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Special Issue 2023

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ORIGINAL ARTICLE **LIFE SCIENCE**

BANGLADESH WILDLIFE: A CALL TO ARREST ITS DECLINATION THROUGH REMEDIAL POLICIES AND MANAGEMENT

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Abstract

Bangladesh, located between latitudes 20°34' to 26°38' north and longitudes 88°01' to 92°41' east, is the most densely populated country in the world with 1,252 people per one square kilometre; this is almost three times as dense as its neighbor, India, other than the island countries like Singapore, and others. So, Bangladesh is not likely to have a large array of wildlife, including both megafauna and macrofauna, and flora. However, because of its very zoo-geographic location in the Indo-Malayan realm of the Oriental Region, Bangladesh supports at least three major terrestrial habitats and similar number of aquatic environments. These have allowed the country to have astounding number of wildlife and plants, e.g., about 125 species of Mammals, 718 species of Birds, 2500 species of arthropods and 5700 species of vascular plants. However, it is not satisfied status because already lost few charismatic megafaunas such as, all three species of Asian rhinoceroses, water buffalo, swamp deer, two species of peafowls, swamp partridge, *Bhadi Hans* (white-winged duck), marsh crocodile, etc. Existing study posits that the country has already lost about 10% of its mammals, 3% birds and 4% of reptile species and an unknown number of amphibians, fishes, and invertebrates as well as plants. Another 14% of animal species are endangered. The same may very well be true for the plants. Currently the Forest Department, which has its own forestry policy from as early as 1979, has failed to develop such a policy for wildlife although it is trying to manage the wildlife of the country from the inception of Bangladesh in 1971.The department lacks in right workforce trained in wildlife with proper degree in relevant subjects. A few people that work there does not see progressions in their service carrier. The wildlife conservation policy followed round the world does not conform to the forestry policy of Bangladesh. So, Bangladesh must have a concrete wildlife and its sustainable management policy to conserve the wildlife wealth through creating a proper wildlife department to implement such a policy and save the wildlife from further killing or loss of wildlife through managerial failures or mismanagement.

Keywords: Wildlife, Bangladesh, conservation, policy, forest management

Introduction

Bangladesh is just half a century old South Asian nation but with past and long history of archaeological, cultural, national emancipation and freedom struggle footprints . It lies between the massive landmass of the Himalayas in the north and the vast body of water of the Bay of Bengal in the south (Figure 1). The land area of Bangladesh extends over 1, 47,570 sq. km., when the territorial water is limited to 12 nautical miles and the economic zone to 200 nautical miles measured from the base lines.'Bangladesh won in Arbitral Tribunal/PCA more than 1, 18,813 square kilometers of waters comprising territorial sea, and seabed extending as far as 354 Nautical Miles from Chattogram coast in the Bay of Bengal with all the living and non-living resources'(BBS 2020). Its west, north and south-east are bordered by Indian territories. Extreme southeast corner borders with Myanmar. Bangladesh is a deltaic flood plain lying at the junction of the river Ganges (Padma), Brahmaputra (Jamuna), and Meghna along with their tributaries. As a result, the country is rich in fertile alluvial soil. However, due to monsoonal climate, Bangladesh suffers from frequent flood and drought. The hill country lies in the Greater Districts of Sylhet and Chittagong in the north-east, east, and south-

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east of the country. There are few low undulating lands or hillocks in a few districts of Dhaka, Mymensingh, Rajshahi and Rangpur revenue divisions.

Being in the Tropic of Cancer, Bangladesh is heavily impacted by the south-western monsoonal rains, thunderstorms, and lightning followed by over flooding, high humidity, and warmer temperatures, usually lasting from June to September. The country does not have a uniform average rainfall, temperature and humidity that usually varies from drier north-western parts of the country to the moderate central, southern, north-eastern, and eastern sides. The average annual rainfall ranges from 1,329 mm in the north-west to 4,338 mm in the northeast. The annual temperature fluctuates from 17 to 20.6 ^{0}C during winter to 26.9 to 31.1 ^{0}C during summer (Shahid et al, 2005).

The hottest day temperature has been recorded as 45.1ºC on 30 May 1972 in Rajshahi and the lowest was 2.60 degrees in Tetulia, Panchagarh on January 8 in 2018(LGC, 2018).

Wildlife in Bangladesh lives in terrestrial forest ecosystems, man-made environments, including crop fields and homestead gardens, and aquatic ecosystems such as freshwater rivers, wetlands, and marine environment.

Wildlife Habitats

Grossly, there are seven major terrestrial and aquatic habitats available for all wildlife living within Bangladesh territory. These are-

Terrestrial Habitats

1.1 Mixed evergreen or evergreen forests
1.2 Mangrove forests
1.3 Moist deciduous Sal (Shal) Forest and
1.4 Man-altered areas, such as agricultural or crop fields, village groves and backyard vegetation in human habitations.

Habitats from 1.1 to 1.3 are the natural forests, managed by the government forest department with a few exceptions. Items under 1.4 are privately managed having little or no control from the government.

Aquatic Habitats

2.1 Marine environment, including coastal and estuarine areas.
2.2 Freshwater Rivers
2.3 Natural and man-made waterbodies such as haors, baors, beels, ponds, lakes, etc.

Terrestrial Habitats

1.1 **Mixed evergreen or evergreen forests-**These occur only in the revenue divisions of Chittagong and Sylhet that corresponds to hill countries of Bangladesh. These forests occur in the north-east, east, and south-east of the country. These are sometimes also called hill forests.

Table 1. Status of the state-owned forest land (in ha) vide Banglapedia (2021a)

Forest type	Reserve forest	Protected forest	Vested forest	Acquired forest	BWDB* and khas	Unclassed state forest	Total
Hill (Mixed evergreen forest)	594,383	32,303	2,636	11,004	---	721,344	1361,670
Inland (Shal Forest)	68,140	2,689	19,985	31,198	---	---	122,012
Littoral (Mangrove and coastal forest)	656,579	---	---	6	101,526	---	758,111
Total	13,19,102	34,992	22,621	42,208	101,526	721,344	22,41,793

BWDB- Bangladesh Water Development Board*

Condition of forests in Sylhet Division was always bad because all were nearby city centres from early 1950s first, and then after independence in 1971, when our nation became Bangladesh through 9-month long bloody guerrilla warfare.

The loss of government forests in the hill areas, under the administrative divisions of Chittagong and the Sylhet, are mentioned in a paper by the FAO that sometimes provide funds to forest department or NGOs, recognised by it, to perform forest related tasks. Based on such studies, the FAO (2006) evaluated that over a period of 10 years from 1990 to 2000 forest department has lost 21,19% of the forest that existed in 1990 to 27.67% by 2005.

FAO (2006) shows systematic loss of natural forest in the hilly regions of the Chittagong and Sylhet Revenue Divisions (Table-2).

Table-2. Area of forest in Hectares (square kilometres)

Location	Year		
	1990	2000	2005
Kassalong	55,010	46,070	41,600
Rankhiang	6,230	350	350
Sita Pahar	650	650	650
Chittagong	26,111	19,631	16,390
Cox's Bazar	29,081	25,391	23,547
Sylhet	3,060	2,597	2,366
	120,142	94,689	86908
	12,014km^2	9,489km^2	8,690km^2
		78.81%	72.33%
Forest lost between 1990 and 2005		21.19 % (10 years)	27.67% (15 years)

The deterioration of natural forests in Bangladesh is highlighted in other FAO projects related to Bangladesh forests that have been prepared by the former and current employees of the forest department. One such project resulted in this document by the FAO (Choudhury & Hossain 2011). In this paper, the authors have mentioned that 'the natural high forests all over the country have depleted alarmingly. The National Biodiversity Strategy & Action Plan for Bangladesh (October 2006) has pointed out that the forest cover has come down to 6% from 10% of the area of the country. The fact remains that depletion of forests is an ongoing process. The Global Environmental Outlook 3 (UNEP, 2002) has also highlighted the alarming rate of deforestation. Annual forest loss in Bangladesh is estimated at about 0.015 Mha (million hectares) (Chowdhury & Hossain, 2011) or 15,000 hectares or 150 km^2.

In an earlier report, the FAO mentioned that the forests were lost in the past too. 'Bangladesh's natural forests controlled by the Forest Department and fall under three classes: hill forests (48%), inland Sal forests (9%) and mangrove forests (43%). Rural inventories show an overall depletion in forest resources in all the major forests. For example, the growing stock in the Sundarbans fell from 20.3 million m^3 in 1960 to 13.2 million m^3 in 1984, a 35% decline over 25 years. In the reserved forests of Chittagong Hill Tracts, growing stock decreased from 23.8 million m^3 in 1964 to below 19.8 million m^3 in 1985' FAO (2011).

The present condition of the wildlife in the Hill forests is the most precarious. In the past, an array of wildlife lived in the mixed evergreen forests. Along with them also lived the most ethnic minorities of the country. But when the plain-dwelling Bangalees were settled in the hills, during the tenure of ex-president Mr. Ziaur Rahman, it triggered a major civil war between the Shanti Bahini of the ethnic groups and the government leading to a wholesale destruction of wildlife habitats and along with these, most megafaunas, and populations of major wildlife species.

Gone are the two species of rhinoceros, banteng, leopard, green or Javan peafowl or peacock and white-winged wood duck. Nearly extinct species are gaur, Bengal tiger, all three species of Asian bears, stump-tailed, dhole or wild dog, Assamese and pig-tailed macaques, clouded leopard, golden cat, marbled cat, hog deer, hornbills, brown hill tortoise, Malayan box turtle and many other species. These species are recorded from the border areas with Myanmar or India through camera-trap mechanism and do not represent any viable population.

Maximum number of species of amphibians and hill stream fishes used to be present in the mixed evergreen forests too. Although there are no concrete figures on the number of species of invertebrates, dominated by the insects and other arthropods, were or are likely to be present in the hill regions of the country.

However, most large mammals, birds, reptiles, amphibians, and hill fishes of the country are still present in the hilly areas, barring those that are already extinct, but none seemed to have a viable population to withstand the continuous loss of forest habitats that government is apparently failing in halting.

Invariably these forests have the highest number of plant diversity that is likely to range from 2000 to 2500 species of the 6000 species reported for Bangladesh in Banglapedia (2021b). Of course, these forests have been ruined

over the last few decades (Choudhury& Hossain, 2011: FAO 1998, 2011: Khan 2003, 2012) and there is no recent evaluation of the status of plant species in the CHT and Greater Sylhet areas.

1.2 Mangrove forests

Basically, mangrove forests are known largely as the Sundarban. It covers more than 6,000 km^2 or 91% of the mangroves present in the three south-western districts of Satkhira, Khulna and Bagherhut. It is contiguous with the Sundarban forest of the West Bengal state of India having 4000 km of mangroves (Hussain & Acharya, 1994). The remaining 8.6% include all coastal forests from the western coastal areas in Bagerhat District to the easternmost one in Saint Martin's Island in Cox's Bazar. The coastal forests are mainly man-made, as over the last half a century or so, Bangladesh forest department did not allow natural mangrove to grow in our coastal areas but planted such areas mainly with saplings of keora and baen (*Sonneratia apetala* and *Avicennia alba*).

Bangladesh Sundarban supports the highest number of plant species in the world. In 1903, scientist Prain published a list of 334 species of plants from the Sundarban (Prain,1903). Over the years, at least another 50 plant species have been added to the flora of the Sundarban of Bangladesh and India.

Despite loss of the largest megafaunas, such as Great Indian Rhinoceros, Javan Rhinoceros, Buffalo and Barasingha, Sundarban is still the finest natural forest of the country that supports the only viable population of Internationally Endangered Bengal Tiger, Threatened Masked Finfoot, Salt-water Crocodile, King Cobra and nearly a dozen species of kingfishers. Our popular deer, the Chitra Horin or spotted deer only survives in the Sundarban and nowhere else, barring introduced ones in coastal areas. It is the last refuge for the monkey- rhesus macaque, wild boar, barking deer, leopard cat, fishing cat, mangrove pitta, mangrove whistler, white-tailed sea eagle, mangrove snake, ring-tailed lizard, crab-eating frog, several species of fishes, and many species of invertebrates.

Sundarban is the largest mangrove forest in the world. As such, it is so in Bangladesh too and still the best forest as it has never seen the wholesale destruction of hill forests caused by the subsequent forest departments from 1860s to till date and by ruthless burning of primary forests by the ethnic people for ages and converting forest land into human dwellings by the settled Bangalees from the plains land over the last few decades.

1.3 Moist deciduous Sal forest or Inland Forest

Popularly known as the Madhupur Tract is the Sal or Shal forest that is present in the greater Dhaka, Mymensingh, Tangail and Jamalpur districts with bits and pieces in the Rajshahi and Rangpur revenue divisions. Of the three forest ecosystems, the Shal forest has suffered from the highest destruction as is evidenced from Rahman et al (2010). 'The anthropogenic impacts on Sal Forest have increased rapidly over past decades. The Food and Agricultural Organization (FAO) estimated that about 36% of the Sal Forest cover existed in 1985; while in 1990 only about 10% of the forest cover remained (Haque, 2007). It has been reported that central Sal forests are the most threatened ecosystem of Bangladesh (Alam et al., 2008). Currently, these important ecosystems are deteriorating due to anthropological and natural threats.

The Shal forest has lost the maximum number of wildlife, in addition to scores of plant species. There is not a single century old Shal tree anywhere in Bangladesh. The current day Shal forests contain Shal saplings planted by forest department and others as well as some grown from the old stumps.

Of the megafaunas- the Great Indian one-horned rhinoceros is the first to become extinct, followed by the elephant and gaur. Subsequently lost are the Bengal tiger, leopard, sambar, hoolock gibbon, stump-tailed and pig-tailed macaques, slow Loris, pangolin or scaly anteater, Indian peafowl or peacock, hornbills, hill myna, reticulated python, Burmese python, tree-snake and other species of reptiles and amphibians.

The remnant populations of elephants occurring along the Indian border with Mymensingh, Netrokona, Jamalpur and Sherpur districts have stuck inside Bangladesh border due to the electric fence erected all along the border line by India. This is preventing free movements of elephants between forested lands in Meghalaya, Assam and Tripura States of India and Bangladesh. As a result, there is serious casualty on both the elephants and the humans, and their properties.

1.4 Man-altered areas, such as agricultural or crop fields, village groves and backyard vegetation in human habitations.

Considering that the country is about 147,570km2, of which 116,727 km2 (79.1%) is floodplain,12,248 km2 (8.3%) is terraced land and 18,593 km2 (12.6%) is hilly area (Biswas et al., 2004). So, man-made areas are within the floodplain

region of the country, where most human activities are restricted. These include human habitations in towns and villages, major human activity centres, administration, education, culture, business, and commercial operations.

As is known to all, in the olden days, at least up to the 1950s, that I can recollect, all villages and towns, agricultural fields, fallow lands, abandoned houses, dilapidated buildings, prayer places, etc., supported some kinds of wildlife and many species of plants providing food and shelter to them. Each house, from the tiniest hamlet, thatched house to the compound of village head or a zamindar, everybody had a backyard garden of several to dozens of fruit trees, timber trees and many unwanted or untilised species of plants that grew naturally. Most trees in the compound of mosques, temples, other prayer places, schools, madrasas, and others used to remain as 'sacred' and people will usually not cut those trees.

As far as my memory goes, we used to see jungle cat, fishing cat, jackal, Bengal fox, civets, mongoose,100s of fruit bats hanging from banyan, fig and other large trees, insect bats, rodents, c. 100 species of birds, 40-50 species of reptiles, nearly a dozen species of amphibians and at least 50 species of freshwater fishes in the countryside, outside the major forest ecosystems. The moths, butterflies, beetles, bugs, grasshopper, flies, bees, ants, and other invertebrates occurred as 100s of species.

However, things have changed so drastically that there is literally no fallow land in the country now. All such lands are occupied by human habitations, used for agriculture and infrastructural developmental activities. Cropping patterns have changed too. So, all lands around a house are used for growing crops, vegetables, fruits, or timber trees, and all for commerce only.

Thus, we now encounter only the commonest species of mammals, birds, reptiles, and amphibians that are generalists and can thrive in the company of humans. Example being mongoose, jackal, rats-mice, and bats among mammals, sparrows, crows, bulbuls, mynas, kites, drongo, koel, herons and egrets among birds. The house geckos, garden lizard, monitors, checkered keelback snake among reptiles, and few frogs and toad among amphibians. Among fishes, only those that are cultivated may be present in the countryside. Of course, there will be no dearth of obnoxious, harmful, pests and parasites of crops and fruits and those invertebrates that can thrive at the cost of agricultural resources.

So, overall, the countryside does not hold good as a compact habitat for any important or threatened species of wildlife or plants.

Aquatic Habitats

2.1 Marine environment, including coastal and estuarine areas.

Marine areas of the country, estuaries and coastal areas are within the perimeters of the Bay of Bengal that covered the whole of the southern portion of Bangladesh, starting from the Sundarban in the west to the Saint Martin's Island in the east. Bangladesh has immense coastal and marine resources along its south edge at the northernmost part of the Bay of Bengal. The country has a coastline of about 710 km and 121,110 sq. km of Exclusive Economic Zone (EEZ) (Habib & Islam, 2020).

Most dolphins, whales, and porpoise are restricted to the marine ecosystem with extension of some of their ranges into the southern segments of the Sundarban. Only Ganges Susu does not occur in the marine area but northern parts of the Sundarban that are connected to freshwater rivers.

Of the baleen whales, only Bryde's Whale is still present in the Bangladesh portion of the Swatch of No Ground in the Bay of Bengal. All others have disappeared or there is no available information on them such as the Blue Whale. Out of 13 species of toothed whales or dolphins and porpoises mentioned in the red list of the IUCN (IUCN, 2015) most are data deficient, and few have better populations in the Sundarban than that in other parts of the world.

Of the birds, only gulls and terns are common when rest of the oceanic birds are rare.

Among reptiles, all the five species of marine turtles and ten species of sea snakes are in Endangered and Vulnerable categories.

An updated checklist of Marine Fishes of Bangladesh by Habib & Islam (2020) covers a total of 740 species belonging to 389 Genera of 145 Families and 30 Orders. Among the fish species, 53.38% exclusively marine and 46.62% found in both brackish and marine water. Besides, 296 species of fish are reef associated and 204 of these are recorded from the Saint Martin's Island. Further, 271 species of brackish water and/or marine fishes are commonly observed in the Sundarbans mangrove ecosystem and its adjacent sea area. About 7% of the total marine fishes of Bangladesh are identified as threatened as per global IUCN Red List.

This vast area although supported hundreds of species of marine fishes and marine animals during the early 1970s, the fish stock has declined severely due to overfishing by Bangladeshi fishers and those that overseas fishing trawlers that pirated our fish stocks from the Bay of Bengal parts within our territory.

2.2 Freshwater Rivers

The freshwater river systems with their tributaries and distributaries comprise 8,300 km or 5.76% of the area of the country (Table 3).

Table 3. Major physiographic areas of Bangladesh (Hoq, 2009)

Description	Area (km²)	% Of total area
Rivers, canals, streams	8,300	5.76
Estuaries, brackish- waterbodies	1,828	1.27
Floodplains	112,010	77.76
Wetlands	2,930	2.03
Freshwater ponds and tanks	794	0.55
Artificial lakes	906	0.63
Hill areas	17,286	12
Total Bangladesh	144,054*	100

**BBS (2020) mentioned the total area of the country as 1, 47,570.*

Of all the aquatic ecosystems, the freshwater rivers have suffered the highest loss of biodiversity. This is because major countryside rivers have become completely silted up or not navigable. Logically when there is no water, there will be no water loving wildlife there too.

From my early childhood in the 1950s to late 1960s I lived in remote areas in greater Dhaka District under Dhamrai and Manikganj police stations. There was Bangshi River in Dhamrai and two mighty rivers the Dhaleshwari and Kaliganga in Manikganj. At the current time, all these rivers are virtually dead getting only little water during monsoonal rainy season, from June to September. This is mainly because all these three rivers have originated at certain points in the Jamuna River that has become silted up due to millions of tons of silt carried by it from its upstream in the Indian States of Meghalaya and Assam where this river is known as the Brahmaputra. The Jamuna has a major tributary as Tista, and both enter Bangladesh from India.

The other two major rivers, the Padma in the west and the Meghna in the east have also originated from India before entering the Bangladesh Territory. All three rivers have originated in the high hills of China and India.

Most popular and widely known hill rivers are the Sangu-Matamuhuri, Karnaphully, Surma and Kushiyara, etc.

From Karnaphully in the east to the Padma in the west, the only fully aquatic mammal Ganges Susu or Ganges Dolphin that used to live in great numbers in the 1950s to early 1970s has become a Critically Endangered species in Bangladesh. It disappeared from most of the river systems. Current populations are disjunct and devoid of any intermingling of genetic materials between different populations.

Of all the known aquatic megafaunas, we have completely lost the Marsh Crocodile from our freshwater river systems. There is no resident or breeding population of its cousin the Gharial that used to be common in the Padma and the Jumna even during 1970s and early 1980s.

Most turtle-tortoise and fish species have either disappeared or those that are present are represented by dwindling populations in our river systems.

As per the IUCN Bangladesh Red List of 2015 (IUCN, 2015), of the 253 species of freshwater fish species assessed, 64 or 25.3% were assigned to Threatened Category. In addition, nine species are evaluated as Critically Endangered, 30 Endangered, and 25 vulnerable.

2.3 Natural and man-made waterbodies such as haors, baors, beels, ponds, lakes, etc.

Bangladesh used to be known as the country of rivers, beels, baors, haors, ponds, tanks, and ditches. However, at the current time, most of the naturally occurring wetlands such as beels, baors and ponds have disappeared barring a few that are still present in Greater Sylhet and Mymensingh Districts in the North and North-East of the country. These freshwater bodies are represented only by 3.21 % of the total area of the country (Table). Excepting the haors in the above two regions the ponds, village tanks and ditches are all used for fish culture or rather farming monoculture of indigenous and exotic species of fishes. To do these, farmers use poison to kill all animals and plants that naturally used to occur in these pre-1970 freshwater wetlands. Another emerging problem is the introduction of the exotic species of fishes, some of which are so aggressive and voracious eaters that they are not allowing natural breeding and population increase of our indigenous freshwater fishes.

IUCN has listed 253 species of freshwater fishes from the rivers and the other freshwater bodies, be that man-made or natural (IUCN 2015). Of these, nine species are Critically Endangered, 30 are Endangered and 25 are Vulnerable amounting to 25.3% of the fish species are under the Threatened Category. Also, 10.7% or 25 species are Near Threatened.

Nearly a dozen species of toads and frogs, all freshwater turtles and tortoises, and water snakes having rapid decline in populations due to overuse of the waterbodies and monoculture of indigenous and/or exotic fishes.

Larger haors in Sunamganj, Moulvibazar, Netrokona and Kishoreganj still support breeding populations of some local fishes and attract many migratory birds.

Ganges Susu and Otter are rarely found in the freshwater bodies.

BACKGROUND FOR A NATIONAL WILDLIFE POLICY FOR BANGLADESH

ENADNGERED WILDLIFE OF BANGLADESH

Bangladesh has lost 31 species or 2 % of its mammals, birds, reptiles, amphibians, fishes, butterflies, and crustaceans, mostly in the last century. About 4% or 56 species are Critically Endangered and can disappear at any time if conservation interventions are not being made on urgent basis. Another 11.18% or 181 species of animals are endangered also needing immediate conservation interventions. The third category among the Threated species is the Vulnerable group represented by 153 or 9.45% of the species evaluated (IUCN 2015, Table 4). It is conjectured that animals from Vulnerable Category soon likely to jump either to EN or CR, if proper management systems are not put in place sooner than later. We need to remember here that IUCN 2015 red listing is over 7 years old (Table 4).

Table 4. IUCN 2015 species red lists

Category		Number of Species	Percentage
Regionally Extinct		31	2 (1.91)
Critically Endangered	These three falls under the Threatened Category	56	3.45
Endangered		181	11.18
Vulnerable		153	9.45
Near Threatened		90	5.55
Least Concern		802	50 (49.53)
Data Deficient		278	17
Not Evaluated		28	2 (1.72)
Total species		1619	

CURRENT WILDLIFE SCENARIO

1. Based on the daily reports appearing in national news, print and electronic media as well as social media we see a very sad situation with our wildlife wealth, be that in nature or in captivity in safari parks, zoos, and private collections.

In this 21st millennium, nobody wants to see eight or more wild elephants die in a matter of fortnight that points to the inaction of the government bodies responsible to protect the wildlife.

A total of ninety elephants were killed in Bangladesh between 2001 and 2017. Meanwhile, around twenty-eight elephants were brutally killed in just 20 months from January 2020 to August 2021. Twenty-three of them were killed in Cox's Bazar alone (Dhaka Tribune, 2022; Prothom Alo, 2022).

2. None expects to see the majestic and intelligent elephants are tortured in the name of training following the practices of the 19th century that have long been discarded by the zoo and captive breeding organisations round the world.

Torture of Asian Elephant in Bangladesh- from a screenshot of the Daily Star- Dhaka (14 February 2022) that is too grisly a scene to be watched.

3. Dolphins are often brought ashore dead boring marks of injury when whales float to the shores of Bay of Bengal in Bangladesh.

At least fifteen dolphins have been found dead at various points along the coast of Cox's Bazar in the past one week, said local fishermen. The bodies of four turtles were also found on the beach. Among these fifteen dolphins, locals saw the bodies of four dead dolphins at Shaplapur beach in Teknaf, three dolphins at Dariya Nagar Point, three dolphins and two turtles at Sun Parachute Point. Locals said the dead dolphins had signs of injury on their bodies. Moreover, the bodies of these dolphins washed up on the shore were severely wounded (Bangladesh Post, 2020).

4. There is almost weekly confiscation of wild animals from animal sellers, shooters, trappers, etc.

All these are happening because there is virtually no authority to control and punish these law breakers. Even when punished, they get a 'rebuke' or just warnings from the authorities and very rarely monetary punishment or jail terms. So, law breakers take this as casual.

The current scenario presented above is sufficient to demonstrate that Bangladesh lacks a wildlife policy and proper management protocols. Also, the relevant existing acts, rules and regulations are not being implemented properly.

Before I propose an outline of a suitable wildlife policy for Bangladesh, let us take few examples from the existing wildlife policies of Sri Lanka, Tanzania, and FAO (Food and Agricultural Organization).

Wildlife policy of Sri Lanka

Sri Lankan wildlife policy revolves round the "Conservation of Wildlife Heritage for Present & Future Generation (DWLC, 2021) and when Kotagama (2021) gave the detailed policy having four main objectives-

1. Objectives of the National Wildlife Policy having seven elements., the number one of which is "To conserve wildlife resources, through protection, research, education, sustainable use, and benefit sharing, for the benefit of present and future generation".
2. Policy on Protected Area Management and wildlife Conservation that includes twelve elements.
3. Policy on institutional Support for Wildlife Conservation having 5 elements and
4. Policy on Inter-sectoral linkages covering four elements.

To implement all the wildlife related policies and for the sustainable management of the wildlife of Sri Lanka, the government has created an independent wildlife department with the following mission-"To conserve wildlife and nature by the sustainable utilization of men, material and land through participatory management, research, education and law enforcement and ensure the maintenance of biodiversity and forest cover as exist today" through a Biodiversity Conservation Action Plan, supported by such legislative measures as may be necessary to achieve harmony and success among all those who seek to promote conservation and sustainable development in Sri Lanka.

An excerpt from the Wildlife Policy of Tanzania is as follows:

The wildlife policy of Tanzania (TURT,1998) has twelve major elements that include

(a) to conserve areas with great biological diversity which are representative of the major habitats of Tanzania.

(b) to continue to support and where necessary, enlarge the PA network as the core of conservation activities.

(c) to promote involvement of local communities' participation in wildlife conservation in and outside the PA network, and others.

FAO has given a broad guideline for wildlife policy that stands as: -

"a policy should describe the status and role of wildlife in the country and articulate the variety of reasons for the regulation of its use. These should include food values, economic motivations, aesthetic and moral concerns and cultural and historical reasons such as the protection of national animals, human health, and the conservation of genetic resources", McHenry (1993).

Proposed outline of Bangladesh Wildlife Conservation and sustainable management policy.

1. Historically wildlife conservation is a part and parcel of the human society in Bangladesh vis-à-vis the Asian continent. As such, this wealth along with the ecosystems in which they live in, needs to be conserved in their entirety through a rational policy that will conserve all wildlife species and ecosystems as well as ensure their sustainable use and aesthetic values.
2. Like any other conservation conscious country, wildlife in Bangladesh indicates the health of its ecosystems. Thus, "the maintenance of viable natural populations of wildlife and ecological functions always takes precedence over any human use of wildlife", McHenry (1993).
3. The wildlife policy to cover aspects, such as wildlife habitats and ecosystems, wildlife populations, sustainable uses and values of wildlife, public access to wildlife, wild animals in captivity, partners in stewardship, wildlife research and education, public awareness, incorporating international wildlife and biodiversity protocols and conventions., developing wildlife study curricula for schools, colleges, universities, kindergartens, and madrasas, etc.
4. The proposed wildlife policy can be further modified taking elements mentioned in the Lankan, Tanzanian and FAO guidelines.

5. The implementation of the new wildlife policy and management of the wildlife wealth of the country will need a full-fledged wildlife organization, most likely a wildlife department or dividing the current forest department into a division of commercial forestry operations and a second one as the wildlife conservation department with proper professional progression of employees of both divisions to be ensured and be eligible for the highest position slated for the department.
6. This proposed wildlife policy needs to be further expanded by competent wildlife biologists of home and abroad.

Conclusion

Bangladesh does not have a wildlife policy although it has one for the forestry and the other for the environment. Even smaller countries like Sri Lanka have a wildlife Policy from the 1990s or so. In the absence of such a well-defined wildlife policy, the management of wildlife in the country is not moving in the right direction as every now and then either the land giant the elephant, wild cat or marine mammal gets killed. On the other hand, people get killed; crops and properties get damaged by the elephants. So, it is high time Bangladesh has its own wildlife policy, implement the same through a well-established wildlife department to save wildlife and lessen the human-wildlife conflicts, and manage this limited resource in a sustainable way.

Conflict of interest

No conflict of interest exists.

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Khulna University Studies Special Issue ICES: 11-24: 2023

ORIGINAL ARTICLE **LIFE SCIENCE**

ORNAMENTAL PLANTS FOR ENVIRONMENTAL MANAGEMENT AT ROADSIDES IN KATHMANDU VALLEY, NEPAL

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Abstract

Plant species growing along roadsides in Kathmandu gives the development which is necessities of the good environment. This study determines plantation, coverage, leaves condition of important ornamental species at different sites of Kathmandu. Many Plant species are growing and applicable for bio-indicator and maintaining greenery. *Bougainvillea glabra*, *Callistemon citrinus*, *Duranta erecta*, *Euphorbia pulcherrima*, *Jasminum mesnyi*, *Lagerstroemia indica*, *Malvaviscus penduliflorus*, *Nerium oleander* and *Rose indica* are documented. The heavily polluted sites shows dominance of *C. citrinus* 73.33±2.08 and *N. oleander* has 73.7±1.6. The coverage of *C. citrinus*, *D. erecta*, *J. mesnyi*, *L. indica* and *N. oleander* has been observed as excellent along the roadside. However *B. glabra*, *E. pulcherrima, M. penduliflorus* and *R. indica* are seen at some places. These trees have the capacity to absorb carbon dioxide and reduce pollution levels. The study sites are heavily, moderately and less polluted sites. Plants in the period of study (2017 to 2022) from the observation and comparison are in further development. Elements analysis of soil and leaves are from ICP-OES (Inductively coupled plasma-optical emission spectrometry) were done. Subsequently, following elements Aluminium, Barilium, Cadmium, Cobalt, Chromium, Copper, Iron, Manganese, Nickel, Lead, Vanidium and Zinc were determined. The dust deposition on plants, chlorophyll content and anatomy of *N. oleander* was with less deposition of dust and chlorophyll values is increased (0.001547±0.000788 and 2.397±0.299). The stomata and stomatal index of *C. citrinus* are calculated (33.607± 4.509 and 15.699± 0.898). Evergreen and broad leaf trees have advantageous in environment. The summer in Kathmandu is gorgeous with *L. indica* flowers while flowers of *C. citrinus* and *N. oleander* observed in all seasons of the year. The success programs are recommended from the authority that is evaluated and continued for long term benefit.

Keywords: Air quality, environments, floral diversity, plants, pollution, roadsides

Introduction

The urban area of capital city consists of designed planted roadsides in Kathmandu. Different selected ornamental plants are conserved. They are trees, shrubs and grasses as well. The ornamental plants with leaves, flowers are giving good scenic environment at different sites. Vehicles, population, education, economic, business, political, national importance are in many places (Nepal Government, 2017). The management of environment, controlling of unwanted in the cities are for the civilization and a long term programs. Urban roadsides plants management is in importance to make a city more attractive, peaceful and environmental protections.

Biodiversity makes the Earth habitable, and is necessary for our existence. Biodiversity provides various other important goods and services such as cultural, recreational, and spiritual nourishment that play an important role in maintaining environments, urbanization as well as social life. Chaudhary, R. P. et. al. (2020). Biodiversity includes fundamental things to our health like fresh water, clean air and food products, as well as the many other products such as

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timber, fiber, medicinal plants, etc. Ambient air quality is relates at different sites in the capital of Nepal. This promotes the plantation and it brings floral diversity.

Figure 1. The road site vegetation, Koteshwor

Figure 2. Ornamental plants, Kathmandu

Green vegetation, the roadside ornamental plants in city area; Kathmandu

Objectives

General objective

Study of plants for the environmental management through plantation program at roadsides in Kathmandu valley

Specific objectives

The study determine the flora with environment conservation, coverage of vegetation for beauty environment in the roadsides

1. Study the plantation and coverage of important ornamental plant species at different sites of Kathmandu.
2. Determine the leaves dust condition by air pollution and its chlorophyll analysis
3. Determine the leaves and soil elements in study areas.
4. Analyze green vegetation, morphology and anatomical structural values of plants leaves.

Significance of the Study

The conservation of environments and make appropriate for human society has different useful aspects. It is an important task for the country. Conservation of biodiversity plays active role for present and coming days. Study arranges plans and conserves results for academic programs which have economic, ecological, social and spiritual progress. This is the real benefits of large exploration and established greenery roadside designed in the development.

Statement of the problems

The study is necessary to analyze the resources. Development is giving further movement. Along this, arise unnecessary harmful and makes difficulties. These cases should identify and need to bring good plans. This is the ways for highly appreciation in the field of work and achievements. The environmental factors and biodiversity plays an important role in maintaining and sustaining the supply of good air and services (MEA, 2005). So, it is also important to preserve every species of plants and animals as each one has some advance role to play in our nature and make assessments of biodiversity.

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Hypothesis

- Plant species growing along roadsides in Kathmandu vary in their sensitivity to roadside pollution and environment.
- Plant species growing along roadsides in Kathmandu are giving the advantageous beautiful scenario.

Materials and Methods

The study plants are *Bougainvillea glabra* Choisy, *Callistemon citrinus* (Curtis) Skeels, *Duranta erecta* L, *Euphorbia pulcherrima* Willd, *Jasminum mesnyi* Hance, *Lagerstroemia indica* L., *Nerium oleander* L., *Malvaviscus penduliflorus (M. arboreus)* and *Rose indica*.

Many plant species are growing and applicable for bio-indicator and maintaining greenery.

The trees are *Callistemon citrinu*s, *Lagerstroemia indica* and shrubs are *Bougainvillea glabra, Duranta erecta*, *Euphorbia pulcherrima*, *Jasminum mesnyi*, *Nerium oleander*, *Malvaviscus penduliflorus (M. arboreus)*, *Rose indica*.

Methods

i) Survey and selection of the study area
ii) Ambient air quality monitoring in different sites.

Based on different reports in field study, sites were categorized into 3 different types. These are,

A: *Heavily polluted sites*: Urban area
Koteshwor-Airport area, Tudikhel-Ratna Park area, Kalanki area.
B: *Moderately polluted sites*: Bhudhanilkantha area
C: *Less polluted sites*: Sub urban area, Tribhuvan University, Kirtipur Campus area is taken as for the comparative study.

iii) The methods for this piece of work are applied specially by field visit. All plants are available in all selected sites but the distribution pattern and coverage are not similar. The botanical description and their natural phenomenon are also different.

The field study is carried through the years.As the observation, comparison, plants, among the streets, in the period of 5 years and now the work and environment is in further development. Some samples experimental works are analyzed in Central Department of Botany, Tribhuvan University and some were in School of Environmental Science in Jawaharlal Nehru University, Delhi. The work is related with respect to plant and environment.

1 The coverage of different plants. Field documentation of plants, comparative study and statistical analysis were followed.

2 Formula in different work analysis are,

i) Total Chl = $\frac{A\ 652 \times 1000 \times V}{34.5 \qquad 1000 \times W}$ (Arnon, D. J. 1949)

ii) $W = \frac{(W_1 - W_2)}{A}$

iii) W (Dust %) = $\frac{W_1 - W_2}{W_1} \times 100$

(Prajapati, S. K. and Tripathi, B. D. 2008)

iv) ICP-OES methods were used to determine the different elements, Anju, 2016

v) Stomatal Index = No. of stomata present per unit area of leaf /
No. of stomata + no. of epidermal cells within a unit area of leaf
× 100

Results and Discussions

Biodiversity conservation, its scope in research and in environment is presented. The coverage of *C. citrinus*, *D. erecta*, *J. mesnyi*, *L. indica* and *N. oleander* are very good. The coverage of *B. glabra*, *E. pulcherrima M. penduliflorus* and *R. indica* are also in different places. *L. indica* is flowering in summer. *C. citrinus* and *N. oleander* is flowering in different seasons. The plants are well in heavily and less polluted sites equally. The plants are recommended. The plants are good with beauty environment in the roadside. Some of experimental results are also good as the elements analysis of the soil and plants leaves.

1) Plantation, coverage of important ornamental plant species at different sites of Kathmandu which results green roadsides environmental management.

The concept of environment, civilization and decoration built the ideas for the green streets in the Kathmandu. The research is in Kathmandu city. The general environmental condition of the city can be considered as environmental, social, economic and the green floral conservation in the country.

i) This study determine; floral conservation, coverage, beauty environment in the roadsides. In general, plantation and greenery is the primary work in study area (Kathmandu**).**

Table 1. Description and documentations

S.N.	Study Sites	Places	Road Plants Trees	Coverage of Plants	Road Plants Shrubs	Coverage of Plants
1	Less Polluted	Kirtipur	Good plantation, normal coverage	68±3	Good plantation, normal coverage	64±3.60
2	Moderately Polluted	Bhudhanilkantha	Good plantation, normal coverage	61.33±3.05	Good plantation, less coverage	59.66±4.50
3	Heavily Polluted	Kathmandu	Good plantation, good coverage	71.33±3.51	Good plantation, good coverage	72±3

The above table explains the plants and its coverage in the different sites. The heavily polluted sites shows comparatively maximum plantation (trees 71.33±3.51 and shrubs 72±3).

ii) Selected plants in study areas (Kathmandu).

Bougainvillea glabra, *Callistemon citrinus*, *Duranta erecta*, *Euphorbia pulcherrima*, *Jasminum mesnyi*, *Lagerstroemia indica*, *Malvaviscus penduliflorus*, *Nerium oleander* and *Rose indica* are documented. These trees and shrubs have the capacity to absorb carbon dioxide and reduce pollution levels.

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Table 2. Description and documentations

S.N.	**Study Sites**	Places	**Study Plants** Trees	**Coverage of Plants**	**Study Plants** Shrubs	**Coverage of Plants**
1	Less Polluted	Kirtipur	Good plantation, normal coverage	66.33±1.52	Good plantation normal coverage	62.33±2.52
2	Moderately Polluted	Bhudhanilkantha	Good plantation,normal coverage	64.662±.51	Many plants less coverage	56.33±2.30
3	Heavily Polluted	Kathmandu	Good plantation good coverage	69.66±1.52	Good plantation good coverage	70.332±.08

The above table explains the study plants and its coverage in the different sites. The heavily polluted sites shows comparatively maximum plantation (trees 69.66±1.52 and shrubs 70.332±.08) in Kathmandu.

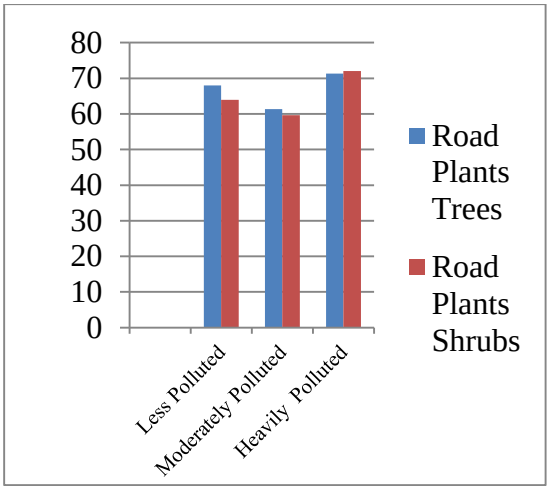

Figure 3. Plants in different sites

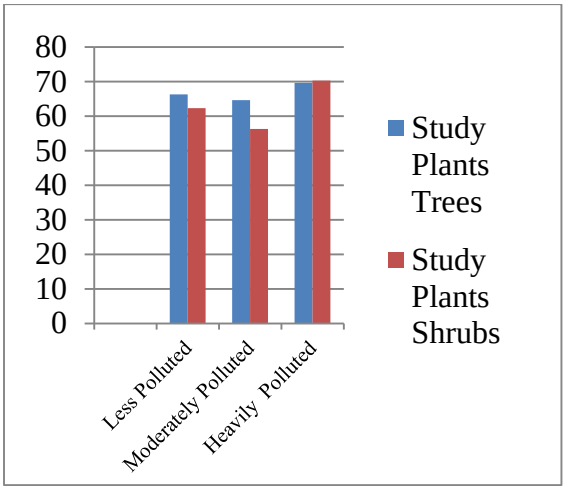

Figure 4. Plants in different sites

Table 1 and Table 2 are represented in Bar Diagrams Figure 3 and Figure 4.

The coverage of plants in different sites is good (Table 1 and Table 2; Bar Diagrams Figure 3 and 4). Field observation describes evergreen plants, many flowers and capacity to survive in the different environment with ornamental purpose.

Figure 5. Study sites, Koteshwor

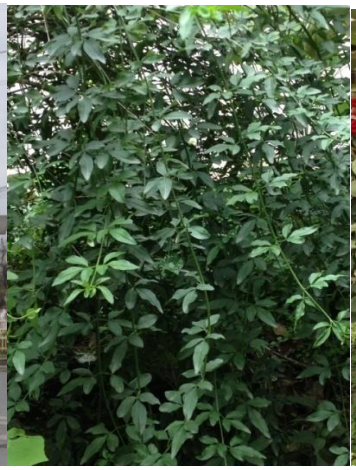

Figure 6. *Jasmine mesnyi*

Figure 7. *Rose indica*

Green street plant coverage makes important results. This gives the ornamental plants for environment management in the roadsides of Kathmandu.

Table 3. Study plants documentations

S. N.	Plants	Heavily Polluted	Moderately Polluted	Less Polluted
1	*B. glabra*	52±2	45±1	41.7±1.7
2	*C. citrinus*	73.33±2.08	52.7±2.08	72±2
3	*D. erecta*	73.33±1.6	51.33±1.52	64.7±1.52
4	*E. pulcherrima*	44±1	51±1.52	55±1
5	*J. mesnyi*	54±1	61.7±1.52	75±1
6	*L. indica*	61±1	54.7±1.52	74.3±0.6
7	*M. enduliflorus*	55±1	44.7±1.52	51±1
8	*N. oleander*	73.7±1.6	55±1	51±1
9	*R. indica*	54±1	51.7±1.52	54.7±0.6

The above table explains the nine study plants and its coverage in the different sites. The heavily polluted sites shows comparatively maximum plantation of species. Tree *C. citrinus* shows 73.33±2.08 and shrubs *N. oleander* has 73.7±1.6.

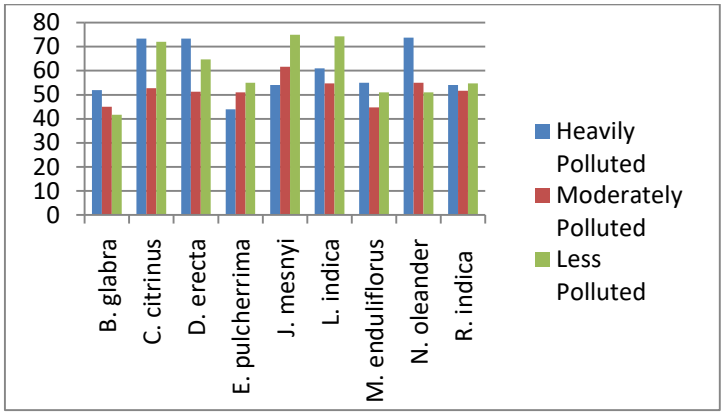

Figure 8. The bar diagrams representation of the coverage of plants

iii) Determine the leaves condition by air pollution on study plants.

Impacts of dust are on normal leaves rather than in glossy leaves. Dust deposition is on leaves as like in different places. The *Duranta species* looks a little clean leaves in the roadsides. Leaves kind and its coverage in the ornamental, environmental management are high in different sites.

The dust deposition on plants in different sites and in seasons

Table 4. Field study, the two plants results are presented.

S.N.	**Study Sites**	**Places**	***Callistemon citrinus*,**	***Nerium oleander***
1	Less Polluted	Kirtipur	Green, leaves and plants are being in good results	Green, leaves and plants are being in good results
2	Moderately Polluted	Bhudhanilkantha	The minimum environmental effects in the dust deposition was obtained	The minimum environmental effects in the dust deposition was obtained
3	Heavily Polluted	Kathmandu	The area is wide. Vehicles, dust were in the environment gives some effects	The area is wide. Vehicles, dust were in the environment gives some effects

Different plants are representative in the fields,Such as trees, shrubs and herbs. Field observation makes beauty, evergreen. The dust in polluted sites, plant with capacity to the environment with ornamental purpose.

