flood). In water samples it was present only at Sonatala, Sariakandi, Gabtali, Kahalu, Adamdighi and Sonatala thana (after flood). But in soil samples orgnochlorine were present only in Samples No. 1 (Sonatala thana), 2 (Sariakandi thana), 3 (Gabtali thana), 7 (Kahalu thana) and 11 (Sonatala thana, after flood). DDT was found in all types of samples. Other organochlorine residues identified were aldrin, dieldrin, lindane, heptachlor and HCH. Among organophosphorus insecticides only temphos was identified. Maximum concentration of DDT was found in water, mussel and soil samples were 13.46 ng/g, 4.92 ng/g and 11.73 ng/g respectively.

CHAPTER ONE

INTRODUCTION

1.1 General

Pearl is a valuable gem known to mankind since ancient times. The pearl, in fact, is of aquatic animal origin and produced by certain mussel of Mollusca but the most valuable natural pearls are produced by pearl producing mussels *Lamellidens marginalis* (Lamarck, 1819), which significantly declined from our aquatic ecosystem with time. On the other hand mussels are a great source of human food in various parts of the world (Preston, 1915). Soft inner part of mussels are eaten in China, Japan, Malaya, Europe and America being regarded as a delicacy.

We have plenty of unknown aquatic resources which can lead to economic development of our country, of which pearl culture can be a superior one. Pearl enhances the beauty of ornaments. Pearl jewelry has well acceptance in aristocratic society of women of the world. In the evolution of human society, possession of the pearl in a family was marked as honour and enhanced the dignities of the families. In Bangladesh, pearls are produced in mussel, which are known as pink pearl that are used as an attractive ornament. Previously abundant mussels were present in the freshwater ecosystem out of which some produced pearl naturally. Recently, decline of mussels caused marked reduction in pearl availability in the natural resources.

Pearl producing mussels are available in freshwater and marine ecosystem that is environmental friendly. Normal fish production is not hampered by culture of mussels in the same water bodies, for which fish and mussel can be cultured at the same time. If we can successfully spread the technology across our country, it will create new job opportunities for women and unemployed youth resulting in economic development.

The class Bivalvia also called Pelecypoda or Lamellibranchia is comprised of molluscs and includes common forms such as Clams, Oysters and Mussels.

Bivalves first appeared in rocks of Ordovician period in the form of Oysters. Some of them were remarkably similar to the present day forms becoming abundant in the Jurassic and Cretaceous periods, about two hundred million years ago (Walne, 1979).

The Unionidae, the largest freshwater family, containing the familiar important genera like *Unio*, *Lamellidens* and *Anodonta* along with eighty five genera and some 1000 species, accounting for one-fifth of all living Lamellibranches. The three sub-families of the group are Lampsilinae of America, Anodontinae of Europe, America, East Asia and Unioninae of Asia, Indian subcontinent and also from America and Africa. The small family Aetheriidae has three genera of specialized

freshwater oysters in the Amazon basin, tropical Africa and India (Morton and Bushek, 1963).

The specific origin of molluscan aquaculture is lost in antiquity. Bardach *et al.* (1972) suggested that the culture of clams predated oyster in Japan. Bourne (1981) stated that clam culture was made in Japanese literature as early as eighth century. During World War II considerable numbers of mussels were harvested and canned (Scattergood and Taylor, 1949).

Bivalve culture in China has a long history and an important position in modern agriculture. Mussel culture in China began in the late 1950s. Mussels grow quickly, have a short culture cycle and produce a high yield. There are three commercial species of mussels in China; the common mussel (*Mytilus edulis*) the black mussel (*M. crassitesta*), and the green mussel (*M. smaragdinus*), (Nie Zhong-Qing, 1982).

Bivalve culture in Indonesia is largely experimental at present, although five companies in Eastern Indonesia are in the business of producing pearl oysters. Recently, the experimental culture of green mussels (*Perna viridis*) has begun at Ancol, (Jakarta Bay), Mauk-Tangerang (West Jakarta), and Banten Bay (Unar *et al.* 1982).

Freshwater pearl comes from freshwater mussels which are produced mostly by Japan, China, Italy, France, Greece and United States. Most famous type of freshwater pearl is Biwa (pronounced bee-wah) which came from mussels grown in Lake Biwa, Japan's largest freshwater lake. Freshwater shell and pearl are from the family Unionidae, from which about 20 different species are commercially harvested. Worldwide mussel production, export and import is shown in Table 1.

Multiple of the freshwater pearls are dictated by the mother shell. White, most common, followed by pink, other colours depends on the type of mussel. Big washboard mussels (*Megaloniaias nervosa*) and warty back mussel (*Quadrula nodulata*) usually have pink pearls. Ridge mussels (*Amblema plicata*) have pearls in shades of blue-green and lavender, muckets (*Actinoniaias ligamentina*) produce fine pink pearls, and sand mussels (*Lasmigona costata*) have salmon-pink pearls. Other mussels of freshwater produce pearls that include- bony, heel splitter, pimple back, elephant ear, maple leaf, three-ridge pig toe, pistol grip, and butterfly. In the last five years, Chinese freshwater pearl has greatly improved in quality and the price is 10 higher than the Japanese salt water pearls.

Mussels are popular and inexpensive protein source for Thai people as well as a feed for poultry, prawn, shrimp and fish. The history of mussel culture in Thailand was not documented, but at one time, fishing families harvested mussels from fishing gear such as bamboo-stake traps and from rocks or stones in natural beds. The demand for mussel products increased with human population (Saraya, 1982).

The gross national income per capita is \$370; Bangladesh was 167 positions in the year 2000 which was very close to Vietnam and India. High population density, low per capita income and poor resources resulted in high level of poverty in Bangladesh. Bangladesh is a riverine country; it has many ponds, lakes, beels, canals and rivers containing freshwater mussels. If we culture freshwater mussels there we can earn lots of foreign currency to enrich our per capita income annually.

Table 1. Worldwide mussel production, export and import

Country		Production			Imports			Exports		
		1998	1999	2000	1998	1999	2000	1998	1999	2000
Grand Total	Q	31228	35158	37495	185170	192509	178827	189287	202678	209390
	V				226788	235189	253874	220797	240664	266058
	u.v				1225	1222	1420	1166	1187	1271
New Zealand	q	261091	278271	30400	1	18	11	26284	28085	26691
	v				3	49	26	60177	58913	73492
Netherlands	q	---	---	---	14099	19863	7143	30108	44834	38290
	v				7044	10104	6269	44238	62971	65840
Belgium	q	---	---	---	25054	26394	27417	174	426	639
	v				44573	46812	59607	452	748	1258
USA	q	587	933	348	15467	16862	19569	649	937	743
	v				35675	37618	52155	1696	2501	1842
France	q	---	---	---	54866	45946	39042	3809	4574	4888
	v				45633	43899	35715	4881	5846	5719
Italy	q	---	---	---	31620	34478	27324	5901	4988	6029
	v				30901	35261	26026	4721	4917	5680
Spain	q	---	---	---	8081	7796	7751	23678	26594	23211
	v				13926	12443	12726	22866	26526	19866
Germany	q	---	---	---	19252	23619	27105	24083	15372	16843
	v				10072	8244	10445	17840	12318	12626
China	q	---	---	---	393	277	1063	8395	12719	14714
	v				377	380	1337	9883	14681	17742
Canada	q	---	---	---	1973	2027	2206	5993	6628	8695
	v				4479	4836	5628	10401	11750	15860
Denmark	q	---	---	---	117	102	179	26383	23820	27275
	v				414	444	503	11904	10475	13428
UK	q	---	---	---	3148	4050	3612	9219	7074	7247
	v				7782	8592	8086	8399	5756	5190
Others	q	4532	6398	6747	11099	12077	16405	24611	26627	34125
	v				25909	26507	35351	23339	23262	27515

q= mt v= US\$ 1000 u.v. =US\$/mt

Source: F. A. O yearbook, 2000.

According to a survey conducted by the East Pakistan Small Industries Corporation in 1964 about 165 Kg of natural pink pearls were produced worth about Tk. 1.4 million (Tk. 19 = US \$1). In 1964, the number of pearl and mussel collection centre in Bangladesh was 95; 8 in Bogra, 4 in Rajshahi, 9 in Pabna, 7 in Faridpur, 3 in Dhaka, 40 in Mymensingh and 24 in Sylhet (Ahmed, 1982).

Pink pearls are obtained from some species of bivalves *Lamellibranch* molluscs belonging to the family Unionidae (Zobairi, 1956). In Bangladesh, freshwater mussel culture presents a good opportunity to produce poultry food and pearls increasing export possibilities to different countries of the world (Ali and Begum, 1976).

In the early 1960's, the Government realized the potential of pearl and mussel culture and launched a project to investigate windowpane oyster fisheries and

artificial culture of pearl oysters at Cox's Bazar. At the same time, a malacological unit was set up under the freshwater fisheries research station at Chandpur in Comilla district. During 1975-80 a pilot project on the culture of pearl bearing mussels was set up in Tangail district (Ahmed, 1982). Later, in 1983 another project was undertaken by Fisheries Research Institute at Mymensingh. Both the research to date has not produced essential information on the biology, ecology, and population dynamics of the bivalves in Bangladesh.

The mussels may be used for the following purpose:

- a) pearl and shells are used for ornament (Ahmed, 1958)
- b) as an indigenous medicine for heart, bone, dental, pox and in some types of eye diseases (Ahmed, 1958)
- c) the shell is an important source of calcium oxide (CaO), which is widely used in cement, ceramic, paper, bleaching powder, soda, urea industries and fish culture
- d) to control the plankton in fish culture pond – Algae, Diatoms, etc
- e) some fishes lay eggs in the mantle cavity of freshwater mussels e.g. *Bitterling* (Lagler *et al.* 1963)
- f) lime from the shells and crushes are used for feeding poultry for formation of their egg shells
- g) shells are used to make knife handles, lime, fertilizer, face powder and different types of ornaments, toys and decorations
- h) shells of freshwater mussels are used in the pearl button industry in all parts of the world, they are made from the nacreous layer of shells
- i) shells of mussels are mixed with tar for making roads in America
- j) lime from the shells is used in building, in Bangladesh

The first taxonomical description on the freshwater mussels was made by Lamarck in 1779 later reviewed by Preston (1915).

In developed countries where the people are very much conscious about the molluscan fauna a vast records of malacology are available. The most important works done by some authors in the Indian subcontinent and other countries on the taxonomy and ecology of freshwater molluscan are: Preston (1915), Annanda (1918), Satyamurti (1960), Brown (1965), Warwick (1968), and Johnson and Boss (1972).

Ahmed (1968) reported the frequency and location of seeds of freshwater mussels of East Pakistan. Ali and Begum (1976) described only four species of freshwater bivalves from Dhaka City to study the taxonomy of the bivalves. Later Begum *et al.* (1989) reported 5 genera 10 species belonging to 4 families of class Pelecopoda. The culture of freshwater mussel has a great economical scope in the country. Besides Pearl production it may be utilized for poultry, fish meal and lime industry (Lubitz *et al.*, 1947).

In Bangladesh rice, wheat and vegetables of various kinds are being damaged in huge quantities by the molluscan pest for which they need protection to ensure the maximum production. The Production of food grain has increased by more than twice from 11.08 million tons in 1970-71 to 24.30 million tons in 1999-2000 and thus had led the country to attain self sufficiency in food production (Rahman, 2000). Such a success has been possible due to use of pesticides for effective protection against pests and disease, besides other modern agricultural attributes. The pesticide consumption in the country has reached 1516 tons of formulated product (FP) and 2443.11 tons of active ingredients (a.i) in 1999-2000 as against only 3134 tons of FP in 1976-77 (PAB, 2000).

Agriculture is the largest sector of economy in Bangladesh, contributing nearly half of the economy's output (Islam, 1993). The attacks of pest and diseases on crops destroy the economy of agricultural productions. In addition to flooding, drought, thunder, storms, etc. 20% of agricultural products of Bangladesh are destroyed every year (Talukder, 1995). The widely cultivated high yielding varieties of crops are very vulnerable to pest and diseases. Moreover, the tropical climate results in high pest pressure, which necessities the application of pesticide (Rahman *et al.*, 1995). Following Tables are showing the utilization of pesticides to enhance production for the major crops of Bangladesh (Table 2).

Table 2. Consumption of pesticides in Bangladesh

Table 2. Consumption of pesticides in Bangladesh									(mt/kl)
Period	Pesticide			Fungicide		Herbi- -cide	Roden- ticide	Public Hygiene	Total
	Granular	Liquid	Powder	Liquid	Powder				
1998	9139.10	1298.80	107.11	---	734.71	239.15	91.74	---	11610.66
1999	11192.91	1524.171	123.32	---	1065.42	315.14	119.22	---	14340.18
2000	11915.67	1789.41	80.99	---	1430.01	271.10	122.06	---	15632.24
2001	10788.37	1426.45	86.04	18.85	2170.60	838.00	70.30	---	15398.61
2002	12335.35	1496.85	142.12	2418.80	---	963.99	36.34	---	17393.45
2003	11781.36	1830.82	155.02	2940.68	---	1354.02	18.56	---	18080.46
2000								---	
Jan- Mar	5392.44	284.85	31.25	---	632.38	140.78	21.47	---	6703.17
Apr- Jun	1711.42	294.13	38.24	---	171.81	34.55	30.07	---	2280.22
Jul-Sep	3351.96	450.21	25.26	---	333.07	33.62	40.45	---	4234.57
Oct- Dec	1459.85	560.22	9.24	---	292.75	62.15	30.07	---	2414.28
2001								---	
Jan- Mar	5037.60	370.19	20.04	4.14	1084.16	484.55	28.60	---	7029.28
Apr- Jun	1947.92	374.99	38.58	9.42	99.55	52.12	14.18	---	2536.71
Jul-Sep	2233.38	369.66	20.48	3.06	490.32	219.96	14.11	---	3350.97
Oct- Dec	1569.47	311.61	6.99	1.51	496.57	81.27	13.40	---	2480.82
2002									
Jan- Mar	4692.98	407.27	26.66	1127.33	---	590.39	10.40	---	6855.03
Apr- Jun	2670.46	322.56	52.04	147.19	---	81.75	7.05	---	3281.80
Jul-Sep	3388.10	439.47	40.62	550.88	---	108.93	9.90	---	4537.90
Oct- Dec	1583.81	327.55	22.80	593.40	---	182.92	8.98	---	2719.46
2003									
Jan- Mar	5182.96	521.35	40.24	1428.56	---	1027.14	4.10	---	8204.34
Apr- Jun	1490.31	361.81	51.53	190.15	---	66.71	2.16	---	2162.67
Jul-Sep	3261.43	549.13	44.59	757.48	---	153.16	3.94	---	4769.73
Oct- Dec	1846.67	398.53	18.66	564.49	---	107.01	8.29	---	2943.65
2004									
Jan- Mar	5891.53	599.77	50.58	2212.30	---	1804.33	9.23	---	10567.74
Apr- Jun	1561.59	349.90	85.03	374.25	---	186.41	2.72	---	2559.90

Source: BCPA, 2004

Pesticides are used to control undesirable target organisms, but there is ample evidence, which shows that many of these pesticides and its by-products are toxic to other non-target organisms including mussels and man (Murphy, 1980)

A good number of information is available on the effect of pesticides on non-target animals. Notable among them are Hotchkiss and Pugh (1940) who reported mass mortality of birds due to the spraying of DDT on forest trees to control the gypsy moth. Dewitt (1956) reported that large number of mortality of hatched chicks occurred as a result of feeding the hens with dieldrin contaminated food.

Rudd and Genially (1956) reported death of song birds due to dieldrin, duckling and redwings black birds due to aldrin, which was sprayed to control mosquitoes. Reduce population of several species of birds due to reproductive failure and thinning of eggshell occurred as a result of pesticide accumulation (Peakall and Ratcliffe 1967, Peakall 1970, Ratcliffe 1967, 1970, Hickey and Anderson 1968, Bulsa 1970, Keith *et al.*, 1970, Cad *et al.*, 1971, Anderson and Hickey, 1972)

There are also reports on fish poisoning due to pesticides. Harrington and Bidlingayer (1958) reported mortality of 25 tons of fish belonging to 30 species of Florida, after dieldrin was applied to control sand fly, *Calicoides sp.*, Crouter and Vernon (1959) noticed the complete elimination of the young stage of Coho salmon and sleeted minnows after application of DDT over a coniferous forest in British Columbia. Keenleyside (1959) said that Salmon fries were eliminated in New-Burnwick following the spraying of adjoining forestland with DDT. Cottam (1956) and Taylor (1970) reported several instances of the biomagnifications of the pesticides in the food chain, leading to the death of fish.

In Bangladesh, studies relating to toxicity of pesticides on aquatic ecosystem are very meagre but a good number of works have been done in this subcontinent. Notable among them are Mathur (1959, 1962-66, 1969 and 1975), Sreenivasan and Sounder Raj (1968); Konar (1969); Bhatia (1971); Aurora *et al.*, (1971-72); Toor *et al.*, (1973); Amminikutly and Rage (1977)

Generally three types of synthetic pesticides are widely used for pest control such as organochlorine, organophosphorus and carbamate are highly toxic but less persistent. Some organochlorine pesticides are notoriously persistent in the environment that may cause a potential hazard to man and environmental ecosystem. DDT and other organochlorine pesticide at a concentration of 10 ppm inhibit the photosynthesis of 4 species of phytoplankton (Cremlyn, 1984). Since, all living organisms depends on photosynthesis, this observation was alarming for which organochlorine pesticides have been banned and replaced by organophosphorus compound in many countries.

Among the above-mentioned pesticides and pesticides, the chlorinated hydrocarbons are more toxic to fish than carbamates (Macek and Mcallister, 1970)

In the year of 1991, more than 7500 metric tons of pesticides belonging to carbamates, parathroids, organochlorines and organophosphates worth of Tk. 1000 million were marked in the country for agriculture sector. Even though uses have gradual increased every year, for the last 5 years

Tan *et al.* (1972) has pointed out that only the utilization of organophosphorus pesticides are responsible for the rapid decline in the production of fishes in paddy fields. Islam *et al.* (2000) mentioned that recommended dose of malathion pesticide is not acute for catfishes (*Heteropneustes fossilis*).

1.2 Scope of the present research

In Bangladesh, studies in relation to toxicity of pesticides on mussel, snail, crab, shrimp and prawn are very meager. But a good number of works have been done in this sub-continent about toxicity on mussels. Among them Das and Jana (2003) reported that freshwater mussel *L. marginalis* exposed by cadmium. Shaheed *et al.* (1997) observed that mollusc *L. marginalis*, accumulated higher concentration of polonium-210 and it could serve as an abominator of (210) radioactive unraveling system. Methyl parathion toxicity on freshwater mussel, changes in respiration, ionic and carbohydrate metabolism in tissue Moorthy *et al.*, (1984). Report of Matheson Trigas Inc. 959 Route 46 East Parsippany, New Jersey 07054-0624 (1989) indicate that component ethylene glycomonomethyl ether is toxic to invertebrate *L. marginalis*. Basham *et al.*, (1988) showed that the activities of ctenidial cilia and the heart in *L. marginalis* is exposed to methyl-parathion [parathion-methyl, presumably technical grade and metacid-50].

To do the above mentioned research, Institute of Food and Radiation Biology Division, Atomic Energy Research Establishment (AERE), Savar, Dhaka has extended their hand and the present research work is an outcome of such a co-operation.

The increase of industrial effluent, pesticide dependable agriculture practice and elevated contaminant levels from anthropogenic sources are probable causes of mussel declines.

During the past 20 to 30 years the use of pesticide has become an integral part of most crop production system and a vast array of pesticide, fungicides and herbicides is now available to farmers and growers in Bangladesh. On the other hand farmers and growers do not have clear idea about the recommended doses of pesticide and its fate on our ecosystem. That's why we are sufferer of the bio-accumulation and toxic problems associated with the several of the older organochlorine, carbamate and organophosphorus pesticide. We should have considerable information on concerning toxicity, toxicology, degradation, stability, volatility, mobility, and adsorption/desorption relationships, water/sediment and animal interactions and transfer between the various environmental compartments about pesticides before its commercialization. In spite of this wealth of information, occasional problems are encountered.

In order to evaluate the degree of pollution and its impact on the freshwater ecosystem, the present study on the mussel (*Leamellidens marginalis*) which is an abundant species and is also well known for its role as a bio-indicator of pollution (Sindermann 1988; Porte *et al.*, 1990; Narbonne *et al.*, 1991b) has been taken.

1.3 Aim and Objectives of the study

The objectives of the study were as-

1. Toxicity and lethal concentration (LC₅₀) of carbosulfan and malathion on *Lamellidens marginalis*;
2. Influence of temperature and pH on toxic water;
3. Fate and pathway of pesticide degradation in the freshwater model ecosystem;
4. Bio-chemical relation with toxicity;
5. Persistence of pesticide residue in different organs of the *L. marginalis* such as gill, stomach, foot, mantle tissue, and also soil and water;
6. The interrelationship between pearl production and pesticide residual effects;
7. To find out the *L. marginalis* as a field application of mussels as environmental sentinel of contaminates effect and ecosystem health;
8. To develop and validate of battery bio-indicator for freshwater mussels which assess health, reproductive status and contaminant exposure or effects;

CHAPTER TWO

LITERATURE REVIEW

2.1 Mussel

Reviewing the literature from Bangladesh, it was noted that no such work on the proposed topic was available. However, for the benefit of the present study papers from home and abroad had been reviewed.

Churchill and Lewis (1924) studied on the food and feeding habits of freshwater mussel of United States of America. Loosanoff and Marak (1951) reported that the species of bivalve molluscs *Ostrea*, *Crassostrea*, *Venus*, *Mya*, *Macra*, *Mytilus*, *Petricola*, *Pitra* and *Ensis* can survive easily with only the algae, *Chlorella* species. Davis (1953) Loosanoff *et al.* (1955) and Davis and Guillard (1958) reported that the plankton of the following class Chlorophyceae, Chytassophyceae, Bacillariophyceae, and Cryptophyceae are the most suitable food for mussels. Donald (1961) described the various types and formation process of pearls and also the conditions suitable for pearl culture in Australia. Roscoe and Susan (1964) studied the ecology of freshwater pearl mussel (*Margitifera margaritifera*). It includes the ecology, habitat relations, behavior of mussels, work on freshwater pearl fisheries, etc.

Harris (1965) studied the ecology of the freshwater mollusca of Iraq. He found that freshwater mollusca faunas could be used for determining current velocity, certain aspects of water chemistry and origin of the water and associated deposits when they are collected from alluvial deposits. Ahmed (1968) worked on frequency and location of seed pearls in freshwater mussels (*Lamellidens*) from East Pakistan. He found that the seed pearls located in an area of mussels where the shell could be drilled easily and nuclei can be embedded in its proper place. Nagabhushanam and Lomte (1971) studied some aspect of biochemical process in the body of freshwater mussels, *Parreysia corrugata* and observed that glycogen and fat showed seasonal variations which were correlated with spawning season as well as with gonadal changes. Shafee (1976) studied on the various allometric relationships in the green mussel *Perna viridis* in Emore Estuary, Madras. He observed that the immature mussels (below 40 mm in length) showed negative allometry while mature mussels (above 40 mm in length) showed positive allometry. The mature and immature mussels showed significant differences in all relationships except in those involving measurements of weights of one part with weight measurements of another part. Sherman and Sherman (1976) express that bivalves are principally ciliary mucus feeders. The ctenidium is the principal organ of food capture in these sedentary animals and also is responsible for some selection of materials. Langton *et al.* (1977) studied the effect of ration size on the growth and growth efficiency of the bivalve mollusc (*Tapes japonica*). It was found that this mollusc growth better when feed on phytoplankton than when feed on Zooplankton. Sivalingan (1977) analyzed the intestinal content of green mussel, *Mytilus viridis*, and observed that this species selectively ingested the

diatom, *Coscinodiscus nodulifer* the dominant phytoplankton in the water around the culture station.

According to Winter (1978), for maximum growth mussels should be maintained at a constant optimum food level by replacement of food removed per unit time. The inference of low loads of suspended silt on the filter feeding activity is quantified. Asif (1979) observed reproductive cycle and changes in associated parts of gonad in *Crassostres glomerata* from the coast of Karachi. The species attains maturity of gonad in majority of specimens in April, which is continued in fluctuating manner through October. Active spawning continues from May/June to November. Comparison among the different size groups shows that spawning in youngest size group is very restricted. No "inactive phase" is observed but "indifferent phase" generally occurs in winter.

Kunigelis and Saleuddin (1983) experimental studied on shell repair rates and carbonic anhydrase activity during shell repair in mollusca. They observed that mantle tissue carbonic anhydrase titres were found to vary with shell deposition rates. Following shell injury at the growing edge, linear shell deposition rates and mantle tissue anhydrase specific activity were found to increase significantly. This suggests that the process of shell regeneration does not only include the patching of actual injury but also allows for the establishment of a new growing edge to correct for the displacement of the plane of shell deposition.

Moorthy *et al.* (1984) observed that oxygen consumption and sodium, potassium and calcium concentration were determined in the freshwater mussel, *Lamellides marginalis*, and its tissue during methyl parathion (MP) toxicity. A transient increase followed by decrease in whole animal or tissue respiration as a function of time was observed. Greater loss of calcium by mantle, sodium by the gill, and calcium and sodium by the hepatopancreas and foot was observed. As compared to calcium and sodium, the loss of potassium by the tissues was less.

Sreenivasa *et al.* (1984) observed in vitro of methyl parathion on some kinetic constants of succinic dehydrogenase (SDH) in hepatopancreas of freshwater mussel, *L. marginalis* were studied. Altered pH vs. specific activity curves for SDH demonstrated significant inhibition by methyl parathion in buffered acidic, neutral and alkaline ranges. At high pH ranges IC₅₀ (12.5 microM) of methyl parathion did not cause 50% inhibition enzyme as it did at neutral and acidic pH. Activation energies (ΔE) were found to be increased suggesting decreased efficiency of enzyme in presence of methyl parathion. Non-competitive inhibition with respect to activation by succinate was indicated by decreased maximal velocity (V) without change in Michaelis Menten constant (K_m). Pyridine-2-aldoxime (25 microM), pyridine-4-aldoxime (15 microM) and L-cysteine (40 microM) neutralized the inhibition of SDH by methyl parathion (12.5 methyl parathion). The kinetic data suggests that inhibition of SDH by methyl parathion was pH and temperature independent.

Alam and Patra (1985) described a preliminary study of the freshwater molluscan fauna of Chittagong division with their ecology. All together 10 families, 14 genera and 15 species were recorded. Seven species were found to be reported from the entire place while 4 species were restricted.

Alagarwami and Sreenivasan (1987) studied on chromosome complement of Indian pearl oyster *Pinctada fucata*. It is suggested that as in the case of *Crassostrea* spp., genus *Pinctada* may exhibit conservativeness in chromosome number.

Jayabal and Kalyani (1987) showed the hard clam *Meretrix meretrix* has an extended breeding period from February to September and the maturation of gonads takes place immediately after spawning. Both males, which are slightly more dominant and females, attain maturity at the same size. Among the ecological factors salinity is found to play an important role in the reproductive cycle.

Bailey and Green (1988) studied on morphology and growth rate of a freshwater mussel. Two growth rate analyses showed that the mussels from high exposure areas also grew faster. The results demonstrate the importance of (i) a multivariate consideration of shell form (as opposed to the use of ratio variables) when assessing form-habitat correlations, (ii) quantitatively defining habitat variation in examining such relationships and (iii) measuring form-habitat relationships among more than two sites in a given basin or stream reach.

Hossain (1988) studied on the food and feeding habits in different size groups, of freshwater mussels, *Lamellidens marginalis*.

Begum *et al.* (1989) studied on pearl culture in freshwater mussels *Lamellidens marginalis* in two rearing ponds of aquaculture experiment station FRI at Mymensingh in Bangladesh.

Smith and Green (1992) studies on age determination of three species of freshwater mussels. They found that age estimates based on internal bands were most suitable for *Elliptio complanata*. Estimates based on external rings were more precise than those based on internal bands for *Anodonta imbecilis*, but were believed to include disturbance rings. Shell length and weight were similarly correlated with age for a given species and population.

Sreedevi *et al.* (1992) state that the levels of soluble, structural and total protein, and the activities of ALAT and AAT decreased along with an increase in the levels of free amino acids and the activity of protease in the ctenidium, hepatopancreas and foot of the freshwater mussel *L. marginalis* after 1, 2, 3 and 4 d of exposure to a lethal concentration (115 mg. L^{-1}) of nickel. But the activity of GDH and the level of urea decreased in the hepatopancreas and increased in the ctenidium and foot. A reverse trend was observed in the level of ammonia. In a sub lethal concentration (23 mg.L^{-1}), the levels of soluble, structural and total proteins and ammonia decreased in these three organs of the mussel after 1, 5, 10

and 15 d of exposures, with an increase in the levels of free amino acids, urea and in the activities of protease, ALAT, AAT and GDH. The extent of these changes differed in degree depending on exposure period in the lethal and sub lethal concentrations. The results are discussed in order to arrive at the degree of metal stress on the overall nitrogen metabolism of the mussel according to the period of exposure to lethal and sub lethal concentrations of nickel.

Das and Jana (2003) observed the freshwater bivalve, *Lamellidens marginalis* was experimentally exposed to 10 and 30 glycogen level of liver and gills for health assessment for their reuse in the reclamation of cadmium intoxicated environments. In situ experiment was also performed for better appraisal of the filtration rate in the lake. Oxygen uptake in the treated group exceeded that of control by 15-22% during the early 24h after cadmium exposure, but followed an essential decline (23-30%) thereafter. The reduction of filtration rate ranged from 12-62% in laboratory to 83-85% in field trials. At the tissue level, glycogen content was reduced by 61-72% in liver and 52-63% in gill. In both tissues, glycogen content was inversely proportional to the cadmium contents of the animal. Critical appraisal of data suggests that the threshold values of cadmium in gill and liver were 50-80 microg/g dw for oxygen uptake and 50-60 microg/g for filtration rate because of marked reduction of these parameters beyond the value of cadmium. It is concluded that filtration rate, oxygen uptake of the freshwater bivalve, *L. marginalis* can be used as biomarker for animal health assessment and for possible reuse of the stock animals.

Das and Jana (2003) observed that biofilter potential of the freshwater bivalve, *Lamellidens marginalis* was examined in cage experiments conducted in a river canal (Ichhaorem 24 Parganasm West Bengal, India) receiving industrial effluents from steel and metal factories as well as from an ordinance factory. Cadmium is one of the major contaminants in this river canal. *Lamellidens* collected from polluted free natural ponds, were sorted into three size groups (large: 59 $\pm$ 3.2 g, 10 $\pm$ 2.3 cm; medium: 30 $\pm$ 2 g, 6 $\pm$ 1.7 cm and small: 13 $\pm$ 1.5 g, 4 $\pm$ 1.2 cm) were held in cages at three different sites along a cadmium concentration gradient. Concentrations of cadmium were measured from water, sediment and different tissues of *Lamellidens* at weekly intervals using atomic absorption spectrophotometric methods. Cadmium uptakes by *Lamellidens* in all media were highly concentration dependent in both summer and winter months. For all three size groups, cadmium uptake was maximum in the gills at the beginning of experiment, and liver at the later phase. Cadmium uptake was 11-67% higher during summer than during the monsoon season for all tissues and size groups. Estimation of concentration factor revealed that tissues were saturated with cadmium during the 13-14th week after *Lamellidens* introduction during summer, but remained unsaturated during the monsoon season. It is concluded that *Lamellidens* might be considered as an efficient biofilter for reclamation of aquatic environment having sub-lethal concentration of cadmium.

Das and Jana (2004) water and sediment samples collected from 18 wetland ponds within and outside industrial areas were examined for cadmium

concentration and water quality parameters during the period of January to July 1996. The Cd contents in gill, liver, mantle and shell of freshwater mussel (*Lamellidens marginalis*) as well as leaves and roots of water hyacinth *Eichhornia* those occurred in these ponds also estimated. Cd concentration ranged from 0.006 to 0.7025 mg/l in water and from 1-7 to 77 microg/gdw in sediments of all the ponds investigated. The amount of Cd occurring in water and sediment was much higher in concentration in the ponds located in Captain Bheri and Mudiali farm close to industrial areas, compared to remaining ponds located outside the industrial belt. *Lamellidens marginalis* procured from Mudiali and Captain Bheri ponds showed regardless of size, tissue and season of collection significantly higher Cd concentration than did those from other ponds. Likewise, tissue Cd in *Eichhornia* collected from Mudiali pond was as high as 125-152 microg/gdw in root and 21-63 microg/gdw in leaves compared to 40-108 microg/gdw in root and 9-43 microg/gdw in leaves in the remaining ponds. Seasonal variability of Cd was clear-cut; the concentration was relatively higher in water and sediment in all ponds during summer than during monsoon season or winter. Size-wise, smaller groups showed the highest concentration of Cd in all tissues of *Lamellidens* compared with medium and large size groups. Concentration factor for all tissues of *Lamellidens* regardless of size and season was inversely proportional with the ambient Cd concentration. Concentration factor estimated for all tissues in all ponds and all seasons was in the order: liver>gill>shell>mentle. As all ponds located outside the industrial belt showed Cd concentration ranging from 0.006 to 0.049 mg/l, it is suggested that these wetlands do not pose serious risk to the environment.

2.2 Pesticide

Pesticides are commonly designed for the control of pest populations. The word pest means any destructive noxious or harmful organisms. So, pesticides are used for killing or controlling pest and controlling diseases caused by them. Precisely pesticides are defined under the Federal Environmental Pesticide Control Act "any substance or mixture of substances included for preventing, destroying repelling or mitigation any pest (insects, rodents, nematodes, fungus, weeds and other forms of terrestrial and aquatic plant or animal like bacteria or other microorganism)" or "any mixture of substances intended of reuse as a plant regular, defoliant or desiccant" (Mishra *et al.*, 1987)

More than 10000 species of insects, 600 species of weed, 1500 plant diseases and 1500 species of nematodes which are injurious and are being controlled by pesticides (Brady, 1990). Through designed to eradicate harmful agents for crops, pesticides are often criticized for their ill effects on non-target organisms. Pesticide residues also pose considerable problem of injury to non-target organisms, movement out of the intended zone of activity, and the threshold levels of hazard in other segments of the environment. There is continuing concern that these chemicals may also adversely affect various non-parasitic segments of soil micro flora.

Modern pesticides are mostly organic chemicals. Inorganic pesticides are only sparsely used. Discussion, therefore, will be centered on organic pesticides. Now-a-days it is an enormous challenge to use pesticides while maintaining water quality (Gan, 2002). On the other hand, we all recognize the significant roll of pesticides in ensuring a high quality of life. At the same time, we also need to be aware that pesticide application has affected and still can affect water quality.

The potential of pesticides to contaminate groundwater depends on a number of soil and pesticide properties that directly or indirectly affect the mobility and persistence of the pesticides in the soil environment. While a number of studies have been carried out in attempts to understand behavior and fate of pesticides in soil (Singh *et al.*, 1989, Pennel *et al.*, 1990; Kookana *et al.*, 1992) the high expenses of these studies combined with the large number of pesticides make it prohibitive to study every pesticide under various soil and environmental condition. Increasingly scientists and environmental managers use computer models to study the complex pesticide processes in soil and to provide management guidance (Jury *et al.*, 1987; Mullins *et al.*, 1993).

2.3 Classification of pesticides and their chemical nature

2.3.1 Classes of chemical insecticides

The majority of the chemical insecticides consist of an active ingredient (the actual poison) and a variety of additives which improve the efficacy of its application and action. The chemical and physical properties of the technical product, for example, its state, melting point and vapour pressure, will to some extent influence the type of additives that can be used and subsequently the overall formulation of the insecticide. The formulation of an insecticide will influence the method by which it is applied, its persistence in the field and also in its toxicity. These two aspects then, the chemistry of the technical product and the insecticide formulation, are central to an understanding of chemical insecticides. Chemical insecticides are usually divided into four major classes, the organochlorines, the organophosphates, carbamates and pyrethroids. In addition to these there are now a number of new compounds representing other 'smaller' classes e.g. formamidines; insect growth regulatory, imidates, phenylpyrazoles

2.3.2 Organochlorine insecticides

The organochlorines are insecticides that contain carbon (thus organo-), hydrogen, and chlorine. They are also known by other names: chlorinated hydrocarbons, chlorinated organics, chlorinated insecticides, and chlorinated synthetics. The organochlorines are mostly of historic interest, since only a few survive in today's arsenal.

In 1874 a German chemist, Dr. O. Zeidler, synthesized a chemical with the rather daunting name I,I,I,trichloro-z,z,di-(4-chlorophenyl) ethane. It was a white powder of no apparent use. It was put into a bottle, labelled, and put on the laboratory shelf where it was to lie practically untouched for 65 years until the

demands of war in 1939 brought it to the fore, not as a weapon of war, but as a means of saving lives (Wright *et al.*, 1972).

Its insecticidal powers were discovered by a Dr. Paul Muller, working for J. R. Geigy in Switzerland. It was found to be effective against the Colorado beetle, fleas, lice, mosquitoes, flies and many other insects. Samples were sent secretly to Britain and to the United States of America where further tests were carried out. Volunteers went for days in underclothes treated with this chemical, now known as DDT (Fig. 1), and three weeks after treatment clothes were still lethal to lice. No volunteer showed any signs of poisoning. It was used for treating soldiers' uniforms, and it is of interest to note that at that time the rumors went round that it had an adverse effect on male virility. This was completely unfounded; the birth rate shot up at a staggering rate after the war and has been going up ever since. One of DDT's triumphs was in 1944 when it controlled a major outbreak of typhus in Naples.

And so the first of the organochlorine was born. Despite the fact that DDT was one of the first organic insecticides to be used and is still the most widely used insecticide, it is only recently due to the work of Drs. Matsumura and O'Brien that we have come to have an understanding of how it works. Apparently, it complexes with the energy-producing enzyme system at nerve endings, causing the characteristic tremors and convulsions that are well-known features of DDT poisoning. The site of action is established as the peripheral nervous system, since it has been shown that the isolated central nervous system is insensitive to DDT.

Following on the discovery of the insecticidal action of DDT, other organochlorine compounds were examined and synthesized. One of these, benzene hexachloride, was first prepared by Faraday in 1825; but its insecticidal properties were not discovered till about 1940 when British (I.C.I.) and French workers independently developed it. A purified form, gamma benzene hexachloride or gamma-BHC (Fig. 7), is sometimes known as lindane after Van der Linden (who first isolated it without knowledge of its insecticidal powers in 1912). It has a broadly similar action to DDT. Both compounds can induce resistance in insects but resistance to gamma-BHC is more likely to be coupled with resistance to the cyclodienes than to DDT. The taint caused by crude BHC, which was evident even at very low concentrations, has led to its being condemned for use on food crops and although the purified form gamma-BHC has little taint the prejudice remains. It is used as a soil pesticide, as a seed treatment, for grasshopper control and against cotton insects. Its mode of action is unknown, though it is thought that it may be similar in action to DDT. It is one of the less persistent of the organochlorine compounds and has only a moderate toxicity to mammals. Related to gamma-BHC is toxaphene which is a mixture of several compounds which have always been formulated without separation. It has similar biological properties to gamma-BHC.

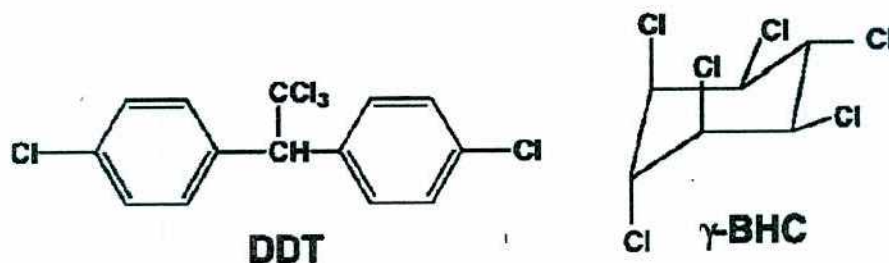


Fig. 1. Chemical structures of DDT and Gamma-BHC

The cyclodienes appeared after World War II: chlordane, 1945; aldrin and dieldrin, 1948; heptachlor, 1949; endrin, 1951; mirex, 1954; endosulfan, 1956; and chlordecone (Kepone(r), 1958 (Ware, 2000). The insecticidal properties of chlordane were first described in 1945 by Kearns and co-workers, and this was the first of a series of compounds which were made using the so-called 'diene' synthesis. This reaction was discovered by two chemists, Diels and Alder, and for this reason the most important members of this group of insecticides were given the names dieldrin (Fig. 1) and aldrin.

