

Recent Trends in Environmental Science and Applied Ecology

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Editors

Mr. Debkanta Ghosh

M.Sc, B.Ed

Assistant Professor in Zoology,

Vidyasagar College for Women (Calcutta University)

Dr. Sanjib Saha

M.SC., M.Ed., Ph.D., Post Doc, SRFIASC, FASC, FIARA

Assistant Professor in Zoology,

Vidyasagar College for Women (Calcutta University)

Former Project Assistant (UGC, DRDO) and Guest Lecturer in BMC and BSC (CU)

Assistant Coordinator in IGNOU (LSC)



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RECENT ADVANCES IN FISH AND SHRIMP HEALTH MANAGEMENT FOR SUSTAINABLE AQUACULTURE

Debajyoti Pradhan and Gadadhar Dash*

Department of Aquatic Animal Health, Faculty of Fishery Sciences,

West Bengal University of Animal and Fishery Sciences,

5-Budherhat Road, Panchasayar, Kolkata-700 094

*Corresponding author E-mail: dashgadadhar@gmail.com

Abstract:

Aquaculture is the fastest growing animal food producing industry and it contributes significantly to food and nutrition security in the society. Disease (both infectious and non-infectious) is recognized as one of the major constraints to global aquaculture production and infectious diseases take the lion share causing billion-dollar loss annually. Viral and bacterial diseases are of major concern in fish and shrimp farming systems. FAO/NACA (2000) has recommended the use of three levels of diagnostics- Level-I, II and III. A good health management practice in fish and shrimp culture requires anamnesis, onsite observation of habitat, onsite examination of feed, and onsite physical examination of aquatic animal. Novel technologies with potential for use in aquaculture include immunoassays (FAT, IHC, ELISA and blot), molecular tests (Real Time PCR, NASBA, nested PCR, rcb-PCR, RAPD and LAMP) and nanotechnology for rapid and accurate detection of various diseases. Feed additives like prebiotics, probiotics, enzyme additives and nucleotides are used for sustainable fish and shellfish aquaculture. Various immunostimulants also provide effective protection against diseases. Successful health management begins with prevention of disease that includes GAP, BMP and strict biosecurity measures.

Keywords: Aquaculture, Anamnesis, Biosecurity, Probiotics, Nanotechnology

Introduction:

Aquaculture is considered as the fastest growing animal food producing sector and it contributes significantly to food and nutrition security in the society. The achievements of highest yields by the farmers have transformed the aquaculture system from extensive to semi-intensive, intensive and super intensive. According to the United Nations Food and Agriculture Organization (FAO, 2019), approximately 84% of aquaculture is comprised of aquatic plants, molluscs and freshwater fish. Aquaculture is a dynamic activity and new techniques are to be continuously developed for successful and sustainable aquaculture development.

The aquatic animals are very efficient in producing animal protein in comparison to the terrestrial ones. Protein retention efficiencies of aquatic animals, such as salmon, are notably higher than those of terrestrial animals, such as beef and pork (Marine Harvest, 2017). Salmon is considered as an attractive and alternative source of meat protein because it produces twice the amount of protein as beef per unit of protein fed. In addition, each kilogram of salmon produced represents a reduction of 27 kg of CO₂ discharged into the environment relative to the production of beef (Table 1), thereby reducing the production of green house gases (GHG).

Table 1: Environmental factors associated with meat production (farm gate) from different species (adopted from Marine Harvest, 2017).

	Protein Retention %	Edible Meat per 10 kg feed	Carbon footprint kg CO ₂ /kg meat	Freshwater consumption L/kg edible meat
Beef	15	4-10	30	15,400
Pork	18	17	5.9	6,000
Chicken	21	21	2.7	4,300
Salmon	31	61	2.9	2,000

Aquatic organisms are more efficient in biomass production because they are poikilothermic and have no energy requirements to maintain body. The efficiency of biomass production realized through monoculture intensively fed (MIF) model (an aquaculture model) is much higher than that of terrestrial animals. According to Tidwell (2012), generally, to produce 1 kg of cattle takes 7–8 kg of feed, 1 kg of swine 3–4 kg, and 1 kg of poultry 2–3 kg, whereas 1 kg of fish is produced using 1.5 kg of feed.

Aquaculture has three phases- the hatchery, nursery and grow-out phases. Most hatchery operators use tanks to hold the young organisms that they grow. In the nursery and grow-out phases, tanks, ponds, and floating cages hold the farmed animals until harvest time. There are extensive, semi-intensive, or intensive production system in aquaculture and choice of a system depends on the desired density of animals to be farmed in a particular area. In the intensive system, farming of aquatic animals occurs with high stocking density. High stocking density result in exposure of the animals to stress that often leads to disease. Disease outbreaks in turn cause production losses due to lower harvests or poor quality of aquatic products. Fish and shellfish diseases are a substantial source of monetary loss to farmers. Production costs are getting increased by disease outbreaks in the farms due to increased treatment costs and decreased growth during convalescence. There are several factors like nutrition, environment, physiological status etc. that are either directly or indirectly related to fish health status (Fig 1).

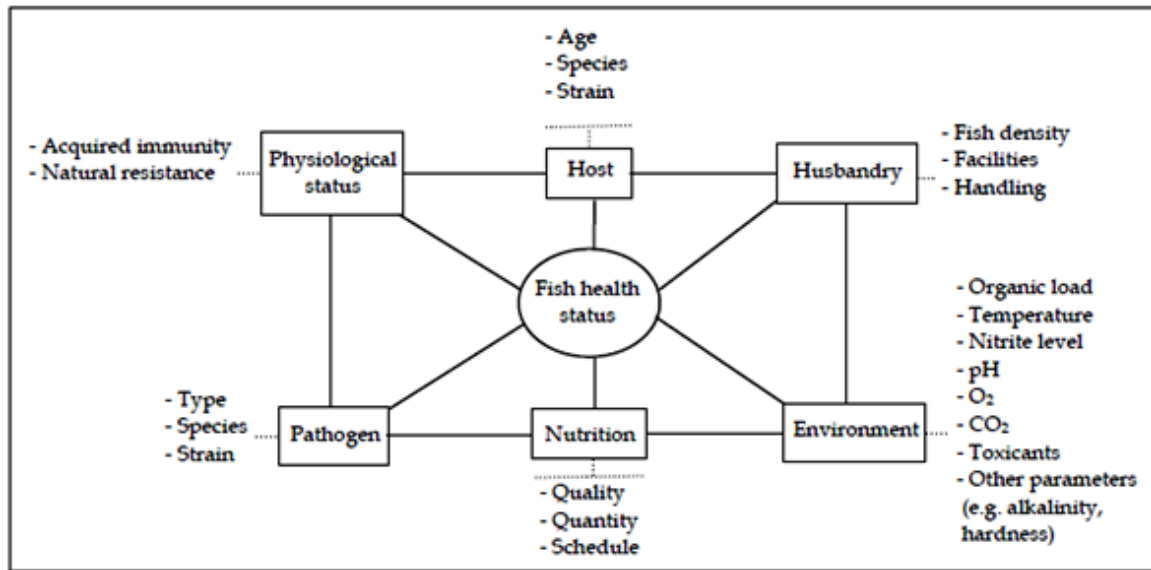


Figure 1: The relationship of various factors in fish health status
(Modified from Magnadóttir, 2006; Plumb and Hanson, 2011)

Health management of fish and shellfish is a term used in aquaculture to describe management practices which are designed to prevent fish and shellfish diseases. Implementation of an effective health management programme that consists of well organized management and husbandry practices, efficient biosecurity and hygiene measures, and improved resistance to disease through vaccination can help to reduce and control disease at farm sites (Thompson and Adams, 2004; Adams, 2009).

Sustainable aquaculture:

The sustainable aquaculture is dynamic concept and the sustainability is achievable when both the integrity of the environment and the socioeconomic benefits are maintained in the absence of animal health issues (Fig 2.A and 2.C). Depending upon the species, location, technology and societal norms, the concept of sustainability of an aquaculture system will vary. A truly sustainable aquaculture system must have environmental sustainability, economic sustainability and social and community sustainability.

Disease:

Disease is recognized as one of the major constraints to global aquaculture production and it has severe impact on both the economic and socio-economic development. Disease is defined as any abnormality in structure or function displayed by living organisms through a specific or non-specific sign (symptom). The farmed aquatic animals may suffer from diseases by infectious organisms due to environmental problems and wrong management practices. Diseases in fish and shellfish are indicated by tissue or organ damage, reduced growth rate, or

death. The aquaculture ventures may be collapsed due to the occurrence of persistent disease and it may threaten the sustainability of the industry as a whole.

There are two broad categories of diseases- infectious and non-infectious diseases. Infectious diseases are contagious and broadly categorized as parasitic, bacterial, viral, or fungal diseases. On the other hand, non-infectious diseases are not contagious and caused by environmental problems, nutritional deficiencies, or genetic anomalies. Infectious diseases required some kind of treatment to control the outbreak of diseases but non-infectious diseases cannot be cured by medications.

How disease develops?

Several factors are involved for the development of disease in a particular aquaculture system: the farmed fish (host), the disease-causing organisms (pathogens) and the surroundings (environment). There is a complex interaction that exists among these three factors as represented in the diagram of three overlapping circles (Fig 2B). For a disease situation to exist there must be either increased virulence of the pathogen, or decreased resistance of the host and it is possible if there are potential pathogen, susceptible host, and required environmental conditions at the same time. According to Assefa and Abunna (2018), up to 50% of production loss in aquaculture is caused by various diseases. The optimal conditions for the infestation and reproduction of parasites are provided by high stocking densities and poor water quality. Furthermore, the inevitable transport of fish and equipment support spreading of infectious pathogens (Subasinghe *et al.*, 2001).

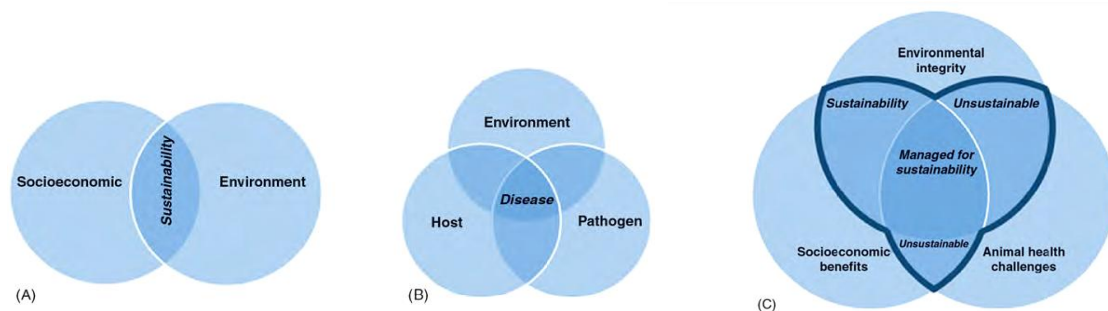


Figure 2: Sustainability and animal health in aquaculture. (A). Sustainability is achieved by fulfilling both socioeconomic needs while maintaining environmental function. (B). Poor animal health and disease is realized when the host and pathogen overlap in an appropriate environment (Snieszko, 1973). (C). Melding these two ideas and overlapping the diagrams on the environment (and acknowledging the animal will always be present), presents the outcome space for aquaculture (area encapsulated by the *dark line*). Sustainability can be achieved when benefits to the socioeconomic and the environment are maintained for the long term, when animal health is not an issue (Tlusty MF, 2020).

Details of OIE listed pathogens of fish and crustacean (2020):

Fish:

▪Epizootic haematopoietic necrosis virus ▪Infectious salmon anaemia virus ▪Salmonid alpha virus ▪Infectious haematopoietic necrosis virus ▪Koi herpes virus ▪Red sea bream irido virus ▪Spring viraemia of carp virus ▪Viral hemorrhagic septicaemia virus ▪*Aphanomyces invadans* ▪*Gyrodactylus salaris*

Crustacean:

▪Acute hepatopancreatic necrosis disease ▪*Hepatobacter penaei*- Necrotising hepatopancreatitis ▪IHHNV ▪IMNV ▪*Macrobrachium rosenbergii* nodavirus ▪TSV ▪WSSV ▪YHV ▪*Aphanomyces astaci*

Transboundary aquatic animal diseases:

Transboundary aquatic animal diseases (TAADs) are highly contagious and can rapidly spread across national borders. They limit the development and sustainability of the aquaculture sector through production losses and other negative consequences. They have direct and indirect impacts on livelihoods (income and employment), increase operating costs, restrict trade, reduce market share and result in investment losses.

Example: Epizootic ulcerative syndrome (EUS), Tilapia lake virus (TiLV), Acute hepatopancreatic necrosis disease (AHPND), Infectious myonecrosis virus (IMNV), Koi herpes virus

Signs of diseases:

The sick fish and shrimp often exhibit some disease signs before they die. Reduced feeding, abnormal changes in body colour and behaviour etc. are the primary indication of affected fish and shrimp. The fish may stay away from the school, or swim at the surface or along the tank sides. Sometimes, certain abnormal behaviour like flashing, scraping on the bottom of projecting objects, darting, whirling or twisting, and final loss of equilibrium are also exhibited by the affected fish. The body surface abnormalities and lesions are also important signs of diseases that may be observed externally or internally.

Diagnosis:

Diagnosis is the determination of the nature of a disease and correct diagnosis is the basis of appropriate and effective disease control measures. Disease diagnosis involves recognizing the occurrence of an abnormality and identifying its cause. Fish and shrimp disease diagnosis follow a format similar to that applied to other animal species. More importance is given to water quality parameters because any disturbance in water quality is directly related to the health of aquatic organism. A good history of the disease should be supported by personal observations before performing post-mortem examinations. Apart from the gross physiological examinations

of fish and shrimp, the disease diagnostic methods include microscopic, histological, microbiological, immunological and molecular diagnosis.

Levels of diagnostics:

FAO/NACA (2000) has recommended the use of three levels of diagnostics (Fig 3) based on the scale of expertise and infrastructure i.e., training, facilities and resources required for disease diagnostics:

Level I: Farm/production site observations and gross clinical observations.

Level II: Laboratory-based analysis of fish (e.g. parasitology, bacteriology, mycology and histopathology) and water samples.

Level III: Advanced diagnostic specializations (e.g. virology, electron microscopy, molecular biology and immunology).

Of the three levels of diagnostics, the level III is considered more advanced and more reliable.

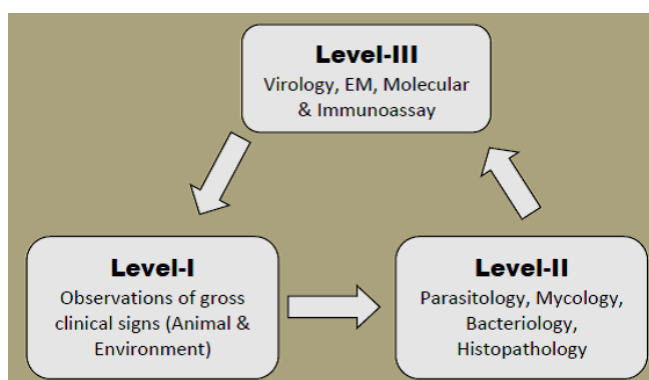


Figure 3: The three levels of diagnostics represent a continuum of observations from simple to sophisticated observations that ensure meaningful interpretation of the disease situation

Disease investigation:

Anamnesis (Case history/ records):

A good health management practice of aquatic animal requires accurate and complete record keeping of animals.

Onsite observation of habitat:

Onsite observation of habitat includes:

- Water and soil quality, plankton (bloom and colour), macrovegetation, number of dead and moribund animals found in the aquatic body.
- Congregation of predatory birds or other animals near the pond premises.
- Behavioural abnormalities of fish and shrimp in the farm.
- For fish: behavioural abnormalities include flashing, circling, gasping, surfacing, whirling, congregation near inlet/ water surface, vertical swimming, vertical hanging, dorsal/ lateral recumbency, rubbing, lethargic movement, isolation, listlessness and anorexia.

- For shrimp: observe for surfacing, congregation near inlet/ water surface, vertical swimming, lethargic movement, coming to the sides of pond during day time, isolation, listlessness, cannibalism, and anorexia.

Onsite examination of feed:

To ascertain the quality of feed following onsite examinations are very much required by the farmers:

- Smell of feed
- Colour changes (if any)
- Presence of fungus on feed (by physical examination)
- Water stability of feed
- Presence of feed crumbs

Onsite physical examination of fish and shrimp:

Visual examination of fish and shrimp is an important and fastest method of detection of disease that requires a well-trained eye. Although it is not always highly reliable but the experts can predict about the disease by observing some important signs and symptoms.

Fish: Some important indicators are given below-

- Physical measurement- Length, weight
- Body- Observe with magnifying glass for discolouration, reddening, haemorrhage, ulcer, wound, abdominal swelling/ dropsy, emaciation, tumour, deformity, presence of parasite/ epizoid growth, nodules
- Eye- Exophthalmia, enophthalmia, cloudy eye, haemorrhage
- Fin- Erosion/ rot, ragged/ torn, haemorrhage at the base, presence of parasite/ epizoid growth
- Scale- Protrusion, loss
- Vent- Redness, swelling, petechiae (pin point haemorrhage), faecal trailing
- Mucus- Increased production, lack of production, colour change
- Gill- Change in colour in both sides (pale/ red/ orange/ yellow/ black), rot, necrosis, gas super saturation, nodules, presence of parasite/ epizoid growth
- Buccal cavity- Haemorrhage, presence of parasite/ epizoid growth.

Shrimp:

- Physical measurement- Length and weight
- Exoskeleton- Discolouration, presence of white/ black/ brown spots, loose shell, wound, deformities, presence of parasite/ epizoid growth and dull appearance
- Rostrum- Broken, bent/ deformation
- Antenna- Cut, loss
- Appendage- Reddening, broken, loss, necrosis
- Telson- Erosion/ rot, presence of parasite/ epizoid growth
- Eye- Cloudy
- Gill- Change in colour (pale/ red/ orange/ yellow/ black), rot, clogging, necrosis, branchiostegal blister, presence of parasite/ epizoid growth

- Muscle- Flaccid, discolouration (pale, opaque, whitish, reddish, yellowish, bluish);
- Gut- Empty, colour change (white)
- Hepatopancreas- Swollen/ shrunken, colour change (yellow/ brown/ pale).

Health control in aquaculture:

Sustainable health management in fish and shrimp should be practiced by the following ways:

▪improved nutrition ▪improved disease resistance ▪quality control of water, seed and feed ▪use of probiotics ▪use of immunostimulants ▪rapid detection of pathogens and ▪application of vaccines to reduce the use of antibiotics

Novel technologies with potential for use in aquaculture:

Immunoassays:

Fluorescent antibody test (FAT/IFAT), immunohistochemistry (IHC), enzyme linked immunosorbent assay (ELISA), and blot (dot-blot/dip-stick/western blot) are advanced immune techniques used to detect fish and shellfish pathogens.

Molecular tests:

Real-time PCR, Nucleic Acid Sequence Based Amplification (NASBA), nested PCR, random amplification of polymorphic DNA (RAPD), reverse transcriptase-PCR (RT-PCR), reverse cross blot PCR (rcb-PCR) and RTPCR enzyme hybridisation assay, Loop-mediated isothermal amplification (LAMP), Lateral flow, LAMP with lateral flow are more advanced among the molecular techniques.

Other technologies:

Nanotechnology: Nanotechnology is considered as an emerging science which is representing a new frontier of the 21st century having the potential to revolutionize agriculture and allied fields including aquaculture and fisheries. It can provide new tools for aquaculture, fish nutrition, fish biotechnology, fish genetics, fish reproduction and aquatic health etc. The Nanotechnology tools are used for rapid disease detection, targeted gene and drug delivery, DNA vaccines, and nutrients. The silver nanoparticles (AgNPs) are the most studied nanomaterial and are potentially used as antiviral agents for the treatment of diseases in aquaculture organisms, particularly the White Spot Syndrome Virus (WSSV) in shrimp (*Litopenaeus vannamei*) culture (Moreno *et al.*, 2017). The potential antibacterial effects of *Bacillus subtilis* silver nanoparticles (AgNPs) from the gut of *Litopenaeus vannamei* were studied by Sivaramasamy *et al.* (2016) and they concluded that the AgNPs showed promising activity against *Vibrio parahaemolyticus* and *V. harveyi*. Oxidative stress is the most common form of stress phenomenon responsible for the retardation of productivity in fisheries. Selenium acts as a potent antioxidant with reduced toxicity in its nanoscale form (Sarkar *et al.*, 2015).

Use of feed additives as contributors to sustainable aquaculture:

In addition to the essential nutrients and energy supplied by feeds, recently a variety of additives are frequently used to supplement the primary nutrients on which they are based. The use of additives has moved to the use of “functional ingredients” that affect metabolism and physiology either directly (e.g., nucleotides) or indirectly (e.g., prebiotics).

Prebiotics:

According to Gibson and Roberfroid (1995), prebiotics are “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon”. Prebiotics are composed of complex carbohydrates that act as a substrate for the fermentation and proliferation of probiotic bacteria farther within the intestinal tract. Prebiotics are typically oligosaccharides and two common oligosaccharides used in aquaculture include mannan-oligosacchrides (MOS) and fructo-oligosaccharides (FOS). The lesser common prebiotics include xylooligosaccharides and galactooligosaccharide. The nonstarch polysaccharides (NSPs) like inulin and glucans can also act as prebiotics (Fig. 4).

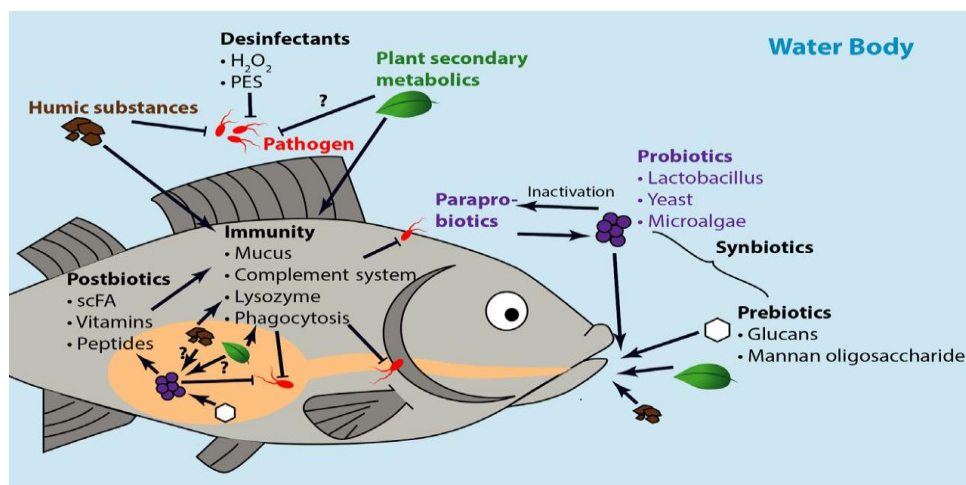


Figure 4: Scheme of environmental friendly treatments used to prevent or treat diseases.

: stimulation; ⊥ : inhibition; ? : proposed pathway (Lieke *et al.*, 2019)

Probiotics:

Probiotics are cultured product or live microbial feed supplements that are used to improve the intestinal balance and health of the host. According to Fuller (1989), probiotics are living bacteria or yeast that are orally consumed by the animal to colonize the intestine for benefit of the host organism (Fig 4). Bacteria species of *Bacillus*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, and *Pediococcus* as well as brewer's yeast *Saccharomyces cerevisiae* are common probiotics applied in aquaculture. Of these probiotics, *Bacillus subtilis*, *Lactobacillus rhamnosus*, and brewer's yeast were the most cited in aquaculture studies with a particular focus

on Nile tilapia and rainbow trout. Most probiotics are gram-positive, lactic acid bacteria. According to Ringo *et al.*, (2018) probiotics are generally considered as favourable microbes due to their abilities to stimulate host GI development, digestive function, mucosal tolerance, immune response, and disease resistance. Probiotic bacteria and yeast are used to increase growth rate, weight gain for several aquaculture species, especially Nile tilapia and rainbow trout. Probiotics interact with the immune cells, such as monocytes, macrophages, neutrophils and natural killer (NK) cells to enhance the innate immune response in fish (Nayak, 2010). Many studies indicated that several probiotics induce significant immune responses of fish that include elevated levels of lysozyme, peroxidase, complement activities, leucocyte counts and gene expression of inflammatory cytokines.

General guidelines for use of probiotics:

Soil Probiotics: 1.5 Kg /Acre or 10 Kg/Ha (100Kg/Ha in a total culture period) depending on concentration of beneficial bacteria and sludge condition (Varies from one company to other).

Water Probiotics: Initial start-up dose followed by weekly doses up to end of the culture recommended by the producing company and also as per the product specification.

▪Dose: 0.5-1.0 kg/ha/week (powder) ▪0.5-2.5 liters/ha/week (liquid)

Feed Probiotics: Administrated through feed on a daily basis from day 1 of culture till harvest. Mostly once a day (peak meal).

▪Dose: 0.01-0.4 g / kg feed with a suitable binder.

Enzyme additives:

Presently, most commercial enzyme additive used in aquaculture feeds is based on one of three primary functions (Castillo and Gatlin III, 2015): increasing the digestion of NSPs (e.g., xylanase), reducing the negative effects of anti-nutritional factors (e.g., phytase), and accentuating protein digestion (e.g., proteases).

Other additives:

Nucleotides are used in fish diets for the stimulation of feed intake and fish feeding behaviour. Recently, nucleotides are used to enhance immunity and disease resistance of a variety of aquaculture species (Li and Gatlin III, 2006; Ringo *et al.*, 2012). Carotenoids are widely used to improve muscle and skin coloration in salmonid, shrimp, and aquarium fish (Sales and Janssens, 2003; Torrissen and Christiansen, 1995; Wade, Gabaudan, and Glencross, 2017). Synthetic astaxanthin is the most widely used pigment in aquaculture but the natural sources are also gaining interest that are obtained from green algae (*Haematococcus pluvialis*), red yeast (*Phaffia rhodozyma*), and crustaceans (Wade *et al.*, 2017). It is reported that dietary carotenoids enhance growth and immunocompetence of shrimp (Wade *et al.*, 2017)

Immunostimulants:

Immunostimulants are dietary additives that enhance the innate (non-specific) defence mechanisms and increase resistance to specific pathogens (Sakai, 1999). Immunostimulants are categorized as chemical agents, bacterial preparations, polysaccharides, animal or plant extracts, nutritional factors and cytokines. There are at least 20 different compounds, including levamisole, lipopolysaccharides, glucan, vitamin C and E etc. that are used as immunostimulants, adjuvants and vaccine carriers in fish (Anderson, 1992). Among these compounds, glucan is one of the most promising and most studied stimulants for nonspecific defence mechanism in aquatic species. Glucan enhances resistance against bacterial pathogens such as *Vibrio anguillarum*, *Aeromonas salmonicida*, *A. hydrophila* and *Yersinia ruckeri* studied in *Cyprinus carpio*, Atlantic salmon *Salmo salar*, rainbow trout *Onchorhynchus mykiss*, yellow tail *Seriola quinqueradiata* and African catfish *Clarias gariepinus* (Jeney and Anderson, 1993; Matsuyama *et al.*, 1992).

Immunostimulants protect fish from several infectious diseases and increase resistance against infectious bacteria such as *Vibrio anguillarum*, *V. salmonicida*, *Aeromonas salmonicida* and *Streptococcus sp.*, viral infections such as IHN (Infectious Hematopoietic Necrosis) and yellow head (YHV) disease, and parasitic infections such as white spot disease and sea lice. Immunostimulants do not increase resistance against *Renibacterium salmoninarum*, *Pseudomonas piscicida* or *Edwardsiella ictaluri* infections due to their resistances to phagocytosis and abilities to survive within macrophages (Jadhav *et al.*, 1906).

Vaccines:

Vaccination is one of the key practices among the effective prevention and control strategies in aquaculture. Vaccination is the process by which a host organism is exposed to organic (biological) molecules to fight subsequent infections of a specific pathogen. A wide range of commercial vaccine is available against bacterial and viral pathogens. Vaccines are classified as killed, attenuated, DNA, synthetic peptide, recombinant vector, genetically modified, and subunit vaccines. However, most of the vaccines do not completely prevent disease (Dadar *et al.*, 2016). The recombinant vaccines like DNA vaccine and subunit vaccine which are developed against IPNV and IHNV respectively (Adams and Thompson, 2006). Fish vaccine is generally orally administered through feed or by dripping or bathing the fish in a vaccine solution or directly by intramuscular/intraperitoneal injection of antigen. Bacterins produced from several strains of *Vibrio* species are used in *Penaeus monodon* against WSSV infection. Bacterin (isolated from *Vibrio harveyi*) applied in shrimps against WSSV and 47% survival is reported (George *et al.*, 2006). Truncated VP28 oral vaccine has been demonstrated against WSSV infection in *Penaeus monodon* (Alabi *et al.*, 1999). Formalin-inactivated *Vibrio* sp. has reported to be effective against vibriosis in the larval penaeid shrimp culture (Alabi *et al.*, 1999).

Shrimp immune system lack immune memory characteristics as that of vertebrates and relies on innate or non-specific response. The defence mechanism in shrimp is largely brought about by the activities of specialized haemocytes that carry out phagocytosis, encapsulation and produce antimicrobial substances to remove or neutralize foreign particles and infectious agents (Lio-Po *et al.*, 2001).

Biosensors for the assessment of fish health:

Biosensors can measure specific target substances easily and rapidly by detecting small changes using signal conversion elements such as electrodes and optical devices, which transform the changes into electric signals (Grieshaber *et al.*, 2008) [Fig 5].

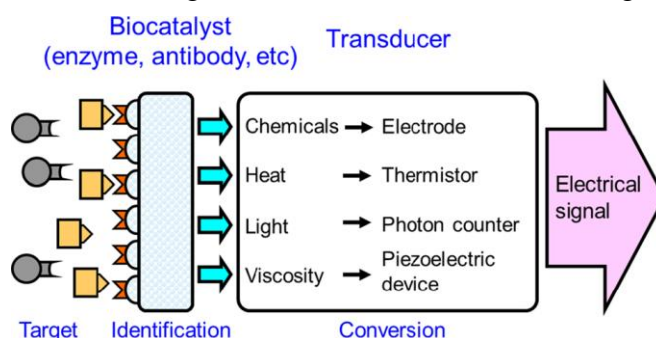


Figure 5: Principles of a biosensor (Endo and Wu, 2019)

Biosensors are characterised by high sensitivity and specificity, and they are used to evaluate fish health, determine their physiological condition and detect abnormalities at an early stage. The types of fish health that can be achieved with biosensors include stress monitoring, prediction of spawning time, and detection of pathogenic bacteria that cause diseases in fish. This technology can also be applied in some research fields, e.g., fish physiology and fish behaviour.

Preventive health management:



Figure 6: Strategies of Preventive Health Management. BMPs: Better Management Practices (Farm level and value chain). CoC: Industry Code of Conduct (Responsible Trade Practices). GAqP: Good Aquaculture Practices (Farm/facility and value chain). BG: Biosecurity Governance (National Strategies: policies and legislation)

Successful fish health management begins with prevention of disease rather than treatment (Fig 6).

Better Management Practices (BMP):

BMPs are innovative, dynamic and improved management guidelines applied to fish and shrimp farming, and production systems. The adoption of these guidelines is relatively easy to achieve without increased costs. It is developed based on population based risk factor studies, in consultation with the practitioners and relevant stakeholders, and on the evaluation of current practices. The application of BMPs in aquaculture minimizes harm to the environment and it ensures a sustainable fish and shrimp production. BMP category includes good pond preparation, quality seed selection, water quality management, health management, feed management, pond bottom monitoring, emergency disease management, harvest and post harvest management and environmental awareness.

Good Aquaculture Practices (GAP):

Implementation of Good Aquaculture Practices (GAP) and biosecurity maintenance in farming systems depend greatly on the system, the species, recommended stocking season and the region where farming is conducted.

Biosecurity:

Biosecurity is any management action to prevent the introduction of disease-causing agents to aquaculture facility. Biosecurity includes effective disease control and management of emergency situations. Biosecurity is the sum total of a country's activities and measures taken to protect its natural aquatic resources, capture fisheries, aquaculture and biodiversity. The spread of serious transboundary aquatic animal diseases (TAADs) should be prevented by applying strict biosecurity principles.

There are two lines of defence against fish and shrimp pathogens: protection and prevention. Protection includes hygienic practices using pathogen-free water, seed/ broodstock and feeds, controlling wild fish, shrimp, vectors and pests, regular monitoring of health and responsible movement of fish and shrimp with strict quarantine (if required). Prevention includes maintaining required water quality, sufficient and standard quality feed, appropriate stocking density/stocking density reduction and stress-free rearing environment.

Use of veterinary medicines:

Antibiotics, chemotherapeutants and disinfectants, drugs or similar agents are used as veterinary medicines to improve on-farm biosecurity. Veterinary medicines are used in aquaculture for treatment of emerging and re-emerging infectious diseases, developing new culture technologies, and maintaining the well-being of fish and shellfish. The use of veterinary medicines should be strictly maintained and always prescribed by qualified aquatic animal health professionals. These medicines are used according to accurate and appropriate diagnostic

procedures, and applied in a responsible manner in all phases of aquaculture production. The veterinary medicines are required in some cases although BMPs are mostly preferred in aquaculture sector.

Brood stock management and hatchery:

Broodstock management is an important aspect of fish and shellfish aquaculture because high quality seeds are required for successful aquaculture operation and quality seeds can be obtained only from healthy and pathogen free broodstock. Strict biosecurity measures are very essential in hatchery phase of aquaculture for handling of sensitive and fragile animals. The hatchery operators are encouraged to establish an effective broodstock management system for secured and improved quality of seeds. Movement of broodstock needs to be conducted in a responsible manner and the imported broodstock must be quarantined.

Where the Farmer can go for help?

The farmers cultivating fish and shrimp can consult with the experts of following government and private organizations regarding various diseases and other guidance.

▪AOC- Aqua One Centers (NFDB) ▪Field Schools (NFDB) ▪NASCA: National Centre for Sustainable Aquaculture (MPEDA) ▪Fisheries Colleges /ICAR Institutes/ State Govt. laboratories (Blocks/District Offices) ▪Private Clinics owned by Fisheries Professionals

Conclusion:

The aquaculture sector has been facing many constraints and challenges, and among these, infectious diseases take the lion share causing billion-dollar loss annually. There should be proper planning for prevention and control strategy based on globally accepted principles and locally applicable strategies. A health management program has several requirements and must cover all levels of aquaculture activity. At the production level, a healthy environment requires healthy seeds and juveniles, appropriate nutrition, proper waste management, optimal water quality, and regular monitoring. At the farm site level, good record keeping is essential that must cover all aspects of farm operation. Farmers should be trained to understand disease outbreak. The rapid and accurate diagnosis of various diseases for better health management of fish and shellfish is highly required. Health management is a shared responsibility, and each stakeholder's contribution is essential to the health management process for sustainable aquaculture development.

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A REVIEW ON ECOSYSTEM BASED FISHERY MANAGEMENT

Srijoni Basak

SACT, Vidyasagar College For Women

Abstract:

In most of the communities of the world fish and fisheries are an unabated part and it provides economic benefit as well as social benefit in many countries. Approximately 12.5 million people from all over the world has taken fisheries as their source of employment and world wide production of capture fisheries vary between approximately from 85 to 90 million tonnes in current years. The products of fisheries are used in wide range of varieties from livelihood uses to international commercial uses. The worth of fish trade is nearly US\$ 40 billion internationally per year. Because of this immense importance the world's fish resources are facing the collective effects of massive exploitation and environmental deterioration. Fisheries should be considered as systems in which social systems and ecological systems are in fact linked. This perspective calls for a new way of managing fisheries, that is, through an ecosystem-based approach which is Ecosystem Based Fishery Management.

Keywords: Fisheries, Ecosystem Based Fishery Management, environmental degradation.

Introduction:

Ecosystem based fisheries management (EBFM) is comparatively recent idea which is introduced by international resource management agreements to maintain a healthy aquatic ecosystem. An ecosystem based fisheries management can be done only when knowledge of aquatic lives, their habitat and human participation on ecosystem are known. EBFM illustrates a multidisciplinary approach which emphasizes the understanding of interplay between human and fish resources by altering the management process which starts from the managing of ecosystem instead of the target species. Management of fisheries is abroad aspect which narrates the process of controlling fishing for exploited fish stocks. Several fish species that inhabit in different ecosystem falls under fisheries management and this also employs various political systems along with local, cultural customs. Thus one can say that fisheries management is a division of larger fishery system. Natural system consist of exploited fish stock, the human system includes economy, fisherman and local cultures and the fishery management system include research, policy, planning – all these together construct the fishery system. The ecosystem-based fisheries management views the connections between human and natural systems and identifies the necessity for management ways that direct these connections. It will also need transformation and innovation. Combinations of both tried and true and new and unfamiliar management

approaches will be used. There will be learning and adaptation. The communities of fishermen, resource managers and researchers will need to work together to decide the best combination of approaches to address their situation.

Objectives of Ecosystem Based Fishery Management (EBFM):

Even if EBFM concerned with illustration of ideas, process and scopes, then also it ensue the following five common goals -

- a. Society participation:** The purpose of resource management is a matter of societal choice, where decentralized resolution should be taken. To balance among economic, cultural, and social objectives it needs collective participation of society.
- b. Ecosystem dynamics:** EBFM needs the understanding of primary processes of ecosystem and relationships among the factors controlling the ecosystem. To describe the ecosystem response towards the internal and external disturbances one must consider the inter relationship between the ecological variables. This perception helps fisheries managers to maintain ecosystem structures and functions which results in the conservation of species and ecosystem.
- c. Spatio: temporal variability:** Ecosystem based fishery management should undertake proper resolutions to represent ecosystem processes that functions on various temporal scales and influence varied spatial extents. It is difficult to consider the factors of change acting between geographic scales and long period of time for the processes with ill defined boundaries like migration and water currents.
- d. The balance of conservation and use:** To sustain a long term socioeconomic advantage without harming the ecosystem an ecosystem based approach should maintain a balance between conservation and exploitation. To attain this balance, EBFM identifies that change is unavoidable, and so, it ought to be adaptive in its development over time as situations change or as new fact becomes obtainable. As the mismanagement of ecosystem leads to deep irreversible consequences over long period of time so the ecosystem must be managed within the limits of their functioning.
- e. Integrated management:** In the context of EBFM, integration implies the outcomes of human activities on intricate processes are learnt. All related sectors of society, scientific principles, local and indigenous ecological knowledges must be involved in integrated management. To surpass the theoretical and idiomatic obstacle among principles engaged in describing ecosystems, three parts of integration are needed, which include : a multidisciplinary approach which include cooperative research ; an inter disciplinary approach include combined works which are mutually developed into prototype from

multiple disciplines; and a trans disciplinary approach include collaborative planning that undergo transcends disciplinary limits.

An ecosystem based fisheries management should include:

Goals, objectives and constraints that characterize the desired state of the fishery, including fish, fishermen, and habitat, should be stated clearly, and be based on societal choice, bounded by an ecological understanding of how the system functions, for the other components of the fisheries management system to be effective.

Conservation of fisheries resources that is precautionary, takes account of species interactions, and is adaptive.

Governance arrangements that are participatory and transparent and address cross-sectoral institutional arrangements at multiple levels/scales with multiple parties.

Management measures that allocate fishing rights and access in a manner that provides incentives for conservation and efficient use of living resources.

Ecosystem protection for habitat, and for species vulnerable to extinction or deemed by society to warrant special protection.

Monitoring of biological and human elements of the ecosystem including measurement of stresses and benefits and behavior of people and their institutions.

Management support that provides information, enforcement and performance evaluation.

The goal of ecosystem-based approach to fisheries management is to sustain ecosystem health, integrity and sustainability. One of the distinguishing features of ecosystem-based approach to fisheries management is an emphasis on protecting the productive potential of the system that produces resource flows, as opposed to protecting an individual species or stock as a resource. For an ecosystem that is already degraded, however, sustainability requires restoring those parts of the ecosystem that will sustain a diversity of species. The restoration of degraded ecosystems poses particularly difficult decisions related to balancing human needs with resource productivity requirements. The human component of marine ecosystems may exhibit irreversible regime shifts with poorly understood thresholds and limits, similar to those more commonly associated with the living marine resource components. The ecosystem approach also recognizes the complexity and uncertainty in predicting responses to management actions.

In simple terms, the objectiveness of fisheries management can be divided into four subtypes: biological; ecological; economic and social (political and cultural goals). The biological and ecological goals may be more correctly thought of as constraints in achieving desired economic and social benefits but for simplicity and consistency with the terminology most commonly used in fisheries management.

Biological: to assure constant productivity target species are maintained at or above the levels.

Ecological: to reduce the influence of fishing on the abiotic environment, non-target and dependent species.

Economic: to increase the net earnings of the participating fisherman.

Social: to increase employment facilities for those who depends on the fishery for their existence.

The goals stated above have two obvious limitations. Firstly, they have clear conflicts in intention as it is impossible, for example, to minimise impacts of the fishery on the ecosystem and simultaneously to maximise net incomes. Similarly, it is very probable that management strategies that aim to maximise net incomes will not also maximise employment opportunities. Some compromise between these goals has to be achieved before an effective management strategy can be devised. Secondly, the goals are too uncertain to provide benefit to the manager. For example, the results of fishing can only decreased by no fishing at all, which is unlikely to happen for those who stated the goal. Maximising employment opportunities could mean allowing as many fishers as possible to participate, regardless of whether or not they could make a living from the fishery, or it could mean maximising the number which could still earn some acceptable income, or many other such targets.

Hence it is very important to filter the goals further and to develop operational objectives for each fishery. Operational goals are very precise and are prepared in such a manner that they should be simultaneously achievable in that fishery. In other words, the trade-offs between the biological, ecological, economic and social goals must have been agreed upon and the conflicts and contradictions resolved.

1. Unexploited stock should be maintain above 50% at all times.
2. In absence of fishing activity all non target, dependent and associated species should be maintained above 50% of their mean biomass levels.

So, the aim of EBFM is to ensure that, despite variability, variations in the ecosystem, the potency of aquatic ecosystem health, both natural and human, is preserved considerably for the welfare of present and future generations.

Conclusion:

Ecosystem-based fisheries management cannot solve all of the fundamental problems of the existing fisheries management problems. Ecosystem-based fishery management is an important complement to the present fisheries management processes and it should be understood in a broader context rather than individual fishing sector and its application should also take into account the impacts of relevant sectors on the ecosystem. To assure continued productivity and sustainable ecosystem managing individual segment like fishing is essential but not enough thereby human activity must be conducted in such a way which consider the effects

on the ecosystem. Marine protected areas (MPA) are increasing by being proposed for use as a fishery and ecosystem management tool. However, for species that are highly mobile, one would expect marine protected areas to be quite ineffective (Sissenwine and Murawski 2004). Marine protected areas is one of important fishery management tools, however, marine protected areas are not synonymous with ecosystem-based fishery management. It is not necessary to implement marine protected areas in order to be successfully implementing ecosystem based fishery management.

Ecosystem based fishery management cannot be attained in a management system which is incapable to control fishing and it's effects. To make ecosystem based fishery management fruitful, the effect of fishing on other fisheries and aquatic ecosystem must be identified. If fishery management is aware of these interplay it has began to follow the pathway of EBFM. Ecosystem based fishery management cannot be implemented if the management ignores ecosystem conservation.

