FATE OF TOXIC METALS THROUGH MARINE FOOD WEB TO HUMAN USING HAIR AND NAIL AS BIOMARKER FROM HIGHLY POLLUTED CUDDALORE COAST, EAST COAST OF INDIA

A Thesis Submitted to

BHARATHIDASAN UNIVERSITY, TIRUCHIRAPPALLI

for the award of the degree of

DOCTOR OF PHILOSOPHY

IN

ZOOLOGY

(Interdisciplinary Marine Science)

By

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Ref. No: 25861/Ph.D. K1/ Zoology (Interdisciplinary Marine Science) /FT/January 2018

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CERTIFICATE

This is to certify that the thesis entitled **"FATE OF TOXIC METALS THROUGH MARINE FOOD WEB TO HUMAN USING HAIR AND NAIL AS BIOMARKER FROM HIGHLY POLLUTED CUDDALORE COAST, EAST COAST OF INDIA"**submitted by **Mr. A. VINOTHKANNAN** for the award of the degree of **Doctor of Philosophy in Zoology (Interdisciplinary Marine Science)** is based on a result of studies carried out by him under my supervision and guidance at Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli – 620 024, Tamil Nadu, India. This thesis or any part of this thesis has not been submitted elsewhere for the award of any other degree or diploma.

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DECLARATION

The research work presented in this dissertation entitled **"FATE OF TOXIC METALS THROUGH MARINE FOOD WEB TO HUMAN USING HAIR AND NAIL AS BIOMARKER FROM HIGHLY POLLUTED CUDDALORE COAST, EAST COAST OF INDIA"** has been carried out under the guidance of **Dr. R. RAJARAM**, Associate Professor, Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli – 620 024, Tamil Nadu, India. This work is original and has been not submitted in part or full for any degree or diploma of this or any other University.

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1	Name of the Scholar	**A. Vinothkannan**
2	Course of the Study	Ph.D., Zoology (Int. dis. Marine Science)
3	Title of the Thesis/Dissertation	Fate of toxic metals through marine food web to human using hair and nail as biomarker from highly polluted Cuddalore coast, East coast of India
4	Name of the Research Supervisor	**Dr. R. Rajaram**
5	Department/Institution/Research Centre	Department of Marine Science School of Marine Sciences Bharathidasan University Tiruchirappalli-620 024
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Dedication

This thesis is lovingly dedicated to
my beloved parents; without whom
I wouldn’t made it this far…

ACKNOWLEDGEMENT

First and foremost, I convey my sincere gratitude to my research supervisor **Dr. R. Rajaram**, Associate Professor, Department of Marine Science, Bharathidasan University for **suggesting this research work and his valuable guidance, timely suggestions, care, patience and encouragement**.

Beside my supervisor, I would like to thank Doctoral Committee Members **Dr. S. Umamaheshwari**, Associate Professor & Head, Department of Zoology, Periyar E.V.R. College, Tiruchirappalli and **Dr. P. Santhanam,** Associate Professor, Department of Marine Science, Bharathidasan University, Trichy for **their valuable suggestions and encouragement.**

I accord my thanks to **Dr. R. Arthur James**, Head, Department of Marine Science, Bharathidasan University, for **providing me with all necessary facilities during the study period.**

I place on record, my sincere gratitude to the department faculty members **Prof. P. Perumal, Dr. N. Manoharan** and **Dr. V. Yoganandan** for **their moral support and for their help in various stages of my research.**

My heartfelt thanks to authorities of **Bharathidasan University** for facilities provided and financial assistance through **University Research Fellowship (URF).**

My special thanks to **Dr. P. Balasubramanian**, Assistant Professor, Department of Zoology, Govt, arts college, Kumbakonam, for **introducing me** to my supervisor

I would like to gratefulness to my seniors, **Dr. K. Mathivanan, Dr. V. Ramalingam, Dr. K. Kaleshkumar, Dr. A. Ganeshkumar, Dr. G. Arun, Dr. K. Prabhu, Dr. M. Santhosh Gokul, Dr. S. Henciya, Dr. S. Dineshkumar, Dr. M. Divya, Mr. P. Raju, Mr. S. Thennarasu, Mr. K. Arjun** for their support, protective critics, advice and timely help during my research work.

I would like thank my lab, Department juniors **Mr. P. Emmanuel Charles, Ms. V. Jebashalomi, Ms, K. R. Priyanka, Mr. A. Aravinth, Mr. R. Manikandan, Mr. T.D Vengateshwaran, Mr. S. Gunabal, Mr. P. Sridhar, Mr. N. Mohankumar,** for their helping me to complete my experiments and thesis.

My heartfelt thanks to my colleagues and friends **Mr. P. Arunkarthik, Mr. S. Saravanakumar, Dr. N. Krishnaveni, Mr. M. Durairaj, Mr. T. Ajaykamal, Mr. S. Dinesh vijay, Mr. R. Sasikumar, Mr. A. Thirumurugan, Mr. A. Ramkumar, and R. Sarathkrishnan** for their constant encouraging and helping me at every stage of my research work and also make me happy during critical situation.

I would like to express my sincere thanks to **Dr. M. Srinivasan**, Post-Doctoral Fellow, ICAR- National Institute of Animal Nutrition and Physiology, Bengaluru for **framing the zebrafish experimental studies and demonstrating the dissection.**

I sincerely thanks to **Dr. P. Muhilvanan,** PAR Life Sciences and Research Pvt, Ltd, Woraiyur, Trichy for the **zebrafish gene expression studies.**

I heart fully thank to **Mr. G. Sathiyaraj,** Assistant technical manager at the Rajiv Gandhi Centre for Aquaculture in Sirkali, for the **analysis of the zebrafish histological studies.**

I thank to **Mr. Kalliyappan, Mr. Arumugam**, fisherman for providing the finfish and shellfish samples, and all the volunteers from fisher folk communities for providing the hair and nail samples then **Mr. S. Prithivraj,** and **Mr. S. Prakashraj** for during the help of sample collections.

My special thanks to my **School teachers,** and **Professors** from A.V.C College, Mayiladuthurai and Govt. arts college, Kumbakonam.

VINOTHKANNAN ANBAZHAGAN

CONTENTS

LIST OF ABBREVIATIONS

% - Percentage
± - Plus, or minus
^{0}C - Degree celsius
AAS - Atomic absorption spectrophotometer
ANOVA - Analysis of variance
BAF - Bio accumulation factor
BCF - Bio concentration factor
BDL - Below detection limit
BMI - Body mass index
BW - Body weight
C deg - Contamination degree
CCC - Continuous criteria concentration
Cd - Cadmium
cDNA - Complementary DNA
CEPI - Comprehensive environmental pollution index
Cu - Copper
DNA - Deoxyribonucleic acid
E_i - Ecological risk index
ERA - Environmental risk assessment
ERL - Effects range low
FA - Factor analysis
FAO - Food and agriculture organization
FIR - Food ingestion rate
HCA - Hierarchical cluster analysis
HI - Hazard index

I_{geo} - Geo-accumulation index

MIBK - Methyl isobutyl ketone

mRNA - Messenger RNA

NEMs - Non-essential metals

Pb - Lead

PCA - Principal component analysis

PCR - Polymerase chain reaction

PER - Potential ecological risk

PERI - Potential ecological risk index

PLI - Pollution load index

QA/QC - Quality assurance and quality control

RfD - References dose

RNA - Ribonucleic acid

rRNA - Ribosomal ribonucleic acid

RT-qPCR - Real-time reverse transcription–polymerase chain reaction

SD - Standard deviation

SIPCOT - Small industrial promotion corporation of Tamil Nadu

SQGs - Small quantity generators

STIs - Sexually transmitted infections

THQ - Target hazard quotients

TNPCB - Tamil Nadu pollution control board

USEPA - United states environmental protection agency

WHO - World health organization

YRE - Yangtze river estuary

Zn - Zinc

LIST OF TABLES

Chapter I

General introduction

Environmental pollution

Environmental pollution has been defined as undesired, unwanted modification in the physico-chemical, and biological properties such as air, water, and soil and that causes hazardous effect to flora and fauna in any ecosystem (Wong, 2013). Pollutants were categorized under three major types which includes inorganic, organic, and biological. Inorganic pollutants are habitually mineral-derived substances such as metals, salts, and minerals, whereas organic pollutants are derived from human waste, food waste, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and pesticides. Bacteria, viruses, fungus, animal dander and cat saliva, mites, cockroaches, and pollens are examples of biological pollution (Masindi and Muedi, 2018). The post-World War II period as the 'New Chemical Age' because had seen a huge growth in world chemicals supply (Arora, 1997). Across all pollutants, heavy metals contamination gets the superior concern due to their hazardous nature among the environmental scientists. Metals are generally present in small amounts in aquatic environments, but several of elements are dangerous even at trace levels. The most important ecological threat is pollution, which has been increased by agricultural development, urbanism, industrialism, and notably in South Asian emerging countries such as India, Pakistan, Bangladesh, and Nepal (Karn and Harada, 2001).

Metal and its impact

Metals are elements with strong conductivity, flexibility, and shine that lose electrons spontaneously to produce cations. Metals are naturally found in the crust of the earth, and its contents change between locations, causing in spatial differences (Jaishankar *et al*., 2014). Heavy metals with a specific density, much over 5 g/cm3 that have causes a negative impact on the environment and life forms (Jarup, 2003). Heavy metals are classified depending on their atomic weight as well as density. Currently, the term "heavy metal" refers to metallic chemical elements, and metalloids or metals. This may be harmful to the ecosystem and humans (Briffa *et al*., 2020). The tremendous rise in metals loadings into the ecosystem has impacted negatively on both terrestrial and marine ecosystems. Metal toxicity increases due to the absorption of a large quantity of the metal, and metals are entered into the human body through due to the intake of food, air, and water (Patil *et al*., 2013). Subsequently, these toxins enter the humans through diet, caused by a wide variety of health impacts including reduced renal function, cutaneous and bladder cancer, low reproductive capability, liver problems, and even death (Wei *et al*., 2014)

Origin of metals

The environment itself is the primary source of environmental pollution because many chemicals are originated from natural origin, but in anthropogenic origin which leads to the production of hazardous chemicals that contaminate the environment. The most of pollution originates from human influence, which increases the quantity of exposure. Anthropogenic sources are provided more hazardous chemicals to the environment than natural sources (Das *et al*., 2014). When compared to other harmful chemicals, metals are heavily influenced because of its frequently generated and introduced into the environment by humans. Both natural and manmade sources can release toxic metals and metalloids into the environment. Weathering of parent rocks, volcanic activity, forest fires, atmospheric deposition, erosion, and geological changes are examples of natural sources. Man-made sources include the rapid pace of industrialization, consumption of energy, and negligent exploitation of resources (Gautam *et al*., 2016).

Natural origin

The rock and sediments are containing naturally a large number of chemicals such as arsenic, iron, chloride, sulphates, manganese, fluorides, and radionuclides. When these compounds are dissolved in water, they pollute it, the organic molecules and natural compounds run off directly to aquatic environment (Mukhopadhyay *et al*., 2005). Volcanic activity, wild fires, and cosmetics particles are important natural sources of pollution; these are releasing CO_2, H_2, H_2O, HF, CO, CH_4, CS, CS_2, HCl, H_2S, and SO_2, as well as many hazardous metals such as lead, cadmium, gold, and mercury (Das *et al*., 2014).

Anthropogenic origin

Anthropogenic origin is a main cause of pollution in the environment. Toxic metals are originated particularly in effluent from the industries like dyeing and pigmentation, metal polishing, electroplating, leather, and mining sectors (Naidu and Bolan, 2008). As a result of agricultural practices such as fertilizer and insecticides, metals were released into the environment. Apart from that, roadways and automobiles are significantly contributed to the environmental load of metals such as lead, cadmium, and arsenic (Sun *et al*., 2001).

Fate of metals

Metals are discharged into many ecosystems due to both natural and anthropogenic origins. The ecosystems are generally categorized into two types: terrestrial ecosystems and aquatic ecosystems (Slaveykova and Cheloni, 2018). Metals pollute water channels, sediment, and biota when they were generated from both natural and man-made sources because they are toxic to the environment and leach into aquatic medium. Metals invading and assembling in living organisms that found in the aquatic biota via bio-concentration and bioaccumulation through the process of food chain, and will become hazardous when accumulation exceeds (Huang, 2003). Major effects on human health result from the contamination of water and other living things with potentially hazardous metals (Ali *et al*., 2019).

Seafood as a source of metal transfer to humans

The coastal community's consumption of seafood is a significant source of toxic metals. Despite the fact that the coastal environment is regarded as an appropriate site for dumping various types of human waste, this is likely to result in ocean pollution and a significant health hazard from seafood consumption. Fish are the most important life forms in the marine food web because they are sensitive to metal contamination and can serve as biological indicators of the hazard assessment of aquatic ecosystems, which has a greatest influence majorly on the food web (Chi *et al*., 2007) as the same time fish are one of the main sources of protein food all over the world (Mansour and Sidky, 2002). Fish have been shown to acquire metals through the consumption of colloidal matter, dietary items, and the continuous ion exchange process of dissolved metals along lipophilic transporters such as gills, as well as the absorption of trace metals on tissues and membranes (Oguize and Okosodo, 2008). As a result, metals bio-accumulation is a primary pathway for raising pollution levels, that are then passed via the food web and impact the human population.

Metals and their significance

Metals have a wide range of uses and play a significant part in our industrialized world. Some metals have key biochemical and physiological activities in living organisms, and their lack or excess can cause metabolic disruption and, as a result, a variety of chronic diseases. Life cannot live without certain metals and metalloids. Since they may be found in biomolecules like enzymes, which catalyze biological events in living creatures, they play crucial biochemical and physiological roles in the human body. (Ali *et al*., 2019).

Essential and nonessential metal

Based on their roles in living things, metals are divided into essential and non-essential categories. For basic metabolic processes to take place in living things, essential metals may be needed at extremely small amounts. Non-essential metals (NEMs) are high-risk pollutants that can affect both human health and the environment. Mn, Fe, Cu, and Zn are examples of essential metals, whereas Hg, Pb, Cr, Cd, and As are toxic and considered nonessential metal (Ramírez, 2013; Türkmen *et al*., 2009).

Purpose of the current research

In India, especially in southern regions the MoEF&CC, GoI recognized polluting industrial parks in the nation based on the CPCB's comprehensive environmental pollution index (CEPI) score. Likewise, in Tamil Nadu, Vellore (Ranipet), Cuddalore, Manali, and Coimbatore have been categorized as severely contaminated areas with CEPI scores greater than 70, while Tiruppur, Mettur, Erode, and Tuticorin have been categorized as highly polluted areas with CEPI scores between 60 and 70 (TNPCB, 2020). Cuddalore (11°40'58.0"N 79°45'11.1"E) district has a land cover of 3,678 km2, which includes a 68-km stretch of coastline. Only the Uppanar estuary, which runs along Cuddalore city and eventually empties into the Bay of Bengal, receives many drainages and industrial waste channels. In this context, we aimed to assess the fate of metal accumulation on the Cuddalore coast. We analysis the source, seasonal variations, accumulation, and effect on human reproductive health. Nowadays, metal exposure is increasing a day by day, and as a result, several emerging diseases will emerge; however, the few examples of metal-causing diseases that we have are Minamata and Itai Itai diseases. Subsequently, researchers are focusing on nephrotoxicity, cardiac toxicity, and carcinogenicity caused by metal, however, the reproductive toxicity studies are insufficient. The possible source of cadmium in Cuddalore is higher due to agricultural runoff in the form of fertilizer and pesticides, corrosion-resistant plating industries, stabilizers in plastic production industries; these industries are more common on the Cuddalore coast. We assume that this cadmium will spread through the food chain and cause infertility in humans therefore, the zebrafish animal model was used to test this hypothesis.

Metal chosen for current research

Among the 119 elements in the periodic table, there are 92 metals, 20 nonmetals, and 6 metalloids. Many metals, especially lead (Pb), mercury (Hg), chromium (Cr), cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), and arsenic (As), have been proven to be hazardous even at low doses and over the permissible limits. Toxic metals are those that are dangerous to the environment and humans, they are Pb, Cr, Ni, Zn, Cu, As, Hg, and Cd (Huang *et al*., 2008). Based on their source of metals, we selected four of the eight toxic metals, including two non-essential metals, Cd and Pb, and essential metal, Cu and Zn.

Cadmium properties, uses, food source and human effects

Cadmium (Cd) is the 64th greatest abundant metal, with a density of 8.69 g/cm3 and a silvery bluish tint. It is commonly detected in combined with zinc and is used in fertilizers, pesticide residues, nickel-cadmium capacitors, glass containers pigmentation, corrosion-resistant plating, plastic production stabilizer, and nuclear reactors. Dietary sources include shellfish, mussels, dried seaweed, shrimp, and mushrooms. When cadmium builds up in people, the major consequences occur: Infertility due to biological system failure, calcium metabolism changes, psychological disorders, gastrointestinal disorders, central nervous system complications, immune response deficiencies, genetic material (DNA) impairment, tumor, Itai-Itai disease, which has already been noted to be genotoxic and ecotoxic in species of animal (Briffa *et al*., 2020).

Lead properties, uses, food source and human effects

The density of lead is 11.3 g/cm3, and dull silver-grey metal, 37th a mineral ore called galena, which is made up of lead sulphide and may be coupled with silver, zinc, and copper. It contains the most abundant metal, which is commonly found in association with zinc. Additionally, it is utilised in corrosive liquid canisters, sporting goods, diver weight belts, insecticides, lead-acid batteries, lead-glazed pottery, beauty products, computer monitors that shield against radioactivity, lead-acid capacitors for cars, lead-acid batteries, lead-acid piping, projectiles, ammunition, lead crystal glass, cable sheeting, and sports equipment. The food groups that make up a diet include fruits and vegetables, grains, seafood, red meat, alcohol, and soft drinks. When lead accumulate in humans, the following consequences occur: Miscarriages, Hypertension Stillbirths, premature and low births renal dysfunction, injuries to the brain, pica, abdominal ache, damage to the peripheral nerves, iron insufficiency as a result of hemoglobin synthesis interruption impaired cognition among children. The way the brain and central nervous system grow has been altered. Reduced intelligence, worse educational performance, reduced attention span, and an increase in antisocial behaviour (Briffa *et al*., 2020).

Copper properties, uses, food source and human effects

The density of copper is 8.96 g/cm3, and Reddish-gold metal, 26th the most common metal is usually found among minerals like chalcopyrite, which contains copper, iron, and sulphur, and bornite, which is also known as peacock ore and contains copper, iron, and sulphur. Also utilised in copper cables, plating, coins, pipes, organic manure, wood protection, fabric retention, barrier lotion, chemical analysis for sugar detection in Fehling's solution, copper sulphate used as an algicide in water treatment, and copper sulphate to control mildew in agriculture. Copper is also used in copper alloys also including bronze and brass. Oyster, shiitake mushrooms, spirulina, lobster, nuts and seeds, dark chocolate, leafy greens, are the dietary sources. When copper accumulate in humans, the following consequences occur: Metal fever presenting itself with flu-like symptoms, includes vomiting, diarrhoea, dizziness, irritation of the eyes, kidney disease, irritation caused in the mouth cavity, anxiety, insomnia, speech impairment, agitation, Wilson's disease, difficulty in swallowing, which indicates that copper was not eliminated through bile, but rather collected in the organs (Briffa *et al.*, 2020).

Zinc properties, uses, food source and human effects

The density of zinc is 7.134 g/cm3, and blue tinge found with silvery-white metal with, 24th abundant metal, found frequently among minerals known as the peacock ore includes iron, chalcopyrite containing copper, sulphur, bornite also containing copper. The combination of zinc oxide used in as cosmetics, paints, deodorants, soaps, weapons, anti-dandruff shampoo, batteries, electrical equipment, ink, plastic, textiles, rubber, pharmaceutical products in the combination of zinc sulphide as luminous paint, X-ray screens, fluorescent lights. Beef, lamb, herring, sunflower seeds, and cheese, are the dietary sources. When copper accumulate in humans, the following consequences occur: stomach cramps, nausea and vomiting, stomach cramps, cholesterol, stomach cramps, anaemia, fatigue, pancreatic complications, copper deficiency, epigastric pain, neutropenia, impaired immune function (Briffa *et al.*, 2020).

Human hair and nail as biomarker

Metals may easily accumulate in the human body through a variety of routes, but the most common or massive accumulation occurs through dietary consumption via food chain (González-Muñoz *et al.*, 2008). In this perspective, the determination of metal concentrations in human is important. Hair, nail, blood, urine, and breast milk are now commonly used biomarkers in the research of metal pollution hazards to human health (Dursun *et al.*, 2016; Qin *et al.*, 2015; Sani and Abdullahi 2017; Were *et al.*, 2008). However, hair and nail are regarded as an ideal noninvasive resource for assessing the metal concentration in the human body, not only because it has a strong attraction to metals (Morton *et al*., 2002; Sukumar and Subramanian, 2007).

Environment and its reproductive health impact

According to scientific evidence, early life exposure to toxic chemicals is directly associated to an increase in the disease rate of humans. Asthma, breast and prostate cancer, cognitive and behavioral issues, Parkinson's disease, hormonal changes, obesity, infertility, and also some other disorders have grown the most in the past twenty years (Novikova *et al*., 2008; Perera *et al*., 2009). Over 80,000 chemicals in use, and they can cause environmental pollution as they disintegrate and persist in the air, soil, water, consumer goods, food, and residential waste (Brumovsky *et al*., 2017). Prenatal exposure to these environmental contaminants, which are now widely used, have been related to a greater range of unfavorable reproductive health impacts on future generations. The dysregulation of hormones that control healthy development of human reproduction causes significant adverse health consequence of widespread exposure to environmental contaminants (Di Renzo *et al*., 2015). Endocrine disrupting substances found in the environment include pesticides, phthalates, bisphenol A, plasticizers, polyhalogenated compounds, alkylphenols, dioxins, furans, and metals (Encarnação *et al*., 2019).

Causes of infertility

Infertility can be caused by a wide variety of factors among male and female reproductive systems. Even though, explaining the causes of infertility is not a single specific reason. Infertility as in reproductive system of female have also been induced by: abnormalities found among tubal region, such as fallopian tubes obstruction, were caused by uncontrolled Sexually Transmitted Infections (STIs) or consequences of unsafe abortion, ovarian abnormalities such as polycystic ovarian syndrome and various follicular disorders. Among the reproductive system of male, infertility can also be caused by: Obstruction of the reproductive system, testicular sperm production failure, resulting in abnormal ejection of sperm, caused by medicinal therapies that damage sperm-producing cells or varicoceles, and causes issues in function and quality of sperm (Gore *et al*., 2015; Rutstein and Shah, 2004). Apart from that, environmental and lifestyle characteristics like obesity, smoking and alcohol usage, can also have an impact on fertility factor. Furthermore, the contaminants present in the environment and chemicals that directly hazardous to gametes (eggs and sperm), subsequently reduces quantities and poor quality, eventually causing to infertility (Segal and Giudice, 2019). Several environmental pollutants may possibly play a role in infertility, however evidence on metals causing infertility is limited. Various metals have the potential to induce infertility in living organisms, with cadmium being the most possible source (Benoff *et al*.,1997).

Cadmium caused infertility

Each metal causes different disorders, but most possibly cadmium may induce infertility. As a result, the current research concentrated on cadmium-induced infertility. Fertility is defined as "the specific reproductive outcomes achieved, a couple, a group, or a population", and therefore infertility is described as the failure to reproduce (Savitz *et al*., 2002). Among the four metals chosen, all metal accumulation has a negative effect on human health. Cd can accumulate in humans over up to 40 years and induce major health issues such as infertility. The current research uses zebrafish as an animal model to demonstrate that continuous cadmium exposure can induce infertility in humans.

Zebrafish animal model

Since the 1960s, the zebrafish (*Danio rerio*) has played a growing role in scientific studies. It possesses several features that make it an excellent model for understanding human genetics and diseases (Goldsmith and Jobin, 2012). The genetics of zebrafish is close to that of humans. We share 70% of our genetic material with this animal and 84% of genes related with human disease. The zebrafish, being a vertebrate, possesses the same primary organs and tissues as humans. Their muscles, blood, kidneys, and vision have significant similarities to human systems. Zebrafish might be employed in environmental monitoring studies and several evaluations of contaminant, includes organic pollutant assessments, hazardous metals, and endocrine disruptors (Goolish *et al*., 2000). By monitoring the activity of an enzyme or the expression of a gene in wild-type embryos or adult zebrafish, contamination of metals in the aquatic environment may be indirectly detected. Zebrafish might be used as a model animal to identify endocrine disruptor concentrations in aquatic environments as well as to assess the damaging impact these substances have on the nervous and reproductive systems (Dai *et al*., 2014). Therefore, utilizing zebrafish as an animal model, the current study has focused on the destiny of metal accumulation to humans from the Cuddalore coast and its detrimental influence on human reproductive component.

Chapter II

Review of Literature

Massive scientific and technological progress, which has resulted in fast urbanization, industrialization, and population increases, has led to qualitative and quantitative degradation of water, sediment, and biota in the environment. Metals generated in the terrestrial ecosystem eventually reach the coastal environments, and metal contamination occurs in the coastal ecosystem as well. This chapter contains an overview of previous scientific work on metal contamination of coastal water, sediment, biota, human hair, nail, and zebrafish animal model studies with relation to Cd, Cu, Pb, and Zn, and the risk they imply on human health.

Aquatic ecosystems occupy over two-thirds of our world and play an important role in climate stabilization while also providing several advantages to a rapidly growing human population. Anthropogenic activities, on the other hand, are having an increasingly negative impact on aquatic habitats. The major contamination factors in the aquatic environment include sewage, fertilizers and terrigenous materials, crude oil, heavy metals, and plastics (Häder *et al*., 2020). Environmental impacts have a considerable influence on aquatic ecosystems that are also affected by climate change, urban and recreational growth, and unethical use of aquatic resources. Water contamination by agricultural runoff, industrial wastes, sewage drainages, and improper waste disposal has negative effects on marine and freshwater species (Verhougstraete *et al*., 2015).

Coastal ecosystem is considered to be the storehouse for commercially significant flora and fauna. However, deterioration process has severe consequences against living and non-living resource, as well as humans. Enormous amounts of nonessential elements are present in the coastal environment originating from industries and human activities that are uncontrolled (Rajaram and Ganeshkumar, 2019). The biota in the estuarine ecosystem is extremely effective in productivity owing to the large amount of nutrients obtained from river discharge. One among the most significant functions of the estuaries is that it acts as a nursery bed for several newborn marine species, resulting in a population group of flora and fauna that are well-adapted to living in coastal habitats (Lakshmanasenthil *et al*., 2013).

Many works have been conducted to monitor the various parameters of water and sediment along with the aquatic life in different aquatic ecosystems all over the world. Various attempts have been made to study the different components of water bodies at selected locations in India also. Coastal zone is vital for a variety of important biological processes. However, due of vast human populations and interactions between coastlines, it is also vulnerable to environmental changes (Crossland *et al*., 2005).

Zhao *et al*. (2018) estimated the heavy metal concentrations from the surface water and sediments collected in the Sheyang Estuary and evaluated their source attribution and associated environmental hazards. The relative concentration of heavy metal present in the sediments was categorized as follows: Zn > Cr > Cu > Pb > As > Cd > Hg. Except for Hg and Cr, heavier metal average concentrations in sediment were higher than their background values, despite the fact that heavy metal levels in surface water seemed lower.

A qualitative and the quantitative study has been conducted by Zhao *et al*. (2012). The concentration of metals in crab legs and head, and skin of fishes exhibited strong connections with those in respective biological habitats. *Collichthys lucidus* functioned as a highly reliable ecological indicator for determining metal concentration in water. By predicting daily consumption and targeting risk quotients assessments revealed that there were no major health risks involved.

A study by Liu *et al*. (2016) on sediment samples (30 sites) collected from the Yangtze River Estuary (YRE) assessed the risk factor against the geographical and ecological dispensation patterns of heavy metals. According to I_{geo} and risk index, arsenic, chromium, and cadmium were considered to be the principal pollutants. Moreover, the most polluted region was around the mouth of the river (YRE's south branch). Mn emerged as the most significant contaminant, whereas As did not contribute much to YRE contamination.

Li *et al*. (2019) conducted a study on the correlation of heavy metal contamination and sediment deposits found in various estuarine aquatic habitats. The primary goal of their research was to evaluate concentrations of heavy metals and the geographic distribution patterns of surface sediment samples obtained from 20 stations in the Yangtze River estuary during 2012 to 2016. The findings revealed that a significant decreasing tendency in the proportion of these heavy metals as distance from the coastline increased. Muddy regions often had a greater accumulation of heavy metals. The principle component analysis (PCA) and factor analysis (FA) have identified significant components in which three different groups of contaminants were identified Also, the sources of metals and the spatial and temporal differences were directly impacted by environment and man-made activities in accordance with seasonal variation.

Liu *et al*. (2011) worked on sediment samples obtained from the coastal zone in Pearl River Estuary's near Qiao Island. They evaluated for cumulative concentration of metals, chemical partition, and physico-chemical parameters. The use of geoaccumulation index to calculate the contamination of heavy metal in the sedimentary sample revealed that Cr, V, Be, Se, Sn, and Tl were pollution-free, but Cu, Ni, Pb, Zn, Cd, and Co have been contaminated to varying levels throughout the samples. It was fascinating to see various levels of pollution among the metals ranging from medium (for Cu, Pb, and Zn) to high (for Cd) in the top 45 cm of the profiles. The potential sources of Cu, Pb, Zn, and Cd were associated with greater residential and industrial sewage water discharge, agricultural runoff, earth's atmospheric inputs, and surface runoff from upstream mining or metal processing activities, which may also be related with the Pearl River Delta region's sustainable growth over the last decade.

In a study by Castillo *et al.* (2013), the concentrations of metals present in the sediment and water around Málaga Bay had been examined (South Spain). Coastal pollutants have been sourced from ancient anthropogenic sectors, as well as modern urbanization and coastal habitat development, leisure and tourism, and facilities of wastewater treatment. Bio-availability of metals based on their magnitude was as follows: Cd > Ni > Pb > Cu > Cr. The water samples collected from the sea show that the concentrations of cadmium and lead have been the major problematic metals, because these metals violated the Continuous Criteria Concentration (CCC) of US EPA (United States Environmental Protection Agency).

Based on largescale sample data, the spatial-temporal fluctuations in metal content and health risk of dissolved heavy metals (eight) were recorded in the Yangtze Estuary over a five-year period by Yin *et al.* (2015). Differentiation of metal sources received special attention. The order of metal concentrations was as follows: Zn > As > Cu > Cr > Ni > Pb > Cd > Hg. However, hazard quotient indices were clearly split into three gradients. Source of metal correlations and hierarchy cluster analysis findings were in good agreement with spatial-temporal pattern observed. Cu and As in the estuary came mostly from Yangtze River runoff. Under the influence of the salt-out effect, Cd and Cr contamination were tightly linked to sediment release.

Abdolahpur *et al.* (2013) analyzed the metal concentration (Co, Cd, Ni, Pb and Cu) in sediment samples collected from Musa Estuary. The heavy metal concentration order in sediments was as follows: Ni > Co > Cu > Pb > Cd >. Cd and Ni concentrations in the collected fish samples were as follows *Johnius belangerii* > *Euryglossa orientalis* > *Liza abu*, whereas the levels of Cobalt and Copper were *L. abu* > *E. orientalis* > *J. belangerii* and *E. orientalis* > *L. abu* > *J. belangerii*. Apart from Nickel concentration found in the *J. belangerii* species, reverting analyses indicated that there are insignificant associations between the concentration of metal among the tissues obtained from fishes and soil samples. The amounts of the metals examined in fish tissues were less than the FAO, WHO, and EC acceptable limits.

Caçador *et al*. (2012) proposed numerous benthic macro-invertebrates and some species of fish as bio-indicators for pollution of heavy metal in the Seixal Bay, (Tagus estuary, Portugal). During four selected sampling campaigns, 266 specimens of 16 taxa were collected, and the heavy metals (Cr, Pb, Cu, Ni, Cd, Zn, and Co) concentrations on fish tissues were measured, including full soft bodies of invertebrates, as well as suspended particle matter found in the aquatic ecosystem and soil sediments. Based on the results, they suggest that *N. diversicolor, P. serratus, C. maenas, S. plana,* and *Pomatoschistus* sp., might be employed as bio-indicators of heavy metal contamination in sustainable environment in future programs.

Zohra *et al*. (2016) investigated the heavy metals in soil and fish samples from southern coast of Sfax, Tunisia. Copper, Lead, Mercury, Iron, Nickel, and Cadmium were among the heavy metals investigated, as well as one metalloid, Arsenic. The concentrations of the metals tested in sediment samples violated the quality-control requirements (SQGs). According to the Potential Ecological Risk Index (PERI), the examined region may be posed by a moderate risk to aquatic life. Bioaccumulation of metal in fish tissues differed across species. Target Hazard Quotients (THQ) of particular heavy metals in fish samples indicated tolerable values for direct human utilization.

Despite numerous advantages of eating aquatic foods, hazardous metal bioaccumulation in fish can increase the health risk for humans (Ahmed *et al*., 2019). Heavy metal concentrations were noted in edible tissues of commercial fish species including *Otolithoides pama, Latis calcarifer, Tenulosa ilisa, Rhinomugil corsula, Planiliza subviridis, Silonia silondia, Clupisoma garua, and Aila coila* in the Meghna River estuary. According to this research finding, to safeguard customers from health hazard, the discharge of harmful materials should be verified in a suitable monitoring process. Veerasingam *et al*. (2015) investigated the amounts of seven trace metals in three sediment cores to determine metal depositional patterns and pollution levels in the Mandovi estuary. The estuary is moderately contaminated with Lead, but unpolluted-to-moderately polluted with iron, manganese, copper, chromium, cobalt, and zinc as per the values obtained from the geoaccumulation index (I_{geo}).

Kumar *et al*. (2011) stated that heavy metal contamination is of special concern since it has negative impacts on biodiversity in the Cochin estuary and two adjacent rivers due to involvement and deposition in different trophic levels. According to the research, heavy metal concentrations were reported to be excessive in the Periyar River near the industrially developed areas, as well as in the pre-monsoon season. Chemical plants release their waste into the Periyar River, which flows into the Cochin estuary.

The concentrations and distribution patterns of trace metals from sediment samples collected from the Cochin backwaters were examined across two seasons. The man-made input of industrial, household, and agricultural effluents is primarily responsible for trace metal enrichment, which is exacerbated by metal settling owing to flocculation precipitation (organic and inorganic) associated with variations in salinity. Sediment samples were classified as slightly polluted with copper and lead, mildly-to-heavily polluted with zinc, and heavily-to-extraordinarily contaminated with cadmium based on the degree of contamination (Martin *et al.*, 2012).

According to Shynu *et al.* (2012), metal contaminants may have negative effects on benthic creatures. In their work, Mn showed substantial-to-heavy pollution in all the seasons. During the monsoon season, chromium, nickel, and zinc showed minor contamination, and during the post-monsoon season, only chromium contamination was higher. The main trace metals were predominantly impacted by a combination of river flow, resuspension, Fe–Mn particle releases, and seasonal pollution induced by human activities.

Srichandan *et al.* (2016), investigated different metal concentrations in seawater and zooplankton at Rushikulya estuary in northwestern Bay of Bengal. Indonesian seas have become major components of the world ocean environment system due to their unique features, as well as their placement between the Pacific Ocean and the Indian Ocean. For marine cycling and sea surface temperature level, mass and temperature flows from the Pacific into the Indian Ocean are critical (Firdaus *et al.*, 2015).

Certain trace metal concentrations have been recorded from the Brahmani, Baitarani, and Dhamara river complexes, as well as the Dhamara estuary. A quantitative technique was developed using several risk factors such as enrichment factor, geoaccumulation index, and pollution load index. Oxides or hydroxides of Fe–Mn appear to have an essential part in scavenging activity of metals in the riverine region, but not in the estuarine zone, according to statistical factor analysis (Asa *et al.*, 2013).

Mohan *et al.* (2012a) conducted a study in the Cochin estuary, which is one among the most contaminated aquatic environments in India's southwest coast. The research examines the presence of heavy metals (Zn, Cd, Pb, and Cu) in the sediment samples collected from Periyar River and Cochin estuary. According to the results of sequential extraction, cadmium concentration was higher in the first weakly coupled fractions (exchangeable and carbonate bound) than in the remaining and organic bound fractions.

According Venkatramanan *et al.* (2015), risk-assessment code analysis and environmental indices reveal that cadmium contaminated the sediments significantly. At the Coleroon river estuary in Tamil Nadu, India, environmental risk management activities were conducted to explore the pollution sources and ecological concerns of surface sediment samples. The massive quantities of heavy metal concentrations found in the surface sediment samples were detected in the following descending order: Fe > Mn > Zn > Cu > Pb > Cr > Ni > Co.

Suja *et al.* (2017) studied the distribution patterns of numerous heavy metals from surface sediments of Surat’s Tapti Hazira estuary. Their analysis indicated that Cd contamination ranged from mild to severe, Pb, Zn, and Cu contamination ranged from moderate to severe, while Co and Cr contamination ranged from severe to aggressive. Trace metals were analyzed during the different seasons, including premonsoon, monsoon, and postmonsoon seasons to conclude on the estuarine processes and source. Both natural and human-caused alterations in the river were observed.

In a study by Sulieman and Suliman, (2019), metal contamination was reported in three aquatic species (*Mugil cephalus, Penaeus indicus,* and *Portunus pelagicus*) obtained from the Vellar and Uppanar estuaries. When compared with marine species gathered from the Uppanar estuary, the marine species recovered from the Vellar estuarine had much lower quantities of heavy metals. Cd levels were found to be extremely low in tissues taken from the specimens.

De *et al.* (2010) analyzed the muscles of many major marine fishes from in and around the Hooghly estuary coastal region for heavy metals such as Cu, Ni, Cr, Cd, Zn, and

Pb. The toxicity of metals (Pb and Cd) varied more than that of important metals (Cu, Zn, and Ni) found in that biota. According to Karthikeyan *et al*. (2020), metal concentrations reported in biota, water, and sediment samples obtained from the Ennore estuary were investigated regionally over a two-year period to identify their possible influence and environmental threat. Copper, chromium, lead, nickel, and cadmium concentrations present among the water, sediment, and aquatic life differed. According to EQI, the Ennore estuary was heavily polluted by Cd and Pb from anthropogenic sources.

Jayaprakash *et al*. (2015) reported the accumulation of heavy metals in water and sediment samples (n=20), as well as six fishes from different feeding guilds (*Gerres oyena, Liza parsia, Etroplus suratensis, Sillago sihama, Arius parkii, and Oreochromis mossambicus*) from Ennore creek in Chennai. Similarly, Velusamy *et al*. (2014) collected seventeen economically important marine fishes from Mumbai Harbor and analyzed heavy metals using AAS and ICP-OES. Although, diverse heavy metal concentration was found in the fish specimens collected, the metal levels of toxic trace elements in the edible fishes studied in this study were far below the threshold levels suggested for human consumption.

Mohan *et al*. (2012b) indicated in their study that heavy metals accumulated in the aquatic environment might endanger the health of the biota. Some of the common edible fishes (*Arius arius, Mugil cephalus, Etroplus suratensis,* and *Lutjanus ehrenbergii*) were gathered from the backwaters of Cochin, Southwest India, and tested for Hg and other heavy metals (Cd, Zn, Cu, and Pb). As a result, the study brought attention to the possibility for heavy metal bio-accumulation in fish, which are a major group of species in this saline water habitat.

Achary *et al*. (2017) assessed heavy metal concentrations in water, sediment, zooplankton, and fish in Kalpakkam's coastal zones to help us understand their distribution and bioaccumulation patterns. Metal concentrations in plankton were significantly greater than in water, soil, or fish. The amount of heavy metal content in the fish species studied were low enough that the fish tissue could be consumed. The findings of this investigation showed that cadmium and manganese levels in both stations are much beyond permissible standards, posing a risk to human health. As per their suggestion, substantial research is required to examine the potential biota risk factors for concentration of heavy metals in the area of estuarine ecosystem.

Sundaramanickam *et al*. (2016) reported the contamination of heavy metal in surface sediments from several habitats, including the Vellar-Coleroon estuary, the Pichavaram mangrove, and the coastal area of Parangipettai in India's Southeast coast. The level of heavy metals was found to be highest in the mangrove environment, showing that the abundant organic matter works as a powerful metal binding agent. The current study demonstrated that sediments from the whole Vellar-Coleroon estuarine and Pichavaram mangrove habitats were moderately contaminated with Cd.

The research work of Rajaram *et al*. (2020a) focused primarily on the disposal of heavy metals (Cd, Cu, Pb, and Zn) in economically significant finfish and shellfish gathered from the Tuticorin coast of the Gulf of Mannar in Southeastern India. The findings of the study have raised public awareness regarding heavy metal pollution in marine seafood. The study concluded that continuous ecosystem monitoring is critical to the preservation of the pristine environment and the quality of the seafood. Similarly, the heavy metal concentrations in the environment, water, and sediment samples of the Ennore estuary were regionally monitored over a two-year period to better understand their possible impact and ecological danger (Karthikeyan *et al*., 2020).

According to Thiyagarajan *et al*. (2012), the levels of hazardous metals (cadmium, chromium, and manganese) present in Cuddalore and Mudasalodai were much higher than acceptable limits. Chromium, cadmium, mercury, and manganese concentrations in *C. arel* were over the permissible limit, which is compatible with their environment and diet. Heavy metal concentration in the research area was varying in accordance with environment season and geographical settings. The concentrations of heavy metal present in water and sediment samples from the research region were greater during the monsoon season in comparison with other seasons.

Mathivanan and Rajaram (2014) investigated the heavy metal contamination in Cuddalore coastal area based on the anthropogenic sources as a result of dispensing of waste from industries, agricultural activities, and urban wastewater into estuarine regions, which transports the wastes into coastline during tidal action. Also, Kesavan *et al*. (2013) reported that the heavy metal concentrations found in sediments, shells, and tissues of the mollusks (*Meretrix meretrix, Crassostrea madrasensis,* and *Cerithidea cingulata*) collected from two sites in Uppanar Estuary in India's southeast coast. The concentrations of Zn and Cu were not concerning. However, Mg content was observed to be greater in shell and tissue in the investigation. The Fe concentration of sediment was found to be greater in both locations, yet it was the second highest metal observed in shell and tissue. Cd and Co were the least accumulating metals in sediment and animals.

Juberg *et al*. (2008) stated that analytical developments had made it feasible to quantify trace amounts of both natural and synthesized chemicals in human tissue and bodily fluids. Biomonitoring helps the researcher to estimate the extent to which humans have been affected to certain chemical pollutants, as well as how exposures vary considerably, more accurately than before. Hair was used as a biomarker to assess human dietary habits. A thorough review of the relationship between the mineral content of hair and physiological disorders was explored by Wołowiec *et al.* (2013).

Heavy metals are mostly transferred to people, animals, and plants through diet, water, and sediment. Since metal are non-biodegradable, they remain in the environment continuously and generate major ecotoxicological issues (Leblanc *et al.*, 2000). Urinary and blood samples might be used as biomarkers of rapid, instantaneous exposure, while human hair or nails might be employed as indicators of prolonged exposure (Chojnacka *et al.*, 2010).

Marcinek-Jacel *et al.* (2017) focused to determine the effect of several factors on the level of mercury in human hair samples, such as gender, age, seafood diet, hair colouring, and smoking habits. The study included 444 samples (102 men and 342 females) gathered from the community of persons residing in the Lodz region (central Poland). Chojnacka *et al.* (2005) collected hair specimens (n = 83) from residents of Wrocaw, situated in South Silesia, during 1996 and 2003 from both urbanized and industrialized regions. Inter-element relationships were investigated using correlations among two elements and regression analysis. A report of Liang *et al.* (2017) on human hair from various age groups, as well as dietary samples, were obtained in Beijing, China. The metals in enquiry – Cr, Pb, As, and Hg – were examined, and metal levels were compared with age, gender, and food consumption.

Grashow *et al.* (2014) worked on forty-eight participants using biomarkers and detected transitional exposure periods in populations exposed to high level of metals. They wanted to assess whether there was a link between toenail metal levels and previous 12-month job activity in welders who had varied, metal-rich welding exhaust exposure, while

Laohaudomchok *et al*. (2011) examined the effect of nails as a marker of Mn exposure in a population of steel workers and welders with varying exposures.

Salcedo-Bellido (2021) suggested that sociodemographic factors such as age, sex, socio-economic status, body mass index (BMI), lifestyle detriments, smoking status, and environmental contaminants are the causes of metals in nail samples. Parizanganeh *et al*. (2014) have used human nail used as a biomarker to understand the heavy metal contamination in Dizajabaad, Zanjan Province in Iran.

Sera *et al*. (2002) attempted to resolve worldwide environmental challenges which have been effectively applied to many types of bio-samples. A technique for unprocessed hairs has been used in many polluted places to assess human exposure to harmful substances. Apart from hair, nails are believed to provide vital information regarding human exposure to hazardous elements.

Janbabai *et al*. (2018) investigated the trace elements in the nails and hair of people with stomach carcinoma, and the results indicated that an increase in trace elements might be a possible marker for cancer development and origin. Rashed *et al*. (2007) reported that human materials are commonly employed as a bio-indicators for heavy metal contamination, and human hair and nails are examples of this.

Cadmium is a natural element found in the earth's crust. It is usually associated with other metals, but due to the impacts caused by human activity, its concentration has increased in the aquatic environment. This metal may damage aquatic animal reproduction, decreasing the rate of fertilization of organisms such as fish (Acosta *et al*., 2016). Cadmium bioaccumulates in phytoplankton and complex food webs involving aquatic animals like mollusks, fish, and crustaceans (Cardoso and Chasin, 2001). *Danio rerio*, zebrafish, has been widely utilized as an experimental model due to its desirable characteristics such as its tiny size, which allows for easier handling, and its quick absorption of chemicals (Spence *et al.*, 2008).

D. rerio, like most teleost fish, employs external fertilization; male and female gametes are discharged into the aquatic environment and must come into contact for fertilization to occur (Coward *et al.*, 2002). *D. rerio* is a freshwater tropical fish. It was formerly a well-known aquarium fish at home, but it has quickly evolved into an invaluable animal model for today's biologists. The various benefits and qualities of this little animal have never failed to entice academics to use it as an animal model in their scientific research endeavors. Perhaps, the popularity of this animal is due to its low cost and ease of care in the laboratory (Darrow *et al.*, 2004; Zhao *et al.*, 2011).

Comprehensive analyzes of biochemical biomarkers, histopathological observation, and functional gene expression firstly demonstrated that the presence of microplastics (MPs) enhanced the toxicity of Cd on zebrafish and the combined exposure caused oxidative damage and inflammation in zebrafish tissues. Collectively, the results highlight the chronic effects of combined exposure to MPs and heavy metals (Lu *et al.*, 2018).

Cadmium has been linked to a variety of harmful consequences in aquatic creatures as a result of waterborne exposure. The adult zebrafish (*Danio rerio*) was used in an in vivo investigation to explore Cd toxicity over a wide range of doses, from ambient to toxic (Renieri *et al.*, 2017). Zebrafish has been used as a vertebrate model for Cd toxicity studies; however, the majority of the literature focuses on its early life stages (embryo and larvae), and just a few research papers have employed adult fish. The primary acute toxic impact of Cd in zebrafish larvae and adults appears to be ion loss, particularly Ca^{2+} and Na^{+} (Alsop and Wood, 2011).

The effects of maternal Cd exposure on female zebrafish (*Danio rerio*) and their progeny were detected. Cd interfered with fertility and other reproductive functioning in females. It slowed the gamete formation and growth and affected gene expression in their embryos. There was a favorable connection between Cd levels in female ovaries and Cd treatment dosages ranging from 0 to 8.9 μM. When females were exposed to 8.9–35.6 μM of Cd over 72 h, their mating rate reduced by 60% compared to the control group. Growth of maternal Cd embryos were delayed by one somite stage compared to control embryos, which develop at the sixth somite stage (Wu *et al*., 2013).

According to Szczerbik *et al*. (2006), the maximum dose of dietary Cd (10 mg/g) considerably reduced the gonadosomatic index in goldfish, while it had no effect after 0.1–1.0 mg/g of Cd exposure. According to Fraysse *et al*. (2006), the hatching process in zebrafish is not synchronized and there is a large variance in the hatching rate. Kimmel *et al*. (1995) also reported that zebrafish embryos that had not been exposed to Cd appeared to have varied hatching rates as well. Another study discovered that dietary Cd adaptation had physiological consequences in the form of renal impairment (Chowdhury and Wood, 2007).

