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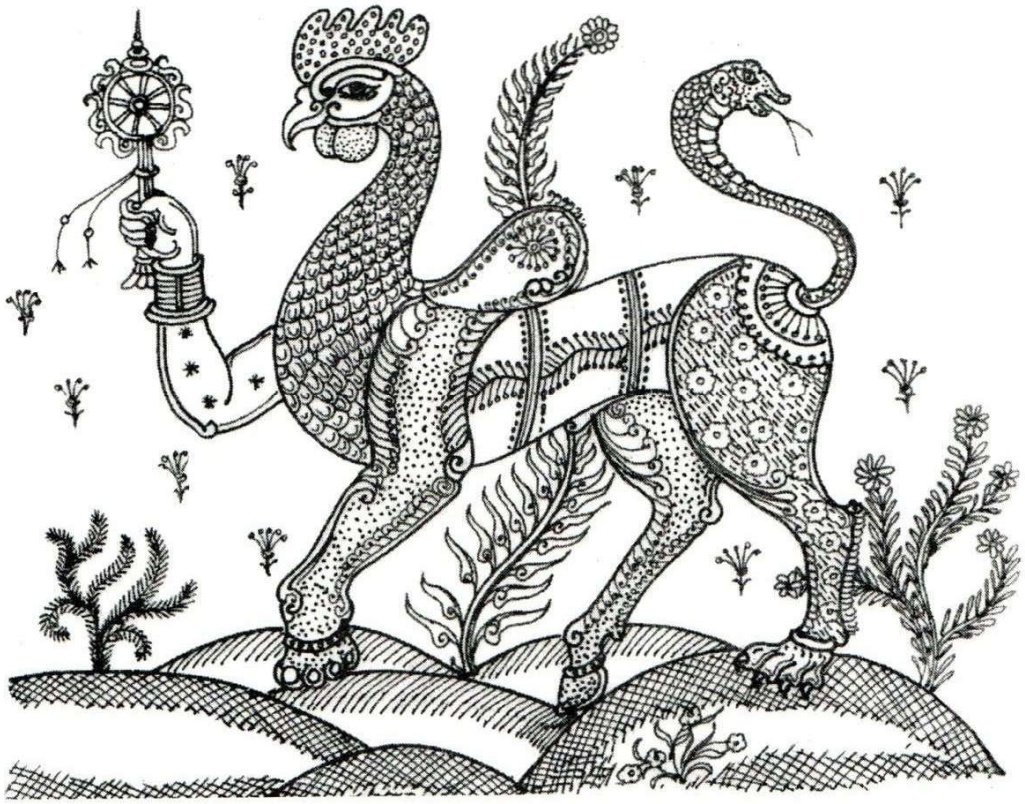

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The emblem of Pranikee

The emblem "***NABAGUNJARA***" is a chimeric animal and a common motif of Odishan art and literature. It literally means "Nine form". This form has been described by poet Sarala Das in the Odia version of the epic Mahabharata. Apparently, Lord Krishna appeared in Nabagunjara form consisting of the body of an elephant, a leg each of a horse, a deer and a tiger respectively; throat of a peacock, tail in the form of a serpent, waist of a lion, hump of a bull and head of a cock, to fool his friend Arjuna. The Chimera was holding a lotus flower in a human hand. Arjuna had never seen such a creature in his life and guessed that this could not be a real animal but a form assumed by Lord Krishna and immediately bowed down at his feet. It is said that the human hand with the lotus provided the clue. In the paintings and sculptures however, the lotus is often replaced by a "Chakra" or the "stylized discus" of Lord Krishna. Chimeric forms are encountered in literature and art all over the world. However, a chimera of nine animals is uniquely Odishan. Therefore, it was considered to be an appropriate emblem for the Journal of Zoological Society of Odisha.

Padma Shri Prof. Priyambada Mohanty-Hejmadi

Former Editor

From the Editor's desk

The present edition of the Journal (Volume XXXV) covers different aspects of Zoology. One of the major conservation concerns related to wildlife protection is human-elephant conflict. The first article describes human elephant conflict and status of habitat in Chaibasa forest division of India. The second article discusses association of different fingerprint patterns with ABO blood groups and gender among the students as human fingerprint has been considered as the most trustworthy criteria for identification of an individual and remains unchanged throughout one's lifetime. Considering the present status of water pollution as one of the global challenges and bioaccumulation of heavy metals in aquatic animals exposed to contaminated water leading to oxidative stress, "higher Cr and Pb contamination in fish (*Labeo bata*) muscle with enhanced lipid peroxidation: a threat for food chain and food web" has been discussed in the third article. The fourth article entitled "Chronic effects of fluoride exposure on growth and ossification of bones in the tadpoles of the ornate frog, *Microhyla Ornata* (Anura: Microhylidae) covers the impact of fluoride as a developmental toxicant on a frog model. Taking into account the judicious disposal of the huge amount of prawn wastes generated in our country, this volume is concluded with an article that describes extraction and characterisation of chitin and chitosan from fresh water prawn wastes**.**

I hope that this volume will add to the prevailing knowledge in Zoology.

PK Mahapatra

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CONTENT

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HUMAN ELEPHANT CONFLICT AND STATUS OF HABITAT IN CHAIBASA FOREST DIVISION, WEST SINGHBHUM DISTRICT, JHARKHAND, INDIA

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ABSTRACT

The Asian elephant population has declined due to intense human activity and changes in land use and land cover, causing habitat loss and fragmentation. As facultative migrants, elephants need a healthy ecosystem with connected habitats. Effective conservation efforts require understanding forest loss and fragmentation, as forest quality influences their distribution. In this study, we analysed land use land cover (LULC) changes, deforestation, and forest fragmentation patterns from 2000 to 2020, along with a survey on the human-elephant conflict in the Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand, India. CFD offers limited habitat for elephants, with only 9.85% of the core area being over 500 acres, 3.09% between 250-500 acres, and the remaining 24.75% consisting of fragmented forest patches. Additionally, these forests have a 9.62% perforation level, with barren areas and sparse vegetation increasing by 5.03% and 1.48%, respectively, and dense vegetation decreasing by 8.06%. The rising habitat loss and fragmentation over the past decades likely contributed to the human-elephant conflict, with 65 human casualties recorded between 2012 and 2020 and the highest crop raids occurring in 2014. During the study, the highest number of house damages occurred in the Noamundi range (n=685), followed by Hatgamariah (n=328), both of which lie along elephant movement routes. There were 11 poaching incidents, including three cases where tusks were missing. Paddy, the main crop cultivated from July to November, was also the most frequently raided. Most people believed that agricultural lands near forests were more prone to crop raids, with solitary male elephants seen as the biggest threat. The largest crop raids occurred during the harvesting and ripening stages, followed by the reproductive stage. In the Chaibasa Forest Division, where agriculture dominates and the remaining habitat is fragmented, elephants raid crops to meet their nutritional needs, leading to Human-Elephant Conflicts (HEC). Successful elephant conservation depends on fostering human-elephant coexistence, requiring strategies like greening corridors to reduce forest perforation, eliminating invasive species, rejuvenating water bodies, and providing nutritional supplements in the forest to minimize crop raids.

Keywords: Coexistence, Crop Damage, Human-Elephant Conflicts, Wildlife.

INTRODUCTION

Human-wildlife Conflict is as old as the existence of human civilization (Anand and Radhakrishna, 2017) but has been found to be escalating for four decades. The extremity of human-wildlife conflict is observed in conservation areas that are under huge anthropogenic threats due to the demand for natural resources to sustain human livelihood (Barua et al., 2012; Rakshya, 2016; Githiru et al., 2017). However, a wide range of species is responsible for the conflict, with the principal culprits being primates, rodents, ungulates (including antelope, bushpig, elephant, hippo, buffalo, and zebra), lions, leopards and hyenas (Naughton-Treves, 1998; Naughton-Treves et al., 1998; Hill, 2000 and O'Connell-Rodwell et al., 2000). However, conflict with elephants receives more publicity and evokes stronger emotions (Santiapillai et al., 2010). Land use land cover (LULC) changes have led to the decline of the Asian elephant population due to habitat loss and fragmentation. Loss of habitats or fragmentation can cause serious impacts on biodiversity (Raghubanshi and Tripathi, 2009) as well as ecological patterns and processes by increasing the number of forest patches, reducing the patch size, and interrupting connectivity within the ecological network, thus impacting several species. Elephants are facultative migrants moving in search of water, forage availability, and mates (Ngene, 2010; Purdon et al., 2018) due to their high nutritional and ecological requirements. To facilitate its migratory behaviour, the existence of a functional ecosystem and connectivity between habitat patches is essential. Conservation

of elephants requires a clear understanding of forest loss and fragmentation, as forest cover quality determines the distribution of elephants. The fragmented landscape of India, with the growth of the human population, has increased human-elephant conflict in most parts of the country. Conflict situations can arise anywhere, but they are frequently concentrated at the fringes of the reserves where wildlife enjoys protection and land is often fertile, leading to a wealth of agriculture (Nelson et al., 2003). Spatial patterns of HEC are somehow positively related to human usage and the presence of settlements, and agricultural fields in India, Nepal (Sukumar, 1991; Gubbi, 2012; Acharya et al., 2016), Thailand (Chen et al., 2016; Van de Water and Matteson, 2018) and Africa (Hoare and Du Toit, 1999). Distance from the forest edge determines the intensity of crop raid (Naughton-Treves, 1998). There are various proximate factors that influence crop raiding by wild elephants, such as elephant density in the vicinity of the villages, crop cover area, the density of certain preferred browse species, phenophase of the agricultural crops, availability of shade, incidence of wood cutting, water availability, rainfall, cattle grazing, abundance of weed and occurrence of forest fire (Daniel, 1995). Crop depredation is regarded as the stimulus of HEC (Webber et al., 2011; Mumby and Plotnik, 2018), as animals that damage crops may also injure or kill farm workers. Apart from agricultural damage, restrictions on movement, competition for water sources, the need to guard property (which may lead to loss of sleep), reduced school attendance (through loss of sleep, or fear of travel), poor employment opportunities, increased exposure to malaria, and psychological stress (Sukumar, 1990; Naughton-Treves, 1998; Hoare, 2000) may also act as a major component of conflict for local people (Dublin et al., 1997). Crop loss is the most commonly reported form of damage, yet a rise in human injuries and deaths reduces social tolerance towards elephants in Asia and Africa, thus imposing a threat to the conservation of wildlife (Sitati et al., 2003; Lenin and Sukumar, 2011; Lamichhane et al., 2018; Van de Water and Matteson, 2018).

Central India faces serious conservation challenges due to HEC. Changes in land use patterns, expansion of agricultural land, increase in mining activities, and man-made disturbances in the West Singhbhum district of Jharkhand have resulted in many incidences of human and elephant casualties in recent times. The Chaibasa Forest Division (CFD), rich in mineral resources such as Iron Ore and Manganese Ore and dominated by agricultural practices, not only offers a surplus opportunity to elephants and migratory routes, but the conservation efforts of elephants face several threats. Despite increasing threats to elephant conservation and landscape-specific information on the change in forest cover and habitat fragmentation, there are no systematic studies on HEC in Chaibasa Forest Division, West Singhbhum District, Jharkhand, except for a few stray information.

In view of the above, the present work has been designed focusing on studying the nature and causes of HEC in Chaibasa Forest Division, West Singhbhum district, Jharkhand, and mitigation measures that need to be considered for a successful coexistence and conservation of wild animals.

MATERIALS AND METHODS

Study Area

Chaibasa Forest Division (CFD) located within Latitude 21°57′30″N and 20°30′00″E and Longitude 85° 20′ 00″ and 86° 02′ 30″ E, is the oldest forest division of Jharkhand that shares a border with the forests of Saranda, Kolhan, and Saraikela and Mayurbhanj and Keonjhar districts of Odisha state. The entire forest area of the division comprises three Forest Ranges: Chaibasa, Hatgamariah, and Noamundi. West Singhbhum is rich in mineral reserves with major minerals like Iron, Manganese, and Limestone. There are about 12 granted mining leases of iron ore and manganese and 22 leases of limestone (all outside forest areas). According to the revised classification of forest types in India, Chaibasa Forest Division falls into following major types (Champion and Seth, 1968):

TYPE II: Northern Dry tropical forests
GROUP-5: Tropical Dry deciduous forests
SUB GROUP 5B- Northern type mixed deciduous forests subtype.

i. 5B/C1: Dry Peninsular Sal Forests.
ii. 5 B/C2: Northern dry mixed deciduous forests.

The Annual rainfall is about 1433.50 MM, and the temperature ranges from 45.2 °C to 6.9 °C. The main perennial rivers that drain this division are Baitarani in the south, Karo and Deo in the southwest and the west, and Kharkai in the Northeast.

To carry out the study, the following methodological procedures were adopted.

1. The preparation of the LULC map for three years, 2000, 2011and 2020 was done using techniques such as image processing and supervised classification techniques (Cihlar and Jansen, 2001). For the preparation of the LULC map, Satellite data of CFD, West Singhbhum, Jharkhand was collected from various primary sources, i.e. Landsat 7 for 2001, Landsat 5 for 2011, and Landsat 8 for 2020 (USGS, 2022) (Table 1). Secondary data on crop raiding, house damage, human death/injury and elephant census was collected from the Office of the Divisional Forest Officer, Chaibasa Forest Department. Other data e.g., the topographic map (scale: 1:50,000) of the study area of 2009, was collected from the website of Survey of India (https://onlinemaps.surveyofindia.gov.in/FreeMapSpecification.aspx)

2. The landscape fragmentation analysis was carried out for three years (2000, 2011, and 2020) by running landscape fragmentation tools for two classes: forest and non-forest (Vogt et al*.,* 2007).

3. A study on Human-elephant conflict was carried out first by collection of secondary data from the Forest Department, Jharkhand. Based on the secondary data, a survey on the human-elephant conflict scenario through key informant interviews, focus group discussions, and interactions with the victims in the Chaibasa Forest Division was done. Victims' surveys, as well as crop raids and places of house-damage, were visited during the period.

4. The Elephant Census data of Chaibasa Forest Division, Jharkhand, during 2010, 2012, and 2017 were collected from the records of the Forest Division.

We have considered the study area's climate pattern, particularly the monsoon's influence on the image's spatial signature, and satellite imagery captured during the dry season between December and February (winter) as less than 10% cloud cover.

RESULTS

1. Elephant Habitat

1.1 LULC and Forest Fragmentation in CFD

The CFD has a good population of elephants. As per the elephant census data, the population of elephants was 33 in 2010, 38 in 2012, and 27 in 2017. In CFD, only 17.77% of the land is under forest cover [52016.80 ha under protected forest (PF) and 31.36 ha under reserve forest (RF)] and 82.22 % of Non-Forest Land. The CFD provides an inadequate habitat for nurturing elephants due to high anthropogenic population pressure and fragmentation in the forest areas. From 2000 to 2020, the core forest area has been decreased which is presented in Table 2. The good chunk of forest with a core area of >500 Ac. has decreased to 9.85 % in 2020, from 19.58% in 2011 and 22.70% in 2000 with a net decrease of 12.85% in the last 20 yrs. The core area of 250-500 Ac. has decreased to 3.09% in 2020, from 4.83% in 2011 and 3.70% in 2020. On the other hand, the patch forest, edge, perforated area, and forest with a core area of <250 Ac. have increased from 2000 through 2011 to 2020 (Table 2, Figure 2).

During the last two decades (2000 to 2020), dense vegetation decreased 8.06% from 40.82% in 2000 to 32.76% in 2020. On the other hand, the sparse vegetation has increased by 1.48 % from 17.93 % in 2000 to 19.41 % in 2020. The agricultural land area has increased by 3.98% in the last 20 years, from 33.35 % in 2000 to 37.33 in 2020 (Table 3, Figure 3). The barren land has also increased by 5.24 % from 0.03 % in 2000 to 5.27 % in 2020. The area under mines also increased minimally by 0.02 %, from 0.14 % in 2000 to 0.16 % in 2011 and 2020. The area of the water body, which is very important for elephants as a megaherbivore mammal, has decreased by 0.44 % from 1.33 % in 2000 to 0.89 % in 2020 (Table 3, Figure 3). Elephants require large forest areas for food and shelter and enough water for their survival; the study indicates that these have decreased over the years. When food is scarce in the forest and readily available in nearby agriculture fields and human settlements, they resort to raid crops.

Table 1 LULC Map Preparation for the Year 2000, 2011 and 2020.

A. SATELLITE DATA								
Sl. No.	Year	Satellite	Sensor Name	Path/Row	Spatial Resolution	Date of Acquisition	Sources	Map
1.	2000	Landsat 7	ETM	140/(44/45)	30m	17/12/2000	USGS	LULC LFRAG
2.	2011	Landsat 5	TM	140/(44/45)	30m	6/11/2011	USGS	
3.	2020	Landsat 8	OLI	140/(44/45)	30m	30/11/2020	USGS	
B. SECONDARY DATA								
			SOURCE		DATA COLLECTED			
1.	Secondary Data		Chaibasa Forest Division Office, West Singhbhum District of Jharkhand		i. Crop raiding Data ii. House Damage Data iii. Human Death/Injury Data iv. Elephant Census report			
2.	Primary Data		Field Visit		Questionnaire Survey of Victims			

Table 2 The Fragmentation Status of Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand during 2000. 2011 and 2021

LULC Class	2000		2011		2020	
	Area (Ha)	Area (%)	Area (Ha)	Area (%)	Area (Ha)	Area (%)
Patch	26703.05	24.22	15635.70	19.58	19997.70	24.76
Edge	33096.81	30.01	26465.95	33.14	25379.10	31.42
Perforated	13312.94	12.07	15832.61	19.83	17524.26	21.69
Core (<250 Ac.)	8046.47	7.30	7033.04	8.81	7427.35	9.19
Core (250-500 Ac.)	4080.68	3.70	3855.29	4.83	2498.59	3.09
Core (>500 Ac.)	25032.06	22.70	11028.72	13.81	7954.10	9.85

1.2 Forest Fire

Incidence of forest fire was recorded in the years 2010 (n=7), 2012 (n=35), 2013 (n=22), 2015 (n=10), and the maximum number of forest fires in 2016 (n=44). Data on percent of the burn was available for only three years, 2010 (5.66%), 2013 (29.5%), and the maximum in 2012 (44%).

Table 3 The LULC status of Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand during 2000, 2011 and 2021.

LULC Category	2000		2011		2020	
	Area (Ha)	Area (%)	Area (Ha)	Area (%)	Area (Ha)	Area (%)
Agricultural land	241309.27	33.35	302968.8	41.87	270154.2	37.33
Barren land	243.75	0.03	5557.46	0.76	38097.42	5.27
Dense Vegetation	295350.23	40.82	194910	26.93	237041.1	32.76
Sparse Vegetation	129762.92	17.93	181395.7	25.06	140449.7	19.41
Mines	1001.93	0.14	1192.52	0.16	1154.85	0.16
Railway Line	617	0.09	586.76	0.08	534.98	0.07
Road	25894.8	3.58	25448.25	3.51	25208.83	3.48
Sand	19730.88	2.73	4146.09	0.57	4538.51	0.63
Water Body	9673.94	1.33	7378.57	1.02	6404.22	0.89

1.3 Corridors and Movement Pathway of Elephants

Elephants take refuge in patchy habitats in the daytime and raid crops in the nighttime. Although the Chaibasa Forest Division (CFD) cannot harbour a large number of elephants, it provides a migratory pathway to the elephants of about 275 km, of which only 90 km falls inside the protected forest while the rest falls under non-protected forest areas. There are five corridors (Figure 4) in this division i.e. Bhagabilla-Ratnasai, Jampani-Bhagabilla, Siyaljoda-Dhobadhobin, Sangajata-Haldipokhar and Lepang-Dumuria (MoeFCC Report, 2023).

The movement of elephants into CFD is recorded through the following major route: 1: West Bengal via Dalma Sanctuary – Saraikela to Chaibasa Division; 2: Saranda Forests to Noamundi Range of Chaibasa South Division then to Kolhan Forest Division; 3: Mayurbhanj Forest Division of Odisha to Hatgamariah range of Chaibasa South Division; and Keonjhar Forest Division of Odisha to Chaibasa South Division. The total forest land diverted for various anthropogenic development activities such as mines, roads and railways etc., (1980-2019) was 730.366 ha (Table 4).

2. Human-elephant conflict (HEC) scenario

2.1. Victims of HEC

The total number of victims of HEC in CFD during the period of 2012 to 2020 was 65 in number with 36 cases of death, 24 suffered from severe permanent injury while only five (05) cases of recoverable injury. Among the victims, 56 were males, and only nine (09) were females (Figures 5A-C).

Among the three forest ranges of CFD, the highest number of victims were from Noamundi Range (n=23), followed by Hatgamariah Range (n=22) and Chaibasa Range (n=14) (Figure 6A). The month of December noticed the highest cases of human injury/death followed by August and May. No cases were reported during March and April (Figure 6 B). From the year 2012 to 2020, the number of elephant attacks varied between five (05) to 10, and the highest numbers (n=10) of attacks were in the years 2014 and 2016, followed by the year 2020 (n=8) (Figure 6C).

During elephant attacks on human beings, they were either involved in guarding their house or field (n=13), sleeping (n=7), collecting firewood in the forest (n=5), grazing cattle in the forest (n=4), collecting Non-Timber Forest Produce (NTFP) in the forest (n=3), defecation inside the forest (n=2) and crowd watching (n=1) (Figure 6D). During the period 2012-2020, the time of elephant attacks was restricted mostly to the night time, with the maximum occurring

during 2000-2200 hrs (n=19), followed by 1800-2000 hrs (n=19), 2200-2400 hrs (n=7) and in early morning from 400- 600 hrs (n=8). There was only one (n=01) instance in the morning 800-1000 hrs and no attack during the daytime (Figure 6 E). During elephant attacks, the human victims were either in a drunken state or in a normal state. During the study, a maximum number (n=33) of victims were found in a heavily drunken state, followed by drunken (n=23), and a few (n=9) in a normal state (Figure 6 F).