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SCOPE OF SMART PHONE WITH INTERNET CONNECTION

TOWARDS THE DEVELOPMENT IN FISHERY-

A STUDY IN BIRBHUM, WEST BENGAL, INDIA

Md. Ekramuddin

Department of Zoology,

Shibpur Dinobundhoo Institution(College), Shibpur, Howrah, West Bengal, India

Corresponding author E-mail: ekramuddinzoology@gmail.com

Abstract:

Fishery in India is still not fully organized and devoid of application of Information Communication Technology (ICT). The country has recently witnessed a telecommunication revolution initiated by a famous service provider company. 4G Internet connection is available in the Smartphone. Data availability is now cheap in affordable cost causing involvement of large number of citizens resulting into digital literacy. Many have own the phone and the internet connection is available even in distant corners of the country. Many are capable to use the internet even some of them have no institutional degree. There is scope to utilize this ICT tool to improve the productivity in fishery particularly in low productive areas like Birbhum district. The persons involved in fishery practices can be acknowledged about advanced fishing technologies and processes, various problems in the practice and way to solve or overcome these. The Government of India is trying to realize this possibility and has taken the initiative.

Keywords: Birbhum, fishery development, ICT, internet, Smartphone.

Introduction:

Information communication technology (ICT) is the most significant aspect in present day and covers all kinds of life activities from getting up from bed to going to bed and even when in sleep. This technology can be established in rural India through government and different related organizations that can give a magical output by creating knowledge based society (Omar and Chhachhar, 2012). Information communication technologies (ICT) are set of tools that assist the capturing, storage, processing, transmission and display of information by electronic mean of technologies. It covers both traditional tools (radios, telephones) and recently developed advanced tools(computer, smart phones, internet system, GPS technology etc). Nowadays it is an important element that determines the current presentation and the future situations for the different communities. This is at present efficiently applied in urban areas, not found in a parallel growth in villages due to shortage of infrastructure and network to reach the

rural communities. But situation is currently changing by implementation of smart phone based internet system that is available to every people in most part of the country reached by eminent service provider. The Reliance Jio that has been launched and has made a revolution in the field of information and communication. Fast speed internet service is available to citizens in very cheap rate that makes the people interested and even addicted in using android based smart phones. ICT could be used by the concern parties to transform information to the community and provide the information about upcoming events and different activities. It may be possible through the communication technology the distance among government and community can be reduced. The societies are struggling to grow their economies and emphases for rural development and fully combined in the global knowledge economy for rural community. In present study I have tried to find out the actual scenary of use of ICT in economic growth choosing the fishermen community in some villages of Birbhum District, West Bengal.

This is relatively an economically backward district with a population size of about 3.5 million. There are 2455 villages under 169 gram panchayat. The district has fresh water bodies (ponds, ditches, canals, bills and baors, reservoirs etc) suitable for fishery. There is an annual demand of 67699 metric tons fishes every year but production still does not reach it. There is a deficit of about 2606 metric tons every year that can be possible to reach. There are 51350 fisherman families and also persons from non-fisherman families play crucial role in fish production and marketing. Modern Information Communication Technology (ICT) may play a vital role to enhance the production. Smart phones with internet connection is at present situation is more significant ICT tool. Hardy (1980) assessed role of telephones in economic development at a time when communication standard was very poor. Abraham (2007) tried to find out the relationship between mobile phones and economic development. In Srilanka ICT application in fishery development has been experimented (Wimalasena, 2016). Kopaska (2014) worked out on useful role of smart phones in development of fishery. Nigeria (Eucharia *et al.*, 2016) and Malayasia (Omar *et al.*, 2011) have also made their interest in application of ICT towards development in fishery. In Ghana impact of mobile phones in artisanal fishing market efficiency and enhancement in livelihood have been studied (Salia *et al.*, 2011). Gangadhar (2011) studied the possibilities of application of ICT and policy making to improve fishing sector in Indian context. Jahan and Viswanathan (2014) performed a deep study in coastal fishery in Bangladesh improved through advanced communication methods. In India the picture of digital communication has been changed considerably after launching of Reliance Jio on 5 September 2016. This is a telecommunication company provided free 4G internet service to the consumers if they have a smart phone i.e. capable 4G connections. The aim was to make large number of Indian to literate digital communication and web browsing. They have simply made a revolution in the Indian telecom industry (Haq, 2017, Arora and Arora, 2017). Srivastava and Mishra

(2016) studied on possibilities of use of smart phones in forecasting which as an important factor in successful culture and capture fishery as well as marketing. Information technologies are trying to make interesting among the fishermen who are involved in offshore fishery in bay of Bengal (Shuva, 2017). The smart phone is more popular among the young and hence if they are used properly economic development can be expected. We may find it as economic revolution. Ohzzabieh and Samah (2015) made a study and found the interest of the youth towards different ICT tools. From a study it is found that smart phones are useful but still difficult to use by the fishermen mainly for their under literacy and financial constraints (Chachhar *et al.*, 2014). The Government of India has developed several portals to provide digital supports to all kinds of farmers by providing valuable consultations, financing, information supply etc. The National Fishery Development Board is also ready to provide their support towards a sustainable development in fishery. Hence I have studied the possibilities of use of smart phone in Birbhum district towards an enhanced fishery development-both in production and marketing. We have to analyze both the goods and bads of present day digital literacy.

Methodology:

A qualitative method has been employed in this research to provide an in-depth description of mobile and internet usage, benefits and problems among fishermen in five villages in Birbhum. The study was performed based on both primary and secondary data. Primary data was collected from respondent as a pre-set interview questionnaire and also capability testing of internet using by ten fishermen. Secondary data was collected from competent source to interpret the observation.

Study area and period:

The study was performed during March and April, 2021 in five significant villages from five different Development Blocks of Birbhum district of West Bengal, India (Table 1). I have selected the villages in random manner but observation on opportunity and possibility in fishing practice.

Table 1: Villages where survey performed

Name of village	Development Block	Latitude	Longitude	Population as per census report 2011
Sahapur	Dubrajpur	23°47'16"	87°30'08"	5621
Patadanga	Suri I	23°55'07"	87°25'47"	1104
Haraipur	Suri II	23°52'34"	87°33'48"	1506
Kunuri	Sainthia	23°55'13"	87°36'40"	3217
Chandrapur	Rajnagar	23°55'30"	87°23'06"	2012

Courtesy: Google Earth and Census Report of India, 2011

Annexure 1: Set of questions for interview

Sl. no	Questions/ Enquiry	Reply
1.	Name	
2.	Age	
3.	Education status	
4.	Male/Female	
5.	How involved in fishery	
6.	Annual income from fishery	
7.	Cost of handset	
8.	How long the smart phone using	
9.	Why/How influenced to buy smart phone	
10.	What is the internet bandwidth 2G/3G/4G	
11.	Name of Internet service provider	
12.	Whether comprehend English in the mobile phone input/output	
13.	Whether mother tongue language supported in the handset	
14.	Whether capable to use search engine	
15.	Chief interest area in smart phone (Social media, Game, Net search, online service)	
16.	What type of social media followed	
17.	How the mobile phone is used in fishery related work (communication/ market information/ weather information)	
18.	Whether aware about any government e-service portal	
19.	Whether interested in learning about use of search engine	
20.	Whether interested in communication with e-service portal or websites to get information related to fishery	
21.	What type of information may require	
22.	Whether any improvement after launching of easy available and cheap data service in smart phone in recent months	
23.	If yes, then what type of improvement than feature phone?	
24.	Do you think smart phone can improve the fishing sector and how?	

Data collection:

The interview of twenty persons from each village (total 100 respondents) was carried out during the study. The respondents are randomly selected and all are directly related to production and marketing in fishery. The multistage sampling method was followed. All of them have smart phone handset of different company and different prices. The use connection of different network Provider Company and all have internet data connection. The secondary data was studied collected from several authentic website sources.

Data presentation:

Collected data was presented in Tables, Histogram, Frequency polygon and Pie diagram for easy comprehension of the research matter.

Result and Discussion:

Basic information of Birbhum District:

The district is located in between 23°23'30" and 24°35'00"N latitudes and 87°5'25" and 88°1'40"E longitudes (Figure 1). This is featured by a diversified topography and demography and a mixture of diversified profession. This is populous and larger district (Table 2). There is enough water body having good potency in fishery development (Table 3).

Table 2: Birbhum district at a glance

Area in sq.km	4545
Community Development Block	19
Number of Villages	2455
Number of Gram Panchayat	169
Number of Municipalities	6
Number of total households	817899
Total population	3502387
Density of population per sq.km	771
Male/Female ratio	955.52

Source: Census Report, NPR. Govt. of India, 2011

Table 3: Fishery status in Birbhum district during 2015-16

Total ponds/tanks	21376.87 ha
Private ownership	20134.51 ha
Culturable	14833.80 ha
Beel and baor	632.16 ha
Reservoir	9416.53 ha

River	795.63 ha
Canal	5896.85 ha
Total annual production	65092.8 metric tons
Total annual demand	67699 MT
Total annual deficit	2606.2 MT
Total fisherman families	51350
Functioning co-operative societies	15
Total hatcheries	13
Total seed production	600 million spawn

Source: Portal of Assistant Director of Fisheries, Birbhum

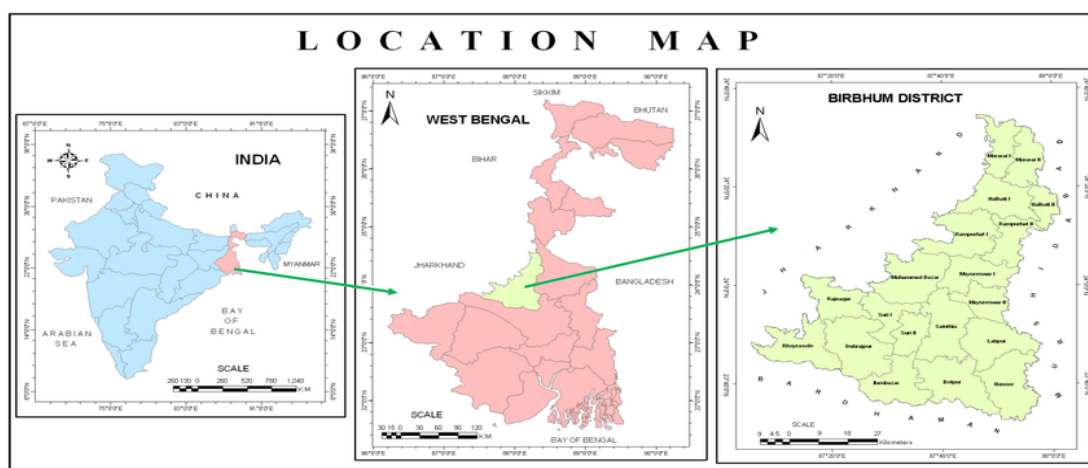


Figure 1: Location of Birbhum district in India (Courtesy: Google map)

Analysis of data:

It was found that only 38 % of the respondents have mobile phone with internet connection and 84 % of them use their phone for entertainment purpose like mobile games, photography, music and video playing. Only few can browse internet who are all matric passed. Some can use app based service that also have a formal education. There is age related biasness in using smart phones among the respondents (Figure 2). None of the respondent has heard about any Government portal offering online service or assistance regarding fishery development. They do not take part in any awareness related or training programme. Only 15 % of the respondents are interested in mobile phone related management in fishing practices mainly for the governmental assistance. Most respondent use local language in their phone as there is that kind of facility in the handset. Most use low cost handset but fine to them and are useful. They have eagerness and curiosity to use the phone in professional development as replied by 42 % respondent. 86 % told that they faced a remarkable change in mobile phone use after launching

the Reliance Jio service. Most of the respondent having the smart phone have bought the hand set after 5th September 2016 i.e. the launching date of Jio service. Some of the respondent are eager to collect a smart phone by replacing the old feature phone but unable for financial insufficiency. From secondary data it is found that the district Birbhum has a good potency in fishery but the production is yet unsatisfactory (Table.1). There is a lot of fisherman family who are still not updated in advanced fishing practice.

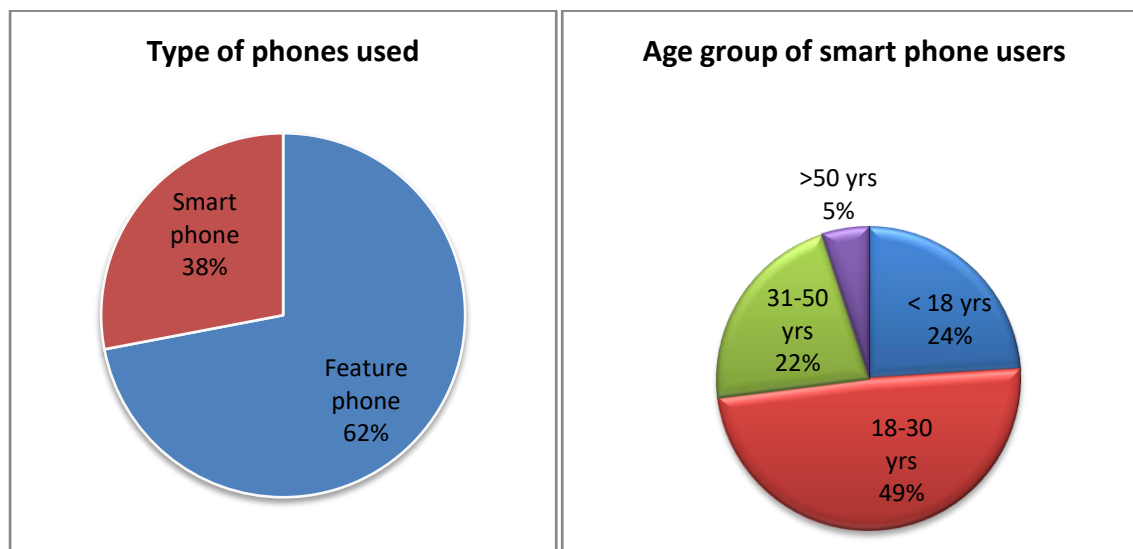


Figure 2: Age wise trend in using the smart phone in the respondent

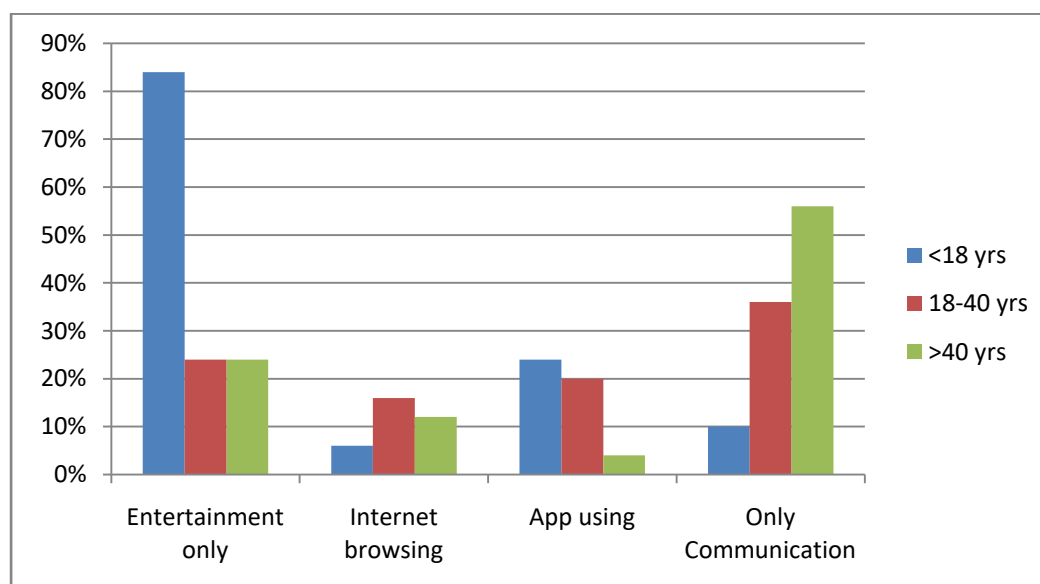


Figure 3: Trend in use of Smart phones among the respondents

Discussion:

The data shows that young generation has a curiosity in using new and advanced digital gadget. They can use phones with internet connection in a smart way. There are many incidences

to diversion of the students and youth by using smart phones from their right and productive way of career. If they are oriented in a proper pathway they are useful resource in social development as they are simply addicted in smart phone. They insist to get a phone and in most cases parents can arrange so. There is still no use of such phones in fishery development but Joshi and Ayyangar (2010) described it as a boon for fishermen community that means a grand possibility is awaiting in near future. Only in needs a government policy to implement to train the fishermen community as they can use smart phone in development of fishery. They can collect information about weather forecasting, searching better seed availability in minimum effort and saving valuable time, can communicate each other in smart way, can search market trend and over all can avail government assistance in both financial support and valuable consultation. In several decade ago introduction of telephone cased a revolution in economy (Hardy, 1980) and india is now waiting for another more impacting economic revolution. Introduction of digital banking has increased the possibility of development. Aftershock effect of digital revolution and ICT medium improvement will improve the fishery sector also.

Conclusion:

Launching of Jio as internet service provider and cheap rate of availability of internet service and also gradual digital literacy, digital banking arises a possibility in digital development of fishery sector in Birbhum as well as in the country. There are still several constraints like lack of proper knowledge and education in fisher men communities, financial burden, lack of easy comprehension of government portals, lack of enthusiastic, efficient and active officers and workers in sufficient number etc. but there is hope. If the stake holders think positively, these constraints will stand no more. The effect and result will be encouraging soon.

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A STUDY OF MORPHOLOGY OF FRESH WATER PRAWN ZOEAL LARVA *MACROBRACHIUM LAMARREI LAMARREI* (H. MILNE EDWARDS, 1837)

Indrani Sarkar

Department of Zoology (UG and PG),

Vidyasagar College, 39 Sankar Ghosh Lane, Kolkata 700006

Corresponding author E-mail: sarkarindrani.biswas@gmail.com

Abstract:

The present study describes the morphological changes and development of telson, mouth parts, rostrum appendages and biramous pleopods of fresh water prawn *Macrobrachium lamarrei lamarrei*. The results indicate that the first zoeal larvae have reduced setae and unsegmented telson and uropod. Pleopod is uniramous. At 3rd zoeal larva pleopods become biramous and biramous telson also formed. At 1st zoeal stage larvae are inactive and benthic in habit attach with substratum. At 3rd zoeal stage larvae are active and taking food.

Keywords: Zoea, telson, pleopod, uropod, rostrum.

Introduction:

Fresh water prawn genus *Macrobrachium rosenbergii* completes their life cycle in fresh water environment, but larval phase requires brackish water, about 20% to 40% seawater for complete metamorphosis, growth and development (Kurian and Sebastian, 1976). But small freshwater prawn *Macrobrachium lamarrei lamarrei* (H. Milne Edwards, 1837) and complete their lifecycle in freshwater completely. Saline water is not needed for their larval development. Mating always occurred in between soft pelagic newly moulted female which contain fully matured ovary with hard shelled male. After 12 – 14 hours of mating fertilized eggs were held within “brood pouch”. Within the brood pouch cell division took place and embryo gradually became fully mature to larvae. It required more or less 20 – 24 days in *M. lamarreilamarrei*. The Larvae hatched out from the brood pouch when they were at zoeal stage. The larval rearing technique of *M. rosenbergii* (De Man) has been described by Ling (1961, 62). Newly hatch larvae of *M. lamarreilamarrei* are inactive settled on the wall of the aquaria and did not take any food. It started to take feed after first moult but larvae of *M. dayanum* were active from the time of hatching.

Fielder (1970) described the larval development of *Macrobrachium australiense*. Complete larval development of the Palaemonid shrimp, *Macrobrachium acanthurus* was described by Choudhury (1970). Embryonic and larval development of *M. kistnensis* described

by Nagabhushanam and Kulkarni (1979). White shrimp post larvae in Southern Gulf of Mexico studied by Mario *et al.* (2018). Rocha *et al.* (2017) studied the morphological structure of larvae and juvenile of fresh water prawn *Macrobrachium acanthurus*. The present paper deals with description of larval morphology of *M. lamarreilamarrei*.

Materials and Methods:

Live specimens of mature *M. lamarreilamarrei* were captured from the local pond of behala, Kolkata, West Bengal during pre-spawning period i.e. month of February, 2009 and brought to the Vidyasagar College Post- graduate campus, Fisheries and Aquaculture Department, Kolkata.

Rearing of mature and berried prawns:

Matured males and female prawn of *M. lamarreilamarrei* with an average size range 2.5 cm to 3.5 cm and 4.5 cm to 5.8 cm with weight 0.35 g to 0.45 g and 0.83 g to 1.12 g respectively were brought to the Vidyasagar College Post Graduate Campus, Fisheries and Aquaculture Department, Kolkata. After arrival at Vidyasagar College, prawns were given a dip bath in 0.5 ppm potassium permanganate solution (KMnO₄) for two minutes then released in a separate large transparent 35 liter glass jar aquaria, measuring 45 cm x 25 cm x 25 cm (stocking aquaria) in the laboratory provided with good aeration numbering 25 in each group with 2:1 (male: female) ratio. Prawns were fed twice daily with frozen dried tubifex, cultured Cyclops, daphnia. Spawning occurs after 12-14 h of fertilization. After spawning 'the berried prawns' were carefully transferred into another 10 L transparent glass jar aquaria measuring 25 cm x 10 cm x 10 cm having fresh water (P^H 7.4 DO₂ – 5.47ppm). Artificial aerator was added for proper oxygenation. The temperature of water varied from 25⁰ C - 28⁰ C. Water was replaced weekly.

Larval rearing and development:

When larvae were fully developed inside the egg within brood pouch, berried prawn was transferred to a bottle shaped, short necked, 3.5 L transparent glass jar aquaria. Aerator was connected to the aquaria for additional oxygen supply. Hatching took place from midnight to late night in *M. lamarreilamarrei*. After completion of hatching, mother was transferred to the stocking aquaria. The larvae were transferred to a fresh container every day. Dead larvae were removed from the aquarium if any. Everyday water was aerated artificially for 6 hours (3 hours at day time and 3 hours at night) by air pump (SHENG ZHE, Model BS – 410). The newly hatched larvae were fed (crushed frozen dried tubifex) twice daily to ensure proper growth and development. Two or three larvae were collected daily up to 30 days in a small 5ml bottle with cap and preserved in 70% alcohol. The bottle was leveled properly to mark the age of larvae. Larvae were passed through graded alcohol (70%, 90%, 100%) for dehydration, stained with 2% eosin, cleared with xylene and mounted in DPX. Then observed the development of larvae day

wise under compound light microscope (Motic B1 SERIES) and photographed with camera (OLYMPUS Magnus MIPS – USB No. 700847).

Results:

i) Embryonic and larval development of *M. lamarreilamarrei*:

Berried female continuously agitated its pleopod for better oxygenation of the egg to ensure proper growth and development of the embryo. Initially light green or fluorescent green colour of the berried eggs gradually turned to light grey with the progress of embryonic development. Berried eggs of *M. rosenbergii* were initially deep yellow which gradually became light grey (Prasad and Singh, 2007). Opaque eggs were unfertilized or dead which removed by female with its first chelate legs. Incubation period of eggs lasted for 20-22 days at the temperature of 27-30⁰ C. But at low temperature incubation period became more longer. Below 18⁰ and above 34⁰ C embryonic development became arrested and hatchlings were not hatched out. Incubation period of *M. rosenbergii* was 15-18 days at water temperature 30⁰ (Prasad and Singh, 2007). Incubation period of *M. kistnensis* was found to 29 to 30 days (Nagabhusanam and Kulkarani, 1979).

Larval Development:

1st Zoea (Age 0 to 24 h):

The total length of first Zoea was 4.5mm and the age was about 24 h from hatching. The abdomen and carapace lack spines. The carapace length was 1.8mm and small rostrum without any teeth (Fig 2). Eyes were large, sessile and held beneath the anterior part of carapace. The abdomen consists of five segments. The last segments bear 12 hairy long setae (Fig 3). Colour of the larva was transparent. The abdomen having somites, the last one was not separated from telson.

Eyes: Eyes large, sessile. Antennules, antenna and mouth parts developed (Fig 1).

Antennules: Peduncle was unsegmented with two flagella distally.

Antenna: It was biramous, and unsegmented with scale like lateral exopod and median rod like endopod.

Maxillule: Endopod unsegmented armed with two small spines. Maxillule was uniramous with three distinct lobes.

Maxilla: Flattened, bilobed structure. The endopod was unsegmented with numerous hairs laterally and two spines.

Mandible: Mandibles were without a palp and with a spine.

Maxilliped I: Maxilliped was biramous. Exopod was longer than endopod. Exopod unsegmented carrying four setae. Endopod was small with three terminal setae.

Maxilliped II: It was biramous. Endopod 3 – segmented, last segment carrying a small setae. Exopod unsegmented with 4 setae.

Maxilliped III: Endopod four segmented last segment with one small setae. Exopod unsegmented with four setae. It was also biramous.

Abdomen: Five segmented, 3rd segment were largest, 6th segment fused to telson. Spines was absent from all segments.

Periopods: All pereopods were rudimentary, unsegmented and uniramous. First two pereopods developed without chelae.

Pleopods: Unilobed buds of five pleopods existed. Exopod and endopod were not separated and unarmed.

Telson: Telson was not separated from the last abdominal segment, bear 12 long hairy plumose terminal setae (Fig 3).

2nd Zoea (2-3 days):

Although there was no significant increase in size, the larvae had undergone some morphological change. Length of the larva was 4.5 – 5.0 mm and carapace length was 1.8 mm to 2.00 mm. Still eyes are sessile, separated a little from carapace . Telson with 12 hairy setae (Fig 3). Outlines of the uropods are visible within the telson in the 3 days old larva, still it was not separated (Fig 2). In the 2nd day, single spine present on the rostrum (Fig 6), on the next day i.e. 3rd day 2 spines present on the rostrum. On the 3rd day supraorbital spines developed on the rostrum. Carapace with prominent branchiostegal spines. Chromatophore was more prominent.

Antennule: Antennular peduncle two segmented, flagellum present distally.

Antenna: Antennal scale with only 12 long setae distally.

Mandible: Small teeth now visible, presence of two spines.

First Maxilla or Maxillule: Endopod unsegmented devoid of setae; very similar to previous stage.

2nd Maxilla: Endopod unsegmented with 3 setae. Scaphognathite present with 7 setae.

Maxilliped I: Exopod unsegmented with 6 setae. Endopod with 2 setae.

Maxilliped II: Second segment of the endopod consisted two setae.

Maxilliped III: 1st segment of the endopod with two setae, second segment with three setae and last segment with two setae.

Abdomen: A pair of lateral spines were present on posterior border of abdominal segment 5.

Pereopods: All pereopods were rudimentary, non-segmented. First two pereopods with non-functional chelae.

Pleopods: Bilobed buds of five pleopods existed (Fig 5).

3rd Zoea (4-6 days):

Length of the larva was 5.0 mm to 6.0 mm; carapace length was 2.0 – 2.5 mm. Age 4 to 6 days.

Telson: This stage was easily made out by the distinct appearance of uropods. Uropod and telson become separated from each other. Remnant of triangular telson contained 8 plumose setae. Tip of the telson clawed and each uropod consisted of more or less 12 long setae. Exopods as well as endopods were clearly differentiated. Endopodite was smaller than exopodite completely separated from each other. Exopodite dumbbell shaped. Telson articulated with sixth abdominal segment (Fig 7).

Carapace: Rostrum having 3 spines and carapace had a supra-orbital spine. Eyes were stalked. Now rostrum was elongated up to the length of eye .

Antennules: Two flagellae of the antennules were of equal length, biramous with 5 plumose setae Laterally it carried one plumose setae on inner side, four on outer side.

Antenna: 18 anterolateral spine were developed on the scale. Flagellum segmented, as long as $\frac{1}{2}$ of the body length and was more than twice as long as the scale.

Mandible: It was similar to that of previous stage.

Maxillule: Coxal endite with three spine and two setae.

2nd Maxilla: Nearly similar to previous stage. The exopod bears a large number of setae.

1st Maxilliped: It had lost two lateral setae from its endopod.

2nd maxilliped: It was unchanged except a setae are present on the distal segment of endopod.

3rd maxilliped: The basipod consists of 2 setae. 3rd segment contained 3 setae.

Pereiopods: 1st and 2nd pereiopods were chelate. Chela was non functional. 2nd pereiopods were larger than first one. Rest pereiopods are nonchelated and segmented.

Pleopods: Pleopods are long biramous leaf like. Exopod was larger than endopod.

Table 1: The length, weight, and age of different stages of Zoea larva

Sl. No.	Length (mm)	Weight (gm)	Length of the carapace	Age (day)	Developmental stage
1.	4.5	0.001	1.8	1	1 st Zoea
2.	4.5-5.0	0.001	1.8-2	2-3	2 nd Zoea
3.	5.0-6.0	0.001	2-2.5	4-6	3 rd Zoea

Discussion:

Berried eggs of *M. lamarrei lamarrei* was initially eight green, but changed to light grey as the embryonic development proceeded. Similar observation was made by Prasad and Singh, (2007) in *M. rosenbergii*, where berried yellow eggs turned to light grey as the days passed by.

Incubation period of *M. lamarrei lamarrei* was 20-22 days at 27-30° C which varied with low and high temperature. In *M. rosenbesgii* the incubation period was 15-18 days at 30° C (Prasad and Singh, 2007), where as it was 29-30 days in *M. kistnensis* (Nagabhusanam and Kulkarni, 1979). The fertilized eggs of *M. lamarrei lamarrei* were spherical shaped and 1.0 mm in diameter (both length and breadth). But in *M. kistnensis* it was oblong in shape and 0.90x0.50 mm. (Nagabhusanam and Kulkarni, 1979), in *M. rosenbergii*, measured 0.6 – 0.7 mm, slightly elliptical in shape (Ling, 1969). At the end of the 15th day of incubation period, the embryo became nauplius larvae. Eyes were prominent and chromatophores began to appear at 16th day of incubation. Appendages became more visible at 18th day. The zoea larvae formed to be hatched out at the end of 20th day. In the larval development of *M. lamarrei lamarrei*, there are four larval stages before it attained the post larval condition. The length of first zoea was 4.5mm. It was very similar to *M. kistnensis* where four zoea stages was found and length of the first zoea was 4.2 – 4.4mm (Nagabhushanam and Kulkarni, 1979). Frelder (1970) described the therezoaeal stages in *M. austrailense*. In our study, the four zoeal stages extended their life up to 9th days before they metamorphosed into post larval stage, which was up to thirteen days in *M. kistnensis* (Nagabhusanam and Kulkarni, 1979).

In *M. lamarrei lamarrei*, the pereopods became well developed and functional. The pleopods became biramous and well developed. Eyes were stalked and rostrum contained three teeth on dorsal surface. The development of these stages of larvae was also very similar to *M. kistnensis* (Nagabhushanam and Kulkarni, 1979). The post larval stages of *M. lamarrei lamarrei* contained single strong spine in telson and appendix interna became more prominent on 24th day of post larval stages. The post larval life contained up to 30 days before they became fully independent. In *M. kistnensis*, the post larval life extended up to 13th days. The rostrum contained 4 – 5 dorsal teeth (Nagabhusanam and Kulkarni, 1979).

According to Solland (1923) types of larval development, *M. lamarrei lamarrei* closely conformed second type of fresh water species, which produced fewer and larger eggs having an abbreviated larval life, usually of these stages.



Figure 1: Cephalothorax of one day larva of *M. lamarrei lamarrei* (1st Zoea)

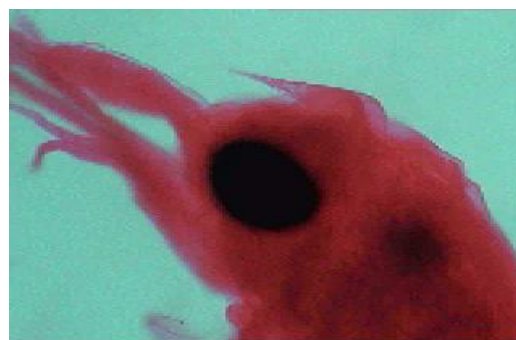


Figure 2: Rostrum of one day larva of *M. lamarrei lamarrei* (1st Zoea)



Figure 3: Unsegmented telson of one day larva

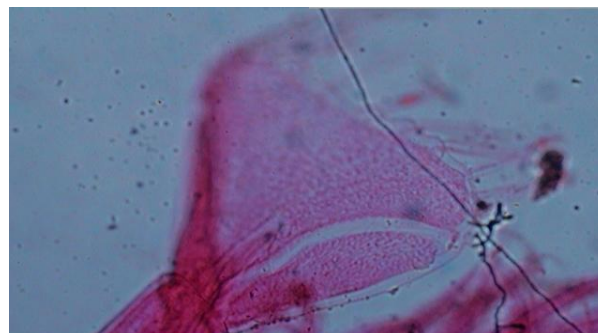


Figure 4: Telson showing outline of uropod (3 days) of *M. lamarrei lamarrei* (1st zoea)

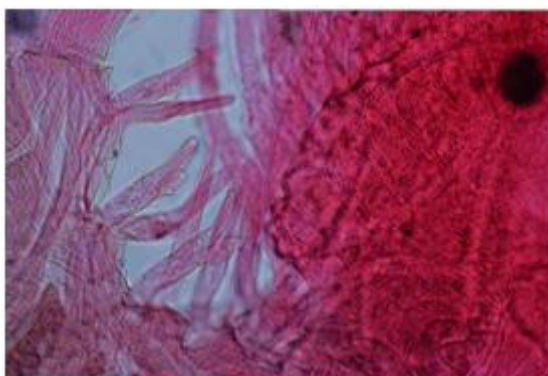


Figure 5: Initiation of formation of biramous pleopod (3 days)

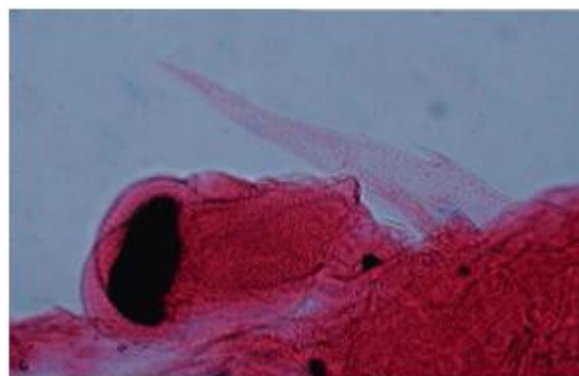


Figure 6: Rostrum showing single spine (2 days)



Figure 7: Abdomen of 6 day larva of *M. lamarrei lamarrei* (3rd Zoaea)

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HISTOCHEMICAL CHARACTERISTICS AND FUNCTIONAL ASPECTS OF THE OLFACTORY EPITHELIUM IN AN ASIAN SCHILBEID CATFISH, *CLUPISOMA GARUA* (HAMILTON, 1822)

Saroj Kumar Ghosh

Department of Zoology,

Bejoy Narayan Mahavidyalaya, Itachuna, Hooghly-712 147, West Bengal, India

Corresponding author E-mail: saroj.fisherylab@gmail.com

Abstract:

The localization of silver reaction appropriate to axons, acid and neutral mucopolysaccharides pertaining to mucous cells, biologically active glycogen, protein and lipid constituents as well as alkaline phosphatase (ALPase) and adenosine triphosphatase (ATPase) in the cells lining the olfactory mucosa of *Clupisoma garua* (Siluriformes, Ailiidae) have been described by employing different histochemical methodology. The silver stain is well marked in the primary and secondary receptor cells as well as rod receptor cells. The highest magnitude of acid and neutral mucin is discernible in the secretory and non-secretory mucous cells. The ciliated and non-ciliated supporting cells contain much glycogen. Variant of protein and lipid localization in the cellular elements of olfactory lining have been correlated with feeding niches of the organism. Acute ALPase and ATPase activity have been observed in the extended dendrite processes of receptor cells, ciliated supporting cells, labyrinth cells and basal cells. The functional significance of the olfactory system has been discussed in relation to the life of fish concerned.

Keywords: *Clupisoma garua*, Olfactory system, Cell types, Histochemical feature, Chemoreception

Introduction:

Olfaction has profuse functions in the life of fish. Olfactory and gustatory are the main chemosensory pathways that allow the fish to sustain in an aquatic environment. Olfaction is paramount mediator of chemical signals and considered to be of the first range of the behavioural adaptations (Hara, 1994). Olfactory cues perform momentous role in the behavioural activities of fishes such as procurement of food, selection of mate, location of breeding site, run away from predators, parental behaviours, orientation and in many other approaches (Singh *et al.*, 1995). Chemical information is detected by the sensory neurons on the olfactory mucosa which transmit signals directly to the central nervous system by the olfactory tract. The morphoanatomy and

cellular organization of olfactory organ variegates considerably among teleosts belonging to different taxonomic levels and varying habits. The structure and function of the olfactory organs of teleosts have been studied by many workers. Very limited attention has been paid to histochemical investigation of the olfactory mucosa of fishes (Dorshenko, 2007; Sinha, 2008; Bettini *et al.*, 2009; Ghosh and Chakrabarti, 2015; Kim *et al.*, 2019). The knowledge of the enzyme histochemistry of the olfactory epithelium of schilbeid catfish is almost unknown.

An attempt has been made to evaluate the chemical complexion and functional importance of various cells lining the olfactory mucosa of river catfish, *Clupisoma garua* (Hamilton, 1822) by histochemical analysis.

Materials and Methods:

Fish sampling:

Mature specimens of *C. garua* (measured 16 ± 1.67 cm in total length) were collected from the Bhagirathi-Hooghly River at Kalyani surrounding areas of West Bengal using traditional fishing gear throughout the year 2019. The specimens were anaesthetized and euthanized with an overdose of Benzocaine approved by intuitional animal care and use committee. The olfactory organs were dissected out thoroughly from the olfactory pits under stereoscopic binocular microscope (Magnus MS24) and processed for relevant techniques.

Histochemical methods:

Olfactory tissues were fixed in 10% neutral formalin for 16-18 hour immediately after collection. After fixation, the tissues were washed well in 70% ethanol and dehydrated in graded ethanol series, cleaned in xylene and embedded in paraffin wax of 52-54°C (Merck) by the usual method. Serial paraffin sections were cut at 8 µm thick using a rotary microtome (Weswox MT-1090A). The following histochemical tests were applied to the sections: Silver Impregnation Method (SIM) for detection of axons (Marsland *et al.*, 1954), Periodic Acid Schiff's (PAS) reaction in combination with Alcian Blue (AB) (PAS-AB) for detection of neutral and acid mucins (Mowry, 1956), Best's Carmine (BC) method for detection of glycogen (Best, 1906), Mercury-Bromphenol Blue (MBB) method for detection of basic protein (Mazia *et al.*, 1953) and Sudan black B (SB) method for detection of bound lipid (Berenbaum, 1958). All the slides were examined and photographed using a light microscope (Zeiss Primo Star) equipped with a Tucsen 5.0 MP camera at different magnification.

Enzyme histochemical methods:

The olfactory organs were removed and quickly fixed in cold absolute acetone (Merck) for 16 hour at 4°C. The tissues were dehydrated in two changes of absolute acetone each for 45 minute. After clearing in methyl benzoate, the tissues were embedded in paraffin wax at 48°C in vacuum medium for 30 minute. Serial paraffin sections of 8 µm thick were cut and followed

Calcium-Cobalt method for detection of Alkaline Phosphatase (ALPase) (Gomori, 1951) and Calcium method for detection of Adenosine Triphosphatase (ATPase) (Padykula and Herman, 1955). The staining sections were photographed under Zeiss Primo Star trinocular microscope.

Results:

Olfactory lamella of *C. garua* emitted from raphe is consisted of two layers of olfactory epithelium enclosing central lamellar space, the central core. The central core is distinguished from the epithelium by a basement membrane and composed of loose fibres, connective tissues, bundle of nerves and blood vessels. The olfactory epithelium comprises of the following cell types: primary receptor cells, secondary receptor cells, rod cells, microvillous cells, labyrinth cells, mucous cells, basal cells, mast cells, ciliated and non-ciliated supporting cells.

Different histochemical techniques have been carried out for locating and distribution of axons, mucopolysaccharides, glycogen, basic protein, bound lipid, alkaline phosphatase and adenosine triphosphatase in the olfactory cells lining the mucosa concerning their chemical component and utility. The results procured from the present investigation are mentioned below.

Histochemistry:

Detection and localization of axons:

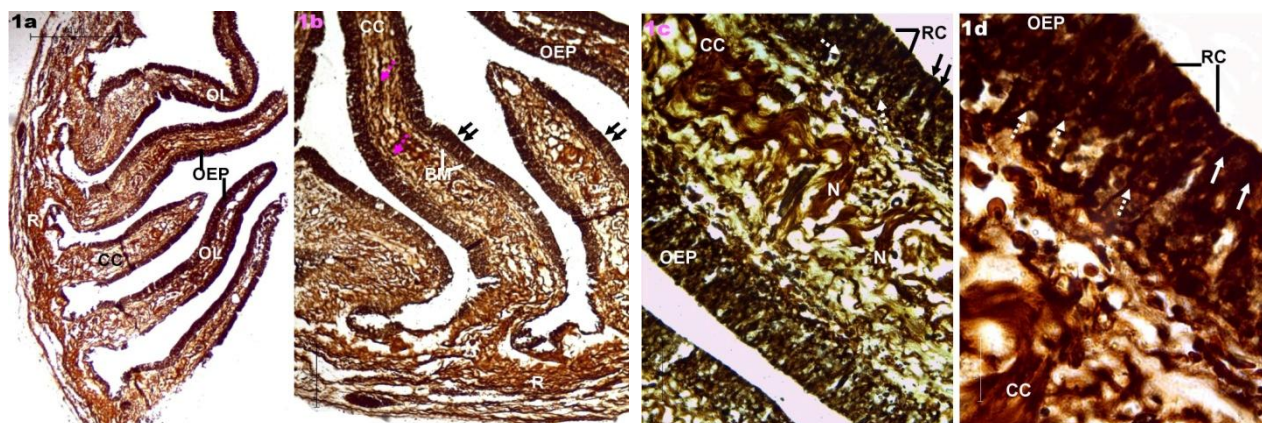


Figure 1: Photomicrographs of the section of different regions of olfactory lamella of *C. garua* showing silver deposition in the neurons by Silver Impregnation Method (SIM). (a) Olfactory lamellae (OL) furnished silver reaction in the olfactory epithelium (OEP) and central core (CC) (4X). (b) Showing pronounced silver reaction in the dendrite process of primary receptor cells (solid arrows) of OEP. Note positive reaction in nerve fibres (broken arrows) of CC and basement membrane in between OEP and CC (10X). (c) Intense localization of silver stain in primary receptor cells (RC), secondary receptor cells (broken arrows) and rod cells (solid arrows) of OEP. Noted bundle of nerve fibres (N) in CC (40X). (d) Exhibited silver staining in primary receptor cells (RC) along with synaptic connection of secondary receptor cells (broken arrows) and rod cells (solid arrows) of OEP.

CC marked central core (SIM) (100X).

Silver reaction shows profound brown colour and distinguishes the occurrence of receptor cells in the epithelial lining. Different magnitude of silver deposition is observed in the various layers of the olfactory lining in *C. garua* (Figs. 1a-b). Intense reaction of silver staining is associated with dendrite process of primary receptor cells (Figs. 1b-d). The silver reaction is also discernible in the rod cells and synaptic association in between primary and secondary receptor cells (Figs. 1c-d). Bundle of nerve fibres in the central core and the basement membrane also arduously stained with silver reaction (Figs. 1b-c).