The starting-point for the production of chlordane is chlordene, which was discovered by a Dr. Hyman in 1945. Chlordane contains heptachlor as an impurity, and this has been found to be a more effective insecticide than chlordane itself. Other variations of the Diels-Alder reaction produce aldrin, which can in turn be oxidized with hydrogen peroxide to yield yet another powerful insecticide (dieldrin). Dieldrin is also formed from aldrin in living tissue; another close chemical relative is endrin.

Chlordane and heptachlor are used for the control of cockroaches, ants, termites and other household pests (Luckmann and Metcalf, 1975). Aldrin, dieldrin and endrin are the most powerful general insecticides known. Aldrin is volatile, and this makes it particularly useful for soil application; but it is also used as a broad spectrum insecticide for the control of insect pests of fruits and vegetables, and as a seed treatment. Dieldrin is particularly active against the parasites found on the skins of sheep and cattle and it has been used for this purpose as a dip against lice, ticks, and blowflies. It is also used for the mothproofing of materials. Endrin is very useful for the control of caterpillars attacking field and vegetable crops and it is one of the few pesticides which is effective against certain species of mite otherwise difficult to control. The symptoms of poisoning by all of these cyclodiene insecticides are similar. Affected insects show convulsive activity with final complete prostration. The insecticides are readily absorbed through the cuticle of the insect and the site of action appears to be the central nervous system rather than the peripheral nervous system which is affected by DDT. Treated insects show a characteristic 'fanning' of the wings. There are interesting variations of toxicity within the group; thus endrin is relatively non-toxic to the housefly whereas aldrin is highly toxic. The biochemical mode of action is not known. Most of these compounds, especially aldrin, dieldrin, and endrin, are very

stable and resistant to breakdown and this has led to the introduction of other cyclodienes which are less persistent. Endosulfan, for example, has been produced by the Hoechst Chemical Company in Germany and telodrin by Shell in Britain.

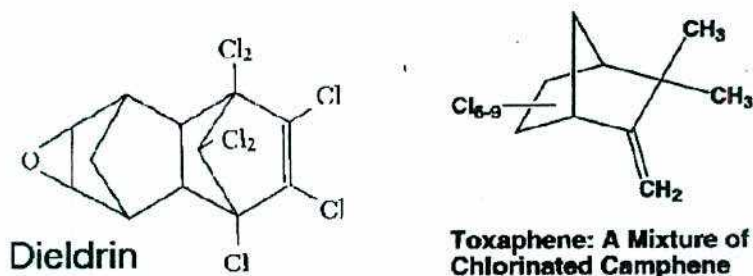


Fig. 2. Chemical structures of Dieldrin and Toxaphene

2.3.3 Organophosphorus insecticides

The first indications that organophosphorus chemicals were toxic came not from insecticides but from bootleg liquor in the United States of America during the prohibition period. A chemical called tricresylphosphate was manufactured originally as a plasticizer but was also used occasionally as an adulterant of cooking fat and more widely as a ginger flavouring for some rather unpalatable and questionable 'whiskeys'. Some of the illegal liquors were no doubt poisonous enough in themselves but the addition of tricresylphosphate made even the most innocuous liquids deadly. Many deaths and many cases of paralysis of the limbs resulted.

Organophosphates (OPs) is the currently used generic term that includes all insecticides containing phosphorus. Other names used, but no longer in vogue, are organic phosphates, phosphorus insecticides, nerve gas relatives, and phosphoric acid esters. All organophosphates are derived from one of the phosphorus acids, and as a class are generally the most toxic of all pesticides to vertebrates. Because of the similarity of OP chemical structures to the "nerve gases", their modes of action are also similar. Their insecticidal qualities were observed in Germany during World War II in the study of the extremely toxic OP nerve gases sarin, soman, and tabun. Initially, the discovery was made in search of substitutes for nicotine, which was heavily used as an insecticide but in short supply in Germany

Research into the possible use of organophosphorus compounds as war gases was begun during the Second World War in the laboratories of Britain, France, Germany, and the USA. The research was very successful, but the products were fortunately never used. Among them were some of the most potent nerve gases known to man. But among them too were some which, while not so toxic to man, were very potent against insects. And so a whole new group the organophosphorus insecticide was born. In 1943 Dr. Gerhard Schrader of the Bayer Company's laboratories in Germany produced the first organophosphorus compound available for crop protection parathion. There followed in rapid

succession schradan and demeton (the first practical systemic insecticide), azinophos-methyl (a broad spectrum insecticide), and trichlorphon, a stomach poison.

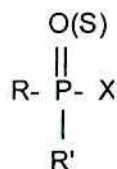
Many of the early organophosphorus compounds including parathion (trichlorophor is an exception) are very toxic to mammals, including man. Research has therefore been directed towards finding compounds that are highly toxic to insects but which are of low mammalian toxicity. Such a chemical is malathion which was introduced in 1950 by the American Cyanamid Company. Although only about a half to a twentieth as toxic to insects as parathion, it is only about one two hundredth parts as poisonous as parathion to mammals. Malathion which is an outstandingly successful insecticide, and is probably the most widely used insecticidal organophosphorus compound, has a low mammalian toxicity because it is rapidly broken down in the livers of animals. In the insect, on the other hand, it is very stable and can therefore exert its toxic effect; it is widely used in the tropics for mosquito control. At the other end of the scale it is also widely used by amateur gardeners for the control of pests. Its worldwide use in million pound quantities, in the home and in the garden, in the orchard, on animals and in insect eradication campaigns indicates that it is one of the safest of insecticides.

Organophosphates are derived from phosphoric acid and are some of the most toxic insecticides. As opposed to the chlorinated hydrocarbons, they are unstable (in the presence of light) and quickly breakdown into non-toxic compounds. This class of insecticide inhibits the action of several esters splitting enzymes. The organophosphorous insecticides are particularly effective against cholinesterase. This enzyme is widely distributed in the animal kingdom. Adrian *et al.* (1947) first observed the inhibition properties of organophosphorous esters against cholinesterase. Balls and Jansen (1952) found that the inhibition was attributed to the phosphorylation of the esteratic site, which was initially demonstrated by the action of DFP with chymotrypsin. It is well known that actual thion type insecticides do not inhibit the easterase *in vitro*, but act to increase the efficiency of anticholinesterases *in vivo*. Gage (1953) demonstrated that the inhibition produced from parathion *in vivo*, was the oxoanalogue paraoxan. It has been demonstrated that, when paraoxon is presented to chymotrypsin in a dilute solution, the phosphorous of one molecule of the inhibitor becomes locked up in every molecule of the enzyme inhibited and at the same time one free nitrophenate ion appears. The active site of the enzyme evidently splits the molecule of the inhibitor, which is then unable to release the phosphoryl moiety. This causes blockage of the further action, whereas the unchanged enzyme can attach, split and release some thousands of its normal substrate molecules per second.

In animals acetylcholine is believed to be the transmitter in the synapses of the nervous system (Smallman and Mansingh, 1969). Acetylcholine is synthesized in the nerve ending by the action of the cholinacetylase from choline and acetyl co-enzyme-A, which is stored in synaptic vesicles. These vesicles burst automatically

to generate a miniature potential without giving any action to the associated cells. However, by the stimulation of the action current, the burst and release of acetylcholine increases quickly by 100 to 1000 times. This generates synapse or end-plate potential and consequently, the excitation of the postsynaptic membrane. The released acetylcholine is then rapidly hydrolysed into inactive acetic acid and choline by the action of acetylcholinesterase before the nerve impulse arrives. The original state of the postsynaptic vesicle cannot reach till the acetylcholine remains in the region of the synaptic cleft. So, acetylcholine inhibition results in the disturbance of the nervous function leading to severe and often-lethal damage in the animal body. Generally acetylcholine inhibition interferes with the co-ordination of muscular response in the vital organs with serious symptoms and eventually death.

Organophosphates are characterized as having different alcohols attached to their phosphorous atoms, and the various phosphorous acids produced are termed esters. These esters have different combinations of oxygen, carbon, sulphur and nitrogen, and organophosphates formed from them can be divided into three groups of derivatives: aliphatic, phenyl and heterocyclic. In spite of the enormous structural diversity of organophosphorus insecticides, all the compounds can be represented by the classical hypothetical structure as:



Where R and R' is short chain alkyl, alkylthio or amide groups and X is labile as ion leaving group.

It is estimated that more than 100,000 different organophosphorus compounds have been synthesized and tested out as insecticides. At present more than 40 are commercially successful, a number of them being manufactured in multimillion pound quantities. All OPs are esters of phosphorus having varying combinations of oxygen, carbon, sulfur and nitrogen attached, resulting in six different subclasses: phosphates, phosphonates, phosphorothioates, phosphorodithioates, phosphorothiolates and phosphoramidates. These subclasses are easily identified by their chemical names.

The OPs are generally divided into three groups--*aliphatic*, *phenyl*, and *heterocyclic* derivatives.

Aliphatics—The aliphatic OPs are carbon chain-like in structure. The first OP brought to agriculture, TEPP (1946) belonged to this group. Other examples are malathion, trichlorfon [Dylox(r)], monocrotophos [Azodrin(r)], dimethoate [Cygon(r)], oxydemetonmethyl [Meta Systox(r)], dimethoate [Cygon(r)],

dicrotophos [Bidrin(r)], disulfoton [Di-Syston(r)], dichlorvos [Vapona(r)], mevinphos [Phosdrin(r)], methamidophos [Monitor(r)], and acephate [Orthene(r)].

Phenyl derivatives--The phenyl OPs contain a phenyl ring with one of the ring hydrogens displaced by attachment to the phosphorus moiety and other hydrogens frequently displaced by Cl, NO₂, CH₃, CN, or S. The phenyl OPs are generally more stable than the aliphatics, thus their residues are longer lasting. The first phenyl OP brought into agriculture was parathion (ethyl parathion) (Fig. 3) in 1947. Examples of other phenyl OPs are methyl parathion, profenofos [Curacron(r)], sulprofos [Bolstar(r)], isofenphos [Oftanol(r), Pryfon(r)], fenitrothion [Sumithion(r)], fenthion [Dasanit(r)], and famphur [Cyflee(r), Warbex(r)].

Heterocyclic derivatives--The term heterocyclic means that the ring structures are composed of different or unlike atoms, e.g. oxygen, nitrogen or sulfur. The first of this group was diazinon (Fig. 3) introduced in 1952. Other examples in this group are azinphos-methyl [Guthion(r)], azinphos-ethyl [Acifon(r), Gusathion(r)], chlorpyrifos [Dursban(r), Lorsban(r), Lock-On(r)], methidathion [Supracide(r)], phosmet [Imidan(r)], isazophos [Brace(r), Triumph(r)], and chlorpyrifos-methyl [Reldan(r)].

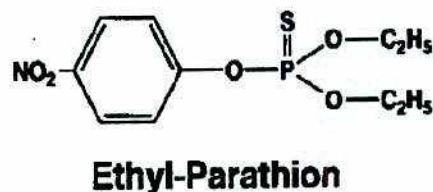
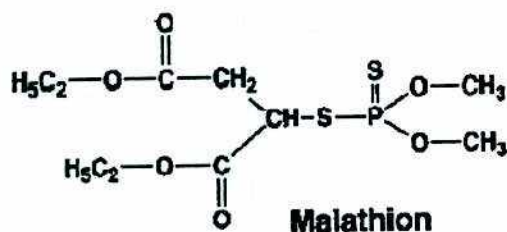


Fig.3. Chemical structures of some common organophosphate insecticides.

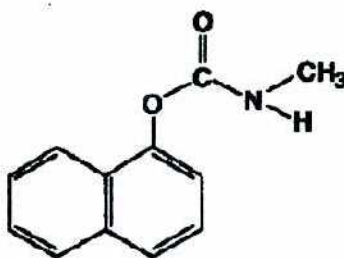
2.3.4 Carbamate insecticides

The successful development of the organophosphates as pesticides directed attention to other compounds, which act as anticholinesterases. This led to discovery of carbaryl (Fig. 4) with its broad spectrum activity. It appears that the action of the carbamates on both insects and mammals is an inhibition of the cholinesterase. The carbamate insecticides are derivatives of carbamic acid (as the OPs are derivatives of phosphoric acid). And like the OPs, their mode of action is that of inhibiting the vital enzyme cholinesterase (ChE).

The carbamates are broad-spectrum insecticides that have had wide application in agriculture. They were developed by the Geigy Corporation in 1951 but, because of initial problems, were not practical until 1956. These insecticides are produced from carbamic acid and are similar in environmental persistence to the organophosphates. A distinct limitation of carbamate in pest management is their toxicity to Hymenoptera, including both pollinators and parasitoids.

Carbamates have similar effects on nerve function as organophosphates and cause similar symptoms of poisoning. However with carbamate poisoning, cholinesterase blood levels return normal within a few hours of the incident. Therefore cholinesterase levels are not reliable for diagnosing carbamate poisoning. As with organophosphates the greatest danger from exposure to carbamates and their residues is acute illness (poisoning). Unlike those due to organophosphates, few carbamate poisonings are life threatening (with the exception of aldicarb, which is extremely toxic) (Hill, 1983). These drugs purposely made quaternary salts to obtain water solubility.

The first successful carbamate insecticide, carbaryl (Sevin(r)), was introduced in 1956. More of it has been used worldwide than all the remaining carbamates combined. Two distinct qualities have made it the most popular carbamate: its very low mammalian oral and dermal toxicity and an exceptionally broad spectrum of insect control. Other carbamates are methomyl [Lannate(r)], carbofuran [Furadan(r)], aldicarb [Temik(r)], oxamyl [Vydate(r)], thiodicarb [Larvin(r)], methiocarb [Mesuro(r)], propoxur [Baygon(r)], bendiocarb [Ficam(r)], carbosulfan [Advantage(r)], aldoxycarb [Standak(r)], promecarb [Carbamult(r)], and fenoxycarb [Logic(r), Torus(r)].



Carbaryl

Fig.4. Chemical structures of Carbaryl.

2.3.5 Formamidine insecticides

The formamidines comprise a small group of insecticides. Three examples are chlordimeform [Galecron(r), Fundal(r)], which is no longer registered in the U.S., formetanate [Carzol(r)], and amitraz (Fig. 5) [Mitac(r), Ovasyn(r)]. Their current value lies in the control of OP- and carbamate-resistant pests.

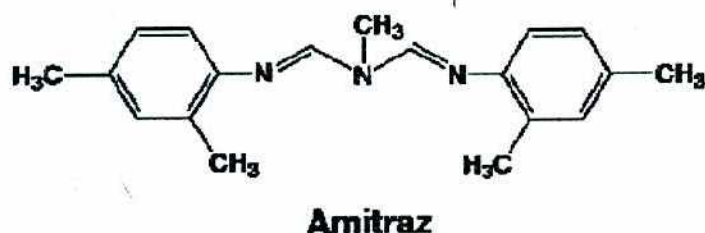


Fig.5. Chemical structures of Amritaz.

2.3.6 Pyrethroid insecticides

Natural pyrethrum has seldom been used for agricultural purposes because of its cost and instability in sunlight. Over the past two decades many synthetic pyrethrin-like materials have become available. They were originally referred to as synthetic pyrethroids. Currently, the better nomenclature is simply pyrethroids. These are very stable in sunlight and are generally effective against most agricultural insect pests when used at the very low rates of 0.01 to 0.1 pound per acre.

Pyrethroids are a class of insecticides of great importance for the protection of man's crops and his health. The large differential toxicity to mammals and insects means that these compounds can be used safely by man to generate very unsafe conditions for insects. Pyrethroids also have very many of the properties required for a deleterious effect on the environment, i.e., ready degradation in soil, virtually zero mobility in soil and rapid metabolism and excretion by animals. No insecticide is perfect but the pyrethroids appear to be more acceptable than many other standard insecticides (Leahey, 1984).

The characteristics of high knockdown, lethal activity, wide spectrum, good residual activity together with repellent and antifeeding activity of the pyrethroids have enabled them to become widely used for plant protection and public health and vector pest control (Hirano, 1989).

The pyrethroids are active against a wide range of insect pests and have been used on a variety of crops as experimental evidence has shown that they are non-phytotoxic. Their major use in agriculture has been for control of bollworms and leaf worms in cotton, but they have also been successful with lepidopterous pests in fruits and cereals, aphids in cereals and other minor outlets. Further pyrethroids are being introduced for use in soil (Hirano, 1989).

The control of public health pests involves a variety of techniques using pyrethroids, these include, (a) Domestic aerosols/sprays: these generally use powerful knockdown pyrethroids such as tetramethrin, bioallethrin combined compounds providing good kill activity such as remethrin, permethrin and the alpha (α) -cyano pyrethroids (e.g. cypermethrin). (b) Mosquito coils/mats: Bioallethrin gives a 50% knockdown at time of 5.5 minutes. (c) Large scale space sprays: Especially important in urban fly control. Where control of 90% to 100% has been achieved with all pyrethroids tested. (d) Surface treatments: Applied as a residual spray to resting sites has given efficient control. (e) Larvicidal: As pyrethroids are highly effective against mosquitoes, they can be use as surface treatments without toxic effects to fish. (f) Special treatments: pyrethroids have been used for bed nets, clothing impregnation and targets (Carter, 1989).

The symptoms of pyrethroids poisoning are indicative of an attack on the insect nervous system. Insects are not dependent on continuous nervous control of respiration and circulation. Because of this death appears to be due to the irreversible damage to the nervous system occurring when poisoning lasts for more than a few hours.

Pyrethroids interact with sodium channels, potassium channels, GABA- activated chloride channels and membrane bound ATPases (Leahey, 1984). The most important effect of pyrethroids in their interaction with sodium channels. Type-1 Pyrethroids [non- alpha (α) - cyano substituted pyrethroids] tend to cause repetitive firing while type-2 [alpha (α) - cyano substitutes pyrethroids] depolarize the nerve membrane and lead to action potential block. The range of pyrethroids has increased dramatically over the last 10 years. Allethrin (commercially launched in 1953) and Tetramethrin (launched in 1965) both showed good knockdown properties have been succeeded more recently by pallethrin (launched in 1968), which is faster and more efficient (Elliot, 1989).

Many pyrethroids (e.g.- cypermethrin, cyhalothrin) are now used in the animal sector as ectoparasiticides and in the household insecticide market, laboratory tests have indicated that pyrethroids are highly toxic to bees. However, in the field this toxicity is diminished by the repellent activity e.g. alpha (α) -cypermethrin. Fluvalinate has been used to control mites in honeybee colonies without adversely affecting the bees (Elliot, 1989).

In 1986 the market share of pyrethroid reached 25% of the total insecticide market for plant protection and can be expected to increase in the future (Hirano, 1989).

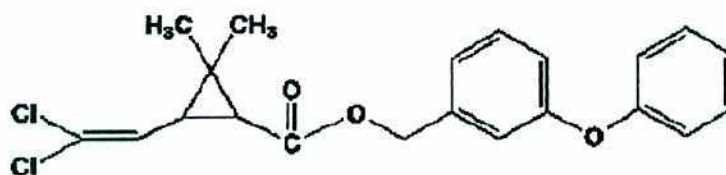
The pyrethroids have an interesting evolution, which is conveniently divided into four generations. The **first** generation contains only one pyrethroid, allethrin (Pynamin(r)), which appeared in 1949. Its synthesis was very complex, involving 22 chemical reactions to reach the final product.

The **second** generation includes tetramethrin [Neo-Pynamin(r)] (1965), followed by resmethrin [Synthrin(r)] in 1967 (20X as effective as pyrethrum), then bioresmethrin (50X as effective as pyrethrum) (1967), then Bioallethrin(r) (1969), and finally phonothrin [Sumithrin(r)] (1973).

The **third** generation includes fenvalerate {Pydrin(r) (discontinued), Tribute(r), & Bellmark(r)}, and permethrin [Ambush(r), Astro(r), Dragnet(r), Flee(r), Pounce(r), Prelude(r), Talcord(r) & Torpedo(r)] which appeared in 1972-73. These became the first agricultural pyrethroids because of their exceptional insecticidal activity (0.1 lb ai/A) and their photostability. They were virtually unaffected by ultraviolet in sunlight, lasting 4-7 days as efficacious residues on crop foliage.

The **fourth** and current generation, is truly exciting because of their effectiveness in the range of 0.01 to 0.05 lb ai/A. These include bifenthrin [Capture(r), Talstar(r)], *lambda*-cyhalothrin [Demand(r), Karate(r), Scimitar(r) & Warrior(r)], cypermethrin [Ammo(r), Barricade(r), Cymbush(r), Cynoff(r) & Ripcord(r)], cyfluthrin [Baythroid(r), Countdown(r), Cylense(r), Laser(r) & Tempo(r)], deltamethrin (Decis(r)) esfenvalerate [Asana(r), Hallmark(r)], fenpropathrin [Danitol(r)], flucythrinate [Cybolt(r), Payoff(r)], fluvalinate [Mavrik(r), Spur (r), discontinued], prallethrin (Etoc(r)), *tau*-fluvalinate [Mavrik(r)] tefluthrin [Evict(r), Fireban(r), Force(r) & Raze(r)], tralomethrin [Scout X-TRA(r), Tralex(r)], and *zeta*-cypermethrin [Mustang(r) & Fury(r)]. All of these are photostable, that is, they do not undergo photolysis (splitting) in sunlight. And because they have minimal volatility they provide extended residual effectiveness, up to 10 days under optimum conditions.

Recent additions to the **fourth** generation pyrethroids are acrinathrin (Rufast(r)), and the still experimental imiprothrin [Pralle(r)].



Permethrin

Fig.6. Chemical structures of Permethrin.

2.4 Behavior of pesticides in soil

The behavior of pesticides in soil is complicated by numerous interactions between the soil, liquid and gaseous phases of soil and between living and non-living components.

Soil characteristics are very important in determining how a pesticide breaks down and whether it leaches into groundwater (Jackson *et al.*, 1989). Because most breaks down occur in the soil, there is a greater potential for groundwater contamination in areas where substances such as pesticides move quickly through the soil. Sandy soils have large "pore" spaces between individual particles, and the particles provide relatively little surface area for "sorption" or physical attachment of pesticides. Large amounts of rainfall or excessive irrigation water can percolate through these soils, and dissolved pesticides can move rapidly down through the soil and into groundwater.

Clay soils, on the other hand, are made up of extremely small particles that provide a vast surface area for "sorption" the small pores between clay soil particles slow the movement of water and dissolved pesticides through the soil.

The following are the primary factors that affect the behavior of pesticides in the soil.

1. Properties of the pesticides
2. Properties of the soil
3. Site conditions including rainfall and depth to groundwater
4. These processes are dynamic and interrelated.

2.5 Movement of pesticides

Movement of a pesticide through soil can occur by two processes, diffusion and mass flow. Mass flow occurs as a result of external forces. The pesticide will be considered to either dissolved or suspended in water, present in the vapor phase, or adsorbed on solid mineral or organic components of the soil. Mass flow of the pesticides result from the mass flow of water and/or soil particles, which the pesticide molecule is associated. Pesticides, which have high adsorption coefficients, are relatively immobile in the soil (Lately and Farmer, 1999). Mass transfer of pesticides can occur through leaching of the pesticides, through runoff with soil and rainwater or upward movement in drying soils, laterally from water to drier soil as well as downwards in the soil. Mass transport by water flowing through the soil. Profile is dependent upon the direction and rate of water flow and the sorption characteristics of the pesticide with soil.

Diffusion processes are involved either directly or indirectly in the fate and behavior of pesticides in soils. Diffusion is the process by which matter is transported as a result of random molecular motion caused by the molecule's thermal energy. Dispersion of a treatment band is the intent of many pesticide applications and will often occur through diffusion. In the case of broadcast application, diffusion also becomes a mechanism for more complete distribution

of the pesticide in soil. While water may serve as a principal carrier for moving some pesticides into the soil profile, distribution into smaller pores, aggregates, and blocked pores depend on diffusion. Diffusion of pesticides through soils depends on a number of soil properties including water content, bulk density, air porosity and temperature and on certain chemical properties of the pesticides such as solubility, vapor density and diffusion coefficient (Farmer and Spencer 1973).

This incomplete list of possibilities suggests that the movement of pesticides in the environment is very complex with transfers occurring continually among different environmental compartments. In some cases, these exchanges occur not only between areas that are close together but also may involved transportation of pesticides over long distances. The worldwide distribution of DDT and the presence of pesticides in bodies of water such as the Great Lakes far from their primary use areas are good examples of the vast potential of such environment.

While all of the above possibilities exist, this does not mean that all pesticides travel along long distance or that all compounds are threats to groundwater. In order to understand which ones are of most concern, it is necessary to understand how the pesticides move in the environment and what characteristics must be considered in evaluation contamination potential. Two things may happen to pesticides, one they are released into the environment or they may be broken down, or degraded, by the action of sunlight, water or other chemicals, or microorganisms, such as bacteria. This degradation process usually leads to the formation of less harmful breakdown products but in some instances can produce more toxic products.

The second possibility is that the pesticide will be very resistant to degradation by any means and thus remain uncharged in the environment for long periods of time. The ones that are most rapidly broken down have the shortest time to move or to have adverse effects on people or other organisms. The ones, which last the longest, so called persistent pesticides, can move over long distances and can build up in the environment leading to greater potential for adverse effects to occur.

2.6 Studies on pesticide transport

2.6.1 Bangladesh aspects

The plant-protecting wing, of the Government, first imported chemical fertilizer and pesticide in Bangladesh in 1956. The Government of Bangladesh gave hundred percent subsidies in case of chemical fertilizer and pesticides up to 1974 and in that year it was reduced to fifty percent. In 1979 the support was totally withdrawn and the private import of chemical fertilizer and pesticides was recommended in the next year. According to a report, prepared by Philip Grain from Dhaka based Non Government Organization (N.G.O.) and Society for Environment and Human Development (SEHD) the use of chemical fertilizer and pesticides in Bangladesh has increased two folds during the time 1985 to 1990 (Gain, 1993). The amount of chemical fertilizer and pesticides used in

Bangladesh was 3063 MT in the year 1965-1986 and reached to 6948 MT in the year 1990-1991. If this rate of increase continues, this amount would be 16000 MT in the year 1995-1996. It is to be noticed that, 80% of the imported chemical fertilizer and pesticides have only been used in the production of rice. About 80% of 9.1 million hectare of agricultural land of Bangladesh is under rice cultivation.

Water quality aspects of pesticide and fertilizer use in Bangladesh were studied by NMIDP (1994 and 1995). They reported that pesticide and fertilizer use in Bangladesh is low compared to other developing countries. Water quality aspects studied were mainly related to possible groundwater contamination by pesticide and fertilizer residues and their impact on the environment. Their choice of monitoring sites was based on the maximum pesticide and fertilizer consumption areas and on the cropping intensity. They performed the groundwater sample test in the Chemistry Department, Atomic Energy Commission, Dhaka for general parameters and in the Institute of Food and Radiation Biology (IFRB) under Atomic Energy Research Establishment (AERE), Savar, Dhaka for pesticide residues analysis. They found groundwater quality in Bangladesh is good in terms of mineralization, except in the southern regions due to intrusions of salinity from tidal rivers and the sea. Pesticides residues were found only in 10 samples out of 78 samples. All the pesticides detected were longer-lived organochlorine, no traces of the moderately persistent organophosphorous, which account for the bulk of pesticide use, were found. Critical zones were identified based on rate of pesticide and fertilizer use and evidence of contamination by agrochemicals as detected.

Begum and Begum (1973) carried out experiment and found that of the organochlorines DDT was the most toxic to the fourth instars larvae of *Aedes aegypti* (L.) followed by aldrin, dieldrin, gammexene and heptachlor and of the organophosphorus temephos (Abate) was very toxic in comparison to imidan and dipterex. They concluded that temephos is 12 times as toxic as DDT, 23 times that of dieldrin, 15 times that of aldrin, and 93 times that of dipterex to the larvae.

Qadri *et al.* (1982) tested toxicities (LC_{50}) of topically administered organophosphates to *Apis cerana* and fishes. In the inhibition of bee and fish brain cholinesterase was also examined. The low toxicity of technical carboxin to honey bees may be due to the lower sensitivity of bee cholinesterase to inhibition by this carbamate, monocrotophos, quinalphos, diazinon and dimethoate were toxic to honey bees in technical and/or formulation grades.

Azam *et al.* (1985) evaluated that the availability of soil-bound ^{14}C residues of malathion to maize plants and microbes was investigated under laboratory conditions. Maize plants were grown in clay loam soil treated with ^{14}C malathion and it was found that 2.5% of the total bound ^{14}C and 6.6% of the freshly added ^{14}C was removed by the maize plants. In both cases the shoots contained more radioactivity than the roots and the uptake rate from freshly treated soil was three times higher than soil containing bound residues. The analysis of the residual soil after harvesting the plants showed that 6.1% and 12.02% of the residual ^{14}C from

bound and freshly applied malathion, respectively, could be extracted. This indicated that a part of the bound ^{14}C residues was released during plant growth.

Mostafa and Zayed (1986), in their experiment allowed sweet potato tubers to be absorbed either radioalabelled methomyl or radiolabelled carbofuran. After extraction, the mounts of residues bound to plant tissues were determined. The data indicated that binding of two chemicals was more or less equal. When the extracted tissues were fed to rats both chemicals were found to be bioavailable. The amount of methomyl taken up by rats was twice that of carbofuran. The major part of radioactivity derived from mehtomyl was rapidly eliminated from the animal. From the ^{14}C activity in the feed, 8.9% ^{14}C CO_2 , 7.9% organic volatile ^{14}C CO_2 fraction, 24.1% urinary ^{14}C metabolite and 21% ^{14}C material in the faeces could be recovered. The organic colatile fraction is believed to be methylmercaptan. In the case of carbofuran, the elimination of radioactivity was 10.7% ^{14}C - CO_2 9.1% urinary ^{14}C metabolites and 66% ^{14}C material in faeces. The amount of radioactivity found in the analyzed body organisms was less than 4% and 1% for methomyl and carbofuran respectively. They indicated that the pesticide was used by *Penicillium notatum* as carbon source.

Ibrahim (1992) reported that although DDT is banned all over the world, the manufacture's of Bangladesh used DDT in the coils produced by them which is harmful to human, animals as well as surrounding environments.

2.6.2 International aspect

Lockhart *et al.* (1985) studied on a population of young walleye (*Stizostedion vitreum*) in a shallow pond in Winnipeg, Manitoba, was exposed to malathion after 2 aerial applications of this pesticide for mosquito control. The fish population was sampled frequently throughout the season for survival and growth and also for brain cholinesterase measurements since that enzyme was known to respond to malathion poisoning. Cholinesterase activity fell to out 25% of prescribed values within 12 h. after the 1st spray, and then gradually recovered to about 80% of prescribed values over 2 weeks. The same pattern was evident after the 2nd spray, but the inhibition of cholinesterase activity was not quite as large. Fish captured for analysis showed no indication of the symptoms of organophosphorus pesticide poisoning. There have been a number of reports published linking cholinesterase inhibition with lethal and sub lethal responses in exposed groups, but the authors are unaware of the previous cases in which strong cholinesterase inhibition has been observed in field population for which growth and survival statistics were available. This case, then offered an opportunity to evaluate the biochemical response in terms of population parameters. The population statistics indicated small temporary decreases in both catch per unit effort and weight gains after the 1st spray. Evidently a reduction in cholinesterase activity to about 25% of pre-exposure levels was the approximate threshold for population effects.

Dec *et al.* (1986), also mentioned that the ^{14}C carbofuran was applied to sterile and non sterile soil at a rate of 5 mg/kg and incubated for 16 weeks. Soil samples were sokhlet extraction with methanol, the ^{14}C in the extracts and in dried

extracted soil was determined by combustion and liquid scintillation counting. The percentages of ^{14}C bound, expressed as a fraction of the quantity applied, were 2.4% immediately after application and 65.7% after 16 weeks period, wheat plants, seeded in an extracted soil: sand mixture (1:4) and grown for three weeks, took up 1.44% of the bound ^{14}C .

Formation and distribution of bound residues of ^{14}C chlorfenvinphos in winter rape and in soil, and their availability to rape and wheat plants were investigated by Dec *et al.* (1986). After application of ^{14}C chlorfenvinphos to rape plants, no bound radioactivity was detected either in the flowers and siliques or in the roots. In the leaves and /or stems the bound ^{14}C residues ranged from 0.02 $\mu\text{g/g}$ one hour after treatment to 0.18 $\mu\text{g/g}$ at harvest. Bound ^{14}C was located in lignin (12.1%) and other material that had been exhausted with methanol and water excreted via the faces. No radioactivity was excreted in urine of expired air CO_2 . A steady increase of bound radioactivity was observed in soil treated with ^{14}C chlorfenvinphos. Bound ^{14}C residues, expressed as percentage of the applied ^{14}C , were 13.7% in soil of higher organic matter content and 11.2% in soil of lower organic matter content. Soil sterilization resulted in a significant reduction of bound ^{14}C residues, indicating that binding was related to the activity of soil micro-organisms. The presence of bound ^{14}C residues did not affect the growth of wheat.

Raghu and Drego (1986), detected the bound residues in unflooded and flooded soils, even year after the application of ^{14}C lindane, the higher occurrence being in were formed in flooded soils which had been amended with green manure. Bound residue formation was higher in neutral and alkaline soils and appeared to be related to the organic matter content of these soils. The bound residues were associated with the fulvic acid and humic acid fractions. When unflooded and flooded soils containing bound residues were extractable residues were bound in unflooded soils then in flooded soils. The uptake of ^{14}C residues from soils containing bound ^{14}C residues by barley plants was observed. The bound residues appeared to have no effect on general soil microbial activities.

Zorgani *et al.*, (1986), studied the finding of ^{14}C endosulfan in three types of Sudanese agriculture soils under laboratory conditions. Binding of endosulfan did not exceed 4% of applied radioactivity after the incubation period of ten weeks. Edwards (1987) stated that the persistence and fate of pesticides in the soil depends on their physico-chemical characteristics, soil and climatic factors. They may affect micro-arthropods, myriapods, earthworms and insects and can be taken up into invertebrate tissues. Ghosh and Chakrabarty (1987) studied on effectiveness of organophosphorus compound on fish (*Barbus stigma*). He found that *B. stigma* was exposed in the laboratory to sub lethal doses (0.21mg/liter) of Nuvan (dichlorvos), Ekalux (quinalphos or suquin (of unstated composition) at 27°C and pH 7.2. This caused hyperglycaemia, hypocholesterolaemia, depressed free fatty acids levels and glycogenolysis in liver and skeletal muscles.

Arshad (1988) reported that (i) to study the persistence of malathion residues in the stored milled rice following multiple applications of ^{14}C malathion to the bagged rice and (ii) to determine the degradation of malathion in stored milled rice and stored paddy following direct and single application of ^{14}C malathion. The storage conditions were similar to those found in the local rice godown. Three applications of malathion to the bagged mill rice resulted in the accumulation of malathion and its metabolites in and / or on the rice grain over the storage period. After 9 months of storage, Ca. 4% of the total applied radioactivity of ^{14}C residues, were found in and / or the grain. About one fourth of the residue remained as the unchanged parent compound. On the other hand when ^{14}C malathion at the 10 ppm level was mixed directly with the milled rice or paddy prior to storage, the amount of malathion decreased from 6.2 $\mu\text{g/g}$ at zero time to 2.9 $\mu\text{g/g}$ after 3 months storage for the mill rice samples. The major metabolite in the milled rice and paddy samples was malathion monocarboxylic acid with trace amounts of malathion dicarboxylic acid and malaoxon.

Basham, *et al.* 1988, experimented the activities of ctenidial cilia and the heart in *L. marginalis* exposed to methyl-parathion (parathion-methyl, presumably technical grade) and Metacid-50 (parathion) showed a proportionate response to the concentrations of the pesticide in the medium. The use of ciliary and cardiac activity as an indicator of respiratory metabolism is discussed in the context of assays for organophosphate pollution.

Chakrabarty and Banerjee (1988) found that individuals of ^{14}C . *punctatus* were exposed for 15-90 days to sub lethal concentration of Thimet (phorate) 10 G (0.2 mg/litre), Elsan (phenthoate) 2% dust (2.0 mg/liter or Bazonon (diazinon) 5 G (5.0 mg/liter) in the laboratory, and their effects on blood parameters were recorded. Erythrocyte size shrunk initially and then expanded. There were marked fluctuations in total erythrocyte count, haemoglobin levels and packed cell volume percentage. Total leukocyte counts decreased before increasing. Thrombocyte counts and coagulation time fluctuated markedly. The effected parameters did not return to normal even after 3 months.

Dickinson *et al.* (1988) developed a new HPLC method to determine temephos in water. The method was tested and found to be reliable for measuring concentrations of 0.005-5.00 ppm. The detection limit at 254 nm was 1ng. The standard curve was not linear over the entire range; separate curve were required for concentrations above and below 0.1 ppm.

Kugler *et al.* 1988, reported on the bio-accumulation of cyanuric acid in bivalve molluscs and histological effects of toxicity in the freshwater mussel *Anodonta cygnea*.

Reddy and Bashamohideen (1988) individuals (8-12g) of the carp ^{14}C . *carpio* were exposed in the laboratory to malathion 50 E.C. over 48h. The LD_{50}S ranged from 9.8 to 10.04 ppm, with a maximum safe concentration of 3-2 ppm.

Mane and Muley 1989, showed individuals of the freshwater mussel *L. marginalis* were exposed to 0.0036 p.p.m endosulfan (10% of 96h LC₅₀) for 96h at pH 7.55 and 34°C without pesticide. Mussels kept at 34°C (without endosulfan) had elevated glycogen levels in the gills and hepatopancreas, increases amounts of protein in the gills and higher levels of lipids in the mantle gills and siphons. Glycogen content of the siphons and protein content of the mantle decreased. In the presence of endosulfan, glycogen levels in the gills and hepatopancreas decreased, but increased in the siphons. The mantle, gills and foot protein levels decreased; it increased in the gonads. Lipid levels in the siphons decreased, but it increased in the mantle, gonad and foot.

Moitra and Lal (1989) reported that the intestinal mucous secreting epithelium of *P. sarana* was completely sloughed off after exposure to BHC (HCH) (0.015 ppm/liter) for 15 days. A higher concentration (0.03 ppm/liter) of HCH was less deleterious for mucosa. Malathion proved to be less injurious; it reduced the number of mucus secreting cells by causing their contents to enter the lumen. Both pesticides affected the sub-mucosa, due to the vacillation of connective tissue and the widening the lumen.

Shankara *et al.* 1989 viewed biochemical and physiological responses to sub lethal dose (15 ppm.) of parathion-methyl were studied in the laboratory over 4 weeks at 27°C, pH 7.1-7.3, water hardness of 61 mg/liter HCO₃ and a photoperiod of LD 12:12 with regard to ammonia detoxification in the freshwater muller *L. marginalis* (25g). Parathion-methyl augmented tissue protein degradation by triggering acid and alkaline protease activity. Glutamine oxidation increased in these tissues. resulting in increased ammonia production in order to maintain the acid-base balance.