Figure 9. *C. citrinus*

Figure 10. *M. penduliflorus*

Figure 11. *Nerium indica*

Figure 12. *N. oleander*

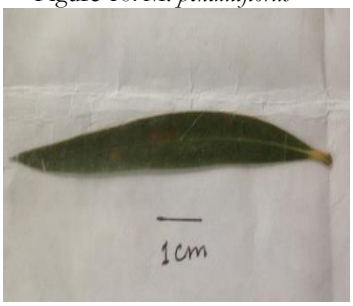

Figure 13. *C. citrinus*

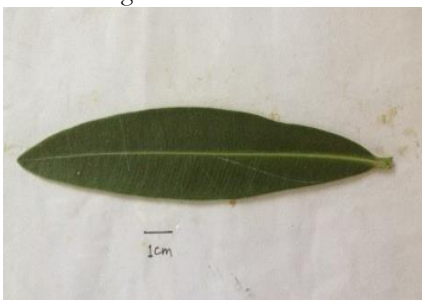

Figure 14. *N. oleander*

The experimental works and the results were presented in the tables. Leaves are for physiological activities of the plant body. The important part, biochemical process and survival, development depends on it. The leaves surface, anatomical structure, purpose and study keep very good in different research for plants and environment. The different pigments and chlorophyll are available in plants

The dust deposition and total Chlorophyll content of *Callistemn* and *Nerium* plants leaves.

Table 5. Study of *Callistemn citrinus*

Study Sites	**Places Name**	**Dust Deposition**	**Total Chlorophyll**
Study sites	Kathmandu	0.004155±0.003513	1.48±0.02309

Table 6. Study of *Nerium oleander*

Study Sites	**Places Name**	**Dust Deposition**	**Total Chlorophyll**
Study sites	Kathmandu	0.001547±0.000788	2.397±0.299

The study of dust deposition and chlorophyll content in these two plants are given in the tables.

Nerium oleander was with less deposition of dust and chlorophyll values is increased (0.001547±0.000788 and 2.397±0.299).

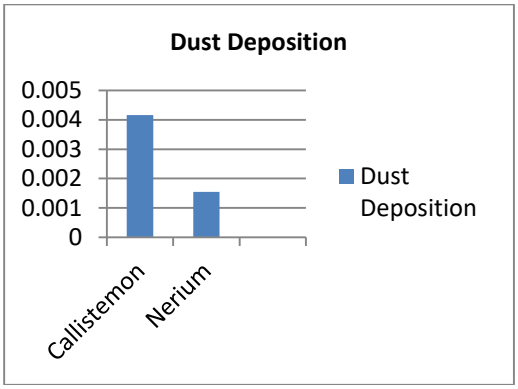

Figure 15. Dust deposition of 2 plants

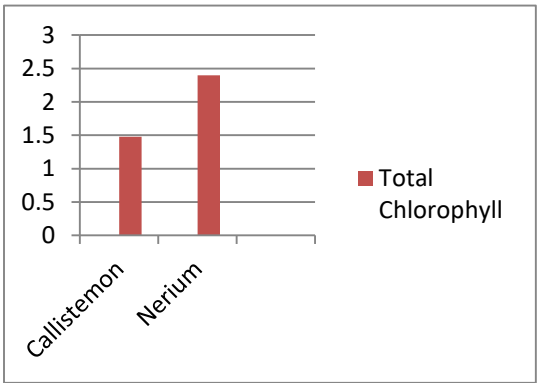

Figure 16. Chlorophyll content of 2 plants

The dust deposited on the surface and chlorophyll results are related with different activities in the leaves and in environment (Table 5 and 6; Fig 15 and 16). It affects in selection of the plants and comparative advantageous in the development programs.

Flagella et. al. (1995) explains chlorophyll concentration to provide rapid and accurate techniques of detecting and quantifying plant tolerance to stress. This implies that trees with higher foliar chlorophyll content could have the ability to withstand adverse weather conditions as would increase their adaptive capacity. Suitable ornamental plants in some areas can be planted to reduce problem as chlorophyll degradation in air polluted conditions. As in international airport sites *L. indica* will be the good work for the development in roadsides. Because it has very nice flowers as suitable in Asian countries.

Morphology and anatomy give some results in floral diversity. Field observation is pleasant, evergreen, capacity to survive in the environment with tolerance resistant and ornamental purpose are collectively for the plants and environments.

Table 7. The description of the morphological structure of plants.

Study Sites	**Places**	***C. citrinus***	***N. oleander***
Less, moderately and heavily Polluted	Kirtipur, Bhudhanilkantha and Kathmandu	Green, long leaves and red flowers are good. Leaves are thick and numerous in plants.	Green, long, broad leaves and pink flowers are good. Leaves are thick, quality, flowers are excellent.

The heavily polluted sites, area is also good and is showing the affirmative influences in the plants and in environments. The coverage of *C. citrinus*, *D. erecta*, *J. mesnyi*, *L. indica* and *N. oleander* has been observed as excellent along the roadside. However *B. glabra*, *E. pulcherrima, M. penduliflorus* and *R. indica* are seen at some places.

Some experimental results of Elements analysis of soil and leaves are also good. Comparatively in different seasons in general different elements are important. Some are benefitted and some are not good in the environment in their higher percentage.

Sample: Soil Season: Winter 017

Table 8. Elements (Metal ions) in the soil collected from different study sites.

Sites	**Al**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Aver age of 3 sites	44996.68 3 ±14998. 92671	1.6±0.026 457513	8.1 13 ±3. 97	81.027 ±43.9 3084	25.47 ±12.12 93253	21033.28±105 91.46343	343. 61 ±24 2.38	30.303±4.29 0108779	41.61± 5.98	58.6 7± 25.1 8	117.23 ±6.92

Al, Fe, Mn, Zn and Cr values are more. Other 6 elements values are also in environment. Co and Cd values are normal. Aluminium value is high (44996.683 ±14998.92671). Iron value is also good.

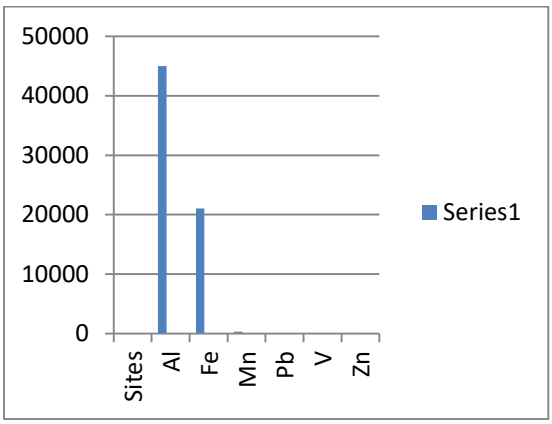

Figure 17. Soil elements values

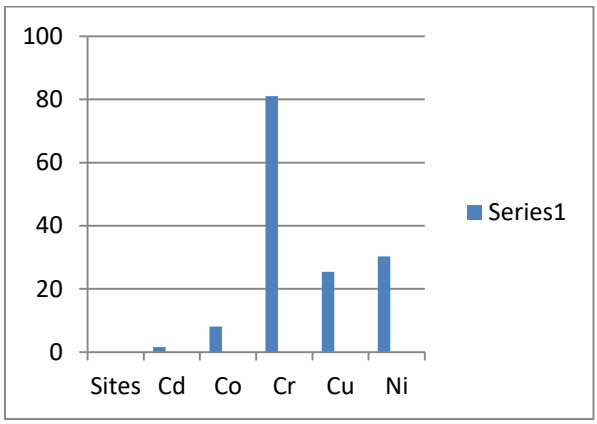

Figure 18. Soil, elements values

Plant: *Callistemon* **Season: Winter 017**

Table 9. Elements (Metal ions) in the leaves collected from different study sites.

Sites	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Average of 3 sites	6.46±0.2967	0.097±0.0445	0.0143±0.0140	1.163±0.3667	5.87±1.7548	139.55±19.938	26.64±9.6034	2.163±0.5300	5.43±2.9154	0.933±0.0208	19.7±4.9450

Fe, Mn, Zn, Ba and Cu values are normal. Other 6 elements values are also in environment. Cd and Co values are normal.

Fe>Mn>Zn>Ba>Cu>Pb>Ni>Cr>V>Cd>Co

The lowest values showed to Cobalt (**Co)** in ***Callistemon*** is 0.0143mg/kg at polluted site and in winter/summer season. The values of Cadmium (Cd) and Cobalt (Co) elements are low in all study plants, in different season and at different sites.

Plant: *Nerium* **Season: Winter 017**

Table 10. Elements (Metal ions) in the leaves collected from different study sites

Sites	Ba	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
Average of 3 sites	3.99±1.28	0.476333±0.53	0.032667±0.022	2.231±0.65	10.2±2.420792	325.93±121.7286	74.85±45.46	4.7333±5829	1.84±0.650	1.35±0.23	26.44±8.82

In comparison with seasons Fe, Mn, Zn and Cu value is more in different study sites.

The elements are in these series, Fe>Mn>Zn>Cu>Ni>Ba>Cr>Pb>V>Cd>Co

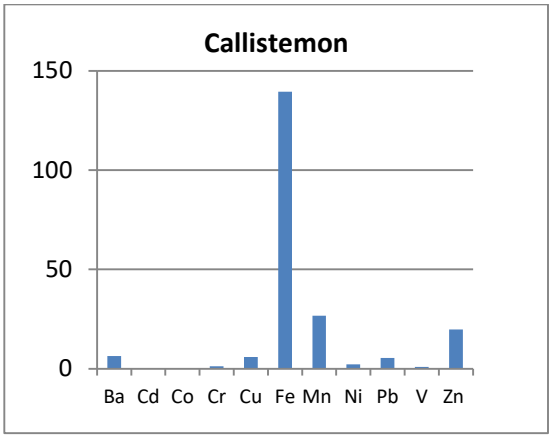

Figure 19. *Callistemon*, elements values

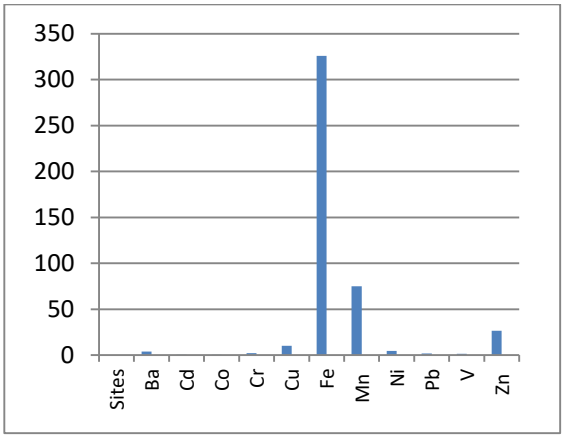

Figure 20. *Nerium,* elements values

The *Callistemon* and *Nerium* leaves were used in the elements analysis. Different values of 11 elements are pr Iron and manganese are in maximum values (*C. citrinus* 39.55±19.938 and 26.64±9.6034) and (*N. oleander* 10.2±2 and 325.93±121.7286).

The study of heavy metal (HMs) contamination of environment is of great interest due to health .and environmental impacts. The leaves of common trees i.e. *Azadirachta indica, Butea monosperma, Eucalyptus, Ficus religiosa, Mangifera indica* and *Tectonia grandis* were selected for assessment of the HMs contamination as bioindicator. The concentration of HMs (i.e. As, Fe, Cr, Mn, Cu, Zn, Cd, Pb and Hg) in the tree leaves was observed, ranging from 2.8 - 43, 728 - 5182, 8.6 - 49, 48 - 1196, 43 - 406, 79 - 360, 1.12 - 1.65, 1.6 - 16.4 and 0.13 - 0.76 mg/kg, respectively. The concentration, enrichment and sources of the HMs in the leaves are described. *Azadirachta indica* leaves, accumulating higher concentration of the HMs, showed a higher efficiency as bioindicator for the urban pollution, Khageshwar, S. P. et.al., 2015. The different values promote the research results and sites environments.

Need to be aware and make proper choices concerning which Plants are best suited for the surrounding urban environment. We need evergreen and broad leaf trees, these trees have the capacity to absorb carbon dioxide and reduce noise levels. The country has diverse environmental resources; biodiversity, water, forest, land, climate and weather. Landslide, soil erosion, deforestation, forest fires have caused the land to deteriorate, water sources to decreased away, rivers to flood, biodiversity to deplete and people to migrate into urban areas and elsewhere, Poudel, K. 2010. Many Plant species are growing and applicable for bio-indicator, remove unwanted environmental problems and maintaining greenery.

2) Morphological structure, *Callistemon citrinus* and *Nerium oleander*. Structural analysis of leaves: and anatomical structure (micromorphology; epidermal cells, stomatal index / frequency, stomatal size).

1. Plant*: Callistemon* Season: Winter 017 / 018
   Within the two seasons, winter and spring seasons have taken and different anatomical structures of plant leaves were studied, Figures 21-23.

Table 11. Different anatomical structures of plant leaves were studied.

Sites	**No. of Stomata**	**Clogged Stomata**	**Stomatal Pore Size Length (L)**	**Stomatal Pore Size Breadth (B)**	**Subsidiary Cells**	**Epidermal Cells**	**Stomatal Index**
Kathmandu	33.607± 4.509	2.633±1.055	5.11± 1.067	4.093± 0.502	6.503± 0.335	181.687± 14.535	15.699± 0.898

The number of stomata and stomatal index are calculated (33.607± 4.509 and 15.699± 0.898).
Structural analysis of leaves of selected plants, morphological structure is being analyzed.

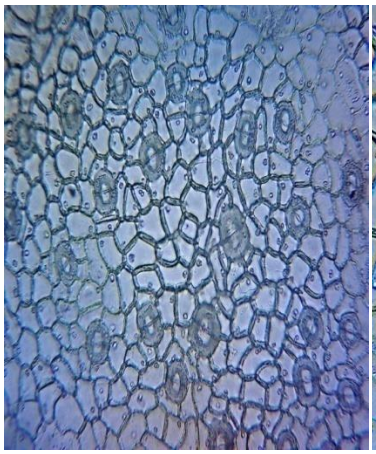

Figure 21. *Callistemon citrinus*

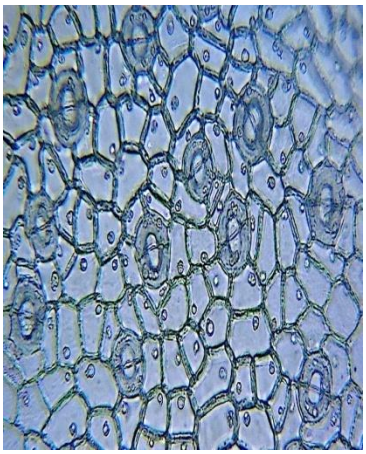

Figure 22. Enlarge cells

Figure 23. Plant diversity richness

Figure 24. *Nerium oleander.*

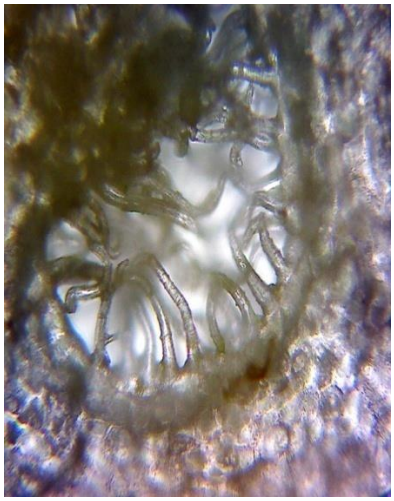

Figure 25. Sunken stomata

Figure 26. Floral diversity in a good planning, Kathmandu.

Study of anatomical, cells and its different measurements values for the plants.

Sarma et. al. (2017), leaf thickness was found to increase in all the roadside plants; however, maximum leaf thickness was observed in *C. sofera* than other plant species. It is also evident that plant under the environmental stress, produces thicker leaves as an adaptive response (Gostin, 2009) and to cope with the stress of vehicular dust; roadside plants may produce thicker leaves.).

Light capturing capacity of leaf via lowering photosynthetic rates, clogged stomata and increased foliage temperature are the cause behind dust deposition reported earlier. Stomatal index has been proven to be an indicator of

environmental stress (Gostin 2009). (Reduction of the stomata index of the roadside plant may be due to shading effect of dust layers, which may block the stomata and reduce the photosynthesis rate of roadside plants, Khan et al. 2015).

Conclusions

The ornamental plants of Kathmandu are giving green vegetation streets. In general, greenery is the achievements in study area; Kathmandu. Along with this, the work is useful in the other industrial cities of the country. Planting trees is not enough to beautify the roads and clean the air. It needs to be aware and make proper choices concerning which trees are best suited for the surrounding urban environment. Evergreen and broad leaf trees have the capacity to absorb carbon dioxide and reduce noise pollution levels. Research results and findings in biodiversity, environments, pollutant effects, tolerance index of plants, structure, plants beauty and adaptation in the roadsides of urban area are appropriate in different highly organizes government programs. Analysis of plants and soil in elements has different adaptive values in environment.

The government programs, managements are excellent. Ornamental environment of the plant species with proper management in the main city is encouraged. In different places either in sides of the road or in the central; plantation and greenery program is suitable. The civic important in the national and international exploration are useful. The work, implementation, popularity of the programs with is successful. The responsibilities of roadside plants are of Department of Metropolitan Traffic Police Office and Department of Road, Municipality (Metropolitan Office), Government of Nepal are with united efforts.

Recommendations

1) Study programs are very useful. The distribution pattern, floral diversity and coverage of selected species have good places.
2) It is very important to be in the coming days with still beauty as possible. In this context, the scientific results and the program are nice. The perfect results and appreciation are available in different sites of the Kathmandu valley.
3) The purpose for ambient air quality, bioindicators, monitoring and evaluation in different sites resulted environmental management is increased.
4) The successful results for documentation, presentation, uplift and influences are existed.

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Conflict of Interest

The author declares no conflict of interest.

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Khulna University Studies Special Issue ICES: 25-29: 2023

ORIGINAL ARTICLE **LIFE SCIENCE**

HEALTH AND ECONOMIC IMPACTS OF CLIMATE CHANGE IN RURAL BANGLADESH AND OPTIONS TO GO THROUGH

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Abstract

Bangladesh is one of the most vulnerable countries due to its unfavorable geographic location, flat and low-lying terrain, dense population, and high levels of poverty. This research aims to evaluate the effects of climate change on the availability of water, housing, financial support, sanitation, and health status in Bangladesh's southwest coastal area and to investigate adaptation options. A descriptive cross-sectional study was conducted in a disaster-prone village *Pratapnagar* of Assasuni Upazila in Satkhira, Bangladesh from September to October 2021. A questionnaire survey was conducted to 100 male respondents 30-70 years of age who were suffering because of a breach of the embankment and could not recover from the loss due to supercyclone Amphan (category 5) from May 16 to May 21, 2020. The mean age of the respondents was 42 years (SD = 9.14). Fishing is the main source of income for 46% of the respondents. The study also revealed that 40% of the respondents had lost their dwellings and are still submerged in water, and 32% of the respondents have lost their agricultural land. 55% of respondents have lost their livestock and 35% of the respondents use riverside open toilets; while 55% do use toilets but water submerges the toilets during high tide. During the last 2 months of the study period, 82% of respondents had recurrent diarrhea, 42% had respiratory diseases and 32% have been infected with skin diseases. Assasuni was the victim Upazila, where cyclone Amphan hit, and most of the drinking water sources were devastated. This socio-economic impact falls not only on the people in the coastal belt but also on the people of the whole country.

Keywords: Climate change, Health impact, Protapnagar, Assasuni, Bangladesh

Introduction

Satkhira is one of the most vulnerable districts in Bangladesh and is linked to salinity intrusion, tidal flooding, water logging, cyclones and storm surges, and drought. Satkhira's main sources of revenue include agriculture, fishing, and livestock. However, climate-induced rapid and slow-onset disasters damaged the natural ecology in this area, making life and livelihoods more difficult for the inhabitants (Rahaman et al., 2019; Islam et al.,2013). Temperature variations, unpredictable rainfall patterns, salinity intrusion, droughts, extreme heat waves, and cyclones during the last decade such as Cyclone Sidr (2007), Cyclone Bijli, Cyclone Aila (2009), Cyclone Mahasen (2013), Cyclone Roanu (2016), Cyclone Fani and Cyclone Bulbul (2019), and Cyclone Amphan (2020), has made their lives and livelihoods of the inhabitants miserable (Kabir et al., 2014).

The effects of climate change might be both direct and indirect. It has detrimental effects on human health, fishing biology, aquatic ecosystems, and freshwater resources and also increased incidences of water-borne diseases e.g., diarrhea, cholera, dysentery, etc. The most recent super cyclonic tropical storm, Amphan, caused significant damage in Bangladesh and West Bengal. Amphan began as a low-pressure area on May 13, 2020, around 300 kilometers (200 miles) east of Colombo, Sri Lanka. It soon strengthened into an exceptionally violent cyclonic storm on May 17, 2020. Over a million people were affected in Bangladesh across nine districts in the divisions of Khulna and Barisal, with deaths and damage to houses, infrastructure, livelihoods, and water and sanitation facilities, with

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approximately 18,235 water points and 40,894 latrines destroyed in the most impacted districts. (IFRC., 2020). Therefore, this study's goal is to provide information on how people may protect themselves from climate change and determine its harmful effects on health.

Materials and Methods

A descriptive cross-sectional study was carried out in a most vulnerable coastal village, namely, *Pratapnagar* of Assasuni Upazila in Satkhira district during September-October, 2021. Cyclone Amphan washed away several points of the Kopotakkho and Kholpetua river embankments in May 2020. Approximately 30,000 residents in Assasuni Upazila in Satkhira are still living in temporary huts on the embankments of the upazila's Protapnagar union after losing their homes to the Kopotakkho River. One hundred male respondents 30-70 years of age were selected through judgmental sampling and a structured questionnaires survey was done to understand the financial losses the respondents suffered in the wake of the natural catastrophe, how they dealt with such climate change-related disasters, how the natural calamities affected their patterns of livelihood, their health, and other aspects.

Results and Discussion

The study focuses on coastal households residing at *Protapnagar* village of Assasuni Upazila under the Satkhira district. The result showed that Among 100 respondents, the mean age of the respondents was 42 (SD = 9.14). Fishing is the main source of income for 46% of respondents.

Table 1. Socio-demographic characteristics

Age interval	**Number of respondents**
30-39	49 (49%)
40-49	35 (35%)
50-59	08 (08%)
60-69	08 (08%)
Total	100 (100%)
Source of income	
Fishing	46 (46%)
Brickfield	24 (24%)
Van puller	14 (14%)
Labour	16 (16%)
Total	100 (100%)
Monthly income (Taka)	**Numbers of family**
<10000	62 (62%)
>10000	38 (38%)
Total	100 (100%)

Table 2. Economic loss for breaking the embankment

Economic loss	Number of respondents
Loss of Home	
Submerged underwater	40 (40%)
Washed away in the river	18 (18%)
Tidal water comes into the homes	30 (30%)
Being repaired	22 (22%)
Total	100 (100%)
Loss of agricultural land	
Yes	32 (32%)
No	68 (68%)
Total	100 (100%)
Loss of livestock	
Yes	55 (55%)
No	45 (45%)
Total	100 (100%)
Loss of ponds	
Yes	25 (25%)
No	75 (75%)
Total	100 (100%)

Water, sanitation, hygiene, and healthcare facilities are crucial for human health and well-being because their absence impairs quality of life and impedes basic human rights. In the present study, 40% of the respondents' houses are still underwater, and 32% of respondents have lost their agricultural land. 55% of respondents have lost their livestock. Around 30,000 residents in Assasuni Upazila in Satkhira are still living with high tides nearly 15 months after supercyclone Amphan. Coastal embankments are important for protecting human habitats and croplands. As well they are important in increasing agricultural yields and enhancing coastal livelihoods. The coastal region has a larger proportion of the population living below the absolute poverty line than the rest of the country (Imam et al., 2021).

The result showed that during the last 2 months after Amphan hitting the region people in the *Protapnagar* and *Kurikaunia* villages have been more affected by various diseases. Due to several natural calamities, the weather was not favorable for keeping people healthy. In the present study, 82% of the respondents had suffered from diarrhea during the last 2 months after Amphan presumably because of contamination of potable water due to salinity (Kabir et al., 2014). Similar findings were recorded in West Bengal's Midnapur area, where diarrhea was the most prevalent morbidity among flood-prone residents. The incidence of diarrhea, other enteric disorders, and respiratory infections was found to be considerably greater ($p<.05$) among cyclone-affected residents compared to those who were not. (Biswas et al., 1999).

Table 3. Number of responders who had a sickness in the previous two months

Disease	Number of respondents (%)
Diarrhea	
Yes	82 (82%)
No	18 (18%)
Fever and respiratory infection	
Yes	
No	42 (42%)
	58 (58%)
Skin disease	
Yes	32 (32%)
No	68 (68%)
Conjunctivitis	
Yes	40(40%)
No	60(60%)
Ear infection	
Yes	35 (35%)
No	65 (65%)
Total	100 (100%)

In the present study, 75% of respondents developed fever and respiratory infection during the last 2 months after the Amphan respectively. They did not classify the fever whether that was dengue or typhoid fever. Overcrowding, inadequate ventilation, and poor nutrition can all raise the risk of respiratory illnesses, especially in crowded shelters during the winter. The current study shows, that 62% of respondents had skin disease where in most cases, superficial fungal infection and bacterial skin infection were responsible. Several of these illnesses were identified clinically and lack microbiologic or laboratory validation. Bacterial colonization on macerated skin may have risen due to excessive exposure to polluted water, friction, high humidity, and an unsanitary atmosphere.

Table 4. Number of respondents according to the source of drinking water and condition of latrine

Source of drinking water	Number of families (%)
Tubewell	75 (75%)
Pond+Tubewell	19 (19%)
Rain water	06 (06%)
Condition of latrine	**Number of families (%)**
Riverside open toilet	35 (35%)
Tidal water comes into the toilet	55 (25%)
Repaired but unhygienic	10 (10%)
Total	**100 (100%)**

The sanitary status of the coastal region differs from that of the rest of the country in many ways. Many villages are still far from having complete sanitary coverage. Assasuni represents one of the rural communities with inadequate sanitation. According to the field study results, 35% of respondents used the riverbank open toilet, and 55% utilized the toilet, but water enters the toilet during high tide. The coastal area's drinking water supplies are harmed by topographical disadvantages and are frequently subjected to natural hazards. According to the current study, tube wells constitute the primary drinking water source for 75% of the households in the study region. Despite the fact that these

individuals face several issues as a result of natural disasters, they travel long distances seeking better drinking water in locations where tube wells are plentiful.

Conclusions

The coastal zone of Bangladesh is very prone to natural disasters. People on the coast almost yearly lose their valuable property and lives due to the cyclone. Early recovery and reconstruction efforts will be required for a medium to long term (06 to 12 months) to repair damaged infrastructure, particularly embankments, roads, buildings, safe drinking water supplies, sanitary facilities, etc. Priority should be given to restoring livelihoods, rehabilitating agricultural land affected by saline water, and enhancing preparedness and community support mechanisms.

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Conflict of Interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE **LIFE SCIENCE**

LEAF PIGMENT AND TOTAL CARBOHYDRATE CONTENT AT EARLY STAGES OF HERITIERA FOMES BUCH. SEEDLINGS GROWN AT THREE SALINE ZONES OF THE SUNDARBANS, BANGLADESH

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Abstract

The experiment was conducted to find the leaf pigments and carbohydrate content in *Heritiera fomes* at different growth stages exposed to different salinity levels in the Sundarbans areas, Bangladesh. Three leaf pigments, viz., chlorophyll a, chlorophyll b, and carotenoid, total chlorophyll, and total carbohydrate content of *Heritiera fomes* Buch. Ham seedlings were determined in the leaves grown in the oligohaline, mesohaline, and polyhalite zones at early (6 and 9 months) and survival ages (30 months) following standard methods. Leaves from seedlings at the age of 30 months (survival aged) show relatively high leaf pigments than that of the 6 and 9-month-old seedlings grown in the three saline zones. Total chlorophyll content in leaves was found low in the oligohaline zone at the early and survival ages, but higher in the polyhalite zone at the early ages, and higher in the mesohaline zone at the survival age. Chlorophyll b was relatively higher than chlorophyll a. Leaves of 30 months seedlings showed relatively higher chlorophyll a than chlorophyll b at the polyhalite zone, but higher chlorophyll b was noted at the mesohaline zone. The highest content of total carbohydrates was observed in the shoots of 6, 9, and 30 months ages *H. fomes* seedlings in the polyhalite zone, while the lowest was in the mesohaline zone. At the same age periods in the mesohaline zone, the roots of the *H. fomes* seedlings showed relatively higher carbohydrate content and lower at the 30 months seedlings in the all saline zones. Chlorophyll a was found to be more sensitive than chlorophyll b and carotenoid at different salinity levels tested. Higher carbohydrate content was discovered as an additional mechanism to prevent salt toxicity at early ages of high saline conditions (polyline zone).

Keywords: Leaf pigment, chlorophyll, carbohydrate, oligohaline, mesohaline, polyline salinity zones

Introduction

The Sundarbans are the largest and most unique natural mangrove forests in the world and have been shown as the outer deltas of the Ganges, Brahmaputra, and Meghna rivers. About 10,000 km^2 of total forest area is located in the southwestern parts of Bangladesh and West Bengal, India. Among them, 62%, or 6017 km2, of the Sundarban forest in the south-western part of Bangladesh between latitudes 21°31' N and 22°30' N and between longitudes 89°18' E and 90°18' E lies in the Khulna region (Katebi, 2001). In this area, about 4016 km^2 is occupied as forestland, and the remaining 1874 km^2 is made up of rivers, creeks, and canals (Wahid, 1995). The natural vegetation of the Sundarbans is composed of sixty-six halophytic species, mainly herbs, shrubs, climbers, and tree species (Chaffey et al., 1985). However, the trees and shrubs are dominant in the ecosystem. Among them, tree species are belonging to 30 genera under 22 families. Generally, members of Rhizophoraceae, Avicenniaceae, and Sonneratiaceae family are dominating among the most mangrove forests.

The Sundarbans is a region of transition between the freshwater of the rivers originating from the Ganges and the saline water of the Bay of Bengal (Wahid et al., 2007). So, the growth and distribution of the mangroves depend on different levels of salinity (Chanda & Dutta, 1986). Though mangroves are remarkably adapted to tidal and coastal land

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through their ability to live in poorly oxygenated sediment and can tolerate inundation by salt water through physiological and chemical mechanisms *(*Morley, 2000). In general, wetland ecosystems are physical and dynamic links between terrestrial and aquatic resources developed by integrated relationships (Erickson, 1994; Curry & Meguire, 2002). In this development, they show a combined relationship between the atmosphere, soil, and water by developing mineral cycling in wetland ecosystems (Dykyjova & Ulehlova, 1998; Mitsch & Gosselink, 2000). However, increasing sediment salinity generally affects the growth of mangroves, but extremely high salinity has been found to negatively influence the growth of mangrove seedlings (Clough et al.,1982). The osmotic adjustment of water uptake of mangrove seedlings applied distinct strategies or physiological adaptation among the different stages of seedling's growth with their differential ability of salt tolerance (Shan et al., 2008). The condition of the juvenile or early stage of the tree is quite different from the adult stage (Khan et al., 2006). At the adult stage survivability of mangroves occurred due to the presence of sequester and compartmentalization in the presence of a high concentration of ionic compounds (salt, including metals) in the soil. However, at the early or juvenile stage, they are affected by high ionic content. Biochemical mechanisms as well as various metabolites of the species counter the high osmoregulatory of salts that accumulate from compatible solutes (Taskemura et al., 2000). zone. It was reported that at the high saline condition, higher carbohydrate content was regarded as an additional mechanism to prevent salt toxicity (Rathert, 1982). However, leaf pigment content was found to decrease with the increase in salinity (Mitra & Banerjee, 2010; Shinde & Bhosale, 1985; Alamgir et al., 1992). So, in unfavorable environmental conditions, mangroves accumulate different types of ions or osmolytes like proline or carbohydrate, which allow them to grow and survive by preventing water loss and ionic toxicity of the plant cells (Rajaravindran & Natarajan, 2012).

Several factors contribute to the productivity of a forest for future stocking and its continuity. Although propagules of mangroves can exploit their habitat (the intertidal zones) by developing physiological, anatomical, and morphological adaptations to waterlogged or saline environments (Saenger, 2002), they are negatively influenced by excessive salinity (Wang et al., 2007). Due to changing environmental conditions, they face obstacles to their physiological activities and the early natural growth of the species (Ungar, 1995; El-Keblawy & Al-Rawai, 2005). Under these circumstances, the present paper tried to determine leaf chlorophyll and carbohydrate content at the early and mature growth stages of *Heritiera fomes* seedlings as well as the best salinity level for the establishment of such mangroves.

Materials and Methods

Heritiera fomes Buch-Ham species were selected for the present investigation. Three different areas of the Sundarbans mangrove forest were chosen as an experiment sites viz. Chandpai, Jungra, and Munshiganj as oligohaline (4-8 dS-1m), mesohaline (8-15 dS-1m), and polyline (> 15 dS-1m) zones (SRDI, 2010). The Chandpai and Jungra zones are in the Chandpai range and the Munshiganj zone is in the Satkhira range of the Sundarbans forest. *Heritiera fomes* seedlings were collected from naturally regenerated sample plots randomly at the ages of 6 months, 9 months, and 30 months old as an early stage and a survival stage was collected from the selected field of three saline zones of the Sundarbans mangrove forest.

Determination of leaf pigment

Leaf pigment (chlorophyll a, chlorophyll b, and carotenoid) was determined using leaves from 6, 9, and 30 months old seedlings collected from three saline zones of the Sundarbans. After collection, 0.5g of leaves were chopped into small pieces and homogenized in a mortar with 10 mL of 100% acetone, and the supernatant was decanted into a centrifuge tube. To the residue, 10 ml of acetone was added for further extraction, and the process was repeated for total extraction of the pigments. The combined extract was balanced and centrifuged in the cold (0-4°C) at 1000g for 10 minutes, and the clear supernatant was taken and volume was made up to 50 ml in a volumetric flask by adding more 100% acetone. The concentration of chlorophyll a, b, and carotenoids were determined following Wettstein (1957) by taking absorbance in a spectrophotometer at 662 nm, 644 nm, and 440.5 nm for chl a, b, and carotenoids, respectively, after proper dilution against 100% acetone.

The formula used for calculation was:

Concentration of chlorophyll-a, ca = 9.784×E662 - 0.990×E644 mg/L

Concentration of chlorophyll-b, cb =21.426×E644 - 4.650×E662 mg/L

Concentration of carotenoids-car, c car =4.695×E440.5 - 0.268 (chl.a+chl.b) mg/L

$$A = \frac{C \times U \times V}{1000 \times Wt}$$

Where,

A = Pigment content mg/g fresh weight
C = Concentration of the corresponding pigment in mg/L
V = Volume of the pigment
U = Dilution factor
Wt = Freash weight of the leaf,g.

Determination of carbohydrates in the seed and the plant materials

Total carbohydrates content was determined at the root and shoot of the 6 months, 9 months as early stage, and 30 months as survival stage of the *Heritiera fomes* seedlings.

The anthrone method (Hedge and Hofreiter 1962) was used for the determination of the total carbohydrate content of *H. fomes* seedlings. This compound forms a green-colored product with an absorption maximum of 639 nm.

Analysis the samples

By adding extra distilled water, all of the sample and standard solution tubes were filled to 1ml. Then they added 4 ml of anthrone reagent and heated them for eight minutes in a boiling water bath. Then, after cooling, read the OD at 630nm. A standard curve was drawn by plotting the concentration of the standard on the x-axis versus absorbance on the y-axis.

Co-efficient was calculated following the formula.

$$\text{Co- eff.} = \frac{\text{Concentration of the standard solution}}{\text{OD for each standard solution}}$$

Results and Discussion

a. Leaf Pigment (Chlorophyll a, Chlorophyll b, and Carotenoid) content at the early (6 and 9 months) and survival age (30 months) of *Heritiera fomes* seedlings grown at the three saline zones of the Sundarbans

Three leaf pigments like chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b content were determined in the leaves of *Heritiera fomes* seedlings at the ages of 6, 9, and 30 months in three saline zones viz., oligohaline, mesohaline, and polyline of the Sundarbans. At the three saline zones, 30-month-old Heritiera fomes seedlings had a higher content of chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b. But at the ages of 6 and 9 months, the leaves of the species contain relatively higher chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b at the polyhaline zone (Munshiganj) but lower at the oligohaline zone (Chandpai). In 30 months, *Heritiera fomes* had relatively higher chlorophyll a and carotenoid content in the Munshiganj (strong saline) zones, but the Jungra (moderate saline) zone had relatively higher chlorophyll b and chlorophyll a+b content. As observed by many authors (Mitra & Banerjee, 2010; Shinde & Bhosale, 1985; Alamgir et al., 1992), chlorophyll content in the leaf was less affected in less saline conditions than that in the leaf under high salinity. Although leaf pigment content decreased as salinity increased, similar results to the present findings are also observed in other mangroves by many authors (Rajesh et al., 1998; James et al., 1999). In the current study, chlorophyll b content was found to be relatively higher than chlorophyll a. in the leaves of *H. fomes* grown in the three saline zones. Higher chlorophyll b content is also noticed by previous findings (Rajaravindran & Natarajan, 2012; Ramani *et al.*, 2006) and also remarked by other findings that Chlo. b found comparatively higher saline tolerance than Chlo.a (Singh & Dubey, 1995)

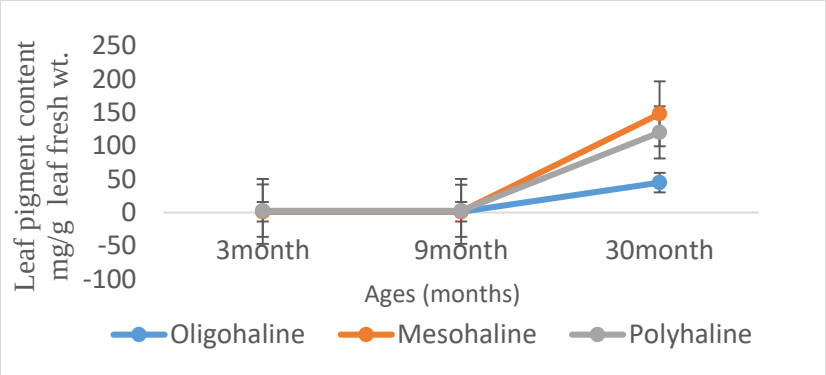


Figure 1. Total Chlorophyll content in the leaves of *Heritiera fomes* seedlings at the three saline zones of the Sundarbans

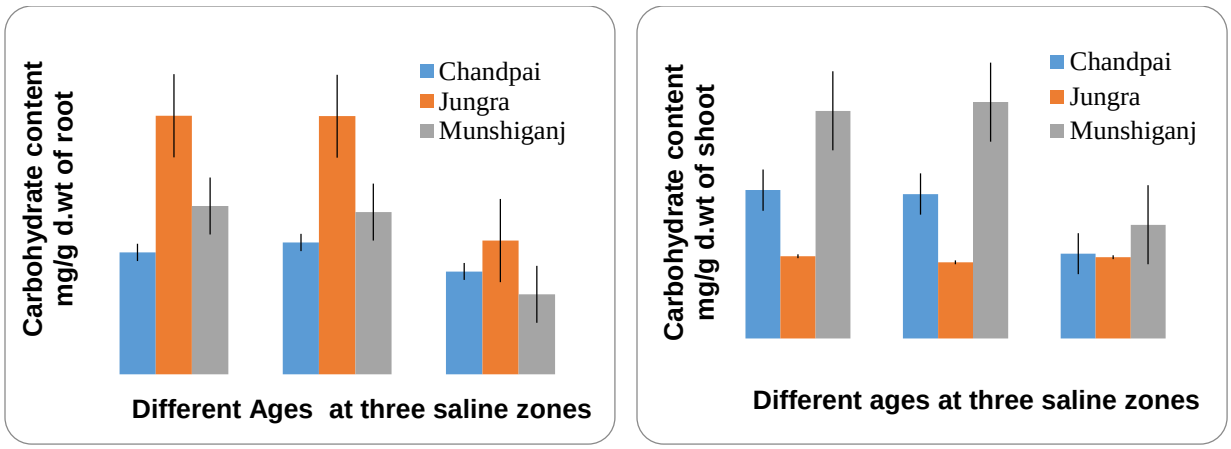
Table 1. Chlorophyll a, Chlorophyll b, and Carotenoid content in the leaf of *Heritiera fomes* Buch, Ham seedlings at the early (6 months) and the survival (30 months) ages at three saline zones of the Sundarbans

Ages	Saline zones	Leaf pigment content mg/g leaf fresh wt.		
		Chlorophyll a	Chlorophyll b	Carotenoid
6 month	Oligohaline	0.50 ±0.01	0.98 ±0.01	0.52 ±0.02
	Mesohaline	0.52 ±0.02	1.30 ±0.01	1.07 ±0.01
	Polyhaline	0,59 ±0.01	2.44 ±0.01	1.28 ±0.01
9 month	Oligohaline	0.48 ±0.02	0.94 ±0.01	0.44 ±0.01
	Mesohaline	0.51±0.01	1.24±0.01	0.90 ±0.05
	Polyhaline	0.61±0.01	2.33±0.01	1.26 ±0.12
30 month	Oligohaline	44.47±0.87	67.32±1.51	12.48 ±0.08
	Mesohaline	60.50±0.33	87.62 ±0.33	15.66 ±0.34
	Polyhaline	77.84±1.03	42.43±0.40	26.56 ±0.63

b. Carbohydrate content in the seedlings of *Heritiera fomes* at the early (6 and 9 months) and survival ages (30 months) grown at three saline zones of the Sundarbans

Total carbohydrate content was determined in the root and shoot of early (6 and 9 months) and survival age (30 months) seedlings of *Heritiera fomes* Buch. Ham is grown at three saline zones viz. oligohaline, mesohaline, and polyhaline zones of the Sundarbans.

At the early (6 and 9 months) and survival (30 months) both ages, roots of the *H. fomes* seedlings showed relatively higher carbohydrate content grown in the mesohaline zone. However, among all ages (early and survival ages) relatively lower carbohydrates were found in the roots of 30 months old seedlings grown at the all saline zone. The highest content of total carbohydrates was found in the shoots of early (6 and 9 months) and survival (30 months) both ages *H. fomes* seedlings grown in the polyline zone, while the lowest and almost similar carbohydrate content was found in the mesohaline zone. At the high saline condition as a non-tolerant of high salinity *H. fomes* seedlings faced many obstacles like disturbances in metabolism. Previous findings show that in that situation plant tissues accumulate more carbohydrates as a dominant osmoregulation compound to prevent salt toxicity by acting as an additional mechanism. (Popp et al., 1985; Rathert, 1982).



(A) (B)

Figure 2. Carbohydrate content in the root (A) and the shoot (B) of *Heritiera fomes* seedlings at early (6 and 9 months) and survival (30 months) ages in three saline zones of the Sundarbans

Conclusion

Heritiera fomes Buch-Ham, as a moderately salinity-tolerant species, influenced the leaf pigments, viz., chlorophyll a, chlorophyll b, carotenoid, total chlorophyll, and carbohydrate content at the early and survival ages with three different salinity levels in the Sundarbans mangrove forest. The survival age (30 months) showed a relatively higher content of leaf pigments (chlorophyll a, chlorophyll b, and carotenoid) in the three saline zones. However, at the same ages, total chlorophyll (leaf pigments) content in leaves and carbohydrate content in roots of *H. fomes* species were comparatively higher in the mesohaline zone. Furthermore, in the shoots of early and survival, both ages of *H. fomes* species remarked the highest content of total carbohydrate grown in the polyline zone as a dominant osmoregulation compound to prevent salt toxicity by acting as an additional mechanism for survival.

Conflict of Interest

The authors declares no conflict of interest.

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ORIGINAL ARTICLE

LIFE SCIENCE

INSECT-INDUCED FOLIAR GALLS: A CROSS-TALK AMONG PHYTOHORMONES FOR TISSUE GROWTH AND ENDOGENOUS DEFENSE

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Abstract

Insect-induced gall tissue has a unique ability to influence its hosts' phenotypic expression. When plants are stressed by insects, phytochemical manipulations in galling tissue strengthen the plant's resilience to subsequent herbivore attacks, and as a result, the damaged plant tissue regenerates and rejuvenates. Gall tissue development and differentiation are initiated by changes in and modulation of a variety of phytohormones in the affected galling sites. Such hormonal changes ultimately boost the plant's ability to respond to herbivore invasions. In this study, the insect-induced gall tissues of three model plants—mature and immature galls and non-gall tissue—were evaluated for five phytohormone gradients. Phytohormone gradients are continually altered and compared from non-differentiated (non-gall) tissue to moderately (immature gall) and highly (mature gall) developed tissue. The results indicate that phytohormones serve a dual role in stimulating the plant's endogenous defense and promoting tissue growth, pointing to a complex chemogenesis process in galling tissue associated with developing neoplasm and plant defensive responses. Tissue abnormalities in galls are thought to have resulted from the herbivore's interactions with the plant on which it infests. Insects' ovipositing fluids or oral discharge may have also contributed to the accumulation of phytohormones in the stressed and wounded tissue. Foliar galls are thus a sign of manifestation of insects' adaptation since the herbivore and their hosts are likely to co-evolve in the context of chemical adaptation.

Keywords: Foliar gall, phytohormone gradients, plant-herbivore co-evolution.

Introduction

Insect-plant interaction is a dynamic process during which both sides often create defensive mechanisms to deceive one another (Mello & Silva-Filho, 2002). Plants and insects have evolved a number of defense strategies against one another over time (War et al., 2012; Agrawal & Konno, 2009). Plants can "directly defend" themselves by changing the herbivore's physiology and behavior or "indirectly defend" themselves by luring in the herbivore's natural predators (Tiku, 2021). According to War et al. (2012), the direct defense mechanism employs physical or chemical barriers to impede herbivores from grazing, resulting in immediate adverse consequences for the herbivores. On the other hand, the phenomenon of "indirect defense" diminishes the frequency of herbivore consumption by attracting natural competitors such as parasitoids and predators (Agrawal & Konno, 2009; Tiku, 2021). As a result, the involvement of external intervention leads to an augmentation of indirect defense mechanisms in plants, concurrently strengthening their inherent ability to withstand herbivorous attacks directly (Ullah et al., 2018).