Detection and localization of mucopolysaccharides:

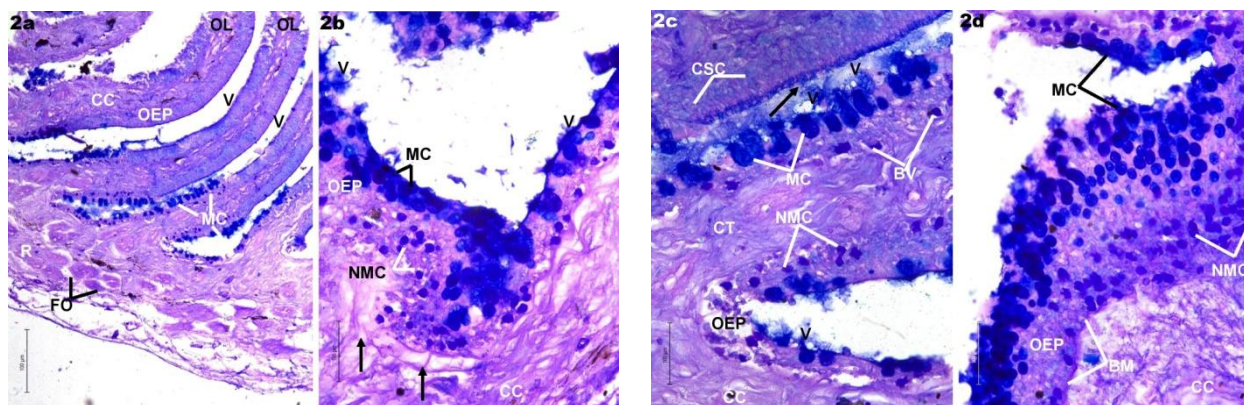


Figure 2: Photomicrographs of the vertical section of different regions of olfactory lamella of *C. garua* showing Periodic Acid Schiff's (PAS) reaction in combination with Alcian Blue (AB) (PAS-AB). (a) Localization of exclusively acid mucin in the secretory mucous cells (MC) of olfactory epithelium (OEP). Moderate reaction occurred in the central core (CC) and epithelial lining (arrow heads) of olfactory lamella (OL). Noted fila olfactoria (FO) in raphe (R) (10X). (b) Intense acid mucin in the secretory mucous cells (MC) and neutral mucin in non-secretory mucous cells (NMC) of OEP. CC having connective tissue fibres showing moderate reaction. Arrows indicated luminal secretion (40X). (c) Showing localization of acid mucin in secretory mucous cells (MC) and neural mucin in non-secretory mucous cells (NMC). Displaying the localization of mixture of acid and neutral mucin in the ciliated supporting cells (CSC) and epithelial border (solid arrow). Connective tissues (CT) and blood cells (BC) of CC furnished moderate reaction. Arrow heads marked secreted mucin from vacuolated and coarsely reticulated MC (40X). (d) Displaying the intense acid mucopolysaccharide reaction in peripheral mucous cells (MC) and neutral mucopolysaccharide reaction in non-secretory mucous cells (NMC) of OEP. Noted moderate reaction in basement membrane (BM) and CC (40X).

Among the various type olfactory cells in the epithelial lining of *C. garua*, the mucous cells possess the mucosubstances. The mucous cells at their various stages of maturity containing different degrees of mucopolysaccharides have been identified by employing the PAS-AB

histochemical technique. They are categorized as secretory, mature non-secretory and basal non-secretory ones. The former category is capable of secreting mucins and obviously located along the peripheral layer of the epithelial lining. On the contrary, the non-secretory mucous cells whose mucins are yet to be secreted are embedded either in the superficial layer and/or in the deeper layer of the olfactory mucosa. The latter type, therefore, may be termed as the peripheral non-secretory mucous cells which are generally mature enough for discharging their content to the exterior and basal non-secretory ones which are not yet mature to secrete their content. However, secretory and non-secretory mucous cells in the olfactory epithelium of the experimental fish species differ greatly in their distribution and chemical nature which have been studied by employing PAS-AB histochemical technique and described foregoing.

The combined PAS-AB reaction furnishes a purple-bluish colour of varying intensities in accordance with the neutral and acid mucin content of the various mucous cells in the olfactory epithelium. This combined test imparts a bright red colour due to PAS for neutral mucin and bright blue colour for AB reaction due to the presence of alcinophilic mucin exclusively. The secretory mucous cells and peripheral non-secretory mucous cells of the olfactory epithelium exhibit bluish and purple colour confirming the presence of acid and neutral mucins respectively (Figs. 2a-d). The basement membrane shows moderate reaction to this test (Fig. 2d). The secretory mucous cells are vacuolated and coarsely reticulated, situated mainly at the apical region of the lamella (Fig. 2c). The ciliated supporting cells shows moderate reaction to PAS-AB test (Fig. 2c). The connective tissues, collagen fibres and blood cells of the central core are also positively reacted (Figs. 2c-d). The secreted epithelial mucins also positively react with this test. The receptor cells however, display negative reaction to PAS-AB test.

Detection and localization of glycogen:

The results of Best's carmine test indicate an intense to moderate red colour in the receptor cells, basal cells, ciliated supporting cells, non-ciliated supporting cells and mast cells in the olfactory epithelium of *C. garua* (Figs. 3a-d). However, maximum glycogen content is observed in the ciliated and non-ciliated supporting cells (Figs. 3c-d). The epithelial lining of lamella also positively reacts with this reaction. The central core containing blood vessels and connective tissue fibres show different shades of staining deposition of glycogen.

Detection and localization of basic protein:

Mercury-Bromphenol Blue reaction has been employed for the detection of protein material associated with the different cells of the olfactory epithelium (Figs. 4a-b). The presence of protein material associated with the mucopolysaccharide content of secretory mucous cells in the olfactory epithelium of *C. garua* is well documented (Fig. 4d). The non-secretory mucous cells in the deeper region of the epithelium stained more intensely due to the accumulation of higher amount of protein. Moreover labyrinth cells, basal cells, ciliated supporting cells, non-

ciliated supporting cells and receptor cells along with extended dendrite processes of surface epithelium also positive to this test (Figs. 4c-d). This reaction has been encountered in the blood cells and connective tissues in central core (Figs. 4c-d).

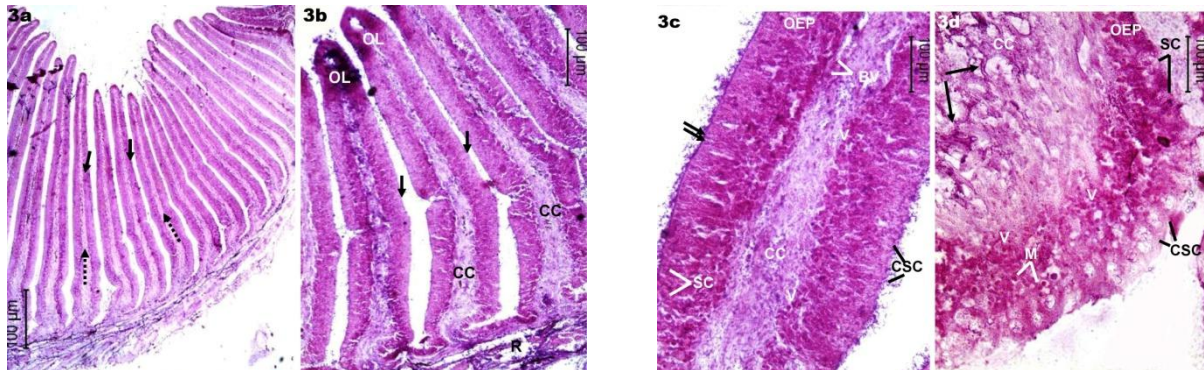


Figure 3: Photomicrographs of the vertical section of different regions of olfactory lamella of *C. garua* showing Best's Carmine (BC) reaction for glycogen. (a) Showing positive Best's Carmine reaction in olfactory epithelium (OEP) (solid arrows) and moderate reaction in central core (CC) (broken arrows) (4X). (b) Intense localization of glycogen in the epithelial border (arrows) of olfactory lamella (OL). Central core (CC) and raphe (R) displayed moderate reaction (10X). (c) OEP exhibited glycogen content in ciliated-supporting cells (CSC), non-ciliated supporting cells (SC), receptor cells (solid arrows) and basal cells (arrow heads). CC with blood vessels (BV) showed moderate reaction (40X). (d) Showing intense localization of glycogen in SC and CSC. Mast cells (M) and basal cells (arrow heads) of OEP and connective tissues (arrows) of CC contained different shades of staining deposition of glycogen (40X).

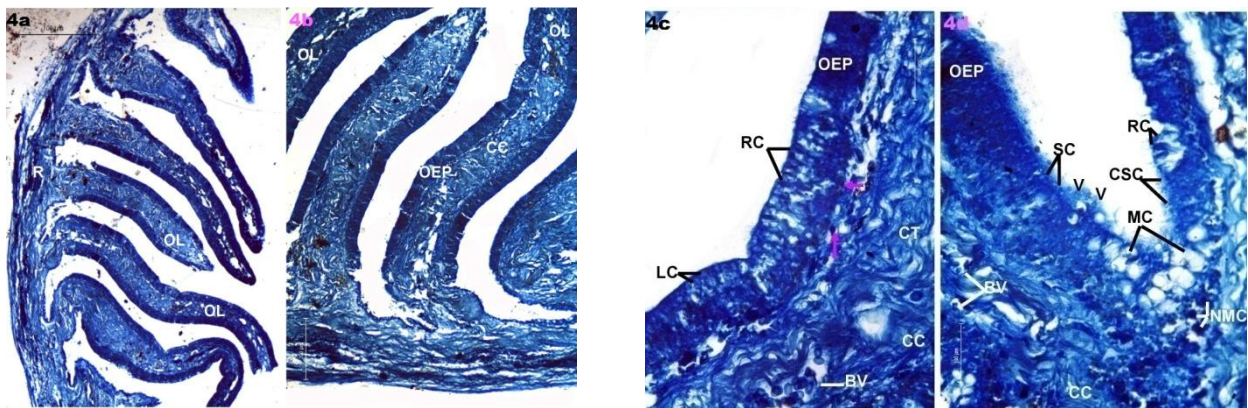


Figure 4: Photomicrographs of the vertical section of different regions of olfactory lamella of *C. garua* showing Mercury-Bromphenol Blue (MBB) reaction for basic protein. (a) Localization of protein in various layers of olfactory lamellae (OL) based on (R) (4X). (b) Showing intense protein reaction in various cells lining the olfactory epithelium (OEP) and central core (CC) of OL (10X). (c) Showing intense localization of protein in receptor cells (RC) labyrinth cells (LC) and basal cells (BC) of olfactory epithelium (OEP). Noted positive reaction in blood vessels (BV) and connective tissues (CT) of central core (CC) (40X). (d) Localization of protein in luminal secretion (arrow heads) of mucous cells (MC), non-secretory mucous cells (NMC), ciliated supporting cells (CSC), non-ciliated supporting cells (SC) and RC. Note intense reaction in BV of CC (40X).

Detection and localization of bound lipid:

Sudan black B staining has been performed for distinguishing bound lipid among the various cells of the olfactory epithelium of *C. garua*. Deposition of sudanophilic material imparts bluish-black colour. Considerable reaction with Sudan black staining is found to be associated with the ciliated receptor cells including dendrite processes and ciliated supporting cells (Figs. 5a-d). Labyrinth cells and basal cells furnish moderate lipid content (Fig. 5c). The blood vessels and nerve fibres of central core show intense reaction (Figs. 5b-d).

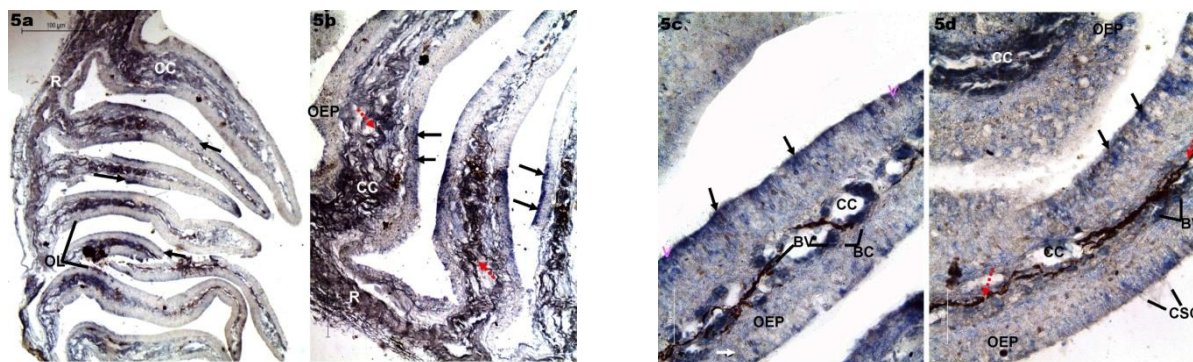


Figure 5: Photomicrographs of the vertical section of different regions of olfactory lamella of *C. garua* showing Sudan black B (SB) reaction for bound lipid. (a) Showing lipid reaction in epithelial border (arrows) and central core (CC) of olfactory lamella (OL) radiated from raphe (R) (4X). (b) Exhibiting strong sudanophilic reaction in ciliated receptor cells (solid arrows) of olfactory epithelium (OEP) and nerve bundles (broken arrows) of CC. R for raphe (10X). (c) Showing lipid reaction in receptor cells (solid arrows), labyrinth cells (arrow heads) and basal cells (BC) of OEP. Noted positive reaction in blood vessels (BV) of CC (40X). (d) Localization of lipid content in receptor cells (solid arrows) and ciliated supporting cells (CSC) along OEP. Nerve fibres (broken arrows) and BV of CC exhibited acute reaction (40X).

Enzyme histochemistry:

Detection and localization of alkaline phosphatase (ALPase):

ALPase activity furnishes dark brown colour in the epithelial lining. Maximum ALPase activity is discernible in the extended dendrite processes of receptor cells, ciliated supporting cells, labyrinth cells and basal cells (Figs. 6b-d). Diffused granules are observed among basal cells. Positive enzyme activity is noticeable in microvillous cells of epithelial border (Fig. 6d). Moderate localization of this enzyme is observed in the blood cells and nerve fibres of the central core (Figs. 6c-d). Mucus cells and their secretion show negative activity.

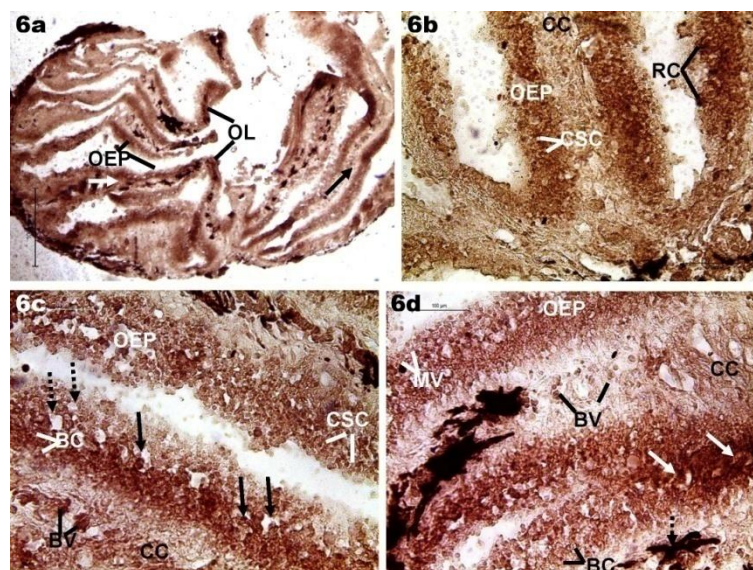


Figure 6: Photomicrographs of the vertical section of different regions of olfactory lamella of *C. garua* showing Alkaline Phosphatase (ALPase) activity. (a) Showing ALPase actiity in the epithelial lining (OEP) of olfactory lamella (OL) based on raphe (R). CC marked central core (4X). (b) Displaying the localization of ALPase in receptor cells (RC) and ciliated supporting cells (CSC) of OEP. CC for central core (10X). (c) Positive ALPase activity in CSC, labyrinth cells (solid arrows) and basal cells (BC) of OEP. Noted moderate enzyme activity in blood vessels (BV) of CC and negative activity in mucous cells (broken arrows) (40X). (d) Exhibited ALPase activity in microvillous cells (MV), labyrinth cells (solid arrows) and basal cells (BC) of OEP. CC containing BV (arrow heads) and nerve fasciculus showed positive activity (40X).

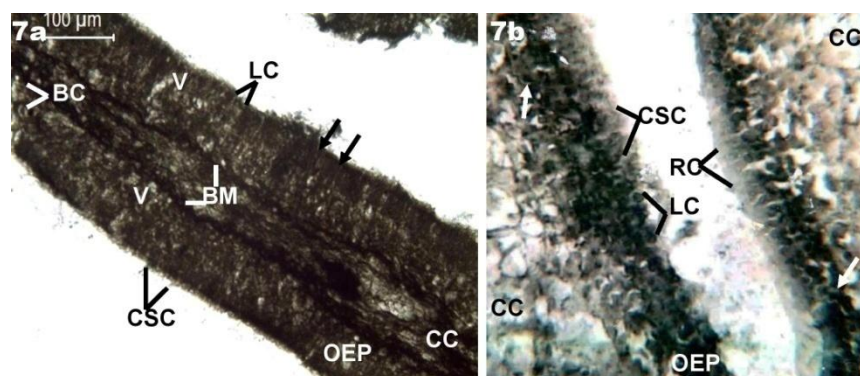


Figure 7: Photomicrographs of the vertical section of different regions of olfactory lamella of *C. garua* showing Adenosine Triphosphatase (ATPase) activity. (a) Showing intense ATPase activity in receptor cells (solid arrows), labyrinth cells (LC), ciliated supporting cells (CSC), mast cells (arrow heads) and basal cells (BC) of OEP. Basement membrane in between OEP and central core (CC) furnished strong enzyme activity (10X). (b) Localization of ATPase in receptor cells (RC), CSC, LC and basal cells (arrows) of OEP. CC for central core (40X).

Detection and localization of adenosine triphosphatase (ATPase):

The site of ATPase activity takes blackish-brown colour in the different portions of the lamella. Intense ATPase activity is observed in the receptor cells, ciliated supporting cells, labyrinth cells and mast cells (Figs. 7a-b). The nucleus and nucleolus in all cells are diffusely stained. Moderate enzyme activity is associated with the dense granules of basal cells and the large nuclei are strongly positive (Fig. 7a). The basement membrane below the basal cells is also positive. The nerve fibres and blood cells in the central core show moderate to intense ATPase activity (Figs. 7a-b).

Discussion:

Chemical communication is associated with the development of chemosensory organs and chemical signals are effused in aquatic environments by different teleosts (Hara, 2000). Chemical stimuli are detected by the apical dendritic parts of sensory cells lining olfactory epithelium and admissible behaviours revealed by organisms. In the present study, the intense localization of silver reaction in the olfactory epithelium in *C. garua* provide a direct evidence of synaptic connection of primary and secondary neurons as well as orientation of dendrites of the receptor cells, along the most superficial layer of the olfactory epithelium. Silver staining in the knob like structure of the primary receptor cells related to the transmission of various nerve impulses precisely to the dendrites of secondary receptor cells. In some places of the olfactory epithelium intense silver deposition in the axons of the primary receptor cells make synaptic contacts with the dendrites of the secondary neurons and the axons of the secondary neurons which enter in the central core are also intensely stained with silver reaction. This clearly advocates that the impulses received by the dendrites of primary receptor cells ultimately send impulses to the central core for final transduction of impulses to the brain (Ghosh and Das, 2020). Further study is needed to fully describe the morphology of the neuron, their connection with the central core and olfactory bulb.

The histochemical nature of the olfactory epithelium mucin in *C. garua* have been studied by employing PAS-AB histochemical test to establish its chemical nature as well as finding out the importance of its secretion with olfaction of fish. Chemically mucins are hexoseamine-containing polysaccharides which are bounded covalently with varying amounts of proteins. Mucins secreted by the mucous cells of the olfactory epithelium are of mucoprotein or glycoprotein in nature (Datta Munshi and Singh, 1975). Secretory and non-secretory mature mucous cells situated at the surface of the olfactory epithelium contain a mixture of neutral and acid mucins as confirmed by PAS-AB histochemical test. The secretion of mixture of acid and neutral mucopolysaccharides from the mucous cells probably helps to prevent friction against microscopic debris and also helps the smooth flow of water in the olfactory chamber. This is in

conformity with the findings of Rahmani and Khan (1980) in the olfactory mechanism of *Anabas testudineus* and Chakrabarti (2005a) in the olfactory epithelium of *Puntius javanicus*. Zalewsky and Moody (1979) also reported the presence of heterogenous nature of mucus and its secretion as a mosaic of neutral and acidic mucopolysaccharides in the canine gastric mucosa. Peripheral secretory mucous cells and their luminal secretion are strongly reacted with AB for acid mucopolysaccharide. This is due to the fact the acid mucin content of the secretory mucous cells keep the epithelial surface of the olfactory rosette moist and helps in easy flow of water and prevent friction during onward transmission of water through olfactory nares. On the other hand, the non-secretory mucous cells are provided with exclusively neutral mucin as advocated by PAS-AB test. This is probably due to the fact that the mucous cells involve in the synthesis of complex glycoprotein macromolecules during their formation from the multipotent cells. However, Sinha (1975) reported that during the early stages of development of the mucous cells *i.e.*, in the mucous mother cells there is a primary synthesis of neutral mucopolysaccharide granules which in the course of development of the same either transformed into acid mucopolysaccharide granules and/or the cells synthesizes new acid mucopolysaccharides present in the subsequent stages in the mature non-secretory and secretory mucous cells. These polysaccharide granules (acidic and neutral) ultimately fuse together in the mature non-secretory mucous cells which are gradually pushed towards the periphery and give rise to a complex mixture of both acidic and neutral mucins (Gona, 1979). Intense bluish-purple colour in PAS-AB reaction in the empty mucous cells is due to the presence of profuse amount of heparin which are thought to cause fluctuations in the production of mucus in the olfactory mucosa. Moulton and Beidler (1967) reported that the terminal mucus film in the olfactory mucosa is believed to be an important factor in the olfactory process which influences the variations in the olfactory sensitivity.

The various degrees of glycogen localization occur in the cytoplasm and nuclei of receptor cells, basal cells, mast cells, supporting cells in *C. garua*. The movement of cilia of the supporting cells maintains a continuous directional flow along the surface epithelium, which is an active process, requires energy and presence of glycogen particles is the main source of such energy. The glycogen reaction in the receptor cells may be related to the transduction of various impulses and glycogen probably act as a substrate for the source of energy required for the activities (Chakrabarti, 2005b). Glycogen in the basal cells and mast cells under study may help in maintaining their metabolic status as well as provide energy so essential for sustaining their motility.

The mucous cells, receptor cells, labyrinth cells and basal cells of the olfactory epithelium of *C. garua* exhibited intense protein reaction in the present study. The contents of the mucous cells exhibit positive reaction for protein confirming the proteinaceous nature of the

aforesaid cell which synthesis neutral glycoproteins. Therefore, it is concluded that the secretion from the mucous cells is at least in part, proteinaceous. The labyrinth cells and basal cells in the olfactory epithelium of experimental fish exhibit intense to moderate reaction for protein probably for various metabolic as well as physiological activities (Ghosh and Chakrabarti, 2015).

The occurrence and localization of bound lipid in the dendrite process of receptor cells, ciliated supporting cells, labyrinth cells and basal cells in the olfactory epithelium of *C. garua* have been confirmed. The presence of relatively higher quantity of sudanophilic lipid material in the receptor cells may be a sign of myelinated sheath in the axons of receptor cells and probably help in the impulse transduction process. The concentration of lipid material in blood cells of central core needed as a source of endogenous energy including its involvement for the physiological activities (Chakrabarti and Ghosh, 2013).

ALPase reaction has been found to occur in the various cells lining the olfactory epithelium in *C. garua*. The occurrence of ALPase activity in microvillous receptor cells for mediating responses to olfactory cues. The dendrite process of the receptor neurons display strong ALPase reaction in is probably due to its positive role in transportation of various chemicals and nerve impulses from olfactory epithelium to the central core. Further, the ALPase activity in the nuclei of basal cells, labyrinth cells and supporting cells in the olfactory epithelium may be involved in the degradation of nucleotides and may be coupled with some kind of metabolic processes. Banerjee and Mittal (1975) while dealing with the histochemistry of giant cells located in the skin epidermis of *Clarias batrachus* reported that the perinuclear areas of the aforesaid cells are metabolically active. The intense ALPase activity in the blood capillaries of the central core of olfactory epithelium probably helps the transportation of various macromolecules. Moog (1946) however, confirmed the role in transportation of ALPase in the blood vessels of the ovulatory follicles in domestic duck.

During the course of present study, it has been observed the deposition of ATPase reaction in the olfactory cells lining the mucosa of *C. garua*. However, intense ATPase activity the basal cells in all fishes may be involved in the process of mitotic activity for the replacement of regenerating receptor and other cells of the olfactory epithelium. Andres (1966; 1969) also suggested that basal cells are the precursors of regenerating receptor cells. Evans *et al.* (1982) also observed increased mitotic figures in the basal region in a constituting epithelium after degeneration. ATPase activity in the receptor cells including their dendrite processes of the fish under study is related to the transmission of various nerve impulses. Shantra and Nakajima (1970) reported that the presence of ATPase in the olfactory cell axons of the olfactory mucosa of monkey possibly involved in the process of olfactory sensations elicited by the contact of odour particles with these receptor cells. ATPase activity in mast cells and labyrinth cells for

performing different metabolic and physiological roles. Cuschieri (1974) remarked pungent ATPase activity in supporting cells pointed to high metabolic activity.

C. garua possesses acute sense of smell and depend on their olfactory organ for exploring the surrounding aquatic environment in which they subsist. Olfaction is accomplished predominantly by olfactory cells on the epithelial lining. Occurrence of different sensory neurons on the olfactory mucosa response to chemical changes in the surroundings aquatic environment. For better understanding, the chemoreception in affinity with reproductive behaviour and necessary life processes of the organism, more investigations are to be suggested.

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MICRONUCLEUS ASSAY IN HUMAN BUCCAL CELLS AS A TOOL FOR BIO-MONITORING NUCLEAR ANOMALIES

Gargi Dutta

Mentor of Biotechnology Laboratory of Birla Industrial and Technological Museum,

19A, Gurusaday Road, Kolkata – 700019

Corresponding author E-mail: gargidutta9@gmail.com

Abstract:

The micronucleus assay in human buccal cells is an innovative, sensitive, simple, fast and non-invasive technique which is used as biomarker for genomic damage. MN assay acts as a tool for bio-monitoring nuclear anomalies as it gives the opportunity to evaluate the genotoxic damage by quantifying frequencies of micronuclei, binucleated cell, broken egg, karyolysis, karyorrhexis, pyknosis and condensed chromatin. This article reviews the MN assay with respect to exfoliated buccal cells of individuals irrespective of age, gender, genotypic diversity exposed to different carcinogenic mixtures and the cytogenetic effects of environmental and occupational pollutants like pesticides, trivalent chromium, derivatives of petroleum, lifestyle factors like smoking, chewing of tobacco, using of alcoholic mouthwash, medical procedures and different diseases. It is proposed to enhance the advancement and reliability of evaluation of genomic instability by micronucleus assay on a large scale as it can be applied in human biomonitoring, cancer risk assessment and ecotoxicology.

Keywords: Micronucleus, environmental pollutant, buccal cells, nuclear anomalies, DNA.

Introduction:

The most important fundamental cause of developmental and degenerative disease is genomic damage. Gradual damage to DNA can be caused by environmental exposure to genotoxins, lifestyle habits (e.g. alcohol, smoking, drugs, etc.), treatment related genotoxins (e.g. radiation and chemicals), micronutrient deficiency and genetic factors. These damaged DNA are not naturally repaired and can lead to genomic instability and can increase risk of cancer.

There is an increasing effort worldwide to determine the impact of environmental, genetic and lifestyle factors on genomic stability in human populations. One technique that has been adopted by numerous laboratories is the measurement of the Micronuclei (MN) in peripheral blood lymphocytes and to a lesser extent epithelial cells, erythrocytes and fibroblasts. MN provide a measure of both chromosome breakage and chromosome loss and it has been shown to be at least as sensitive and indicator of chromosome damage as classical metaphase

chromosome analysis. The key advantage of the MN assay is the relative ease of scoring and the statistical power obtained from scoring larger numbers of cells than are typically used for metaphase analysis (1).

The buccal cell was first proposed by Stich et al. (1,2), is useful as a biomarker of genetic damage caused by lifestyle habits (e.g. consumption of tobacco products, alcohol, micronutrient deficiency), exposure to environmental pollutants (e.g. pesticides, arsenic, formaldehyde), medical procedures (e.g. radio and/or chemotherapy) as well as inherited genetic defects in DNA repair (1-5).

The non-invasive nature of this technique makes it an attractive candidate for the bio-monitoring of the human populations or individuals. However, the increased use of the MN assay in buccal cells has led to a wide diversity in techniques, timing for cell collection relative to exposure period, cell and nuclear staining procedures, numbers and types of cells analysed, and scoring criteria for micronuclei (MN) and other nuclear anomalies (e.g. karyorrhexis, condensed chromatin, pyknosis, karyolysis, “broken egg” or nuclear buds; 4,5).

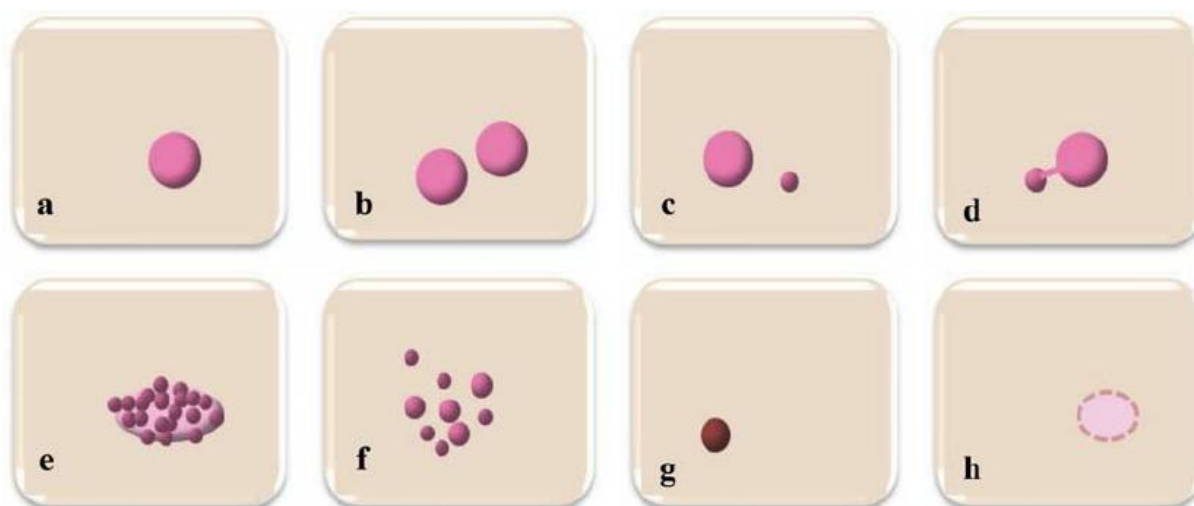


Figure 1: Schematic diagram of different types of buccal cells and the possible mechanisms for their origin; (a) normal differentiated cell (normal genome); (b) binucleated cell (cytokinesis defect); (c); micronucleated cell (chromosome breakage or loss); (d) nuclear bud (gene amplification); (e) condensed chromatin (apoptotic cell death); (f) karyorrhexis (apoptotic cell death); (g) pyknosis (apoptotic cell death); (h) karyolysis (necrotic cell death)

Different issues related to the buccal cell MN assay were reviewed in several publications over the last decade (6,7,11-13). However, most of these articles focused primarily on either broad evaluation of non-invasive methods for biomonitoring (12), the associations of micronuclei (MNi) or other markers with cancer in various types of exfoliated cells (7), or are

limited to effects of a specific type of exposure, such as smoking and smokeless tobacco (13) or formaldehyde (11).

Occupational exposure:

A comparative study on micronuclei of buccal mucosa of carcinoma patients and petrol pump attendants:

An elevated frequency of micronuclei of buccal cells was observed in oral carcinoma group and then followed by the **petrol pump workers** of 20 different petrol pump stations who were working five years or more in petrol pumps (14). Subjects were all non-alcoholics and non-smokers and they were not exposed to any radiation within past one year and were not suffering from any systemic diseases, endocrine and known immunological diseases since past 1 year.

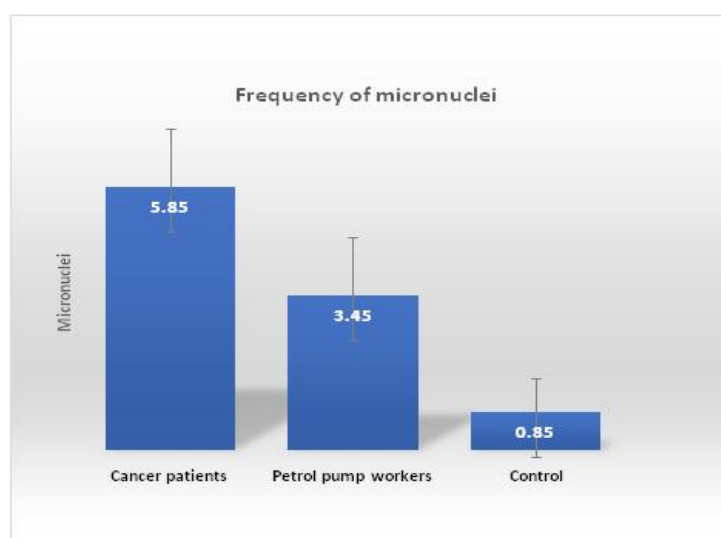


Figure 2: Micronuclear frequency of petrol pump workers in comparison to cancer patients and control group

Micronucleus test on buccal epithelial cells of automobile spray painters:

Frequency of buccal cell micronuclei that resulted from chromosomal breakage was more in **automobile spray painters of Nigeria** than in the control group of exposed volunteers. Increased micronucleus frequency was also observed with the increased age, smoking and alcohol consumption habits. All the participants of the study were male. The nuclear abnormalities showed a sign of cytogenetic damage from occupational exposure to genotoxic chemicals (15).

MN assay of buccal cells of chromium exposed tannery workers:

Tannery workers showed a significant increase in the micronucleus when compared with the control group. Smokers and alcoholic tannery workers showed a highly significant increase in the micronucleus frequency when compared to the control group smokers and

alcoholic individuals. Study showed the adverse effects of occupational exposure to trivalent chromium in tannery workers (16).

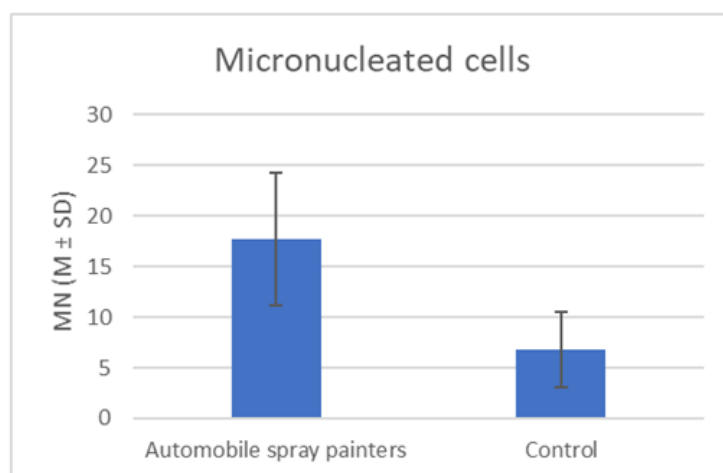


Figure 3: Frequency of MN in automobile spray painters

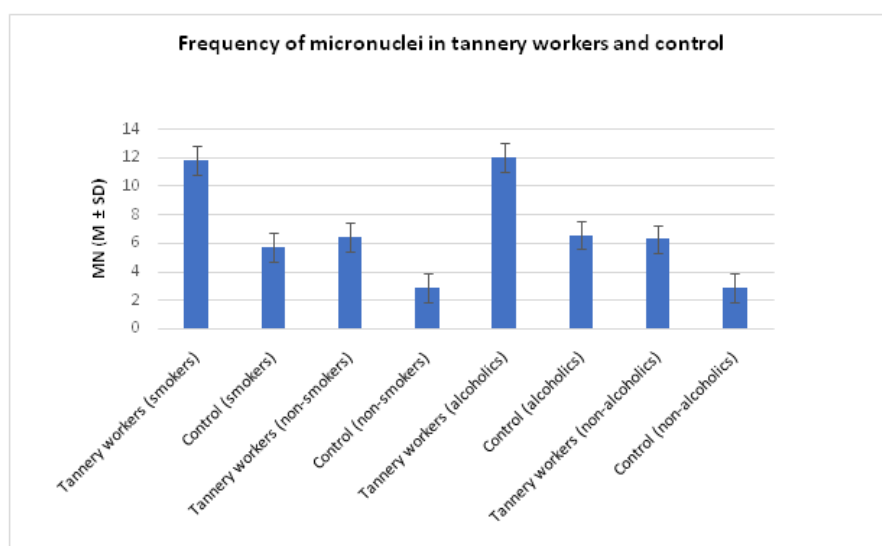


Figure 4: Frequency of MN in tannery workers who have different life-style exposure

MN and nuclear anomalies in buccal mucosa of Calcite factory workers:

Increased micronuclei and nuclear abnormalities frequencies in Turkey calcite factory workers showed that they are under the risk of significant cytogenetic damage (17). Smokers and nonsmokers were both included in both the groups. The study helped to reveal the harmful effects of calcite dust formed during its processing on the health of calcite factory workers.

MN and nuclear anomalies in petrol station workers:

Another study revealed that petrol station attendants of Tamil Nadu have a higher degree of nuclear anomalies for long occupational exposure (18). Age and sex ratio were same in both

the groups of petrol station attendants and in the control group. The genotoxic effect of derivatives of petroleum exposure is observed in subjects.

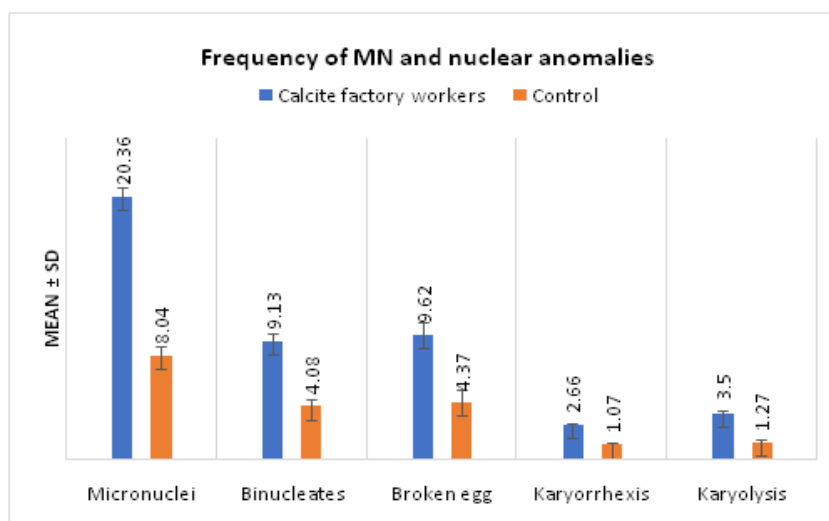


Figure 5: Frequency of MN and nuclear anomalies in buccal mucosa of Calcite factory workers exposed to environmental contaminant, trivalent chromium

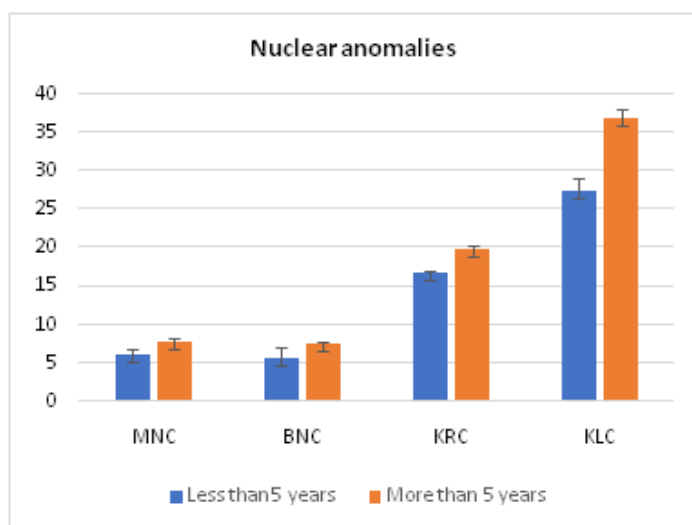


Figure 6: Frequency of nuclear anomalies in petrol pump workers in respect to duration of exposure

Observed an increase of nuclear abnormalities other than micronucleated cells, like binucleated cells, karyolysis, karyorrhexis in the exfoliated buccal cells of petrol pump workers in respect to duration of exposure.

Petrol station workers are always exposed to petroleum derivatives and the product of the fuel fumes and combustion (18). Duration of working hours for over a long period also affect human health which is clearly seen in the significant difference of nuclear anomalies when compared with the male workers to the female workers. Less nuclear abnormalities were found

in female workers who do not work for late hours on night shifts and almost of the female attendants in petrol stations do not serve for more than three years due to various socio-economic reasons in India.

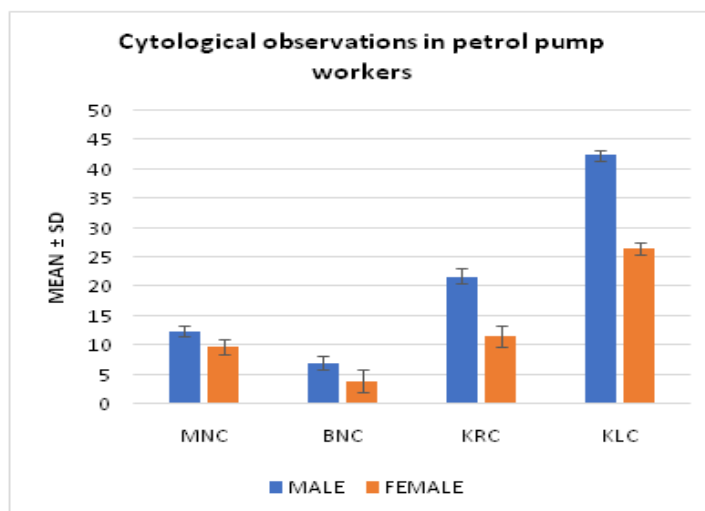


Figure 7: Frequency of micronucleated cells, binucleated cells, karyorrhexis, karyolysis with respect to male and female petrol pump workers

Environmental pollutant exposure:

Nuclear abnormalities in buccal epithelial cells of farmers exposed to pesticides:

Different **agricultural activities** farmers in peninsular Malaysia showed the nuclear abnormalities in their exfoliated buccal epithelial cells (19). Farmers are at risk due to the exposure of genotoxic agent like pesticides. Graph showing the higher frequency of micronuclei in farmers as compared to controls with respect to duration of years (1 – 10 years).