2.2 **Crop Depredation**

During the survey period of 2012 to 2020, elephants raided crops every year (Figure 5G). The year 2014 noticed the greatest crop damage over an area of 638.859 ha, followed by 234.03 ha in the year 2017, and the lowest crop raid was recorded in 2022 over an area of 30.22 ha (Figure 7A).

The frequency of crop raids was also maximum (n=538) in the year 2014, followed by the year 2015 (n=421) and the year 2020 (n=340). The area of crop damage was maximum in the month of November (412.25 ha) when paddy was in a harvesting stage, followed by September (324.4 ha). During the study period (2012-2020), the frequency of elephant visits to land was high in the month of December (n=425), followed by October (n=383), September (n=314), and November (n=309) (Figure 7B). The total area of crop damage during the study period was 1801.23ha. Due to the rain-fed system of irrigation in CFD, paddy is the most-grown crop in the region as well, and this crop is also the most damaged one. Most of the paddy crop was found affected during the ripening or the harvesting stage when the crops were fully grown.

3. House Damage and Grain Loss by Elephants

3.1 House Damage

One of the most important features of HEC is the damage to houses by elephants in the affected area (Figures 5D-F). From the year 2012 to 2020, the total number of houses damaged was 1109, with the highest frequency of house damage occurring in the Noamundi Range (n=685), followed by Hatgamariah (n=328), as these ranges fall in the elephant movement routes. In the month of August, we noticed the highest house damage. Among the houses damaged, the majority i.e., 95.04 % (n=1054) were Kacha Houses, compared to only 4.06% (n=55) of Pacca Houses in the affected area (Figure 8A).

3.2 House Damage Associated with Grain Loss

In CFD, elephant attacks resulting in house damage along with grain loss (Figure 5F) occurred maximally in the year 2016 (n=97), followed by 2012 (n=93) and 2013 (n=82) (Figure 9A). House damage associated with grain loss was found to be high in the post-harvesting months of February (n=66) and January (n=59) (Figure 9B). CFD noticed 380 cases of House damage that were associated with grain damage. The severity of the house damage was high in the off-paddy season when paddy was kept inside houses. Sometimes liquor storage served as an attractant. Kaccha mud houses were the most affected ones (Figure 8B).

4. Wildlife Mortality

In CFD, cases of poaching were reported in the years 2008 (n=1), 2011 (n=2), 2012 (n=3), 2013 (n=1), 2014 (n=2) and 2017 (n=2) (Figure 9 A). Out of the 11 cases of poaching, three cases with missing tusks were reported. Besides, one case of un-natural death (calf) and four (04) cases of natural death of adult elephants (Figure 9 B) were reported during the period 2008-2019 (Figure 9 A).

5. Questionnaire Survey Report

5.1 Social Profile of the Respondents

Out of 100 respondents (Male-66, Female-34), 35 numbers of victims were also surveyed. The number of respondents in the different age groups were: 20-29:18, 30-39:34, 4-49:39, 50-59: 05 and 60-69: 04, respectively. The respondents in the family size group of <5 members were 69, 5-10 members: 24 and > 10 members: 07, respectively. Coming to the educational qualifications of respondents, the number of illiterates was 14, and in the educated category, a maximum of 39 were in the Lower Secondary category, 21 in the Secondary category, 17 had primary level education, 08 had Secondary level education while only one person was a Graduate (Table 5A).

5.2 Economic Profile of the Respondents

The economic profile of the respondent in the study includes the nature of work, Average number of livestock per household, sources of cooking energy, and purpose of livestock rearing. Among the respondents, the majority were involved in general labour (n=33) and in agriculture activities (n=28). The rest of the respondents were involved in the cottage industry (n=15), business (n=13), and job (n=11), respectively. The source of cooking energy includes firewood (n=45), followed by use of kerosene (n=31) and LPG (n=24) (Table 5B).

5.3 People's Perception on HEC

People found decreasing trends (54%) in Human Elephant Conflict (HEC) due to a decrease in forest cover (72%) and a simultaneous shift in agricultural practices. People's perception on HEC has been presented in Table 6.

The reason stated by the respondents for wild animals visiting crop fields varied from place to place depending upon the geographical location of the field. The area that was close to the forest witnessed habitual crop raiding, and the area that fell in the movement pathway was suddenly raided occasionally (Table 6). CFD is the first in Jharkhand to install both active and passive Animal Intrusion Detection and Repellent Systems (ANIDERS) (TOI, 2021), and we found that these were installed in Chaibasa and Noamundi Forest Range. Most of the respondents didn't find it effective in repelling elephants. The movement of cattle and heavy vehicles created unnecessary panic among people as a false warning of an elephant's visit to the land. Most of the ANIDERS were not functional and unmaintained while a few were found to be broken or stolen (Table 6).

Places that were not frequently raided were satisfied with the functioning of the Forest Department (Hatgamariah Range followed by Chaibasa Range). However, places that were highly prone to conflict and suffered regular disturbance from elephants were found to be dissatisfied with the assistance of the Forest Department. The communication network was quite efficient in places of regular conflict. Most of the respondents were dissatisfied with the nonpayment of wages for night duty and the supply of dragon torches and crackers for scaring away elephants (Table 6).

Most of the respondents believed that the decrease in forest cover was the reason behind change in the movement of elephants. Developmental activity along the pathway was also the reason behind shift in routes (Table 6). People perceived tremendous danger from solitary males. In fact, most (69%) of the victims were attacked by a single bull. Those areas that were close to a good chunk of forest were raided by elephants that halted and visited at night time in consecutive days. People perceived that elephants have become more aggressive and retaliate and were hardly scared of human interactions (Table 6).

5.4 People's perception on payment of compensation

People belonging to places that were highly prone to conflict were dissatisfied with time of compensation paid but were satisfied with the process of claiming payment. Only those respondents living in places less frequently visited by elephants were unaware of the scheme (Table 7).

Most of the respondents received compensation within six (06) months of time while few respondents' payment was under process. There were also respondents who did not claim compensation due to family loss or other personal issues (Table 7).

According to the survey carried out, most of the respondents believed grain storage inside houses was the main reason for elephants visiting near settlements, followed by local liquor called Handiya (Table 7). During the study period, Jack Fruit was the fruiting tree near the settlement that was raided most of the time, followed by Mango, Peepal, and Kendu (Table 7).

Table 4 Forest land diverted for other purposes in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand, India

Purpose of Diversion	Forest Land Diverted (Ha)
Mines	433.22
Roads	3.41
School	0.73
Transmission line	0.05
Railways	0.90
Multipurpose Subarna-Rekha Project	273.30
Mineral Company	18.69

Table 5 Socio-Economic Profile of the Respondents in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand, India.

A. SOCIAL PROFILE							
Gender	**Number**	**Age-Group**	**Number**	**Family Size**	**Number**	**Qualification**	**Number**
Male	66	20-29	18	<5	69	Graduate	1
Female	34	30-39	34	5 - 10	24	Higher Secondary	8
		40-49	39	>10	7	Secondary	21
		50-59	5			Lower Secondary	39
		60-69	4			Primary	17
						Illiterate	14
B. ECONOMIC PROFILE							
Nature of Work		**Avg. No. Livestock/HH**		**Source of Cooking Energy**		**Purpose of Livestock Rearing /HH**	
Work	**Number**	**Livestock**	**No. of HH**	**Source**	**Number**	**Purpose**	**No. of HH**
Labour	33	Chicken	88	Kerosene	31	Labour force	72
Business	13	Goat/ Sheep	59	LPG	24	Money after selling milk and egg	57
Agriculture	28	Buffalo	45	Firewood	45	Milk	66
Cottage Industry	15	Cow/Ox	68			Manure	89
Job	11					Meat	28

Table 6 People's Perception on Human-Elephant Conflict (HEC) in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand

People's Perception on Trends of HEC	
HEC Rate	**Number**
Increasing	31
Decreasing	54
Same As Before	15
Forest Status	
Status	**Number**
Dense	16
Thin	72
Same as Before	12
Towards the Anti-Depredation Scheme	
Anti-Depredation Scheme	Satisfied
Dragon Torches (Quantity)	15
ANIDERS (Performance)	4
Communication Network (Alarm)	79
Wages	11
Solar Street Light	78
Flying Squad (Active)	73
Assistance from Forest Dept.	56
Change in the Movement of Elephant	
Status	**Number**
Changed	22
Not Changed	44
Remain Same	34
Rate of Aggression Level of the Elephant while Chasing	
Behaviour	**Number**
Retaliatory	61
Indifferent	29
Scared of Human Interaction	10

Wild Animals Visiting Crop Field/ Nearby Settlements	
Purpose	**Number**
In Search of Palatable Food	88
Deforestation	89
In Search of Water	67
Food Deficiency in the Forest	77
Movement Pathway	49
Efficiency of the ANIDERs in Warning Presence of Elephant	
Status	**Number**
Useful	88
Not Useful	78
Sometime Useful	24
Reason for Change in the Movement of Elephant	
Reason	**Number**
Increase In Vehicular Movement	17
Water Availability	13
Change In The Pattern of Driving Away Elephant	20
Developmental Activity Along Pathway	58
Decrease in Forest Cover	78
Agricultural Practices Near the Pathway	23
Perceived Danger from Herds or Solitary Male	
Perceived Danger	**Number**
Herds	31
Solitary Male	69
Halt Time of Elephant before Next Crop Raid	
Halt Time Inside Forest	**Number**
Doesn't Halt	23
1-2 Days	36
3-7 Days	32
>7 Days	9

Table 7 People's Perception on Compensation Payment in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand

Time of compensation payment	
Time of Payment	**Respondent**
Bearable	45
Delayed	37
Over Delayed	18
People's awareness on availability of compensation	
Awareness	**Respondent**
Aware	87
Unaware	13
Reason for elephant coming near settlements	
Reason	**Respondent**
Agricultural Interest	16
Fruiting Tree	26
Water Availability	22
Handia	32
Stored Grains	54
Chased From Other Place	29
Mitigation strategies	
Traditional Strategies	**Frequency**
Bow and arrow	15
Guarding at night	34
Noise making tools	65
Stone throwing	60
Mashaal	80
Chasing	89
Using noise by people	78

Procedure of claiming payment	
Procedure	**Respondent**
Hectic	27
Smooth	36
Satisfactory	37
Status of compensation payment	
Payment Status	**Respondent**
Paid	67
With Held	27
Claimer Unavailable	6
Preferences of trees by elephants near settlements	
Tree Name	**Preference**
Jack Fruit	9
Mango	7
Papaya	2
Banana	3
Bamboo	6
Peepal	7
Kendu	7
Berries	2
Mahua	5
Wood Apple	3

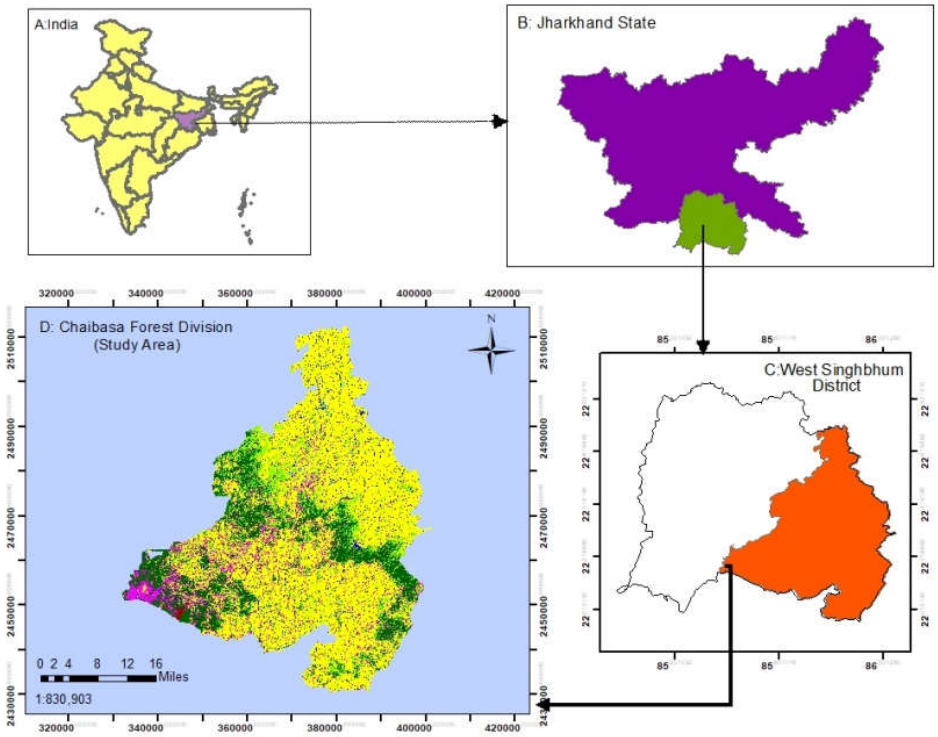

Figure 1 Map of the Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand, India.

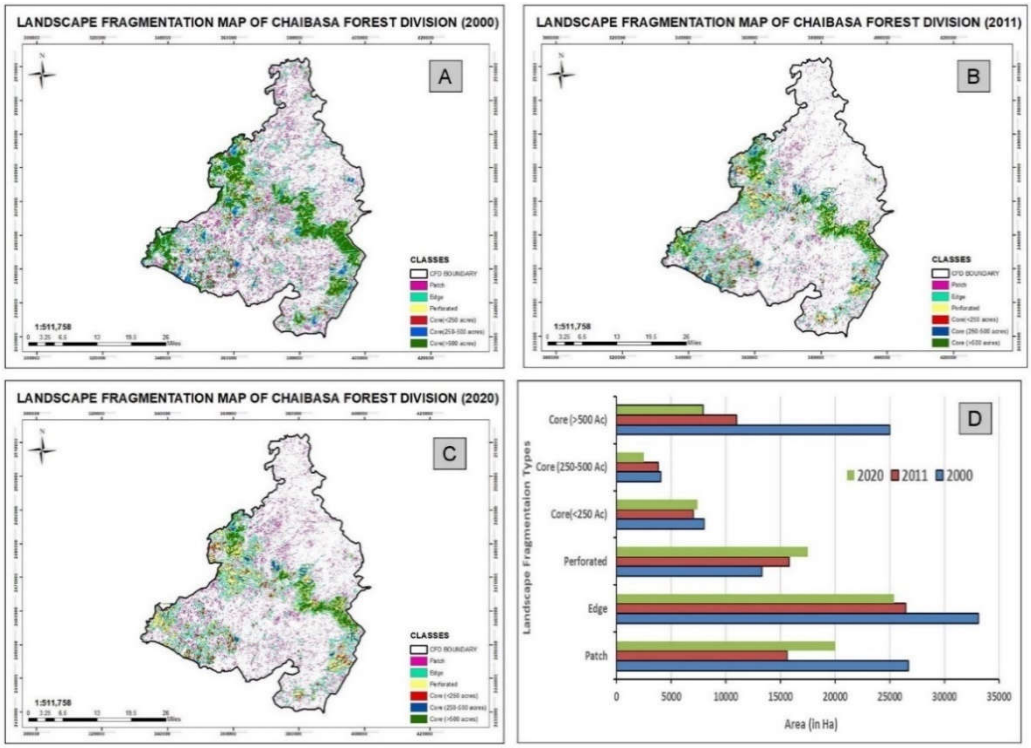

Figure 2 Landscape Fragmentation Map of Chaibasa Forest Division (CFD). **A.** Landscape Fragmentation Map (2000), **B.** Landscape Fragmentation Map (2011), **C.** Landscape Fragmentation Map (2020), and **D.** Figure showing Area of different Forest Fragmentation Classes.

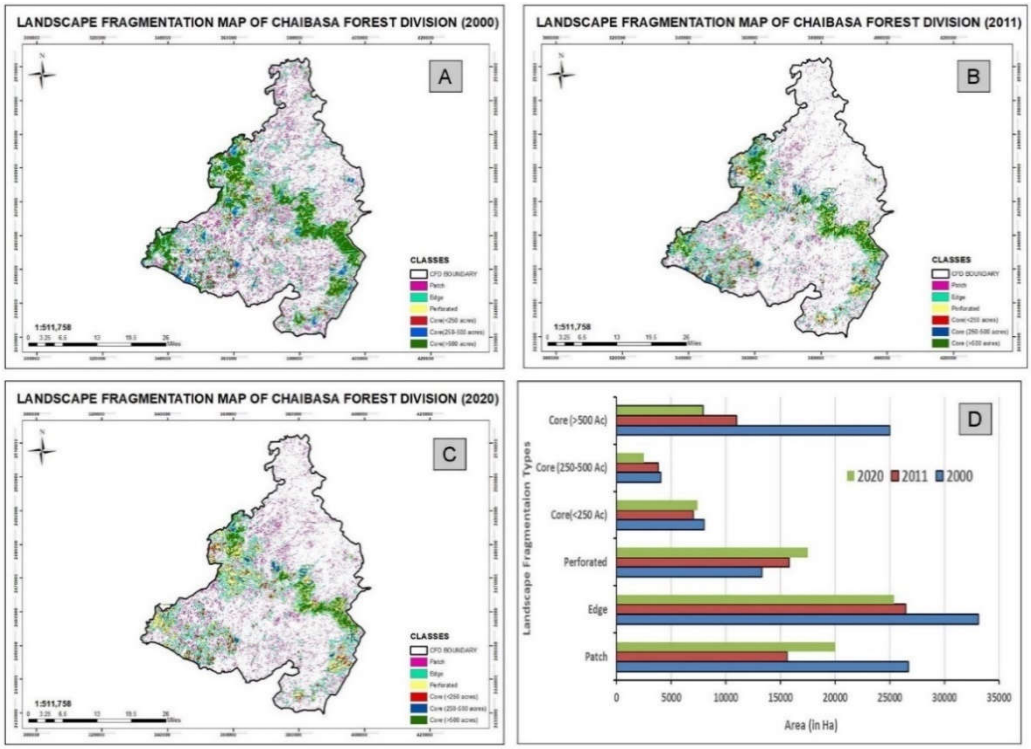

Figure 3 LULC Map of Chaibasa Forest Division (CFD). **A.** LULC Map of CFD (2000), **B.** LULC Map of CFD (2011), **C.** LULC Map of CFD (2020), **D.** Figure showing area of different LULC Classes in 2000, 2011 and 2020.

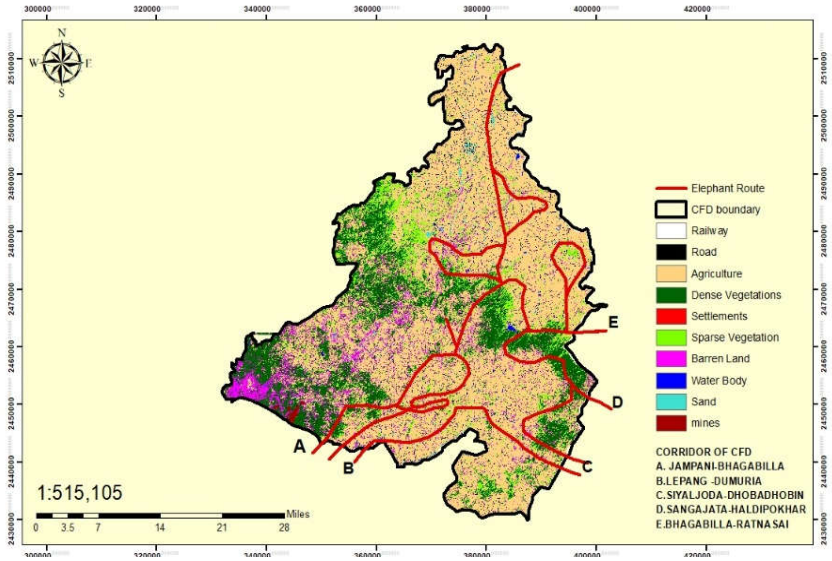

Figure 4 Elephant Corridors and movement routes of elephants in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand.

Figure 5 Human-Elephant Conflict (HEC) in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand. **A**-Severity of Human injury suffered in HEC during 2012-2020. **B**- Death caused due to Elephant attack near settlements (Victim: Late Gurua Tiria), **C**-Injury caused due to elephant attack while chasing Elephant from an agricultural field (Victim: Soma Chatar), **D**- Case of house damage of Shri Lomsa Gagrai, Bichaburu, Noamundi Range, **E**- Case of house damage of Shri Menjo Hessa, Nuagaon, Noamundi Range, **F**- Case of house damage associated with grain loss of Shri Bahadul Paan, Buruikuti Hatgamariah Range., and **G**- Case of crop damage of Shri Lomsa Gagrai,Bichaburu,Noamundi Range.