Sun *et al.* (1993), stated that carbofuran applied to paddy soil was transferred to all the components in the ecosystem. Just over 50% of the radioactivity was found in the upper soil, mostly in the 0-10 cm from daus 5 to 93 although the amount below 10 cm increased to 12% by the end of the experiment. The total residues in the paddy soil 12 days after treatment (0.22 mg kg), more than 87% was in the form of bound residues. The concentration of ¹⁴C residues in water decreased from the initial 0.84 mg/l to 0.1 mg/l within 3 weeks. Rice plants accumulated radioactive carbofuran slowly and the level increased until harvest. The concentration was always higher in the shoots than in the roots. It appears that rice plants took up carbofuran residues mainly via the paddy water. At harvest time (93 days) brown rice contained 0.3 mg kg of extractable carbofuran. The fish, *Tilapia nilotica*, contained 1.54 mg kg of carbofuran equivalents of which 1.51 kg were in the form of bound residues. Carbofuran was degraded in paddy water and soil mainly to carbofuran phenol where as in rice plants and fish the major metabolites were 3 hydroxy-carbofuran. Carbofuran, was the major component of extractable residues.

Mohamad *et al.* (1990) observed that the amount of carbofuran and metabolites in the water of the ecosystem declined from 0.98 mg L⁻¹ to 0.27 mg L⁻¹ after 14

days. Ninety days after treatment, only 15 mg L⁻¹ of carbofuran equivalents were detectable. There was an increase in the amount of residues in the soil corresponding with the loss from the water. 70% of the applied radioactivity was found in the soil 21 days after treatment and about 60% after 90 days. The amount of extractable radioactivity decreased with time while the non-extractable fraction increased. Accumulation of radioactivity in rice plants was slow but increased with time. About 3% of the total applied radioactivity was detected in the plants 14 days after treatment. 90 days after treatment, dehusked rice grains contained 0.25 mg kg⁻¹ total residues. All the Gouramy fish, *Trichogaster trichopterus*, died within 24 hours in winter containing 0.8 mg L⁻¹ carbofuran equivalents, indication that this species is extremely sensitive to carbofuran. Metabolites of carbofuran, 3-keto and/or 3-hydroxycarbofuran, were found in the water and/or rice plants and were not present 90 days after treatment where as carbofuran phenol was present in both soil and paddy water.

Rani *et al.* (1990) found that in liver, gill and brain tissue of ¹⁴C. batrachus exposed to malathion at 5 ppm for 96h, the levels of glucose and lactic acid increased, and those of glycogen and pyruvic acid decreased and the percentage changes in liver, gill and brain were 23.4, 51.06 and 47.22 respectively. Similarly, succinate dehydrogenase activity was reduced and the percentage changes in liver, gill and brain were 70.56, 53.66 and 26.74, respectively. Significant alterations in metabolite content and enzyme activity under malathion toxicity may suggest a transient shift from aerobic to anaerobic metabolism.

Tejada and Bajet (1993) carried out experiment with monocrotophos, cypermethrin and parathion methyl applied to rice-fish culture in the field at Los Banos, Philippines, had no apparent effect on fish survival. In laboratory tests, toxicity to fish was in the order cypermethrin > parathion-methyl > monocrotophos. Residues of parathion-methyl in fish accumulated only up to 4 days but were nondetectable there after. There were no residues on rice grains at harvest time. Harvesting rice 50 days after pesticide application is considered safe for human consumption.

Lou *et al.* (1991) observed that the pesticide fenitrothion was applied at various concentrations to rice fields for control of rice borers and to study its effects on the rice/fish aquatic ecosystem in Sichuan, China. A 50% fenitrothion emulsion at 25-50 g/mu (1 mu=0.067 ha) gave 96-98% control of rice borers but had no significant toxic effects on fish raised in the paddy fields. The 86.2% residues on rice plants degraded 1 day after application. Fenitrothion half-life was 2 days in rice, fish and paddy soils, and 1 day in the water. The resulting mortality of spiders was 9.4%. It is concluded that fenitrothion had no significant influence on the rice/fish aquatic ecosystem.

Medina *et al.* (1991) reported that the persistence of endosulfan in a rice field and its potential for adverse effects on rice-fish culture, was evaluated. Endosulfan was applied at the rate of 0.5 kg ai/ha. 30 days after transplanting. Residues were at a maximum at 2 h. Degradation of total residues followed pseudo-first order

kinetics with half-lives of 1.2, 1.3 and 1.0 day in paddy soil, water and rice leaves, respectively. In paddy soil, maximum residues were 0.48 and 0.58 mg/kg for the alpha and beta isomers, respectively. In paddy water, the maxima for both alpha and beta isomers were 0.12 mg/L. In the rice leaves, the maximum for alpha mg/kg, respectively. Both the alpha and beta isomers dissipated at similar rates and did not accumulate in these substrates. The residues in water were still toxic to *Oreochromis mossambicus* up to 18 days after spraying (17% mortality). In fish, the bioconcentration factor was determined to be 9 (head) and 82 (flesh) for the alpha isomer and 171 (head) and 279 (flesh) for the beta isomer.

Bajet *et al.* (1992) conducted experiment the corn cob based formulation of thiobencarb released maximum concentration in water at 7 day after application (DAA) and sustained up to 56 DAA as compared to the alginated based formulation which slowly released the active ingredient and attained the maximum at 56 DAA. The corn cob based formulation was more useful since release coincided with the critical period for weed competition in rice. The LC_{50} of thiobencarb to Tilapia (*Oreochromis niloticus*) was found to be 0.234 mg/liter. Bioaccumulation of fast (TA1) and slow (TA2) corn cob based formulation at 0.2 microgram/g respectively. Average concentration of thiobencarb in dead fish fillet was 0.312 and 0.332 microgram/g respectively for TA1 and TA2 low level of uptake of thiobencarb from TA1 and TA3 was found for both young and old leaves of kangkong, *Ipomoea aquatica*. The stalk absorbed most of the ^{14}C thiobencarb and no significant yield difference was found between commercial and controlled release formulations applied at 1.5 and 0.75 kg ai/hectare.

Jebakumar and Jayaruman (1993), treated 0.5 mg kg radioactive carbofuran in the soil of rice-fish ecosystem. *Lepidocephalichthys thermalis* fish were added on the 7th day. On the 1st day only 63% of the applied radioactivity was recovered from the soil, and the concentration decreased to 8 $\mu\text{g kg}$ on the 14th day, Rice shoot contained 0.2 mg kg of ^{14}C carbofuran residues on the 3rd day of exposure which increased to 0.5 mg/kg at the time of harvest (90th day). Root concentrations were highest, with a maximum of 0.28 mg/kg on the 56th day decreased to 0.11 mg/kg at harvest. The concentration of radioactivity in the paddy water was highest on the 3rd day (6 $\mu\text{g/l}$) and then decreased to 10 mg/l. Fish initially accumulated 5 $\mu\text{g/kg}$ carbofuran equivalents and on the 14th day contained 20 mg/kg. At the time of harvest, 3 $\mu\text{g/kg}$ carbofuran equivalents were detected. No data were represented on bound residues.

They also stated that the levels of endosulfan deposition on rice plant leaves were low compared to reported field values. Even these values reduced to very low levels (75% or 0.06 mg/kg) in about 11 days. The half-life was 5 days. Endosulfan was strongly bound to the soil and extractability was very low. The compound percolated from the field area through soil at the rate of about 5 $\mu\text{g cm}$ day. Metabolism takes place in the soil endosulfan sulphate and alcohol were detected. However, there was no variation in the concentration of alpha and beta isomers, but specific correlation was not possible. There was no tendency to bioaccumulation. Endosulfan either was detected in *Hydrilla verticillata*.

Barredo-Medina *et al.* (1993), evaluated the persistence of endosulfan in a rice-field and its potential adverse effects on rice-fish culture. Endosulfan was applied at the rate of 0.5 kg ai/ha 30 days after transplanting. Residues were at a maximum at 2h. Degradation of total residues followed pseudo-first order kinetics with half-lives of 1.2, 1.3 and 1.0 day in paddy soil, water and rice leaves respectively. In paddy soil maximum residues were 0.48 and 0.58 mg/kg for the alpha and beta isomers respectively. In paddy water, the maximum for both alpha and beta isomers were 0.12 µg/L. In the rice leaves, the maxima for alpha and beta endosulfan were 2.75 and 1.56 mg/kg, respectively. Both the alpha and beta isomers dissipated at similar rates and did not accumulate. The residues in water were still toxic to *Oreochromis mossambicus* up to 18 days after spraying (17 & mortality).

El-Arab (1993), also mentioned the highest concentration of carbofuran residues occurred in the paddy water 7 days after the addition of the pesticide (0.225 mg/l) and decreased to 0.04 mg/l after 90 days. Fish, *Tilapia militia*, were placed in the ecosystem 7 days after pesticide treatment. At harvest time (90 days), only 0.6 mg/l of carbofuran equivalents were found in the fish. Carbofuran accumulated in fish to a lesser extent than did lindane. Of all the constituents of the model ecosystem, rice-plants contained the highest concentration of ¹⁴C residues and the amount remained relatively constant in spite of dilution caused by new growth. Soil residues were relatively low 7 days after treatment and did not change appreciably.

Gonzales and Bello. (1993), ¹⁴C carbofuran granules were incorporated into the soil at 0.75 kg a.i./ha. Radioactivity reached a maximum in the water 9 days after flooding when it amounted to 27% of the total application. The level declined after 12 days and at day 145 it accounted for 3.7% of that applied. One week after flooding 58% of the radioactivity remained in the soil, about two thirds of it being in the top 5 cm. Corresponding figures for 56 day were 2.9% and 1.8% concentrations of radioactivity in plant parts were low. Residues in the harvested grain were variable ranging from 0-1.5 mg/kg carbofuran equivalents. There was no fish (*Tilapia nilotica*) mortality following their addition 7 days after flooding. Concentrations in fish tissue were high at day 14 (equivalent to 23-37 mg/kg carbofuran) but they declined subsequently to about 0.23 mg/kg. The proportion of the residue in bound form was not established.

Alpha and beta ¹⁴C endosulfan residue data were generated by Gonzales and Bello (1993) from experiments conducted in a model rice-fish ecosystem. All fish put into the ecosystem 10 days after spraying, died within 24 hours. Replacement continued to die until six weeks after spraying beta endosulfan and until five weeks after spraying alpha endosulfan. However, a rapid decline in ¹⁴C activity with time was observed in all the components of the ecosystem. At harvest time, grain contained ¹⁴C level equivalent to less than 0.001 mg/kg alpha and beta endosulfan. If fish is to be cultivated in an endosulfan-treated farm, they will have to be introduced at least six weeks after spraying.

Keller (1993) showed acute toxicity of several pesticides, organic compounds, and a waste water effluent to the freshwater mussel, *Anodonta imbecilis*, *Ceriodaphnia dubia*, and *Pimephales promelas*.

Samudra (1993) studied on pesticides furadan 3G, diazinon 10G, quinalphos 5G and fonophos 5g at the rate of 0.60 kg, 1.50kg, 1.25kg active ingredient per hectare were applied at one day before transplanting, 30 and 60 days after transplanting. Fishes were released one week after transplanting to the field. The fishes were kept in the field for 75 days. The pesticide residues detected from the field water were the following carbofuran, 0.006-0.0051 ppm, diazinon, 0.002-0.0036 ppm, quinalphos 0.0006-0.0516 ppm and fonophos 0.003-0.0043 ppm. In the soil the range of pesticide residues detected were carbofuran, non detected 0.0236 ppm diazinon 0.0062-0.0216 ppm, quinalphos 0.0178-0.9125 ppm, and fonophos, 0.0579 ppm-0.1412 ppm. The residues detected from dead fish three days after being released were: diazinon 0.0340 ppm, fonophos 0.6735 ppm and carbofuran was not detected. Three days after the second pesticide application the residues found in dead fish were carbofuran 0.1919 ppm, diazinon 15.9023 ppm, fonophos 13.9465 ppm, and quinalphos 11.30401 ppm residues detected from fish meat were carbofuran 0.0031 ppm, diazinon 0.0027 ppm, fonophos 0.0049 ppm and quinalphos not detected. In hulled rice the residues of all pesticides were not detected while in the straw the residues found were diazinon 0.0079 ppm; fonophos 0.0400 ppm, quinalphos 0.0991 ppm, and carbofuran not detected.

The fate of ^{14}C fenitrothion was studied by Sun *et al.* (1989), in a rice/fish/azolla ecosystem. Most of the applied fenitrothion was intercepted by plants and decreased with time. Fenitrothion residues in the flesh of fish were low and most of them were in the bound form. Fenitrothion concentration in water decreased rapidly with formation of water soluble products. In soil, most of the fenitrothion was retained in the upper layer largely as bound residues. At harvest, the concentration of fenitrothion in cargo rice and of the fish were very low, much lower than the permitted maximum residue level, 0.2 mg/kg for rice (China). 1 $\mu\text{g/kg}$ for rice (codex Alimentarius), and 0.05 mg/kg for flesh of fish (in Japan). Fenitrothion residues in early season rice-fish azolla ecosystem had little effect to the next paddy field.

Tayaputch *et al.* (1993), studied the behavior of endosulfan in a simulated rice-paddy fish ecosystem, ^{14}C endosulfan sprayed directly on rice plant was absorbed more by the shoot than the root and endosulfan sulphate was the major metabolite found at all growth stays, ^{14}C endosulfan was highly toxic to fish causing 100% mortality from day after spraying to 14 days. The alpha and beta isomers of ^{14}C endosulfan degraded rapidly into endosulfan sulphate. Alpha-Endosulfan degraded faster than the beta isomer was in water and rice plants where as beta isomer was faster in soil.

The distribution of ^{14}C fenitrothion residues in a model, paddy and fish ecosystem was studied by Zhang *et al.*, (1993). The pesticide was sprayed over the rice

plants at two levels; 116 mg kBg (as x dose) and 232 mg a.i./tank, 8288 kBg (as x dose) and 232 mg a.i./tank 15576 kBg (as 2x dose). The results from the study showed that the residues of the pesticide in paddy water and rice stems and leaves decreased with time and those in the soil, rice roots and fish body increased soon after application and decreased there after. About 60-90% of the pesticide residue in the soil and rice plants existed in bound form and to increase with time. No toxicity to fish was observed when the 2x dose of the pesticide had been applied which implies that the tested dose of fenitrothion could be used safely in the paddy fish ecosystem.

Giouranovits-Psyllidae *et al.* (1994) observed that Organochlorine residues are significant as environmental contaminants because of their stability, bio-accumulative capacity and global occurrence.

Wan *et al.* (1994) observed that the basic pH promote degradarion of OPs pesticides in water samples by stabilizing the more phenolic structures.

Anwar (1995) conducted a study on water quality aspects related to possible groundwater contamination by pesticide residues on Gangschara thana, Rangpur. The choice of monitoring sites was based on Leaching Potential Index (LPI) methodology. Based on higher LPI values, eight groundwater samples were collected from the wells of varying depth 35 ft to 85 ft. Samples were analyzed for Pesticide residues in the c under Atomic Energy Research Establishment (AERE), Savar, Dhaka. Tests were performed for organochlorine and organophosphorous pesticide but none of the standard organochlorine and organophosphorous pesticides were detected. Samples were collected from the most vulnerable sites but the tube well depth was not representative.

Pagiola (1995) studied on the environmental and natural resources degradation in Bangladesh due to intensive agriculture. He carried out his research on environmental damage from intensive agricultural practice. He investigated pesticide use in agriculture like rice, vegetables and other crops, which may in turn create health problems, water pollution and degradation of fisheries. He suggested integrated pest management (IPM) practices, which would allow the low current levels of pesticide use to be reduced substantially without adverse consequences for agriculture. But IPM is very knowledge intensive and relatively expensive to extend to farmers. He suggested reforming the regulatory framework, which would help ensure that the pesticides that are used are safe both for their users and for the environment.

2.7 Bionomics of *Lamellidens marginalis* (Lamarck, 1819)

2.7.1 *Lamellidens marginalis* (Lamarck, 1819)

Lamellidens marginalis (Lamarck, 1819) is found in Indian subcontinent and other parts of the world. Different countries of Asia are producing pearl by creating mussel culture technology due to its recent commercial importance. At present, China and Japan has established themselves as the main pearl producing countries. The increase in demand of pearls in the world trade market has earned them lot of foreign currency. There has been marked decrease in production of pearl and pearl producing mussels (*Lamellidens marginalis*) in natural waterbeds of Bangladesh due to environmental pollution, changes in their habitat, indiscriminate harvesting and other reasons.



Fig. 7. Pearl Jewelry

2.7.2 Characteristics of freshwater mussel

Anatomically *L. marginalis* (Lamarck, 1819) has three characteristics,

1. It has bivalves which contains soft body.
2. This soft body contains respiratory, digestive and other essential organs.
3. It has muscular foot for locomotion and by which it makes hole and remain still in the bottom of marsh land.

There are some identifying characteristics as shell oblong – ovate, thin, very smooth, periostracum blackish-brown and shiny, light brown border along the ventral margin, posterior side broad, rounded angular, wing very narrow, dorsal margin a little curved, anterior side short and narrow, ventral margin slightly contracted in the middle, hinge with tow cardinals in the right valve, lower better developed, a single lateral and trace of a second one, left valve with one feebly developed cardinal, tow curved, lateral teeth. The inside, bright layer of mussel is called nacre or pearl layer. The mantle has contact with the shell, it has branches of blood vessels, and inside this is connective tissue with blood vessels, nerve

cells and fibers, unicellular mucous glands and nacreous glands which secrete nacreine into the nacreous layer or pearl layer of the shell. From above study the taxonomic position of species was identified as

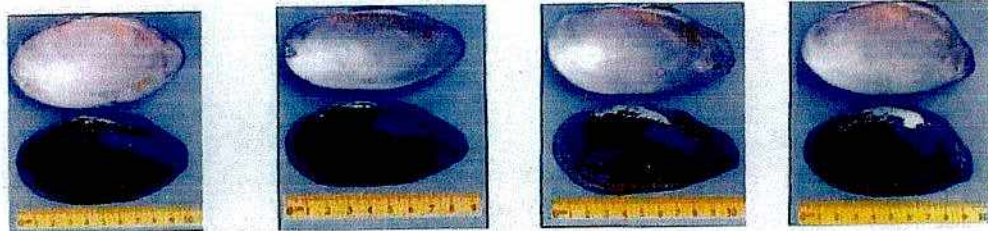


Fig. 2. *Lamellidens marginalis* (Lamarck, 1819)

The animal is sedentary but ploughs slowly through the mud or sand by its wedge-shaped muscular foot at the bottom of the pond or river. *L. marginalis* usually stays in shallow water during night but migrates to deeper water during day time. The food of *L. marginalis* comprises microscopic organisms, both plants and animals such as diatom, gold algae, green algae, etc, which are bed upon by filter feeding mechanism (Mazid, 1996 and Hossain, 1988).

The body is laterally flattened. The anterior side of the body is roughly oval in an outline and the posterior end is slightly narrower. It has a bilaterally symmetrical body. The size varies from 5 to 10 cm in length.

The soft body of the animal is completely enclosed by a hard calcareous shell. Shell is composed of two symmetrical and equal halves called vales and known as right and left valves, the two vales are united by a dorsal elastic band called a hinge ligament, which is continues with the two shell valves but is made of uncalcified conchiolin, it is elastic and causes the valves of the shell to open. At the anterior end of the hinges ligament on ach side, is a swelling called umbo which is the oldest part of the shell and is first formed in a young animal. Below the umbo are concentric lines of growth of the shell valves.

The shell valves are rounded anteriorly and pointed posteriorly. The umbo is directed anteriorly marking it possible to determine the right and left shell valves of the animal. The two shell valves are similar and equal in size but in some sessile families (Oysters) the upper or left valve is always larger than right valve by which the animal is attached.

If a shell valve is removed from the mantle lobed, its inner surface is seen, which shows marks of insertion of muscles running transversely between two valves.

The shell has three layers, and outer brown, horny layer, the periostracum, which is protective and is made of horny organic material called conchiolin. Below it the middle layer is a thick prismatic layer made of vertical crystal or prisms of CaCO_3 separated by conchiolin. The inner most nacreous layer or the *mother-of-pearl* layer is made of alternate layers of CaCO_3 and conchiolin. The nacreous layer is thickest at the umbo and thinnest at the shell margin.

The body is elongated but laterally compressed. The head is lost; in the upper half is a visceral mass, which passes into a mid-ventral, wedge-shaped, laterally compressed foot directed anteriorly, this is adaptation for burrowing. The foot becomes swollen and turgid; it is then used for plugging through mud in burrowing.

On each side the body is produced into a mantle lobe, the space between the mantle lobe is the mantle cavity from which hangs a ctenidium on each side. The mantle cavity is large and extends on each side of the body, it protects the ctenidia and prevents their closing with silt, and it allows a current of water to pass in and out in definite directions.

The Pallium lining of inner surface of the shell valves is a semi-transparent mantle. It is like skin; it closes the soft parts and also hangs down like a skirt. The mantle cavity can be divided into two chambers, a large ventral infrabranchial chamber and a small dorsal suprabranchial chamber. The mantle encloses the body in the upper half, and a muscular foot on the mid-ventral side. The border or edge of each mantle lobe is thickened and contains muscles. The thickened lower border of the mantle has three parallel lobes or folds, the inner most fold is largest and it is this fold which contains muscle which are both radial and circular it controls the flow of water. The middle fold is sensory in function. The outer fold secretes the shell, the inner surface of the outer fold lays down the periostracum, while its outer surface secretes the prismatic and nacreous layers, but the nacreous layer is also secreted by the entire outer surface of the mantle. The mantle secretes a pearl in many bivalves when any foreign particle lodges between the shell and the mantle, the pearl is formed in concentric layers around foreign particle.

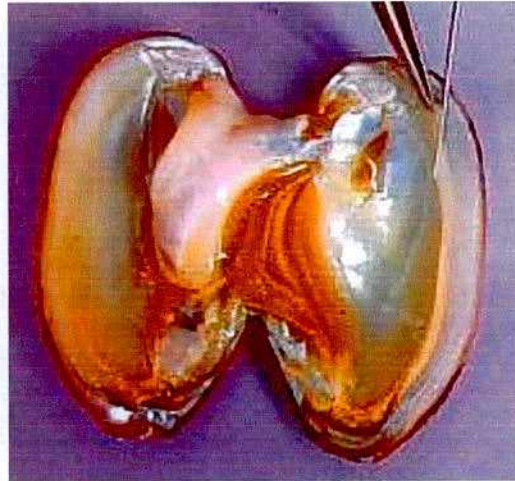


Fig. 9. *Lamellidens marginalis* (Lamarck, 1819) with internal structure

The muscular foot is the chief locomotory organ. When the *L. marginalis* walks, the foot is thrust forward between the two valves of the shell and burrows like plough-share through the mud. This permits blood to flow into the many sinuses of the foot, causing it to swell and, thus forms an anchor. As the retractor muscles contract, the *L. marginalis* is drawn forward an inch or so. The blood is then forced out of the foot so that it thins down again and can be withdrawn from the mud or sand. The process is repeated with each step and wedge-shaped path is left behind.

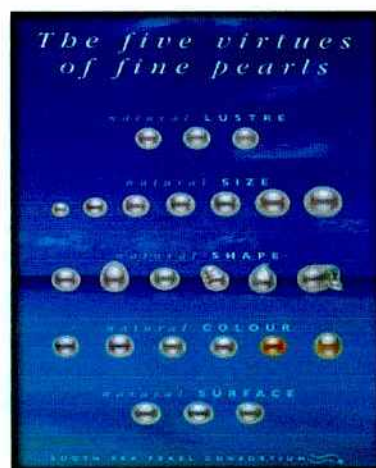
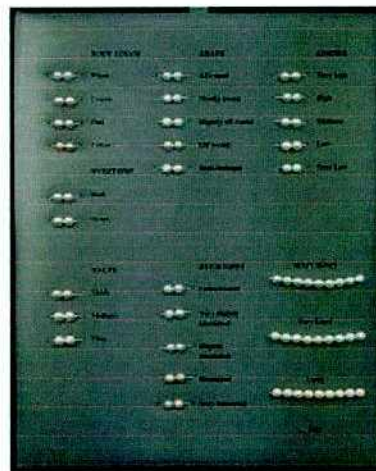
The gonads are testes in male and ovaries in female. The gonads are paired, large, highly branched structures lying in and around the intestinal coils in the visceral mass above the foot. During breeding period they become greatly enlarged and conspicuous; the tests are brightly whitish and ovaries are reddish. The lining of the gonads proliferates to give rise to spermatozoa in male and eggs in female. Each gonad has a short duct, vas deference in male and oviduct in female, opening into the suprabranchial chamber of the mantle cavity near the excretory pore.

In male the sperms pass out through the exhalant siphon and are carried to the surrounding water from where they enter the inhalant siphon of the female and reach its ctenidia. In female the eggs are shed into the suprabranchial chamber of mantle cavity and are carried into water tubes of ctenidia where fertilization and early development occur. Fertilized eggs generally develop in the outer gill plates of ctenidia which become enlarged to form a broad pouch or marsupium.

In *L. marginalis* (Lamarck, 1819) the sex cells are discharged externally into water where fertilization occurs, and the zygote develops into a free swimming trochosphere larva.

The zygote undergoes complete but unequal cleavage to form a morula having small micromeres and large macromeres. Gastrulation occurs by macromeres

invaginating into micromeres. The gastrula has micromeres or ectoderm, macromeres or ectoderm, macromeres or endoderm, a large blastocoele and small archenteron. It is enclosed in vitelline membrane. Some cells of the gastrula are budded into the blastocoele to form mesoderm. A deep invagination occurs to form a shell gland, which is the characteristic of Mollusea. The shell gland marks the dorsal surface of the embryo; the posterior end is marked by a tuft of long cilia. The shell gland secretes an unpaired shell which is soon replaced by a triangular bivalve shell; the shell valves enclose a larval mantle. The lower parts of shell valves are curved to form hooks beset with spines. The embryo is cleft in the middle to form a dorsal body and two mantle lobes. On each mantle lobe four brush-like sense organs arise, each having a cluster of bristles. The mesoderm forms a large adductor muscle which runs between two shell valves anteriorly. On the body a byssus gland is formed which secretes a sticky thread called byssus. The embryo is now known as a glochidium larva. The embryo is nourished on yolk present in the egg. The larva has no mouth or anus and the digestive tract is not yet formed. Thousands of glochidia may be formed by single *Lamellidens marginalis* (Lamarck, 1819).



Literature Review

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area and data collection

3.1.1 Selection of the study area (Bogra)'

Bangladesh forms the largest delta in the World. Bangladesh lies in the northeastern part of South Asia between 20°C 34' and 26°C 38' north latitude. The country is bounded by India on the west, the north and the northeast of Bengal on the south. The area of the country is 147570 km² (SRDI, 1990). Bogra district has been selected as the study area. The district of Bogra is situated between 20°02' and 25°08' North Latitude and between 85°56' and 89°44' East Longitude.

Bogra is bounded on the north by the district of Rangpur and Dinajpur, on the south by Pabna and Rajshahi, on the east by the river Jamuna which separate it from the district Mymensingh and west by the district of Rajshahi and west Dinajpur (West Bengal, India).

The general aspect of district is that of a great plan unbroken through out its whole extend. Its landscape, being an absolute dead level, only by a slight slope, amounting in the whole length of the district to a few from the northwest towards the south and southwest. The river Karatoya and Phuljur divide it longitudinally into two portions, whose characteristics are distinct. The eastern portion is smaller, containing some what less than two-fifths of the entire area of the district, may be regarded as forming part of the flood plane of the Jamuna. This tract is generally low lying, and is intersected by the numerous khals or natural drainage, channels and jhil or shallow swamps and marshes. It is subjected to yearly inundations from the Brahmaputra, the Bengali, and the Katakali, the last of which now drain much of the southern Rangpur.

The soil is of whitish color and is known locally as Poli (Literally, clay covered). There is a little jungle and a few trees, except on the sandy islets and accretion on the banks of the Daokoba (Brahmaputra) where a stunted species jhau tree (*Tamarix dioica*) is found.

Materials and Methods

Materials and Methods



Fig. 13. River of the study area



Fig. 14. Dead mussels in pesticide used paddy field



Fig. 15. Dried river of the study area



Fig. 16. Paddy field of study area

3.1.2 Climate

The climate of Bogra is not much different from other north-western districts of Bangladesh. Most of the precipitation occurs during the monsoon season from May to October, followed by a cool and dry winter from November to February. The pre-monsoon season from March to May is hot and the highest temperatures and evaporation rates are recorded during this period.

Bogra district receives the mean annual rainfall within the flood plain varies from 1100 mm in the south to 2300 mm in the north, with about 1300 mm to 1900 mm falling within the monsoon season. The heaviest rainfall about 400 mm per month occurs during June and July. Average maximum temperature range from 24 °C in winter to 34°C in summer; while minimum temperature varies from 5 °C in winter to about 25 °C in summer. The annual and monthly rainfall and monthly temperature in Bogra district are shown in respectively.

3.1.3 Soil

There are two divisions of soil, Poli and Khaiyar which are again subdivided according to the fertility of the soil and its level. Most of the high lands are bastu lands, on which they are grown. Lands lower than the bastu grow paddy, chiefly Aus. Thanas like Guptali sugar cane is grown. Still lower lands, which are not above flood level, grow Aman paddy and jute, where as in beel lands boro paddy is largely grown lastly in Charlands Kahan, pulses, mustard and water-melons are the principal crops. The soil which is whitish in colour is known locally as *Poli*. It is extremely fertile and most of the fields bear two crops and some even three. Jute and paddy grow (luxuriantly, but the soil seems to be specially suited for jute. The largest acreage under jute is to be found in the thanas of Dhunot of and Shariakandi. Paddy is very often sown after jute, and where this is not done robi crops such as pulse, mustard, etc., are almost invariably grown. These later crops are also sown in field, which have yielded a crop of paddy before and there are fields that produced jute, paddy Kahan during the same agricultural year.

The soil for the western portion is locally known as a *Khiyar*. It is hard compact clay of reddish colour apparently rainy season it becomes stickly mud. In the cold weather it dries hard as bricks. Along its western boundary, however where it is watered by the Jamuna, it is overlaid by recent alluvial deposit of Poli, due to the overflow of the atria. The Khiyar soil is generally suited to the growth of paddy. Jute, however, is also grown to some extent in the Sibganj than and in the Poli tract of Adamhighi thana. The soil is perhaps not so fertile as it is to the east of the river Karatoya and it requires manuring on a much more liberal scale. Continuous cultivation has severely depleted the fertility. Poor soil yields low and poor quality crops.

3.1.4 River system

There are quite a few rivers in the district of Bogra. Taking the Karatoya as the central dividing water-channel of the district, the other rivers may be classified into the eastern and the western systems. The course of all the rivers is, with such

allowances as must be made for beds and windings, nearly uniform north and south.

The eastern rivers are Monas, Charkadaha and Khamati besides a few other smaller ones. Through the Khair tracts in the western part of the district flow the Nagar, Tulshiganga, Nagar and other minor streams. All the western rivers are the tributaries of the river Atrai which itself flows into the Jamuna 12 miles north of the confluence of that river with the Ganges (Padma) at Goalunda.

The upper portion of Bengali river comes from Rangpur district and enters Bogra at Salika in the Shariakandi thana about three miles to the south-east of Sonatala railway station.

3.1.5 Agricultural practices

Country's need to increase agricultural production and thereby attain self-sufficiency in food grain led to an increased cultivation of high yielding varieties (HYV) of rice and other crops since 1960's. As a consequence, there has been an increase in the total cropped area during the dry season. Many farmers, who once cultivated local Aus and Aman varieties of rice during the rainy season and left their fields fallow during the dry seasons now cultivate HYVs Aus and Aman rice as well as HYV Boro rice during the dry season. In 2000 approximately 75% of the total cropped area was under rice. The area cultivated in Bangladesh in 2000 is shown in Fig. 17a and 17b (BBS, 2000).

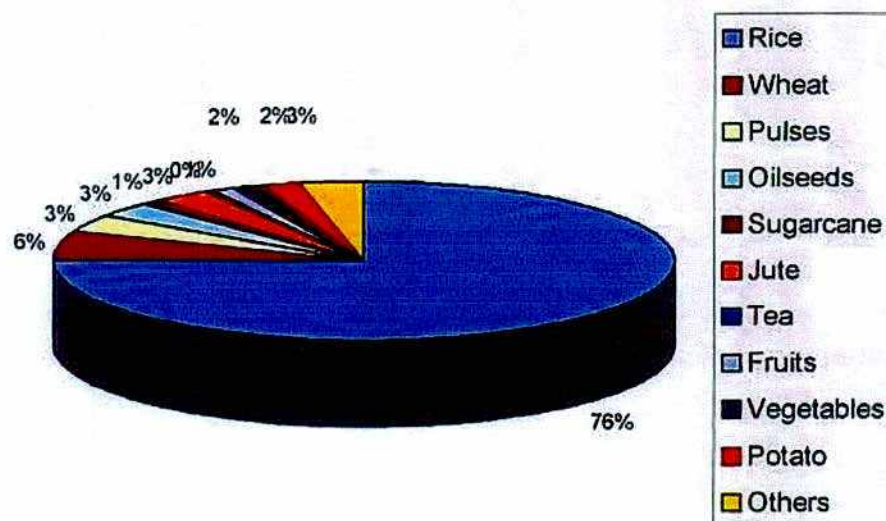


Fig. 17a. Area under cultivation in Bangladesh 2000

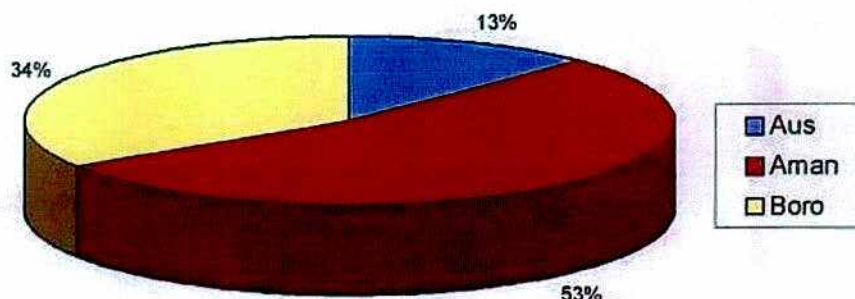


Fig. 17b. Area under cultivation in Bangladesh 2000

The change in acreage and production under different varieties of rice in Bogra. From the figure it is seen that the total crop area estimated for Aus decreases compared to Aman and Boro in Bogra. The decline may be due to unfavorable weather condition during sowing period of the crop and to remarkable increased cultivation of Boro crop.

Increases in total crop area do not represent increases in the actual acreage under production. Rather this increase reflects an increase in the cropping intensity. Cropping intensity is calculated as the total area cropped during the year divided by the net cultivable area. The changes in the cropping intensity in the study area Bogra and whole Bangladesh from the year 1996 to 2001. On the average cropping intensity increased by 2.7% in Bogra and 2.15% in Bangladesh between 6 years. Except rice, another major crop is wheat. Weather condition of this area is favorable for this crop. Many crops other than rice and wheat are grown in this region. These include sugarcane, pulses, oil seeds, vegetables, cotton and etc. selected irrigated crops in acres from the year 1996 to 2002 in the study area Bogra. However these crops are accounted for almost 25% of all cropped acreage in the 2000 crop year.

3.1.6 Use of fertilizer and pesticide in the study area

Physiographic features of the region, in particular a high water table, frequent flooding, high recharge rates, and unconfined of the aquifer, suggest the presence of a relatively high potential for surface water contamination and degradation. Agricultural and industrial wastes together with these physiographic features will also influence the potential for surface water contamination and degradation. Irrigation increases recharge rates and the soil water velocity, thus increases the amount of leaching and the speed at which contaminants may reach groundwater and surface water (Anwar, 1995).

Table 3. Selected irrigated crops (acres)

Year	Bogra					Bangladesh				
	1996	1997	1998	1999	2000	1996	1997	1998	1999	2000
Aus	55261	57794	39482	32000	27000	283770	276358	250853	237000	238000
Aman	36329	38001	31920	25000	26000	730334	752166	827788	721000	743000
Boro	549539	602240	612282	676000	762000	6251916	6430021	6803463	7086000	7506000
Wheat	134905	133011	152645	162000	155000	741168	791796	854019	894000	896000
Other Cereals	3201	4110	2110	2000	2000	9084	9901	8147	9000	8000
Pulses	501	440	678	1000	1000	4032	7803	8367	9000	9000
Oil seeds	3429	2450	2133	3000	3000	46768	54344	62761	73000	79000
Potato	31823	32359	35482	37000	43000	248543	268271	280093	300000	318000
Vegetable	15017	15051	11769	15000	16000	218299	230227	247085	258000	271000
Sugarcane	3295	3305	3153	3000	3000	34480	38085	39297	47000	44000
Cotton	448	910	975	1000	1000	22073	22760	22924	17000	22000
Others	83142	73593	72201	69000	74000	187661	242572	272864	196000	211000

Source: BBS (2000)



Fig. 18a. Name of the pesticide selling shop



Fig. 18b. Poultry feed made from mussel's shell

Through the cost of production of HYV rice is higher than that of local varieties and less resistant to pests, it requires relatively heavy application of pesticides. In 1987 report, Master Plan Organization (MPO), now the Water Resources Planning Organization (WARPO), estimated a 30 percent increase in pesticide use per cropped acre of HYV rice by the year 2005. Bangladesh Bureau of Statistics (BBS, 2000) show that the use of pesticides and fertilizers have increased significantly. Achieving self-sufficiency in food production and sustaining such production level is major objective of the Bangladesh government. But the heavily populated country has a total land surface of 14.4 million hectare of which 9.1 million hectare are used for agriculture and 80% of this agriculture land are used for growing rice. Because of humid climate for many month of the year, a large number of insect pest are present on crops. For the same reason a big population of beneficial parasites and predators of insect and pest are also present on the crops.

In south Asian context Bangladeshi farmers use little pesticides. While, the pesticide market in Bangladesh began with 3 tons of pesticides and 500 hard sprayers in 1956, currently the average expenditure per irrigated hectare is about US \$5 in Bangladesh as against US \$8 in India and US \$27 in Indonesia (Gain, 1993). But indications are there that on one hand, pesticide consumption is increasing in relation to acreage of irrigated agriculture and on the other hand, use of pesticide is very indiscriminate in Bangladesh. In some district such as Comilla, Dhaka, Chittagong, Bogra, Rangpur, Jessore farmers use pesticides in excessive quantities. This, among other reasons makes monitoring and assessment of pesticide contamination quite difficult.

The majority of pesticides used fall into following three groups and some of its examples are given in the Table below.

1. Organochlorine
2. Organophosphorous
3. Carbamate

Organochlorines are effective against a large number of insect and pests. While this property is highly desirable for effective pest control, it is equally harmful to the beneficial organism. Organochlorines are highly soluble in fat and are highly persistent. Endosulfan and heptachlor are widely used for tea and sugarcane respectively. Through farmers are only authorized to use heptachlor on sugarcane, several farmers of Bogra were found using heptachlor on their potato and wheat fields.

Carbamate and organophosphorous pesticides are in general, less persistent than the organochlorines and due to which they are preferred to the organochlorines. Diazinon, chlorpyrifos, malathion, cabofuran and carbaryl are mainly used for rice. They are sometimes also used for potato, vegetables, pulse and oilseeds.

3.1.7 Dirty dozen pesticides in Bangladesh

Some pesticides are banned due to acute toxicity, environmental persistence and bioaccumulation. These are known as "Dirty Dozen". Most organochlorines pesticides fall into this category. The pesticides listed in the "Dirty Dozen" are Parathion, BHC, Endrin, DDT, Heptachlor, Endosulfan and etc. are banned for agricultural use in Bangladesh. DDT was allowed to use by the Health Department Services until November 1991 for mosquito control (Gain, 1993).

3.1.8 Pesticides use data

Pesticides use data are taken from Bangladesh Agricultural Statistical Yearbook, 2000. Recommended dose of various pesticides are found from Department of Agricultural Extension (DAE) Bogra sadar. Chemical properties of pesticides are available in literatures (Hornsby, 1995; Olesen *et al.*, 2001; Windel *et al.*, 1999; Kookana and Aylmore, 1993; Pang and Letey, 1999; Huntush *et al.*, 2000). The major parameters of different pesticides used in the model are shown in Table 4.