Chapter- III

Aim of the research

3.1 Scope and objective

Metal pollution is a worldwide issue; hence most global researchers are focusing on this topic. Consequence, many researchers have concentrated on environmental monitoring and assessment of metal pollution, and although remedial aspects are limited, monitoring the fate of metal pollution and its influence on human health, with a special emphasis on reproductive health, remains unexplored. As a result, the current study examines how metals (Cd, Cu, Pb, and Zn) are transported from marine food source to human via food web in the Cuddalore coastal environment by analyzing water, sediment, fish, and human hair and nail samples. The cadmium in the chosen four metals has the potential of causing infertility in vertebrate organisms; this hypothesis was further tested using a zebrafish animal model. Now with factors stated above in consideration, the next objectives were validated.

- Identification of the source of metals in water and sediment from Cuddalore coastal environments with respect to different seasons.
- To investigate the accumulation of metals in locally captured and often consumed finfish and shellfish
- To assess the metal concentration in human hair and nail samples as biomarkers
- To determine the level of health risk caused by metal accumulation in sediment and fish samples.
- In order to understand the reproductive toxicology of cadmium, the zebrafish animal model was used.

3.2 DESCRIPTION OF THE STUDY AREA

Cuddalore district has a land cover of 3,678 km2, which includes a 68-km stretch of coastline. Multiple drainages and industrial waste channels drain exclusively into the Uppanar River which flows along the Cuddalore city and finally drains into the Bay of Bengal. The Uppanar estuary is approximately 5-km long with an average width of 30 m and a mean depth of about 2.5 m. The study area has an average elevation of about 1 m above the mean sea level. Tidal interaction can reach about 6 km upstream from the estuary to the river (Rajaram *et al.*, 2005). In Cuddalore, the southwest and northeast monsoons play a key role in the precipitation. The southwest monsoon is less intensive, whereas the northeast monsoon is more intensive which is from October to December. The average annual rainfall reported here is 1902 mm (Gopal *et al.*, 2018). The summer season witness rising temperatures in the period from April to June with temperatures surpassing 40°C. The Cuddalore district in Tamil Nadu is an important coastal city with several large-scale industries, agricultural land, fishing activities, harbour and tourism activities (Rajaram and Ganeshkumar, 2019). There are three main rivers flowing through the coastal region across the Cuddalore district — Uppanar in the south and Then Pennai and Gadilam in the north, all of which are adjacent to the Bay of Bengal. In ancient days, this coastal zone had a healthy ecosystem with much more marine fauna and flora. The western part of the Uppanar River is influenced by many activities like agriculture, harbour activities, industries such as chemicals, paint, tanneries, pharmaceuticals, etc. Additionally, Perumal Lake which holds ash discharged from the surrounding thermal power station (Jonathan *et al.*, 2008) is one of the pollution sources for the Uppanar River.

Sampling Sites

Three rivers stretch in this study area, but the Uppanar River and Gadilam River have several sources of pollution identified in comparison with Then Pennai. Hence, the three sampling sites around the Uppanar and Gadilam estuaries are chosen to collect water and sediment samples based on the pollutant source. There are about 55 major and minor industries that represent approximately 520 acres of land area, and they operate under the State Industries Promotion Corporation of Tamil Nadu (SIPCOT) industrial hub located in the western part of the study area (Rajaram and Ganeshkumar, 2019). Fishing harbours and urban settlements are found in the northern region. Watering by monsoon and stream supplies were observed in the lower margin of this estuary. Agricultural lands are present, but most of the people utilize this estuary for fishing activities. The three sampling sites selected here have different sources of pollution

Agricultural Runoff (Station 1)

Station 1 (St 1) chosen on the basis of agricultural runoff was Sami nagar (11°39'24.7"N, 79°44'55.7"E) situated about 14.8 km northeast of Perumal Lake. The Uppanar River until this station is dotted with various agricultural farms and on either side. Most to the agricultural runoff water reach the Uppanar River and are mainly influenced by the agricultural wastes and the water from the Perumal Lake which has loads of ash from a major thermal power plant.

Industrial waste and fishing activities (Station 2)

Station 2 (St 2) chosen on the basis of industrial activities and fishing activities was Thaikkal thoni thurai (11°41'33.5"N, 79°46'01.1"E) situated about 3 km north of Station 1. The important industrial hub State Industries Promotion Corporation of Tamil Nadu (SIPCOT) with comprising about 55 major and minor industries is located in this study area. The industries here dispose their untreated wastes into the Uppanar River (Rajaram and Ganeshkumar, 2019). This station also has marked fishing and boat activities which further pollute the environment with their fumes generated from boat engines and other waste spillages.

Tourist activities (Station 3)

Station 3 (St 3) chosen on the basis of marked tourism activities is Silver Beach (11°44'15.1"N, 79°47'02.4"E) situated on the northern part of the estuary. This site is popular for its tourist position in the district of Cuddalore. The Cuddalore port is also present near this station. This site is situated at about 12.3 km north of Thaikkal thoni thurai. This site is influenced by pollution from tourism and harbour activities.

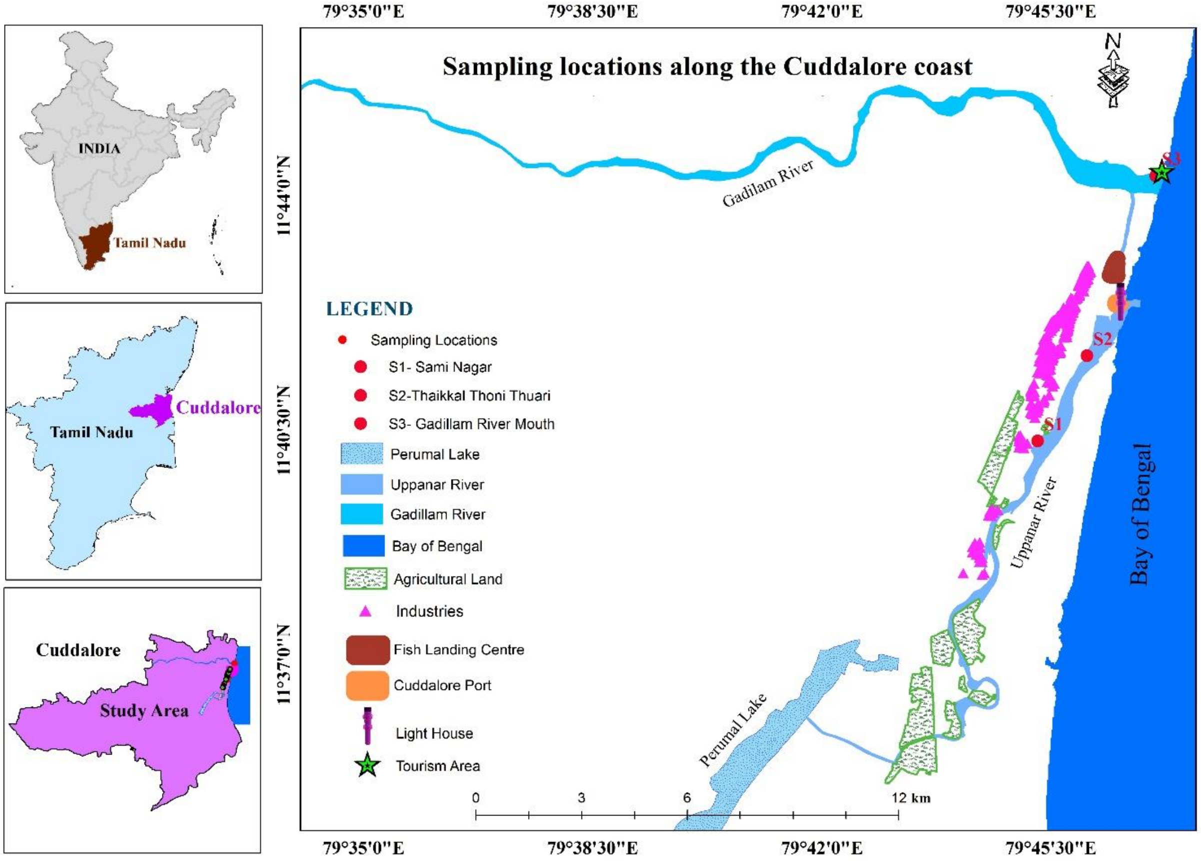

Figure 1. Study area map of sampling stations along the Cuddalore coast

Chapter IV

Seasonal variations of metal concentration (Cd, Cu, Pb, and Zn) in water and sediment samples

4.1 INTRODUCTION

Oceans cover more than 70% of our planet and are considered as one of the earth's most precious natural resources. However, these precious resources are slowly disintegrating of their prosperity due to coastal pollution, increasing human population, and emerging industrial areas which highly utilize the ocean resources (Le Tissier *et al*., 2006; Nour and Nouh, 2020). The environmental impacts of different stressors in India are gradually becoming very serious and even posing threats to the associated biotic community. Massive volumes of hazardous waste contaminants, distributed worldwide, are affecting the world ocean. Contemporary and historical manufacturing processes have contaminated the natural environment including air, water, and sediment. The effect of this pollution is felt even in the most rural areas making them lose their pristine nature (Ramesh Kumar and Anbazhagan, 2018). The geologic analysis of aquatic life describes the essence of the geochemical characteristics helping us to determine the exogenous metal cycle in the marine environment (Giridharan *et al*., 2010). India has 8,129 km of coastline with prominent features like coastal backwaters, rivers, streams, and wetlands. In the Indian coastline, the southeast coast plays a prominent role with its diverse characteristics. There are several rivers trenching into the Bay of Bengal in the southeastern region. Moreover, the flora and fauna are higher in the eastern coast compared with the western coast of India (Ayyamperumal *et al*., 2006).

Heavy metals can degrade the environment by polluting air, water, and soil, subsequently initiating adverse health effects in the ecology and living forms, when they get concentrated as a result of various industrial activities (Rajaram *et al*., 2020b; Stankovic and Stankovic, 2013). Heavy metals are not only accumulated in the water column, but also aggregate in colloidal matter and sediments afterwards, eventually reaching the food chain by travelling to higher consumers. Depending on the composition of the sediment layer, by various physical and chemical adsorption routes they aggregate in the sediments (Ghrefat and Yusuf, 2006; Khaled *et al*., 2006; Nour, 2015). These metals exist in marine environments and are distributed between various components (Linnik and Zubenko, 2000).

The river streams transport large amounts of suspended particulate matter, in both organic and inorganic forms, to the ocean (Bainbridge *et al*., 2018). This biomass carries heavy metals which tend to accumulate in the marine ecosystem and cause modifications in biological and chemical parameters of the oceans. The geochemistry of dissolved trace metals is regulated by an intricate balance of hydrodynamic factors, industrial effluents, municipal wastewater discharges, and biogeochemical cycles (Sun *et al.,* 2018). Dissolved metal contaminants in the estuarine waters are mostly influenced by water–particle interactions, such as flocculation, adsorption, organic and inorganic conglomeration, and resuspension of particulate matter by means of agitation (Jonathan *et al*., 2008).

The examination of trace metals helps in finding the relationship between the distribution in the sediments and water column. Sediment and water interaction might be important for the accumulation of metals in sediments (Silva *et al*., 2014; Singh *et al*., 2005; Wan *et al*., 2012). The investigation of dispersed metals in sediments adjoining regions of colonization and agriculture can provide proof of their anthropogenic effect on the ecosystem and thus aid in the determination of the potential risks due to the heavy metals in the environment. Trace metal contamination is considered as a major problem among various types of coastal pollutions because of the toxicity and deposition of metals in the environment by high adsorption rates and their property to bio-magnify easily through the food chain (Nour and Nouh, 2020; Yeardley *et al*., 1998). Heavy metals, including Pb, Cd, Cr, and Hg, found in sediments and water are harmful to marine organisms. However, they may emerge from raw sewage, industrial, agricultural, urban, domestic, and mining practices, and penetrate through river and brackish water into the marine environment (Anandkumar *et al*., 2018, Rajaram *et al*., 2017). In the marine environment, various metals are generated by natural and manmade processes. Among these metals, the essential metal groups include Zn, Cu, Cr (III), Fe, and Mn. The excessive deposition of these essential elements can also be toxic, however useful for metabolic activities in lower concentrations. The nonessential metals, including toxic metals like Cd, Hg, Pb, and Cr (VI), are harmful even in lower concentrations (Cohen *et al*., 2001; Fergusson, 1990).

The key objective of this chapter was to provide the source identification of toxic metals of two essential (Cu and Zn) and two nonessential (Cd and Pb) metals across four seasons in the water and sediment collected from the Cuddalore coast of Tamil Nadu, Southern India. Metal accumulation and evaluation will explain the source of declining conditions in estuary habitats and interpret modifications in temporal and spatial patterns. Once we have identified the sources of these heavy metals, strict policies and environmental conservation strategies can be incorporated to recover the marine ecosystem. Regular long-term seasonal monitoring of this region will aid to control pollution and implement better management practices for the ecosystem protection.

4.2 MATERIALS AND METHODS

4.2.1 Collection and analysis of water samples

A total of 36 surface water samples from the estuaries were collected for four seasons (premonsoon, monsoon, postmonsoon, and summer) from July 2018 to June 2019. Samples collected from surface of the water were stored in 1000-mL sterile containers with 1 mL of concentrated HNO3 for preservation and to eliminate cross contamination by debris. The stored water samples were filtered using the Millipore filter unit (0.45-μm) and transferred into a separation funnel. Then, 10 mL of 1% APDC (ammonium pyrrolidine dithiocarbamate) solution was added and shaken well, and 25 mL of MIBK (iso-butyl methyl ketone) was added and subjected to vigorous shaking for 10–15 min. From the two separated layers formed, the upper organic phase was collected. The organic stage obtained was extracted with 50% HNO3. The organic layer was collected and made up to 25 mL using double-distilled water (Arumugam *et al*., 2018b; Jonathan *et al*., 2008). The resulting sample solution was analyzed using the atomic absorption spectrophotometer (AAS) instrument (Shimadzu AA-7000, Japan).

4.2.2 Collection and analysis of sediment samples

A total of 36 sediment samples across four seasons were collected from the study area. In all three stations, the top 2-cm layer of sediments were collected from the intertidal zones using acid-cleaned PVC pipes (with an outer diameter of 3.5 inches). The pipes were pressed in the mud to extract the topsoil as per the method of Arumugam *et al*. (2018b). The collected sediment samples were stored in zip-lock covers and transported to the laboratory for analysis. The sediment samples were dried out at room temperature and were powdered to a fine texture with an agate mortar and pestle. The fine powder was sieved through a 63-μm sieve. Then, one gram of sieved sample was transferred into the beaker and subjected to acid digestion process. About 10 mL of mixed reagent in the ratio of 5:2:1 [HNO_3 (72% strength), $HClO_4$ (70% strength), and H_2SO_4 (97% strength)] was used for the digestion. The beakers containing sample and the reagent mixture were heated on a hot plate at 60 °C. At the end of the digestion, a few drops of 2N HCL were added. The final extract was collected and filtered with Whatman Grade 1 filter paper to remove any residual contaminants for AAS analysis. The filtered sample was analysed using the atomic absorption spectrophotometer (AAS) instrument (Shimadzu AA-7000, Japan) (Arumugam *et al.*, 2018b) with the lowest detection limit of 0.01 mg/L for the analysed elements.

4.2.3 Statistical Description

The data obtained from the AAS analysis provided the essential information about the metal distribution in the water and sediments of Cuddalore coast. Data were processed using Microsoft Excel 365 (Windows 10) and presented as mean ± standard deviation. Two-way ANOVA was used to determine significant variation in heavy metal concentrations in water and sediment in view of different sampling sites and seasons at 0.05% levels. Principal component analysis (PCA) revealed the interrelationship between the heavy metal concentrations based on the correlation matrix. To understand the correlation between the study sites, hierarchical cluster analysis (HCA) was applied. The results of PCA are expressed in biplot, and HCA is portrayed using a dendrogram. Statistical analyses were performed using PAST tool (version 3.0).

4.2.4 Quality Assurance and Quality Control

The instrument model AA-7000 model spectrophotometer (make: Shimadzu, Japan) was used to analysis the whole experimentation of this study. For quality control and quality assurance, triplicate readings of the samples, blanks, and standardized reference materials were logged throughout the analysis. The standard metal solutions were procured from

Merck Genei, Bangalore. All reagents used in the procedures were prepared with metal-free double-distilled water and chemicals of analytical grade. The standard curve was set up using a prepared standard with different concentrations and calibration using the automated software of the instrument. The instrument was rinsed with double-distilled water after each 10 samples and a blank reading was taken to confirm lack of contaminants. The working wavelengths were 324.75 nm, 228.80 nm, 217.00 nm, and 213.85 nm, for Cu, Cd, Pb, and Zn, respectively.

4.3 RESULTS

4.3.1 Metal concentrations in surface water samples

4.3.1.1 Cadmium

Overall cadmium concentrations ranged from BDL to 0.87 mg/L. The monsoon season at station 1 had the highest concentration, whereas the postmonsoon season at station 3 and summer season at stations 1 and 2 had the lowest. The cadmium concentration in water samples was descending from Monsoon > Premonsoon > Postmonsoon > Summer seasons, while the station wise order was descending from Station 1 > Station 2 > Station 3. During the premonsoon season, station 2 had the highest concentration (0.86 mg/L), while station 3 had the lowest value (0.07 mg/L). During the monsoon season, the highest concentration (0.87 mg/L) was recorded at station 1 and the lowest concentration was recorded at both stations 2 and 3 (0.01 mg/L). During the postmonsoon season, the highest concentration was recorded at station 1 (0.78 mg/L) and the lowest concentration was recorded at station 3 (BDL). During the summer season, the highest concentration was recorded at station 3 (0.03 mg/L) and the lowest concentration was recorded at both stations 1 and 2 (BDL) as shown in **Figure 2.**

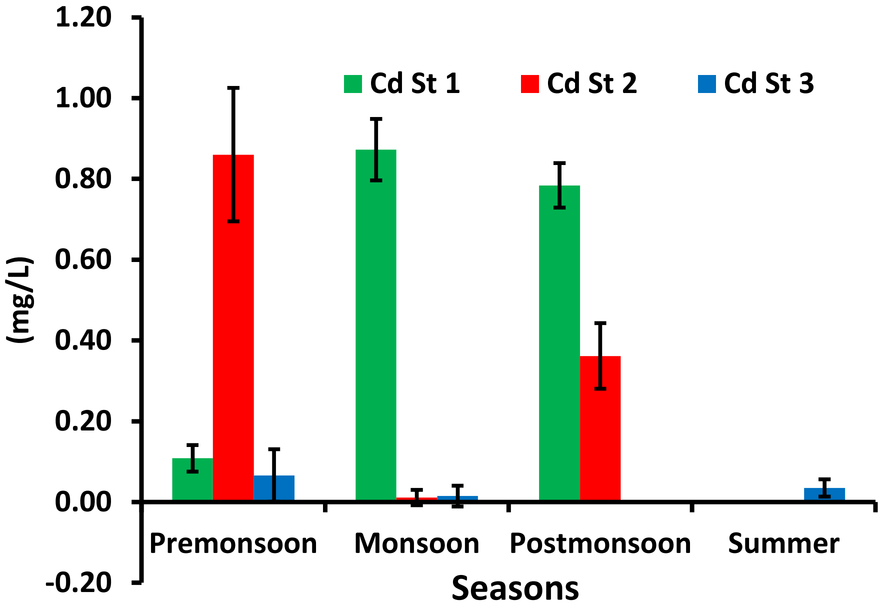

Figure 2 Cadmium concentrations in water samples with respect to different seasons and stations

4.3.1.2 Copper

Overall, copper values range from 0.33 to 0.47 mg/L. The summer season had the highest concentration at station 1, whereas the postmonsoon season had the lowest. The copper concentration in water samples was descending from Summer > Premonsoon > Monsoon > Postmonsoon seasons, while the station wise order was decreased from Station 1 > Station 2 and Station 3. Station 1 had the highest concentration (0.45 mg/L) during the premonsoon season, whereas station 2 had the lowest (0.43 mg/L). During the monsoon season, the highest concentration (0.41 mg/L) was found at station 1 and the lowest concentration (0.37 mg/L) was found at station 2. During the postmonsoon season, the maximum value (0.37 mg/L) was recorded at station 2 while the lowest concentration (0.33 mg/L) was reported at station 1. The maximum concentration was observed at station 1 (0.47 mg/L) and the minimum concentration was obtained at both stations 3 (0.45 mg/L) throughout the summer season as shown in **Figure 3.**

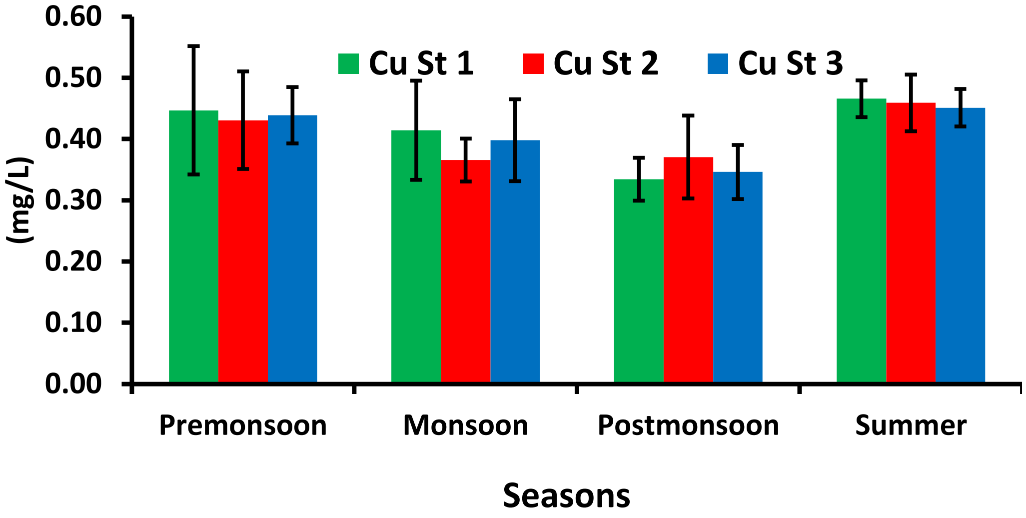

Figure 3 Copper concentrations in water samples with respect to different seasons and stations

4.3.1.3 Lead

Overall, lead concentrations range from BDL to 1.02 mg/L. At station 3, the postmonsoon season had the highest concentration, while the summer season had the lowest. The lead levels in water samples reduced as the seasons proceeded from Postmonsoon > Monsoon > Premonsoon > Summer, whereas the station-wise order fell from Station 1 > Station 2 and Station 3.During the premonsoon season, station 1 had the highest concentration (0.29 mg/L), while station 3 had the lowest value (0.03 mg/L). During the monsoon season, the highest concentration (0.87 mg/L) was recorded at station 3 and the lowest concentration was recorded at both stations 1 and 2 (0.77 mg/L). During the postmonsoon season, the highest concentration was recorded at station 3 (1.02 mg/L) and the lowest concentration was recorded at station 2 (0.88 mg/L). During the summer season, the highest concentration was recorded at station 1 (0.07 mg/L) and the lowest concentration was recorded at both stations 2 and 3 (BDL) as shown in **Figure 4**.

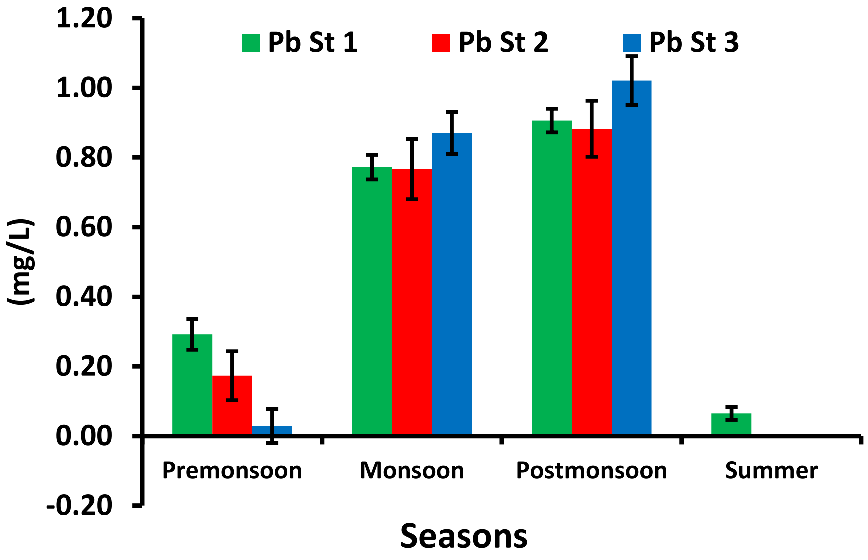

Figure 4 Lead concentrations in water samples with respect to different seasons and stations

4.3.1.4 Zinc

Overall zinc concentrations ranged from 1.33 to 3.28 mg/L. The premonsoon season at station 2 had the highest concentration, whereas the monsoon season at station 3 had the lowest. The zinc concentration in water samples was descending from Premonsoon > Summer > Postmonsoon > Monsoon seasons, while the station wise order was descending from Station 2 > Station 1 > Station 3. During the premonsoon season, station 2 had the highest concentration (3.28 mg/L), while station 3 had the lowest value (1.36 mg/L). During the monsoon season, the highest concentration (1.41 mg/L) was recorded at station 2 and the lowest concentration was recorded at station 3 (1.33 mg/L). During the postmonsoon season, the highest concentration was recorded at station 3 (1.45 mg/L) and the lowest concentration was recorded at station 2 (1.36 mg/L). During the summer season, the highest concentration was recorded at station 1 (1.50 mg/L) and the lowest concentration was recorded at station 3 (1.48 mg/L) as shown in **Figure 5.**

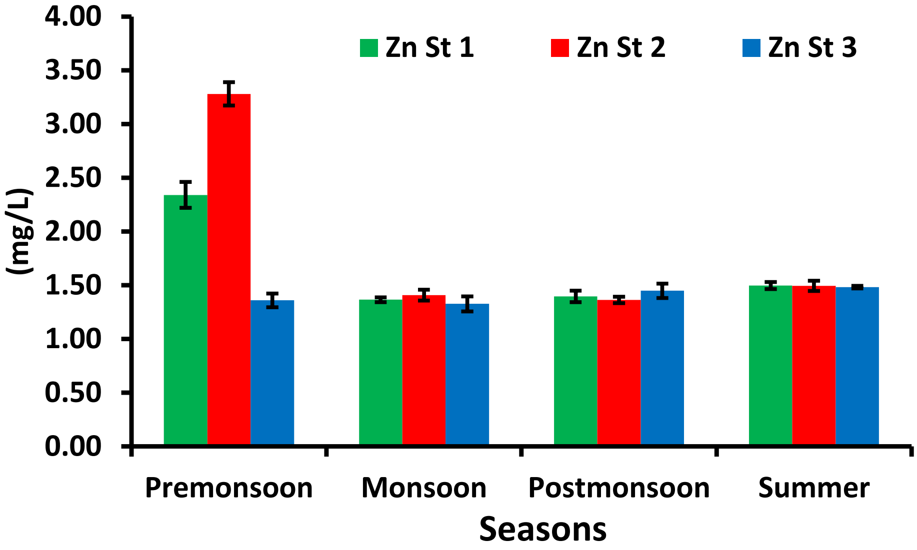

Figure 5 Zinc concentrations in water samples with respect to different seasons and stations

4.3.2 Metal concentrations in sediment samples

4.3.2.1 Cadmium

Overall cadmium concentrations range from BDL to 1.61 mg/kg. The postmonsoon season at station 3 had the highest concentration, although the summer season at all three stations was reported in BDL. Cadmium concentrations in sediment samples fell from Postmonsoon > Monsoon > Premonsoon > Summer seasons, whereas station wise order decreased from Station 3 > Station 1 > Station 2. During the premonsoon season, station 3 had the highest concentration (0.79 mg/kg), while station 2 had the lowest value (0.04 mg/kg). During the monsoon season, the highest concentration (0.77 mg/kg) was recorded at station 2 and the lowest concentration was recorded at station 3 (0.23 mg/kg). During the postmonsoon season, the highest concentration was recorded at station 3 (1.61 mg/kg) and the lowest concentration was recorded at station 2 (0.14 mg/kg). During the summer season, all the three stations values were recorded at BDL as shown in **Figure 6.**

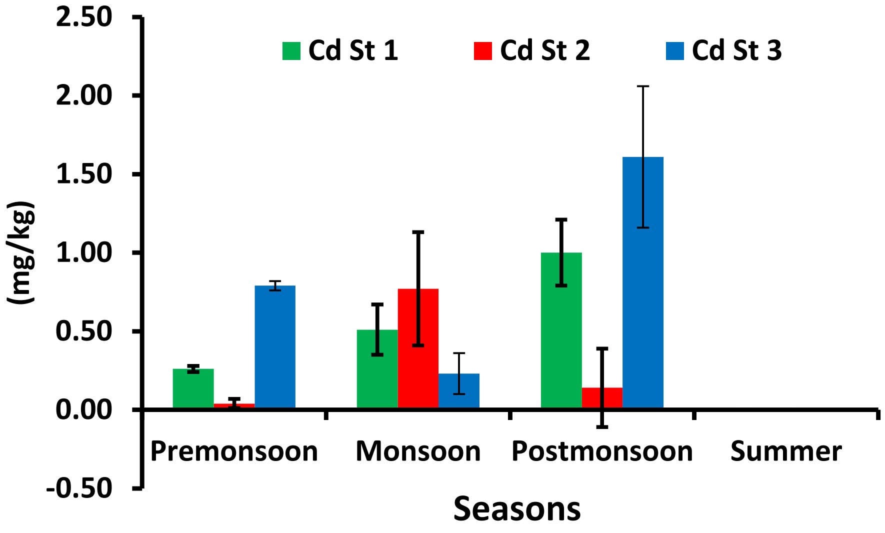

Figure 6 Cadmium concentrations in sediment samples with respect to different seasons and stations

4.3.2.2 Copper

Overall copper concentrations ranged from 2.43 to 28.49 mg/kg. The summer season at station 2 had the highest concentration, whereas the premonsoon season at station 3 had the lowest. The cadmium concentration in sediment samples was descending from Summer > Postmonsoon > Premonsoon > Monsoon seasons, while the station wise order was descending from Station 2 > Station 1 > Station 3. During the premonsoon season, station 1 had the highest concentration (5.01 mg/kg), while station 3 had the lowest value (2.43 mg/kg). During the monsoon season, the highest concentration (3.93 mg/kg) was recorded at station 1 and the lowest concentration was recorded at station 3 (2.81 mg/kg). During the postmonsoon season, the highest concentration was recorded at station 2 (13.64 mg/kg) and the lowest concentration was recorded at station 1 (5.61 mg/kg). During the summer season, the highest concentration was recorded at station 2 (28.49 mg/kg) and the lowest concentration was recorded at station 1 (24.47 mg/kg) as shown in **Figure 7.**

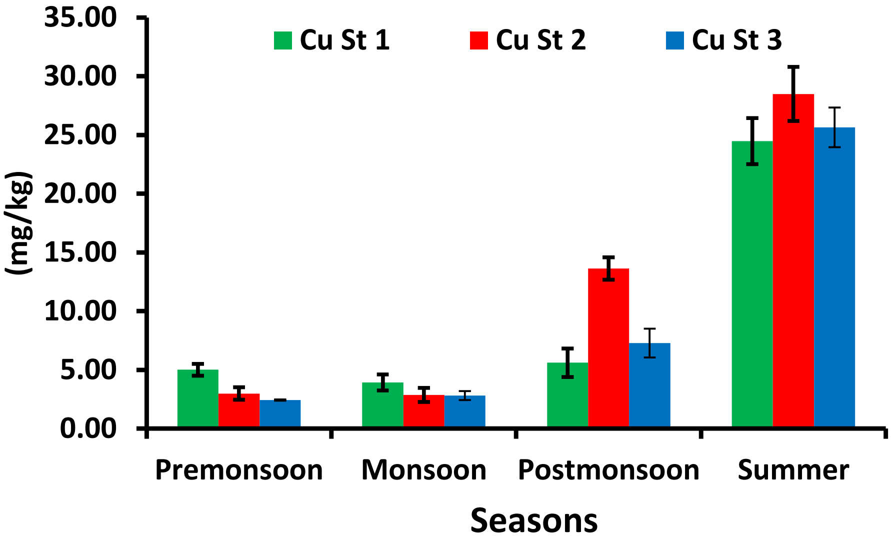

Figure 7 Copper concentrations in sediment samples with respect to different seasons and stations

4.3.2.3 Lead

Overall lead concentrations range from 2.37 to 8.40 mg/kg. The summer season at station 2 had the highest concentration, although the premonsoon season at station 3 was reported in lowest value. Lead concentrations in sediment samples fell from Summer > Postmonsoon > Monsoon > Premonsoon seasons, whereas station wise order decreased from Station 2 > Station 1 > Station 3. During the premonsoon season, station 2 had the highest concentration (3.44 mg/kg), while station 3 had the lowest value (2.37 mg/kg). During the monsoon season, the highest concentration (4.26 mg/kg) was recorded at station 1 and the lowest concentration was recorded at station 3 (2.87 mg/kg). During the postmonsoon season, the highest concentration was recorded at station 2 (3.80 mg/kg) and the lowest concentration was recorded at station 1 (3.40 mg/L). During the summer season, the station 2 was reach the maximum value (8.40 mg/kg) and the lowest value were recorded at station 3 (3.64 mg/kg) as shown in **Figure 8.**

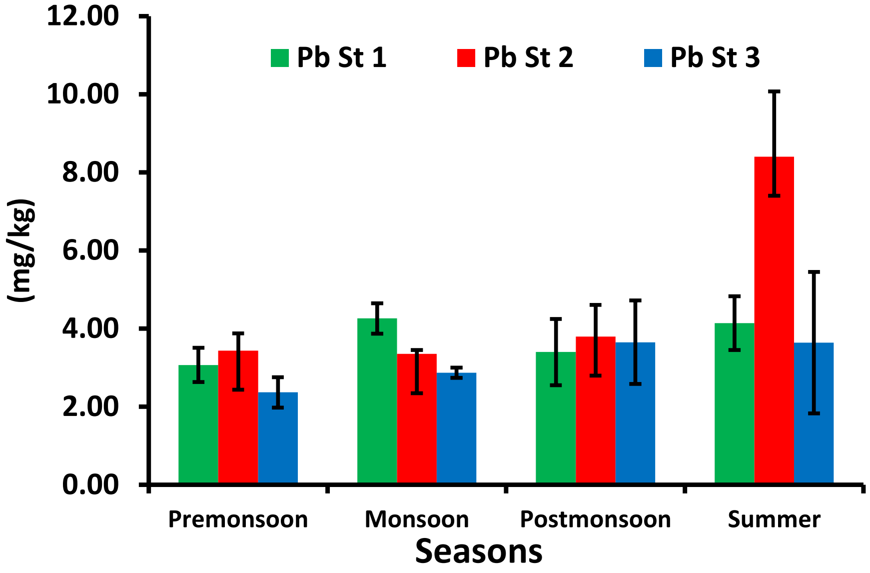

Figure 8 Lead concentrations in sediment samples with respect to different seasons and stations

4.3.2.4 Zinc

Overall zinc concentrations ranged from 3.02 to 32.33 mg/kg. The postmonsoon season at station 3 had the highest concentration, whereas the monsoon season at station 3 had the lowest. The zinc concentration in sediment samples was descending from Postmonsoon > Summer > Monsoon > Premonsoon seasons, while the station wise order was descending from Station 2 > Station 1 > Station 3. During the premonsoon season, station 1 had the highest concentration (13.79 mg/kg), while station 3 had the lowest value (3.60 mg/kg). During the monsoon season, the highest concentration (16.14 mg/kg) was recorded at station 1 and the lowest concentration was recorded at station 3 (3.02 mg/kg). During the postmonsoon season, the highest concentration was recorded at station 3 (32.33 mg/kg) and the lowest concentration was recorded at station 1 (16.30). During the summer season, the highest concentration was recorded at station 2 (22.72 mg/kg) and the lowest concentration was recorded at station 1 (16.62 mg/kg) as shown in **Figure 9.**

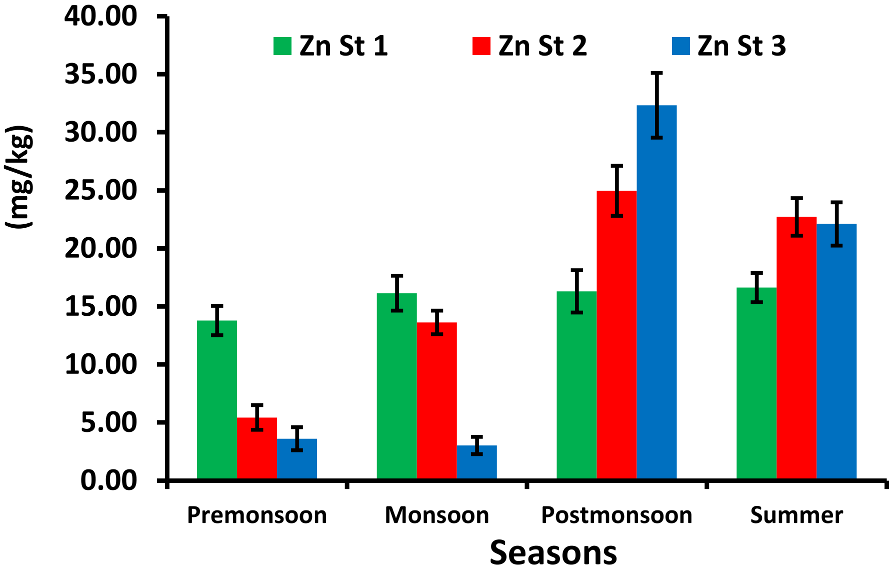

Figure 9 Zinc concentrations in sediment samples with respect to different seasons and stations

4.3.3 Statistical approaches

4.3.3.1 Two-way ANOVA

Two-way ANOVA results reveal that metal concentrations in all water samples of different stations does not differ significantly at 5% significance. In water samples, there is no significant difference with respect to the seasons for Cd and Zn at 5% significance. However, there is a significant difference in the concentrations of Cu and Pb in water samples at 5% significance level with respect to season. Two-way ANOVA results for water samples are tabulated in **Table 1**. In sediment, two-way ANOVA results reveal that metal concentrations of different stations do not differ at 5% significance. There is no difference with respect to the seasons as far as a Cd, Pb, and Zn are concerned at 5% significance. Nevertheless, a significant difference in the levels of Cu was noted in sediments at 5% significance with respect to season. Two-way ANOVA results for sediment samples are tabulated in **Table 2**.

Table 1. Seasonal variations and two-way ANOVA results of metal concentrations in water samples in the study area.

Element	Station	Season				ANOVA					
		Premonsoon	**Monsoon**	**Postmonsoon**	**Summer**	**Factor**	***df***	***SS***	***MS***	***F* value**	***P* value**
						Water					
Cu	St-1	0.45 ± 0.10	0.41 ± 0.08	0.33 ± 0.03	0.47 ± 0.03	Station	2	0.000	0.000	0.179	0.840
	St-2	0.43 ± 0.08	0.37 ± 0.04	0.37 ± 0.07	0.46 ± 0.05	Season	3	0.022	0.007	22.385	0.001
	St-3	0.44 ± 0.05	0.4 ± 0.07	0.35 ± 0.04	0.45 ± 0.03						
Cd	St-1	0.11 ± 0.03	0.87 ± 0.08	0.78 ± 0.06	ND	Station	2	0.355	0.177	1.268	0.347
	St-2	0.86 ± 0.17	0.01 ± 0.02	0.36 ± 0.08	ND	Season	3	0.257	0.086	0.613	0.631
	St-3	0.07 ± 0.07	0.01 ± 0.03	ND	0.03 ± 0.02						
Pb	St-1	0.29 ± 0.04	0.77 ± 0.04	0.91 ± 0.03	0.07 ± 0.02	Station	2	0.006	0.003	0.374	0.703
	St-2	0.17 ± 0.07	0.77 ± 0.09	0.88 ± 0.08	ND	Season	3	1.866	0.622	76.778	0.000
	St-3	0.03 ± 0.05	0.87 ± 0.06	1.02 ± 0.07	ND						
Zn	St-1	2.34 ± 0.12	1.36 ± 0.02	1.4 ± 0.05	1.5 ± 0.03	Station	2	0.461	0.230	0.995	0.424
	St-2	3.28 ± 0.11	1.41 ± 0.05	1.36 ± 0.03	1.49 ± 0.05	Season	3	1.874	0.625	2.696	0.139
	St-3	1.36 ± 0.06	1.33 ± 0.07	1.45 ± 0.07	1.48 ± 0.01						

SS - sum of squares, *MS* - mean square, *df* - degrees of freedom, significance level 0.05, ND - not detected/below detection limit

Metal concentration data are presented as mean ± standard deviation in mg/L for water and in mg/kg (dry weight) for sediment.

Table 2. Seasonal variations and two-way ANOVA results of metal concentrations in Sediment samples in the study area.

Element	Station	Season				ANOVA					
		Premonsoon	**Monsoon**	**Postmonsoon**	**Summer**	**Factor**	***df***	***SS***	***MS***	***F* value**	***P* value**
					Sediment						
Cu	St-1	5.01 ± 0.51	3.93 ± 0.67	5.61 ± 1.22	24.47 ± 1.96	Station	2	14.819	7.410	1.303	0.339
	St-2	2.99 ± 0.54	2.87 ± 0.6	13.64 ± 0.95	28.49 ± 2.31	Season	3	1055.35	351.784	61.870	0.000
	St-3	2.43 ± 0.06	2.81 ± 0.38	7.28 ± 1.23	25.64 ± 1.69						
Cd	St-1	0.26 ± 0.02	0.51 ± 0.16	1.00 ± 0.21	ND	Station	2	0.353	0.176	0.896	0.456
	St-2	0.04 ± 0.03	0.77 ± 0.36	0.14 ± 0.25	ND	Season	3	1.292	0.431	2.187	0.190
	St-3	0.79 ± 0.03	0.23 ± 0.13	1.61 ± 0.45	ND						
Pb	St-1	3.07 ± 0.44	4.26 ± 0.39	3.4 ± 0.85	4.14 ± 0.69	Station	2	5.348	2.674	1.604	0.277
	St-2	3.44 ± 0.44	3.35 ± 0.10	3.8 ± 0.81	8.4 ± 1.67	Season	3	10.064	3.355	2.012	0.214
	St-3	2.37 ± 0.39	2.87 ± 0.13	3.65 ± 1.07	3.64 ± 1.81						
Zn	St-1	13.79 ± 1.26	16.14 ± 1.51	16.3 ± 1.81	16.62 ± 1.27	Station	2	4.233	2.116	0.042	0.959
	St-2	5.44 ± 1.06	13.62 ± 1.03	24.97 ± 2.15	22.72 ± 1.61	Season	3	566.997	188.999	3.742	0.079
	St-3	3.6 ± 1.00	3.02 ± 0.75	32.33 ± 2.78	22.11 ± 1.86						

SS - sum of squares, *MS* - mean square, *df* - degrees of freedom, significance level 0.05, ND - not detected/below detection limit
Metal concentration data are presented as mean ± standard deviation in mg/L for water and in mg/kg (dry weight) for sediment.

4.3.3.2 Hierarchical cluster analysis (HCA)

The interrelationship between sampling locations based on metal concentrations in water samples and sediment for different seasons and their outcome is illustrated in **Figures 10 and 11** respectively. The dendrogram was obtained based on the interrelationship of the dataset. Accordingly, sampling locations were separated into the following clusters: Clusters 1–5 for water samples and Cluster 1–4 for sediment samples, which are mostly connected by the different anthropogenic influences across different seasons.

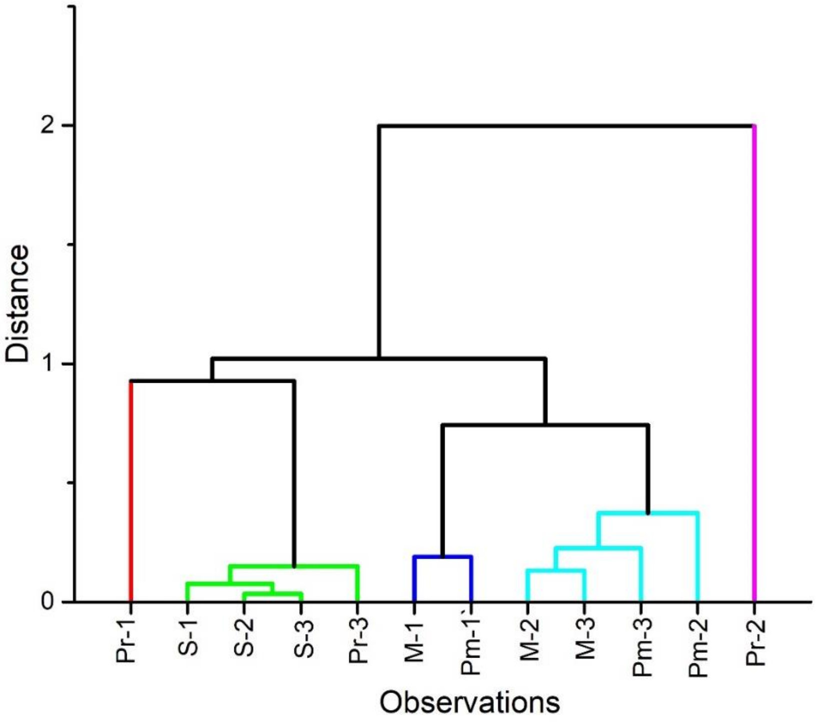

Figure 10 Dendrogram showing the interrelationship of heavy metals in water samples. Pr (1–3) refer to samples from premonsoon in stations 1–3, M (1–3) refer to samples from monsoon in stations 1–3, Pm (1–3) refer to samples from postmonsoon in stations 1–3, and S (1–3) refer to samples from summer in stations 1–3.

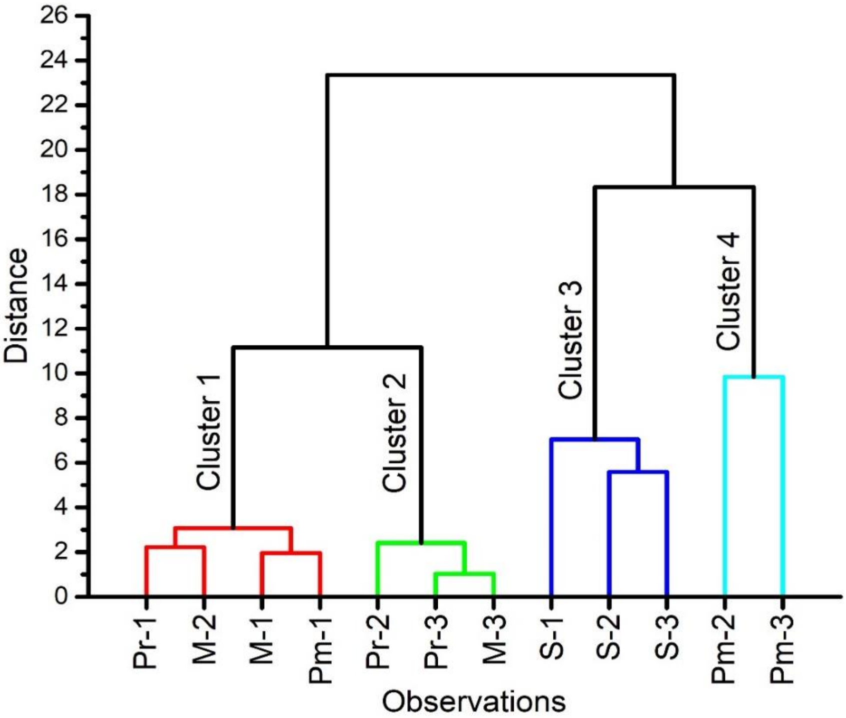

Figure 11 Dendrogram showing the interrelationship of heavy metals in sediment samples. Pr (1–3) refer to samples from premonsoon in stations 1–3, M (1–3) refer to samples from monsoon in stations 1–3, Pm (1–3) refer to samples from postmonsoon in stations 1–3, and S (1–3) refer to samples from summer in stations 1–3.