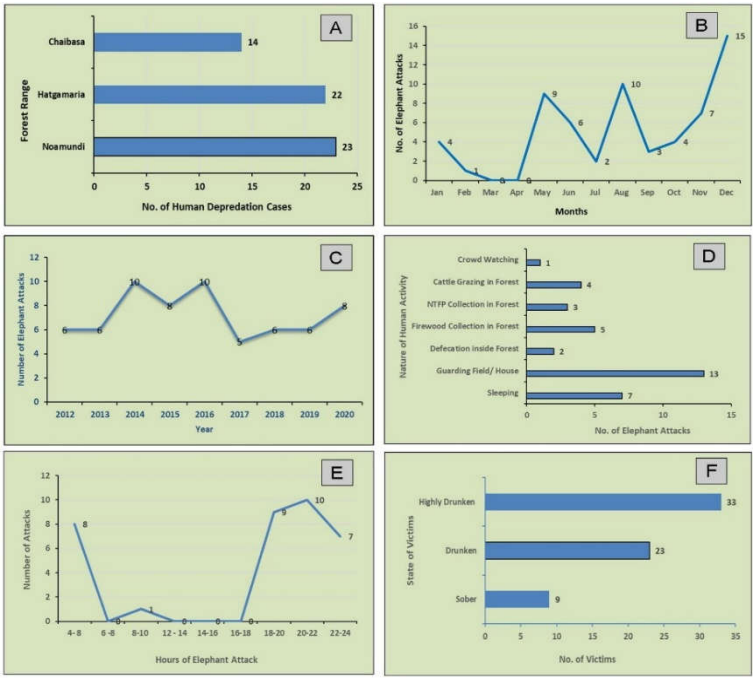

Figure 6 Human depredation cases in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand during 2012 -2000. **A.** No. of human depredation cases in three Forest Ranges of CFD, **B.** No. of elephant attacks in different months of a year, **C.** No. of elephant attacks from 2002 to 2020, **D.** Nature of human activity during elephant attack, **E.** Hours of elephant attack, **F.** Intoxication level of victim during elephant attack.

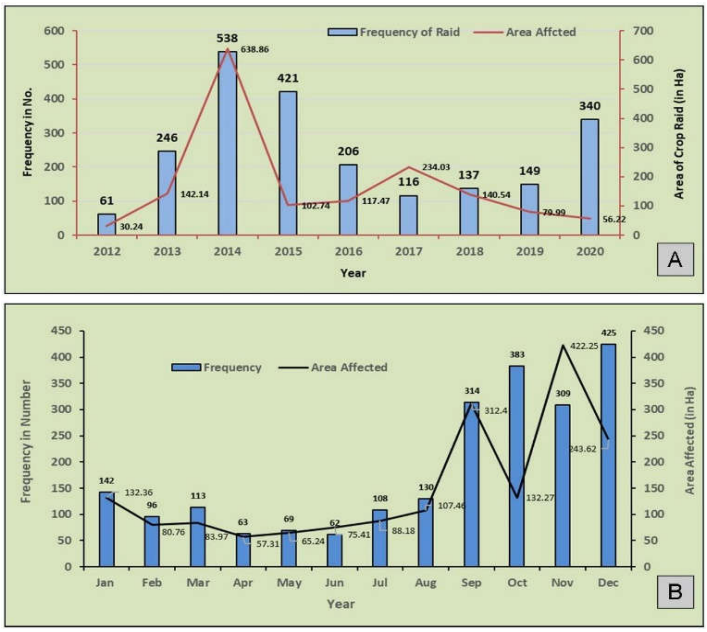

Figure 7 Crop raid by elephants in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand during 2012 to 2020. **A.** Yearly frequency and crop raid by elephants, **B.** Month-wise frequency of crop raid and crop area damaged.

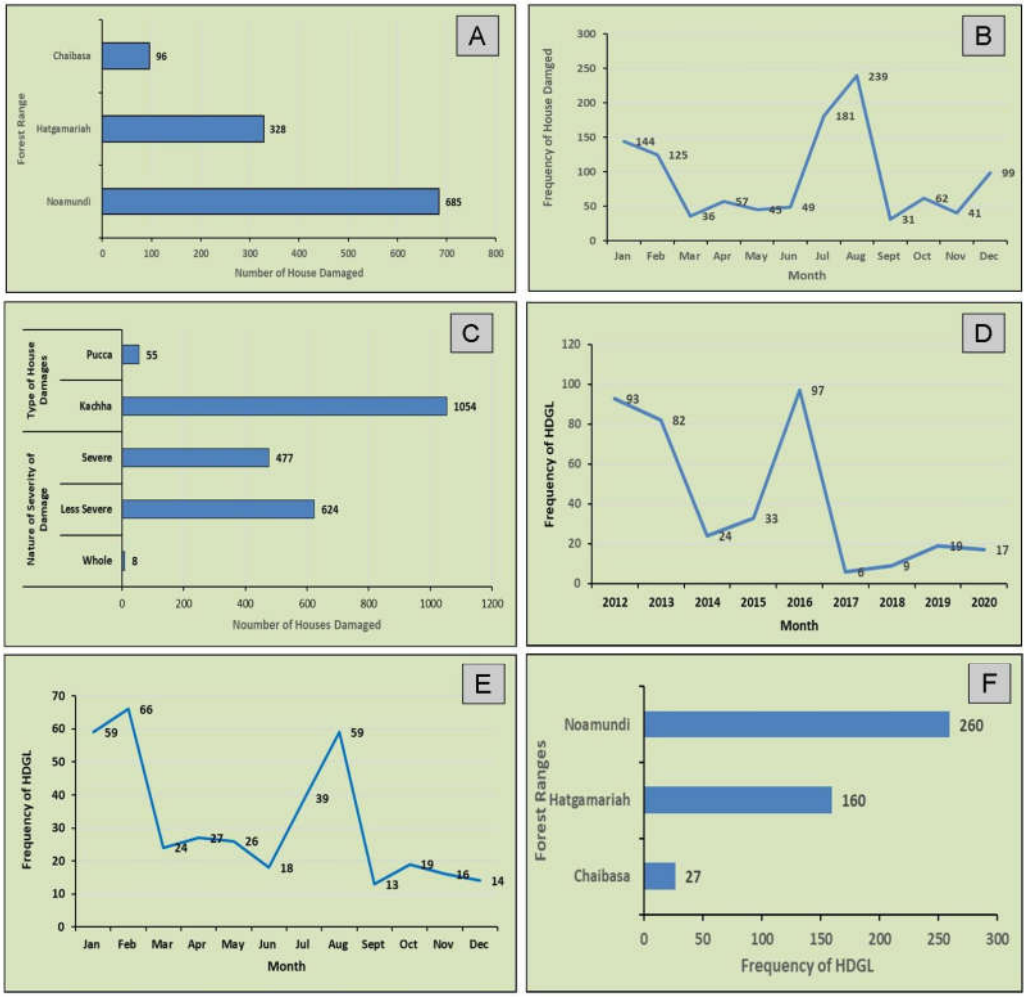

Figure 8 Incidence of House damage and Grain Loss by elephants in Chaibasa Forest Division (CFD), West Singhbhum, Jharkhand, from 2012 to 2020. **A.** No. of House Damage in three Forest Ranges of CFD, **B.** Monthly variation in the frequency of house damage, **C.** Severity and nature of house damage, **D.** Frequency of House Damage with Grain Loss (HDGL) during 2012 to 2020, **E.** Monthly variation in the frequency of HDGL, **F.** Frequency of HDGL in three Forest Ranges of CFD.

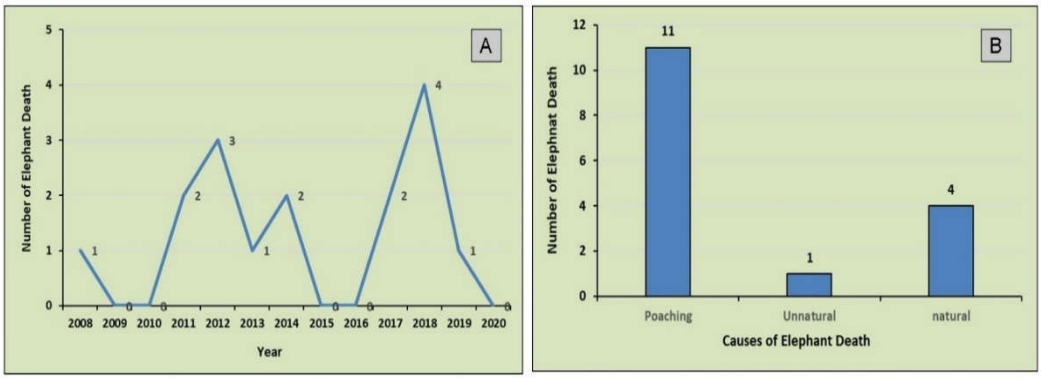

Figure 9 Elephant deaths in Chaibasa Forest Division (CFD) and causes of deaths during 2008 to 2020. **A.** Cases of death of elephants in different years, **B.** Causes of Death of Elephants in CFD.

DISCUSSION

An elephant has a large home range size and requires a large contiguous tract of forest and grassland habitat for their long-term survival as they travel 1000 square kilometres every year in search of food and water resources (Sukumar, 2003). CFD has only 17.77% forest cover, with 82.22% of its area being non-forest land, dominated by agriculture (37.33%). Due to human pressure, CFD offers very limited habitat for nurturing elephants, with only 9.85% core forest with an area >500 acres, 3.09% with an area of 250-500 acres, and 21.69% of the forest area is perforated. Elephants shelter in patchy forests during the daytime and raid crops at night. The elephant census report shows minimal population changes over the past decade. As a long-lived species, elephants expand their home range in response to resource scarcity, rather than experiencing a sharp population decline. They also compensate for a lack of natural forage by feeding on crops (Madhusudan and Mishra, 2003; Madhusudan, 2003). LULC data shows a 6.58% reduction in vegetation, a 6.8% increase in human-dominated areas, and a 0.45% decrease in water bodies. CFD cannot support a large elephant population but offers a 275 km migratory route, with only 90 km within the protected forest and the rest in non-protected areas. Most of the CFD lies within Human Elephant Coexistence (HECZ) and Elephant Exclusion Zones (EEZ), where habitat is insufficient for elephant conservation.

Globally, LULC degradation is recognized as a serious issue, significantly affecting wildlife distribution, populations, and ecosystems (Sukumar, 1989). In CFD, barren areas and sparse vegetation have increased by 5.03% and 1.48%, while dense vegetation has decreased by 8.06%. Between 1980 and 2019, 730.36 ha of land were diverted for development projects such as mines, roads, schools, transmission lines, railways, and the Subarnarekha Project. Since 2011, the core forest area (>500 acres) has decreased by 12.08%, with a 9.62% increase in forest perforation compared to 2000. From 2010 to 2016, 156 forest fires occurred, affecting 173.32 hectares. Fires, often caused by grazers and forest produce collectors, reduce elephant food resources and significantly alter habitats (Sukumar, 2003). The loss of forests, which may be key elephant habitats, leads to fragmentation, pushing elephants into shrinking habitat patches and heightening human-elephant conflicts (Sukumar, 1989; Desai, 1991; Johnsingh,1983). Most elephant ranges are highly fragmented and lack sufficient wild areas, which threatens their long-term viability unless connectivity is enhanced. Due to fragmentation, wildlife struggles to find adequate resources, leading to frequent encounters with human settlements and resulting in human-animal conflicts. A large portion of elephant habitat overlaps with human-dominated areas, creating strong competition for resources. As a coping strategy, elephants often raid crops to meet their nutritional needs.

Cropland acts as the main stimulus of the human-elephant conflict. While spatial drivers of HEC are shaped by land-use patterns and human activities, the seasonality of these conflicts is tied to the agricultural calendar (Naha et al., 2020). In Chaibasa, most crop damage (412.25 ha) occurred in November during the paddy harvest (n=655). Crop raiding by elephants may be driven not only by a lack of natural fodder but also by the availability and preference for crops. Most raids took place during the rainy season, more so than in the dry season. Elephants damaged crops at all growth stages, though in varying proportions. From 2012 to 2020, elephant visits to croplands peaked in December (n=425). Crop raids were most frequent during monsoon and winter months, coinciding with the harvest of maize and paddy (Sukumar, 1990; Bhima, 1998; Sitati et al., 2003; Campos-Arceiz et al., 2009; Wilson et al., 2015 and Chen et al., 2016). Despite abundant wild forage in the post-rainy season, the preference for highly nutritious, mature crops better explains the increased crop damage during this period, rather than a shortage of wild food. In 2014, crop damage reached its peak at 638.859 ha. This damage is particularly severe from a socio-economic standpoint, as many households rely on subsistence farming with medium-sized landholdings (Neupane et al., 2017; Prins et al., 2022). The economic strain on rural farmers in elephant-affected areas can lead to heightened resentment, potentially resulting in poaching and local efforts to exterminate elephants (Sukumar and Gadgil, 1988; Sukumar, 1991; Santiapillai and Ramono, 1993; Williams et al., 2001). During the study period, of the 16 total elephant deaths, 11 were adult males due to poaching, and one was an unnatural case death of a calf. According to the 2017 Elephant Census, the elephant population of Jharkhand has declined to 555 compared to 688 of 2012 Elephant Census (Mukesh, 2017), with 14 in Chaibasa Forest Division, representing 2% of the state's population. In that year, Chaibasa experienced 7% of the human casualties related to elephant incidents.

An adult Asian elephant feeds on a variety of diets, such as grasses, fibres, aquatic plants, foliage, bamboo, roots, bark, dry twigs, the pith of bananas, and fruits (Sukumar, 2003). They need about 150 to 300 kg of forage per day (Fernando et al*.,* 2008). They spend 40 to 75% of their time on feeding. During the study period, jack fruit was the fruiting tree that was raided the nearest settlement, followed by Mango, Peepal, and Kendu. Although elephants are considered to be generalist feeders, in the study area, 39 species were identified as food plants foraged mostly.

While economic losses from agriculture are a primary driver of human-wildlife conflict, other socio-economic costs can also be significant and may surpass the direct damage to crops. These costs, perceived by local people, include human fatalities, restricted movement, competition for water, property guarding leading to loss of sleep, reduced school attendance, diminished employment opportunities, increased malaria risk, and psychological stress (WWF, 1997; Sukumar, 1990; Naughton-Treves, 1998; Hoare, 2000). From 2012 to 2020, 65 human casualties were reported in CFD. Elephants driven from nearby states or forest divisions caused significant damage, particularly in areas like Noamundi (n=23) and Hatgamariah range (n=22), where the elephant corridor passes through agricultural land. The division has extensive cropland, increasing the likelihood of conflict. Blocking traditional elephant routes due to settlements and agriculture can lead to aggressive behaviour in elephants (Naughton et al., 1999). December saw the highest number of human injuries and deaths. Seasonal patterns show that crop-raiding and fatal elephant attacks peak during the monsoon and winter months (Naha et al., 2019). In most elephant attack cases, the victims were intoxicated with local liquor (n=56), and the majority of casualties occurred at night (n=26). Elephants are known to leave forested areas for crop raiding at night when human activity is minimal (Wakoli and Sitati, 2012). Additionally, attacks often occurred in villages where alcohol production, such as Handia (rice beer), was common. Alcoholism and drunkenness are significant factors in fatal elephant attacks, as intoxicated individuals sometimes harass and chase elephants from villages and crop fields (Naha et al., 2019). Over the past 10 years, more than five hundred people have been killed by elephants (Naha et al., 2019). Similar patterns have been observed in Assam (India) and the Terai region of Nepal, where victims of HEC were often drunk and chasing elephants (Lahkar et al., 2007; Lenin and Sukumar, 2011; Neupane et al., 2013). In CFD, respondents (n=69) reported that most damage and casualties were caused by adult males or bulls. While the specific identity of the elephants involved in casualties is often unknown, deaths inside the

forest are typically attributed to both bulls and herds, whereas those near settlements are usually linked to sub-adult males and bulls (Sukumar, 2003). Herds generally visit villages when crops are ripe, while bulls can cause conflict throughout the year (Lahkar et al., 2007).

From 2012 to 2020, the highest frequency of house damage occurred in the Noamundi range (n=685), followed by Hatgamariah (n=328), both of which are key elephant movement routes. August saw the highest number of house damages (n=239). Studies suggest that elephant herds generally avoid high-density settlements during migration to reduce anthropogenic risks, unlike solitary elephants (Douglas et al., 2005; Joshi, 2009; Songhurst et al., 2016; Khanal, 2022). In CFD, there were 380 cases of house damage linked to grain damage. The severity of house damage was greater during the off-paddy season when paddy was stored in homes. Additionally, the pungent smell of fermented rice beer can attract elephants, leading to further damage (Ghosh and Das, 2004).

The majority of respondents (n=77) perceived those agricultural lands close to forests experienced more frequent crop raids and generally felt more threatened by solitary males (n=69). Elephants tended to stay longer in areas where the forest provided refuge, with most respondents believing they did not halt for more than seven days. In CFD, victims or their families who received timely compensation had a relatively positive attitude towards wildlife and the government. However, those who did not receive full compensation for crop losses (n=27) due to departmental delays or missing land documents, developed lower tolerance towards the department. Effective management of compensation payments can help mitigate negative perceptions towards wildlife. Ensuring timely and accurate disbursement of compensation is crucial for conflict management. Madhusudan (2003) highlights that official corruption in India hampers the compensation system, leading to widespread dissatisfaction and unreported cases (Nath and Sukumar, 1998). To manage conflicts effectively, it is essential to understand both people's perceptions and the scientific aspects of wildlife damage (Manfredo et al., 1998; Marker et al., 2003; Naughton-Treves et al., 2003; Naughton-Treves and Treves, 2005).

The traditional methods for deterring elephants are affordable and can help reduce crop loss, but they also pose risks of direct confrontation, which can be dangerous for people (Nath and Sukumar, 1998; Desai, 2002; Nelson et al., 2003; Boafo et al., 2004; Fernando et al., 2008). In Assam, NGOs have set up Anti-Depredation Squads that employ strategies such as observing from elevated platforms, using spotlights, and setting off firecrackers to prevent elephants from entering croplands.

The locals use various traditional methods to mitigate elephant conflicts, such as vocal sounds, noise-making tools (drums, stones, dust), chasing with fire (mashaal), and monitoring from elevated platforms (machan). During periods of high crop vulnerability, farm households often band together to drive away elephants. While crackers have been effective in deterring elephants, these short-term methods lose their effectiveness when used repeatedly without variation (Parker et al., 2007). Villages near protected forests frequently suffer from habitual crop raids, while agricultural lands in elephant corridors experience sudden, one-time destruction. Many respondents (n=61) felt that traditional methods were ineffective when elephants retaliate aggressively.

CONCLUSION

Conserving elephants in Chaibasa Forest Division, Jharkhand, is challenging due to high anthropogenic pressure on the fragmented forest land. While sufficient habitat to support the large home ranges of elephants is not available in CFD, it provides a migratory pathway. Improving landscape connectivity between forest patches is crucial for coexistence. In the coexistence zone, effective conservation strategies are needed, such as greening corridors to reduce forest perforation, eradicating invasive species, rejuvenating water bodies, constructing check dams, and providing nutritional supplements in the forest to minimize crop raids. In the Elephant Exclusion Zone (EEZ), preventive and site-specific mitigation strategies are essential to reduce conflict intensity. These strategies may include involving

local communities in managing human-elephant conflicts (HEC), using beehive fencing, planting buffer crops in areas with frequent crop raiding, and deploying expert teams to handle emergency HEC situations. These strategies implemented successfully can convert Human-Elephant Conflict to Human-Elephant Coexistence.

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ASSOCIATION OF FINGERPRINT PATTERNS WITH BLOOD GROUPS AND GENDER AMONG THE STUDENTS OF UTKAL UNIVERSITY, VANI VIHAR, BHUBANESWAR, ODISHA: A DERMATOGLYPHIC STUDY

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ABSTRACT

Fingerprint is undoubtedly the most distinctive and trustworthy kind of personal identifying proof today. Because of the unique capability of fingerprint to serve as an important tool for identification of individuals for multiple purposes, an attempt has been made in the present work to analyse their association with blood group of a person. The present study was completed within a period of 2 months among the students of Utkal University campus, Bhubaneswar, Odisha in the year 2022. A total of 384 randomly selected students (169 male) and (215 female) having blood group of O" (n=138), "B" (n=131), "A" (n=85), and "AB" (n=30) that comprised of 350 subjects with Rh +ve and 34 with Rh -ve antigens. The objectives of the study were (i) to study the distribution pattern of fingerprint among the students having "ABO" and "Rh" blood group, (ii) to correlate any association between pattern of fingerprints and blood groups. Results demonstrate that, each finger impression is specific. Loops are the most frequently occurring pattern, while arches are the least common. Male subjects show higher incidence of blood group "O", while females show higher frequency of blood group "B". Loop pattern frequently occurs in blood group "A" and "O", whereas, group "B" and "AB" shows higher incidences of whorl pattern. Whorls are predominantly more in thumb as well as ring fingers, while, loops are common in middle fingers and little fingers. This implies that there is an association between blood group and fingerprint patterns. This study can be further confirmed by involving different molecular markers in future which can be useful to identify individuals based on blood groups and fingerprints during exigency.

Keywords: Fingerprint Pattern, Blood Group, Correlation.

INTRODUCTION

Identification of any individual is essential for different reasons and purposes. Biometric identification is an important aspect of identification and verification of individuals based on unique biological features (physical, behavioural, or physiological characters) possessed by them. Biometrics (Gr. *bio* = life, *metric* = to measure) is the measurement and statistical analysis of the unique characteristics of people. The primary use of this biometric technology is to identify a person based on fingerprints, facial recognition, iris recognition, retina scanning, handwriting, DNA fingerprinting etc. of a person (Qureshi and Bhatti, 2003). Biometric identification characters are unique and individualistic, and they remain constant from birth to death. Hence, it is preferred as a more significant and authentic identification technique. It is the reason that biometric technology has become a topic of interest and an emerging field of study these days.