The gall complex serves as a notable example of the co-evolutionary relationship between herbivores and plants (Meyer, 1987; Shorthouse & Rohfritsch, 1992; Winde & Wittstock, 2011; Melnyk, 2017; Minelli, 2018). As a consequence, the plant-generated galls accommodate the invaders; some of them are friends, but the majority of them are foes. Plant galls, or 'cecidia', develop into tumor-like organs where their cells lose adhesion to surrounding tissues and multiply faster than non-gall tissues. As a result, the development of galls can lead to new growths in various plant organs, which are typically the result of hypertrophic and hyperplastic processes. A variety of parasitic organisms known as parasitic gall-inducers, or cecidozoans, are responsible for this neoplasia (Orlovskis & Hogenhout, 2016). The gall-inducer stimulates plant tissue regeneration in response to damage by modifying the developmental pathway through physiochemical mechanisms (Pfunder & Roy, 2000; Goethals et al., 2001). The concept of the "resource sink" model (Larson & Whitham, 1991; Fay et al., 1993) describes gall tissue as a "dynamic reserve" that has the potential

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to exert substantial influence on plant growth and metabolism. Auxin (IAA) and cytokinin (CK) are two plant hormones that have been observed to enhance the growth of abnormal outgrowths in gall tissues (Hartley & Miles, 1999; Tooker & De Moraes, 2009; 2011; Jameson, 2000; Davies, 2004). When herbivores attack, jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) elicit plant defense responses (Robert-Seilaniantz et al., 2007; Howe & Jander, 2008). This information shows a complicated mutualistic relationship between the injured plant and the herbivore during gall formation. Scientists have different opinions on the roles of phytohormones in gall development because the chemical physiology of gall tissue varies from plant to plant (Tooker & Helms, 2014). As a result, the role of phytohormones in promoting tissue growth at galling sites needs to be better known. It is, therefore, critical to determine whether galling tissue accumulates a higher concentration of phytohormones than non-galling tissue or whether they work independently. The current study focuses on how phytohormone gradients rush in galling tissue compared to non-gall tissue; as a result, quantitative adjustments of phytohormones at the galling site were compared to those of normal non-gall tissue. This research provides insight into how phytohormones promote cell aggregation in gall formation while avoiding plant defense.

Materials and methods:

Sample collection:

The comparison of phytohormone accumulation and depletion in galling tissue versus non-galling tissues was done using three tropical plant species: *Alstonia scholaris* L. (Apocynaceae) (Milkwood Pine), *Mangifera indica* L. (Anacardiaceae) (Mango), and *Ficus racemosa* L. (Moraceae) (Fig). Mango and Fig exude latex, while milkwood pine secretes resins as a defense chemical against herbivore attacks (Kim et al., 2003). Such plant species were chosen to investigate how herbivore infestations may occur despite the presence of defensive compounds in those plants. Three kinds of plant leaves were chosen: a) Non-Gall leaves: leaves that did not have any gall infestation (NG); b) Immature-Gall leaves: leaves that showed early gall formation (IG); and c) Mature-Gall leaves: leaves that showed only mature galls (MG). The proportions and arrangement of galls on a leaf were the basis for categorizing IG and MG leaves. Gall structures with a diameter of less than 1 mm were classified as IGs, while those with a 2–5 mm diameter were classified as MGs (Price & Clancy, 1986; Rehill & Schultz, 2001). Five plants of a species having both galled and non-galled leaves were collected during 2020–2021 from various locations in and around Kolkata, India, and returned to the laboratory for further studies. The NG samples were considered as 'control'. At least 20 leaves from various branches of each plant (of a species) were randomly picked for the NG, IG, and MG samples. Foliar tissue from IG and MG was removed from the leaves by fine blades, classified by plant species, and examined biochemically. Foliar NG tissues were collected at random from a non-gall leaf as the control sample.

An amount of 40 mg of plant tissue was collected for each tissue type (NG, IG, and MG) by extracting 2 mg of sample tissue from a leaf. For the biochemical study, specific tissue samples (either NG/IG/MG) from all leaves of an individual plant (40 mg) were mixed together. For the phytohormone analysis, there were five replicates for each tissue type, with each replicate consisting of 40 mg of tissue. An individual plant was considered a separate replicate. To minimize inaccurate calculations resulting from the potential uneven distribution or depletion of phytohormones in specific leaves, if any, all foliar tissues were homogenized for phytohormone analysis. Insects from each gall chamber were collected with soft forceps and needles for taxonomic identification.

Biochemical analysis:

For conducting phytohormone analysis, NG, IG, and MG foliar tissues were rinsed with distilled water and stored at -80ºC for 24 hours to eliminate any excess moisture. Following this, the tissues were subjected to drying in a micro-oven for 48 hours at 40ºC. Following previously established protocols (Yamaguchi et al., 2012; Tanaka et al., 2013; Silva et al., 2017), the following phytohormones were quantified for each tissue type (NG, IG, and MG) of each plant species: a) auxin, b) cytokinin, c) ethylene, d) jasmonic acid, and e) salicylic acid. Three separate runs of each test were conducted.

Tissue extraction:

Double volume cold extraction buffer [0.1M Tris-HCl, pH 8.0; 1μM ethylene diamine-tetra-acetic acid (EDTA); 0.5% polyvinyl pyrrolidone; 2% polyvinyl-poly-pyrrolidone; 10 ml 2-mercapto-ethanol and 10% SDS] was used to homogenise the finely ground foliar tissues during phytohormone assays (Dubois et al., 1956). Each assay sample (a-e) was extracted chemically, centrifuged (10000 rpm, 30 min, 4°C), and the supernatants were saved for future analysis.

Phytohormone estimation:
The concentrations of plant hormones like auxin (indole acetic acids, IAA) and cytokinins (CK) were quantified using stable isotope-labelled standards in each tissue type (NG, IG, and MG) following the protocols outlined by Yamaguchi et al. (2012) and Tanaka et al. (2013). Ethylene (ET) was quantified using Gas Chromatography-Mass Spectrometry (GC-MS) technology, as described by Tooker et al. (2009). The LC-electrospray ionisation (ESI)-MS/MS technique was employed to quantify the levels of Jasmonic acid (JA) and Salicylic acid (SA) by using a mass-mass-spectroscopy (Agilent 6410; ZORBAX Eclipse XDB-C18 column). Fresh tissue samples weighing approximately 100 mg were used for the analysis. The quantification process was carried out using the Mass Hunter v.B.01.02 spectrometer software (Agilent, Santa Clara, CA, USA), as reported by Tooker and De Moraes (2009). All measurements were taken in ng/g FW.

Statistical analysis:
The collected samples were represented as mean ± SD. The data were compared using parametric tests (one-way ANOVA) followed by Tukey's HSD post hoc test at 5% significance level. The R software (R Core Team, 2013) was used for all statistical analyses.

Results:
Gall-inducing insect:
The identified gall-inducing insects in the studied plants were: *Pauropsylla tuberculata* (Hemiptera: Psyllidae) from Milkwood Pine (Alstoniascholaris, Apocynaceae); *Indodiplosis mangifoliae* (Diptera: Cecidomyiidae) from Mango (*Mangifera indica*, Anacardiaceae); and *Horidiplosis mathuri* from Fig, (Ficus racemosa, Moraceae).

Auxin (IAA), Cytokinin (CK), Ethylene (ET), Salicylic acid (SA) and Jasmonic acid (JA):
Across all plant species tested (*A. scholaris, M. indica, and F. racemosa*), the auxin concentration (mean value in ng/g FW) showed a steady increase from non-gall tissues (NG) to immature gall tissues (IG), and then from immature (IG) to mature gall tissues (MG) (Fig. 1). All of the plants under investigation demonstrated an approximately two-fold rise in auxin levels from newly formed non-gall (NG) to mature gall tissues (MG) (Fig. 2a). Overall, cytokinin levels were nearly three times higher in MG samples than in NG samples (Fig. 2b). Furthermore, the levels of ethylene and salicylic acid were found to be elevated by a factor of three to four in the MG group compared to the NG group. However, the increase in these compounds was only one to two times higher in the MG group compared to the IG group (Figs. 2c and 2d). The concentration of jasmonic acid (JA), a stress-responsive hormone, exhibited a gradual decline in all plant samples, starting from NG to IG and subsequently from IG to MG, in contrast to ethylene and salicylic acid (Fig. 2e). The Post-hoc (Tukey HSD) analysis results indicate that there were statistically significant increases in the levels of auxin, cytokinin, ethylene, and salicylic acid in all foliar tissues, specifically from the NG to the IG and from the IG to the MG stages. Conversely, the concentrations of jasmonic acid exhibited a significant decrease concurrently. IAA, CK, ET, and SA, which promote neoplastic growths in galling tissues, were observed in substantially higher quantities in the gall tissues of all model plants. The levels of different phytohormones in immature galled tissues (IG) exhibited an approximately two-fold elevation (ranging from 90–120%) in comparison to normal (NG) tissues. However, these levels may increase by as much as fourfold (about 200–300%) in mature gall tissues (MG). Furthermore, the study demonstrated that the levels of JA in IG and MG were significantly reduced by 35–45% and 65-75%, respectively, compared to the levels observed in normal tissues (NG). The data presented in Figure 1 indicates that the JA gradient was inverted across all plant species. The presented data in Table 1 illustrates the variations in phytohormone gradients across different tissue types (NG, IG, and MG) within individual plant species with statistical differences.

Discussion:
The phenomenon of galling insects manipulating the metabolism of galling tissue to regulate the rate of growth and development of the affected tissue is widely acknowledged (Hartley & Lawton, 1992; Weis et al., 1988). Based on this perspective, the chemical interaction between gall and non-gall tissues is a multifaceted and varied phenomenon wherein numerous cellular metabolic processes facilitate the conversion of normal cells into gall tissue. The host plant and its associated organism engage in a mutually beneficial interaction, resulting in enhanced nutritional benefits and the accumulation of compounds that contribute to wound defense. While it may not be appropriate to construct an exhaustive framework specifically focused on the relationship between phytohormones and gall formation, it is

plausible to hypothesise that galling sites exhibit an increased concentration of growth hormones that play a crucial role in facilitating tissue growth and differentiation (Hartley, 1998). Based on the findings of Wu and Baldwin (2010), it has been observed that an insect larva assimilates nitrogen compounds by entering the host plant's vascular system and consuming phloem fluids. The statement aligns with the findings of Abrahamson and McCrea (1986), who noted that the survival of larvae that induce gall formation is contingent upon their ability to obtain nitrogen from the host plant. Gall tissues have been described as "nitrogen sinks" or "physiological sinks" due to their ability to reduce the relative nitrogen content of adjacent leaves, thereby impeding nutrient transport to the leaves (Wu & Baldwin, 2010). The observation that the presence of gall causes a reduction in tissue nitrogen levels provides evidence supporting this perspective. The development of abnormal tissue growth at galling sites may be impacted by plant hormones or chemicals with similar hormonal properties, as suggested by Veselov et al. (2003). Turlings et al. (1993) also observed comparable findings, indicating that phytohormones play a predominant role in the induction of plant galls. The precise origins of the hormones in question, however, remain undetermined.

Gall-inducing insects may raise the concentration of phytohormones in galling tissues due to their activities and fluid output (saliva, reproductive fluids, etc.). Phytohormones probably play a dual role in the growth and prevention of galls caused by herbivores; plants are expected to boost their hormone synthesis and tissue expansion, resulting in a cascade of signal transduction in direct and indirect defensive systems. Phytohormones exhibit various interactions with both abiotic and biotic factors, functioning as signalling molecules that regulate the growth and development of plants. Plants may produce extra hormones in response to these obstacles. Multiple phytohormones at work in gall tissue have been hypothesised to allow for simultaneous growth and defense, as suggested by Dicke and Van Poecke (2002) and Kessler and Baldwin (2002). Hence, "cross-talk" between active and interfering phytohormones can be attributed to synergistic or antagonistic activities, depending on their respective mechanisms of action. The current investigation demonstrated a progressive elevation in growth-stimulating phytohormones across all plant specimens as they transitioned from non-galled to immature gall tissue and subsequently to mature gall tissue. The study results provide evidence in favour of the hypothesis that herbivore-induced galls promote the growth and differentiation of plant tissues by releasing phytohormones. Auxin (IAA) is believed to play a crucial role in the anomalous development of tissues, characterised by hypertrophy and hyperplasia, observed in galling regions (Byers et al., 1973; Dorchin et al., 2009). Tooker and Helms (2014) have posited that the presence of a defective auxin receptor gene can impede or halt the formation of gall tissue by diminishing the hormone's responsiveness at the site of gall formation. The results of this investigation align with previous research, which also reported elevated levels of indole-3-acetic acid (IAA) and IAA-oxidase in galls. Additionally, it was observed that the auxin concentration in galled tissues may be approximately 2.5 times greater compared to non-galled tissues (Byers et al., 1973; Dorchin et al., 2009). The elevated auxin levels in juvenile galls (IG), according to Tooker and De Moraes (2011), suggest that gall-inducing larvae may manage IAA levels in affected tissues. The precise role of cytokinin (CK) in plant defense remains uncertain. According to a theory proposed by Veselov et al. (2003), CK may regulate plant defense mechanisms against invasive gall-causing insects to promote neoplastic growth. The findings indicate that the levels of CK exhibit a greater increase, up to twice as much, compared to the levels of auxin in galling tissues. It suggests that CK might have a role in the differentiation of tissues and the formation of galls (Weiler & Spanier, 1981; Jameson, 2000). The higher levels of CK in gall locations may offer evidence that gall insects may have combined IAA and CK actions (Dorchin et al., 2009). The role of jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) in plant defense is thought to be indirect, although the specific mechanisms by which they contribute to defense remain unclear (Erb et al., 2012; Zhang et al., 2015). The investigation revealed that the tissues of the present galling plants exhibited a threefold increase in salicylic acid (SA). This finding suggests that SA may be able to regulate plant growth and development, just like auxin and CK. There are two mechanisms through which an elevated concentration of SA can effectively enhance a plant's resistance against herbivore infestation: the production of volatile emissions and the attraction of natural enemies to the plant (Anand et al., 2008; Vidhyasekaran, 2015). Elicitors created by herbivores are thought to cause higher plant-herbivore resistance (Gatehouse, 2002; Howe & Jander, 2008; Schmelz et al., 2003). The current observation demonstrates that, regardless of the plant type, the levels of JA were roughly two times lower in galling tissue than in non-galling tissue. The empirical data and research findings from this analysis support the notion that JA and SA interact negatively, as observed previously (Tooker & De Moraes, 2009). According to Lee et al. (2009), the interaction known as "cross-talk" between jasmonic acid (JA) and salicylic acid (SA) can have a negative impact on plants, potentially increasing their susceptibility to insect infestation. In contrast, it has been observed that indirect plant defense mechanisms against herbivores exhibit synergistic interactions between jasmonic acid (JA) and

salicylic acid (SA). Jasmonic acid (JA) and salicylic acid (SA) exhibit antagonistic properties, which necessitate an induced response after herbivorous consumption (Moran & Thompson, 2001; Morkunas & Gabry, 2011). In order to effectively synchronise the activation of the defensive mechanism in response to the elicited reaction, both entities may partake in a competitive process. According to Bari and Jones (2009), herbivore attacks boost ethylene (ET) production at galling sites. Similar to the current study, the "ET burst" phenomenon, which is triggered by insect oral secretions, has been seen in a variety of plant species (Akitt et al., 1980). The results of the present investigation suggest that ethylene production at galling sites may be more than twice that of non-galled tissues. This observation supports the notion that ET could potentially play a role in the indirect defense of plants by producing volatile blends (Schmelz et al., 2003). The ethylene (ET) production in galls has been observed to exhibit a threefold increase compared to non-galling tissues. The levels of jasmonic acid (JA) are commonly elevated by ethylene (ET), which acts as an inhibitor of salicylic acid (SA), a JA antagonist, thereby impacting the defensive reaction (Zander et al., 2010). Therefore, the interaction between jasmonic acid (JA) and ethylene (ET) is crucial for direct and indirect plant defense mechanisms in response to herbivore attacks. Recent investigations have shown that galling tissue can better regulate phytohormones than fresh, healthy leaves that do not gall. Phytohormone interaction was higher in mature gall tissues than in non-gall or immature gall tissues. According to this finding, galling tissues create multiple phytohormones and their various gradients to promote tissue growth and differentiation towards neoplasia or build a defense mechanism in response to the plants. The predominant phytohormones responsible for inducing gall formation exhibit dual functionality, as they facilitate tissue proliferation and activate internal defense mechanisms. The study highlights the impact of gall-inducing insects on delivering nutrients and chemicals to the host plant. The observed phenomenon can be attributed to the insects' ability to regulate phytohormones within injured tissues, thereby influencing plant tissue growth. As a result, the coevolutionary dynamics between gall-producing insects and their host plants have evolved along a convoluted evolutionary path.

Conclusion:

The gall-inducing insect initiates plant tissue differentiation by releasing chemical secretions that interact with the induced phytohormones produced by the plants. The natural formation of galls is attributed to the co-evolutionary process between gall inducers and their host plants, wherein both organisms undergo simultaneous adaptations. This adaptation is thought to have been influenced over time by changes in the production and release of several substances, including phytohormones.

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Conflict of Interest

The authors declare no conflict of interest.

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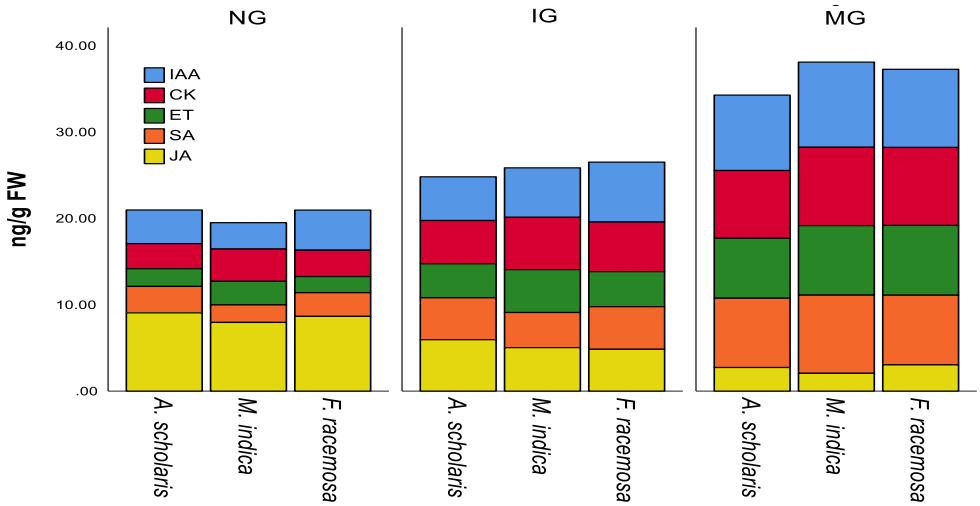

Figure 1. Phytohormone gradients among non gall (NG), immature gall (IG), and mature gall (MG) tissues for three model plants (*A. scholaris*, *M. indica* and *F. racemosa*). IAA: Indole acetic acid; CK: Cytokinine; ET: Ethylene; SA: salicylic acid; JA: Jasmonic acid

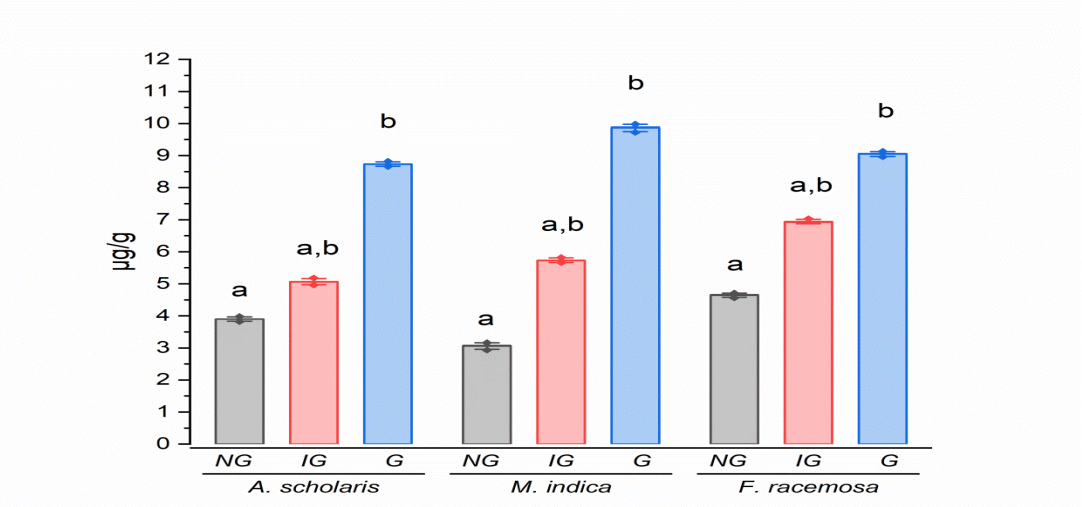

Figure 2a. Auxin (IAA) gradients from NG to IG and from IG to MG in foliar tissues for *A. scholaris*, *M. indica* and *F. racemosa*. Similar letters indicatenon-significant difference and non-similar letters indicate significant difference of phytohormone surge in three gall types for a plant species.

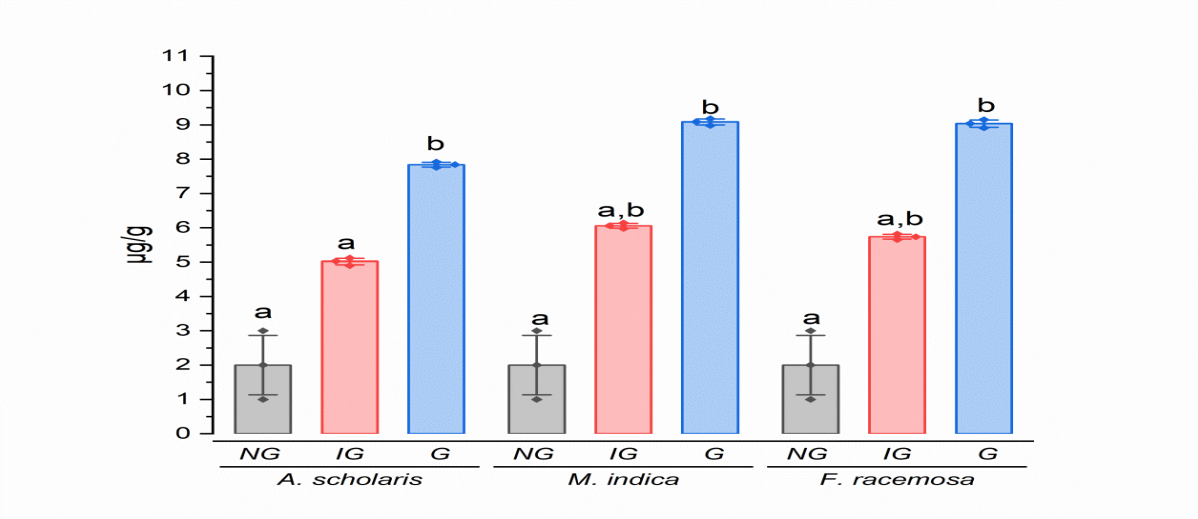

Figure 2b. Cytokin concentrations from NG to IG and from IG to MG in foliar tissues for *A. scholaris*, *M. indica* and *F. racemosa*. Similar letters indicate non-significant difference and non-similar letters indicate significant difference of phytohormone surge in three gall types for a plant species.

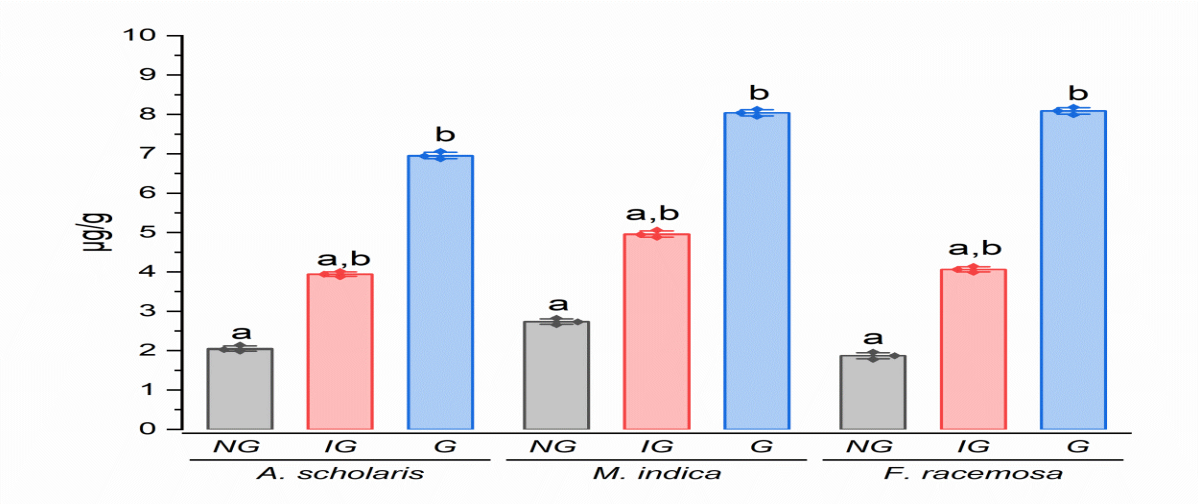

Figure 2c. Ethylene gradients from NG to IG and from IG to MG in foliar tissues for *A. scholaris*, *M. indica* and *F. racemosa*. Similar letters indicate non-significant difference and non-similar letters indicate significant difference of phytohormone surge in three gall types for a plant species.

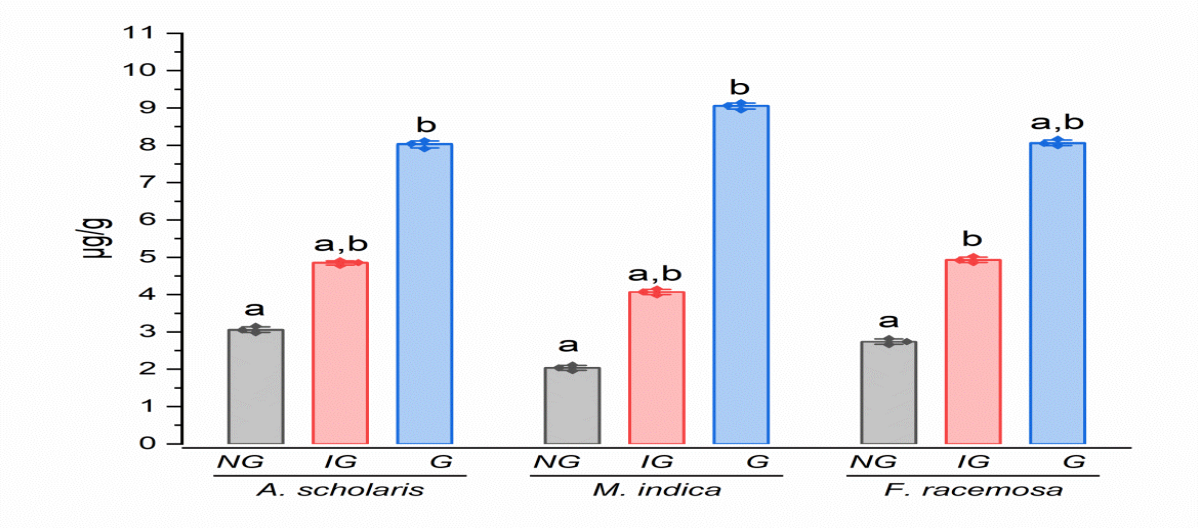

Figure 2d. Trends of phytohormone salicylic acid changes from NG to IG and from IG to MG in foliar tissues for *A. scholaris*, *M. indica* and *F. racemosa*. Similar letters indicate non-significant difference and non-similar letters indicate significant difference of phytohormone surge in three gall types for a plant species.

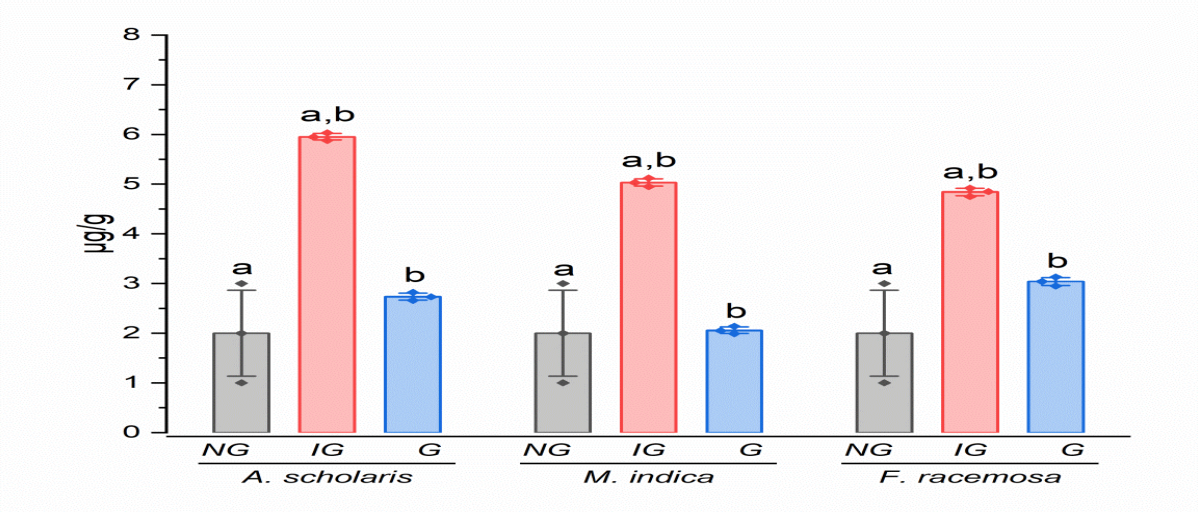

Figure 2e. Trends of Jasmonic acid changes from NG to IG and from IG to MG in foliar tissues for *A. scholaris*, *M. indica* and *F. racemosa*. Similar letters indicate non-significant difference and non-similar letters indicate significant difference of phytohormone surge in three gall types for a plant species.

Table 1. Phytohormone variations among non-gall (NG), immature gall (IG) and mature gall (MG) leaves of *Alstonia scholaris*, *Mangifera indica* and *Ficus racemosa* (*p* values are as obtained from ANOVA). Values followed by same letters (a, b, c) are statistically non-significant at 5% level by Tukey's post hoc analysis.

Plant	Tissue type	Phytohormone				
		Auxin (ng/g FW)	Cytokinin (ng/g FW)	Ethylene (ng/g FW)	Jasmonic acid (ng/g FW)	Salicylic acid (ng/g FW)
Alstoniascholaris	NG	3.9±0.08[a]	2.9±0.08[a]	2.05±0.07[a]	9.06±0.08[c]	3.06±0.08[a]
	IG	5.06±0.11[b]	5.01±0.11[b]	3.94±0.06[b]	5.95±0.07[b]	4.85±0.06[b]
	MG	8.73±0.08[c]	7.84±0.08[c]	6.95±0.09[c]	2.73±0.08[a]	8.02±0.10[c]
	$F_{(2,6)}$	2296.463	2131.721	2822.689	4872.255	2490.361
	p	0.000	0.000	0.000	0.000	0.000
Mangiferaindica	NG	3.05±0.11[a]	3.74±0.08[a]	2.73±0.08[a]	7.95±0.10[c]	2.03±0.07[a]
	IG	5.73±0.08[b]	6.06±0.08[b]	4.96±0.16[b]	5.03±0.08[b]	4.07±0.08[b]
	MG	9.86±0.13[c]	9.08±0.10[c]	8.04±0.15[c]	2.06±0.07[a]	9.05±0.09[c]
	$F_{(2,6)}$	2821.7	2722.383	2803.392	3264.363	5544.915
	p	0.000	0.000	0.000	0.000	0.000
Ficusracemosa	NG	4.64±0.07[a]	3.06±0.10[a]	1.87±0.09[a]	8.65±0.10[c]	2.74±0.08[a]
	IG	6.94±0.07[b]	5.74±0.08[b]	4.06±0.07[b]	4.84±0.08 [b]	4.93±0.08[b]
	MG	9.05±0.08[c]	9.03±0.12[c]	8.08±0.09[c]	3.04±0.09[a]	8.06±0.08[c]
	$F_{(2,6)}$	2281.05	2525.436	3929.75	2786.502	3167.077
	p	0.000	0.000	0.000	0.000	0.000

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ORIGINAL ARTICLE **LIFE SCIENCE**

OLFACTORY RESPONSES OF *AULACOPHORA FOVEICOLLIS* LUCAS – AN ECOLOGICALLY IMPORTANT PEST - FROM WEST BENGAL, INDIA

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Abstract

Pumpkin, *Cucurbita maxima,* is severely harmed by *Aulacophora foveicollis Lucas* (Coleoptera: Chrysomelidae), as a major insect pest of the plant. Based on Scanning electron microscopic analysis, compared to mechanosensilla, prevalence of antennal olfactory sensilla, was observed. The number of olfactory sensilla in the circumferential band of IXth antennal segment was higher in female species. Stimulation of the sensilla present on the antennae of *A. foveicollis* was analyzed by electroantennogram (EAG) at four different concentrations *viz.* 10.0, 5.0, 1.0, and 0.1 mg/ml in both sexes. The male species displayed greatest peak amplitude at 5mg/mL for heneicosane, whereas the female species displayed maximum peak amplitude at 10.0 mg/mL for 2-methyl phenol followed by 5.0 mg/mL for 1,4-dimethoxybenzene. However, pooled EAG data revealed that GLV elicited the strongest reactions, followed by aliphatic chemicals, oxygenated monoterpenes, hydrocarbon monoterpenes, and sesqueterpenes. These findings univocally shows that the pest's antennal responses clearly displayed sexual dimorphism.

Keywords: Electroantennogram, volatile organic compounds VOCs, SEM

Introduction

Plants interact with insects through release of various allelochemicals or volatile compounds. It is generally the highly developed olfactory sensory system of the antennae of insects, used for the perception of these volatile compounds in the environment. Electroantennogram (EAG) is being used to register these responses to the varied classes of plant odor volatiles (Park et al., 2002).

Previously few studies have been performed on *Aulacophora foveicollis* Lucas that reported the affinity of the pest to alkanes in *Momordica cochinchinensis* floral surface waxes (Mukherjee et al., 2013; 2014a). Host suitability, feeding behavior and food preference of this beetle was studied (Sarkar et al., 2016; Sing et al., 2000). This study emphasizes the ecological relationships between the insect pest, *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae) the red pumpkin beetle, and host plant, *Cucurbita maxima*. *A. foveicollis*, a polyphagous pest, primarily feeds on cucurbits besides a several different fruit crop. Lifespan, fecundity, and survival rates of *A. foveicollis* on *Cucurbita maxima* are comparable to the wide range of variations recorded on other host plant species (Pavlakos, 1943; Al-Ali et al., 1982; Sinha and Krishna, 1971; Alikhan and Yousuf, 1985; Mukherjee and Barik, 2014b; Mukherjee et al., 2015). It has been recorded on several continents, including Asia, some parts of Europe, and Africa. (Doharey, 1983; Butani and Jotwani, 1984; Raman and Annadurai, 1985; Kumar and Nadarajan, 2007; Abe and Matsuda, 2005; Khan et al., 2011; Khan et al., 2012). Young and senescent leaves were less likely to be consumed than mature leaves and blossoms. Flowers and mature leaves showed low sugars, high nitrogen and protein content, narrow C/N ratio and moderately high phenols compared to other parts of plant (Beck, 1956; Hartfield et al., 1982). An individual plant is heterogeneous to any plant eating insect, depending on the nutritional content of the plant parts. Basically, both larval and adult stages of *A. foveicollis* are harmful to the crops, though adult beetles can do greater harm. They feed on flowers, cotyledons and leaves while the grubs mainly feed on plant roots. The pest occurs throughout the year and is capable of causing great

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economical loss (Khan et al., 2012). Presently, in the absence of effective ecological sustainable control methods, extensive damage by the pest attracts the use of chemical pesticides which besides their effects on the environment and soil, leave pesticide residues on the crop and increases the pesticide resistance on insect pest. Hence an alternative sustainable control measure is required.

This study is focused on the detail structure of antennal chemosensillae, olfactory stimulation and sensitivity to a wide range of chemicals, applying electroantennogram technique, that have been investigated in both the sexes of *A. foevicollis*. This finding from the understudied North 24 Parganas region of West Bengal aids in understanding the role of olfaction by the pest and its host plant.

Materials

From 2016 to 2019 throughout the months of February to May, adult species of the insect pest were collected from the fields in North 24 Parganas, West Bengal and reared under laboratory condition on leaves of *Cucurbita maxima* (Family: Cucurbitaceae).

Methods

Scanning electron microscopy (SEM)

Antennal sensillum was characterized by SEM. The protocol for SEM were followed as already stated by Bhowmik et al., 2016. It was based on the nomenclature by Zacharuk, 1985, sensilla categorization on the distal segments was determined and illustrated.

Electroantennograms (EAG) and calculation of EAG data

EAG (version 2.6c, 1998, Syntech, Hilversum) analysis were measured as mentioned earlier from adult species of *Aulacophora foveicollis* (Bhowmik et al., 2016, 2017). Total 40 insects, and at a time one antenna for ~ 12 VOCs for 30 min, were used in the experimental procedure for both male and female. The information from EAG was used to calculate the mean values, standard deviation, standard error, and $\log_{10}$ values as well as the relative mean amplitudes in percent. Odorant and air flow were carried out at 0.6 L/min and 1.8m L/min, respectively, using an air stimulation controller for 5 seconds.

Results and Discussion

Antennal chemosensilla distribution and variation in the pest

Adult *A. foveicollis* pest's antennae include nine segments, where each antenna has a scape, a tiny pedicel, and a flagellum, as its three main components. Sensillae of the male and female species did not differ significantly in terms of morphology, rather, the sensilla's distribution (density), notably in the circumferential band, differed, which is the apical band area of IXth segment. The antenna's circumferential band has sensilla on the dorsal and ventral sides that showed varied distribution and subjected to a t-test analysis as collated in Table 1. SEM images showed sensilla of three types *viz*., Sensilla Basiconica (SB), Sensilla Chaetica (SC), and Sensilla Trichodea (ST). On the dorsal and ventral sides of the antennal segment of female pests, more Sensilla Chaetica Type-I have been seen. While, males have more Sensilla Chaetica Type-I on the ventral side of the circumferential band than females do, and more number of Sensilla Basiconica Type-I on the dorsal side.

EAG Dose –response studies in Aulacophora foveicollis

A total of 50 chemicals, obtained from several chemical agencies, were examined for their effects on EAG dose-dependent stimulus in both females and males. Figure 1-4 showed the mean normalized responses in EAG at 0.1, 1.0, 5.0 and 10.0 mg/mL concentrations of plant volatiles in both female and male pest. Compounds were chosen for the EAG study based on their presence in considerable quantities in the cucurbita plant volatile extract, along with a few other normal plant volatiles. SPSS 16.0 software was used to analyse the data in 2-way ANOVA utilising POSTHOC TUKEY ALPHA (0.05).

Table 1. T-test analysis *(at $p<0.05$)* for the sensilla distribution in the apical band area of IXth segment in *Aulacophora foveicollis*

Dorsal side										
	SB-I	SB-II	SB-III	SB-IV	SB-V	SB-VI	SB-VII	SCH-I	SCH-II	ST
Male species	8±0.3	6±0.3	3±0.3	2.2±0.2	4.2±0.5	5±0.3	4±0.3	5.2±0.3	6.2±0.2	4.2±0.3
Female species	7±0.3	6.2±0.2	5±0.3	4±0.3	4±0.3	8±0.3	6±0.4	8±0.3	8±0.3	2.2±0.2
t-test value	2.23	0.53	**4.47***	**4.81***	0.30	**6.7***	**3.65***	**5.71***	**4.81***	**4.71***
p-value	0.055	0.6	0.002	0.001	0.77	0.0001	0.006	0.0004	0.001	0.001
Ventral side										
	SB-I	SB-II	SB-III	SB-IV	SB-V	SB-VI	SB-VII	SCH-I	SCH-II	ST
Male species	4±0.3	5..2±0.3	2.2±0.2	2.2±0.2	3.2±0.2	2.6±0.2	4±0.3	7.2±0.3	5.4±0.2	2.4±0.2
Female species	6±0.4	5.6±0.2	4±0.3	3±0.3	4±0.3	4.6±0.2	6.2±0.4	8.8±0.2	6.2±0.3	3.6±0.2
t-test value	**3.65***	0.89	**4.81***	**2.13***	**2.13***	**5.7***	**3.77***	**3.77***	1.78	3.46
p-value	0.006	0.39	0.001	0.06	0.06	0.0004	0.005	0..005	0.11	0..008

Green leaf volatile compounds (GLV)

Females responded to cis-3-hexen-1-ol to 113.4± 1.23% at 10.0 mg/mL proceeded by 103.32± 1.89% at a dosage of 5.0 mg/mL. 94.45±0.39% at 1.0 mg/mL and 67.06±1.11% at concentration of 0.1 mg/mL. Where as decanal showed minimum responses of 17.80±0.13% at 0.1 mg/mL, while 29.15±2.63%, 68.363±2.46% and 96.84±1.62% relative mean amplitude was shown at 1.0, 5.0 and 10.0 mg/mL. With rest of the GLV molecules like trans-2-Hexenal, relative mean amplitude of 51.95±1.26%, 73.84±2.52%, 61.052±1.64% and 57.59±1.17% were observed at 0.1, 1.0, 5.0 and 10.0 mg/mL concentrations respectively. While trans-2-hexenyl acetate elicited 45.25±1.86% at 0.1 mg/mL, 71.52±2.60% at 1.0 mg/mL, 60.558±1.93% at 5.0 mg/mL and 64.13±1.73% at 10.0 mg/mL. Hexanal showed 82.68±6.95%, 72.80±1.61%, 60.363±1.61% and 68.63±0.56% relative mean amplitude at 0.1, 1.0, 5.0 and 10.0 mg/mL, respectively.

Males had a similar pattern of responses, although their dosage responses varied. Higher responses were induced by cis-3-hexen-1-ol at 10.0 mg/mL, which were 121.702.44%, followed by 94.95±2.46%, 86.5±3.34% and 62.33±3.34% at doses of 1.0, 5.0 and 0.1 mg/mL, while decanal gave minimum responses of 10.96±1.42% at dose of 1.0 mg/mL, while at 0.1, 5.0 and 10.0 mg/mL concentrations it responded 22.97±0.87%, 17.47±0.77% and 33.55±4.84% relative amplitude, respectively. With other GLVs the responses at different doses have been summarized as bar graphs in Fig. 1-4.

Aliphatic compounds

Among the aliphatic compounds, trans-2-hexen-1-ol elicited in females the highest EAG stimulation (119.21.87%) at 5.0 mg/mL, followed by 90.481.16%, 86.622.47% and 65.680.84% at 1.0, 10.0 and 0.1 mg/mL, respectively. The response of female *A. foveicollis* with other aliphatic compounds like 1-Heptanol, maximum response of 102.50±0.85% was observed at 10.0 mg/mL followed by 90.52±1.96%, 88.45±1.07% and 85.75±1.97% at 5.0, 0.1 and 1.0 mg/mL. On the other hand, Hexyl acetate demonstrated 61.50±5.13%, 43.53±1.18%, 48.59±0.99% and 76.09±1.93% relative amplitudes at doses 0.1, 1.0, 5.0 and 10.0 mg/mL, respectively. 1-Octene-3-ol elicited greatest response at 10.0 mg/mL (98.93±6.14%) followed by 77.656±3.08%, 46.89±1.27% and 12.25±0.94% at 5.0, 0.1 and 1.0 mg/mL doses.1-Pentanol, elicited highest response of 97.68±2.02% at 10mg/mL, while responses at 0.1, 1.0 and 5.0 mg/mL were 57.67±1.04%, 71.45±2.56% and 72.005±2.08%. 2-Nonanone showed highest response of 52.645±1.84% at 5.0 mg/mL while other values read as 41.74±1.02%, 29.65±0.61% and 19.13±0.71% at doses 10.0, 0.1 and 1.0 mg/mL. 1-hepten-3-ol elicited amplitudes of 84.33±0.43%, 76.85±2.39%, 76.05±2.98% and 63.126±2.10% at concentrations 10.0, 0.1, 1.0 and 5.0 mg/mL. Nonanal elicited maximum EAG response of 63.20±2.67% at 0.1 mg/mL followed by 62.22±1.78%, 38.69±2.91% and 31.00±1.83% at 10.0, 5.0 and 0.1 mg/mL. The highest EAG response to nonadecane was at 10.0 mg/mL (96.96±1.82%), followed by 66.21±1.82% at 5.0, 51.72±2.11% at 0.1 and 41.29±2.01% at 1.0 mg/mL of concentration. 2-Heptanone gave 50.165±1.93%, 49.94±1.35%, 45.57±1.05% and 16.53±0.72% responses at 5.0, 10.0, 0.1 and 1.0 mg/mL doses. The greatest response for heptadecane, on the other hand, was 65.101.72% at 10.0 mg/mL, with subsequent results showing 39.721.72%, 41.721.32%, and 63.792.32% at 0.1, 1.0 and 5.0 mg/mL, respectively.

Of all the aliphatic compounds, heneicosane had the highest reaction in males at 5 .0 mg/mL (129.30±1.9%), compared to its responses at 0.1, 1.0 and 10.0 mg/mL (65.00±1.72%, 101.21±1.21%, and 111.99±1.81%). While maximum stimulation of 126.11±2.82% at concentration of 10.0 mg/mL followed by 116.01±1.22%, 103.27±2.865% and 63.28±1.82% at doses 5.0, 1.0 and 0.1 mg/mL was responded with 1-hepten-3-ol. At 10.0 mg/mL concentration, acetic acid was observed to have a minimum amplitude of 2.48±1.38%. Hexyl acetate showed maximum responses at 0.1 mg/mL (44.54±1.71%). 1-Pentanol elicited maximum response of 76.87±1.34% followed by 61.53±0.39%, 57.64±0.33% and 52.53±0.43% at 1.0, 0.1 and 5.0 mg/mL concentrations. Trans-2-hexen-1-ol showed highest amplitude of 116.1±1.60% at 5 mg/mL followed by 111.70±2.80%, 108.46±2.89% and 65.17±1.80% at 10.0, 1.0 and 0.1 mg/mL doses, respectively. 1-Octene-3-ol showed maximum response at 0.1 mg/mL dose (34.84±2.3%). 1-heptanol, on the other hand, displayed the greatest EAG mean amplitude at 5.0 mg/mL (111.571.22%), followed by concentrations at 1.0, 10.0, and 0.1 mg/mL (102.99±2.03%, 94.82±3.02%, and 68.91±1.02%, respectively). 2-Nonanone elicited maximum stimulation of 66.94±3.60% at 5.0 mg/mL, while nonanal showed highest stimulation of 45.40±1.48% at 10.0 mg/mL. 2-Heptanone gave 33.23±2.31% as maximum stimulus at 10.0 mg/mL dose. Nonadecane produced a maximum stimulation at 5.0 mg/mL (105.0±2.11%) and a lowest response at 0.1 mg/mL (66.21±1.55%). Tetradecane showed highest response at 10.0 mg/mL (106.92±1.99 %), while at 0.1, 1.0 and 5.0 mg/mL of doses it responded to 60.72±0.62 %, 87.60±2.11% and 97.32±1.78%, respectively. Pentacosane showed highest response of 100.11±1.11% at 10.0 mg/mL followed by 90.21±1.72%, 82.32±2.31% and 62.71±1.72 % at 5.0, 1.0 and 0.1 mg/mL doses, respectively. The largest EAG mean amplitude was seen for heptadecane at a dosage of 5.0 mg/mL, where it was 81.3±1.98%, followed by responses of 78.62±1.32%, 72.89±0.99%, and 50.32±0.88%. 3-Methyl butanol showed maximum amplitude at 5.0 mg/mL (51.62±2.62%), while at 0.1, 1.0 and 10.0 mg/mL of doses it responded to 42.19±2.62%, 21.28±2.24% and 40.97±3.62%. With Eicosane highest stimulation was observed at 10.0 mg/mL (82.40±1.48 %) and lowest response was shown at 0.1 mg/mL (49.99±2.01 %). 3-Hydroxy-2-butanone showed maximum response at 0.1 mg/mL (22.22±0.56%). With rest of the aliphatic compounds, nonanoic acid, hexanoic acid, butyric acid, 2,3-butanedione and Heptane, maximum response of 51.90±2.82%, 55.67±3.9%, 36.27±2.39%, 55.84±1.78% and 15.41±3.73%, respectively, was elicited at 0.1 mg/mL. Responses with rest of the aliphatic compounds at four different concentrations have been summarized in Fig. 1-4.