4.3.3.3 Principal component analysis (PCA)

Considering the interrelationship between metals, seasons, and stations, four distinctive principal components were extracted (PC1, PC2, PC3, and PC4) with eigenvalues (2.124, 1.390, 0.391, and 0.095) for water samples. The percentages of variance for the four factors are 53.11%, 34.75%, 9.78%, and 2.37%. Among the four principal components based on the eigenvalues, PC1 and PC2 alone (**Figure 12)** are considered for further investigation. which show the eigenvalues > 1 with the cumulative variance of 87.85% (**Table 3**). The third and fourth principal components are suggestively (eigenvalue < 1) less significant, explaining only 9.78% and 2.37% of the variance, respectively. For the sediment samples, to portray the interrelationship between metals, seasons, and stations, four distinctive principal components were obtained (PC1, PC2, PC3, and PC4) with eigenvalues (2.212, 1.262, 0.392, and 0.134). The percentages of variance for the four factors are 55.31%, 31.55%, 9.80%, and 3.34%. Among the four principal components based on the eigenvalues, only PC1 and PC2 (**Figure 13**) are considered. which show the eigenvalues > 1 with the cumulative variance of 86.86% (**Table 3**). These positive factors in PCA show that the sediments are impacted by the close proximity of parameters that are significantly stacked with the particular factor. Also, negative scores are suggestive that sediment prevalence is unaffected by this parameter. PC3 and PC4 are suggestively of (eigenvalue < 1) lower significance with only 9.80% and 3.34% of the variance, respectively.

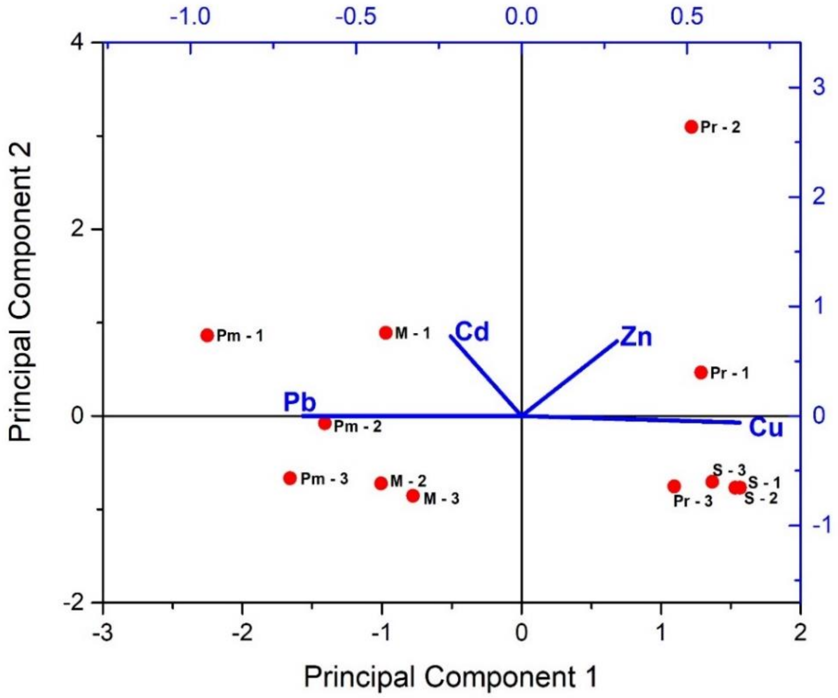

Figure 12 Biplot showing the distribution of heavy metals in water samples (Pr – premonsoon; M – monsoon; Pm – postmonsoon; S – summer; and numerals 1–3 represent stations 1–3, respectively)

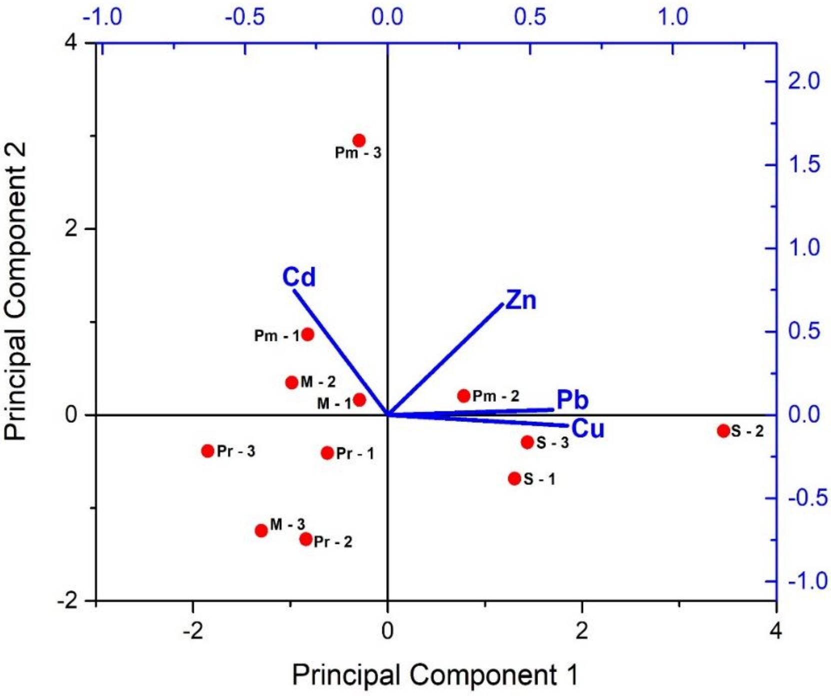

Figure 13 Biplot showing the distribution of heavy metals in sediment samples (Pr – premonsoon; M – monsoon; Pm – postmonsoon; S – summer; and numerals 1–3 represent stations 1–3, respectively)

Table 3. Loading, eigenvalues, and parameters of four heavy metals on principle components (PCs) for water and sediment samples.

	Water	
	PC1	**PC2**
Cu	**0.659**	-0.061
Cd	-0.214	**0.728**
Pb	-0.661	0.001
Zn	0.288	**0.683**
Eigenvalue	2.124	1.390
Variance (%)	53.11%	34.75%
Cumulative Variance	53.11%	87.85%
	Sediment	
	PC1	**PC2**
Cu	**0.630**	-0.064
Cd	-0.327	**0.745**
Pb	0.578	0.031
Zn	0.402	**0.663**
Eigenvalue	2.212	1.262
Variance (%)	55.31%	31.55%
Cumulative Variance	55.31%	86.86%

Values in bold represent strong positive loading.

4.4 DISCUSSION

Metal concentrations in sediment are higher on the Cuddalore coast than in water in all stations and seasons because sediments are a mixture of organic materials and minerals with charged surface and the potential to absorb dissolved heavy metals inherent in the ecosystem. (Langston, 1986). Heavy metals have the ability to settle, precipitated, deposit, and bind strongly to sediments, hence metal levels in sediment are typically greater than in water. (Schertzinger *et al.*, 2018), this could also contribute for the higher heavy metal concentrations in our study area sediment. The results show that the water column is impacted by increased metal levels during in the monsoon seasons due to the massive discharge of the waters caused by rains, which flushes all the heavy metals into the estuary (Hossain *et al.*, 2020). The summer season exhibits increased amounts of metals in the sediments, indicating that the combination between the water and the sediment, in the lacking of any input of freshwater, leads the muddy sediments to accumulate along the estuary terminal regions. (Pande and Nayak, 2013).

Cu values are much lower than those found along the coast Tamil Nadu (Arumugam *et al.*, 2018b; Kayalvizhi *et al.*, 2013; Rajaram *et al.*, 2021). The maximum amount of Cu was reported in sediment samples during in the summer season and the lowest level during in the premonsoon season, which agrees with a similar trend seen in the study area in a previous report by Rajaram *et al.* (2021). In comparison to the other three elements, Zn concentrations are higher including both water and sediment samples along the Cuddalore coast. The concentration of Zn responds differently to anthropogenic and natural sources. The higher concentration of Zn is observed in the estuary midstream near Station 2, indicating waste disposal from the nearby SIPCOT industrial zone (Gopal *et al.*, 2018). However, Zn concentrations in both water and sediment samples do not above WHO permitted limits, with the exception of a water sample taken during the premonsoon season at Station 2. Cu and Zn are essential metals that play important roles in the development of both terrestrial and aquatic life, yet excessive amounts of these elements can be hazardous to life (Singare *et al*., 2012). Cu and Zn may have manmade and lithogenic origins. The uniform distribution of Cu in all water column samples could indicate to a geogenic source. The increased concentration of Cu in Station 3 might be due to differences in sediment depositional features. Stations 1 and 2 are located upstream of the river and have mixed impacts from river water and tidal invasions, but Station 3 is located in the estuary and has increased boating activity. Because of the application of Zn in battery anodes and Cu in antifouling coatings, increased boat movements in estuarine waters may be a significant source of metals. (Boyle *et al*., 2016; Schiff *et al*., 2004). As per Rajaram and Ganeshkumar (2019), Copper emission levels from antifouling paint used to coat recreational ships to prevent fouling organisms were found to be considerable. Cd concentrations in water samples collected from all three locations throughout the premonsoon, monsoon, and postmonsoon seasons were generally higher than the acceptable limits of WHO (WHO, 1989). The Cd levels were comparable with previous research in this area by Dhinesh *et al.* (2014). The concentrations of Cd in water were below permissible limits in the summer season in Stations 1 and 2 but was in the baseline limit in Station 3. Cd levels in sediment samples reached the permissible limit at Station 3 during the premonsoon season and in Station 2 during the monsoon season. Throughout the postmonsoon season, the Cd limits were higher than the standard limits in Stations 1 and 3; however, Cd levels in the sediments were not detected during the summer season. The higher Cd levels during the monsoon and postmonsoon seasons suggest that Cd is mostly derived from the plastic manufacturing sectors. (Mathivanan and Rajaram, 2014) and other anthropogenic influences that deposit their pollutants into the Uppanar estuary during the monsoon season when the river water flow is higher. Cd concentrations in the water samples from all three stations across the premonsoon, monsoon, and postmonsoon seasons were mostly higher than the permissible limits of WHO (WHO, 1989). Pb levels in water samples from all three locations were considerably higher than the acceptable limit. of WHO (WHO, 1989) with the exception of summer season where Pb was not detected in Stations 2 and 3. Pb values in sediment samples were all within permitted levels in all stations and seasons. Increased Pb concentrations in sediments were found in the Uppanar estuary's upstream and midstream areas, which might be attributed to the presence of industrial effluents in the area and additional possible sources of Pb in the aquatic environment include fertilisers, herbicides, air dust from fuel burning, lead-based paints, and sewage. (Mathivanan and Rajaram, 2014). Higher quantities of Cd and Pb, both non-essential elements, are found in the water during the monsoon and postmonsoon seasons, indicating anthropogenic causes. The risk level of Cd is higher than that of other metals, followed by Pb, and the elevated levels of Cd and Pb demonstrate that the coastal environment here has been impacted by diverse anthropogenic activities.

Chapter V

Seasonal variations of metal concentration (Cd, Cu, Pb and Zn) in finfish and shellfish collected from Cuddalore coast.

5.1 INTRODUCTION

Marine fish are considered to be a key source of protein in the human diet. Contamination of fish with metals has become a major source of concern, not only because of the risk to health of the fish, but also to humans who consume these metal-contaminated marine fish (Gu *et al.*, 2016). Toxic metal concentrations in the marine ecosystem can pose a health risk to fish. Metal pollution is mainly sourced from agricultural runoff, sewage effluents, industrial discharge, toxic waste dumps, and fuel from boats (Mishra *et al.*, 2007; Satheeshkumar and Senthilkumar, 2011). Various contaminants, including harmful elements and biomolecules, have been released into the coastline of Southern India by several metal-based industries (Anandkumar *et al.*, 2018; Mathivanan and Rajaram, 2014). The progressive levels of metals seem to have a harmful effect on the food chain because of the biomagnification from the primary producers to the consumers (Heng *et al.*, 2004). Several researchers have explored the accumulation of metals and their adverse effects on seawater, sediments, and biological species in the last few decades (Arumugam *et al.*, 2018a; Kumar *et al.*, 2017). Metals, from both anthropogenic and natural inputs, are considered to be prominent contaminants in the environment since they are easily absorbed and rapidly deposited in organisms, posing a serious hazard to public health through consumption of metal-contaminated food (Copat *et al.*, 2013). The main route for metals entry into fish tissues is through adsorption and absorption. Metal deposits in body tissues are mainly from the absorption process through gills, kidneys, liver, and especially the digestive tract (Annabi *et al.*, 2013). Metals reach the marine food chain in two major ways including direct water consumption and food intake via the gastrointestinal tract, and non-dietary routes, such as muscle and gills, via osmoregulation (Ribeiro *et al.*, 2005). Humans require essential metals like iron (Fe), cobalt (Co), and manganese (Mn) for a number of physiological and metabolic processes. Further, metals toxic to humans including mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr), and nickel (Ni) can cause dermatitis, infertility, lung damage, coronary and renal failure, also pulmonary and sinus carcinoma (Renieri *et al*., 2014). Biomarkers are biological indicators that act as early warning signs (Annabi *et al*., 2013). Fish occupy top positions in the marine food chain and are vulnerable to accumulation of metals through their diet and the surrounding medium water and sediments (Yılmaz *et al*., 2007; Zhao *et al*., 2012); hence, they can act as good biomarkers (Rahman *et al*., 2012; Rajaram and Devendran, 2013). In comparison to other pathways like respiratory and dermal exposure, food consumption is the predominant pathway which constitutes about 90% for human exposure to metals. Fish is a commonly consumed food and a primary source of protein in many fishing towns. It helps to maintain a healthy lifestyle by delivering important omega-3 fatty acids, amino acids, and nutrients. Consumption of fish is indeed beneficial for preventing arrhythmia and thrombosis, as well as decreasing bad cholesterol (Korkmaz *et al*., 2019). In opposition to the advantages mentioned earlier, aquatic species play a significant role in the transmission of contaminants to humans (Ferrante *et al*., 2018). While fish is rich in many nutrients, consuming too much of it can have health implications due to bio-accumulated metals that exceed permitted limits (Rahman *et al*., 2012). Renal problems, bone damage, neurological issues, reproductive problems, cardiac failure, and cancer impacts are among the health hazards posed by metal toxicity (Renieri *et al*., 2019). Several metals can bond to the sulphur found in enzymes, causing them to dysfunction (Ali and Khan, 2018). Due to rapid urbanisation and industrial activities in and around Cuddalore city, mainly due to the runoff from SIPCOT (Small Industrial Promotion Corporation of Tamil Nadu, with 52 industries spread over 520 acres) industrial complex, the Uppanar River that flows through the Cuddalore Town in Tamil Nadu is recognized among the most polluted waterways on India’s southeast coast.

Evaluation of harmful compounds from the industrial sources in Cuddalore coastal waters would cover a wide range of potential environmental pollutants. The higher levels of pollutants in these waters may be a contributing factor for the regular death of fish and degradation of aquatic habitats in this area. The frequent release of untreated or partially treated industrial effluent into the coastal environment has an impact on both the biotic and abiotic systems, and eventually gives way to negative consequences for humans via the food chain (Dar, 2013). In this context, the main objectives of this chapter are to: (1) provide baseline data on levels of Cd, Cu, Pb, and Zn in the muscles of the ichthyofaunal resources, and (2) evaluate the possible risk to human health associated with consumption of these fish.

5.2 MATERIALS AND METHODS

5.2.1 Collection and analysis of fish samples

Over a year, from July 2018 to June 2019, a total of 12 species of finfish and eight species of shellfish were collected across three sampling stations and across four seasons (premonsoon, monsoon, postmonsoon, and summer). At least six individuals per each species were collected for each sampling for obtaining the average metal concentrations for each species. The collected finfish speciemen are as follows: *Iniistius cyanifrons, Carangoides malabaricus, Mugil cephalus, Sardinella longiceps, Rastrelliger kanagurta, Saurida thumbil, Cynoglossus sp., Lagocephalus sapadiceus, Siganus canaliculatus, Stolephorus indicus, Sphyraena acutipinnis, and Trichiurus lepturus* as shown in **Figure 14.** Followed by the collected shellfish samples are as follows: *Calappa lophos, Portunus sanguinolentus, Charybdis natator, Penaeus indicus, Loligo duvauceli, Perna viridis, Meretrix casta, and Ficus variegata* as shown in **Figure 15.** The fish species chosen for this study were frequently consumed by the local population and had commercial importance. The finfish species were split into two groups depending on the feeding habitat, according to Keshavarzi *et al*. (2018). The sampled fish were classified into two categories based on the habitats they thrive as pelagic and benthic fish. Pelagic organisms are mostly found in the water column, while benthic organisms dwell predominantly on the seafloor. Out of the 12 species, nine species were pelagic fish, including *Iniistius cyanifrons*, *Carangoides malabaricus*, *Mugil cephalus*, *Sardinella longiceps*, *Rastrelliger kanagurta*, *Saurida thumbil*, *Stolephorus indicus*, *Sphyraena acutipinnis*, and *Trichiurus lepturus*, and three were benthic fish, including *Cynoglossus* sp., *Lagocephalus sapadiceus*, and *Siganus canaliculatus.* According to Venugopal and Gopakumar (2017), the shellfish species were divided into two categories based on their salient features. Out of the eight species of shellfish, four were crustaceans including *Calappa lophos, Portunus sanguinolentus, Charybdis natator, and Penaeus indicus,* and four were mollusks including *Loligo duvauceli, Perna viridis, Meretrix casta, and Ficus variegata*. The fish specimens from the Uppanar coastal waters were collected and preserved in an ice-filled box before being transported to the laboratory, where all finfish and shellfish specimens were identified using taxonomic key from the fisheries survey of India (FSI), WoRMS (Mees *et al*., 2015), and FishBase (Froese, 2009). The fish were washed in the laboratory using deionized water to remove any external contaminants, and the muscle tissue was dissected and dried at 70 °C until completely dry. The dry tissue was crushed with a mortar and pestle. For AAS (atomic absorption spectrophotometry) analysis according to Arumugam *et al*. (2018b), 1 g of dry powdered of each individual fish sample was digested using 10-mL mixture of HNO_3 (72%), $HCLO_4$ (70%), and H_2SO_4 (98%) in a 5:2:1 proportion at 60 °C. Any undigested sample was further subjected to digested using 5 mL of 2N HCl. Once the samples were digested and only about 1 mL of the final extract remained, the sample was diluted with distilled H_2O and made up to 25 mL by volume. Finally, the extract was filtered with Whatman filter paper (grade 1) and transferred into a metal-free container for further AAS analysis (Arumugam *et al*., 2018b).

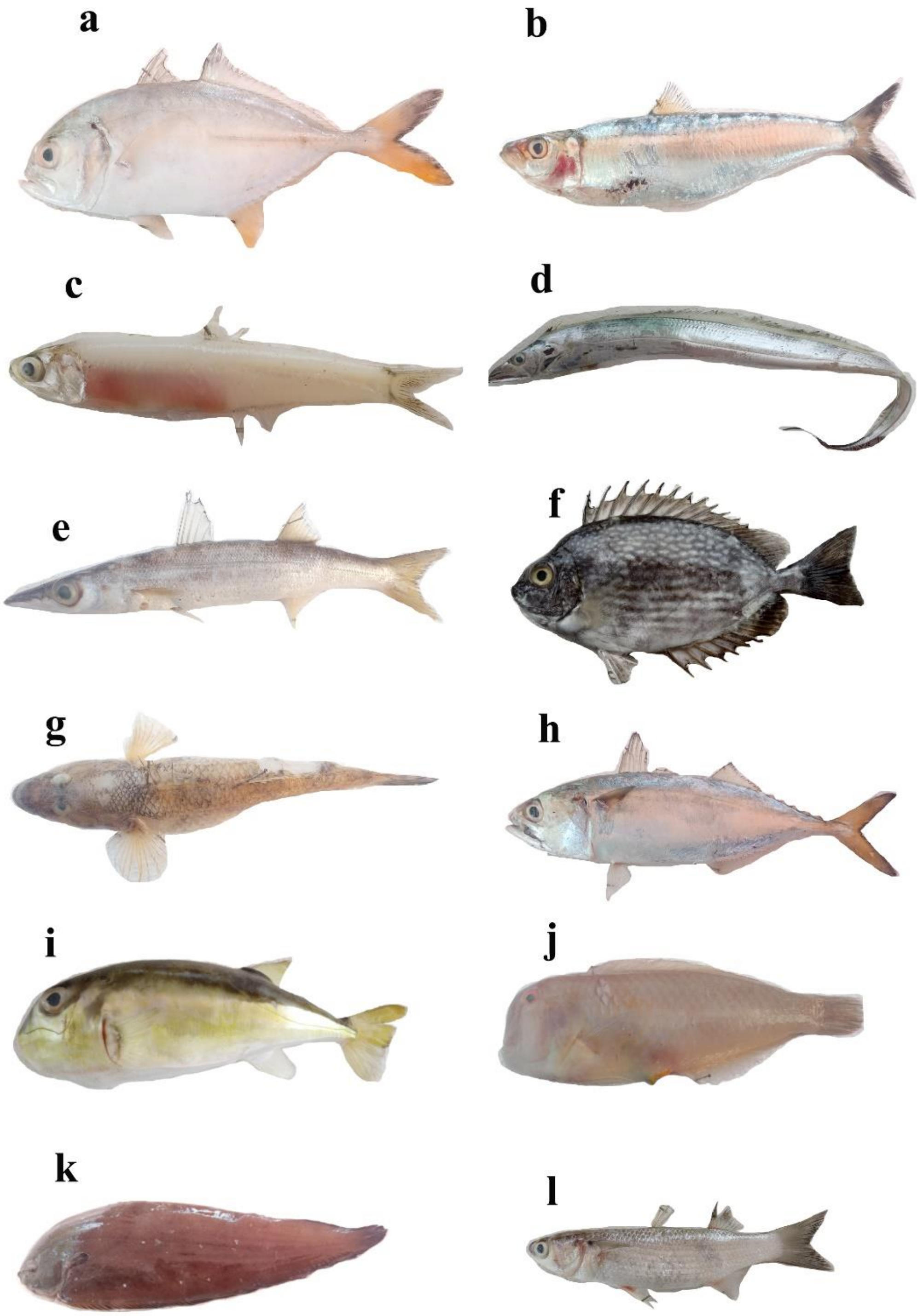

Figure 14 Plate showing the collected finish. a) *Carangoides malabaricus*, b) *Sardinella longiceps*, c) *Stolephorus indicus*, d) *Trichiurus lepturus*, e) *Sphyraena acutipinnis*, f) *Siganus canaliculatus*, g) *Saurida thumbil*, h) *Rastrelliger kanagurta*, i) *Lagocephalus sapadiceus*, j) *Iniistius cyanifrons*, k) *Cyanoglossus* sp, l) *Mugil cephalus*

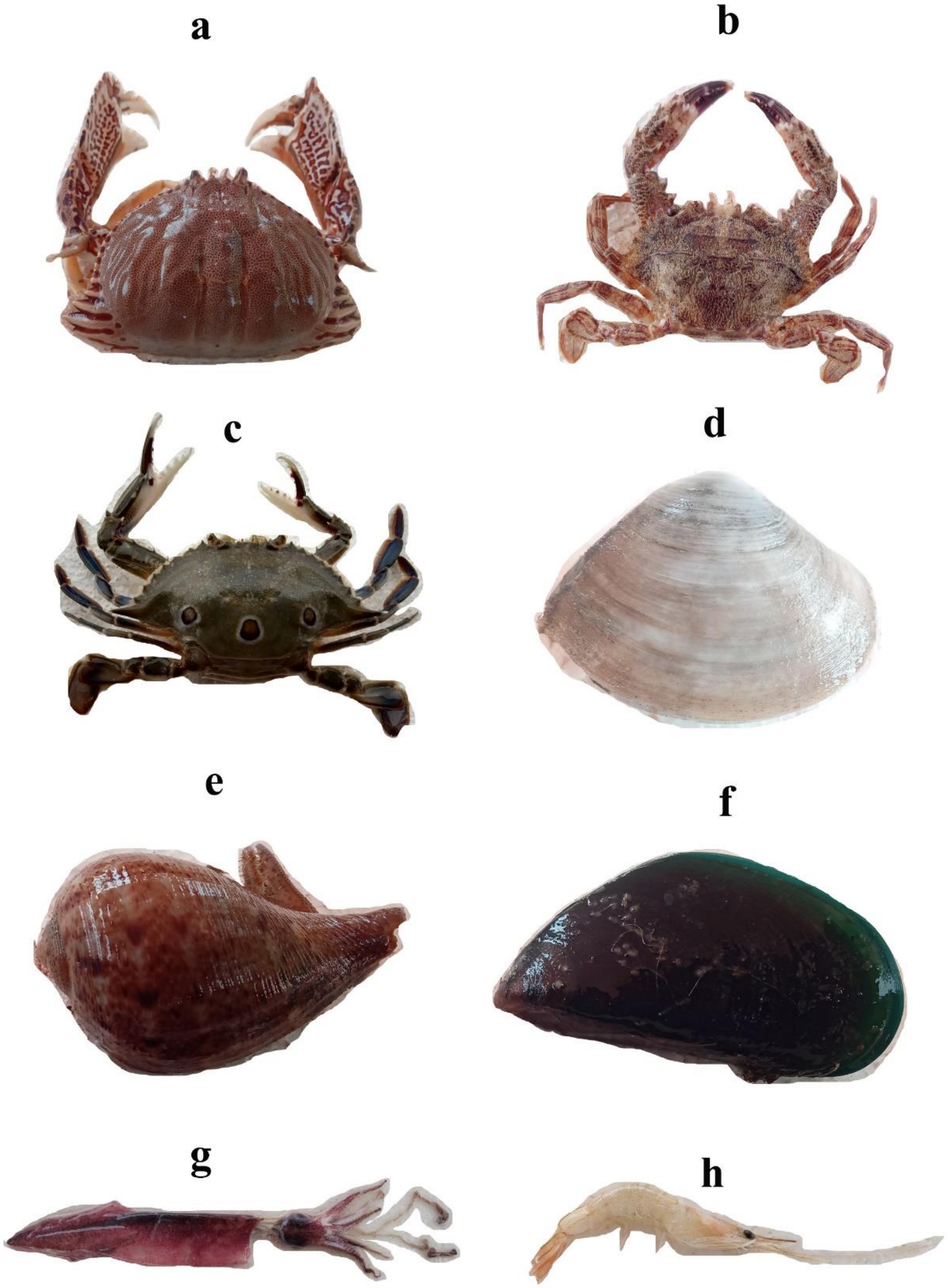

Figure 15 Plate showing the collected shellfish. a) *Calappa lobhos*, b) *Charbdis natator*, c) *Portunus sanguinolentus* d) *Meretrix casta*, e) *Ficus variegata* f) *Perna viridis*, g) *Loligo duvauceli*, h) *Penaeus indicus*.

5.2.2 Statistical Description

The raw data of metal concentrations came from the AAS analysis, which was used for determining the metal distribution in the finfish throughout the four seasons. All the data were systemically analyzed and represented as mean and standard deviation using MS Excel (Version 365, Windows 10). Two-way ANOVA was used to evaluate the species–season differences at 0.05% levels for each element. The source identification of the metals in the Cuddalore coastal zone was performed using multivariate analysis, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA). These results provided the biplot for expressing the correlation between the metal, season, and species of fish, and the dendrogram depicting the relationship between the metals.

5.2.3 Bio concentration factor for finfish

The potential of an organism to aggregate the various constituents from its surrounding environment or medium water, sediment, etc., is known as bioconcentration factor (BCF) (Achary *et al*., 2017). For assessing the BCF, the following formula is used.

$$\mathrm{BCF} = \frac{\text{Metal concentration in tissue}}{\text{Metal concentration in surrounding medium}}$$

Where, the background concentration of the surrounding medium (water) for Cd is 0.26, Cu is 0.41, Pb is 0.48, and Zn is 1.65 as per our simultaneous study Vinothkannan *et al*. (2022a). These values are the average metal concentrations in the water samples collected in concurrence with the fish samples.

5.2.4 Bio accumulation factor for shellfish

Bioaccumulation factor (BAF) refers to an organism's means to obtain different substances out of its external environment or medium, i.e., sediment, because shellfish found in sediment habitats can clearly reflect the accumulation of metals. (Barron, 2003; Zhang *et al*., 2016). The following formula was used to calculate the BAF.

$$\text{BAF} = \frac{\text{Metal concentration in tissue}}{\text{Metal concentration in surrounding medium}}$$

As per our contemporary work Vinothkannan *et al.* (2022a), the baseline level of the surrounding medium (sediment) for Cd is 0.45, Cu is 10.43, Pb is 3.87, and Zn is 15.89 µg/g. These are the average metal values in sediment samples collected in concurrence with the shellfish samples.

5.2.5 Quality Assurance and Quality Control

The processed samples were analyzed for metals using an Atomic Absorption Spectrophotometer (Model: AA7000; Make: Shimadzu Company, Japan) at the following operating wavelengths: 213.85 nm for Zn, 324.75 nm for Cu, 228.80 nm for Cd, and 217.00 nm for Pb (Rajaram *et al.*, 2017). The below detection limit (BDL) for the instrument for all metals analyzed was 0.01 µg/g. All sample processing and analysis were done in a sterile and metal-free environment to ensure quality assurance across the entire analysis. Triplicate readings of the samples were made and the mean and standard deviation was computed to present the results. AAS metals reference standards procured from Sigma-Aldrich, Bangalore, were used for plotting the standard curves. Standard calibration curves for each element had a regression coefficient (R^2) value greater than 0.98. The quality assurance and quality control (QA/QC) for the AAS instrument were maintained by checking the readings with the metal standards of known concentration in the range of 0.1–10 µg/g. The recovery percentages for the metals analyzed using the known standards were in the range of 92–106%. Blanks were run once for each ten sample readings to ensure the accuracy of the instrument readings for quality control and quality assurance of the metal data.

5.3 RESULTS

5.3.1 Metal concentrations in finfish samples

Seasonal variation of four metals (Cd, Cu, Pb, and Zn) was assessed in 12 different species of finfish divided into two categories based on their habitat including 9 pelagic fish and 3 benthic fish as shown in **figure 16** and **17**. shows the seasonal variation of four metal concentrations in the 12 species of finfish **Table 4.** Overall, the range of metal concentration across all four seasons was as follows: Cd (<BDL–0.95 µg/g ¬), Cu (<BDL–47.98 µg/g), Pb (<BDL–5.12 µg/g), Zn (9.75–42.92 µg/g). The distribution of metals across the 12 species of fish for the different seasons was statistically significant at 0.05 level ($P < 0.05$): premonsoon ($P < 0.0001$; $F = 62.25$), monsoon ($P < 0.0001$; $F = 95.00$), postmonsoon ($P < 0.0001$; $F = 184.50$), and summer ($P < 0.0001$; $F = 55.48$) values were shown in (Table). Cd was not detected or generally low in many species of fish during the premonsoon and monsoon seasons. Cd was detected in many species in the postmonsoon and summer seasons. Out of the detected Cd values, the lowest concentration of Cd was observed in *Sardinella longiceps* (0.06 ± 0.01 µg/g) during the summer season, and the highest concentration was recorded in *Rastrelliger kanagurta* (0.95 ± 0.24 µg/g) during the postmonsoon season. Cu concentration was not detected in *Iniistius cyanifrons* during the postmonsoon and summer season, and the highest concentration of Cu was noted in *Mugil cephalus* (47.98 ± 1.74 µg/g) during the summer season. Many fish species were devoid of Pb during the premonsoon and monsoon seasons; however, the Pb content increased in the postmonsoon and summer seasons. Out of the detected values, the minimum was in *Rastrelliger kanagurta* (0.06 ± 0.05 µg/g) during the premonsoon season, and the maximum was also noted in *Rastrelliger kanagurta* (6.82 ± 0.40 µg/g) during the summer. Zn was detected in all species across all seasons. The least concentration of Zn was noted in *Stolephorus indicus* (9.75 ± 1.51 µg/g), and the maximum level was recorded in *Siganus canaliculatus* (42.92 ± 1.65 µg/g). The overall order of mean concentration of metal in different season is as follows: premonsoon (Zn > Cu > Pb > Cd), monsoon (Zn > Cu > Pb > Cd), postmonsoon (Zn > Cu > Pb > Cd), and summer (Cu > Zn > Pb > Cd). Considering only the 9 pelagic fish, the mean concentrations of Cd, Cu, Pb and Zn were 0.27, 9.87, 1.22, and 24.72 µg/g, respectively. The ranges of the four metals in the pelagic fish species were as follows: Cu (<BDL–0.95 µg/g), Cu (<BDL–47.98 µg/g), Pb (<BDL–5.12 µg/g), and Zn (9.27–38.46 µg/g). In the 3 benthic fish, the mean concentrations of Cd, Cu, Pb and Zn were 0.24, 9.00, 1.08, and 31.84 µg/g, respectively. The ranges of the four metals in the benthic fish species were as follows: Cu (<BDL–0.75 µg/g), Cu (1.28–30.21 µg/g), Pb (<BDL–4.55 µg/g), and Zn (19.57–42.92 µg/g). In both benthic and pelagic fish, the mean metal concentrations were in the following descending order: Zn > Cu > Pb > Cd. A comparison of the metal concentrations in the present study with the permissible limits set by various international agencies for metals in the fish tissues are presented in Table 1. Average Cd concentration was higher in pelagic fish (0.27 µg/g) compared with benthic fish. Similarly, the Cu and Pb levels were also higher in the pelagic fish in comparison with benthic community. The average level of Cu in pelagic fish was 9.87 µg/g, whereas it was only 9.00 µg/g in the benthic fish. Pb value in 1.22 µg/g and 1.08 µg/g in the pelagic and benthic fish, respectively. But there was a converse scenario in Zn, where the benthic fish had higher levels of Zn compared with pelagic fish. The mean value of Zn in the pelagic group was 24.72 µg/g and 31.84 µg/g in the benthic group.

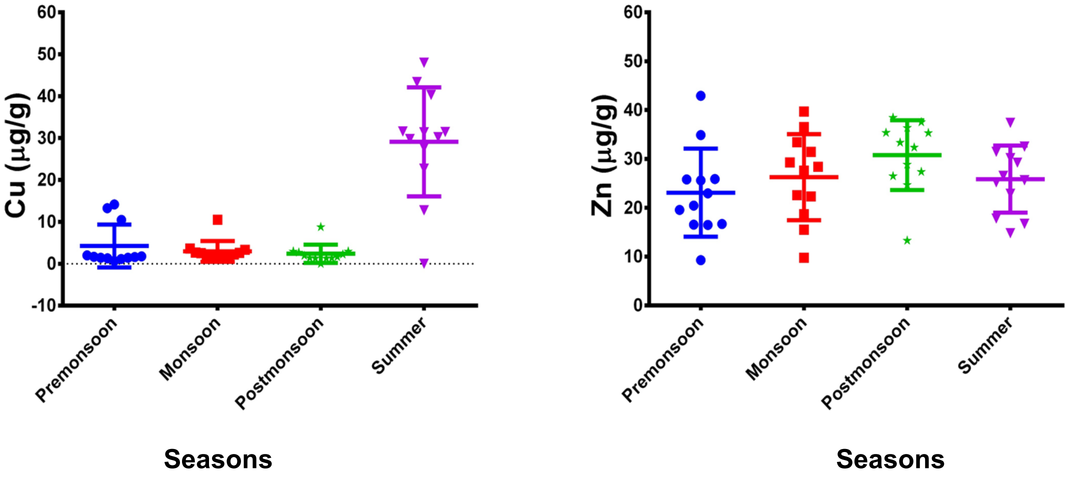

Figure 16 The concentration of Cu and Zn in the twelve species of finfishes collected from Cuddalore coast.

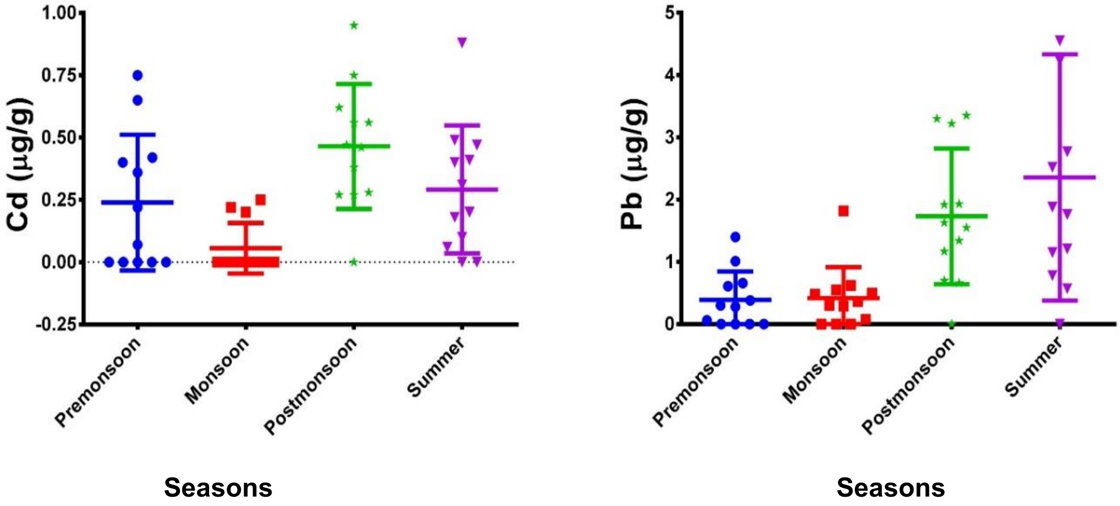

Figure 17 The concentration of Cd and Pb in the twelve species of finfishes collected from Cuddalore

Table 4. Seasonal distributions of four metals (Cd, Cu, Pb, and Zn) and two-way ANOVA for the ichthyofaunal resources from the Cuddalore coast.

Season	**Habitat**	**Species**	**Metal Concentration**				**ANOVA**					
			Cd	**Cu**	**Pb**	**Zn**	**Factor**	***SS***	***df***	***MS***	***F***	***P-value***
Premonsoon	Pelagic	*Iniistius cyanifrons*	BDL	0.66 ± 0.19	BDL	20.43 ± 2.05	Species	430.6078	11	39.15	1.71	0.11
		Carangoides malabaricus	BDL	14.14 ± 1.43	1.01 ± 0.2	25.59 ± 2.01	Metal	4269.883	3	1423.29	62.25	0.00
		Mugil cephalus	0.36 ± 0.16	13.25 ± 1.85	1.4 ± 0.35	25.79 ± 1.39						
		Sardinella longiceps	BDL	1.41 ± 0.46	0.3 ± 0.12	16.68 ± 2.00						
		Rastrelliger kanagurta	0.65 ± 0.19	1.75 ± 0.23	0.06 ± 0.05	22.95 ± 1.45						
		Saurida thumbil	0.22 ± 0.09	1.13 ± 0.26	BDL	16.49 ± 1.06						
		Stolephorus indicus	0.42 ± 0.43	1.98 ± 0.41	0.61 ± 0.06	25.89 ± 1.43						
		Sphyraena acutipinnis	0.4 ± 0.11	1.57 ± 0.26	0.28 ± 0.15	16.55 ± 1.08						
		Trichiurus lepturus	BDL	1.29 ± 0.16	BDL	9.27 ± 0.87						
	Benthic	*Cynoglossus sp.*	0.75 ± 0.15	1.42 ± 0.26	0.66 ± 0.16	19.57 ± 0.97						
		Lagocephalus sapadiceus	0.07 ± 0.06	1.66 ± 0.15	0.38 ± 0.13	34.9 ± 1.58						
		Siganus canaliculatus	BDL	10.46 ± 0.96	BDL	42.92 ± 1.65						
Monsoon	Pelagic	*Iniistius cyanifrons*	BDL	2.57 ± 0.57	0.30 ± 0.03	33.44 ± 1.88	Species	260.8122	11	23.71	1.18	0.34
		Carangoides malabaricus	BDL	3.30 ± 0.69	BDL	18.67 ± 1.82	Metal	5743.525	3	1914.51	95.00	0.00
		Mugil cephalus	BDL	2.53 ± 0.38	0.36 ± 0.16	36.54 ± 1.70						
		Sardinella longiceps	BDL	2.65 ± 0.17	0.08 ± 0.01	27.59 ± 1.41						
		Rastrelliger kanagurta	BDL	2.22 ± 0.40	0.29 ± 0.05	22.58 ± 1.44						
		Saurida thumbil	BDL	1.67 ± 0.14	0.55 ± 0.15	15.5 ± 2.02						
		Stolephorus indicus	0.22 ± 0.06	1.27 ± 0.36	0.62 ± 0.21	9.75 ± 1.51						
		Sphyraena acutipinnis	BDL	1.32 ± 0.19	BDL	29.27 ± 2.00						
		Trichiurus lepturus	0.2 ± 0.07	1.78 ± 0.16	1.82 ± 0.20	31.45 ± 2.15						
	Benthic	*Cynoglossus* sp.	BDL	1.77 ± 0.15	BDL	22.33 ± 2.05						
		Lagocephalus sapadiceus	0.25 ± 0.1	3.60 ± 0.65	0.48 ± 0.13	39.69 ± 1.54						
		Siganus canaliculatus	BDL	10.49 ± 1.54	0.50 ± 0.13	28.39 ± 2.00						

Postmonsoon	Pelagic	*Iniistius cyanifrons*	BDL	BDL	BDL	32.34 ± 2.06	Species	168.913	11	15.36	1.10	0.39
		Carangoides malabaricus	0.27 ± 0.13	1.34 ± 0.21	1.92 ± 0.28	13.28 ± 2.21	Metal	7728.685	3	2576.23	184.50	0.00
		Mugil cephalus	0.46 ± 0.09	2.21 ± 0.37	1.34 ± 0.19	37.54 ± 1.87						
		Sardinella longiceps	0.47 ± 0.15	1.30 ± 0.24	3.35 ± 0.70	26.47 ± 2.57						
		Rastrelliger kanagurta	0.95 ± 0.24	2.67 ± 0.4	3.3 ± 0.35	24.70 ± 2.06						
		Saurida thumbil	0.38 ± 0.14	1.39 ± 0.18	1.17 ± 0.26	35.31 ± 1.87						
		Stolephorus indicus	0.75 ± 0.15	2.96 ± 0.26	3.22 ± 0.30	38.46 ± 2.01						
		Sphyraena acutipinnis	0.56 ± 0.15	1.91 ± 0.29	1.63 ± 0.21	28.84 ± 1.37						
		Trichiurus lepturus	0.62 ± 0.20	1.63 ± 0.21	1.55 ± 0.15	27.37 ± 1.96						
	Benthic	*Cynoglossus* sp.	0.28 ± 0.15	1.28 ± 0.2	1.93 ± 0.20	33.34 ± 2.00						
		Lagocephalus sapadiceus	0.56 ± 0.15	2.93 ± 0.36	0.66 ± 0.16	36.36 ± 1.90						
		Siganus canaliculatus	0.27 ± 0.14	8.76 ± 1.50	0.7 ± 0.11	35.37 ± 1.06						
Summer	Pelagic	*Iniistius cyanifrons*	BDL	BDL	BDL	14.79 ± 1.37	Species	1023.504	11	93.05	1.79	0.10
		Carangoides malabaricus	0.10 ± 0.01	31.48 ± 1.99	1.88 ± 0.13	25.64 ± 1.66	Metal	8647.207	3	2882.40	55.48	0.00
		Mugil cephalus	0.18 ± 0.03	47.98 ± 1.74	4.23 ± 0.36	29.25 ± 1.92						
		Sardinella longiceps	0.06 ± 0.01	31.37 ± 2.08	1.76 ± 0.14	37.39 ± 1.98						
		Rastrelliger kanagurta	0.88 ± 0.11	40.32 ± 1.19	6.82 ± 0.4	30.16 ± 2.00						
		Saurida thumbil	0.47 ± 0.15	29.78 ± 1.47	1.15 ± 0.22	22.85 ± 1.56						
		Stolephorus indicus	0.20 ± 0.02	43.36 ± 2.41	2.77 ± 0.15	26.53 ± 1.84						
		Sphyraena acutipinnis	0.49 ± 0.12	27.66 ± 1.80	0.78 ± 0.14	17.8 ± 1.47						
		Trichiurus lepturus	0.41 ± 0.04	31.6 ± 1.84	1.21 ± 0.26	16.71 ± 1.46						
	Benthic	*Cynoglossus* sp.	0.40 ± 0.13	22.68 ± 1.45	2.52 ± 0.22	25.23 ± 0.64						
		Lagocephalus sapadiceus	BDL	30.21 ± 1.01	4.55 ± 0.35	31.46 ± 0.97						
		Siganus canaliculatus	0.31 ± 0.09	12.74 ± 1.47	0.57 ± 0.14	32.52 ± 1.97						

BDL – below detection limit
All metal concentration values are represented as mean ± standard deviation in µg/g (dry weight).

The PCA results are shown in **Figure. 18** With eigenvalues of 1.86 and 0.96, respectively, only two main components i.e., PC1 and PC2 were considered for discussion. Owing to the low eigenvalue (<1), the principal components PC3 and PC4 were not considered. PC1 contributed 46.56% of variance with significant positive loadings for Pb (0.67) and moderate loading for Cu (0.53). PC2 contributed 23.89% of variance with significant positive loading of Zn (0.94) (**Table 5)**. PC1 exhibited a close collaboration with Pb followed by Cu, with the results indicating that greater levels of Pb in the environment are due to anthropogenic activities rather than geogenic sources. The possible sources of lead in the study areas are paint industries, automobile emissions, lead-acid batteries, and other industrial products. The scenario observed in this study has a strong correlation with an earlier study of Arumugam *et al.* (2018b). The loading of other metals in PC1 denoted the sources of the metals from a combination of both anthropogenic and natural sources. For PC2, only Zn had a strong positive loading and all other metals were negatively loaded. This indicates a natural source; nevertheless, an overabundance of Zn on the other hand, has the potential to harm organisms. Our PCA results revealed that first principal component is influenced by both manmade and natural sources, while the second principal component is influenced by natural source. This trend can be explained by the fact that monsoon dilution is a prominent factor in the Cuddalore coast.

Table 5. Percentage of variance, Eigenvalues, and principal component values of metal concentration in finfish species across two significant principal components.