Fingerprints have been used by humans as a means of identification for a very long time (Sudikshya et al,, 2018). The most reliable basis for identification of individuals is fingerprints because they do not change during lifetime and is specific to each person. A fingerprint is a representation of all the friction ridges on the finger. Every Person is born with their own individual unique fingerprints. In fact, identical twins having the same "DNA fingerprint" have different fingerprints (Sudikshya et al., 2018). When we look at these fingerprints and their characteristics, they

reveal different patterns. The distribution and arrangement of the papillary ridges or the epidermal ridges on the fingertip create patterns that are constant and particular to each person. It bears for the whole of life. Development of fingerprints starts from the intrauterine life during the first four months of gestation period (Bose and Kabir, 2017). Development of ridges in the palm (both primary and secondary) marks the beginning of fingerprint formation. The pattern ridge configurations are determined by both hereditary and environmental influences. The fingerprints are grouped under three broad categories such as Arch, Loop and Whorl (Fig. 2a). These are further sub categorised into radial loop, ulnar loop, plain arch, tented arch, plain whorl, central pocket loop whorl, double loop whorl and accidental whorl (Herschel, 1880) (Fig. 2a).

Blood group system was discovered by Karl Landsteiner in the year 1901. Only 'ABO' and 'Rhesus' groups are given major importance clinically. 'Rhesus' system is classified into 'Rh + ve' and 'Rh – ve' according to the presence or absence of 'D' antigens. 'ABO' system is further classified as A, B, AB, O blood group types according to presence of corresponding antigen upon red blood cell (RBC) plasma membrane (Yin-Jiang et al., 2023). Objectives of the present investigation was to study the distribution pattern of fingerprints among the students having “ABO” and “Rh” blood group and correlate the association between fingerprints and different blood groups.

MATERIALS AND METHODS

A period of two months (March and April, 2022) was spent in conducting this survey among the students of the Utkal University campus in Bhubaneswar, Odisha, India. A total of 384 participants (169 males, 215 females) with age group between 20 to 24 years, took part in the study. The written consent of these students was taken before the work. Students with any hand deformity, birth defects and those having worn out fingerprints were excluded from this study. A straight line was drawn in the centre of the white sheet of paper crossing it from top to bottom, placing the paper horizontally. This line divides the paper into right and left column for right and left hand respectively (Fig. 1). Both of these columns were again divided into 5 sub-columns for finger prints of five fingers of left and right hands respectively (Fig. 1). The top of each column was marked as thumb, index finger, middle finger, ring finger and little finger of right and left hand, respectively. Each subject was explained the purpose of taking their fingerprints. He/she was then asked to press their fingertip into rubber stamp ink pad (blue ink) and roll their finger across the ink pad and transfer it into the sheet of paper (Fig. 2b). The same procedure was repeated for every finger and ten fingerprints of each person was recorded. After taking fingerprints, details of every individual such as name, age and “ABO” and “Rh” blood groups were noted. A total of 3840 fingerprints were collected from 384 students. Out of which 2150 prints belonged to girls and 1690 prints from boys. With the use of a hand lens, these recorded fingerprints were studied, and their patterns were divided into three fundamental types: arches, loops, and whorls. The “ABO” and “Rh” blood types (Fig. 3) were studied in relation to fingerprints. GraphPad Prism Ver 5.0 (USA) software was used to tabulate, and analyse the data statistically. Data were analysed by X^2 at a significance threshold of p value < 0.05.

RESULTS

Number of female participants were more than males with female to male ratio 1.2:1. It was found that blood group “O” is highest in case of males and group “B” was predominant among the females. The prevalence of blood group with respect to gender in decreasing order was “O” (n=138, 36 %), “B” (n=131, 34.11%), “A” (n=85, 22.13%), and “AB” (n=30, 7.8%) (Table 1). The relationship between sex and blood group was found to be statistically significant ($p < 0.05$). Distribution of Rh antigen according to ABO blood groups is presented in Table 2. Out of total 384 cases, 350 (96.1 %) subjects were Rh +ve and only 34 (3.84 %) were Rh -ve. Among all the Rh +ve cases, 128 (35.1 %) individuals had “O” blood group followed by blood group “B” (n=124, 34.6 %), blood group “A” (n=76, 20.9%)

and "AB" blood group (n=22, 6.04 %). A total of 14 subjects were Rh -ve which was highest in blood group "O" (1.37 %) followed by blood group "A" (1.09 %), blood group "AB" (0.82 %) and (0.54%) of were observed in this study. The distribution "ABO" and "Rh" blood groups with respect to gender was found to be statistically significant ($p < 0.05$).

Table 1. Distribution of blood groups according to gender (n=384).

Blood Group → / Gender ↓	A	B	AB	O	Total
Males	34	51	9	75	169
Females	51	80	21	63	215
Total	85	131	30	138	384
Statistics	**$X^2 = 10.3, p < 0.05$**				

Table 2. Distribution of Rh antigen according to "ABO" blood groups (n=384).

Blood group	Rh +ve	Rh -ve	Total (384)
A	76	9	85
B	124	7	131
AB	22	8	30
O	128	10	138
Statistics	**$X^2 = 14.55, p = 0.0022$**		

Since the primary pattern of the fingerprints is classified into arches, loops, whorls (Table 3), it was observed that loops were predominant among all fingerprints, (42.8 %), followed by whorls (40.5%) and arches (16.75 %). The distribution of different fingerprint patterns with respect to "ABO" and "Rh" blood groups is presented in Tables 4.1, 4.2, 4.3, and 4.4.

Table 3. Distribution of finger print patterns in all fingers of both hands (n= 3840)

Pattern of fingerprint	Total	Percentage
Arches	643	16.75%
Loops	1644	42.8%
Whorls	1553	40.5%
Total	3840	100%

The distribution of fingerprint pattern in "A" blood group is presented in Table 4.1. It was observed that among the "A" blood group and Rh +ve individuals, the incidences of loops were the highest (51.8%), followed by whorls (39 %) and arches being the least (14.5 %). Whereas, in the Rh -ve cases of "A" blood group, arches were the highest (42 %) followed by whorls (32 %) and the loops (26 %). This difference was statistically significant (p-value < 0.0001).

Table 4.1 Distribution of Fingerprints among subjects of "A" blood group with respect to "Rh" blood group.

Types of prints	Blood Group "A"		Total
	Rh +ve	Rh -ve	
Arches within Rh	110 (14.5%)	21(23.3%)	131
Loops within Rh	394 (51.8%)	13 (14.4%)	407
Whorls within Rh	296 (39%)	16 (17.7%)	312
Statistics	$X^2 = 30.0$, $p < 0.0001$		

The distribution of fingerprint pattern in blood group "B" is presented in Table 4.2. Among the group "B" individuals, Rh +ve cases reveal predominance of whorl pattern (54.6%) followed by the loops (31.6%) and arches (16.2%). The fingerprint pattern of Rh-ve cases of blood group "B" shows highest incidence of loops (31.4%), followed by whorls (20%) and arches (5.7%). This difference was found to be statistically significant ($p = 0.005$).

Table 4.2 Distribution of Fingerprints among subjects of "B" blood group with respect to "Rh" blood group.

Types of prints	Blood Group "B"		Total
	Rh +ve	Rh -ve	
Arches within Rh	201 (16.2%)	4(5.7%)	205
Loops within Rh	392(31.6%)	22(31.4%)	414
Whorls within Rh	677(54.6%)	14(20%)	691
Statistics	$X^2 = 10.45$, $p = 0.005$		

Table 4.3 displays the fingerprint pattern distribution among subjects of blood group "AB". In case of Rh +ve subjects, whorl pattern was predominant (64%) followed by loops (43.2%) and arches (10.4%). Whereas, in Rh-ve cases loops were found to be the highest (31.2%) followed by whorls (11.2%) and arches (7%). This difference was also found to be statistically significant ($p < 0.05$).

Table 4.3 Distribution of Fingerprints among subjects of "AB" and "Rh" blood group.

Types of prints	Blood Group "AB"		Total
	Rh +ve	Rh -ve	
Arches % within Rh	23(10.4%)	7(8.75%)	30
Loops % within Rh	95(43.2%)	25(31.25%)	120
Whorls % within Rh	141(64%)	9(11.2%)	150
Statistics	$X^2 = 15.07$, $p = 0.0005$		

The distribution of fingerprint pattern in "O" blood group is presented in Table 4.4. Out of 138 subjects of blood group "O", 128 were Rh +ve and 10 were Rh -ve. Among the Rh +ve cases, the frequency of the loop pattern was the highest (53 %), followed by whorls (31 %) and arches (19%). However, the frequency of arches remained the highest (25%) in the Rh -ve cases. Using the chi square test, this difference was found to be statistically significant with a p-value < 0.05. As a result, the observation was that those with blood group type "A" and "O" are more likely to experience loops. The occurrence of whorls was found to be higher in Rh +ve blood group "B" and "AB" persons.

Table 4.4 Distribution of Fingerprints among subjects of "O" and "Rh" blood group.

Types of prints	Blood Group "O"		Total
	Rh +ve	Rh -ve	
Arches % within Rh	244(19%)	25 (25%)	269
Loops % within Rh	679 (53%)	22(22%)	701
Whorls % within Rh	397 (31%)	13(13%)	410
Statistics	$X^2 = 19.65$, $p < 0.0001$		

Among the students with blood group "A" and "O", the incidence of loops was high in their middle fingers and little fingers (Fig. 4 and 7). In individuals with blood group "A", loop was predominant in middle finger (28%), arch in thumb (29.3%), whorl in thumb (28.3%), whereas Arch (33.8%), loop (32%) and whorls (28%) were predominantly found in index, middle and thumb respectively in individuals with blood group "B" (Fig. 4 and 5). Predominance of both arch (27.3%) and whorl (30.4%) in thumb but loop (34.4%) in middle finger were observed in individuals with "AB" blood group (Fig. 6). On the other hand, individuals with blood group "O" revealed predominance of arch in index (31.6%) finger, loop (27.6%) in little finger and whorl (33.5%) in thumb (Fig. 7).

The distribution of the subject's primary pattern of fingerprints in all of their fingers on both the right and left hand was observed to be statistically significant. However, the distribution of primary finger print patterns remained more significant, for blood groups type "A" and "AB" ($p < 0.0001$).

Figure 1 Fingerprint sampling on a plain paper (T=Thumb, IF= Index Finger, MF=Middle Finger, RF= Ring Finger and LF=Little Finger)

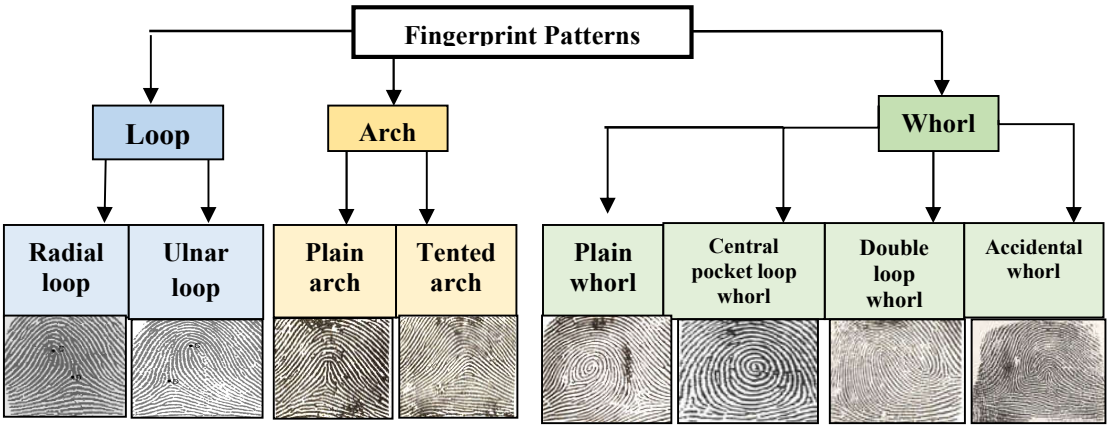

Figure 2a Different types of fingerprint patterns (After Herschel, 1880)

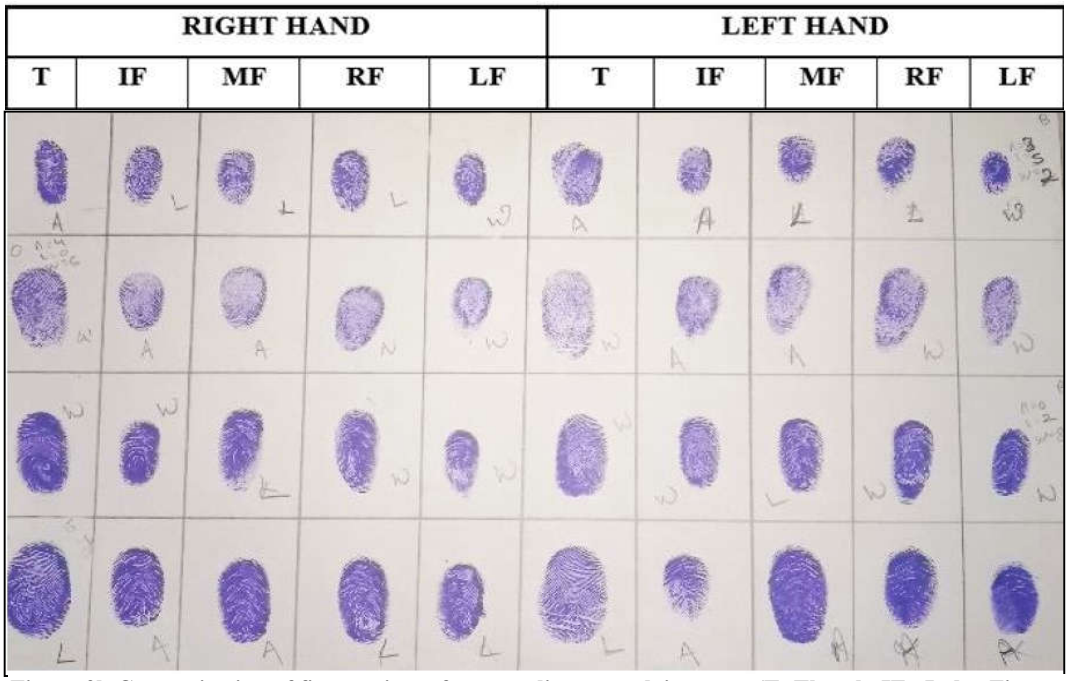

Figure 2b Categorisation of fingerprints after sampling on a plain paper (T=Thumb, IF= Index Finger, MF=Middle Finger, RF= Ring Finger and LF=Little Finger)

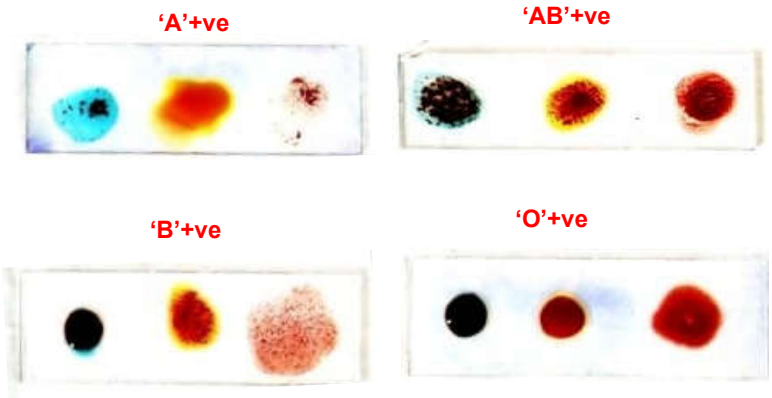

Figure 3 ABO Blood group categorisation

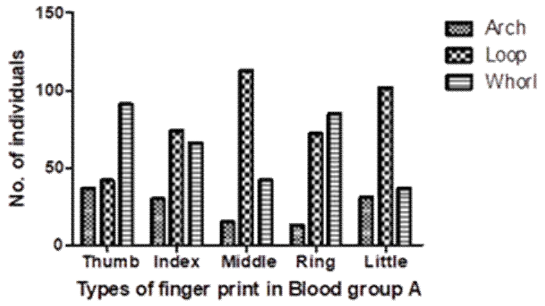

Figure 4 Distribution of pattern of fingerprints (Blood Group A)

Fig.5.2

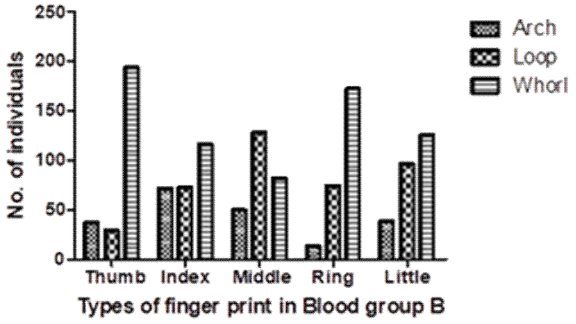

Figure 5 Distribution of pattern of fingerprints (Blood Group B)

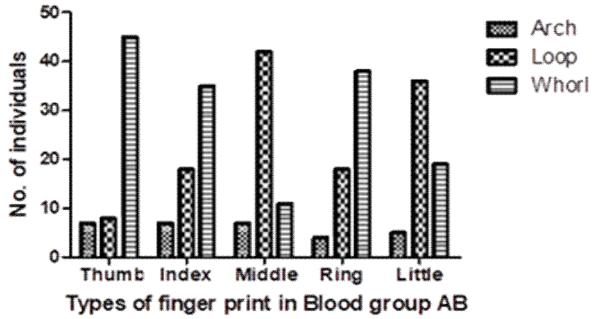

Figure 6 Distribution of pattern of fingerprints in (Blood Group AB)

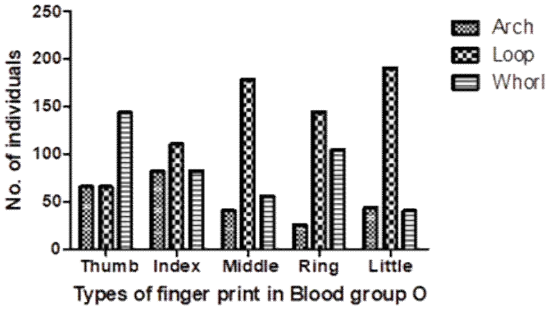

Figure 7 Distribution of pattern of fingerprints in (Blood Group O)

DISCUSSION

Fingerprint is unique reliable evidence for personal identification even though each community has a different distribution of fingerprint patterns (Sahu et al., 2017). This study sought to relate a person's blood group to his/her fingerprint pattern. According to our survey, the occurrences of Rh +ve was high compared to Rh -ve type. Similar results have been observed in a study in Sriganganagar district of Rajasthan (Sandhu et al., 2017). Among the ABO blood groups, the blood group "O" is the most common, followed by "B", "A", and "AB." In India, O +ve is the most common blood group type, followed by B +ve (Bharadwaja et al., 2004 and Srilekha et al., 2014). It has been confirmed by earlier studies on 300 Nepalese citizens (Sudikshya et al., 2018), 200 medical students of Kasturba Medical College, Mangalore, India (Rastogi and Pillai, 2010 and Srilekha et al., 2014) and 150 dental students

studying at Vivekananda Dental College for Women, Elayampalayam, Tiruchengode, India (Manikandan et al., 2019). According to a study conducted among 233 undergraduate dental students of Visnagar of Gujarat state, both “O” +ve and “B” +ve were reported to be predominat (Patel et al., 2015). Result of present study is conforms to earlier similar study by Patil V and Ingle DR, (2021) where the frequency of blood group “O” +ve was high but “O” -ve and “AB” -ve were the rarest. But present finding is in contrary to some reports that indicated higher incidences of the occurrences of Blood group “B” followed by blood group “O”, “A” and “AB” (Mehta and Mehta, 2011) and (Khalid and Qureshi, 2006).

The predominant finger prints among the students in decreasing order were found to loops, whorls, followed by arches. This result is consistent with many studies by different groups of people belonging to different places like Rajasthan, (Bharadwaja et al., 2004), Mangalore (Rastogi and Pillai, 2010 and Bansal et al., 2013), Karnataka, (Bhavana et al, 2013), Gujrat (Raloti et al., 2013) and Sriganganagar, Rajasthan, (Sandhu et al., 2017; Shrestha and Malla, 2019; Srilekha et al., 2014 and Patil and Ingle, 2021). In the present study, blood groups "A" and "O" exhibited more loops, while blood groups "B" and "AB" exhibited more whorls. This finding is consistent with the findings of Bharadwaja et al. (2004), who conducted a study in Rajasthan in 2000 - 2001 on 300 medical students having varied “ABO” blood groups and found that those with blood group “A” had more number loops, while those with blood group “AB” had a greater number of whorls. Present report finding is also similar to the findings of Mehta and Mehta (2011) where loops were dominant pattern in blood group “O” and least in the “AB”.

Prevalence of Rh +ve (96.15%) was found to higher than Rh -ve (3.84%) subjects in all blood types (100%). This is similar to earlier report obtained from different nations, such as India (Bharadwaja et al., 2004), Pakistan (Khalid and Qureshi, 2006) and Libya (Fayrouz et al., 2012). The present finding revealing that thumb and ring fingers had a greater incidence of whorls, whereas the middle fingers and little fingers had a larger incidence of loops, was also seen in earlier study conducted in India (Bharadwaja et al., 2004) and Libya (Fayrouz et al., 2012).

In a society with increasing crimes and criminals, fingerprint sensing technology has to be advanced with more improvement and accuracy. This is the sole reason, the demand for automatic personal identification is rising day by day. As, fingerprints, lip prints and “ABO” blood groups are determined genotypically and persistent from birth to death, correlating these evidences at a crime scene can give a more accurate result to find the suspects. Besides correlating the fingerprint distribution with blood group need to be further refined by associating these findings wuth different molecular markers. Therefore, fingerprints will always be an essential component of the widely known biometric based identifying strategies in the coming years. The association between fingerprint pattern and blood group offers opportunity for extra added identification information that can be used as proof of identity.