Table 4. Major parameters on different pesticides properties

Parameters	Values for Pesticide									Reference
Chemical Name	Diazinon	Chlorpyrifos	Malathion	Cypermethrin	Fenitrothion	Carbaryl	Carbofuran	Heptachlor	Endosulfan	
Trade name	Digmol 60 EC	Dursban 20 EC	Limithion 57 EC	Rapcord 10 EC	Sumthion 50 EC	Sevin 85 SP	Furadan 5 G		Thiodan 35 EC	
Organic Carbon Partition Coefficient, K _{OC}	1000 cm ³ /g	6070 cm ³ /g	1800 cm ³ /g	10000 cm ³ /g	2000 cm ³ /g	300 cm ³ /g	22 cm ³ /g	24000 cm ³ /g	12400 cm ³ /g	Hornsby, 1995
Half life, t _{1/2}	40 days	30 days	1 days	30 days	8 days	10 days	50 days	250 days	50 days	Horn by, 1995
Diffusion Coefficient in Water, D _{1w}	0.000043 m ² /day	0.000043 m ² /day	0.000043 m ² /day	0.000043 m ² /day	0.000043 m ² /day	0.000043 m ² /day	0.000043 m ² /day	0.000043 m ² /day	0.000043 m ² /day	Jury et al., 1987
Longitudinal Dispersivity, α _L	0.1 m	0.1 m	0.1 m	0.1 m	0.1 m	0.1 m	0.1 m	0.1 m	0.1 m	Jury et al., 1987
Ratio of transverse to longitudinal dispersivity	1	1	1	1	1	1	1	1	1	Fetter, 1993
Sorption Isotherm	Linear	Linear	Linear	Linear	Linear	Linear	Linear	Linear	Linear	Olesen et al., 2001; Windel et al., 1999; Kookana and Aylmore, 1993; Pang and Liley, 1999; Hantush et al., 2000
Recommended dose	2 ml/l	2 ml/l	2 ml/l	1.7 ml/l	2 ml/l	3.44 g/l	9.9 g	2 Kg	1.68 l	Department of Agriculture Extension Bangladesh

3.1.9 Income generating source based on *Lamellidens marginalis* (Lamark, 1819) at community level.

Hindu community of Horikhali village situated at the bank of Bangali river under Sonatala thana, Dist, Bogra. Producing CaCO₃ of *L. marginalis* is the main income generating source of this community. Approximately three thousand people of Bogra district are strongly related with this profession. Note that, women are associated with this profession. Generally they serve their duties as house wife but at the same time they also share with their male partners on this profession. Most of the people of this area are habituated to eat CaCO₃ with betel leaf (*Piper betle* L.) and makes trading to other markets as well. So that Bogra district and other districts of Bangladesh have lots of demand for this quick lime (CaCO₃). Most of the families in this community maintain their expenditure smoothly from this income generating program. Not only are they earning money

by selling CaCO_3 but also sells soft inert part muscle and shell of the *L. marginalis* to poultry farms. On the basis of the above reasons poultry farm are built there, that's why economy of this village is strengthening and improving. Recently, poultry farming has obtained highest position among the other industries of the rural level in Bogra district.

To fulfill the increasing demand of poultry feed, some new entrepreneur take initiatives to collect *L. marginalis* with other species of mussel sold to the poultry feed producing entrepreneur for earning lots of money that's why small entrepreneur to CaCO_3 producing at grass root level of Hindu community going to be extinct, at the same time *L. marginalis* treated as an endanger species of our wet land ecosystem.



Fig. 19. Pile of harvested mussel's shell



Fig. 20. Production of calcium carbonate (CaCO_3) by manually from mussel's shell

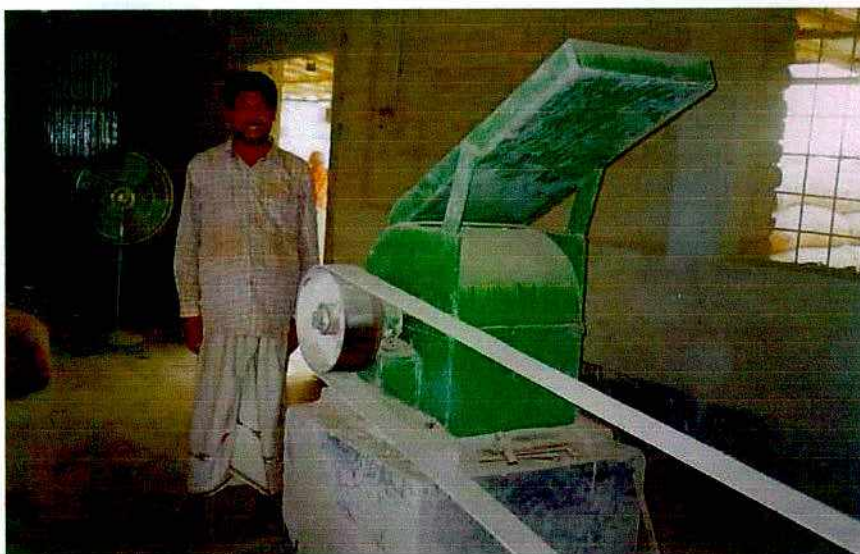


Fig. 21. Production of poultry feeds from mussel's shell in industry



Fig. 22. Women working in the poultry feeds industry

3.1.10 Data collection for pesticides residues analysis

One of the objectives of this research is to collect *L. marginalis*, soil and surface water samples from the most vulnerable site for the pesticide residue analysis. Facilities for determining pesticide residues in internal organs of *L. marginalis*, soil and surface water are available only in the Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, under the Ministry of Science and Technology. For this purpose several field trip was made to Bogra to collect samples.

Several pesticides shops were visited to get the data on pesticide use in the area. One of the shops is **Tayab Enterprise**, shown in Fig. 39a and 39b, formation about pesticide, their uses and costs are taken from these shops. Pesticides (pesticides) sales during one year period July 01, 2002 to June 2003 in Sonatala thana of Bogra district are shown in Table 5. Mostly used pesticides in Bogra and their recommended dose for different crops are given in Table 6. Different types of tools and machines, which are used for pesticides application.

Table 5. Pesticides sales from July 01, 2001 to June 30, 2002 in Sonatala thana

No.	Chemical Name	Trade Name	Amount (kg or Litre)
1	Carbofuran	Biesterien 5G	8000 Kg
		Furadan 5G	85000 Kg
		Sunfuran 5G	1700 Kg
		Furataf 5G	800 Kg
		Furwafuran 5G	15000 Kg
		Brifur 5G	1500Kg
		Vitafuran 5G	700 Kg
2	Chlorpyrifos	Pyriban 20 EC	350 lit
		Dursban 20 EC	3000 lit
		Classic 20 EC	200 lit
		Luxan 20 EC	300 lit
		Pyrifos 20 EC	1800 lit
3	Cypermethrin	Ralathrin 10 EC	100 lit
		Cymbush 10 EC	120 lit
		Ripcord 10 EC	500 lit
4	Diazinon	Diazinon 60 EC	500 lit
		Diginol 60 EC	300 lit
		Basudin 10G	12000 Kg
		Rison 60 EC	500 lit
5	Fenitrothion	Sumithion 50 EC	500 lit
6	Malathion	Limithion 57 EC	300 lit
		Maladan 57 EC	600 lit
		Fyfanon 57 EC	500 lit
		Hyfanon 57 EC	100 lit
		Malataf 57C	200 lit
7	Fiprolin	Regent 50 EC	300 lit
		Regent 3GR	4000 Kg

Source: DAE, Sonatala thana, 2000

Table 6. Pesticides used in Sonatala thana

No.	Trade Name	Chemical Name	Group	Recommended Crops	Dose	Cost
1	Suntap 50 Sp	Cartap	Carabamate	Rice	16 gm/10 l for 5 decimal land	34 Tk per 25 gm
2	Sevin 85 SP	Carbaryl	Carabamate	Rice	34.4 gm/10 l for 5 decimal land	70 Tk per 100 gm
3	Brifur 5G	Carbofuran	Carabamate	Rice, Sugarcane	10 kg/ha	54 Tk per 500 gm
4	Diginol 60 EC	Diazinon	Organophosphorus	Rice	2 ml/l for 5 decimal land	68 Tk per 100 ml
5	Regent 50 EC	Fiprolin	Carabamate	Rice, Sugarcane	1 ml/l for 5 decimal land	68 Tk per 100 ml
6	Classic 20 EC	Chlorpyrifos	Organophosphorus	Rice	2 ml/l for 5 decimal land	58 Tk per 100 ml
7	Ripecord 10 EC	Cypermethrin	Organophosphorus	Cotton, Mango, Jute, Tea	1.7 ml/l for 5 decimal land	110 Tk per 100 ml
8	Ralathrin 10 EC	Cypermethrin	Organophosphorus	Rice	2 ml/l for 5 decimal land	106 Tk per 100 ml
9	Dursban	Chlorpyrifos	Organophosphorus	Rice, Tea, Potato, Cotton, Sugarcane	2 ml/l for 5 decimal land	58 Tk per 100 ml
10	Fastak-2 EC	Cypermethrin	Organophosphorus	Brinjal, Tea	1 ml/l for 5 decimal land	66 Tk per 100 ml
11	Sumithion 50 EC	Fenitrothion	Organophosphorus	Rice	2 ml/l for 5 decimal land	248 Tk per 100 ml
12	Shobicon 10 EC	Cypermethrin	Organophosphorus	Brinjal, Cotton, Guava	2 ml/l for 5 decimal land	66 Tk per 100 ml
13	Limithion 57 EC	Malathion	Organophosphorus	Rice	2 ml/l for 5 decimal land	25.26 Tk per 100 ml
14	Furadan 5G	Carbofuran	Carabamate	Rice	9.9 kg/he	187 Tk per 100 ml
15	Marsal 20% EC	Carbosulfan	Carabamate	Rice, Vegetable	1.5 l/he	115 Tk per 100 ml
16	Hilthion 57 EC	Malathion	Organophosphorus	Rice, Jute, Vegetable	1000 ml/he	106 Tk per 100 ml

Source: DAE. thana. 2000

Sonatala upazilla officers establishes “Krishi Kendra” in order to train the farmers to use the different pesticides, its doses and functions in crop cultivation. There are widespread press reports that numbers of inferior quality pesticides are smuggled from neighbouring country. The dishonest traders, takes the advantages of ignorance of the farmers because in our country 90% farmers are illiterate. The imported pesticides that are smuggled into Bangladesh include banned organochlorines pesticide, which are not recommended to use in Sonatala thana (Table 6). The Department of Agriculture Extension (DAE) circulated leaflets in the areas where foreign substandard pesticides are seen, to draw the attention of the pesticide buyers about the adverse impact of the banned and highly toxic pesticide and asked the farmers not to use these brand items.

3.2 General

Toxicity of pesticide, its fate on *Lamelliden marginalis* (Lamarck, 1819) and pesticides used in the agriculture that transports to surface water are important environmental aspect. Due to frequent detection of pesticides and their breakdown products in surface water, soil, if and animal bodies, much attention has been paid to pesticide induced contamination of surface and sub-surface environments (Chu and Marino, 2004). In this research, the methodology to predict the fate of such chemicals released into the environment can be divided into two parts.

1. The methodology for toxicity test
2. The methodology for analyzing the pesticide residues in soil, water and different organ of *Lamelliden marginalis* (Lamarck, 1819)

The methods are described below.

3.3 Toxicity

The experiment was carried out in one of the outdoor laboratory facilities of agrochemical residue research and analysis division, Institute of Food and Radiation Biology (I.F.R.B), Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka. Analytical and other laboratory works were done in the laboratories of Agro-chemical Residues Research and Analysis Division, I.F.R.B., Savar, Dhaka.



Fig. 23. Insecticide applied into the aquarium for toxicity test

3.4 Toxicant used

In the present study malathion (hilthion 57 EC of Cheminova, Denmark) and carbamate (marshal 20 % EC of carbosulfan, FMC Corp., U.S.A.).

3.5 Test animal and their source of supply

Lamelliden marginalis (Lamarck, 1819) were used as test animal in the present study. The test animals were obtained from different beels, lakes and rivers of Bogra district, Bangladesh. The mussels were brought to the aquarium house in oxygenated polythene bags as recommended by Jhingran (1985) for the transport of living fishes.

3.6 Acclimation of test animal

On arrival at the laboratory, the mussels were immediately transferred to a glass tank containing tap water, they were maintained there for about 6-7 days in a state condition. The mussels were fed with artificial feed twice daily. After 24 hours interval, the water was changed to remove the metabolic pollutants. Air compressor with air stones was used for oxygenation of water. The water quality parameters of acclimation medium were monitored at times. However, after acclimation, only healthy mussels approximately of similar size were transferred to the experimental system. During this time, length and weight of mussels were recorded.

3.7 Glass tank

A small glass tank, 45 cm long, 30 cm wide and 31 cm high was used for the toxicity test. Total amount of water was 20 lt.

For the experiment four glass tanks were used for each insecticide, where there were three received the toxicant in three different concentrations and one served as control for comparison. Suitable batch of mussel (20 mussels) were released

into the each glass tank. The insecticides were weighted out in term of active ingredient per acre and uniformly dispersed directly into the water control glass tank received equivalent quantities of tap water minus the toxicant. Twelve mussels were released into each glass tank and similarly treated. Three replications were made for each treatment. All insecticidal treatments were made after the release of the animals. Incase of low or no mortality the same batch of mussel continued to be exposed to the action of the toxicant for variable period's up to 45 days. Water temperature and pH were recorded before and after the application of the insecticide. In all experiments reapplication of insecticide was made after 30 days of the first treatment.

3.8 Analysis for pesticide residues

In the study compression the transportation and degradation of pesticides and analysis of their reduces in soil, water and different parts of mussels are carried out at the Agro-Chemical and Environmental Research Division (AERD) Institute of Food and Radiation Biology (IFRB) Atomic Energy Research establishment (AERE) Ganakbari, Savar, Dhaka. Chemical properties of pesticides were available in literatures (Hornsby, 1995; Olesen *et al.*, 2001; Di and Aylmore, 1997; Huntush *et al.*, 2000).

3.9 Sample collection

The soil samples are collected from location considered as the most vulnerable site. More than 500g soil sample are collected from the bottom of the surface and at a depth of 1', 2', 3', below the water surface. Prior to sampling, glass bottles are cleaned by detergent and then distilled water. After collection of samples, bottles are marked by site number and depth and sealed by leucoplast. After drying the sample inside the fume hood on a petri dish then stones, twing and other debris were removed from the same collected samples are shown in Fig.24.



Fig. 24. Collected soil samples

Surface water from all the location were collected as water sample.

Mussels were collected from beel and river during flood, after flood and different season of the year. After collection of mussel, container are marked by site number and brought to IFRB for making model ecosystem and pesticide residual accumulation analysis.



Fig. 25. Collected mussels in aquarium

3.10 Preparation of toxicant concentration

Test solution of insecticide was made by adding requisite amount of chemical, calculated in terms of active ingredient, to tap water. The test chemical was measured by micropipette. Initially, stock solution of desire insecticide was prepared. Then calculated amount of stock solution was then added to the experimental tanks to obtain the desired concentrations of the chemical.

The tap water was free from any fertilizer or insecticide contamination. As mentioned earlier that tap water used in the present study was of underground source.

3.11 Experimental procedure

Present study consisted of a series of experimental trials. All trials were carried out with various concentrations of each chemical. The description of each individual experimental trial is given in Table 7 and Table 8 for convenience, it may further be mentioned that prior to the actual trial, a preliminary trial was carried out with each chemical using arbitrary concentration of the toxicants. On the basis of observed mortality in the preliminary trial, a series of closely spaced concentrations were selected to be used in the actual trials. Usually, 6-10 toxicant concentrations were used and each concentration was tested in duplicate. In all cases, control groups of mussels in each experimental trial were maintained.

Before setting any experimental trial the glass tank were washed and cleaned and further dried in the sun. Each glass tank was filled with 20 lt of tap water. Ten randomly selected mussels were transferred to each glass tank. The mussels were not fed during the experimental trials. During the experiment water quality parameters, mortality, behavior and other changes of the mussels were observed and recorded separately.

Table 7. Showing data on the average length, weight of experimental mussels and concentrations of malathion (organophosphorus) insecticide used

Name of the experiment	Length of mussels (cm)	Weight of mussels (g)	Concentration used (ppm)
Preliminary trial with malathion insecticide	8.19	97.00	0, 0.54, 0.74, 1.33, 2.22
Trial IA with malathion insecticide	8.15	109	2.67, 2.97, 3.96, 4.95
Trial IIA with malathion insecticide	8.17	84.00	5.94, 6.93, 7.99, 8.94
Trial IB with malathion insecticide	9.14	76.00	9.90, 10.10, 10.30, 10.40, 10.59
Trial IIB with malathion insecticide	9.20	73.00	10.89, 12.80, 13.86, 14.85

Table 8. Showing data on the average length, weight of experimental mussels and concentrations of carbosulfan (carbamate) insecticide used

Name of the experiment	Length of mussels (cm)	Weight of mussels (g)	Concentration used (ppm)
Preliminary trial with carbosulfan insecticide	9.15	84.00	0, 0.54, 0.59, 0.69, 0.79
Trial IA with carbosulfan insecticide	9.20	87.05	1.28, 1.78, 2.27, 2.77, 3.26
Trial IIA with carbosulfan insecticide	9.00	76.00	4.25, 6.24, 9.21, 9.90
Trial IB with carbosulfan insecticide	8.00	78.00	9.95, 10.05, 10.15, 10.40
Trial IIB with carbosulfan insecticide	8.17	77.19	10.89, 11.39, 11.58, 11.88, 12.38

3.12 Organism loading and media renewal

An organism loading approximately 1.0 g/l water was maintained in all the tests as recommended by American Society for Testing and Materials (ASTM, 1970) and American Public Health Association (APHA, 1980). Test media was renewed regularly during the experimental period.

3.13 Duration of the test

Each test; whether preliminary or actual was conducted for a period of 96 hours to determine the median lethal concentrations (LC_{50}) as recommended by Sprague, 1969 and 1973; Alabaster and Lloyd (1982) and American Public Health Association (APHA, 1980). According to sprague 96 hours LC_{50} in most reproducible. The LC_{50} of an agent is the concentration, which will kill or inactive 50% of the test animal. LC_{50} is inversely proportional to the toxicity of a compound i.e, the lower is the LC_{50} , the higher is the toxicity.

3.14 Observation on mortality and criterion for death

Records of mortality were made at logarithmic time intervals (Sprague 1973; Hasan and Macintosh, 1986 a.b) i.e after 6, 12, 24, 48, 72 and 96 hrs. of exposure. The open of valve was used as the criterion for death animal. During inspections, dead mussels were removed from the glass tank as soon as possible to prevent the deterioration of water quality.

3.15 Measurement and analysis of water quality

The physical and chemical characteristics of test water measured before and during the experimental trial. Temperature was measured by a N-filled mercury thermometer in °C. Dissolved oxygen, pH, total hardness, carbon dioxide, conductivity of test water were measured by using portable water kit (Mettler-Toledo Ltd. U.S.A.).

3.16 Concentration used

The various concentration used in the different experimental trials during the present study are given in Table 7 and 8.

3.17 Statistical analysis of data

The median lethal concentration (LC_{50}) values for different exposure times were estimated by using computerized EPA PROBIT, analysis program (Version 1.5). Before performing any computer analysis, the heterogeneity of data was tested by Chi-square. When the difference was significant log transformation of data was done.

In order to demonstrate the asymptoticity, logarithmic LC_{50} values were plotted against logarithmic time period and regression line plotted by the log probit method.



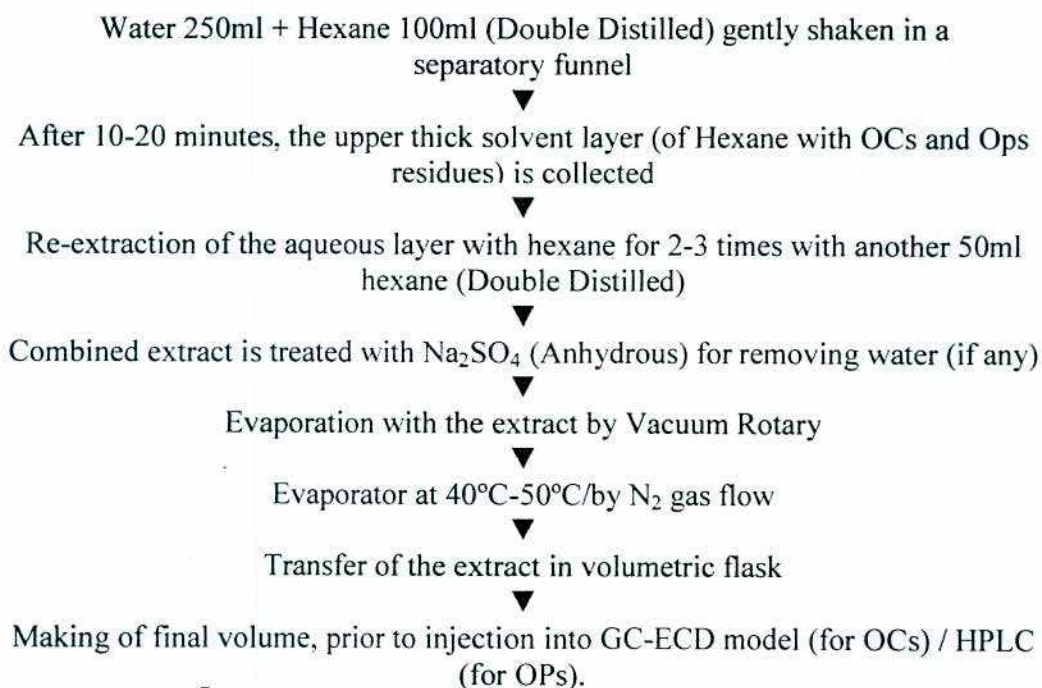
Fig. 26. Chemical and reagents used in laboratory

In the field-collected samples were preserved/stored (in situ) carefully. If samples hadn't been preserved accurately, quality of samples could be deteriorated. Different types of samples were preserved according to their type and variety, i.e. Mussel samples were put in glass tank as early as possible but it is not essential to store soil sample in ice. This preservation prevented further degradation of OCs and OPs residues until these are taken to the laboratory for analysis. Water samples were analyzed readily in the laboratory after collection.

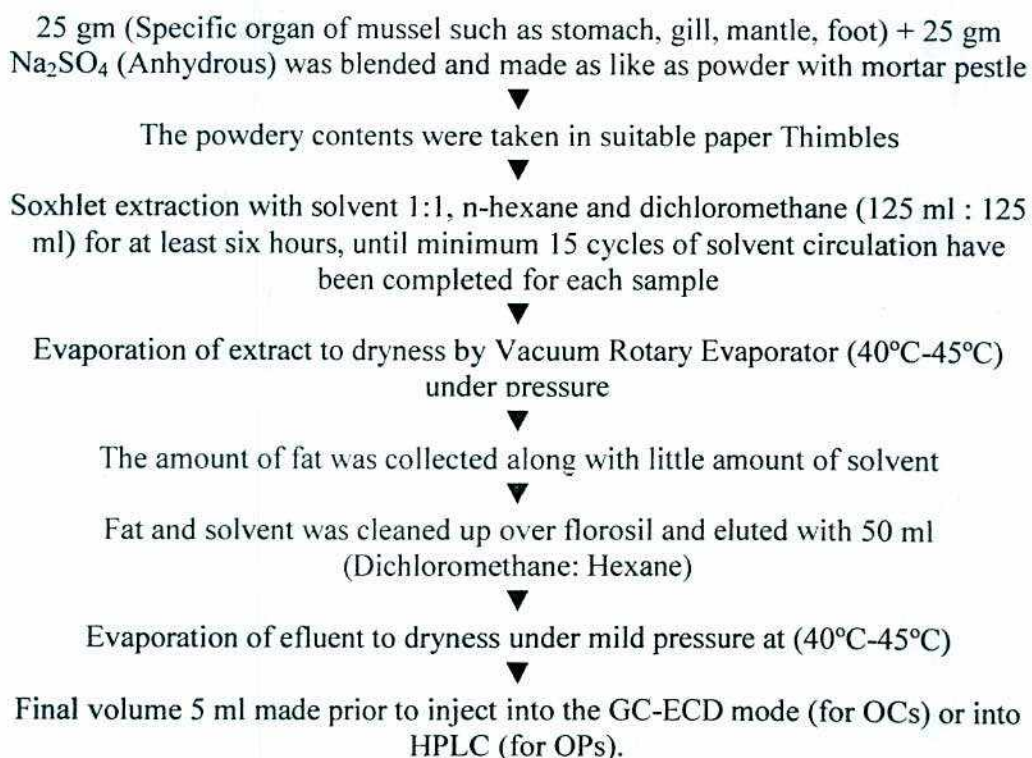
3.18 Laboratory works

The determine OCs and OPs residues of all the collected samples were prepared according to the following different procedures.

3.18.1 Brief method for extraction of OCs and OPs residues from water samples



3.18.2 Brief method for extraction of OCs and OPs residues from different organs of mussel



3.18.3 Brief method for extraction of OCs and OPs Residues from soil samples

Two different procedures were applied to extract OCs residues from soil samples—

Method -1: In shaker, using only Hexane.

Method -2: In shaker, using Hexane 95% + Diethyl ether 5%

The first method was followed for this purpose and method is as follows.

20 gm dried and powdered soil from each samples were mixed with 25 ml of DD

Hexane



Contents were taken in 250 ml Conical flask opening were blocked with Aluminium Foil



Flasks with soil and Hexane mixture were set accurately in Rotator and it was continuously rotated @ 225 RPM for 12 hours



After 12 hours solvents were let to settle down and Na_2SO_4 (Anhydrous) for removing water (if any)



The clean upper layer was separated to volumetric flask carefully



Extracts were concentrated by Rotary vacuum Evaporator at (40°C-45°C) under pressure in N_2 gas flow



Cleaning up the extract through glass column florisil (Magnesium Silicate) if color appeared



Final volume was made to 5 ml prior to injection into the GC-ECD mode (for OCs) or HPLC (for OPs).

3.18.4 Detail analytical scheme of pesticide residues for water, mussel's organ and soil samples

The residues of organochlorine compounds in experimental samples were determined by gas-chromatography (GC) using Electron Capture. Detector (ECD) and the residues of organophosphorus compounds were determined by High Performance Liquid Chromatography.

The following steps were performed for analysis of pesticide residues from collected samples.

3.19 Extraction procedure

All extraction procedures were followed by "D.F.G. manual of pesticide residue analysis volume-1" (Hanspcter theie and Hans Zenmer, 1987).

3.19.1 Extraction of water

Following the Liquid-liquid separatory funnel method all the glass wires washed carefully with the relative extraction solvent(s) for this purpose to maintain the procedure accurately. Descriptions of the methods are given below:

1. 250ml water + 100ml Hexane (Double Distilled) was taken in a separatory funnel and was vigorously shaken for 5 minutes. The separatory funnel was kept in a suitable stand for a certain time for water-Hexane separation; lower water portion was removed in a round bottom flask.
2. The remaining aqueous layer was extracted with hexane for 2-3 times with another 50ml, hexane (Double Distilled).
3. The combined extract was treated with Anhydrous Sodium Sulfate (Na_2SO_4) to remove any few amount of water, otherwise this matter can make critical problem during injection to GC or HPLC. Extracts was evaporated by Vacuum Rotary Evaporation or by N_2 gas flow dryer.
4. Extraction was transferred to the 5ml volumetric flask by repeated washing the Rotary Vacuum Evaporator with hexane, as some little amount of pesticide residues could be attached with the glass wall.
5. Clean up will be required if color is appeared.
6. Final volume was made with hexane as required prior to injection Gas Chromatograph (GC) with Electron Captured Detector (ECD) or to HPLC.



Fig 27. Extraction of water sample

3.19.2 Extraction of sediment

Soil Sample Preparation—The soil samples were mixed properly by a glass rod. The samples were taken in glass wares and kept in the oven at 150°C until it becomes dry. The dried samples were made powder. Sample powdered soils are shown in Fig.28.



Fig. 28. Powdered soils

3.20 Solvent preparation

Solvent n-hexane was made double distilled to remove impurities. The boiling temperature of hexane is 65°C. At this temperature hexane starts to vaporize and after coming in contact with continuous water supply it concentrates and it was collected in conical flask connected with standard solvent preparation is shown in Fig.29. Mentioned that, this solvent is needed for water samples, soil samples and different organs of mussels samples.



Fig. 29. Distillation of solvent

3.21 Analytical scheme of soil sample

50 gm powdered sample, 50 ml double distilled hexane and 50 ml double distilled acetone were taken in a conical flask. After shaking 8 hours on an electric shaker it was being to settle down for 10 minutes. This process is known as extraction. Extraction of sediments using electric shaker is shown in Fig.30. After extraction, the extracted layer was transferred in another conical flask. This was re-extracted 3 times adding 10 ml double distilled hexane and hand shaking for 5 minutes each time. Then about 5 gm anhydrous Na_2SO_4 was added. After Na_2SO_4 salt treatment the extract layer was transferred in to a flat bottom flask and evaporated by Rotary Vacuum Evaporator at 40°C temperature up to 2-3 ml volume. That concentrated extract was transferred into vial and cleaned up with florosil eluting 100 ml double distilled hexane. If again any of the sample showed color then extracts were clean-up through glass column florosil (Magnesium silicate) 2-3 ml by rotary vacuum evaporator was done. Then cleaned concentrated extract was transferred into vial and dried fully by Na_2 gas blower. After all processes final volume was made (5 ml) for injection into GC and HPLC.



Fig.30. Extraction of sediment using electric shaker

3.22 Preparation of mussel's samples

Different organs of mussel was obtained by the dissection. ^{14}C was taken in separating different organs of mussel. Raw mussels organ were mixed for subsequent dissolution and analysis (Attar et. al 1992). The mussel's organ samples were grinded in high-speed blender until a paste like consistency formed. Procedure was followed by U.S. Food and Drug Administration (Mansado, 1987).

After making the mussel paste homogenate, 25 gm of tissue was mixed with around 25 gm of anhydrous sodium sulfate (Na_2SO_4), previously Na_2SO_4 was heated to 200°C in the oven for 24 hrs. at 95°C - 110°C . Then homogenate was grinded in a mortar and pastel until a free flowing powder had been formed.



Fig. 31. Organs of mussel obtained by dissection

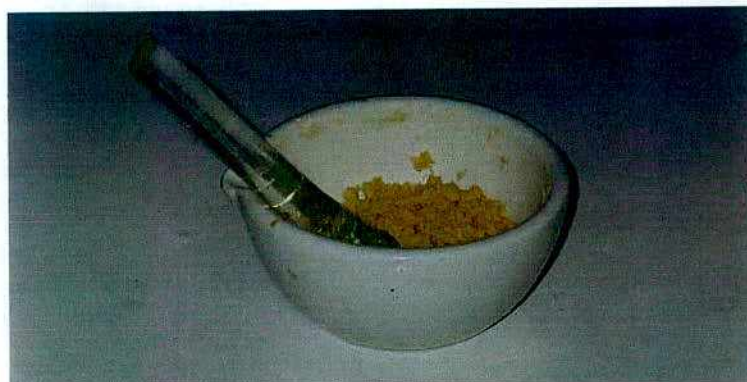


Fig. 32. Mussel's sample (stomach) grinding in a mortar

3.22.1 Extraction of fat from mussel's organ

Prepared samples (free flowing powder) were taken in a paper thimble and then setup in soxhlet apparatus using 250 ml, solvents (125 ml Dichloromethane + 125 ml Hexane). Prepared samples in thimbles were extracted through soxhlet apparatus for 6 hours, but it is obvious that at least 15 cycles for each sample should be completed. The dichloromethane and hexane were BDH analytical grade prior to use, the hexane was double distilled at 60°C. After around 6 hrs. when 15 cycles for each sample has been completed, the combined extracts were evaporated by a Rotary Vacuum Evaporator (40°C-45°C) under pressure (Fig. 35). The remaining fat was weighted if it required determining the fat content of the sample for other purpose.



Fig. 33. Fat extracted through soxhlet apparatus



Fig. 34. Grinding mussel's sample in solvent

3.23 Clean up procedures

The combined extracts were filtered to avoid un-dissolved materials. The collected extract was then evaporated to 5 ml with a stream of nitrogen. To avoid the co-extractives from the extract, the florisil column chromatograph was applied where necessary. Electrons Capture Detector of Gas Chromatograph (GC) and Ultra Violet Detector of High Performance Liquid Chromatograph (HPLC) are very sensitive to co-extractives and would otherwise contaminate the detector following procedures were taken out for florisil column chromatograph.



Fig. 35. Sample evaporated by Rotary Vacuum Evaporator

3.24 Florosil preparation

Florosil, 60-100 mesh (Magnesium Silicate) was heated overnight at 550°C, then cooled and stored in tightly stopper desiccators. Prior to use (if used after a long time of preparation) the florosil should be activated by heating in an oven at 200°, for 6 hours and cooled in a desiccators. The freshly activated florosil was then partly deactivated with drop wise addition of distilled water (2% v/v water/florosil) at 40°C, with constant stirring for 2 hours. Florosil was partly deactivated to increase the polarity of the absorbent, which is very essential for perfect clean up.

3.25 Column preparation

A freshly chromatographic glass column (accurately washed rinsed) was set with a stand; the column was rinsed with double distilled hexane. About two-third of the column was filled with double distilled hexane and then 10 g of deactivated florosil was slowly applied in the column by gently tapping the tube with a non contaminated glass rod to avoid the formation of any bubble in the column packing. The absorbent was allowed to settle down. Then the stopcock of the column was opened to drain out the hexane up to a level of 1 cm above florosil layer. The florosil of the column was covered with an approximate 2 cm layer of anhydrous sodium sulfate to remove the water (if any) from the extracts. Column preparation and elution procedure are shown in Fig.36.



Fig. 36. Column preparation and elution procedure

3.26 Elution procedure

All the 5 ml extracts derived from the above mentioned extraction procedure were pipette on to the chromatographic column and allowed the solution to percolate to a level of around 1-2 mm above the top of the florosil to the column. The vial was rinsed with a small portion of elution mixture and the rinsed content was again applied to the column and was allowed to percolate. The column was eluted with 100ml double distilled hexane (1:1) at a flow rate of 1-2 drops per second (5 ml/min). The elute extract was collected in a stopper flask. The elution was made to volume with or without evaporation depending on the initial injection at the appropriate mode of GC and HPLC.

3.27 Chromatographic determination

From the final solution derived after clean up, aliquot (usually 0.5 μ l-1 μ l) was injected by micro liter syringe into the Gas Chromatograph (GC) fitted with the column Electron Capture Detector and into the High Performance Liquid Chromatograph (HPLC) fitted with the Ultra Violet Detector.

3.27.1 Operating condition of GC (ECD Mode)

Apparatus (Type)	: HP 5890, Series 2
Detector (Type)	: Electron Capture Detector
Injection technique	: Manual by Micro syringe
Injection volume	: 0.5 μ l or 1 μ l
Carrier gas	: N ₂ , purity 99.99%
Carrier gas flow	: 8 cm ³ /min
Column (type)	: Capillary column
Column initial temperature	: 175°C
Initial time	: 4 min
Final temperature	: 255°C

Final time	: 10 min
Injector temperature	: 290°C
Detector temperature	: 275°C
Detector make up gas	: N ₂
Make up gas flow	: 75 cm ³ /min
Recorder/Injector (Type)	: HP 3396, series 3
Column head pressure	: 6 psi
Attenuation of Integrator	: 1 mm/sec
Threshold	: 5

Gas Chromatograph and printer for detecting pesticide residues are shown in Fig.37.



Fig. 37. Gas Chromatograph and printer

3.27.2 Operating mode of HPLC

The High Performance Liquid Chromatograph (Waters 486) equipped with UV detector along with the output device computing integrator, waters was used for determination of the level of organophosphorus and carbamate pesticide residues as data graphics along with dialogues and report i.e. peak, area%, time retention and are Basement Count (BC). HPLC is shown in Fig. 38.

Operating condition of HPLC (UV mode)

Apparatus (Type)	: Water 486
Detector (Type)	: Ultra Violet Detector
Injection technique	: Manual by Micro syringe
Injection volume	: 0.5µl or 1µl
Mobile phase	: (65% Acetonitrile)
Column (type)	: Capillary column
Recorder/Injector (Type)	: Waters
Column head pressure	: 5000 psi
Attenuation of Integrator	: 4 mm/sec

Flow rate : 0.5 ml/min
Absorbance : 254 mm wave length



Fig. 38. High Performance Liquid Chromatograph and printer

3.27.3 Chromatographic process

The heart of every chromatograph is the column. The samples are normally introduced into the carrier gas stream using a micro-liter syringe via a rubber septum in an injection port at the front end of the column. The injector column and detector are in close proximity to each other and all electrically heated temperature. When the components elute, they pass into a detector. Where, assuming they are above a threshold level, their presence is detected and the electrical signal produced amplifies to usable level. The response of the detector is displayed on a recorder or printer as a chromatogram.

- a. Column – There are various columns that are secondary to the separating column or stationary phase. They are: Guard, Derivating Capillary, Fast, and preparatory columns in HPLC and there are two types of columns in GC i.e packed column and capillary column. In GC and HPLC capillary column is used. Capillary column is more efficient than any other column.
- b. Carrier gas – In GC carrier gas brings the total flow into a detector. The carrier gas must be chemically inert. Commonly used gases include nitrogen, helium, argon and carbon dioxide. The choice of carrier gas is often dependent upon the type of detector, which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities. For Electron Capture Detector (ECD), nitrogen is used as a carrier gas. In GC make up gas is also required make up gas in capillary column brings the total flow into a detector up to the rate for optimum sensitivity. In HPLC no carrier gas required the mobile phase acts as a carrier for the sample solution. The mobile phase in HPLC refers to the solvent being continuously applied to the column or stationary phase. The

stationary phase in HPLC refers to the solid support contained within the column over which the mobile phase continuously flows. A sample solution is injected into the mobile phase of an assay through the inject port. As a sample solution flows through a column with the mobile phase, the components of that solution migrate according to the non-covalent interactions of the compound with the column; determine the degree of migration and separation of components contained in the sample. For example those samples, which have stronger interactions with the mobile phase than with stationary phase, will elute from the column faster, and thus have a shorter retention time, which the reverse is also true. The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

- c. Detector – It has been established that the purpose of a detector is to produce a response that is proportional to the concentration (or amount) of compound passing through it, in the form signal which can be measured through the output device, the printer.
- d. Threshold – Threshold controls sensitivity. Increasing the threshold value makes the integrator less responsive to noise in the signal. The range is 6 to 28.
- e. Attenuation – Attenuation sets the scale for the chart width. Low number expands the chromatogram's width. Large numbers compress it. The valid range is 8 to 36.

3.27.4. Identification and quantification procedures

Tentative identification of the suspected pesticide was carried out in relation to the Retention Times (RT) of the pure analytical standard of that pesticide, standard DDT, DDE, DDD, lindane, BHC, heptachlor, aldrin, dieldrin and Endrin for organochlorines and Diazinon, chlorpyrifos, Fenitrothion, Malathion, cypermethrin for organophosphorus and carbofuran and carbaryl for carbamate, were purchased from chromopack.

Quantification of the identified pesticides was performed by the calibration technique. For this purpose, injection equal volumes of differently concentrated standard solutions into GC and HPLC prepared calibration curve for each pesticide. The time retention featured is changed a little bit which of course will not make any confusion for identification and 0.09% difference is acceptable.