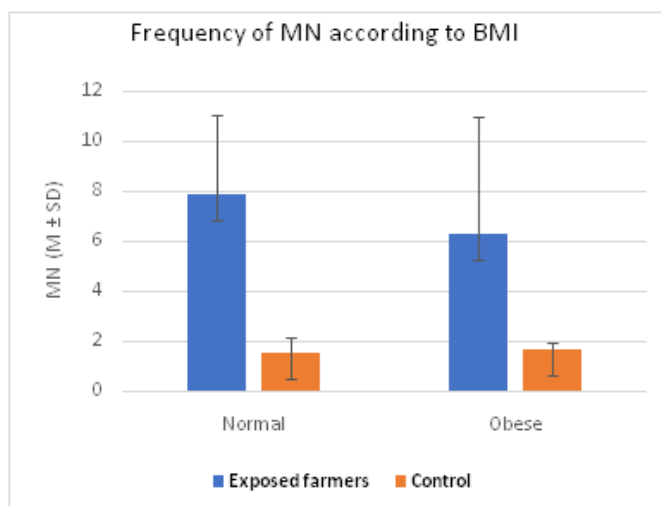


Figure 8: Frequency of MN in Malaysian farmers exposed to pesticides for 1 – 10 years

The intra-groups comparison of MN in respect to BMI showed no significant difference whereas inter groups (exposed farmers and control) comparison revealed a significantly higher

frequency of MN among farmers as compared to control (19). Irrespective of normal and obese conditions farmers exposed to pesticides showed more micronuclei which is a sign of genetic damage.

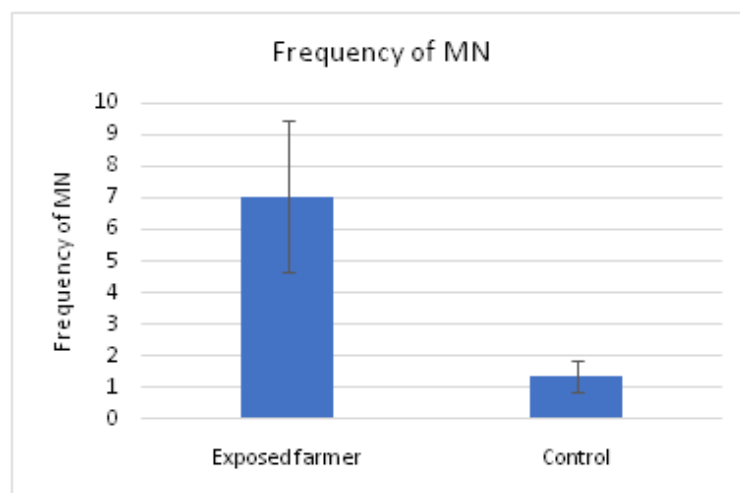


Figure 9: Frequency of MN of farmers exposed to pesticides with respect to different BMI

Micronucleus test on patients of chronic diseases and cancer:

Increased frequency of micronucleated cells, binucleated cells and karyolytic cells in the buccal mucosa of **breast cancer patients** compared to healthy control group suggests that micronucleus assay act as a tool for biomonitoring nuclear abnormalities and detection of genomic instability at an early stage (20). The study was done to evaluate the frequencies of micronuclei and other nuclear anomalies in buccal mucosa cells of Iraqi women with breast cancer.

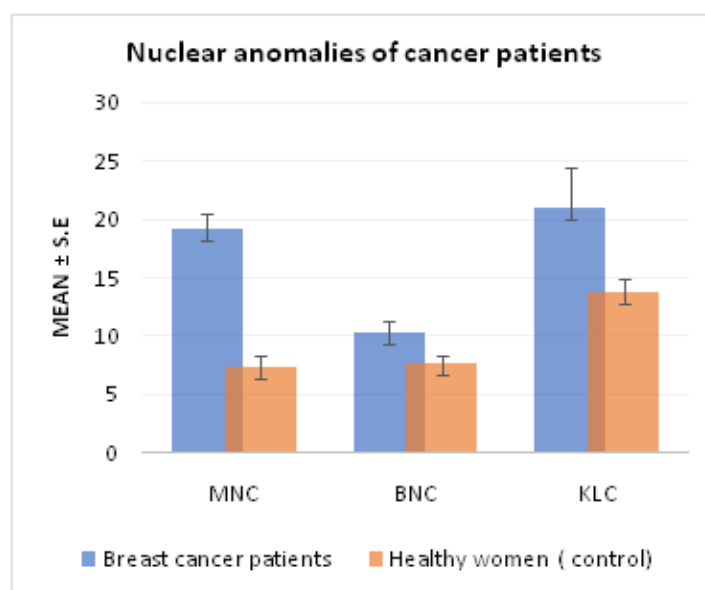


Figure 10: Frequency of micronucleated, binucleated and karyolytic cells of breast cancer patients in comparison to control group

When micronuclei and other nuclear anomalies of buccal epithelial cells were compared with **chronic kidney disease (CKD) patients** with healthy control group then buccal mucosa showed higher number of micronucleated cells, binucleated cells, condensed chromatin cells karyorhectic cells (21). All the individuals of both the study groups consisted of children of 2 to 19 years. Micronuclei assay of buccal epithelial cells helped to evaluate the genetic susceptibility of patients having chronic kidney disease.

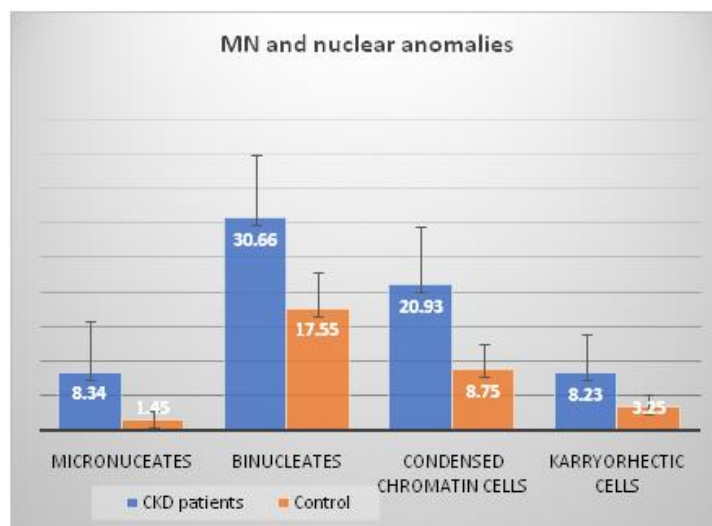


Figure 11: Frequency of MN and nuclear anomalies in chronic kidney disease patients in comparison to the healthy control group

MN assay of buccal mucosa on subjects having different lifestyle habits:

MN assay on tobacco users:

More number of micronuclei was found in exfoliated buccal epithelial cells of chewers than smokers and least number of micronuclei were found in control groups. Individuals of all the groups were not having any oral diseases (22). Tobacco chewing induce more genotoxic effect which increases as the frequency and duration of habit increases with time.

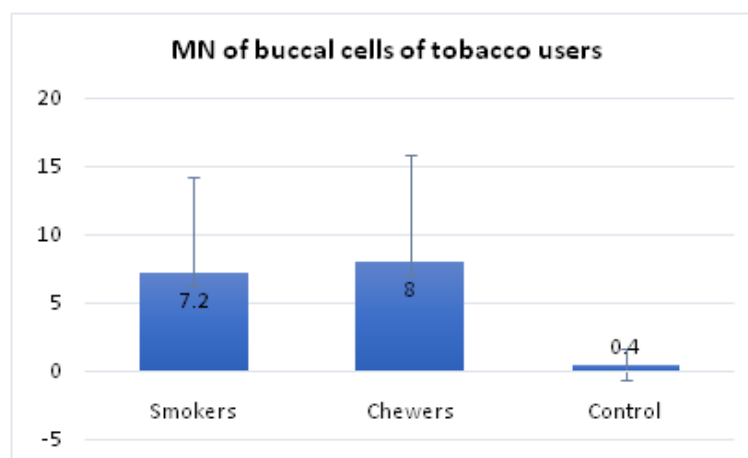


Figure 12: Frequency of micronuclei of smokers, tobacco chewers and control group

MN assay on individuals using alcoholic mouthwash:

Micronucleus assay of buccal mucosa was done among the groups having **individuals using alcoholic mouthwash, individuals using non-alcoholic mouthwash** and control group. Increased number of micronucleus and nuclear abnormalities was seen in the group using alcoholic mouthwash rather than individuals using non-alcoholic mouthwash. Ages, genders and tobacco exposure in all the groups were similar (23). Alcohol containing mouthwash 26% (v/v) ethanol concentration possesses a risk to human health.

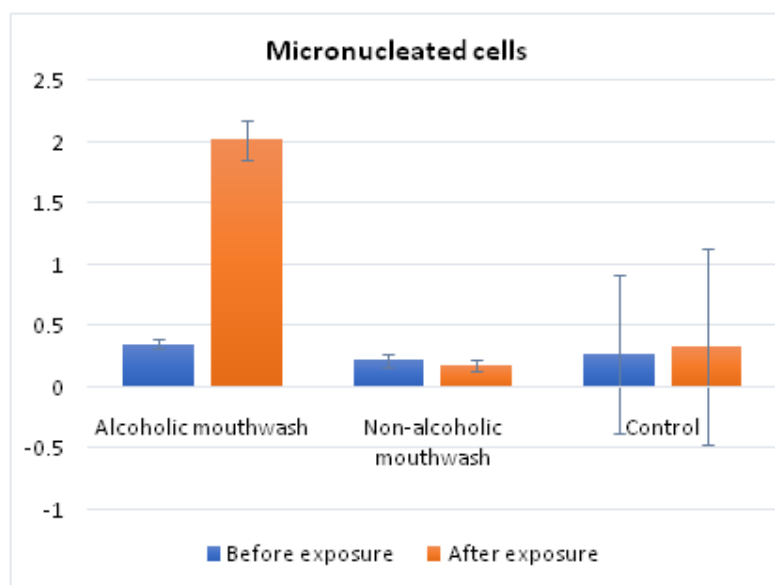


Figure 13: Frequency of MN of individuals who use alcoholic mouthwash in comparison to individuals who use non-alcoholic mouthwash and control group

MN assay on patients exposed to CT scan, a medical procedure

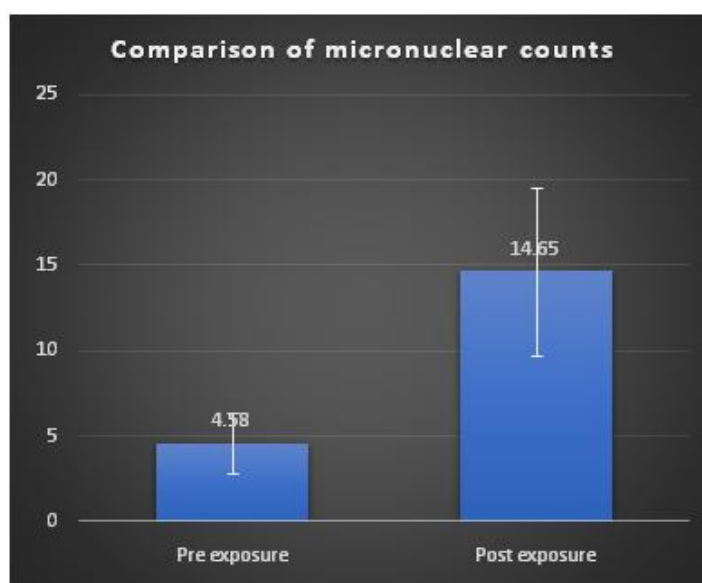


Figure 14: Micronuclear counts of individuals with respect to pre-exposure and post-exposure to head and neck CT scans

Significant difference in the micronuclear count along with nuclear abnormalities was noticed in buccal epithelial cells in patients before and after Head and neck CT scans (24). Patients with habits of smoking, drinking, chewing tobacco or having malignant conditions and previous radiation exposure were not included for this study. Micronucleus assay helped to detect the DNA damage caused due to medical exposure like CT scan. It is predictive that long and repetitive medical diagnostic exposure may lead to genomic instability.

Discussion:

Micronuclei act as the internal dosimeter which reveals the genomic instability in individuals exposed to carcinogenic mixtures. As buccal cells are in immediate contact with inhaled and ingested genotoxic agents, and metabolites of the chemicals, epithelial tissues are the first to express the genotoxic effects of these agents and buccal cells have limited DNA repair capacity relative to peripheral blood lymphocytes, and therefore, may more accurately reflect genomic instability event in epithelial tissue.

The above studies reveal that,

- Micronucleus assay with respect to exfoliated buccal cells irrespective of age, gender, genotypic diversity, demonstrates the genomic damage due to occupational exposure, environmental pollutants like pesticides, medical procedures like radiation, lifestyle factors like smoking, chewing of tobacco, using of alcoholic mouthwash, chronic diseases, cancer, etc.
- The presence of MN and other nuclear anomalies within the buccal epithelial cells are known to be associated with genetic defects.
- Micronuclei and other nuclear abnormalities increase with –
 - duration of exposure in terms of years / working hours with carcinogens or mutagens
 - smoking habit and alcohol consumption
 - chronic diseases
 - exposure to medical procedure like radiation
 - exposure to environmental pollutants like pesticides
- Individuals working in petrol stations, tanneries, calcite factories, etc are constantly exposed by nasal inhalation or oral inhalation of volatile organic compounds emitted during refuelling and genotoxic agents during processing. A significant increase in the micronuclei frequency in exfoliated buccal cells of petrol pump attendants, factory workers, and automobile spray painters was observed in the above studies. Occupational exposure to such derivatives poses genotoxic risk.

- Environmental pollutants like pesticides, lifestyle habits like smoking, chewing tobacco, using alcoholic mouthwash also showed higher frequency of micronuclei and nuclear anomalies.

More than 90% of human cancers are of epithelial origin, MN assay with buccal epithelial cells is the most suitable biomarker used for the detection of increased cancer risk in humans due to exposure to genotoxic carcinogens or mutagens. Buccal cell micronuclei have been identified as useful biomarkers in clinicopathologic investigations. Micronucleus assay is the reliable and relevant non-invasive biomarker which can improve the implementation of biomonitoring, diagnostics and treatment of diseases caused by, or associated with, genetic damage. MN assay is an outstanding marker for human biomonitoring and genotoxicity studies. Therefore, automation of MN and nuclear anomalies analysis for speedy and fairly more authentic detection of anomalies is needed immediately to allow its applicability on a wide-ranging scale.

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PLASTIC PERSONAL PROTECTIVE EQUIPMENTS (PPE)-

A SAVIOUR OR CURSE TO MANKIND

Debolina Sinha

Department of Zoology,

Vidyasagar College (Affiliated to University of Calcutta), West Bengal 700006, India

Corresponding author E-mail: debolina008@gmail.com

Introduction:

World Health Organization declared the outbreak of SARS-CoV-2 virus as pandemic in March 2019, popularly referring it as COVID-19 (1). Following the announcement there was an unprecedented situation ever faced in the recent history of human civilization. Various public health measures were taken worldwide to contain the spread of the contagion. With the ever-increasing uncertainty and concern, governments around the world have introduced special measures to prevent the spread of the virus. The regulations include major guidelines of social distancing while imposing several restrictions on our daily activities along with the use of personal protective equipment (PPE) to limit the spread of the virus by individuals in a population (2). Thus, face masks, gloves, goggles and gowns were widely used in public health facilities, hospitals, clinics, nursing homes and in specific industries. In major countries, this requirement has also been applied to public spaces. With an historic surge in demand of PPE, resulted into establishment of new market for its manufacturing. Though it has evidence, that PPE can control the transmission of the SARS-CoV-2 virus such widespread use and necessity of PPE kits in several circumstances is still a matter of debate. A survey showed an increase in PPE production, including an estimated 11% increase in global production of gloves in 2020 (3). As PPE kits are made up of mostly plastics, after brief use these are the potential source of micro-plastic fibres in the environment. Therefore, PPE kits need proper disposal. Polypropylene is the common material of protective equipment like N-95 masks and Tyvek is used for protective suits, hand gloves and medical face shields, which can persist for a long time and pollute the environment (4). Hence, growing usage of simple PPEs emerged as a new waste category a major cause of concern.

One can compare a standard box of nitrile gloves has the same CO₂ footprint as driving 20 miles in a petrol car (5). It is only in 2018, the whole world became conscious about the toxic impact of plastics on environment and were banning on plastic use. Now thanks to the pandemic, we are heavily using the same without properly disposing it. Countries like India fighting with merciless plastic use and improper waste management system this sudden rise of COVID-19-

related plastic waste load turned in to a threat to environment. More of its inappropriate disposal increased the risk of re-transmission and had big consequences on the planet earth as well. Appropriate disposal of these wastes is vital for the control of the recurrence of viral infection and environmental protection to meet the Sustainable Development Goals.

Mark Benfield, an oceanographer who researches on plastic pollution made a preliminary survey where he found that gloves are the most common PPE waste, followed by wipes and masks. He is presently researching to find the trail of PPE in water courses and eventually into the ocean (6). Usually, PPE is not recyclable or biodegradable and it falls under the category of waste. But sadly, PPEs are thrown in the recycle bins due to lack of public awareness. According to the Centers for Disease Control and Prevention guidelines, there is no indication to propose that waste related with COVID-19 needs any extra decontamination preceding disposal. Hospitals and other related institutions that produce potentially communicable wastes labelled as biohazards must endure to custom precautions to avoid transmittion of disease. These institutions should be conscious of their wastes that might be subject to special packaging, labelling, transportation, or treatment requirements. Typically, single-use PPE generated as a result of routine activities at these places may be disposed off in the regular solid waste stream like municipal trash. Used PPE related to COVID-19 needs no diverse treatment except when it becomes soaked with body fluids that can drift easily or be squeezed from the PPE. Standard protocols should be used to determine whether used PPE requires any special handling requirements or it may be discarded into the regular solid waste line. Municipal trash should be the right place to dispose PPE generated by businesses and residences in relation to COVID-19 (7). For additional safeguard, used PPE may be bagged rather than placed loosely in wastebins or trash vessels. This will help to safeguard the custodial staff, family members, solid waste workers and others potential persons to Coronavirus exposure. Creased waste containers should meet the requirement for extra protection. Bags should be knotted before inserting them in wastebins or garbage vessels.

Inspite of all the above guidelines we see, PPE do litter on the ground in our neighbourhood with limited disposal. Due to the concerns of possible virus transmission from the surfaces of face masks or gloves and increasingly common use of PPE in society, management of PPE waste has emerged as a challenging task that includes legal, business and social aspects. A study carried out in Bangladesh shows since the COVID-19 outbreak, biomedical waste generation rate has increased in the country which creates extra burden on public health and becomes a challenge to waste management authorities (8). It is reported that, on average, 1.63–1.99 kg/bed/day medical waste is produced in the capital of Bangladesh, Dhaka where there are approximately 141,903 hospital beds (9). Due to lack of adequate protection, nearly 40,000 casual waste collectors, work across the country is at high risk of COVID-19 infection (8).

Another six-month study in UK on the environmental impact by the PPE used by medical personnel shows the carbon footprint of PPE distributed during the study period totalled 106,478 tonnes CO₂, with major contributions from gloves, aprons, face shields and Type IIR surgical masks. They estimated damage to human health was 239 disability adjusted life years, effect on ecosystems was 0.47 losses of local species per year and resource depletion was costed at US \$12.7m (GBP £9.3m). They used scenario modelling and found UK manufacture would have reduced the carbon footprint by 12% by limiting gloves by 45%, reusing gowns and gloves by 10% and maximal recycling by 35%. (5).

Thus to optimise the sustainability of PPE and minimise the generation of waste many pointed on the combination of the "5Rs": **reduce, reuse, recycle, research and rethink** (10).

Reduction in the use of PPE is the most actual approach to minimise its environmental impacts. While the use of PPE is essential in many clinical settings during the pandemic, measures to less unnecessary and repetitive use of it especially in the non-clinical settings like hospitality and service is never highlighted. Wearing of PPE such as nitrile gloves is redundant in their case and must be controlled. Organisations should take steps to focus the deployment of PPE to where it is absolutely required for safety, rather than agreeing to its use for reasons of public image. Furthermore, the extensive use of plastic packaging like disposable visors should be curtailed (11).

Then comes the reuse, as per Zhang *et al.* we should concentrate on reusable alternatives rather than vouching for single-use face masks (11). Respirator masks that can be cleaned after use, with one-time use part being the filters which are routinely changed after several months is a good alternative. Though they create hindrances to the users in communication and discomfort, still use of those must be encouraged. Powered air purifying respirators are generally more comfortable and allow the wearer's face to be seen, aiding communication among the team and with the patient, however they are more complex and expensive than masks so may not be suitable for every situation. Another best substitute is the reusable surgical gowns which are presently adopted in many centres that are washed before returning to circulation, reducing the environmental impact by around 65% compared to disposable gowns (11).

The third R, Recycling of single use PPE renders numerous challenges as some mentioned above in this review too. These comprise matters with unscrambling mixed materials and the possible risks of contamination as a consequence of handling communicable material. To handle these, the manufacturers of single usage PPE need to understand and emphasise on the importance of using single recyclable materials which can feed back into a closed loop recycling system. However, to thrive this, it must be aided by regulatory approvals and systems that enable the safe recycling of equipment that has been used in the clinical setting (10).

Epidemics and pandemics are forecasted to happen more frequently in the future and if the COVID-19 pandemic subsides sooner, we must maintain focused research on PPE to reduce its contribution to substantial environmental impacts of healthcare. This may comprise the advancement of reusable equipment that better suits healthcare work and the study of the roles of plastic alternatives like starch-based biopolymers in the manufacture of PPE (11).

The scientific base for PPE usage demands continual re-examination as the evidence base knowledge on transmission and contamination advances. For instance, there is growing reports that SARS-CoV-2 transmission is mostly air borne with relatively low risks of surface transmission provided that effective handwashing is maintained. Still now contact precautions such as gloves, aprons and gowns have been a constant feature of PPE policy since the start of the pandemic. Though some reports of an increasing number of healthcare organisations opting to reduce the usage of these items despite Public Health England's guidance remaining unchanged shows some hope. Yet mass change will be seen only after the new emerging studies were properly incorporated in the national guidance(12).

Irony is, we have witnessed that we can develop multiple vaccines for COVID-19 in one year but failed to make a better sustainable PPE. The environmental impact of PPE is extensive and requires urgent attention to lessen detrimental effects on planetary health. The most fitting and impactful strategies may be through reduced use of gloves by using hand washing alone, domestic manufacture of PPE and extended use or reuse of PPE such as masks and gowns. Sustainable management of plastic-based PPE is challenging, and further research on green materials are the need of the hour. We should be seeking multidisciplinary input from biomedical, environmental and material scientists to focus on the reuse and recycling of plastic-based PPE where possible within our working environment to prevent contamination and minimise the potential risk.

More importantly, general public should be actively encouraged to wear washable, reusable face masks and shift towards the use of sustainable alternatives wherever possible. The present review is aimed to give a clear picture of the over-all pros and cons of using plastic PPE to facilitate public awareness that may save mankind from turning it in to an irreversible curse.

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IMMUNOPATHOGENESIS OF COVID-19 ASSOCIATED MUCORMYCOSIS

Mrittika Dasgupta and Suchandra Chowdhury*

Applied Immunology Laboratory,

Department of Zoology,

Bethune College, 181, Bethune Sarani, Kolkata – 700006, West Bengal, India

*Corresponding author E-mail: suchandra79@gmail.com

Abstract:

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus causing COVID-19 in man. SARS-CoV-2 induced pandemic has claimed millions of human lives not only through pneumonia or other associated disorders but also by paving path for some of the opportunistic pathogens like the Mucorales to strengthen the misery. India, being a tropical country has hot and humid climate. Such climatic condition favours the growth and prosperity of several fungal infections. Consequently, the second wave of the pandemic invited such a fungal infection commonly known as the black fungal disease or Mucormycosis. Mucormycosis is predominantly caused by the members of the subphylum Mucoromycotina and order Mucorales. The fungi are readily available in our environment. Mucormycosis is an unusual but fatal fungal infection that frequently affects patients with compromised immune condition. Since the COVID-19 patients are terribly immunocompromised due to the disease or prolonged treatment by steroids, they are easy targets of the fungi. This chapter reviews the immunopathogenesis of Mucormycosis in normal individuals and the factors that make COVID-19 patients, either during or post treatment, susceptible to this opportunistic pathogen.

Keywords: SARS-CoV-2, COVID-19, immunopathogenesis, Mucormycosis

Introduction:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus causing COVID-19 in man. It was first observed in December 2019 in Wuhan, China. Although the symptoms of the disease are mild to moderate in majority of the cases, innumerable people have exhibited morbidity and mortality (Huang *et al.*, 2020, Chung *et al.*, 2021). Coronavirus infections can be asymptomatic or symptomatic in man conveyed with fever, cough, breathlessness, pneumonia and gastrointestinal irritation. It was declared a pandemic in the early 2020 (Sharma *et al.*, 2021).

Early in 2021, India encountered the second wave of this pandemic. Along with the severity of the COVID-19, an associated opportunistic infection mucormycosis took its toll on

human health (Adil, 2021). India, being a tropical country has hot and humid climate. Such climatic condition favours the growth and prosperity of several fungal infections. Consequently, the second wave of the pandemic summoned this fungal infection commonly known as the black fungal disease or Mucormycosis. Mucormycosis is an unusual, fatal fungal infection that usually affects patients with compromised immunity. The factors assisting the germination of Mucorales in patients suffering or recovered from COVID-19 are a milieu of hypoxia, high blood sugar level (diabetes or steroid-induced hyperglycemia), acidic medium (metabolic acidosis, diabetic ketoacidosis [DKA]), increased iron level (increased ferritins) and diminished phagocytosis due to immunosuppression (Singh *et al.*, 2021).

Mucormycosis (also known as zygomycosis) is a sporadic fungal infection caused by contact with mucormold, found in soil, manure, plants, decaying fruits and vegetables, air and even in the mucus of healthy people. It affects the sinus, brain and lungs and can be life-threatening in people with comorbidity like diabetic or immune-compromised individuals (Mahalaxmi *et al.*, 2021). Mucormycosis caused by fungi belonging to the subphylum Mucoromycotina and order Mucorales (Ribes *et al.*, 2000). The species belonging to the family *Mucoraceae* are more potent in causing mucormycosis. The most common cause of mucormycosis is by far *Rhizopusoryzae* (*Rhizopusarrhizus*) belonging to the family the *Mucoraceae* (Spellberg *et al.*, 2005, 2009). Other species of the *Mucoraceae* family causing similar infections include *Rhizopus microspores* var. *rhizopodiformis*, *Absidia corymbifera*, *Apophysomyces elegans*, *Mucorspecies*, and *Rhizomucor pusillus* (Ribes *et al.*, 2000). Increasing mucormycosis cases have been also reported due to infection with *Cunninghamella* spp. (in the *Cunninghamellaceae* family) (Spellberg *et al.*, 2005).

In this chapter, we discuss the virulence factors of Mucorales, the immunopathogenesis of mucormycosis in normal individuals and how the patients of COVID-19 become more susceptible to this fatal fungal infection.

Virulence factors of the Mucorales:

In order to cause disease, the agents of mucormycosis have to ensure three attributes. They must sequester sufficient iron from the host for their growth. They must evade the hostphagocytic defense mechanism and must have access to the vasculature to disseminate. The virulence factors of the mucorales play a pivotal role in the accomplishment of the damage process. Some of these factors are discussed below.

FTR1, a high-affinity iron permease plays a role in uptake and transport of iron in Mucorales fungi especially when there is a lack of iron in the environment (Ibrahim *et al.*, 2007). *Rhizopus* spuse iron from ferrioxamine (iron-rich form of deferoxamine). They have special receptors for iron uptake namely Fob1 and Fob2 proteins on the Mucorales surface, which are highly expressed in the presence of up regulated ferrioxamine (in deferoxamine treated patients).

The receptors are involved in the transfer of iron to fungal body through the reductase/permease system without interference with siderophore-iron complex into the fungal cells. They are vital in the pathogenesis of *R. oryzae* (Liu *et al.*, 2015). Another virulence factor is the spore coat (CotH) protein identified on all the spore surface of Mucorales. It plays a key role as invasins in the pathogenesis of mucormycosis, disrupts and damages immune cells (Gebremariam *et al.*, 2014).

Arp (Alkaline *Rhizopus* protease enzyme) isolated from culture filtrate of *Rhizopus* sp. enhances coagulation in patients suffering from mucormycosis (Speer *et al.*, 2006). Arf (ADP-ribosylation factor) is a virulence factor obligatory for growth, virulence and fungal dimorphism in *M. circinelloides* (Patiño-Medina *et al.*, 2018). Dihydrolipoyl dehydrogenase is the most abundant antigen in the serum of patients who suffered from Farmer Lung Disease (Rognon *et al.*, 2016). Calcineurin (CaN) is another crucial virulence factor in Mucorales. It is calcium and calmodulin-dependent serine/threonine protein phosphatase that has a substantial role in yeast to hyphae transition in *M. circinelloides* (Lee *et al.*, 2013). Presence of heat shock transcription factors in the genome of these fungi explains the thermotolerance of Mucorales sp (Schwartz *et al.*, 2016). In addition, the CAZymes (carbohydrate active enzymes) represented the majority of the secreted proteins which possibly has a role in the host- environment interaction (Prakash *et al.*, 2017; Mohamed *et al.*, 2019).

Immunopathogenesis of Mucormycosis in normal individuals:

Mucormycosis infection in man can be predominantly rhinocerebral, gastrointestinal, cutaneous, subcutaneous, pulmonary, and disseminated zygomycosis. The less frequently occurring forms include cystitis, gastrointestinal or vaginal colonization, allergic disease and external otitis. It is caused due to inhalation of the filamentous (hyphal form) fungi. The distinguishing feature of this disease is the development of hyphae in and around blood vessels, leading to lethal consequences in immunocompromised patients. Once the fungal spores invade the human system, the hyphae encroach on the blood vessels, resulting in tissue infarction, necrosis and thrombosis. Mucormycosis occurs in the host primarily by evading the immune system and surviving inside the host cell and then by agitation of the immune system leading to further damage of the host (Brunke *et al.*, 2016, Iyer *et al.*, 2021).

An enhanced level of iron is noted in patients with compromised immunity and this creates an environment suitable for the growth of Mucorales fungi. Additionally, these fungi consume iron by means of high-affinity iron permease and transport it to development inside the host cell (Artis *et al.*, 1982). The virulence factors CotH protein, present on the spore surface of the Mucorales helps in penetrating, disturbing and damaging the immune cells (Gebremariam *et al.*, 2014). Initially, the pathogen comes in contact with the host epithelial cells, damaging it through increased signalling of platelet-derived growth factor receptor B (PDGFRB). PDGFRB provides the appropriate growth factors to the fungi. Neutrophils, being an important component

of the innate immune system release chemotactic factors to combat infection. However, in diabetic or steroid treated patients, due to ketoacidosis or hyperglycemic conditions, the secretion of these chemotactic factors decrease, thereby increasing the fungal hyphae in human hosts (Roilides *et al.*, 2017). After entering the host cell, the Mucorales, produce pathogen-specific T-cells which generates interleukins (IL-4, IL-10 and IL-17) and IFN- γ . These pro-inflammatory cytokines further stimulate CD4+ T cells and damage the host cell (Castillo *et al.*, 2018). The release of various immunomodulatory molecules like RANTES (regulated upon activation, normal T-cell expressed and secreted) and IFN- γ are reduced due to pathogenic invasion, providing selective advantage to the latter in evading the host immune system (Figure 1; Iyer *et al.*, 2021).

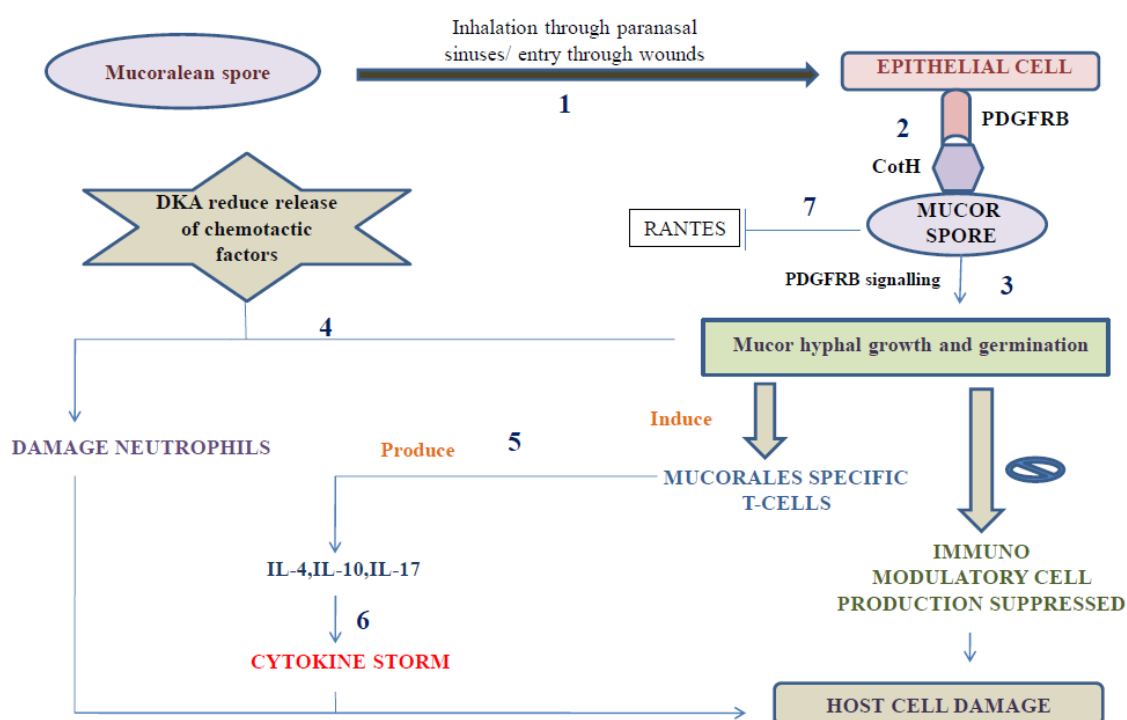


Figure 1: Immunopathogenesis of Mucormycosis in normal individual. 1. Mucormycosis infection is occurs due to inhalation of hyphal form fungi; 2. The hyphae of the fungus, through its virulence factors CotH protein attacks the epithelial cells,damaging it through increased signalling of platelet-derived growth factor receptor B (PDGFRB); 3.PDGFRB provides the growth factors to the fungi.; 4. Neutrophils release chemotactic factors to battle infection. In diabetic or steroid treated patients, due to ketoacidosis (DKA), thesecretion of these chemotactic factors decrease, increasing the fungal hyphae in human hosts; 5. Inside the host cell, the Mucorales, produce pathogen-specific T-cells which generates interleukins (IL-4, IL-10 and IL-17) and IFN- γ ; 6. These pro-inflammatory cytokines further stimulate CD4+ T cells and damage the host cell;7. The release of various immunomodulatory molecules like RANTES (regulated upon activation, normal T-cell expressed and secreted) and IFN- γ are reduced due to pathogenic invasion, providing selective advantage to the latter in evading the host immune system.

Immunopathogenesis of Mucormycosis in COVID-19 affected individuals:

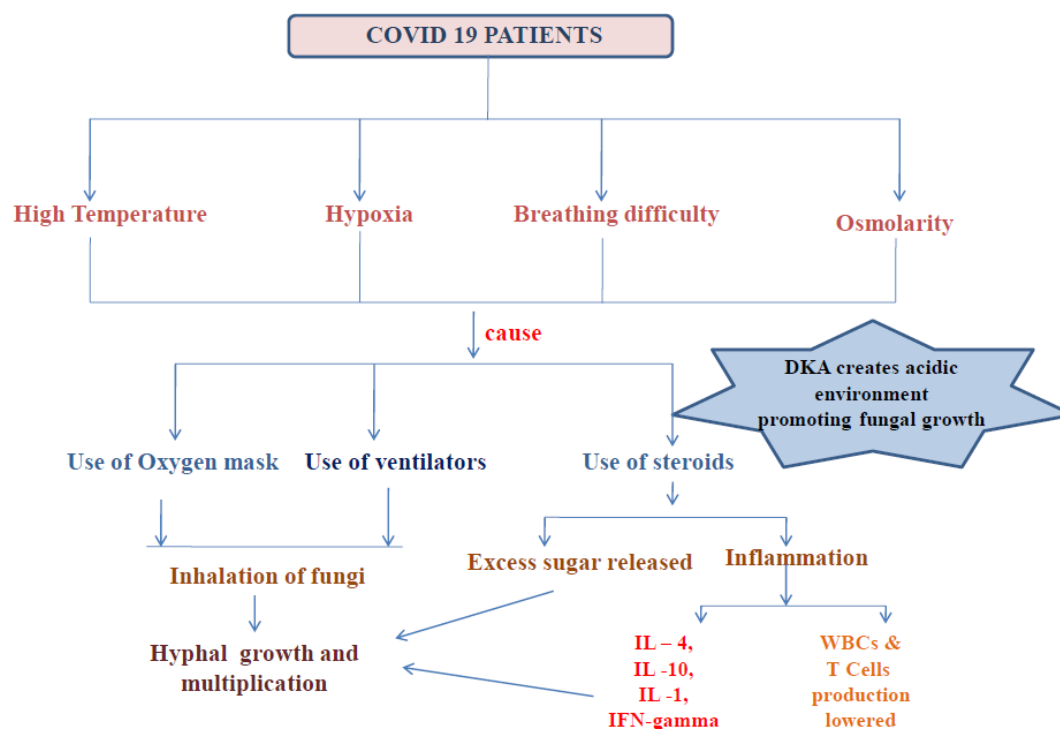


Figure 2: Conditions favouring the development of Mucormycosis in COVID-19 patients

The primary symptoms of COVID-19 include a rise in body temperature, osmolarity, hypoxia and breathlessness (Balachandar *et al.*, 2020a). As a treatment regimen, steroid is administered to these patients. Steroids diminish both inflammation and the activity of the immune system, where the production of leukocytes and T-helper cells are decreased, enabling pathogens to invade and disintegrate the immune system of the host. Additionally, steroids trigger the uncontrolled release of sugar, which also enables the Mucorales to grow, multiply and invade at a rapid rate. This problem is also triggered by extreme usage of oxygen masks and ventilators which makes these patients more susceptible to mucormycosis. Thus the main symptoms of COVID-19 create a flawless milieu for the growth and development of Mucorales inside the human body ((Figure 2; Iyer *et al.*, 2021).

Discussion:

Mucorales are readily available in the environment from soil to near rotting food. It is a side-effect of the treatment of COVID-19 that makes patients suffer from this ailment. Since the mucorales are angio-invasive, once inhaled they can easily manifest the symptoms of the disease, especially in those patients who are immunocompromised. Thus early detection of their presence in the host system can reduce morbidity and mortality to a significant level.

Abbreviations used:

diabetic ketoacidosis (DKA); platelet-derived growth factor receptor B (PDGFRB)
regulated upon activation, normal T-cell expressed and secreted (RANTES)
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); spore coat protein (CotH)

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BIODIVERSITY AND CONSEQUENCES OF ITS DEPLETION: AN INDIAN PERSPECTIVE

Prosenjt Ghosh

Department of Zoology,

Government General Degree College at Kaliganj, Debagram, Nadia, West Bengal, 741137

Introduction:

The term biological diversity was used first by wildlife scientist and conservationist Raymond F. Dasmann in the 1968 lay book *A Different Kind of Country*. The scientific societies follow the definition of biodiversity given by Wilson (1992) as: “...all hereditarily based variation at all levels of organization, from the genes within a single local population, to the species composing all or part of a local community, and finally to the communities themselves that compose the living parts of the multifarious ecosystems of the world.” It represents the most important working component of a natural ecosystem. It helps maintain ecological processes, creates soils, recycles nutrients, exerts considerable effect on climate, degrades waste, controls diseases and above all, provides an index of health of an ecosystem. It is the source of food, medicines and a variety of important products. Thus it exists as natural wealth in terrestrial and aquatic ecosystems.

Levels of biodiversity:

Biodiversity is generally described at three levels: Ecosystem diversity, species diversity and genetic diversity.

1. Ecosystem diversity or habitat diversity:

- It is the differences between ecosystem types and the diversity of habitats and ecological processes that occurs within each ecosystem type.

Habitat diversity may be terrestrial habitat (forests and grass lands), marine habitat, fresh water habitat and wetlands.

2. Species Diversity:

- This refers to the variety of species.
- It relates to the number of species in a defined area.
- The diversity of species can be measured through its richness, abundance and types.

Areas rich in species diversity are called hotspots of diversity. Species diversity may be terrestrial diversity (plant and animal diversity), marine diversity and freshwater diversity.

3. Genetic Diversity:

- Genetic biodiversity refers to the variation of genes within species.

- It is very crucial for healthy breeding of population of species.

India as a mega diverse country:

The mega diverse countries are a group of countries that harbor the majority of the Earth's species and are therefore considered extremely biodiverse. Conservation International identified 17 mega diverse countries in 1998. Together, these 17 countries harbour more than 70% of the earth's species (Kumar and Verma, 2017).

A. Floral diversity in India:

Though India harbours more than 5% of the total known animals and species of the world, it encompasses only 2.4% of the total land area of the world. India has secured 10th position in the worldwide ranking on botanical diversity, 11th based on limited regional variations and 6th on crop production. India is a home to about 18,660 flower plants (angiosperm), 82 gymnosperm, 7411 algae, 15396 fungus, 1302 pteridophytes and 1223 viruses. Till now, nearly one third of all known angiosperms are present only in India. About 2200 varieties of mango, 50000 varieties of rice, 500 varieties of oats and pepper each are found in India, of which 167 crop species including rice, cane, banana, lime mustard, ginger, cardamom, turmeric, black pepper, etc were first grown in India. In addition, 51 different varieties of grain coarse cereals, 104 species of fruits, 55 species of vegetable and beans, 24 species of fibers containing cereals and 24 species of oilseed are found in India. Out of 109 varieties of spices widely used in the world, 75 are grown in India (Agnihotri1 *et al.*, 2020).

With world's 34 leading biodiversity sites, the biodiversity of India is very sensitive. The Himalayas, the Sundarbans, the Western Ghats are some of the highly sensitive biodiversity areas of the world found in India. The north eastern region of India is considered as the home of world's most ancient native flowering plants (Agnihotri1 *et al.*, 2020).

B. Animal diversity in India:

India is also one of the leading countries in the world in terms of diversity of fauna. So far, about 89317 types of animals have been identified in India, including 390 types of mammals, 1225 types of birds, 456 types of snakes, 2546 types of fishes, 2577 types of protozoa, 68389 types of insects and arthropodes, and 8329 species of other animals. Two third of the world's tiger population, 60% of wild buffalos and one horned rhinoceros are found in India only. The Gir sanctuary in Gujarat is the only habitat of Asiatic lion. Many species of birds are also found only in India (Agnihotri1 *et al.*, 2020).

Loss of biodiversity:

The biological wealth of the planet has been declining rapidly. Important causes are as follow (Kumar and Verma, 2017):

1. Natural causes like floods, earthquakes and other natural disasters.

2. **Habitat loss and fragmentation:** It is considered as the principal reason leading to extinction of animals and plants. Tropical rain forests are the best examples of the habitat loss. Once covering more than 14% of the earth's land surface, these rain forests now cover no more than 6%. Besides total loss, pollution related degradation of many habitats causes serious threat to the survival of many species. With the broken up of large habitats into small fragments due to various human interventions, mammals and birds requiring large territories and many animals with migratory habits are being badly affected, resulting in the decline of their population. The reasons of habitat loss are deforestation, over population, pollution, global warming etc.
3. **Over-exploitation:** Over-hunting, over-fishing or over-collecting of a species can quickly result in its decline and even extinction. Many species like Steller's sea cow, passenger pigeon have now become extinct because of the changing consumption behaviour of humans, which is considered as one of the key reason for unsustainable exploitation of natural resources.
4. **Alien species invasions:** When alien species are introduced, some of them turn invasive, causing depletion or extinction of indigenous species.
5. **Co-extinctions:** When a species becomes extinct, the plant and animal species having associated with it in an obligatory way also become extinct. When a host species becomes extinct, its unique assemblage of parasites also experiences the same destiny.
6. **Global climate change:** Changes in climate and climate variability have been found to cause biodiversity loss.
7. **Hunting and Poaching:** Hunting and poaching pose great threat of extinction not only to a particular species but also the other species dependent on that species.