Aromatic compounds

Both the male and female species of *Aulacophora foveicollis* responded to dose-dependent aromatic chemicals in a similar manner that showed varied amplitudes of their EAGs. The EAG amplitude in female was dramatically increased by 2-methyl phenol at 10.0 mg/mL (136.51±0.97%). While the same for benzoyl alcohol in the female pest at dosages of 10, 5, 1.0 and 0.1 mg/mL was 100.99±1.83%, followed by 92.71±0.98%, 74.59±2.80%, and 66.76±2.34%. The response to 4-methyl phenol on the other hand was greatest at 5mg/mL (88.90±0.73%), following at 10mg/mL (66.49±1.59%), 1 mg/mL (28.18±0.54%), and 0.1 mg/mL (7.71±0.75%). The highest amplitudes for rest of the aromatic compounds, such as phenethyl alcohol, isoeugenol, 2-methyl phenol, ethyl benzoate, and 3-methyl phenol, were 119.37±1.09%, 40.08±0.99%, 136.51±0.97% and 120.08±2.26%, respectively, at 10 mg/mL. Another aromatic molecule, ethyl benzene displayed largest EAG response, measuring 100.51±1.21% at 10 mg/mL, and lowest response of 50.43±1.29% at 1.0 mg/ mL concentration. Furthermore, the female pest showed highly significant EAG response to 1,4 dimethoxybenzene at a concentration of 5.0 mg/mL (122.4 1.23%), followed by 119.82 ±1.89% at 10 mg/mL, and then 95.50± 0.49% and 69.16±1.11% at 1.0 and 0.1 mg/mL dosages. Other aromatic compounds showed the responses as summarized in Fig. 1-4.

Males elicited maximum changes of 92.07±5.24% in EAG amplitudes with 2-methyl phenol at 10 mg/ml followed by 78.10±5.34%, 26.90±3.52% and 20.01±5.54% at 5.0, 1.0 and 0.1 mg/mL. Acetophenone caused maximum stimulation of 37.62±4.07% at 0.1 mg/mL. Benzaldehyde showed maximum amplitude of 29.16±6.84% at 5 mg/mL while phenylacetaldehyde elicited 35.88±1.05% as maximum EAG amplitude at 10 mg/mL. Benzyl alcohol showed maximum stimulation of 80.25±1.23% followed by 71.35±1.43%, 64.99±1.53% and 52.14±2.61% at 10.0, 5.0, 0.1 and 1.0 mg/mL. Isoeugenol, Phenethyl alcohol, 2 methyl phenol and 3-methyl phenol, all four compounds, produced the highest amplitude of 29.39±1.08%, 81.50±2.82%, 92.07±5.24% and 86.30±1.58%, respectively, at 10 mg/mL. Ethyl benzoate and 4-methyl phenol on the other hand, at 0.1 mg/ mL elicited maximum EAG amplitude of 27.43±5.90% and 25.81±2.13%. 1,4, dimethoxybenzene caused response of 80.25±1.11% at 5.0, 69.14±0.61% at 1.0 and 62.99±1.02% at 0.1 mg/mL, while highest stimulus was showed at 10 mg/mL (100.50±1.25%). Furthermore, Ethyl benzene produced the lowest response of 26.40±1.31% at 10 mg/mL and the maximum EAG stimulus of 69.00±1.39% at 5 mg/mL.

Responses of several other molecules from oxygenated monoterpenes, sesquiterpenes and hydrocarbon monoterpenes varied in both the sexes of the pest as observed with other compounds and described in the Fig. 1-4 at different concentrations.

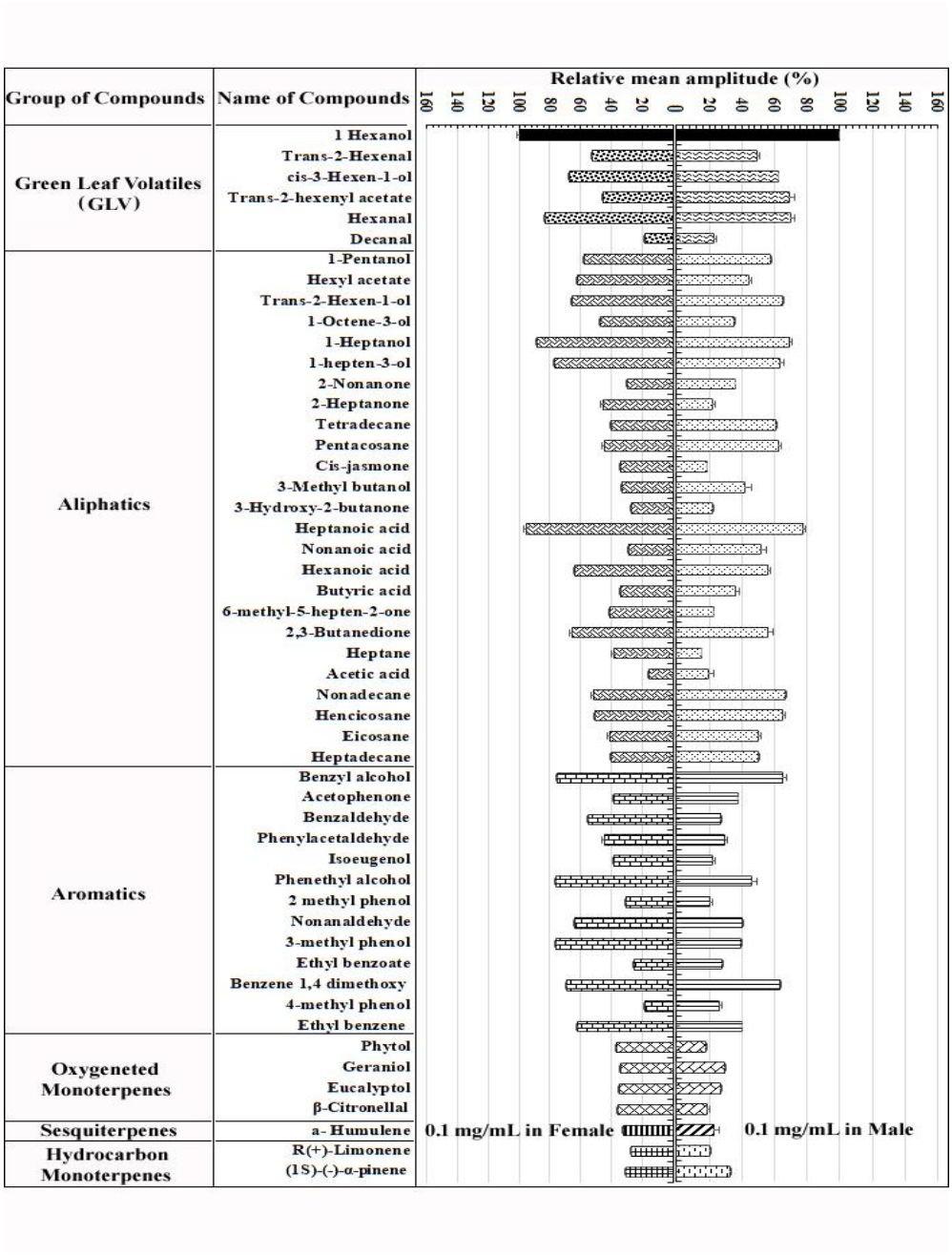

Figure 1. Mean normalized EAG responses of male and female species at 0.1 mg/mL concentration of plant volatiles.

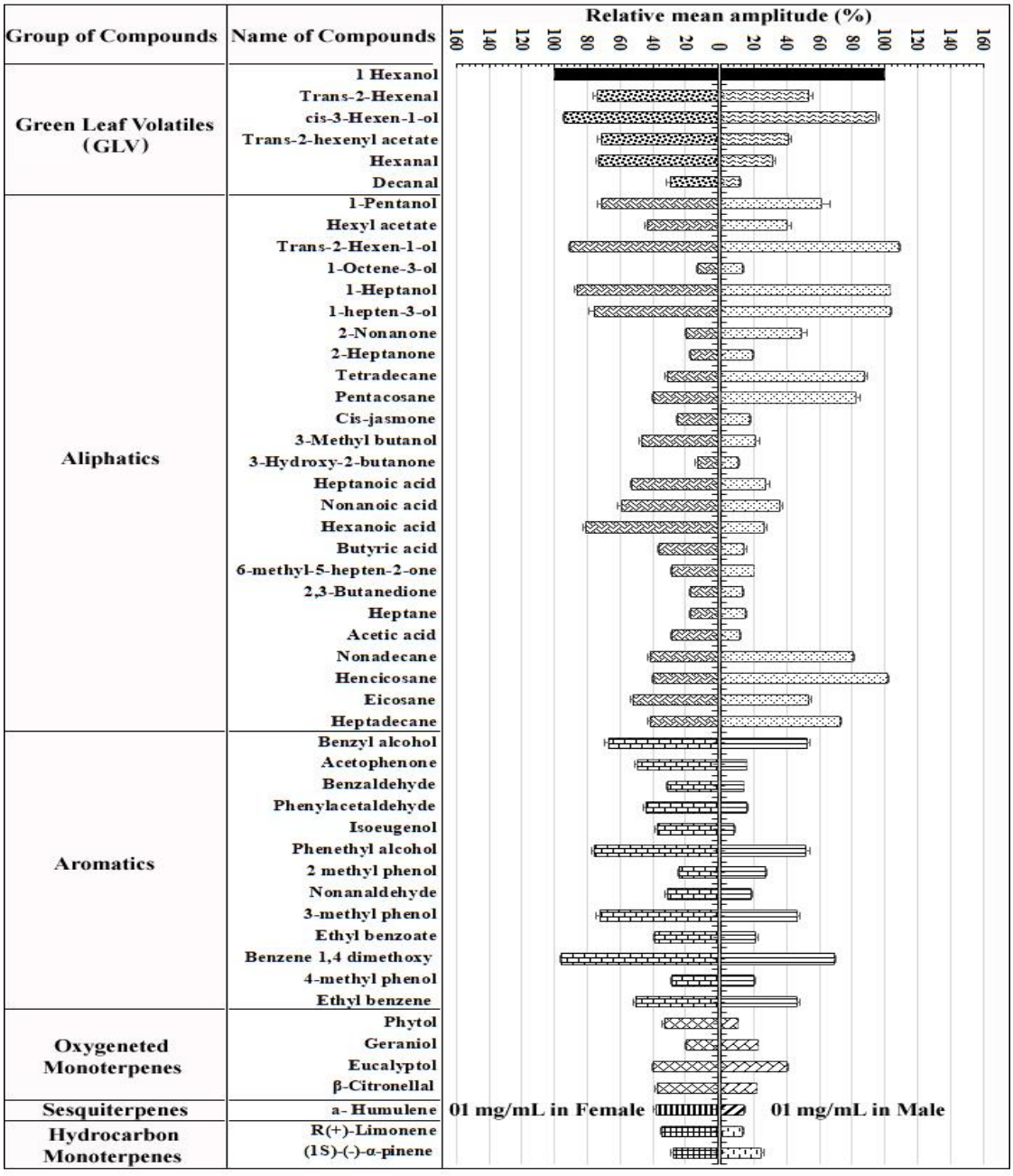

Figure 2. Mean normalized EAG responses of female and male species at 1.0 mg/mL concentration of plant volatiles.

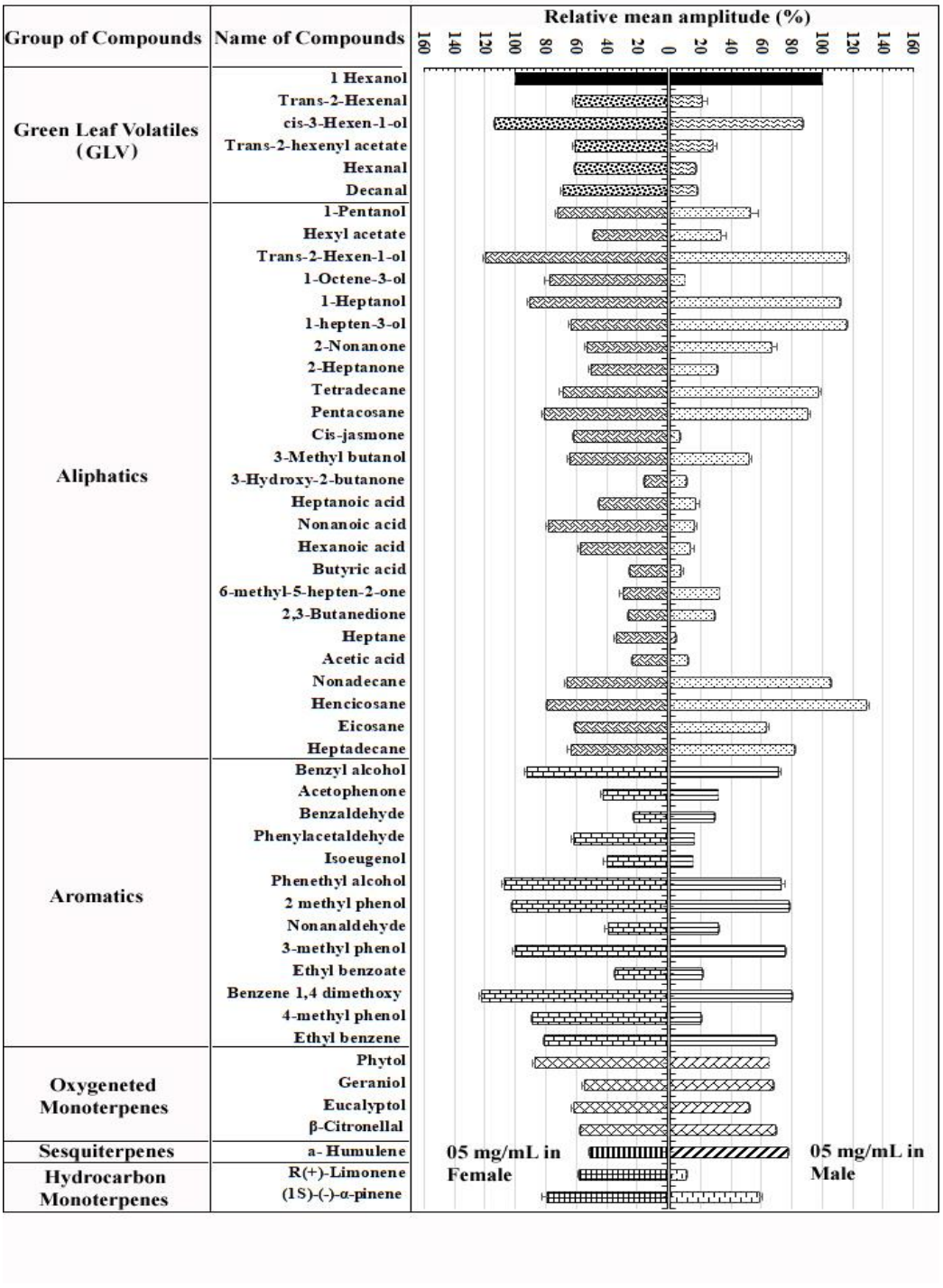

Figure 3. Mean normalized EAG responses at 5.0 mg/mL concentration of plant volatiles in male and female

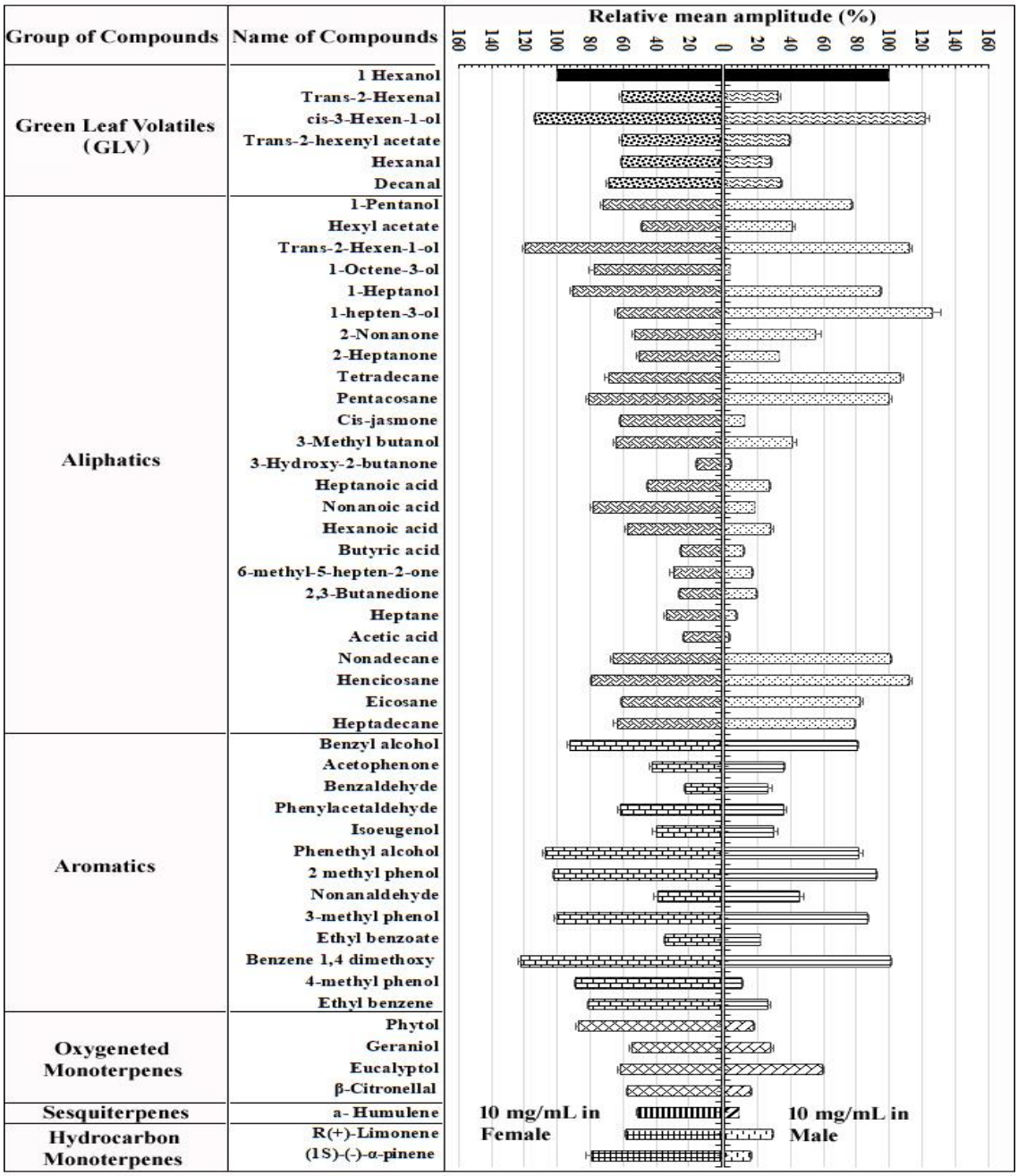

Figure 4. Mean normalized EAG responses at 10.0 mg/mL concentration in *A. faveicollis*

Pooled electroantennogram responses

At the end, individual groups of chemicals were pooled and averaged for comparisons (Fig.5). Responses were, in general, much higher in females than in males. The largest mean EAG amplitudes in both males and females were obtained with GLV, followed by aromatic and aliphatic compounds. The lowest responses were obtained with sesquiterpenes < hydrocarbons monoterpene < oxygenated monoterpenes.

Both men and females of this insect pest may detect the same fragrance because they use the same signals to locate identical host plants for survival and reproduction in similar environments. It has been established that the choice of a certain host plant depends on the bouquet of chemicals emitted in a specific ratio in different species of phytophagous insects (Visser et al., 1986; Zhang et al., 1999; Das et al., 2007). Based on pooled EAG data, maximum EAG responses was found with green leaf volatile (GLV), aromatic compounds, aliphatic compounds, followed by oxygenated monoterpenes, hydrocarbon monoterpenes and least with sesqueterpenes, that reflected the wide range of feeding habitat of the insect pest. The pumpkin beetle's predilection for GLV chemicals seemed to be a significant determinant in the host plant it chose, pointing to the beetle's polyphagous nature (Das et al., 2007). The species also displayed sexual dimorphism that can be seen based on the variations in responses by EAG at various dosages, in addition to differences in the sensilla distribution in the circumferential bands. Female species showed highest peak amplitude for 2-methyl phenol (at 10mg/mL, 136.51±0.97) followed by 1,4, dimethoxybenzene (at 5mg/mL, 122.4±1.23) and cis 3-hexene-1-ol (at 5 mg/mL, 113.4±1.23) where as males showed maximum amplitude for heneicosane (at 5mg/mL, 129.3±1.99) followed by 1-hepten-3-ol (at 10 mg/mL, 126.11±2.82) and cis 3-hexene-1-ol (at 10 mg/mL, 121.70±2.44). The fact that aromatic chemicals are significant components of pheromones in these insect pests may explain why females respond to them more strongly than aliphatic compounds. Additionally, differences shown in dose-response studies may result from changes in the rates at which various substances are released (Park et al., 2001) and in the sensitivity of olfactory receptor system, which would represent variable tuning of receptors (Visser et al., 1996). Sesquiterpenes, among the group of terpenoids, produced a substantially lower EAG response than oxygenated and hydrocarbon monoterpenes. Previous report by Sinha and Krishna,1971 showed that terpenoid of optimum concentration is an important parameter to stimulate the feeding activity of *A. foveicollis* in cucurbita plant. Higher quantities of these chemicals, however, operate as a feeding deterrent whereas lower concentrations of these compounds will not trigger any feeding activity.

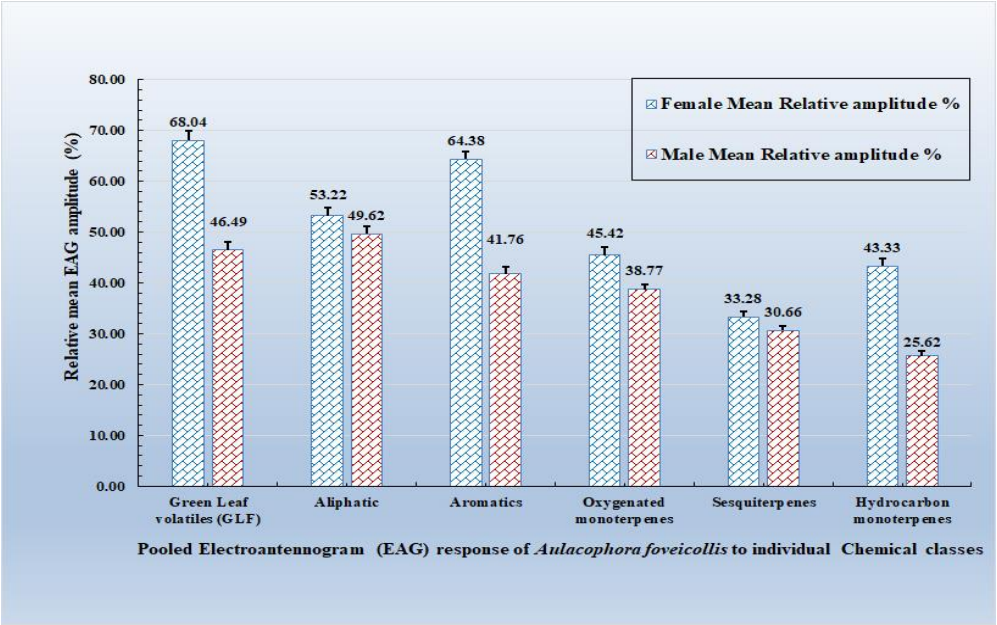

Figure 5. Pooled EAG response of *A. foveicollis* to individual chemical classes

Conclusions

The morphology and olfactory responses of antennal chemosensilla of the pest, examined by SEM and EAG revealed prominent sexual dimorphism in the distribution and responses of the sensilla, the predominance of olfactory sensilla with the presence of few mechanosensilla and greater responses was observed in females for Green leaf volatile GLV followed by aromatic compounds and aliphatic compounds.

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Conflict of interest

No conflict of interest exists.

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ORIGINAL ARTICLE

LIFE SCIENCE

EXPOSURE UNDER CHOLINE CHLORIDE EXHIBITS SUCCESSFUL GONADAL MATURATION OF INDIAN MAJOR CARPS AND AIR-BREATHING TELEOSTS IN A SEMI-INTENSIVE PISCICULTURE SYSTEM: A HISTOTECHNOLOGICAL INTROSPECTION

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Abstract

Surveillance under direct field-pond application of choline chloride in addition to farm-made-aqua-feed under semi-intensive culture system was investigated on the gonadal maturity in two Indian Major Carps *Catla catla* (Catla) and *Labeo rohita* (Rahu) and in two air-breathing teleosts, e.g., *Clarias batrachus* (Magur) and *Anabas testudineus* (Koi) reared in a ratio of 2:5:1:1:: Catla:Rahu:Magur:Koi for a period of 90-d both during dry [November to January as control-dry (CD) and treatment-dry (TD)] and in breeding seasons [June to August as control-breeding (CB) and treatment-breeding (TB)]. Results were compared with control [C: pond (C) fed only with farm-made-aqua-feed] and treatment [T: ponds (P1 and P2) fed with farm-made-aqua-feed plus feed-grade choline chloride]. The histological observations of ovary under control condition in both the seasons (CD and CB) depicted the follicular layer separation, follicular atresia, resulting into non-fertile oocytes, and ovarian tissue necrosis, declination of yolk granules, while under choline supplementation in both the seasons (TD and TB), the fish species showed ripe and developed oocytes resulting into excellent reproduction performance and steroidogenesis as well as ovulation especially in breeding season. Besides, the exposure of choline (TB) has improved manifolds in the seminiferous tubules of testis of the experimental fish species with the development of increased sertoli cells, development of mature spermatozoa within the epidermis resulting into successful maturation of the sperm and occurrence of better sperm quality having increased motility especially in the breeding season. Finally, choline can trigger the successful ovarian maturation depicting better yield, causing substantial profit to fish farmers.

Keywords: Indian major carps, air-breathing teleosts, semi-intensive culture, choline chloride, ovary, testis

Introduction

Choline, a rediscovered vitamin B_4 that mostly exists in the form of phospholipids, plays a critical role in several biological functions. It is essential for building and maintaining the cell membranes and organelles, such as mitochondria and microsomes, and is also needed for normal maturation of the cartilage matrix of the bone (Calderano et al., 2015). It is also an essential component of acetylcholine, involved in the transmission of nerve impulses across synapses (Wauben & Wainwright, 1999). The distinguished structural feature is the presence of biologically active methyl groups labile as a methyl donor in the formation of methionine from homo-cysteine after being oxidized to

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betaine (Zhang et al., 2012). It is a lipotropic factor preventing the abnormal accumulation of lipid and development of fatty liver (Halver, 2002). Unlike other vitamins, choline can be synthesized through *de novo* synthesis, and its deficiency resulting in growth retardation and perosis in developing animals; moreover, the bioavailability of native choline varies largely and its abundance is also restricted (NRC, 1994; Ghazalah, 1998; Workel et al., 2002), while, a substantial amount of choline in the animal diets results in hygroscopicity, acceleration of oxidative loss of vitamins, and the formation of trimethyl amine (TMA) in the gastrointestinal tract of the animals (Zeisel et al., 1989). Direct application of choline chloride into the pond water under a semi-intensive culture system resulted into increased yield, quality food-fish comprising less fat in liver and muscle, decreased muscular cholesterol and finally making a conducive aquatic body for sustainable aquaculture (Das et al., 2020, 2021, 2022).

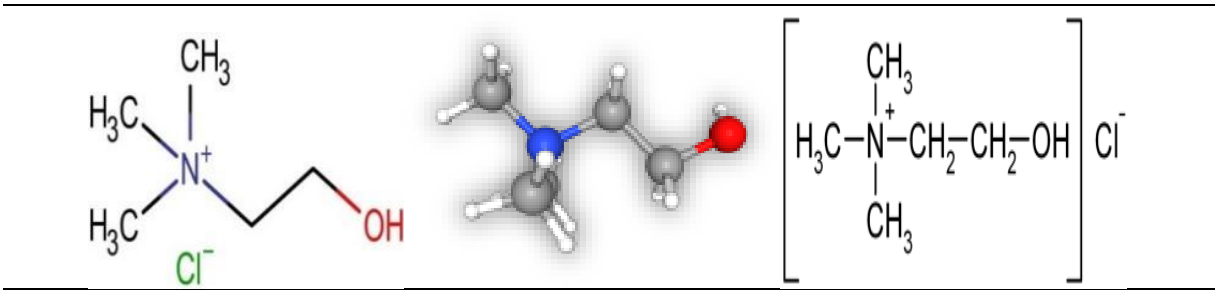

Figure 1. The Chemical structure of choline chloride (Sheard & Zeisel, 1989)(3D structure of choline chloride: iStock by Getty images).

So, there are number of studies on dietary supplementation of choline on growth and development of all animals starting from human to fish, e.g., in yellow perch (*Perca flavescens*) (Twibell & Brown, 2000), in juvenile cobia (*Rachycentron canadum*) (Mai et al., 2009), in channel catfish (*Ictalurus punctatus*) (Zhang & Wilson, 1999), in lake trout (*Salvelinus namaycush*) (Ketola, 1976), in juvenile Atlantic salmon (*Salmo salar* L.) and in juvenile Jian carp (*Cyprinus carpio*) (Wu et al., 2011) etc.

Interestingly, study on gonadal maturation due to the direct application of choline into the pond water is scanty, so, the present work had been framed to meet up this research gap for not only exploring the gonadal maturation but also the upgradation of reproductive quality of experimental fish species under the choline exposure into a semi-intensive aquaculture condition by using histotechnology.

Materials and Methods

Experimental design

The experiment was conducted under field conditions in two seasons (Dry: November to January and Breeding: June to August) for the period of ninety days in three semi-intensive ponds, where the experimental fish species (*Catla catla, Labeo rohita*, *Clarias batrachus* and *Anabas testudineus* reared in a ratio of 2:5:1:1) in the two ponds (P1 and P2) were treated with choline chloride along with 'farm-made aqua-feed, in short, 'farm-feed' and another pond was considered as control (C: fed only with 'farm-feed'). The experimental diet, study area, culture pond preparation, procurement of fish species, culture procedure, and the dose of choline chloride (feed grade: 98 % pure) directly into the treatment ponds had been followed as described earlier (Das et al., 2021, 2022). While, the analytical part of the present study was carried out in the Eco-toxicology laboratory, the University of Burdwan, Burdwan, West Bengal, India. Moreover, the present experimental protocol has also been summarized in the Figure 2.

Sampling

After the end of the experiments in both the seasons the experimental fish species both from the treatment and control ponds were taken after hauling and selected randomly (species-wise) [For treatment (T): n = 5 (3 number fishes from P1 and 2 number of fishes from P2) while, for control (C): n = 5, 5 number of fishes from C]. Finally, those were anesthetized with tricaine methanesulphonate (MS 222) and sacrificed for collecting desired tissues of gonads. Moreover, during the end of the dry season, both (treatment and control) of the gonads were collected on the 1st day of February, while in the breeding season, the desired tissues were taken on the 1st day of September.

Tissue preparation, fixation, and staining

For the purpose of general histological study, desired tissues were being fixed for about overnight in aqueous Bouin's fluid solution. Then, those were processed for paraffin sections and were cut into at 3-4 micron. The tissue sections were stained by using Haematoxylin-Eosin [(H-E: for histological study (Ghosh, 1991)] for understanding the histological orientation of cells. Finally, the stained slides were mounted with the help of the DPX solution for future use of image capturing.

Image capturing and image processing

Tissue histological studies were captured under a Leica microscope (Model no. DM 2000) with an attachment of EC3 digital camera and finally processed under 'Adobe photoshop 7' for presentation.

Results and discussion

Histotechnological observations

Ovary

In the natural condition, there are almost six stages of oogenesis (development of oocytes) depending on time, season, age and sex. Usually, according to the changes in size, nucleus, ooplasm and egg membranes of the developing ova, the developmental stages are being categorized as follows: (a) Stage 1 (oogonia), (b) Stage 2 (early oocyte or chromatin nucleus stage), (c) Stage 3 (late oocyte or perinucleolar stage), (d) Stage 4 (vacuolated follicles or yolk vesicle or cortical alveolar stage), (e) Stage 5 (yolk globule stage or vitellogenesis), (f) Stage 6 (mature follicles). During dry season we have seen up to stage 4 and in breeding season the ovary undergoes stage 5 and stage 6. Here, observations were also categorized according to developmental stages.

During dry season under farm-feed condition (CD), in the ovary of *C. batrachus* (Plate 1) revealed the presence of cortical alveolus (CA) oocytes with the content of cortical alveoli in the cytoplasm. Nucleus (N) in the cytoplasm was noticed. Nucleoli (NU) were observed in the periphery of the nuclear membrane. Cortical vacuoles appeared and those were gradually increased. A thin layer of zona radiata (ZR) was formed and was noticed under observation. Moreover, yolk vesicles (YV) were also noticed in the cytoplasm and fish under this cortical alveolar (CA) stage depicted quite thin and less condensed yolk vesicles (YV) in the cytoplasm under farm-feed exposure with the presence of small yolk globules (YG) appeared in the ooplasm around the nucleus (Photo 1.1). Under the choline exposure in the dry season (TD) the fish displayed a large number of yolk vesicles (YV) and also a moderate number of yolk globules (YG) in the cytoplasm around the nucleus. Zona radiata (ZR) was prominent and distinct in nature and the oocytes under this cortical alveolar stage (CA) the ZR was well-compacted, well-articulated, quite thickened and well-bordered (Photo 1.2). On the other hand, in *C. batrachus*, the ovary under breeding season in farm-feed condition (CB) showed a greater number of residual primary oocytes (RPO) and atretic follicle (AF) as the experimented fish were harvested in the spawning phase/stage, i.e., under the stage 6 (mature follicles). The extra vascular space (EVS) was found to be maximum during farm-feed condition. The ova were held together by the stroma (vascular collagenous connective tissue with a few smooth muscle fibres). The stroma (ST) was consisted of finger-like ovarian (ovigerous) lamellae, which contained ovarian follicles of different stage of oogenesis. Ovigerous lamellae (OL) protruded into ovocoel (ovarian cavity) from the ovarian wall and the oogenesis generally occurred in this lamellae. The stroma was found in this condition extremely pressed due to enlargement of ova (Photo 1.3).

Under the farm-feed condition in the dry season (CD), the *A. testudineus* (Plate 2) was also characterized by the appearance of cortical alveoli (CA) and small amount of yolk globules (YG). The centrally positioned nucleus (N) in the cytoplasm appeared as oval in the stage of cortical alveolus (CA) with the abundance of numerous peripherally located nucleoli of various sizes. A thin layer of ZR was appeared in this stage (Photo 2.1). Whereas, under choline-exposed condition in the same season (TD), the *A. testudineus* depicted condensed cytoplasmic and nuclear volumes which were comparatively higher. The yolk vesicles (YV) of nucleus were increased under choline exposure rather than fishes under farm-feed exposure. The nucleoli were tending to lie close to the nuclear envelop. The yolk globules (YG) were deeply noticed in this CA stage. The ZR was quite thick and predominant (Photo 2.2). Moreover, the *A. testudineus* under aqua-feed situation in the breeding condition (CB) depicted maximum number of RPO and AF. The stroma (ST) was reduced between distended ovigerous lamellae (OL) under this condition. Some yolk nucleus (YN) was found at this stage of experiment. The nucleus was found to begin to move to the animal pole just beneath the surface of oocytes and breakdown of germinal vesicles (GVBD) indicating the end of vitellogenesis, which had resulted into rapid growth of oocytes (Photo 2.3).

Catla catla (Plate 3) in farm-feed condition in the dry season (CD) depicted nucleoplasm with acidophilic reaction as revealed in the H-E stain and the nuclear membrane was irregular. Ooplasm became slightly acidophilic and few of small yolk vesicles were arranged in the periphery of the cytoplasm. Later these became cortical alveoli and took part in the formation of perivitelline space. Few small yolk globules were also observed in the ooplasm around the nucleus. Nucleus was not densely arranged in presence of nucleoli, having different sizes (Photo 3.1). But in *C. catla* under choline exposure during the same season (TD) showed a dense arrangement of yolk vesicles in presence of lager amount of small yolk globules in the cytoplasm. Zona radiata became thick and persistent. Nucleus was densely arranged and well-articulated in presence of nucleoli, having uniform sizes (Photo 3.2), whereas, in the breeding season under farm-feed condition (CB) ovary produced a sufficient number of RPO and AF. The ovocoel was noticed under the same condition. Content in the yolk vesicles was found to become thinner in presence of some nucleolus into the oocytes with a thin layer of the theca. The matured oocytes under this condition displayed slight distortion and damage (Photo 3.3).

Distortion in the membranes of the cells in most of the cortical alveolar oocytes was observed under the only farm-feed supplemented *L. rohita* (Plate 4) in the dry condition (CD). Nucleus was not arranged compactly and the nucleoli were bigger in size, but altered and deformed as noticed in the present experiment (Photo 4.1). Whereas, in the choline-supplemented condition in the same season (TD) the number and size of the nucleoli decreased in the yolk globule and thus leading to the maturing phase to form matured oocytes. A large number of yolk vesicles along with the presence of higher quantity of yolk globules were also dominant under exposure of choline in this cortical alveolar stage (Photo 4.2). Under the farm-feed condition in the breeding season (CB) the experimental *L. rohita* depicted mature oocytes, characterized by its large size and absence of nucleus. Ovigerous lamellae (OL) were prominent and distinct, but the presence of atretic follicle was maximum in absence of choline and the non-fertile residual primary oocytes (RPO) were abundant during only farm-feed condition in the present experimental species. A thin fibrous layer of basal lamina (BL) was noticed between follicular epithelium and theca layer (Photo 4.3).

Testis

Testes possess almost five developmental stages, viz., (a) resting phase or early immature condition, (b) late immature phase, (c) maturing phase, (d) mature phase, and (e) spent phase; present study intended to focus on it.

C. batrachus under choline exposure at breeding season (TB) [Plate 1]: Histologically, Testis is enclosed completely by a thin tunica albuginea. Histologically it is consisted of few collagenous fibres, some smooth muscles and elastic fibres. The testicular parenchyma is consisted of branching tubular seminiferous tubules and interstitial tissues and the seminiferous tubules contained the germinal epithelium which gives rise to spermatozoa (SP). Under this condition the spermatozoa were observed to remain in the lumens of seminiferous tubules. These seminiferous tubules were found to be lager under the breeding condition and also had a central lumen surrounded by the germinal epithelium. The sertoli cells (SC) were situated near the outer rim of the seminiferous tubules. The spermatocytes were formed by the cytoplasmic projections of sertoli cells. The spermatocytes contained primary spermatocytes (PS), secondary spermatocytes (SS) and spermatids (SD), while spermatozoa (SP) were found in the lumen of the seminiferous tubules and the content of secondary spermatocytes (SS) was quite dense and thick, spermatogonia were less and dense number of spermatozoa was also observed under choline exposure in the mature phase. The decreased volume of interstitial tissues (IT) was noticed in the triangular space between the seminiferous tubules, contained interstitial cells (IC), fibroblasts, blood vessels (BV) and collagen fibres. Interstitial cells of Leydig was bigger in size during the breeding season under choline exposure and these were located in the fibrous supporting connective tissues and formed the groups near the blood capillaries in-between the seminiferous tubules (Photo 1.4).

A. testudineus under choline exposure at breeding season (TB) [Plate 2]: Histological observations revealed presence of interstitial cells (IC), a thick bulbous content of sertoli cells (SC). Interstitial tissues were also noticed, where IC was observed as small or large clusters and contained spherical nuclei and maximum development was found in this stage of maturation under choline exposure. Moreover, abundance of SS in higher quantity with huge spermatozoa (SP) in larger volume depicted the maximum maturity of the fish under the choline exposure. Occurrence of spermatid and well-articulated sperm (S) also displayed the gross development of testis under this mature phase. A thick layer of basement membrane (BM) was also noticed under choline supplemented condition (Photo 2.4).

C. catla under choline exposure at breeding season (TB) [Plate 3]: Fish under choline-supplemented condition depicted a well-organized content of spermatogonial cells within tunica albuginea. A thick layer of basement member was also observed which was surrounded by each testicular lobe. SC, PS, SS, IC and SP were clearly visible and well-organized under choline exposure. A large number of matured SS, having dense basophilic nuclei (as observed under

H-E stain) were produced by the meiotic division and the SS were somewhat smaller than PS. The spermatids metamorphosed into spermatozoa (Photos 3.4, 3.5 & 3.6).

L. rohita under choline exposure at breeding season (TB) [Plate 4]: Under choline exposure in breeding season *L. rohita* revealed that the seminiferous tubules were comprised of primary spermatocytes, secondary spermatocytes, and spermatids. The lobules of the testis were well-articulated bearing a thick layer of basement membrane. The volume of the interstitial tissues was decreased where interstitial cells were noticed (Photo 4.4).

Histological conditions of gonads due to the application of dietary feed supplements viz., cotton seed meal (free from gossypol), commercial feed additives, e.g., Therigon®; Nuvisol Hatch®; gibberellic acid; L-carnitine, energy diets, containing soya-acid oil etc., were studied in many fishes like Nile tilapia (*Oreochromis niloticus*), juvenile common carp (*Cyprinus carpio*), sharp tooth catfish (*Clarias gariepinus*) by various researchers (Tope-Jegede et al., 2019; Wang et al., 2014; Abdelhamid et al., 2013; Cek & Yilmaz, 2008).

The farm-feed fed diet in the present experimental aliquots during dry (CD) and breeding (CB) conditions disclosed similar observations as on Nile tilapia (*Oreochromis niloticus*) under the feeding arrangement of 1.0 g Therigon®/kg basal diet showing coagulated necrosis in yolk granules, follicular layer separation, follicular atresia, resulting into non-fertile oocytes, and ovarian tissue necrosis. Degenerative changes like atretic follicles and declination of yolk granules were also noticed when the fish fed with 2.0 g Therigon® along with per kg basal diet, but 0.5 g Therigon® with per kg basal diet did not affect the fish ovary too much alike the fish of the present experiment in TD condition during choline-supplementation (Abdelhamid et al., 2013). Whereas, Nuvisol Hatch® at the rate of 1.0 g per kg basal diet depicted ripe and developed oocytes with few atretic follicles which showed resemblance to the TD condition of the present experiment in choline exposure of the experimental fish. The present experiment in the CB condition under farm-feed exposure showed similar results when they were fed with 2.0 g and 3.0 g of Nuvisol Hatch® with per kg basal diet (Abdelhamid et al., 2013). In the present experiment, in the CD and CB conditions, the vitellogenic oocyte depicted several alterations, e.g., cell wall erosion in some oocytes, yolk sphere liquefaction, containing vacuoles in the ripe oocyte of *O. niloticus* when fishes fed with 60 mg gibberellic acid with per kg basal diet (Abdelhamid et al., 2013). Moreover, the occurrence of such alternations as well as degradation of ovary in farm-feed fed system both in the CD and CB conditions might be resulted from the environmental stress, thrust of agricultural waste and bacterial invasion, which caused disruption and development of germ cells, finally, reproductive ability of the experimental fish became reduced (Cek & Yilmaz, 2008; Lye et al., 1998). Furthermore, choline into the water [as GnRH (Gonadotropin releasing hormone) stimulant] might result in an excellent reproduction performance in females and the hypothalamic neurosecretory decapeptide exhibited gonadotropin secretion, steroidogenesis and ovulation in breeding season alike in salmon as studied by Van Der Kraak et al. (1984); Haraldsson and Sveinsson (1993); El-Sebai et al. (2003). But the dietary gossypol (a toxic crystalline compound present in cotton-seed oil) present in the cotton-seed meal (CM) did not affect the ovary (development or degradation) and gonado somatic index (GSI) of *C. carpio* and *O. niloticus* (Wang et al., 2014; Rinchard et al., 2002) when fed with the basal diet, even in the high level of the dietary CM (52 %). Whereas, dietary gossypol caused a negative impact on the gonad maturity of male fish, viz., decreased sperm concentration with the increase of dietary gossypol (0.22 - 0.95 %) in rainbow trout (Dabrowski et al., 2001), depletion of sperm cells in seminiferous tubule at dietary CM of 24 % in tilapia (Salaro et al., 1999), lowered sperm cell ratio at a level of 54 % CM in *C. carpio* (Wang et al., 2014). A gross development of testes under the choline-supplemented condition in the breeding season was observed in Nile tilapia, fed with L-carnitine (dipeptide amino acid), synthesized from methionine (a metabolite of choline) and lysine (Zeyner & Hameyer, 1999). On the other hand, extensive seminiferous tubules degeneration, necrosis in the seminiferous tubules and also in the focal areas with major haemolysis in testicular tissue were observed when the same fishes (Nile tilapia) were fed with 1100 mg with per kg of basal diet (Msiska, 2002; Abdelhamid et al., 2013). Actually, choline may procure requisite substrate for the spermatozoa within the epidermis; it can trigger the successful maturation of the sperm in the breeding season and also initiated the motility of the sperm and thus, resulted in better sperm quality (Chatzifotis et al., 1995; El-Damrawy, 2007). On the other hand, the ovarian development was sharply affected by the exposure of high energy diets, containing soya-acid oil in higher concentration in the sharptooth catfish, depicting a smaller number of yolky oocytes and follicular atresia by hindering the metabolic function in the liver, while the development of vitellogenic follicles is directly related with the synthesis of yolk (vitellogenin) done by the liver (Cek & Yilmaz, 2008; Guraya, 1986). But an optimum concentration of the energy diet resulted in the best weight gain as well as best gonadal maturity, with the highest mean number of yolky oocytes in the ova in sharptooth catfish (Cek and Yilmaz, 2008), similar to the present experiment in choline-supplemented condition. Moreover, the choline into pond water along

with the basal diet improved the seminiferous tubules of testis as the number of sertoli cells increased a lot which has a direct co-relation with sperm production as also reported by Tope-Jegede et al. (2019) in Nile tilapia.