3.27.4.1. Calculation of residues

The residue R' , expressed in ng/ml for water, ng/gm for soil and mussels were calculated from the following equations:

$$R' = \frac{H_A V_{End} W_{St}}{H_{St} V_i G} \quad \text{(DFG Manual of Pesticide Residue Analysis, 1987)}$$

Where,

- G = Sample weight (gm)
- V_{End} = Terminal volume of the sample solution (ml)
- V_i = Portion of volume V_{End} injected into GC or HPLC (μ l)
- W_{St} = Amount of standard pesticides injected with standard solution - (ng)
- H_A = Peak area obtained from V_i (mm^2)
- H_{St} = Peak area obtained from W_{St} (mm^2)

CHAPTER FOUR

RESULTS

4.1 Water quality

Table 24 shows the physico-chemical characteristics of tap water used as dilution water in the experiments. The average range of temperature recorded was 26-29 °C. The dissolved oxygen content range from 4.2 mg/l to 6.20 mg/l. The hydrogen ion concentration (pH) in dilution water varied from 7.12-8.0 during study period. Ammonia concentration were ranged from 0.10-0.14 mg/l. The physico-chemical characteristics of exposure media monitored during preliminary trials with carbosulfan and malathion insecticides and are shown in Appendix Table I and IV, respectively. The results of water quality of experimental Trail IA and Trial-IIA are presented in Appendix Table II and Table III, respectively and that of Trial IB and IIB are given in Appendix Table V and Table VI Respectively. During any Experimental Trial there was slight fluctuation in any water quality parameters among different treatment tank except for pH, ammonia, alkalinity during experiment with malathion insecticide. The pH value showed a concentration dependent changes ranging from 5.9 to 8.1, ammonia increased with increases in carbosulfan concentration in the exposure media which varied from 2.8 to 3 mg/l (showed in Appendix Table II and III). Consequently, alkanity value also showed a decrease with the increasing carbosulfan contents in the water, the level which varied from 152 to 206 ml/l (Appendix Table I). Low values of dissolved oxygen range from 3.7-3.9 mg/l⁻¹ were observed in Trial IIA with carbosulfan (Appendix Table I).

Table 9 Physico-chemical characteristics of the tap water used as experimental dilution water in different trials

Parameters	Mean	Range
Temperature (°C)	27 ± 0.87	26 – 29
Dissolved oxygen (mg/l)	5.7 ± 0.31	4.2 – 6.20
pH	7.6 ± 0.44	7.12 – 8.0
Carbon dioxide (mg/l)	19.8 ± 0.52	18.5 – 20
Total hardness (as CaCO ₃ , mg/l)	97.50 ± 3.1	86.10 – 103.0
Total alkanity (mg/l)	78.02 ± 3.21	73.0 – 107.0
Ammonia (mg/l)	0.110 ± 0.0002	0.100 – 0.140
Nitrile –N (mg/l)	0.04 ± 0.0001	0.03 – 0.05

4.2 Behaviuor

Both carbosulfan and malathion insecticide caused behavioral abnormalities in the test mussels. However, the degree and extent of these changes varied with the exposure concentration of the applied chemicals and duration of exposure. The various behavioral activities observed in the test mussel, *L. marginalis*, following exposure to carbosulfan and malathion insecticide described below—

4.3 Carbosulfan

The control group of mussel showed normal behavior as was observed in the acclimation period, during the entire experimental period. The mussel exposed to 9.90 ppm carbosulfan insecticide showed increased activity as manifested in the sudden move and orientation. The two valve of mussel is strongly tight in condition. But no sloughing off of mucus was observed. However, toward the end of the experiment, the abnormality disappeared. When mussel exposed to 12.87 ppm and 13.86 ppm carbosulfan caused substantial changes in behavior. There was an abundant secretion of mucous both on gills and mantle. Immediately after following exposure, the mussel showed heightened activity, frequent orientation, escape tendency and air gulping. Foot is out from the body. This continued up to the end of exposure (96h) in mussel group exposed to 13.86 ppm carbosulfan while mussel became lethargic toward the end of exposure.

The most heightened reaction was observed at the highest concentrations 13.86 ppm to 14.86 ppm within few minutes of exposure. Mussel (*L. marginalis*) at this concentration become hyperactive, frequently tried to foot out from the body. Mantle and gill observed to be covered with mucous. Suffocation of mussel (*L. marginalis*) manifested in the form of air bubbles on the water surface. Mussel (*L. marginalis*) died with open shell, gill and mantle is covered with mucous.

4.4 Malathion (OP)

The first visible reaction to malathion exposure was observed at the highest exposure concentration 11.86 -12.87 ppm within few minutes of exposure. The mussel (*L. marginalis*) at this concentration rapidly lost normal condition frequent spiral movement with form a air bubble, foot is out from the body were commonly seen. When first period of passing, the mussels were partially open the shell and foot settled on the bottom. The loss of sensivity (response) was seen by extended of foot from the body. Subsequently a vigorous and resistant of the mussel were set in and respiration became rapid. The body weight of the mussel appeared to be lighter than the previous time due to excessive mucous secretion. When the mussel lost the normal activities due to paralysis the mussel became dull colour.

The mussel released in the lower concentration, i.e. below 1.28 ppm showed no or little behavioral changes depending on the organophosphorus concentration in the exposure media. During the later period of exposure, the body of the mussel appeared to be more light than normal. Heightened activity with rapid foot release from the body and air bubble were formed, were observed when exposed to 9.90- 10.89 ppm.

4.5 Mortality and lethal concentrations

4.5.1 Carbosulfan insecticide

The cumulative mortality (%) data of preliminary trial is given in Appendix Table VII. The result show that no mortality caused to mussel exposed up to the exposure concentration of 12.87 ppm.

Table 10. Comparison of cumulative percent mortality of *L. marginalis* during an exposure period of 96h at different concentration of carbosulfan insecticide

Treatment Number	Trial IA		Trial IIA	
	Concentration of carbosulfan ppm	% Mortality	Concentration of carbosulfan ppm	% Mortality
Control	0	0	0	0
1	9.90	0	10.40	0
2	11.39	0	12.38	0
3	13.07	40	13.07	30
4	13.86	100	14.06	100
5	14.85	100	14.36	100

Table 11. Lethal concentrations and confidence limit for *L. marginalis* exposed to carbosulfan insecticide for various time periods

	Exposure (hour) time	LC ₅₀ ppm (carbosulfan)	95% confidence limit
Trial IA	12	15.02	14.05-15.99
	24	13.60	12.83-14.37
	48	13.47	12.70-14.24
	72	13.33	12.56-14.10
	96	13.20	12.43-13.97
Trial IIA	12	14.52	14.26-15.00
	24	14.26	13.97-14.55
	48	14.09	13.80-14.38
	72	13.57	12.60-14.54
	96	13.35	12.38-14.32

Equation for 95% confidence limit ($X - S_d d_{0.05} \leq \mu \leq X + S_d d_{0.05}$)

Carbosulfan insecticide during the preliminary experimental period, however, mussel exposed to 14.85 ppm malathion insecticide induced 20% mortality within 6 hrs of exposure and a complete (100%) mortality was obtained by 24 hrs of exposure (Appendix Table VII). The cumulative mortality (%) of *L. marginalis* exposed to various concentrations of malathion at different time intervals are presented in Appendix Table VIII and shown graphically in Fig. 39 and 40 for Trial-IA. The mussel exposed to the concentration of 11.39 ppm did not cause any mortality during the experimental period. However, 13.07 ppm malathion induced 25% mortality by 72 hrs. of exposure and this group of mussel showed

only 40% mortality by the end of exposure. 100% mortality was first observed in mussel group exposed to 14.85 ppm malathion by 24 hrs. However, mussel exposed to 15.84 ppm carbosulfan insecticide caused a complete (100%) mortality within 6 hrs. of exposure.

The mortality results of Trial-IIA are shown in Appendix Table IX and Fig.41. There was no mortality either in control mussel or in mussel exposed up to the concentration of 10.40 ppm carbosulfan insecticide during the experimental period. The lowest mortality (30%) after 96h exposure was observed in mussel group exposed to 12.38 ppm carbosulfan, although the same group of mussel recorded only 15% mortality at 72h of exposure. However a complete mortality (100%) was observed at carbosulfan concentrations of 14.06 ppm, 14.36 ppm and 14.85 ppm by the end of 96h, 48h and 12h of exposure. For the visual comparison, the data, for cumulative percent mortality of mussel after 96h exposure period is provided in Table 12. In case of Trial-IA the median lethal concentration value could be computed for 12h, 24h, 48h, 72h and 96h exposure period and estimated to be 15.02 ppm, 13.60 ppm, 13.47 ppm, 13.33 ppm and 13.20 ppm (CL 14.05-15.99, 12.83-14.37, 12.70-14.24, 12.56-14.10, 12.43-13.97). This happened probably due to the use of wide spaced malathion insecticide level. The estimated median lethal values and confidence limit for malathion at various exposure times for Trial-IA and IIA are presented in Table 11. In case of Trial IIA the 12h, 24h, 48h, 72h and 96h values were 14.52 ppm, 14.26 ppm, 14.09 ppm, 13.57 ppm and 13.35 ppm (CL-14.03-15.00, 13.97-14.55, 13.80-14.38, 12.60-14.54, 12.38-14.32).

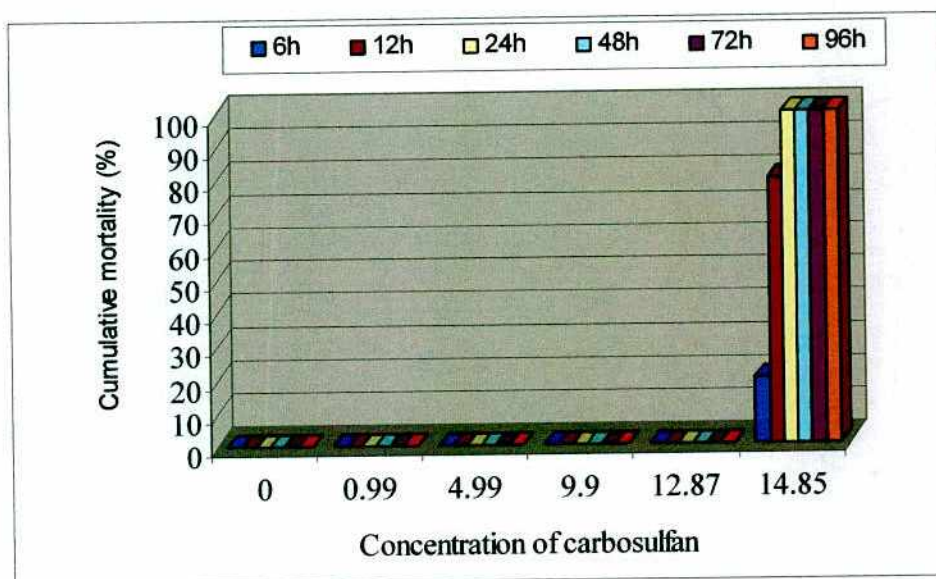


Fig. 39. Cumulative mortality (%) of *L. marginalis* at different concentration of carbosulfan insecticide during an exposure period of 96h (Preliminary Trial)

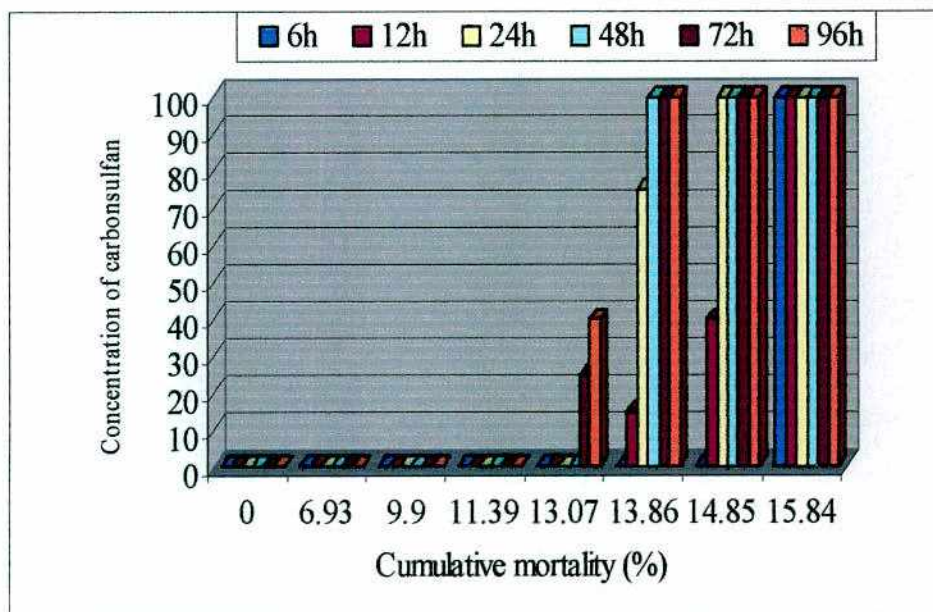


Fig. 40. Cumulative mortality (%) of *L. marginalis* at different concentration of carbosulfan insecticide during an exposure period of 96h (Trial IA)

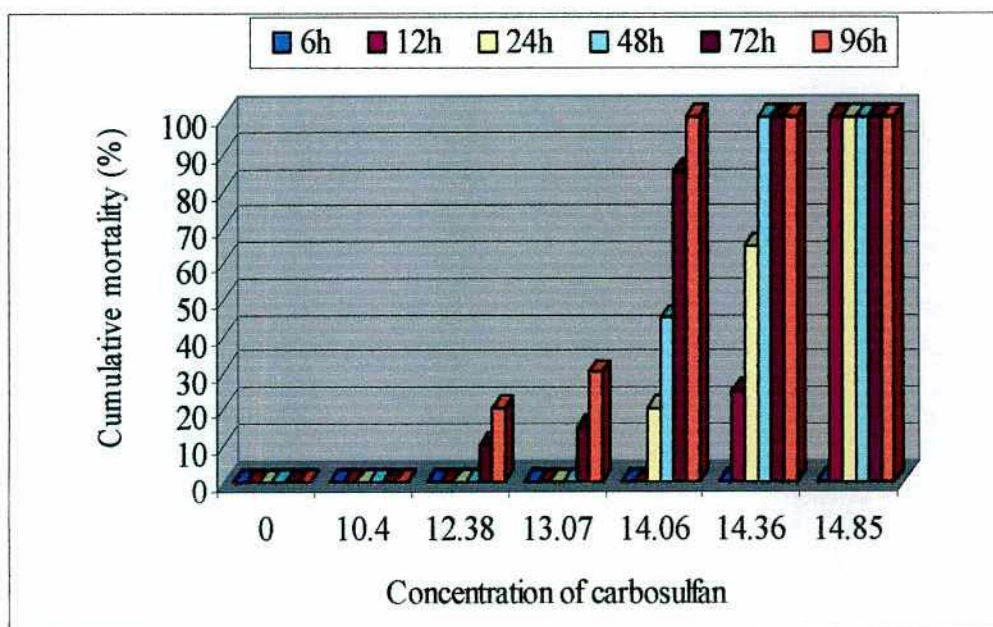


Fig. 41. Cumulative mortality (%) of *L. marginalis* at different concentration of carbosulfan insecticide during and exposure period of 96h (Trial IIA)

4.5.2 Malathion (OP) insecticide

The data on cumulative mortality (%) of the preliminary trial are given in Appendix Table X. The results show that there is no mortality in mussel groups exposed up to 0.99 ppm during 96h exposure. However, 14.85 ppm malathion insecticide induced mussel mortality (20%) by 72h of exposure and 35% mortality occurred in the same treatment group by 96h of exposure. A complete mortality (100%) however was observed in mussel at 24.76 ppm malathion insecticide within 72h of exposure.

During Trial IB, no mortality occurred in mussel exposed to 4.95 ppm malathion insecticide on the other hand, a complete (100%) mortality was observed in mussel group (*L. marginalis*) at 19.80 ppm, 21.90 ppm and 24.28 ppm of exposures, respectively. However, the lowest mortalities (60%) were experimental period 96h at the concentration of 14.85 ppm (Appendix Table XI and Fig. 43). For convenience, the cumulative mortalities (%) of the mussel at different concentrations of malathion insecticide after 96h exposure are presented in Appendix Table XI.

The cumulative mortality % of *L. marginalis* exposed to various concentration of malathion insecticides at different time periods are presented in Appendix Table XII and shown graphically in Fig. 44 for Trial IIB. No mortality was observed in mussel (*L. marginalis*) exposed to any concentration below 9.90 ppm malathion insecticide. Cumulative mortalities of 35% and 40% were observed in mussel exposed to 11.88 ppm at 72 and 96h, respectively. 50% mortality was recorded during 96h of exposure at the concentration of 13.86 ppm malathion insecticide. However, 60% mortality was found in 15.84 ppm at 72h exposure. A complete mortality (100%) was observed at the dose of 19.80 ppm concentration within 48h of exposure (Appendix Table XI and XII).

The median lethal concentration (with 95% confidence limits) for malathion insecticide at various exposure periods in Trial IB and IIB are presented in Table 13. In Trial – IB, 24h, 48h, 72h and 96h LC_{50} values were 21.20 ppm, 17.82 ppm, 15.30 ppm and 13.75 ppm (95% CL 19.14-23.26, 12.97-22.67, 10.45-20.15, 9.90-17.60) malathion insecticide respectively (Table 13).

In Trial IIB: 24h, 48h, 72h and 96h LC_{50} values were 19.36 ppm, 17.49 ppm, 14.52 ppm and 13.86 ppm (95% CL 17.42-21.30, 15.55-19.43, 12.58-16.46 and 11.92-15.80) malathion insecticide respectively (Table 13).

In both trials, the LC_{50} values for malathion insecticide gradually decreased with time which is indicated by the toxicity curve shown in Fig.48 and 49.

Table 12. Comparison of cumulative mortality (%) of *L. marginalis* during an exposure period of 96 hours at different concentrations of malathion insecticide

Treatment Number	Trial IB		Trial IIB	
	Concentration of malathion (ppm)	% Mortality	Concentration of malathion(ppm)	% Mortality
Control	0	0	0	0
1	4.95	0	5.94	0
2	9.90	0	7.92	0
3	14.85	60	9.90	40
4	19.80	100	11.88	55
5	24.76	100	13.86	70
6	29.71	100	15.84	100
7	-	-	17.82	100
8	-	-	19.82	100

Table 13. Lethal concentrations and confidence limit for *L. marginalis* exposed to malathion insecticide for various time periods

	Exposure (hour) time	LC ₅₀ ppm (malathion)	95% confidence limit
Trial IA	12	24.28	-
	24	21.20	19.14-23.26
	48	17.82	12.97-22.67
	72	15.30	10.45-20.15
	96	13.75	9.90-17.60
	12	-	-
Trial IIA	24	19.36	17.42-21.30
	48	17.49	15.55-19.43
	72	14.52	12.58-16.46
	96	13.86	11.92-15.80
	12	-	-

Equation for 95% confidence limit ($\bar{X} - S_d d_{05} \leq \mu \leq \bar{X} + S_d d_{05}$)

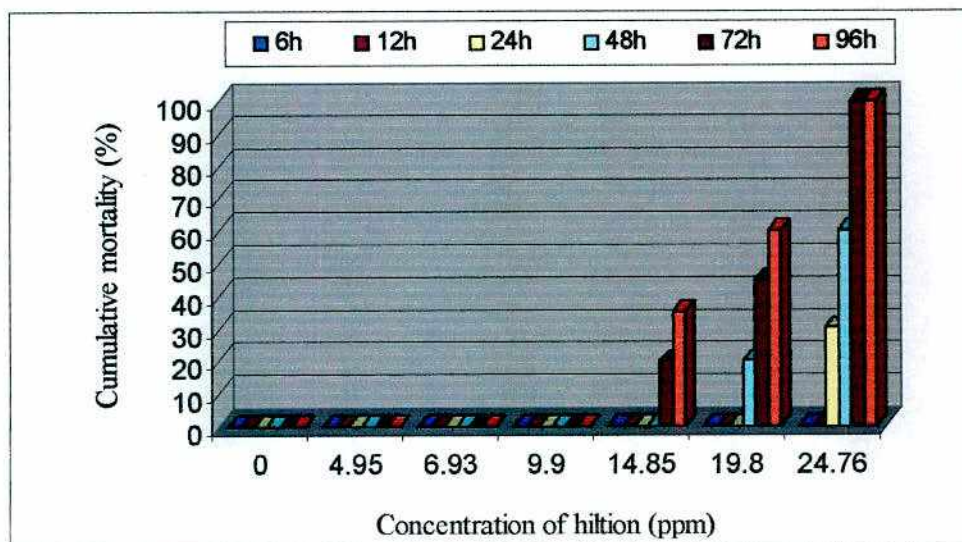


Fig. 42. Cumulative mortality (%) of *L. marginalis* at different concentration of malathion insecticide during and exposure period of 96h (Preliminary Trial)

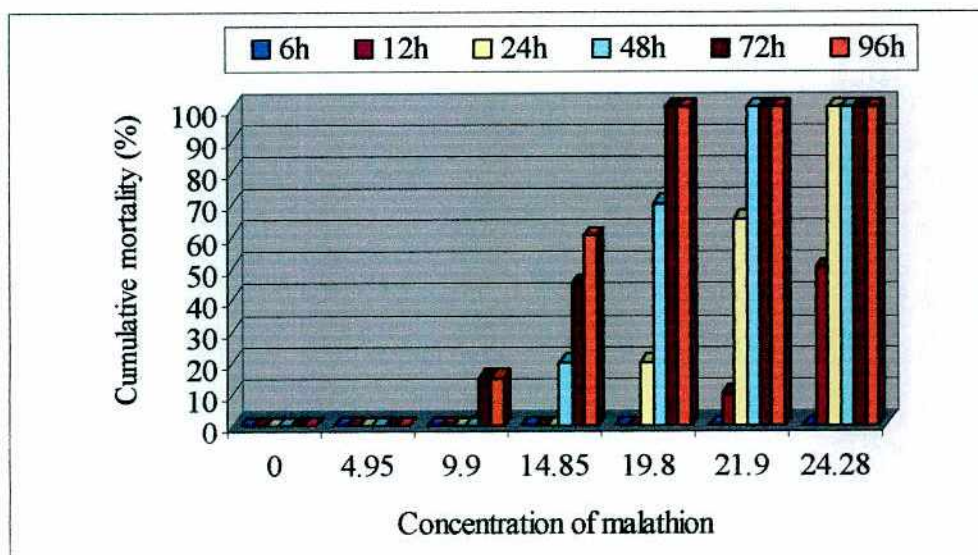


Fig. 43. Cumulative mortality (%) of *L. marginalis* at different concentration of malathion insecticide during and exposure period of 96h (Preliminary Trial IB)

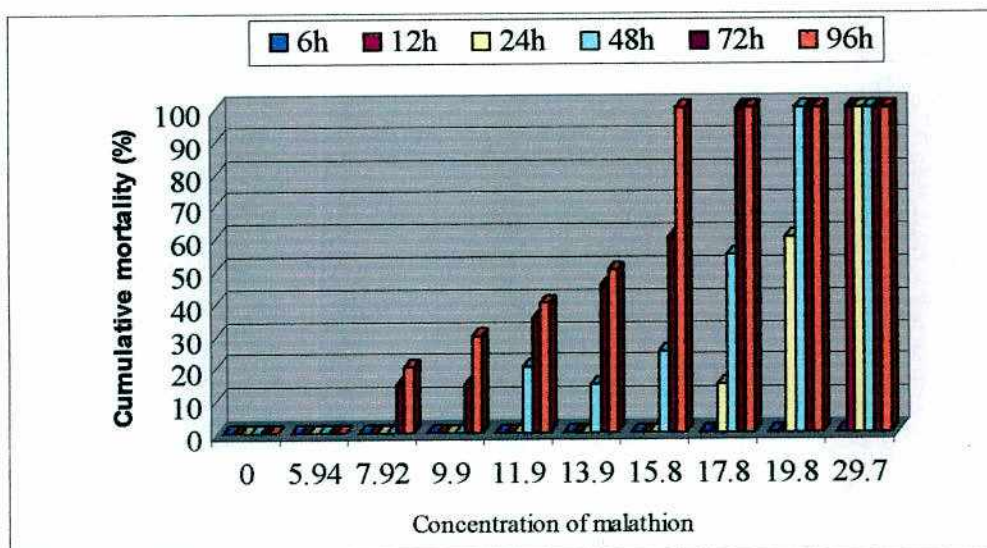


Fig. 44. Cumulative mortality (%) of *L. marginalis* at different concentration of malathion insecticide during and exposure period of 96h (Trial IIB)

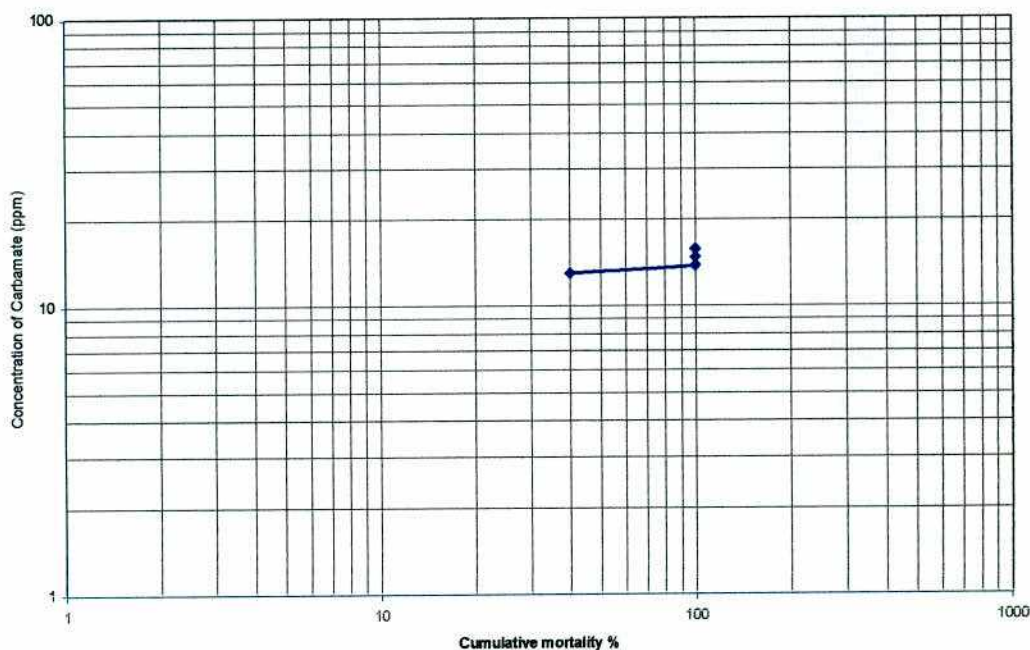


Fig. 45. Mortality (%) of *L. marginalis* at different concentration of malathion insecticide during an exposure period of 96h, Trial IA (regression line plotted by the log probit method)

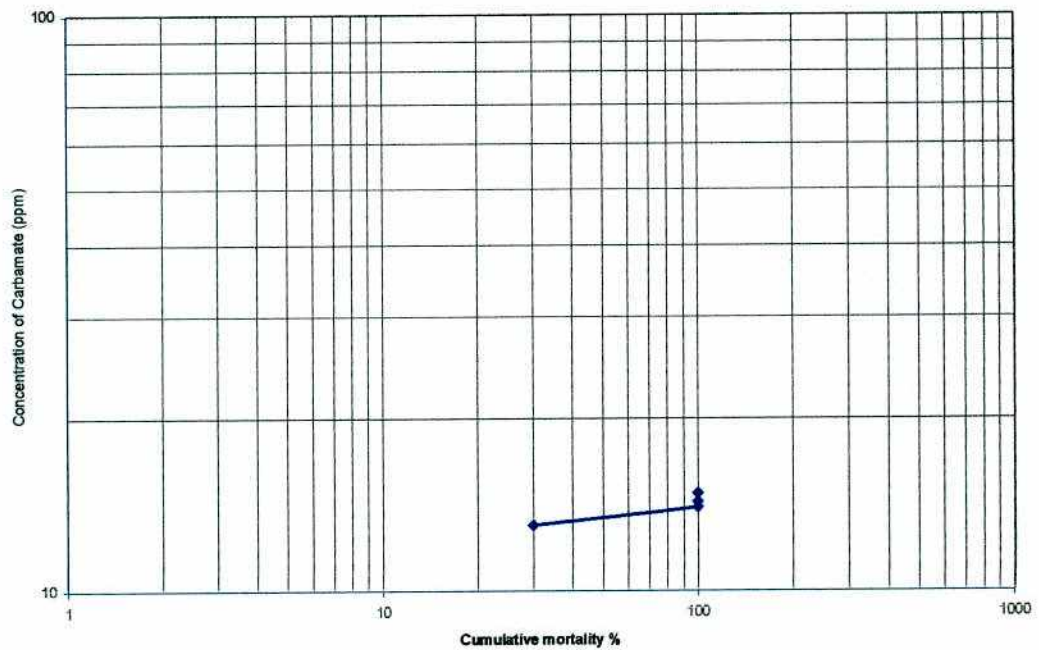


Fig. 46. Mortality (%) of *L. marginalis* at different concentration of malathion insecticide during an exposure period of 96h, Trial IIA (regression line plotted by the log probit method)

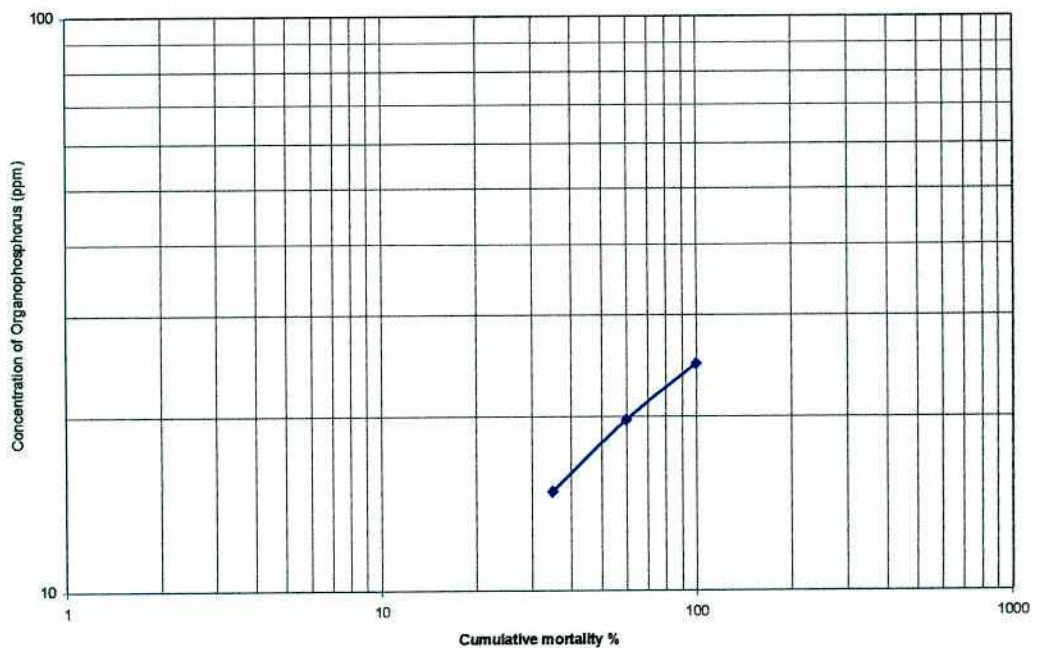


Fig. 47. Mortality (%) of *L. marginalis* at different concentration of malathion insecticide during an exposure period of 96h, Preliminary Trial (regression line plotted by the log probit method)

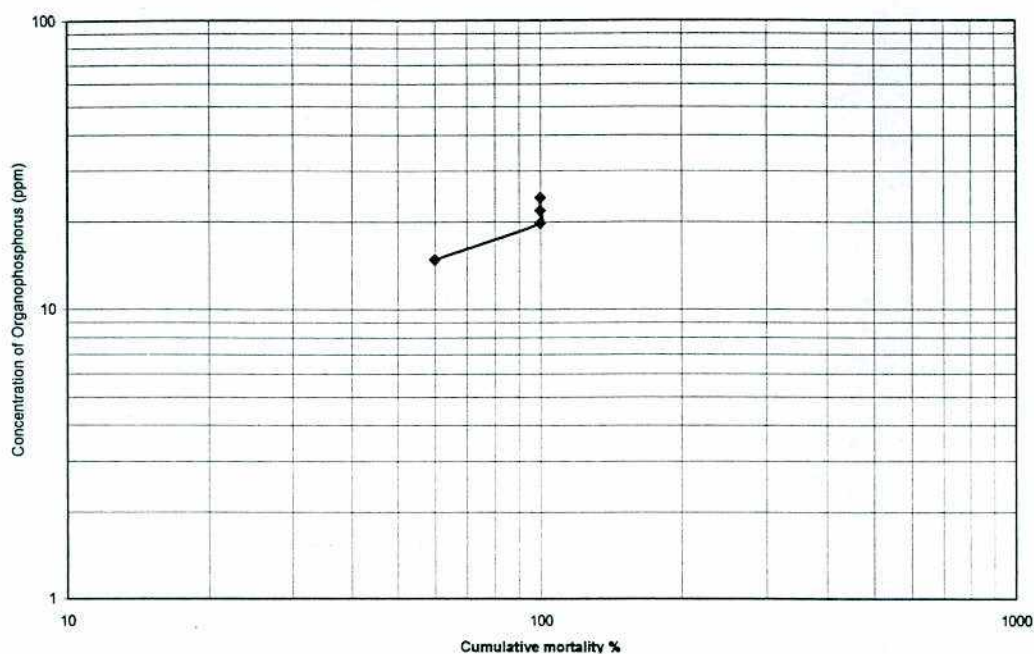


Fig. 48. Mortality (%) of *L. marginalis* at different concentration of malathion insecticide during an exposure period of 96h, Trial IB (regression line plotted by the log probit method)

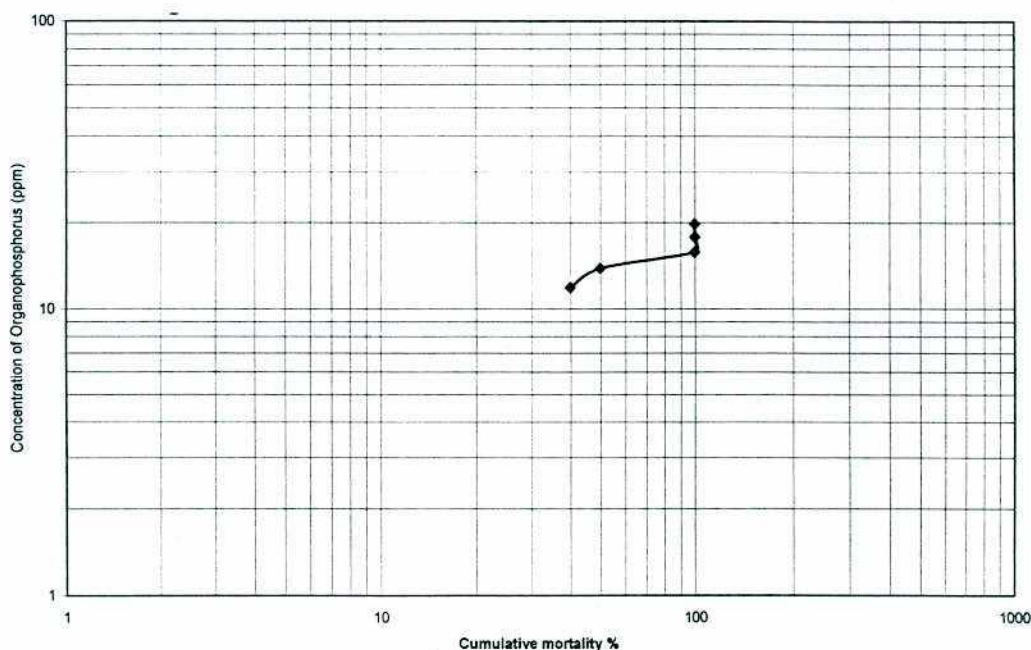


Fig. 49. Mortality (%) of *L. marginalis* at different concentration of malathion insecticide during an exposure period of 96h, Trial IIB (regression line plotted by the log probit method)

4.6 Pesticide residue analysis

Water, Mussel and Soil samples collected from 9 thanas of Bogra district were analyzed for the presence of organochlorine and organophosphorus residues. Samples of each location coded as sample number 1 to 11 i.e. location number 1 means sample no. 1 etc. It should be noted that samples no 1, 2, 3 were the bell samples of three thanas namely Sonatala, Sariakandi and Gabtali respectively, samples no 4, 5, and 6 were river samples of other three thana name as Sibganj, Bogra sadar and Sherpur respectively; samples no 7, 8 and 9 were the rest of the thanas namely Kahalu, Adamdighi and Dhunot and samples no 10 and 11 was the only samples which was collected during the flood and after the flood.

Results of the studies on water, mussel and soil samples are presented under the following headings:

4.7 Water samples

Concentrations (ng/ml) of organochlorine pesticide residues in water samples are presented in Table 14. p,p'- DDT compound were found in all samples except sample no. 4, 5 and 6. p,p'- DDT was found in sample no. 1, 7, 8 and 11 ranged from 0.33 ng/ml to 11.93 ng/ml. p,p'- DDD was found in the three samples (sample no. 1, 7, 11) ranged from 1.14 ng/ml to 2.30 ng/ml where p, p'- DDE was found in range of 0.10 ng/ml to 1.75 ng/ml. Among organochlorine residues, DDT, aldrin, dieldrin and heptachlor were detected. Aldrin was found in 3 samples (sample no. 3, 8, 11) ranged from 0.02 ng/ml to 0.05 ng/ml and dieldrin was detected in the range from 0.08 ng/ml to 1.25 ng/ml. Lindane and heptachlor were detected in samples 7, 1 respectively as the concentration of 0.10 ng/ml and 1.20 ng/ml successively. Endrin and HCH were not found in any of the water samples analyzed.

Among all the water samples, sample 7 had the highest concentrations of DDT followed by the sample no 11.93 (Fig.54), where the total concentrations of organochlorines were also found in the maximum level (Appendix Table XIV).

Fig 50 shows higher concentration of p,p'- DDT (11.93 ng/ml) among the composition of the DDT compounds which is followed by p,p'- DDD (2.30 ng/ml).

The permissible limit of p,p'- DDT and lindane in non-portable water recommended by FAO/WHO (1993) are 0.042 ng/ml and 0.56 ng/ml respectively. Concentrations of these two organochlorine residues found in the water samples of Kahalu, Adamdighi and Sonatala thana (after flood) are much higher than the suggested level. The persistent nature of organochlorines and their translocations from agricultural land and urban sewage can cause residues in food commodities.

Concentrations of organophosphorus residues in water samples are shown in Appendix Table XIII. Several organophosphorus pesticides (temephos, S-bioalletrin, pyraminforte diazinon and malathion) were analyzed. Only temephos

was identified and qualified in water samples. Temephos is cauterized as highly hazardous in Table 19 (Corbett, 1974). Results showed that only three locations namely Sonatala, Sarokandi and Gabtali contained temephos as the concentration of 0.04 ng/ml, 0.03 ng/ml and 0.04 ng/ml respectively.