Elements	**PC1**	**PC2**
Cd	0.441	-0.313
Cu	0.538	-0.066
Pb	0.676	-0.083
Zn	0.243	0.944
Eigenvalue	**1.862**	**0.956**
Percentage of variance	46.56%	23.89%
Cumulative	46.56%	70.45%

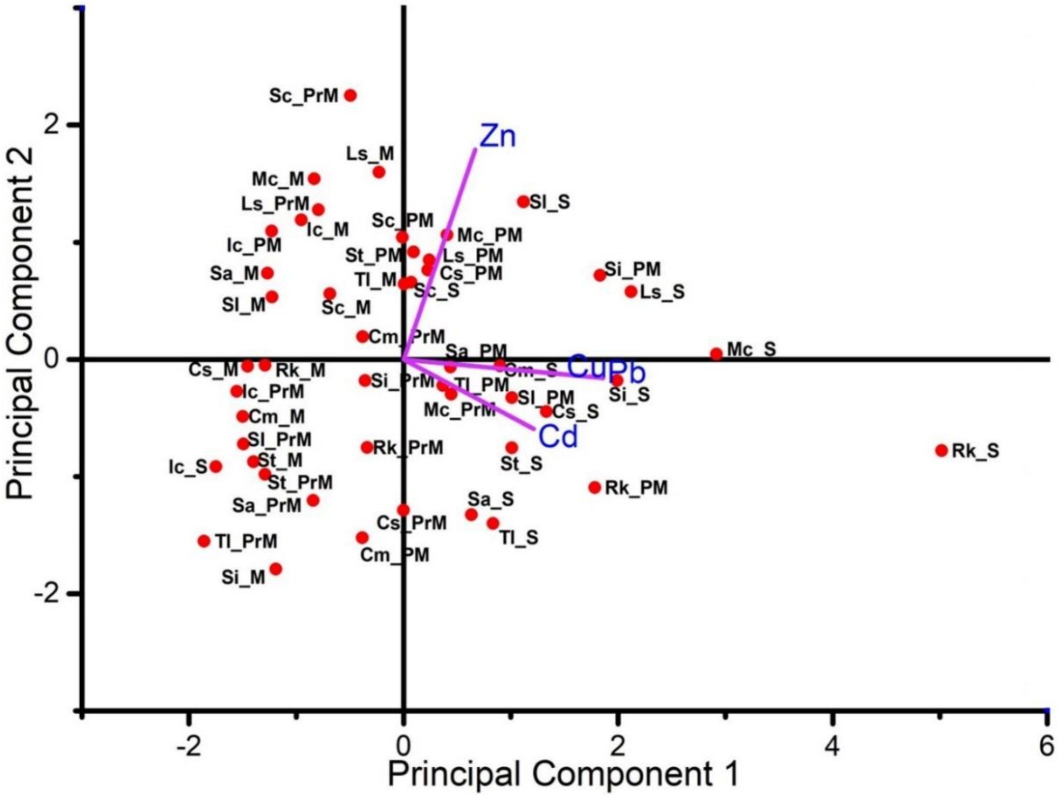

Figure 18 Biplot showing the interrelationship between metal, season, and finfish species. Species — Ic - *Iniistius cyanifrons*; Cm - *Carangoides malabaricus*; Mc - *Mugil cephalus*; Sl - *Sardinella longicep*; Rk- *Rastrelliger kanagurta*; St - *Saurida thumbil*; Cs - *Cynoglossus* sp.; Ls - *Lagocephalus sapadiceus*; Sc - *Siganus canaliculatus;* Si - *Stolephorus indicus*; Sa - *Sphyraena acutipinnis*; and Tl - *Trichiurus lepturus*. Season — Prm - premonsoon; M - monsoon; PM – postmonsoon; and S – summer. Labels of scores in the biplot are expressed as 'Species_Season'

The BCF was determined to better understand how different elements accumulated in different species with respect to season (**Figure 19**). The order of BCF value in pelagic fish was Cu > Zn > Pb > Cd, and the range of BCF for different metals was Cd (0–3.65), Cu (0–117.02), Pb (0–10.67), and Zn (5.62–23.31); while in benthic fish, the order was Cu > Zn > Pb > Cd, and the range was Cd (0–2.88), Cu (3.12–73.68), Pb (0–9.48), and Zn (11.86–26.01). The highest BCF value of Cd (3.65) in pelagic fish was observed for *Rastrelliger kanagurta* during the postmonsoon season. Maximum BCF value for Cu (117.02) was noted in *Mugil cephalus* during the summer season. Highest BCF value for Pb (10.67) was noted in *Rastrelliger kanagurta* during the summer season. BCF value for Zn (23.31) was high in *Stolephorus indicus* during the postmonsoon season. Coming to the benthic, the highest BCF for Cd (2.88) was noted in *Cynoglossus* sp., during the premonsoon season. BCF for Cu (73.68) was highest in *Lagocephalus sapadiceus* during the summer season. Maximum BCF value for Pb (9.48) was also observed in *Lagocephalus sapadiceus* in the summer season. Highest BCF value for Zn (26.01) was seen in *Siganus canaliculatus* during the premonsoon season. The higher BCF values for the four metals were recorded in the postmonsoon and summer seasons in the pelagic fish. And in the benthic group, the highest values were observed in the summer and premonsoon seasons. For most species, values of BCF fell within the threshold level (BCF<1) in the premonsoon and monsoon seasons. Compared with premonsoon and monsoon seasons, the BCF values reported during the postmonsoon and summer seasons were higher than the threshold level (BCF>1). The results indicate that the monsoon clearly influences the Cuddalore coast and has a massive impact on the ecosystem with respect to the distribution of metal contaminants in the marine food web.

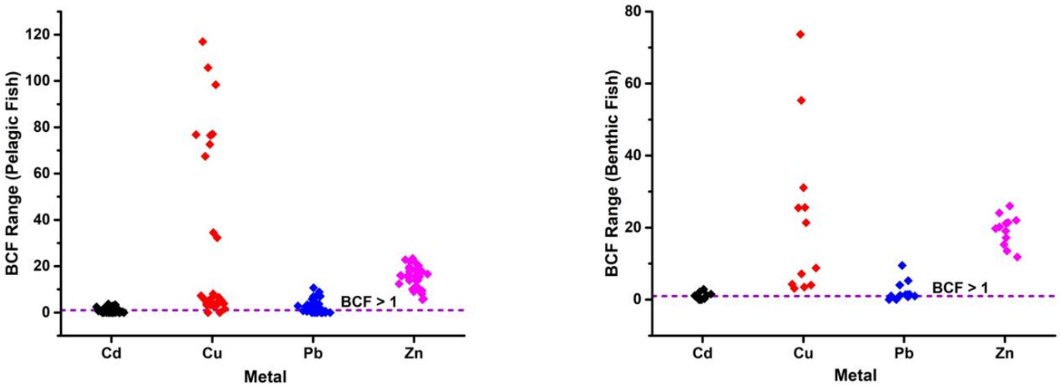

Figure.19 Variations in the bio concentration factors (BCF) in the pelagic and benthic finfish species.

5.3.2 Metal concentration in shellfish

Seasonal variation of Cd, Cu, Pb, and Zn was studied in eight different shellfish species, which were divided into two groups based on their salient features: four crustaceans and four molluscs. The seasonal fluctuations in four metal concentrations in eight shellfish species are illustrated in **Table 6.** The metal concentration ranged across all four seasons as follows: Cd (0.97–1.45 µg/g), Cu (3.53–26.32 µg/g), Pb (0.37–2.39 µg/g), and Zn (32.07–39.71 µg/g). Likewise, the lower and higher bounds of metal concentration for the different seasons are as follows: premonsoon, Cd (0.22.–2.81 µg/g), Cu (1.02–8.59 µg/g), Pb (BDL–0.61 µg/g), and Zn (15.35–55.90 µg/g); monsoon, Cd (BDL–3.00 µg/g), Cu (0.56–20.69 µg/g), Pb (BDL–0.81 µg/g), and Zn (27.95–50.67 µg/g); postmonsoon, Cd (BDL–3.31 µg/g), Cu (BDL–21.44 µg/g), Pb (BDL–0.81 µg/g), and Zn (10.92–46.82 µg/g); summer, Cd (BDL–3.32 µg/g), Cu (1.80–49.67 µg/g), Pb (BDL–4.01 µg/g), and Zn (22.90–47.78 µg/g). The metal distributions across the eight shellfish species were statistically significant at the 0.05 level: Cd (*P* <0.05, F = 67.31); Cu (*P* <0.05, F = 122.15); Pb (*P* <0.05, F = 38.67); and Zn (*P* <0.05, F = 27.66) (**Table 6**). Cd levels were below the detection limit (BDL) in certain species, including *Portunus sanguinolentus* in the postmonsoon and summer seasons, and in *Loligo duvauceli* and *Ficus variegata* in the monsoon season.

During the summer season, the highest concentration of Cd was found in *Loligo duvauceli* (3.32 ± 0.19 µg/g), followed by *Calappa lophos* (3.31 ± 0.24 µg/g) in the postmonsoon season. During the premonsoon and monsoon seasons, *Meretrix casta* and *Ficus variegata* the Cd concentrations were considerably high. Coming to Cu, only during the postmonsoon season, *Portunus sanguinolentus* species showed values below detection limit. In all other species, the values were above the detection limit, with *Calappa lophos* (49.67 ± 1.50 µg/g) having the highest concentration, followed by *Loligo duvauceli* (49.62 ± 0.93) and *Calappa lophos* (21.44 ± 1.17 µg/g) during postmonsoon season. On the other hand, the lowest Cu concentration was observed in *Perna viridis* (0.56 ± 0.16 µg/g) during the monsoon season. Overall, in the premonsoon season Cu concentration were generally lower. Pb values were BDL in *Meretrix casta* in premonsoon, *Ficus variegata* in monsoon, *Portunus sanguinolentus* in postmonsoon, and *Charybdis natator* and *Portunus sanguinolentus* in summer. *Loligo duvauceli* (4.01 ± 0.17 µg/g) had the highest Pb concentration during the summer season, followed by *Ficus variegata* (03.45 ± 0.2 µg/g) during the postmonsoon season. *Penaeus indicus*, *Loligo duvauceli*, *Perna viridis*, *Meretrix casta*, and *Ficus variegata* are had slightly increased level of Pb in the postmonsoon season compared with other seasons. During the premonsoon season, Zn values were higher in *Portunus sanguinolentus* (55.90 ± 2.40 µg/g) followed by *Ficus variegata* (50.95 1.53 µg/g). During the postmonsoon season, *Penaeus indicus* (10.92 ± 0.26 µg/g) had the lowest Zn concentration. In all four seasons, Zn values were found to be evenly distributed. The order of the metals across the different seasons is as follows: premonsoon (Zn > Cu > Cd > Pb), monsoon (Zn > Cu > Cd > Pb), postmonsoon (Zn > Cu > Pb > Cd), and summer (Zn > Cu > Pb > Cd). The order of seasonal average for the four metals is as follows: Cu (summer > postmonsoon > monsoon > premonsoon), Cd (postmonsoon > summer > monsoon > premonsoon), Pb (postmonsoon > summer > monsoon > premonsoon), and Zn (premonsoon

> summer > monsoon > premonsoon). **Figure 20 and 21 represents** the seasonal distribution of four metals in eight different species of shellfish.

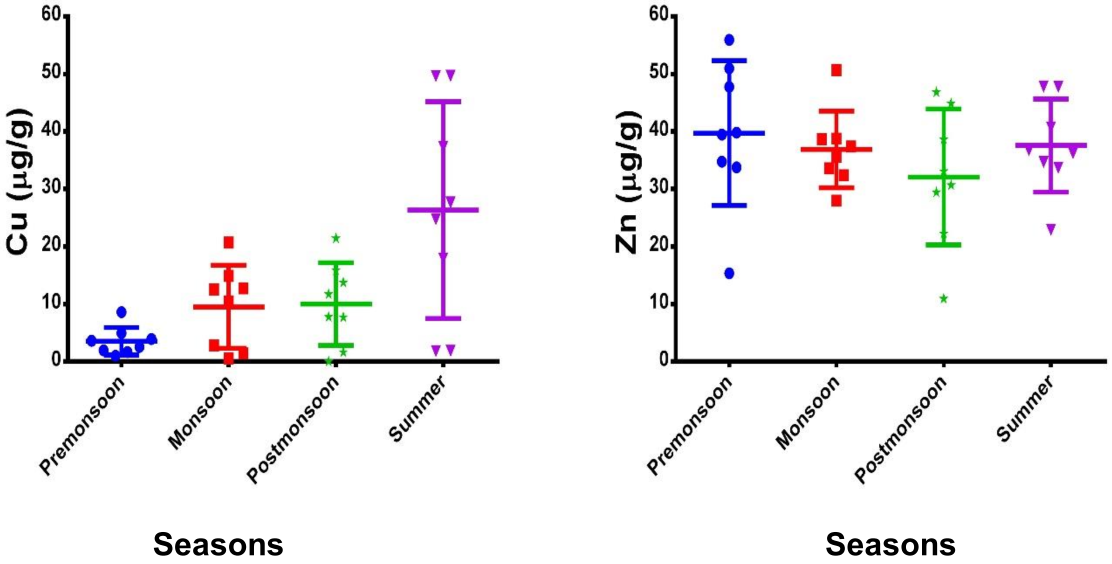

Figure 20 The concentration of Cu and Zn in the nine species of shellfishes collected from Cuddalore coast.

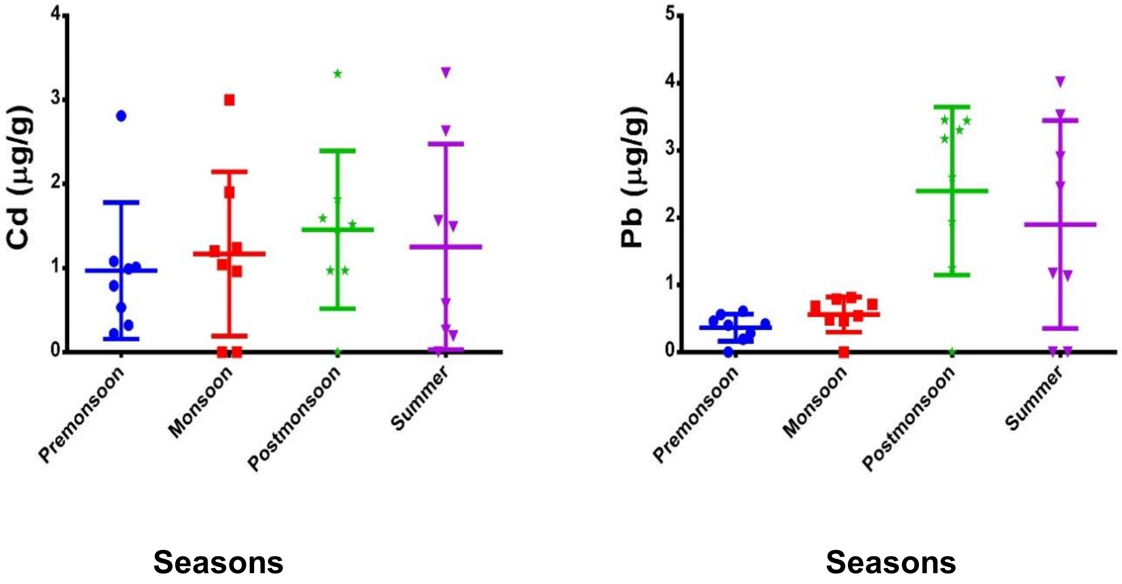

Figure 21 The concentration of Cd and Pb in the nine species of shellfishes collected from Cuddalore coast.

Table 6. Seasonal distributions of four metals (Cd, Cu, Pb, and Zn) and two-way ANOVA for the shellfish species from the Cuddalore coast.

Metal	Species (N=3)	Metal Concentration (Mean ± SD)				ANOVA					
		Premonsoon	**Monsoon**	**Postmonsoon**	**Summer**	**Factor**	***SS***	***df***	***MS***	***F value***	***P value***
Cd	*Calappa lophos*	0.32 ± 0.05	1.90 ± 0.31	3.31 ± 0.24	0.26 ± 0.08	Species	242.68	7	34.67	0.80	0.60
	Portunus sanguinolentus	0.99 ± 0.20	0.96 ± 0.15	BDL	BDL	Metal	8748.81	3	2916.27	67.31	0.00
	Charybdis natator	0.53 ± 0.11	1.04 ± 0.24	1.46 ± 0.15	2.63 ± 0.26						
	Penaeus indicus	1.01 ± 0.20	1.20 ± 0.23	1.59 ± 0.16	0.19 ± 0.02						
	Loligo duvauceli	0.22 ± 0.03	BDL	0.97 ± 0.14	3.32 ± 0.19						
	Perna viridis	0.79 ± 0.12	1.24 ± 0.26	0.97 ± 0.04	1.49 ± 0.11						
	Meretrix casta	1.08 ± 0.11	3.00 ± 0.12	1.52 ± 0.10	1.56 ± 0.15						
	Ficus variegata	2.81 ± 0.39	BDL	1.82 ± 0.11	0.57 ± 0.01						
Cu	*Calappa lophos*	8.59 ± 0.65	20.69 ± 1.67	21.44 ± 1.17	49.67 ± 1.50	Species	281.23	7	40.18	2.11	0.09
	Portunus sanguinolentus	4.94 ± 0.32	12.73 ± 0.50	BDL	17.9 ± 1.65	Metal	6982.33	3	2327.44	122.15	0.00
	Charybdis natator	1.02 ± 0.04	14.88 ± 1.21	15.86 ± 0.55	27.64 ± 0.92						
	Penaeus indicus	2.55 ± 0.52	10.47 ± 0.72	07.72 ± 0.62	37.3 ± 1.04						
	Loligo duvauceli	3.59 ± 0.70	2.82 ± 0.15	11.71 ± 1.5	49.62 ± 0.93						
	Perna viridis	1.68 ± 0.41	0.56 ± 0.16	07.75 ± 0.62	01.80 ± 0.17						
	Meretrix casta	3.93 ± 0.10	12.52 ± 0.87	13.76 ± 1.55	24.73 ± 0.46						
	Ficus variegata	1.95 ± 0.19	1.43 ± 0.11	01.63 ± 0.09	01.88 ± 0.13						
Pb	*Calappa lophos*	0.19 ± 0.06	0.47 ± 0.12	01.25 ± 0.09	1.17 ± 0.07	Species	474.83	7	67.83	1.61	0.19
	Portunus sanguinolentus	0.56 ± 0.14	0.54 ± 0.10	BDL	BDL	Metal	4875.77	3	1625.26	38.67	0.00
	Charybdis natator	0.28 ± 0.05	0.68 ± 0.15	01.94 ± 0.27	BDL						
	Penaeus indicus	0.46 ± 0.13	0.79 ± 0.12	03.30 ± 0.36	1.13 ± 0.11						
	Loligo duvauceli	0.40 ± 0.10	0.48 ± 0.05	02.60 ± 0.33	4.01 ± 0.17						
	Perna viridis	0.61 ± 0.10	0.71 ± 0.09	03.17 ± 0.24	3.52 ± 0.14						
	Meretrix casta	BDL	0.81 ± 0.08	03.44 ± 0.32	2.45 ± 0.19						
	Ficus variegata	0.42 ± 0.04	BDL	03.45 ± 0.2	2.90 ± 0.13						
Zn	*Calappa lophos*	15.35 ± 1.81	50.67 ± 1.61	44.86 ± 1.34	47.78 ± 1.65	Species	973.91	7	139.13	1.46	0.23
	Portunus sanguinolentus	55.90 ± 2.40	27.95 ± 1.50	22.23 ± 0.40	40.69 ± 1.34	Metal	7882.56	3	2627.52	27.66	0.00
	Charybdis natator	47.74 ± 1.56	37.4 ± 0.94	30.68 ± 0.64	34.69 ± 1.20						
	Penaeus indicus	39.79 ± 1.51	38.73 ± 0.45	10.92 ± 0.26	22.9 ± 1.34						
	Loligo duvauceli	33.76 ± 1.36	32.38 ± 0.77	29.41 ± 0.74	47.77 ± 0.55						
	Perna viridis	34.74 ± 1.50	38.66 ± 0.48	46.82 ± 1.67	33.68 ± 1.42						
	Meretrix casta	39.45 ± 0.63	35.57 ± 0.75	33.06 ± 0.56	36.74 ± 0.45						
	Ficus variegata	50.95 ± 1.53	33.57 ± 1.38	38.62 ± 0.56	36.22 ± 1.33						

BDL – below detection limit; *SS* – sum of squares; *df* – degrees of freedom; *MS* – mean square
All metal concentrations are in µg/g (dry weight)

Figure 22 illustrates the PCA data. Only two principal components, PC1 and PC2, were considered for discussion because their eigenvalues were 1.63 and 1.07, respectively. The principal components PC3 and PC4 were not considered because of their low eigenvalue (<1). PC1 was responsible for 40.86% of the variation, with strongly positive loadings for Cd (0.64) and moderate loadings for Cu (0.5). PC2 was responsible for 26.64% of the variation, with considerable positive loading in Zn (0.75) as shown in **Table 7** and PC1 has a strong relationship with Cd, followed by Cu, demonstrating that higher levels of Cd in the ecosystem are primarily due to human activities instead of natural earth crust sources. The occurrence of other metals in PC1 demonstrates that the metals may have originated from both man-made and natural sources. Only Zn had a significantly positive loading in PC2, followed by Cd, which was also marginally positive, while the other two metals were negatively loaded. PC2 similarly includes both man-made and natural sources. Zn despite being an essential element, excess concentrations of Zn also can be harmful to organisms.

Table 7. Percentage of variance, eigenvalues, and principal component loading values of four metal concentration in shellfish species in two significant principal components (PC1 and PC2).

Elements	**PC1**	**PC2**
Cd	0.64	0.05
Cu	0.5	-0.05
Pb	0.44	-0.66
Zn	0.38	0.75
Eigenvalue	1.63	1.07
Percentage of variance (%)	40.86	26.64
Cumulative (%)	40.86	67.50

The purpose of BAF was to understand more about how different metals accumulated in different biota as a consequence of season, and the BAF results are shown in **Figure 23.** The overall BAF average for the different metals was as follows: Cd (2.69), Cu (1.18), Pb (0.34), and Zn (2.30). Based on the average, Cd > Zn > Cu > Pb was the order of BAF values in shellfish and the range of BAF for different metals was Cd (0–7.38), Cu (0–4.76), Pb (0–1.04), and Zn (0.97–3.52). *Calappa lophos* had the highest BAF value for Cd (7.35) among the shellfish during the postmonsoon season. The maximum BAF value for Cu (4.76) was observed in two species — *Calappa lophos* and *Loligo duvauceli*, both during the summer season. During the postmonsoon season, *Loligo duvauceli* had the maximum BAF value for Pb (1.04), while during the premonsoon season, *Portunus sanguinolentus* had the highest BAF value for Zn (3.52). BAF values greater than one is usually considered to have a higher bioaccumulation of metals than what is present in the surrounding medium. If the number is less than one, it means there is lesser accumulation in the biota compared with the surrounding medium. As per this hypothesis, BAF values for Zn were found to be higher than one in many species throughout all seasons, followed by Cd values that reached the threshold values in many species across all seasons, and Cu values also reached the threshold values in the monsoon, postmonsoon and summer seasons; however, BAF value for Pb was greater than one in only one species crossing the threshold level during the summer season.

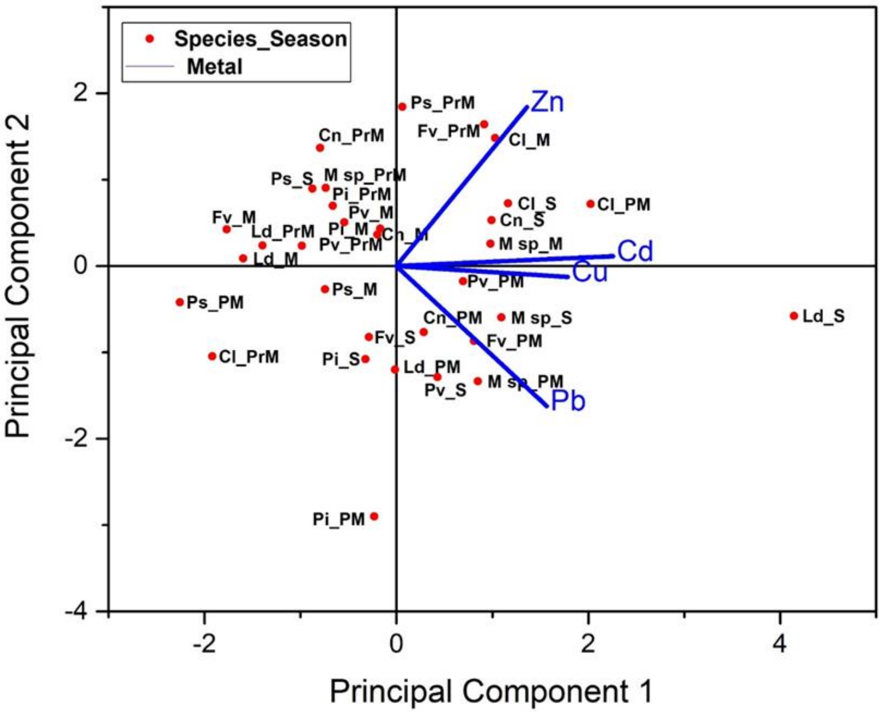

Figure 22 Biplot showing the interrelationship between metal, season, and shellfish species. Species — Cl is *Calappa lophos,* Ps is *Portunus sanguinolentus,* Cn is *Charbdis natator,* Pi is *Penaeus indicus,* Ld is *Loligo duvauceli,* Pv is *Perna viridis,* Mc is *Meretrix casta, and* Fv is *Ficus variegata.* Season — Prm is premonsoon; M is monsoon; PM is postmonsoon; and S is summer. Labels of scores in the biplot are expressed as 'Species_Season'

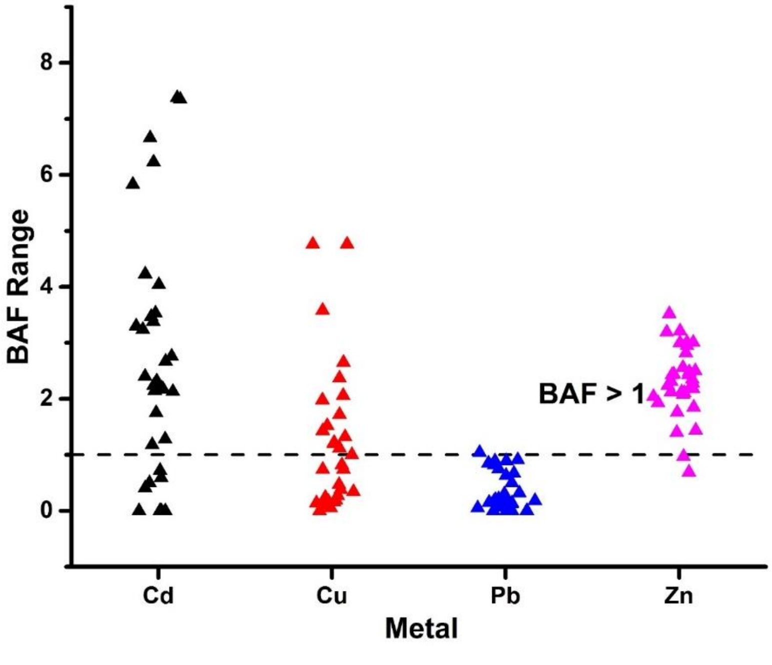

Figure 23 Variations in the bio concentration factors (BAF) in the shellfish species.

5.4 DISCUSSION

The average levels of metals found in the tissue samples differ with the species of fish. A variety of factors influence the metal accumulation; however, season may play a significant role (Kargin, 1996). As per the report of Vinothkannan *et al.* (2022a), more metal contaminants may get flushed and diluted into the rivers and estuaries during the monsoon seasons due to increased runoff caused by the rainwater (Hossain *et al.*, 2020). The higher levels of metals observed in the summer season might be due to the lack of any influx of freshwater causing the muddy sediments loaded with metals to get deposit in the estuarian ecosystem (Pande and Nayak, 2013). The variation in metal levels shows how each species' feeding habit and accumulating capacity changes over the seasons. The primary route of transition for many aquatic species is a direct transfer of metals from sediment to aquatic organisms. Metals tend to accumulate in the benthic species and transmit upwards through the food chain via biomagnification (Jovanovic *et al.*, 2017). In a similar study, Pourang (1995) has already explored the effect of dietary habits on metal deposition in various fish organs and tissues, and the relative significance of water and food to the accumulation of metals by fish. Cadmium and arsenic are metals that are strongly linked with sediments which can act as long-term pollutants for benthic fish (Noel *et al.*, 2013).

Shellfish have been used as biomarkers to assess the metal contamination for two major reasons: (1) their ability to accumulate metals from the environment and easily accumulate them in their bodies, and (2) they can be used in determining the health hazards towards humans who consume these contaminated shellfish (Saher and Kanwal, 2019). Leung *et al.* (2014) broadly classified the shellfish into two categories — crustaceans and molluscs. We collected eight different shellfish species and evaluated the metal concentrations in their tissues. Among these eight species, four were crustaceans, including three crab and one shrimp species (VanHook and Patel, 2008), and the other four species were mollusks, including one squid one gastropod and two bivalve species (Dolorosa and

Dangan-Galon, 2014). Molluscs and crustaceans, both inhabit the benthic region, and crustaceans are mainly scavengers and are omnivorous in diet, which implies that they may be consuming more contaminants (Ip *et al*., 2005). As these organisms thrive in the benthic floor, they are more associated with the contaminated sediment, which might cause excessive metal accumulation (Zhao *et al*., 2012). For mollusks, bivalves tend to absorb and store inorganic elements of anthropogenic sources over long periods of time by filtering enormous amounts of water (Arumugam *et al*., 2020; Chelomin *et al*., 2022). The Cephalopoda group, on is a top trophic level since they are carnivores, which is one of the sources of metal accumulation (Kojadinovic *et al*., 2011). Crustaceans are fed by sediments, which are thought to be the major source of pollution (Younis *et al*., 2014). Except Cu, the concentrations of Cd, Pb, and Zn in the mollusc species were higher in our study site, and our findings correlate well with the statement of Chelomin *et al*. (2022). Metal levels in sediment are generally much higher than in water because metals have the ability to deposit and bind to sediments (Schertzinger *et al*., 2018). Shellfish are so closely associated with marine sediments leading to more metal deposition and accumulation in them. As per our previous report (Vinothkannan *et al*., 2022a), marine sediment from Cuddalore coast has been found to be contaminated in the recent decades with various metal loadings from treated and untreated industrial effluents and agricultural runoff that flows into the Uppanar estuary during the monsoon. The stormwater and floodwaters flow during the monsoon season are also increased. The present results show that Cd, Cu, and Pb concentrations are higher in the postmonsoon and summer seasons. According to Vinothkannan *et al*. (2022a), the results clearly illustrate that season is a major contributor to metal accumulation as it follows an increasing trend from the premonsoon season towards the summer season.

Among the twelve fish species assessed in our study, there are no reports elsewhere on metal accumulations for two fish species — *Iniistius cyanifrons* and *Sphyraena acutipinnis*. Cd and Pb levels in *Carangoides malabaricus* in our study had considerably lower value compared with a study in the Qatari coast, Doha (Kureishy, 1993). Cu and Zn values in *Carangoides malabaricus* are higher the current study in comparison with the values reported in the Andaman Sea and Gulf of Mannar, Chinnamuttom region (Kureishy *et al*., 1981; Nagarani *et al*., 2020).

The average Cd concentration in *Mugil cephalus* reported in our study was similar to several studies conducted worldwide (Frias-Espericueta *et al*., 2011; Genc and Yilmaz, 2018; Ouali *et al*., 2018); however, in a study from the creek in Woji, Southern Nigeria (Ihunwo *et al*., 2020), Cd values about hundred times higher than the present study were reported. In the case of Cu, the present study recorded several folds' higher concentration when compared to the report of Yilmaz (2005). The Pb concentrations for *Mugil cephalus* in the current report were similar to values reported in the coastal lagoons of NW Mexico Frias-Espericueta *et al*. (2011). The Zn values were slightly similar to Krishna (2014) and Kalay *et al*. (1999), but three-fold higher than North African coasts of the Mediterranean Sea (Ouali *et al*., 2018).

In *Sardinella longiceps* the values for Cd were comparable to the values reported all over world, except for the value from Abeokuta in Nigeria which was slightly higher (Akinhanmi *et al*., 2021). The Cu values were higher in present study compared with the data elsewhere. Pb level was lower in the present study compared with that reported in the Arabian Sea by Tariq *et al*. (1991) and in the Gulf of Mannar by Rameshkumar *et al*. (2018). Zn values were similar to that reported by Bristy *et al*. (2021), and lesser than that reported in the Balochistan coast in Pakistan by Ahmed *et al*. (2016). In *Rastrelliger kanagurta*, the Cd value in our study was ten-fold lesser than the values reported in the Olavakkode Fish Market in Kerala, India (Rini *et al*., 2020). Nevertheless, the values reported by us are similar to that of Kumar *et al*. (2012). The Cu and Pb values were higher when compared with the other works reported globally (Heba *et al*., 2014; Khandaker *et al*., 2015; Mziray and Kimirei, 2016; Praveena and Lin, 2015; Rejomon *et al*., 2010). In *Saurida thumbil*, the

Cd, Cu, and Pb values in the current study were higher than the values reported by Yesser *et al*. (2013) in Basra city, Iraq, but were almost similar to the data of Yesser *et al*. (2013). Zn values were higher in the present study compared with globally reported data.

In *Cynoglossus* sp., the present values were on average comparable to the data reported from different regions of the world (Charisma *et al*., 2013; De *et al*., 2010; Taiwo *et al*., 2017). In *Lagocephalus sapadiceus* the Cd, Pb, and Zn showed higher values were noted in the female fish at Berdan River and Yeşilovacık Bay (Kosker *et al*., 2019) compared with the present study; however, the Cu values were higher in the current study. In *Siganus canaliculatus*, the Cd and Pb values were higher in the Arabian Gulf Coast as reported by Zyadah and Almoteiry (2012). In *Stolephorus indicus*, all the four metal values were higher than those reported by Alizada *et al*. (2020) and Arulkumar *et al*. (2017). In *Trichiurus lepturus*, the Cd value was comparatively higher in the Miri coast of Borneo (Anandkumar *et al*., 2018) and lower in Mumbai Harbor, India (Velusamy *et al*., 2014). The Cu and Pb values were higher in the present study in comparison to an earlier report (Anandkumar *et al*., 2018). The Zn value was notably higher in Mumbai Harbor, India (Velusamy *et al*., 2014). In comparison to our data, lower level of Zn was reported in Karachi fish harbour in Pakistan (Ahmed *et al*., 2018).

There are no other reports on metal accumulations in *Ficus variegata*, one of the eight shellfish species studied in our study. Yang *et al*. (2021) studied *Calappa lophos* in the Pearl River Estuary of the South China Sea. When comparing the current research values to that study, the Cd, Cu, and Pb values were higher, but the Zn values were lower in *Calappa lophos*. In the Thoothukudi coast, Shalini *et al*. (2020) reported Cd and Pb levels in *Portunus sanguinolentus*, and our findings were higher than that reported in their study. In the case of *Charybdis natator*, the specimen from the Cuddalore coast had higher Cd level than other report of *Charybdis natator* from Thoothukudi in South India, Red Sea in Egypt, and Lagoons in South China (Feng *et al*., 2020; Sallam and Gab-Alla, 2009; Shalini *et al*., 2020), whereas the other three metals (Cu, Pb, and Zn) in *Charybdis natator* were higher in the study conducted by Sallam and Gab-Alla (2009). Furthermore, for *Penaeus indicus*, several studies around the world shows, Cd concentration have found higher values in Sagar Island of Kolkata (Basu *et al*., 2021), hence the present study also indicated that, Cd concentration was higher in Cuddalore coast, compared with the study done in Vellar estuary, Uppanar estuary, Pulicat lake, Visakhapatnam, Malad Market of Mumbai (Batvari *et al*., 2016; Pragnya *et al*., 2021; Sivaperumal *et al*., 2007; Sulieman and Suliman, 2019; Zodape, 2014). Pb concentration was higher in the region of Sagar Island of Kolkata and Pulicat lake (Basu *et al*., 2021; Batvari *et al*., 2016). In Cuddalore, Pb concentration was higher when compared with Vellar estuary, Uppanar estuary, Visakhapatnam, Malad Market of Mumbai (Pragnya *et al*., 2021; Sivaperumal *et al*., 2007; Sulieman and Suliman, 2019; Zodape, 2014). The Pb level in *Loligo duvauceli* was higher in the Arabian Sea, India (Tariq *et al*., 1991), and the present study Cd, Cu, and Zn levels were higher in *Loligo duvauceli.* Mahat *et al*. (2018) reported higher concentrations of Cd, Cu, and Pb in *Perna viridis* in Pasir Gudang coastal area, Malaysia; however, Zn value was not reported in that study. The higher Zn values were present in west coast of Peninsular Malaysia (Yap *et al*., 2004). When compared to other studies, in the present study, the concentration of Zn in *Perna viridis* was higher (Rojas de Astudillo *et al*., 2002; Sasikumar *et al*., 2006). The *Meretrix casta* was used to assess metal concentration in many studies, among which the present study found a higher Cd level, followed by Cu and Pb, on India's Southeast coast (Muthukumar *et al*., 2014). Higher Zn values were found in the Cochin backwaters of Kerala, India (Lakshmanan and Nambisan, 1983).

Chapter VI

Ecological and human health risk assessment of trace metal pollution in Cuddalore coast.

6.1 INTRODUCTION

Contamination has a significant influence on ecosystem health and habitat compatibility for both flora and fauna. Environmental Protection Agency (EPA) requires an ecological risk assessment (ERA) to determine the possible negative effect of any anthropogenic actions on the environment (USEPA, 1997). Several contaminants are involved in polluting the ecosystem and human health, with metal contamination being a major threat to people around the world. Metals are inert in sedimentary environments, but when they interact with the water medium, they pose a harm to ecosystems and are typically categorised as conservative pollutants (Agarwal *et al.*, 2005; Chow *et al.*, 2005; Olivares-Rieumont *et al.*, 2005)**.** Metals can affect aquatic species intentionally or unintentionally and the effects of pollutants on sediment can also be identified as a result of bioaccumulation in the food web (Zhang and Ke, 2004). As a result, an assessment of the accumulation of metals in sediment in urban centers could be used to explore anthropogenic influence on the environment and contribute in the evaluation of hazards caused by human pollutants released (Zheng *et al.*, 2008).

Ever 1980s, ecological risk assessment has developed over the years, as have new concerns such as global climate change, habitat loss and biodiversity loss, and the effects of various human pollutants on ecological systems that must be included in risk assessment procedures. There is also a continuous trend away from assessing adverse health consequences on specific, typically small-scale ecosystems and toward more complicated ecological analysis of large people and organizations across ecologically important regions

(Hope, 2006). Ecological risk assessment is indeed a versatile method for gathering and analysing information, presumptions, and uncertainly in order to assess the possibility of negative ecological effects that could have happened or may happen as a result of exposed between one or more sources of stress associated with human activities (Suter *et al*, 2000).

Previously, ecological risk assessments have intended to evaluate the risk involved with a receptor's exposed to hazardous stimuli in abiotic (sediment, water, etc.) and biotic (predatory animals) media. A free living receptor, on the other hand, is continually challenged to avoid or minimize the negative effects of physical (loss of habitat) and biological (lack of adequate nutrition) stressors that are already a persistent and natural part of its daily life (Hope, 2006). The index of geo-accumulation (Porstner, 1989), the potential ecological risk index (Hakanson, 1980), and the excess regression analysis (ERA) (Hilton *et al*., 1985) are the most often calculations used to assess the ecological risk caused by metals in sediments. The first two indexes are the most widely used.

Potentially toxic contaminants such as metals are commonly found in Cuddalore water, finfish and shellfish resources (Chapter V). These contaminants can threaten to human health and thus provide a considerable restriction to the consumption of these food resources. The main purpose of this chapter was to provide an approach for determining the risk to public health posed by the accumulation of metals in seafood resources from Cuddalore coast. This assessment study will lead to further decisions on risk management of contaminated fish consumption in the future, and appropriate treatment methods will be necessary.

There are several techniques to analysing various risks of metals in fish to public health. It can be estimate the cancer-causing and non-carcinogenic effects. Measured or expected exposure levels for carcinogenic pollutants are compared to adverse effect thresholds calculated using the dose-effect relationship (Solomon *et al.*, 1996). Several studies have used the probability of risk assessment approach to fully exploit existing dose and toxicity evidence (Hall *et al.*, 2000; Solomon *et al.*, 1996; Wang *et al.*, 2002). These methods are only relevant for measuring the health risks provided by carcinogenic pollutants. These approaches do not assess the risk of obtaining non-cancer effects from contamination and do not measure non-carcinogenic risk. Non-carcinogenic risk calculation methods were identified in order to calculate the target hazard quotient (THQ). Despite the fact that the THQ-based approach to human health risk assessment does not give a quantitative analysis of an affected population. It has a negative impact on health and acts as an indicator of the level of contamination exposure (Wang *et al.*, 2005; Yi *et al.*, 2011).

The main purpose of this chapter was to establish ecological assessment methods including the Geo-accumulation index, contamination factor and degree, pollution load index, and potential ecological risk. Human health risk assessment of toxic metals contamination, including estimated daily intake and target hazard quotients of two essential (Cu and Zn) and two non-essential (Cd and Pb) metals in sediment and fish samples collected from the Cuddalore coast of Tamil Nadu, Southern India.

6.2 MATERIALS AND METHODS

6.2.1 Metal concentrations in sediment and fish samples

The sediment results were used for the ecological risk assessment in the chapter IV, and the finfish and shellfish results (Chapter V) were applied to estimate the human health risk in the second chapter. The sample collection and preparation methods are explained in the previous chapters. The equations and formulae used in this assessment study are detailed below.

6.2.2 Ecological risk assessment

6.2.3 Pollution monitoring indices

To evaluate the pollution levels in the study area, we have used pollution monitoring indices like geo-accumulation index (I_{geo}), contamination factor (C_f^i), contamination degree (C_{deg}), pollution load index (PLI), and potential ecological risk (PER).

6.2.4 Geo-accumulation index

As per Muller (1969), the geo-accumulation index (I_{geo}) was assessed to know the pollution levels in surface sediments of the study area. The below equation was employed to compute the geo-accumulation index:

$$I_{geo} = log_2 \left(\frac{C_n}{1.5 \times B_n} \right)$$

where C_n is the concentration of metal (n) in the sediment samples and B_n denotes the geochemical background value of the respective metal (n) in the Earth's crust. In our study area, the background values for these metals are not available; hence, the B_n values as suggested by Taylor and McLennan (1985) were considered for computation, and a factor of 1.5 was introduced to recompense the background content due to various lithogenic effects. The pollution classification of I_{geo} based on its values (Nethaji *et al.*, 2017) is as follows: $I_{geo} < 0$ signifies lack of pollution, 0–1 indicates no pollution to moderate pollution, 1–2 denotes moderate pollution, 2–3 represents moderate to strong pollution, 3–4 indicates strong pollution, 4–5 represents strong to extreme pollution, and $I_{geo} > 5$ denotes extreme levels of pollution.

6.2.5 Contamination factor and contamination degree

Hakanson (1980), in his work, has stated four grades for classifying the contamination factor (C_f^i) and contamination degree (C_{deg}) in order to assess the heavy metal pollution levels. The factor C_f^i were calculated using the formula:

$$C_f^i = \frac{C_s^i}{C_b^i}$$

where C_s^i represents the mean concentration of heavy metal i in the sample, and C_b^i is the reference concentration of heavy metal i, signifying the earth crust background (Taylor and McLennan, 1985). Following a modified method of Hakanson (1980), the degree of contamination for four heavy metals in sediment samples was determined as follows:

$$C_{deg} = \sum C_f^i$$

The classification for degrees of contamination suggested by Hakanson (1980) was based on eight elements, while we consider only four elements in this study. Thus, the original degree of contamination classification was altered accordingly and is presented in **Table 8**.

6.2.6 Pollution load index

The degree of heavy metal pollution present in sediments was evaluated by computing the pollution load index (PLI) as per the method set forth by Tomlinson *et al.* (1980):

$$PLI = \left(C_f^1 \times C_f^2 \times C_f^3 \times \ldots C_f^i\right)^{1/i}$$

where i is the number of heavy metals, and C_f^i is the contamination. Here, we assess the pollution load index for four metals (Cu, Cr, Fe, and Zn). PLI can basically segregate the site quality by categorizing pollution levels into 3 groups (**Table 8**), viz., PLI < 1 meaning lack of pollution, PLI > 1 suggesting deterioration of site quality, and PLI = 1 denoting baseline levels of pollutants (Tomlinson *et al.*, 1980).

6.2.7 Potential ecological risk

The potential ecological risk (PER) of heavy metal contamination is quantitatively evaluated using potential ecological risk index (E_i) (Hakanson, 1980; Zhu *et al.*, 2008), which is derived by multiplying contamination factor (C_f^i) and toxic response factor (T_i). The potential risk index can be calculated as follows:

$$E_i = T_i \times C_f^i$$

$$PER = \sum E_i$$

where E_i and PER symbolize the ecological risk index for a single element and potential ecological risk for multiple elements, respectively. T_i is the toxic response factor of each metal which is equal to 1 for Zn, 5 for Cu and Pb, and 30 for Cd (Xu *et al.*, 2015). The degree of ecological risk can be categorized as follows (Xu *et al.*, 2015): low for $E_i < 15$, moderate for $15 \leq E_i < 30$, considerable for $30 \leq E_i < 60$, high for $60 \leq E_i < 120$, and very high for $E_i \geq 120$. The grade of ecological risk can be classified as follows (Xu *et al.*, 2015): low for PER < 50, moderate for 50 ≤ PER <100, considerable for 100 ≤ PER < 200, and high for PER ≥ 200 (**Table 8**).

6.2.8 Human health risk assessment

Majority of the human population consumes fish as a source of protein. Hence, fish muscles tissues were utilized to evaluate the risk to human health using an estimated daily intake (EDI) of elements and target hazard quotients (THQ). The below calculation was used to estimate the daily intake of metals (Pal and Maiti, 2018).

$$\mathrm{EDI} = \frac{\mathrm{FIR} \times \mathrm{C}}{\mathrm{BW}}$$

Where FIR represents the food ingestion rate, C stands for metal concentration in fish samples, and BW is for average body weight. The regional daily intake rate for fish in human was considered as 57.5 g/person/day, and the average body weight was considered as 55.9 kg (Siddiqui *et al*., 2019).

The target hazard quotients (THQs) were used to estimate the health risks associated with the consumption of fish species, and the calculations were done using the standard hypothesis for an integrated USEPA report (USEPA, 2000). The formulae for calculating the THQ and the resultant hazard index were as follows:

$$\mathrm{THQ} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{C}}{\mathrm{RfD} \times \mathrm{BW} \times \mathrm{TA}} \times 10^{-3}$$

$$\text{Hazard index (HI)} = \sum \mathrm{THQ}$$

Where EF stands for exposure frequency (365 days/year), ED stands for exposure duration (70 years), which was the life expectancy as per Bennett *et al.* (1999), FIR stands for food ingestion rate (57.5 g/person/day), C is for metal concentration in fish tissues in µg/g, RfD is the oral reference dose in mg/kg/day, BW is the body weight (55.9 kg), and TA is the exposure time (365 days/year × ED). The RfD values for the metals as per USEPA (2015) are as follows: Cd (0.001), Cu (0.04), Pb (0.00357), and Zn (0.3).

6.3 RESULTS

Risk levels of toxic metals

6.3.1 Geo-accumulation index (I_{geo})

Geo-accumulation index values were determined based on the observed metal concentrations in sediments with reference to the background values. The results of geo-accumulation index are presented. The results indicate that all the metals fall between the classes of 0 and 4. The geo-accumulation index is also represented as a bar graph in **Figure 24**. The reported levels of Cu, Pb, and Zn fall under unpolluted Class 0 in all the stations for all seasons. Only Cd levels fall in the classes 1–4 depicting the sites are moderately to strongly contaminated in the premonsoon, monsoon, and post monsoon seasons. Cd contamination is absent in the summer season. The highest I_{geo} value (3.45, strongly contaminated) was observed for Cd in Station 3 in the postmonsoon season.

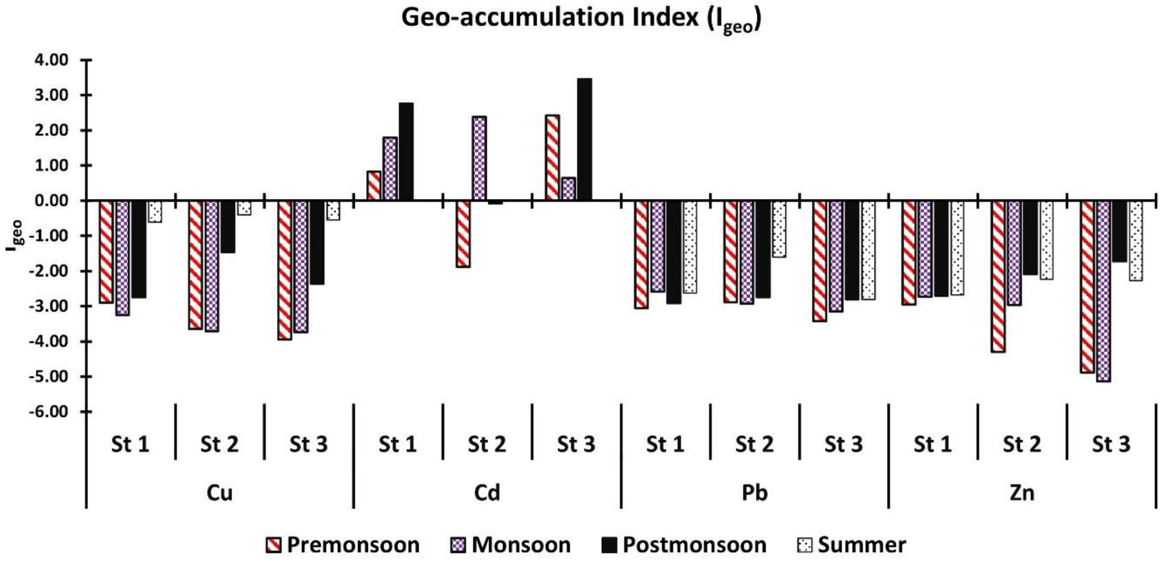

Figure 24 Geo-accumulation index for four metals in different stations across the seasons.