CONCLUSION

Fingerprints are one of the most important tools for identifying a person for a wide range of purposes. Based on the present study it is reasonable to conclude that loops are the most frequent type of fingerprint, whereas arch pattern are the least frequent. Loops are the predominant pattern in blood group type “A” and “O”, whereas, whorl was predominant among blood group type “B” and “AB”. Whorls are more common in the thumb and ring fingers, while loops are more prevalent in the little fingers and middle fingers of all blood group types. With our present study we conclude that, fingerprint and blood group of an individual shows certain relationship and one can predict the blood group of a person based on his/her fingerprint and vice versa. However, this correlation between fingerpring and blood group need to be validated by expression pattern of different genomic markers among the individuals in future. Besides, the reliability and accuracy of the prediction can be increased by conducting more such studies with a larger sample size across different communities and populations.

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HIGHER Cr AND Pb CONTAMINATION IN FISH (*Labeo bata*) MUSCLE WITH ENHANCED LIPID PEROXIDATION: A THREAT FOR FOOD CHAIN AND FOOD WEB

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ABSTRACT

The Talcher industrial zone, is one of the largest and oldest coal mining and thermal power generation complexes in the country, situated in the state of Orissa, India along the banks of the river, Brahmani. Mining activity and industries in this area have been releasing various pollutants such as effluents, sewage, and domestic wastes (i.e. plastics garbage bags, unused household dyes/ paints from local garages, post festival immersion of idols) into the Brahmani River and its tributaries. The Brahmani River is a significant waterway in Odisha, covering a catchment area of 39,000 square kilometres, and it significantly impacts human activities both directly as well as indirect. This study focuses on comparing the physico-chemical characteristics of water samples obtained from two distinct sampling locations, i.e., the upstream (Rengali multipurpose dam) and downstream (Kamalanga and Khargprasad villages) of the Talcher industrial zone. Several parameters including temperature, pH, conductivity, alkalinity, dissolved oxygen (DO), biochemical oxygen demand (BOD), and total dissolved solids (TDS) were compared between the two stations. Besides this, the toxic metal concentration was also measured in the water along with their level in the muscle of fish (*Labeo bata*) samples collected from these two sites and compared. High levels of toxic transition metals like Cr and Pb were detected in the muscle tissue which are known to generate free radicals and reactive oxygen species subsequently leading to oxidative stress in the fish. Therefore, lipid peroxidation, the index of oxidative stress was measured in the edible part of the fish tissues (muscle) which has the potential for bioaccumulation in the food chain upon consumption. The concentration of Cr and Pb were above the permissible limits in the water collected from downstream station which was corroborated with their level of accumulation in the fish tissues. The level of lipid peroxidation was observed to be 1.5 fold higher in the muscle of *Labeo bata* collected from downstream of River Brahmani as compared to the upstream fishes and 10 fold higher than the farm bred fishes. Based on the present finding it is suggested that the deterioration in water quality of the river in the downstream of industrial complex possesses a significant threat to aquatic life. The study also revealed that some parameters of the sampled surface water like pH, conductivity, TDS, total alkalinity remained within the tolerance limits set by organizations like the World Health Organization (WHO) and the Indian Standards (IS), while certain other parameter exceeded these limits such as BOD.

Keyword: Brahmani River, Water quality, Toxic Metal, Fish

INTRODUCTION

Water, a vital natural resource, is indispensable for the sustenance of human civilization. Sustainable management of water resources is crucial for the economic growth and well-being of communities. According to the National Water Policy (2002), development and management of water resources should consider hydrological units like drainage basins or sub-basins, incorporating quantity, quality, and environmental aspects. Even though water quality is a significant concern for economic development, degradation of water resources has worsened due to rapid industrialization, urban sprawl, and anthropogenic influences, including urbanization, agricultural activities, and

changing precipitation inputs and erosion. The heightened presence of contaminants in river water, stemming from the discharge of municipal sewage and industrial byproducts, constitutes a noteworthy peril to aquatic ecosystems (Carpenter et al., 1998; Jarvie et al., 1998; Singh et al., 2005; Sundaray et al., 2006).

Water quality encompasses complex interactions among physical, chemical, hydrological, and biological characteristics, and its acceptability is determined by various factors depending on its intended use, such as drinking, irrigation, or recreational purposes. However, water resources face significant pollution from various sources, including sewage, industrial waste, and heavy metals. Many studies have assessed water quality in different regions, highlighting the impact of industrialization and human activities on water degradation (Bartarya and Bahukhandi, 2012; Patel et al., 2020; Gani et al., 2023).

The Brahmani watershed in Odisha is a highly developed and urbanized region facing deteriorating water quality primarily due to improper disposal of solid and toxic waste from industrial effluents, agricultural runoff, and other human activities. This pollution not only degrades water quality but also poses threats to human health, aquatic ecosystems, and the state's economic development. While certain individual investigations have been conducted regarding water pollution in the Brahmani River resulting from major industries in the Rourkela region (Dey et al.,2005), the evaluation encompasses aspects such as human bathing along the Brahmani River at Vedvyas (Naik and Purohit, 1998), the impact of industrial effluent discharge on the water quality of the Brahmani River in Rourkela (Nanda and Tiwari, 1999), an appraisal of water quality in the Koel River (Sundaray, 2010), and the ambient air quality within the Rourkela industrial complex (Sundaray, 2005). Nath et al. (2018) reported a fourteen years (2000-2014) data regarding potability of water of Bramhani River. Although the previous reports documented the water quality of Bramhani River system including its tributaries and branches none of them reported its impact on the biological system particularly on the edible fishes where there is a chance of bioaccumulation of toxic metals due to presence of effluents from mining activity and industries. Therefore, in the present study an attempt has been taken to find out not only the water quality of the Bramhani River but also the concentration of toxic metals in the water and the muscle of the edible fish *Labeo bata.*

MATERIALS AND METHODS

Sampling sites

The Brahmani River, the second largest river in Odisha, boasts a drainage basin spanning 39,035 square kilometres. It stretches a total length of 800 kilometres and exhibits a peak discharge of 22,640 cubic meters per second. The selection of monitoring stations was primarily based on the proximity to major industries and urban areas that were expected to have a significant impact on pollution levels. The industrial complex at Talcher is located with latitude 20.951542°N and longitude 85.215668°E along the banks of the Brahmani River, approximately 8 to 10 kilometres away from the riverbank. This study focuses on evaluating the water quality of the Brahmani River, which has been affected by the wastewater discharged from the Talcher industrial complex. This waste water is channeled through the Nandirajhor and released into the river. The industrial complex was established in close proximity to the coal mines of Talcher and encompasses various facilities including thermal power stations, a coal-based fertilizer plant operated by The Fertilizer Corporation of India Ltd (FCIL) that produces urea/ chemicals, Captive Power Plant (NALCO) for electricity power generation, Smelter Plant (NALCO) for Aluminum production and Talcher Heavy Water Plant (Department of Atomic Energy) for special chemicals i.e. Boron Trifluoride (BF_3 gas), Calcium fluoride complex (BF_3CaF_2). The effluent from the fertilizer plant is directed through the Deojhar Nallah, which eventually joins the Nandirajhor, carrying effluents from other units and wastewater from the Talcher township. Ultimately, the combined waste from the NandiraJhor finds its way into the Brahmani River. Both treated and untreated municipal

and industrial wastes are discharged directly into the river from different points. For this study, two specific regions were chosen for sampling: the relatively less affected upstream region of the Rengali Dam with latitude 21.271341°N and longitude 85.017319° E (control site), the Talcher industrial complex (experimental site including Kamalanga with latitude 20.61021°N and longitude 85.2010639°E and Kharagprasad with latitude 20.610213°N and longitude 85.2010609°Eboth located downstream of the industrial area) (Figure 1). The samples were collected during day time from 10 AM to 5 PM at a distance of about 5 meters inside the river from the bank and at a depth of about 0.25 meter.

Measurement of physico-chemical parameters of water samples

The physical and chemical parameters were estimated according to methods described in standard methods for the examination of water and wastes water (APHA, 1992).

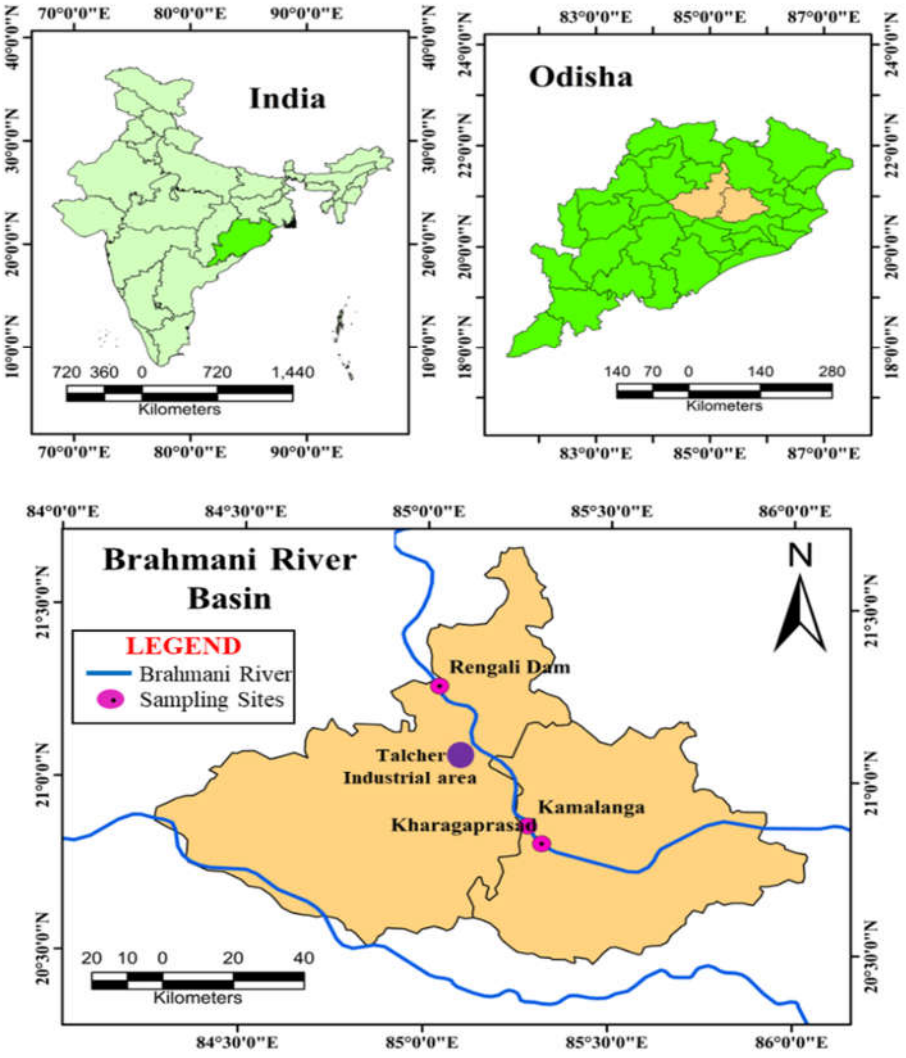

Figure 1 Map of the study area and location of sampling sites in the Brahmani River.

Fish sampling and estimation of toxic metals

Sampling of fish was conducted between the months of August to October (post-monsoon period) using fishing nets and the captured fish were immediately placed in clean, isolated polythene bags containing aerated river water. These samples were swiftly transported to the laboratory at the Department of Zoology, Ravenshaw University, Cuttack, within an hour of collection. Control samples were obtained from ICAR-Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar. Fish were selected for the study from each site, with an average body weight of 105.75 ± 33.21 grams and a total length of 24.22 ± 1.98 centimetres (n = 15 per site). Toxic metals (Fe, Cr and Pb) concentration in the water samples and muscle of *L. bata* were estimated using atomic absorption spectrophotometer after overnight digestion with a solution of concentrated HNO_3 and $HClO_4$ (3:1) at 80 °C (Mohanty and Samanta, 2016).

Measurement of oxidative stress biomarker

Lipid peroxidation (LPx) in the muscle of the fishes collected was estimated following the method of Ohkawa et al. (1979) and Mohanty and Samanta (2016). The molar extinction coefficient of thiobarbituric acid reactive substance (TBARS) 156 mM^{-1} cm^{-1} (Wills, 1969) was used to determine the results, which were expressed as nmoles equivalent of malondialdehyde (MDA) produced per mg protein. As a reference of oxidative stress under optimal conditions, LPx was also assessed in fish tissues obtained from the farming sites of ICAR-Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar.

RESULTS

The physico-chemical characteristics in the water samples collected from the study sites are presented in Table 1. The dissolved oxygen concentration of the downstream (Kamalanga and Kharagprasad) was significantly lower than the upstream region (Rengali dam). Although the total alkalinity of water from both the regions were within the limits approved by the Bureau of Indian Standars (BIS), its level is substantially higher in the downstream water. A significant decline in dissolved oxygen concentration with concomitant augmentation in biological oxygen demand (BOD) was also noticed in the downstream water samples (Table 1).

The concentrations of Cr and Pb in both the sampling sites are higher than BIS approved standards and their concentrations in the downstream is significantly higher than the upstream (Table 2). However, concentration of Fe was found to be within limit in both sites.

The muscle tissues of *Labeo bata* fish collected from upstream and downstream sites showed higher levels of Cr i.e., 0.089 mg/kg dry weight and 0.12 mg/kg dry weight, respectively than the permissible limits (0.05 mg/kg dry weight) recommended by National Research Council (1989) and World Health Organization (2017). Although the level of Pb (0.032 mg/kg dry weight) was low in upstream, its level was significantly higher in the downstream i.e, 0.043 as compared to upstream. On the other hand, Fe concentration in the muscle of *L. bata* in both sites were found to be within the limits (186 mg/kg dry weight) recommended by National Research Council (1989) and World Health Organization (2017) (Figure 2).

The levels of LPx (Figure3) in the muscle showed significant elevation in both the upstream and downstream sites in comparison to reference i.e., 0.6248 ± 0.13 nmol TBARS formed per mg protein while the downstream fishes showed the highest level i.e., 6.73 ± 1.02 nmol TBARS formed per mg protein.

Table 1 Physico-chemical parameters of Brahmani River water collected from upstream (Rengali Dam) and Down Stream (Kamalanga and Kharagprasad) of Talcher Industrial complex. Data are expressed as Mean ± sd of 5 observations taken in duplicate. *p<0.05 with respect to upstream.

Parameters	**BIS (IS 10500 (2012) approved limits**	**UP Stream**	**Down Stream**	**Status**
Temperature (°C)	38-43 (Max) 10-15(Min)	24.67±3.50	30.67±2.15	Ambient
pH	6.5 to 8.5	7.42±0.29	7.763±0.041	Within limit
Conductivity ($\mu S\ cm^{-1}$)	300 – 800	133 ± 15	358.33 ± 23.28*	Within limit
TDS (mg/L)	500	160.273±1.87	232.93±4.39*	Within limit
Total alkalinity $CaCO_3$ (mg/mL)	50	22.423±1.53	36.353±5.38*	Within limit
DO (mg/L)	5 mg/L or more	8.533±0.17	4.67±0.23*	Below permissible limit
BOD	3 mg/L or less (3 days 27^0C for bathing) and 2 mg/L (5 days at 20^0C for drinking)	0.753±0.12	2.816±0.07*	Above permissible limit

Table 2 Concentration of toxic metals in Brahmani River water collected from upstream (Rengali Dam) and Down Stream (Kamalanga and Kharagprasad) of Talcher Industrial complex. Data are expressed as Mean ± sd of 5 observations taken in duplicate. *p<0.05 with respect to upstream.

ELEMENTS (mg/L)	**BIS (IS 10500 (2012) approved limits**	**UP Stream**	**Down Stream**
Cr	0.05	0.01 ± 0.0001	0.10 ± 0.0001*
Pb	0.01	0.016 ± 0.004	0.022 ± 0.008*
Fe	0.30	0.05 ± 0.0001	0.05 ± 0.0001*
Cu	0.05	0.019± 0.0015	0.017 ± 0.002
Mn	0.1	0.049± 0.001	0.051 ± 0.003
Ni	0.02	0.0048 ± 0.0002	0.0047 ± 0.0003
Cd	0.003	0.003± 0.001	0.002 ± 0.0001

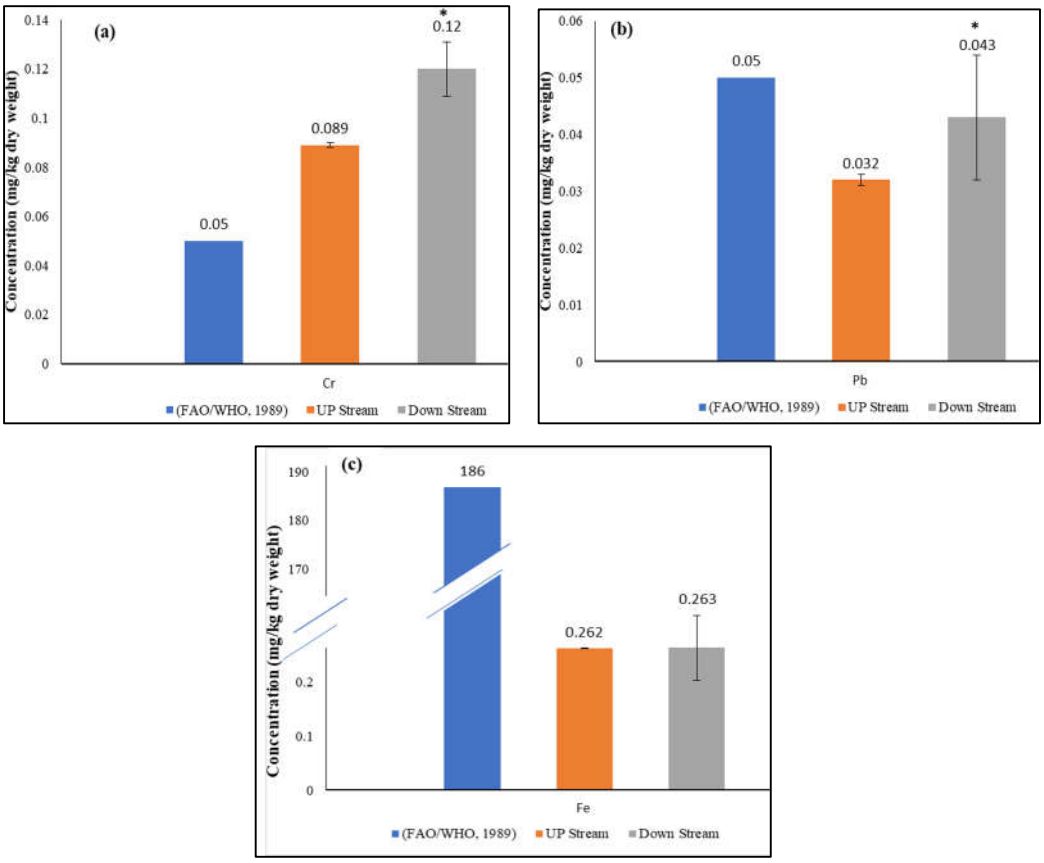

Figure 2 Concentration of toxic metals (**a**) Cr, (**b**) Pb, (**c**) Fe in the muscle of *Labeo bata* fish collected from upstream (Rengali Dam) and Down Stream (Kamalanga and Kharagprasad) of Talcher Industrial complex of the Brahmani River. Data are expressed as Mean ± sd of 5 observations taken in duplicate. *p<0.05 with respect to upstream.

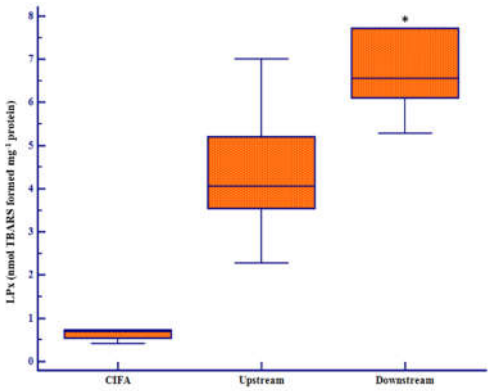

Figure 3 Lipid peroxidation (LPx) in the muscle tissues of *Labeo bata* collected from upstream (Rengali Dam) and Down Stream (Kamalanga and Kharagprasad) of Talcher Industrial complex of the Brahmani River and CIFA (Reference). Data are expressed as Mean ± sd of 5 observations taken in duplicate. *p<0.05 with respect to upstream.

DISCUSSION

River contamination by harmful substances is a global concern, especially in developing countries, causing severe environmental and health impacts. Various sources contribute to river pollution, including industrial waste, mining activities, agricultural runoff, sewage discharge, and improper waste disposal. Monitoring water bodies is crucial for policy-making and remediation. Although water quality assessments for the Brahmani River have been conducted by different labs (Nayak and Mohanty, 2018; Nayak 2020), many reports focus on the Rourkela Industrial Complex (Dey et al 2005). Notably, the Brahmani River, the state's second-largest, requires attention. The Central Pollution Control Board (CPCB) reported in 2020 to the National Green Tribunal (NGT) that the river is among the three most polluted in the state. Thus, our study aimed to analyze physicochemical parameters and the bioaccumulation of toxic metals in fish muscles to assess health risks in the food chain.

Fish and other organisms thrive within a specific temperature range; deviations result in decreased populations. Water temperature influences chemical reactions, impacting fish growth, reproduction, and immunity. It also affects water chemistry; warmer water holds less dissolved oxygen. Our findings align with the findings of Nayak (2020), indicating the highest temperature at Kamalanga (downstream station of our study).