Conclusion

Present investigations revealed the number of ripe and developed oocytes with few atretic follicles resulting into excellent reproductive performance in female, and the hypothalamic neurosecretory decapeptide exhibited gonadotropin secretion, steroidogenesis and ovulation especially in breeding season during additional choline supplementation. Moreover, choline can trigger the successful maturation of the sperm in the breeding season and it can also able to initiate the motility of the sperm in testis and thus, resulted in better sperm quality under its exposure as revealed in the present experiment. Thus, it can be assumed from the present observations that the maiden attempt of administrating choline chloride directly into the pond water under field condition resulted in better quality of brood fishes that can be utilised for spawn production with higher success rate under this semi-intensive pond culture system which may support rural, poor fish farmers to a greater extent.

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Conflict of Interests

The authors declare no conflict of interest with the contents of this article

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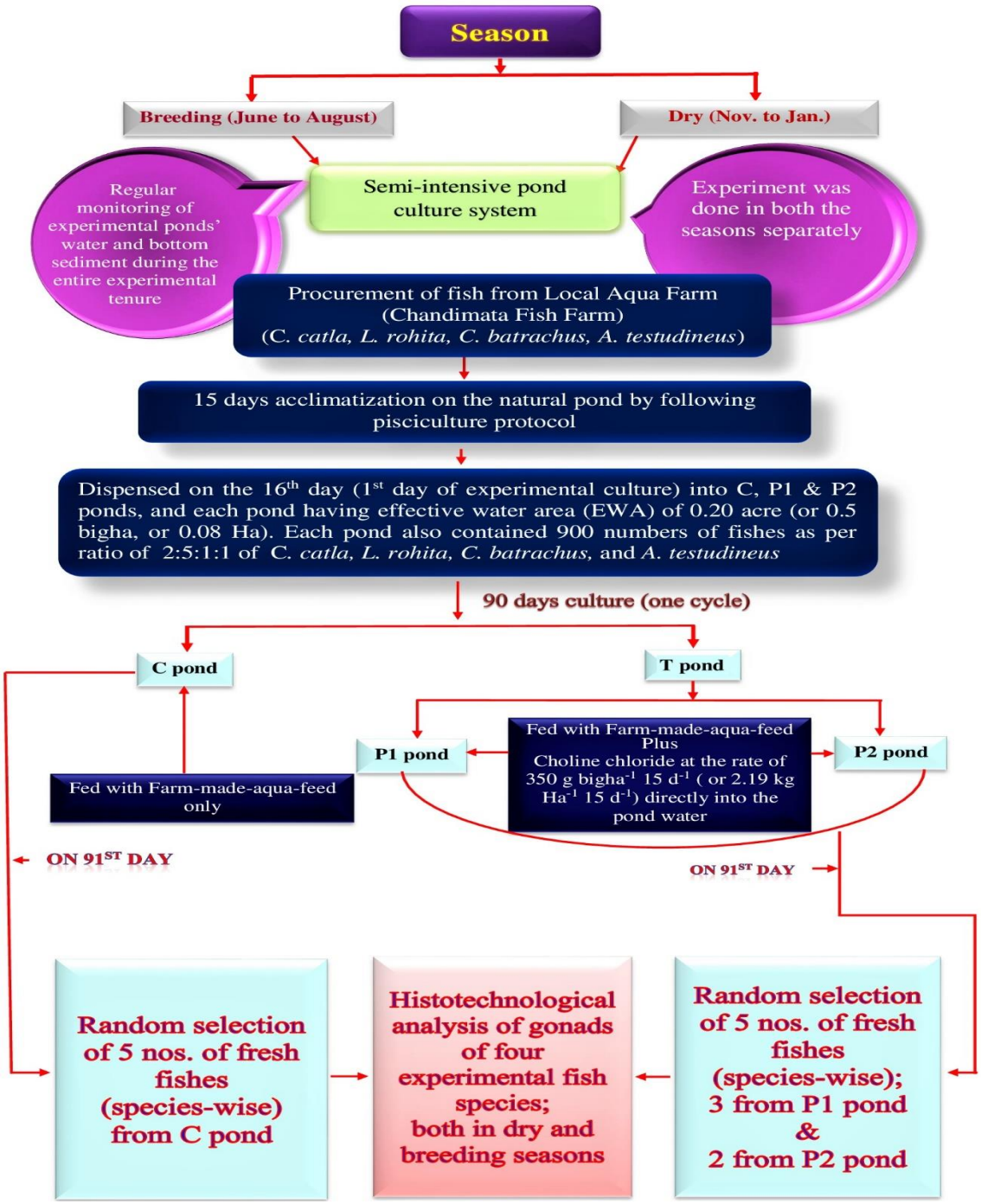

Figure 2. Experimental design at field.

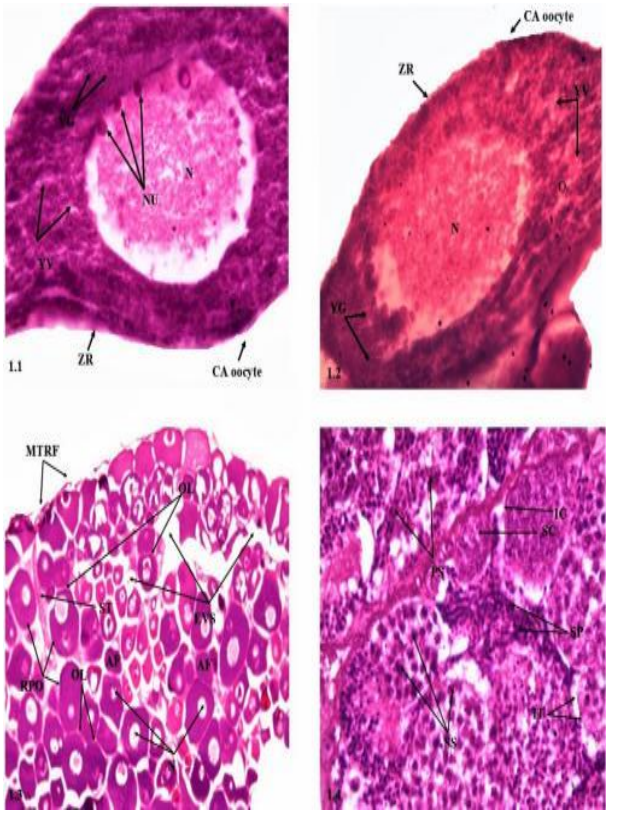

Plate 1: Gonads of *C. batrachus*

Plate 2: Gonads of *A. testudineus*

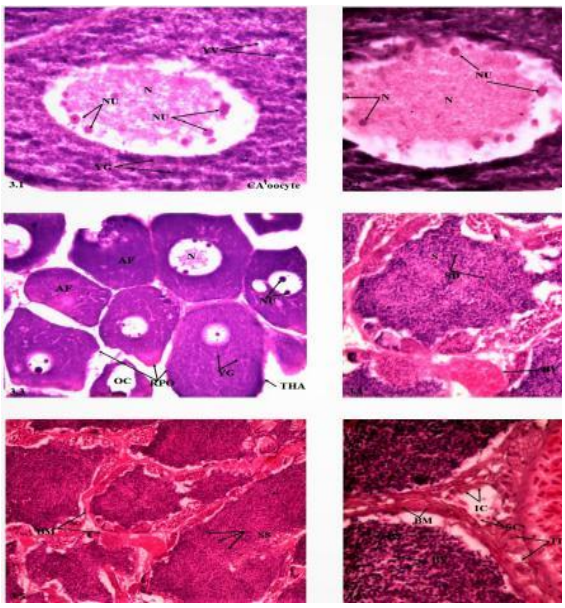

Plate 3: Gonads of *C. catla*

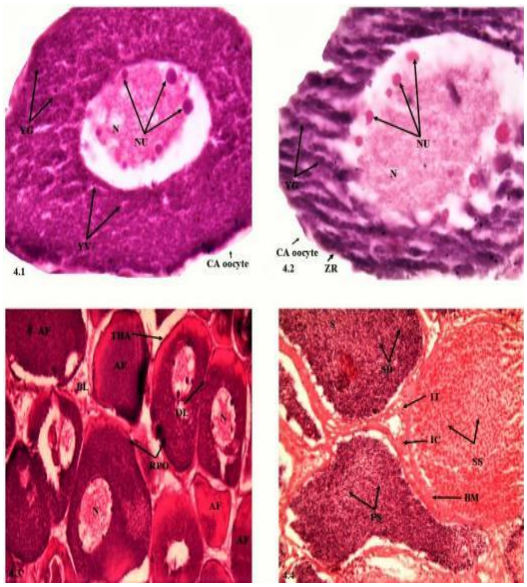

Plate 4: Gonads of *L. rohita*

PLATE 1

Photos 1.1 - 1.4: Photomicrographs of transverse section of gonads in *C. batrachus* under control-dry (CD), treatment-dry (TD), control-breeding (CB) and treatment-breeding (TB) conditions under field exposure showing cortical vacuoles of cortical alveolus (CA) oocytes of ovary, thin layer of zona radiata in the ovary; well-compacted, well-articulated and thickened zona radiata; residual primary oocytes and atretic follicle, stroma consisting of finger-like ovarian lamellae in the ovary; dense number of spermatozoa in the lumens of seminiferous tubules, sertoli cells, primary spermatocytes (PS), dense and thick secondary spermatocytes (SS), spermatids (SD), interstitial tissues (IT) and interstitial cells of Leydig in the testis. [H-E; X 400, 1000]

PLATE 2

Photos 2.1 - 2.4: Photomicrographs of transverse section of gonads in *A. testudineus* under CD, TD, CB and TB conditions under field exposure showing deformed appearance of cortical alveoli stage of ovary; well-compacted and densely arranged yolk globules and thick layer of zona radiata of ovary; residual primary oocytes with atretic follicle in mature follicular stage of the oocyte, reduced stroma in the ovary; interstitial cells and interstitial tissues, bulbous content of sertoli cells, secondary spermatocytes in higher quantity with huge spermatozoa, healthy spermatid and well-articulated sperm under mature follicular stage of the oocyte in the matured testis. [H-E; X 200, 400, 1000]

PLATE 3

Photos 3.1 - 3.6: Photomicrographs of transverse section of gonads in *C. catla* under CD, TD, CB and TB conditions under field exposure showing small yolk vesicles and yolk globules in the ooplasm around the nucleus under cortical alveolar stage of ovary; presence of thick and persistent zona radiata, and densely arranged well-articulated nucleus in the ovary; occurrence of sufficient number of residual primary oocytes and atretic follicles, thinner content in yolk vesicles with a thin layer of theca under mature phase of ovary; occurrence of well-organized spermatogonial cells within tunica albuginea, well-articulated sertoli cells, secondary spermatocytes, primary spermatocytes, interstitial cells, spermatozoa and numerous blood vessels with thick layer of basement membrane (BM) in the testis. [H-E; X 200, 400, 1000]

PLATE 4

Photos 4.1 - 4.4: Photomicrographs of transverse section of gonads in *L. rohita* under CD, TD, CB and TB conditions under field exposure showing occurrence of non-compacted nucleus with altered and deformed nucleoli under cortical alveoli stage of ovary; decreased numbered and reduced sized nucleoli in the yolk globules, but presence of higher quantity of yolk globules in the ovary; presence of maximum number of atretic follicle and non-fertile residual primary oocytes, thin fibrous layer of basal lamina in the matured ovary; occurrence of numerous healthy primary spermatocytes, secondary spermatocytes, and spermatids, well-articulated lobules, and reduced volume of interstitial tissues with abundant interstitial cells in the matured testis. [H-E; X 200, 400, 1000]

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ORIGINAL ARTICLE **LIFE SCIENCE**

ASSESSMENT OF WATER QUALITY PARAMETERS OF AN ABANDONED OPENCAST COAL PIT (OCP) OF ASANSOL-RANIGANJ COALFIELD (ARCF), PASCHIM BARDHAMAN, WEST BENGAL, INDIA

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Abstract

Coal mining and its auxiliary actions have already been proved to cause potential pollutions to ecosystems. This research work assesses the surface water quality of Samdihi abandoned Opencast Coal Pit (OCP) of Asansol-Raniganj Coalfield Areas (ARCF). Twenty seven water samples were collected maintaining temporal variability and were analyzed for physicochemical attributes. The pH was slightly alkaline with lowest of 7.8 during winter. The water temperature varied between 20 and 35°C. Conductivity was highest during winter (601±3.51 µS/cm) and lowest during monsoon (333±2.8 µS/cm). The Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) ranged from 212 to 2738 mg/L and 170 to 413 mg/L respectively. The low values of TSS (212 mg/L) and TDS (170 mg/L) were observed during winter and the higher values of TSS (2738 mg/L) and TDS (413 mg/L) were observed during monsoon. The dissolved oxygen concentration was moderately high (between 6.89 and 8.43 mg/L) but comparatively higher (8.5 mg/L) in monsoon. The estimated dissolved concentration of phosphate, sulphate, sodium and potassium were 4.2±0.17, 98.6±10.48, 8.6±0.47 and 2.5±0.75 mg/L respectively. Zooplankton population in the OCP was found to be 8±1.76 ind/mL in monsoon and 14±1.12 ind/mL in winter with an impressive abundance of 157/L and 274/L in monsoon and winter respectively. The water chemistry suggests the non-potability compared to prescribed standards but the chemical attributes showed promising traits for pisciculture, aquaculture, horticulture and/or other recreational practices that may assist in improving the socio-economic condition of the community habituating in and around the area.

Keywords: Samdihi OCP, water quality, aquaculture, agriculture, pisciculture

Introduction

In India, coal is the key fuel resource and primary source of energy and the opencast coal mining process is one of the best and economically viable methods of extraction of coal in India. Mining operations usually create an adverse environmental impact, both during the mining activity and subsequently after its closure. Mineral resources are essential materials, because it provides more than 95% of primary energy, 80% of industrial raw materials and 70% of agricultural production materials. After surface mining, it forms a big pit, i.e., void, locally called *khadans*. After coal extraction and dewatering ceases, opencast mining resulting into a void which is eventually filled up by surface runoff and groundwater seepage and becomes a pit-lake or large reservoir. Pit lakes can represent significant liabilities after closing mining activities. These pit lakes are the potential water reservoir for local population of Asansol-Raniganj Coalfield Areas of Paschim Bardhaman (Ghosh, 2012). Transformation of *khadans* into fish ponds or means of aquaculture is one of the most productive processes of ecological restoration and vertical expansion of a productive resource (Pal et al., 2014). Use of mine water for aquaculture is a practical way to avoid pathogens and their hosts and enhance the nutritive value of the concerned fish practised in this pisciculture (Pal et al., 2013). The water scarcity is a major problem in this coalfield area which can be solved by these permanent water reservoir of this area and at the same time these water bodies can be used for different purposes like pisciculture, agriculture, horticulture, recreation,

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aquatic and wildlife conservation, industrial purposes, flood control and waste management (McCullough & Lund, 2006).

Keeping in mind the constant growth in population with the trailing surge in demand of food, shelter and employment, the present study aimed to investigate the water quality of an opencast pit lake and to explore the possibilities of transformation of these water bodies into a site of specific impoundments for speculated fish farms and other vertical and horizontal developmental probabilities. The hydrological quality obtained from this open cast coal pit is highly variable, depending on the type of mine, the environment or the climate and local geochemistry. The physicochemical properties of pit lake aquatic system depend on the composition and nature of the overburden and bottom sediment, and the growth and development of biological organisms. Several studies on ARCF areas revealed that mining of this area is free from acid mine drainage (Ghosh et al., 2005). The present study undertaken in the Samdihi abandoned OCP of Asansol Coal Block, under Eastern Coal Field Limited (ECL), thus, foreshows the intense possibilities of socioeconomic development of local population based on the safe uses of this large water reservoir through fisheries, agriculture, horticulture and allied purposes.

Materials and Methods

Description of study site

Samdihi khadan (or *dihi/dighi* in Bengali means large reservoir) is located at Samdihi colliery (Samdihi Opencast Coal Mine) in Lohat region of ARCF areas of Eastern Coalfields Limited under Paschim Bardhaman District of West Bengal and around 20.0 km from Asansol. It is about 5.0 km of south east to Rupnarayanpur Railway Station of Eastern Railways and about 8.5 km of north east of Salanpur Railway Station of Eastern Railways within the latitudinal and longitudinal extension of about 23°47'06"N to 23°47'07"N and 86°55'37"E to 86°55'40"E. Total land cover of this area is around 114.0 km² with an outstanding population of around 2.50 lakh according to 2011 census. The nearest human habitat is in Sangramgarh, Samdihi, within 0.5 km from the experimental pit lake.

The Samdihi OCP is very significant in regard to its location and recent closure not more than about 5-7 years ago, it is a perfect experimental site with lowest anthropogenic interventions. The area of this OCP is currently near 1,80,433.62 m² with an irregular perimeter run of about 2,286.25 m and average mean sea level (MSL) of about 14.0 ft. Samples were collected from four distinct sites, named Spot 1 (23°47'06.5"N and 86°55'27.03"E), Spot 2 (23°47'07.02"N and 86°55'37.8"E), Spot 3 (23°47'10.05"N and 86°55'43.75"E) and Spot 4 (23°47'08.49"N and 86°55'40.04"E).

Figure 1. Map of Samdihi OCP with four distinct sampling sites.

Collection of samples and methodology

Water samples were collected in laboratory grade, non-reactive sampling bottles of 1L capacity each from all the four spots in triplicate and were preserved properly for further studies. For dissolved oxygen and biochemical oxygen demand, water samples were collected in clean and clear glass BOD bottles of about 300mL each from four spots.

Zooplankton population and abundance were assessed by collecting by plankton net of mesh size 22 micron by horizontal hauling process and were collected and condensed into 50mL collection tube and were fixed with 5% formaldehyde solution for further studies.

Table 1. Methods of analysis of physicochemical parameters of Samdihi OCP.

Parameters	Methods	Reference (s)
pH	Electrode Method	APHA-AWWA, 2005
Temperature	Electrode Thermometer Method (Total Immersion Thermometer)	APHA-AWWA, 2005
Dissolved Oxygen (DO)	Titrimetric (Winkler's Method)	APHA-AWWA, 2005
Conductivity	Electromagnetic Induction Method	APHA-AWWA, 2005
Biochemical Oxygen Demand (BOD)	Titrimetric (Winkler's Method)	APHA-AWWA, 2005
Alkalinity	Titrimetric (Sulphuric Acid Method)	APHA-AWWA, 2005
Acidity	Titrimetric (Sodium Hydroxide Method)	APHA-AWWA, 2005
Hardness	Complexometric Titration (EDTA Method)	APHA-AWWA, 2005
Combined Carbon Dioxide	Titrimetric (Free CO_2 Method)	APHA-AWWA, 2005
Salinity	Electrical Conductivity Method	APHA-AWWA, 2005
Total Dissolved Solids (TDS)	Gravimetric Method	APHA-AWWA, 2005
Total Suspended Solids (TSS)	Gravimetric Method	APHA-AWWA, 2005

Table 2. Methods of analysis of anions and cations of Samdihi OCP.

Parameters	Methods	Reference (s)
Chloride	Argentometric Method	APHA-AWWA, 2005
Sodium	Flame Emission Photometric Method	APHA-AWWA, 2005
Potassium	Flame Photometric Method	APHA-AWWA, 2005
Nitrate-nitrogen	UV-Spectrophotometric Screening Method	APHA-AWWA, 2005
Ammoniacal-nitrogen	Titrimetric (Sulfuric Acid Method)	APHA-AWWA, 2005
Phosphate	Spectrophotometric (Stannous Chloride Method)	APHA-AWWA, 2005
Sulfate	Gravimetric (Barium Chloride Method)	APHA-AWWA, 2005

Method for plankton population and abundance

Zooplankton count was done by 'Sedgewickrafter cell count method' and the plankton abundance index was calculated using the formula (Agus Tjahjono & Hartanto, 2020):

$$N = \frac{1}{A} x \frac{B}{C} x \frac{D}{E} xF$$

Where,
N – Plankton abundance/L (ind/L)
A – Volume of filtered water (L)
B – Volume of water in the sample (mL)
C – Volume taken during identification (1 mL)
D – Area of glass cover or preparation space (mm^2)
E – Field of view (25 mm^2)
F – Average number of observed individuals

Results and Discussion

Physicochemical parameters

Table 3. Analysis of Physicochemical parameters of Samdihi OCP water in monsoon and winter.

Parameters	Seasons	
	Monsoon	Winter
pH	08.12±0.79	07.80±0.56
Temperature (^oC)	35.00±2.10	20.00±1.98
DO (mg/L)	08.50±0.13	06.89±0.11
Conductivity ($\mu S.cm^{-1}$)	333.00±2.80	601.00±3.51
BOD (mg/L)	01.90±0.06	01.10±0.01
Alkalinity (mg/L)	180.00±6.60	207.23±4.90
P-acidity (mg/L)	106.00±8.01	106.00±6.97
M-acidity (mg/L)	11.20±4.15	11.20±4.88
Hardness (mg/L)	260.00±2.73	199.90±2.53
Combined CO^2 (mg/L)	171.30±4.60	102.10±3.90
Salinity (mg/L)	00.04±1.20	05.00±2.60

N.B.: Data represented as average value ± standard deviation

pH

pH is the good indicator for determination of the quality of water. For a productive ecosystem, pH ranges from 6.7 to 8.4 and is considered to be safe for aquatic life, and pisciculture can be done under the pH ranging from 6.0 to 9.0 (Swingle, 1967). In this study average pH of all the sampling sites (spot 1 to 4) of Samdihi OCP were between 7.0 and 9.0, with the highest of 8.12±0.79 in monsoon and lowest of 7.80±0.56 in winter. The pH is impacted not just by the response of carbon dioxide yet additionally by the natural and inorganic solutes present in water, thus, any change in water pH is joined by the progressions alongside other physicochemical boundaries (Kulshrestha et al., 1992). Basic nature of pH of the OCP water body of this study communicated its cushioned and high trophic circumstances, which was comparative with the examinations made by Chandrasekhar (1996) on Saroornagar Lake of Hyderabad and Jumbe and Nandini (2010). Kulshrestha et al. (1992) also expressed similar kind of results as in this study, that higher the pH during hot seasons were presumably due to excessive photosynthesis resulting into the precipitation of carbonate of calcium and magnesium from bicarbonates causing higher alkalinity in water bodies. In this manner, the pH in the OCP was found reasonable for endurance and culture of fish in the re-established biological system of water body.

Temperature

Water temperature regulates the biological activity of aquatic organisms like survival, metabolism, physiology, growth, development and reproduction (Welch, 1952). In this study the average temperature was noted as 20±1.98and 35±2.1^oC in winter and monsoon respectively. Temperature around 30^oC is considered to be optimum for biological activities and thus the observed temperature of the Samdihi OCP being in that range can signify the conduciveness of fish culture.

Dissolved Oxygen

Dissolved oxygen concentration plays a key role in aquatic ecosystem and regulates the health of aquatic organisms. Dissolved oxygen (DO) is one of the most important indicators of water quality and is essential for the survival of fish and other aquatic organisms (Santhosh & Singh, 2007). In Samdihi OCP, in winter season DO value was 6.89±0.11 mg/L, but in monsoon it increased upto 8.5±0.13 mg/L. Higher DO in rainy season is suitable for fish culture. Higher DO during monsoon may be because of the effect of downpour water bringing about air circulation and agitation, which was supported by Hannan et al. (1979). Crampton et al. (2003) described that the hotter limnic climate can cause downfall of dissolved oxygen content of the water bodies, but in this study, the DO was noted to increase with rise in temperature, which is due to increased rate of photosynthesis by macrophyte and planktonic

community, and wind action during monsoon. Thus, the observed concentration of DO in Samdihi OCP showed the optimum condition of the OCP for fish culture.

Conductivity

Conductivity is sensitive to variation in dissolved solids, mineral salts dissociation, ion mobility and the temperature. The electrical conductivity values of Samdihi OCP water was under the range of 330.0and 605.0µS/cm. In the current study, highest value of conductance was recorded in winter (601±3.51 µS/cm) and lowest during rainy season (333±2.8 µS/cm). Although, Shariatpanahi and Anderson (1987) described the increased conductance with higher temperature and dissolved solids, the consistency of higher conductance throughout the colder time of year (580 to 601µS/cm) can be explained by the phenomenon of decomposition of organic matter increasing the dissolved solids and ion mobility of the OCP water. Also, the event of low conductance in winter was explained and supported by Salodia (1996).

Biochemical oxygen demand

Biochemical oxygen demand (BOD) can get reduced in heavy oxygen availability and consuming condition. In this study, the BOD was 1.9±0.06 and 1.1±0.01mg/L during monsoon and winter respectively. Higher BOD was seen in rainy season because of oxygen availability and oxygen dissolving capacity is higher in high temperature also; air activity and agitation tend to increase dissolved oxygen in water which then increased the BOD values of Samdihi OCP. The ideal BOD level for fish culture must not be more than 10.0 mg/L (ICAR, 2007) and water with BOD more than 35.0 mg/L are not considered as commodious quality of water for fish culture (Pande & Sharma, 1999). In the present study, BOD of 1.9±0.06 mg/L in monsoon months was due to presence of a load of organic matters coming from degrading aquatic organisms in OCP, and thus provides a great base for pisciculture (Wurts & Durborow, 1992).

Alkalinity, total acidity, total hardness and combined carbon dioxide

Total alkalinity, total acidity, total hardness and combined carbon dioxide (CO_2) are the interrelated biological parameters in an aquatic framework. These parameters can influence the actual stress level of freshwater fishes, oxygen availability in the aquatic system, and other harms.

Total alkalinity is the amount of bases present in natural water and alkalinity comprises carbonate (CO_3^{2-}) and bicarbonate (HCO_3). The present study revealed the alkalinity of the Samdihi OCP as of 180.00±6.60 mg/L and 207.23±4.90 mg/L in monsoon and winter respectively. The average value of alkalinity of the OCP water was 193.62±5.75, ranging a higher value in winter and lower in monsoon because of impact of buffering of pH, copper disintegration and dissociation of other metals which was supported by Boyd & Tucker (2012). An alkalinity value of 20.0 mg/L and more can be sustainable for aquaculture, although a range of 50 - 200 mg/L as $CaCO_3$ is generally suitable for fish culture (Wurts & Durborow, 1992). In this context, it can be established that the OCP water under present condition may be proved reasonable for fish culture and sustainability.

Likely, total acidity is the sum total of titrimetric value of phenolphthalein acidity (P-acidity) and methyl orange acidity (M-acidity). Hydrolyzing salts and mineral acids can contribute in increasing the acidity of a water body (Apha, 2005). In this study, the concentrations of P- and M-acidity were 106±6.97 and 11.2±4.15 respectively, which defines the total acidity range to be in favour of pisciculture (Wurts, 2000). Also, there was no significant change in the values of acidity during the winter and monsoon seasons.

Total hardness is a significant parameter in determining the probability for fish culture and is a significant viewpoint of estimating water quality. In this OCP water, total hardness was maximum in monsoon (260±2.73 mg/L as $CaCO_3$) because of less retention by aquatic plants and degradation of organic materials, while in winter, the value decreased upto199±2.53 mg/L as $CaCO_3$ due to increased bioabsorption and same was explained by Silva et al. (2003). However, Wurts (1993) described the availability and dissociation of calcium and magnesium in higher temperature to be the factor behind the variations of hardness.

In Samdihi OCP, monsoon season bears greatest measure of carbon dioxide load (171.3±4.6 mg/L) and winter showed the low value of CO_2 (102.1±3.9 mg/L). Aquatic combined CO_2 appears mainly from the respiratory course of aquatic organisms, some elemental dissolution and organic matter decomposition (Mandal et al., 2021). Thus, heavy organic load and greater algal bloom in higher temperature causes rise in aquatic carbon in monsoon as compared to winter (Hargreaves et al., 1996).

Salinity, total dissolved solids and total suspended solids

Salinity of water is a significant factor in deciding numerous aspects of water chemistry and organic cycles inside it. In the present study, during winter the salinity of the OCP was much higher (5±2.6 mg/L) compared to that of monsoon (0.0318±1.2 mg/L) due to lower temperature and higher conductance of the water in winter (Mondal et al., 2015).The total dissolved solids (TDS) and total suspended solid (TSS) refer to the solid matter substances dissolved and suspended in water respectively, both of which significantly define the productivity, particulate load and water quality. In this study, the TDS and TSS values of the samples were higher in monsoon (413±6.3 mg/L and 2738±2.1 mg/L, respectively) due to high rate of water drainage and overburden runoff, while in winter, the values were much lower (170±4.7 mg/L and 212±2.3 mg/L, respectively). Similar results were denoted by Pal et al. (2013).

Anions and cations

Table 4. Analysis of anions and cations of Samdihi OCP water in monsoon and winter.

	Seasons	
Parameters	Monsoon	Winter
Chloride (mg/L)	16.99±5.70	16.90±7.10
Sodium (mg/L)	08.60±0.47	06.66±0.55
Potassium (mg/L)	01.27±0.53	02.50±0.75
Nitrate-nitrogen (mg/L)	00.81±0.16	00.16±0.29
Ammoniacal-nitrogen (mg/L)	00.25±0.19	00.19±0.08
Phosphate (mg/L)	04.50±0.17	00.02±0.21
Sulfate (mg/L)	98.60±1.48	23.25±2.00

N.B.: Data represented as average value ± standard deviation

Chloride (Cl)

Chloride is a significant parameter of water quality and is helpful to fish in keeping up with their osmotic equilibrium. In the OCP, the chloride concentration was found to be in a steady minimum average of about 16.99±5.7 mg/L. Though, there is no particular standard of chloride content for freshwater fish culture, but value near 100.0 mg/L is suitable (Ghosh et al., 2005). This low concentration (16.99±5.7 mg/L) of chloride in this OCP may be due to the lack of the chloride rich rocks which were the primary providers of the chloride in OCP water.

Sodium (Na^+) and potassium (K^+)

Sodium and potassium, the micronutrients are very important for aquatic life. In this study, the sodium concentration in OCP water was high in rainy season (8.60±0.47 mg/L) and low in winter (6.66±0.55 mg/L). But, the potassium concentration followed opposite traits with higher value of 2.50±0.75 mg/L in winter and as low as 1.27±0.53 mg/L in monsoon. Being micronutrients, the concentrations of sodium and potassium are usually low in freshwater but value near 5.0 mg/L is suitable for the sustainability and proper cellular metabolism of aquatic organisms (Tepe & Mutlu, 2004).

Nitrate-nitrogen (NO_3-N) and ammoniacal-nitrogen (NH_3-N)

In the present study, the nitrate-nitrogen (N-Nitro) was recorded within the normal range, measuring highest in monsoon (0.807±0.16 mg/L) and lowest in winter (0.16±0.29 mg/L). Nitrate is the most non-harmful of the major inorganic nitrogen compounds and is found as the finished result of the nitrification interaction of the aquatic system. For pisciculture <80 mg/L nitrate can be the suitable as reported by Svobodová (1993), but according to Pillay (2008) and Zweig et al. (1999) <3.0 mg/L nitrate is admissible for aquaculture.

In this study, the values of ammoniacal-nitrogen (A-Nitro) were found 0.25±0.19 mg/L as the highest in monsoon and 0.19±0.08 mg/L as lowest in winter. Unlike nitrates, ammonia is the underlying result of the deterioration of nitrogenous natural materials, organic substances and digestion of microorganisms, and may demonstrate the presence of decaying urea, excrement, and organics. Accordingly, the present study indicated the availability of phytoplankton and water plants to absorb those as also evidenced by Wurts (2003). Zweig et al. (1999) proposed that the value of ammoniacal-nitrogen within 0.1 mg/L is suitable for fish culture practices which were later additionally supported by Pillay (2008).

Phosphate (PO_4^{3-}) and sulfate (SO_4^{2-})

Phosphate permits plants and animals to develop and keep up with the proper physiological functioning. From the current study, in OCP water, higher phosphate value was recorded during monsoon as 4.5±0.17 mg/L, while in winter, it was lowest 0.019±0.21 mg/L. Sayre et al. (1983) and USEPA (1986) recommended that for sustainable freshwater fish cultivation, the phosphate-phosphorus value should be less than 5.0 mg/L, otherwise excess of it might cause eutrophication in water body which is most unsafe for fish culture.

Sulfate adds to osmotic tension, and sulfur is a part of protein in organisms' body. In the present study, OCP water portrayed greatest sulfate content in rainy season (98.6±1.48 mg/L) and lowest in winter (23.25±2 mg/L). Sulfate in OCP water is mostly coming from sulphur containing rocks, though there is no such evidence found in this case. Nonetheless, freshwater fish evidently don't have any explicit prerequisite for sulfate in water and they get sulphur mostly from their food. Sulfate had positive relation with EC, thus, higher EC may be the conceivable justification for the higher sulphate content in OCP water bodies during rainy season (Cole, 1979).

Plankton

Zooplankton population count

Plankton is the different assortment of life form tracked down in water that can't drive them against a water current momentum. In the aquatic system, they give an urgent wellspring of food to numerous little and huge aquatic living beings, like bivalves, fish, whales and other aquatic organisms. In this study, the average zooplankton count of samples was 8±1.76 and 14±1.12 ind/mL in monsoon and winter respectively. Zooplankton plays an important role in bio-diminishing pollutants and other contaminating metals due to their high rate of bioaccumulation, and thus is a very important factor for health of any aquatic system consideration (Rossi & Jamet, 2008). Zooplankton of the order Cladocera and Calanoida was found to be plentiful in Samdihi OCP dominated by *Cyclops* sp. along with the community abundance of 7.91±0.21 U/L.

Plankton abundance

Plankton abundance of the present study attributes highest in winter (274/L) and lowest in monsoon (157/L). Thus, with the higher value of plankton abundance, the rate of bioaccumulation of heavy metals gets increased due to the special ability of plankton to take up metals much faster than other organisms, which results into a steady aquatic system suitable for aquaculture (Saygı & Yiğit, 2012). Maintenance of the nutrient cycle through polyculture is the new era of sustainable aquaculture and for that maintaining a feed pool of zooplankton and phytoplankton is necessary even though they don't have any market value, but still contributes a lot to the aquatic ecosystem (Wurts, 2000).

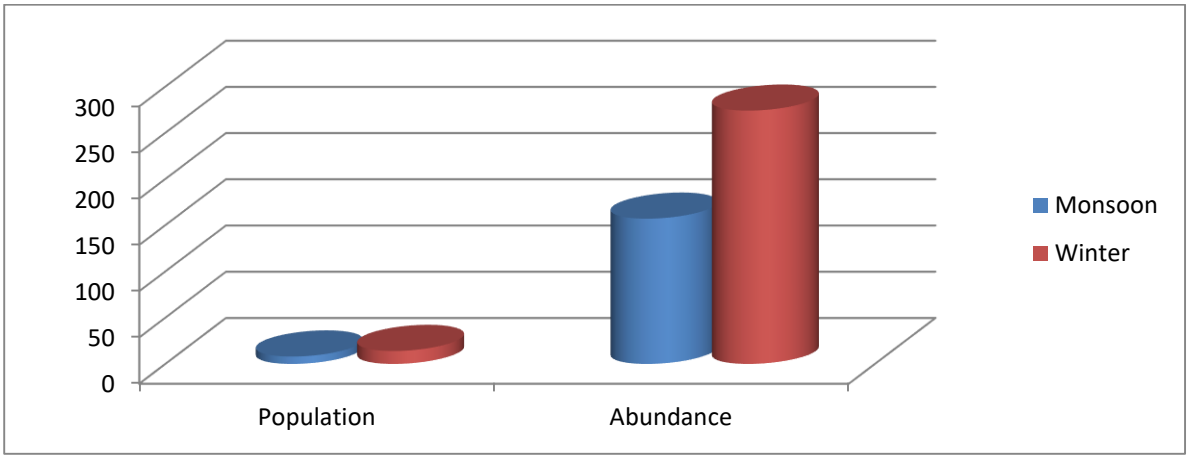

Figure 2. Graph showing zooplankton population and plankton abundance in monsoon and winter.

Conclusion

Present study intended to assess the nature of mine water comparing with a standard portrayal. This study includes assurance of physical, natural and compound boundaries of surface water of Samdihi OCP. Outcome of this

investigation revealed that, the water is substantially unsuitable for the end goal of drinking, but for other different utilization, actually could be thought of as very satisfactory. The water was viewed as marginally basic in nature, suitable level of alkalinity and DO, and low range of BOD values showed the evidence of commodious productivity. Because of high pace of precipitation nearby during rainy season and with dispersed, irregular, and heavy rain practically all the year round, the agitation caused the OCP water to be more turbid in monsoon than in winter. The other physicochemical parameters being either within prescribed limits or only somewhat more, alongside the suitable concentrations of sodium, potassium, nitrate, sulfate, phosphate, ammonia and chloride and higher planktonic overflow, the water probably won't be appropriate for drinking, but may be an excellent base for hydroponics, pisciculture, agriculture and horticulture. The increased water quality parameters in monsoon showed that during the rainy season the pit lake water is more impacted than winter. This could be because of the way that the microbial movement get diminished because of low temperature, consequently keeping higher DO during whole winter season, and higher DO indicates the higher productivity. Thus, the non-consumable OCP water (according to BIS standards) can be utilized in different purposes, such as pisciculture, agriculture and horticulture, which will help in development of financial state of the area, and furthermore dealing with abandoned and deserted mines for recovery, restoration and natural reclamation as much as possible and also makes valid toward sustainability and conservation of biological system.

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Conflict of Interests

It is hereby declared that the authors have no such conflict of interests.

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ORIGINAL ARTICLE **LIFE SCIENCE**

HEAVY METAL CONTENT IN WATER AND SEDIMENT OF SHRIMP FARMS OF SATKHIRA, BANGLADESH

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Abstract

Toxic contaminants moving along with runoff usually contaminates shrimp farms in most areas of Satkhira district, southwest Bangladesh. An investigation was carried out to assess the Physico-chemical properties and heavy metal concentration of sediments and water in shrimp farms from Satkhira district, southwest Bangladesh during December 2020 to March 2021. Twelve water and sediment samples were collected from six *Ghers* of two Upazilla e.g., Debhata and Assasuni of Satkhira. Physico-chemical attributes such as temperature, DO, BOD_5, pH, TDS, EC, turbidity, salinity, HCO_3^-/CO_3^-, SO_4^-, NO_3^-, PO_4^- were estimated. The concentration attributes such as TDS (2280-5250 mg/l), EC (4380-9670 µs/cm), Turbidity (50.8-348 NTU), HCO_3^-/CO_3^- (219.6-445.3 mg/l), NO_3^- (0.3635-1.94 mg/l) were observed to be higher than the allowable standards recommended by WHO and FAO. Five trace metals such as chromium (Cr), cadmium (Cd) copper (Cu), lead (Pb) and Zinc (Zn), were measured in sediments and water by Atomic Absorption Spectrometer. The range of metal concentrations in sediment were as follows: Cr (1.95–3.43 mg/l), Zn (205.76-265.71 mg/kg), Cu (0.60–1.1mg/kg), Cd (3.8-4.1 mg/kg), and Pb (0.92-1.11mg/kg wet weight. The range of metal concentration in water were as follows: Cr (0.1-0.8 mg/l), Zn (0.003-0.34 mg/l), Cu (0.10-0.13 mg/l), Cd (0.07-0.08 mg/l), and Pb (0.23-0.34 mg/l). Higher concentrations of metal were recorded in most of the sediment and samples of water of the studied *Ghers* except Cr and Cu, those were below standard as prescribed by WHO and FAO. The concentration of Cu ranges from 0.602 to 1.113 mg/kg and 0.10 to 0.13 mg/l and range of Cr concentration were 1.95 to 3.43 mg/kg and 0.156 to 0.807 mg/l in sediment and water respectively. However, Cd, Zn and Pb were higher than the allowable standard as recommended by WHO and FAO. The higher heavy metal concentrations in water and sediment of shrimp *Ghers* suggest that shrimp could be contaminated by these heavy metals as well.

Keywords: Contamination, Heavy metal, Sediments, *Gher* water, Satkhira

Introduction

Due to their toxicity, persistence, and bio - accumulative characteristics, heavy metals are typically considered to be environmentally dangerous. "Naturally occurring metals with an atomic number greater than 20 and an elemental density greater than 5 $g.cm^{-3}$" are categorized as heavy metals (Khan and Ali 2018). They come from varieties natural and anthropogenic sources that release them into the environment. Agricultural runoff, residential sewage, and industrial effluents are examples of anthropogenic sources of heavy metal pollution. Industries degrade ecosystems and the environment by releasing pollutants into the air and water. (Bangash and Alam 2004). Metal contamination today poses a threat to almost every area of the aquatic environment, and its impacts are inevitable. The current situation of global water contamination is mostly caused by the expansion of industrialization, expansion of urbanization, and population boom (Tchounwou et al. 2012).

Toxic heavy metals have become more transportable in the environment and their biogeochemical cycles have changed as a result of industrialization and rapid economic development. Various human activities have made

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freshwater ecosystems, such as rivers, lakes, and streams susceptible to heavy metal pollution. A significant environmental problem is the toxic heavy metal contamination of freshwater ecosystems and as a consequence of aquatic species like fishes accumulate relatively high levels of heavy metals because of their high trophic position in food webs (Siraj et al. 2014). Human health is significantly impacted by the trophic transmission of potentially hazardous heavy metals in human food chains, particularly in fish. The Minamata disease, which was driven by consuming Hg-contaminated fish in Japan, is a well-known case. A significant environmental chemical disaster of the previous century was the development of Minamata disease in Japan in the 1950s (Ali and Khan 2017).

These metals are non-biodegradable, so they accumulate in environmental components like water and sediment before moving easily into living systems like fish and shellfish and participating in bioaccumulation and biomagnification, which is prevalent in the aquatic food web and ultimately causes of disorders of various biochemical in humans and other animals (Zhao et al. 2012, Olmedo et al. 2013, Kumar et al. 2015, Dadar et al. 2016, Dhanakumar et al. 2015, Duruibe et al. 2007).

As freshwater fish play a significant role in the human food chain, it is crucial to routinely examine them for heavy metal pollution (Ali et al. 2017). Through the gills, skin, and stomach of the fish, heavy metals may enter the body either directly from the abiotic environment (ambient water and sediments) or indirectly from the fish's diet or prey. Because they are directly exposed to the outside environment, fish gills are vulnerable to contaminants in the ambient environment (Waheed et al. 2014). Heavy metals and other chemical pollutants, particularly those caused by industrial and agricultural expansion and urbanization, are constantly released into rivers. Plants, animals, and people may all be affected by heavy metals. They function as metabolic toxins (Csuros et al. 2002).

Due to their toxicity, longterm persistence, bioaccumulation, and biomagnification in the food chain of ecosystems, metals of natural and anthropogenic sources may represent a severe hazard (Papagiannis et al. 2004). Therefore, residues of heavy metal in polluted ecosystems can build up in bacteria, fish, and aquatic animals, which could enter the food chain of human and create health hazards (Gupta et al. 2009). The heavy metals quantity in fish and crustaceans along the northern Bay of Bengal shoreline has apparently enlarged and posing a threat to human health (Borrell et al. 2016).

In Bangladesh, the second-largest export material is shrimp due to its high commercial importance, tiger shrimp are referred to as the country's "white gold" (Ahmed and Diana 2015). In Bangladesh, the coastal areas particularly Bagerhat, Khulna, Satkhira, Chittagong, Cox's Bazar and other districts, are where shrimp are primarily raised (Matin et al. 2016). Heavy metals are more harmfull to crustaceans (Ahsanullah et al. 1981). Baki et al. (2018) recorded the levels of heavy contamination of As, Cd, Cr, Cu, Fe, Hg, Mn, Pb and Zn in six most consumed fish, a lobster, three crabs, a *P.sculptilis* shrimp, and other crustaceans taken from the Saint Martin's Island, Bangladesh. In aquatic ecosystems, such as water, sediments, fish, and shrimp, heavy metals may accumulate. These are subsequently absorbed into the human body via the food chain. (Yohannes et al. 2013 and Maceda-Veiga et al. 2013) Increased concentrations of heavy metals including Pb, Cd, and Cr in shrimp could be dangerous for human health. However, heavy metal pollution in shrimp has grown to be a significant global concern due to both the damage it poses to shrimp and the non-cancerogenic health hazards associated with shrimp eating. For instance, the presence of lead in food may result in renal failure and liver damage (Lee et al. 2011). Long-term lead exposure can cause comas, mental impairment, and even death (Al-Busaidi et al. 2011). Cadmium damages the kidneys and causes chronic toxicity symptoms such as tumors, hepatic dysfunction, infertility, hypertension, and reduced renal function (Rahman et al. 2013). Similarly, Chromium could destroy proteins and membrane lipids, hence disturb cellular integrity and activities. (O'Brien et al., 2001; Mattia et al., 2004)

One of the major shrimp-farming regions in Bangladesh's south-west is Satkhira. Studies on the physico-chemical characteristics of this location as well as the concentration of heavy metals in water and sediment have been conducted in very small numbers. Regarding the assessment of heavy metal concentrations in water and sediment, there is still a dearth of knowledge. The physico-chemical characteristics of the water and the level of heavy metal in the surrounding sediments and water have become valuable knowledge in this context. It is possible that the results will increase public knowledge of the safety of consuming shrimp and other *Gher* fish, which absorb heavy metal from water and sediments.

Materials and Methods

Study Area

In the south-west region of Bangladesh, Satkhira is an important shrimp farming location. The majority of Satkhira's residents work in agriculture, fishing, and shirmp farming. The study is conducted in six different *Gher* situated in Satkhira District, Bangladesh (Figure 1); which were selected randomly to carry out the present study.