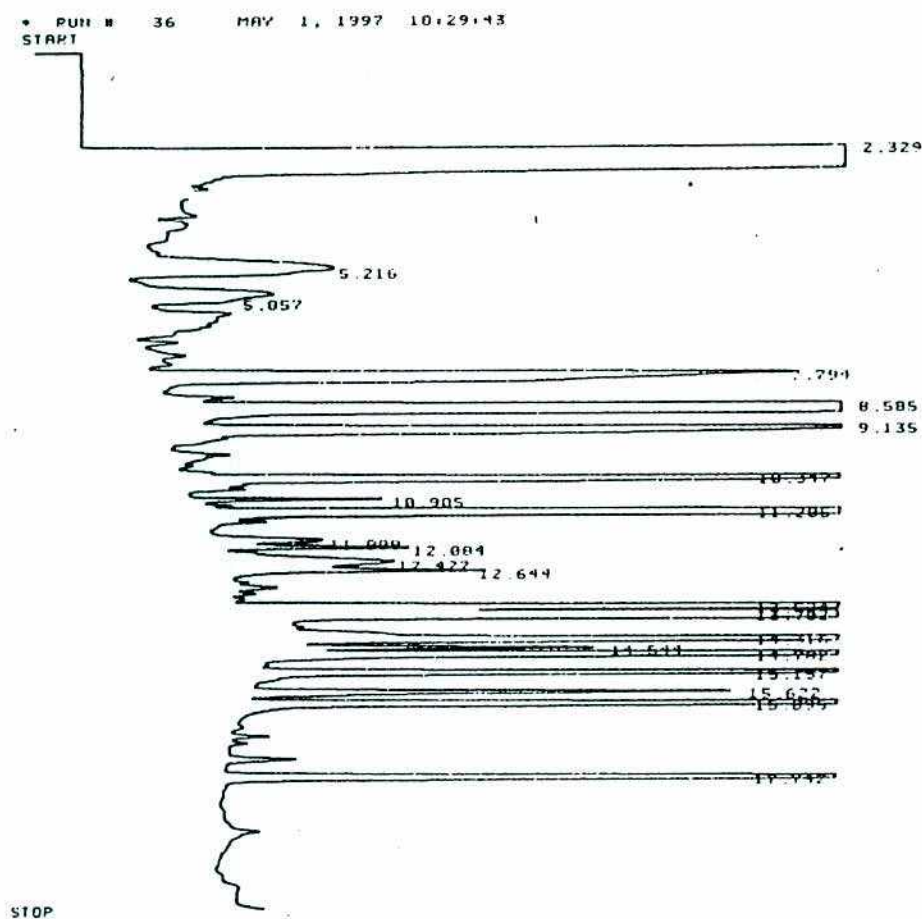
Table 37 Organochlorine residues in water samples collected from different locations (in ng/ml)

Sample No.	Locations	p,p'-DDT	p,p'-DDE	Aldrin	Dieldrin	Endrin	Lindane	Heptachlor	p,p'-DDD	HCH
1	Sonatala	0.33	0.10	ND	ND	ND	ND	1.20	1.14	ND
2	Sariakandi	ND	2.2	ND	0.08	ND	ND	ND	ND	ND
3	Gabtali	ND	ND	0.03	ND	ND	ND	ND	ND	ND
4	Sibganj	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	Bogra Sadar	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	Sherpur	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	Kahalu	11.93	0.03	ND	0.15	ND	0.10	ND	1.20	ND
8	Adandighi	1.19	0.06	0.05	1.25	ND	ND	ND	ND	ND
9	Dhunat	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	Sonatala (during flood)	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	Sonatala (after flood)	3.12	1.75	0.02	ND	ND	ND	ND	2.30	ND

ND - Not Detected

425599

ঢাকা
বিশ্ববিদ্যালয়
গ্রন্থাগার



Pesticides	Retention time
DDT	17.742
DDE	14.544
Dieldrin	14.702
Aldrin	12.084
Heptachlor	11.205
Lindane	9.135
BHC	10.347
Endrin	15.895
TDE	15.157
DDD	15.622
Endosulfan	13.702

Fig. 50. Standard chromatogram for known organochlorine pesticides (DDT, DDE, Dieldrin, Aldrin, Heptachlor, Lindane, BHC, Endrin, TDE, DDD, Endosulfan)

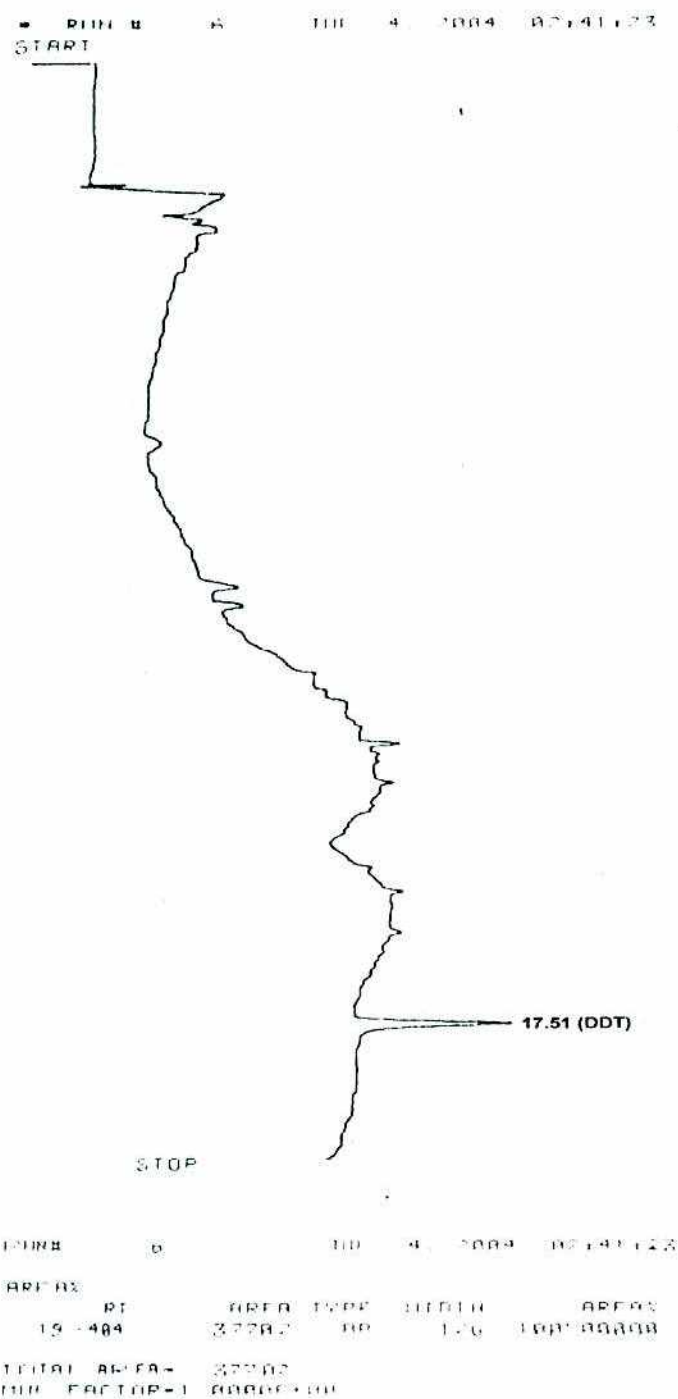
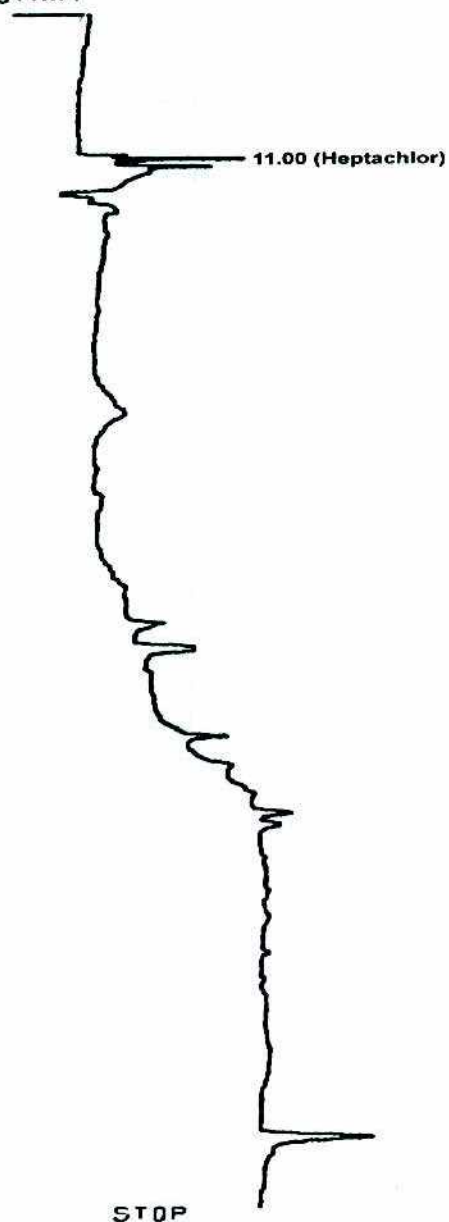


Fig. 51. Organochlorine test: chromatogram for soil sample (Kahalu thana)

* RIIN # 15 JIII 4, 2004 07:21:12
START



STOP

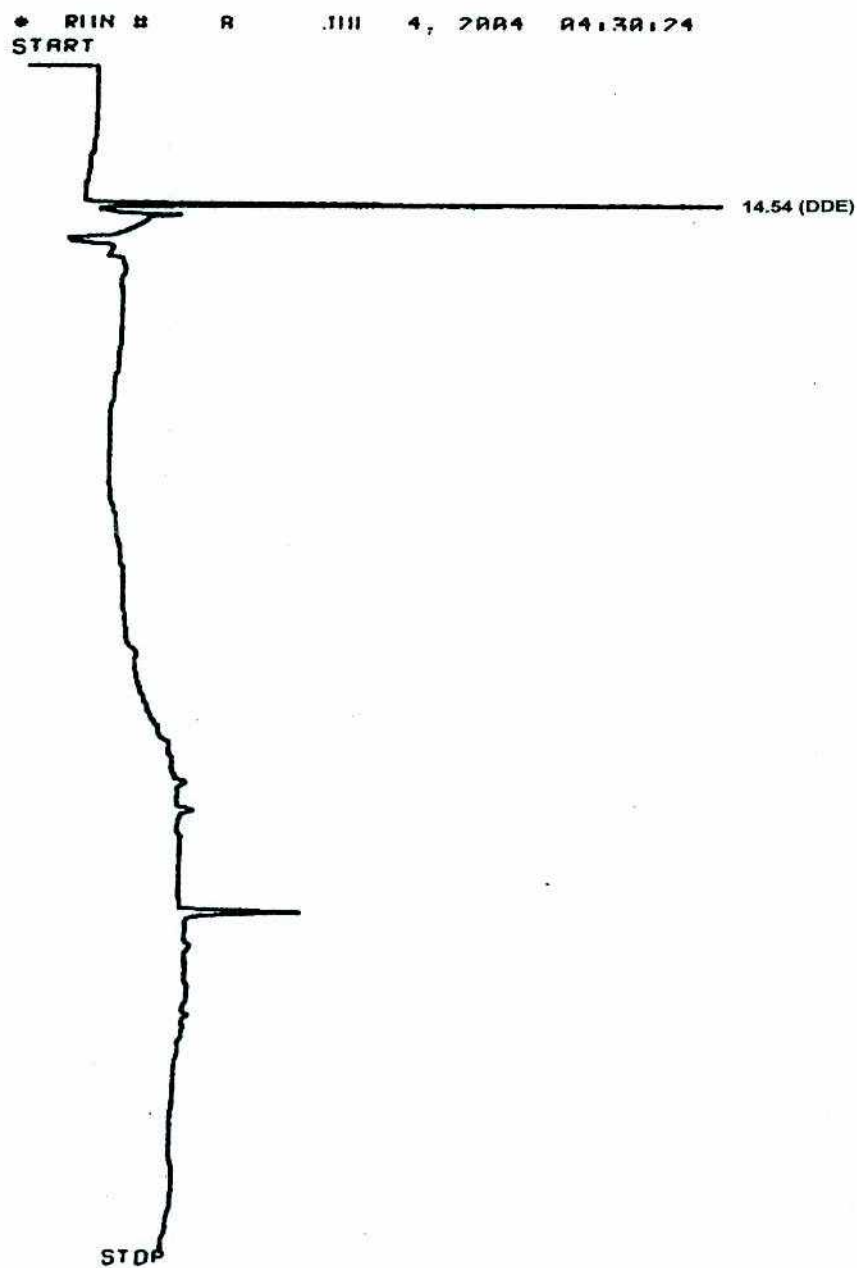
RIIN# 15 JIII 4, 2004 07:21:12

AREA%

RT	AREA	TYPE	LITATH	AREA%
2'5 15	A902	PP	039	100.00000

TOTAL AREA= 8902
MULTI FACTOR=1 000000.00

Fig. 52. Organochlorine test: chromatogram for water sample (Sonatala thana)



RIIN# R .III 4, 2004 04:30:24

AREA#

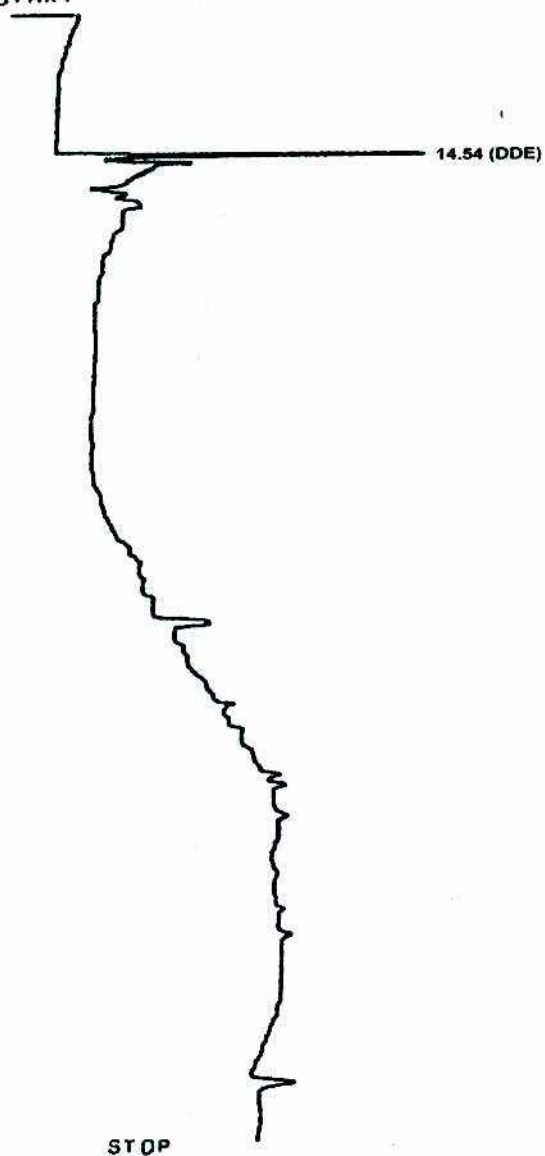
RT	AREA	TYPE	WIDTH	AREA%
2.447	37174	PR	0.33	100.00000

TOTAL AREA= 37174

III FACTOR=1 00000+00

Fig. 53. Organochlorine test: chromatogram for water sample (Sonatala thana, after flood)

* RUN # 11 .III 4, 2004 05:31:04
START



RUN# 11 .III 4, 2004 05:31:04

AREA#

RT	AREA	TYPE	WIDTH	AREA%
2.519	30726	PU	045	100.00000

TOTAL AREA= 30726
MULTI FACTOR=1 000000.00

Fig. 54. Organochlorine test: chromatogram for water sample (Sariakandi thana)

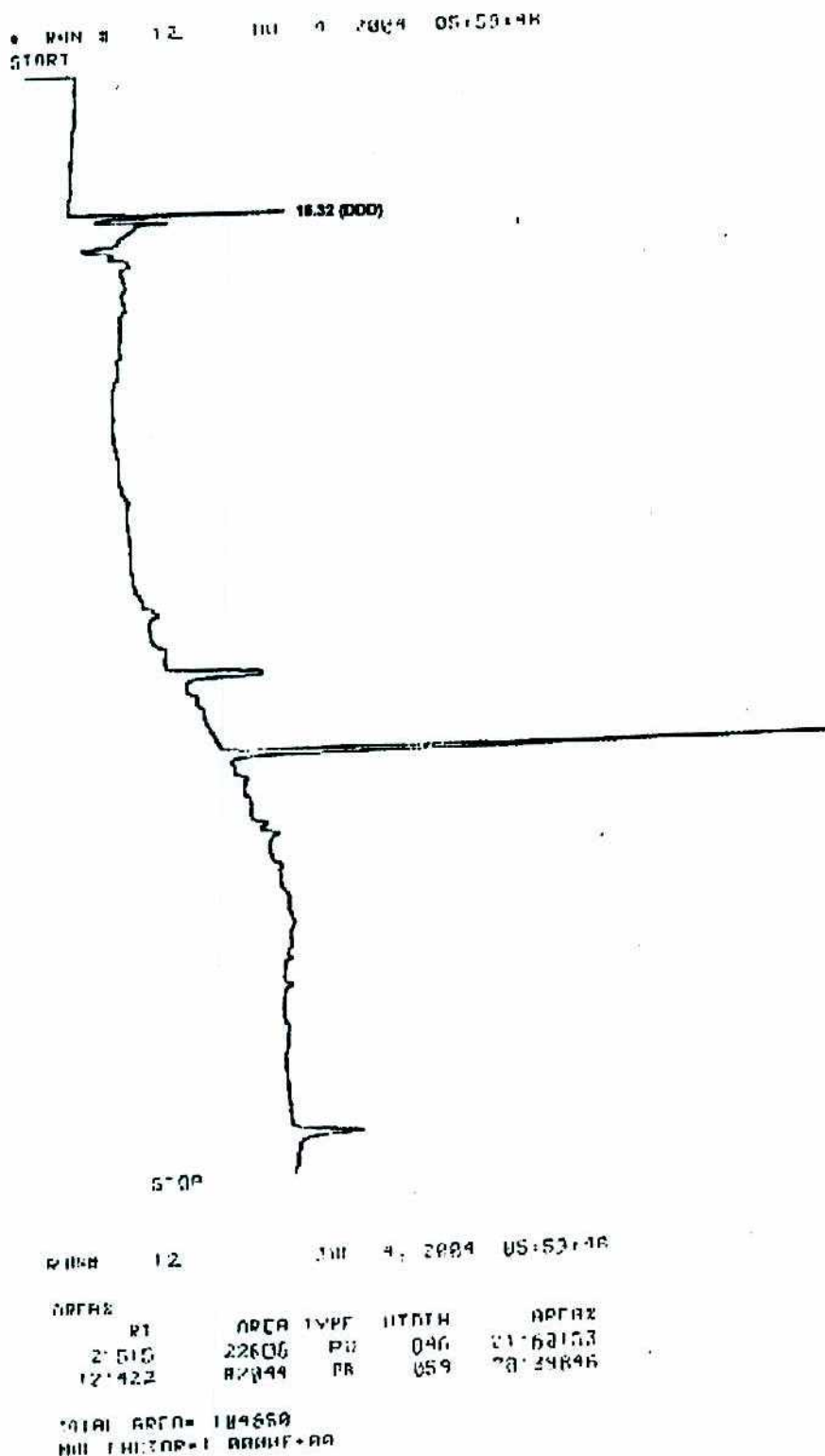
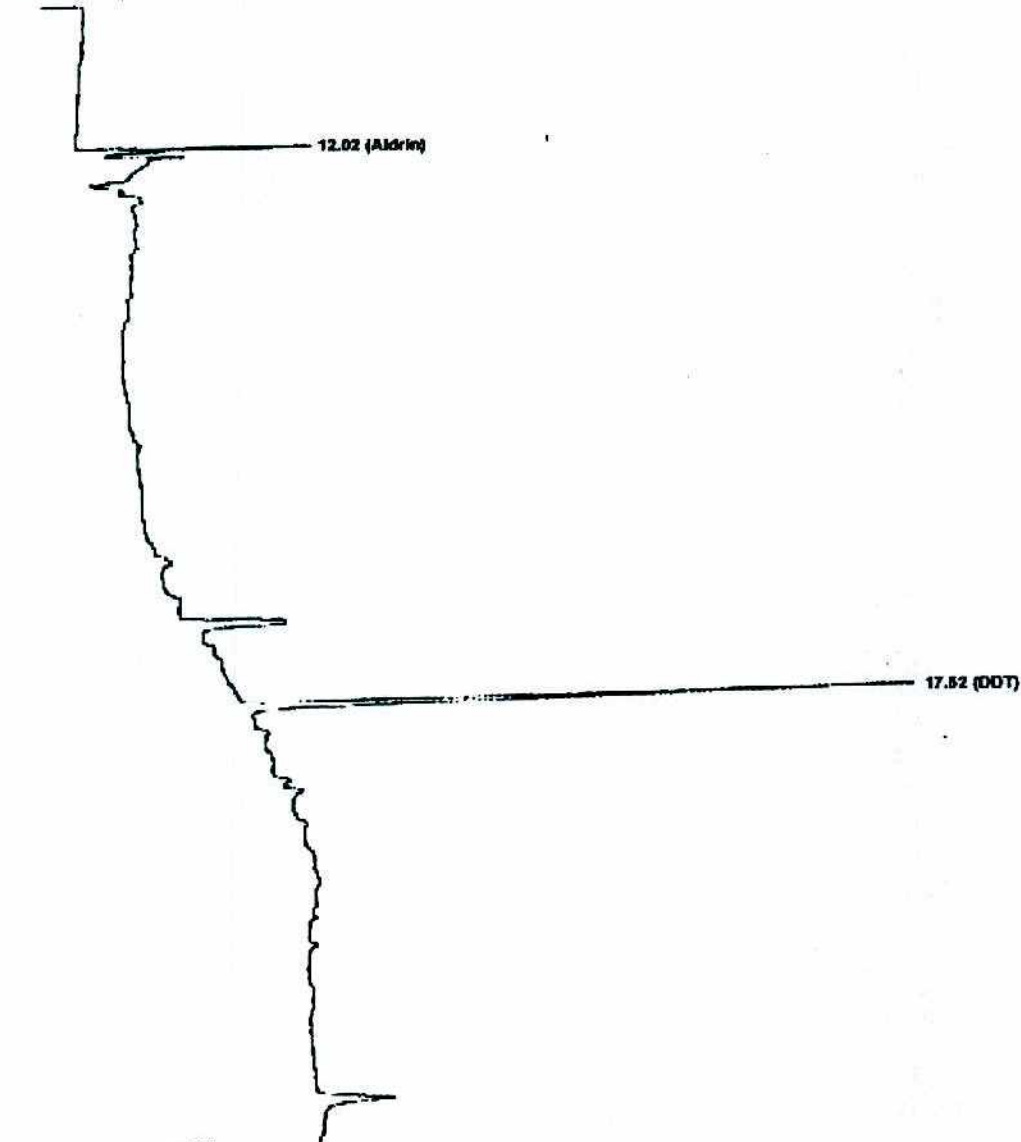


Fig. 55. Organochlorine test: chromatogram for water sample (Kahalu thana)

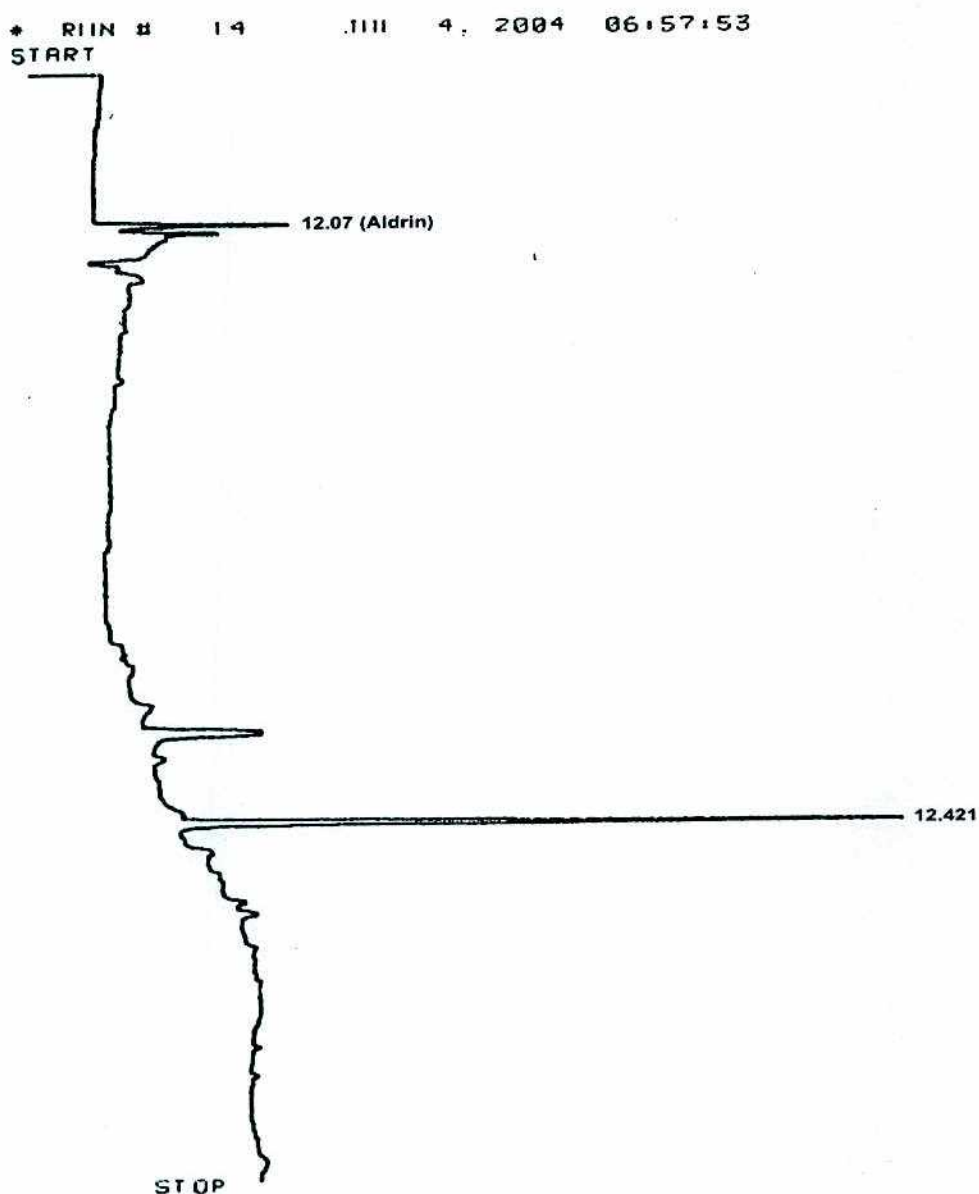
[illegible]

2025 12 10 4:27:24 05:53:46

DATE	DESCRIPTION	AMOUNT	BALANCE
12-1-82	DEPOSIT	100.00	100.00
12-15-82	WITHDRAWAL	50.00	50.00
12-31-82	DEPOSIT	75.00	125.00

MAIL ROOM - 10-659
ALL THE COR-1 AUGUST 1971

Fig. 56. Organochlorine test: chromatogram for water sample (Adamdighi thana)



RIIN # 14 .III 4, 2004 06:57:53

RT	AREA	TYPE	WTOTH	AREA%
2.516	15076	PU	049	17.61892
12.421	70530	PA	059	82.38906

TOTAL AREA= 85606
MII FACTOR=1 0000E+00

Fig. 57. Organochlorine test: chromatogram for stomach sample (Gabtali thana)

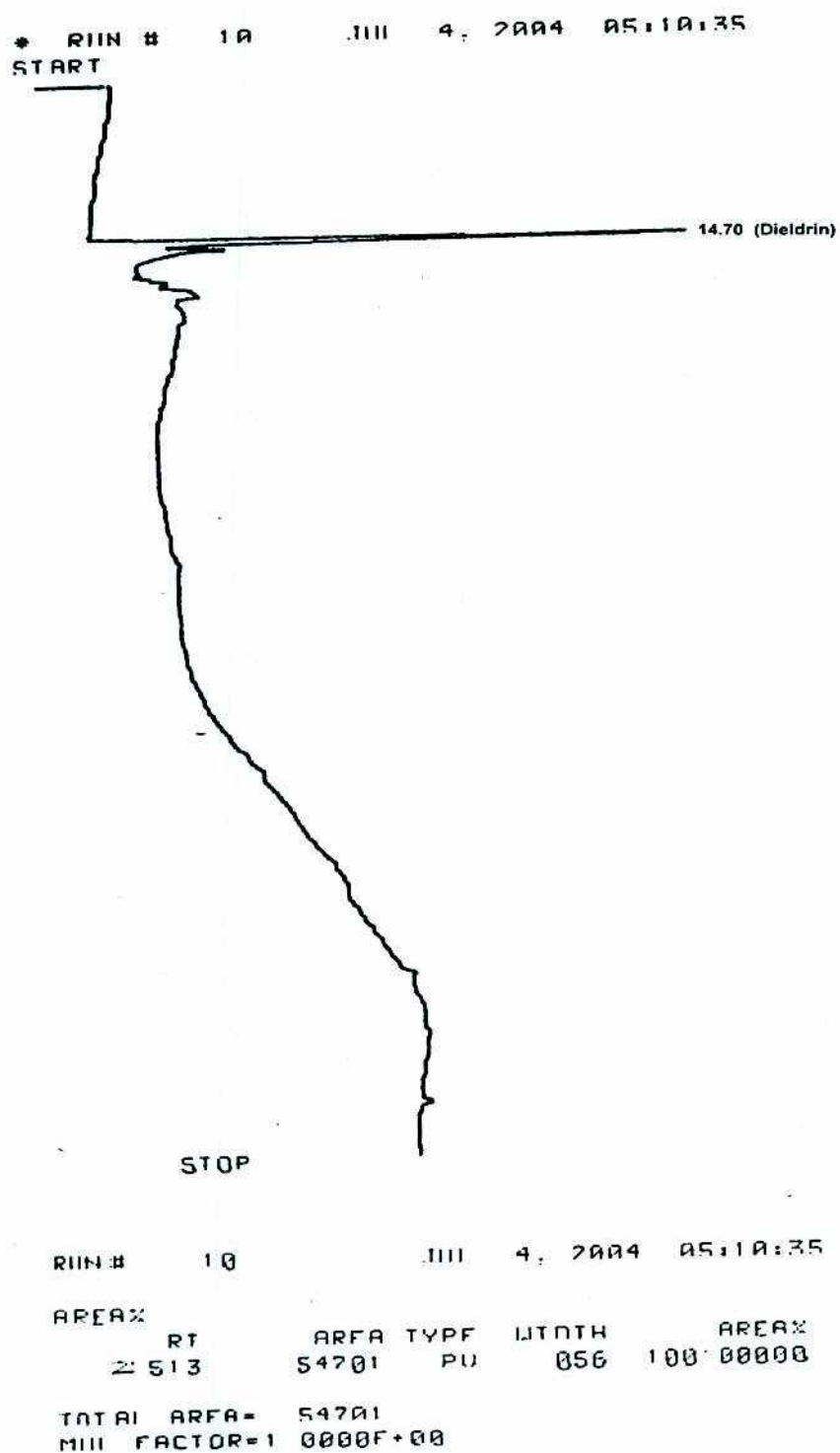


Fig. 58. Organochlorine test: chromatogram for gill sample (Sonatala thana)

4.8 Soil samples

4.8.1 Soil quality

Soil samples of Sonatala, Gabtali, Sibganj, Bogra Sadar and Sherpur thana were silty clay type and pH range were 6.7 to 7.4. Soil of Kahalu and Adamdighi thana were clay silty type. Rest of thana like Sariakandi and Dhunot were sandy silty clay and sandy loam. Lowest pH value was 6.8 of Dhunot and Sherpur thana. Beside these, other components such as organic carbon, organic matter and other organic matters are shown in Table 15.

Table 15. Physico-chemical characteristics of soil and water samples

Sample No.	Name of the thana	Moisture (%)	pH	Organic Carbon (%)	Organic Matter (%)	Other Organic Matter (%)	Water pH
1	Sonatala	5.2	7.2	2.84	4.67	90.24	7.20
2	Sariakandi	4.6	7.5	2.90	1.90	91.10	7.30
3	Gabtali	4.5	7.4	1.36	2.24	94.25	7.40
4	Sibganj	5.5	7.3	0.97	1.64	97.30	6.70
5	Bogra Sadar	5.3	6.7	1.26	1.25	95.82	6.70
6	Sherpur	5.8	6.8	1.35	2.23	96.78	7.43
7	Kahalu	6.5	7.4	1.78	4.80	95.73	6.80
8	Adamdighi	6.3	7.2	2.15	2.36	96.23	7.10
9	Dhunot	5.6	6.8	2.00	2.15	95.00	6.70
10	Sonatala (during flood)	---	---	---	---	---	---
11	Sonatala (after flood)	---	---	---	---	---	---

Table 16. Textural composition of soil (in present)

Sample No.	Name of the thana	Sand (%)	Silt (%)	Clay (%)	Textural Class
1	Sonatala	15	43	47	Silty Clay
2	Sariakandi	64	45	35	Sandy Silty Clay
3	Gabtali	14	47	36	Silty Clay
4	Sibganj	15	45	30	Silty Clay
5	Bogra Sadar	16	48	32	Silty Clay
6	Sherpur	19	46	30	Silty Clay
7	Kahalu	24	30	49	Clay Silty
8	Adamdighi	18	28	48	Clay Silty
9	Dhunot	45	35	10	Sandy Loam
10	Sonatala (during flood)	---	---	---	---
11	Sonatala (after flood)	---	---	---	---

The results of the concentration of organochlorine residues in soil samples of 11 samples are given in Table 17 which shows that six organochlorine residues are found in soil samples from four (4) locations they were p,p'- DDT, p,p'- DDD, p,p'- DDE, aldrin, dieldrin and hexachlor. After flood soil sample of Sonatala thana contained aldrin and dieldrin having the concentration 0.21 and 1.34 ng/g

respectively where as during the flood soil samples of same location contained no organochlorine pesticide.

p,p'- DDE was found in 3 samples ranged from 1.25 ng/g to 3.10 ng/g and p,p'- DDT and p,p'- DDD was found in the range of 5.4 ng/g and 3.23 ng/g respectively.

A number of organophosphorus pesticides were analyzed but except temephos no other organophosphorus and carbamate pesticides were detected in soil sample collected from different water bodies of Bogra district.

Table 17 Organochlorine residues in soil sample collected from different locations (in ng/g)

Sample No.	p,p'-DDT	p,p'-DDD	p,p'-DDE	Aldrin	Dieldrin	Endrin	Lindane	Heptachlor	Hexachlor cyclohexane
1	ND	ND	1.25	ND	ND	ND	ND	ND	ND
2	ND	ND	2.15	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	0.23	1.56	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	5.4	3.23	3.10	1.32	ND	ND	ND	ND	1.42
8	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	ND	ND	ND	0.21	1.34	ND	ND	ND	ND

ND - Not Detected

4.9 Mussel samples

Concentrations of organochlorine pesticide residues in mussel samples are given in Table 18. Five organochlorine pesticides were detected in mussel samples of which p,p'- DDD is categorized as relatively non hazardous, the rest are categorized as moderately hazardous (Table 19) (Corbett 1974).

Results showed that p,p'- DDT, p,p'- DDD, p,p'- DDE, aldrin, dieldrin and heptachlor were detected in mussel samples. Endrin, lindane and HCH were not found in any of the samples. p,p'- DDD was found in the mussel samples from 2 locations, namely Sonatala (sample no 1) and Kahalu (sample no 7) ranged from 1.44 ng/ml and 2.15 ng/ml. p,p'- DDE residues in 11 samples ranged from 1.14 ng/ml to 2.56 ng/ml. Aldrin in three samples ranged from 0.23 ng/ml to 1.20 ng/ml.

p,p'- DDT was only found in the mussel from Kahalu thana (sample no 7) and concentrations is 1.30 ng/ml which is the maximum concentration found in mussel sample (Fig. 52). Mussel from Sonatala thana (sample 1) contained no p,p'- DDT but p,p'- DDD and p,p'- DDE at the concentration of 1.44 and 2.20 ng/ml in stomach respectively. Mussel from Gabtali thana (sample no 3)

contained maximum p,p'- DDE 1.87 ng/ml and the gill of the mussel from Kahalu thana (sample no 7) contained the minimum concentration, 1.14 ng/ml. Alam (1996) found only DDE in the fish (*Macrocephalus punctatus*) as 2.10 ng/ml. Georgakopoulos – Gregoriadas *et al.* (1991) found p,p'- DDT, p,p'- DDD, p,p'- DDE as 1.9 ng/ml respectively in red Mullet from Greek water.

The maximum concentration of p,p'- DDT in Sonatala thana after flood is probably due to settling down of all types of pollutants from adjacent agricultural field samples 4 and 5 contained no organochlorine residues because these area are fully close water body. Absence of river and canal, and other water inlet system hence no chance of pesticide contamination.

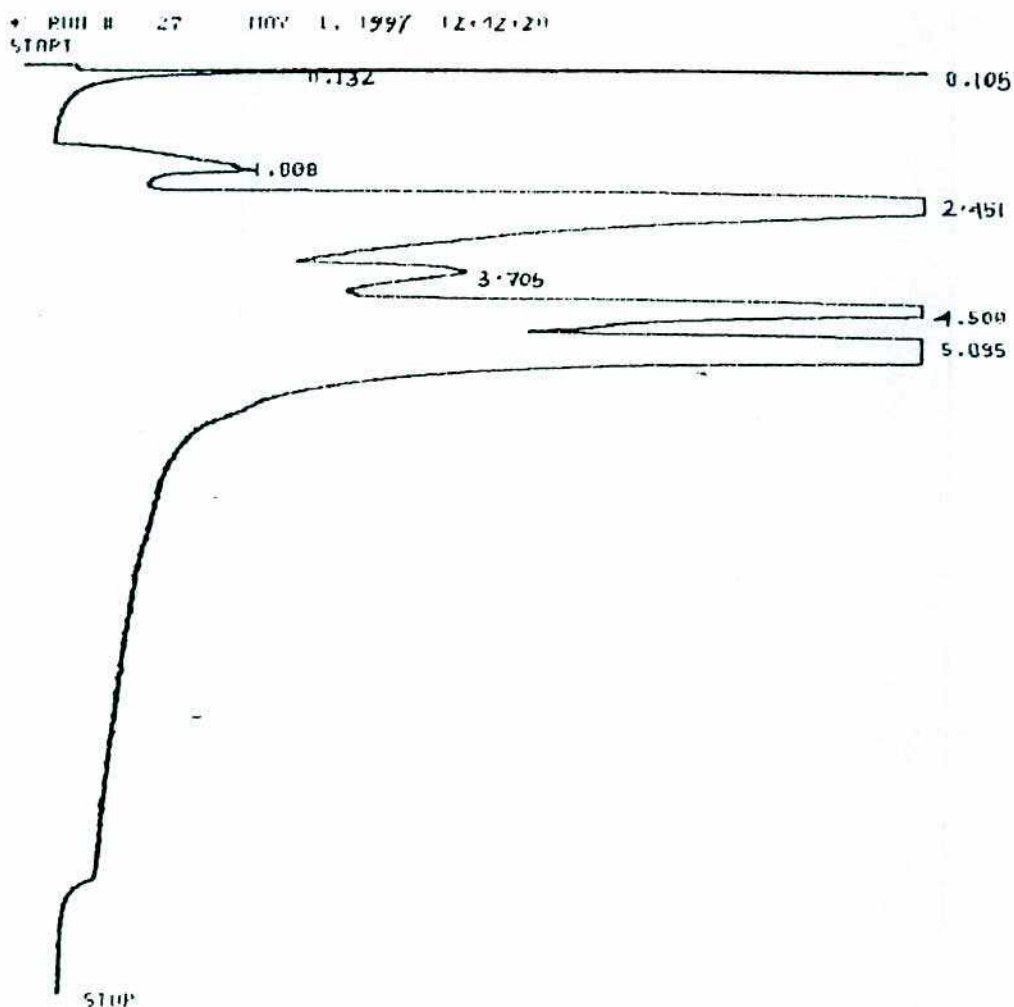
DDT is highly persisted compound by heating or cooking it becomes as DDD and by enzymatic action p,p'- DDT becomes p,p'- DDE. So, the containing DDT is not safe for consumption.

The result of the organophosphorus residue study given in Appendix Table XIII shows that no organophosphorus and carbamate pesticide was found in mussel samples.