Conductivity (EC) and total dissolved solids (TDS) are common water quality indicators (Marandi et al., 2013). Their values often influenced by dissolved ions, average ion activity, and ionic strength (Hayashi, 2004). Dissolved oxygen is negatively correlated with Biological Oxygen Demand (BOD), TDS, and dissolved heavy metals, reflecting organic pollution and microbial activity (Sharma et al., 2021). Rising pollution-related parameters reduce dissolved oxygen. In the present study the BOD level exceeded drinking water standards but remained suitable for bathing and irrigation. The contamination of aquatic ecosystems by toxic metals from both natural and human sources has emerged as a global concern (Ibemenuga, 2013). These heavy metals, due to their toxicity, persistence, and ability to accumulate in food chains, present potential risks to both ecological systems and human health (Salem et al., 2014). The assessment of the aquatic environment is essential not only to halt biological degradation but also to identify the sources that disrupt the ecological equilibrium (Ashraf et al., 2012; Salam et al., 2019). Notably, the concentrations of chromium (Cr) and lead (Pb) in both water and fish muscle were significantly disparate between the two sites, with notably higher levels downstream. While the concentration of Pb and iron (Fe) in muscle adhered to acceptable limits, the Pb levels were relatively elevated in 3 out of 5 samples, warranting analysis of a larger fish samples to ascertain the extent of bioaccumulation of this extremely toxic metal. Furthermore, the concentration of Cr in the muscle collected downstream exceeded permissible limits. It is evident that metals manifest toxicity in fishes by inducing oxidative damage by generating highly reactive hydroxyl radicals (•OH) via Fenton type reaction (Sakuragui et al., 2013, Mohanty and Samanta, 2016). Hydroxyl radicals have the ability to attack nearly all biomolecules such as lipids, protein, and nucleic acids within one atomic radius, resulting in lipid peroxidation, protein carbonylation, and DNA strand breaks (Halliwell and Gutteridge, 2015). As a result, it would not be out of place to state that water from the river collecting urban runoff and wastewater from industry on its banks would increase the rate of ROS generation in living organisms. Our result showed a substantial increase in the level of the oxidative stress indicator, LPx, suggesting the existence of higher amount of ROS in the tissues of fish collected from the downstream site of Brahmani River corroborating with higher levels of redox acting transition metals Cr and Pb. The Pb level is within the acceptable limits set by WHO (2022), there's a notable disparity in Pb concentrations between downstream and upstream fish populations, with downstream fish exhibiting significantly higher levels, which along with high Cr content potentially leading to elevated lipid peroxidation (LPx) in their muscle tissue.

Fish serve as apex consumers in aquatic food chains, offering not only crucial protein but also vitamins, essential minerals, and unsaturated fatty acids. However, their capability to accumulate heavy metals from water, food, and sediment can undermine their beneficial attributes (Sarah et al., 2019). The detrimental effects of heavy metals on human health, including kidney failure, cardiovascular disorders, liver damage, and even fatality, have been well-documented (El-Moselhy et al., 2014). Additionally, heavy metal accumulation in fish can have enduring impacts on biogeochemical cycles (Yi et al., 2011; Gu et al., 2017). These metals accumulate as they ascend the natural food chain, potentially reaching hazardous levels for human consumption (Ip et al., 2005; Yi et al., 2011). Consequently, it is imperative to evaluate the accumulated chemical content, particularly pollutants such as toxic metals, in commonly consumed fish species.

CONCLUSION

The depletion of oxygen and the elevated value of BOD suggest that the pollution level at various locations along the Brahmani River is trending upward. A water body that experiences a depletion of DO will be unable to sustain aquatic life and will lose its inherent ability to purify water. Water quality has been noted to be declining from upstream following the mixing of Talcher Industrial Complex effluents. Additionally, mixing industrial waste raises the concentration of heavy metals in downstream water, which eventually causes these metals to bioaccumulate in fish tissue and subsequent entry into the food chain. It can affect fish health and lower the quality of fish meat by contributing to the generation of free radicals, which is evident from high LPx levels.

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CHRONIC EFFECTS OF FLUORIDE EXPOSURE ON GROWTH AND OSSIFICATION OF BONES IN THE TADPOLES OF THE ORNATE FROG, *MICROHYLA ORNATA* (ANURA: MICROHYLIDAE)

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ABSTRACT

Increase in fluoride toxicity has been constantly identified as a critical stressor, leading to the arrival of deformities resulting in the rapid waning of the global amphibian population. The current study sets out to ascertain how different doses of long-term exposure to fluoride (1mg NaF/L, 5mg NaF/L and 10 mg NaF/L) affected the tadpoles of the ornate frog, *Microhyla ornata*, including their overall body growth and ossification of skeleton. Development of tadpoles exposed to NaF exhibited retardation of growth as evident from the body weight, snout vent length and total body length in comparison to the tadpoles of the control group. Ossification of bones and onset of metamorphosis was delayed in the experimental tadpoles in a dose dependant manner. NaF exposure had dose dependant toxic effect on survival of tadpoles as 10 to 40% tadpoles of the treated group died prior to onset of metamorphosis. Based on the present findings it is reasonable to conclude that chronic exposure to NaF not only exhibits toxic effect on the tadpoles but also retards general growth, metamorphosis and ossification of bones.

Keywords: *Microhyla ornata*, fluoride, metamorphosis, mortality, ossification

INTRODUCTION

Fluorine is a negatively charged, non-metallic halogen existing widely in the environment as an organic and inorganic compound (Chen et al., 2016a, b; Zuo et al., 2018). It is a ubiquitous element in the earth's crust and never exists in its elemental state due to its high reactivity (Camargo, 2003). The increase in anthropogenic inputs has been proportionally increasing fluoride concentration in aquatic environments. Various kinds of anthropogenic activities like fertilizer production, aluminium smelting, and production of bricks, ceramics, and glass, induce a significant rise in fluoride concentration in water either by direct deposition or by deposition to soil and subsequent runoff into water (Metcalfe-Smith et al., 2003; Jha et al., 2011; Chai et al., 2016). In general, the unpolluted freshwaters show fluoride concentration ranging from 0.01 to 3 mg/L, while its natural concentration may exceed even upto 50mg/L (Camargo, 2003). Higher fluoride content has been reported in many aquatic environments with values ranging from 2.3 to 49 mg/L (Brahman et al., 2013; Rafique et al., 2015). Fluoride toxicity have been documented in plants, animals and humans (Choubisa et al., 2022). Based on the adverse effects of fluoride, World Health Organization (WHO, 2017) and Bureau of Indian Standards (BIS, 2012) have recommended maximum permissible limit of fluoride in drinking water to be 1.5 mg/L.

Fluoride contamination has been constantly identified as a critical stressor in aquatic ecosystems (Cao et al., 2015). Anuran amphibians have a large potential as bioindicators and they serve to be an excellent model to access the chronic effect of fluorides on aquatic environments because of their highly permeable skin, unshelled eggs, and exposure to aquatic environments at two different early life periods, embryos and larvae (Babini et al., 2015; Chai et al., 2016). Amphibians show both the role of prey and predators, making up a fundamental element in the accumulation and transfer of pollutant substances between aquatic and terrestrial environments (Marques et al., 2013). Several studies have explained the adverse effect of fluoride on amphibians. In amphibians, high fluoride

concentrations can cause negative effects including growth inhibition, metabolism alterations, behavioural changes, bone abnormalities, generalized edema, stunted growth, and ruffled dorsal and/or ventral tail fins (Camargo, 2003; Chai et al. 2016, 2017 and Chen et al., 2016a). The teratogenic effect of sodium fluoride inhibiting the metamorphosis of *Bufo gargarizans* at a concentration of 50 mg/L was explained by Goh and Neff (2003). Dysmorphosis of *Xenopus* and *Rana chensinensis* embryos and anomalies in endochondral ossification and lipid metabolism of *Bufo gargarizans* were also reported (Goh and Neff, 2003; Chai et al., 2016; Bo et al., 2018 and Wu et al., 2018). Pollo et al. (2019) have described diets and morphological indicators of anuran tadpoles inhabiting a fluoride-rich stream. In an interesting study, osteofluorosis was documented in New Zealand native frogs (*Leiopelma spp*) from reduced ultraviolet B (UVB) light exposure and a diet low in the Ca:P ratio after the use of fluoridated water with 0.94 ppm F. However, the captive frogs were made to recover with increased UVB light exposure, a diet with a higher Ca:P ratio, and the use of defluoridated water with 0.06 ppm F (Spittle, 2012). These frogs warn that fluoridated water may not be safe for humans. The significance and beneficial effects of fluoride ion on human health when present in a trace amount has well been documented. However, at higher concentration, fluoride ion causes acute and chronic health problems including incurable impacts on human beings (Tiwari et al., 2023). Woefully, to date, there are negligible studies about the toxic effect of fluoride on anurans tadpoles from Indian context. The tadpoles of the ornate frog, *Microhyla ornata* was selected as the model animal for assessment of fluoride toxicity due to the local availability in abundance and this species has been categorised as least concerned according to IUCN red list (2023). The findings will be beneficial in predicting the population-level effects of fluoride toxicity and can contribute towards establishing a long-term amphibian protection programme.

MATERIALS AND METHODS

Collection of tadpoles, rearing and experimental set up

Tadpoles of *Microhyla ornata* were collected from various breeding grounds within the Utkal University campus (Lat: 20.30811, Long: 85.84127) during the month of July- September. They were taken from their native environment using a hand net and reared in the laboratory following standardised procedure (Mohanty-Hejmadi, 1977). The collected tap water was aerated for 72 hours and conditioned (CTW) for the rearing of tadpoles. The tadpoles were fed with smashed egg yolk *ad libitum* by scattering across the water surface in each container. For the study, the limb bud stage tadpoles i.e., Gosner stage 26 to 28 (Gosner, 1960) were selected. The tadpoles were split up into 8 experimental tubs, 2 of which were used as controls while the other 6 contained treated water with the required concentration (1mg/L, 5 mg/L and 10 mg/L) of sodium fluoride. Each treatment was triplicated with 5 tadpoles per replicate. The LC50 value for the tadpoles was found to be 12mg NaF/L at 48 hr. Therefore, tadpoles were exposed to three different fluoride treatments: 1mg NaF/L, 5mg NaF/L and 10 mg NaF/L. A litre of dechlorinated water was added to the aforementioned concentration in which five tadpoles were reared. To preserve water quality and fluoride concentration, the treated water was replaced every 48 hours.

Morphometrics Study

The total body length, Snout-vent length (SVL) and body weight of the tadpoles, were taken on the 7^{th}, 14^{th}, $21^{st,}$ and 28^{th} day from the onset day of the experiment. By using a caliper, the body length and snout to vent length were measured and noted. The body weight was recorded using Mono Pan balance. The above measurements were done for both treated and control group tadpoles. The data were recorded and statistically analysed to study the variations at different concentrations.

Determination of the skeletal development

The double staining method of Wassersug (1976) was used to examine the skeletal development of tadpoles. From each treated concentration and control group 2 tadpoles were selected randomly. Tadpoles were selected from 28th day from the onset of the experiment. Then tadpoles were fixed by 4% formalin and were stained using the alcian blue-alizarin red double staining method.

RESULTS

Survivability and onset of metamorphosis

Control tadpoles showed better (99%) survivability compared to NaF-treated tadpoles during the experiment period of 28 days (Figure 1). Chronic exposure to NaF showed a slow and sluggish rate of development of tadpoles. Mortality of treated tadpoles was dose dependant. 40% tadpoles died prior to onset of metamorphosis (emergence of forelimbs) in the 10mg/L NaF-treated tadpoles (Figure 1) on chronic exposure as compared to exposure to low dose (10% in 1mg/L and 20% in 5mg/L NaF treated tadpoles). During the twenty-eight days experimental period, onset of metamorphosis (emergence of fore limbs) did not occur in any tadpole. However, in 90% of the control tadpoles, fore limbs emerged within the experimental period.

Length and body weight of tadpoles

Fluoride exposure resulted in reduced total length, snout to tail base length (SVL), and the weight of the tadpoles compared to the control group (Tables 1 to 3). At the start of the experiment, the mean total length (mm) of the tadpoles was 10.69 ± 0.0236, the mean SVL (mm) was 8.92 ± 0.245 and the mean body weight (mg) was 0.027 ± 0.001. The mean body weight, mean total length and mean SVL were significantly (one-way ANOVA, $p < 0.05$) affected by experimental treatments relative to untreated control. Significant reductions in total length, SVL and body weight relative to controls ($p < 0.05$) were observed at higher concentration (10 mg NaF/L) on day 7, day 14, day 21 and day 28 of treatment (Figures 2 to 4). Increase in total length (Figure 2) of tadpoles were significantly inhibited by fluoride concentration of 1mg Na F/L relative to untreated controls (14.82±0.507, 17.00±0.270, 21.15±0.032, 22.01±0.260 on day 7, 14, 21, and 28, respectively). In 1mgNaF/L treated group, the total length was significantly reduced on day 14 (15.05±0.263), day 21(18.72±0.352) and day 28 (19.26±0.236) ($p < 0.05$). Total length of 5mg NaF/L treated tadpoles (Figure 2) were also significantly reduced on day 14 (13.03±0.188), day 21 (16.05±0.277), and day 28 (18.55±0.246) compared to control. Total length of 10mg NaF/L treated tadpoles was significantly reduced on day7 (10.73±0.319), day 14 (12.90±0.216), day 21(15.53±0.290), and day 28 (17.44±0.171). The SVL (Figure 3) of 1 mg NaF/L treated tadpoles was significantly reduced compared to control (10.22±0.285, 11.04± 0.245, 13.16±0.416 and 14.97±0.275 on day 7, 14, 21, and 28, respectively) at day 21 (12.95±0.462) and day 28 (14.81±0.307), but the reduction at day 14 (10.85±0.246) was not significant. 5mg NaF/L treated tadpoles had significant reduction in SVL on day 21 (12.61±0.472) and day 28 (14.47±0.298). 10mg NaF/L treated tadpoles had significant reduction in SVL on day 7 (9.09±0.264), day 14 (10.17±0.182), day 21 12.11±0.499), and day 28 (13.97±0.216). Moreover, body weight (Figure 4) was significantly lower at 1mg NaF/L compared to control (0.034±0.002, 0.039±0.001, 0.049±0.001, and 0.068±0.001 on day 7,14,21, and 28 respectively) on day 7 (0.0298±0.004), day 14 (0.037±0.001), day 21 (0.041±0.001) and day 28 (0.055±0.002) ($p < 0.05$). Body weight of 5mg NaF/L treated tadpoles was also significantly reduced compared to control on day 7 (0.272±0.002), day 14 (0.032±0.003), day 21 (0.036±0.001) and day 28 (0.045±0.002). In 10mg NaF/L treated tadpoles body weight was significantly reduced on day 7 (0.0217±0.001), day 14 (0.022±0.002), day 21 (0.027±0.001), and day 28 (0.035±0.002), respectively.

Ossification in Control and treated tadpoles on 28th day

A comparative account of ossification of different skeletal elements of the control tadpoles was assessed on day 28 of chronic exposure to different concentration of NaF/L. In the control group tadpoles (Figure 5A), frontoparietal, exoccipital bones of skull were observed clearly with ossification. All the arches and transverse processes of bones of vertebral column were completely ossified. The pectoral girdle bones (the clavicle, the coracoid, the scapula) and forelimb bones (humerus, antibrachium, carpus, ossa metacarpalia and the phalanges) were well ossified. Ossification of the pelvic girdle bone (ilia, ischia, pubis) and hindlimb bones (femur, tibia fibula), and the pes (tarsals and phalanges) were distinct.

In 1mg NaF/L treated group (Figure 5B), the exoccipital bone was clearly ossified, hindlimb bones (femur and tibia fibula) were ossified. The pelvic girdle bones were also ossified. The arches and transverse process were less ossified compared to control. In 5mg NaF/L treated group there was no distinct ossification of frontoparietal, exoccipital, or prootic skull bones. The girdle and the limb bones were cartilaginous. Slight ossification was observed in the transverse process. In 10 mg NaF/L treated group (Figure 5C) the first vertebrae only showed very little ossification. The transverse process was completely cartilaginous. The chondrocranium was lightly stained suggesting they were not well chondrified. Also, no obvious ossification was observed in the vertebral column, pelvic girdle and hind limb bones.

Table 1 Total body length (mm) of control and NaF -treated tadpoles. Values are Mean ± SE

Sample	Day 7	Day 14	Day 21	Day 28
Control	14.82 ± 0.507	17.00 ± 0.270	21.15 ± 0.302	22.01 ± 0.260
1 mg NaF/L	12.93 ± 0.569	15.05 ± 0.263	18.72 ± 0.352	19.26 ± 0.236
5 mg NaF/L	11.81 ± 0.597	13.03 ± 0.188	16.05 ± 0.227	18.55 ± 0.246
10 mg NaF/L	10.73 ± 0.319	12.90 ± 0.216	15.53 ± 0.290	17.44 ± 0.171

Table 2 Snout to vent length (SVL) (mm) of control and NaF-treated tadpoles. Values are Mean ± SE

Sample	Day 7	Day 14	Day 21	Day 28
Control	10.22 ± 0.285	11.04 ± 0.245	13.16 ± 0.416	14.97 ± 0.275
1 mg NaF/L	9.62 ± 0.379	10.85 ± 0.246	12.95 ± 0.462	14.81 ± 0.307
5 mg NaF/L	9.39 ± 0.317	10.58 ± 0.248	12.61 ± 0.472	14.47 ± 0.298
10 mg NaF/L	9.09 ± 0.264	10.17 ± 0.182	12.11 ± 0.499	13.97 ± 0.216

Table 3 Body Weight (mg) of control and NaF-treated tadpoles. Values are Mean ± SE

Sample	Day 7	Day 14	Day 21	Day 28
Control	0.034 ± 0.002	0.039 ± 0.001	0.049 ± 0.001	0.068 ± 0.001
1mg NaF/L	0.0298 ± 0.004	0.037 ± 0.001	0.041 ± 0.001	0.055 ± 0.002
5 mg NaF/L	0.0272 ± 0.002	0.032 ± 0.003	0.036 ± 0.001	0.045 ± 0.002
10 mg NaF/L	0.0217 ± 0.001	0.022 ± 0.002	0.027 ± 0.001	0.035 ± 0.002

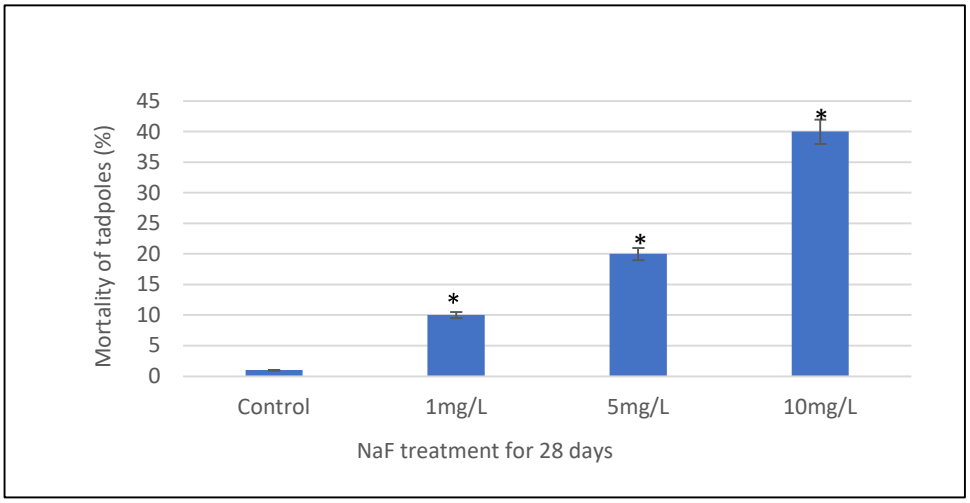

Figure 1 Mortality (%) of control and NaF treated tadpoles on 28th day. Values are Mean ± SE. Compared to control group (one-way ANOVA): *p<0.05.

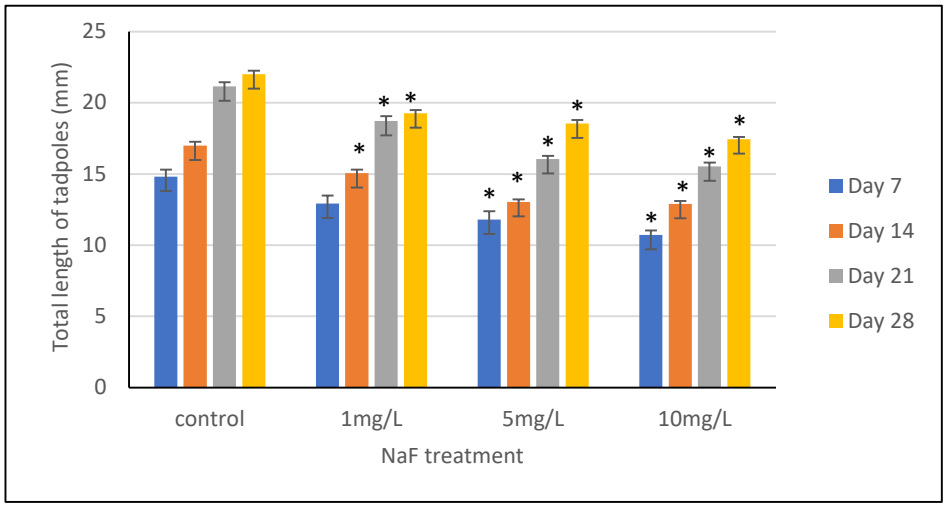

Figure 2 Total length (mm) of control and NaF treated tadpoles on 7 th, 14th, 21st and 28th day. Values are Mean ± SE. Compared to the control group (0ne-way ANOVA): *p<0.05.