Consequences of Biodiversity Loss:

1. **Impacts on the Ecosystems:** One major consequence of biodiversity loss is the alteration and decline in species compositions, which may in turn lead to local and global extinctions (*unescap.org*). Large scale ecosystem changes may occur due to the removal of key herbivore and predator species. For instance, removal of triggerfish was found to result in the outburst in their prey population such as burrowing urchin, which subsequently accelerates reef erosion through feeding activities (*unescap.org*). Furthermore, the loss of top predators or dominant herbivores of any region is mostly damaging as it can lead to a cascade of disruptions in the ecological relationships among species that maintain diversity and proper functioning of ecosystems. Loss of biodiversity through bioinvasion of exotics also has a serious consequence as it could trigger loss or alteration of genetic purity or genetic uniformity. Exotics can pose a kind of internal threat to natives as they may cause the mixing of genetic stocks (*unescap.org*). Such genetic invasions can weaken the uniqueness or stability of a native population by swamping in it foreign genes. Biodiversity loss on different Indian floral and faunal population has been summarized below.

A. Floral Species:

India is blessed with wide variety of floral species. With increasing biodiversity depletion, several floral species have become endangered and are moving towards extinction. In the table below there is a list of reported endangered flora in India.

Table 1: Endangered flora, causes for loss of biodiversity and places last found

Species Endangered	Place of interest	Causes
<i>Rauvolfia serpentine</i> , <i>Terminalia chebula</i> , <i>Sapindus laurifolius</i> and <i>Jatropha curcas</i>	Western Ghats (Kamalappa, 2003)	Destructive harvesting followed by unscientific handling
<i>Catuneregam spinnosa</i> , <i>Garcinia cambogea</i> , <i>Acacia pinnata</i> , <i>Ficus benghalensis</i> , <i>Zanthoxylum rhesta</i> , <i>Hemidesmus indicus</i> , <i>Terminalia chebula</i> , <i>Wrightia zeylanica</i> , <i>Cinnamomum verum</i> , <i>Bombax ceiba</i> , <i>Sapindus laurifolius</i> , <i>Alangium salvifolium</i> and <i>Calophyllum inophyllum</i>	Maradavally, Shimoga district (Kamalappa, 2003)	Medicinal use and deforestation
<i>Abrus precatorius</i> , <i>Adenanthera paronina</i> , <i>Aegle marmelos</i> , <i>Caesalpinia bonducella</i> , <i>Cardiospermum halicacabum</i> , <i>Corallocarpus epigaeus</i> , <i>Gloriosa superba</i> , <i>Andrographis paniculata</i>	Devrayanadurga forests, Tumkur, Deccan Plateau (Kamalappa, 2003)	Destructive harvesting and medicinal use
<i>Lichen</i> genera <i>Parmotrema</i> , <i>Everniastrum</i> , and <i>Rimelia</i>	Ramnagar and other places in India (Upreti <i>et al.</i> , 2005)	Commercial use
Arunchal Hopea Tree (<i>Hopea shingkeng</i>)	Arunachal Pradesh	Construction of house posts
<i>Hubbardia heptaneuron</i>	Karnataka	Construction of the Linganamakki reservoir
<i>Sapria himalayana</i>	Himalayas (Myers <i>et al.</i> , 2000)	Human influx

B. Wild life:

The following table illustrates the reported endangered wild life in India (Anil *et al.*, 2014).

Table 2: Endangered wild life, causes for loss of biodiversity

Species Endangered	Causes
Indian/ Asiatic cheetah, Javan <i>Rhinoceros</i> and Sumatran <i>Rhinoceros</i>	Exploitation of land and forest resources
The cheetah (<i>Acinonyx jubatus</i>) and the pink headed duck (<i>Rhodonessa caryophyllacea</i>)	Annihilated, unrecorded
The Asiatic lion, the Bengal tiger, and the Indian white rumped vulture	Feeding on the carrion of diclofenac treated cattle
Asian elephant (<i>Elephas maximus</i>)	Ivory poaching
The Indian tiger	Making of beauty products
<i>Muntiacus putaoensis</i> (leaf deer)	Hunting

C. Birds:

In India Birds play important role in the traditional lifestyle and dressing habits of many tribes. The table below describes some endangered Indian birds (Anil *et al.*, 2014).

Table 3: Endangered birds, causes for loss of biodiversity and places last found

Species Endangered	Place of interest	Causes
Seychelles Parakeet (<i>Psittacula wardi</i>)	Indian ocean islands	Intense persecution by farmers and coconut plant owners.
Pink headed Duck (<i>Rhodonessa caryophyllacea</i>) and the Himalayan Quail (<i>Ophrysia superciliosa</i>)	Not reported	Annihilated, unrecorded
Great Indian Bustard (<i>Ardeotis nigriceps</i>), Bengal florican (<i>Houbaropsis bengalensis</i>), Jerdon's courser (<i>Rhinoptilus bitorquatus</i>), Forest owlet (<i>Heteroglaux blewitti</i>), White bellied (<i>Heron Ardea insignis</i>) (IUCN endangered red list)	Not reported	Not reported
Narcondam Hornbill (<i>Aceros narcondami</i>) (IUCN vulnerable species list)	Not reported	Not reported

Sarus crane	Himalayas	Hunting
Great Indian hornbill (<i>Buceros bicornis</i>)	Arunachal Pradesh	Human traditions
Long billed vulture (<i>Gyps indicus</i>),	Northern and central	Pesticides
Slender billed vulture (<i>Gyps tenuirostris</i>), and Oriental white backed vulture, (<i>Gyps bengalensis</i>)	India	

D. Insects and Amphibians:

Among the insects, butterflies occupy a vital position in ecosystems and their occurrence and diversity are considered as good indicators of the health of any given terrestrial biotope (Thomas, 2005). Recent reports reveal that about 100 out of 1500 butterfly species occurring in India are on the verge of extinction (Raju and Rao, 2002).

E. Mammals:

The important large mammals facing extirpation in Himalaya are black bear (*Ursus thibetanus*), musk deer (*Moschus*), bharal (*Pseudois schaeferi*), Himalayan tahr (*Hemitragus jemlahicus*), serow (*Capricornis sumatraensis*) and common leopard (*Uncia uncia*) (Pandit *et al.* 2007). In Kudremukha, at least 26 species of mammals were hunted and facing extinction. In Nagarhole, 6 out of 9 focal species of large mammals were present at considerably lower densities at the heavily hunted site (Anil *et al.*, 2014).

2. Impacts on humankind:

As a result of continuous loss of terrestrial, freshwater and marine biodiversity, fish, grains and other food and medicinal products which are derived from the ecosystems are also under increasing pressure. Loss of biodiversity is often associated with a decrease in the quality of diet and/ or intake of food for the poor, causing incidence of malnutrition and sickness, especially amongst children. In addition, the rich biodiversity of the world is the source of a number of medicinal and industrial products for human civilization. With the loss of plant and animal species having medicinal properties, primary healthcare for millions of people across the region, and in particular the poor, is at risk (Campbell and Schlarbaum, 1994).

Conclusion:

A stable biodiversity is very much important for human survival, its economic security as well as for the ecosystem function and stability. Large scale public awareness regarding the importance biodiversity as well the impact of its depletion both at regional and global levels is of great importance. For the conservation of biodiversity, active public participation is needed which is lacking in many cases. Despite such shortcomings, India has a rich tradition of

conservation, and with increasing contribution from the Government, scientists and NGOs should come forward in developing appropriate methodologies and strategies for assessment of biodiversity and its conservation.

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**A PRELIMINARY REPORT ON FAUNAL MORTALITY BY
WILDLIFE-VEHICLE COLLISION IN TRANSPORT
NETWORKS OF WEST BENGAL, INDIA**

Kanad Roy^{1, 3} and Subhendu Mazumdar^{1,2 *}

¹Department of Zoology, University of Calcutta, Kolkata, India

²Shibpur Dinobundhoo Institution (College), Shibpur, Howrah, India.

³Wildlife Institute of India, Dehradun, India

*Corresponding author E-mail: subhendumazumdar@gmail.com

Abstract:

Wildlife–vehicle collisions on the extensive network of roads (national highways, state highways, expressways, etc.) and railway tracks cutting across the Indian landscape, lead to mortality of a range of animal taxa both within the forests, as well as around wildlife corridors. With rapid globalization, enlarging economies, and increasingly extensive road networks, the need to quantify and understand the impact of roads on wildlife mortality are essential for identifying vulnerable taxa in order to ensure their suitable dispersal. The present review was done to quantify the incidences of wildlife mortalities of different species due to vehicular collisions in West Bengal over a period of four years (2015-16, 2016-17, 2017-18 and 2019-20). A total of 752 incidences of mortality belonging to 17 species were recorded, of which road and railway accidents (17.287%) were observed to be one of the major causes. Mortalities due to accidents were highest during 2015-2016 (37) and lowest during 2019-2020 (22). Overall, 96.923% of the road kills were mammals and 3.076% were reptiles. Wild elephants (*Elephas maximus*) were the major victims of vehicular collisions (39 individuals died). We found that the number of wildlife mortalities by accidents showed no significant differences between years. Conservation and management initiatives are essential to prevent the local extinction of these fauna. Hence, while designing roads, safe animal movements need to be ensured, particularly where roads pass through forests. Moreover, sensitizing highway authorities and forest officials is essential to reduce wildlife mortalities and make roads more wildlife-friendly in West Bengal. The present study provides an overview of species mortality caused by wildlife-vehicle collisions. We feel it is important to carry out in-depth research to assess when, where and how wildlife-vehicle collisions are occurring in the network.

Keywords: Transport networks, roadkill, forest corridors, wildlife-vehicle collisions, wildlife mortality, West Bengal

Introduction:

Roads symbolize one of the most extensive forms of modification of the natural landscape associated with expansion in transportation infrastructure that is often justified for facilitating linkages, enhancing mobility, improving accessibility, and primary way of connecting people. To meet the needs of an ever-expanding human population and rapidly enlarging economies, increasingly extensive road networks now penetrate into even the most remote regions, covering a considerable proportion of the land area in some countries (Laurance *et al.*, 2014). Currently roads cover over 21 million kilometers, and this overall length is anticipated to increase by 25% by 2050 (Meijer *et al.*, 2018). Through direct mortality on the roads, or indirect effects such as modification of adjacent aquatic and terrestrial communities through vehicle exhaust or runoff, or for species dispersal across a range of taxa acting as physical barriers for movement (Van der Ree *et al.*, 2009), or modification of animal behavior, alteration of the physical and chemical environment, spread of exotic species (Trombulak and Frissell, 2000), or increased predator activity near roads (Van der Ree *et al.*, 2009), roads contribute to reduced average heterozygosity and genetic polymorphism (Van der Ree *et al.*, 2009). The negative impact of roads on wildlife in protected areas was first reported in 1935 (Stoner, 1935). It has been well documented that the collision of wildlife with trains and other vehicles, aquatic transportation vessels and even aircrafts have caused mortality of a wide range of species leading to significant biodiversity loss (Baskaran and Boominathan, 2010). Roadkills are primarily influenced by features of the road (such as length, width, state of the road surface), the amount of traffic and the habitat features of the adjoining areas (Seshadri *et al.*, 2009). Roadkill has increased significantly in recent years, both in rural and urban areas (Laurance *et al.*, 2014). Seasonal dispersal, particularly during heavy rainfall and flood, as well as during the reproductive season (Vijayakumar *et al.*, 2001) leads to the mortality of several species on these anthropogenic thoroughfares.

India, with more than 3.31 million kilometers of existing road length, has the world's second-largest road network after the United States (Transport Research Wing, 2016). Besides, India possesses 121,000 kilometers of railway tracks (Indian Railways, 2017). Vehicular densities on Indian roads have increased from 0.3 to 30 million in the last 50 years and approximately 26,000 kilometers of this network cuts across various natural landscapes, including protected areas through as many as 30 tiger reserves spread across the country resulting in a large number of wildlife-vehicle collisions each year (Seshadri *et al.*, 2009; Roy and Sukumar, 2017). Globally, as well as in the Indian context, road-related mortality has been widely documented on different taxa, like mammals (Kumawat and Purohit, 2020) including primates (Pragatheesh, 2011), birds (Siva and Neelanarayanan, 2020), reptiles (Das *et al.*, 2007) including snakes (Vijayakumar *et al.*, 2001) and Mugger Crocodile (*Crocodylus palustris*; Vyas

and Vasava, 2019), amphibians (Seshadri *et al.*, 2009), insects and butterflies (Sony and Arun, 2015). Deaths of wild animals due to train collisions are also fairly common (Roy and Sukumar, 2017). Road mortalities have been found to act as a major limiting factor during the dispersal of large herbivores and carnivores, with demonstrated detrimental effects, particularly on elephants (*Elephas maximus*; Roy *et al.*, 2009; Roy and Sukumar, 2017), tigers (*Panthera tigris*), and leopards (*Panthera pardus*; Baskaran and Boominathan, 2010). In India, a total of 42.6% of wild faunal death is caused by road and railway accidents of which West Bengal accounts for 6.1% (ADSI, 2012). For the state of West Bengal, except for herpetofauna and large mammals like elephants (*Elephas maximus*), studies on road and railway inflicted mortality and ecological loss of many other species of wild fauna are still lacking. The present study is an attempt to summarize the road kills of faunal species in West Bengal, in order to suggest a few mitigation measures to prevent and/or reduce road-induced mortality.

Materials and Methods:

Study area:

West Bengal (22° 58' 43.0464" N and 87° 44' 52.0908" E) with over 91 million inhabitants is the fourth-most populous and covering an area of 88,752 km² (34,267 sq mi), is the fourteenth-largest state in the eastern region of India along the Bay of Bengal. Out of the total length of road in West Bengal (92,144 kilometers), national highway, state highway, expressway and district and rural road comprises 2,578 kilometers, 2,393 kilometers, 121 kilometers and 87,052 kilometers respectively (National Highways, 2007) (Fig. 1a) with 103.69 kilometers per 100 square kilometers road density.

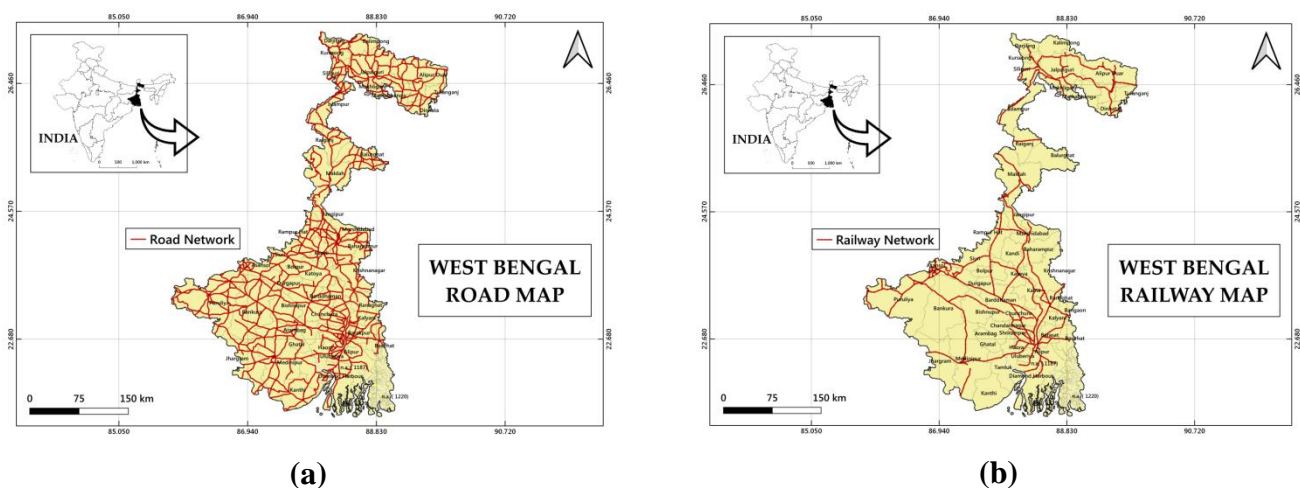


Figure 1: (a) Road Map and (b) Railway Map of West Bengal

(Map source: Google Earth Pro and QGIS 3.6).

Industrial areas and cities are well-connected with the port of the state by national and state highways. The Railway network system of West Bengal is operated by two zones of Indian Railways; the Eastern Railway zone which is further divided into the Howrah (266 stations covering a length of 906 kilometers) and Sealdah (105 stations covering a length of 254 kilometers) divisions and the South Eastern Railway zone with Kharagpur division (87 stations covering a length of 341 kilometers) (Indian Railways, 2017) (Fig. 1b).

Data collection and analysis:

Data on wildlife mortality for the period of 2015-2020 was collected from the Annual administrative reports of West Bengal published by the Directorate of Forests, Government of West Bengal (Annual administrative report West Bengal, 2015-2016, 2016-2017, 2017-2018, 2019-2020). The recorded number of wildlife casualties in West Bengal during the period of 2015-2020 due to *Accidents* (Shapiro-Wilk's test: $W=0.5581$, $p<0.05$) showed non-normal distribution. So, non-parametric statistical tests were performed for data analyses. We performed nonparametric Kruskal-Wallis test to find out if significant differences exist between the numbers of wildlife mortalities by accidents in different years. The statistical tests were performed using RStudio (Ver. 1.3.959) and data were tested at $p < 0.05$.

Results:

In West Bengal, a total of 752 individual kills belonging to 17 wild species were recorded between 2015 and 2020 in West Bengal, of which road and railway accidents accounted for 17.287% (130) of total wildlife casualties. Wildlife mortalities due to accidents were recorded to be highest during 2015-2016 (37 incidences) and lowest during 2019-2020 (22 incidences) (Fig. 2).

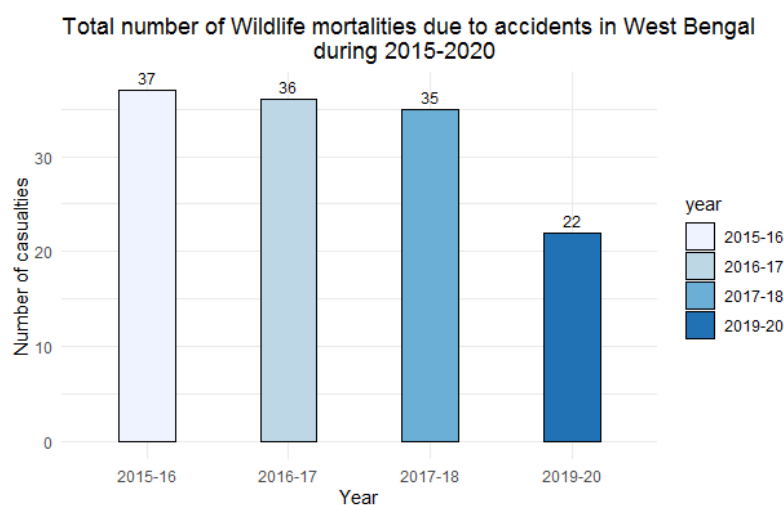


Figure 2: Total number of wildlife mortalities due to accidents in West Bengal during 2015-2020



Figure 3: Images of wildlife mortalities due to road and rail accidents.

(a) **Leopard - *Panthera pardus fusca*** (Uttar Dhupjhora Road connecting Murti Bridge to NH31C on northern side of GNP; photo credit: HEAL and Gorumara Wildlife Division; Image source : https://in.docworkspace.com/d/sICupt-4_q9OxiQY);

(b) **Gaur - *Bos gaurus*** (NH717 (formerly NH 31) that goes through Gorumara, north of the Uttar Dhupjhora road stretch between Murti bridge and NH 17 (formerly NH 31C); photo credit: HEAL and Gorumara Wildlife Division; Image source: https://in.docworkspace.com/d/sICupt-4_q9OxiQY);

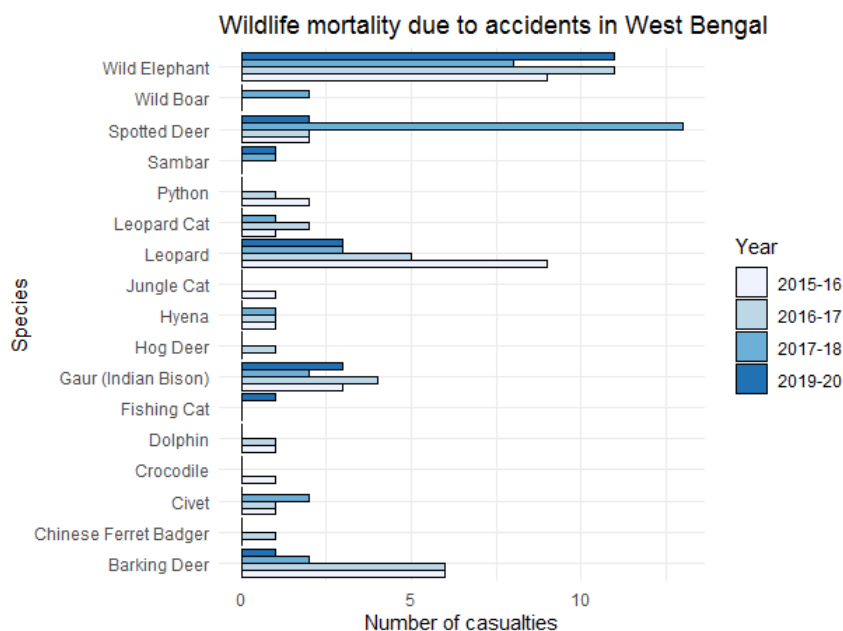


Figure 4: Number of mortalities of various wild fauna due to accidents in West Bengal during 2015-2020

Besides, spotted deer (*Axis axis*) mortality incidences due to vehicle collision were individual highest in a single year (13 individuals in 2017-18) and wild elephants (*Elephas*

maximus) faced the most number of overall deaths (39 individuals) due to accidents between 2015 to 2020 in West Bengal, followed by leopard (*Panthera pardus fusca*; Fig. 3a) (20 individuals), spotted deer (*Axis axis*) (19 individuals), barking deer (*Muntiacus muntjak*) (15 individuals), gaur (*Bos gaurus*; Fig. 3b) (12 individuals), leopard cat (*Prionailurus bengalensis*) (4 individuals) and civet (*Paradoxurus hermaphroditus*) (4 individuals). However, we did not find any significant differences in the number of wildlife mortalities by accidents between different years (Kruskal-Wallis Test: $H_c = 2.2599$, $df = 3$, $p\text{-value} = 0.5202$; Fig.4).

Discussion:

It is alarming to note that around 130 individuals of different species (including reptiles and mammals) died due to wildlife-vehicle collisions between the period of 2015-2020 in West Bengal. Mammalian mortalities accounted for the highest proportion of occurrences (96.923%). On the other hand, ungulates (such as Barking deer *Muntiacus muntjak*, Hog Deer *Axis porcinus*, Spotted deer *Axis axis*, Sambar *Rusa unicolor*, Wild boar *Sus scrofa* and Gaur *Bos gaurus*) were found to come in the vicinity of the national and state highways cutting through the national parks and wildlife sanctuaries during night foraging and in search of elevated lands during monsoon floods (Habib *et al.*, 2020), where they often get killed by speeding vehicles. Anthropogenic and other disturbances from roads such as noise, light, etc. might create wariness in most of the wildlife that leads to their displacement from roads leading to the disruption of their community composition (Pragatheesh, 2011).

Large number of wild elephants (*Elephas maximus*) die every year in the railway tracks being hit by the speeding trains during their dispersal from one forest patch to the adjacent forest which accounted for 30% of all mammalian mortalities due to vehicular collisions (Roy and Sukumar, 2017). Such unfortunate incidences particularly take place where the railway tracks intersect their dispersal routes (Roy and Sukumar, 2017; Menon *et al.*, 2017). It is pertinent to mention that, conversion of many of these tracks from meter gauge to broad gauge has increased the number of trains and also their speed which has aggravated the problem alarmingly (Roy *et al.*, 2009). On the other hand, a completely different reason has been put forward by various authors to explain the mortalities of our national aquatic mammal Ganges river dolphin (*Platanista gangetica gangetica*) due to collisions with various water transport vehicles where the echolocating clicks of the species are masked by the high intensity sounds produced by the propellers of heavy motorized vessels like ships, which causes disorientation and ultimately leads to their death by propeller hit (Dey *et al.*, 2019).

Vehicular collision mortalities of Chinese ferret-badger (*Melogale moschata*), Civet cat (*Paradoxurus hermaphrodites*), Jungle cat (*Felis chaus*), Fishing cat (*Prionailurus viverrinus*), and Leopard cat (*Prionailurus bengalensis*) could be either due to the greater width of roads

connecting species' habitats where the confusion is caused by vehicles moving at very high speed or because certain food items offered or thrown by tourists alongside roads (Jeganathan *et al.*, 2018). Large carnivores (like Hyena *Hyaena hyaena* and Leopard *Panthera pardus*) also fall victims of road accidents (Jeganathan *et al.*, 2018). Reptilian roadkill accounted for about 3.076% of the total number of roadkill incidences. Due to their smaller body size reptiles are often hard to notice by the drivers, especially during night, which results in being hit by the speeding vehicles (Baskaran and Boominathan, 2010; Vyas and Vasava, 2019). It is also a fact that the drivers are less compassionate towards these tiny and less charismatic species (Baskaran and Boominathan, 2010; Vyas and Vasava, 2019). Slow movement and use of the roads as a substrate for thermo-regulation are other reasons for reptilian road mortality (Das *et al.*, 2007). Due to their nocturnal habit and slow dispersal, many species of amphibians are the worst victims of road kills (Seshadri *et al.*, 2009). Surprisingly, there were no incidents of amphibian mortality and least occurrences of reptile mortality in the official record of the Department of Forest (Govt of West Bengal) for the period assessed during the present study. Possibly, the amphibians and other victims of roadkill with small body size are often underrepresented as the dead individuals are displaced by the speeding vehicles and not noticed by the surveyors due to their smaller body size (Seshadri *et al.*, 2009), or else their mortal remains are devoured by the scavengers, even before being noticed and recorded by the respective authorities.

In the present study, we did not notice any significant reduction in the incidents of wildlife mortalities by road and railway accidents over the years. This indicates that the mitigation measures to reduce the roadkill incidences in West Bengal have either not been implemented in many places, or they have not been very much successful. The total number of accidents was lowest in 2019-20 as compared to the previous four years of study, possibly due to the lesser number of traffic on roads due to lockdown imposed to prevent COVID-19 pandemic. However, we did not find any significant difference in the number of wildlife casualties due to vehicular collisions in different years indicating that there has been no overall reduction in the incidents. Lack of awareness and empathy for wildlife among the drivers and lapse in the strict implementation of existing laws for prevention of accidental killing of wildlife (Talukdar, 2003) are responsible for the consistent scenario of wildlife mortalities by vehicle collisions year after year.

Conclusion and management implications:

The impact of road on wild animals and plants may be attributed to human developmental activities (Selvan *et al.*, 2012). The present study portrays the overall scenario of wildlife mortalities in roads and railway tracks of West Bengal between 2015 and 2020. The findings clearly indicate that as the roads fragment habitats, wildlife mortality by vehicular traffic during

movements across roads in search of resources or foraging will be inevitable. We emphasize that the national highways, state highways, expressways, and railway networks passing through wildlife corridors connecting two forest landscapes have devastating impacts on wildlife. Therefore, it is essential to monitor the vehicular traffic of roads crossing forest corridors and implement mitigation measures to reduce wildlife-vehicle collisions, in order to increase habitat connectivity of various wild fauna and increase their genetic diversity (Habib *et al.*, 2020; Kumawat and Purohit, 2020).

Utmost priority needs to be given while designing the roads and railway tracks to resolve the burning issue of wildlife mortality due to vehicle accidents. In order to successfully implement the mitigation measures, first, the hotspots of wildlife mortality due to accidents need to be identified. Some of the general recommendations include:

- Construction of V-shaped deep trenches or erection of guard walls or fences along the roads and railway tracks in the accident hotspots, so that no animal suddenly comes on roads.
- Roadside bushes, particularly at the verges, should be removed to increase visibility so that drivers can avoid accidents as well as increase the shyness for animals to cross the roads (Selvan *et al.*, 2012; Kumawat and Purohit, 2020).
- To reduce the incidences of rail collision kills especially for large mammals like wild elephants, the drivers have to be more cautious on the railway tracks. Two or more engaged train drivers with a full view of the frontal side, should negotiate curves skillfully and at a low speed on the tracks while passing through forest areas where elephants pass through frequently.
- Good communication network in the moving train to allow forest and railway patrolling staff to pass on necessary information along with strong barriers on both sides of the track except a few passages where the elephants pass through frequently may reduce elephant and other wildlife deaths due to collision with train (Roy *et al.*, 2009).
- Construction of underpasses, fliers, and shining display boards with cautionary instructions, regular speed breakers, particularly in the biodiversity rich road accident-prone areas can minimize the road killing of large bodied animals or small amphibians and reptiles.

Despite these general recommendations, it has to be understood that "one size fits all" cannot be the solution for all accident-prone areas. Rather, the management interventions are likely to be different in different areas. Appropriate strategies may be designed for each of such accident-prone areas only after duly considering the cause, nature, and magnitude of the problem. Concerted efforts are needed by the conservation community, the state forest departments, and civil society in raising awareness about the threats of wild animals due to

vehicular collisions (Pragatheesh, 2011). The present study is based on the available official data and only gives a glimpse of the incidences of wildlife mortalities among different species and discusses its implications towards wildlife management. Nevertheless, the situation is alarming, emphasizes the need for in-depth research, and calls for due attention from the concerned authorities. We agree that the roads are an important component of ever-expanding urban areas and necessary for human well-being, but the alleviation of the threats to wildlife also needs to be given the highest priority in the days to come.

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SEASONAL VARIATIONS OF ALPHA RADIOACTIVITY IN THE RIVER ICHAMTI , INDIA

Arnab Basu^{1*}, Sheela Roy¹, Siddhartha Datta² and Dipak Ghosh³

¹Department of Zoology (P.G.), Vidyasagar College,

Bidhannagar Campus, Block CL, sector-II, Saltlake city, Kolkata-700091, India

²Department of Chemical Engineering,

Jadavpur University, Kolkata-700032, India

³Department of Sir CV RAMAN Centre for Physics and Music,

Jadavpur University, Kolkata-700032, India

*Corresponding author E-mail: arnab.basu3@rediffmail.com

Abstract:

The radioactivity due to alpha radiation detected usually originates from U^{238} and Th^{232} series those present in everywhere in the earth-crust. Present study indicated that the amount of alpha radioactivity recorded was the maximum in pre-monsoon in all stations (upper reaches: 55.78 Bq/L, middle reaches: 79.51 Bq/L and down reaches: 116.40 Bq/L), then it gradually decreased in monsoon (upper reaches: 42.45 Bq/L, middle reaches: 18.55 Bq/L and down reaches: 106.80 Bq/L) followed by post-monsoon season (upper reaches: 21.79 Bq/L, middle reaches: 29.09 Bq/L and down reaches: 66.25 Bq/L) thus in middle reaches a slight increase in alpha radioactivity in post-monsoon was observed. Concentration of lead was the maximum during pre-monsoon in upper (0.3ppm) and down reaches (0.89ppm) of Ichamati while it was highest in monsoon (0.28ppm) in middle reaches. Minimum value was obtained in post-monsoon (Middle reaches: 0.11ppm and down reaches: 0.52ppm) but in upper reaches lowest value was estimated in monsoon (0.15ppm). The results showed that the lowest amount of radio-activity present in the up-stream (mean: 40.01 Bq/L) then in the middle- (mean: 42.38 Bq/L) and down- streams (mean: 96.50 Bq/L). As the river progressed, it is expected that the river gathered more of radioactivity in its course.

Keywords: Ichamati, alpha radiation, lead

Introduction:

Rivers are precisely balanced aquatic ecosystem existing in close proximity to man. The presence of radio-nuclides in water has added a new problem to environmental hazards. The radioactive materials found in the river (bed) originating from U^{238} and Th^{232} series are the ones

those present in everywhere in the earth-crust. The main component of natural uranium is U^{238} , and its half-life is 4.5 billion years that are almost equal to the age of the earth. U^{238} itself is a radioactive nuclide and the generated nuclides are also radioactive and repeat to decay one after another. It finally becomes a lead (Pb^{206}) after changing through 14 different radioactive nuclides. Those radionuclides were called as “daughters” or “progenies”. Moreover, among the ‘daughters’ some nuclides such as radium and radon have unique danger of higher activity. When mining of radioelements and their subsequent use, for example, in the nuclear reactors are undertaken a major fraction of radionuclides in effluents from reactors and reprocessing plant during normal operations are associated with particles and colloids which distributes in the environment. Hence the presence of radioactive particles or colloids in releases from nuclear sources occurs more frequently than usually expected. After deposition, weathering of particles occurs and associated radionuclides are mobilized with time to the river ecosystem. As the radioactive elements decay, alpha radiation continues to be released into groundwater as well as surface water of ponds and rivers. Various other sources including nuclear power plants, use of radioactive isotopes in medical and industrial research are also responsible for the alarming level of radioactivity in freshwater. Moreover, the runoff from the fly ash deposits (which contains radioactive materials) of the coal fired brick kilns standing on the river banks enriches the radioactive contaminations (Koide, 2004). Ghosh *et al.*, 2008a) pointed out that in water, radionuclides are of two types - short lived (e.g., Na^{24} , P^{32} , Mn^{56} and Np^{239} , as well as Cr^{51}) and long lived (e.g., U^{238} , Ra^{226} , Pb^{210} , Ra^{228} , Th^{238} etc.). Short lived can build or decay during processing and storage prior to consumption. Long lived goes into at rock water interface and are detectable during sampling. Alpha radiation in water varies with the varying amounts of radioactive elements in the earth’s bed-rock. It continues to be released into groundwater as well as surface water. Based on limited literatures in the subject, there are presumably no immediate health risks from drinking such alpha radiation contaminated water. However, it may cause health problems over time (Ghosh *et al.*, 2008b). Studies have also revealed a possible link between alpha radioactivity in water and gastric cancer (Ghosh *et al.*, 2008c). Winters *et al.* (1982) stated that alpha radiation is thousands times more carcinogenic than gamma radiation. Radon in air and water has also been reported as one of the most significant sources of cancer (Gabler, 1988). EPA (1996) estimated that 11% from stomach cancer caused by consuming radon containing water.

A number of literatures are available on the radioactivity study on the water bodies. Carroll and Moore (1993) collected data on the Ganges-Brahmaputra mixing zone, uranium and salinity during a period of low river discharge. The uranium distribution with salinity showed that in waters less than 12 ppt salinity, uranium activities were significantly lower than predicted from conservative mixing of river and seawater. This suggested that uranium was being removed

within the mixing zone. Bioaccumulation of Pb^{210} in the Kaveri river ecosystem, India was conducted by Hameed *et al.* (1997) showed the highest level of Pb^{210} activity in the sediment (15.5 Bq kg^{-1} dry) and the lowest activity in water (2.7 mBq l^{-1}). The natural radiation level and the concentration and spatial distribution of heavy metals (Pb, Cr, Cu, Zn and Ni) were studied to understand the heavy metal contamination and its level of toxicity in the sediment samples of the Ponnaiyar river with an aim of evaluating the radiation hazard (Suresh *et al.*, 2011). Systematic studies on radiation level and distribution of radionuclides have been carried out in riverine environments of three major rivers, namely, Kali, Sharavathi and Netravathi of coastal Karnataka. It was calculated that the representative level index values are high in sediment samples of the Netravathi River (Narayana *et al.*, 2007). The rivers Vouga, Dão, Távora and Mondego in Portugal were contaminated by waste waters from uranium ore milling. A study indicated that discharge was not alarming enough to pose a significant radiological risk either to aquatic fauna or to freshwater fish consumers (Carvalho *et al.*, 2007).

Fertilizer industries with its vast extension of phosphogypsum piles sited in the estuary formed by the Odiel and Tinto river mouths (southwest of Spain), were producing an unambiguous radioactive impact in their surrounding aquatic environment through radionuclides from the U-series. The obtained results indicate that the main pathway of radioactive contamination of the estuary was through the dissolution in its waters of the radionuclides released by the industrial activities and their later fixation on the particulate materials. It was concluded that the tidal activity also played an important role in the transport and homogenization along the estuary of the radioactivity released from the fertilizer plants (Bolivar *et al.*, 2002). The discharge of radioactive waste, from nuclear fuel reprocessing facilities, into the coastal waters of north-west Europe was discussed by Kershaw and Baxter (1995). Sellafield (U.K.) has dominated the supply of Cs^{134} , Cs^{137} , Sr^{90} , Tc^{99} and Pu^{238} , whereas La Hague (France) has contributed a larger proportion of I^{129} and Sb^{125} in the Barents Sea. In another interesting article Amano *et al.* (1999) focused on the transfer capability of long lived Chernobyl radionuclides from surface soil to river water in dissolved forms in Sahan River within 30 km zone of Chernobyl, Russia to suggest that the transuranic elements such as Pu and Am are associated with mobile high molecular weight materials like fulvic acids in water leachates. On the other hand, saline waters from underground coal mines in Poland often contain natural radioactive isotopes, mainly Ra^{226} from the uranium decay series and Ra^{228} from the thorium series. It is observed in coal mines, that as a result of precipitation of radium from radium-bearing waters, highly radioactive deposits are formed. Sometimes the radioactivity of such materials is extremely high and such deposition takes place underground, but sometimes co-precipitation of radium with barium takes place on the surface, in settling ponds and in rivers (Chalupni *et al.*, 2001). Contamination of river water may be induced by many different ways.

To remind a tragic accident of recent years in nuclear power plant in Japan it was recorded that due to a gigantic earthquake and resulting tsunami that hit Japan In March 2011, Fukushima Daiichi Nuclear Power Plant (FDNPP) of Tokyo Electric Power Company was severely damaged. Eventually, a large amount of radioactive material was released from the reactor in the environment to contaminate adjacent river water. Radioactivity concentrations of I^{131} , Cs^{134} and Cs^{137} were determined for the river water sampled from the Edogawa River, the Arakawa River and the Tamagawa River, three major rivers spreading in or surrounding Tokyo Metropolis to record a high level of contaminations (Oura and Ebihara, 2012). A recent study on the river Cauvery, India clearly indicated from the data of the gamma ray spectroscopic analysis of sediment samples that the levels of mean activity concentration of U^{238} , Th^{232} and K^{40} for Cauvery river is lower than the international recommended limit (Murugesan *et al.*, 2011).

On the heavy metal contamination, the Krishna in India is seen to be a minor contributor of heavy metals (V, Cr, Fe, Co, Ni, Cu, Zn and Pb) to Bay of Bengal (Ranesh *et al.*, 1990). Ghosh *et al.* (2008a) investigated the alpha radioactivity of Ganga river. Ghosh *et al.*, 2004, worked on measurements of alpha radioactivity of ground water in West Bengal. They stated that drinking water that contained alpha radiation may cause health problems overtime. Ghosh *et al.* (2008b) analyzed alpha radioactivity of some ponds near industrial area in Maheshtala, West Bengal. Basu *et al.* (2013) studied the alpha radioactivity of some managed and unmanaged ponds at East Calcutta wetlands, India as well.

Our present study aimed to find out the distribution of radioactive elements and amount of alpha radioactivity within the basin of the river Ichamati as this river is a cross-border river catering for the livelihood for a large population needed a thorough investigation on possible radioactive contamination for the general safety of the people. A good number of people use its water for drinking purposes too. The lack of data on the radioactivity of this river prompted the authors to study the river water to assess and create a base-line resource.

Material and Methods:

Description of the sites:

Three (3) locations had been chosen along the entire length of the river Ichamati in India - one from up-stream near its origin at Majhdia ($23^{\circ}41' 88''$ N latitude and $88^{\circ} 72' 52''$ E longitude), then middle-stream at Tetulia (latitude $22^{\circ} 66'78''$ N and longitude $88^{\circ}60'23''$ E) and lastly the down-stream near the mouth of the river at Hasnabad (latitude $20^{\circ} 34' 60''$ N, longitude $88^{\circ}55' E$).

At upstream, it was about 150 meters wide and depth of water became 1.5-2.0 m during summer. The area was covered with aquatic vegetation or macrophytes throughout the summer

and winter. Only in monsoon the rain water flushed the macrophytes and more or less cleaned the river.

Ichamati at middle stream was 200 meters wide while the depth varied from 3-4.5 m seasonally. The river was free flowing with low current and the bottom bed contained fine sand, silt and small pebbles. No macrophyte was found here.

At down stream, the depth and broadness of the river increased more as compared to the middle-stream. The width of the river here was about 400 meters and the depth was 9 m respectively during summer. The volume of water increased during monsoon and had a free flow and strong current in all seasons. The bottom bed was consisted of fine sand, silt and clay. People dwell in both sides of the bank. There was a regular boat services to ferry people from each side of the river banks. In recent years this area became a popular tourist spot and a large number of people gather to enjoy the immersion of idols in the river especially during the Durga puja idol immersion festival each year.

Estimation of alpha radioactivity and lead:

1) Alpha radioactivity

Estimation of alpha activity from the river water has been performed using CR-39-Solid State Nuclear Track Detector (SSNTD), procured from Page Mouldings (Pershore) Ltd., England following the standard methodology (Henahaw, 1989; Ghosh *et al.*, 2008).

2) Lead

Along with Alpha radioactivity lead content of water was also estimated as lead was considered an important heavy metal indicating pollution of water (Ranesh *et al.*, 1990). Lead concentration was measured using the standard protocol as recommended by APHA (2005). The sample filtrates were analyzed to determine the lead concentration quantitatively by atomic absorption spectroscopy.

Results:

The results were presented in Fig.1 to show the variations of alpha radioactivity with seasons. It indicated that the amount of alpha radioactivity recorded was the maximum in pre-monsoon in all three sites (up-stream: 55.78 Bq/L, middle-stream: 79.51 Bq/L and down-stream: 116.4 Bq/L), then it gradually decreased in monsoon (up-stream: 42.45 Bq/L, middle-stream: 18.55 Bq/L and down-stream: 106.8 Bq/L) followed by post-monsoon (up-stream: 21.79 Bq/L and down-stream: 66.25 Bq/L) but middle-stream experienced a slight rise in alpha radioactivity (29.09 Bq/L) (Table 1).

Concentration of lead was the maximum in pre-monsoon in upper (0.3ppm) and in down reaches (0.89ppm) in post-monsoon period (Fig.2) while it was the highest in monsoon (0.28ppm) in middle reaches. Minimum value was obtained in post-monsoon in Middle reaches:

0.11ppm and down reaches: 0.52ppm) but in upper reaches lowest value was obtained in monsoon (0.15ppm).

Concentrations of lead recorded the maximum in up-stream (pre-monsoon) followed by middle-stream (monsoon) and down-stream (post-monsoon). It was minimum in up-stream (monsoon) followed by middle-stream (pre-monsoon) and down-stream (pre-monsoon) (Fig.2).