6.3.2 Contamination factor and contamination degree (C_{deg})

The contamination factor and degree are classified into four classes each starting from Class 1 representing low contamination to Class 4 signifying very high contamination. Station-wise contamination factor and contamination grades for the different elements across four seasons. Overall, the contamination factors for all the metals are from Class 1–4 ranging from low–very high contamination. The contamination factors for the different metals across all seasons for the different stations fall in the ranges of Cu, 0.10–1.14; Cd, 0–16.43; Pb, 0.14–0.49; and Zn, 0.04–0.46. The highest contamination factor was detected for Cd in Station 3 during the postmonsoon season. The station-wise contamination degree (C_{deg}) for all metals across the four seasons is tabulated in **Table 9** and represented as a graph in **Figure 25**. Overall, the contamination degrees for all the metals also fall in the classes 1–4 ranging from low–very high contamination. The overall contamination degree for all stations across the seasons fall in the range of 0.81–17.39. The highest contamination degree was detected in Station 3 during the postmonsoon season. The lowest value of contamination degree was observed in Station 2 during the premonsoon season.

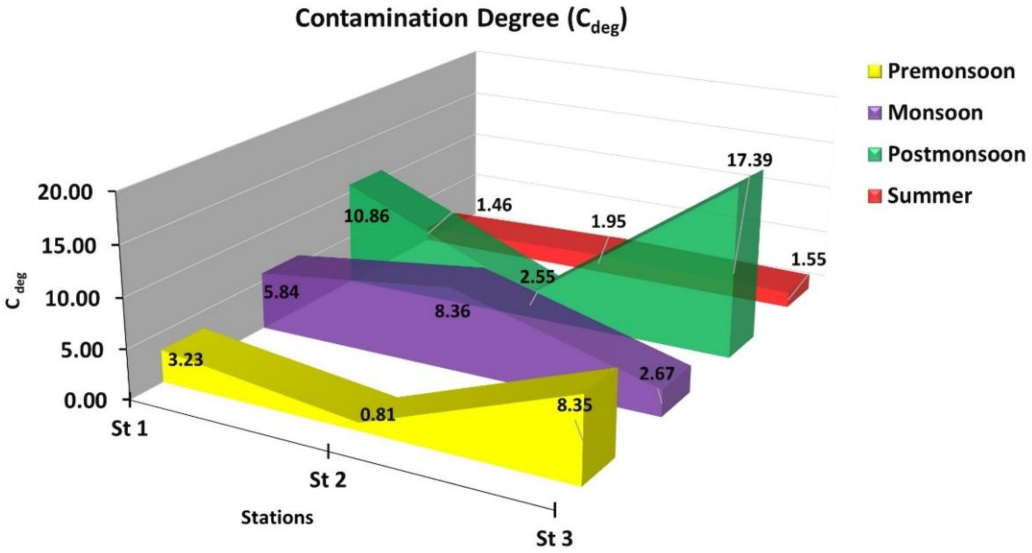

Figure.25 Contamination degree of sediment across different stations and seasons.

6.3.3 Pollution load index

Pollution load index (PLI) was computed using the assessed contamination factors to find out the joint contamination effect from different locations by various elements across different seasons. The PLI values are shown in **Table 9** and **Figure**.**26.** All values of PLI for the different sites across the four seasons are less than 1 indicating that there is no considerable pollution in the sites. The range of the pollution load index is from 0 to 0.83.

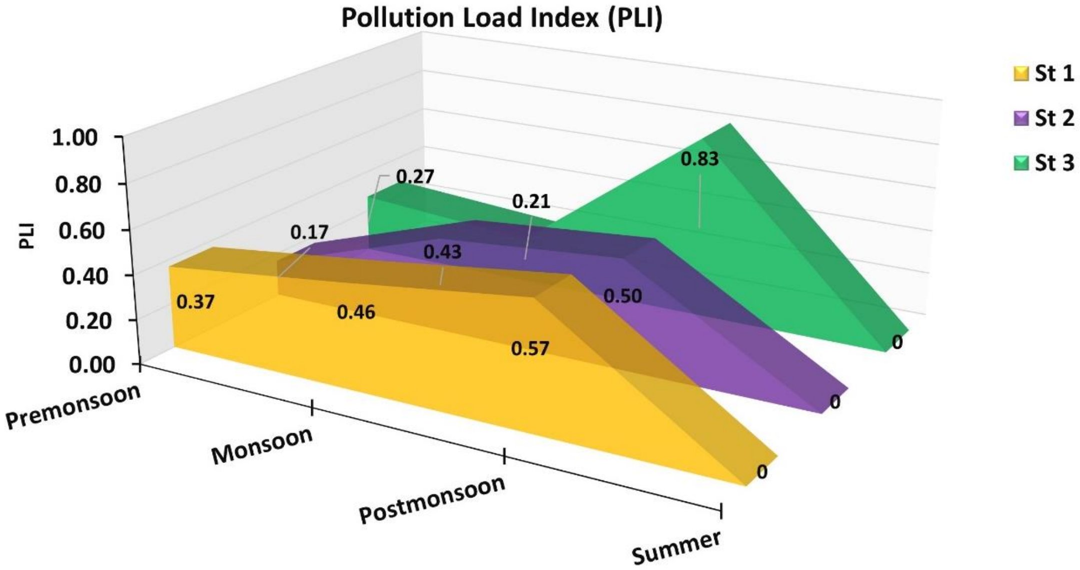

Figure 26 Pollution load index of across different stations and seasons.

6.3.4 Ecological risk and potential ecological risk

Ecological risk index (E_i) and potential ecological risk (PER) were applied to determine the risk level of metals present in the study area across different seasons, and the results of ecological risk index are shown in **S3**. The ecological risk index for the elements falls in the following ranges: Cu, 0.49–5.70; Cd, 0–492.86; Pb, 0.70–2.47; and Zn, 0.04–0.46. The ecological risk ranges from low to very high. The highest ecological risk index for Cd in Station 3 during the postmonsoon season. PER was computed from the E_i values, and the PER values are tabulated in **Table 9** and graphically illustrated in **Figure 27.** PER values ranged from 6.35 to 495.84. The highest PER value was reported for Cd in Station 3 in the postmonsoon season. The average potential ecological risk is very high for Station 3 (204.32), followed by considerable risk for Station 1 (138.72), and a moderate risk for Station 2 (76.73).

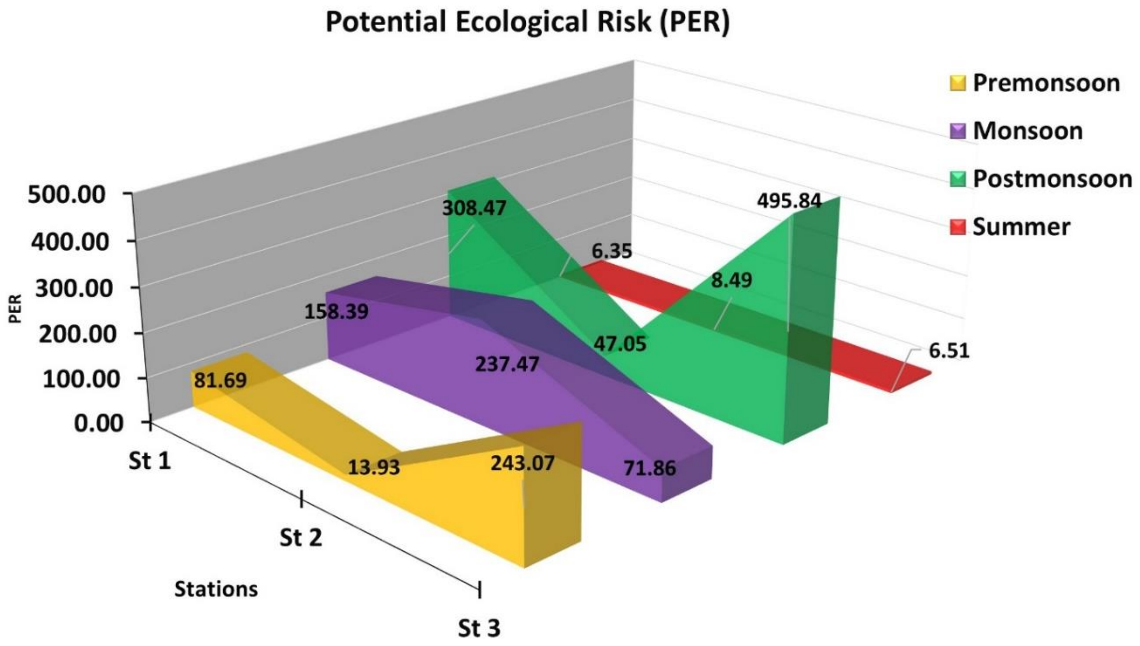

Figure 27 Potential ecological risk of across different stations and seasons.

Table 8 Classification and grading for the values of contamination degree, potential ecological risk, and pollution load index.

CLASSIFICATION OF CONTAMINATION DEGREES (C_{deg})

C_{deg} value	Degree of contamination
$C_{deg} < 4$	Low
$4 < C_{deg} < 8$	Moderate
$8 < C_{deg} < 16$	Considerable
$16 \geq C_{deg}$	Very high

GRADE OF POTENTIAL ECOLOGICAL RISK (PER)

PER value	Ecological risk
PER < 50	Low
50 ≤ PER < 100	Moderate
100 ≤ PER < 200	Considerable
PER ≥ 200	Very high

POLLUTION LOAD INDEX (PLI)

PLI value	Level of pollution
PLI < 1	No pollution
PLI = 1	Baseline levels of pollution
PLI > 1	Polluted

Table 9 Station-wise contamination degree, potential ecological risk, and pollution load index for four seasons.

Station	Seasons			
	Premonsoon	**Monsoon**	**Postmonsoon**	**Summer**
Contamination degree (C_{deg})				
St 1	3.23	5.84	10.86	1.46
St 2	0.81	8.36	2.55	1.95
St 3	8.35	2.67	17.39	1.55
Potential ecological risk (PER)				
St 1	81.69	158.39	308.47	6.35
St 2	13.93	237.47	47.05	8.49
St 3	243.07	71.86	495.84	6.51
Pollution load index (PLI)				
St 1	0.37	0.46	0.57	0.00
St 2	0.17	0.43	0.50	0.00
St 3	0.27	0.21	0.83	0.00

Human health risk assessment

6.3.5 Risk to human health from finfish and shellfish

Metal concentrations in finfish and shellfish may have a direct impact on public health, particularly for people who consume finfish and shellfish on a regular basis; hence, the health hazard evaluations for consumption of fish from contaminated environments are essential. The finfish hazard index (HI) for the pelagic fish was 0.97 and for the benthic community was 0.90, the hazard index was less than the threshold level (HI>1). While the shellfish hazard index for crustacean was 1.88 and for the molluscan community was 2.25, the hazard index was greater than the threshold level (HI<1). The health risk evaluations showed a threshold reaction. The ED, THQ, and HI of finfish from the Cuddalore coast are shown in **Table 10** and **11**

Table 10. Estimated exposures and risk assessment due to consumption of finfish from the Cuddalore coast based on the USEPA guidelines.

Finfish Type	**Metal**	**Mean concentration**	**di**	**ed**	**bw**	**at**	**Exposure Dose (ED)**	**RfD**	**THQ**
Pelagic Fish (n=9)	Cd	0.27	57.5	70	55.9	70	0.28	1	0.28
	Cu	9.87	57.5	70	55.9	70	10.15	40	0.25
	Pb	1.22	57.5	70	55.9	70	1.25	3.57	0.35
	Zn	24.71	57.5	70	55.9	70	25.42	300	0.085
								HI =	**0.97**
Benthic Fish (n=3)	Cd	0.24	57.5	70	55.9	70	0.25	1	0.25
	Cu	9	57.5	70	55.9	70	9.26	40	0.23
	Pb	1.08	57.5	70	55.9	70	1.11	3.57	0.31
	Zn	31.84	57.5	70	55.9	70	32.75	300	0.109
								HI =	**0.9**

Table 11. Estimated exposures and risk assessment due to consumption of shellfish from the Cuddalore coast based on the USEPA guidelines.

Shellfish Type	**Metal**	**Mean concentration**	**di**	**ed**	**bw**	**at**	**Exposure Dose (ED)**	**RfD**	**THQ**
Crustacean (n=4)	Cd	1.09	57.5	70	55.9	70	1.12	1	1.12
	Cu	15.84	57.5	70	55.9	70	16.29	40	0.41
	Pb	0.8	57.5	70	55.9	70	0.82	3.57	0.23
	Zn	35.52	57.5	70	55.9	70	36.54	300	0.122
								HI =	**1.88**
Mollusk (n=4)	Cd	1.33	57.5	70	55.9	70	1.37	1	1.37
	Cu	8.83	57.5	70	55.9	70	9.08	40	0.23
	Pb	1.81	57.5	70	55.9	70	1.86	3.57	0.52
	Zn	37.59	57.5	70	55.9	70	38.67	300	0.129
								HI =	**2.25**

6.4 DISCUSSION

Toxic fluctuations in sediment composition are a sign of water contamination. Sediment serves as a geochemical cycling zone and the base of the food chain and Sediments were used extensively to evaluate the health of aquatic environments (Burton *et al*., 2001). Increased amounts of contaminated human waste are clearly connected to an increasing concentration of trace metals along the Tuticorin coast. In contrast, industrial effluents from chemical, fertilizers, and automobile industries include trace elements such as copper, cadmium, iron (Wake, 2005). The current findings of the study are also related to the wake (2005) statement.

Geo-accumulation index clearly portrays the harmful levels of Cd that are polluting the Cuddalore coast. The I_{geo} values for all metals except Cd fall below zero and denote lesser degree of contamination because of these metals. But the alarming levels of Cd in the premonsoon, monsoon, and postmonsoon season are indicative of excessive Cd pollution persistent in the Cuddalore coast mainly due to the industrial wastes that are dumped into the coastal waters. The fact that the I_{geo} value for Cd is highest in Station 3 which is the river mouth area is suggestive that the metal loads are brought downstream and distributed more in the river mouth areas as suggested by Gopal *et al.* (2018). Cd poses the highest threat to the environment compared with other three metals in the Cuddalore coast. This vast metal contamination is mainly sourced from multiple stimulants from the wide-ranging industrial activities and inappropriate transfer of metal-containing fluid wastes into the environment. An analogous effect was explained by Cevik *et al.* (2009). Affirming the geo-accumulation index results, contamination factor also points out the higher contamination levels for Cd compared with other metals. After Cd, Cu levels pose a marginal moderate level of risk during the summer season. The contamination degree and potential ecological risk together denote higher contamination levels in Station 3 near the estuary mouth, and the C_{deg} and

PER values are highest for the postmonsoon season. This could be explained by the excessive loads of metals that are washed downstream and deposited in the river mouth areas. PLI is below 0 for all instances indicative of lack of pollution. However, the fact that we have taken only four metals here for our study could under value the PLI values. Still, the highest observed PLI value (0.83) is almost very close to achieve the baseline pollution mark (1). The PLI values can increase and point to a polluted status in the near future, with additional elements being assessed for computation of PLI.

The marine ecosystem, which is contaminated by toxic metals and hazardous wastewater, is the main sources of metals in fish tissue (Fakhri *et al*., 2018). Fish can be used as a biomarker for metal accumulation and potential human health risks (Teffer *et al*., 2014). Several local populations in industrial areas may be at risk from dietary toxic metals because they consume more fish meat than the average person, consume polluted locally produced food sources, breath contaminated air, or drink polluted water (Zhuang *et al*., 2013). According to Miri et al. (2017), the metals under evaluation pose no risk to human health in the Sistan population. However, of all the metals studied, Cd poses the highest harm to human health, followed by Pb. The current study demonstrates a similar trend since the risk assessment of finfish does not pose any health risk to humans, while the results of shellfish suggest a higher risk to humans. The BCF and BAF data further show that Cd and Pb are the most significant pollutants in fish resources.

The THQ, which is calculated as the proportion of intake to reference doses is used to represent the risk of non-carcinogenic impacts. If the hazard index rate is less than 1, there will be no alarming sign. The results of finfish HI values show that even a minimal increase in metal content in finfish can be harmful to human consumption. HI values in shellfish are already beyond the threshold level. An assessment of the health hazards associated with finfish consumption indicates that people are not exposed to a serious possible health risk from finfish consumption. The THQ for shellfish was around twice as higher than finfish and may have some negative health impacts. As a result, consumption of fish from the Cuddalore coast may pose a significant health risk to humans. According to Copat *et al.* (2013), metals are major contaminants in the ecosystem that are easily consumed and accumulated in organisms, posing a health risk through the consumption of metal-contaminated food. Because the shellfish HI values in our study are all over the threshold level, shellfish collected in the Cuddalore coast pose serious health risks to those who ingest them. Our results are supported with Copat *et al.* (2013).

Exclusively seafood consumption was evaluated to estimate the health risk owing to trace metal absorption, which accounts for only 3% with per capita each day calorie consumption by dietary items. Additional sources of food, including essential grains, fruits and vegetables, cereal sources, must be included in order to determine the specific health hazards associated with dietary trace metal consumption. Furthermore, continuous monitoring on all food products is required to assess whether any possible health concerns from metal exposure occur, to prevent contamination, and to safeguard target consumers from food that may impact their health (Ullah *et al.*, 2017).

Chapter VII

Analysis the metal concentrations in human using hair and nail as biomarkers.

7.1 INTRODUCTION

Metal exposure from a variety of sources and activities may be harmful to human health. Metal pollution and its effects for health have become a problem in developing countries. Metal toxicity tests are complicated and expensive to analyse implying that regulations, guidelines, legislation, and institutional managements are prohibited (Were *et al.*, 2008). Whereas many important trace elements are required in trace levels for various biological mechanisms, at higher concentrations of these elements are toxic and disrupt multiple physiological processes, resulting in diseases. As a result, it is necessary to quantify metal concentrations in humans in order to monitor and assess their influence on human health (Nath, 2000). Several factors contribute to metal accumulation in the human body response to exposure, which might have negative health consequences. metal exposure can occur through three routes: ingestion, dermal, and inhalation. Metal exposure is mostly obtained by ingestion via water and food, with inhalation and dermal transfer serving as secondary routes (Wongsasuluk *et al.*, 2021). Particularly in fish and other seafood are important sources of metal exposure in the general public. Food containing hazardous metals over permissible levels are deemed dangerous to human health and are prohibited from export under several national and international legislation (Djedjibegovic *et al.*, 2020).

The human body may avoid metal accumulation by a variety of mechanisms such as elimination through urine, faeces, sweating, breast milk, hairs, nails, and so on. Urine is a significant pathway for metals to exit the human body (Marchiset-Ferlay *et al.*, 2012). To detect exposure to toxic metal, biomarkers can be used. Non-invasive biomarkers, including urine, hair, and nails, likely to be more accessible indications of exposure than invasive biomarkers like blood or internal organ tissues. Furthermore, urine is the primary route of excretion of previously consumed contaminated food and water (Mikulewicz *et al*., 2011). Urine, on the other hand, was used to assess daily exposure, and hair and fingernails were evaluated as biomarkers to assess long-term exposure (Calderon *et al*., 2013; Wongsasuluk *et al*., 2018). Additionally, blood and other biological samples are unsuitable for determining Cd levels since the metal only exists temporarily in the medium (Okoro *et al*., 2015).

The elemental composition of hair provides detailed information regarding the concentration of elements in the body of internal organs. Hair has a higher element content than other tissues and organs, particularly urine and blood, therefore the distribution of metals in the hair reflects the concentration throughout the entire body (Chojnacka *et al*., 2010). As a result, metal concentrations in hair may indicate the metabolic condition of the human body as well as the impact of environmental influences on it (Zhou *et al*., 2017). The high metal attraction of hair is mostly related to the availability of cystine, which accounts for around 14% hair (Morton *et al*., 2002). Furthermore, choosing hair to analyse metal concentration in the human has the additional benefits of simple sample collection, no harm to the human body, and easy to storage or transfer for clinical examination, diagnosis, and environmental pollution monitoring (Xue *et al*., 2015). Many researchers have carried out considerable study on metal content in hair (Anwar, 2005; Gonzalez-Reimers *et al*., 2014; Massaquoi *et al*., 2005; Mohammed *et al*., 2008; Mosaferi *et al*., 2005). According to Zhou *et al*. (2016), metal concentrations in hair are associated to age, gender, environmental variables, nationality, city resident health variations, dietary habits, and diagnostic technique. Considering these multiple findings, the concentration of metals extracted from hair has varied widely, sometimes between locations.

Human nails grow at a continuous rate of around 0.05–1.2 mm each week, with toe nails growing at a slower rate of 30–50%, providing a longer absorption phase for the metals. Toenail clippings are likely to reflect metal exposures accumulated over the past 6–12 months, with a monthly growth rate of about 1.6 mm and an average length of 20 mm (Longnecker *et al.*, 1993; McCarthy, 2004; Yaemsiri *et al.*, 2010). Metals such as lead, copper, cobalt, cadmium, and zinc may enter the fingernails as a result of recreational activities, poorly maintained hygiene habits, and active metabolic activity (Okoro *et al.*, 2015). Nail also can be obtained noninvasively and painlessly, and are simple to transport and store, nail metal content should be explored as a possible biomarker of internal dosage for both environmental and occupational exposures. Toenails, as contrast to blood or urine, may indicate longer-term exposure to harmful metals because to its slow rate of development (Adair *et al.*, 2006; Yaemsiri *et al.*, 2010), whereas these metals are not affected by metabolic processes once absorbed into the keratin structure of the nails (Sukumar, 2006). Toenail interaction with potential factors such as demographical, biomechanical, or lifestyle parameters, as well as exposure sources such as food or environmental pollution (Salcedo-Bellido *et al.*, 2021). The majority of research has concentrated on the metal transfer pathway of occupational and environmental exposure, whereas research on the dietary route is minimal. As a result, the current chapter established the source of metal transfer in personal and dietary characteristics using human hair and nail as biomarkers.

7.2 MATERIALS AND METHODS

7.2.1 Collection and analysis of hair and nail samples

Participants in the year 2019–2020 donated hair and nail samples after completing out a questionnaire on their personal characteristics such as gender, lifestyle, food, health status etc. The participant was asked to complete the following questionnaire in **Table 12**

Table 12. The survey questionnaire provided to the participants

Survey questionnaire		
Personal parameters		**Volunteer's information**
Gender	Male	
	Female	
Age	10-20	
	21-30	
	31-40	
	41-50	
	>50	
Habit/Habitat		
Smoker	Yes	
	No	
Alcoholic	Yes	
	No	
Distance of home/habitat from industry or estuary	≤ 500 m	
	≥ 500 m	
Health Status		
History of chronic disease	Yes	
	No	
Dietary parameters		
Diet preference	Vegetarian	
	Non- vegetarian	
(If non-vegetarian)		
Preferences	Poultry	
	Red meat	
	Sea food	
(If seafood)		
Frequency of Finfish	Never	
	Daily	
	Weekly	
	Fortnightly	
	Monthly	
Frequency of Shellfish	Never	
	Daily	
	Weekly	
	Fortnightly	
	Monthly	
(If Poultry)		
Frequency of Poultry	Never	
	Daily	
	Weekly	
	Fortnightly	
	Monthly	
(If Red Meat)		
Frequency of meat	Never	
	Daily	
	Weekly	
	Fortnightly	
	Monthly	

The current research included the hair samples from 80 people (47 men and 33 females), with an average age of 18 ± 4.4 years and the nail samples from 40 people (21 men and 19 females), with an average of 9 ± 4.1. The population was comprised of individuals which inhabited around the Cuddalore coastline, mainly in the fishermen community. Because the experimental group of donor hair and nail samples were people who lived in the same village and did consume the same food, the participants chosen for metal quantification in hair and nail analysis were differ in terms of personal and dietary exposure. Hair samples were carefully treated with detergents and rinsed four times with 15 ml acetone and after being washed four times with distilled water to remove adhesion on the surface of the hair and external pollutants. The samples have also been oven dried at 80°C. The dried samples were cut into 1–3 mm pieces before being stored in plastic bags at room temperature. 1 g of dried hair was digested with 15 ml of mixed solution (HNO_3, $HClO_4$, and H_2SO_4, 5:1:1) at 80 °C until a transparent solution was formed to extract metals (Michalak *et al*., 2014; Wang *et al*., 2017).

The nail specimens in the containers were cleaned free of metallic material after being immersed in non-ionic detergent for 2 hours in a marked glass container. Then they were immersed in acetone for 1 hour until being rinsed five times with distilled water. The samples were stored in labelled containers and oven dried at 60 °C. Especially polished nails were cleaned in 4-methyl pentan-2-one before being oven dried. The dried samples were weighed and placed in labelled glass beaker. They were given 10 mL of a 6:1 mixture of concentrated nitric acid and perchloric acid and were maintained on a hotplate until the digestion was complete. In a polypropylene container, the digested sample solution was diluted with distilled water, filtered using whatman filter paper, and made up to 25 mL for AAS analysis (Were *et al*., 2008)

7.2.2 Statistical Description

The raw data of metal concentrations came from the AAS analysis, which was used for determining the metal distribution in the hair and nail samples. Based on the questionnaire the data were segregate into the personal and dietary characteristics. All the data were systemically analyzed and represented as mean and standard deviation using MS Excel (Version 365, Windows 10).

7.2.3 Quality Assurance and Quality Control

Metals were detected in the processed samples using an Atomic Absorption Spectrophotometer (Model: AA7000; Make: Shimadzu Company, Japan) at the following operating wavelengths: Zn has a wavelength of 213.85 nm, Cu has a wavelength of 324.75 nm, Cd has a wavelength of 228.80 nm, and Pb has a wavelength of 217.00 nm (Rajaram *et al.*, 2017). The instruments below detection limit (BDL) for all metals examined was 0.01 g/g. To provide quality assurance throughout the study, all sample preparation and analysis were performed in a clean and metal-free area. The samples were analyzed in triplicate, and the mean and standard deviation were calculated to provide the results. The standard curves were produced using AAS metals reference standards obtained from Sigma-Aldrich in Bangalore. Standard calibration curves for each element had a regression coefficient (R^2) value greater than 0.98. The quality assurance and quality control (QA/QC) for the AAS instrument were maintained by checking the readings with the metal standards of known concentration in the range of 0.1–10 μg/g. The recovery rates for the metals examined using recognized standards ranged from 92 to 106 percent. To assure the accuracy of the instrument readings for quality control and quality assurance of the metal data, blanks were run once in every 10 sample readings.

7.3 RESULTS

7.3.1 Metal concentrations in human hair and nail samples

The mean values and standard deviations of four metals in the hair of people from the Cuddalore coastal community are divided into two groups. Personal and dietary parameters are revealed in **Tables 13** and **14**. From the obtained results the personal parameters such as gender, age, occupation, habit, distance of habitat from industry, and chronic disease. Dietary parameters such as diet preference, if non-vegetarian, if sea food/ frequency of finfish, if sea food/ frequency of shellfish, if poultry/ frequency of poultry, if Red Meat/ frequency of Meat. Among the 80 individuals of hair samples, Cd ranged from 0.00 to 1.75 µg/g with a mean of 0.29 µg/g **(Figure 29)** Cu range was fall in 0.02 to 25.46 µg/g with 7.78 mean **(Figure 28).** Pb range was fall in 0.00 to 2.64 µg/g with 0.41 mean **(Figure 29)**, and Zn range was fall in 0.00 to 102.24 µg/g with 49.10 mean as shown in **(Figure 28).** Among the 40 individuals of nail samples, Cd range was fall in 0.00 to 0.35 µg/g with 0.06 mean **(Figure 31)**, Cu range was fall in 0.00 to 6.33 µg/g with 1.20 mean **(Figure 30)**, Pb range was fall in 0.00 to 12.32 µg/g with 2.26 mean **(Figure 31)**, Zn range was fall in 35.20 µg/g to 107.16 with 67.65 mean **(Figure 30).**

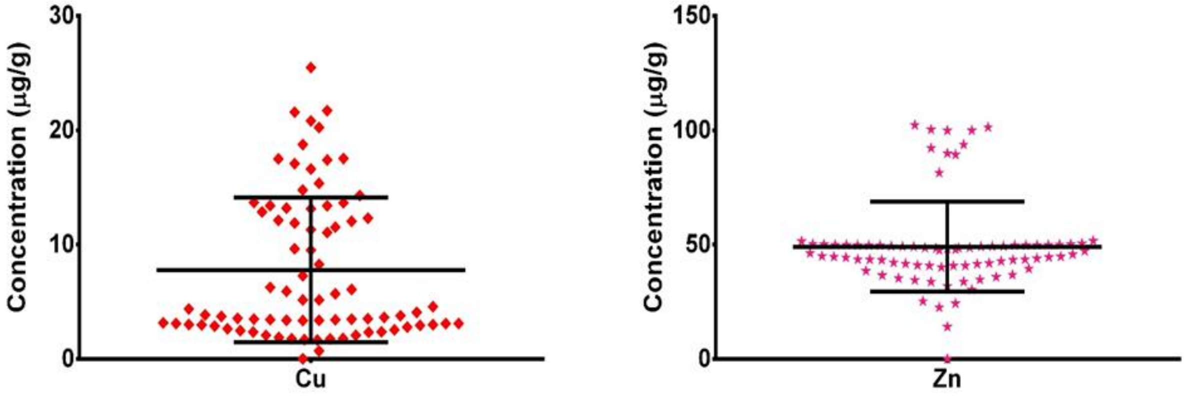

Figure 28 Distribution of Cu and Zn in the human hair collected from Cuddalore fisher folks.

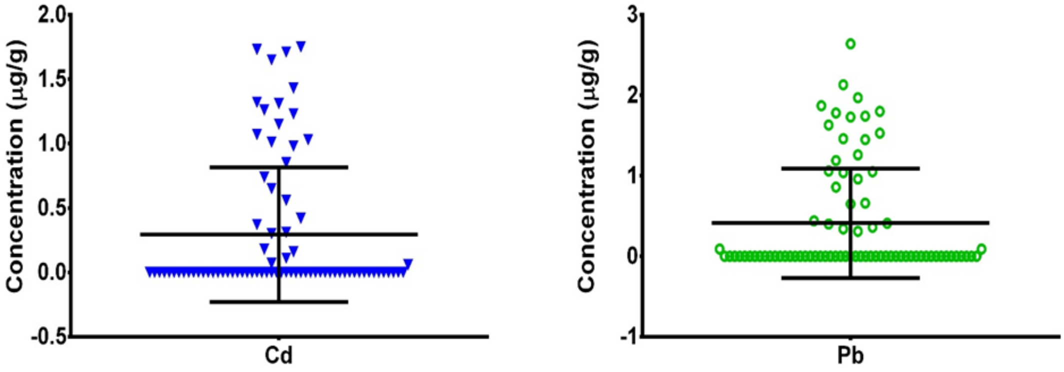

Figure 29 Distribution of Cd and Pb in the human hair collected from Cuddalore fisher folks.

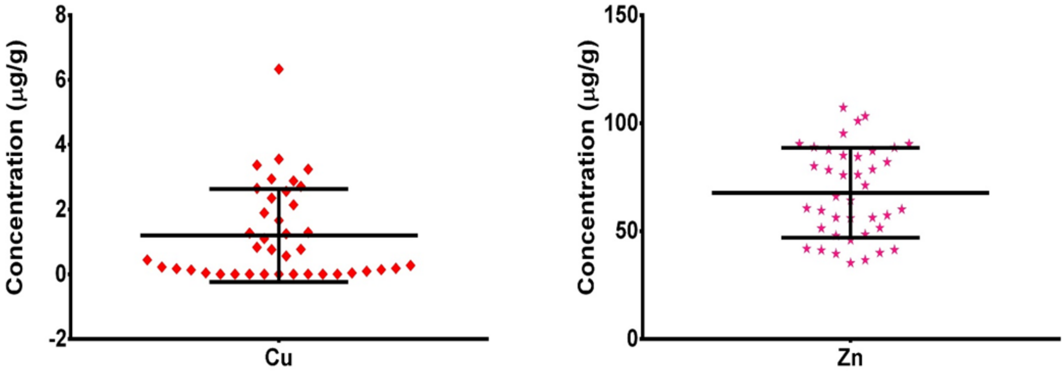

Figure 30 Distribution of Cu and Zn in the human nail collected from Cuddalore fisher folks.

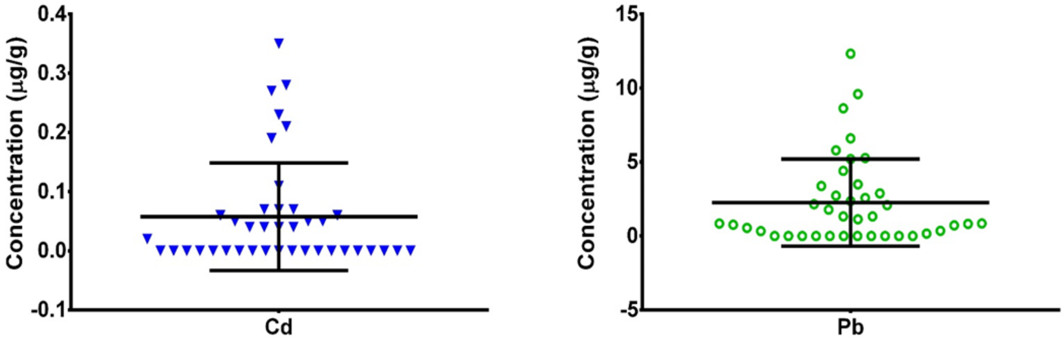

Figure 31 Distribution of Cd and Pb in the human nail collected from Cuddalore fisher folks.

7.3.2 Metal concentration in human hair with personal parameters

Higher Cd levels were observed in the male population (0.48 µg/g), people above 50 age group is 0.84 µg/g, fisherman community (0.33 µg/g), distance of habitat from industry ≥ 500 m (0.33 µg/g), and people with chronic disease (0.85µg/g) respectively. Among male Cd levels in smokers is detected as 0.81 µg/g and alcoholics (0.75 µg/g), was recorded. Higher Cu levels were observed in the female population (7.93 µg/g), people above 50 age group is 9.49 µg/g, fisherman community (8.6 µg/g), distance of habitat from industry ≥ 500 m (0.32 µg/g), and people with chronic disease (0.38 µg/g) respectively. Among male, Cu levels in smokers is detected as 0.37 µg/g and alcoholics (0.38 µg/g), was recorded. Higher Pb levels were observed in the male population (0.42 µg/g), people above 50 age group is 0.92 µg/g, fisherman community (0.49 µg/g), distance of habitat from industry ≥ 500 m (0.44 µg/g), and people with chronic disease (0.7 8 µg/g) respectively. Among male, Pb levels in smokers is detected as 0.69 µg/g and alcoholics (0.66 µg/g), was recorded. Higher Zn levels were observed in the female population (51.26 µg/g), people 41-50 age group is 53.06 µg/g, fisherman community (51.7 µg/g), distance of habitat from industry ≥ 500 m (50.09 µg/g), and people with no chronic disease (49.2 µg/g) respectively. Among male, Zn levels in non-smokers is detected as 49.3 µg/g and non-alcoholics (49.55 µg/g), was recorded. The hair personal parameter results show **Table 13.**

7.3.3 Metal concentration in human hair with dietary parameters

Higher Cd levels were found in the non-vegetarian population (0.31 µg/g), with consumed red meat showing higher level 0.57 µg/g when compared with poultry and seafood. In the case of seafood, the frequency of weekly consumption of finfish with the highest concentration 0.47 µg/g. Similarly, the daily consumption community of shellfish had a higher concentration of 0.85 µg/g. In the case of poultry consumption fortnightly, the maximum value was 0.45 µg/g. Also in the red meat scenario the fortnightly consumption having the higher concentration of Cd (1.65 µg/g). Higher Cu levels were found in the non-vegetarian population (7.81 µg/g), with the consumption of both seafood and poultry shows similar level 7.81 µg/g when compared with red meat. In the case of seafood, the frequency of weekly consumption of finfish with the highest concentration 8.25 µg/g. Similarly, the weekly consumption community of shellfish had a higher concentration of 9.15 µg/g. In the case of poultry consumption weekly, the maximum value of Cu was 9.42 µg/g. Also in the red meat scenario the fortnightly consumption having the higher concentration 11.05 µg/g. Higher Pb levels were found in the non-vegetarian population (0.44 µg/g), with consumed red meat showing higher level 0.6 µg/g when compared with poultry and seafood. In the case of seafood, the frequency of daily consumption of finfish with the highest concentration 1.06 µg/g. Similarly, the weekly consumption community of shellfish had a higher concentration of 0.58 µg/g. In the case of poultry consumption fortnightly, the maximum value was 0.56 µg/g. Also in the red meat scenario the weekly consumption having the higher concentration 0.72 µg/g. Higher Zn levels were found in the non-vegetarian population (49.52 µg/g), with the consumption of both seafood and poultry shows similar level 49.52 µg/g when compared with red meat. In the case of seafood, the frequency of weekly consumption of finfish with the highest concentration 52.68 µg/g. Similarly, the weekly consumption community of shellfish had a higher concentration of 51.85 µg/g. In the case of poultry consumption fortnightly, the maximum value was 49.95 µg/g. Also in the red meat scenario the fortnightly consumption having the higher concentration 49.69 µg/g. The dietary parameter of hair results shows in **Table 14.**

Table 13. Concentration (μg/g) of metals with Mean ± SD respect to various personal parameter in human hair

Personal Parameter	Criteria	n	Cd	Cu	Pb	Zn
Gender	Male	47	0.48 ± 0.03	7.68 ± 0.29	0.42 ± 0.03	47.58 ± 2.1
	Female	33	0.02 ± 0.00	7.93 ± 0.35	0.39 ± 0.02	51.26 ± 2.11
Age	10 to 20	20	0.00 ± 0.00	7.03 ± 0.34	0.00 ± 0.00	43.7 ± 1.97
	21 to 30	16	0.04 ± 0.00	7.66 ± 0.3	0.13 ± 0.01	48.5 ± 2.26
	31 to 40	13	0.09 ± 0.01	8.65 ± 0.34	0.02 ± 0.01	50.21 ± 2.05
	41 to 50	23	0.65 ± 0.04	7.43 ± 0.27	0.09 ± 0.06	53.06 ± 2.12
	above 50	8	0.84 ± 0.05	9.49 ± 0.39	0.92 ± 0.06	50.61 ± 2.22
Occupation	Fisherman	76	0.33 ± 0.02	8.06 ± 0.35	0.49 ± 0.03	51.7 ± 2.2
	Non Fisherman	4	0.00 ± 0.00	0.26 ± 0.03	0.00 ± 0.00	6.48 ± 0.11
Habit	Smoker	26	0.81 ± 0.05	10.02 ± 0.37	0.69 ± 0.05	48.68 ± 2.18
	Non smoker	54	0.04 ± 0.00	6.71 ± 0.29	0.28 ± 0.02	49.3 ± 2.07
	Alcoholic	28	0.75 ± 0.05	10.04 ± 0.38	0.66 ± 0.05	48.7 ± 2.12
	Non Alcoholic	52	0.04 ± 0.00	6.79 ± 0.29	0.31 ± 0.02	49.55 ± 2.11
Distance of habitat from industry	≤ 500 m	71	0.33 ± 0.02	7.96 ± 0.32	0.44 ± 0.03	50.09 ± 2.14
	≥ 500 m	9	0.00 ± 0.00	6.4 ± 0.27	0.17 ± 0.01	41.26 ± 1.84

Table 14. Concentration (µg/g) of metals with Mean ± SD respect to various diet parameter in human hair

Diet Parameter	Criteria	n	Cd	Cu	Pb	Zn
Diet preference	Vegetarian	5	0.00 ± 0.00	7.32 ± 0.32	0.00 ± 0.00	42.72 ± 1.84
	Non-vegetarian	75	0.31 ± 0.02	7.81 ± 0.32	0.44 ± 0.03	49.52 ± 2.13
If Non-vegetarian	Poultry	75	0.31 ± 0.02	7.81 ± 0.32	0.44 ± 0.03	49.52 ± 2.13
	Red Meat	15	0.57 ± 0.03	7.25 ± 0.29	0.06 ± 0.04	47.98 ± 2.06
	Sea Food	75	0.31 ± 0.02	7.81 ± 0.32	0.44 ± 0.03	49.52 ± 2.13
If sea food/ frequency of finfish	Never	5	0.00 ± 0.00	7.32 ± 0.32	0.00 ± 0.00	42.72 ± 1.84
	Daily	3	0.38 ± 0.02	4.7 ± 0.16	1.06 ± 0.07	43.04 ± 1.98
	Weekly	43	0.47 ± 0.03	8.25 ± 0.33	0.66 ± 0.04	52.68 ± 2.02
	Fortnightly	27	0.07 ± 0.00	7.82 ± 0.32	0.06 ± 0.01	45.57 ± 2.02
	Monthly	2	0.00 ± 0.00	2.86 ± 0.15	0.00 ± 0.00	44.62 ± 2.19
If sea food/ frequency of shellfish	Never	5	0.00 ± 0.00	7.32 ± 0.32	0.00 ± 0.00	42.72 ± 1.84
	Daily	1	0.85 ± 0.06	3.43 ± 0.12	0.00 ± 0.00	41.83 ± 1.41
	Weekly	26	0.78 ± 0.05	9.15 ± 0.36	0.58 ± 0.04	51.85 ± 2.23
	Fortnightly	37	0.06 ± 0.00	7.78 ± 0.32	0.43 ± 0.03	49.31 ± 2.11
	Monthly	11	0.00 ± 0.00	5.14 ± 0.23	0.17 ± 0.01	45.45 ± 2.00
If poultry/ frequency of poultry	Never	5	0.00 ± 0.00	7.32 ± 0.32	0.00 ± 0.00	42.72 ± 1.84
	Daily	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Weekly	22	0.06 ± 0.00	9.42 ± 0.39	0.18 ± 0.01	48.78 ± 2.11
	Fortnightly	48	0.45 ± 0.03	7.39 ± 0.29	0.56 ± 0.04	49.95 ± 2.18
	Monthly	5	0.07 ± 0.01	4.74 ± 0.19	0.46 ± 0.04	48.7 ± 1.7
If Red Meat/ frequency of Meat	Never	65	0.23 ± 0.01	7.91 ± 0.32	0.37 ± 0.02	49.35 ± 2.12
	Daily	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Weekly	2	0.00 ± 0.00	3.12 ± 0.14	0.72 ± 0.04	42.75 ± 2.01
	Fortnightly	1	1.65 ± 0.09	11.05 ± 0.48	0.00 ± 0.00	49.69 ± 2.52
	Monthly	11	0.62 ± 0.04	7.99 ± 0.31	0.68 ± 0.05	49.43 ± 2.08

7.3.4 Metal concentration in human nail with personal parameters

Higher Cd levels were observed in the male population (0.08 µg/g), people between 41-50 age group had 0.11 µg/g, fisherman community (0.07 µg/g), distance of habitat from industry ≥ 500 m (0.06 µg/g), and people with chronic disease (0.15µg/g) respectively. Among male, Cd levels in smokers and alcoholics were detected as 0.22 µg/g, was recorded. Higher Cu levels were observed in the male population (1.53 µg/g), people between 21-30 age group had 3.32 µg/g, fisherman community (1.25 µg/g), distance of habitat from industry ≤ 500 m (1.64 µg/g), and people with no chronic disease (1.24 µg/g) respectively. Among male, Cu levels in smokers is detected as 1.45 µg/g and alcoholics (1.25 µg/g), was recorded. Higher Pb levels were observed in the male population (2.42 µg/g), people above 50 age group is 8.57 µg/g, fisherman community (2.45 µg/g), distance of habitat from industry ≥ 500 m (2.39 µg/g), and people with chronic disease (6.32 µg/g) respectively. Among male, Pb levels in smokers were detected as 2.58 µg/g and alcoholics (2.95 µg/g), was recorded. Higher Zn levels were observed in the female population (69.04 µg/g), people above 50 age group is 78.94 µg/g, non-fisherman community (79.6 µg/g), distance of habitat from industry ≤ 500 m (81.15 µg/g), and people with no chronic disease (67.85 µg/g) respectively. Among male, Zn levels in non-smokers had detected as 70.02 µg/g and non-alcoholics (69.19 µg/g), was recorded. The personal parameter of nail results shows in **Table 15.**

7.3.5 Metal concentration in human nail with dietary parameters

Higher Cd levels were found in the non-vegetarian population (0.06 µg/g), with consumed red meat showing higher level 0.11 µg/g when compared with poultry and seafood. In the case of seafood, the frequency of daily consumption of finfish with the highest concentration 0.21 µg/g. Similarly, the weekly consumption community of shellfish had a higher concentration of 0.15 µg/g. In the case of poultry consumption fortnightly, the maximum value was 0.07 µg/g. Also in the red meat scenario the monthly consumption having the higher concentration 0.09 µg/g.

Higher Cu levels were found in the non-vegetarian population (1.26 µg/g), with the consumption of poultry shows similar level 1.28 µg/g when compared with red meat and seafood. In the case of seafood, the frequency of daily consumption of finfish with the highest concentration 2.88 µg/g. Similarly, the fortnightly consumption community of shellfish had a higher concentration of 1.46 µg/g. In the case of poultry consumption monthly, the maximum value was 1.62 µg/g. Also in the red meat scenario the people never consumed had the higher concentration 1.26 µg/g.

Higher Pb levels were found in the vegetarian population (2.47 µg/g), with consumed poultry showing higher level 2.42 µg/g when compared with red meat and seafood. In the case of seafood, the frequency of weekly consumption of finfish with the highest concentration 3.05 µg/g. Similarly, people never consume shellfish had a higher concentration of 2.47 µg/g. In the case of poultry consumption fortnightly, the maximum value was 2.76 µg/g. Also in the red meat scenario the people never consumed had the higher concentration 2.48 µg/g.