Table 18 Organochlorine residues in different organ of mussel samples collected from different location (ng/g)

Sample No.	Sample Specimen	p,p'-DDT	p,p'-DDD	p,p'-DDE	Aldrin	Dieldrin	Endrin	Lindane	Heptachlor	Hexachlor cyclohexane
1	Stomach	ND	1.44	2.20	1.20	ND	ND	ND	ND	ND
	Gill	ND	ND	1.28	ND	2.52	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	Stomach	ND	ND	1.26	ND	ND	ND	ND	ND	ND
	Gill	ND	ND	2.14	ND	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	Stomach	ND	ND	ND	0.23	1.96	ND	ND	ND	ND
	Gill	ND	ND	1.87	ND	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	Stomach	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Gill	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	Stomach	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Gill	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	Stomach	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Gill	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	Stomach	ND	2.15	ND	ND	ND	ND	ND	ND	ND
	Gill	1.30	ND	1.14	ND	2.56	ND	ND	1.74	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	Stomach	ND	ND	2.56	ND	3.36	ND	ND	1.50	ND
	Gill	ND	ND	1.23	ND	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	Stomach	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Gill	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	Stomach	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Gill	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	Stomach	ND	ND	1.15	ND	ND	ND	ND	ND	ND
	Gill	ND	ND	1.50	0.25	ND	ND	ND	ND	ND
	Mantle	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Foot	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND - Not Detected



Pesticides	Retention time
Diazinon	0.105
Malathion	1.808
Fenitrothion	4.5
Chlorpyrifos	5.095
Carbaryl	2.451
Carbofuran	3.705
Cypermethrin	0.132

Fig. 59. Standard chromatogram for known organophosphorous pesticides (Diazinon, Malathion, Fenitrothion, Chlorpyrifos, Cypermethrin) and Carbamate pesticides (Carbaryl, Carbofuran)

HANNEL A INJECT 13-05-84 23:11:01 STORED TO BIN # 1



DATA SAVED TO BIN # 1

Fig. 60. Organophosphorous and carbamate test: chromatogram for stomach sample (Sonatala thana)

HANNEL A INJECT 13-05-84 23:19:00 STORED TO BIN # 3



DATA SAVED TO BIN # 3

Fig. 61. Organophosphorous and carbamate test: chromatogram for gill sample (Kahalu thana)

NNEL A INJECT 13-05-84 23:27:08 STORED TO BIN # 2



A SAVED TO BIN # 2

Fig. 62. Organophosphorous and carbamate test: chromatogram for mantle sample (Sariakandi thana)

NNEL A INJECT 13-05-84 23:35:15 STORED TO BIN # 4



SAVED TO BIN # 4

Fig. 63. Organophosphorous and carbamate test: chromatogram for foot sample (Adamdighi thana)

CHANNEL A INJECT 13-05-84 23:42:34 STORED TO BIN # 6



DATA SAVED TO BIN # 6

Fig. 64. Organophosphorous and carbamate test: chromatogram for soil sample (Kahalu thana)

CHANNEL A INJECT 13-05-84 23:39:13 STORED TO BIN # 5



DATA SAVED TO BIN # 5

Fig. 65. Organophosphorous and carbamate test: chromatogram for water sample (Sonatala thana)

Table 19. Characteristics of common insecticides detected in water, soil and mussel samples

Common insecticides	Hazard category	Type	Class	Water solubilities	Vapor pressure mg/hg at 20°C
p,p'- D DT	2	Insecticide	Organochlorine	Soluble	2×10^{-7}
p,p'- DDD	3	Insecticide	Organochlorine	Soluble	---
p,p'- DDE	2	Insecticide	Organochlorine	Soluble	---
Aldrin	2	Insecticide	Organochlorine	Insoluble	---
Dieldrin	2	Insecticide	Organochlorine	Insoluble	1.8×10^{-7}
Endrin	1	Insecticide	Organochlorine	Soluble	----
Lindane	2	Insecticide	Organochlorine	Soluble	9×10^{-6}
Heptachlor	2	Insecticide	Organochlorine	Soluble	3×10^{-4}
HCH	2	Insecticide	Organochlorine	Insoluble	3×10^{-5}
Temephos	1	Insecticide	Organophosphorus	Soluble	---

1 = Highly toxic; 2 = Moderately toxic; 3 = Relatively non toxic (Based on LD₅₀ / LC₅₀ values)

Table 20. Standard of Temephos

Volume of standard solution per injection (μl)	Quantity of Temephos (μg)	Peak area
5	5.40	90557
10	10.80	223126
15	16.20	346295
20	21.60	453891

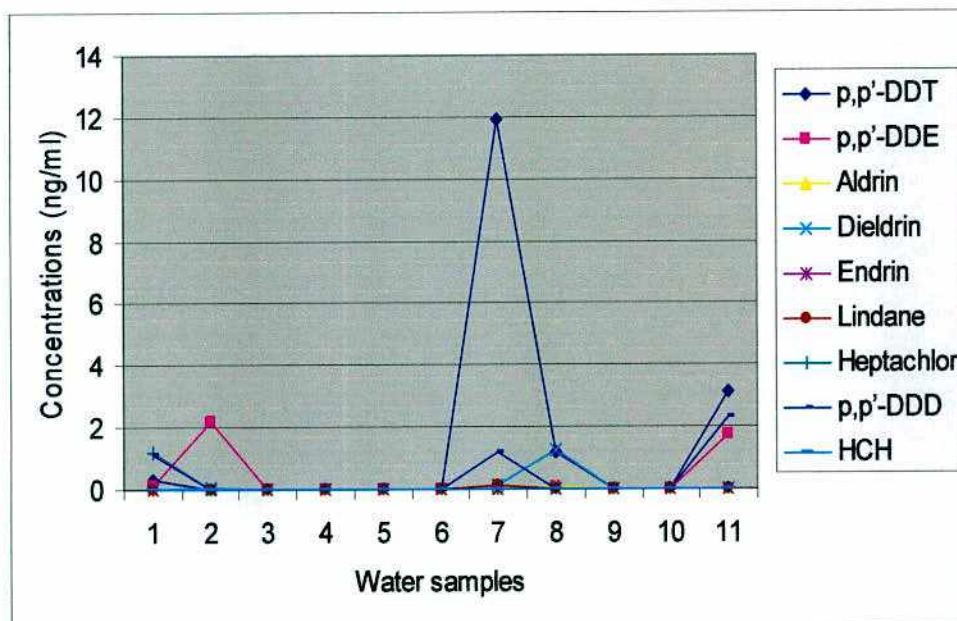


Fig. 66. Organochlorine residues in water samples collected from different locations (in ng/ml)

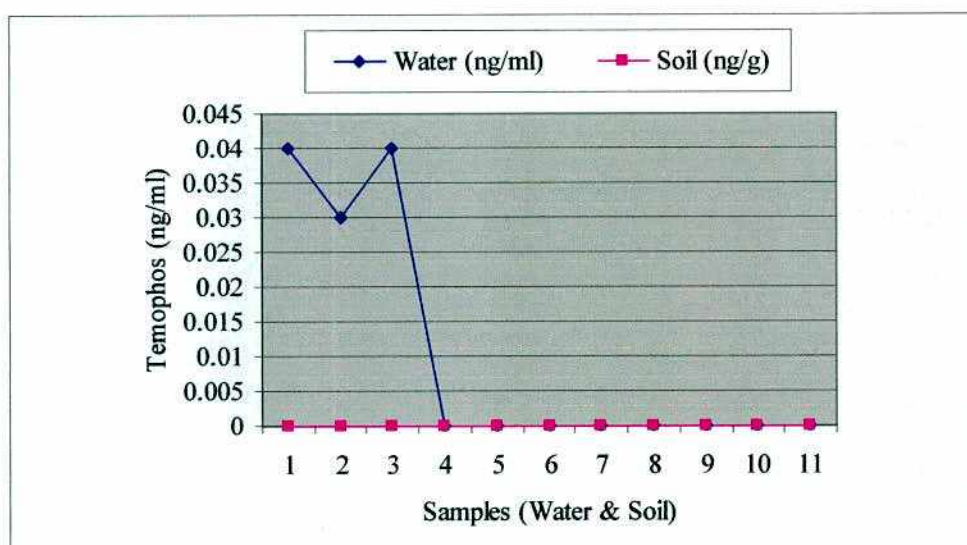


Fig. 67. Organophosphorus (temophos) residues in water and soil samples from different locations

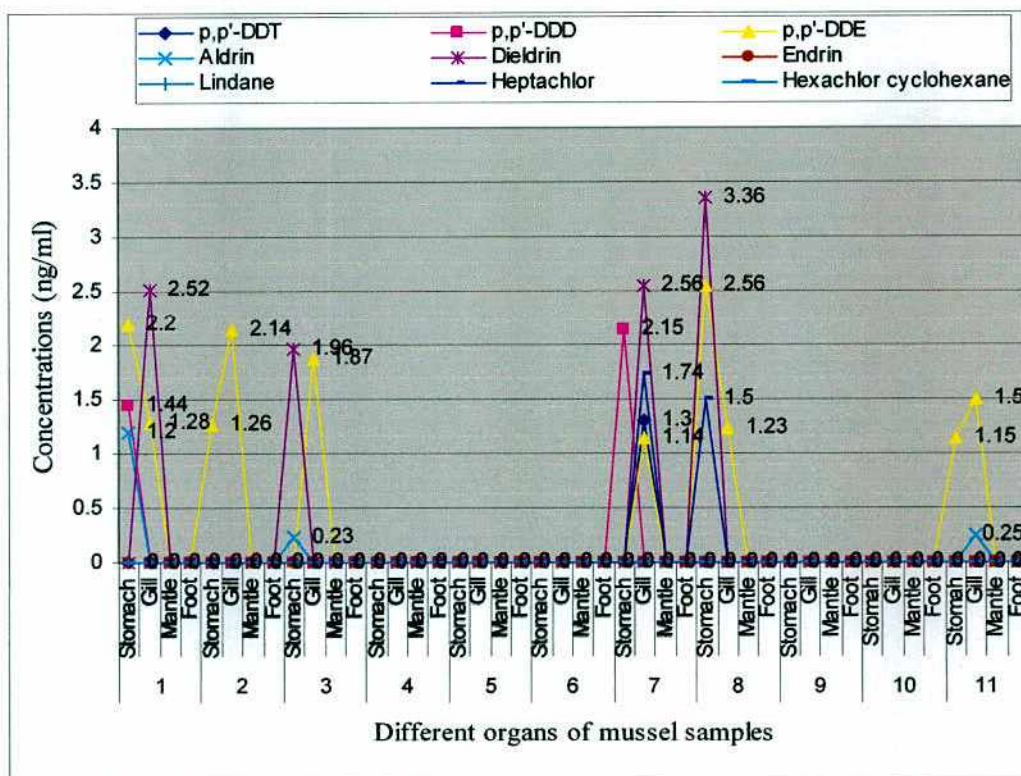


Fig. 68. Organochlorine residues in different organs of mussel samples collected from different location (ng/g)

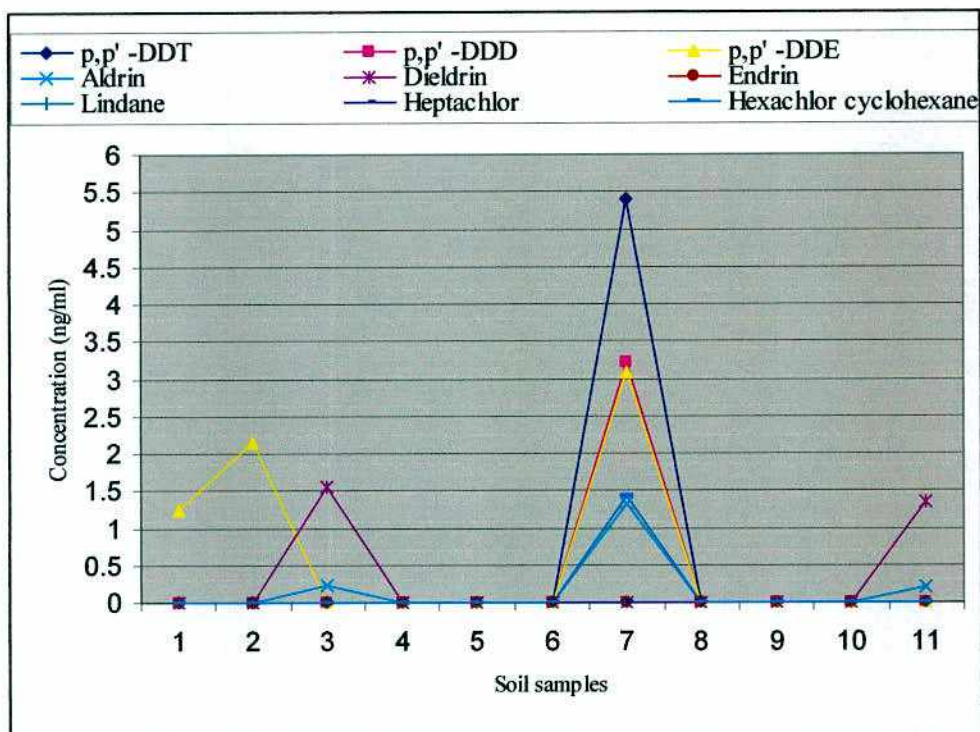


Fig. 69. Organochlorine residues in soil sample collected from different locations (in ng/g)

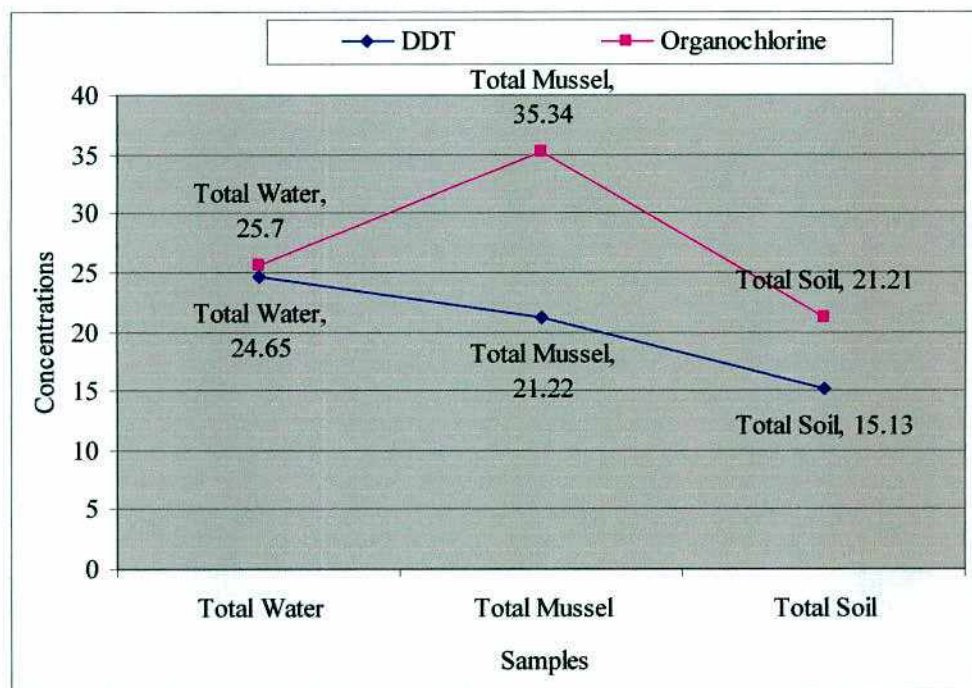


Fig. 70. Amount of total DDT and OCs in mussel, water and soil (ng/g)

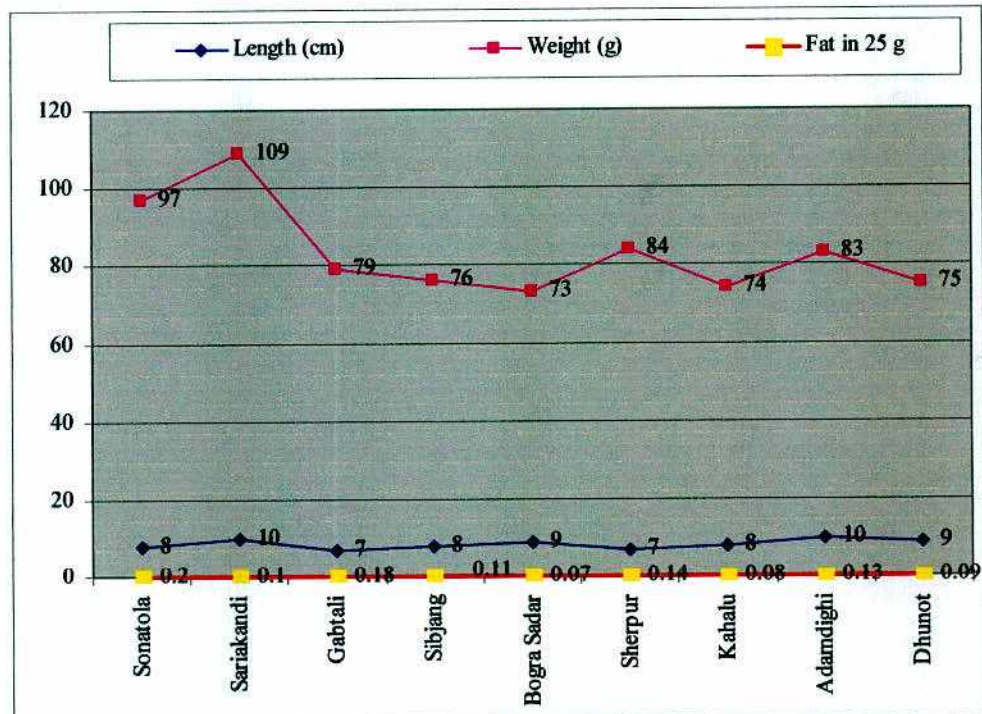


Fig. 71. Length, weight and fat content of mussel of different locations

CHAPTER FIVE

DISCUSSION

5.1 Water quality

The range of water quality values measured for different parameters were within the normal range of water quality suitable for mussel (Alabaster and Lloyd, 1982 APHA, 1980). The data on water quality parameter showed that there were no or little fluctuations between the different treatment glass tanks. The experimental trial glass tanks treated with carbosulfan showed fluctuations of the data. Therefore, the results obtained through the present experiment are attributable to the toxicant.

It is evident from the results of the experimental trial with carbosulfan that pH, alkalinity and ammonia levels in the exposure media varied greatly. This is in agreement with Rahman *et al.*, (1993). According to the author, pH and alkalinity level decreased when ponds, beels and rivers are toxicated with various types of toxicant. The reduction in pH could be the consequence of release of NH_3 from fertilizer or any other toxicant (Rahman *et al.*, 1993). The changes in alkalinity could be accompanied by the changes in pH. The higher amount of CO_2 measured in tanks having higher dose of carbosulfan is probably due to the breakdown of toxicant into ammonia and carbon dioxide (Metlev, *et al.*, 1983). However, the changes in the above parameters were dependent on the concentrations of the media. This was unavoidable within the framework of the experimental trials. It seems that the changes at this extent probably exerted little influence on the toxic action.

The present investigation was carried out in a static test with daily media renewal. Static test technique is most preferable with non-volatile toxicant (APHA, 1980; Alabaster and Lloyd, 1982; Hasan and Macintosh, 1986). Therefore, investigation with both carbosulfan and malathion should not be a problem since both are non volatile. Since carbosulfan and malathion are biodegradable carbosulfan and organophosphorus compound, so it may be lost from the system with time and with daily change of exposure media probably minimized that loss. As for the best result, the density of mussel in the test media was kept low, so that, high metabolic pollutants probably did not build up as it was evident from water quality measurements. The present experimental trial of acute lethal toxicity was carried out for a period of 96h. However, there is a tendency to continue the test beyond 96h (Hasan and Macintosh, 1986; Alam *et al.*, 1990).

According to APHA (1980), 96h test are usually preferable for static test technique. Sprague (1969) reported in his review on pollutant toxicity studies, only in 42 cases, lethal threshold level reached after 96h. He recommended that a 96h LC_{50} is, therefore, a useful substitute and is often equivalent to the incipient LC_{50} . He, however, suggested that test should be continued until the shape of the toxicity curve is clearly established. In the present study since the toxicity curve

became asymptotic by 96h of exposure logically the test was not continued beyond that period.

5.2 Toxicity

The study type defines the toxicity endpoint used for a particular study. For the U.S. National Toxicology Program (NTP) data, the only endpoint evaluated is mortality. The toxicity endpoints used in the U.S. NTP data are LD₅₀, LC₅₀, LDLo and LCLo. (CHSUS, 2002.)

The LD₅₀ is the dose of the insecticide in milligram (mg), microgram (μg) or nanogram (ng) per kilogram (kg) of body weight that is lethal to 50% of the test organisms. This designation is used for routes of exposure where a known dose is administered (oral, dermal, intravenous, etc). Unit used are ppm (mg/kg), ppb (μg/Kg), and ppt (ng/kg) (USEPA, 2002.)

The LC₅₀ is only used for inhalation studies in the NTP data and is defined as the concentration of insecticide in air that is lethal to 50% of the test organisms within the stated study time. According to NTP, the LC₅₀ is given in milligrams of insecticide per liter of air (mg/l), milligrams of insecticide per cubic meter (mg/m³) and grams of insecticide per cubic meter (g/ m³). Other units used are parts per million (ppm) or parts per hundred (pph). In the present study, we used ppm unit. This is conversion from μl/l (0.52 ppm is equivalent to 525 μl of carbosulfan and malathion insecticide).

The exposure route is the route by which the test animals were exposed to the chemical. The most common exposure routes are oral (by mouth), dermal (applied to the skin), inhalation (by breathing the chemical). Since three routes are the standard ones used for rating toxicity. In the present study, the common exposure routes are oral (by mouth/siphon).

The acute toxicity of a particular chemical to organism groups (amphibians, fishes, molluscs, zooplankton, etc). Narrative descriptions of acute toxicity were assigned based on LC₅₀, according to the guidelines in Kamrin, 1997.

Since any single summery rating will inherently not be representative of the extremes of the sensitivity of organisms to chemical exposure, we also provided the toxicity range for species within the organism group, as well as the raw data for the individual studies.

The analyst can evaluate the studies and make his or her own decision about toxicity to particular species. According to above instruction in the present study we applied various categories of doses on *Lamellidens marginalis* such as very highly toxic, highly toxic, moderately toxic, slightly toxic and not acutely toxic.

The different types of observed effects of a particular insecticide on aquatic organisms. Effects include mortality, behavioral, bioaccumulation, biochemical, developmental, enzymatic, feeding behavior, genetic, growth, histological,

intoxication, morphological, mobility, physiological, population and reproductive (USEPA, 2002). In the present study we observed similar observation.

The average LC_{50} value calculated for each chemical/species combination by adding the LC_{50} values for each study and dividing by the number of studies. The LC_{50} is the concentration of toxicant that is lethal to 50% of the test organisms. The average toxicity of a chemical to a particular species. Narrative descriptions of toxicity were assigned based on average species, according to the guidelines in Kamrin (1997). This technique used to determine these rating in our present study.

The age of the animal used in the toxicity study include: juvenile (juv), early life (erlylf), adult, gravid, sperms, embryo, larval, worker (for bees) or not reported (N.R.). Other terms used are self-explanatory. Age is also given by reporting the average mass of the test organism, (Rand 1995). In the present study, we used adult group of mussel *Lamellidens marginalis* in the toxicity rest.

In the present study, many behavioral abnormalities were recorded in response to carbosulfan and malathion intoxication, revealing anomalies that were in conformity with other workers. A behavioural change may, in fact be, the first response of an organism to environmental perturbation. The abnormal behavior of experimental mussels exposed to malathion, such as, to and fore movement, orientation, air gulping tendency, violent spasm, loss of equilibrium, mucus secretion, more or less support the studies of other worker who performed experiment with various toxicants (Andrews *et al.*, 1966; Roa *et al.*, 1967; Verma *et al.*, 1977).

According to Metelev *et al.* (1983) at a toxic concentration of carbosulfan mussel are quiescent, their mantle and gill highly covered with mucous, respiration became slow; death occurred slowly. Only in concentrated solution a brief stage of excitation is observed. The gills of dead mussel are polluted and the respiratory epithelium altered. This also supports the observation of the present study made with carbosulfan.

In the present study, hyperactivity was only observed at the early stage of exposure. Similar observation was also made by Rao, *et al.*, (1967) for malathion, methyl parathion and BHC treated *Elliptio buckleyi*. Metals (cadmium, Chromium and zinc) also produced a concentration dependent hyperactive locomotors responses in the blue gill (Ellgaard *et al.* 1978). However, Weiss and Weiss (1974) found that after 3 day of exposure to 1.0 ppb DDT, groups of mussel moved faster, turned more often and occupied a great concentration. Toxicant increased the air gulping and oxygen demand and is consistent with observation made by Kadama *et al.* (1955).

In the present study secretion of mucus following exposure to carbosulfan 20% was an important observation. According to Holden (1962) pesticide accumulates in the external mucous of river plata mussel and it probably entered into mussel

through mantle. Excess mucous secretion may be the adaptive effort to prevent entry of pesticides through mantle and gill (Holden, 1962; Verma *et al.* 1977).

Slow movement, loss of equilibrium and other symptoms observed in present study could be due to consequences of impairment in nerve function caused by the toxicant. According to Meteleev *et al.*, 1983 organophosphorus compound are poison with a neuromuscular and enzymatic action. The basis of their toxic action lies in their selective effect on cholinesterase which is a nerve tissues enzyme, leading to excessive accumulation of acetylcholine in the organism, giving rise to complex poisoning symptoms. In salmonoids, inhibition of this enzyme alters respiration (Klaverkamp *et al.*, 1977) and movement (Matton and Lattam, 1969).

In variance, Rand (1977a) also demonstrated that exposure to sub lethal concentration of parathion for 24h resulted in an overall decline in activity of river plata mussel. It is interesting to note that during the 24h parathion exposure, mussel remained quiescent on the bottom of the holding tank. A similar decline in activity also observed in blue gill and large mouth bass exposed to sub lethal concentration of parathion (Rand and Petrocelli, 1985).

The abnormal activities observed by carbosulfan and malathion exposure in the present study were also recorded by Begum, 1975; Ahmed, 1976 in *H. fossilis*, *Anabas testudineus* and *Tilapia mossambica* respectively. However those were observed in fish at the higher concentrations and at the later stage of exposure.

5.3 Length- weight variation in respect of carbosulfan insecticide and malathion insecticide

The present study has clearly demonstrated a negative effect of carbosulfan and malathion insecticide, mussels receiving same amount of both insecticides (14.85 ppm), were 13% lesser weight than the control mussels, in 126 days and about 17% lesser weight during in the 210 days period. A study with diazinon, an organophosphorus pesticide, Keller (1993) also recorded a concentration dependent loss in weight reaching a 21% loss at the highest concentration (16 ppm) used during a 95 day exposure period.

The observed depression in growth in mussel treated with carbosulfan and malathion insecticide possibly might be due to starvation or less food consumption caused by the disturbance in the normal digestion of the mussel caused by the insecticide. Abada (1983) and Boutbib and El Boudrari (1984) found that mussels of a certain size had lower growth rate due to their presence in polluted water. This might be due to the direct effect of pollutants on some physiological processes of the mussels (Bayne *et al.*, 1988) or to their indirect effect, changing the physico-chemical characteristics of the environment (Tendergren *et al.* 1988; Kautsky *et al.* 1990).

The present study has demonstrated the toxic effect of carbosulfan and malathion exposure on locomotor activity of *L. marginalis* and that are consistent with the visual observations made during the earlier experimental trials. The increase in

locomotory activity following chemical exposure was also observed by a number of authors with varieties of chemicals, including organophosphorus. Mane, and Muley (1989) reported that exposure to sub lethal concentrations of endosulfan 35 EC elicited hyperactive locomotors responses in *L. marginalis* and the degree of the responses was concentration dependent. Similarly Vonhof *et al.* (1999) and Kaandorp *et al.* (2001) found that after 3 days exposure to 1.00 ppb DDT bivalve moved faster and turned more often. Rand (1977a) demonstrated that exposure for 24h resulted in an over all decline in activity of gold fish. A similar decline in activity was also observed in bluegill and large mouth-bass exposed to sub lethal concentration of parathion (Rand, 1977b). These differences in observations may arise from the differences in time period of exposure. In the present study, the increased activity was observed after 2 hours of exposure and probably the activity declined with exposure time and was found significantly lower when measured on day 15 of exposure. Therefore, the present results should not contradict. Changes in orientation frequency and pattern also affected by carbosulfan and malathion insecticide.

The reason for initial increase in locomotors activity is not clearly understood. However, it seems that it might have resulted from stimulation of nervous system and or irritation of perceptive organs surface receptor. According to Gardner and Laroche (1973) that neurotoxic substances like organophosphate may causes irritation to the perceptive system including behavioral reactions, organophosphorus is also known to cause stimulation of the central nervous system and may cause excitation until stage of fatigue has reached and may be reflected in the activity of mussel. Another explanation could be that the carbosulfan and malathion may be noxious to the mussel which can be avoided actively.

The observed decrease in locomotors activity after prolonged exposure may be a consequence of impairment in the nerve action. Loss of equilibrium in mussel observed in this study (visually observed) is an indicative of neural toxicity (Mount *et al.*, 1962; Rao, 1989; Matton and Lattam 1969) observed marked inhibition in acetylcholineesterase, a nerve enzyme, by organophosphorus has also been reported by other workers (Holland *et al.*, 1967; Morgan *et al.*, 1973). Inhibition of this enzyme's activity causes paralysis to mussel and eventually may lead to its death (Matton and Lattam, 1969). The lethargic condition attained in mussel at the highest concentration may also be due to the exhaustion of the physiological and biological capabilities. However, more work is required to demonstrate this.

It is evident from the results of the present study that carbosulfan treatment increased oxygen consumption rate in mussels during acute exposure. In consistent with the present findings Moorthy *et al.*, (1984) also measured changes in respiration or oxygen uptake and ionic content in tissues of freshwater mussels exposed to methyl parathion toxicity, whereas no effects were obtained by Kugler – Laffont and Rouquier (1988) exposure in bivalve molluscs (*Anodonta cygnea*) to a organochlorine, endrin. The result with carbosulfan is varied. Pyrethroids

exposure at 0.1 and 5 mg/l increased oxygen consumption which agrees well with present results in a bivalve *L. marginalis* Shankara *et al.*, (1989). On the other hand, the author also recorded reduced oxygen uptake at intermediate concentration.

In the present study, although oxygen consumption rate increased during acute exposure but the rate decreased after 15 days of exposure, being greatly reduced at the highest concentration of carbosulfan insecticide. In agreement, Das and Jana (2003) observed decrease oxygen uptake in gills, liver and muscle tissue (hence whole body) for health assessment in the freshwater bivalve, (*L. marginalis*) after a 15 days exposure to 0.5 mg/l⁻¹ pyrethroids. Other than pesticide, while working with steel and metal factories effluent Das and Jana (2004) found a similar response, oxygen uptake increased in *L. marginalis* on initial exposure to 20% of the 96h LC₅₀ to the effluent, oxygen consumption decreased and tended to approach pre-exposure levels. The author further commented that the oxygen might eventually go below the normal level. Such a phenomenon probably has occurred in the present study with carbosulfan insecticide.

The initial elevation in oxygen consumption observed in the present study could be due to the increased activity of the mussel with malathion treatment. Routine metabolism (oxygen consumption) is proportional to mussel (*L. marginalis*) activity, during methyl parathion (MP) toxicity (Moorthy *et al.*, 1984); the results of the present study are thus explicable from the above point of view. Decrease oxygen consumption by the mussel after a 15 day exposure observed in the present study again could be the consequences of several factors. First the mussel probably became exhausted due to stress effect caused by toxicant (as assessed by visual observation and locomotion measurement). The mussel became lethargic, foot release from the body shell is partially open at the bottom of the aquarium for most of the time. This behavior of mussel signifies the hypoactivity resulting in the less oxygen consumption. Alternatively, this may also be attributed to decreased activity of oxidative enzymes. Effects *in vitro* of methyl parathion on some kinetic constants as succinic dehydrogenase (SDH) in hepatopancreas of freshwater mussel, *L. marginalis* were studied. Altered pH vs. specific activity curve for SDH demonstrated significant inhibition by methyl parathion in buffered acidic, neutral and alkaline ranges. At high pH ranges LC₅₀ (12.5 micron) methyl parathion did not cause 50% inhibition enzyme as it did at neutral and acidic pH. Activation energies were found to be increased suggesting decreased efficiency of enzyme in presence of methyl parathion. The kinetic data suggests that inhibition of SDH by methyl parathion was pH and temperature independent (Sreenivarsa Moorthy, *et al.*, 1984). Natarajan (1984) found hypo activity of LDH, SDH enzyme during a 30 day exposure period to metasystox. Similarly, *in vitro* exposure to endrin reduced activity of lactate dehydrogenase, succinate dehydrogenase and cytochrome oxydase. Similar phenomenon might have occurred in the present case. The reduce activity of the above oxidative enzyme usually means that less oxygen being used in the body and probably being reflected in the oxygen consumption by the mussel.

The mussel exposed to lower concentration (3.5 mg l^{-1} and 5.20 mg l^{-1}) of carbosulfan and malathion insecticide showed essentially the same trend in oxygen consumption data but changes in the response was little but significant. With further extended exposure the trend may change. The mussel either adapts to the altered environment by modifying its homeostatic mechanism or the situation may further deteriorate leading to death of mussel.

The mean survival time of mussel and oxygen tolerance (oxygen threshold) level were also affected by carbosulfan exposure. Several authors working with various chemicals have shown that under conditions as low dissolved oxygen mussel either dies earlier or lower levels of poisons are required to cause lethality of fish (Hick and Witt, 1971; Adelman and Smith, 1972; Cairus and Scheier, 1957).

In the present study, during acute exposure, the mussel exposed to carbosulfan died earlier than control mussel, although oxygen threshold values were almost similar for all treatment groups. This indicates that the mussel at the highest carbosulfan concentration consumed oxygen at a higher rate depleting oxygen level in the aquarium quickly and consequently the mussel died earlier. The observed oxygen consumption rate confirmed the view.

5.4 Lethal concentration (LC_{50}) and mortality

In the present study, the 96h LC_{50} values of carbosulfan for *L. marginalis* was estimated to be 13.56 ppm for Trial IIA and for Trial IA is 13.37 ppm. No such comparable works of carbosulfan toxicity on *L. marginalis* available in the literature. However, Dineshs *et al.* (1986) mentioned that with medium dose (300 l/ha) and high dose (600 l/ha) produced high concentration of ammonia (2.4-3.0 ppm) which killed all the mussels on 14 days of stocking.

According to Rahman (1992) it is very likely that the treated mussels were unable to carry out their normal functions. Carbosulfan pesticide increase acidity in a considerable amount, so after its application alkalinity and pH are reduced sometimes seriously, when high dose is applied.

The death of mussel following carbosulfan pesticide exposure has been mentioned by Metelev (1983). According to him, a saturated amide of carbonic acid is a toxic compound for mussel. However, depending on pH, after enzymatic hydrolysis carbonic acid, anhydride acid and ammonium ions release free form of ammonia. Ammonia, as is known, is quite toxic to mussel, it causes mortality of mussel during disintegration of carbosulfan pesticide. At a toxic concentration of carbosulfan to mussel are quiescent, their mantle and gill highly covered with mucus, and respiration slows down causing death to occur slowly. The gills of dead mussels are polluted and the respiratory epithelium altered. In hyper toxic solutions of carbosulfan mussels are severely dehydrated, the mechanism of toxic action is expressed it damages the gills.

Bulkley *et al.* (1976) have established that lowest vital concentration of carbosulfan in mussel 1.0 g/l. *Lamellidens marginalis*, *L. jenkinsianus* were used as experimental mussel. It was demonstrated that the eggs of *L. marginalis* have a high degree of resistance to carbosulfan. At concentration around 10 g/l the development of larvae after hatching did not differ from that of control; at higher concentrations a rapid onset of pigmentation was noted. *L. corrianus* egg could tolerate a concentration up to 10 g/l; a further increase in concentration leads to death. Larve after hatching died at 10 g/l carbosulfan concentration but remained alive at 0.5 g/l. Carbosulfan is toxic in concentration of 15g/l and above for *L. marginalis* larva. Juveline *L. marginalis* was more sensitive to carbosulfan than larvae. At a lower concentration of carbosulfan, oxygen consumption decreases and with an increase in concentration, the oxygen consumption will increase, according to Bulkley and his co-workers.

In the present study, the 96h LC₅₀ values of malathion for *L. marginalis* estimated to be 13.75 and 13.86 ppm for Trial IB and IIB respectively. The above values are more or less agreed with the report on *Lamellidens marginalis* given by Orme and Kegley (2004). They obtained similar LC₅₀ value of organophosphorus and endosulfan for mussel. Much lower value was reported by HITC (2002) for *Lamellidens marginalis* of mercury chloride, methyl parathion and phosphomidon. This variation could be due to the differences in species variation. Heavy metal also show lower values of LC₅₀ for *Lamellidens marginalis* Kamrin (1997) and Orme and Kegley (2004) found a higher LC₅₀ value (174 ppm), for *L. marginalis* this higher variation is due to different group of pesticide/chemical.

5.5 Concentration of pesticides residues in water samples

Seven organochlorine pesticides were found. Table 22 shows that only p,p'- DDD is categorized as relatively non hazardous and the rest are moderately hazardous (Corbett, 1974).

The permissible limit of p,p'- DDT and lindane in non-portable water recommended by FAO/WHO (1993) are 0.042 ng/ml and 0.56 ng/ml respectively. Concentrations of these two organochlorine residues found in the water samples of Kahalu, Adamdighi and Sonatala thana (after flood) are much higher than the suggested level. The persistent nature of organochlorines and their translocations from agricultural land and urban sewage can cause residues in food commodities.

Sources of DDT and other organochlorine compounds in the water bodies of Bogra district might be from its use to kill mosquito (*Anopheles sp*), plague vector (*Xenopsylla cheopis*), etc. DDT is also used in mosquito coils manufactured in our country (Ibrahim, 1992) detected p,p'- DDT, p,p'- DDE, p,p'- DDD and dieldrin as the concentration of 25 g/dm³, 2 g/dm³ and 38 g/dm³ respectively. Shaheen (1995) working on organochlorine pesticide in Bay of Bengal found the maximum concentration of p,p'- DDT as 1.85 ng/ml and maximum concentrations of lindane as 0.32 ng/ml. Alam (1996) found the maximum amount of p,p'- DDT as 5.7 ng/ml and lindane as 5.084 ng/ml in the water samples collected from

Meghna- Dhanogoda Irrigation Project. Cai *et al.* (1997) showed the influence of salinity, dissolved oxygen, chemical oxygen dispersion, etc, is not correlated with the distribution of DDT, HCH and other compounds. Alam (1996) showed correlation between organochlorine residues and suspended particles of water. However, these findings were not examined during the present study.

p,p'- DDT > p,p'- DDE > p,p'- DDD > heptachlor > dieldrin > lindane > aldrin.

Concentrations of organophosphorus residues in water samples are shown in Appendix Table XIII. Several organophosphorus pesticides (temephos, S-bioalletrin, pyraminforte diazinon and malathion) were analyzed. Only temephos was identified and qualified in water samples. Temephos is cauterized as highly hazardous in Table 19 (Corbett, 1974). Results showed that only three locations namely Sonatala, Saroakandi and Gabtali contained temephos as the concentration of 0.04 ng/ml, 0.03 ng/ml and 0.04 ng/ml respectively.

The fate of organophosphorus pesticides in the aqueous environment has lead to numerous investigations in recent years. In this regard, it has been reported that degradation is influenced by hydrolysis, particularly at a pH of 7, by photolysis and by microbial degradation (Larcrote, 1996).

Sonatala, Sariakandi and Gabtali thanas water pH is above 7 (Table 15) and this may enhance the degradation of organophosphorus pesticides. Wan *et al.* (1994) showed that basic pH promotes degradation of temephos in water samples by stabilizing the more phenolile structures, which in addition show a tendency to absorb more sunlight, thus increasing the overall degradation rate. Besides, through oxidation temephos is converted to oxon and sulfoxide (Barcelo 1977). These isomers were not analyzed in this present study.

High temperature (30°C) enhances thermal degradation and hydrolysis and increases microbial activity (Barcelo 1997).

The estimated safe concentration of the pesticide on *L. phenehooganjens* was 3.0 ng/ml and 0.2 ng/ml for *Neurocorelia virginensis* (Anadu *et al.*, 1996). Results reveal that findings of the present study were below the safety level during the sampling period. If they are not applied in recommended dosage they may cause serious hazard to the aquatic life especially to phytoplankton and zooplankton.

Five organochlorine pesticides were detected in mussel samples of which p,p'-DDD is categorized as relatively non hazardous, the rest are categorized as moderately hazardous (Table 19) (Corbett 1974).