Table 1: Seasonal variations of alpha radioactivity (Bq/L) in Ichamati

Ichamati	Pre-monsoon	Monsoon	Post-monsoon
Up-stream	55.78	42.45	21.79
Middle-stream	79.51	18.55	29.09
Down stream	116.4	106.8	66.25

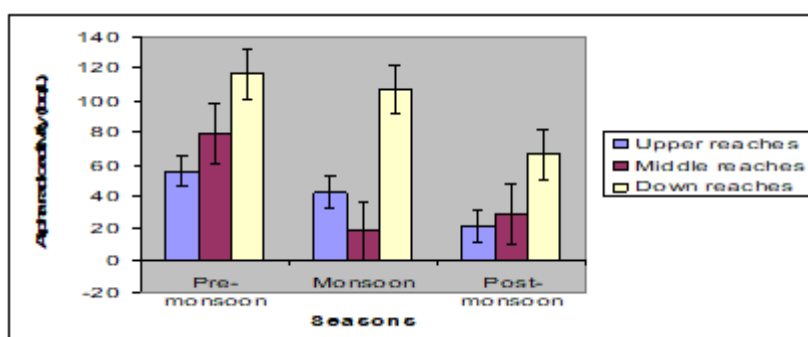


Figure 1: Seasonal variation of alpha radioactivity in the river Ichamati

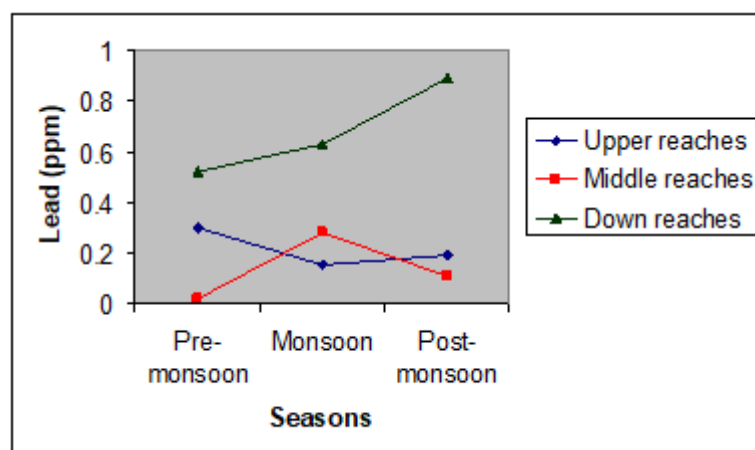


Figure 2: Seasonal variation of lead concentration in the river Ichamati

Discussion:

The results showed that the lowest amount of radioactivity was present in the up-stream (mean: 40.01 Bq/L) then in the middle- (mean: 42.38 Bq/L) and down- (mean: 96.50 Bq/L) streams. As the river progressed, it probably collected increasing amounts of radionuclides in its

course. This perhaps was a direct consequence of the number of brick kilns present on the river bank. The number was more in the banks of middle- and down- streams than up-stream. The runoff of fly ashes produced from brick making industries, presumably was one of the main reasons for the increase in radionuclides in middle- and down- streams (Koide, 2004).

Further, the middle- and down- streams were the mixing zones of freshwater and brackish water making the water more saline than the up-stream. The increase in salinity enhanced the radioactivity of water (Carroll and Moore, 1993) bolstered the observation during this study that the activity increased in middle-stream and more so in down- streams in Ichamati. Investigation by Tomza and Lebecka (1981) revealed that the concentration of radium in water was correlated with its salinity. As the salinity of water usually increased with depth, water with higher radium concentration occurred in deeper levels. The depth of water in Ichamati was always lower in up-stream while in down-stream as the river progressed was higher. Hence the radioactivity in down-stream was the maximum. This observation was further supported by Chalupnik *et al.* (2001) and Oura and Ebihara (2012). Data revealed that in water, a maximum level of 11.1 Bq/L was considered as contamination (maximum level of contamination, MCL) for public health (UNSCEAR, 1993., WHO, 1996). All the locations of Ichamati studied contained radioactivity higher than the MCL value. The radioactivity was recorded the highest in pre-monsoon period in all three sites probably due to decrease in water level during summer (i.e., pre-monsoon) as a result of which the concentration increased.

One of the heavy metals, namely, lead, showed a great variation in concentrations (Fig.2). This may be due to the variations in discharged effluents from industries and other sources (boat paints, paints from idols immersed in Ichamati) situated near middle and down reaches of the river. Thus the Pb concentrations (up-stream: 0.31 mg L^{-1} ; middle-stream: 0.34 mg L^{-1} and down-stream: 0.95 mg L^{-1}), independent on the site locations or seasons were always higher than the accepted limits (of Drinking water: 0.01 mg L^{-1} – IS 10500, 2012; EPA, WHO, 2012: 0.015 mg L^{-1}).

Conclusion:

The result of the study and its subsequent discussion brings out the fact that river Ichamati is contaminated by both alpha radiation activity and lead. Both the parameters have exceeded the standard safety limits. Depending on this preliminary data it is, therefore, extremely necessary to carry out a further thorough investigation on the water status of Ichamati for these two parameters in particular to suggest a remedial measure.

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VARANASI CITY AND ITS SACRED ECOLOGY

Shouvonik Bala

Vidyasagar College for women

Abstract:

Sacred ecology is an emerging field of religion, conservation, and education that acknowledges that there is a spiritual dimension to all problems related to conservation, environmentalism, and world governance. Sacred ecology examines the knowledge held by indigenous and other rural peoples around the world and how we can apply this knowledge and knowledge acquisition methods and solve problems between learning and gaining experience. Berks has shown the importance of local cultural knowledge as a complement to scientific ecology, and by using this knowledge for indigenous groups, he has sought solutions to problems and questions of cultural and political significance. Actually no person or subject exists in isolation; they are involved on the basis of multiple causal relationships. In this case, a tradition, a way of life, a way of thinking is formed and guided by the interaction and work with different or one's own community and this is the essence of the social ecology. In the Hindu mythology Mahaupanishad, the sky is described as the father and the earth as the mother, thus the whole world is a family (Vasudhaiva Kutumbakam) and we are all brothers and sisters.

Keyword: Sacred ecology, sacred landscape, pilgrimage, cosmology

Introduction:

According to Indian traditional Hindu beliefs, the sacred place is not isolated from the realm of material (ecological) ecology. Rather it is one of the ways to understand the truth of the created physical world and build environment. Historical and mythological interpretations of the sacred space combine divine power and the physical or geographical world. Hindu mythological scriptures help to understand the interrelationship between Sacred and natural ecological phenomena and indicate the dynamics of Indian culture. Descriptions of the process of creation, cosmic activities, time cycles, and creation of various deities, their position and spiritual significance all indicate the relationship between man and nature in a metaphorical sense (Singh Rana, 1993). From the point of view of practice and thought, sacred ecology is generally divided into two parts: deep ecology that refers to feelings, emotions, and sensory activities, and shallow ecological experimental observations. Varanasi was originally a sacred place and later developed as a holy city. Varanasi is considered to be a good example of the sacred ecosystem due to its

pilgrimage circuit, interconnected temples and shrines and the powers of expression imposed on it to varying degrees.

Importance of the study:

Contributors to the field of sacred ecology claim that there are spiritual elements at the root of environmental problems. Those working in spiritual ecology further suggest that there is an important need to acknowledge and address sacred and divine dynamics at the root of environmental degradation (Sponsel, 2014). Concerning the environmental crisis, (Meadows, 1992), he said that the whole world is facing a kind of cosmic loneliness that we have not seen before. Historian of Science Lynn White (1967) said that what we discuss about ecology depends on our perceptions of nature. Until we find a new religion, or rethink the old religion, science and more technology will not be able to bring us out of our current environmental crisis. The scientist community has now confirmed that the world is interconnected and interdependent, that is why ecology has become so popular today. Sacred ecology considers the whole universe as a single system and presents it as a worldview. A state of harmony and unity exists throughout the world, called cosmic unity or law, which consists of visible or invisible, tangible or non tangible, earth, sky, water, atmosphere, man and other organism (Oates, 1989). James Gustav Speth said that the top environmental problems were depletion of biodiversity, ecological degradation, climate change, etc., and that the correct solution to these problems could be found through thirty years of good science practice, but his ideas changed. The top environmental problems are selfishness, greed and apathy, which are the root causes of other aforementioned problems, and he speaks of the need for a cultural and spiritual transformation to deal with them.

Location of the study area:

Varanasi city is India's oldest city encircled by the three Rivers Ganga in the east, Varuna (Varna) in the north and Assi in the south. Actually the name "Varanasi" which was derived from the two tributaries of the holy River Ganga named Varuna (Varna) and Assi. Varanasi city is an important urban area of Varanasi district of eastern Uttar Pradesh. Geographical location of the city is between $25^{\circ}15'$ to $25^{\circ}23.5'$ North latitude, $82^{\circ}57'$ to $83^{\circ}1'$ East longitude and it covers an area more than 83 sq km. However, the territory of religious areas of Varanasi is much larger than the current administrative boundaries of the city, as the boundaries of any religious area do not correspond to the administrative boundaries of that place, but for the convenience of discussion we have completed the work by taking the present Varanasi area as our study.

Objectives:

As a traveler without a path loses his way, the path takes the traveler to his destination, so the path is considered here as a living entity and insight of the mind. Which brings the traveler to the right destination and this is the main objective of sacred Ecology. That is why the main

purpose of this study is to introduce the concept of sacred ecological cosmology in the context of sacred Varanasi city.

Methodology:

Ralph (1976) presents three basic elements for place such as space (Physical installation), activities and the importance of the place. Cantor (1977) again describes these elements as physical features, activities, and concepts respectively. The Panchakroshi pilgrimage route fully expresses the above three elements. The physical setting of this journey refers to the territorial boundaries of cosmic cities through the spatial expression of the whole world. In fact, the structure of the man-environment relationship of that place is determined by the interaction of certain people and the environment. People make pilgrimages to get rid of all kinds of sins, which create an orderly bond between man and nature. So the Sacred Ecology of this holy city has been explained by combining the structure of the sacred region of this city and the short description of the Ganga ghats, through the holy religious pilgrimage of Varanasi.

Sacred ecology of Varanasi:

Pilgrimage connects the natural world with human spiritual thought. Pilgrimage is an inspiration that reveals nature's long-standing and deep-rooted relationship with man. The manifestation of the divine power of a place turns that place into a holy land and later that place acquires a different character and unique image through human thought and practice. In other words, the religious symbol is meaningfully adjusts and projects on that place or the landscape. Sacred places are not equal in all respects in terms of sanctity, but different orders have been created depending on the spiritual closeness of the space and the magnitude of holiness, people symbolized for that particular place (Cohen, 1976). Once a place recognized by the society as a sacred place, it shows order, wholeness, and power of holiness (Tuan, 1978). Various pilgrimage journeys (yatras) in Varanasi are the examples of varieties of order. Varanasi has a variety of sacred and religious territory in different frame of reference, five of which have gained the most importance and popularity in mythology. They are Chaurashikroshi Yatra, Panchakroshi Yatra, Nagara Pradakshina and Avimukta Yatra. The five major religious circuits or areas of Varanasi are comparable to the *Panchamahabhuta* or five heavenly gross elements of Hindu cosmology, (such as the sky, the earth, the air, the water and the fire), and also it is parallel to the five basic parts of the human body, (such as the head ,the legs,the face,the blood and the heart) and it is also comparable to mystical or transcendental power, associated sheath or chakra (the food, the mind, the breath, the intellect, the bliss) and commensurate sanctified number of temple on the route (Singh and Rana, 2006).

The sacred space of Varanasi can be understood in terms of a geomantic frame of above mentioned five sacred territories that are connoted as five sheaths and are depicted as five

concentric rings. Though in reality all the territory have irregular shapes, except the outer one, which runs as a circle (Chaurashikroshi) but religious geomancers and cartographers, eliminates all these irregularities and express easily through circles or other geometric shapes or in a combination of geometric shapes. The spiritual greatness of these religious circuits indicates the interconnectedness between man and the universe.

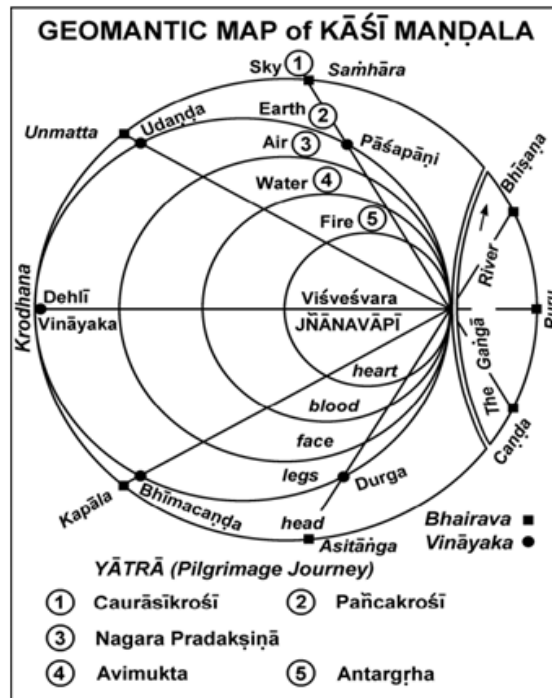


Figure 1: Geomagnetic map of Kashi (Varanasi) (Singh, 1994)

Table 1: Five major sacred circuite of Varanasi (Source: Singh Rana, 2016)

Layer	Macro cosm/ <i>Pancha mahabhuta</i>	Mesocosm/ Pilgrimage rout	Microcosm/Body parts	Transcendental power	Sheath (Cakra)	No.of temple on the route
1	Sky	Chaurashikroshi	Head	Consciousness	Food	144
2	Earth	Panchkroshi	Legs	Action	Mind	108
3	Air	Nagar pradakshina	Face	Cognition	Breath	72
4	Water	Avimukta	Blood	Wisdom	Intellect	72
5	Fire	Antargha	Heart	Bliss	Bliss	72

Varanasi is the ideal celestial archetype where material expression to that of parallelism between macrocosmos and microcosmos are still present today. The cosmogram of Varanasi becomes alive due to the corresponding movement of worshiper. Pilgrims are the soul of Varanasi cosmogram and without it cosmogram is not more than the archaic relic (Lannoy,

2002). If we arranged the above mentioned five sacred space of varanasi according to their size from large to small (Chaurashikroshi Yatra to Avimukta Yatra), then we can see that though the size decreases, the religious greatness increases. There is a negative relationship between area of the sacred space and manifestive power. (Singh Rana, 1994).

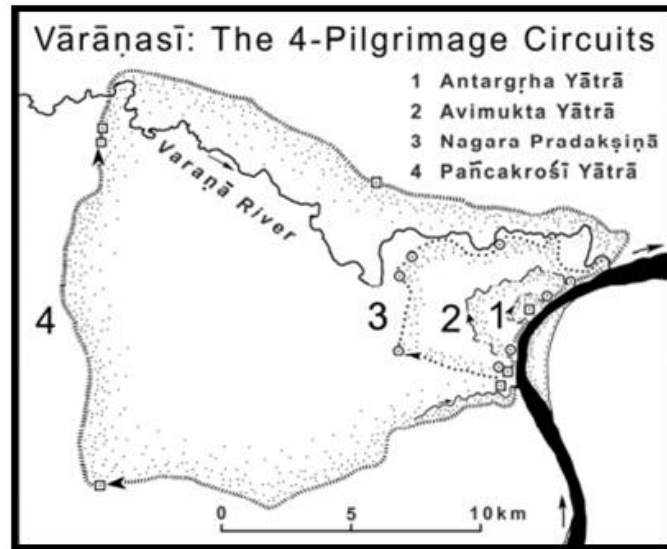


Figure 2: Varanasi: The Pilgrimage circuits (Singh, 1994)

One of the popular ancient names of the Varanasi city is Kashi, i.e., Kashya eti Kashi. That means where the light of cosmos concentrates and illuminates in disc-shaped region is known as Kashi. This idea is glorified in the Puranic literature describing the limits and boundaries of the city. Chaurashikroshi pilgrimage route is the outermost boundary which encircles the cosmic territory of the sacred realm of Varanasi city and also referred to as Kashi Mandala. Thus covering a circumbulatory circuit of 168 miles (269 km), known as Chaurashikroshi yatra. The shrine of the Dehli (gate) Vinayaka identifies the entrance into the cosmic Mandala; the shrine of Dehli Vinayaka identifies “the gate of entrance having Ganesha as guardian (Singh, 1988). The route is divided into eight directions and is run by 12 directional guardians or deities on each direction (Shakti or Devy). For this reason, the city of Varanasi is believed to be guarded by $8 \times 12 = 96$ Devy. Apart from Devy, Kshetra Devi, Bhairab, Dikpal (directional deity), three Vetals (demi god) etc. are protecting Kashi. The first historical reference to this area was made in a 7th century text of mythological import, the Padma Purana (Sristhi khanda) Pilgrimage along this territorial edge symbolizes circumambulation of the entire cosmos; but this journey is now rarely performed. Over the passage of time the Chaurashikroshi yatra shrunk to the present path of Pancakroshi yatra.

During the 16th century the Panchkroshi yatra had become quite popular. Thus five krosha equals to about 11 miles (17.6km). The Panchkroshi yatra (pilgrimage) circular path, at a

radius of Panchkrosha (80km) demarcates the territorial limit of the cosmic territory of Varanasi, generally known as Kashi mandala.

Nagar pradakshina is the third sacred loop or pilgrimage route which covers a distance of 25 km (r 4km) and connects 72 shrine and sacred spots. Nagar Pradakshina Yatra, which travels around the outer part of the city.

Avimukta is said to be defined by a circle that radius of which only one krosha, again Madhyamesvara at the centre. One krosha represents 3.2 km (.509 km). Avimukta Yatra, travels around the inner city of Varanasi.

Antargrha yatra radius is 400 meters and r is 63.62 meters. This is the inner inner sanctum of the city. Antargriha Yatra which circles the core or the "inner sanctum" (Kasi Viswanath temple) of Varanasi.

Sacred and heritage ecology of Ghats:

The Ganges is always regarded by Hindus as the "mother" and is worshiped as an essential power in life-force. Like the five gross elements of organic (Panchabhuta), rivers are also made up of these elements. These traits call for a re-establishment of a sacred relationship with nature or with space. Gestalten rightly said that the riverfront landscape should be viewed not as an isolated element, but as a holistic subject of meaningful relationships where life and experience are intertwined with the everyday world. The geological structure affects the flow of the Ganga from south to north in a semicircular shape near Varanasi. This sharp-river bend and unique geological structure helped the city to grow into a crescent, which is also symbolically described as the crescent of the forehead of Lord Shiva. From the point of view of river ecology, this feature is also considered as one of the indicators of energy quantity and direction of energy flow and speed. Indeed, this whole flow of the Ganga River adjacent to Varanasi is an example of natural heritage. A geomantic sequence has been formed on the banks of the Ganga by reverence, devotion and regular rituals in terms of deep sacred ecology.

The multiculturalism of India is observed along the Ganga ghats of Varanasi as people from different parts of India have settled in the ghats adjoining the city of Varanasi since ancient times. There are 84 important ghats and 96 holy shrines from Ganga-Varna confluence north of Varanasi to Asi- Ganga confluence in the south. These 84 ghats have been compared with 84 lakh organic species (yoni) according to Hindu mythology. It is believed that each ghat frees man from the pains of being born in one million organic species (yoni) and helps in salvation by washing away the sins of the people. Therefore, to get rid of 84 lakh organic species, to purify the soul and to be free from the cycle of rebirth, it is necessary to take a bath in every ghat of Varanasi city (84ghats x 10, 00000 organic speies or yoni = 84,000000 organic speies). Further 12 months / 12 Zodiac x 7 layers of atmosphere is equal to 84 in this way one can complete the annual cycle of cosmic journey by bathing all 84 ghats and can attain salvation by being

completely free from the bondage of birth and death. On the other hand, 96 holy water-shrines near the ghats have been explained $12 \times 8 = 96$, (12 is division of time/Zodiac and 8 is direction) so it is also represents manifestive transcendental dimension of Varanasi (Singh Rana, 2009). According to The Kashikhanda (KKh 84.107-10, 114) among all the 84 ghats, five ghats have been given more importance in terms of spirituality. To the north of these ghats are "Adikeshab, then gradually to the south are Panch Ganga ghat, Manikarnika ghat, Dashaswamedh ghat and Asi ghat respectively. These most sacred five ghats are called "Panch tirthas". It is believed that by bathing in these five ghats, one attains the same virtue of bathing in all the ghats and one is freed from the cycle of life and death and attains salvation.

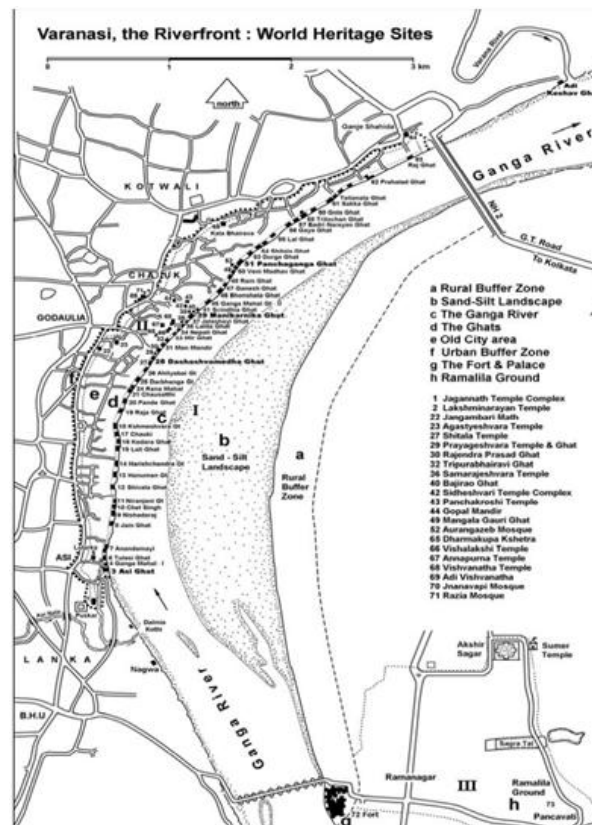


Figure 3: Varanasi Riverfront (Singh, 2009)

Findings:

In order to experience the spiritual experience of the city of Varanasi, we need to be directly involved in certain rituals, only then we can understand the underlying psychological ecology of the Mother Nature, which will contribute to sustainable and dignified development. The concept of sacred ecology not only refers buildings and artifacts of culture; it also indicates a spatio-territorial point of view to integrate the monuments with people's faith and performance system (sacred ecology). The basic idea behind this approach is "place making."

A pilgrim becomes part of an expanded larger ecosystem, experiencing a unified reality of a larger ecology-nature-spirit and human psyche. Programs and plans must be taken to

encourage pilgrimage and tourism. Because of the re-establishment and rediscovery of the values involved in experiencing the living sacred world and the revival of such traditions so that more people can get the opportunities to realize the spiritual relationship between pilgrimage and Mother Nature.

Heritage scape symbolizes the progress, prosperity, growth and potentiality of mankind, which we must preserve. This conservation idea can also be adopted as a conservation policy or environmental ethics having heritage ecology at the core. The growing pace of modern tourism and the lack of traditional ecological maintenance and the deterioration of the heritage environment have become a major threat to Varanasi due to the competition of modernization of the city. So, when planning or developing, we need to be aware of whether our own culture is being compromised and whether our culture is being lost or extinct, somewhere between historical continuity and the acceptability of modernity. The planning strategy should therefore be followed by following a middle path while maintaining deep harmony.

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ENVIRONMENT AND FUTURE GENERATION: SOME PHILOSOPHICAL STRANDS

Molly Ghosh

Abstract:

The issue of protection and preservation of natural resources is a crucial one when it is seen from ethical perspective. There are two opposite kinds of doctrines popular in this realm, such as, anthropocentrism and non-anthropocentrism. It is, though appears to be obvious, but difficult to determine why human being should be responsible and dutiful to their next generation as well as to the nature. Whether it is only the matter of sustainability of human existence in a known and comfortable way or is it connected with the idea of essence of human life and self? In this way the ethical issues turn into the investigation of their philosophical foundations and this endeavor may disclose the greater understanding of our duties towards future generations.

Keywords: Nature, Anthropocentric, responsibility, reverence for life, ecology. Human existence

Introduction:

Since last two years almost we are facing a new threat to our survival. Though we do not have clear idea about the source of emergence of this deadly corona virus, still most of the environmental issues are effects of human activities. The severity of each problem is so intense that sometimes we feel helpless and sometimes we just avoid the role that could prevent to some extent. When governments and other different org try to find out some solutions and seek our support, we do not always act responsibly. Sustainability is the key to prevent or reduce the problems prevailing in some country or other. Now it is almost definite that we are living unsustainably, and hardcore effort of every individual is needed to pay back to the nature against the use of natural resources to sustainable limit. Still the question comes whether the problems will be resolved in pleasant ways of our own choice or we have to fall through more dangerous pandemics and all. What is the future? What are we going to leave for our future generations? Shouldn't we leave some precious and necessary resources for future human race as well as for the planet itself?

The western tradition of environmental thoughts:

We can discuss the western ideas from two basic perspectives, firstly from the perspective of Holy Bible or basic Christian theological doctrines and then from the ancient Greek ideas of the great Aristotle. The Holy Bible states that God is the creator of all things, rules over all and deserves our worship and adoration. This planet is the manifestation of

omniscient and omnipotent God, consequently governed and sustained by His unending eternal power. He has created men and women reflecting his own image and has given the privilege to command over other creatures of the world and to exercise stewardship over the earth. We must focus on the idea of Stewardship. According to the doctrine, God has given us knowledge and righteousness and the power to dominate the earth. But our Stewardship under the surveillance of God includes our moral accountability and best service to fulfill the objectives of God. Hence we have some virtues in one hand and some free choice on the other, as our attributes. God's moral law can be revealed in the conscience of human being. Now if we judge the doctrine in the name of one anthropocentric view, we may be challenged by the view of those who may claim this to be theo-centric by nature. Here in this discussion we can regard this traditional western view based on Bible, as anthropocentric view towards environmental moral discourse.

To quote a famous passage from Aristotle's *Politics*, "...after the birth of animals, plants exist for their sake, and that the other animals exist for the sake of human beings, the tame for use and food, the wild, if not all at least the greater part of them, for food and for the provision of clothing and various instruments. Now if in nature nothing is incomplete, and nothing in vain, the inference must be that she has made all animals for the sake of human beings." (*Politics* 1.8) Hence, it is clear that the flavor of anthropocentrism had always been present in western traditional ideas which never teach our mind to genuine concern for environment. Even defining moral virtue and vice, fixing the boundaries of moral duty and responsibility towards environment may be distorted if we rest on these doctrines. The question that comes naturally is, if human being is superior than all other creatures then do we get the right not to care for other creatures of the environment on the basis of our innate superior qualities?

A closer observation into the writings of Aristotle may disclose that there are several strands of Aristotle's thoughts that support his concern about genuine intrinsic value of nature and environment. It should not be taken for granted that Aristotle's philosophical strand is not bio-centric. As Aristotle has supported teleological doctrine of creation of Universe which admits that every particle of this creation has some goal to realize. The creation of this universe is the manifestation of a divine plan. All small planets and animals strive, by nature, to become fully developed and well functioning creatures. Their activities to achieve excellence include them in the discourse of moral life. In his *Metaphysics* he says that each life and their activities are interconnected. There is no strict boundary line between human life and non-human entities. So it is not very difficult to find out a domain of holistic approach of his thoughts which is overlapping with his anthropocentric ideas and this altogether may lead us to think that the anthropocentric view towards nature does not exclude moral responsibility of human being towards nature.

The dichotomy of anthropocentric and non-anthropocentric approach:

A sustainable environment is essential for the living of present and future generation of human race. This truth is undeniable. But the issue of development of environmental ethics rests in the question whether preservation and protection of environment is necessary only for the benefit of human being? Are cutting trees and killing animals wrong only because it hampers the ecological balance which is ultimately harmful to our future generation? Do the elements of nature have only instrumental values? Are they valuable for causing good livelihood and enjoyment of human being? Are our responsibilities towards preservation and protection of natural resources and natural entities determined by the claim of our future generation? The view that admits that the needs and necessities of human being is the determining factor of assigning values to natural non-human creatures is known as Anthropocentrism.

It is true that we do prefer to save and protect those things that we think to be precious, for our future generation. We may afford a level of sacrifice for that too. The question is what is wrong in this doctrine of anthropocentrism? The latest trend of human thoughts finds that this doctrine is based on an inferior presupposition regarding moral superiority of human beings to members of other species on this planet. This presupposition is not only wrong but it could be dangerous in consequence. Immanuel Kant in his Lectures on Ethics ("Duties to Animal and Spirits) suggests that cruelty towards any non-human creature or entity might encourage a person to develop a character which could be desensitized to cruelty towards other humans who are not my friend. Hence cruelty or mentality of destruction towards non-human entities would be considered to be instrumentally wrong, not intrinsically. Thus the doctrine of anthropocentrism involves non-intrinsic wrongness of anthropogenic devastation of nature. Such destruction might hamper the well being and virtue orientation of human being of present time as well as of future. After 1970, a new philosophy about environmental ethics developed and on the basis of newly emerged philosophical approach various doctrines towards environment started challenging such anthropocentric views and offered non-anthropocentric values and ideas. In the first place it questioned the fundamental presumption of anthropocentrism i.e., moral superiority of human being in comparison with other non-human elements. Next it developed some rational arguments to establish intrinsic value of each element of nature. It proposes that each and every element of nature has its own worth and no one else has any right to show disrespect to them. They are valuable as they are the part of the creation, neither for their utility nor for any other issue. This is to recognize their intrinsic value and to respect the feature of being a part of the creation. If the forest is cut once, the link with the past gets lost forever. And the cost will be borne by the upcoming future generations that succeed us on this planet. According to the environmentalists the value of wilderness can be defined in terms of 'world heritage'. What we have inherited from our ancestors, we must preserve those for our descendants, irrespective of any other issue. This is

certainly called the non-anthropocentric view. Very clearly the difference between two of these views are based on two different culture of thoughts, the first one keeps man as the determining factor of all others and the second one considers man as only a part of the nature and mostly equally valuable comparing to non-humans. In other words it rejects man as superior than others but believes in inter-connectedness of lives and elements of nature. Such a philosophical idea equates care for nature and care for oneself.

Some questions crop up from this dichotomy obviously. Two most important of those are to be discussed here as they are connected to the welfare of future generation. The first is : Can we avoid destruction of natural situation totally such as, deforestation , killing animals , making dams, using electricity, at all? The second is: Can we be sure that future generation will appreciate our endeavor of preservation of wilderness?

This is quite obvious that we cannot imagine living back in forest. Hence we need to cut some trees and built some dams but while using and destroying natural resources we should be very careful about the idea of reservation of true wilderness on the earth for the future generation of near and far. Our decisions should consider the scenic and biological significance of the forest and other relevant resources. This argument does not anyhow justify our decisions and our actions taken in terms of our earning more and to create huge amount of employment facility or something like that. In other words utilitarian approach is the worst justification for deforestation and perhaps for this reason only we have crossed the limit and already have created problems to our own sustainability as well as to the nature. Secondly, this might come true that future generation people would not appreciate and value such preservation to be very precious, especially of those nations who have no poverty and hunger. They might feel that preservation of nature is necessary only for aesthetic enjoyment and for scientific discoveries. Hence understanding the idea of intrinsic value of natural element is neither easy nor clear, when it comes to the question of benefiting future generations.

The idea of reverence for life:

The idea of reverence for life is found in the ethical theory of Albert Schweitzer who claims this to be the basic moral principle and the highest moral value. Which is a variation of non-anthropocentric view? This idea says that life has an intrinsic value. This idea of intrinsic value decodes a sense of responsibility which characterizes a genuine ethical attitude oriented with spiritual significance. It claims the fundamental similarity and unity of all creatures which is helpful to overcome anthropocentric point of view and also to realize that there is no strong ethical ground on which we can prefer one life comparing to any other.

Albert Schweitzer defends his own view by saying that “true philosophy must commence with the most immediate and comprehensive facts of consciousness.” If we introspect our own will-to-live, which is innate in us, we may find that we want to live in the lap of nature with other

creatures which do possess will-to-live for themselves. Whether I am able to understand their sound and voice is uncertain but I must respect their life in terms of respecting their will-to-live. It seems to me an evil to destroy and to check life. This includes fundamental principle of morality and we need not seek this moral foundation outside our own existence but within our own existence as in-built in consciousness. Hence the idea of Reverence for Life is intrinsically valuable and the guiding principle of the sustainability. This view goes against traditional western thoughts about environment and may face criticisms too. One may ask that if we start living on this principle of reverence for life we have to admit that the entire civilization is based on evil thoughts of mankind. We do show respect for some lives more than other lives. We acknowledge importance of some plants and do not care about others. How can we justify these facts? Perhaps we do not have any strong strand in justifying this other than opting anthropocentric grounds and utilitarian arguments. Hence I feel that anthropocentrism is real but non-anthropocentrism is ideal.

Paul Taylor, another contemporary American philosopher has been defended non-anthropocentric view and rejects anthropocentric view as groundless because he discards the superiority of human being. However, he acknowledges that mankind has a moral responsibility to act in the best interest of other creatures. He argues that man is a member of Earth's community of life. The system of nature is a complicated interconnected web about which we are not fully acquainted. But like man every single creature, every life and non-living element of nature is a centre of telos of autonomous choice. Consequently each element is an end in itself, and worthy of moral consideration. These non-anthropocentric ideas actually tend to establish a mystic interconnectedness of all creatures. It also tends to overcome the narrowness present in anthropocentrism. Undoubtedly this kind of view reminds me the ancient Indian thoughts and values of Veda and Upanishads. These scripts teach us that realization of own self is the ultimate goal of human life. Knowingly or unknowingly life is a journey to know this lesson and it includes an all pervasive nature of life, discarding the separated identity and discovering the non-duality of the ultimate reality.

The idea of deep ecology:

The view of Aldo Leopold is also commendable in this regard, especially as a non-anthropocentric view. He redefined the idea of wilderness on the basis of his own experience and realization. His idea is known as Land Ethic which states that the relation between people and land are interconnected. His idea is not only a matter of discussion in philosophical discourse but also in biological studies too. He says that people's value regarding environment may develop directly from their experiences. He believes that direct contact with nature works as a significant factor in shaping our ability to realize and apply ethical intuitions crossing our self-interest. It has been recognized by him that relationship between people with each other along with land is a

complex matter and he describes this as a social evolution for which we need to do thoughtful and hopeful dialog to chalk out our vision about our future generation and future of the nature as well.

It has been observed that Leopold's idea tends to preserve the integrity and stability of the environment without caring the benefit that may come to the account of human life. Hence, for him, the ethical value of non-human is completely independent of their utilitarian value. The natural richness and diversity of the creations of this planet hold intrinsic value and realization of these diverse life-forms man can realize the higher ethical values that, in turn, help to preserve the nature and environment. The philosophical implication of such an idea is to consider that the species or ecosystem not as a collection of individuals but an entity as a whole having moral worth. Otherwise the question may arise how can we assign equal intrinsic value to each bigger and smaller species? The fact that all organisms are part of an inter-related whole does not suggest that they are all of intrinsic worth. They may be having worth as they are needed for the existence of the whole. And the whole may be having worth as it supports the existence of conscious being. So the attempt to establish non-anthropocentric doctrine may include the rival ideas within itself. The actual philosophical hindrance is to assign individuality or self to a species. Do the plants and other ecosystems have morally significant interest to afford moral worth in them independently of their important role in sustaining conscious life? Hence, what is the outcome of the discussion is to understand that non-anthropocentric view is philosophically weaker than its competitor. Also if we argue in this way we have to rest on the argument based on the interest of conscious being.

Developing environmental awareness:

Besides environmental movements and campaigns what is found in reality is lack of awareness among mass. They have the scientific information regarding the state-of-affairs and ethical knowledge regarding codes of conduct but still they are careless and insincere about their actions. The probable reason lies in lacking the true emotional attachment along with the spiritual awakening. A true philosophical idea has the power to discern the ability to control oneself-one's impulse, tendencies and attributes. That is why without philosophical clarity of thoughts we fail to survive as organically responsible and therefore neglect our duties. However, the love towards nature will be etched in the minds of each when there will be an awakening of self and people will be compassionate beings truly understanding the greater interconnectedness with self and environment. Every individual has to accept consciously that each person is an entity of the cosmos itself. And the role of apparently insignificant creature may be of some subtle and significant importance. This reminds me the ancient Indian thoughts. Also I do remind the ancient doctrine of Machiavelli namely end justifies the means. To achieve our goal is most important. If we set a goal which requires excellence of man and his thoughts, it must be a

value-in –itself. It is not to allow misdeeds or evils. If we can maintain all natural resources in their natural way of life and can create the world full of flowers and fruits, rivers and forests, fountains and oceans, birds and animals and we can manage to fill our life with unlimited happiness and mutual trust, what else do we need? What else would be better to achieve the Goal?

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RECENT ADVANCEMENTS IN ECO-FRIENDLY MOSQUITO CONTROL STRATEGIES

Debraj Biswal

Department of Zoology,
Government General Degree College at Mangalkote,
Burdwan 713132, West Bengal, India

Corresponding author E-mail: debhraj@gmail.com

Abstract:

Mosquitoes transmit several diseases and are therefore a major cause of global health concern. Global climate changes has made situations even worse by facilitating the mosquitoes to cross continental borders and carry the diseases to those parts of the world where they did not exist earlier. Absence of suitable treatments and/or vaccines against most of them aggravates the situation further. Under these circumstances, vector control seems to be the most effective way to control these diseases. However, application of conventional control regimes has failed to achieve the targets. Moreover, the chemicals despite being cheap and effective leave behind toxic residues with deleterious effects on the environment. This has encouraged substantial research across the globe to develop innovative strategies of mosquito control with environment friendly approaches. Some of them include novel larvicides, mosquito traps and genetic manipulations. The current paper discusses recent advancements in this field and highlights their importance.

Keywords: Mosquito; Vector control; Innovative strategies of vector management; Eco-friendly approach; Public health

Introduction:

Mosquitoes are medically important arthropods because the female members of this insect species feed on animal blood and in the act of doing so they are able to transmit the causative agents of several diseases of global concern (Jones *et al.*, 2021). They need blood to nourish their developing ovum making them inseparable from their animal hosts including man. Thereby, they are ubiquitously found close to human habitations and over the years they have adapted themselves to breed in almost every kind of habitat - natural or man-made (Becker *et al.*, 2012). This has boosted their breeding success enabling them to proliferate at higher rates. Moreover, the global climate change has made conditions favourable for them to populate regions where they were not initially found (Mahgoub *et al.*, 2017). The warmer climates have

facilitated them to cross the continental borders further threatening public health (Mahgoub *et al.*, 2017). According to the reports of World Health Organization, 2021 about 229 million people were diagnosed with malaria from across the world in 2019. The report further states that approximately 100 to 400 million dengue infections are reported worldwide every year. Comparing the current report with earlier ones shows evidence of increases in global burden of mosquito-borne diseases despite the vector control strategies being undertaken from time to time (Achee *et al.*, 2019).

The failure of conventional methods of mosquito control has encouraged researchers in this field to develop innovative strategies of mosquito control. These studies have been found to be beneficial because they have enabled to overcome the eco-toxic effects of chemical-based insecticides and the development of vector resistance to several chemicals (Araújo *et al.*, 2015). The current paper discusses about some of the recent developments in this field.

Eco-friendly strategies of mosquito control:

Use of novel larvicides

Application of chemical larvicides is still popular because they are cheap and efficient. However, the chemical residues left behind have been demonstrated to exert eco-toxic effects. In the recent years some innovative larvicides like the entomopathogenic fungi (e.g. *Metarhizium anisopliae*, *Beauveria bassiana*) have been found to be effective against the larval stages of dengue vectors (Scholte *et al.*, 2004). Using different strains of *Bacillus thuringiensis* as mosquito larvicides have been tested with encouraging results (Zhang *et al.*, 2010). Currently, the use of soil bacteria like *Bacillus cereus*, *Aneurinibacillus aneurinilyticus* and others are being worked upon (Chatterjee *et al.*, 2015). Use of various formulations of plant products either as tablets or as nano-emulsions in the natural habitat water of mosquitoes have given positive results and are being explored further (Pavoni *et al.*, 2021). Lately, biosynthesized silver nanoparticles (i.e., using plant extracts as reducing agents) have yielded promising results for controlling mosquito larvae (Soni and Prakash, 2014). These novel larvicides are more efficient than the chemical ones because of their reduced adverse effects on environment (Wilson *et al.*, 2020). Furthermore, most of them have been found not to induce resistance in mosquito larvae (Pavoni *et al.*, 2021). This offers an added advantage. However, the major disadvantage of these larvicides lies in their longevity and affectivity in natural conditions (Achee *et al.*, 2019). Scientific research is currently focussed on developing stable novel larvicides that can effectively reduce vector populations.

Spatial and/or Oviposition repellents

Commonly, spatial repellents are used to keep away mosquitoes from indoor settings to decrease the contact between hosts and vectors (Achee *et al.*, 2012). The repellents mainly work by releasing volatile substances to the air that somehow modify the behaviour of the mosquitoes preventing from staying indoors (Achee *et al.*, 2012). The mode of their action and their heritability in mosquitoes has not yet been studied in details (Achee *et al.*, 2019). Though pyrethroids are mostly used in such repellents recently essential oils from plants have also been found to be effective (Maia and Moore, 2011). Some phytoproducts like neem oil derivatives have already been shown to modify the egg laying behaviour of female mosquitoes by making the habitat water non-preferable for such activities (Anjali *et al.*, 2012). For this purpose, intensive research on habitat water characterization and its role in oviposition is being carried out recently (Panigrahi *et al.*, 2014; Dida *et al.*, 2018).

Mosquito traps

The use of traps as agents of mosquito control is a newly emerging area of research. Some modern traps use carbon dioxide plumes and counter flow geometry (Kline, 1999) or even propane to lure the mosquitoes and kill those (Achee *et al.*, 2019). Small plastic tubes called ‘eave tubes’ with insecticide-treated electrostatic nettings when implanted in the walls of houses have been observed to kill the adult mosquitoes efficiently when they enter homes in search of human hosts (Knols *et al.*, 2016). Development of attractive baited lethal ovitraps or ALOT has been reported to effectively attract and kill mature fecund female mosquitoes (Wesson *et al.*, 2012). Research on peridomestic lethal ovitraps is presently being encouraged because it could be an efficient way to reduce mosquito population by interfering with their life-cycle (Achee *et al.*, 2019).

Wolbachia

Wolbachia is a naturally occurring symbiotic bacteria found in several insect species but not usually encountered in mosquitoes (Wilson *et al.*, 2020). It has been experimentally demonstrated that establishment of *Wolbachia*-infected male mosquitoes in wild-type mosquito population can significantly reduce the vector population (Flores *et al.*, 2018). This is because copulation between *Wolbachia*-infected males and normal females produce non-viable eggs (Achee *et al.*, 2019). The trial programs for this innovative technique of mosquito control are being undertaken by the ‘World Mosquito Program’ (Achee *et al.*, 2019).

Genetic control of mosquitoes

This strategy involves production of transgenic mosquitoes using genetic engineering techniques and is based on two main principles. The first one requires the introduction of a lethal trait with the aim of reducing the life span of mosquitoes. The second one includes manipulation of their reproductive fitness to reduce the number of offspring produced. The sterile insect technique (SIT) is based on the second principle where the males are irradiated to render them

sterile (Hammond and Galizi, 2017). With improved techniques it has become possible to introduce mutations in the female germ cells rendering them incapable of being fertilized (Achee *et al.*, 2019). RIDL techniques or release of insects with dominant lethality uses gene alterations such that the larvae obtained as a result of mating between mutant parent populations do not survive beyond the pupal stages (Carvalho *et al.*, 2015). Though they seem to be very promising they are expensive and the scientific community raises doubts on their applicability in field conditions.