Higher Zn levels were found in the vegetarian population (84.49 µg/g), with the consumption of poultry shows similar level 66.64 µg/g when compared with seafood and red meat. In the case of seafood, people never consume finfish with the highest concentration 84.49 µg/g. Similarly, people consume shellfish had a higher concentration of 84.49 µg/g. In the case of poultry weekly consumption, had the maximum value was 72.87 µg/g. Also in the red meat scenario, people never consume had the higher concentration 69.23 µg/g. The dietary parameter results show in **Table 16.**

Table 15. Concentration (µg/g) of metals with Mean ± SD respect to various personal parameter in human finger nail

Personal Parameter	Criteria	n	Cd	Cu	Pb	Zn
Gender	Male	21	0.08 ± 0.00	1.53 ± 0.06	2.42 ± 0.13	66.39 ± 4.78
	Female	19	0.03 ± 0.00	0.82 ± 0.03	2.08 ± 0.01	69.04 ± 4.48
Age	10 to 20	11	0.02 ± 0.00	0.55 ± 0.02	0.72 ± 0.04	69.7 ± 5.29
	21 to 30	3	0.04 ± 0.00	3.32 ± 0.21	0.91 ± 0.03	70.51 ± 4.29
	31 to 40	10	0.03 ± 0.00	1.44 ± 0.04	2.09 ± 0.11	65.27 ± 4.17
	41 to 50	12	0.11 ± 0.00	1.16 ± 0.04	2.05 ± 0.01	63.28 ± 4.37
	above 50	4	0.09 ± 0.00	0.87 ± 0.02	8.57 ± 0.42	78.94 ± 5.09
Occupation	Fisherman	34	0.07 ± 0.00	1.25 ± 0.05	2.45 ± 0.12	65.54 ± 4.58
	Non Fisherman	6	0.01 ± 0.00	0.89 ± 0.03	1.16 ± 0.07	79.6 ± 4.98
Habit	Smoker	7	0.22 ± 0.01	1.45 ± 0.04	2.58 ± 0.15	56.51 ± 4.27
	Non smoker	33	0.02 ± 0.00	1.14 ± 0.04	2.19 ± 0.11	70.02 ± 4.71
	Alcoholic	6	0.22 ± 0.01	1.25 ± 0.03	2.95 ± 0.17	58.96 ± 4.35
	Non Alcoholic	34	0.03 ± 0.00	1.19 ± 0.05	2.14 ± 0.11	69.19 ± 4.69
Distance of habitat from industry	≤ 500 m	36	0.06 ± 0.00	1.15 ± 0.04	2.39 ± 0.12	66.15 ± 4.49
	≥ 500 m	4	0.05 ± 0.00	1.64 ± 0.05	1.1 ± 0.08	81.15 ± 6.00
If any chronic disease	Yes	4	0.15 ± 0.01	0.83 ± 0.03	6.32 ± 0.33	65.84 ± 4.68
	No	36	0.05 ± 0.00	1.24 ± 0.05	1.81 ± 0.09	67.85 ± 4.63

Table 16. Concentration (μg/g) of metals with Mean ± SD respect to various diet parameter in human finger nail

Diet Parameter	Criteria	n	Cd	Cu	Pb	Zn
Diet preference	Vegetarian	4	0.00 ± 0.00	0.61 ± 0.02	2.47 ± 0.07	84.49 ± 4.72
	Non-vegetarian	36	0.06 ± 0.00	1.26 ± 0.05	2.24 ± 0.12	65.78 ± 4.63
If Non-vegetarian	Poultry	32	0.07 ± 0.00	1.28 ± 0.04	2.42 ± 0.13	66.64 ± 4.57
	Red Meat	5	0.11 ± 0.00	1.03 ± 0.03	1.21 ± 0.06	53.04 ± 3.9
	Sea Food	35	0.07 ± 0.00	1.3 ± 0.05	2.3 ± 0.13	65.17 ± 4.51
If sea food/ frequency of finfish	Never	4	0.00 ± 0.00	0.61 ± 0.02	2.47 ± 0.07	84.49 ± 4.72
	Daily	1	0.21 ± 0.01	2.88 ± 0.09	0.00 ± 0.00	47.83 ± 4.17
	Weekly	22	0.06 ± 0.00	1.22 ± 0.04	3.05 ± 0.16	71.17 ± 4.88
	Fortnightly	13	0.06 ± 0.00	1.21 ± 0.05	1.02 ± 0.06	57.64 ± 4.14
	Monthly	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
If sea food/ frequency of shellfish	Never	4	0.00 ± 0.00	0.61 ± 0.02	2.47 ± 0.07	84.49 ± 4.72
	Daily	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Weekly	8	0.15 ± 0.01	1.23 ± 0.04	1.79 ± 0.1	51.34 ± 4.18
	Fortnightly	18	0.05 ± 0.00	1.46 ± 0.06	2.07 ± 0.11	65.66 ± 4.29
	Monthly	10	0.02 ± 0.00	0.93 ± 0.03	2.9 ± 0.17	77.04 ± 5.47
If poultry/ frequency of poultry	Never	7	0.03 ± 0.00	0.97 ± 0.05	1.86 ± 0.06	69.51 ± 4.33
	Daily	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Weekly	12	0.06 ± 0.00	1.37 ± 0.04	2.2 ± 0.13	72.87 ± 4.79
	Fortnightly	18	0.07 ± 0.00	1.18 ± 0.05	2.76 ± 0.14	65.31 ± 4.6
	Monthly	2	0.04 ± 0.00	1.62 ± 0.06	0.66 ± 0.04	41.19 ± 3.05
If Red Meat/ frequency of Meat	Never	34	0.05 ± 0.00	1.26 ± 0.05	2.48 ± 0.13	69.23 ± 4.62
	Daily	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Weekly	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Fortnightly	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Monthly	6	0.09 ± 0.00	0.86 ± 0.02	1.01 ± 0.05	58.71 ± 4.72

7.4 DISCUSSION

According to this chapter results, metal accumulated considerably in human hair and nail samples from various parameters depending on their exposure. The overall Cd mean value for personal parameters was 0.32 µg/g, while the dietary parameter value was 0.29 µg/g. The mean value of Pb personal parameter was 0.42 µg/g, while the value of dietary parameter was 0.31 µg/g. Cd was higher and Pb was lower when compared to other worldwide studies such as Poland (Chojnacka *et al.*, 2005), Sweden (Rodushkin and Axelsson, 2000), Italy (Senofonte *et al.*, 2000), Brazil (Miekeley *et al.*, 1998), Spain (González-Munoz *et al.*, 2008), and Beijing (Liang *et al.*, 2017). The overall Cu mean value of the personal parameter was 7.63 µg/g and the dietary parameter was 6.43 µg/g, while the Zn value of the personal parameter was 46.38 µg/g and the dietary parameter was 43.22 µg/g. Both the essential metal values were lower than Tianjin, China (Wang *et al.*, 2017). Only the non-essential metal Cd was found to be significantly higher in human hair sample of this study.

Metal concentrations in nail samples collected from the Cuddalore coast. Personal and dietary parameters were also observed from obtained results. The overall Cd mean value for personal parameters was 0.08 µg/g, while the dietary parameter value was 0.05 µg/g. The mean value of Pb personal parameter was 2.58 µg/g, while the value of dietary parameter was 1.50 µg/g. The overall Cu mean value of the personal parameter was 1.28 µg/g and the dietary parameter was 0.91 µg/g, while the Zn value of the personal parameter was 68.47 µg/g and the dietary parameter was 47.88 µg/g. When compared to Dizajabaad Village, the nonessential metal Cd and Pb readings were lower in both personal and dietary parameters (Parizanganeh *et al.*, 2014). The personal parameter Zn results were similar to those reported by Parizanganeh *et al.* (2014), but the dietary parameter Zn levels were lower.

Cu levels in Khyber Pakhtunkhwa, Pakistan, were higher than in the current study of both personal and dietary Cu values (Khan and Muhammad, 2018).

In hair samples, non-essential metal Cd and Pb levels were greater in male populations because smokers (n=26) and alcoholics (n=28) are male populations smoking and alcoholism are two main sources of this non-essential metal. This scenario is supported by Galażyn-Sidorczuk *et al.* (2012). Except for Zn, all four metals had higher levels in the over-50 age group. Non-essential metal levels in nail samples are higher in male populations because smokers (n=7) and drinkers (n=6) are male populations. The hair samples followed the same pattern. Except for Cu, all two metal (Pb & Zn) higher values were fall in above 50 and the Cd higher was noted in above 50. It indicating that long-term exposures are causing more accumulation. Were *et al.* (2008) stated that increased amounts of toxic metals in humans might be caused by industrial inhabitants; our findings are in accordance with his statement since less than 500 meter residents accumulate more metals in hair samples. Non-essential metal levels were found to be higher in people residing within 500 metres of an industry in nail samples.

Non vegetarian foods from both terrestrial and marine source, play an important role in the human nutrition because they contain proteins, vitamins, and other essential nutrients with associated health benefits (Pieniak *et al.*, 2010). However, its consumption is the primary mode of transfer to toxic materials such as metals and organic chemicals, posing a major public health concern (Chen *et al.*, 2017). Because animals are frequently exposed to toxins in the environment and have the potential to accumulate the pollutants at high concentrations in their tissues (Authman *et al.*, 2015). This statement is also confirmed by the previous chapter. Our previous studies revealed the consumption of finfish from the Cuddalore coastline does not currently pose a health risk; however, hazard index values (pelagic = 0.97; benthic = 0.90) are marginal. Even slightly increases of metal content in fish can be harmful to human consumption. But in the case of shellfish hazard index values (crustacean= 1.88; molluscan= 2.25). It is clearly evident that shellfish consumption from Cuddalore have negative health consequences for humans (Vinothkannan *et al*., 2022b; Vinothkannan *et al*., 2022c).

We identified seafood as one of the major sources of metal accumulation, the results of our survey questionnaire were confirmed. According to the questionnaire, if you are a vegetarian or non-vegetarian, if you are non-vegetarian prefer red meat, poultry, and seafood. The seafood was further divided into finfish and shellfish, with all preferences are documented. Based on diet preference in hair samples, all four metal higher values were recorded in non-vegetarians since the majority of them (n=75) are non-vegetarians, and among the 75 persons, poultry and seafood (n=75), but red meat (n=15) were higher in cadmium. Simultaneously, the Pb content in seafood and poultry was higher. The major source of the cadmium levels increased in red meat, however, is the difference between the averages of 15 and 75 people. In respect of nail sample diet preference, non-vegetarians had higher Cd and Cu levels, whereas vegetarians had higher Pb and Zn values. According to Di Bella *et al*. (2020) consumption of animal meat from terrestrial and seafood sources should pose a health hazard to regional public health. As a result of the findings, consumption of Cuddalore water finfish and shellfish is a major source of metal accumulation in regional population.

Chapter VIII

Effects of cadmium exposure on male and female infertility in a zebrafish animal model.

8.1 INTRODUCTION

The Cuddalore coastal environment has been polluted by metals, as supported by PCA, and the seasonal influence of metal concentrations has been detected by HCA (Chapter IV). The copper source is higher in finfish (BCF study), whereas lead is higher in several species, and shellfish species were highly contaminated by cadmium (BAF study) (Chapter V). The geo-accumulation index values of Cd levels fall into classes 1-4 (Ranges Cd, 0-16.43; overall 0.81-17.39; ecological risk index Cd, 0-492.86), indicating that the sites are moderately to strongly polluted throughout the premonsoon, monsoon, and postmonsoon seasons (Chapter VI). Metal accumulations in human hair and nails were found in those who consumed a non-vegetarian diet, particularly those who consumed seafood on a regular basis. Although Cd and Pb were found in both hair and nail samples, Cd levels were higher in human hair samples collected from the Cuddalore coast. This source might have originated from the consumption of Cuddalore water finfish and shellfish (Chapter VII). Above all, the results of the chapter indicated that, among the four metals, Cd had the strongest influence on the Cuddalore coast.

Cadmium (Cd; atomic number 48; relative atomic mass 112.40) is indeed a hazardous element in the periodic table and a ubiquitous ecological contaminant in several industrial applications, as well as in smoking and residue of other metal manufacturing, such as zinc, lead, or copper, and is mostly exploited in electrodes, paints, coatings, smelting, plastic stabilizers, fertilizers, and other uses. (Faroon *et al*., 2012). Humans absorb Cd from contaminants in the air, water, and food through the food web (Chirinos-Peinado and Castro-

Bedrinana, 2020). The average daily Cd intake of humans is 1.06 g/kg body weight (Wan *et al.*, 2013). In the Pan *et al.* (2010) report, which associated environmental pollution around Europe, dietary exposure is linked to the rates of breast cancer in females and prostate cancer in males. It represents the continuous alarm about the possible effects of Cd on human health. Cd is accumulated in considerable amounts from smoking and is recognized to have several negative impacts on health, including both experimental organisms and humans, affecting the renal, hepatic, and cardiovascular systems. Furthermore, a diverse variety of negative impacts on the reproductive system as well as the embryo have been documented (Thompson and Bannigan, 2008). Previous reports stated that Cd, when ingested and accumulated in the organism, is mostly toxic to the renal, especially the renal tubules cells, and induces skeletal skeletal fluorosis as well as increased exposure to airborne contaminants. Cadmium can damage pulmonary function by increasing the cancer risk (Messaoudi *et al.*, 2010).

Reproduction is a biological function that produces new generations and is necessary for a species' existence as well as survival and growth (Roychoudhury and Massanyi, 2014). The sexual system regulates the healthy activity and physiological changes among males and females, and the organism's behavioral activity. Hazardous metal exposure in the environment, workplace, and diet causes diverse changes in the living system and causes infertility. It has been one of the world's largest social health issues, affecting 15% of people of reproductive life (Anyanwu and Orisakwe, 2020). Cd does have the ability to alter reproductive and growth in a number of ways and at all development stages. In the case of males with higher experimental dosages, the impacts on the testicles include disintegration of the blood-testis membrane due to harmful effects on cell adhesion, oxidative stress, and necrosis. Contribution in the developing spermatozoa genome also has been documented. Followed by a female higher experimental dosage, oocyte growth is reduced, steroidogenesis is decreased, ovary bleeding and necrosis occur (Thompson and Bannigan, 2008). The most recent evidence on the possible harmful effects of Cd on human fertility. The hypothesized connection between Cd exposure and sexual health problems is erectile dysfunction, testosterone decrease, motility, viability, sperm production, and male infertility. In female early puberty, longer menstruation period, hormonal imbalance, spontaneous abortion, preterm delivery (Kumar and Sharma, 2019). Considering this knowledge, zebrafish were chosen as an animal model in this chapter to reveal the acute toxic effects of Cd on the testis and ovaries by evaluating histopathological and molecular alterations. The zebrafish (*Danio rerio*) is a vital fish in scientific investigation, and it is commonly used as a human model system in genomics, reproductive toxicology, and other field experiments (Dai *et al*., 2014; Meunier, 2012), as well as an effective model organism for fertility studies (Hoo *et al*., 2016). Additionally, as compared to other models, the usage of the zebrafish model system saves time. It also provides better knowledge and prediction accuracy when compared to in vitro studies (Mani *et al*., 2016). It enables uncomplicated handling and efficient absorption of compounds introduced directly into the water (Spence *et al*., 2008).

As a consequence, the current chapter experiment was conducted to analyse if Cd exposure in the water medium causes infertility in both male and female zebrafish. The hypothesis of this chapter is confirmed by metal accumulation in tissues, histological variation in testis and ovaries, and subsequently confirmation with gene expression studies.

8.2 MATERIALS AND METHODS

8.2.1 Experimental animal

Adult male and female zebrafish (*Danio rerio*) were procured from a fish aquarium retailer and acclimated for 1 month in very well holding glass aquaria (45 L), provided with tap water (pH = 7.05; dissolved oxygen = 10.5 mg/L; salinity = 0.5 ppt), with a light/dark cycle of 14 h:10 h and a temperature of (25) C. Zebrafish were fed a balanced diet of fish twice daily (optimum, the highly nutrious food for all aquarium fish). The water medium was refilled on a daily basis. During the acclimatisation period, twice-daily inspections were carried out to remove unhealthy, sick, and dead fish.

8.2.2 The structure of an experiment

The experiment was carried out using 250 zebrafish (n = 125 males and n = 125 females) over a three-week period (21 days) with a male wet weight of 0.68 ± 0.12 cm and a length of 3.82 ± 0.26 cm and a female wet weight of 0.86 ± 0.14 cm and a length of 4.32 ± 0.27 cm. To eliminate interference caused by reproductive processes, male and female fish were separated. The fish were separated into ten groups (five male groups and five female groups) and placed in different glass aquaria. Each of the five groups was as follows: the first group was Cd-free water as a control for both male and female groups. The remaining four groups as Cd exposed for both male and female populations, 0.25ppm concentration as the second group, 0.50ppm concentration as the third group, 0.75ppm concentration as the fourth group, and 1ppm concentration as the sixth group, as shown in the **Figure 32**. These Cd concentrations were chosen to be reflective of what may be found in a polluted environment. In this experiment, we are evaluating cadmium contamination on the Cuddalore coast and its impact on reproductive health. So, in the first chapter, Cd levels in water and sediment were found to be as high as 1 ppm. As a result, the maximum concentration was fixed at 1 ppm. Throughout the experiment, the water was continually aerated and maintained at a 45-liter rate. During the experiment, all subgroups of fish were fed twice a day at a rate of 0.5% of the fish population. This low amount was decided to avoid Cd absorption of residual aquarium feed (Cambier *et al.*, 2010; Chouchene *et al.*, 2011). All experimental techniques have been authorised by the institutional ethical committee (Ref no: BDU/ IAEC/ P13/ 2021).

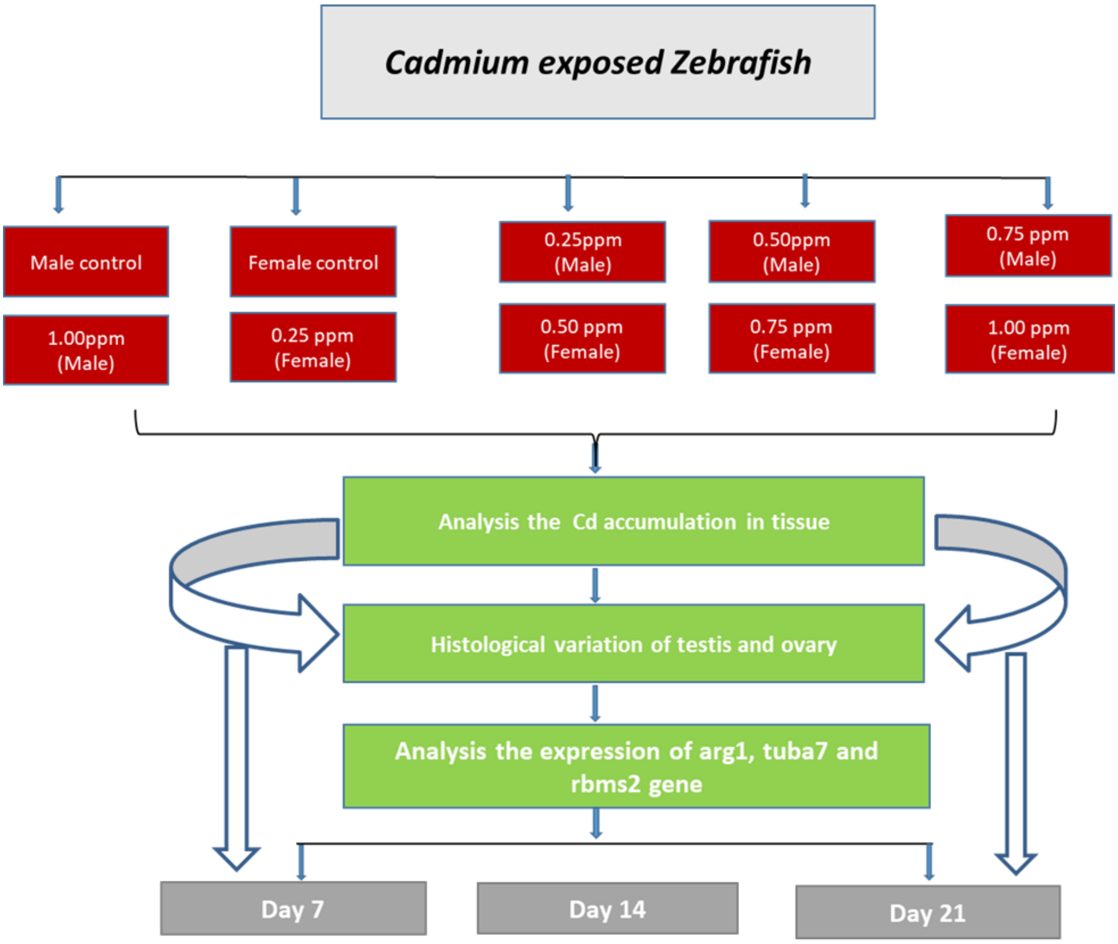

Figure 32 The schematic design of the zebrafish animal model study

8.2.3 Analytical methods

8.2.3.1 Collection of samples

The total number of experimental days was 21, and the sample collection periods for analytic purposes are days 7, 14, and 21. The histology and metal accumulation studies were carried out in all three sampling periods and in all experimental groups, but the gene expression study was carried out in the group that had the lowest and highest amount of exposure at the end of the day of the experiment. The gene expression group was determined based on the results of the accumulation and histology studies.

8.2.3.2 Cd examination

Due to minimal animals, each sample week, the dead fish from the sampling day and the previous day were collected for the accumulation study. One gram of dry tissue was collected for acid digestion to AAS analysis. The procedure and methodology used by the chapter II methods.

8.2.3.3 Histological examination

Dissected testis and ovaries from zebrafish were dried in ethanol before being fixed in paraffin. Sequential sections were cut at 5 μm and placed on glass slides, each paraffin block produced five microscope slides (anterior, mid anterior, central, mid posterior, and posterior gonads). Where they were examined with haematoxylin and eosin reagent, after which it was observed with a light microscope. Each group had three males and three females examined, and each individual had three dorso-ventral portions from various levels studied. The oocyte development in zebrafish ovary does not differ with location, the centre of each ovary was histologically examined. The animals used to identify the effects of each group and the morphological change were used to undertake a qualitative assessment of the histology results in the testis and ovaries (Chouchene *et al*., 2011; Petrovici *et al*., 2020).

8.2.3.4 Molecular examination

Isolation of RNA and production of cDNA

Total RNA was isolated from approximately 10 mg frozen ovarian tissues as per the manufacturer's instructions using the Trizol reagent (Sigma-Aldrich, St. Louis, USA). The OD260/OD280 absorbance ratio (>1.8) was used to confirm RNA purity. The purity of the RNA was determined by comparing the 18S and 28S peak on electrophenograms for each sample was determined. For further analysis, only undamaged RNA was employed. In a 20 lL mixture, 1.5-2 lg of total RNA was reversed transcription with randomly hexamers primer (Roche) and 200 U of M-MuLV HRT (Fermentas, Vilnius, LI), 0.5 mM dNTPs (Roche), and

1X M-MulV RT buffer as reported by Dondero *et al*. (2005). In brief, synthesized RNA was damaged by boiling it for 5 minutes at 70°C, cooling it on ice, and then incubating this with the reverse transcription reaction medium. During transcription, tubes were then incubated at 42°C for 60 minutes before being rapidly cooled. Its quantity of the RT solution was increased to 100 ll using nuclease-free deionized water, and 6 lL was employed for gene target amplification.

Real-time quantitative PCR

In a real-time quantitative PCR (RT-qPCR) equipment (iCycler, BioRad Laboratories), 1X QuantiTect Sybr Green PCR Master Mix (Qiagen), 10 nM fluorescein, and 0.2 lM of each gene Q-PCR primer were used **(Table 17)**. The data on, beta actin, and as internal reference genes because of their relatively high expression stability in zebrafish tissues. The expression level stability of the two reference genes in our experimental systems was determined using geNorm, as described by Vandesompele *et al*. (2002). For beta actin, our results demonstrated expression stability values of 0.32. The thermal procedure was as follows: 10 minutes at 95°C, followed by 40 cycles (10 seconds at 95°C, 20 seconds at 60°C, and 30 seconds at 72°C, when the signal was captured). On the basis of a single peak in the melting curve (60-90°C) and a single band of the expected size identified on agarose gels in preliminary testing, all primers were proven to yield just one gene product. The PCR efficiency rate for all amplifications ranged between 92 and 110 percentages. RT-qPCR was carried out in triplicate for each sample, and mRNA levels were calculated using a mean value. For each, three biological replicates were assessed.

Table 17. Target gene and specific primer pairs for the genes used in our study

Target Gene	Forward Primer	Reverse Primer
β-actin	GTG GGC CGC TCT AGG CAC CAA	CTC TTT GAT GTC ACG CAC GAT TTC
arg1	GGTGTGCAGGAAGGAGCAGA	CGATGGACTCGTCGTTGGGA
tuba7	CACACTGCTCTCTGGACTTTG	GGTGCCCAAGGATGTCAAC
rbpms2	CATACTCTTCTCTCCCCATATAC	TTTGTCTTGTTTGTGTTTGTTAG

8.3 RESULTS

8.3.1 Cd concentration in male zebrafish tissue

The control group was below the detection limit for the whole experimental day. On day 7, 0.25 ppm and 0.50 ppm were also below the detection limit. On day 7, the highest concentration was found in the 1 ppm group (0.37 µg/g), whereas the lowest concentration was found in the 0.75 ppm group (0.19 µg/g). Except for the control group, all of the experimental groups began accumulating on day 14; the highest concentration was reported in the 1 ppm group (1.09 µg/g), followed by the lowest concentration in the 0.25 ppm group (0.34 µg/g). Except for the 1 ppm group on day 21, all of the remaining groups are below the detection limit. On Day 21, the 1 ppm group (1.13 µg/g) had the highest concentration during the whole experimental period, as shown in the **Figure 33.**

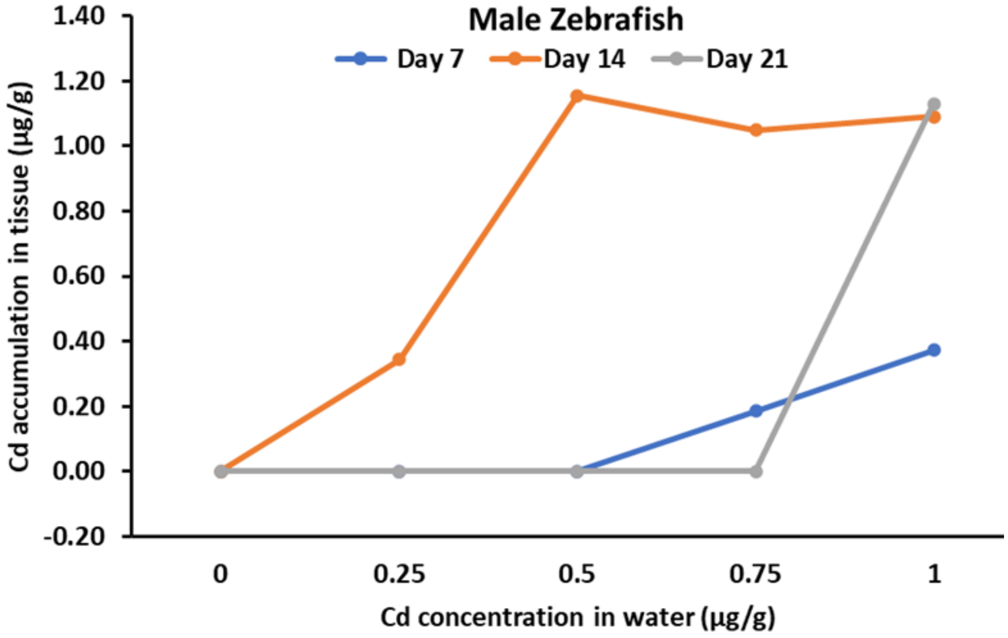

Figure 33 Cadmium accumulation in male zebrafish during the experimental period

8.3.2 Cd concentration in female zebrafish tissue

The control group was below the detection limit for the whole experimental day. On day 7, 0.25 ppm group also below the detection limit. On day 7, the highest concentration was found in the 1 ppm group (0.34 µg/g), whereas the lowest concentration was found in the 0.50 ppm group (0.02 µg/g). On day 14, the control group, 0.25 ppm group, and 0.75 ppm group are all below the detection limit. The remaining experimental groups started accumulating within that the highest concentration (2.72 µg/g) was recorded in the 1 ppm group, followed by the lowest concentration (0.15 µg/g) in the 0.50 ppm group. Except for the 1 ppm and 0.50 ppm groups on day 21, the remaining groups are all below the detection limit. On Day 21, the 1 ppm group (5.30 µg/g) had the highest concentration during the whole experiment, as shown in the **Figure 34.**

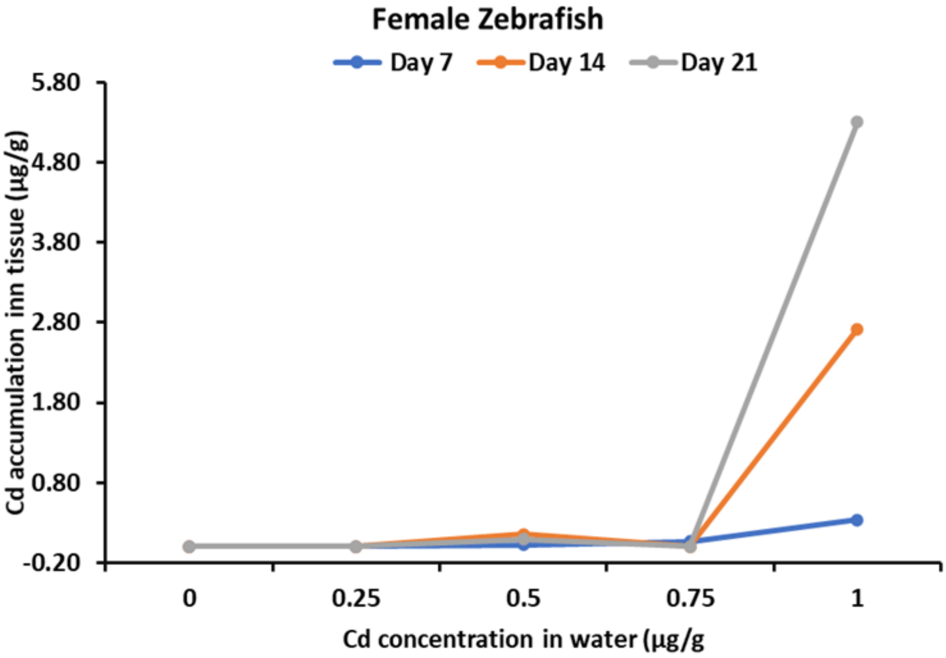

Figure 34 Cadmium accumulation in female zebrafish during the experimental period

8.3.3 Control and Cd exposed animal histological comparison

Control and Cd exposed testis

The seminiferous tubules in the control group revealed all phases of spermatogonial maturation in an adequate quantity, from spermatogonia through spermatocytes to sperm filling the lumens of seminiferous and efferent ducts. The 0.25 ppm group exhibited a small but noticeable decrease in aspects of cell count and size, whereas spermatogonia showed a minor decrease in quantity. The third group exposed to 0.5 ppm showed a transitional phase between the gonad toxicity shown in groups IV and V. Thus, the 0.25 ppm group showed considerable cyst involution as well as a decrease in the quantity of spermatocytes, with many spermatocyte clusters scattered among them. Mature sperm cells were revealed in the lumen of seminiferous tubules in the control group. The group that received the highest dose of 1 ppm had the most noticeable effects. The number of degenerated spermatogenic cell clusters increased from low to high concentration groups. The pattern of spermatogenic cluster alteration started on day 7 and increased on day 14, with the maximum damage occurred on day 21 as shown in **Figure 35.**

Control and Cd exposed ovary

Control group having normal ovarian histology and oocytes at various stages of development and primary oocytes contained round cells including an oval shape nuclei and a prominent nucleolus. The follicular sections were not fully established during the initial development phase, but they have been evident. The 0.25 ppm group showed minimal ovarian changes, however we could identify an increase in atretic oocytes and slightly reduced the number of previtellogenic and vitellogenic oocytes. The 0.5 ppm group had initial rupture with atretic oocytes, followed by the 0.75 ppm and 1 ppm groups, which had maximal cell rupture. When compared to the exposure day, the 21 day caused higher injury, particularly in the 1 ppm group as shown in **Figure 36.**

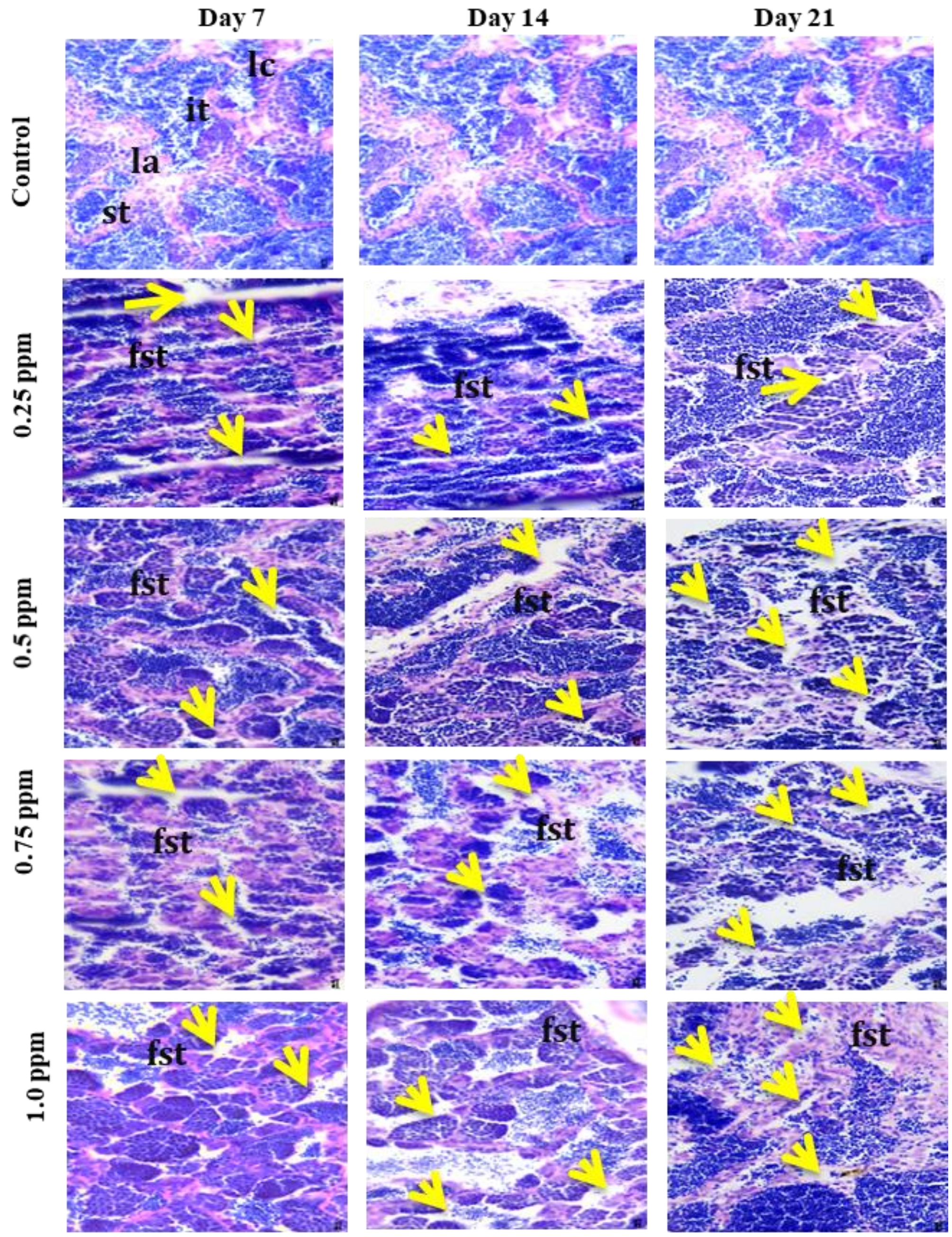

Figure 35 Histological analysis of the zebrafish exposed to cadmium (Cd) for the male testis. Cells marked with yellow arrows represent degenerated spermatogenic cell clusters, separation and vacuolization (scale from images represents 10 µm). Control showing testicular histology with testis; Cd-exposed zebrafish with different concentration exhibiting seminiferous tubule (st); luminal area (la); intersitial tissue in intertubular area (it); Leydig cells (lc).

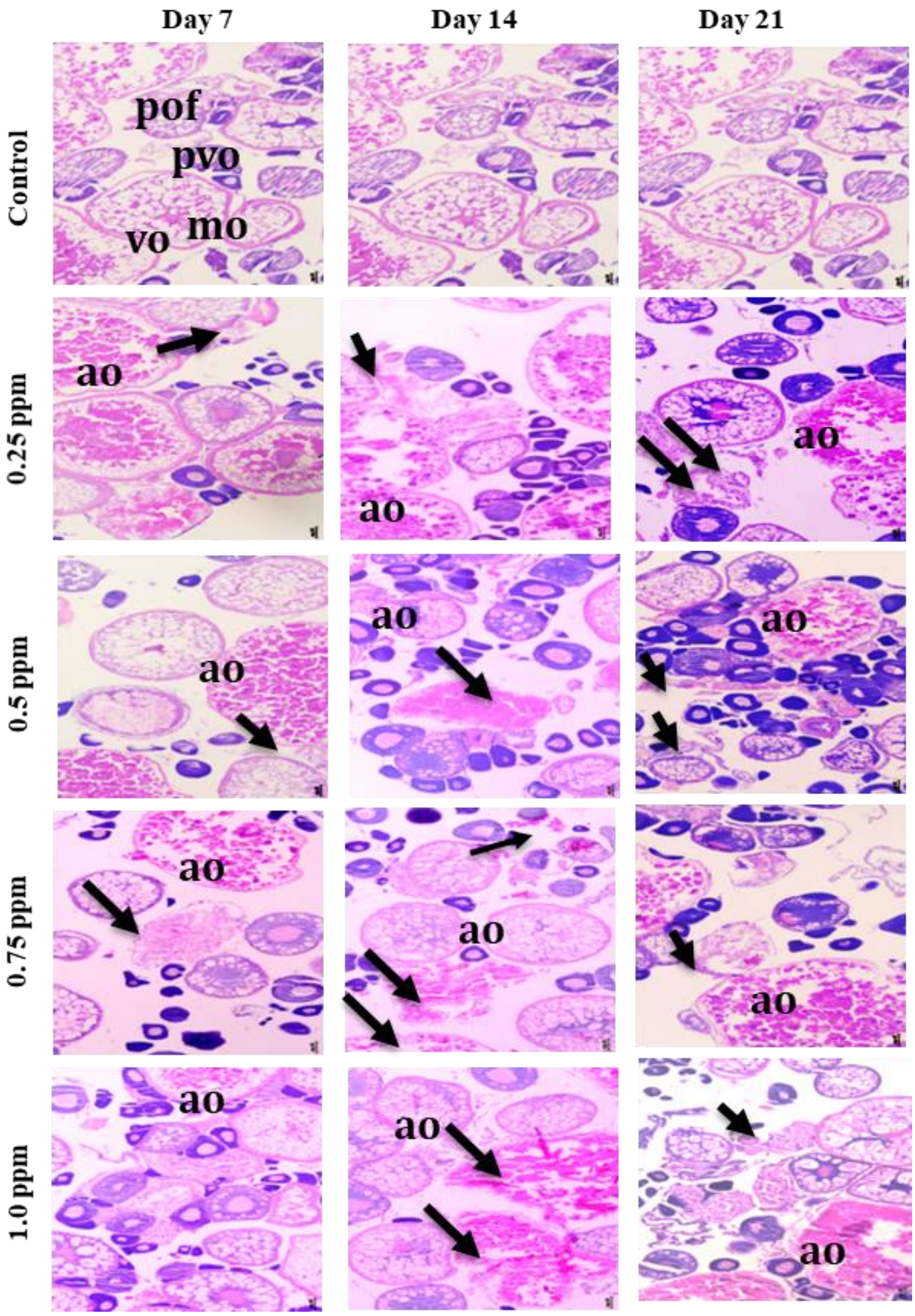

Figure 36 Histological analysis of the zebrafish exposed to cadmium (Cd) for the female ovary. Cells marked with black arrows represent examples of the positive activity of the cadmium (scale from images represents 50 µm). Control showing normal ovarian histology with oocytes of all different maturation stages; Cd-exposed zebrafish with different concentration exhibiting atretic oocytes (ao); previtellogenic oocytes (pvo); vitellogenic oocytes (vo); mature oocytes (mo); post-ovulatory follicle (pof).

8.3.4 Effects of cadmium on mRNA expression

Arginase 1 (arg1)

Arginase 1 (arg1) is an important gene in transcriptome analysis, as well as toxicological study, significantly influenced in inflammatory and immunological responses. Since oxidative stress is a possible consequence of cadmium toxicity, we used real-time qPCR to examine the mRNA expression of the gene arg1 in cadmium-exposed and control zebra fish. **Figures 37** and **38** shows the quantitative expression of the mRNA level of arg1 gene in male and female zebrafish. RT-qPCR analysis revealed that cadmium treated groups had higher mRNA levels than controls. Despite the fact that the quantity of arg1 mRNA over expression differs with exposed groups, the results clearly demonstrate the effectiveness of cadmium exposure in cellular oxidative activation. The fish was exposed to cadmium in both male and female group at lower and higher dosage exposure groups.

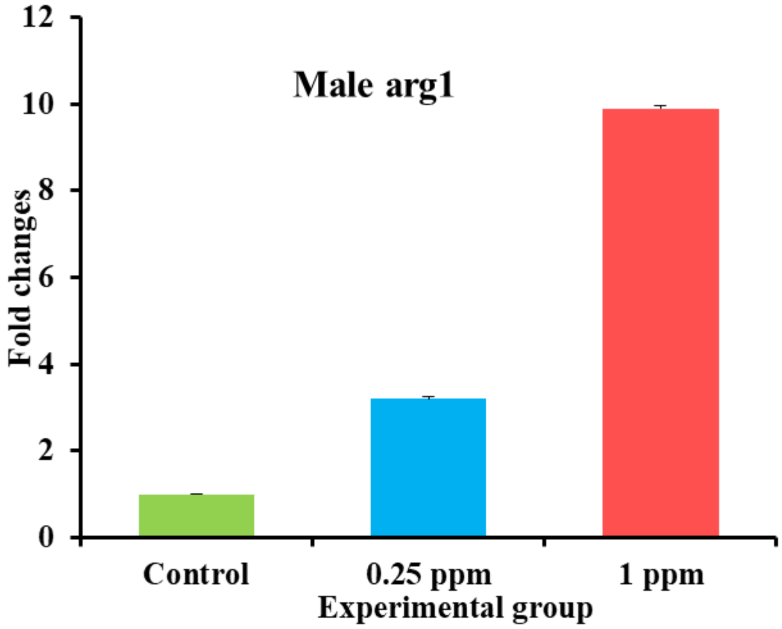

Figure 37 Quantitative expressions of arg1 mRNA in zebrafish testis by qPCR showing the expression ratio in comparison with control

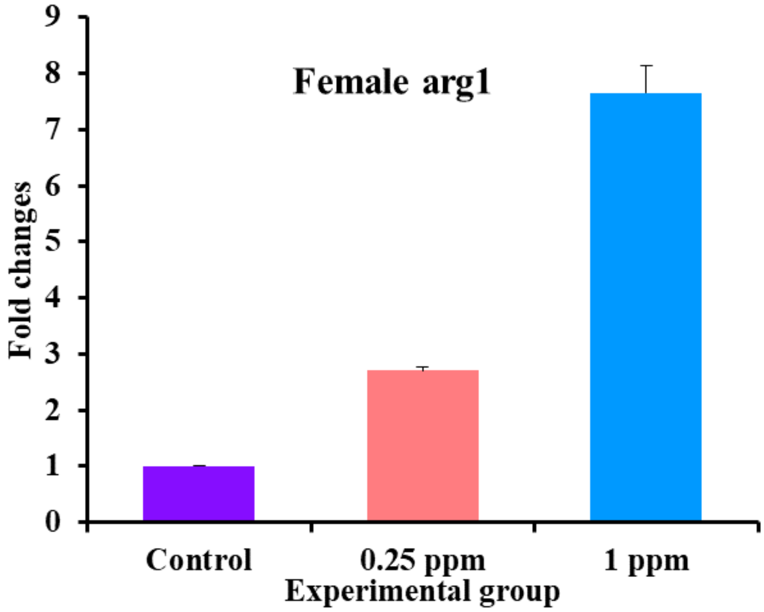

Figure 34 Quantitative expressions of arg1 mRNA in zebrafish ovary by qPCR showing the expression ratio in comparison with control

Tubulin alpha 7 (tuba7)

Tuba7 is a gene that produces male gamete testis, is active in male metabolic activities, and plays an important role in male zebrafish reproduction. We used real-time qPCR to examine the mRNA expression of the tuba7 gene in cadmium-exposed and control zebra fish. The quantitative expression of the tuba7 gene mRNA is shown in the **Figure 39**. In RT-qPCR analysis, upregulation of tuba7 mRNA expression is observed in the control group, while down regulation of tuba7 mRNA expression is observed in the exposed groups of 0.25 ppm and 1 ppm. These findings clearly indicate the significant toxic effect of cadmium exposure in both low and high dosage exposed groups**.**

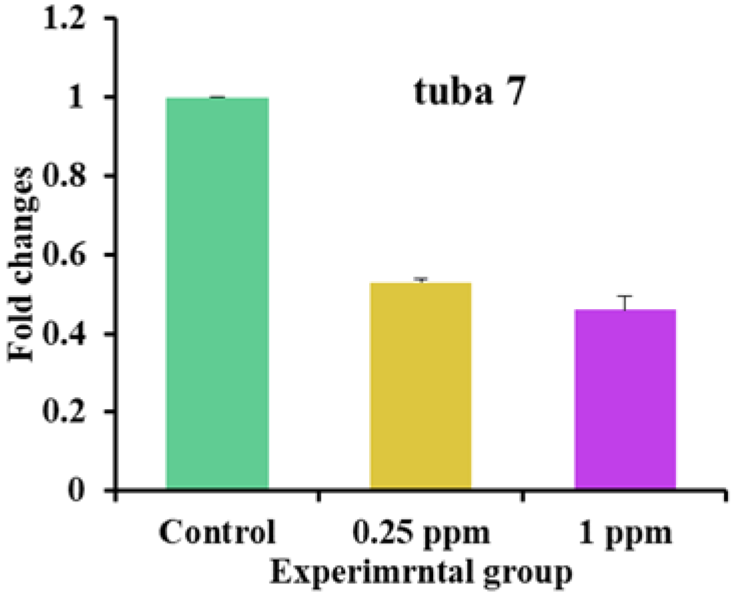

Figure 39 Quantitative expressions of tuba7 mRNA in zebrafish testis by qPCR showing the expression ratio in comparison with control

RNA binding protein with multiple splicing 2 (rbpms2)

Rbpms2 is a gene that produces female gamete ovary, is active in female metabolic activities, and plays an important role in female zebrafish reproduction. We used real-time qPCR to examine the mRNA expression of the rbpms2 gene in cadmium-exposed and control zebra fish. The quantitative expression of the rbpms2 gene mRNA is shown in **Figure 40.** In RT-qPCR analysis, upregulation of rbpms2 mRNA expression is observed in the control group, downregulation of rbpms2 mRNA expression is observed in the exposed groups of 0.25 ppm and 1ppm. However, the group with 1ppm have seen a significant decrease. The results clearly indicate that cadmium exposure has a considerable harmful effect in both low and high dose exposed groups, with highest concentration having the strongest effect.

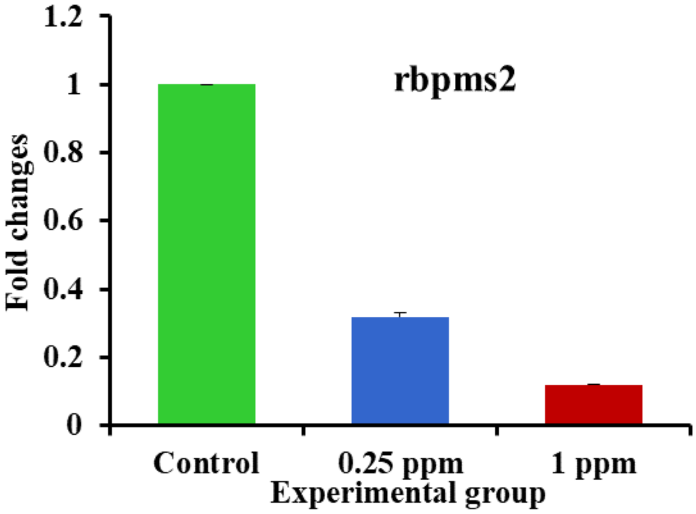

Figure 40 Quantitative expressions of rbpms2 mRNA in zebrafish ovary by qPCR showing the expression ratio in comparison with control

8.4 DISCUSSION

We explored how zebrafish respond to different levels of Cd exposure in order to provide insight on potential differences throughout the range of Cd reproductive toxicity. The study focused on cadmium accumulation in zebrafish tissues and its relationship to fish reproductive health. In addition, histopathological variation and qPCR analysis were performed in an effort to correlate Cd effects with infertility. Different concentrations were exposed to various groups of fish. Cd accumulates equally throughout their tissues, while Cd accumulation in male and female zebrafish tissues was observed in higher concentration groups. For any of the exposure levels, accumulation was not time dependent (Renieri *et al*., 2017). Following chronic Cd exposure, several researchers reported that Cd accumulates in zebrafish in a dosage and time dependent mechanism in both the whole body and particular organs (Arini *et al*., 2015; Cambier *et al*., 2010).

AAS results indicate that Cd accumulation was slightly higher on day 7 in the 0.75 ppm and 1 ppm groups in males. Females had minor accumulation in the 0.50 ppm and 0.75

ppm groups, whereas the 1 ppm group had higher accumulation. During this period, linear mortality was noted. After that, the mortality rate was reduced in the second and third weeks of the experiment. However, the male group's Cd accumulation was severely affected by the second week of the experiment, but still the female group, with the exception of the 1 ppm group animals, had less accumulation. Male mortality rates were higher than female groups during this time period. However, the highest accumulation was observed in the 0.75 and 1 ppm concentration groups, whereas the highest mortality was observed in the lower doses of 0.25 and 0.50 ppm in both male and female animal groups. Renieri *et al.* (2017) stated that the mortality of zebrafish reveals a variance in toxic reactions along a range of Cd doses, resulting in a nonlinear response. Zebrafish may have developed a defensive mechanism against Cd exposure. These diverse reactions, along with the above-mentioned literature evidence, might be connected to hormesis phenomena. Our findings about the cadmium accumulation reveal similar patterns to previous reports. When cadmium deposition in mosquitofish during chronic exposure was studied, two distinct accumulation trends were discovered: Cd deposition increased significantly until 20 days following exposure, then decreased by the 30th day (Annabi *et al.*, 2011).

The histological findings show that even low dose Cd has significant histopathological effects in zebrafish tissue, with higher dose exposure in the long term causing severe damage to zebrafish testis and ovarian cells. According to histology results of the gonads, exposure to different concentrations of cadmium is responsible for a decrease in fertility in zebrafish. We observed an immediate difference in the ovarian dispersion of the various types of ovarian follicles in female gonads, which resulted in the predominance of atretic follicles, a massive reduction of different phases, and an enormous number of pre-nucleolar oocytes due to reduced oogenesis in its most exposed group. The current results reveal that the gradual decrease in spermatogenic clusters, which support the male reproductive function, was disrupted. The latest article also concludes that the fibrosis and a decrease in spermatozoa volume, as well as a significant and gradual decrease in spermatogenic cluster (Petrovici *et al*., 2020).