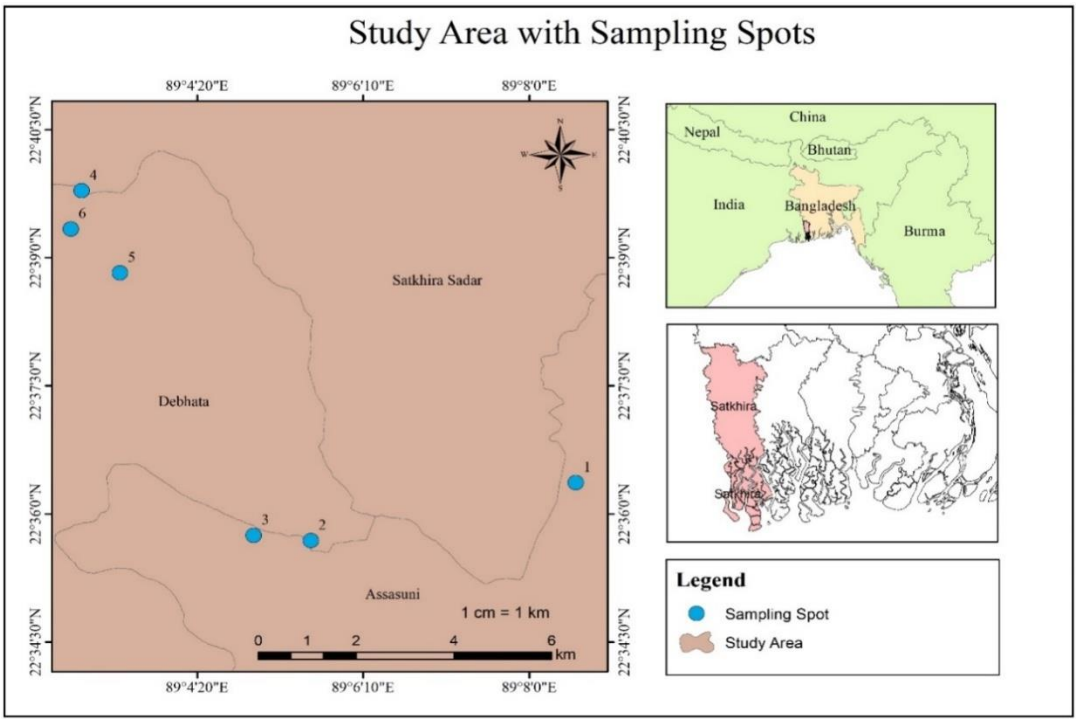

Figure 1. Study location with the sampling sites

Water and sediment collection

Using 1000 mL bottles labeled with a code number and the sampling date, water samples were taken from six shrimp culture ponds (*Gher*) at various locations. Water samples were stored in a plastic bottle containing 1% HNO_3 for the purpose of determining the presence of metal ions, and they were transported from the field to the lab using an ice box. Similar to this, from December 2020 to March 2021, six sediment samples were taken from the bottom sediment of particular shrimp farms (*Gher*) at a depth of 0–15 cm. To keep the collected sediment samples moist, they were stored in zip-top bags.

Water and sediment analysis

Physico-chemical attributes such as temperature, DO, BOD_5, pH, TDS, EC, turbidity, salinity, HCO_3^-/CO_3^-, SO_4^-, NO_3^-, PO_4^- were estimated (APHA,1998). In 100 ml plastic bottles that had been washed with diluted hydrochloric acid and then filled with distilled water, then water samples were collected. All bottles were transported to the lab following the collection of wetland water samples. To separate unwanted suspended and solid contaminants, filter paper (Whatman No. 1) was used to filter the water samples. The next step was to transfer 6 water samples to new 100 ml glass bottles that contained 10 ml of 2M HCl. To prevent air exposure, all bottles of the 100 ml solution were promptly sealed. Each sample taken in a plastic bottle was labeled independently with a unique identification number in order to provide the necessary information for each sample, such as the date of collection, location, sources of water, etc.

The amount of metal that dissolves in water after passing through a 0.45 m filter is included. The fresh samples were collected and rapidly filtered by using a membrane filter (0.45 m) in Millipore Filtration Assembly. Prior to use, 1:1 HNO_3 acid solution was used to wash the Millipore Filtration Assembly. The solution was then

accurately measured (10–20 ml) and thoroughly mixed before being placed in a beaker or conical flask. After placing boiling chips on a hot plate, 5 ml of concentrated HNO_3 was added and vigorously heated until the amount reached 10 to 20 ml. HNO_3 was added and the boiling process was repeated until the solution was clear or light in color. After cooling, the volume was increased to the appropriate level (Ali et al. 2012). Ready samples were analysis through Atomic Absorption Spectrophotometry, the recorded value was calculated as follows:

Concentration of metal in sample (ppm) = Observed concentration (ppm)× Concentration factor

After drying the sediments, the samples were grinding and sieved. 1.0 g sediment sample was taken in the digestion vessel, and 5 ml HNO_3 and 3 ml $HClO_4$ were added. After that digestion vessel placed on a heating block and heated at 120 ^oC temperature (two hours) and 180 ^oC (one hour). Digested cooled sample was filtered and diluted to 50 ml with distiled water in a plastic bottle. Another 1g sediment sample was mixed with 17 ml Triacid mixture (14 ml HNO_3:2ml $HClO_4$:1 ml H_2SO_4) and placed in a digestion tube; it was heated at 150^o C for 2 hours. Digested sample (colorless or faint color) was filtered and washed with 2% HNO_3 solution and made it 50 ml volume. (Ali et al. 2012). The concentration was calculated as follows:

$$\text{Concentration of metal (ppm)} = \frac{\text{Concentration observed (ppm)} \times \text{Final volume(ml)}}{\text{Sediment weight(g)}}$$

Results and discussion

Concentrations of Pb, Cu, Zn, Cd and Cr in samples of water and sediment collected from the selected *Ghers* under study are summarized in Table 1 and Table 2. The Concentration of Cu and Zn in water were negligible (0.107±0.002 to 0.137±0.006 mg/l) and (0.232±0.006 to 0.345±0.009mg/l) in all localities while concentration of Pb, Cd, Cr were very high (0.232±0.006 to 0.345±0.009 mg/l), (0.0723±.0007 mg/l to 0.0885±.0005 mg/l) and (0.15±0.091 to 0.807±0.006 mg/l) in water in most of the sampling stations.

Table 1. Heavy metal conditions of the waterbodies of *Gher*

Sampling Spot	Heavy Metal Concentration in Water (mg/l)				
	Lead (Pd)	Copper (Cu)	Zinc (Zn)	Cadmium (Cd)	Chromium (Cr)
Gher 1	0.235±0.003	0.137±0.006	0.0056±0.006	0.0809±.0009	0.314±0.007
Gher 2	0.262±0.005	0.128±0.009	0.0065±0.007	0.0853±.0006	0.15±0.091
Gher 3	0.257±0.003	0.119±0.007	0.0033±0.003	0.0777±.0004	0.561±0.006
Gher 4	0.29±0.006	0.118±0.009	0.3466±0.347	0.0723±.0007	0.807±0.006
Gher 5	0.345±0.009	0.107±0.002	0.0121±0.012	0.0858±.0008	0.167±0.003
Gher 6	0.232±0.006	0.123±0.006	0.0042±0.004	0.0885±.0005	0.357±0.007
Maximum	0.345±0.009	0.137±0.006	0.3466±0.347	0.0885±.0005	0.807±0.006
Minimum	0.232±0.006	0.107±0.002	0.0033±0.003	0.0723±.0007	0.15±0.091
FAO/WHO Standard	0.01	2	5	0.01	0.05

Svobodová and Vykusová (1993) recorded that, the highest admissible copper concentration was in the water from 0.001 to 0.01 mg/l depends on the fish species and physico-chemical condition of water. Present study founded that highest value for Cu concentration was found 0.137 mg/l in *Gher*-1 and the lowest concentration value was found 0.107 mg/l in *Gher*-5. WHO and FAO recommended a permissible limit for Cu concentration in water for shrimp farming, which is 2 mg/l. Cu content of the selected six shrimp *Gher*was lower than the maximum permissible concentration limit. Guhathakurta and Kaviraj (2000) documented that, Zn concentration of the selected six shrimp *Gher* was lower than the maximum allowable concentration limit. The concentration of Zn in water ranged from 0.21–13.6 $\mu gm.L^{-1}$. Present study founded that the highest concentration value was found 0.346 mg/l in *Gher*-4 and the lowest concentration were found 0.232 mg/l in *Gher*-3. WHO and FAO recommended a permissible limit for Zn concentration in water for shrimp farming, which is 5 mg/l. Guhathakurta and Kaviraj (2000) studied that, Concentrations of Cd in water was negligible which range is 0.03-0.08 μgmL^{-1} in all the localities.

Present study founded that the highest concentration value was found 0.346 mg/l in *Gher*-6 and the lowest concentration value was found 0.0723±.0007 mg/l in *Gher*-4. WHO and FAO recommended a permissible limit for Cd concentration in water for shrimp farming, which is 0.01 mg/l. Cd concentration of the selected six shrimp *Gher* was always higher than the maximum permisible concentration. WHO and FAO recommended a permissible limit for Pb and Cr concentration in water for shrimp farming, which is 0.01 mg/l and 0.05 mg/l. The concentration Pb and Cr of the selected six shrimp *Gher* was higher than the maximum allowable concentration limit.

The Concentration of Cu and Cr in sediment were negligible (0.602±0.04 to 1.176±0.04 mg/kg) and (1.957±0.09 to 3.433±0.09 mg/kg) in all localities while concentration of Pb, Cd, Zn were very high (0.921 ± 0.10 to 1.113 ± 0.11 mg/kg), (3.805±0.08 to 4.10±0.07 mg/kg) and (205.76±1.14 to 265.716±2.29 mg/kg) in sediment in most of the sampling stations.

Table 2. Heavy metal conditions of the sediment of *Gher*

Sampling Spot	Heavy Metal Concentration in Sediment ($mgkg^{-1}$)				
	Lead (Pd)	Copper (Cu)	Zinc (Zn)	Cadmium (Cd)	Chromium (Cr)
Gher 1	0.921 ± 0.10	1.030±0.05	205.76±1.14	4.045±0.01	1.957±0.09
Gher 2	1.113 ± 0.11	1.054±0.06	265.716±2.29	3.805±0.08	2.368±0.15
Gher 3	1.031 ± 0.06	0.905±0.03	224.16±1.01	3.885±0.10	2.87±0.07
Gher 4	1.004±0.06	0.801±0.02	223.51±0.90	4.155±0.08	3.316±0.16
Gher 5	0.976±0.07	1.176±0.04	230.8±0.91	3.887±0.10	3.271±0.04
Gher 6	1.058±0.05	0.602±0.04	240.235±2.99	4.10±0.07	3.433±0.09
Maximum	1.113 ± 0.11	1.176±0.04	265.716±2.29	4.10±0.07	3.433±0.09
Minimum	0.921 ± 0.10	0.602±0.04	205.76±1.14	3.805±0.08	1.957±0.09
FAO/WHO Standard	0.3	40	40	0.2	5

Himadri and Kaviraj (2000) recorded that the range of Cu concentration in sediment was 15.02 to 205.236 µg.g-1. Present study founded that the highest concentration was found 1.176 mg/kg in Gher-5 and the lowest concentration were found 0.602 in Gher-6. WHO and FAO recommended a permissible limit for Cu concentration in water for shrimp farming, which is 40 mg/kg. Cu concentration of the selected six shrimp Gher was lower than the maximum allowable concentration limit. Swarna Das et al. (2017) reported that, the highest mean concentration of Chromium (Cr) in sediment was 9.16 mg/kg ± 4.87 mg/kg found in farm 3 and lowest concentration of Cr was 6.60 mg/kg ± 1.77 mg/kg found in farm 1. Present study founded that the highest concentration was found 3.433 mg/kg in Gher-6 and the lowest concentration were found 1.957 mg/kg in Gher-1. WHO and FAO recommended a permissible limit for Cr concentration in sediment for shrimp farming, which is 5 mg/kg. Cr concentration of the selected six shrimp Gher was lower than the maximum allowable limit. Swarna Das et al. (2017) reported that, the range of concentration of lead (Pb) was to 6.34 mg/kg to 7.92 mg/kg found in farm 2 and 3 in the bottom sediment. Present study found that the highest concentration was found 1.113 mg/kg in Gher-2 and the lowest concentration were found 0.921 in Gher-1. WHO and FAO recommended a permissible limit for Pb concentration in water for shrimp farming, which is 0.3 mg/kg. Pb concentration of the selected six shrimp Gher was higher than the maximum allowable concentration limit. Aktaruzzaman and Hossain (2013) reported that, the level of cadmium (Cd) varied in sediment samples from 0.116 - 0.160 mg/kg. Present study founded that highest concentration was found 4.10 mg/kg in Gher-6 and the lowest concentration was found 3.805 mg/kg in Gher-2. WHO and FAO recommended a permissible limit for Cd concentration in sediment for shrimp farming, which is 0.2 mg/kg. Cd concentration of the selected six shrimp Gher was higher than the maximum allowable concentration limit. Guhathakurta and Kaviraj (2000) documented that, concentration of Zn in the surface layer of the sediment (upto 10.0 cm depth) ranged from 40.8 to 3448.3 µg.g-1. Present study founded that the highest concentration was found 265.716 mg/kg in Gher-2 and the lowest concentration were found 205.76 mg/kg in Gher-1. Zn concentration of the selected six shrimp Gher was lower than the maximum allowable concentration limit. WHO and FAO recommended a permissible limit for Zn concentration in sediment for shrimp farming, which is 40 mg/kg.

Table 3 indicates the outcomes of the physioco-chemical conditions of various shrimp Gher water samples. The values of temperature, pH and DO of shrimp Gher water were determined to be adequate for cultivation of shrimp, whereas the high values of TDS, turbidity and EC were recorded. On the other hand salinity levels were suitable for cultivating shrimp. The temperature was roughly within the range of the ideal temperature (32°C) for shrimp culture (Chiu et al. 1988). The present study founded that the mean temperature of the waterbody is

approximately (23°C). Ramanathan et al. (2005) reported that ideal pH range (6.8 to 8.7) should maintained for maximum growth and production of shrimp. The recorded values of pH of all studied shrimp Gher water were slightly deviating from the ideal range (7.5-8.5), that range recommended by Chiu et al. (1988) for ideal shrimp culture. The present study founded that the mean pH of the Gher is 8.52. The Dissolve oxygen in Gher water range from 4.85 mg/l to 8.72 mg/l.

The average water Dissolve oxygen is 6.88 mg/l. The minimum water Dissolve oxygen is found in *Gher*-1 and maximum Dissolve oxygen is found in *Gher*-2. An optimum range of DO for shrimp farming is >5 mg/l which was recommended by WHO and FAO. Except *Gher*-1, All the selected farm of DO are exceeded the permissible allowable limit. The salinity of all the shrimp *Gher* water was measured to be between 8.78 and 10.48 ppt, despite the fact that the ideal salinity is 15 to 25 ppt for the culture of shrimp (Boyd, 1995), which is essential in pond dynamics. The six mixed shrimp *Gher* water samples had their turbidity analyzed, and the results ranged from 50.8 to 348 NTU, which was outside of the ideal range (7 to 30) suggested by Lin et al. (1993). The total dissolve solids in *Gher* water range from 2280 mg/l to 5250 mg/l. The average water total dissolve solids are 4331.63 mg/l. The minimum water total dissolve solids are found in *Gher*-1 and maximum water total dissolve solids is found in *Gher*-3. The optimum level of TDS value is 1000 mg/l which is recommended by WHO and FAO. The Electrical conductivity in *Gher* water range from 4380 to µs/cm 9570 µs/cm. The average water Electrical conductivity is 8053.33 µs/cm. The minimum water Electric conductivity is found in *Gher*-1 and maximum water Electric conductivity is found in *Gher*-3. The optimum EC value is 1000 µs/cm which is recommended by WHO and FAO. The Bicarbonate in *Gher* water range from 219.6 mg/l to 445.3 mg/l. The average water Bicarbonate is 345.67 mg/l. The minimum water Bicarbonate is found in *Gher*-1 and maximum water Bicarbonate is found in *Gher*-5. The optimum level of HCO_3^- value is 200 mg/l which is recommended by WHO and FAO. The selected all farms of HCO_3^- are above the standard. The Sulphate in *Gher* water range from 0.38 mg/l to 5.72 mg/l. The minimum water Sulphate is found in *Gher*-2 and maximum water Sulphate is found in *Gher*-5. The optimum level of SO_4^- value is 8 mg/l which is recommended by WHO and FAO. The selected all farms of SO_4^- value are lower than permissible allowable limit. The Nitrate in *Gher* water range from 0.356 mg/l to 1.94 mg/l. The minimum water Nitrate is found in *Gher*-1 and maximum water Nitrate is found in *Gher*-3. The permissible standard of NO_3^- for shrimp farming is 100 mg/l which recommended by WHO and FAO. The $NO_3^-$of all six farms are very lower than the standard values. The Phosphate in *Gher* water range from 0.132 mg/l to 0.826 mg/l. The minimum water Phosphate is found in *Gher*-1 and maximum water Phosphate is found in *Gher*-5. The optimum level of PO_4^- value is 0.2 mg/l which is recommended by WHO and FAO. The selected all farms of PO_4^- value are higher than permissible allowable limit.

Conclusion

The value of physico-chemical parameters (EC, TDS, Turbidity, HCO_3^-/CO_3^-, NO_3^-) are higher than internationally recommended WHO and FAO allowable limit. The concentration values of Cadmium (Cd), Chromium (Cr), Lead (Pb) in water and Cadmium (Cd), Zinc (Zn), Lead (Pb) in sediment samples are higher than internationally recommended permissible limit WHO and FAO. A sustainable environmental management is necessary to control the heavy metal pollution in the studied water bodies as well as sediment too.

Table 3.Physico-chemical conditions of the waterbodies of *Gher*

Sampling Spot	Temp (°C)	pH	DO (mg/l)	BOD_5 (mg/l)	Salinity (ppt)	Turbidity (NTU)	EC (µs/cm)	TDS (mg/l)	HCO_3^-/CO_3^- (mg/l)	SO_4^- (mg/l)	NO_3^- (mg/l)	PO_4^- (mg/l)
Gher-1	21.3	8.19	4.85	1.36	9.25	63.6	4380	2280	219.6	1.48	0.37	0.13
Gher-2	21.2	8.62	8.72	1.45	9.2	218	9570	5180	274.5	1.38	1.45	0.6
Gher-3	21.9	8.75	6.8	1.56	8.78	75.6	9670	5250	402.6	5.72	1.94	0.45
Gher-4	23.2	8.33	5.8	1.42	9.93	348	9490	5150	347.7	1.08	1.16	0.14
Gher-5	23.6	8.51	7.55	1.46	10.48	81.9	6060	3180	445.3	0.39	0.94	0.83
Gher-6	23.5	8.69	7.58	1.45	9.89	50.8	9150	4950	384.3	5.14	0.8	0.38
Mean	22.45	8.52	6.88	1.45	9.59	139.65	8053.33	4331.67	345.67	2.53	1.11	0.42
Maximum	23.6	8.75	8.72	1.56	10.48	348	9670	5250	445.3	5.72	1.94	0.83
Minimum	21.2	8.19	4.85	1.36	8.78	50.8	4380	2280	219.6	0.39	0.37	0.13
Std. Deviation	1.11	0.22	1.39	0.07	0.62	118.88	2264.83	1276.77	84.41	2.28	0.55	0.27
WHO/FAO standard	28-31	7-8.5	>5	<50	10-30	5	1000	1000	100-200	3-8	10	<0.2

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Conflict of interest
No conflict of interest exists.

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ORIGINAL ARTICLE **LIFE SCIENCE**

BIOACCUMULATION OF HEAVY METAL IN FARMED TILAPIA AND SHRIMP IN SATKHIRA DISTRICT, SOUTHWEST BANGLADESH

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Abstract

Fish farmers in the Satkhira district, southwest Bangladesh, are progressively moving towards intensive aquaculture, and heavy metal contamination of the feed may frequently occur. This study explores the bioaccumulation of heavy metal in farmed tilapia *(Oreochromis mossambicus)* and shrimp species such as bagda *(Penaeus monodon)*, golda *(Macrobrachium rosenbergii)*, randomly collected from 3 *gheer* at Assasuni and Debhata Upazilla in Satkhira, Bangladesh from December 2020 to March 2021. The levels of elements including Fe, Mn, Zn, Cu, Cr, Cd, Pb, and As were evaluated using the flame-AAS and HG-AAS methods followed by Nitric Acid (HNO_3)-Perchloric Acid ($HClO_4$) digestion. The results revealed that the concentrations of Fe, Mn, Cr, and As were higher than the WHO and FAO recommended levels. Maximum concentrations (mg/kg) of Fe, Mn, Cr, and As were observed as 104.00 ± 8.30 in Tilapia (*O. mossambicus*) from *gheer* #3, 3.40 ± 1.71 in Tilapia (*O. mossambicus*) of *gheer* #3, 12.80 ± 1.45 in golda (*M. rosenbergii*) of *gheer* #1, and 1.96 ± 0.09 in Tilapia (*O. mossambicus*) from *gheer* #3, respectively. But on average, *M. rosenbergii* was discovered to contribute more to the maximum level of almost every metal compared to the other two species, whereas species from *gheer* #3 were found to be more contaminated. Overall, tilapia and shrimp from all three *gheer* were considered unsafe for consumption. More research is recommended for estimating the levels of heavy metal accumulation in fish and shrimp over a longer time period and across a wider geographic range.

Keywords: Heavy metal, bioaccumulation, shrimp, Tilapia, fish feed, Satkhira.

Introduction

Bioaccumulation is the process of deposition of a pollutant with increasing concentration inside the tissue of living organisms as a result of rapid consumption or unambiguous absorption from the water rather than excretion (Fatema et al. 2019 and Nyamete *et al.* 2020). According to Ali and Khan (2018), naturally formed elements having an atomic number higher than 20 as well as an elemental density higher than 5 g/cm^3 are referred to as heavy metals, while the bioaccumulation of heavy metals in biological tissues is the transmission of contaminants through different hierarchical levels in an ecosystem (Fatema *et al.* 2019). Bangladesh is one of the countries with the densest populations in the world, and the majority of its population depends either directly or indirectly on agriculture. According to the FAO report, *The State of World Fisheries and Aquaculture 2020*, Bangladesh has achieved a significant milestone in the development of aquaculture and is ranked 3rd in inland open water capture production and 5th in world aquaculture production (FAO 2020). Fish is a vital component of many natural food webs and a valuable source of protein for humans, as well as being high in biologically important proteins, fats, and fat-soluble vitamins. Shrimp consumption has grown as people have come to recognize the need for a balanced diet and the nutritional significance of elements like high protein content, vitamin D, vitamin B_3, and zinc, all of which are advantageous to one's health (Aremu and Ekunode 2008 and Copat et al. 2011). However, pollutants in aquatic environments have grown to be a serious problem because they may accumulate in aquatic food acquired from the adjoining environment (Ahmed et al. 2019).

Impetuous industrial expansion and economic advancement have led to the continuous discharge of several synthetic and geogenic substances into aquatic environments. Heavy metals are assigned higher precedence as contaminants among the potentially harmful components that enter these systems because of their toxic effects, endurance, and ability to enter the food webs through bioaccumulation and biomagnification. As a result, they constitute a significant global public health issue that endangers both aquatic life and human health as well as the environment as a whole (Sun et al. 2018 and Raknuzzaman et al. 2016). Health hazards from ingesting tainted aquatic foods are growing globally at an alarming rate, especially in poor third-world nations like Bangladesh (Ahmed et al.

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2019). An accumulation of these trace elements can have severely adverse effects on the liver, kidneys, central nervous system, mucosal tissues, digestive tract, and reproductive systems. Aside from their poisonous and cancer-causing effects on people and animals, several metals are crucial for maintaining appropriate cell function (Fatema et al. 2015). Fish farmers in Bangladesh are gradually transitioning from no feed to factory-made feeds by using farm-made feeds, while the use of suitable feeds is essential for the success of both exaggerated and semi-exaggerated fish production (Nasim et al. 2012). Some feed manufacturers those who produce feed commercially have fallen short to meet the required standard needed for fish meal. The raw materials used to make the feeds, such as tannery and poultry wastes, which are regularly employed as an affordable source of fish food, are frequently contaminated with heavy metals. The usage of these kinds of feed sources might potentially raise the amounts of hazardous components like Pb, Cd, and Cr in farmed fish and endanger public health (Shamshad et al. 2009 and Kundu et al. 2017).

Certain authors in Bangladesh have examined the aquatic species, water, and sediment (Sarkar et al. 2016, Bhuyan et al. 2016, Kundu et al. 2017, Das et al. 2017, Ahmed et al. 2019, and Akter et al. 2020) to find the levels of some heavy metal in specific rivers for a number of certain organisms. Information on the contamination of metal in farmed shrimp and tilapia in the Satkhira region, which are produced using contaminated water and other artificial feeds, is scarce. This important gap forced us to find the heavy metal contamination in the farm-reared tilapia and shrimp. The present investigation was therefore designed to determine the degree of metal contamination in farm-produced bagda shrimp *(P. monodon)*, golda shrimp *(M. rosenbergii)*, and tilapia *(O. mossambicus)* regarding bioaccumulation of metals such as Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Cadmium (Cd), Chromium (Cr), Lead (Pb), and Arsenic (As).

Materials and Methods

Study area

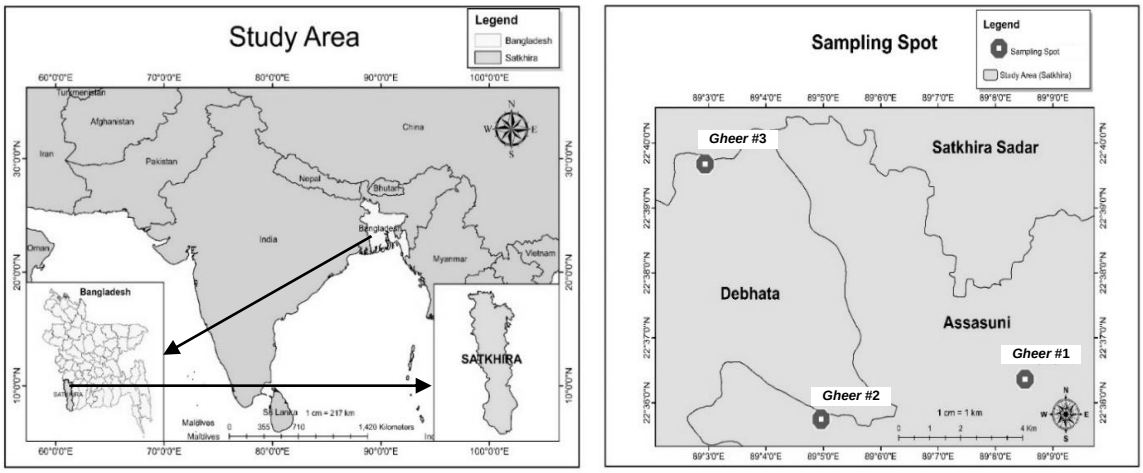

Figure 1. Location of the Study Area

Three different *gheer* such as *gheer* #1 (22° 36' 21.79" N and 89° 08' 30.78" E), *gheer* #2 (22° 35' 42.76" N and 89° 04' 57.42" E) and *gheer* #3 (22° 39' 47.05" N and 89° 03' 03.42" E) situated at Assasuni and Debhata Upazilla of Satkhira District, were randomly selected for sample collection to perform this study.

Collection and preservation of sample

Cultivated fish and shrimp sample collection, preparation, and storage were conducted by following Islam et al. (2016), Sarkar et al. (2016), Bhuyan et al. (2016), Kundu et al. (2017), Das et al. (2017), and Ahmed et al. (2019). Samples of fish and shrimp were properly cleansed with distilled water straight immediately following collection. And the edible parts of the fish and shrimp (muscle tissues) were diced and oven dried at (70-80) °C to get a consistent weight. Prior to laboratory testing, the dried fish and shrimp samples were crushed and powdered before being screened through a 2 mm nylon mesh and preserved in sterile, sealed plastic containers in the refrigerator.

Preparation and digestion of sample

Digestion of fish and shrimp samples was done by following the Nitric Acid (HNO_3)-Perchloric Acid ($HClO_4$) digestion method (APHA 1998). Further treatment was conducted with 3 ml of Sulfuric Acid (H_2SO_4) and 3 ml of 30% H_2O_2 until adequate digestion was completed and the solution turned colorless (Islam et al. 2016). After being digested, the solution was poured into immaculate volumetric flasks. To precisely create 100 ml of each solution, double-distilled water was added. The solutions were filtered with Whatman Filter Paper No. 42. All experimental supplies and equipment were subsequently washed by immersing them overnight in a 10% nitric acid solution, followed by a deionized water rinse.

Determination of heavy metal concentration

By using the flame-AAS technique (AA-7000, Shimadzu, Japan), the concentrations of Fe, Mn, Zn, Cu, Cr, Cd, and Pb were directly measured, while the hydride vapor generation (HG-AAS) method was employed to determine the level of As (APHA 1998). The average findings of the triple analyses of each sample were utilized to represent the data. In order to determine the concentrations of heavy metal, the formula shown below was utilized (Ali *et al.* 2012):

Metal concentration in a sample (mg/kg) =

$$\frac{Concentration\ observed\ (ppm) \times Final\ volume\ of\ sample\ (ml)}{Weight\ of\ sample\ tissue\ taken\ (g)}$$

Results and Discussion

A diverse variety of concentrations of heavy metal were recorded in the 3 tested species. The level of heavy metal (Fe, Mn, Zn, Cu, Cr, Cd, Pb and As) bioaccumulation (mg/kg) in bagda shrimp (*P. monodon*), golda shrimp (*M. rosenbergii*), and tilapia (*O. mossambicus*) collected from three different fish farms (*gheer*) is presented in Table 1.

Iron (Fe)

Iron (Fe) is essential for most life on Earth, including humans, since it plays a part in a number of metabolic functions, including the production of red blood cells, transportation of oxygen, DNA synthesis, and electron transport but excess intake of Fe may affect the human body in various ways such as DNA damage, gastrointestinal problems (Gupta 2014). On a dry weight basis, the highest Fe content was (104 ± 8.30) mg/kg in tilapia (*O. mossambicus*) from the *gheer* #3 and the minimum value was recorded (39.40 ± 2.75) mg/kg in bagda shrimp (*P. monodon*) of the *gheer* #2 (Figure 2). Except for two species from *gheer* #3 like *O. mossambicus* and *M. rosenbergii*, the average concentrations of Fe in all the species from all the *gheer* were reasonable and not particularly dreadful.

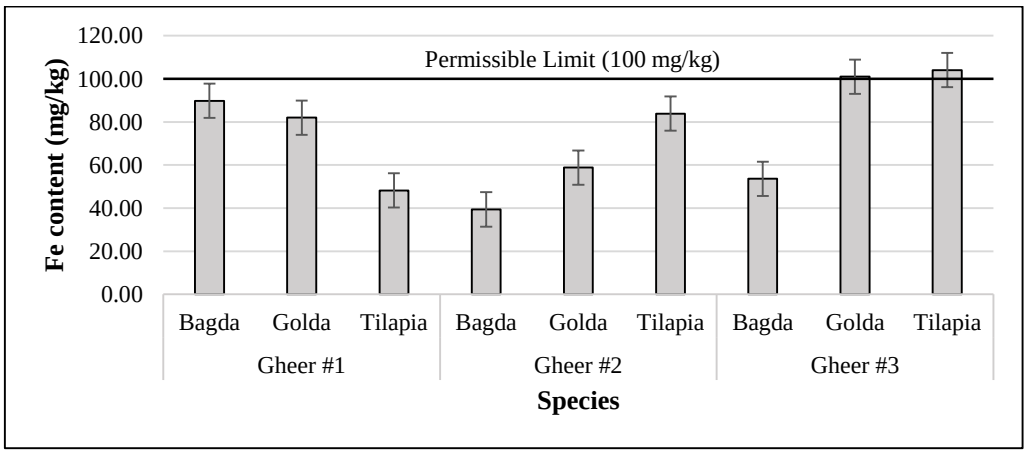

Figure 2. Fe bioaccumulation (mg/kg) in species

The recommended guideline value for Fe concentration in food by the WHO (1989), as mentioned in Mokhtar et al. (2009), is 100 mg/kg. Golda shrimp (*M. rosenbergii*) contributed to the uppermost concentration level of Fe (80.60 ± 21.13 mg/kg) which is still below the highest allowable level, whereas the lowest value of Fe (60.93 ± 25.99 mg/kg) went to bagda shrimp *(P. monodon)* (Table 1). Iron content in fish fluctuated between (136.241 - 200.26) mg/kg in an investigation carried out in Mymensingh by Kundu *et al.* (2017), which is higher than our findings. Variations in the physicochemical state of the water, sediment, and feeding practices of various regions may also be responsible for the

concentration variance observed in this research and other investigations. Kundu et al. (2017) reported the level of Fe (475.878 - 10004.855 mg/kg), which seems higher than the outcome of the present investigation. According to Mokhtar et al. (2009), intensive agricultural activities may contribute to higher concentration of Fe in species in this study.

Manganese (Mn)

Mn, a trace metal, is a crucial element for human life, and the typical human body has roughly 12 mg of Mn (Teodorovic et al. 2000). While Mn insufficiency causes skeletal and fertility issues, excessive Mn consumption can lead to psychiatric and neurological ailments (Ahmed et al. *2016*). In this investigation, Mn levels in several samples of farmed species exceeded the WHO and FAO-recommended maximum allowable limit of 1 mg/kg (WHO 1989, FAO 1983), as mentioned in Mokhtar et al. (2009) and Ahmed et al. (2016), respectively. Mn concentration was measured and ranged from 0.60 ± 0.38 to 3.40 ± 1.71 mg/kg (Fig. 3). The highest (3.40 ± 1.71 mg/kg) and lowest (0.60 ± 0.38 mg/kg) Mn content were recorded in tilapia (*O. mossambicus*) collected from the *gheer* #3 and bagda shrimp *(P. monodon)* collected from the *gheer* #2, respectively.

The findings denote that the maximum Mn level (2.27 ± 1.21 mg/kg) was determined in tilapia (*O. mossambicus*), and golda shrimp *(M. rosenbergii)* represented the lowest Cd concentration (1.27 ± 0.46 mg/kg) (Table 1). Consumption of fish having an excess amount of Mn may cause harm to the human body. In the earlier studies conducted by Bhuyan et al. (2016), the highest concentration of Mn was recorded in *Tetraodon cutcutia* (19.07 mg/kg) and the lowest was found in *Ctenopharyngodon idella* (0.96 mg/kg) collected from the Meghna river in Narsingdi district, and it was concluded that the area around the Meghna River was contaminated by numerous heavy metal released from diverse manufacturing, municipal, and agricultural activities. Mn exists instinctively in nature as mentioned by Ahmed et al. (2016) that can enter into aquatic systems while extensive use of agrochemicals may contribute to the higher recorded level of Mn in this study.

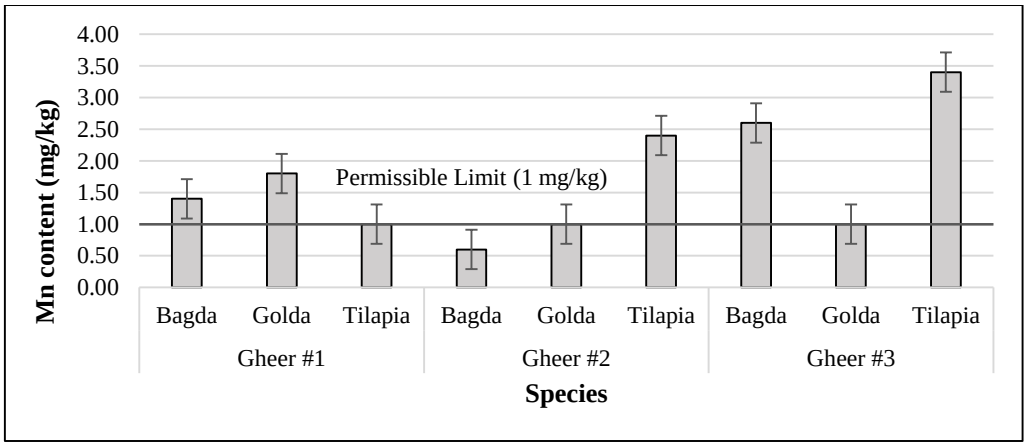

Figure 3. Mn bioaccumulation (mg/kg) in species

Table 1. Metal content (mg/kg) in selective species from *gheer* in Satkhira area, guideline values and literature review

	Species		Metal concentration (mg/kg)							
	Local Name	Scientific Name	Iron (Fe)	Manganese (Mn)	Zinc (Zn)	Copper (Cu)	Chromium (Cr)	Cadmium (Cd)	Lead (Pb)	Arsenic (As)
Present Study	Bagda	*P. monodon*	60.93 ± 25.99	1.53 ± 1.01	25.27 ± 0.50	10.37 ± 5.61	8.53 ± 4.45	0.03 ± 0.01	0.15 ± 0.04	0.84 ± 0.41
	Golda	*M. rosenbergii*	80.60 ± 21.13	1.27 ± 0.46	25.33 ± 0.31	16.60 ± 9.17	9.47 ± 3.89	0.04 ± 0.01	0.18 ± 0.07	0.91 ± 0.17
	Tilapia	*O. mossambicus*	78.67 ± 28.25	2.27 ± 1.21	21.80 ± 0.53	1.87 ± 0.42	9.13 ± 5.15	0.04 ± 0.01	0.16 ± 0.02	1.39 ± 0.65
Guidelines										
FAO Standard (FAO 1983) as mentioned in Ahmed *et al.* (2016), Ahmed *et al.* (2019)			-	1	30	70	0.15	0.10	0.50	1
WHO Standard (WHO 1989) as mentioned in Mokhtar *et al.* (2009)			100	1	100	30	50	1	2	-
Bangladesh Standard (Fish) (MOFL 2014) as mentioned in Ahmed *et al.* (2019)			-	-	-	5	1	0.25	0.30	5
New Zealand (CEPA 1995-97) as mentioned in Raknuzzaman *et al.* (2016), Ahmed *et al.* (2019)			-	-	40	30	-	1	2	1
Turkey Standard (TFC 2002) as mentioned in Raknuzzaman *et al.* (2016), Ahmed *et al.* (2019)			-	-	50	20	-	0.10	0.30	-
Literature										
Fish farm, Noakhali (Das *et al.* 2017)			-	-	-	1.77 ± 0.74 - 2.70 ± 0.38	1.29± 0.54 - 0.42 ± 0.22	0.28 ± 0.16- 0.42 ± 0.22	2.76 ± 0.42 - 4.98 ± 1.27	-
Fish farm, Mymensingh (Kundu *et al.* 2017)			136.2-200.3	-	-	19.08-25.34	< 0.0001	9.08-10.45	-	-
Fish farm, Tala, Satkhira (Sarkar *et al.* 2016)			-	-	-	-	0.19 ± 0.01	0.96 ± 0.38	<0.1	<0.1
Fish farm, Tala, Satkhira (Fatema *et al.* 2017)			-	-	-	-	<0.1	<0.1	<0.1	<0.1
Fish market, Satkhira and Bagherhat (Islam *et al.* 2016)			-	-	-	-	<0.54	0.42-0.67	4.57-4.60	<0.30

Zinc (Zn)

Zn being an integral part of various enzymes (Ahmed et al. 2016), the majority of human metabolic activities are known to require such as synthesis of several metallo-enzymes and physiological development (Akter et al. 2020), while its shortage can cause lack of appetite, delayed puberty, skin abnormalities, and immunological irregularities (Tuzen 2009). Akter et al. (2020) mentioned that after Fe (approximately 4 g), Zn (about 2.5 g) is the next element found in every system of the human body, with muscle tissue containing half of it. The levels of Zn in analysed farmed species ranged from (21.4 ± 3.69 to 25.8 ± 2.45) mg/kg (Figure 4).

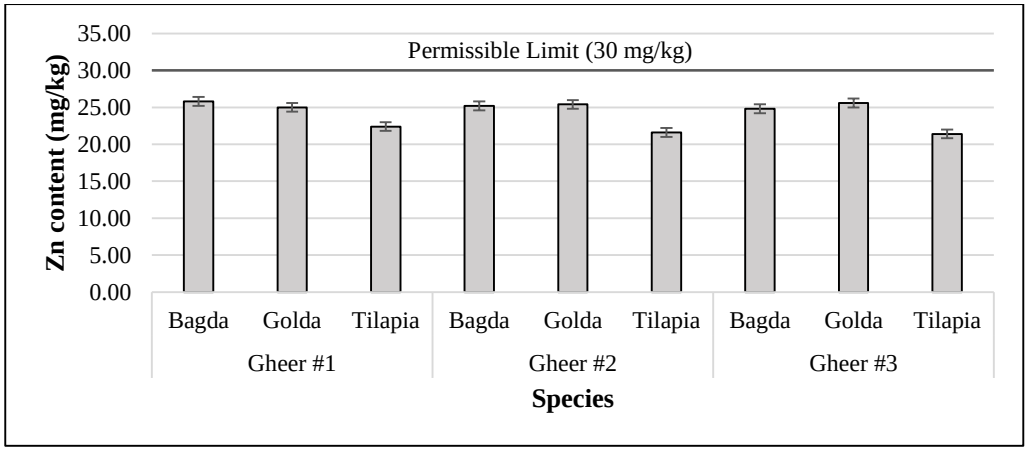

Figure 4. Zn bioaccumulation (mg/kg) in species

Moreover, the mean Zn concentrations (mg/kg) for golda shrimp (*M. rosenbergii*), bagda shrimp *(P. monodon)*, and tilapia (*O. mossambicus*) were (25.33 ± 0.31), (25.27 ± 0.50) and (21.80 ± 0.53), respectively (Table 1), which means that the Zn concentration of all the species under the current study is still below the maximum permissible limit (100 mg/kg, 30 mg/kg) recommended by WHO (1989) and FAO (1983) as mentioned in Mokhtar et al. (2009) and Ahmed et al. (2016), respectively. The highest Zn content (25.8 ± 2.45 mg/kg) was found in bagda shrimp (*P. monodon*) collected from the *gheer* #1 while the lowest (21.4 ± 3.69 mg/kg) was determined in tilapia *(O. mossambicus)* collected from the *gheer* #3. Regarding the Zn content, none of the species falling under this investigation were discovered to be frightful.

Copper (Cu)

Despite being a necessary component for maintaining health, particularly in the production of hemoglobin and a few necessary enzymes, Copper overdose can impair the activities of the liver and kidneys (Vu et al. 2017 and Baki et al. 2018). Copper (Cu) concentration was varied on a dry weight basis from (1.40 ± 0.21 to 24.00 ± 2.91) mg/kg. The highest and lowest value were measured in golda *(M. rosenbergii)* collected from the *gheer* #3 and tilapia *(O. mossambicus)* collected from the *gheer* #1, respectively. The variation of Copper (Cu) concentration (mg/kg) in studied species from different fish farms is shown in Figure 5.

The mean Cu content for all of the specimens were rational and not overly alarming. The fact is that they met the minimum level, which was less than the suggested level (30 mg/kg) given by WHO (WHO 1989) as mentioned in Mokhtar et al. (2009). It is clear that the maximum contamination level of Cu (16.60 ± 9.27) mg/kg was observed in golda shrimp (*M. rosenbergii*) and the minimal level (1.87 ± 0.42 mg/kg), was detected in tilapia (*O. mossambicus*) (Table 1). The MOFL (2014)-established acceptable value of 5 mg/kg is exceeded by the species bagda shrimp (*P. monodon*) and golda shrimp (*M. rosenbergii*), as mentioned in Ahmed et al. (2019). TFC (2002) and CEPA (1995–1997) established further acceptable limits of 30 mg/kg and 20 mg/kg for Cu, respectively (Raknuzzaman et al. 2019 and Ahmed et al. 2019).

According to ATSDR (2004), Cu has a maximum suggested level of 1.0 mg/day for children (1–3 years old) and 10 mg/day for males and females (19–70 years old) due to its status as a trace metal, while the liver and kidneys may be damaged if the dose is exceeded. In the prior research, the mean Cu concentration (4.97 ± 1.13 mg/kg) was measured in fish collected from the Meghna river, conducted by Ahmed et al. (2019), and a range of 1.77 ± 0.74 to 2.70 ± 0.38 mg/kg was estimated in fish collected from different fish farms in Noakhali (Das et al. 2017), were less than our readings. In contrast, a range, 19.073–25.343 mg/kg was estimated for the fish farms in the Mymensingh district of Bangladesh (Kundu et al. 2017), which was higher than our results.

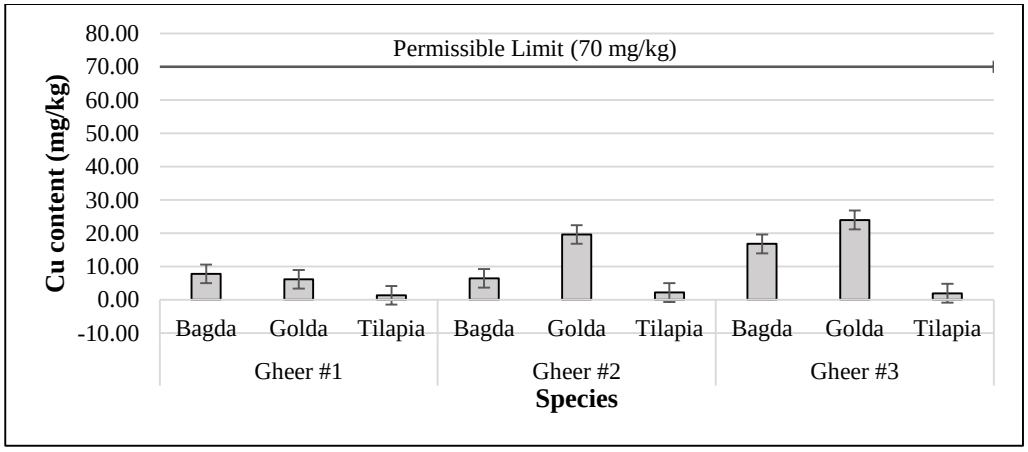

Figure 5. Cu bioaccumulation (mg/kg) in species

Chromium (Cr)

Dietary Cr is inevitable to properly metabolise lipids and glucose (Mertz 1969 and Velusamy et al. 2014). Cr insufficiency can impede growth and hinder the synthesis of lipids, proteins, and glucose and on the other hand, excessive ingestion of Cr has been linked to acute respiratory problems as well as organ damage in the liver, lungs, and kidneys (Forti et al. 2011). Each of the studied species surpassed the acceptable values of 0.15 mg/kg (FAO 1983), and 1.0 mg/kg MOFL (2014) as mentioned in Ahmed *et al.* (2019) and Mokhtar et al. (2009), respectively. The level of Cr content (mg/kg) was measured on a dry weight basis and ranged from 3.20 ± 0.18 to 12.80 ± 1.45 (Figure 6).