Alam (1996) found only DDE in the fish (*Macrocephalus punctatus*) as 2.10 ng/ml. Georgakopoulos – Gregoriades *et al.* (1991) found p,p'- DDT, p,p'- DDD, p,p'- DDE as 1.9 ng/ml respectively in red Mullet from Greek water.

The maximum concentration of p,p'- DDT in Sonatala thana after flood is probably due to settling down of all types of pollutants from adjacent agricultural

field samples 4 and 5 contained no organochlorine residues because these area are fully close water body. Absence of river and canal, and other water inlet system hence no chance of pesticide contamination.

DDT is highly persisted compound by heating or cooking it becomes as DDD and by enzymatic action p,p'- DDT becomes p,p'- DDE. So, the containing DDT is not safe for consumption.

Alam (1996) found DDT in Meghna – Dhonagoda irrigation project. He found maximum concentration of p,p'- DDT in *Mastacembelus pancalus* (5749.84 ng/g) and minimum in *Glossogobius giuris* (14.45 ng/g), which is the higher than the maximum concentration found in the present study. Alam (1996) found p,p'- DDE in *Channa punctatus* (0.13 ng/g) and endrin in *Tilapia nilotica* (3.3 ng/g).

Giouranovits – Psyllidou *et al.*, (1994) showed a positive relationship between lipid content and organochlorine concentrations. The mussel species studied were the same species and were not from the same location. For this the correlation between fat contents and organochlorine residues showed in Fig. 62 is not reported in the study concerning red mullet (Stasmakies *et al.*, 1988, Georgapoulos – Gregoriades *et al.*, 1991; Vassilopoulous and Georgakapolos-Grrgoriades, 1993). Mussel size is known to play a role in pollutant accumulation. When organochlorine concentration is generally increases as mussel growth is reduce (Vassilopoulou and Georgakopolos – Grrgoriades, 1993).

Result of this study shows that mussel of two thanas and specially of Kahal thana (sample no 7) contained much higher concentration of organochlorine pesticide than FAO/WHO (1993) recommended MRs. Consumption of this mussel may cause serious hazard to human. The persistent of pesticides may cause biological changes in the mussel which may lead to its extinction.

The result of this study given in Appendix Table XIII shows that no organophosphorus and carbosulfan pesticide was found in mussel samples. Information is not available on its presence in contents of mussel in Bangladesh context.

Mareus *et al.*, (1990) studied different organophosphorus pesticides namely diazinon, malathion, parathion, ethion, guthion, methyl parathion and ronnel in American Oyster *Crassostrea virginical* (Gmelin) and in the blue crab, *Callinectes sapidus* (Rathun), but all of them were below the determination level.

Organophosphorus pesticides are increasingly used through the world not only for their high pesticidal activity and less toxicity but also for their low persistent in aquatic environment (Aly *et al.*, 1982), resulting in relatively less residual effect to the aquatic life (Sarker and Gupta 1986).

5.6 Concentration of pesticide residues in soil samples

Shaheen (1985) studied on the OC residues in the surface sediment of Bay of Bengale and found maximum concentration of DDT as 4.56 ng/g. Mettal *et al.*, (1980) found (0.06 ng/g to 0.09 ng/g) studied on the DDT residues in surface sediment of the river Jamuna at Dehli. Sarker and Gupta (1986) found p,p'- DDD concentration as 17.6 ng/g. Alam (1996) worked in Meghna Dhanagoda irrigation project and found maximum concentration of DDT and DDD as 2.84 ng/g and 3.56 ng/g respectively. Guenzi and Beard (1967) reported that in reduced organic matter condition DDT is degraded to p,p'- DDD and other metabolites. Present study shows that p,p'- DDD is only found in sample 7, which is lower than the concentration of p,p'- DDT (Fig. 53) percentage of organic matter is more or less high in the sample no. 7 (Table 15) which supports Guenzi and Beard's idea. Among DDT compounds p,p'- DDT was found as 21.21 %. The maximum concentration of aldrin and dieldrin was found in the sample no. 7 and sample no. 3 as the concentration of 1.32 ng/g and 1.56 ng/g respectively. Sarkar and Gupta (1986) found 0.95 ng/g of aldrin in the Arabic sea sediment. Larsen (1989) found aldrin as 3.29 ng/g to 11.20 ng/g.

Sarkar and Gupta (1986) could not detect the DDT compounds in the sediment with percentage of sand where they found maximum concentration of DDT compounds in sediment with lower percentage of sand. The percentage study shows a similar result in case of sample 7 (Table 17)

A number of organophosphorus pesticides were analyzed but except temephos no other organophosphorus and carbosulfan pesticides were detected in soil sample collected from different water bodies of Bogra district. Only one of the organophosphorus pesticides is persistent to the sediment. Readman *et al.* (1992) identified chlorpyrifos and parathion as comparatively persistent organophosphorus pesticides in the soil.

CHAPTER SIX

CONCLUSIONS

6.1 The following conclusions were made from the present research

Acute toxicity of carbosulfan and malathion on *L. marginalis* carried out with indication of lethal effect and determination of LC_{50} on mussel population during the 12h, 24h, 48h, 72h and 96h of exposure period. Higher LC_{50} value indicated that the malathion and carbosulfan insecticide is slightly toxic to the test animal.

The great diversity of environments within the study area is reflected by the large variation of insecticide residual accumulation rate. Sample sensitivity analysis revealed that chemical properties were also a major factor in evaluating the vulnerability of hydrological settings. Therefore, it is important to consider the chemical properties of insecticides while selecting and implementing insecticide management practices for agricultural areas.

Low persistent insecticides are generally none threatening to health and insecticides with low sorptivity should be taken as a serious concern as they pose a significant impact on surface water.

During the analysis of soil, water and mussel sample by GC and some unknown compound was detected. This might be other compound or any living organism under organochlorine group or a combination of different insecticide or some unknown brand of insecticides.

Model results showed that because of being low persistent malathion insecticides, very negligible amount of temophos remain in the surface water and in other sample like soil and mussel, which cannot be traced out by HPLC.

6.2 Suggestions for insecticide management practices

Insecticides by nature are poisonous. They can be difficult and expensive to remove, and, if inhaled or consumed, can be hazardous to human health. Based on the analysis, following suggestions can be made for the best possible insecticide management practices.

1. An effective insecticide should be selected that are less likely to leach and that pose lower human and environmental risks i.e., low persistence.
2. Recommended application rates of pesticides should not exceed and application equipment should be calibrated to apply desired rates. When insecticides are used, the potential for surface water contamination can be minimized if applicators follow label directions, use irrigation scheduling and follow handling, storage and disposal guidelines because the insecticide label reflects the best scientific knowledge on the safe and environmentally sound

use of insecticide. Irrigation scheduling increases irrigation efficiency and minimizes leaching of water and insecticides.

3. When chemicals are generally components of any pest control program, other methods or the combination of methods might sometimes be more appropriate. In recent years, attention has been directed toward the coordinated use of multiple methods of pest control, an approach known as Integrated Pest Management (IPM). IPM is the use of all means of pest control (chemical and non-chemical) in a compatible fashion to reduce crop losses. Insecticides are the last line of defense and are used only when pest levels are causing sufficient damage to offset the expense of application. Effort should be made to maximize the benefits of naturally occurring biological controls and use of insecticides when necessary.

CHAPTER SEVEN

SUMMARY

The experiment was carried out at the Agro-chemical and Environmental Research Division (AERD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Ganakbari, Savar, Dhaka. The aim of the study was to test the acute lethality of carbosulfan (carbamate) and malathion to *Lamellidens marginalis* (Lamarck, 1819). The pesticide residue analysis was also evaluated.

Acute lethality test

The physico-chemical characteristics of exposure media monitored during different trial with carbosulfan and malathion insecticide. During experimental trial there was slight fluctuation in water quality parameters among different treatment tank except for pH, ammonia and alkalinity. The pH value showed a concentration dependent changes ranging from 6.93 to 15.84, ammonia increased with increases in carbamate concentration which varied from 2.8 to 3 mg/l. Alkalinity showed a decrease level, which varied from 152 to 206 ml/l.

The result show that no mortality caused to mussel exposed up to the exposure concentration of 12.87 ppm. Carbosulfan (carbamate) insecticide during the preliminary experimental period, mussel exposed to 14.85 ppm carbosulfan insecticide induced 20% mortality within 6 hrs. of exposure and a complete (100%) mortality was obtained by 24 hrs. Mussel exposed to 15.84 ppm carbosulfan (carbamate) insecticide caused a complete (100%) mortality within 6 hrs. of exposure.

For the malathion, the results show that there is no mortality in mussel groups exposed up to 0.99 ppm during 96hrs. exposure. But 14.85 ppm malathion insecticide induced mussel mortality 20% by 72h of exposure and 35% mortality occurred in the same treatment group by 96h of exposure. A complete mortality 100% was observed in mussel at 24.76 ppm malathion insecticide within 72h exposure.

Acute lethality tests were conducted with a view to determine the LC_{50} values for carbosulfan and malathion in separate trials to *L. marginalis* at different exposure time. Each of the trials was conducted for a period of 96h in static system with daily media renewal.

A preliminary trial was carried out with each toxicant with a view to find out a suitable range to be used in actual lethality trial. Actual trial was performed twice with each chemical. The mortality of mussel was recorded at 6h, 12h, 24h, 48h, 72h and 96h of exposure. The mortality data were used to calculate the LC_{50} value for each of the chemicals for different exposure times. For calculation of LC_{50} value EPA Probit Analysis Program, version 1.5, was used.

Various behavioral anomalies such as foot release from body, to and fro movement, orientation, air gulping tendency, violent spasm, loss of equilibrium, mucous secretion, etc. were observed in carbosulfan and malathion exposed mussel.

During Trial IA with carbosulfan LC_{50} values at 12h, 24h, 48h, 72h and 96h were 15.02 ppm, 13.60 ppm, 13.47 ppm, 13.33 ppm and 13.20 ppm (95% CL 14.05-15.99, 12.83-14.37, 12.70-14.24, 12.56-14.10, 12.43-13.97). During Trial IIA, the LC_{50} values were 14.26 ppm (95% CL 13.97-14.55) for 24h, 13.57 ppm (CL 12.60-14.54) for 72h, 13.35 ppm (CL 12.38-14.32) for 96h of carbosulfan respectively.

Similarly, LC_{50} values for malathion at 24h, 48h, 72h and 96h of exposure times were 21.20 ppm (95% CL 19.14-23.26), 17.82 ppm (CL 12.97-22.67), 15.30 ppm (CL 10.45-20.15) 13.75 ppm (CL 9.90-17.60) respectively. During Trial IIB, with the same chemical the values obtained were 19.36 ppm (95% CL 17.42-21.30), 17.49 ppm (95% CL 15.55-19.43), 14.52 ppm (95% CL 12.58-16.46) 13.86 ppm (95% CL 11.92-15.80) at 24h, 48h, 72h and 96h exposure times respectively.

Pesticide residues analysis

Water, mussel and soil samples were collected from nine thanas of Bogra district and their analysis for determination of organochlorine, organophosphorus and carbamate pesticides residues were performed.

Laboratory works were carried out mainly at the Institute of Food and Radiation Biology (IFRB), Bangladesh Atomic Energy Establishment, Savar, Dhaka. Gas Chromatography, (Electron Capture Detector mode) and High Performance Liquid Chromatography were used to determine the concentration level of pesticide residues.

Organochlorine residues were present in different organs of mussel samples collected from different locations, except Sample 4 (Sibganj thana), Sample 5 (Bogra sadar), Sample 6 (Sherpur thana), Sample 9 (Dhunat thana) and Sample 10 (Sonatala thana, during flood). In water samples it was present only at Sonatala, Sariakandi, Gabtali, Kahalu, Adamdighi and Sonatala thana (after flood). But in soil samples organochlorine were present only in Samples No. 1 (Sonatala thana), 2 (Sariakandi thana), 3 (Gabtali thana), 7 (Kahalu thana) and 11 (Sonatala thana, after flood).

DDT was found in all types of samples. Other organochlorine residues identified were aldrin, dieldrin, lindane, heptachlor and HCH. Among organophosphorus insecticides only temphos was identified. Maximum concentration of DDT was found in water, mussel and soil samples were 13.46 ng/g, 4.92 ng/g and 11.73 ng/g respectively.

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APPENDIX A

Appendix Table-I Physico-chemical characteristics of the test solution monitored during preliminary test with carbosulfan

Solution	Concentration of carbosulfan (ppm)	Temperature (°C) Mean (Range)	Dissolved Oxygen mg ^l ⁻¹ Mean (Range)	pH Mean (Range)	Carbon-dioxide (CO ₂) mg ^l ⁻¹ Mean (Range)	Alkalinity mg ^l ⁻¹ Mean (Range)	Hardness (as CaCO ₃) mg ^l ⁻¹ Mean (Range)	Ammonia mg ^l ⁻¹ Mean (Range)	Nitrite-N mg ^l ⁻¹ Mean (Range)
1	0	27 (26.0-28.0)	5.7 (4.1-4.3)	8.2 (8.1-8.3)	17.2 (17.1-17.3)	206 (205-207)	207 (206-208)	0.124 (0.091-0.13)	0.003 (0.002-0.004)
2	0.99	21 (20.0-22.0)	5.0 (4.9-5.1)	8.1 (7.9-8.3)	20 (19.0-22.0)	203 (202-208)	210 (209-211)	1.2 (0.091-0.13)	0.013 (0.002-0.014)
3	4.99	21 (20.0-22.0)	5.2 (4.9-5.3)	8.0 (7.9-8.1)	22 (20.0-23.0)	202 (201-203)	211 (210-212)	1.4 (1.0-1.5)	0.25 (0.1-0.31)
4	9.90	21 (20.0-22.0)	4.7 (4.6-4.8)	7.6 (7.5-7.7)	25 (24.0-26.0)	190 (189-192)	221 (220-222)	2.8 (2.6-2.9)	0.3 (0.1-0.4)
5	12.87	21 (20.0-22.0)	4.9 (4.6-5.9)	6.1 (5.9-6.2)	52 (50.0-53.0)	152 (150-153)	248 (247-249)	3 (2.9-3.1)	0.3 (0.1-0.4)

Appendix Table-II Physico-chemical characteristics of the test solution measured during trial with carbosulfan (Trial IA)

Solution	Concentration Of carbosulfan (ppm)	Temperature (°C) Mean (Range)	Dissolved Oxygen mg ^l ⁻¹ Mean (Range)	pH Mean (Range)	Carbon-dioxide (CO ₂) mg ^l ⁻¹ Mean (Range)	Alkalinity mg ^l ⁻¹ Mean (Range)	Hardness (as CaCO ₃) mg ^l ⁻¹ Mean (Range)	Ammonia mg ^l ⁻¹ Mean (Range)	Nitrite-N mg ^l ⁻¹ Mean (Range)
1	0	27 (26-28)	5.32 (5.0-6.2)	8.0 (7.2-8.2)	17.5 (17.3-21.2)	210 (150-220)	205 (200-220)	0.130 (0.12-0.17)	0.3 (0.01-0.04)
2	6.93	21 (20-22)	4.8 (4.7-4.9)	7.6 (7.5-7.7)	25 (24-26)	195 (194-196)	220 (219-221)	>3 (2.8- >3)	0.3 (0.01-0.04)
3	9.90	21 (20-22)	4.9 (4.8-5.0)	7.5 (7.4-7.6)	27 (26-28)	192 (190-193)	222 (220-223)	>3 (2.8- >3)	0.3 (0.01-0.04)
4	11.39	21 (20-22)	4.7 (4.6-4.9)	7.4 (7.2-7.6)	30 (29-31)	190 (189-191)	225 (224-226)	>3 (2.8- >3)	0.3 (0.01-0.04)
5	13.86	21 (20-22)	4.4 (4.2-4.9)	6.9 (6.8-7.1)	35 (34-36)	184 (183-185)	236 (235-237)	>3 (2.8- >3)	0.3 (0.01-0.04)
6	14.85	21 (20-22)	4.2 (4.1-4.3)	6.8 (6.6-6.9)	39 (38-40)	178 (177-179)	240 (239-241)	>3 (2.8- >3)	0.3 (0.01-0.04)

Appendix Table-III Physico-chemical characteristics of the exposure media trial with carbosulfan (Trial IIA)

Solution	Concentration of carbosulfan (ppm)	Temperature (°C) Mean (Range)	Dissolved Oxygen mg l ⁻¹ Mean (Range)	pH Mean (Range)	Carbon-dioxide (CO ₂) mg l ⁻¹ Mean (Range)	Alkalinity mg l ⁻¹ Mean (Range)	Hardness (as CaCO ₃) mg l ⁻¹ Mean (Range)	Ammonia mg l ⁻¹ Mean (Range)	Nitrite-N mg l ⁻¹ Mean (Range)
1	0	27 (26-28)	5.22 (5.0-6.3)	8.1 (7.5-8.3)	17.5 (17.2-20.1)	205 (150-210)	202 (200-205)	0.122 (0.11-0.15)	0.003 (0.002-0.004)
2	10.40	21 (20-22)	5.1 (5.0-5.3)	7.9 (7.7-8.0)	19 (18.9-19.1)	200 (199-201)	219 (200-220)	2.6 (2.4-2.7)	0.25 (0.01-0.30)
3	13.07	21 (20-22)	5.0 (4.9-5.1)	7.57 (7.6-7.8)	22 (20-23)	197 (196-198)	220 (219-222)	2.8 (2.6->3)	0.25 (0.01-0.27)
4	14.06	21 (20-22)	4.7 (4.6-4.8)	7.4 (7.3-7.5)	30 (29-31)	191 (190-192)	222 (210-226)	>3 (2.6->3)	0.3 (0.01-0.40)
5	14.36	21 (20-22)	4.2 (4.1-4.3)	6.98 (6.7-6.9)	39 (38-40)	177 (176-178)	241 (235-249)	>3 (2.6->3)	0.3 (0.01-0.40)
6	14.85	21 (20-22)	3.8 (3.7-3.9)	6.2 (6.1-6.3)	50 (49-51)	160 (159-161)	246 (238-249)	>3 (2.6->3)	0.3 (0.01-0.40)

Appendix Table -IV Physico-chemical characteristics of the test solution measured during preliminary test with malathion insecticide

Solution	Concentration of malathion (ppm)	Temperature (°C) Mean (Range)	Dissolved Oxygen mg l ⁻¹ Mean (Range)	pH Mean (Range)	Carbon-dioxide (CO ₂) mg l ⁻¹ Mean (Range)	Alkalinity mg l ⁻¹ Mean (Range)	Hardness (as CaCO ₃) mg l ⁻¹ Mean (Range)	Ammonia mg l ⁻¹ Mean (Range)
Control	0	27.0 (26.0-28.0)	5.42 (5.00-6.00)	7.22 (7.20-7.40)	18.5 (17.20-19.00)	68.0 (66.0-71.1)	242 (230-242)	0.11 (0.09-0.15)
1	9.90	27 (26.0-28.0)	5.34 (5.00-6.00)	7.20 (7.14-7.40)	18.8 (17.40-19.40)	67.0 (66.0-70.1)	234 (220-247)	0.12 (0.10-0.15)
2	11.39	27 (26.0-28.0)	5.83 (5.00-6.40)	7.31 (7.23-7.55)	19.5 (18.5-20.1)	69.0 (66.0-73.0)	230 (220-235)	0.16 (0.12-0.17)
3	13.07	27 (26.0-28.0)	5.51 (5.00-6.00)	7.25 (7.10-7.34)	19.2 (17.3-20.20)	67.0 (66.0-70.2)	237 (231-240)	0.15 (0.096-0.159)
4	13.86	27 (26.0-28.0)	5.20 (5.00-5.90)	7.19 (7.17-7.34)	20.6 (18.2-22.4)	66.0 (65.0-67.1)	226 (210-220)	0.140 (0.096-0.17)
5	14.85	27 (26.0-28.0)	5.63 (5.00-6.00)	7.23 (7.01-7.60)	20.1 (18.7-22.5)	67.0 (65.0-69.1)	229 (210-230)	0.18 (0.086-0.20)

Appendix Table-V Physico-chemical characteristics of the test solution measured during trial with malathion insecticide (Trial IB)

Solution	Concentration of malathion (ppm)	Temperature (°C) Mean (Range)	Dissolved Oxygen mg l ⁻¹ Mean (Range)	pH Mean (Range)	Carbon- dioxide (CO ₂) mg l ⁻¹ Mean (Range)	Alkalinity mg l ⁻¹ Mean (Range)	Hardness (as CaCO ₃) mg l ⁻¹ Mean (Range)	Ammonia mg l ⁻¹ Mean (Range)
Control	0	27.0 (26.0-28.0)	5.5 (6.0-6.0)	7.40 (7.2-7.5)	19.2 (18.1-20.1)	68.0 (67.0-69.1)	224 (220-228)	0.13 (0.092-0.17)
1	4.95	27 (26.0-28.0)	5.7 (5.0-6.7)	7.32 (7.1-7.4)	19.3 (18.2-20.5)	67.0 (65.1-69.1)	230 (225-236)	0.14 (0.091-0.19)
2	6.99	27 (26.0-28.0)	5.6 (5.0-6.2)	7.42 (7.1-7.5)	18.9 (11.5-19.2)	67.2 (65.3-69.4)	235 (220-239)	0.14 (0.091-0.19)
3	9.90	27 (26.0-28.0)	5.9 (5.3-6.7)	7.50 (7.0-7.9)	18.5 (17.2-19.9)	68.1 (65.3-69.3)	239 (230-241)	0.12 (0.091-0.17)
4	14.85	27 (26.0-28.0)	5.80 (5.0-6.9)	7.2 (7.0-7.3)	18.6 (17.4-19.9)	67.5 (65.1-69.5)	240 (231-245)	0.13 (0.093-0.19)
5	19.80	27 (26.0-28.0)	5.5 (5.0-6.0)	7.3 (7.0-7.5)	18.9 (17.1-19.1)	67.3 (66.1-69.5)	242 (230-249)	0.12 (0.091-0.17)
6	24.76	27 (26.0-28.0)	5.82 (5.0-6.5)	7.6 (7.0-7.7)	20.1 (19.1-20.5)	67.5 (66.7-68.0)	247 (240-290)	0.13 (0.092-0.17)

Appendix Table-VI Physico-chemical characteristics of exposure media during trial with malathion insecticide (Trial IIB)

Solution	Concentration of malathion (ppm)	Temperature (°C) Mean (Range)	Dissolved Oxygen mg l ⁻¹ Mean (Range)	pH Mean (Range)	Carbon- dioxide (CO ₂) mg l ⁻¹ Mean (Range)	Alkalinity mg l ⁻¹ Mean (Range)	Hardness (as Ca CO ₃) mg l ⁻¹ Mean (Range)	Ammonia mg l ⁻¹ Mean (Range)
Control	0	27.0 (26.0-28.0)	5.60 (5.5-6.1)	7.22 (7.0-7.5)	18.9 (18.1-19.1)	67.1 (66.1-67.25)	218 (210-220)	0.12 (0.081-0.16)
1	4.95	27 (26.0-28.0)	5.70 (5.4-6.3)	7.31 (7.1-7.8)	18.9 (17.0-19.0)	67.3 (66.1-68.0)	217 (215-221)	0.15 (0.91-0.17)
2	9.90	27 (26.0-28.0)	5.5 (5.3-6.1)	7.21 (7.1-7.9)	18.5 (17.2-18.9)	65.3 (64.0-65.1)	224 (220-230)	0.13 (0.082-0.16)
3	14.85	27 (26.0-28.0)	5.8 (5.3-6.3)	7.33 (7.0-7.9)	18.4 (17.3-18.9)	66.1 (65.7-67.2)	225 (220-225)	0.12 (0.085-0.17)
4	19.80	27 (26.0-28.0)	5.9 (5.4-6.1)	7.25 (7.0-7.9)	18.0 (17.1-18.9)	67.3 (65.3-68.3)	220 (213-224)	0.15 (0.093-0.19)
5	21.90	27 (26.0-28.0)	5.5 (5.2-5.9)	7.31 (7.1-7.6)	18.3 (17.0-18.6)	67.2 (65.2-68.4)	230 (220-231)	0.13 (0.094-0.17)
6	24.28	27 (26.0-28.0)	5.3 (5.1-5.9)	7.23 (7.1-7.4)	18.5 (17.1-18.9)	67.5 (66.1-68.3)	229 (220-230)	0.14 (0.095-0.18)

Appendix Table-VII Cumulative mortality (%) of *L. marginalis* at different concentration of carbosulfan insecticide during an exposure period of 96h (Preliminary Trial)

Treatment Number	Concentration of carbosulfan (ppm)	Number of mussels release	Exposure time (hour)					
			Cumulative mortality %					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	0.99	20	0	0	0	0	0	0
2	4.99	20	0	0	0	0	0	0
3	9.90	20	0	0	0	0	0	0
4	12.87	20	0	0	0	0	0	0
5	14.85	20	20	80	100	100	100	100

Appendix Table-VIII Cumulative mortality (%) of *L. marginalis* at different concentration of carbosulfan insecticide during an exposure period of 96h (Trial IA)

Treatment Number	Concentration of carbosulfan (ppm)	Number of mussels release	Exposure time (hour)					
			Cumulative mortality %					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	6.93	20	0	0	0	0	0	0
2	9.90	20	0	0	0	0	0	0
3	11.39	20	0	0	0	0	0	0
4	13.07	20	0	0	0	0	25	40
5	13.86	20	0	15	75	100	100	100
6	14.85	20	0	40	100	100	100	100
7	15.84	20	100	100	100	100	100	100

Appendix Table-IX Cumulative mortality (%) of *L. marginalis* at different concentration of carbosulfan insecticide during and exposure period of 96h (Trial IIA)

Treatment Number	Concentration of carbosulfan (ppm)	Number of mussels release	Exposure time (hour)					
			Cumulative mortality %					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	10.40	20	0	0	0	0	0	0
2	12.38	20	0	0	0	0	0	0
3	13.07	20	0	0	0	0	15	30
4	14.06	20	0	0	20	45	85	100
5	14.36	20	0	25	65	100	100	100
6	14.85	20	0	100	100	100	100	100

Appendix Table-X Cumulative mortality (%) of *L. marginalis* at different concentration of malathion insecticide during and exposure period of 96h (Preliminary Trial)

Treatment Number	Concentration malathion (ppm)	Number of mussels release	Exposure time (hour)					
			Cumulative mortality %					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	4.95	20	0	0	0	0	0	0
2	6.93	20	0	0	0	0	0	0
3	9.90	20	0	0	0	0	0	0
4	14.85	20	0	0	0	0	20	35
5	19.80	20	0	0	0	20	45	60
6	24.76	20	0	0	30	60	100	100

Appendix Table-XI Cumulative mortality (%) of *L. marginalis* at different concentration of malathion insecticide during and exposure period of 96h (Preliminary Trial IB)

Treatment Number	Concentration malathion (ppm)	Number of mussels release	Exposure time (hour)					
			Cumulative mortality %					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	4.95	20	0	0	0	0	0	0
2	9.90	20	0	0	0	0	0	0
3	14.85	20	0	0	0	20	45	60
4	19.80	20	0	0	20	70	100	100
5	21.90	20	0	10	65	100	100	100
6	24.28	20	0	50	100	100	100	100

Appendix Table-XII Cumulative mortality (%) of *L. marginalis* at different concentration of malathion insecticide during and exposure period of 96h (Trial IIB)

Treatment Number	Concentration malathion (ppm)	Number of mussels release	Exposure time (hour)					
			Cumulative mortality %					
			6	12	24	48	72	96
Control	0	20	0	0	0	0	0	0
1	5.94	20	0	0	0	0	0	0
2	7.92	20	0	0	0	0	0	0
3	9.90	20	0	0	0	0	0	0
4	11.88	20	0	0	0	20	35	40
5	13.86	20	0	0	0	15	45	50
6	15.84	20	0	0	0	25	60	100
7	17.82	20	0	0	15	55	100	100
8	19.80	20	0	0	60	100	100	100

Appendix Table-XIII Organophosphorus (Temophos) residues in water, soil and mussel samples from different locations

Sample No	Locations	Water (ng/ml)	Soil (ng/g)	Mussel			
				Stomach	Gill	Mantle	Foot
1	Sonatala	0.04	ND	ND	ND	ND	ND
2	Sariakandi	0.03	ND	ND	ND	ND	ND
3	Gabtali	0.04	ND ¹	ND	ND	ND	ND
4	Sibganj	ND	ND	ND	ND	ND	ND
5	Bogra Sadar	ND	ND	ND	ND	ND	ND
6	Sherpur	ND	ND	ND	ND	ND	ND
7	Kahalu	ND	ND	ND	ND	ND	ND
8	Adamdighi	ND	ND	ND	ND	ND	ND
9	Dhunat	ND	ND	ND	ND	ND	ND
10	Sonatala (during flood)	ND	ND	ND	ND	ND	ND
11	Sonatala (after flood)	ND	ND	ND	ND	ND	ND

ND - Not Detected

Appendix Table-XIV Amount of total DDT and OCs in mussel, water and soil

Sample No.	DDT			Organochlorine		
	Water	Mussel	Soil	Water	Mussel	Soil
1	0.57	4.92	1.25	1.21	7.44	1.25
2	2.20	3.40	2.15	2.28	3.40	2.15
3	ND	1.87	ND	0.03	4.06	1.79
4	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND
7	13.46	4.59	11.73	13.71	8.89	14.47
8	1.25	3.79	ND	1.30	8.65	ND
9	ND	ND	ND	ND	ND	ND
10	ND	ND	ND	ND	ND	ND
11	7.17	2.65	ND	7.19	2.90	1.55
Total	24.65	21.22	15.13	25.72	35.34	21.21

Appendix Table-XV Length, weight and fat content of mussel of different locations

Sample Nos.	Locations	Species	Length (cm)	Weight (g)	Fat in 25 g
1	Sonatala	<i>L. marginalis</i>	8	97	0.20
2	Sariakandi	<i>L. marginalis</i>	10	109	0.10
3	Gabtali	<i>L. marginalis</i>	07	79	0.18
4	Sibganj	<i>L. marginalis</i>	08	76	0.11
5	Bogra Sadar	<i>L. marginalis</i>	09	73	0.07
6	Sherpur	<i>L. marginalis</i>	07	84	0.14
7	Kahalu	<i>L. marginalis</i>	08	74	0.08
8	Adamdighi	<i>L. marginalis</i>	10	83	0.13
9	Dhunat	<i>L. marginalis</i>	09	75	0.09

Appendix Table-XVI List of registered pesticides in Bangladesh

Serial Number	Common Name	Number of Trade Name Product	Serial Number	Common Name	Number of Trade Name Product
Public Health Insecticides			Miticide		
1	Permethrin and Reasmethrin	1	1	Bromopropylate	1
2	Permethrin+ Tetramethrin	3	2	Dicofol	1
3	S-Bioallethrin+ Permethrin	7	3	Ethion	2
4	Newpynamin Fort+ Goki Light	1	4	Fenbutain Oxide	2
5	Propxur+ Pyrethroid	1	5	Sulphur	5
6	Allethrin	1	6	Properrgite	2
7	Feenitrothion	1	7	Chinimethhionate	1
8	Deltamethrin	3	8	Fenpropathrin	1
9	Permethrin	7	9	Tetradeefon	1
10	Tetramethrin+ S-Bioalthrin	1		Sub-total	16
11	Agamethiphos	1	Fungicides		
12	Deltamethrin	1	1	Carbendazim	2
13	S-Bioalthrin	1	2	Carboniz+Thiram	1
14	Chlorpyrifos methyl	1	3	Edifenphos	2
15	Chlorpyrifos	4	4	Brodeaumix + Curfraneb	1
16	Pirimiphos Methyl	1	5	Pyroquilon	1
17	Temiphos	1	5	Pyroquilon	1
18	Diazinon	2	7	Iprodione	1
19	Phenthoate	1	8	Mancozeb	6
20	Fenitrothion	3	9	Triademefon	1
21	Alpha Cypermethrin	3	10	Metiram Complex	1
22	Propxur	1	11	Propiconazole	1
23	Cypermethrin	4	12	Propineb	1
24	Bendiocarb	1	13	Metalexyl+Mancozeb	2
25	Phenthion	1	14	Triademefon	1
26	Malathion	4	15	Trademmol	1
27	Lambda Cyhalothrin	2	16	Thipenate Methyl+Thiram	1
28	D-Allethrin (Pynanin Fort)	16	17	Thipenate	2
29	S-Bioithrin	11	18	Dezomet	1
	Sub-total	86		Sub-total	26

Serial Number	Common Name	Number of Trade Name Product	Serial Number	Common Name	Number of Trade Name Product
Insecticides					
1	BPMC	5	36	Tetrachlorvinphos	1
2	Carbaryl	4	37	CCA Type C	4
3	Carbofuran	18	38	Propxur	1
4	Carbosulfan	2		Sub-total	157
5	Cartap	3			
6	Chlorpyrifos	2	Stored products pesticides		
7	Chlorpyrifos methyl	1	1	Primiphos methyl	2
8	Cypermethrin	12	2	Methacriphos	3
9	Profenfos + Cypermethrin	1	3	Alluminium Phosphide	7
10	Alpha Cypermethrin	1		Sub-total	12
11	Cyhalothrin	1			
12	Cyfluthrin	1	Rodenticides		
13	Deltamethrin	1	1	Brodifacoum	1
14	Diazinon	18	2	Bromadeolon	2
15	Dichlorvos	6	3	Komatetrilyl	1
16	Dimethoate	11	4	Zinc Phosphide	1
17	Endosulfan	1	5	Diapiesinon	1
18	Ethofenfox	1	6	Flocumafen	1
19	Phenthion	2	7	Zinc Phosphide Bait	1
20	Fenitrothion	11		Sub-total	8
21	Fenitrothion+BPMC	1			
22	Fenvalerate	2	Herbicides		
23	S-Fenvalerate	1	1	Glyphosate	10
24	Isazofos	1	2	Glyphosate + Terbutyl	1
25	Formothion	1	3	2,4-D	7
26	Malathion	19	4	Dalapon Sodium	2
27	Heptachlor	1	5	Paraquat	2
28	Monocrotophos	6	6	Propanil	1
29	MIPC	2	7	Oxadizon	1
30	Oxydemeton methyl	1	8	Glufocinate	1
31	Phosalone	1		Sub-total	25
32	Phenthoate	7			
33	Primicarb	1		Total	330
34	Phosphamidon	2			
35	Quinalphos	3			

Chemical and Environmental Properties of Pesticides and Corresponding LPI Calculation

Diazinon

Organic carbon partition coefficient $K_{OC} = 1000$ cc/gm; Half-life, $t_{1/2} = 40$ days; Decay rate; $\lambda = 0.693/t_{1/2} = 6.324/\text{year}$; Adsorption partition coefficient, $K_d = K_{OC} * f_{OC} = 52.63$ cc/gm; Retardation factor, $R = 1 + (p_b K_b)/\theta_c = 178.62625$; Finally, $LPI = 1000 * V/(R \lambda z) = 1.270132$.

Malathion

Organic carbon partition coefficient, $K_{OC} = 1800$ cc/gm; Half-life, $t_{1/2} = 1$ days; Decay rate; $\lambda = 0.693/t_{1/2} = 252.95/\text{year}$; Adsorption partition coefficient, $K_d = K_{OC} * f_{OC} = 947.34$ cc/gm; Retardation factor, $R = 1 + (p_b K_b)/\theta_c = 3198.2725$; Finally, $LPI = 1000 * V/(R \lambda z) = 0.001774$.

Chlorpyrifos

Organic carbon partition coefficient $K_{OC} = 6070$ cc/gm; Half-life, $t_{1/2} = 30$ days; Decay rate; $\lambda = 0.693/t_{1/2} = 8.43/\text{year}$; Adsorption partition coefficient, $K_d = K_{OC} * f_{OC} = 319.464$ cc/gm; Retardation factor, $R = 1 + (p_b K_b)/\theta_c = 1073.19134$; Finally, $LPI = 1000 * V/(R \lambda z) = 0.15771$.

Cypermethrin

Organic carbon partition coefficient $K_{OC} = 100000$ cc/gm; Half-life, $t_{1/2} = 30$ days; Decay rate; $\lambda = 8.433/t_{1/2} = 6.324/\text{year}$; Adsorption partition coefficient, $K_d = K_{OC} * f_{OC} = 5263$ cc/gm; Retardation factor, $R = 1 + (p_b K_b)/\theta_c = 17763.625$; Finally, $LPI = 1000 * V/(R \lambda z) = 0.009578$.

Fenitrothion

Organic carbon partition coefficient $K_{OC} = 2000$ cc/gm; Half-life, $t_{1/2} = 8$ days; Decay rate; $\lambda = 0.693/t_{1/2} = 31.625/\text{year}$; Adsorption partition coefficient, $K_d = K_{OC} * f_{OC} = 105.26$ cc/gm; Retardation factor, $R = 1 + (p_b K_b)/\theta_c = 356.2525$; Finally, $LPI = 1000 * V/(R \lambda z) = 0.12735$.

Carbofuran

Organic carbon partition coefficient $K_{OC} = 22$ cc/gm; Half-life, $t_{1/2} = 50$ days; Decay rate; $\lambda = 5.059/t_{1/2} = 6.324/\text{year}$; Adsorption partition coefficient, $K_d = K_{OC} * f_{OC} = 1.15786$ cc/gm; Retardation factor, $R = 1 + (p_b K_b)/\theta_c = 4.9077$; Finally, $LPI = 1000 * V/(R \lambda z) = 57.78785$.

Carbaryl

Organic carbon partition coefficient $K_{OC} = 300$ cc/gm; Half-life, $t_{1/2} = 10$ days;
 Decay rate; $\lambda = 0.693 / t_{1/2} = 25.295/\text{year}$; Adsorption partition coefficient, $K_d =$
 $K_{OC} * f_{OC} = 15.789$ cc/gm; Retardation factor, $R = 1 + (p_b K_b) / \theta_c = 54.287875$;
 Finally, $LPI = 1000 * V / (R \lambda z) = 1.0448$.

Heptochlor

Organic carbon partition coefficient $K_{OC} = 24000$ cc/gm; Half-life, $t_{1/2} = 250$ days;
 Decay rate; $\lambda = 0.693 / t_{1/2} = 1.012/\text{year}$; Adsorption partition coefficient, $K_d =$
 $K_{OC} * f_{OC} = 1263.12$ cc/gm; Retardation factor, $R = 1 + (p_b K_b) / \theta_c = 4264.03$;
 Finally, $LPI = 1000 * V / (R \lambda z) = 0.332495$.

Endosulfan

Organic carbon partition coefficient $K_{OC} = 12400$ cc/gm; Half-life, $t_{1/2} = 50$ days;
 Decay rate; $\lambda = 0.693 / t_{1/2} = 5.0599/\text{year}$; Adsorption partition coefficient, $K_d =$
 $K_{OC} * f_{OC} = 652.612$ cc/gm; Retardation factor, $R = 1 + (p_b K_b) / \theta_c = 2203.5655$;
 Finally, $LPI = 1000 * V / (R \lambda z) = 0.128682$.

APPENDIX B

List of Publications of Author

1. Islam, N., Khatoon, J.A., Salam, A. and Rahman, M. 1998. Persistence and distribution of ^{14}C - malathion in rice-fish ecosystem. *Bangladesh J. Zool.* **26** (2): 91-96.
2. Islam, M.N., Nabi, M.R. and Khatoon, J. 1999. Toxicity and translocation of malathion in a stimulated rice-fish ecosystem. *Bangladesh J. Life Sc.*, **11**(1&2): 7-14.
3. Islam, M.N., Nabi, M.R., Salam, M.A., Khatoon, J., Malek, M.A. and Haque., E. 2000. Toxicity and persistence of malathion to catfish (*Heteropneustes fossilis*) in the model paddy-fish ecosystem. *Bangladesh J. Life Sc.*, **12** (1&2): 7-13.
4. Khatoon, J.A. and Islam, M.N. 2001. Magnitude of ^{14}C carbofuran in stimulating tropical marine environment. *Bangladesh J. Life Sc.*, **13** (1&2): 83-90.