The current study gene was selected for its metabolic role; the first gene, arg1, was chosen to determine whether the fish group had been exposed to cadmium. Song *et al*. (2020) qPCR analysis confirmed the arg1 gene in zebrafish, PHMG-P may have lethal and cardiotoxic impacts, and important transcriptional alterations have been related to immunological and inflammatory responses. In the past decades, it has been reported that abnormal expression of ARG1 is increasingly connected to a variety of diseases, including cardiovascular diseases, inflammatory bowel diseases, and Alzheimer's disease. Thus, upregulated arginase is a potential biomarker of progression and severity in these diseases (Niu *et al*., 2022). Based on these findings, the arg1 gene expression results clearly indicate that the fish groups were confirming Cd exposure, as arg1 was downregulated in both male and female control groups. While the exposed groups of both 0.25 ppm and 1 ppm group results indicated that arg1 was upregulated in both male and female group animals, it's because after being exposed to cadmium, the fish produce an immune response against the cadmium, hence the experimental group animals are upregulated for arg1. Intriguingly, ARG1 was detected in the extracellular vesicles (EVs) from the ascites and plasma of ovarian cancer (OvCa) patients and served as the metabolic checkpoint molecule to inhibit antigen-specific T cell proliferation, leading to accelerated tumor progression (Czystowska-Kuzmicz *et al*., 2019).

Santos and colleagues (2007) investigated the molecular mechanism of sex and reproductive potential in breeding zebrafish. After determining the ten transcripts whose gene expression levels change significantly among males and females at the gonad region, the data for sex-related variations in the profiling of genes reported to be associated with reproductive performance between the ovaries and the testis. The present study also found two genes that are significantly expressed with Cd exposure level in both testis (tuba7) and ovary (rbpms2).

Tuba7 and rbpms2 genes were found to be upregulated in the control group, indicating that these animals had a normal reproductive cycle due to the cadmium-free environment. Tuba7 was found to be down regulated in both the 0.25 ppm and 1 ppm Cd exposed male groups. The rbpms2 gene was downregulated in both the higher and lower doses of the experimental group, indicating that cadmium exposure potentially affects the male and female reproductive cycle.

Several publications have demonstrated the action of Cd as an endocrine disruptor (ED) in zebrafish, with the majority of them reporting impacts on vitellogenin (vtg) genes (Chen and Chan, 2016a). Chen and Chan (2016b) state that Cd has anti-estrogenic activities in the ZFL cell line in both embryonic and adult stages. They further claim that Cd's impacts on vtg1 expression of genes in zebrafish are mostly mediated through the endoplasmic reticulum.

Chapter IX

Summary and Conclusion

In the modern world, many anthropogenic activities such as agriculture, industrial, and transportation generate a massive amount of pollution and special types of pollutants. This pollutant has an adverse effect to human health, however, data on reproductive toxicity are insufficient. Consequence, we selected the highly polluted Cuddalore coast as the study area and used zebra fish as an animal model to analyze the effect of cadmium in infertility caused by metal transfer from the marine food web to humans. The chapter wise summary results are described below.

The present study was aimed to understand the metal concentrations and ecological risk assessments for four heavy metals (Cu, Cd, Pb, and Zn) were analyzed. The seasonal variations of metals in water and sediments from sites with different anthropogenic activities were assessed. Outcomes revealed higher concentration of metals in sediments compared to water. Order of metals in water is Zn > Pb > Cu > Cd, and in sediment is Zn > Cu > Pb > Cd. Seasonal distribution of metals in the water was premonsoon > postmonsoon > monsoon > summer, and in the sediment was summer > postmonsoon > monsoon > premonsoon. Monsoon season loaded the estuarian ecosystem with heavy metals from the Cuddalore industries. Later, during summer months, the metals accumulated in the sediments and deposited in the mouths of Uppanar and Gadilam rivers. This study clearly illustrates that the coastal ecosystem in Cuddalore obtains heavy metals by the anthropogenic activities which has been endorsed by PCA and seasonal influence of heavy metal concentrations identified by HCA. The main anthropogenic activities in the study area include disposal of waste from industrial zones, fertilizer and pesticide runoff from agriculture activities, fisheries activity, and boat and harbor activities.

We present seasonal variation of four metals (Cd, Cu, Pb, and Zn) in twelve finfish and nine shellfish species collected from the highly polluted Cuddalore coast in Tamil Nadu, India. Finfish were divided into two categories: pelagic and benthic, whereas shellfish were divided into two categories: crustacean and molluscan. The ranges of the four metals in the pelagic fish species were as follows: Cu (<BDL–0.95 µg/g), Cu (<BDL–47.98 µg/g), Pb (<BLDL–5.12 µg/g), and Zn (9.27–38.46 µg/g). The ranges of the four metals in the benthic fish species were as follows: Cu (<BDL–0.75 µg/g), Cu (1.28–30.21 µg/g), Pb (<BDL–4.55 µg/g), and Zn (19.57–42.92 µg/g). Likewise, the shellfish metal concentration ranged across all four seasons as follows: Cd (0.97–1.45 µg/g), Cu (3.53–26.32 µg/g), Pb (0.37–2.39 µg/g), and Zn (32.07–39.71 µg/g). The metal order in finfish tissue samples, the concentration follows the descending order of Zn>Cu>Pb>Cd. In shellfish, the metal order is Zn>Cu>Pb>Cd. Both the finfish and shellfish multivariate statistical analysis revealed that the metals detected in the fish may have originated from both natural & anthropogenic activities in Cuddalore coast. BCF results indicate that the copper source is higher in finfish, while the lead source is higher in several species, and BAF results show that shellfish species were highly contaminated by cadmium.

The level of geo-accumulation index values of Cu, Pb, and Zn fall under unpolluted Class 0 in all the stations for all seasons. Only Cd levels fall in the classes 1–4 depicting the sites are moderately to strongly contaminated in the premonsoon, monsoon, and post monsoon seasons. Cd contamination is absent in the summer season. The contamination factors for the different metals across all seasons for the different stations fall in the ranges of Cu, 0.10–1.14; Cd, 0–16.43; Pb, 0.14–0.49; and Zn, 0.04–0.46. The highest contamination factor was detected for Cd in Station 3 during the postmonsoon season. The ecological risk index for the elements falls in the following ranges: Cu, 0.49–5.70; Cd, 0–492.86; Pb, 0.70–2.47; and Zn, 0.04–0.46. The highest ecological risk index was for Cd in

Station 3 during the postmonsoon season. Finfish hazard index values (pelagic=0.97; benthic=0.90) are in borderline. Even a slight increase in metal concentration in finfish can prove hazardous for human consumption. Shellfish hazard index values (Crustacean=1.88; Mollusk =2.25) are in above the limit. Hence, consumption of shellfish from Cuddalore coast can pose hazards to human health because HI values are beyond the threshold limit.

The levels of metal concentration in the hair and fingernail were found to be significantly influenced by both personal and diet characteristics. The metal concentration was following the descending order of Zn> Cu >Pb >Cd in hair samples and the nail samples descending order was Zn> Cu> Pb> Cd. The general variance in both personal characteristics and diet characteristics between nail and hair was that Cd, Cu is higher in the hair sample while Pb, Zn was higher in the nail sample. The results from the survey questionnaires revealed that several factors contribute to Cd and Pb exposure, but the frequent intake of finfish and shellfish from Cuddalore water has resulted in higher accumulation when compared to other causes.

In order to understand the reproductive toxicology of cadmium exposed zebrafish. The AAS results show maximum accumulation observed in the group of 1 ppm in both male and female experimental groups at the end of the experimental day. Histological observations are when compared to the Cd exposed group, the control group testis and ovary regions are fully evident; however, in the exposed groups, the damage occurred even at lower concentrations of 0.25 ppm, but the major damage occurred towards the end of the experimental day 21 in both male and female group. Based on the AAS and histological analysis results, cadmium can influence the expression of genes, which we demonstrate using a molecular technique such as gene expression. The arg1 gene, which is expressed after cadmium exposure, was shown to be elevated in both the male and female groups, according to gene expression data. It was confirmed that the animal groups had been exposed to cadmium. The testis-producing gene tuba 7 was found to be downregulated in both 0.25 and 1 ppm groups, and the ovary-producing gene rbms2 was similarly downregulated in both 0.25 and 1 ppm groups. We were able to confirm that Cd exposure caused reproductive system damage in zebrafish using gene expression.

The present study provides evidence of metal accumulation, risk assessment, and its effect on reproductive health. We have detected elevated levels of metal concentrations in the water and sediments of Cuddalore corresponding to the discharge of industrial and urban wastes into the Uppanar estuary. When compared with permissible limits of metal concentrations in the fish tissue as per international agencies the essential metal loadings are under the limit, but the non-essential metal cadmium was cross the permissible limit in shellfish. While in finfish during the post-monsoon and summer seasons the lead values are higher than the permissible limit. The ecological and human health risk assessment results clearly indicated that cadmium loadings had contaminated the Cuddalore coastal sediment, and the consumption of finfish and shellfish also caused harmful effects on humans. The human hair and nail data revealed that several factors are the origin of metal exposure to humans. However, according to the survey questionnaire, people who consume seafood have a high exposure to cadmium and lead, which may have been accumulated from the finfish and shellfish resources. The zebrafish model experiment revealed that cadmium in the environment gets accumulated in the tissue of the organism, causing infertility.

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Chapter XI

List of publications

1. **Vinothkannan, A.**, Charles, P. E., & Rajaram, R. (2022). Ecological risk assessment and seasonal variation of heavy metals in water and sediment collected from industrially polluted Cuddalore coast, Southeastern India. *Regional Studies in Marine Science*, 49, 102134.

2. **Vinothkannan, A**., Rajaram, R., Charles, P. E., & Ganeshkumar, A. (2022). Metal-associated human health risk assessment due to consumption of pelagic and benthic ichthyofaunal resources from the highly contaminated Cuddalore coast in Southern India. *Marine Pollution Bulletin*, 176, 113456.

3. **Anbazhagan, V**., Partheeban, E. C., Arumugam, G., Selvasekaran, V., Rajendran, R., Paray, B. A., ... & Al-Mfarij, A. R. (2021). Avian feathers as a biomonitoring tool to assess heavy metal pollution in a wildlife and bird sanctuary from a tropical coastal ecosystem. *Environmental Science and Pollution Research*, 28(28), 38263-38273.

4. **Anbazhagan, V**., Rajendran, R., Arumugam, G., & Ganeshan, A. (2021). Identification of heavy metal pollution source due to idol immersion activity across the Cauvery river basin, Tamil Nadu, South India. *Current Science*, 120(1), 200.

5. Ganeshkumar, A., Sivaraj, D., Vijayalakshmi, K., **Vinothkannan, A.,** Raju, P., Dineshkumar, S., ... & Rajaram, R. (2022). Sunlight-irradiated bismuth titanate nanoparticles mediated degradation of methylene blue-ecological perspectives. *Environmental Technology & Innovation*, 102749.

6. **Vinothkannan, A**., Charles, P. E., & Rajaram, R. (2022). Consumption of metal-contaminated shellfish from the Cuddalore coast in Southeastern India poses a hazard to public health. *Marine Pollution Bulletin*, *181*, 113827.

7. Suvetha, M., Charles, P. E., **Vinothkannan, A**., Rajaram, R., Paray, B. A., & Ali, S. (2022). Are we at risk because of road dust? An ecological and health risk assessment of heavy metals in a rapid growing city in South India. *Environmental Advances*, 100165.

8. **Anbazhagan, V**., Partheeban, E. C., Arumugam, G., Arumugam, A., Rajendran, R., Paray, B. A., ... & Al-Mfarij, A. R. (2021). Health risk assessment and bioaccumulation of metals in brown and red seaweeds collected from a tropical marine biosphere reserve. *Marine Pollution Bulletin*, 164, 112029.

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11. Rajaram, R., Ganeshkumar, A., & **Vinothkannan, A**. (2020). Health risk assessment and bioaccumulation of toxic metals in commercially important finfish and shellfish resources collected from Tuticorin coast of Gulf of Mannar, Southeastern India. *Marine Pollution Bulletin*, 159, 111469.

Regional Studies in Marine Science 49 (2022) 102134

Contents lists available at ScienceDirect

Regional Studies in Marine Science

journal homepage: www.elsevier.com/locate/rsma

Ecological risk assessment and seasonal variation of heavy metals in water and sediment collected from industrially polluted Cuddalore coast, Southeastern India

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ARTICLE INFO

Article history:
Received 22 July 2021
Received in revised form 23 November 2021
Accepted 8 December 2021
Available online 15 December 2021

Keywords:
Metal pollution
Geo-accumulation index
Pollution load index
Contamination factor
Ecological risk
Anthropogenic

ABSTRACT

Concentrations and ecological risk assessments for four heavy metals (Cu, Cd, Pb, and Zn) were analysed. The seasonal variations of metals in water and sediments from sites with different anthropogenic activities were assessed. Outcomes revealed higher concentration of metals in sediments compared to water. Order of metals in water is Zn>Pb>Cu>Cd, and in sediment is Zn>Cu>Pb>Cd. Seasonal distribution of metals in the water was premonsoon>postmonsoon>monsoon>summer, and in the sediment was summer>postmonsoon>monsoon>premonsoon. Monsoon season loaded the estuarian ecosystem with heavy metals from the Cuddalore industries. Later, during summer months, the metals accumulated in the sediments and deposited in the mouths of Uppanar and Gadilam rivers. Environmental pollution indices, such as geo-accumulation index, contamination factor, and ecological risk index, pointed that Cd posed a greater risk to the ecosystem compared to other metals. Through multivariate statistical analyses, metal sources were linked to industrial wastes, chemical fertilizers, shipping activities, and fishing and boat activities which pollute the Cuddalore coast. Continuous monitoring for heavy metals and their ecological impacts in the Cuddalore coast is essential to remediate the metal pollution effectively.

1. Introduction

Oceans cover more than 70% of our planet and are considered one of the earth's most precious natural resources. However, these precious resources are slowly disintegrating of their prosperity due to coastal pollution, increasing human population, and emerging industrial areas which highly utilize the ocean resources (Le Tissier et al., 2006; Nour and Nouh, 2020). The environmental impacts of different stressors in India are gradually becoming very serious and even posing threats to the associated biotic community. Massive volumes of hazardous waste contaminants, distributed worldwide, are affecting the world ocean. Contemporary and historical manufacturing processes have contaminated the natural environment including air, water, and sediment. The effect of this pollution is felt even in the most rural areas making them lose their pristine nature (Ramesh kumar and Anbazhagan, 2018). The geologic analysis of aquatic life describes the essence of the geochemical characteristics helping us determine the exogenous metal cycle in the marine environment (Giridharan et al., 2010). India has 8129 km of coastline with prominent features like coastal backwaters, rivers, streams, and wetlands. In the Indian coastline, the southeast coast plays a prominent role with its diverse characteristics. There are several rivers trenching into the Bay of Bengal in the southeastern region. Moreover, the flora and fauna are higher in the eastern coast compared with the western coast of India (Ayyamperumal et al., 2006).

Heavy metals can degrade the environment by polluting air, water, and soil, subsequently initiating adverse health effects in the ecology and living forms, when they get concentrated as a result of various industrial activities (Rajaram et al., 2020; Stankovic and Stankovic, 2013). Heavy metals are not only accumulated in the water column, but also aggregate in colloidal matter and sediments afterwards, eventually reaching the food chain by travelling to higher consumers. Depending on the composition of the sediment layer, by various physical and chemical adsorption routes they aggregate in the sediments (Ghrefat and Yusuf, 2006; Khaled et al., 2006; Nour, 2015). These metals exist in marine environments and are distributed between various components (Linnik and Zubenko, 2000).

The river streams transport large amounts of suspended particulate matter, in both organic and inorganic forms, to the ocean (Bainbridge et al., 2018). This biomass carries heavy metals which tend to accumulate in the marine ecosystem and cause modifications in biological and chemical parameters of the oceans. The

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https://doi.org/10.1016/j.rsma.2021.102134
2352-4855/

geochemistry of dissolved trace metals is regulated by an intricate balance of hydrodynamic factors, industrial effluents, municipal wastewater discharges, and biogeochemical cycles (Sun et al., 2018). Dissolved metal contaminants in the estuarine waters are mostly influenced by water–particle interactions, such as flocculation, adsorption, organic and inorganic conglomeration, and resuspension of particulate matter by means of agitation (Jonathan et al., 2008).

The examination of trace metals helps in finding the relationship between the distribution of metals in the sediments and water column. Sediment and water interaction might be important for the accumulation of metals in sediments (Silva et al., 2014; Singh et al., 2005; Wan et al., 2012). The investigation of dispersed metals in sediments adjoining regions of colonization and agriculture can provide proof of their anthropogenic effect on the ecosystem and thus aid in the determination of the potential risks due to the heavy metals in the environment. Trace metal contamination is considered as a major problem among various types of coastal pollutions because of the toxicity and deposition of metals in the environment by high adsorption rates and their property to bio-magnify easily through the food chain (Yeardley et al., 1998; Nour, 2020). Pb, Cd, Cr, and Hg, found in sediments and water are harmful to marine organisms. However, they may emerge from raw sewage, industrial, agricultural, urban, domestic, and mining practices, and penetrate through river and brackish water into the marine environment (Anandkumar et al., 2018; Rajaram et al., 2017). In the marine environment, various metals are generated by natural and manmade processes. Among these metals, the essential metal groups include Zn, Cu, Cr(III), Fe, and Mn. The excessive deposition of these essential elements can also be toxic, however useful for metabolic activities in lower concentrations. The nonessential metals, including toxic metals like Cd, Hg, Pb, and Cr (VI), are harmful even in lower concentrations (Cohen et al., 2001; Fergusson, 1990).

The key objective of this work was to provide the ecological risk assessment of toxic metals contamination because of two essential (Cu and Zn) and two nonessential (Cd and Pb) metals across four seasons in the water and sediment collected from the Cuddalore coast of Tamil Nadu, Southern India. Ecological monitoring and evaluation will explain the source of declining conditions in estuary habitats and interpret modifications in temporal and spatial patterns. Once we have identified the sources of these heavy metals, strict policies and environmental conservation strategies can be incorporated to recover the marine ecosystem. Regular long-term seasonal monitoring of this region will aid to control pollution and implement better management practices for the ecosystem protection.

2. Materials and methods

2.1. Description of study area

Cuddalore district has a land cover of 3678 km^2, which includes a 68-km stretch of coastline. Multiple drainages and industrial waste channels drain exclusively into the Uppanar River which flows along the Cuddalore city and finally drains into the Bay of Bengal. The Uppanar estuary is approximately 5-km long with an average width of 30 m and a mean depth of about 2.5 m. The study area has an average elevation of about 1 m above the mean sea level. Tidal interaction can reach about 6 km upstream from the estuary to the river (Rajaram et al., 2005). In Cuddalore, the southwest and northeast monsoons play a key role in the precipitation. The southwest monsoon is less intensive, whereas the northeast monsoon is more intensive which is from October to December. The average annual rainfall reported here is 1902 mm (Gopal et al., 2018). The summer months witness rising temperatures in the period from April to June with temperatures surpassing 40 °C. The Cuddalore district in Tamil Nadu is an important coastal city with several large scale industries, agricultural land, fishing activities, harbour and tourism activities (Rajaram and Ganeshkumar, 2019). There are three main rivers flowing through the coastal region across the Cuddalore district — Uppanar in the south and Then Pennai and Gadilam in the north, all of which are adjacent to the Bay of Bengal. In ancient days, this coastal zone had a healthy ecosystem with much more marine fauna and flora. The western part of the Uppanar River is influenced by many activities like agriculture, harbour activities, industries such as chemicals, paint, tanneries, pharmaceuticals, etc. Additionally, Perumal Lake which holds ash discharged from the surrounding thermal power station (Jonathan et al., 2008) is one of the pollution sources for the Uppanar River.

2.1.1. Sampling sites

Three rivers stretch in this study area, but the Uppanar River and Gadilam River have several sources of pollution identified in comparison with Then Pennai. Hence, the three sampling sites around the Uppanar and Gadilam estuaries are chosen to collect water and sediment samples based on the pollutant source. There are about 55 major and minor industries that represent approximately 520 acres of land area, and they operate under the State Industries Promotion Corporation of Tamil Nadu (SIPCOT) industrial hub located in the western part of the study area (Rajaram and Ganeshkumar, 2019). Fishing harbours and urban settlements are found in the northern region. Watering by monsoon and stream supplies were observed in the lower margin of this estuary. Agricultural lands are present, but most of the people utilize this estuary for fishing activities. The three sampling sites selected here have different sources of pollution as represented in Fig. 1.

2.1.1.1. Agricultural runoff (Station 1). Station 1 (St 1) chosen on the basis of agricultural runoff was Sami nagar (11°39′24.7″N, 79°44′55.7″E) situated about 14.8 km northeast of Perumal Lake. The Uppanar River until this station is dotted with various agricultural farms and on either side. Most to the agricultural runoff water reach the Uppanar River and are mainly influenced by the agricultural wastes and the water from the Perumal lake which has loads of ash from a major thermal power plant.

2.1.1.2. Industrial waste and fishing activities (Station 2). Station 2 (St 2) chosen on the basis of industrial activities and fishing activities was Thaikkal thoni thurai (11°41′33.5″N, 79°46′01.1″E) situated about 3 km north of Station 1. The important industrial hub State Industries Promotion Corporation of Tamil Nadu (SIPCOT) with comprising about 55 major and minor industries is located in this study area. The industries here dispose their untreated wastes into the Uppanar River (Rajaram and Ganeshkumar, 2019). This station also has marked fishing and boat activities which further pollute the environment with their fumes generated from boat engines and other waste spillages.

2.1.1.3. Tourist activities (Station 3). Station 3 (St 3) chosen on the basis of marked tourism activities is Silver Beach (11°44′15.1″N, 79°47′02.4″E) situated on the northern part of the estuary. This site is popular for its tourist position in the district of Cuddalore. The Cuddalore port is also present near this station. This site is situated at about 12.3 km north of Thaikkal thoni thurai. This site is influenced by pollution from tourism and harbour activities.

2.2. Collection and analysis of water samples

A total of 36 surface water samples from the estuaries were collected for four seasons (premonsoon, monsoon, postmonsoon,

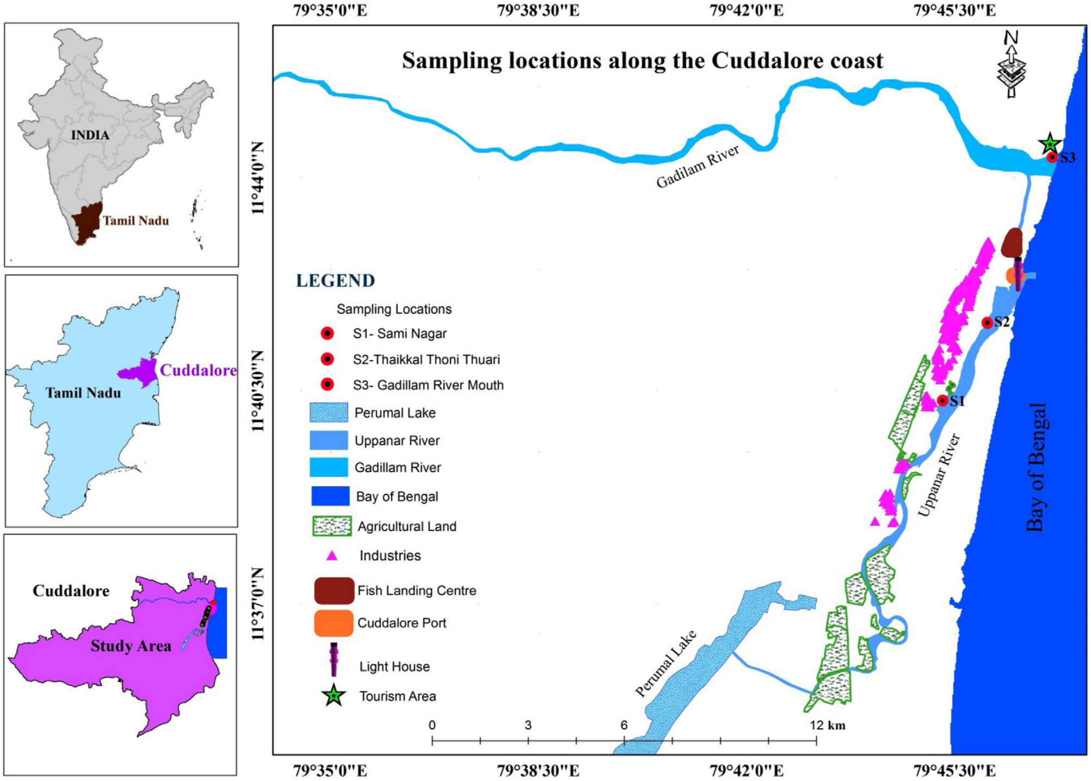

Fig. 1. Study area map of sampling stations along the Cuddalore coast.

and summer) from July 2018 to June 2019. Samples collected from surface of the water were stored in 1000-mL sterile containers with 1 mL of concentrated HNO_3 for preservation and to eliminate cross contamination by debris. The stored water samples were filtered using the Millipore filter unit (0.45-μm) and transferred into a separation funnel. Then, 10 mL of 1% APDC (ammonium pyrrolidine dithiocarbamate) solution was added and shaken well, and 25 mL of MIBK (iso-butyl methyl ketone) was added and subjected to vigorous shaking for 10–15 min. From the two separated layers formed, the upper organic phase was collected. The organic stage obtained was extracted with 50% HNO_3. The organic layer was collected and made up to 25 mL using double-distilled water (Arumugam et al., 2018; Jonathan et al., 2008). The resulting sample solution was analysed using the atomic absorption spectrophotometer (AAS) instrument (Shimadzu AA-7000, Japan).

2.3. Collection and analysis of sediment samples

A total of 36 sediment samples across four seasons were collected from the study area. In all three stations, the top 2-cm layer of sediments were collected from the intertidal zones using acid-cleaned PVC pipes (with an outer diameter of 3.5 inches). The pipes were pressed in the mud to extract the topsoil as per the method of Arumugam et al. (2018). The collected sediment samples were stored in zip-lock covers and transported to the laboratory for analysis. The sediment samples were dried out at room temperature and were powdered to a fine texture with an agate mortar and pestle. The fine powder was sieved through a 63-μm sieve. Then, one gram of sieved sample was transferred into the beaker and subjected to acid digestion process. About 10 mL of mixed reagent in the ratio of 5:2:1 [HNO_3 (72% strength), $HClO_4$ (70% strength), and H_2SO_4 (97% strength)] was used for the digestion. The beakers containing sample and the reagent mixture were heated on a hot plate at 60 °C. At the end of the digestion, a few drops of 2N HCL were added. The final extract was collected and filtered with Whatman Grade 1 filter paper to remove any residual contaminants for AAS analysis. The filtered sample was analysed using the atomic absorption spectrophotometer (AAS) instrument (Shimadzu AA-7000, Japan) (Arumugam et al., 2018) with the lowest detection limit of 0.01 mg/L for the analysed elements.

2.4. Pollution monitoring indices

To evaluate the pollution levels in the study area, we have used pollution monitoring indices like geo-accumulation index (I_{geo}), contamination factor (C_f^i), contamination degree (C_{deg}), pollution load index (PLI), and potential ecological risk (PER).

2.4.1. Geo-accumulation index

As per Muller (1969), the geo-accumulation index (I_{geo}) was assessed to know the pollution levels in surface sediments of the study area. The below equation was employed to compute the geo-accumulation index:

$$I_{geo} = \log_2\left(\frac{C_n}{1.5 \times B_n}\right)$$

where C_n is the concentration of metal (n) in the sediment samples and B_n denotes the geochemical background value of the respective metal (n) in the Earth's crust. In our study area, the background values for these metals are not available; hence, the B_n values as suggested by Taylor and McLennan (1985) were considered for computation, and a factor of 1.5 was introduced to recompense the background content due to various lithogenic effects. The pollution classification of I_{geo} based on its values

Table 1
Classification and grading for the values of contamination degree, potential ecological risk, and pollution load index.

Classification of contamination degrees (C_{deg})	
C_{deg} value	Degree of contamination
$C_{deg} < 4$	Low
$4 < C_{deg} < 8$	Moderate
$8 < C_{deg} < 16$	Considerable
$16 \geq C_{deg}$	Very high
Grade of Potential Ecological Risk (PER)	
PER value	Ecological risk
PER < 50	Low
50 ≤ PER < 100	Moderate
100 ≤ PER < 200	Considerable
PER ≥ 200	Very high
Pollution Load Index (PLI)	
PLI value	Level of pollution
PLI < 1	No pollution
PLI = 1	Baseline levels of pollution
PLI > 1	Polluted

(Nethaji et al., 2017) is as follows: $I_{\text{geo}} < 0$ signifies lack of pollution, 0–1 indicates no pollution to moderate pollution, 1–2 denotes moderate pollution, 2–3 represents moderate to strong pollution, 3–4 indicates strong pollution, 4–5 represents strong to extreme pollution, and $I_{\text{geo}} > 5$ denotes extreme levels of pollution.

2.4.2. Contamination factor and contamination degree

Hakanson (1980), in his work, has stated four grades for classifying the contamination factor (C_f^i) and contamination degree (C_{deg}) in order to assess the heavy metal pollution levels. The factor C_f^i were calculated using the formula:

$$C_f^i = \frac{C_s^i}{C_b^i}$$

where C_s^i represents the mean concentration of heavy metal i in the sample, and C_b^i is the reference concentration of heavy metal i, signifying the earth crust background (Taylor and McLennan, 1985). Following a modified method of Hakanson (1980), the degree of contamination for four heavy metals in sediment samples was determined as follows:

$$C_{deg} = \sum C_f^i$$

The classification for degrees of contamination suggested by Hakanson (1980) was based on eight elements, while we consider only four elements in this study. Thus, the original degree of contamination classification was altered accordingly and is presented in Table 1.

2.4.3. Pollution load index

The degree of heavy metal pollution present in sediments was evaluated by computing the pollution load index (PLI) as per the method set forth by (Tomlinson et al., 1980):

$$PLI = \left(C_f^1 \times C_f^2 \times C_f^3 \times \cdots C_f^i\right)^{1/i}$$

where i is the number of heavy metals, and C_f^i is the contamination. Here, we assess the pollution load index for four metals (Cu, Cr, Fe, and Zn). PLI can basically segregate the site quality by categorizing pollution levels into 3 groups (Table 1), viz., PLI < 1 meaning lack of pollution, PLI > 1 suggesting deterioration of site quality, and PLI = 1 denoting baseline levels of pollutants (Tomlinson et al., 1980).

2.4.4. Ecological risk assessment

The potential ecological risk (PER) of heavy metal contamination is quantitatively evaluated using potential ecological risk index (E_i) (Hakanson, 1980; Zhu et al., 2008), which is derived by multiplying contamination factor (C_f^i) and toxic response factor (T_i). The potential risk index can be calculated as follows:

$$E_i = T_i \times C_f^i$$

$$PER = \sum E_i$$

where E_i and PER symbolize the ecological risk index for a single element and potential ecological risk for multiple elements, respectively. T_i is the toxic response factor of each metal which is equal to 1 for Zn, 5 for Cu and Pb, and 30 for Cd (Xu et al., 2015). The degree of ecological risk can be categorized as follows (Xu et al., 2015): low for $E_i < 15$, moderate for $15 \leq E_i < 30$, considerable for $30 \leq E_i < 60$, high for $60 \leq E_i < 120$, and very high for $E_i \geq 120$. The grade of ecological risk can be classified as follows (Xu et al., 2015): low for PER < 50, moderate for 50 ≤ PER <100, considerable for 100 ≤ PER < 200, and high for PER ≥ 200 (Table 1).

2.5. Statistical description

The data obtained from the AAS analysis provided the essential information about the metal distribution in the water and sediments of Cuddalore coast. Data were processed using Microsoft Excel 365 (Windows 10) and presented as mean ± standard deviation. Two-way ANOVA was used to determine significant variation in heavy metal concentrations in water and sediment in view of different sampling sites and seasons at 0.05% levels. Principal component analysis (PCA) revealed the association in between the heavy metals based on the correlation matrix. To understand the correlation between the study sites, hierarchical cluster analysis (HCA) was applied. The results of PCA are expressed in biplot, and HCA is portrayed using a dendrogram. Statistical analyses were performed using PAST tool (version 3.0).

2.6. Quality assurance and quality control

The AA-7000 model spectrophotometer (make: Shimadzu, Japan) was used to analysis the whole experimentation of this study. For quality control and quality assurance, triplicate readings of the samples, blanks, and standardized reference materials were logged throughout the analysis. The standard metal solutions were procured from Merck Genei, Bangalore. All reagents used in the procedures were prepared with metal-free double-distilled water and chemicals of analytical grade. The standard curve was set up using a prepared standard with different concentrations and calibration using the WizAArd software (Windows 7) of the Shimadzu AA-7000 instrument. It was ensured that all the standard curves were having a regression coefficient (R^2) value higher than 0.99 for accurate readings. The instrument was rinsed with double-distilled water after each 10 samples and a blank reading was taken to confirm lack of contaminants. The working wavelengths were 324.75 nm, 228.80 nm, 217.00 nm, and 213.85 nm, for Cu, Cd, Pb, and Zn, respectively.

3. Results

3.1. Heavy metal concentrations in water and sediment samples

The concentration of heavy metals in water and sediment samples of all three stations for all seasons has varied seasonally as well as spatially as represented in Fig. 2. The metal concentrations observed in water and sediment samples are presented as

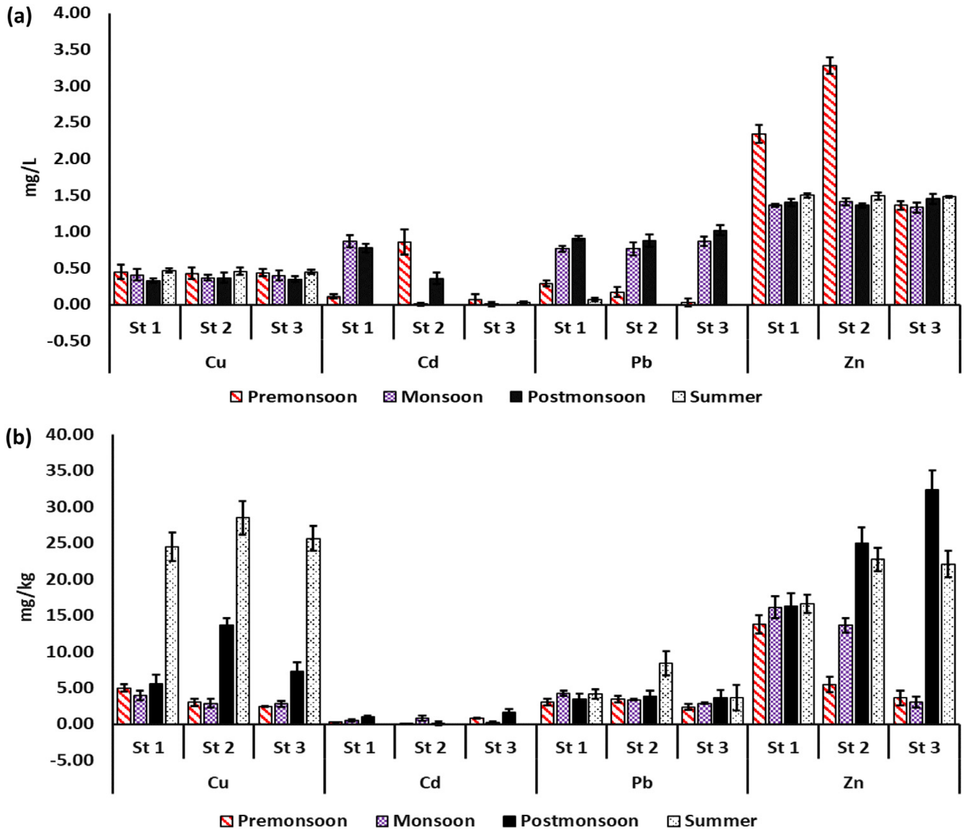

Fig. 2. Seasonal variation in metal concentration of water (a) and sediment (b) samples collected from Cuddalore coast.

mean ± standard deviation (SD) in Table 2, respectively. Overall, sediment samples have higher metal concentrations when compared with water samples. In water samples, the metal concentration follows the descending order of Zn > Pb > Cu > Cd. In sediments, the metal order is Zn > Cu > Pb > Cd. In water samples, seasonal variation of metals follows the order: premonsoon > postmonsoon > monsoon > summer. Whereas, in sediment samples, overall seasonal variation of metals follows the order: summer > postmonsoon > monsoon > premonsoon. The overall contamination for both water and sediment for the stations followed the descending order Station 2 > Station 1 > Station 3.

In the water samples, concentration of Cu ranged between 0.33 and 0.47 mg/L with a mean value of 0.41 mg/L. The maximum concentration was observed in Station 1 in the summer season, whereas the minimum concentration was observed in the same station in the postmonsoon season. The concentration of Cu across stations was more or less similar in all the three stations with only a meagre difference in the average concentration following the descending order Station 1 > Station 2 = Station 3. In sediment samples, concentration of Cu ranges between 2.43 and 28.49 mg/kg with a mean value of 10.43 mg/kg. The maximum concentration was observed in Station 2 in the summer season, whereas the minimum concentration was observed in Station 3 in the premonsoon season. The station-wise concentration of Cu follows the descending order Station 2 > Station 1 > Station 3.

The concentration of Cd obtained in water samples was between 0 and 0.87 mg/L with a mean value of 0.26 mg/L. The higher range of Cd was observed in Station 1 in the monsoon season. Cd levels were below detection limits in Station 3 during the postmonsoon season and in Stations 1 and 2 during the summer season. The station-wise concentration of Cd follows the descending order Station 1 > Station 2 > Station 3. In the sediment samples, concentration of Cd was in the range of 0 and 1.61 mg/kg with a mean value of 0.45 mg/kg. The highest concentration was observed in Station 3 in the postmonsoon season. Cd levels were below detection limits in all three stations during the summer season. The station-wise concentration of Cd follows the descending order Station 3 > Station 1 > Station 2.

The concentration of Pb in water samples was between 0 and 1.02 mg/L with an average of 0.48 mg/L. The highest concentration was observed in Station 3 in the postmonsoon season. Pb levels were below detection limits in Stations 2 and 3 during the summer season. The station-wise concentration of Pb follows the descending order Station 1 > Station 3 > Station 2. In the sediment samples, concentration of Pb was in the range of 2.37 and 8.40 mg/kg with a mean value of 3.87 mg/kg. The highest concentration was observed in Station 2 in the summer season, whereas lowest Pb level was in Station 3 in the premonsoon season. The station-wise concentration of Pb follows the descending order Station 2 > Station 1 > Station 3.

In water samples, the concentration of Zn fell in the range from 1.33 to 3.28 mg/L with a mean value of 1.65 mg/L. The highest concentration was observed in Station 2 in the premonsoon season. Zn level was least in Station 3 during the monsoon season. The station-wise concentration of Zn follows the descending order Station 2 > Station 1 > Station 3. In the sediment samples, concentration of Zn was in the range from 30.2 to 32.33 mg/kg with a mean value of 15.89 mg/kg. The highest concentration was observed in Station 3 in the postmonsoon season, whereas lowest Zn level was observed in Station 3 in the monsoon season. The station-wise concentration of Zn follows the descending order Station 2 > Station 1 > Station 3.

Table 2
Seasonal variations and two-way ANOVA results of heavy metal concentrations in water and sediment samples in the study area.

Element	Station	Season				ANOVA					
		Premonsoon	Monsoon	Postmonsoon	Summer	Factor	*df*	*SS*	*MS*	*F* value	*P* value
Water											
Cu	St-1	0.45 ± 0.10	0.41 ± 0.08	0.33 ± 0.03	0.47 ± 0.03	Station	2	0.000	0.000	0.179	0.840
	St-2	0.43 ± 0.08	0.37 ± 0.04	0.37 ± 0.07	0.46 ± 0.05	Season	3	0.022	0.007	22.385	0.001
	St-3	0.44 ± 0.05	0.4 ± 0.07	0.35 ± 0.04	0.45 ± 0.03						
Cd	St-1	0.11 ± 0.03	0.87 ± 0.08	0.78 ± 0.06	ND	Station	2	0.355	0.177	1.268	0.347
	St-2	0.86 ± 0.17	0.01 ± 0.02	0.36 ± 0.08	ND	Season	3	0.257	0.086	0.613	0.631
	St-3	0.07 ± 0.07	0.01 ± 0.03	ND	0.03 ± 0.02						
Pb	St-1	0.29 ± 0.04	0.77 ± 0.04	0.91 ± 0.03	0.07 ± 0.02	Station	2	0.006	0.003	0.374	0.703
	St-2	0.17 ± 0.07	0.77 ± 0.09	0.88 ± 0.08	ND	Season	3	1.866	0.622	76.778	0.000
	St-3	0.03 ± 0.05	0.87 ± 0.06	1.02 ± 0.07	ND						
Zn	St-1	2.34 ± 0.12	1.36 ± 0.02	1.4 ± 0.05	1.5 ± 0.03	Station	2	0.461	0.230	0.995	0.424
	St-2	3.28 ± 0.11	1.41 ± 0.05	1.36 ± 0.03	1.49 ± 0.05	Season	3	1.874	0.625	2.696	0.139
	St-3	1.36 ± 0.06	1.33 ± 0.07	1.45 ± 0.07	1.48 ± 0.01						
Sediment											
Cu	St-1	5.01 ± 0.51	3.93 ± 0.67	5.61 ± 1.22	24.47 ± 1.96	Station	2	14.819	7.410	1.303	0.339
	St-2	2.99 ± 0.54	2.87 ± 0.6	13.64 ± 0.95	28.49 ± 2.31	Season	3	1055.35	351.784	61.870	0.000
	St-3	2.43 ± 0.06	2.81 ± 0.38	7.28 ± 1.23	25.64 ± 1.69						
Cd	St-1	0.26 ± 0.02	0.51 ± 0.16	1.00 ± 0.21	ND	Station	2	0.353	0.176	0.896	0.456
	St-2	0.04 ± 0.03	0.77 ± 0.36	0.14 ± 0.25	ND	Season	3	1.292	0.431	2.187	0.190
	St-3	0.79 ± 0.03	0.23 ± 0.13	1.61 ± 0.45	ND						
Pb	St-1	3.07 ± 0.44	4.26 ± 0.39	3.4 ± 0.85	4.14 ± 0.69	Station	2	5.348	2.674	1.604	0.277
	St-2	3.44 ± 0.44	3.35 ± 0.10	3.8 ± 0.81	8.4 ± 1.67	Season	3	10.064	3.355	2.012	0.214
	St-3	2.37 ± 0.39	2.87 ± 0.13	3.65 ± 1.07	3.64 ± 1.81						
Zn	St-1	13.79 ± 1.26	16.14 ± 1.51	16.3 ± 1.81	16.62 ± 1.27	Station	2	4.233	2.116	0.042	0.959
	St-2	5.44 ± 1.06	13.62 ± 1.03	24.97 ± 2.15	22.72 ± 1.61	Season	3	566.997	188.999	3.742	0.079
	St-3	3.6 ± 1.00	3.02 ± 0.75	32.33 ± 2.78	22.11 ± 1.86						

SS — sum of squares, *MS* — mean square, *df* — degrees of freedom, significance level 0.05, ND — not detected/below detection limit.
Metal concentration data are presented as mean ± standard deviation in mg/L for water and in mg/kg (dry weight) for sediment.

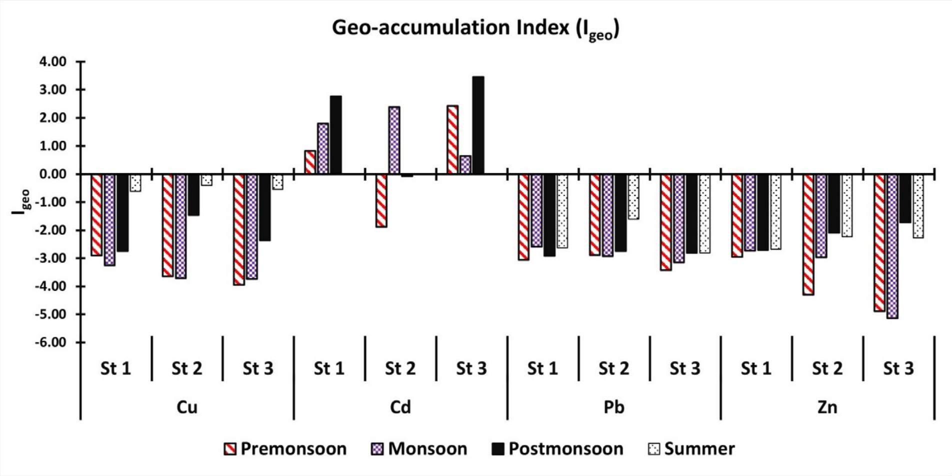

Fig. 3. Geo-accumulation index for four metals (Cu, Cd, Pb, and Zn) in different stations across the seasons.

3.2. *Risk levels of toxic metals*

3.2.1. *Geo-accumulation index (I_{geo})*

Geo-accumulation index values were determined based on the observed metal concentrations in sediments with reference to the background values. The results of geo-accumulation index are presented in **S1** which indicate that all the metals fall between the classes of 0 and 4. The geo-accumulation index is also represented as a bar graph in Fig. 3. The reported levels of Cu, Pb, and Zn fall under unpolluted Class 0 in all the stations for all seasons. Only Cd levels fall in the classes 1–4 depicting the sites are moderately to strongly contaminated in the premonsoon, monsoon, and post monsoon seasons. Cd contamination is absent in the summer season. The highest I_{geo} value (3.45, strongly contaminated) was observed for Cd in Station 3 in the postmonsoon season.

3.2.2. *Contamination factor and contamination degree (C_{deg})*

The contamination factor and degree are classified into four classes each starting from Class 1 representing low contamination to Class 4 signifying very high contamination. Station-wise contamination factor and contamination grades for the different elements across four seasons are tabulated in **S2**. Overall, the contamination factors for all the metals are from Class 1–4 ranging from low–very high contamination. The contamination factors for the different metals across all seasons for the different stations fall in the ranges of Cu, 0.10–1.14; Cd, 0–16.43; Pb, 0.14–0.49; and Zn, 0.04–0.46. The highest contamination factor was detected for

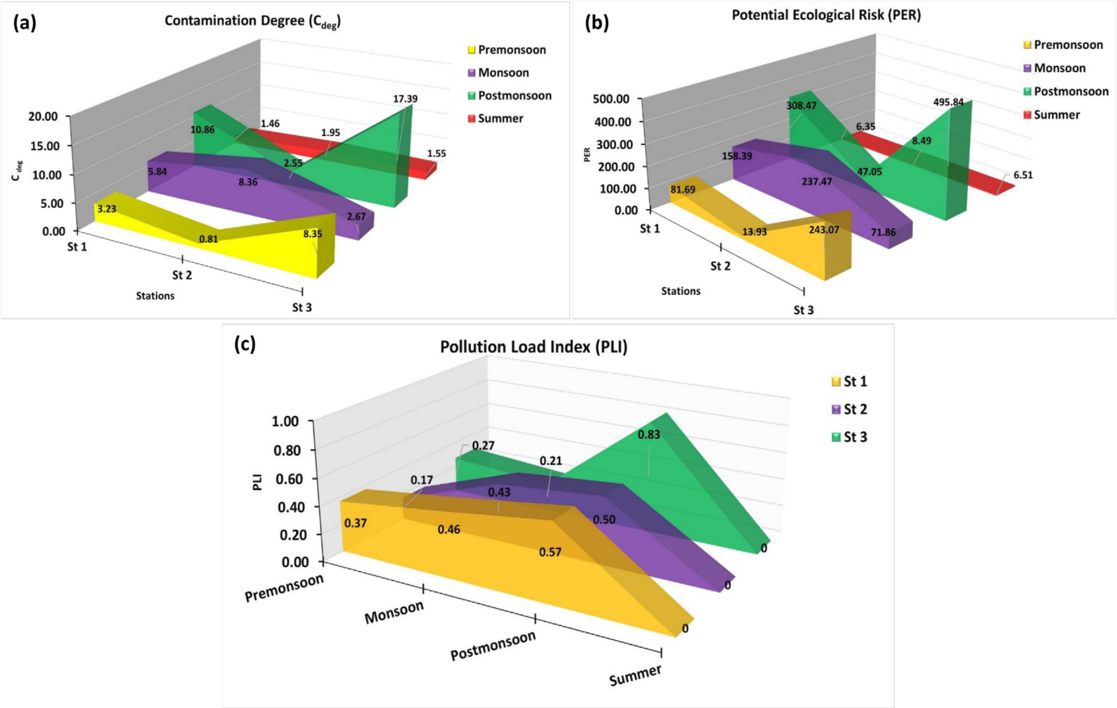

Fig. 4. Contamination degree (a), potential ecological risk (b), and pollution load index (c) across different stations and seasons.