Conclusion:

The increasing populations of vector triggered by global climate change are a thriving problem that needs to be resolved to prevent further spread of deadly diseases. However, application of any one technique cannot effectively control mosquitoes. Current research is focussed on a multi-strategic approach to interrupt the completion of their life cycles and/or reduce their longevity. This requires sufficient knowledge on mosquito breeding behaviour and their habitat ecology that has garnered research interests across the world. In addition to these, community participation and awareness programmes can further help to control vector population.

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IMPLEMENTATION OF PHYTOREMEDIATION AS AN ECO FRIENDLY APPROACH FOR ENVIRONMENTAL CLEAN-UP

Poulami Adhikary Mukherjee

Department of Zoology,

Narajole Raj College, Narajole, West Midnapore, West Bengal, India

Corresponding author E-mail: poulamiadhikary@gmail.com

Abstract:

Environmental contamination due to urbanization, industrialization, anthropogenic activities, heavy metal accumulation, military and agricultural activities etc. is of serious ecotoxicological concern worldwide. Plants are currently used to extract the pollutants and remove them from the environment. This simple cost effective eco-friendly green technology is known as phytoremediation. Phytoremediation is the most promising way that could be successfully used to minimize the concentrations of toxic substances from the environment. Therefore, it is indispensable to focus on the different strategies of phytoremediation in order to enhance its performance. For this, a better understanding of the various phytoremediation processes such as phytoextraction, phytofiltration, phytostabilization, phytodegradation, and phytovolatilization is required. The current review work outlines the general aspects of phytoremediation, along with its advantages and limitations. It also provides a more comprehensive view of the present status of phytoremediation with a much wider range of its applications to environmental pollution control. Furthermore, the recent developments of science and technology related to phytoremediation research are also discussed here to improve and focus on future trends and prospects of global relevance.

Keywords: Phytoremediation, environmental contamination, phytoextraction, phytofiltration, phytostabilization, phytodegradation, phytovolatilization.

Introduction:

Around the world, there is an increasing trend in areas of land and waters that are affected by environmental contamination either due to ignorance, lack of vision, or carelessness. The major cause of environmental pollution is either naturally or due to various anthropogenic activities (Farraji *et al.*, 2020). There has been an increasing amount of heavy metals introduction in the environment mainly due to industrialization and urbanization, which needs significant attention all over the world (Suman *et al.*, 2018; Ashraf *et al.*, 2019; Yan *et al.*, 2020).

In addition, it is also necessary for improving the environmental conditions because of overpopulation that is one of the main causes of industrialization (Farraji *et al.*, 2016).

Generally, for the purpose of decontamination of polluted sites, various physicochemical technologies are employed like chemical precipitation, membrane filtration, adsorption, coagulation-flocculation, photocatalytic degradation, ion exchange, oxidation with ozone and hydrogen peroxide (Fu and Wang, 2011) which are highly expensive and difficult to be used as one complete process in order to successfully accomplish environmental standards (Ahmaruzzaman, 2011; Hasan *et al.*, 2019).

Thus, a cost effective eco-friendly green technology is urgently required for improving the degraded environmental conditions. Phytoremediation is one such environmentally sound technology for pollution prevention, control and remediation. It is the direct use of plants to improve degraded environments (Farraji *et al.*, 2020). Phytoremediation has now been widely applied to water, air, and soil pollution remediation (Mayo *et al.*, 2017; Liu *et al.*, 2020). There are a specific number of mechanisms involved in phytoremediation (Awa and Hadibarata, 2020; Farraji *et al.*, 2020) which are mainly targeted to achieve the actual goal of phytoremediation. Nowadays, numerous of advanced techniques are being added as new subsets for this green technology and additional augmentation and amendment are suitably applied for high-efficiency environmental cleaning even with 100 % pollutant removal in a short time (Gangola *et al.*, 2015).

However, the success rate of phytoremediation depends on the plant selection. There are certain plants having this natural ability to uptake the toxic pollutants and contaminants from air, soil and water. However, the most desirable characters are those plant species that have high biomass and are extremely tolerant to high concentrations of different harmful contaminants present in the environment. The convenient plant species have been found in polluted areas (Farraji *et al.*, 2020), such as plants growing in abandoned mine sites. Suitable plant selection for phytoremediation extremely depends on the nature and concentrations of the contaminants (Pilon-Smits, 2005), period of the area (Hutchinson *et al.*, 2001), its intensity (Cunningham *et al.*, 1996), rate of volatilization (Ghosh and Singh, 2005) and biodegradation (Joner and Leyval, 2003). Furthermore, the selection of a suitable green technology for specific pollution is essential for effective phytoremediation. The main objective of this review work is to highlight the different aspects of phytoremediation, its merits and demerits and their efficiency in environmental clean-up.

Different techniques of phytoremediation:

There are different methods of phytoremediation where specific plants are used to remove the toxic pollutants from the environment.

Phytoextraction:

Phytoextraction, also called Phytoaccumulation, involves absorbing the contaminants from the environment into the plant biomass. In this process, plants are used to absorb, translocate and store toxic contaminants from a soil matrix into their root and shoot tissues for safely removing them from the area. The contaminants are thus eliminated from the environment without degrading them. This is particularly useful for removing metals from soil and, in some cases; the metals can be recovered for reuse, by incinerating the plants, in a process called Phytomining.

Phytostabilization:

Phytostabilization, also called Phytosequestration or Phytoimmobilization involves plant-mediated immobilization of organic wastes into the soil matrix, thereby reducing their bioavailability. The process works by reducing the mobility of organic pollutants into the environment and preventing them from entering the food chain, thus stabilizing them. This technique also has the advantage of preventing humans from inhaling dust that may be hazardous to their health.

Phytovolatilization:

Phytovolatilization involves using specific plants to absorb the pollutants, after which the contaminants are transformed and volatilized into the atmosphere from the leaves. In this process, the pollutants are converted to highly volatile and less toxic substances and thus eliminated from the nature by releasing them into the atmosphere. This process is driven by the high evapotranspiration rate of plants. Initially the plants volatilize the organic contaminants, especially the volatile organic compounds (VOCs). For example, hybrid poplar trees are successfully used to volatilize trichloroethylene (TCE), thus converting it to chlorinated acetates and CO₂. Certain plants can volatilize metals such as Se by converting into dimethylselenide [Se(CH₃)₂]. In this aspect, genetic engineering has gained success in volatilizing specific contaminants by using plants.

Phytodegradation:

Phytodegradation involves the use of enzymes for degrading the harmful organic pollutants like herbicides or trichloroethylene by either releasing them from roots or via metabolic processes of plant tissues. This can occur both inside or outside the plants as the plants can also secrete the enzymes outside. In phytodegradation, the plant parts absorb the various toxic contaminants and metabolize them to less toxic substances. However, the sites of biotransformation vary depending on the type of plants, such as the process can happen in roots, stems or leaves. Poplar trees are frequently used in phytodegradation of toxic organic compounds.

Phytotransformation:

In this process, the harmful chemical substances in the environment are modified due to plant metabolism, because of which they are either inactivated or degraded or immobilized. The technique involves converting the complex molecules into simpler ones, which are comparatively less toxic, and then the plant tissues can absorb these simpler ones.

Phytostimulation:

Phytostimulation is the process where the enzymes in the plant rhizosphere are activated by microorganisms, thus leading to bioremediation. The event occurs in the area around plant roots. This process is also known as Rhizosphere degradation or Rhizodegradation or Rhizosphere bioremediation. Phytostimulation can also involve aquatic plants supporting active populations of microbial degraders, as in the stimulation of atrazine degradation by hornwort.

Phytorhizofiltration:

Phytorhizofiltration, also known as Rhizofiltration, use roots and tissues of plants as a filter to absorb the organic pollutants from water streams or groundwater and store contaminants from an aqueous growth matrix for easily eliminating them. Here, the plant roots filter the water to avoid the contaminants from mixing into the water streams or groundwater. This also helps in minimizing the movement of pollutants in the soil.

Phytohydraulics:

In this process, the deep-rooted trees are used to draw like a pump and sequester or degrade the pollutants of the groundwater contaminants coming near their roots, thus restricting their movement in clean zones.

Table 1: Comparison between different phytoremediation technologies on various groups of contaminants

Technology	Action on Contaminants	Main Type of Contaminant	Vegetation
Phytostabilization	Retained <i>in situ</i>	Organics and metals	Cover maintained
Phytodegradation	Attenuated <i>in situ</i>	Organics	Cover maintained
Phytovolatilization	Removed	Organics and metals	Cover maintained
Phytoextraction	Removed	Metals	Harvested repeatedly
Phytofiltration	Retained <i>in situ</i>	Metals	Cover maintained

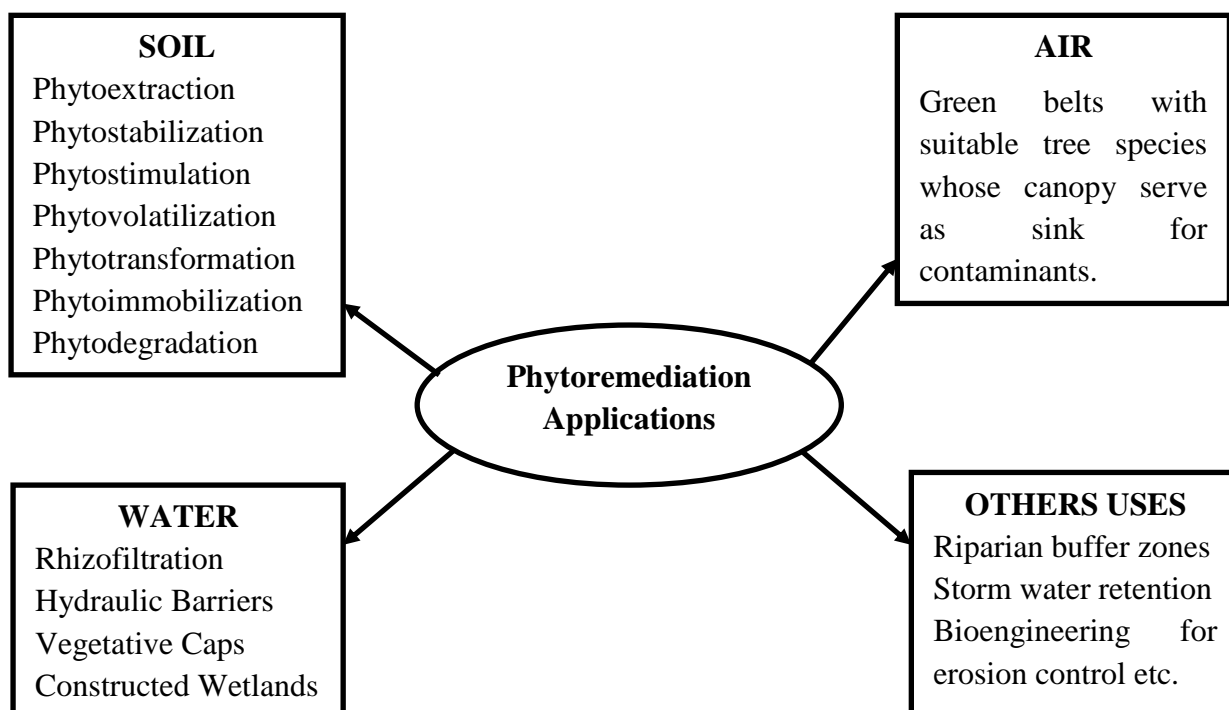
Phytoremediation is effective in the remediation of many different types of contaminants from the environment such as heavy inorganic metal pollutants like Arsenic (As), Antimony (Sb), Gold (Au), Lead (Pb), Molybdenum (Mo), Mercury (Hg), Nickel (Ni), Silver (Ag), Zinc (Zn); other radionuclides include Uranium, Caesium-137, Strontium-90, Anthracene, Atrazine, Trichloroethylene (TCE), Pyrene, Toluene, Phenol, Trinitrotoluene (TNT) and other

contaminants from Polycyclic aromatic hydrocarbons (PAHs), Total petroleum hydrocarbons (TPH), Polychlorinated biphenyls (PCBs), herbicides, pesticides and certain volatile organic compounds which include chlorinated solvents.

Some of the types of plants successfully used in phytoremediation are Alfalfa, hybrid Poplar trees, Tobacco, Spinach, Corn, Stonewort, Black willow, Blue-green algae, Duck weed, Arrowroot, Sudan, Rye, Bermuda, Alpine bluegrass, yellow and white water Lillies etc.

Applications of Phytoremediation:

Most of the phytoremediation methods are commercially successful and developing rapidly in research areas.



Advantages of Phytoremediation:

- Phytoremediation is much affordable in comparison to traditional processes both in situ and ex situ, in large areas.
- The use of plants can be easily monitored in this process, which reduces erosion and metal leaching in the soil.
- It largely depends on solar energy.
- It preserves the topsoil, thereby maintaining the fertility of soil and plant phytochemicals.
- There is possibility of recovery and re-use of valuable metals.
- Phytoremediation sites are low maintenance and more aesthetically pleasing.
- It involves no noisy equipment.
- It is a harmless method because it is eco-friendly using naturally occurring species, thus conserving the environment in its natural state.

Disadvantages of Phytoremediation:

- The process is not successful for sites with high contaminant concentrations.
- It is slower than conventional methods and requires a long-term commitment.
- It is seasonally effective and does not work through the winter.
- It is not always possible to prevent the contamination of groundwater.
- Bio-accumulation of contaminants from plants can enter the food chain directly through the primary consumers.

Conclusion:

Therefore, in conclusion it can be said that despite few disadvantages, phytoremediation is being successfully used to treat a wide range of contaminants in a broad range of environments. The Environment Protection Act (EPA) uses phytoremediation for many reasons. It takes advantage of natural plant processes and requires less equipment and labour than other methods since plants do most of the work. The specific plant species and trees that are used in this technology can also help in controlling soil erosion, thus making an area much attractive, noise free zone, and improve the surrounding air quality.

It is clear that in future days, phytoremediation would be applied increasingly for removing the toxic pollutants from contaminated sites or from process wastes. It is to be noted that the interactive activities of the microorganisms, soil, and plants along with the different techniques of phytoremediation need to be explored further. Additionally, the plants and microbes should be modified genetically so that they may have greater potentiality, however, their consequences on overall environment needs to be investigated before commercialization. The theoretical research of phytoremediation aims at integrating engineering applications in future. With increasing legislation, phytoremediation will be used as a viable alternative to chemical treatment. Phytoremediation is now effectively used across many places all over the country. Phytoremediation offers a slow but low cost, low-risk, attractive and solar energy driven clean-up technique of remediation that is well regarded by the public and has to be fully established before it can be commercially available. Research findings clearly indicate that this is a feasible and novel technology that holds greater promise for future. However, further research is obligatory to observe the economic and ecological competences of phytoremediation.

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VEHICLE POLLUTION AND ITS CONTROL

Sudakshina Ghosh

Department of Zoology

Vidyasagar College for Women,

39 Sankar Ghosh Lane, Kolkata-700006

Abstract:

Vehicles are the fastest growing sector and responsible for two-third of air pollution in urban area. Vehicular pollutants have deleterious effects on human health and ecosystem. Main components of vehicular emissions are carbon monoxide (CO), nitrogen oxides (NO_x), benzene (C₆H₆), aldehydes, 1,3 butadiene (C₄H₆), lead (Pb), particulate matter (PM), hydrocarbon (HC), oxides of sulphur (SO₂) and polycyclic aromatic hydrocarbons (PAHs). But the ratio of these components varies depending on quality of engine, fuels and environment. Steps to be adopted to control vehicle pollution are: purchasing vehicles with advance emission control devices, using cleaner fuels, strengthening the institutions responsible for managing urban air quality, using good fuels, formulation of necessary legislation, and awareness of common people regarding economic and health impacts of the pollution.

Keywords: Vehicle, emission, fuel, pollution, bio-fuel

Introduction:

Air pollution is one of the serious environmental concerns of urban Asian cities where population is exposed to poor air quality. It occurs when harmful or excessive quantities of substances including gases (such as ammonia, carbon monoxide, sulfur dioxide, nitrous oxides, methane and chlorofluorocarbons), particulates (both organic and inorganic), and biological molecules are introduced into the Earth's atmosphere. According to the World Health Organization (WHO), air pollution is responsible for nearly 7 million deaths per year in the world. Nine out of ten individuals currently breathe air that exceeds the WHO's guideline limits for pollutants.

Major pollutants of vehicular emissions:

The emissions from vehicles are responsible for the two-third of air pollution in urban area. Vehicles being the fastest growing sector contributes significantly to the global greenhouse gas emissions (Hasan *et al.* 2019). In India, it is the third most CO₂ emitting sector, and within the transport sector, road transport was found to have contributed more than 90% of the total CO₂ emissions (IEA, 2019). Vehicular pollutants have several deleterious effects on human

health as well as the ecosystem. The release of pollutants from vehicles also includes fugitive emissions of fuel and the source and level of these emissions depending upon the vehicle type, age and also its maintenance. The major pollutants released as vehicle/fuel emissions are, carbon monoxide (CO), nitrogen oxides (NO_x), photochemical oxidants, air toxics, namely benzene (C₆H₆), aldehydes, 1,3 butadiene (C₄H₆), lead (Pb), particulate matter (PM), hydrocarbon (HC), oxides of sulphur (SO₂) and polycyclic aromatic hydrocarbons (PAHs). The predominant pollutants in petrol/gasoline driven and diesel based vehicles are hydrocarbons and carbon monoxide and oxides of nitrogen and particulates respectively.

Quality of emissions:

Automobile pollution is responsible for causing respiratory distress, asthma, cancers, chronic disease and other serious health effects (Dey *et al.*, 2018a). This pollution is also responsible for acid rain and global warming. For running a vehicle internal combustion engine requires mixture of fuels and air. It depends upon the air–fuel ratio. In the lean mixture conditions vehicles produce less CO, less HC and more NO_x gases but in the rich mixture conditions vehicles produce more CO, more HC and less NO_x gases. Compared to diesel engines, petrol engines produce more CO, HC but less amount of NO_x and particulate matter (Jansson, 2000; Keshan *et al.*, 2008). The evaporation from gasoline engine vehicle increases with the increase of temperature and heating of fuel tank, venting of gasoline vapors. Engine modification, fuel pretreatment, fuel additives, exhaust gas recirculation (EGR) and catalytic converters are the controlling techniques of exhaust gas emissions. Catalytic converters are emissions control devices that convert more toxic exhaust gas pollutants into the less toxic ones. Several types of catalysts are used in the automobile vehicles exhaust gas emissions controls like noble metal, base metal, transition metal catalysts etc. The surface area, pore volume and pore size of catalysts are highly effective on catalyst activity. The catalytic converters are highly active and consistent for reducing the poisonous tail pipe emissions. In cold countries, the emissions from automobile vehicles engines are more. On a cold start condition, the petrol takes up nearly 10 km to warm up and the same for diesel engine may be up to 5 km. In cold start conditions compared to petrol engine vehicle, the diesel engine vehicle produces less unburned fuel as (Dey *et al.*, 2017a). The air: fuel ratio may be as lean as 14.64:1 (by mass). To control the air pollution from automobile vehicle exhaust the Bharat stage emissions standards has set the emissions standards instituted by the Government of India that regulate the formation of certain major automobile exhaust pollutants by vehicles from internal combustion engines (Marsh and Acke, 2001).

Control of vehicle pollution:

Medical cost to treat the affected people causes financial burden to the society. It is expected that more and more people will be living in cities within a few years. This rapid urbanization and economic growth in India has also resulted in increase in the number of motor

vehicles. Now it is the high time to find out a solution to reduce the vehicle pollution through vigorous research, survey and mass awareness especially for Indian perspectives.

The major concerns are i) types of vehicles commonly used ii) existing road operation for vehicle movements iii) Strengthening the institutions responsible for managing urban air quality and regular monitoring iv) improvement in the fuel quality v) formulation of necessary legislation and enforcement of vehicle emission standards, vi) improved traffic planning and management etc. Besides these, awareness to the common people regarding the possible economic and health impacts of air pollution and eventually their role in adopting the measures for improving air quality, use of cleaner fuels and purchase of vehicles with advance emission control devices are equally important.

Therefore, possible solutions to reduce the vehicle pollution are:

- i) **Drive Less:** Fossil fuels are exclusively used and the number of vehicles on the roads is increasing day by day. But these vehicular emissions can be reduced by driving less and choosing alternatives such as walking, using the train or public transportation, and even using a cycle.
- ii) **Carpooling:** This is another way to drive less, where people from the same neighborhood or same area can share one vehicle instead of each using their own car. Through the reduction of toll tax or vehicle tax, the Government should encourage carpooling.
- iii) **Vehicle maintenance:** Car users should ensure that their car is in good condition and does not release harmful substances beyond permissible limit. Cars should be maintained on a regular basis by replacing oil filters, changing the engine oil and greasing the moving parts.
- iv) **Using public transports:** Using public transport is a sure short way of contributing to less air pollution as it provides with less gas and energy.
- v) **Governmental intervention:** More emissions are produced by vehicles in urban areas because of the heavy traffic and the fact that people have to drive slowly in towns. To address this issue, Government can order that no vehicles enter the central business district and instead, they be parked on the outskirts.
- vi) **Invest in zero-emission vehicles:** Electric vehicles have moved away from burning fuel and use electrochemical processes to produce the energy required for a vehicle to move. As the by-product of fuel-cell vehicles is water, so these types of cars are known as zero-emission vehicles. They store energy in an onboard battery and emit nothing from their tailpipe. More research needs to be invested on electric cars.
- vii) **Civic education:** Educating common people by both Government and Non- Government organizations (NGOs) can play an important role in awakening the society to the realities of pollution and how reducing it can make the world a better place to live in. This can be done through the education of students from the school level itself. Civic education, with special reference to the short-term and long-term effects of vehicular pollution on our lives should be

brought to the notice of all individuals. It is a task that once imposed can awaken the society to the realities of pollution, its effects and ways to reduce it.

viii) **Progressive policies:** Implementation of legislations that will make people to do the needful for bringing down the levels of vehicle pollution as far as possible. For this, guidelines for standard practices should be framed.

ix) **Discarding old vehicles:** Old vehicles are responsible for more vehicular pollution because their transmission systems are outdated. They may also cause road accidents because of their many complications and replacing them for newer models serves as the most sensible and practical approach. Newer vehicles use new updated technologies and pollute the environment much less than their older counterparts.

Use of alternative source of fuels: An approach to reduce the automobiles exhausts pollution

Any materials or substances that can be used as fuels, other than conventional fuels are referred to as alternative or non-conventional or advanced fuels. Bio-fuels are also taken as a renewable source (Bhave and Kulkarni, 2015; Chand, 2018; Dey and Mehta, 2020e). This renewable energy is used to generate electricity; it is often supposed that some form of renewable energy or a part of it is used to create alternative fuels. Research is ongoing into finding more suitable biofuel crops and improving the oil yields of these crops. Bio-diesel, on the other hand, is made from animal fats or vegetable oils, renewable resources that come from plants such as atrophy, soybean, sunflowers, corn, cottonseed, etc (Mehta *et al.*, 2018a; Ou *et al.*, 2020; Dey and Dhal, 2020d). Fats or oils from these hydrocarbons are filtered and then combined with alcohol like methanol. Thus diesel is formed from this chemical reaction. Methanol and ethanol fuel, the primary sources of energy, is the convenient fuels for storing and transporting energy. As alternative fuels, these alcohols can be used in internal combustion engines (Dey and Mehta, 2020).

Conclusion:

Vehicular pollution is one of the leading causes of air pollution. Every individual should have the responsibility for a cleaner planet. This can be achieved by the combination of cleaner fuels and vehicles that has the potential to reduce emissions by more than 80%.

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URBAN AIR POLLUTION CAUSED BY PHOTOCHEMICAL SMOG

Ranita Dutta Bose

Vidyasagar College for Women, Kolkata

Corresponding author E-mail: ranitabose1983@gmail.com

Abstract:

Unburned hydrocarbons pollute the urban air due to result of uncompleted combustion and the evaporation of solvents and liquid fuels. Volatile organic compounds (VOCs) and other organic substances including hydrocarbons are readily vaporised. Free radicals are added in the urban air with major reactive hydrocarbons which contain a C = C bond. Warmth, ample sunlight, and a stagnant of air are also required for the formation of the Photochemical Smog. Nitrogen dioxide (NO₂), ozone (O₃), PAN (per-oxy acetyl nitrate), and chemical compounds that contain the –CHO group (aldehydes) are the major undesirable components of photochemical smog. Major cities in the World are subjected to smog. The cities are the obvious substantial sources for the emission of sufficient NO_x, different hydrocarbons and other volatile organic compounds into air. Smog makes some plant damage, health hazards, eye irritations to human beings if their concentrations are sufficiently high.

Keywords: PAN (Per-oxy acyl nitrate), Photochemical Smog, Volatile Organic Compounds, Health hazards, Free radicals.

Introduction:

The clarity of the sky is obscure by the dust, smoke and other dry particulate matters, which are actually meant as Haze. There are different codes of different categories of fog, dust, mist, ice fog, steam fog, haze, smoke, volcanic ash and snow. Farming, traffic, industry and wildfires are the different sources of haze particles^[1]. Haze may appear as brownish or bluish, and mist tends to bluish grey, depending on the direction of view with respect to the Sun. Dry air forms haze, whereas humid air forms mist. Wet haze is a form of haze, whereby haze particles act as condensation nuclei for the formation of mist droplets.

The word haze is generally meant for visibility-reducing aerosols of the wet type. Aerosols are formed in the atmosphere by the complex chemical reactions between sulphur dioxides, emitted during combustion and thus converted into sulphuric acid. In presence of sunlight, high relative humidity and stagnant air flow enhance these chemical reactions. There is another small component of wet-haze aerosols as terpenes, released by trees. Therefore, wet haze is a phenomenon of summer season.

Smog is the type of air pollution, derived from vehicular emission from industrial fumes and combustions of engines. These atmospheric pollutants react with sunlight and primary emissions, to form photochemical smog.

Photochemical smog refers to the atmospheric condition which becomes hazy with a poor visibility due to formation of various secondary pollutants or aerosols in atmosphere by photochemical reactions, preferably in metro-politan cities. A typical brownish gray haze formed by the action of solar ultraviolet on atmosphere, which is polluted with hydrocarbons and nitrogen oxides. In the bright sunlight, nitric acid, organic compounds, ozone and other anthropogenic air pollutants are getting trapped at the ground level^[2]. The pollutants have some detrimental effects on plants as well as on human being. Photochemical smog often have an unpleasant odour due to some of its gaseous components. Smog is the word which is represent as a haze near the ground made by a combination of different air pollutants.

Photochemical smog was first observed in Los Angles, USA, in mid 1940s. Then these incidents have been detected in major cities in the world. The word “SMOG” has been derived from smoke and fog. This develops a hazy weather condition with poor visibility with formation of aerosols in atmosphere by photo dissociations and photo reactivations. Since 2002, smog severity is often provoked by straw burning in neighbouring agricultural areas. The ground pollution level of Los Angles, Beijing, Delhi, Lahore, Mexico City, Tehran are often increased by inversion^[4]. Smog is hazardous to human health, can cause severe sickness, respiratory discomfort, shortened life span or premature death^[3]. In India, major metropolitan cities are ideal candidates for formation of photochemical smog.

Conditions for formation of smog:

- Air stagnation
- Abundant sunlight
- High concentration of hydrocarbon and nitrogen oxides in the atmosphere.

Causes:

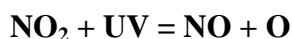
Exhausts of automobiles and major stationary sources perform some photochemical reactions in the lower atmosphere by the interaction of hydrocarbons and nitrogen oxides, create Smog. These in turn results in a series of complex reactions producing different secondary pollutants, some photo-oxidants as ozone's, aldehydes, ketones and per-oxy acyl nitrates (PANs). Ozone in the stratosphere protects us against harmful ultraviolet radiation, but on the ground, it is hazardous to human health.

The emission of nitrogen oxides from internal combustion engines into the air creates photochemical smog. Some chemical reactions occur between free atoms of oxygen (O) and molecular oxygen (O₂) to form the ozone by absorbing visible or ultraviolet sunlight^[2]. Smog formation is totally depending on the chemical reaction of some hydrocarbons, organic compounds and sunlight.

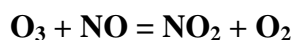
Mechanism of smog formation:

In a typical smog formation:

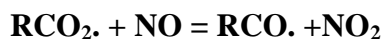
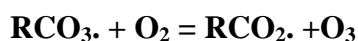
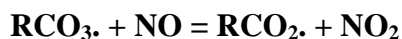
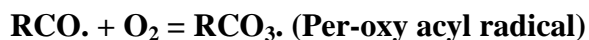
1. The starting mechanism is absorption of UV light from Sun by NO₂, which then decomposes into nitric oxide (NO) and highly reactive oxygen atom (O).



2. Reactive oxygen atom (O) combines with molecular oxygen (O₂), to form ozone (O₃) which itself is reactive and act as an oxidant. An energy absorbing molecule or particle required to stabilize ozone or else it will rapidly decompose.



3. But NO in presence of hydrocarbons (HC) in atmosphere reacts with hydrocarbon radical per-oxy acyl (RCO₃.), as a result O₃ concentration builds up to dangerous levels.



Thus the NO level, that builds up during night and early morning when there is a heavy commuter traffic, drops off eventually later in the day with a concomitant increase of NO₂ and O₃ levels in atmosphere.

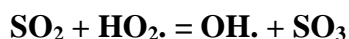
Increased NO₂ concentration initiates reaction with RCO₃· to form per-oxy acyl nitrates (PANs).



Three of the common members of PAN families are:

- a. Per-oxy formyl nitrate (PFN)
- b. Per-oxy acetyl nitrate (PAN)
- c. Per-oxy benzoyl nitrate (PB₂N)

However, CO and SO₂ also play significant role in smog formation:



SO₃ in turn is converted to H₂SO₄ droplets resulting in the formation of a haze.

Smog behaviour:

Formation of photochemical smog is a dynamic process. In the morning, the NO and HC levels are increased followed quickly by increase in NO₂. NO₂ reacts with sunlight leading to various skin reactions and ultimately to the production of O₃ and other oxidants. O₃ concentrations increase until (sometimes in afternoon) it reaches a maximum and then decreases gradually. NO₂ concentrations diminish from its peak as O₃ concentration builds up and is usually low by late afternoon. The typical episode occurs in hot, sunny weather under low humidity conditions.

Effects of “SMOG”:

- Characteristics symptoms of smog are the brown haze in atmosphere with reduced visibility.
- Human health is affected primarily by oxidant species (O₃), PAN, NO₂ and aerosols (containing sulphates, nitrates and ammonium ions)
- Impairment of physical performances at oxidant level (O₃, PAN) above 0.15 ppm.
- Attacks in asthmatics increases at 0.25 ppm.
- Chest pain, cough and headache are reported at a concentration range of 0.25 to 0.30 ppm.
- Eye irritation, as PAN is a powerful lachrymator at 0.1 to 0.45 ppm.
- Respiratory distress, extreme fatigue and lack of coordination have been observed.
- Plant damage occurs as PAN and O₃ are one of the most phytotoxic substances at 0.05 ppm level.
- Complications arise in respiration from the indoor environment and on inhalation of fungi from roof thatch or smoke, when there is no room chimney. Respiratory complications arising from space heaters or gas ovens, inhalation of tobacco smoke in indoor environment by non-smokers, leads to lung cancer.
- Northern India have been covered in a thick layer of winter smog since last few years^[11]. The situation has fallen drastically in capital, New Delhi, where particulate matters i.e. very fine dust particles and toxic gases are concentrated in the stagnant air.
- In India, among various cities, New Delhi is the most polluted one and this air pollution may cause death of about 10,500 people every year. In the year 2013, Delhi pollution was increased by 44% for these peak level of particulate matter (PM) due to high vehicular and industrial emissions, construction work and crop burning. The airborne particulate matter, PM 2.5 is considered as the most harmful one and hazardous to human health with

153 μ gms^[9]. Delhi people specially women and children are supposed to be more affected due to rising of these pollution level with asthma or lung cancer^[5]. During winter season, Delhi weather becomes almost dull of smog, results in the rail and traffic disruption every year^[7]. Since 1998, Delhi has faced a severe problem with its average maximum temperature declining due to rising of the air pollution and smog^[6].

- Delhi residents are mostly unaware about its alarming pollution level in the city and health issues associated with this pollution. Delhi has already taken some urgent measures to curb pollution, as Delhi has enormous quantity of trees and Delhi Transport Corporation operates environment friendly compressed Natural Gas (CNG) buses since mid-1990s. In 1996, a litigation had been started in the Supreme Court of India by Centre for Science and Environment (CSE) for public interest, that ordered the conversion of buses and taxis to run on CNG and banned the use of petrol in 1998^[6]. In the year 2003, Delhi won the first 'Clean Cities International Partner of the Year' award by United States Department of Energy's for its "bold efforts to curb air pollution and support alternative fuel initiatives". Furthermore, a credit also goes to Delhi Metro for significantly reducing air pollutants in the city^[8].
- Stubble burning and rise in market share of diesel cars have been creating high pollution since many years which causes the formation of smog^[10]. Burning of agricultural waste in nearby Punjab, Haryana and Uttar Pradesh regions results in severe intensification of smog over Delhi, stated by CUE and System of Air Quality Weather Forecasting and Research (SAFER). An environmental panel has appealed to India's Supreme Court to impose a 30% cess on diesel cars and the state government of adjoining Uttar Pradesh is considering imposing a ban on crop burning to reduce pollution in Delhi NCR^{[12] [13]}.

Control:

Control of photochemical smog requires:

- Primary emission reduction.
- Nitrogen oxides reduction in a substantial amount produced in urban areas.
- To restrict the release of hydrocarbons from different mobile and stationary sources. Use of catalytic converters in auto exhausts, regular maintenance and decrease in legal speed limits reduce NO_x emission.
- Use of fuels such as hydrogen and methanol eliminates HC problem.

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THE INCREASING ABUNDANCE OF ARSENIC IN THE ENVIRONMENT AND ITS DELETERIOUS EFFECTS

Chaitali Banerjee

Department of Zoology,

Vidyasagar College for Women, 39 Sankar Ghosh Lane, Kolkata 700 006

Abstract:

The age-old potential therapeutic candidate Arsenic (Greek word *arsenikon*, meaning “potent”) deleteriously affects body functioning of aquatic species. The effects are seen across other species as well consuming the arsenic-affected water. This widely prevalent element besides naturally occurring has also been found in elevated concentrations. It has resulted both by natural process like volcanic action and numerous man-made events. Several countries across the globe are affected. It is present both as inorganic and organic forms; the latter being lesser in devastation. Inorganic Arsenic carries out its damage by spreading through contaminated water and food like crops irrigated with arsenic-affected water. Relatively lesser effects are seen in shellfish which harbors the organic form. Be it acute or chronic, the effects are severe. The intricate network of body’s different system is perturbed. Hence, this toxicant has disrupted the homeostasis by interfering and instigating the critical defense players and signaling pathways at the cellular level.

Introduction:

The word arsenic is derived from the *Persian zarnikh* and *Syriac zarniqa*, later incorporated into ancient Greek as *arsenikon*, which meant “masculine” or “potent” and referred primarily to orpiment, or yellow arsenic. The word became *arsenicum* in Latin and *arsenic* in old French, from which the current English term derived (De Bellis *et al.*, 2019). Arsenic has been used as a drug to ailments in various medicinal systems since ages. In different formulations, it has been used for treating different diseases; phenomenal examples being trypanosomiasis, malaria, asthma, eczema, psoriasis, anaemia, hypertension, gastric ulcers, heartburn, rheumatism, syphilis, tuberculosis, cancers of the skin and breast etc. The legacy still continues (De Bellis *et al.*, 2019). Acute promyelocytic leukemia (APL) is a classic example where using Arsenic in its trioxide form miraculously prevented the disease affects and resulting death counts (Rao and Zhang, 2013). However, its physical properties of being tasteless and odorless have also made its use as ideal poison since ages. Arsenic poisoning or arsenicosis, occurs after the ingestion or inhalation of high levels of arsenic (Hughes *et al.*, 2011). Arsenic is

a naturally occurring element; yet its concerning because of the increasing abundance and its presence in the inorganic form resulting from different activities (Cohen *et al.*, 2006).

Biofilms serve as an important source of energy for invertebrates and herbivorous. It works as a site for the transfer of nutrients to higher trophs. The different communities of microorganisms in biofilms also contribute to nutrient cycling. From several reports it is evident that arsenic can devastate multiple trophic levels by disrupting the stability of ecosystem services (Jang *et al.*, 2017). This is primarily done by bioaccumulation and biotransformation at different trophic levels, in turn aggravating and consequently accumulating with the gradual flow of nutrients (Rahman *et al.*, 2012). Cereals, crops, invertebrates, marine and freshwater fish-shellfish species and higher vertebrates are in continuous process of arsenic exposure and toxicity to varied extent. The flowchart depicting the same lies here (Figure 1).

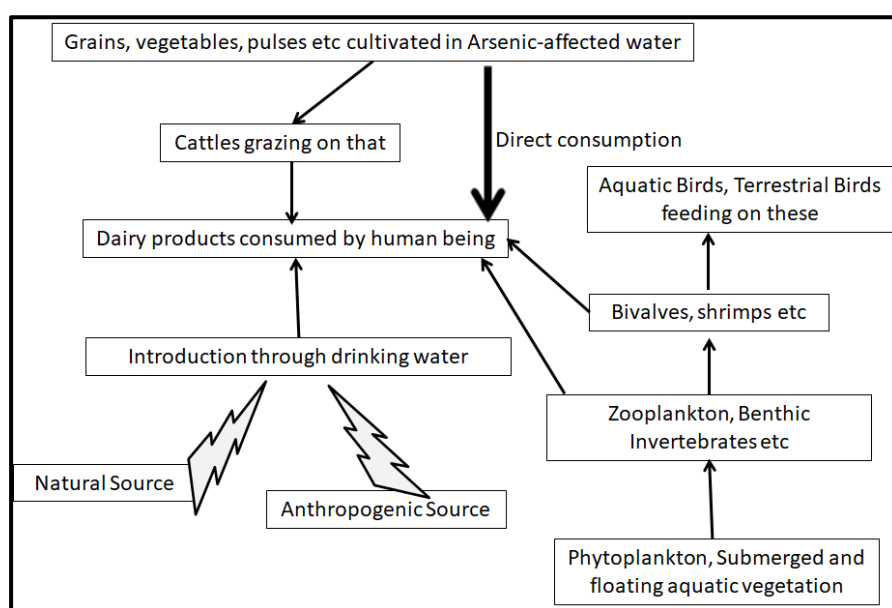


Figure 1: Possible ways of arsenic exposure and routing to different trophic levels

Molecular mechanisms of arsenic toxicity in plants: A glance

Arsenic and its different species adversely effects several essential biological processes and pathways. In case of plants, various extracellular and intracellular mechanisms are involved in detoxification. It includes chelation in the cytosol by high affinity ligands, compartmentalization, biotransformation and cellular repair; alteration of cell membrane or other structural protein. Another major route is passive uptake *via* micropores in the root cell walls. This effectively promotes sequestration or degradation. Thus, immobilization, fixation, and removal either as fixed in soil or accumulated in plant parts effectively reduce arsenic content (Mirza *et al.*, 2013). This property is in practice in several places where soil is severely affected with arsenic. Few examples of such tolerant plants include *Pteris* sp., *Pitrogram macalomelanos*

etc (Zhao *et al.*, 2003). They are very highly evolved group among the lower vascular plants. Basically they are ferns and they specialize in being receptive to the environmental fluctuations.

Specific molecular mechanisms includes AsV reduction to AsIII in roots; high AsIII efflux from cortical cells to the xylem; limited thiol compound complexation of AsIII and sequestration in root vacuoles; in addition to minimal AsIII efflux from roots to the external medium. The capacity to hold large amounts of As in the xylem is an important feature of As hyperaccumulators plants. Reduction of AsV to AsIII is the first and vital step in the detoxification of As in plant tissues which is an enzymatically driven process mediated by arsenate reductase (AR) pathway using GSH. The high expression levels of AR and vacuolar AsIII transporters in shoots justifies the specialization of *P. vittata* to hyper-tolerate and hyper-accumulate As (Shri *et al.*, 2020). Glutathione (GSH) and phytochelatin (PCs) also facilitate its transport into the vacuoles in shoots. The tripeptide GSH (Glu-Cys-Gly) is synthesized by γ -glutamyl cysteine synthetase (γ -ECS) and GSH synthetase (GS). GSH works by binding to AsIII; hence serves as a key metabolite in the cellular redox balance. The overview is schematically represented in Figure 2.

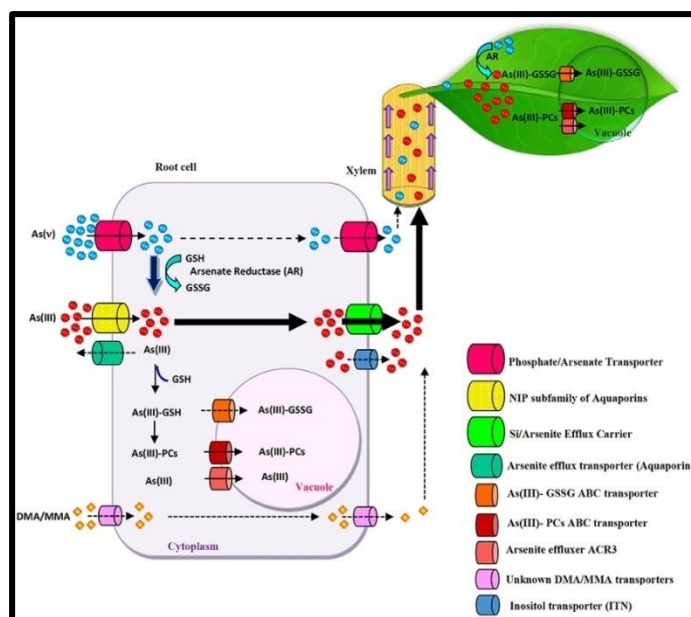


Figure 2: Overview of Arsenic (As) uptake, transport, translocation, and detoxification in plants (Source: Souri *et al.*, 2017)

Arsenic stress induces reactive oxygen and nitrogen species formation through the disruption of electron transport chains (ETC) in mitochondria and chloroplasts, glycolate oxidase activation, antioxidant inactivation, and GSH depletion. They in turn induce the activation of several Mitogen-activated protein kinase (MAPKs) and phosphatases. The interplay of these key players effectively determines metal uptake and homeostasis (Hu *et al.*, 2020).

Molecular mechanisms of arsenic toxicity in invertebrates: A glance

Representatives from several phylum have been used as an established model to study As toxicity like rotifers, copepods, *Drosophila* sp. etc. Arsenic species like DMAsV or MMAsV have been reported from various types of finfish, crabs, molluscs and echinoderms. Arsenocholine (chemically similar to arsenobetaine) is mainly reported in shrimp. Arsenosugars and arsenolipids have been detected from marine molluscs (Zhang *et al.*, 2019). Calcium, and other related supplements primarily derived from seafood also contain high amounts of arsenic. That's being another important entry source to the food web. Seafood that pre-dominantly includes finfish, shellfish, and seaweed contributes immensely as As source to human population. In the context of marine food-web, seaweeds retain the highest quantity of Arsenic (Taylor *et al.*, 2017). Seaweeds are the producer organisms. Hence, a large number of marine molluscs, different fishes feed on them; which eventually reach higher organisms and imparts arsenic-mediated toxic effects (Khandaker *et al.*, 2021).