For species, golda shrimp (*M. rosenbergii*) contributed to the uppermost concentration level of Cr (9.47 ± 3.89) mg/kg), which is almost 9 times higher than the maximum allowable limit, in contrast to the minimum Cr concentration level (8.53 ± 4.45 mg/kg) went to bagda shrimp *(P. monodon)* (Table 1). The golda shrimp *(M. rosenbergii)* collected from the *gheer* #1 led to the highest degree of Cr content (12.80 ± 1.45 mg/kg), while the tilapia *(O. mossambicus)* collected from the *gheer* #1 had the lowest value (3.20 ± 0.18 mg/kg). According to Ahmed *et al.* (2019), the level of Cr content was recorded between 0.62 - 1.19 mg/kg in fish species from the Meghna river and Das *et al.* (2017) observed (1.29 ± 0.54 - 1.93 ± 1.50) mg/kg of Cr content in fish specimens from fish farms in Noakhali district, Bangladesh. According to Fatema et al. (2015), shrimp species from the river and shrimp farms of Tala Upazilla, Satkhira have Cr concentrations ranging from 0.156 - 0.338 mg/kg in bagda and harina shrimp. Thus, our finding surpassed the Cr concentration observed by Ahmed et al. (2019), Das et al. (2017), and Fatema et al. (2015) (Table 1). The application of chemical fish feed contaminated with hazardous metals, as indicated by Shamshad et al. (2009), may be responsible for the elevated level of Cr in the species under this research.

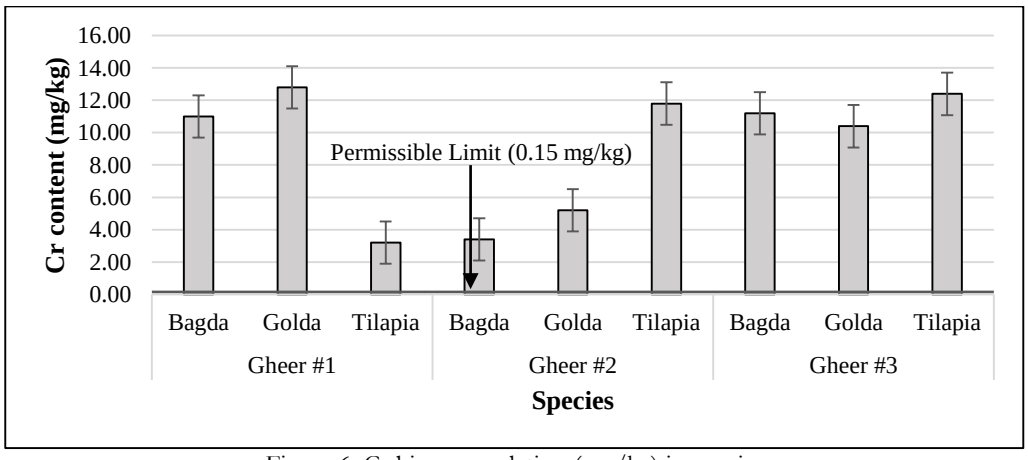

Figure 6. Cr bioaccumulation (mg/kg) in species

Cadmium (Cd)

Usually available at a degree of just 1 mg/kg, Cd can cause persistent poisoning (Roels et al. 1981). As a result of prolonged or excessive contact, Cd may have been linked to collapse of the renal system, skeletal weakening, and prostate carcinoma (Ahmed *et al.* 2016). According to Ahmed *et al.* (2019) the highest allowable limit of Cadmium (Cd) in fish is 0.25 mg/kg MOFL (2014), and 0.10 mg/kg FAO (1983). The content (mg/kg) of Cd in shrimp and tilapia was varied from 0.02 ± 0.002 to 0.06 ± 0.003, which is shown in Figure 7. This research documented that the Cd in the tested species from all fish farms were still lower than the permissible limits suggested by the Codex Committee on Food Additives (FAO 1983). The permitted level (0.05 mg/kg)

specified by the regulations of the European Community was surpassed each of the examined species (EC 2001).

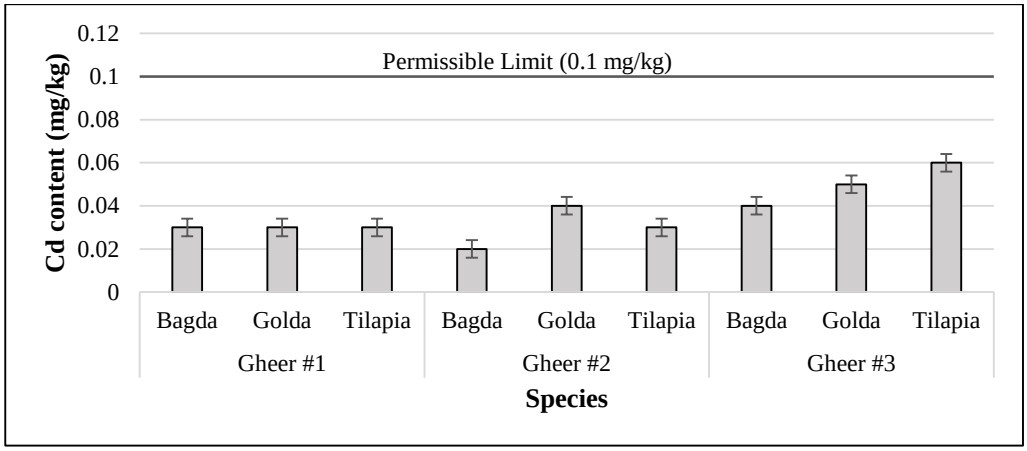

Figure 7. Cd bioaccumulation (mg/kg) in species

Additionally, the mean value of Cd in shrimp and tilapia was lower than the permissible levels conferred by CEPA (1995-97) and TFC (2002), while the induced level is 0.10 mg/kg, and 1 mg/kg, respectively, as mentioned in Raknuzzaman et al. (2016), and Ahmed et al. (2019). In a research by Kundu et al. (2017), Cd was detected in species and fluctuated between 9.083 and 10.453 mg/kg, exceeding our findings. But compared to other coastal locations, Raknuzzaman et al. (2016) demonstrated that the degree of metal content in the fish species was greater. Sarkar et al. (2016) identified an average Cd content of 0.13 mg/kg in the shell as well as 0.09 mg/kg in the muscle of *M. rosenbergii* at fish farm at Paikgacha, Khulna. The *M. rosenbergii* species near the Rupsha River in Khulna had the lowest concentration (0.05 mg/kg) in tissue, which also surpassed our findings. However, the Cd concentration in the studied species is still lower than the permissible levels conferred by FAO (1983). Despite being present in nature at relatively low concentrations instinctively, factory operations (such as casting or electroplating) and the application of agrochemicals may contribute to raising levels (Ahmed et al. 2016 and Kundu et al. 2016). The releasing points of Cd may not have considerably contributed to heavy metal accumulation in the surrounding environment, which decreases the level of contamination in species in this study.

Lead (Pb)

FSANZ (2008) and the European Community (EC 2001) stated that the maximum allowable Lead (Pb) values in fish are 0.5 mg/kg and 0.2 mg/kg, correspondingly. On a dry weight basis, Pb concentration was obtained in a range of 0.11 ± 0.020 to 0.26 ± 0.031 mg/kg (Fig. 8). The maximum Pb content (0.26 ± 0.031) mg/kg in golda shrimp (*M. rosenbergii*) collected from the *gheer* #2, exceeded not only the previous two international standards, but also the national standard (0.30 mg/kg) provided by MOFL (2014). Nevertheless, no discernible variation between the 3 species sampled from 3 different *gheer* was observed. According to this investigation, the highest Pb concentration (0.18 mg/kg) was detected in golda shrimp (*M. rosenbergii*), while the minimum level of Pb content (0.15 mg/kg) was detected in bagda shrimp *(P. monodon)*.

On aggregate, the Pb content of several species was less than the standards of FAO (1983) and MOFL (2014), as well as the permissible levels of CEPA (1995–1997) and TFC (2002). In comparison to prior research, the mean Pb content in all species was lower. The range of Pb concentrations (2.91 mg/kg to 4.63 mg/kg) was found in the fish taken from the Meghna river (Ahmed et al. 2019). The Pb content (mg/kg) fluctuated between 6.787 to 16.386 in fish collected from fish farms in Mymensingh (Kundu et al. 2017). Despite the fact that aquatic species may acquire significant levels of Pb without clear differences to their form or productivity, lead is a poisonous element that can be damaging to them. Results suggest that all species from the *gheer* had Pb concentrations below the allowable limit, making them safe for ingestion. The potential sources of Pb pollution in aquatic systems are the waste discharge from a variety of factories, including printing, dyeing, oil refineries, batteries, and textiles, in the surrounding area, which may not significantly contribute in this area.

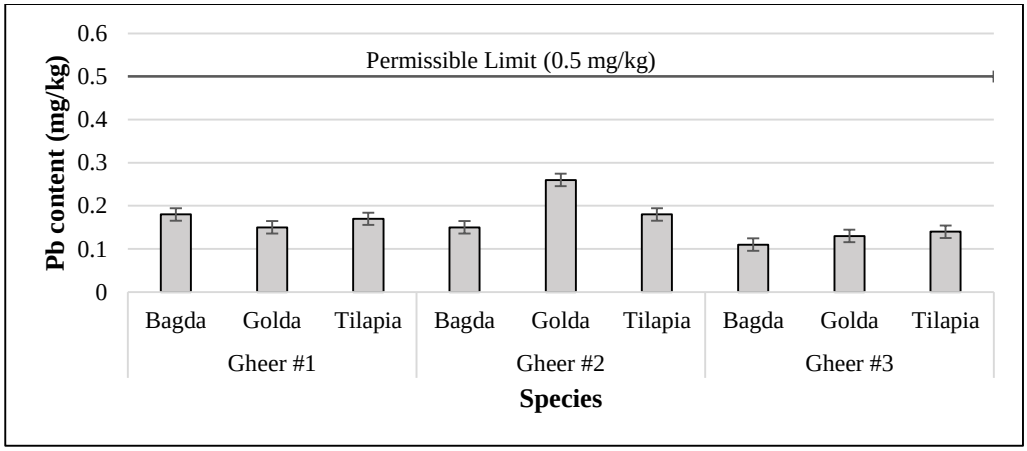

Figure 8. Pb bioaccumulation (mg/kg) in species

Arsenic (As)

The initial signs of acute arsenic poisoning are vomiting, stomach discomfort, and diarrhoea; in severe instances, these are followed by numbness and tingling in the extremities, cramping muscles, and death. Being exposed to As for prolonged term of time can culminate cancer of the skin, lungs, bladder, and kidney; and developmental effects, neurotoxicity, diabetes, and cardiovascular disease are all possible side effects of long-term ingestion of inorganic arsenic (Baki et al. 2018 and IARC 2009). According to this investigation, the level of As content was varied from 0.46 ± 0.035 to 1.96 ± 0.085 mg/kg in the tested species which is shown in Figure 9. The maximum As level was found in tilapia (*O. mossambicus*) (1.96 ± 0.085) mg/kg in *gheer* #3. Meanwhile, the lowest As concentration (0.46 ± 0.035 mg/kg) was present in bagda shrimp (*P. monodon*) from *gheer* #1. The tilapia (O. mossambicus) was discovered to have levels of As well above the allowable level of 1 mg/kg set by CEPA (1995–97) and FAO (1983). All the species were still beyond the maximum allowable limit (5 mg/kg) provided by MOFL (2014). The concentration ranges of As (0.75 mg/kg - 1.48 mg/kg) was observed by Ahmed et al. (2019), which is nearly identical to the outcomes of present research. As, the most hazardous element that is frequently in existence in the ecosystem, can enter a water body through both natural and manmade means (Arisekar et al. 2020). Since shallow groundwater has frequently been fed into aquaculture ponds for cultivation purposes, it is plausible to presume that the elevated level of as in species in the present study might have been caused by the introduction of shallow groundwater. Perhaps the farming water body might be contaminated by tube well discharge wastewater.

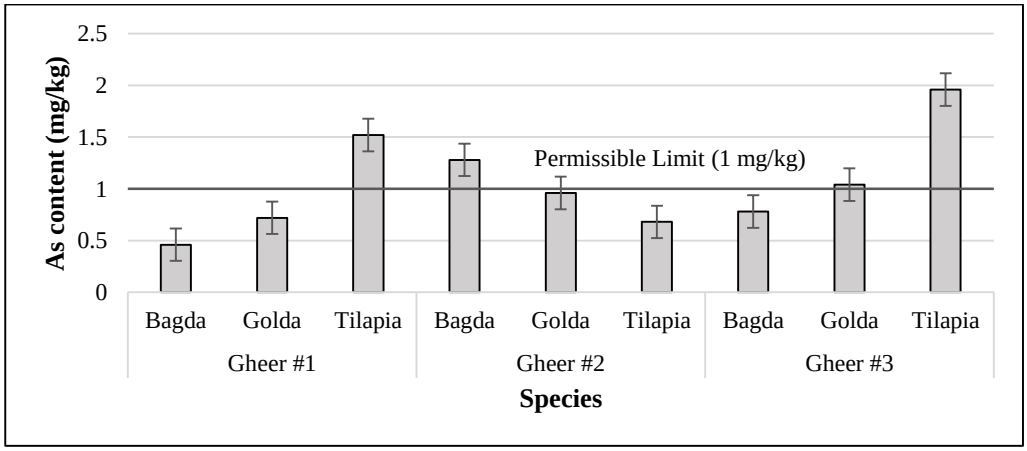

Figure 9. As bioaccumulation (mg/kg) in species

Heavy metal bioaccumulation in tilapia and shrimp

The concentration of all metals in bagda (*P. monodon)* and golda (*M. rosenbergii)* was demonstrated to be still below the standard acceptable level (FAO 1983, WHO 1989), except Mn and Cr. Nevertheless, the level of As, Mn, and Cr in tilapia *(O. mossambicus)* was found to be exceeding the permitted range suggested by FAO (1983) and WHO (1989). In the present study, there were considerable variations in the degree of metal bioaccumulation in different species and different fish farms. This may be because of variations in aquatic ecosystems, feeding sources, and the degree of metal contamination in bottom sediment and water. Alam and Haque (2021) mentioned the capability of fish to bio-accumulate toxic elements from bottom sediments (large metal storage, capturing and depositing >99% of the aggregate amount of deposited elements in the

waterbody) and the water of their habitat. Biological requirements, metabolic processes, and the physico-chemical state of water are the crucial factors influencing the metal accumulation capacity of species (Kundu et al. 2017).

In the previous studies, the mean Cr concentration was documented at 0.186 ± 0.010 mg/kg in bagda collected from the river of Kobadak and <0.1 mg/kg in bagda shrimp from the different farms of the Satkhira area, conducted by Fatema et al. (2017), which were lower than our findings. The concentrations of Pb, Cd, and As were documented as lower than 0.1 mg/kg in the farmed bagda shrimp by Fatema et al. (2017), were much lower than our findings. Prior research done by Islam et al. (2016), found the average concentration of Pb, Cd, As, and Cr in golda shrimp sampled from the fish farms of the Satkhira, and Bagherhat regions. The detected concentrations of Pb, Cd in both studied areas were higher than our findings. But the detected concentration of As in the current research is much higher than the findings of the study conducted by Islam et al. (2016). Moreover, Sarkar et al. (2016) found Pb, Cr, and As concentrations of less than 0.1 mg/kg in farmed golda shrimp (*M. rosenbergii*), which is substantially lower than our findings. On the contrary, the concentration of Cd (0.96 ± 0.38) mg/kg in *M. rosenbergii* under the study done by Sarkar et al. (2016), is higher than the detected concentration of Cd in our study.

The concentration of Mn in tilapia *(O. mossambicus)* was found to be two times higher than the permissible limit, and the concentration of Cr was detected to be almost 10 times higher than the permissible limit recommended by FAO (1983) and WHO (1989). In the previous study conducted by Das et al. (2017) in the district of Noakhali, the average concentration of Cr varied from a range of (0.28 ± 0.16 to 0.42 ± 0.22) mg/kg in tilapia, which was 20 times lower than our findings. On the contrary, Cr was not recognized within the detectable limit (0.0001 mg/kg) in a study in the fish farms of Mymensingh, conducted by Kundu et al. (2017). However, it was discovered that the levels of Cd, Cu, and Fe varied to range from (9.083 to 10.453) mg/kg, (19.073 to 25.343) mg/kg, and (136.241 to 200.26) mg/kg respectively, (Kundu et al. 2017), which are higher than our findings. However, the differences in metal contamination between the current and earlier studies might be related to regional differences in the level of pollution in waterbodies, physicochemical condition of fish habitat (salinity, temperature, total dissolved solids etc.) and feeding practice of different region.

Among the three investigated species, golda (*M. rosenbergii*) accumulated the highest concentrations of almost all metals (Table 1). Fish length and weight were indicated by Kamaruzzaman et al. (2010), Ahmed et al. (2016) and Ghosh et al. (2021) as influencing variables of toxic effect in cultured fish. In addition, Kundu et al. (2016) mentioned a number of inherent characteristics of aquatic species, such as genetical buildup and age, that contribute to the degree of metal accumulation. Moreover, bio-chemical and physical properties of elements may affect the variations in levels of intake of different metals in similar species or even different species in similar habitats. Alam and Haque (2021) reported excess fish feed, human waste, agrochemicals, insecticides, livestock excreta, underground aquifers, wastewater irrigation, and farmland outflows as the potential causes of heavy metal abundance in the bottom sediment of aquatic ecosystems. Since there have been no additional man-made causes of metal pollution in aquaculture practices in this area, it may be presumed that the primary source of metal bioaccumulation in fish is fish feed.

Correlation matrix of heavy metals in species

The Pearson correlation analysis was conducted with the metal concentrations in the studied tilapia and shrimp samples, which are presented in Table 2. The findings from the Pearson correlation test demonstrate that the detected metals showed strong positive relationships. Such correlations reflect that the origins and properties of the metals in the corresponding specimens could be comparable in the aggregate. In the correlations, all metals but Fe-Cr (r=0.780), Mn-Cr (r=0.728), and Zn-Cu (r=0.690) suggested a significant relation ($p < 0.05$). Cd-Cr (r=0.923) and Pb-Cu (r=0.934) strong significant relations were found in previous research, carried by Ahmed et al. (2019) in the estimation of heavy metal content in fishes collected from the Meghna river estuary in Bangladesh.

Even, significant positive correlations were evident, such as As-Pb (r= 0.828), Cu-As (r= 0.833), Cr-Pb (r= 0.839) which indicates that the sources of the corresponded might be similar Ahmed *et al.* (2019). A substantial positive correlation among the heavy metal concentrations was demonstrated, such as follows: Cd-Co (r = 0.733), Fe-Al (r= 0.568), Ni=Co (r= 0.482), Mn-Co (r= 0.395), Cr-Co (r= 0.351), and Pb-Cr (r= 0.283) and concluded that the strong and moderate relationships imply that their origins are similar, such as factory effluents, domestic sewage, agricultural implements and wastewater supply.

Table 2. Pearson correlation matrix of heavy metals in species

	Fe	Mn	Zn	Cu	Cr	Cd	Pb	As
Fe	1							
Mn	0.468	1						
	0.204							
Zn	-0.172	-0.605	1					
	0.657	0.084						
Cu	0.033	-0.309	.690*	1				
	0.933	0.419	0.040					
Cr	.780*	.728*	-0.123	-0.001	1			
	0.013	0.026	0.752	0.999				
Cd	0.628	0.554	-0.250	0.319	0.434	1		
	0.070	0.122	0.516	0.403	0.244			
Pb	-0.167	-0.363	0.081	0.055	-0.402	-0.190	1	
	0.667	0.337	0.836	0.888	0.283	0.625		
As	-0.020	0.192	-0.547	-0.302	-0.313	0.466	-0.165	1
	0.959	0.620	0.128	0.430	0.411	0.206	0.672	

*. Correlation is significant at the 0.05 level (2-tailed).

Conclusion

The recorded data of the present study indicates that among the analysed 8 metals, Fe, Mn, Cr, and As were detected at a higher concentration than the maximum permissible limit recommended by FAO (1983) and WHO (1989). The use of commercial fish feed in fish farms raises the likelihood of aquatic animals accumulating certain heavy metal. To combat heavy metal accumulation in farmed tilapia and shrimp, it is imperative to closely monitor farming practices and develop different preventive actions. The findings could be anticipated to raise public awareness regarding the safety of ingesting shrimp and tilapia produced in aquaculture farm.

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Conflict of Interests

The authors declare no conflict of interest.

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ORIGINAL ARTICLE **LIFE SCIENCE**

CLIMATE CHANGE: ADAPTATION AND PERCEPTION AT COASTAL REGIONS PERIPHERAL TO THE SUNDARBANS, BANGLADESH

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Abstract:

This study demonstrates the adaptation and perception to the climate change by focusing the community's views and experience. This study suggests that majority (90%) of the coastal community proximity to the Sundarbans is experienced to adaption with changing environmental vulnerabilities but the major challenge they face is limitation of financial support. At the same time, most of the community (79%) has no knowledge about climate change but the rest have medium or little knowledge (21%). Results revealed that financial and insufficient livelihood support (76%), overlooking the needs of local communities are the key problem of climate change adaptation and perception. At the same time, most of the respondents are middle aged, less literate, and fishing is the primary occupation, while wood is the main source of their energy. Besides, geographical location, natural and anthropogenic challenges, lack of in-depth knowledge towards climate change adaptation and its vulnerabilities, and inadequate integration of policies and programs are hindering sustainable adaptation. The study suggests that information and knowledge must be improved within the community to find out and practice local scale adaptation options like livelihood diversification, diversify use of resource, community based co-management and financial support. Similarly, relevant training and awareness program could be effective to reduce the vulnerability of most innocent victims of the coastal area

Keywords: Co-management, financial support, livelihood, sustainable adaptation, vulnerable.

Introduction:

Bangladesh is among the nations that are most at risk from climate change and coastal regions of Sundarbans which provides innumerous tangible and intangible benefits in this delta is the hot spot of climate change (Helal Siddiqui & Islam, 2021). The communities of this region are at severe risk in environmentally, socio-economically, culturally due to adverse impact of climate change (Selvaraju et al., 2006; NAPA, 2005). According to the most recent IPCC estimates from their Fourth Assessment Report, warming is predicted to occur over the next 20 years at a rate of 0.2° C each decade. The best predictions indicate that by 2100, the average global temperature will have increased by 1.8 to 4 ° C, while it may reach 6.4° C (Alley et al., 2008; Gregory et al., 2005; Islam et al., 2022). Within the next fifty years or so, it is possible that average global temperatures may rise by 1 to 3°C on current patterns, and if greenhouse gas emissions continue to soar, the Earth will continue to warm by several degrees more (IPCC, 2001; Adger et al., 2007).

The most pervasive and escalating issues in the modern world are climate change, ecosystem degradation, and a rise in natural disasters. This suggests that ecosystem function is under threat at a level that is having an increasingly detrimental effect on human well-being (UNEP, 2008). Bangladesh, where millions of people already suffer, is the only nation and people who are more familiar with this issue. Bangladesh has been considered as one of the nation's most susceptible to the effects of climate change (Siddique, N.A., 2001; Islam et al., 2022). This is a result of its particular geographic location, the dominance of the floodplain, the slow elevation from the sea, the high population density, the high levels of poverty, the inadequate infrastructure, the low level of social development, the lack of institutional capacity, and the extreme reliance on the resources and services of nature (FAO, 2007; Helal Siddiqui et al., 2021; Dey, et al., 2021). A major risk factor for risk aversion is the growing climate

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uncertainty, which poses an additional concern in Bangladesh's disaster-prone regions (Ahmed et al., 2022). Due to the effects of climate change, it is anticipated that the intensity and variability of climatic dangers would continuously grow in the near future (Ahmed, 2004; Sheikh et al., 2021; Islam, et al., 2021).

The majority of coastal settlements near the Sundarbans lack access to electricity and clean drinking water. The region's agricultural output is less than the state average. A low level of development and a high incidence of poverty in the area have been attributed to inadequate infrastructure, poor communication facilities, a lack of access to clean drinking water, health, education, and services (Islam, 2019; Dey et al., 2020). This enormous population depends on the Sundarbans either directly or indirectly for their livelihood (Abdul, 2014). The binding and cohering of soil by plant roots and vegetative matter, the dissipation of erosion pressures including wave and wind energy, and the trapping of sediments are all ways that mangrove plays a significant role in shore line stabilization (Helal Siddiqui & Islam , 2019; Dey, et al., 2021a). The mangroves serve as a natural barrier for the coastal human settlements, reducing the severity of cyclones and tidal surges (Ahmed et al., 2022). The protective effect of the Sundarbans was felt strongly following the super cyclone Sidr. It is generally believed that without the Sundarbans, the damage from cyclones Sidr and Aila would have been much worse (Islam et al., 2020; Helal Siddiqui & Islam, 2020).

The current study is carried out to capture the views of community peoples regarding ecosystem based adaptation so that their views can be recommended to incorporate in policy level. The results of this study may be used to identify possible areas where the community's capacity to adapt to rapidly changing climate variability could be accommodated. Again, the research findings would be supportive to increase the adaptive capability of vulnerable coastal community and to develop strategy for ecosystem based adaptation to climate change proximity to coastal Sundarbans. Furthermore, the findings would be beneficial for planners and policy makers and vulnerable coastal community. The study is carried out to better understand the climate change's effects on the coastal ecosystem as well as on the lifestyle of the local people. However, the objectives of the study are as follows-

1. To clarifies the adaptation and perception to climate change by focusing the respondents's views and experience.
2. To identify problems and provide insightful suggestions to some adaptation preferences for climate-sensitive coastal environments and its resources.

Material and methods

Selection of the Study Area

A World Heritage Site, the Sundarbans (21°39'- 22°30'N, 89°01'-89°52'E) is made up of three wildlife sanctuaries (Sundarbans West, East, and South) that are located on nearby deltaic islands. The Sundarbans, a region of 10,000 km^2 of land and water, is a section of the world's largest delta, which was created when the Ganges, Brahmaputra, and Meghna rivers converged in the Bengal Basin and left behind sediments (Reid et al., 2007; Siddique, N.A., 2001; Helal Siddiqui & Islam, 2020). The study area is located at Symnagor Upazilla of Satkhira district, Soronkhola UpazIla of Bagerhay district and Dhangmari Upazilla of Khulna district, and all of this sampling area located proximity to Sundarbans (Fig. 1). The communities of the area living at embankment are vulnerable to climate induced natural disaster like cyclone, flood, tidal surge, terrorism, tiger attack, river erosion, salinity intrusion by sea level rise and manmade activities (Siddique, N.A. 2001). People are usually collect rain water as drinking purpose and sometimes collect water from tube-wells which are near about 7/8 kilometer far away from their house. Children are frequently afflicted with water-borne illnesses such diarrhea, dysentery, skin conditions, etc. as a result of contamination and salinity (Islam, et al., 2021). People in the studied areas generally have poor socio-economic conditions, making it difficult for them to adapt to climate change and extreme weather (Islam et al., 2020). Therefore, the research region selection was significant in terms of sensitivity to find ecosystem-based adaption options for the study area's local population.

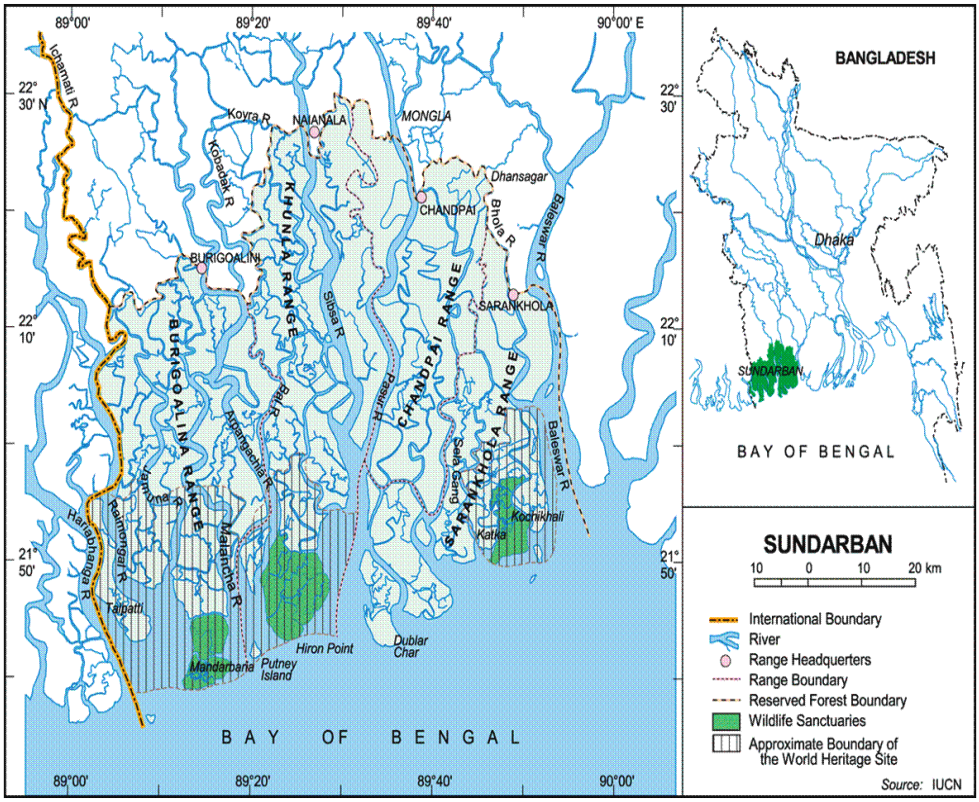

Figure 1. Map of the study area.

Questionnaire preparation and survey

A questionnaire for the selected community was created taking into account the study's objectives. The study's primary data were obtained from a total of 120 sampling units using multistage purposive sampling techniques and a semi-structured questionnaire. Finally, from several government organizations (GOs) and online sources, secondary data and information were gathered.

Data Analysis

The data was processed after it was collected from secondary and primary sources. After being sorted, the information and data were categorized, interpreted in accordance with the objectives, and analyzed with the use of MS Excel.

Results

Effects of climate change on the research area's socio-economic condition

The socio-economic situation in the study area is under pressure due to the effects of climate change. Cyclone and tidal surge, tidal flood etc. natural disasters not only destroy infrastructure but also damage huge amount of production every year. Most of the climate vulnerable coastal communities were male (81%), middle aged (57%) with less family members (82.3%) and possess weak housing type in the study area. Again, their occupation, literacy level, and low income parsed out their miserable condition in the framework of climate variation adaptation and perception (Table 1).

Sources of energy of the respondents in the study area

Source of energy for cooking is another important indicator of the socioeconomic condition for the rural people. In the study area wood, cow dung, dried leaves are the main source of fuel energy. By ranking among the respondents, it was found that 100% respondents used wood as fuel energy for cooking and significant amount of them are

collected from the Sundarbans. In the study area, 65.4% people supported dried leaves as second option for fuel energy, while cow dung was also supported by 29% respondents as second option. Besides, 71 % people supported cow dung as third option while dried leaves were supported as third option by 35.5% people. From the ranking it is evident that, all people in the study area generally used wood for their cooking purpose. The distribution of source of energy for cooking is shown in figure 2.

Table 1. Demographic profile of the respondents in the study area.

Parameter	Categories	Percentage
Gender	Male	81
	Female	19
Age (Years)	<20	17
	20-45	57
	>40	26
Family members (In numbers)	2-6	82.3
	7-10	16.8
	>10	0.9
House type	Kacha	85.6
	Semi-pacca	12.0
	Pacca	2.4
Occupation	Agriculture	40
	Fishing	45
	Others	15
Income (BDT/Month)	<2000	35.6
	2000-6000	58.7
	>6000	5.7
Literacy level	Primary	48
	Above primary	35
	Illiterate	17

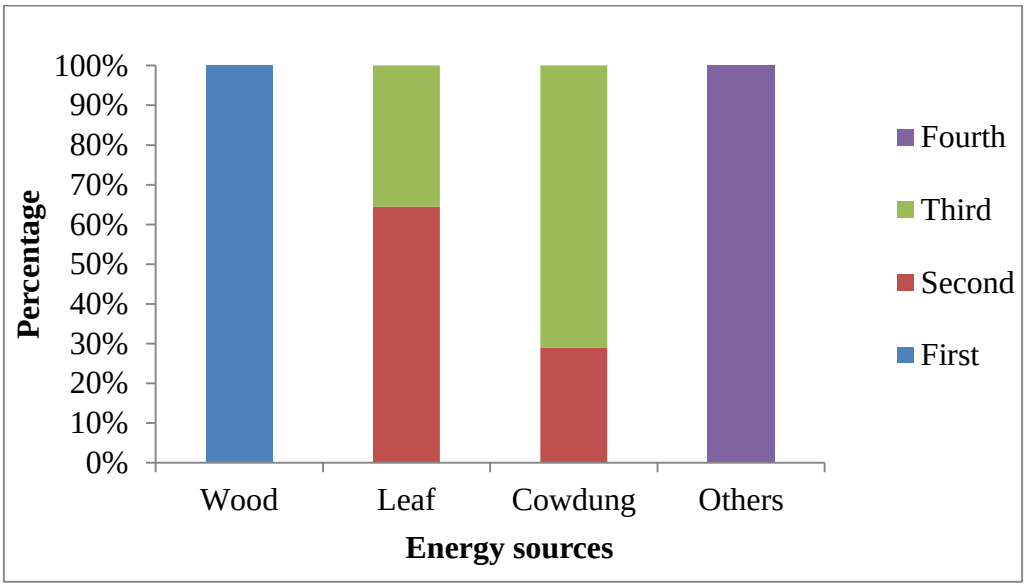

Figure 2.The distribution of fuel energy for cooking.

Primary profession pattern of the respondents in the study area

Majority of the people in the study area mainly depends on natural resources for their livelihood. Land, water bodies, khas land, beels, forests, and livestock are the sources of their livelihood. The major ecosystem based activities in the study area are fishing and aquaculture, wood collection, honey collection. As the Sundarbans is situated near the study area, it is a major source of earning for the habitat. In the study area, 57.9% people are involved with fishing and aquaculture activities, 20.6% and 10.3% are involved in wood collection and honey collection respectively. Despite this, majority of the honey collector and wood collector mainly collect these resources from the Sundarbans. Only 11.2% are related with other activities including small-scale homestead agriculture, trading etc. The distribution of major ecosystem based activities is shown in figure 3.

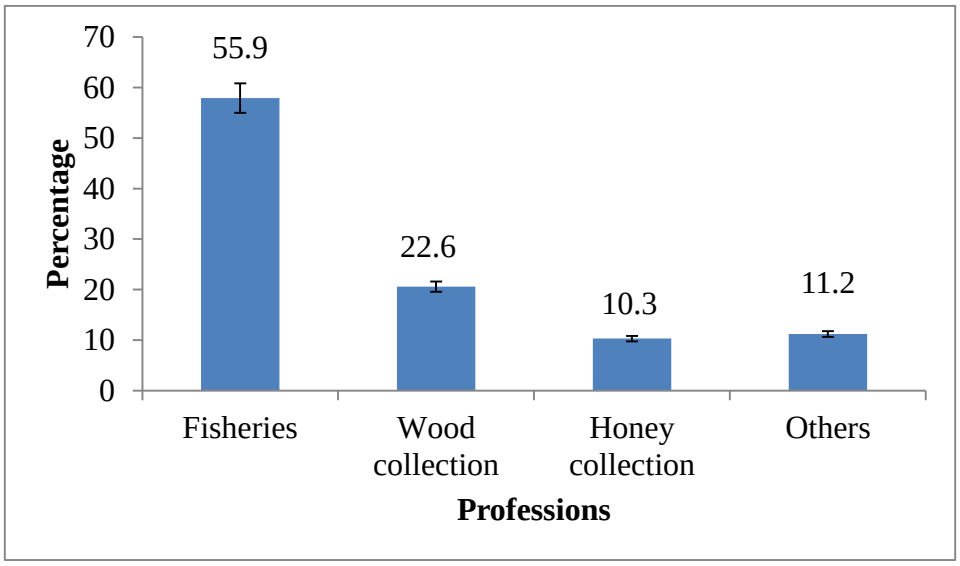

Figure 3. The distribution of major ecosystem based activities.

Perception about climate change by the coastal community

The respondents were divided into three categories based on their level of understanding regarding climate change: those with no knowledge, those with low knowledge, and those with medium knowledge. The majority (79%) of respondents, according to data from the climate-vulnerable coastal community, had little knowledge of climate change. Only 14% people had low knowledge about climate change and only rest 7% people had medium knowledge about climate change and there was no sample found with high knowledge about climate (Figure 4). In the study area all the respondents believe that the concentration of cyclone flood salinity has increased in the contemporary years. Majority of the people disagreed about climate change but they stated that, frequent cyclone, flood are changing their locality. On the other hand, some individuals concur that climate change is a problem, but they are unaware of its causes. This contradictory comment indicates that, they have an extremely negative perception of climate change, making them more susceptible to its impacts.

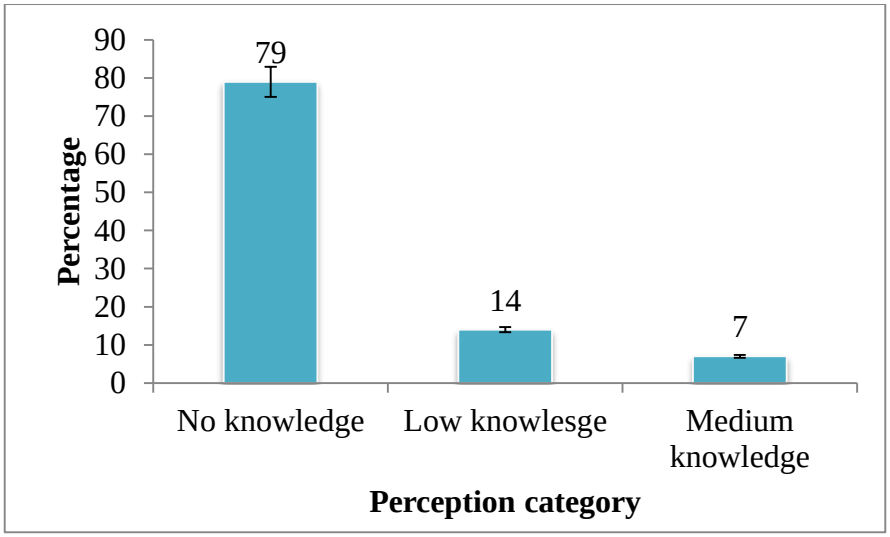

Figure 4. Perception of the respondents about climate change in the coastal area.

Problems faced by the respondents during working period

Most of the population in the study area face different types of problems during their working period. The Sundarbans region and the Bay of Bengal are where the fisherman must travel to fish. In the rainy season they have to catch fish in good and bad weather condition and they face different natural disasters. Wood collector and honey collector also has to go in the Sundarbans and they face different types of problems like disaster, tiger attack, shark attack, crocodile attack, disease etc. In this case, maximum people stated that, they face disaster during their activity and disease with insufficient food is also another severe concern issue. At the same time, a climbing number of people face different types of attack during their working period simultaneously. Coastal people are different types of problems and these are disasters, disease and insufficient food and animal attack. These problems reduce livelihood pattern which ultimately affects basic human needs and thus climate change adaptation and mitigation (Figure 5).

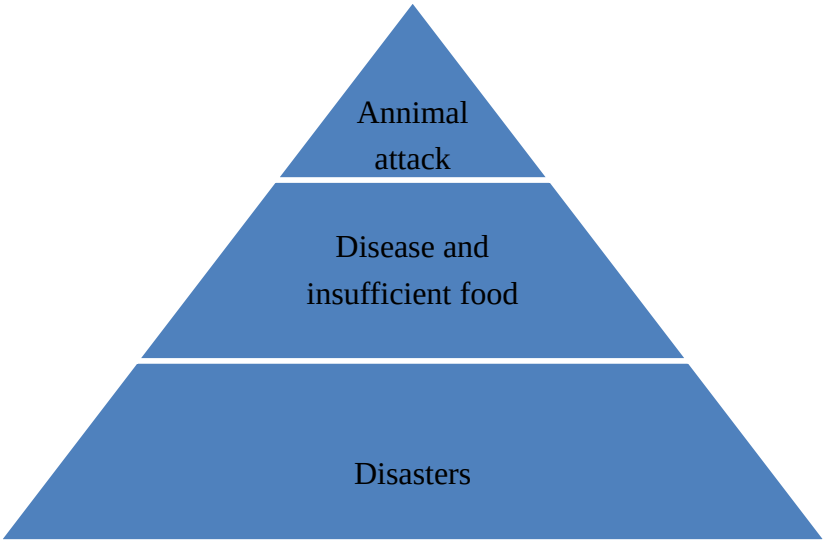

Figure 5. Problems faced by the respondents during working period in the coastal area.

Problems and necessity of support to the adaptation and perception of climate change in the coastal area of Sundarbans

At the present situations most of the people need proper support to maintain their livelihood because they are affected by the recent Aila. Their fish Gher are in water logging condition, they lost their livestock resources and other property. They want different types of support to maintain their livelihood and to adapt with the changing condition. They want training, monetary, educational and technological support. In this regard, in the research area it was found that, maximum 76% people want monetary support. Similarly 5% people want educational support, 6% want instrumental support, 13% people want training and other supports for the adaptation and perception if climate change in the coastal regions of Sundarbans (Fig. 6). This study indicates that the coastal community proximity to the Sundarbans is experienced to adaption with changing environmental vulnerabilities but the major challenge they face is limitation of financial support.

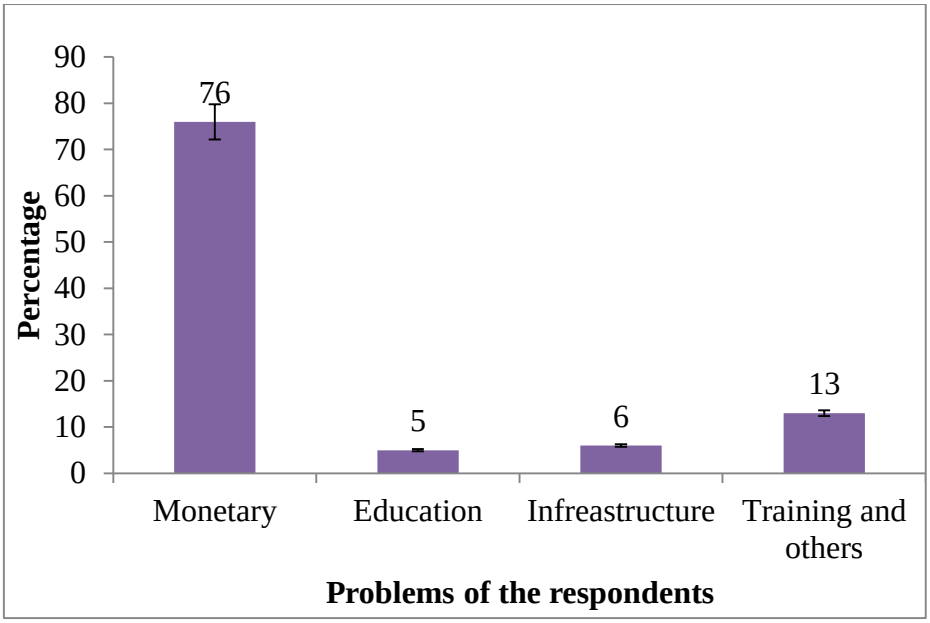

Figure 6. Problems faced by the respondents during working period in the coastal area.

Problems of climate change in coastal regions of Sundarbans

Although for spontaneous adaptation to changing coastal environment, the coastal community required to adapt more fast in a planned way so that they can able to reduce their losses or even able to get benefit from fast changing environment. But in this case they have some barriers. In the study area the common barriers are the lack of awareness or perceptions at all level on global climate change impact. The barriers in adaptation strategies identified are summarized below-

- Due to climate change and sea level rise, impoverished households in the study area are ill-prepared to cope with rising calamities such saline intrusion, tidal surge, and water logging/drainage congestion.
- Most of the households whose livelihood exclusively depends on labor in coastal natural resources collection having very limited capacity to adapt.
- Low socio-economic condition of the households allows them in limited options to adapt with the changing environment.
- The community residents, local authorities, and civil society are not properly informed about the disastrous effects of climate change on the coastal region.
- The climate change issues and its consequences are not yet considered in the development plan of the locality. There is lack of integration in both development plan and implementation.

- Any kind of development activities in the study area generally considered sort term benefits rather than long term effects.
- Lack of proper coordination in the utilization and uses of common natural resources within the community.
- Limited opportunity to diversify livelihoods in the locality due to limited access to facilities.
- Weak disaster management and early warning system within the community.

Discussions

In the study area, majority of farmers are male and middle aged, and their literacy status is at primary level (Table 1) and this are revealed in another study by Uddin et al., (2017) conducted in the coastal region of Bangladesh. On the other hand, most of the houses in the study area are Kacca and majority of the respondents family members are of two to six in numbers (Table 1). The main reason of having such house could be the low income of the respondents as they are in the climate vulnerable areas. However, similar number of family members are observed regarding this climate vulnerability and adaptation in the coastal periphery of Bangladesh (Uddin et al., 2017). Regarding the income and occupation, on the contrary, most of the lower income respondents are massively depends on the fishing (Table 1 and Figure 3) because the study area is located adjacent to the Sundarbans, which could be responsible of such dependency on fishing occupation (Islam et al, 2020). Again, this occupation may not exist in the whole year in the study area and probably this the cause of reducing such income of that vulnerable coastal community and similar phenomenon are repeated in another study conducted by Lázár et al., (2015). Most of the respondents used wood as fuels followed by leaf, cowdung and so on (Figure 2) and this may be happened due to the availability of this resources form Mangrove forest and from the their farming facilities.

The probability of perception of climate change is greater for those who have higher educational attainment compared to less-educated or illiterate farmers. It is apparent that educated farmers have more knowledge, ability to understand and respond to expected changes, able to forecast future scenarios and have greater access to information and opportunities than others (Ndambiri et al., 2012; Amdu et al., 2013; Akanda & Howlader, 2015). So, education as an influencing factor of farmer's perception of climate change but in our study, most of the respondent's educational status is at primary level and they don't have enough knowledge (79%) regarding climate change adaptation (Figure 4). The reason could be behind this is their lack of available facilities such as education, trainings and so on, which are also revealed in this study (Figure 6).

In the study area, perception of climate change adaptation is low (Figure 4), which may make more climate vulnerable community in the coastal region. But, there may be a lot of reasons of having this condition such as proper education, training, and also lack of financial support, and all of these issues are repeated in this study (Figure 6). Again, similar findings are illustrated by Sanog et al., (2012) and Semenza et al., (2008), where they mentioned about potential explanation may be that all farmers have the potentiality but may be lack of proper education, training, poor communication exposures and fail to perceive more.

Recommendations to enhance adaptation and perception of climate change in coastal regions of Sundarbans

Although it is not easy to give any concrete recommendation depending on such a small scale survey but based on the finding some recommendations are presented below.