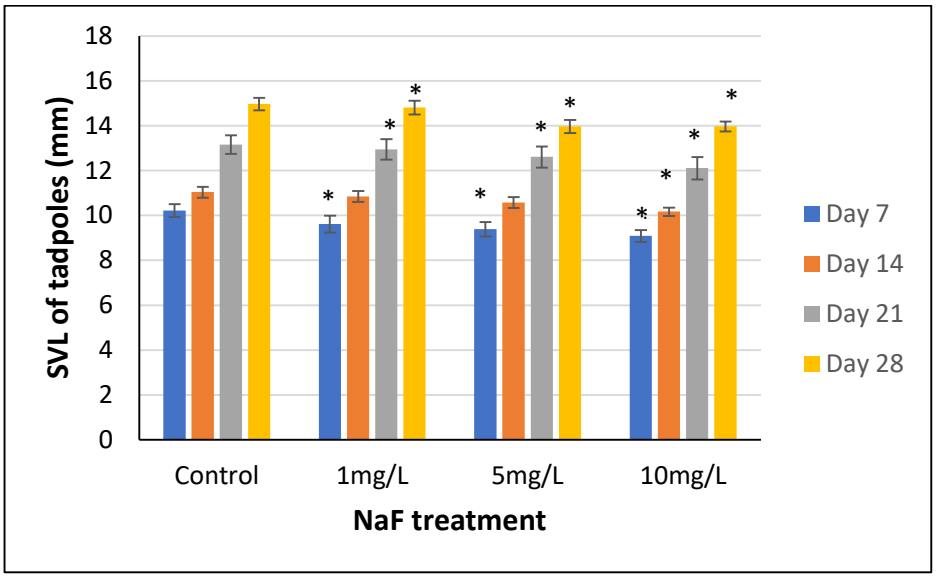

Figure 3 SVL (mm) of control and NaF treated tadpoles on 7th, 14th, 21st and 28th day. Values are Mean ± SE. Compared to the control group (0ne-way ANOVA): *p<0.05.

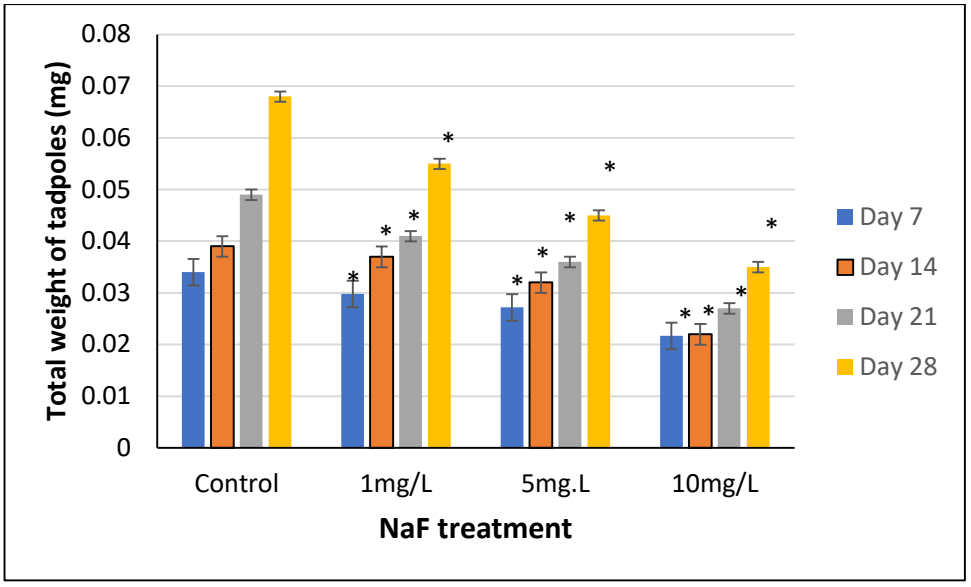

Figure 4 Total weight (mg) of control and NaF treated tadpoles on 7th, 14th, 21st and 28th day. Values are Mean ± SE. Compared to the control group (0ne-way ANOVA): *p<0.05.

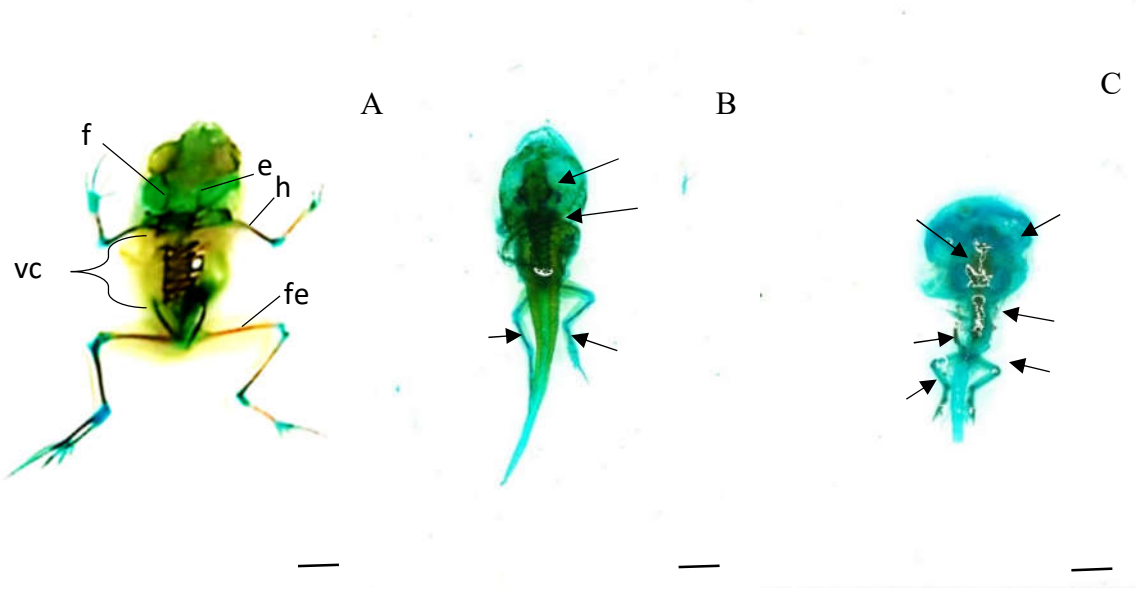

Figure 5 Dorsal view of whole skeleton of double stained tadpoles showing ossification for bone (red) and cartilage (blue) on 28th day (A- control; B- 1mg NaF/L; C- 10mg NaF/L). The arrows indicate ossification differences between treated and control tadpoles, e- exoccipital, f- frontoparietal, fe- femur, h- humerus, vc-vertebral column. Scale bar in panels A-C= 5mm.

DISCUSSION

Our results showed that chronic fluoride exposure significantly inhibited metamorphosis and delayed development in *Microhyla ornata* tadpoles. Mortality increased in a concentration-dependent manner during fluoride exposure. Increased mortality of tadpoles with exposure to high concentrations of fluoride indicates that fluoride may be seriously hazardous for amphibian survival. It is reported that exposure to 50 mg NaF/L had a significant effect on the mortality of *Rana chensinesis* and *Rana nigromaculata* tadpoles (Chen et al., 2016a). Pal et al. (2018) have reported acute toxicity and oxidative stress responses in tadpole of skittering frog, *Euphlyctis cyanophlyctis* to sodium fluoride exposure. Inhibitory effects of other chemicals on amphibians such as fungicide prochloraz, polybrominated diphenyl ethers, and cadmium (Flament et al., 2003; Brande-Lavridsen et al., 2008; Bach et al., 2016) have been documented.

Fluoride exposure resulted in reduced total length, SVL, and weight of tadpoles compared to the control group. Our study showed that mean body weight, mean total length, and mean SVL were significantly affected by treatment with fluoride relative to untreated control tadpoles. Treatment with higher concentration (10 mg/L) showed significant reductions in total length, SVL and body weight. Significant reductions in SVL, total length, and body weight relative to controls ($p<0.05$) were observed at 5 and 10 mg NaF/L on day 21 and day 28 of treatment. Our observation of reduced body weight and total length with NaF exposure is consistent with the finding by Goh and Neff (2003) that NaF exposure reduced the head-tail length and eye diameter of *Xenopus* embryos. Wang et al. (2019) have reported the effect of fluorine exposure on morphological indicators and intestinal microbial community in *Bufo gargarizans* tadpoles. They have described that fluoride declined total body length and body weight of tadpoles.

The normal mineralization of bone mainly involves the deposition of calcium. Usually, alizarin red can combine with calcium ions from bone tissue in a chelation process. With the double staining technique with alcian blue and alizarin red, we found that the ossification of the skeletal system was influenced by fluoride treatment. High dose of fluoride (10mg/L) retarded calcium deposition in the bone as compared to a lower dose (1mg/L). Our observation of reduced ossification with fluoride exposure are consistent with findings by Zhao et al. (2013) that fluoride exposure retarded skeletal development in *Bufo gargarizans* tadpoles. In the NaF-treated group, the ossification of frontoparietal and exooccipital bones was significantly less than that in the control group. The ossification of the vertebral column, girdle bones and limb bones were also less in all experimental tadpoles as compared to the tadpoles of the control group. In a similar experiment on *Rana nigromaculata* larvae it was found that fluoride delayed the development of the skeleton by reducing the apposition rate of calcium ions leading to a prolongation of the mineralization lag time and the bone formation period (Chen et al., 2016a). However, in *Rana chensinesis* tadpoles, fluoride stimulated bone mineralization (Chen et al., 2016a). Hence, it can be inferred that fluoride exposure affects skeletal ossification differently in different species of frog depending upon the dose and duration of the exposure. Chai et al., (2017) have reported adverse effect of Fluoride exposure on growth, metamorphosis, and skeleton development in the tadpoles of *Bufo gargarizans.* It has been suggested that fluoride could damage follicular cells in thyroid gland and induce a sharp reduction in thyroid hormone leading to delayed metamorphosis as well as ossification in bone tissue by inhibiting calcium deposition in the tadpoles of *Bufo gargarizans* (Zhao et al., 2013). Thus, it is proposed that retardation of growth in the NaF exposed tadpoles of *Microhyla ornata* in the present study may be due to the buildup of fluoride in the body, resulting in an inhibition of deposition of calcium ions in the skeletal element of the developing tadpole. In addition, the reason for delayed metamorphosis could potentially be due to thyroid alterations and reduction in synthesis of thyroid hormone. Understanding the molecular mechanism leading to growth retardation, delayed metamorphosis and ossification of skeletal elements in the tadpoles of *Microhyla ornata* exposed to NaF is the futuristic work of this investigation.

CONCLUSION

Based on the findings of the present study it is concluded that chronic exposure to fluoride increased mortality, inhibited metamorphosis, and delayed development of the *Microhyla ornata* tadpole. Higher doses of fluoride had adverse effects on the total length, SVL, weight and metamorphosis rate of the tadpole as compared to lower doses. Based on the differential alizarin red and alcian blue staining, it is suggested that chronic fluoride exposure affected bone mineralization, inhibiting calcium deposition. Our results therefore suggest that high fluoride in aquatic ecosystems may represent a dangerous threat to the fitness of *Microhyla ornata* tadpoles.

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EXTRACTION AND CHARACTERISATION OF CHITIN AND CHITOSAN FROM FRESH WATER PRAWN (*MACROBRACHIUM ROSENBERGII* AND *M. MALCOLMSONII*) WASTES

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ABSTRACT

Commercial production of chitin and chitosan is a multi-billion-dollar business due to their use in diverse fields. Crustacean (crabs and shrimps) shells are the principal source of such production. Chitin and chitosan were extracted from the wastes of fresh water prawns *Macrobrachium rosenbergii* and *M. malcolmsonii* and characterised through XRD. The exoskeleton (shell and carapace) of *M. rosenbergii* is a better source of chitin (40.75%) and chitisan (36.52%) than *M. malcolmsonii* (30.84% and 26.05%, respectively). The protein content, moisture content, ash content, water and fat binding capacity and mineral contents are comparable in both the species. Since the aquaculture industry mostly uses these two prawn species for farming, a potential way for the utilisation of the huge waste generated will go a long way in reducing environmental pollution, employment generation and improvement of economy.

Keywords: Prawn waste, Chitin, Chitosan, Protein, Minerals

INTRODUCTION

The shell fish (prawns, shrimps, crabs and lobsters) industry is operative among all the costal countries and contributes significantly to the food delicacies as well as economy. About 45 to 60% of the whole weight processed is generated as waste (operculum, shells and head) and is used as a feed supplement or is discarded directly on to the land by food industries, seafood markets and capture fisheries (Shahidi et al., 2019). It is estimated that the shell fish industry produces about 1, 25, 000-1, 50, 000 tons of waste per annum in India only (Bhattacharya et al., 2022). The chitinous wastes from the crustacean's operculum and shell could be considered hazardous as a result of uncontrolled dumping. In the sea, it leads to eutrophication and a high load of biological oxygen demand (BOD), while on land it can result in pathogen-borne problems. The rate of bio-degradation of such an enormous amount of waste generated per processing operation is comparatively slow (Vigneshwari and Gokula, 2018). Hence, disposal of such wastes has become a serious environmental concern (Abdel-Shafy and Mansour, 2018; Chakravarty and Edwards, 2022). Prawn waste usually contains, on a dry basis, valuable components such as prawn protein (35–50%), minerals (30-40%), carotenoid (prawn pigment- 58-116 mg/kg), the major component of prawn shell material being chitin (15–25%).

Crustacean shells, the most common source of chitin, are constituted mainly of a matrix made of chitin and protein, hardened by mineral salts. It can be obtained by demineralising and deproteinizing the wastes (Younes and Rinaudo, 2015) and further deacetylated chemically or enzymatically to form chitosan. Chitin is a relatively 'new' polymer in research and food processing applications. Muzzarelli (1977) made an extensive review on chitin, including its sources, preparation, chemistry and applications. Multiple researchers conducted a review of chitin, covering its sources, preparation, chemistry, and applications (Kumar, et al., 2004; Younes and Rinaudo, 2015).

Chitin and its derivatives, including chitosans, are used in agricultural, food and pharmaceutical sectors: as a cationic biopolymer, dietary fiber, antimicrobial coating material, food packaging film, bioactive compound in wound healing and controlled drug release, wastewater treatment aid and plant growth stimulator, just to list a few (Kumar, et al., 2004; Casadidio et al., 2019). Their unique properties, biodegradability, biocompatibility and non-toxicity make them useful for a wide range of applications (Elieh-Ali-Komi and Hamblin, 2016; Jiménez-Gómez and Cecilia, 2020; Abourehab et al., 2022). The importance of chitin and chitosan has grown partly because they represent a renewable and biodegradable source of materials, and partly because of the recent increased understanding of their functionality in various applications. India is the second largest country of fresh water prawn farming after China. All farmed freshwater prawns in India today belong to the genus *Macrobrachium* (includes about 200 species). *M. rosenbergii* (the largest or giant fresh water prawn in the world) and *M. malcolmsonii* (monsoon river prawn*)* are the widely farmed species (Nair and Salin, 2012). The global annual production of freshwater prawns (excluding crayfish and crabs) in 2003 was about 2, 80, 000 tons, of which China produced some 1, 80, 000 tons, followed by India with some 35, 000 tons (FAO Fisheries Global Aquaculture Production Database). A total of 280,000 tons of freshwater prawns (excluding crayfish and crabs) were produced worldwide in 2023. Based on the FAO Fisheries Global Aquaculture Production Database, China produced 180,000 tons, followed by India with 35,000 tons (FAO, 2023).

According to MPEDA (Marine Products Export Development Authority), India, the total shrimp (marine) and scampi (giant river prawn) production in India was 2, 45, 619 tons in 2012. Shrimp exports from India hit a record high of 1,781,602 MT in 2023, according to MPEDA. Considering the fresh water prawns only, some 18, 000 tons of wastes are produced annually in India (Chakravarty and Edwards, 2022; Basawa et al., 2023).

The present study deals with the properties and characterisation of chitin and chitosan obtained from shell and operculum of *Macrobrachium rosenbergii* and *M. malcolmsonii* prawns. This is important as effective prawn waste management ensures safe environment and offers an economical way to earn additional income.

MATERIALS AND METHODS

Shell and operculum were removed from freshly captured prawns (*M. rosenbergii* and *M. Malcolmsonii*). The samples (N = 05 for each species collected from different locations) were washed, oven dried and grinded with a blender into fine powder and used for further studies.

Chitin extraction: Chitin was extracted from the shell and operculum after pigment extraction followed by deproteinization and demineralisation following the methods of Takiguchi, 1991; Oh et al., 2007; Younes et al., 2012. Dried powdered samples were mixed with acetone (1:3 w/v) in a capped flask and shaked on a rotary shaker at 250 rpm for 8-10 hours at ambient temperature, washed to neutral pH, filtered to remove excess water and then dried again. The samples were deproteinised with 10% NaOH (1:10 w/v) by heating at 80°C for 5 hours, washed, filtered and dried in a hot air oven at 60°C for 4 hours. Demineralisation of the samples was done using 2M HCl (1:5 w/v) for 24 hours at ambient temperature. The sample was washed with distilled water to neutral pH, filtered to remove excess water and oven-dried at 60°C for 4 hours. The dried sample was chitin.

Chitosan extraction: Chitosan was extracted from the chitin through deacetylation process (Takiguchi, 1991; Hazeena et al., 2022). The dried chitin was digested in 50% NaOH (5% w/v) for 2 hours on a hot plate to prepare chitosan. The chitosan was thoroughly washed and oven dried. The weight of chitin and chitosan was recorded.

Moisture content (%) of the extracted chitin and chitosan was determined gravimetrically by drying the samples to constant weight and measuring the weight before and after drying (Abolude et al., 2018).

Ash content of chitin and chitosan was determined by heating the samples in a muffle furnace at 650^0C for 4 hr. The crucibles with samples were allowed to cool in the furnace to less than 200^0c and then placed into desiccators with a vented top.

Water binding capacity (WBC) and Fat binding capacity (FBC) of chitin and chitosan were measured using a modified method of Wang and Kinsella (1976) and El-araby et al. (2022). Water or fat absorption was initially carried out by weighing a centrifuge tube (15mL) containing 0.5 g of sample, adding 10mL of water or soybean oil, and mixing on a vortex mixer for 1min to disperse the sample. The contents were left at ambient temperature for 30min with shaking for 5s at every 10 min interval and centrifuged at 3200 rpm for 25 min. After the supernatant was decanted, the tube was weighed again and WBC and FBC percentage were calculated.

Protein estimation: A 10% (w/v) homogenate was prepared in ice-cold 50 mM phosphate buffer (pH 7) by grinding the wet tissues separately in an ice-cold porcelain mortar and pestle. The homogenates were centrifuged at 4000 rpm for 10 minutes in a cold centrifuge. The supernatants were collected for protein estimation (Lowry et al., 1951). To 0.1 ml of suitably diluted homogenate, 0.4 ml distilled water, 5 ml of Biuret reagent containing 2% Na_2CO_3 in 0.1 N NaOH, 0.5% $CuSO_4$ solution and 1% sodium potassium tartarate ($KNaC_4H_6O_6$) were added and vortexed. After 10 minutes of incubation at room temperature, 0.5 ml of Folin-ciocalteau's reagent (1 part of commercially available reagent is diluted with 2 parts of distilled water) was added and vortexed. Absorbance was measured in a UV-VIS double beam spectrophotometer (ANTECH, AN-UV-7000N) at 700 nm against an appropriate blank after 30 minutes of incubation at room temperature. Protein content was expressed as mg/g wet weight of the tissue and aqueous BSA (Bovine serum albumin) was used as standard.

Mineral composition: About 1.0 g of dried powdered sample with 15ml of conc. HNO_3 was heated on a hot plate in a fume hood till dryness. The sample was cooled and reheated with 5ml of the acid till dryness which was repeated once more. After cooling, 8ml of 30% H_2O_2 was added and reheated till dryness. Addition of H_2O_2 with heating was repeated till a clear solution was obtained. The sample was reconstituted with 5 ml of ultra-pure water, filtered into a 100 ml volumetric flask and volume was made up to 100 ml with Millipore water. The digestate was subjected to analyse various elements with an Atomic Absorption Spectrophotometer (Perkin Elmer AA200) using suitable standards.

RESULTS

The average body wet weight (N = 50) of a *Macrobrachium rosenbergii* was 31.264g with an exoskeleton of 2.305g (7.37%) (operculum: 1.299g and shell: 1.005g) on wet wt. basis and 2.007g (6.42%) (operculum: 1.148g and shell:0.859g) on dry wt. basis. *M. malcolmsonii* had an average body wet weight (N = 50) of 11.819g with an exoskeleton of 0.978g (8.27%) (operculum: 0.525g and shell: 0.453g, wet wt.) and dry wt. of 0.668g (5.65%) (operculum:0.359g and shell:0.309g).

Chemical characteristics

Chitin: The yield of chitin from the operculum and shell of *M. rosenbergii* (N=10) was 45.5±2.12% (0.522g) and 34.5±2.12% (0.296g) and from *M. malcolmsonii*, 32% (0.115g) and 29.5±2.12% (0.091g), respectively (Table 1). In both the species, the operculum contained more chitin than the shell. The operculum and shell together (exoskeleton) contained 40.75% and 30.84% chitin in *M. rosenbergii* and *M. malcolmsonii,* respectively.

Chitosan: Chitosan content of the operculum and shell of *M. rosenbergii* was 41.99±1.80% (0.482g) and 29.22±1.89% (0.251g) and that of *M. malcolmsonii* was 31.24±0.13% (0.112g) and 20.21±1.41% (0.062g), respectively (Table 1). The exoskeleton contained 36.52% and 26.05% chitosan in *M. rosenbergii* and *M. malcolmsonii*, respectively.