Arsenic exposure has shown to alter several important cellular functioning like adhesion and aggregation of circulating haemocytes of the freshwater bivalve (Chakraborty *et al.*, 2021). Also processes like non-self adhesion, inter-haemocyte interactions and haemocyte aggregation are severely impaired. The efficiency and functioning of several important enzymes like ATP synthase, succinyl-CoA synthetase and nucleoside diphosphate kinase gets affected implicating the notable interference of As and its derivatives in energy metabolism (Saux *et al.*, 2020).

Molecular mechanisms of arsenic toxicity across vertebrates: A glance

In the earlier part of this review, several ways by which As can reach higher organisms has been discussed. Here, in this section an attempt has been made to focus on the signaling molecules that respond as a result of exposure.

In aquatic animals like teleost, As entering the water-bodies tends to accumulate in their tissues. Often, further metabolism tries to detoxify but at times accumulation of it affects diverse physiological parameters like fish growth, reproduction, immune function, and enzyme activity (Han *et al.*, 2019). The scenario is not much different in amphibians, reptiles, birds and mammals (Death *et al.*, 2019). Generally, Arsenic exposure targets bone marrow thereby affecting blood cells and lymphocytes (Ferrario *et al.*, 2008). This hematopoietic tissue insult results in insufficient erythropoiesis and low concentration of hematocrit and hemoglobin. Importantly, As exposure targets specific organs like liver, spleen, kidney and brain (Hong *et al.*, 2014). The way As and its derivatives work though remains obscure, the role for increased cytotoxicity and altered antioxidant enzymes activity has been vastly studied and implicated. It occurs owing to imbalance between pro-oxidant and antioxidant homeostasis. Besides, Reactive nitrogen species are also implicated. The involvement of Mitogen-Activated Protein Kinases, different caspases,

calcium-dependent proteases, DNA repair enzymes and several signaling molecules have been reported to participate in the signaling cascade and bringing out the effects (Martinez-Finley *et al.*, 2011).

Several enzyme system including diamine oxidase, aldehyde dehydrogenase, tryptophan dual oxidase, liver dehydrogenase and the cytochrome P450 significantly plays role in oxidative stress. Reactive Oxygen and Nitrogen species are the most important group of radical produced in living systems. An excessive amount of ROS or RNS are deleterious to the cells. Superoxide radical (O_2^-), hydroxyl radical (OH^\cdot) and hydrogen peroxide (H_2O_2) are the main forms of ROS. Superoxide radical reacts with NO to produce peroxynitrite ($ONOO^-$). Here the further reaction diversifies. On one hand, its protonated form, peroxynitrous acid ($ONOOH$) reacts with cellular nucleophiles or oxidizes heme proteins to form ferryl-oxo derivatives further. Both $ONOOH$ and ferryl-oxo complexes are potent oxidants. Alternatively, H_2O_2 and O_2^- may react with ferric ion or nitric oxide to generate more potent oxidants. Ferric ion produces ferrous ion by means of a reduction reaction which, in turn, converts H_2O_2 to hydroxide ion and OH^\cdot . Hydroxyl radical (which possesses a redox potential of 2.8 Volts) is highly reactive and can oxidize several molecules that could damage lipids, DNA and proteins leading to cell death. Organisms harbor effective antioxidant system that works to counteract these potential hazards like Enzymes with antioxidant activity, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) or compounds like ascorbic acid (vitamin C), α -tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids etc. SOD carry out the dismutation of superoxide radical into hydrogen peroxide and CAT further converts H_2O_2 to water and molecular oxygen. GPx is an important enzyme in cellular antioxidant defense systems, detoxifying peroxides and hydroperoxides. It principally functions to reduce H_2O_2 to water, oxidizing two molecules of GSH (Figure 3).

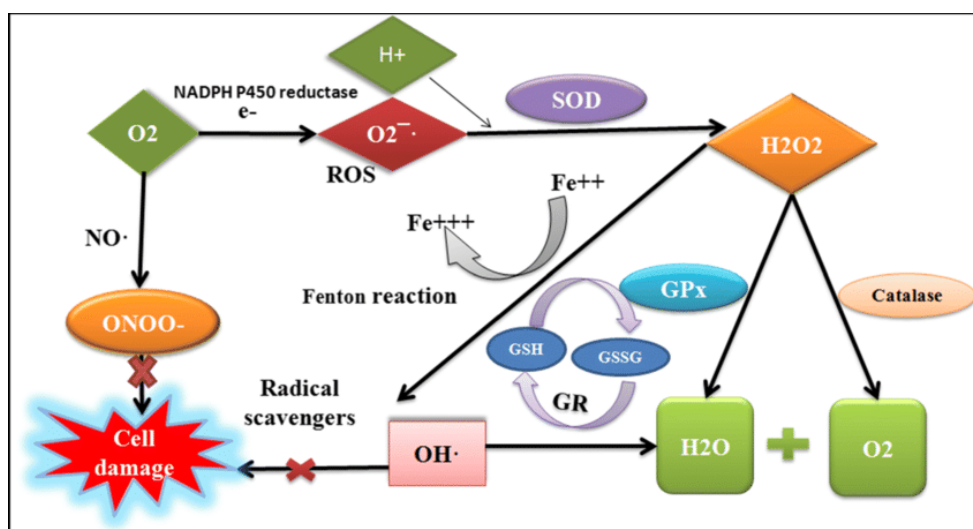


Figure 3: Arsenic-induced oxidative damage (Source: Medda *et al.*, 2020)

Conclusion:

Several property and mechanisms of action exhibited by As and its derivatives we have seen here. Besides this there are other properties as well like being trans-placental, mutagenic, teratogenic and carcinogenic. Arsenic is ubiquitous and gets absorbed by ingestion, inhalation, or through permeation of the skin or mucous membranes. The changes are seen at the cellular levels as they accumulate by using an active phosphate transport system. The toxicity of arsenicals conforms to the following order, from greatest to least toxicity: arsines > inorganic arsenites > organic trivalent compounds (arsenoxides) > inorganic arsenates > organic pentavalent compounds > arsonium compounds > elemental arsenic. Essentially, it's the solubility in water and body fluids that is related to toxicity (the low toxicity of elemental arsenic is attributed to its virtual insolubility in water and body fluids, whereas the highly toxic arsenic trioxide is readily dissolved). The mechanisms of arsenical toxicity differ considerably among arsenic species, although signs of poisoning appear to be similar for all arsenicals. They have been correlated to several disorders like cardiovascular, neural disorders etc. The priority is to have safe drinking water. The best alternative is to rainwater catchment system to collect and store rainwater. No such technology is available to treat arsenic mediated toxic effects. Treatment options advocated relies on vitamin and mineral supplements and antioxidant therapy. "Prevention is better than cure", is an age old phrase. Unfortunately the true meaning was not realized and today we have reached a situation where greed and over-exploitation have put the entire globe on threat. Be it vigorous exposure to different toxicant including Arsenic or inviting unnecessary microbes.

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MERCURY LEVEL IN SUNDARBAN MANGROVE ECOSYSTEM

Rajrupa Ghosh^{1*}, Pallavi Dutta², Shyama Prasad Bepari²,

Manmatha Nath Sarkar², Sufia Zaman² and Abhijit Mitra³

¹Post Graduate Department of Zoology,

Banwarilal Bhalotia College, G.T. Road, Ushagram, Asansol 713303

²Department of Oceanography,

Techno India University, West Bengal, EM 4/1, Salt Lake, Sector V, Kolkata 700091

³Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata 700019

*Corresponding author E-mail: rajrupaphd@gmail.com

Abstract:

During the premonsoon, monsoon, and postmonsoon seasons in 2019, we examined the amounts of dissolved mercury and biologically accessible mercury in surface sediment in 12 selected stations in and around the Indian Sundarbans deltaic region. The Atomic Absorption Spectrophotometer was used to conduct the tests (Perkin Elmer: Model 3030). The highest levels of dissolved mercury were found during the monsoon season, followed by the postmonsoon and premonsoon seasons. In the case of biologically accessible mercury in surface sediment, the highest values were found in the premonsoon, followed by the postmonsoon, and finally the monsoon. There was no trace of mercury in the aquatic phase or sediment at sampling points such as Lothian Island, Jambu Island, Chotomollakhali, Bali Island, Sajnekhali, and Bagmara.

Keywords: Dissolved mercury, biologically accessible mercury, Indian Sundarbans, Atomic Absorption Spectrophotometer

Introduction:

Chemical industry wastewater is usually found in estuaries and coastal zones (Krishna Murti and Viswanathan, 1991; Manahan Stanley, 1994). Industrial and urban garbage, as well as runoff from agricultural and shrimp production units, are deposited in the Indian Sundarbans' estuaries. Beside from runoff from a large catchment area that includes cities like Kolkata, Howrah, and the newly developed Haldia complex, as well as the North and South 24 Parganas districts, the Sundarban estuaries also receive a large amount of waste from industrial, domestic, agricultural, and aquacultural sources. These wastes contain a variety of hazardous heavy metals (Mitra, 1998; Chakraborty *et al.*, 2014; Mitra and Zaman, 2016), which can accumulate in the

body tissues of edible fish and cause problems. Mercury is one of these dangerous heavy metals that have been linked to a variety of physiological problems such as paraesthesia, loss of muscular coordination, trouble speaking, narrowing of the visual field, hearing loss, and blindness (Mitra, 1986). The Indian Sundarbans, as a World Heritage Site, necessitates the monitoring of harmful heavy metals in and around the area in order to assure the quality of the products (ideally edible fishes) obtained from the system, which is the focus of this study.

Materials and Methods:

Site Selection:

Twelve stations were chosen for the current programme: Kakdwip (Station 1), Harinbari (Station 2), Chemaguri (Station 3), Sagar south (Station 4), Lothian Island (Station 5), Jambu island (Station 6), Frasergunge (Station 7), Gosaba (Station 8), Chotomollakhali (Station 9), Bali Island (Station 10), Sajnekhali (Station 11), and Bagmara (S (Table 1). All of the stations are in or near the deltaic region of the Indian Sundarbans.

Table 1: Coordinates of selected sampling stations

Stations	Geographical locations	
	Latitude	Longitude
Kakdwip	21°52'06.00"N	88°11'12.00"E
Harinbari	21°47'01.36"N	88°04'52.98"E
Chemaguri	21°38'25.86"N	88°08'53.55"E
Sagar south	21°38'51.55"N	88°02'20.97"E
Lothian island	21°39'01.58"N	88°22'13.99"E
Jambu island	21°35'42.03"N	88°10'22.76"E
Frasergunge	21°33'47.76"N	88°15'33.98"E
Gosaba	22°15'45.00"N	88°39'46.00"E
Chotomollakhali	22°10'21.74"N	88°53'55.18"E
Bali island	22°04'35.17"N	88°44'55.70"E
Sajnekhali	22°05'13.40"N	88 ° 46'10.8"E
Bagmara	21°39'04.45"N	89°04'40.59"E

Collection of water and sediment samples:

Water samples for dissolved mercury analysis were collected under high tide conditions from all of the selected stations in the premonsoon, monsoon, and postmonsoon seasons of 2019. At least three samples were obtained from the surface during high tide conditions within 500 metres of each observational site. Sediment samples were scraped from the surface (5 cm depth)

with a pre-cleaned and acid washed plastic scale and stored in clean polythene bags that were sealed. The samples were washed in metal-free double distilled water, dried for 5–6 hours at 105°C, cleaned of visible shells or shell fragments, reduced to powder in a mortar, and kept in acid-washed polythene bags. To confirm the quality of our data, three sediment samples were obtained within 200 metres of each other.

Analysis of dissolved mercury:

The dissolved mercury was measured using the APHA's standard procedure (1995). Each water sample collected and stored in clean TARSON bottles was filtered using a 0.45 micron Millipore membrane prior to analysis. The filtrate was treated with diethyl dithiocarbamate and extracted in carbon tetrachloride. The extracted was evaporated to dryness and the residue was mineralized with 0.1 ml of concentrated nitric acid. Analytical blank was prepared and treated with the same reagents. Analyses were done in triplicate by direct aspiration into AAS (Perkin-Elmer Model: 3030) equipped with HGA-500 graphite furnace atomizer and a deuterium background corrector.

Analysis of biologically available mercury:

Sediment samples were cleaned in metal-free double distilled water and dried for 5–6 hours at 105°C, freed of visible shells or shell fragments, reduced to powder in a mortar, and kept in acid-washed polythene bags. After re-drying the materials, 1 gm was extracted and digested with 0.5 (N) HCl according to Malo's usual process (1977). The resulting solutions were then stored in TARSON containers for analysis. The solutions were finally aspirated in the flame Atomic Absorption Spectrophotometer (Perkin Elmer: Model 3030) for the determination of metal concentrations. No detectable trace metals were found in the reagent blank.

Results:

Table 2 shows mercury concentrations in the ambient medium of 12 sampling locations in and around the Indian Sundarbans. In the case of dissolved mercury, there is a distinct seasonal variation, with the highest values occurring during the monsoon season, followed by the postmonsoon and premonsoon seasons. In the case of biologically accessible mercury in surface sediment, the picture is completely different, with the highest values occurring during the premonsoon, followed by the postmonsoon, and finally the monsoon. There was no presence of mercury in the water or sediment at some sampling points, including Lothian Island, Jambu Island, Chotomollakhali, Bali Island, Sajnekhali, and Bagmara.

Table 2: Mercury levels in water (ppm) and surface sediment (ppm dry wt.) in and around Indian Sundarbans

Stations	Dissolved Hg			Biologically available Hg in surface sediment		
	Pre	Mon	Post	Pre	Mon	Post
Kakdwip	0.005 ± 0.002	0.011 ± 0.001	0.007 ± 0.004	0.023 ± 0.006	0.012 ± 0.005	0.021 ± 0.005
Harinbari	0.002 ± 0.001	0.004 ± 0.002	0.002 ± 0.001	0.015 ± 0.009	0.010 ± 0.005	0.012 ± 0.002
Chemaguri	0.002 ± 0.002	0.005 ± 0.001	0.004 ± 0.001	0.015 ± 0.005	0.011 ± 0.004	0.015 ± 0.006
Sagar south	0.004 ± 0.003	0.008 ± 0.002	0.006 ± 0.002	0.041 ± 0.010	0.019 ± 0.006	0.026 ± 0.008
Lothian island	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
Jambu island	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.000 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
Frasergunge	0.009 ± 0.003	0.012 ± 0.003	0.011 ± 0.002	0.088 ± 0.014	0.025 ± 0.009	0.067 ± 0.006
Gosaba	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.013 ± 0.004	0.005 ± 0.002	0.009 ± 0.003
Chotomollakhali	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
Bali island	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
Sajnekhali	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001
Bagmara	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001

Discussion:

Mercury is a global contaminant that circulates in various ways through the air, water, sediments, soil, and organisms (Moreno Fabio *et al.*, 2005). Since the dawn of the industrial age, the amount of mercury mobilised and released into the environment has increased dramatically, with well-known hazardous effects (Boening Dean, 2000). The presence and behaviour of

mercury in aquatic systems is of great interest and importance since it bioaccumulates and biomagnifies through all levels of the aquatic food chain (Lindqvist *et al.*, 1991). Riverine drainage is the main source of metal contamination in coastal areas (Mitra, 1998).

It is interesting to note in this study that the level of mercury in the aquatic system is inversely proportional to that in the surface sediment. This could be called speciation, as evidenced by the highest levels of dissolved mercury in the monsoon and the lowest levels of biologically accessible mercury in the sediment, respectively. The process of speciation is triggered by a drop in pH in the lower Gangetic delta region's aquatic phase (Mitra, 1998; Mitra and Zaman, 2016).

In the near future, the rapid development of electronic businesses in West Bengal, as well as the vast amount of electronic wastes discharged into the environment, may cause major mercury pollution in the Sundarban mangrove ecosystem. These sources and transmission pathways must be identified, quantified, and analysed in order to properly comprehend the fate of total mercury in the Sundarban wetland ecosystem. Such a programme would make it easier to establish long-term remedial measures in the future. Several earlier researchers in the current geographical location have pointed to the pictures of speciation of several heavy metals (Mitra, 1998; Mitra, 2013; Chakraborty *et al.*, 2014; Mitra and Zaman, 2016), but for mercury, this baseline data calls for a long-term research in this domain, as many of the sampling stations show mercury in the ambient aquatic phase and sediment, which may often pave the gateway of bioaccumulation in the resident organisms of this World Heritage Site of India.

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HEAVY METAL CONTENT AND CARCINOGENIC EFFECT IN HUMAN BODY VIA FOOD PRODUCT

Shreya Khan and Subarna Bhattacharyaa*

School of Environmental Studies, Jadavpur University, Kolkata 700 032

*Corresponding author E-mail: barna_kol@yahoo.com

Abstract:

This study was aimed to evaluate the accumulation of heavy metals in human body via food products and their carcinogenic risk factor. To perform that, the work was divided into two phases. In the first phase data on accumulation of heavy metals in various food products was collected. In the second phase, these data are used to calculate the Target Hazard Quotient (THQ) and Target Carcinogenic Risk (TR) for each food products. The result shows that most of the food products listed in the balanced diet chart for Indian people, possess high carcinogenic risks from unwanted heavy metal content.

Introduction:

Heavy metals are naturally occurring elements of the earth's mantle and are long-lasting environmental pollutants since they cannot be decomposed or removed [1]. But there are anthropogenic reasons for accumulation of heavy metals as well. In regions, like periurban eco system where fresh water source is scarce throughout the year, industrial wastewater is used for irrigation in agriculture. Irrigation using wastewater is known to drastically increase the heavy metal content of soil [2]. Wastewater includes significant concentrations of hazardous heavy metals, which leads to such complications [3]. Continuous procedure of irrigation in the crop fields, using this kind of heavily metal accumulated water causes the plants to collect these metal particles and store them inside their body parts. When these plant bodies are consumed by human, on animals, the metal passes through as well. Human consumers not only gather the metals in their body from plant crops, but also when they consume food produced from animals. Hence depending on the quantity of heavy metal present in those food, the daily consumed amount of heavy metals can be quite high, some of which can be carcinogenic as well. Accumulation of carcinogenic heavy metals in human body leads to potentiality of causing various mutations in the consumer body.

A lot of survey works have been done all over the world to curate the hazard level of these heavy metals possess in human body and their carcinogenic risk. Heavy metal contamination has spread across the globe, causing environmental disruption and posing major

risk to human health. The rapid pace of urbanization, land use changes, and commercialization, especially in developing nations with extraordinarily high populations, such as India and China, are widely seen as the primary causes of this situation. The diversity of environmental pollutants has risen enormously since the industrial revolution and economic globalisation, with innumerable anthropogenic causes. As a result, food security challenges, both old and new, have become a global concern, particularly because of their inextricable link to human health [4].

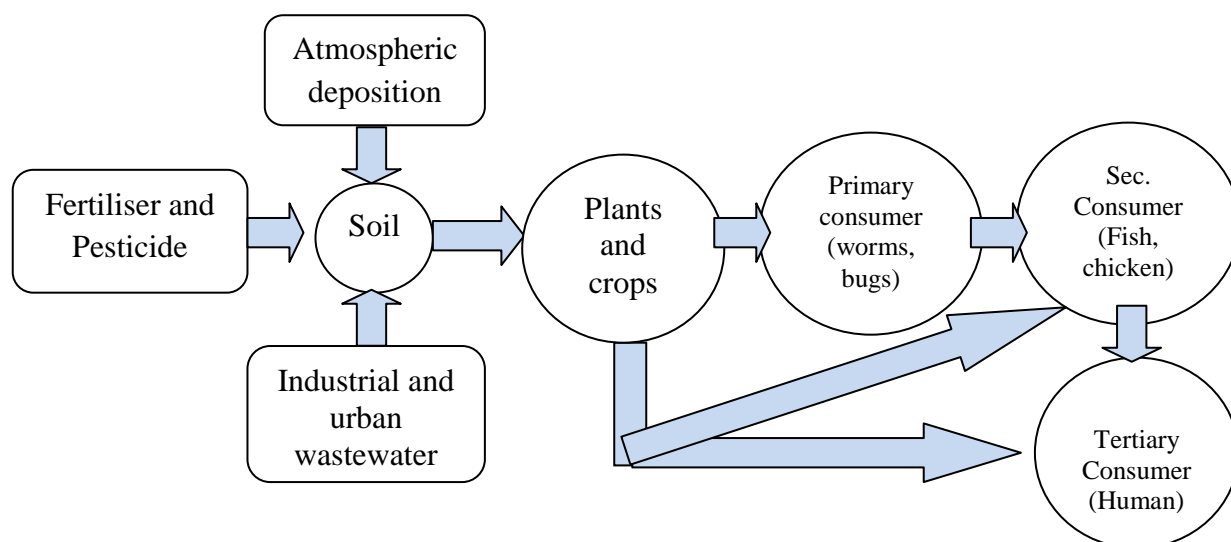


Figure 1: Flow of heavy metal through soil in the food chain

Land degradation with hazardous metals is widespread in urban and peri-urban regions as a consequence of industrial and municipal activity. In urban agricultural lands, waste water is used for irrigation to boost the output of food crops is the primary cause of contamination. These effluents are high in hazardous metals and are a major source of metals in sewage irrigated and modified soil [5]. Depending on the kind of activity, wastewater may contain heavy metals such as zinc (Zn), copper (Cu), lead (Pb), arsenic (As), nickel (Ni), chromium (Cr), and cadmium (Cd) [19]. Several toxic heavy metals and metalloids (for example, As, Pb, Cd, and Hg) are categorised as non-essential to metabolism and other biological activities. Because these metals are harmful in a variety of ways, the United States Environmental Protection Agency and the Agency for Toxic Chemicals and Disease Registry have placed them in the top 20 list of hazardous substances (ATSDR) [5].

In this study the focus is how in India, the heavy metals accumulated in food are possessing carcinogenic risk for the people.

Passage of heavy metal:

Soil is the major repository of heavy metals in the atmosphere, hydrosphere, and biosphere, and so plays a critical part in nature's entire metal loop [6]. Continuous wastewater irrigation has altered the physicochemical characteristics of the soil, resulting in heavy metal absorption by food crops, mostly vegetables. The oxidation state, heavy metal form, and phase all have a significant impact on bioavailability [5]. An explicit comprehension of the pathways and methodologies by which heavy metals endanger human health through the consumption of grains and vegetables allows for the development of appropriate heavy metal management and mitigation strategies for the betterment of the community people, farmers, researchers, pedologists, and policymakers. Heavy metals are transported from soil particles to plants in ionic forms that vary depending on the metal. Heavy metal bio speciation can also differ by food product. Plant roots play the most vital role in the uptake and translocation of heavy metals. The entry of metals into a root depends on its anatomy (especially the cell wall) and environmental adaptability. To manage health concerns, proper research of the processes involved in heavy metal foliar absorption is also required. Heavy metals are carried from the roots to the plant's aerial portions by xylem load capacity, whereas foliar transportation utilises the phloem vascular system. In terms of heavy metal absorption processes in crops, foliar translocation has received less attention than root uptake [4]. The degree of toxicity varied according to metal species and vegetable variety. From plants these heavy metals get passed through consumed food from prey to predator along the food chain and reach the top predators that are humans in the end. Human beings get heavy metals in their bloodstream and body from various sources, but consumption of food heavily laden with them is one of the major sources.

Heavy metals found in Indian food sources:

The food items that are consumed by the majority of the Indian citizen are loaded with various types of heavy metals, from significantly carcinogenic ones to others who can very well cause non carcinogenic issues if consumed and accumulated in the body over the threshold limit. In this study we are considering the heavy metals such as Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Copper (Cu), Mercury (Hg), Manganese (Mn), Magnesium (Mg) and Iron (Fe). We divided the main food items of an ideal diet chart for an Indian citizen into several groups for the more compact analysis of the scenario. We considered the Target Hazard Quotient (THQ) value in case of non carcinogenic risk and the Target Carcinogenic Risk (TR) value in case of determining the carcinogenic level of heavy metals [7]. Since the diet chart for adult men, adult women and children are different regarding the quantity of each kind of food product they consume, we considered these three categories for determining the hazardous levels.

Heavy metal in rice and other grain crops:

Rice is one of the main food items for average Indian people. Heavy metal contamination in rice is very common. Metals like Pb, Cd, Cr, Cu, Fe, and Hg can contaminate paddy field soils and thus, the rice grains.

The THQ values of various heavy metals accumulated in human body via rice is found to be a lot less than the threshold value of 1. The values of Co, Pb and Cu are higher than most but still very much below the threshold value. It can be safely assumed that rice in the daily meal does not possess any non carcinogenic risk for the Indian citizen. In case of TR values only the values of Pb are found to be in the tolerable limit for all three categories. Cd, Cr and Ni values showed carcinogenic risk.

Table 1: heavy metal content in rice [8 -12]

Metal name	Pb	Cd	Ni	Cr	Cu	Zn
Metal concentration (mg kg ⁻¹)	1.020 (0.012-5.177)	0.048 (0.001-0.35)	0.8 (0.01-2.89)	1.32 (0.086-18.29)	6.91 (0.2-30)	32.4 (0.086-114.6)

The THQ values of all the heavy metals for grain crops are found to be lower than the threshold limit as well. The THQ of Pb is visibly higher than other heavy metals followed by Ni and Cd but do not possess any threat. In case of TR values only the Pb values are barely in the tolerable zone and the Cr values are just below the tolerable risk limit. Cd and Ni show high carcinogenic risk for both adults and children.

Table 2: heavy metal content in other grain crops [13, 14]

Metal name	Pb	Cd	Ni	Cr	Cu	Zn
Metal concentration (mg kg ⁻¹)	5.96 (0.85-14.45)	0.78 (0.04-1.74)	17.4 (14.8-20.0)	1.21 (0.20-1.75)	10.15 (5.89-10.84)	34.27 (1.62-78.8)

Heavy metals in vegetables:

We have separated the vegetables in three categories for the clear view of the heavy metal status- 1. Leafy vegetables, 2. Fruity vegetables, 3. Root/tuber Vegetables. The quantity of accumulation of heavy metals is different in various parts of a plant body and so vegetables from different part of the plant, show this variation as well.

First in the leafy vegetables, the THQ values show that Cd has crossed the threshold limit of 1 for hazard quotient in case of both adults and children. Hence leafy vegetables may possess non carcinogenic threat from heavy metal cadmium. Symptoms of slow cadmium poisoning may be observed in the consumers. In case of TR values all four of the heavy metals show

carcinogenic tendency, where $Cd > Ni > Cr > Pb$. This shows high risk factor for cancer from leafy vegetables.

None of the heavy metals are seen to cross the THQ threshold value of 1 in case of fruity vegetables, which is an indication of absence of non carcinogenic risk in the human from consuming this type of vegetables. However, in case of TR value only Pb is in the tolerable carcinogenic risk zone. Ni, Cd and Cr have high values of Carcinogenic risk factor, which makes fruity vegetables prone to cancer risk.

In case of tuber vegetables the THQ values of Pb and Cd are over the threshold limit of 1, which indicates that these two heavy metals in root vegetable possess the threat of non carcinogenic toxicity in human body. The rest of the metals are below 1, so do not show possible non carcinogenic toxicity. In case of TR values all four of the metals are in high carcinogenic risk zone, as $Cd > Ni > Cr > Pb$.

Table 3: heavy metal content in vegetables [13, 15 – 19]

Metal name	Pb	Cd	Ni	Cr	Cu	Zn
Metal concentration (mg kg ⁻¹)	17.36 (0.242-57.63)	6.26 (0.02-17.79)	24.79 (0.375-69.22)	29.14 (0.03-96.3)	12.38 (0.202-34.49)	67.52 (0.289-171.03)

Metals in animal protein:

We have considered egg, fish and chicken as food sources providing animal protein. We found that all the THQ values are way lower than the threshold value of 1, which makes sure there is no non carcinogenic toxicity threat present from egg. All the TR values are within the tolerable risk range as well, which is a very satisfactory result.

Table 4: heavy metal content in eggs [20, 21]

Metal name	Pb	Ni	Cr	Cu	Zn
Metal concentration (mg kg ⁻¹)	0.414 (0.01-1.35)	0.276 (0.18-0.53)	0.584 (0.16-3.9)	1.20 (0.77-1.91)	15.69 (6.58-30.6)

All the THQ values of heavy metals in chicken are below the threshold value, which means the absence of any possible non carcinogenic toxicity from the metals through consumption of chicken. Pb and Cr have TR values in the tolerable risk range, which is safe. But the TR value of Ni is in the possible high carcinogenic toxicity range.

Table 5: heavy metal content in chicken [21]

Metal name	Pb	Ni	Cr	Cu	Zn
Metal concentration (mg kg ⁻¹)	0.93 (0.49-1.59)	1.39 (0.57-2.86)	0.63 (0.24-1.63)	18.11 (8.13-48.79)	43.78 (30.27-72.79)

The THQ values are all fairly below the threshold value, dismissing possibilities of non carcinogenic toxicity. However in case of TR values we can see the Cd and Cr values in significant carcinogenic risk zone, whereas Pb is in the tolerable zone. This also subtly points at the level of heavy metal toxicity in the water bodies and how that is affecting the habitant animals.

Table 6: heavy metal content in fish [22 – 24])

Metal name	Pb	Cd	Cr	Cu	Zn
Metal concentration (mg kg ⁻¹)	3.82 (0.1-32.35)	2.73 (0.02-24.62)	47.73 (2.12-103.25)	15.24 (0.90-92.38)	49.7 (18.80-136.5)

Heavy metal in milk:

In India, milk for daily consumption is collected from a variety of animals. But the main two types are cow milk and buffalo milk.

In case of cow milk the THQ values are basically negligible compared to the threshold value of 1, so possible non carcinogenic toxicity from cow milk can be considered insignificant. In case of TR values as well, all the values are within the tolerable range of carcinogenic risk which is comparatively safe than most food products.

The THQ values for buffalo milk are below the threshold value with a huge difference. Any possible non carcinogenic risk can be considered negligible. The TR value of Pb is in negligible range in terms of carcinogenic effect and the values of Cd are in tolerable range.

Table 7: heavy metal content in milk (25 – 27)

Metal name	Pb	Cd	Cr	Cu	Zn
Metal concentration (mg kg ⁻¹)	0.24 (0.004-5.904)	0.0058 (0.00-0.011)	0.054 (0.013-0.175)	7.43 (0.039-37.29)	0.61 (0.496-0.797)

Conclusion:

For the year 2020, the estimated number of cancer patients in India is 1,392,179[28]. Oral and oesophageal cancer rates in India are among the highest in the world [29]. Several studies

have found a clear connection between exposure to heavy metals and the occurrence and longevity of gastric cancer [30]. This study has found that most of the food products of a daily healthy Indian diet have high level of heavy metal accumulated in them, possessing carcinogenic risk of concerning level.

All of the food products considered here are supposed to be a mandatory part of our daily healthy diet. But with the amount of carcinogenic risk each of them are showing from different heavy metals, the undesirable effects are not to be neglected for the sake of our health. It is important to make more detailed observations regarding this aspect and make sure that the diet which is supposed to be balanced does not cause involuntary harms to the consumers of these particular food products.

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SUSTAINABLE WASTE MANAGEMENT CAMPAIGN AT

MUGBERIA, EAST MIDNAPORE

Sourav Sikdar*¹, Sayanta Sikdar² and T. Senthil Vadivel²

¹Department of Zoology,

Brahmananda Keshab Chandra College, Kolkata – 700 108

² Department of Civil Engineering,

School of Engineering and Technology, Adamas University, Kolkata – 700 126

Corresponding author E-mail: mailsouravin@gmail.com*, sayantasikdar@gmail.com,
tsnsenthu@rediffmail.com

Abstract:

Waste management is required to manage wastes from different resources to its final disposal to maintain a sustainable society. This includes collection of wastes from different resources, transport, scientific treatment and disposal of wastes with proper recycling, reuse and reduces the wastes. Wastes are of mainly solid, liquid, or gaseous in state and each type has specific ways of disposal and management. Wastes can harm the human health caused by extraction and processing of raw materials at different industries that are the resources of all three types of wastes. The authors have organized this waste management programme at Mugberia, Kolkata and made awareness campaign to all the local people to differentiate types of wastes viz. organic or biodegradable and inorganic or non-biodegradable wastes and to manage these scientifically. Different hands-on-training programme, seminars and workshops were organized to the students and teachers nearby the place to manage wastes in both domestic and work places. The local panchayat also been insisted to arrange separate bins at different sites of villages to maintain a proper hygienic condition at local areas. The authors planning to develop more innovative and scientific protocols for the resource management of wastes at this area to make this place as a model village for solid waste management and pollution free nature.

Keywords: Biodegradable Wastes, Non-biodegradable Wastes, Resource management, Solid Waste Management Awareness Campaign.

Introduction:

Waste, a common word has integrated very depth in our daily life. Wastes mainly comprise all types of refuses and garbage that are generally unwanted and/ or useless things. Wastes are some unwanted substances that are discarded by living organisms in the form of urea

and sweat. Management of wastes may be defined as an operation that may lead to resource recovery, recycling, reclamation, and reuse in alternative ways. Globally, various types of wastes are being generated daily due to changing lifestyles, differential consumption patterns, affluence, rapid economic growth, rapid urbanization, increasingly heterogeneous nature of modern products and rapid population growth (Dangi *et al.*, 2017; Malinauskaite *et al.*, 2017). Now-a-days the wastes which are being generated are not properly managed or disposed (NeffaGobbi *et al.*, 2017; Mmerek, 2018). In day-to-day life it is often observed the wastes generation at homes and surroundings and the process of waste disposal. Waste disposal has been done in a haphazard manner in both villages and towns. But today waste generation and management has become a matter of concern to control environmental pollution and threats.

Solid and liquid wastes are the basic two types of wastes, where all plastic materials, foams, metals, glass, etc. are solid wastes and chemicals, oils, domestic industrial waste water, etc. are liquid wastes. On the basis of the properties of wastes there are two types of wastes- biodegradable and non-biodegradable wastes. Bio-degradable wastes like paper, wood, fruits, etc., can be degraded and non-biodegradable wastes like plastics, bottles, old machines, cans, Styrofoam containers, etc. cannot be degraded. On the basis of effects on human health and on environment, wastes again of two types viz., hazardous and non-hazardous wastes. Substances that are unsafe to use and have corrosiveness and toxicity are called hazardous wastes. Non-hazardous wastes are the substances that are safe to use. These substances usually create disposal problems. Municipal bio-medical wastes, solid wastes, agricultural wastes, industrial wastes, radioactive wastes, fishery wastes, e-wastes, etc., are some waste varieties on the basis of their origin.

Now-a-days, Construction and Demolition (CandD) wastes are also a major source of solid waste refers to the waste materials including building material wastes, debris, and rubble generated from any construction activity, demolition and repair work of civil structures such as houses, bridges, roads, dams, large building structures, and other infrastructure (MoEFCC, 2016). This CandD waste usually comprises inert and non-biodegradable material such as concrete, brick aggregates, tiles, plastic, wood, glass, metals, excavated soil and rock particles, etc. (CPCB *et al.*, 2017, Faruqi *et al.*, 2020). A rapid improvement in technology changes lifestyle which has consequences in the fast-growing e-wastes (nearly 41.8 million metric tons (MT) every year) stream worldwide (Balde *et al.*, 2015; Li *et al.*, 2015; Fowler, 2017). Currently, India generates approximately 1, 641 metric kilo tones of e-waste annually (StEP, 2017). According to the United Nations Environment Programme (UNEP) (2009) report discarded computers will increase up to five times more and mobile phone had increased 18 times in 2020. India and other Asian countries will face more increasing amount of environmental damage and health problems incoming future, if e-wastes are not properly managed (Porte *et al.*, 2005; D

Shinkuma and Managi, 2010; StEP, 2010; Wivedy and Mittal, 2010a, 2010b, 2012; Sthiannopkao and Wong, 2013, Awasthi *et al.*, 2018).

Impacts of wastes on animals and aquatics life:

There are different impacts of wastes including gases on environment in a regular basis. CO₂ is emitted by the burning of fossil fuels, wood products, solid wastes, etc., easily mixed in air and hampers the living organisms. CH₄ is produced by the decomposition of organic wastes, livestock, coal mines, natural gas, and oils harm our environment. Different research works reported that US emitted about one-fifth of total global heavy gases in 1977. In recent years there has been increasing concern on environmental sustainability, which has resulted in development of strategies to reduce wastes and improve the waste recovery, resource recycling of wastes (Friedrich and Trois, 2013), and diversion of waste from landfills for a sustainable living environment (Zhuang *et al.*, 2008; dos Muchangos *et al.*, 2015; Mmereki, 2018).

There are so many instances of wastes that are highly toxic and make some serious hazards in the life cycles of animals living in both aquatic and terrestrial environments. The increment of mercury level in fish due to throwing away of mercury in both rivers and seas. Plastic found in oceans ingested by birds caused different disorders and also affects their fertilization capacity. Metals regularly mixed with different water resources resulted in high algal population in rivers and sea. Wastes also degrade the water and soil quality regularly. Open garbage is a common sight in the market, streets or in the vicinity of our homes that emits a foul odor that disturbs our respiratory process. The garbage that is dumped for a long time attracts flies, cockroaches, rats and street dogs. The moist garbage generated by kitchen waste is a perfect breeding place for flies that contaminated our consumable food also. We are affected by different water and food borne diseases like cholera, dysentery and other gastroenteritis that are transmitted by flies.

Waste management:

Managing the waste is a prime target today to protect our family members and the neighbors against dangers that come with poor handling of waste materials. The waste that is disposed of or recycled ethically helps to reduce the negative impacts on environment. Management of wastes also conserves the resources and energy. It reduces water and air pollution and saves landfill space. For successful waste management we should follow three 'Rs'- reuse, reduce and recycle. Reuse by prolonging a product's usable life and by repairing items, selling them or donating them to the charity. Reuse by using durable rather than disposable items (i.e. reusable shopping bags, metal spoons). Reuse of channeled moving boxes internally, reuse the office furniture and supplies including official envelopes, file and folders, paper and other regular usable waste materials. Reuse daily used towels, napkins, cups and

dishes, tablecloths and glasses for different purposes. Reuse incoming packaging materials for outgoing shipments. It is a common duty to everyone to encourage family members, employees, neighbors to reuse domestic and office materials rather than purchase new ones. We have to reduce wastes by reducing daily used office papers unnecessarily by implementing a formal policy to use all daily required documents like training manuals, personnel information, etc. electronically. We have to improve the product designs to use less material. We should redesign the packaging process to eliminate excess materials and have to work with customers to redesign to implement a new frame of packaging return program. On the other hand, recycling is the process of to convert the waste materials into newly formed usable objects. This recycling process can inhibit the waste of highly useful materials and thus reduce the consumption of fresh raw materials. This practice ultimately can control energy usage, air pollution and water pollution.

Waste management programme at Mugberia, East Midnapur:

The campaign for management of wastes started from the class rooms at Mugberia Gangadhar Mahavidyalaya, Mugberia, East Midnapur in which one of the author worked as a teacher. Class room cleaning and management in a regular basis is the prime duty for each teacher, students and non-teaching staffs. Both the laboratories and class rooms of each of the departments were equally cleaned and all wastes were managed scientifically. Every week they have arranged a separate class to demonstrate managing wastes in the area they live. All the students of different semesters are equally involved, separated in different groups to divide their cleaning works. Inside the college campus, the bio-degradable and non-degradable wastes are collected in two different coloured waste bins like green for biodegradable and blue non-biodegradable wastes (Fig 1). The campaigning was started from the college to surroundings of Mugberia. All the teachers and students of Zoology Department, Mugberia Gangadhar Mahavidyalaya was involved for this campaigning programme for waste management. They have made a banner entitled “Environmental Cleaning and Waste Management Campaign” (Fig. 2) and the target was to give some theoretical and practical information to the college staffs and local people for manage wastes in a proper way.

They went to the local hospitals, carpenter shops, tea shops, grocery shops, garages, etc. to observe how the common wastes are managed or wasted. The journey started at Mugberia Gramin Hospital where the sanitation were not clean and were not properly managed (Fig. 3). They have also visited the OPDs, patients' staying rooms, labour rooms, and other utility places and met the hospital staffs also to insist about maintaining the bio-degradable and non-degradable wastes separately in the hospital. In the local shops, the team has demonstrated how to manage bio-degradable and non-degradable wastes separately in a regular basis (Fig. 4 and 5).

There are some bins supplied by local panchayats to manage the wastes, but the local people did not obey the proper rules and regulations rather stored the wastes to the road sides and even in local ponds or lakes. This makes too much pollution in soil and water, its visible by algal blooms in the road side ponds. The team counselled the local people and sellers about the waste management and also demonstrated the side-effects of wastes. The team said the example by cleaning all the road sides nearby areas by wearing gloves and gave a practical demonstration to all the local people to manage wastes properly (Fig. 6).



Figure 1: Degradable and Non-Degradable Waste Disposals at College Campus



Figure 2: Waste Management Campaigning at Mugberia College



Figure 3: Mugberia Gamin Hospital Visit for Waste Management Awareness Campaign



Figure 4: Waste Management Awareness Campaign to the Publics



Figure 5: Demonstration on Biodegradable and Non-Degradable Wastes Disposal



Figure 6: Students Cleaning the Mugberia Streets during Campaign

Discussion:

This paper provides some practical experiences of students and teachers in a college campus and its surroundings to manage wastes by some regular practices and offers a conceptual framework for overcoming the current gaps in waste recovery. It discusses that the transition of solid wastes containing cities into zero-waste sustainable cities requires four inter-related primary strategies like waste avoidance, upstream waste partition, waste collection on time, and proper reuse and management of collected wastes. The aim is to think about the perfect design and development of this waste management framework for smart and sustainable cities with particular emphasis on connecting waste management practices to the whole product life-cycle. More scientific work in future will definitely improve this framework by taking a closer look at the effects of other factors such as regulation, policy, product design strategies, and technology on waste management. In addition, the proposed framework has taken a broad look at waste management and the issues emerging in this field. However, different waste types have different characteristics and management systems, sometimes not compatible with each together.

Furthermore, the proposed framework needs to be validated with different locality-based case studies to test the value of having access to product lifecycle data in solving waste generation and recovery issues in different regions and countries. Traditional views to manage wastes, largely focus on improvement of waste collection efforts, but fail to comprehensively consider the complete product lifecycle and the circular economy opportunities exist over the entire product lifecycle. Waste management efforts should be focused on identifying value chains rather than waste removal chains. The purpose of waste collection and recovery infrastructure should not only be focused on automatizing existing processes, but rather on

implementing best practices with the aim of creating values. Therefore, accessing the city needs and requirements is a required step before making a decision about the type of technology that should be adopted (Esmailian *et al.*, 2018).

Conclusion:

In the Mugberia College and surrounding area the authors have noticed the wastes are not disposed properly. The waste sources are mainly hospitals and local shops. The team of Zoology Department, Mugberia College have maintained the proper campaigning weekly and made some strict rules and regulations for waste management. After a month of this campaign, a survey has been taken again at the same local area including hospital, local shops, ponds, lakes and road sides. There was a good improvement and the local people started disposing biodegradable and non-degradable separately as per the guidelines. The objective of the waste management campaign was really fruitful and solved the purpose in a greater way.

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