- To boost local people's awareness and understanding about climate change, its impact on their way of life, and how to adapt to new climatic conditions, relevant training programs should be undertaken and informal educational facilities level should be raised. The various GOs and NGOs should be involved in the conduction of training programme.
- Information and knowledge for local adaptation must be improved within the community. Supporting the local population's efforts to improve their traditional knowledge systems and management techniques in response to shifting climatic conditions is vital for this reason.
- Necessary steps should be taken to develop effective early warning system, disaster management team weather forecasting practice within the community.

- At the present situation community based safe place for livestock and community based safe drinking water source should be established in the study area.
- As the people in the research area mainly depends on the local resource, training programme could be conducted among them to promote the diversify uses of resources that they collect to maintain their livelihood.
- Saline tolerant fish species can be cultured as fishing and aquaculture is the main occupation at the study area and mangrove afforestation is very much important at the study area to reduce climate change hazards.

Conclusion

Climate change impacts are really high for Bangladesh and the southwest coastal area of Sundarbans is the most vulnerable part of the country. Besides, Bangladesh's coastal settlements can be best defined as being in a precarious state. It is necessary to find out local level ecosystem based adaptation options to save the coastal region, threatened by the upcoming event. The study suggests that information and knowledge must be improved within the community to find out and practice local scale adaptation options like livelihood diversification, diversify use of resource, community based co-management and financial support. Similarly, relevant training and awareness programme could be effective to reduce the vulnerability of most innocent victims of the coastal area. Respective authorities, in particular government and non-government groups, should develop legislative measures that take into account these determining elements in how farmers view climate change. The vulnerability that farmers experience due to the effects of climate change may be significantly reduced as a result.

Conflict of Interest

The author declares no conflict of interest.

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ORIGINAL ARTICLE **SOCIAL SCIENCE**

EFFECTIVENESS OF BANNING PLASTIC BAG IN BANGLADESH FOR ENVIRONMENTAL PROTECTION

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Abstract

Ranging from degradation of air quality to increase in ocean toxicity, plastic bag contributes to disruption of ecosystem processes and the environmental attributes. Plastic bag is the cheapest and most available means to carry goods. However, mass use and improper disposal of this product generate severe environmental issues in developing countries. Bangladesh promulgated nationwide ban on plastic bag in 2002 as a protective measure for safeguarding environment and ecosystem. This study investigates the effectiveness of the ban in the south-west region of Bangladesh. A total of 180 randomly selected retailers from six upazilas of three districts in the south-west region of Bangladesh were surveyed taking one rural and one urban agglomerate from each district. More than three-fourths of the retailers provide plastic bag to the customers and only four percent retailers provide jute bag. Our findings suggest that the efficacy of authority to execute the ban is insignificant that generates the scope of violating the banning efforts of plastic bag. The retailers endorse that lack of alternative bag is the reason behind violating the ban on plastic bag. The logistic regression results suggest that frequency of raid by public authority, non-availability of alternative product, lack of knowledge regarding deteriorating impact of plastic bag on ecosystem and environment, and business license significantly influence the retailers to comply with the enforcement of banning plastic bag, whereas the proximity of shop to the main road provides room for non-compliance. This study suggests for easy availability of environment friendly bag, awareness among mass people and frequent raid from the authority which will contribute in environmental protection and ecosystem restoration through effectively enabling the ban.

Keywords: Plastic bag ban, effectiveness, retailer, consumer awareness, environment, Bangladesh

Introduction

In 1970's plastic bag was first introduced (Derraik, 2002). It gained enormous acceptance amongst consumers and dealers after the introduction. Every year people use and throw around 500 billion plastic bags (Spokas, 2008). The historians and archaeologists defined periods or ages on the basis of materials or technologies those affected mankind the most like: age of stone, bronze and iron. Accordingly, the ongoing period might be termed as the 'plastic period' for the prevalence of plastic (Chandrasekara & Kumar, 2016). Plastic which is made up of synthetic organic polymers were assumed to be harmless. But persistent use and unplanned disposal of it leads to environmental problems. Plastic is now commonly used in a variety of ways in both intermediate and final usages such as in garment industry, medical use, food preservation and packing, electronic products supplies, construction and so on (Lamont, 2005; Proshad et al., 2018). Every year people produce a large percentage of plastic as single-use, for disposable packaging of goods or products that is permanently thrown out within the year. In recent times variety of plastics are used and some of them are recycled as they are disposable in nature (Reddy et al., 2014; Jorgensen et al., 2021). Accumulation of plastic or

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plastic products disturbs wildlife, wildlife habitat as well as human which are major apprehensions for the environment and economies.

Throwing away plastic wastes is now documented widely as a major environmental problem (Proshad et al., 2018; Derraik, 2002), resulting detrimental effects on wildlife (Wilcox et al., 2015), and offers limited plastic removal options (Wilcox et al., 2015). Any type of such wastes such as plastic bags dumping openly at any location like along roadside, park or lands poses a risk to human health and environment because it creates soil pollution (Khajuria et al., 2011). If there is seen littering plastic bag everywhere on the street it is called 'white pollution' in China (Xing, 2009). The Mumbai flood in India is partly resulted from clogging the drains by plastic for which thousands of people died (Halden, 2010). To avoid such adversity Bangladesh has banned the plastic bags in 2002 (Spivey, 2003; Molla et al., 2018).

One of the main reasons of wide usage of plastic bag is its cheapness followed by its convenience to use. It has been investigated that after throwing directly to the environment, such bags persevere up to 1000 years or more to be degraded and thus pose disposal problem without being disintegrated naturally (Shenton & Stevens, 2001; UNEP, 2005). Use of such almost non-degradable substances damages environmental beauty (Verghese et al., 2009; Dikgang & Visser, 2012). In municipalities plastic bags block sewerage systems (Laist, 1997). As a result, tools like levies and taxes or banning are imposed to confine the use and production of plastic bags (Hasson et al., 2007; Ayalon et al., 2009; Clapp & Swanston, 2009). But they are not so effective thus voluntary activities have also been taken in some countries to decrease the use of plastic bag, more specifically to prevent plastic bag induced problems in environment.

To reduce plastic bag usage many countries are using command and control as well as market-based approaches after realizing their potential negative effects. Many studies recommend that the effectiveness of these countless interventions is definite and depend much on their implementation type (Shi & Koné, 2010). Ensuing this universal footstep, the Government of the People's Republic of Bangladesh promulgated a ban on providing single used polythene bags in Dhaka in the year of 2002 and after this a nationwide ban in the year of 2007 (Molla et al., 2018). Use of plastic bag may be controlled via several measures like recycling, ban on the production, usage and distribution or imposing tax. For the sake of economic and quality issues, research has found recycling as impractical (McKinney & Schoch, 2003; Miller, 2005). Given the backdrops, to govern the level of consumer consciousness and find out the reasons behind the non-conformity regarding the phenomenon, it is important to undertake proper strategies and accomplish tasks in a sustainable manner.

Available literature on Bangladesh and all over the world show how plastic bag pollutes environment and why it is so significant to ban this through law and enforcement. Thus, this article intends to trace out the effectiveness of plastic bag ban law in the south-west region of Bangladesh followed by two research questions:

1. To what extent people abide by the plastic bag ban ordinance? and
2. What are the reasons behind the nonconformity (if any) of the plastic bag ban law?

Materials and Method

Data source and study area: Data for this study were collected through structured questionnaire following multi-stage systematic random sampling procedure, picking 30 respondents from each of the 6 villages selected from Khulna, Jeshore and Satkhira district of the southwest Bangladesh. The villages are located in Batiaghata and Khulna Sadar uapzilas in Khulna district, Jashore sadar and Abhaynagar upazilas in Jashore district and Satkhira sadar and Kolaroa upazilas in Satkira district.

Study variables and measurement: The main dependent variable of logistic regression is probability of providing plastic bag by the retailers, measured by 0= retailer does not provide plastic bag and 1= retailer provides plastic bag to customers. The independent variables are: age of the respondent (in years), shop distance from main road (in km), shop size (in km) and so on. There are some independent variables measured in dummy form and Likert scaling to use in the regression. They are: whether shop keeper has license is measured in dummy (1=yes, 0=no), government gives raid and takes punitive measures for providing plastic bag and restrictions faced from consumers while providing plastic bag are measured by 5=very frequently; 4=frequently; 3=sometimes; 2=less frequently; 1=never. Availability of alternative bags, instead of plastic is measured by 5=very much; 4=much; 3=medium; 2=little; 1=very little.

Results

Summary Statistics

Most of the shops are in short distance from main road (Table 1). The minimum distance is only 0.001 km whereas the maximum distance of the shop from main road is 0.091 km. Government official in charge visits the market place to investigate any anomaly in the market regarding the ban. The average number of visits of the government official is around 4 in a year and it deviates by around 5 standard values from the mean. The number of visits vary from none to 24 in a year. The mean percentage of High-Density Polyethylene (HDPE) plastic bag is around 61 percent of the total bags provided by the retailers from his shop and it deviates by around 27 standard values from the mean. This result states that HDPE plastic bags are the most frequently provided to customers by the retailers.

Table 1. Summary Statistics

Variables	Unit of Mesuarement	Obs.	Mean	SD	Min.	Max.
Age of the Respondent	Number of Years	180	43.71	13.63	17	80
Family Member	Number of People	180	4.33	1.69	1	9
Education of the Respondent	Years of Schooling	180	7.76	4.08	0	18
Earning Members	Number of People	180	1.46	0.67	1	3
Shop Size	Square feet	180	176.5	154.4	10	750
Shop Distance from Main Road	Kilometer	180	77.58	79.45	4	300
Experience	Number of Years	180	11.23	8.52	1	40
Number of Government Visit	Number/Year	180	4.39	5.47	0	24
Television Watching Time	Hours/day	180	1.43	1.22	0	5
HDPE Plastic Bag	Percentage	180	61.15	27.42	0	100
LDPE Plastic Bag	Percentage	180	7.75	14.72	0	70
Net Bag	Percentage	180	15.72	20.53	0	95
Paper Bag	Percentage	180	10.28	13.84	0	70
Jute Bag	Percentage	180	3.56	8.16	0	60

SD= Standard Deviation, Min. = Minimum, Max. = Maximum.
Source: Authors' compilation based on field survey, 2020.

The average percentage of Low-Density Polyethylene (LDPE) plastic bag is around 8 percent of the total bags used by the retailers in their shop and it deviates by around 15 standard values from the mean. It indicates that the retailers are providing LDPE plastic bags less frequently to customers (0 to 70 percent cases). The average percentage of net bag is around 16 percent of the total bags used by the retailers in shop and it deviates by around 21 standard values from the mean. It indicates that net bag is hardly provided to customers by the retailers (0 to 95 percent cases).

The mean percentage of environmentally friendly paper bag is around 10 percent of the total bags used by the retailers and it deviates by around 13.84 standard values from the mean. The jute bag is one of the most eco-friendly bags and it is biodegradable. However, it is observed that the jute bag is used least among all the bags available in the market. The average percentage of jute bag is only around 4 percent of the total bags used by the retailers and it deviates by around 8 standard values from the mean. It indicates that jute bag is hardly provided to the customers. Many retailers claim that jute bag is much costly.

Hypothesis Testing

Table 2 shows that the practice of providing plastic bag by retailers for license holders is less than that of license non-holders and the mean difference is statistically significant ($p<0.01$). So, there remains difference between license holder and non-holder in plastic bag providing behavior by the retailers.

Table 2. Hypothesis Testing

Null Hypothesis	Variables	Obs	Mean	Mean Diff.	Std. Error	T Value	Decision
H0	License Non-Holder	93	0.96	0.01***	0.02	7.78	H0 Rejected
	License Holder	87	0.52		0.05		

*N.B: *** p<0.01, ** p<0.05, * p<0.1. Obs= Observation, Diff= Difference, Std.= Standard.*
Source: Authors' Estimation, 2020

Appreciation of Plastic Bag Ban

Around 73 percent of the respondents do appreciate the ban on usage of plastic bags; however, the rest 27 percent do not have any idea of the regulation. Around 30 percent of the retailers have knowledge on the ban. However, instead of having prior knowledge on the ban around 40 percent retailers still provides defying the ban.

Restriction Faced by Retailers in Providing Plastic Bag to Consumers

For understanding the demand side factors it is necessary to find out whether the consumers give any restrictions in receiving plastic bag. Thus, it has been found that 6.67 percent consumers frequently opt for receiving plastic bag in day-to-day purposes. Writing elaborately 55 percent respondents never restricted the shop owners to provide the bag whereas 27 percent respondents once in a while negated receiving the plastic bags while purchasing from them.

Advantages of Using Plastic Bag

The major advantages in using a plastic bag as realized by the respondents are its lower cost (48 percent), lighter in weight (32 percent) and easiness in handling (20 percent). However, licensing, shop distance from main road, government raid, government visit, availability of variety of bags and knowledge regarding plastic bag have statistically significant impact on plastic bag providing behavior (Table 3).

Probability of Plastic Bag Providing

According to regression results, chances of having license has negative significant impact on probability of plastic bag providing behavior. The probability of providing plastic bag is 36 percent lower for license holder than license non-holders, which is statistically significant ($p<0.01$). Again, with one km increase in shop's distance, respondents are more likely to provide HDPE plastic bag ($p<0.1$). The scenario can be that having shop far from the main road give the retailers enough time to hide plastic bags or shut down the shop by the time of governments raid.

Table 3. Logit Model for Probability of Plastic Bag Providing

Dependent Variable Plastic Bag Providing (Yes=1, No=0)			
Explanatory Variables	Measurement Unit	Coefficient	ME
Age of the Respondent	Number of Years	0.02	0.003
Shop Distance from Main Road	Kilometer	0.01*	0.001
Having License	Yes=1; No=0	-2.96***	-0.361
Government Raid	5=VF, 4=F, 3=S, 2=LF, 1=N	-1.35**	-0.196
Restrictions Faced by Consumer	5=VF, 4=F, 3=S, 2=LF, 1=N	-0.40	-0.042
Government Visit	Number/Year	-0.14***	-0.015
Availability of Alternative Bag	5=VM, 4=M, 3=Me, 2=L, 1=VL	-1.10**	-0.147
Knowledge about Negative Environmental Effect of Plastic Bag	Index Value	-0.86**	-0.093
Shop Size	Square feet	0.0006	0.00007
Experience of the Respondent	Number of Years	0.001	0.0001
Constant		6.69***	
N			180
Pseudo R2			0.760

*N.B: *** p<0.01, ** p<0.05, * p<0.1.*
VF= Very frequently, F= Frequently, S= Sometimes, LF= Less frequently, N= Never;
VM= Very much, M= Much, Me=Medium, L=Little, VL=Very little;
SA= Strongly agree, A= Agree, N= Neutral, D= Disagree, SD= Strongly disagree.
Source: Authors' estimation, 2020

For an increase in frequency of govt. raid, probability of plastic bag providing decreases. With one scale increase in govt. raid, probability of providing HDPE plastic bag decreases by 0.196 ($p<0.05$). Number of government visit

has negative impact on probability of plastic bag providing behavior. If number of gov't visit increases by 1 unit, probability of providing HDPE plastic bag decreases by 0.015 ($p<0.1$).

Availability of variety of plastic bag is measured by Likert scale. This factor has negative impact on probability of plastic bag providing behavior. A one scale increase in availability of alternative of plastic bag leads to decrease in the probability of providing HDPE plastic bag by 0.147 ($p<0.05$).

Knowledge regarding plastic bag is measured by calculating index value with the help of some statements which reflects knowledge about negative environmental effect of plastic bag. This factor has negative impact on probability of plastic bag providing behavior. From the regression result, it can be indicated that, for an increase in retailer's knowledge about negative environmental effect of plastic bag use, probability of plastic bag providing decreases. If retailer's knowledge regarding negative impact of plastic bag use increases by 1 unit, probability of providing HDPE plastic bag decreases by 0.093 ($p<0.05$).

Discussion

It is observed that 74 percent of the respondents are providing HDPE bag which is consistent with Musa et al. (2013). In spite of having other bags like paper bag, jute bag and net bag, the retailers mostly use HDPE plastic bag for their trading purpose. If it is assumed that, providing plastic bag is violation of plastic bag ban law, then it may be predicted that 74 percent of the total respondents are avoiding plastic bag ban law. Most of the plastic bag providing retailers are from grocery shops group which is 46 percent.

Plastic bag was banned in Bangladesh in 2002. But surprisingly 73 percent of the respondents do not have knowledge on the ban. Their knowledge gap enhances the usage of plastic bag to a larger extent and is prevalent in rural communities.

Most of the consumers do not raise any objections to retailers in transferring good through plastic bags that reflects unawareness of the consumers and is responsible to certain extent is abiding the ban. Respondents who carry own bag are older in age (between 51 and 60 years) which is consistent with Gupta (2011). It is also observed that consumers between the age of 21 and 30 years don't often carry their own bag.

The study emphasizes that the vital reason for usage of plastic bags is lack of alternatives in the market as has been validated by 60 percent of the respondents. The emergence of new variant of plastic bag is a valid demand which is discussed in literature (Synthia & Kabir, 2014). However, around 25 percent of the respondents observed lack of enforcement of the ban as the cause for unrestricted use of plastic bags.

Conclusion

Plastic waste has been an alarming issue for Bangladesh. On an average, the amount of solid waste formed in major cities of the country is 24,000 tons per day, 10 percent of it is the plastic waste that was 7 percent a few years earlier. In this situation Bangladesh government executes a ban on plastic bags from 2002 in the capital city of Bangladesh which was later extended all over the Bangladesh. However, such ban is basically ineffective because of lack of alternatives and also availability and low cost. Further research may be continued on this issue by extending both its horizontal and vertical space.

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Conflict of Interest

The authors declare no conflict of interest.

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ORIGINAL ARTICLE **SOCIAL SCIENCE**

TREND IN CLIMATE CHANGE-INDUCED MIGRATION: A BIBLIOMETRIC ANALYSIS

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Abstract

Literature argues that numerous climatic factors contribute to migration decisions. To understand the complex interplay between climate change impacts and migration-decision, we need to analyse how the factors affect the said decision. This bibliometric review aims to analyse the climate change and migration literature and assess future research opportunities for exploring climate-induced migration. This review considers 4658 documents extracted from Scopus by performing a search with the words 'migration', 'climate change', 'climatic hazard' and 'coastal region' covering journal articles, review papers, book chapters, books, and conference papers from 2011 to 2020. This study applied VOSViewer for analysis. Results reveal that climate change is a dominant driver of migration, and the literature is deeply rooted in the United States and the United Kingdom. The lexical network shows that the developed countries which are less vulnerable to climatic hazards produce more co-authored documents. Furthermore, in the migration discourse, the co-authors from developed countries have strong ties exhibiting migration and climate change research, mainly concentrated among the collaborative framework of developed countries' researchers. Therefore, more research on migration and climate change issues in collaboration with the global south and north is highly demanding, providing further insights into the existing research arena.

Keywords: Migration; Climate Change; Environmental Hazard; Coastal Region; Lexical Network; VOSViewer

Introduction

Because of its dynamism, migration discourses are significantly rising, which are also complex in nature (Black et al., 2011). In migration discourses, climate change issues appear predominantly because people often choose migration as an adaptation strategy (McAdam, 2012; Farbotko & Lazrus, 2012; Bettini, 2013; Black et al., 2011). Researchers around the globe are contributing with their knowledge, expertise, skills, and experiences to produce scientific papers on climate change and migration. The international communities, including policymakers and researchers, are now tossing light on the migration and climate change nexus to make more effective policies; however, the knowledge is still fragmented (Piguet et al., 2011). The literature argues that climate change effects such as sea-level rise often produce climate refugees (Farbotko & Lazrus, 2012; Hauer, 2017; McLeman, 2014). Migration-induced human resettlement may happen due to climate change which is often treated as a push factor (Piguet et al., 2011). In contrast, migration contributes to adaption from climatic vulnerabilities and enhances social networking through the transfer of knowledge, technology, remittances, and so on (Scheffran et al., 2014). However, the maximum migration fluxes are caused by climate change combined with socio-political governance issues (Black et al., 2011). Nevertheless, migration decisions or relocation planning are also intertwined with multidimensional vulnerabilities, including climate change, which increases social costs (Marino, 2012). However, due to climatic hazards, migration decision is usually preferred by the male person from the household (Djoudi & Brockhaus, 2011). In some studies, climate change effects are treated as environmental stressors. Hence, to increase resilience, households undertake migration decisions as an adaptive strategy (Lu et al., 2016; Kartiki, 2011).

The least-developed countries (LDCs), such as Bangladesh, Bhutan, Nepal, and Cambodia are the worst victims of climatic hazards (Miyan, 2015; Hassani-Mahmooei & Parris, 2012). The literature argues that in Bangladesh, climate change-induced migration mainly occurs from the areas which are environmentally more stressed (Lu et al., 2016). It is predicted that in the subsequent 40 years, due to climatic hazards like droughts, cyclones, and floods, around three to forty million people will migrate from the vulnerable climatic zones of Bangladesh (Hassani-Mahmooei & Parris,

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2012). To avoid adverse climatic shocks, the households of Bangladesh often choose migration as a non-farming adaptation strategy (Alam et al., 2017). Likewise, as an adaption strategy, people from other extremely climatic vulnerable countries such as Sudan and Guinea also choose rural-urban migration (Dumenu & Obeng, 2016).

The literature on migration and climate change dynamics focuses on multidimensional issues where researchers collaboratively share their knowledge and expertise. While exploring the literature, it is found that there exist numerous review papers either on climate change or on migration still, there remains a dearth of Bibliometric analysis that deals with the nexus between climate change and migration. Considering the research gap, this paper aims to analyse the literature on migration and climate change and examine how climate change effects are connected with migration decisions. In addition, this paper aims to assess future research options for exploring climate change issues in migration.

Materials and Methods

Data

The bibliometric analysis considers databases from different sources, including PubMed, Scopus, WoS, and Google Scholar (Biswas et al., 2021). However, due to the inaccessibility to the database, this paper does not explore the WoS database, therefore, it only considers the Scopus database. A total of 5846 documents were initially retrieved using advanced search queries from the online source Scopus. After cleaning up the duplicate documents (1188), the final database covers 4658 documents. For excluding the duplicate copies of documents, manual screening and excluding methods were adopted using Excel. The database was retrieved from the source on December 21, 2021, considering the period 2011-2020. For inclusion, only journal articles, books, book chapters, review papers, and conference papers are considered from the social science subject area. Among all the documents, book chapters and review papers hold the second and third highest positions in terms of citation count, respectively. The advanced search includes words such as 'migration 'and 'climate change' in the keywords, title, abstract and main body. While including the keywords, some other keywords, such as climatic hazard, coastal region, and environmental hazards, are also considered. Because migration may also happen due to environmental and climatic hazards. Moreover, the coastal zone is highly prone to climatic and environmental hazards, pushing people to migrate. All published documents written in English are the only language criteria for inclusion. For extracting data from the online resource Scopus, the advanced search query using the Boolean approach is adopted in this study. The search query includes the keywords migration, reason, decision, climate change, climatic hazard, environmental hazard, and coastal region.

Data processing and analytical tools

This bibliometric analysis uses Excel for temporal network analysis, for example, the number of documents, citations *per* author, citations *per* document, and graphical analysis. In addition, for spatial and lexical network analysis, VOSViewer software (Version 1.6.17) is adopted in this study. In general, bibliometric research combines both quantitative and statistical analyses to analyse journal articles and count citations (Della Corte et al., 2019). Researchers are now also adopting the R package for quantitative data analysis in bibliometric analysis (Priovashini & Mallick, 2021, Zhang et al., 2019; Maretti et al., 2019). Bibliometric analysis is an effective tool to visualize the connections between authors and countries, keywords, and so on (Priovashini & Mallick, 2021). This analysis provides a detailed statistical overview, visualizes the networks, and provides a detailed description of the existing scientific database. VOSViewer software is applied in this study to portray spatial network analysis and lexical network analysis. Using Microsoft excel, descriptive statistics have been produced. In this study, the annual production of documents *per* year (Figure 1) in the social science subject area is considered for temporal analysis. In the second stage, the source network is constructed using the most cited papers on climate change and migration (Table 2**)**. Likewise, the prominent top sources of documents (≥ 21 documents) have been sorted out, which is indicated in Table 3. After that, a list of top contributing authors who commonly research climate change and migration issues (Table 4) has been identified based on the number of documents produced. Later, a spatial network analysis using a graphical presentation (Figure 2) was carried out considering co-authorship of the country (≥ 11 documents per country). In addition, the lexical network analysis of this study consists of co-occurrences of all keywords (≥ 20 co-occurrences) represented in Figure 3**.** This paper also focuses on Bangladesh as a keyword in climate change and migration research (Figure 4).

Results and Discussion

Summary statistics

The descriptive analysis (Table 1) suggests that in the last decade, a growing number of documents (4658) has been produced, which consists of journal articles (3569), books (174), book chapters (547), review papers (243) and conference papers (125). If the average citation *per* year is considered, then the documents received around 5,632 citations, whereas, *per* document, the average citation is around 12. Besides, the average document number of citations each year is around 1.21. There were 20 subject areas where climate change and migration issues were analysed; however, this study includes only social science as a subject area. A total of 14,872 keywords were used in the existing literature, where 3,989 are the author's keywords, i.e., authors who specially worked in the field of migration and climate change. Among 4,658 documents, single-authored and multiple-authored documents are 2,004 and 2,654, respectively. In terms of document production, each of the authors contributed around 1.17 documents. In contrast, the authors *per* document are around 0.86. In migration and climate change issues, 160 organizations sponsored the researchers to explore knowledge. The authors contributing to climate change and migration research are affiliated with a total of 152 universities or organizations and the contribution of those organizations is enormous.

Table 1. Summary statistics of the concerned documents (Scopus database, timespan: 2011-2020)

Description	Data	Description	Data
Timespan	2011-2020	Subject area	20
Documents	4,658	Indexed keywords	6,684
Journal articles	3,569	Total keywords	14,872
Book chapter	547	Authors	3,989
Review papers	243	Single authored documents	2,004
Book	174	Multiple authored documents	2,654
Conference paper	125	Documents per author	1.17
Yearly average citation	5,632	Authors per document	0.86
Per document average citations	12.08	Funding sponsor organization	160
Per document average citations each year	1.21	Affiliated University/organization	152

Temporal network analysis - Trend of annual document production

Considering the yearly production of scientific documents using the time period 2011-2020, it is observed that there is an increasing trend of document production starting from 2011 to 2017 (Figure 1).

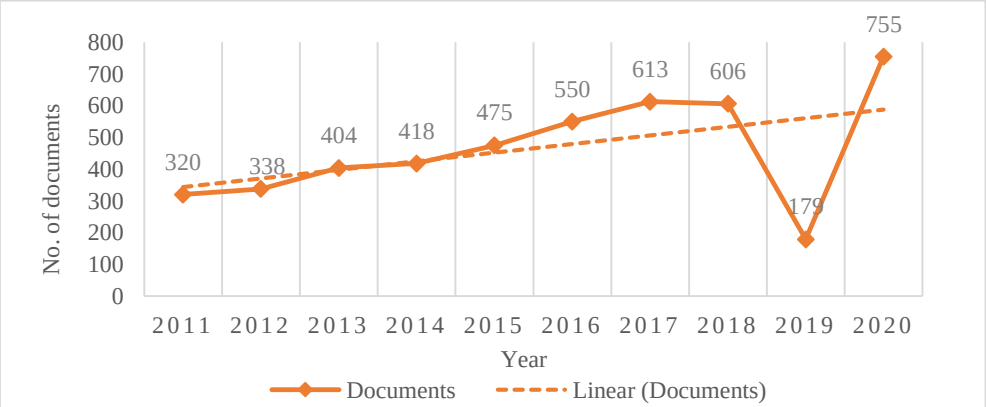

Figure 1. Trend of document production (Scopus database, timespan: 2011-2020)

The declining trend of document production started in 2018 when the annual production of scientific papers declined by seven documents compared to 2017. However, in 2019, the annual production of scientific documents dropped significantly by 427. It might happen due to congestion in the review process which means the reviewers might not give feedback in due time. It is found that the most contributing sources, such as International Migration, Journal of Ethnic and Migration Studies, Population, Space and Place, and Quaternary Science Reviews produced very few pieces of documents in that specific year. During this year, the focus of the journals might be on keywords other than migration and climate change issues. Surprisingly, in 2020, the production of documents regained its increasing tendency, and this year, 755 scientific papers were added to the existing literature. Researchers are currently working more within the collaborative framework than before hence, the blending of ideas and knowledge across and within the countries is contributing more to document production.

Source Network analysis

Top 12 papers with the authors, source, and document type

In this analysis, the papers on climate change and migration cited at least 200 times, are considered (Table 2). The top 10 cited papers were produced within the timeframe of 2011 and 2016. Table 2 demonstrates that the author of the most cited paper is contributed by Black et al. (2011), cited 485 times and published by the Global Environmental Change journal. The paper mainly explored human migration due to environmental change. The second and third most cited papers are contributed by Moreton-Robinson (2015) and Lapola et al. (2014), respectively. A paper on climate refugees (Farbotko & Lazrus, 2012) also attracts the researchers' attraction, therefore, cited 281 times. The top 10 most cited papers that have more than 200 citations were the first three documents combined with articles, books and review papers. It indicates that in migration and climate change issues, different documents like articles, books, and review papers have an almost similar level of contribution to scientific paper production.

Table 2. Top 10 papers based on citation (citations≥ 200) on climate change and migration (Scopus database, timespan: 2011-2020)

Rank	Author (year)	Title	Document type	Source	*TC
1.	Black et al. (2011)	The effect of environmental change on human migration	Article	Global Environmental Change	485
2.	Moreton-Robinson (2015)	The white possessive: property, power, and indigenous sovereignty	Book	University of Minnesota Press	389
3.	Lapola et al. (2014)	Pervasive transition of the Brazilian land-use system	Review	Nature Climate Change	318
4.	Farbotko and Lazrus (2012)	The first climate refugees? Contesting global narratives of climate change in Tuvalu	Article	Global Environmental Change	281
5.	Hazen et al. (2013)	Predicted habitat shifts of Pacific top predators in a changing climate	Article	Nature Climate Change	279
6.	Li et al. (2014)	Community-based rural residential land consolidation and allocation can help to revitalize hollowed villages in traditional agricultural areas of China: Evidence from Dancheng County, Henan Province	Article	Land Use Policy	220
7.	Gray and Mueller (2012)	Drought and Population Mobility in Rural Ethiopia	Article	World Development	215
8.	Giles-Corti et al. (2013)	The influence of urban design on neighbourhood walking following residential relocation: Longitudinal results from the RESIDE study	Article	Social Science and Medicine	208
9.	Hauer et al. (2016)	Millions projected to be at risk from sea-level rise in the continental United States	Article	Nature Climate Change	206
10.	Weiner (2015)	Sons of the soil: Migration and ethnic conflict in India	Book	Princeton University Press	202

** TC indicates total citations*

These documents cover human migration, climate change, environmental change, climate refugees, ethnic conflict, and so on. In the top-cited papers, divergent countries are concerned notably, Brazil (Lapola et al., 2014), China (Li et al., 2014), and India (Weiner, 2015). If the contribution of the journals is in producing top-cited papers, the journal Global Environmental Change contributes two most cited papers (total citations: 485 and 281, respectively). Also, Nature Climate Change has produced five top-cited papers (total citations: 318, 279, and 206, respectively).

Prominent Sources of documents

Table 3 demonstrates the prominent sources of documents, i.e., the top 10 journals which have produced at least 29 scientific papers. Considering the total production of documents, the top journal that appears on the list is international migration (91 documents). In migration research, climate change is a dominant keyword (Figure 3), which may produce more documents in international migration journals. With the highest number of documents, the journal could not secure the top position in the citation count (citations: 1316; citation *per* paper: 14.46). In contrast, with a comparatively smaller number of documents, the Global Environmental Change (IF: 9.523) journal ranks top position in the list in terms of citation count (2435) where citations *per* paper are 62.436. Similarly, the World Development journal produced 32 scientific papers, yet the citation score is 2254 (citations *per* paper: 70.438, IF: 5.278). One of the notable journals in this list is Quaternary Science Reviews which produced 51 scientific papers (citations: 1630; citations *per* paper: 31.961).

Table 3. Prominent sources of documents (journals which have produced documents ≥29) (Scopus database, timespan: 2011-2020)

Rank	Name of Journal	*TP	**TC	Citations/paper	IF** (2020)
1.	International Migration	91	1,316	14.46	1.95
2.	Journal of Ethnic and Migration Studies	79	1,606	20.33	1.51
3.	Population, Space and Place	79	1,835	23.23	3.81
4.	Quaternary Science Reviews	51	1,630	31.96	3.96
5.	Population and Environment	43	924	21.49	2.85
6.	Global Environmental Change	39	2,435	62.47	9.53
7.	Journal of International Migration and Integration	38	254	6.68	1.31
8.	World Development	32	2,254	70.44	5.28
9.	Social Science and Medicine	30	1,267	42.23	4.64
10.	Geoforum	29	667	23	3.91

*Note: *TP implies Total production; **TC indicates total citation; **IF implies impact factor*

Authors who commonly work on climate change and migration issues

Table 4 demonstrates ten dominant authors who produced at least eight papers on the issues of climate change and migration in the corresponding decade. Some authors prefer to produce single-authored papers, whereas other authors prefer to work jointly with different intuitions, universities, and countries. As an author and co-author, Liu appears 16 times (single-authored paper is 1, and 15 are collaborative). Shen commonly worked with Liu (4 papers) among the co-authors. The authors' papers are cited 649 times until accessing the database from Scopus online source. The next top author is Kelman, who has produced 12 scientific papers (single-authored: 4; multiple-authored: 8) and got 191 citations. However, the author produced the articles and chapters with divergent research groups. In contrast, Baldwin (Durham University, UK) contributed 10 ten articles, books, and book chapters. The database provides interesting information about the working nature of the author. The author contributed mainly as a single author (7 papers). Likewise, Warner (United Nations University Institute for Environment and Human Security) has produced nine scientific papers (single-authored papers 4). In contrast, some of the top authors preferably work only within the collaborative framework, such as Black, Gray, Hunter, and Mckenzie.

Table 4. Authors who commonly research climate change and migration issues (Scopus database, timespan: 2011-2020)

Rank	Author's Name	TP*	Percentage (%)	SAP**	MAP***	TC****
1.	Liu, Y.	16	16.84	1	15	649
2.	Kelman, I.	12	12.63	4	8	191
3.	Baldwin, A.	10	10.53	7	3	215
4.	Warner, K.	9	9.47	4	5	363
5.	Black, R.	8	8.42	0	8	939
6.	Gray, C.	8	8.42	0	8	431
7.	Hunter, L.M.	8	8.42	0	8	369
8.	Mckenzie, D.	8	8.42	0	8	427
9.	Mcleman, R.	8	8.42	3	5	166
10	Mcnamara, K.E.	8	8.42	0	8	143
		Total =95	***100%***	***19***	***76***	***3,893***

*Note: *TP indicates Total publication; **SAP designates* Single Authored Paper; ***MAP means Multiple Authored Paper; *and ****TC means Total Citations*

Spatial Network Analysis - Co-authorship network analysis

In this co-authorship network analysis of the countries, among 150 countries, 46 met the threshold level (documents ≥ 20 *per* country). The network visualization indicates the strongest collaboration with the United States, the United Kingdom, Germany, Australia, and Canada. The United States is also performing as a top scientific paper-producing country on environmental drivers (Priovashini & Mallick, 2020). Behind the leading position of the USA, different Universities, notably the University of Sussex (358), University of California (28), Arizona State University (24), Pennsylvania State University (20), and University of Florida (17), played a significant role in this respect. From Figure 2, it is observed that the climate change issue in migration research is concentrated mainly in the developed world, and there is a limited contribution from developing and undeveloped countries. For example, the link strength of the United States with the United Kingdom, Germany, and Canada are 51, 39, and 29, respectively. However, the strength of the links between the United States and Bangladesh is only 9. This collaboration may happen due to the existence of higher funding opportunities, institutional support, language efficiency, heightened knowledge level, and technical expertise of the authors. In contrast, these countries have limited experience in facing climatic vulnerabilities and shocks compared to the highly climatic vulnerable countries. The global north-north collaboration sometimes marginalized the least developed countries like Bangladesh, Nepal, Vietnam, Cambodia, and henceforth. In addition, researchers from the least developed countries usually get less access to resources, which may also be responsible for the lower productivity of scientific documents. Hence, for more effective and policy-oriented research, the heightened connection between climatically vulnerable countries and climatic well-off countries is highly recommended.

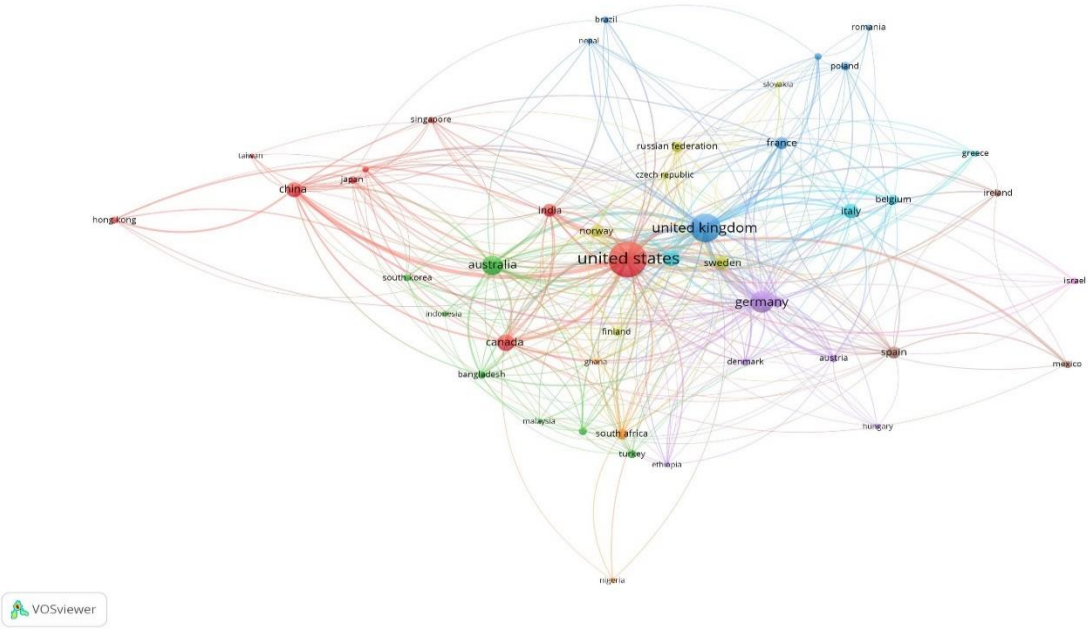

Figure 2. Co-authorship network of the countries (minimum ≥20 occurrences per country) (Scopus database, timespan: 2011-2020)

Lexical network analysis - Co-occurrences of all keywords

Keywords reflect the main themes and explain the contents, which help to get an overall idea of any research paper (Zhang et al., 2019; Lina et al., 2020). Hence, network analysis using visualizing software provides a synopsis of keywords used in the literature. Among all the keywords (14872), 655 meet the minimum threshold level (minimum co-occurrence number ≥ 20). The keywords with the maximum link are selected for graphical presentation. Each of the clusters represents different dimensions and determinants of migration. The networking graphic represents the co-occurrence of the keywords, makes links, and shows the strength of the links among the keywords (Biswas et al., 2021). The greater number of nodes demonstrates the greater the number of keywords available in the concerned field of interest (Lina et al., 2020; Biswas et al., 2021). The links between keywords indicate that the corresponding nodes are used jointly in an identical document (Biswas et al., 2021; Lina et al., 2020; Zhang et al., 2019). The terminology 'migration' is found strongly linked with the keywords such as climate change, vulnerabilities, China, adaption, and so on (Figure 3). From the co-occurrences of all the keywords, migration and climate change dimensions are found dominant. Keyword migration (1351) is strongly connection with the keywords such as climate change (662), international migration, labour migration, decision making, and so on, whereas, climate change is also connected with migration, international migration, population migration, human and henceforth. As a keyword, Bangladesh (77) remains far behind the United States (206), China (178), the United Kingdom (120), Australia (92), and India (91). However, as one of the most climate-vulnerable countries, the researchers on migration and climate change issues should focus more on Bangladesh and other climate-vulnerable countries. In this regard, collaboration with global north and developing countries can generate deeper insights and contribute to effective policy formulation. The co-occurrence of all keywords is concentrated between migration and climate change issues which are expected. This result is also aligned with the findings of Priovashini & Mallick (2020). These two keywords are treated as cause and effect (McAdam, 2012, Hauer & Mishra, 2016; Farbotko & Lazrus, 2012; Bettini, 2013). The literature argues that due to climate change, sea-level rise is a stressor that is also causing displacement of human settlement (Hauer & Mishra, 2016; Black et al., 2011). In Brazil, climate change from the local and international levels, greenhouse gas emissions, deforestation, and agriculture are highly connected. These linkages act as a push factor for rural-urban migration (Lapola et al., 2014). Similarly, Farbotko & Lazrus (2012) also identified sea-level rise as the effect of climate change

which induces people's mobility as refugees (Li et al., 2014). Another climatic effect like draught is responsible for forced migration where poor people are the ultimate victims (Gray & Mueller 2012).

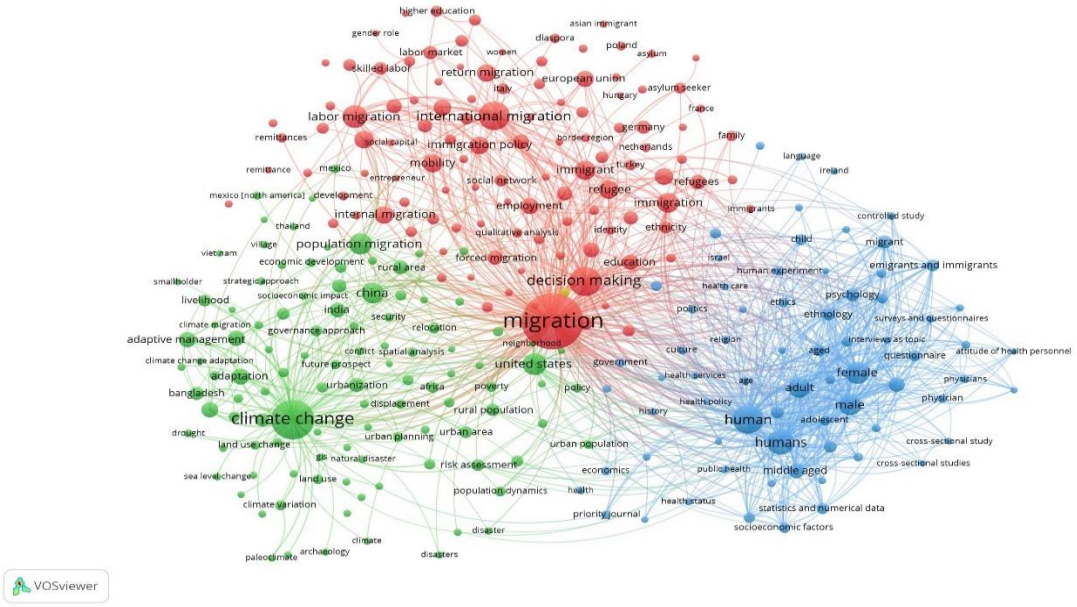

Figure 3. Network of co-occurrences and all keywords (co-occurrences of a keyword ≥20) (Scopus database, timespan: 2011-2020)

Existence of Bangladesh in migration and climate change research

Figure 4 depicts that as a keyword, Bangladesh is still underexplored. As a disaster-prone country and because of the higher prevalence of climatic variability, Bangladesh should be the major concern among the researchers. Moreover, Bangladesh is also a top migrant-sending country. If the researchers focus on the climate change and migration issues concerning Bangladesh, effective policies can be formulated at the national and international levels.

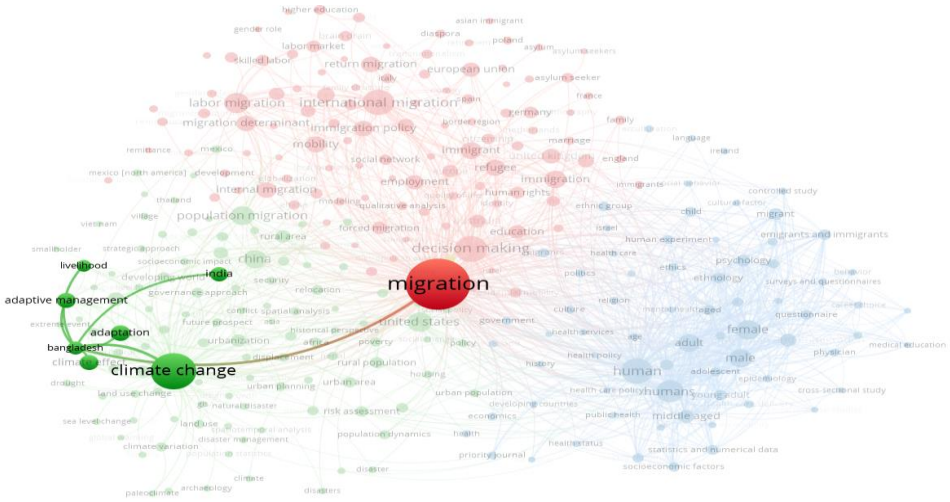

Figure 4. Bangladesh- a keyword in climate change and migration research (Scopus database, timespan: 2011-2020)

Conclusion

Climate change issues are significantly dominating in migration research, especially sea-level rise, drought, and flood. The lexical network suggests that climate change predominantly affects human migration, and the authors from different backgrounds also focused on these two issues intensively. These research areas are given priority by the developed countries' authors and affiliated organizations. They are producing more scientific papers and hiking the total number of citations. In the decade (2011-2020), the United States and the United Kingdom produced many scientific papers where strong networks are predominantly exhibited among developed countries. The United States and the United Kingdom lead the document production hence, total citations are also higher for those countries. More collaboration is extremely demanding to lessen the gaps in scientific research among the developed and least developed countries. A blending of theoretical and imperial analysis from different countries' perspectives can also add value to the existing literature. If the leading countries collaborate with the climatically vulnerable countries, then ideas, thoughts, knowledge, skills, and expertise will be exchanged among the countries which will bring more effective outcomes at the policy level. In addition, the less paper-producing countries should focus on producing more scientific papers and increase their excellence in scientific research arenas. Moreover, researchers from developing countries usually get less/no access to extract databases, funding, and online resources. These may also act as barriers to document production. In this respect, access to different journals, funding and online sources can support the researchers of the global south. Further study options are available using content analysis to examine the drivers of migration, environmental stressors, and socio-economic and political determinants.

Conflict of Interest

The authors declare no conflict of interest.

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