Protein: The protein content in operculum and shell of *M. rosenbergii* was found to be 1.86±1.85% and 6.21±3.32% and in *M. malcolmsonii*, 0.93±0.09% and 1.11±0.12%, respectively (Table 1).

Table 1 Percentage of chitin and chitosan (dry weight) and protein (wet weight) in *M. rosenbergii* and *M. malcolmsonii* (N=10).

	M. rosenbergii				***M. malcolmsonii***			
	Operculum (%)	**Operculum (g)**	**Shell (%)**	**Shell (g)**	**Operculum (%)**	**Operculum (g)**	**Shell (%)**	**Shell (g)**
Chitin	45.50±2.12	0.522	34.50± 2.12	0.296	32.00	0.115	29.50± 2.12	0.091
Chitosan	41.99±1.80	0.482	29.22± 1.89	0.251	31.24±0.13	0.112	20.21± 1.41	0.062
Protein	1.86±1.85	0.024	6.21± 3.32	0.062	0.93±0.09	0.005	1.11± 0.12	0.004

Physico-chemical and functional properties

Moisture content: Chitin from operculum and shell of *M. rosenbergii* contained 0.173% and 0.194% moisture, respectively. The moisture content of chitin from operculum and shell of *M. malcolmsonii* was 0.162% and 0.225%, respectively. The moisture content of chitosan obtained from operculum and shell of *M. rosenbergii* was found to be 1.071% and 0.436% and also chitosan from operculum and shell of *M. malcolmsonii* contained 0.720% and 0.175%, respectively (Table 2).

Ash content: The ash content of chitin from operculum and shell of *M. rosenbergii* was 4.29% and 2.11% and that from operculum and shell of *M. malcolmsonii* was 3.72% and 1.94%, respectively. The ash content of chitosan from operculum and shell of *M. malcolmsonii* was 5.89% and 3.91% and also chitosan from operculum and shell of *M. malcolmsonii* contained 4.85% and 2.12%, respectively (Table 2).

Water binding capacity (WBC): The WBC% of chitin from operculum and shell of *M. rosenbergii* and *M. malcolmsonii* was recorded to be 108.41%, 107.82%, 118.31% and 104.57%, respectively. Chitosan had a similar WBC%.

Fat binding capacity (FBC): The FBC% of chitin and chitosan from operculum and shell of both the species was comparable and that for chitin was 121.68%, 119.03%, 124.72% and 125.53%, respectively.

Table 2 Physicochemical and functional properties of chitin and chitosan extracted from *M. rosenbergii* and *M. malcolmsonii*.

	Chitin				**Chitosan**			
	M. rosenbergii		***M. malcolmsonii***		***M. rosenbergii***		***M. malcolmsonii***	
	Operculum	**Shell**	**Operculum**	**Shell**	**Operculum**	**Shell**	**Operculum**	**Shell**
Moisture %	0.173	0.194	0.162	0.225	1.071	0.436	0.720	0.175
Ash %	4.29	2.11	3.72	1.94	5.89	3.91	4.85	2.12
WBC %	108.41	107.82	118.31	104.57	110.35	114.44	120.28	123.16
FBC %	121.68	119.03	124.72	125.53	106.04	109.39	118.21	118.33

Trace elements: The Cu, Fe, Zn, Ca and Mg contents in operculum and shell of *M. rosenbergii* were 0.98 and 0.67 ppm; 12.4 and 12.6 ppm; 18.7 and 9.3 ppm; 18.3 and 16.4 ppm; 4.8 and 5.7 ppm, respectively. The Cu, Fe, Zn, Ca and Mg contents in operculum and shell of *M. malcolmsonii* were 0.84 and 0.83 ppm; 7.2 and 10.6 ppm; 22.7 and 27.4 ppm; 21.3 and 15.6 ppm; 6.4 and 3.9 ppm, respectively (Table 3).

XRD patterns of chitin and chitosan

XRD patterns of chitin from the operculum and shell of *M. rosenbergii* and *M. malcolmsonii* are illustrated in Figures 1 and 2. The strong reflections in operculum and shell of *M. rosenbergii* were at 2θ=9.0593°, 19.0399° and 2θ=9.2933°, 19.3168°, respectively. In case of operculum and shell of *M. malcolmsonii*, high peaks were recorded at 2θ=9.2616°, 19.1751° and 2θ=9.3694°, 19.2418°, respectively. These peaks matched well with that of the standard chitin. XRD patterns of chitosan from the operculum and shell of *M. rosenbergii* and *M. malcolmsonii* are illustrated in Figures 3 and 4 The high peaks in operculum and shell of *M. rosenbergii* were at 2θ=13.9005°, 19.7152°, 20.0747°, 27.892° and 2θ=10.1985°, 19.6857°, 22.0776°, respectively. In case of operculum and shell of *M. malcolmsonii,* high peaks were at 2θ=10.3124°, 19.8116°, 22.1772° and 2θ=9.96°, 19.74°, 21.92°, respectively.

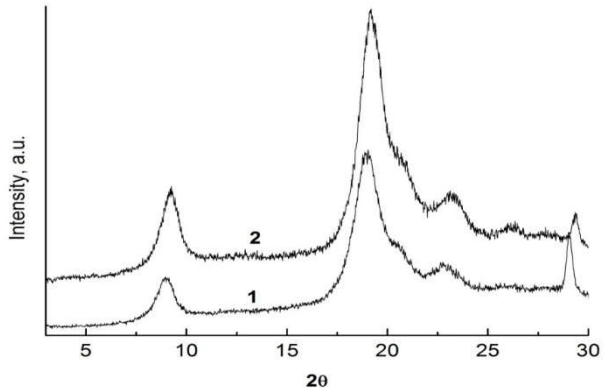

Figure 1 X-ray diffraction patterns of chitin extracted from 1. Operculum (head region) and 2. Shell (body and tail) regions of *M. rosenbergii.*

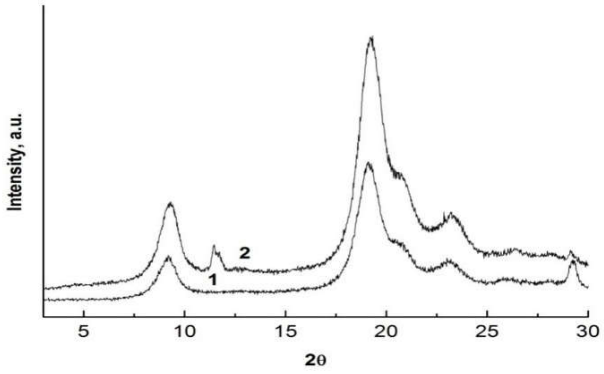

Figure 2 X-ray diffraction patterns of chitin extracted from 1. Operculum (head region) and 2. Shell (body and tail) regions of *M. malcolmsonii.*

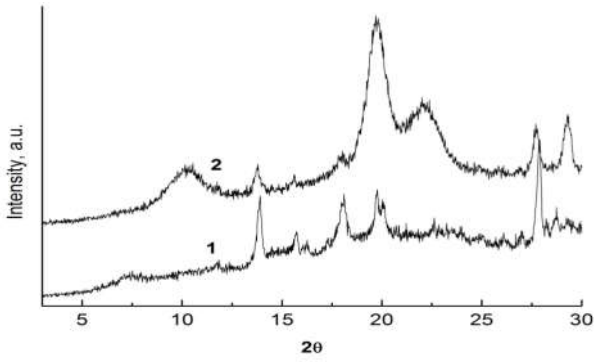

Figure 3 X-ray diffraction patterns of chitosan extracted from 1. Operculum (head region) and 2. Shell (body and tail) regions of *M. rosenbergii.*

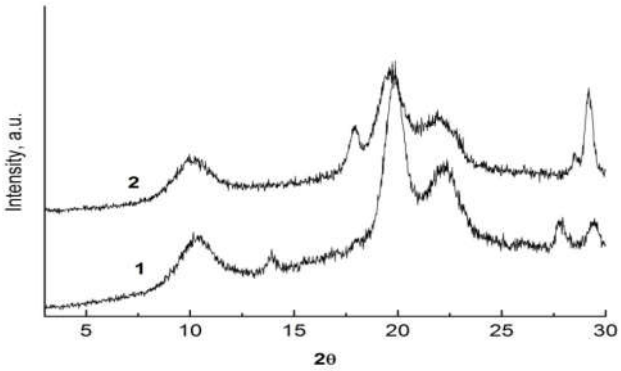

Figure 4 X-ray diffraction patterns of chitosan extracted from 1. Operculum (head region) and 2. Shell (body and tail) regions of *M. malcolmsonii.*

Table 3 Concentration of some trace elements (ppm) in the exoskeleton of *M. rosenbergii* and *M. malcolmsonii.*

Trace elements (ppm)	*M. rosenbergii*		*M. malcolmsonii*	
	Operculum	**Shell**	**Operculum**	**Shell**
Cu	0.98	0.67	0.84	0.83
Fe	12.4	12.6	7.2	10.6
Zn	18.7	9.3	22.7	27.4
Ca	18.3	16.4	21.3	15.6
Mg	4.8	5.7	6.4	3.9

DISCUSSION

Chitin is mainly recovered from crustaceans like prawn, shrimp and crab shells and also from the exoskeleton of gastropods and cephalopods. The major shell components of this group of animals are chitin, proteins, lipids, pigments and trace elements. Chitin, calcium carbonate and proteins account for about 90% of the dry weight of the shell (Rødde et al., 2008; Zaghloul and Ibrahim, 2019). Pure chitin is recovered after deproteinizing and demineralising the shells. Considering the annual Indian shell fish industry waste to be about 1, 25, 000-1, 50, 000 tons (Karuppasamy et al., 2016) which includes 8% exoskeleton (10, 000 – 12, 000 ton), approximately 4, 000 - 4, 800 tons (40%) of chitin and 3, 600 – 4, 300 tons (36%) of chitosan can be produced annually in India only which is a huge resource.

Several reports are available on the quantity of chitin and chitosan extracted from different species (Table 4). In the present study involving two fresh water prawn species, *M. rosenbergii* and *M. malcolmsonii*, the percentage of chitin yield from operculum is higher than the shell. The percentage of chitin yield from *M. rosenbergii* is comparatively more than *M. malcolmsonii* (Table 1). Nair and Madavan (1989) and Das et al. (1996) demonstrated that crabs like *Scylla serrata* and *Portunus pelagicus* had 16.07% and 20.19% of chitin from the body shell waste. Das et al. (1996) reported the same trend in other portunid crabs (*S. serrata* and *P. pelagicus*). In *Nerita Crepidularia*, the percentage of chitin was 35.43% in the operculum and 23.91% in the shell. In case of Shrimp, the percentage of chitin yield was 17%-30% (Naczk and Shahidi, 1990). According to Thirunavukkarasu et al. (2011), *Oratosquilla nepa* had a higher chitin yield, with 20g of the shell producing 2.145g of chitin with a percentage contribution of 10.725%. In most crabs, the chitin yield was 13-15%. But, in the present study, the yield of chitin was comparatively higher both in the operculum (30%-45%) and shell (29%-35%) (Table 1).

The chitosan yield was variable in different exoskeletal parts of the portunid crab, *Scylla tranquebarica* (carapace-6.59, claw-4.12 and legs-8.42%) (Thirunavukkarasu, 2005; Thirunavukkarasu and Shanmugam, 2009). Varghese (2002) reported a chitosan yield of 4.4 to 8.3% in different size groups of mantis shrimp *Harpiosquilla nelanoura*. The exoskeleton of Shrimp yielded 34% of chitosan (Puvvada et al., 2012). The yield obtained for extracted chitosan was 44.57% from mud crabs (*Scylla olivacea*) (Sarbon et al., 2015). The properties of chitosan depend on various intrinsic parameters such as degree of deacetylation (DDA) and molecular weight and thereby on the uses of chitosan (Vaz et al., 2018; Namli et al., 2023). The quality parameters of chitosan vary from producer to consumer. A standard

set of quality parameters for chitin and chitosan is lacking (Palpandi et al., 2009; Alemu et al., 2023). Analyses of different qualities for different commercially available chitins and chitosans had shown marked variations (No et al., 2000; Marzieh et al., 2019; Yadav et al., 2023). Hence, there exists a need for standard analytical parameters and methodologies. Exoskeleton raw materials vary in biochemical composition in terms of the quantity of chitin content and non–chitinous fraction. Besides this, the non– chitinous materials affect the properties of chitin by interacting with it (Palpandi et al., 2009; Jaganathan et al., 2015). The proportion of chitin and the non–chitinous fractions varies with species and with season (Rødde et al., 2008; Jaganathan et al., 2015) apart from different body parts.

The results of the present study revealed that the physicochemical and functional properties of chitin and chitosan extracted from *M. rosenbergii* and *M. malcolmsonii* are variable. The chitin from prawn operculum and shells contain moisture in the range of 0.16%-0.22% with an average of 0.19% and that of chitosan in the range of 0.18%- 1.07% with an average of 0.60% (Table 2). Chitosan obtained from Shrimp shells contained moisture up to 1.25% (Islam et al., 2011) and in case of craw fish, the chitosan moisture content was 0.3%-0.4%. Studies conducted by Naznin (2005), reported a moisture percentage ranging from 6.62 to 8.01% through differential concentrations of the alkaline solution utilized in the deacetylation process. Alishahi et al. (2011) indicated a moisture content of 2.5% in chitosan. Chitosan is hygroscopic in nature (Szymańska and Winnicka, 2015), which results a higher moisture content than that of chitin (Nessa et al., 2010; Qian et al., 2023). Commercial chitosan formulations are said to contain less than 10% moisture (Li et al., 1992). However, chitosan owder moisture levels varies from 5% to 11% (w/w) (Rege et al., 1999; Sreelekshmi et al., 2022).

Ash measurement is an indicator of the effectiveness of the demineralization step for removal of calcium carbonate. Elimination of the demineralization results in products having 31 – 36% ash (Suresh et al., 2013). The ash content of chitosan is an important parameter which may affect its solubility and viscosity (Pădureţu et al., 2019). A high grade of chitosan should have less than 1% of ash content (Kalutharage and Rathnasinghe, 2020). The comparative low ash content in the present case shows effective demineralization steps. Ash content may vary according to the extraction protocol and mineral composition, as crustacean exoskeleton contains high calcium carbonate (Khoushab and Yamabhai, 2010; Mohan et al., 2021).

Water binding capacity (WBC) of chitin of the operculum and shell of the prawns ranged between 104.57%-118.31% with an average of 109.78% and that of chitosan between 110%-123% with an average of 117.06%. There is no remarkable change between WBC% of chitin of operculum and shell of the two prawn species. But, WBC% of chitosan was significantly greater than that of chitin. Water uptake of chitosan was significantly greater than that of chitin and cellulose (Mathew et al., 2009). Cho et al. (1998) reported a WBC range of 458% to 805% for five commercial chitosans prepared from shrimp and crab shells. Water binding capacity of shrimp chitosan was reported to be 537.29% (Hossain and Iqbal, 2014).

The average fat binding capacity (FBC) of chitin (122.74) and chitosan (112.99) in both the prawn species is similar and low. The fat uptake of chitin and chitosan ranged from 315 to 170% with chitosan having the lowest and chitin the highest fat uptake. According to Hossain and Iqbal (2014) shrimp chitosan showed 427.98% FBC.

The level of trace elements (Cu, Fe, Zn, Ca and Mg) in operculum and shell of *M. rosenbergii* and *M. malcolmsonii* were well within the reported ranges. The average Cu, Fe, Zn, Ca and Mg contents in the exoskeleton of the two species were 0.62, 10.7, 19.52, 17.9, 5.2 ppm, respectively.

Table 4. Chitin and chitosan contents in various organisms.

Organism	Chitin %	Chitosan %	References
Shrimp	14-27	-	Iber et al., 2021
Crab	13-15	-	Iber et al., 2021
Snaw crab shrimp	18.7-32.2	-	Naczk and Shahidi, 1990
Dry dead bees	10-20	-	Varlamov et al. 2002
Crab	13-26	-	Synowiecki and Al-Khateeb, 2003
Shrimp	14-42	-	Synowiecki and Al-Khateeb, 2003
Krill	34-49	-	Synowiecki and Al-Khateeb, 2003
Cancer (Crab)	64.2	-	Hobel et al., 2004
Squid pens: *Loligo lessiniana*	36.06	27.59	Chandumpai et al., 2004
Squid pens: *Loligo formosana*	36.55	28.21	Chandumpai et al., 2004
Bee corpses	25	16-25	Nemtsev et al., 2004
Shrimp	14.72	12.03	Naznin, 2005
Callinectes (Blue crab)	28	-	Kurita, 2006
Silkworm pupa	2.59	-	Paulino et al., 2006
Crabs shells	23.72	-	Abdou et al., 2008
Metapenaeus monodon	24.33	15.25	Aranaz et al., 2009
Neritina crepidularia: operculum	35.43	-	Palpandi et al., 2009
Neritina crepidularia: shell	23.91	-	Palpandi et al., 2009
Mussel	23.25	15.14	Alishahi et al., 2011
Shrimp	-	15.21	Islam et al., 2011
Oratosquilla nepa	10.72	-	Thirunavukkarasu et al., 2011
Kuruma shrimp – operculum	13	-	Kazuaki et al., 2012
Kuruma shrimp – shell	8.9	-	Kazuaki et al., 2012
Vannamei shrimp – operculum	15.3	-	Kazuaki et al., 2012
Vannamei shrimp - shell	9.5	-	Kazuaki et al., 2012
Shrimp	-	34	Puvvada et al., 2012
Hawk mouth (*Clani sbilineata*)	-	95.8	Wu, 2012
Mussel shells	23.25	-	Abdulkarim et al., 2013
Metapenaeus monoceros	22.60	21.76	Abdulkarim et al., 2013
Shrimp shells (*P. longirostris*)	26.98	-	Arbia et al., 2013
Shrimp shells	36.43	-	Samar et al., 2013
White-grub cockchafer (*Melolontha melolontha*)	13-14	-	Kaya et al., 2014
Water scavenger beetle (*Hydrophilus piceus*)	19-20	-	Kaya et al., 2014
Colorado potato beetle (*Leptinotar decemlineata*)	7	67	Kaya et al., 2014
Argynnis pandora	22	-	Kaya et al., 2015
Celes variabilis	11.8	58.8	Kaya et al., 2015
Calliptamusbarbarus	20.5	75	Kaya et al., 2015
Ailopus simulatrix	5.3	-	Kaya et al., 2015
Vespa crabro	2.2	-	Kaya et al., 2016
Morocan locust (*Dociostaurus maroccanus*)	14	77.9	Erdogan and Kaya, 2016
Mussel shells	16.73	-	Abdelmalek et al., 2017

Blattela germania	4.4	3.6	Kim et al., 2017
Flour moth	9.5-10.5	-	Mehranian et al., 2017
Musca domestica	9	-	Caligiani et al., 2018
Periplaneta americana	3.4	2.1	Ibitoye et al., 2018
Squid gladius (*Loligo vulgaris*)	31.2	-	Knidri et al., 2018
House cricket (*Brachytrupes potentosus*)	7.1	5.8	Knidri et al., 2018
Cicada lodosi	-	5	Mol et al., 2018
Meal worm (*Tenebrio molitor*)	-	2.5	Song et al., 2018
Apsis melifera	8.8	-	Tsaneva et al., 2018
Hermetia illucens	7	32	Khayrova et al., 2019
Dark black chafer beetle (*Holotricia parallela*)	15	-	Liu et al., 2019
Desert locust (*Schistocerca gregaria*)	22.5	55	Liu et al., 2019
Grasshopper	-	5.7	Luo et al., 2019
Mealworm beetle (Zophobas)	4.6	80	Shin et al., 2019
Silk worm	-	3.1	Battampara et al., 2020
Penaeus monodon	25.33	22.97	Islam et al., 2020
Macrobrachium rosenbergii	26.40	23.63	Islam et al., 2020
Lucanus cervus	10.9	-	Kabalak et al. 2020
Polyphylla fullo	11.3	-	Kabalak et al. 2020
Macrobrachium rosenbergii: operculum	40.50	-	Present study
Macrobrachium rosenbergii: shell	34.50	-	Present study
Macrobrachium malcolmsonii: operculum	32.00	-	Present study
Macrobrachium malcolmsonii: shell	29.50	-	Present study

According to Chandumpai et al. (2004). The Fe, Cu and Zn concentrations in the muscles of squids were 1.79–78.5, 2.74–17.4, 8.39–23.6 μg g−1 wet weight, respectively (Jiao et al., 2018). The XRD patterns of chitin exhibited strong diffraction peaks at 2θ=9° and 19° (Figures 1 and 2), the pattern being similar for all the samples with different intensities and confirm to that of standard chitin (about 2θ=19°). Cárdenas et al. (2004) reported that XRD analysis of α-chitin and β-chitin indicated their main characteristic peak at 19.2–19.3º and 19.3º, respectively. The XRD pattern of chitosan of all the samples of the present study is similar with different intensities. According to Islam et al. (2011), chitosan of shrimp shell shows high diffraction at 10° and 21°. XRD analysis verifies the degree of crystallinity of the extracted chitin and chitosans. The sharpness of the bands is higher in the chitin samples than chitosan as the former is more crystalline than the later.

CONCLUSION

The prawn processing industry is expanding very fast throughout. A large number of co-products generated by numerous processing industries can be an important source of many useful substances, including chitin and chitosan. Improvement of existing technologies would make the industry cost effective, more attractive and would assist in the management of shrimp-industry wastes.

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Chapter in book:
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Conference proceedings:
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