Table 3
Station-wise contamination degree, potential ecological risk, and pollution load index for four seasons.

Station	Seasons			
	Premonsoon	Monsoon	Postmonsoon	Summer
Contamination degree (C_{deg})				
St 1	3.23	5.84	10.86	1.46
St 2	0.81	8.36	2.55	1.95
St 3	8.35	2.67	17.39	1.55
Potential ecological risk (PER)				
St 1	81.69	158.39	308.47	6.35
St 2	13.93	237.47	47.05	8.49
St 3	243.07	71.86	495.84	6.51
Pollution load index (PLI)				
St 1	0.37	0.46	0.57	0.00
St 2	0.17	0.43	0.50	0.00
St 3	0.27	0.21	0.83	0.00

Cd in Station 3 during the postmonsoon season. The station-wise contamination degree (C_{deg}) for all metals across the four seasons is tabulated in Table 3 and represented as a graph in Fig. 4(a). Overall, the contamination degrees for all the metals also fall in the classes 1–4 ranging from low–very high contamination. The overall contamination degree for all stations across the seasons fall in the range of 0.81–17.39. The highest contamination degree was detected in Station 3 during the postmonsoon season. The lowest value of contamination degree was observed in Station 2 during the premonsoon season.

3.2.3. Pollution load index

Pollution load index (PLI) was computed using the assessed contamination factors to find out the joint contamination effect from different locations by various elements across different seasons. The PLI values are shown in Table 3 and Fig. 4(c). All values of PLI for the different sites across the four seasons are less than 1 indicating that there is no considerable pollution in the sites. The range of the pollution load index is from 0 to 0.83.

3.2.4. Ecological risk assessment

Ecological risk index (E_i) and potential ecological risk (PER) were applied to determine the risk level of metals present in the study area across different seasons, and the results of ecological risk index are shown in **S3**. The ecological risk index for the elements falls in the following ranges: Cu, 0.49–5.70; Cd, 0–492.86; Pb, 0.70–2.47; and Zn, 0.04–0.46. The ecological risk ranges from low to very high. The highest ecological risk index was for Cd in Station 3 during the postmonsoon season. PER was computer from the E_i values, and the PER values are tabulated in Table 3 and graphically illustrated in Fig. 4(b). PER values ranged from 6.35 to 495.84. The highest PER value was reported for Cd in Station 3 in the postmonsoon season. The average potential ecological risk is very high for Station 3 (204.32), followed by considerable risk for Station 1 (138.72), and a moderate risk for Station 2 (76.73).

3.3. Statistical approaches

3.3.1. Two-way ANOVA

Two-way ANOVA results reveal that metal concentrations in all water samples of different stations does not differ significantly at 5% significance. In water samples, there is no significant difference with respect to the seasons for Cd and Zn at 5% significance. However, there is a significant difference in the concentrations of Cu and Pb in water samples at 5% significance level with respect to season. Two-way ANOVA results for water samples are tabulated in Table 2. In sediment, two-way ANOVA results reveal that metal concentrations of different stations do not differ at 5% significance. There is no difference with respect to the seasons as far as a Cd, Pb, and Zn are concerned at 5% significance. Nevertheless, a significant difference in the levels of Cu was noted in sediments at 5% significance with respect to season. Two-way ANOVA results for sediment samples are tabulated in Table 2.

3.3.2. Hierarchical cluster analysis (HCA)

The interrelationship between sampling locations based on metal concentrations in water and sediment samples for different

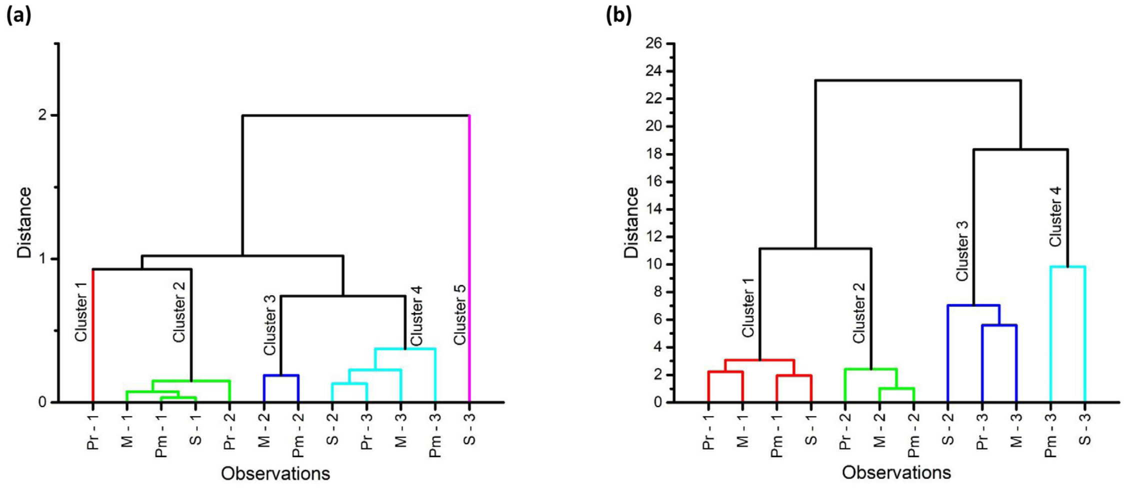

Fig. 5. Dendrogram showing the interrelationship of heavy metals in samples. Pr (1–3) refer to samples from premonsoon in stations 1–3, M (1–3) refer to samples from monsoon in stations 1–3, Pm (1–3) refer to samples from postmonsoon in stations 1–3, and S (1–3) refer to samples from summer in stations 1–3. (a) Water samples and (b) sediment samples.

Table 4
Loading, eigenvalues, and parameters of four heavy metals on principle components (PCs) for water and sediment samples.

	Water	
	PC1	PC2
Cu	**0.659**	−0.061
Cd	−0.214	**0.728**
Pb	−0.661	0.001
Zn	0.288	**0.683**
Eigenvalue	2.124	1.390
Variance (%)	53.11%	34.75%
Cumulative variance	53.11%	87.85%
	Sediment	
	PC1	PC2
Cu	**0.630**	−0.064
Cd	−0.327	**0.745**
Pb	0.578	0.031
Zn	0.402	**0.663**
Eigenvalue	2.212	1.262
Variance (%)	55.31%	31.55%
Cumulative variance	55.31%	86.86%

Values in bold represent strong positive loading.

seasons and their outcome is illustrated in Fig. 5(a) and (b), respectively. The dendrogram was obtained based on the interrelationship of the dataset. Accordingly, sampling locations were separated into the following clusters: Clusters 1—5 for water samples and Cluster 1–4 for sediment samples, which are mostly connected by the different anthropogenic influences across different seasons.

3.3.3. Principal component analysis (PCA)

Considering the interrelationship between metals, seasons, and stations, four distinctive principal components were extracted (PC1, PC2, PC3, and PC4) with eigenvalues (2.124, 1.390, 0.391, and 0.095) for water samples. The percentages of variance for the four factors are 53.11%, 34.75%, 9.78%, and 2.37%. Among the four principal components based on the eigenvalues, PC1 and PC2 alone (Fig. 6(a)) are considered for further investigation (Rajaram et al., 2017), which show the eigenvalues > 1 with the cumulative variance of 87.85% (Table 4). The third and fourth principal components are suggestively (eigenvalue < 1) less significant, explaining only 9.78% and 2.37% of the variance, respectively. For the sediment samples, to portray the interrelationship between metals, seasons, and stations, four distinctive principal components were obtained (PC1, PC2, PC3, and PC4) with eigenvalues (2.212, 1.262, 0.392, and 0.134). The percentages of variance for the four factors are 55.31%, 31.55%, 9.80%, and 3.34%. Among the four principal components based on the eigenvalues, only PC1 and PC2 (Fig. 6(b)) are considered (Rajaram et al., 2017), which show the eigenvalues > 1 with the cumulative variance of 86.86% (Table 4). These positive factors in PCA show that the sediments are impacted by the close proximity of parameters that are significantly stacked with the particular factor. Also, negative scores are suggestive that sediment prevalence is unaffected by this parameter. PC3 and PC4 are suggestively of (eigenvalue < 1) lower significance with only 9.80% and 3.34% of the variance, respectively.

4. Discussion

4.1. Heavy metal concentrations in water and sediment samples

In our study area, metal concentrations are higher in sediment compared to water in all stations for all seasons since sediments are a combination of organic materials and minerals having charged surfaces and the ability to absorb dissolved heavy metals existent in the ecosystem (Langston, 1986). The heavy metal levels in sediment are normally higher than in water for the reason that heavy metals have the ability to settle, precipitate, accumulate, and attach strongly to sediments (Schertzinger et al., 2018), and this may well explain the higher heavy metal loads in our study area. It is clear from the results that the water column is influenced by higher metal levels during the monsoon seasons due to the excessive runoff of the waters due to the rainwater which flush all the heavy metals into the rivers (Hossain et al., 2020). The higher concentrations of metals in the sediments are in the summer season, representing that the interaction between the water and the sediment, in the lack of any influx of freshwater, causes the muddy sediments to deposit along the river mouth areas (Pande and Nayak, 2013).

Cu concentrations in the water samples across all seasons were within the permissible limits (Table 5) as prescribed by the world health organization (WHO) (Arumugam et al., 2018); however, the concentrations exceeded the permissible limits for sediment samples in the summer season in all stations. The values of Cu are considerably lower than the values reported in the coast

Table 5
Permissible limits of heavy metals in water (mg/L) and sediment (mg/kg) as proposed by international agencies.

International agencies	Cd	Cu	Pb	Zn	Reference
Permissible limits for water					
Aquatic life	0.002	0.007	0.01	0.086	Arumugam et al. (2018)
US-EPA Aquatic life protection	0.005	0.004	0.014	3.6	Arumugam et al. (2018)
WHO	0.003	2.00	0.01	3.00	WHO (1989)
Permissible limits for sediment					
CEQG 2001	0.70	18.70	30.20	124.00	CEQG (2001)

US-EPA, US Environmental Production Agency; WHO, World Health Organization; CEQG, Canadian Environmental Quality Guidelines.

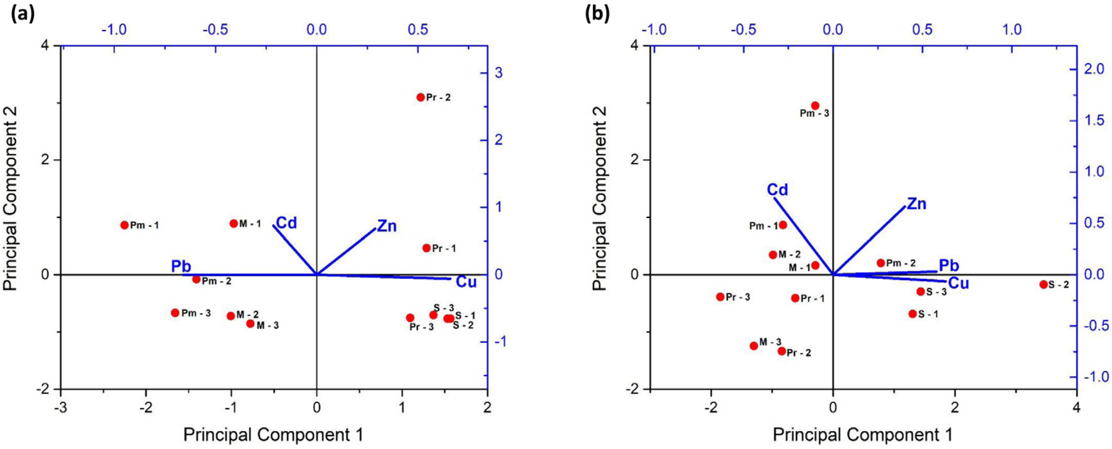

Fig. 6. Biplot showing the distribution of heavy metals in water and sediment samples (Pr — premonsoon; M — monsoon; Pm — postmonsoon; S — summer; and numerals 1–3 represent stations 1–3, respectively). (a) Water and (b) sediment samples collected from the Cuddalore coast.

of Tamil Nadu (Kayalvizhi et al., 2013; Arumugam et al., 2018; Rajaram et al., 2021). The global comparison of the concentrations of heavy metals with the present study is shown in Table 6. In sediment samples, the highest level of Cu was recorded during summer season and the lowest level during premonsoon season which agrees with a similar trend observed in the study area in an earlier report by Rajaram et al. (2021). In Cuddalore coast, overall Zn concentrations are higher in both water and sediment samples compared with the other three metals. The concentration of Zn shows a mixed response from geogenic and anthropogenic sources. The higher concentration of Zn is observed in the midstream of the river near Station 2, which indicates release of wastes from the SIPCOT industrial zone located in the area (Gopal et al., 2018). Nevertheless, the concentrations of Zn do not exceed the WHO permissible limits in both water and sediment samples, except in the water sample during the premonsoon season in Station 2. Cu and Zn are essential metals that play crucial roles in the subsistence of both terrestrial and aquatic life, but exceeding levels of these elements can be harmful to life (Singare et al., 2012). Cu and Zn could have a mixed anthropogenic and lithogenic sources. The even distribution of Cu in all stations in the water column could point to a geogenic source. The higher concentration of Cu in Station 3 might be because of the variations in the sediment depositional characteristics. Stations 1 and 2 are in the upstream of the river and have mixed influences of river water and tidal intrusions, whereas Station 3 lies in the estuary comprising of more boating activities. Increased movements of boats in the estuarine waters may be a noteworthy source of heavy metals because of the use of Zn in battery anodes and Cu in antifouling paints (Schiff et al., 2004; Boyle et al., 2016). As per Rajaram and Ganeshkumar (2019), a significant level of copper emission was observed from antifouling paint used to coat recreational vessels for controlling fouling organisms.

Cd concentrations in the water samples from all three stations across the premonsoon, monsoon, and postmonsoon seasons were mostly higher than the permissible limits of WHO (WHO, 1989). The Cd levels were consistent with an earlier study in this region by Dhinesh et al. (2014). The concentrations of Cd in water were below permissible limits in the summer season in Stations 1 and 2 but was in the baseline limit in Station 3. In the sediment samples, Cd levels marginally exceeded the permissible limit in Station 3 in premonsoon and in Station 2 in the monsoon season. The Cd limits were higher than the permissible values in Stations 1 and 3 during the postmonsoon season; however, Cd levels were not detected in the summer season in the sediments. The increased Cd levels in the monsoon and postmonsoon seasons indicate that Cd is mainly sourced from the plastic manufacturing industries (Mathivanan and Rajaram, 2014) and other anthropogenic activities which dump their wastes into the Uppanar river during the monsoon when the water flow is more in the river. Cd concentrations in the water samples from all three stations across the premonsoon, monsoon, and postmonsoon seasons were mostly higher than the permissible limits of WHO (WHO, 1989). Pb levels in the water samples from all three stations across seasons were mostly higher than the permissible limits of WHO (WHO, 1989) with the exception of summer season where Pb was not detected in Stations 2 and 3. In the sediment samples, Pb concentrations were all below permissible limits in all stations in all seasons. High Pb concentrations in the sediments were observed in the upstream and midstream part of the Uppanar estuary, which may possibly be due to the influence of industrial effluents persistent in the area. Other feasible sources such as fertilizers, pesticides, atmospheric fall out through fuel combustion, lead-based paints, and sewage may also contribute Pb to the aquatic environment (Mathivanan and Rajaram, 2014). Higher concentrations of Cd and Pb, which are non-essential metals, are observed more in the water during

Table 6
Comparison of the metal concentrations obtained in present study with the global data.

Study area	Cd	Cu	Pb	Zn	Reference
Water (mg/L)					
Cuddalore, India	0.26	0.41	0.48	1.65	*Present study*
Cuddalore Coast, India	0.25	0.29	0.38	0.37	Dhinesh et al. (2014)
Cuddalore, India	0.83	5.01	1.36	13.40	Kayalvizhi et al. (2013)
Tuticorin coast, India	0.84	3.73	20.98	3.75	Rajaram et al. (2021)
Muthupet mangrove, India	0.69	1.66	10.92	2.88	Arumugam et al. (2018)
Yalujiang estuary, China	1.18	2.86	0.68	14.93	Li et al. (2017)
Sheyang estuary, China	0.12	3.12	0.55	9.29	Zhao et al. (2018)
Iture estuary, Central Region of Ghana	0.03	–	0.04	1.91	Fianko et al. (2007)
Adyar estuary, Chennai, India	–	4.00	5.10	6.90	Rubalingeswari et al. (2021)
Romanian sector of the Black Sea - 2011	0.41	20.26	8.05	–	Jitar et al. (2015)
Romanian sector of the Black Sea - 2012	2.72	3.06	4.03	–	Jitar et al. (2015)
Daya Bay, South China	0.12	2.40	0.70	10.40	Qiu (2015)
Ennore creek, Chennai, India	ND	3.53	13.89	14.50	Jayaprakash et al. (2015)
South coast of Sfax, Mediterranean Sea	0.10	3.27	2.72	4.59	Ben Salem and Ayadi (2016)
Port Klang, Selangor, Malaysia	0.51	2.59	4.19	53.97	Sany et al. (2012)
Bie′tri Bay in Ebrie′ Lagoon, Ivory Coast	0.10	9.43	3.53	17.04	Coulibaly et al. (2012)
Sediment (mg/kg)					
Cuddalore, India	0.45	10.43	3.87	15.89	*Present Study*
Pearl River estuary, South China	–	40.90	59.50	115.00	Li et al. (2000)
Cuddalore Coast, Southeastern India	0.83	5.84	25.36	6.30	Dhinesh et al. (2014)
Tuticorin coast, India	0.12	15.89	14.64	41.19	Rajaram et al. (2021)
Muthupet, Southern India	0.29	13.47	13.23	27.96	Arumugam et al. (2018)
Yalujiang estuary, China	0.20	9.37	17.95	37.86	Li et al. (2017)
Sheyang estuary, China	0.15	23.51	16.87	62.16	Zhao et al. (2018)
Musa estuary, Persian Gulf	0.38	14.60	4.19	–	Abdolahpur Monikh et al. (2013)
Sangu River estuary, Bangladesh	ND	29.24	19.58	88.97	Hossain et al. (2019)
Adyar estuary, southeast coast of India	–	332.60	77.40	335.50	Rubalingeswari et al. (2021)
Daya Bay, South China	0.28	30.60	52.70	110.40	Qiu (2015)
Ennore creek, Chennai	0.51	102.00	32.00	155.00	Jayaprakash et al. (2015)
Romaniansector of the Black Sea - 2011	1.20	26.68	11.59	–	Jitar et al. (2015)
Romaniansector of the Black Sea - 2012	0.90	17.76	8.42	–	Jitar et al. (2015)
Han river estuary, South Korea	0.22	29.70	35.10	126.00	Kim et al. (2011)
Mediterranean Sea	6.53	13.94	98.15	225.20	Ben Salem and Ayadi (2016)
Port Klang coastal area, Selangor, Malaysia	0.85	18.58	60.64	54.49	Sany et al. (2012)
Bie′tri Bay in Ebrie′ Lagoon, Ivory Coast	0.58	42.15	58.47	187.58	Coulibaly et al. (2012)

Values are expressed as mean.

the monsoon and postmonsoon seasons which is indicative of their anthropogenic sources. Risk level of Cd is more prominent compared with other metals followed by Pb, and the increased levels of Cd and Pb affirm that the coastal ecosystem here is affected by various anthropogenic activities.

4.2. Pollution indices and risk of toxic metals

Geo-accumulation index clearly portrays the harmful levels of Cd that are polluting the Cuddalore coast. The I_{geo} values for all metals except Cd fall below zero (Figure 3 and S1) and denote lesser degree of contamination because of these metals. But the alarming levels of Cd in the premonsoon, monsoon, and postmonsoon season are indicative of excessive Cd pollution persistent in the Cuddalore coast mainly due to the industrial wastes that are dumped into the coastal waters. The fact that the I_{geo} value for Cd is highest in Station 3 which is the river mouth area is suggestive that the metal loads are brought downstream and distributed more in the river mouth areas as suggested by Gopal et al. (2018). Cd poses the highest threat to the environment compared with other three metals in the Cuddalore coast. This vast metal contamination is mainly sourced from multiple stimulants from the wide-ranging industrial activities and inappropriate transfer of metal-containing fluid wastes into the environment. An analogous effect was explained by Cevik et al. (2009). Affirming the geo-accumulation index results, contamination factor also points out the higher contamination levels for Cd compared with other metals. After Cd, Cu levels pose a marginal moderate level of risk during the summer season. The contamination degree and potential ecological risk together denote higher contamination levels in Station 3 near the estuary mouth, and the C_{deg} and PER values are highest for the postmonsoon season (Fig. 4(a) and Table 3). This could be explained by the excessive loads of metals that are washed downstream and deposited in the river mouth areas. PLI is below 0 for all instances indicative of lack of pollution (Fig. 4(c)). However, the fact that we have taken only four metals here for our study could under value the PLI values. Still, the highest observed PLI value (0.83) is almost very close to achieve the baseline pollution mark (1). The PLI values can increase and point to a polluted status in the near future, with additional elements being assessed for computation of PLI.

4.3. Sources and risk level of toxic metals

Cluster analysis identifies sampling stations that have a similar geochemical profile. The cluster dendrogram (Fig. 5) establishes that there exist periodic and spatial differences in heavy metal concentrations for both water and sediments. Five distinct clusters were identified for water, where Clusters 1 and 2 represent the upstream stations, which have an analogous geochemical profile owing to the impact of anthropogenic activities (agricultural activities), whereas Clusters 3 shows the midstream region during the monsoon and postmonsoon season where the pollution level is higher for water due to the excessive effluents from the adjoining SIPCOT industries. Clusters 4 and 5 denote the stations which are located in the downstream region where all the water finally confluences with the sea. For sediment, we have obtained 4 clusters where Cluster 1 clearly distinguishes Station 1 with the similar geochemical profile for all seasons. Cluster 2 comprises Station 2 for all seasons except summer. Clusters 3 and 4 represent the Station 3 where the metal loads are high due to the higher deposition of metal-rich sediments. Cluster analysis shows the influence of freshwater in the monsoon seasons and also the spatial variations with respect to the various anthropogenic sources and geo-chemical profiles.

Factor analysis by PCA alters a large set of correlated data into a lesser number of uncorrelated data, which is termed as the principal component. While considering the two principal components (PC1 and PC2) for water samples, we can see a strong positive loading for Cu and a strong negative loading for Pb in PC1 (Table 4). This indicates that it may be a combination of natural and anthropogenic sources (Rajaram et al., 2017). In Cuddalore coast, explicitly SIPCOT industrial zone and other copper-based industries play significant roles in the raised degree of Cu and Zn in the water samples (Rajaram et al., 2021). In PC2, we see strong positive loadings for Cd and Zn (Table 4). This accounts for the external sources of the pollutants from the industrial and agricultural activities in the Cuddalore district. Coming to the principal components considered for the sediment samples, we considered only PC1 and PC2 as they have eigenvalues greater than 1 like the water component. In PC1, there exists a strong positive loading for Cu followed by a moderate loading for Zn and Pb (Table 4). The loadings point to a mixed source of geogenic and anthropogenic activities. In PC2, there is a strong positive loading observed for Cd and Zn signifying sources from anthropogenic activities.

5. Conclusion

Cuddalore, located in the Southeast coast of India, has been exposed to various industrial, agricultural, and tourism activities. We have detected elevated metal concentrations in water and sediments of Cuddalore corresponding to the discharge of industrial and urban wastes into the Uppanar and Gadilam rivers. This study clearly illustrates that the coastal ecosystem in Cuddalore obtains heavy metals by the anthropogenic activities which has been endorsed by PCA and seasonal influence of heavy metal concentrations identified by HCA. The main anthropogenic activities in the study area include disposal of waste from industrial zones, fertilizer and pesticide runoff from agriculture activities, fisheries activity, and boat and harbour activities. The Cuddalore coast was influenced by freshwater during the monsoon, when the estuarine water was flushed with the metal loads which accumulated into the sediments during dry period in the mouth of the river. In addition, with respect to the risk assessment and pollution indices, Cd was found to pose a higher level of risk to the ecosystem compared with the other metals. Cu accounted for a moderate pollution only in the summer season. The origin of these heavy metals can be sourced to the industrial zones in Cuddalore coast. Furthermore, fishing and boat activities and agricultural wastes add to the metal pollution. In summary, this study presents a seasonal data for the monitoring of heavy metal pollution in the Cuddalore coast. However, continuous monitoring and care should be taken to reduce the threat to the ecosystem arising from heavy metal pollution.

CRediT authorship contribution statement

Anbazhagan Vinothkannan: Conceived and designed the experiments, Collected the samples and carried out the instrumental analysis, and wrote the paper. **Partheeban Emmanuel Charles:** Involved in data analysis, and language editing. **Rajendran Rajaram:** Conceived and designed the experiments, Collected the samples and carried out the instrumental analysis, Wrote the paper, and language editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.rsma.2021.102134.

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Marine Pollution Bulletin 176 (2022) 113456

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

Metal-associated human health risk assessment due to consumption of pelagic and benthic ichthyofaunal resources from the highly contaminated Cuddalore coast in Southern India

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ARTICLE INFO

Keywords:
Biomonitoring
Marine fish
Metal
Hazard index
Bay of Bengal

ABSTRACT

We present seasonal variation of four metals (Cd, Cu, Pb, and Zn) in nine pelagic and three benthic fish species from the highly polluted Cuddalore coast in Tamil Nadu, India. Metals were assessed using atomic absorption spectrometry and detected in all fish species, in at least one season, except *Iniistius cyanifrons* where cadmium was not detected throughout. In both benthic and pelagic fish, order of metal concentration was Zn > Cu > Pb > Cd. Multivariate statistical analysis revealed that metals may have originated from both natural and anthropogenic sources. Health risk assessment revealed that consumption of fish from Cuddalore coast does not pose health risk for now; however, hazard index values (pelagic = 0.97; benthic = 0.90) are in borderline. Even a slight increase in metal concentration in fish can prove hazardous for human consumption. Sooner or later, eating fish from Cuddalore coast may pose a considerable health risk to humans if metal pollution is not held at Bay.

Marine fish are considered to be a key source of protein in the human diet. Contamination of fish with metals has become a major source of concern, not only because of the risk to health of the fish, but also to humans who consume these metal-contaminated marine fish (Gu et al., 2016). Toxic metal concentrations in the marine ecosystem can pose a health risk to fish. Metal pollution is mainly sourced from agricultural runoff, sewage effluents, industrial discharge, toxic waste dumps, and fuel from boats (Mishra et al., 2007; Satheeshkumar and Senthilkumar, 2011). Various contaminants, including harmful elements and biomolecules, have been released into the coastline of Southern India by several metal-based industries (Anandkumar et al., 2018; Mathivanan and Rajaram, 2013). The progressive levels of metals seem to have a harmful effect on the food chain because of the biomagnification from the primary producers to the consumers (Heng et al., 2004). Several researchers have explored the accumulation of metals and their adverse effects on seawater, sediments, and biological species in the last few decades (Arumugam et al., 2018a; Kumar et al., 2017). Metals, from both anthropogenic and natural inputs, are considered to be prominent contaminants in the environment since they are easily absorbed and rapidly deposited in organisms, posing a serious hazard to public health through consumption of metal-contaminated food (Copat et al., 2013). The main route for metals entry into fish tissues is through adsorption and absorption. Metal deposits in body tissues are mainly from the absorption process through gills, kidneys, liver, and especially the digestive tract (Annabi et al., 2013). Metals reach the marine food chain in two major ways including direct water consumption and food intake via the gastrointestinal tract, and non-dietary routes, such as muscle and gills, via osmoregulation (Ribeiro et al., 2005). Humans require essential metals like iron (Fe), cobalt (Co), and manganese (Mn) for a number of physiological and metabolic processes. Further, metals toxic to humans including mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr), and nickel (Ni) can cause dermatitis, infertility, lung damage, coronary and renal failure, also pulmonary and sinus carcinoma (Renieri et al., 2014).

Biomarkers are biological indicators that act as early warning signs (Annabi et al., 2013). Fish occupy top positions in the marine food chain and are vulnerable to accumulation of metals through their diet and the surrounding medium water and sediments (Yılmaz et al., 2007; Zhao et al., 2012); hence, they can act as good biomarkers (Rahman et al., 2012; Rajaram and Devendran, 2013). In comparison to other pathways like respiratory and dermal exposure, food consuming is the

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https://doi.org/10.1016/j.marpolbul.2022.113456
Received 16 December 2021; Received in revised form 8 February 2022; Accepted 9 February 2022

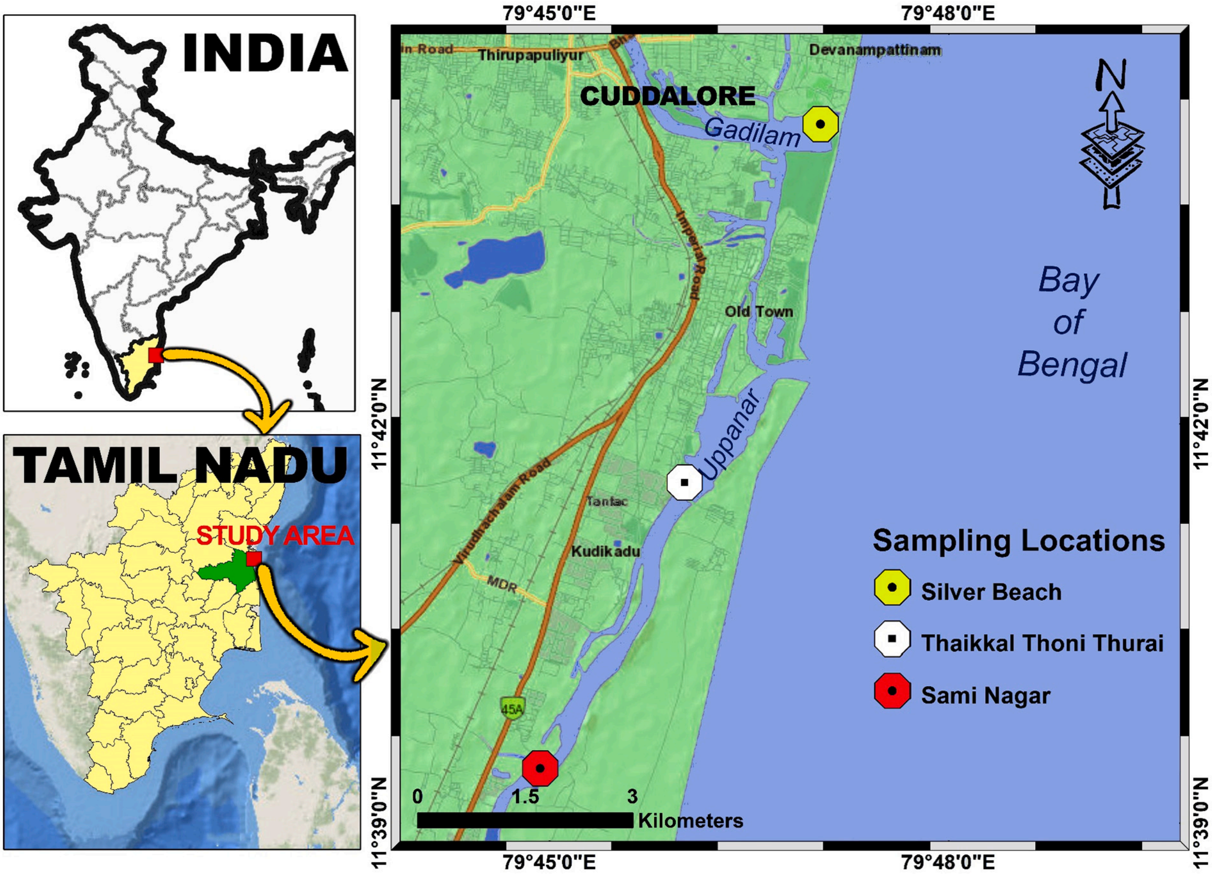

Fig. 1. Map of the sampling locations along Cuddalore coast in Southeast India.

predominant pathway which constitutes about 90% for human exposure to metals. Fish is a commonly consumed food and a primary source of protein in many fishing towns. It helps to maintain a healthy lifestyle by delivering important omega-3 fatty acids, amino acids, and nutrients. Consumption of fish is indeed beneficial for preventing arrhythmia and thrombosis, as well as decreasing bad cholesterol (Korkmaz et al., 2019). In opposition to the advantages mentioned earlier, aquatic species play a significant role in the transmission of contaminants to humans (Ferrante et al., 2018). While fish is rich in many nutrients, consuming too much of it can have health implications due to bio-accumulated metals that exceed permitted limits (Rahman et al., 2012). Renal problems, bone damage, neurological issues, reproductive problems, cardiac failure, and cancer impacts are among the health hazards posed by metal toxicity (Renieri et al., 2019). Several metals can bond to the sulphur found in enzymes, causing them to dysfunction (Ali and Khan, 2018).

Due to rapid urbanisation and industrial activities in and around Cuddalore city, mainly due to the runoff from SIPCOT (Small Industrial Promotion Corporation of Tamil Nadu, with 52 industries spread over 520 acres) industrial complex, the Uppanar River that flows through the Cuddalore Town in Tamil Nadu is recognized among the most polluted waterways on India's southeast coast. Evaluation of harmful compounds from the industrial sources in Cuddalore coastal waters would cover a wide range of potential environmental pollutants. Higher levels of pollutants in these waters may be a contributing factor for the regular death of fish and degradation of aquatic habitats in this area. The frequent release of untreated or partially treated industrial effluent into the coastal environment has an impact on both the biotic and abiotic systems, and eventually gives way to negative consequences for humans via the food chain (Damodharan, 2013). In this context, the main objectives of this study are to: (1) provide baseline data on levels of Cd, Cu, Pb, and Zn in the muscles of the ichthyofaunal resources, and (2) to evaluate the possible risk to human health associated with consumption of these fish.

Cuddalore (11°43′N, 79°49′E) is a coastal town located in the state of Tamil Nadu in South India. Cuddalore has an average elevation of 6 m (20 ft), and the land is completely flat with large black and alluvial soil inlands and coarse sand near the seashore. Three urban rivers including Uppanar in the south, and Ponnair, and Gadilam in the north converge to form an estuary (11°42′N, 79°46′E) and drain into the Bay of Bengal in the Cuddalore coast. The Uppanar Estuary runs behind the SIPCOT complex located at Cuddalore. It originates from the northeastern part of the Shervarayan hills and empties into the Bay of Bengal near Cuddalore. SIPCOT complex houses industries from various sectors including manufacturing, petrochemical, pharmaceutical, biocides, fertiliser, fungicides, chlor-alkali, and metal processing, etc. The Uppanar Estuary gets its pollution load from the industrial complex. Additionally, urban wastes, agricultural runoff, and domestic sewage from Cuddalore's Old Town are discharged into the estuary (Mathivanan and Rajaram, 2013). The Uppanar Estuary is roughly 3.5-m deep near the mouth and 2.5-m deep in the upstream section and serves as a promising fishing area with a yearly average landing of about 2000 tons (Soundarapandian et al., 2009). As shown in Fig. 1, we chose three sampling locations in Cuddalore coast to cover the entire length of the Uppanar Estuary. The three sampling stations with different anthropogenic influences are as follows: (1) Sami nagar (11°39′24.7"N, 79°44′55.7″E — agricultural), (2) Thaikkal Thoni Thurai (11°41′33.5"N, 79°46′01.1″E — industrial), and (3) Silver Beach (11°44′15.1"N, 79°47′02.4″E — tourist activities).

Over a year, from July 2018 to June 2019, a total of 12 species of ichthyofaunae were collected across three sampling stations across four seasons (premonsoon, monsoon, postmonsoon, and summer). At least six individuals per each species were collected for each sampling for obtaining the average metal concentrations for each species. The collected ichthyofaune are as follows: *Iniistius cyanifrons, Carangoides*

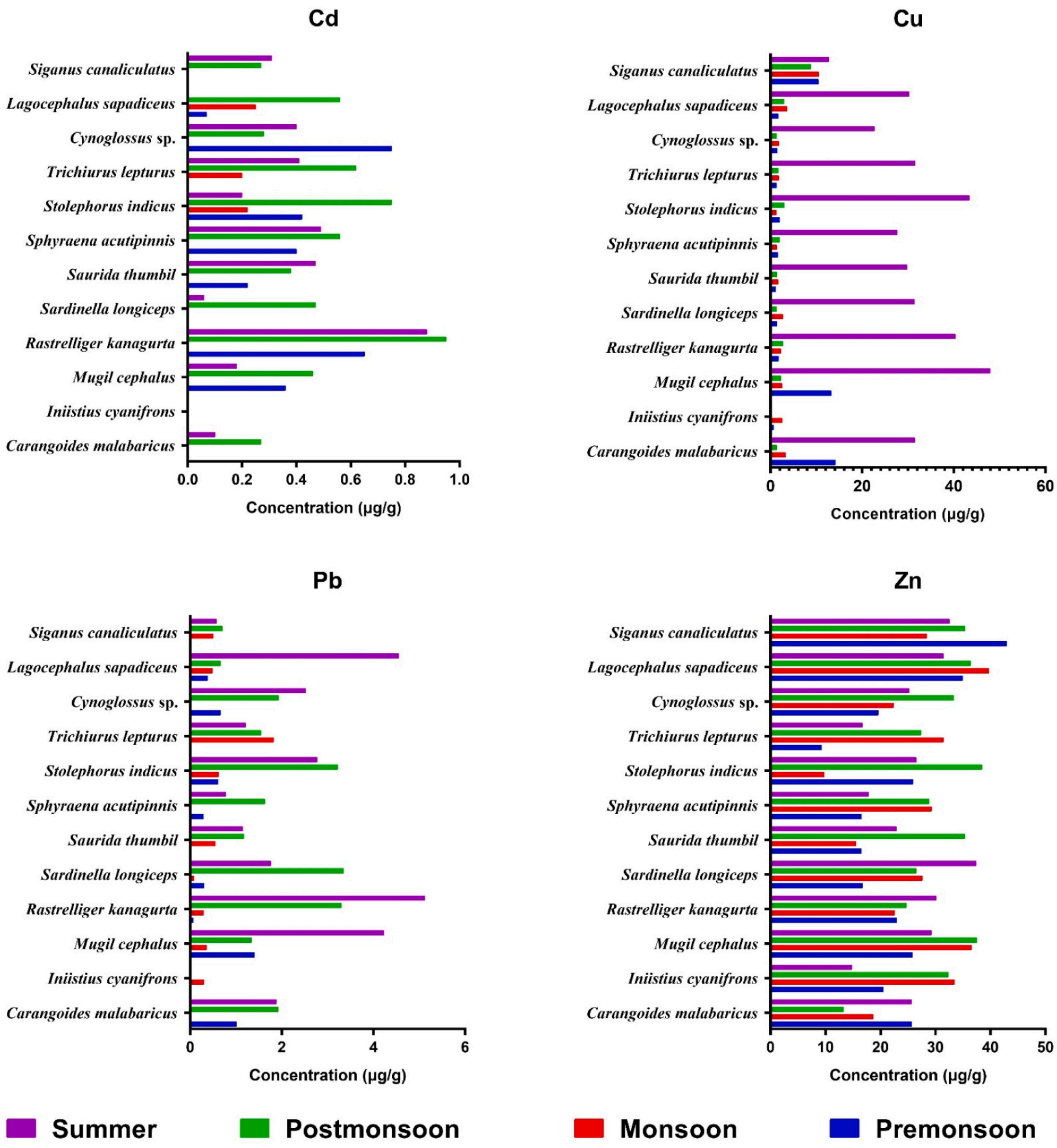

Fig. 2. Season-wise distribution of metals in the twelve species of marine finfish.

malabaricus, Mugil cephalus, Sardinella longiceps, Rastrelliger kanagurta, Saurida thumbil, Cynoglossus sp.*, Lagocephalus sapadiceus, Siganus canaliculatus, Stolephorus indicus, Sphyraena acutipinnis,* and *Trichiurus lepturus*. The fish species chosen for this study were frequently consumed by the local population and had commercial importance. The finfish species were split into two groups depending on the feeding habitat, according to Keshavarzi et al. (2018). The sampled fish were classified into two categories based on the habitats they thrive as pelagic and benthic fish. Pelagic organisms are mostly found in the water column, while benthic organisms dwell predominantly on the seafloor. Out of the 12 species, nine species were pelagic fish, including *Iniistius cyanifrons*, *Carangoides malabaricus*, *Mugil cephalus*, *Sardinella longiceps*, *Rastrelliger kanagurta*, *Saurida thumbil*, *Stolephorus indicus*, *Sphyraena acutipinnis*, and *Trichiurus lepturus*, and three were benthic fish, including *Cynoglossus* sp., *Lagocephalus sapadiceus*, and *Siganus canaliculatus*. Fresh fish samples were collected from local fishermen and packaged in an ice-filled box before being transported to the laboratory. The collected fish species were identified in the laboratory using taxonomic literature from the fisheries survey of India (FSI) and FISHBASE website. The fish were washed in the laboratory using deionized water to remove any external contaminants, and the muscle tissue was dissected and dried at 70 °C until completely dry. The dry tissue was crushed with a mortar and pestle. For AAS (atomic absorption spectrophotometry) analysis according to Arumugam et al. (2018b), 1 g of dry powdered of each individual fish sample was digested using 10-mL mixture of HNO_3 (72%), $HClO_4$ (70%), and H_2SO_4 (98%) in a 5:2:1 proportion at 60 °C. Any undigested sample was further subjected to digestion using 5 mL of 2 N HCl. Once the samples were digested and only about 1 mL of the final extract remained, the sample was diluted with distilled H_2O and made up to 25 mL by volume. Finally, the extract was filtered with Whatman filter paper (grade 1) and transferred into a metal-free container for further AAS analysis.

The raw data of metal concentrations came from the AAS analysis, which was used for determining the metal distribution in the finfish throughout the four seasons. All the data were systemically analyzed and represented as mean and standard deviation using MS Excel (Version 365, Windows 10). Two-way ANOVA was used to evaluate the species–season differences at 0.05% levels for each element. The source identification of the metals in the Cuddalore coastal zone was performed using multivariate analysis, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA). These results provided the

Table 1
Comparison of permissible limits of metal concentrations in the fish tissue as per international agencies and the concentrations reported in the muscle tissue of finfish from the Cuddalore coast.

Metal	Season	Present study (μg/g)[a]	Permissible limits (μg/g)				
			FSSAI (2015)	FAO/WHO (1989)	MAFF (2000)	USEPA (2000)	EC (2014)
Cd	Premonsoon	0.24	0.3	0.5	0.2	2	0.5
	Monsoon	0.06					
	Postmonsoon	0.46					
	Summer	0.29					
Cu	Premonsoon	4.23	–	30	20	120	–
	Monsoon	2.93					
	Postmonsoon	2.37					
	Summer	29.1					
Pb	Premonsoon	0.39	0.3	0.5	2	4	0.3
	Monsoon	0.42					
	Postmonsoon	1.73					
	Summer	2.21					
Zn	Premonsoon	23.09	–	100	50	120	30
	Monsoon	26.27					
	Postmonsoon	30.78					
	Summer	25.86					

WHO - World Health Organization; FSSAI - Food Safety and Standards Authority of India; US-EPA - United States Environmental Protection Agency; EC - European Commission; MAFF - Ministry of Agriculture, Forestry and Fisheries.

[a] Metal concentration in fish muscle tissue.

biplot for expressing the correlation between the metal, season, and species of fish, and the dendrogram depicting the relationship between the metals.

The potential of an organism to aggregate the various constituents from its surrounding environment or medium like water, sediment, etc., is known as bioconcentration factor (BCF) (Achary et al., 2017). For assessing the BCF, the following formula was used.

$$\mathrm{BCF} = \frac{\text{Metal concentration in tissue}}{\text{Metal concentration in surrounding medium}}$$

where the background concentration of the surrounding medium (water) for Cd is 0.26, Cu is 0.41, Pb is 0.48, and Zn is 1.65 μg/g as per our simultaneous study Vinothkannan et al. (2022). These values are the average metal concentrations in the water samples collected in concurrence with the fish samples.

Majority of the human population consumes fish as a source of protein. Hence, fish muscles tissues were utilized to evaluate the risk to human health using an estimated daily intake (EDI) of elements and target hazard quotients (THQ). The below formula was used to estimate the daily intake of metals (Pal and Maiti, 2018).

$$\mathrm{EDI} = \frac{\mathrm{FIR} \times \mathrm{C}}{\mathrm{BW}}$$

where FIR represents the food ingestion rate, C stands for metal concentration in fish samples, and BW is the average body weight. The regional daily intake rate for fish in human was considered as 57.5 g/person/day, and the average body weight was considered as 55.9 kg (Siddiqui et al., 2019).

The target hazard quotients (THQs) were used to estimate the health risks associated with the consumption of fish species, and the calculations were done using the standard hypothesis of an integrated USEPA report (USEPA, 2000). The formulae for calculating the THQ and the resultant hazard index were as follows:

$$\mathrm{THQ} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times \mathrm{C}}{\mathrm{RfD} \times \mathrm{BW} \times \mathrm{TA}} \times 10^{-3}$$

$$\text{Hazard index (HI)} = \sum \mathrm{THQ}$$

where EF stands for exposure frequency (365 days/year), ED stands for exposure duration (70 years), which was the life expectancy as per Bennett et al. (1999), FIR stands for food ingestion rate (57.5 g/person/day), C is for metal concentration in fish tissues in μg/g, RfD is the oral reference dose in mg/kg/day, BW is the body weight (55.9 kg), and TA is the exposure time (365 days/year × ED). The RfD values for the metals as per USEPA (2015) are as follows: Cd (0.001), Cu (0.04), Pb (0.00357), and Zn (0.3).

The processed samples were analyzed for metals using an Atomic Absorption Spectrophotometer (Model: AA7000; Make: Shimadzu Company, Japan) at the following operating wavelengths: 213.85 nm for Zn, 324.75 nm for Cu, 228.80 nm for Cd, and 217.00 nm for Pb (Rajaram et al., 2017). The lowest detection limit (LDL) for the instrument for all metals analyzed was 0.01 μg/g. All sample processing and analysis were done in a sterile and metal-free environment to ensure quality assurance across the entire analysis. Triplicate readings of the samples were made, and the mean and standard deviation were computed to present the results. AAS metals reference standards procured from Sigma-Aldrich, Bangalore, were used for plotting the standard curves. Standard calibration curves for each element had a regression coefficient (R^2) value greater than 0.98. The quality assurance and quality control (QA/QC) for the AAS instrument were maintained by checking the readings with the metal standards of known concentration in the range of 0.1–10 μg/g. The recovery percentages for the metals analyzed using the known standards were in the range of 92–106%. Blanks were run once for each ten sample readings to ensure the accuracy of the instrument readings for quality control and quality assurance of the metal concentration data.

Seasonal variation of four metals (Cd, Cu, Pb, and Zn) was assessed in 12 different species of finfish divided into two categories based on their habitat including 9 pelagic fish and 3 benthic fish. Fig. 2 shows the seasonal variation of four metal concentrations in the 12 species of finfish. Overall, the range of metal concentration across all four seasons was as follows: Cd (<LDL–0.95 μg/g), Cu (<LDL–47.98 μg/g), Pb (<LDL–5.12 μg/g), Zn (9.75–42.92 μg/g). The distribution of metals across the 12 species of fish for the different seasons was statistically significant at 0.05 level ($P < 0.05$): premonsoon ($P < 0.001$; $F = 62.25$), monsoon ($P < 0.001$; $F = 95.00$), postmonsoon ($P < 0.001$; $F = 184.50$), and summer ($P < 0.001$; $F = 55.48$). Cd was not detected or generally low in many species of fish during the premonsoon and monsoon seasons. Cd was detected in many species in the postmonsoon and summer seasons. Out of the detected Cd values, the lowest concentration of Cd was observed in *Sardinella longiceps* (0.06 ± 0.01 μg/g) during the summer season, and the highest concentration was recorded in *Rastrelliger kanagurta* (0.95 ± 0.24 μg/g) during the postmonsoon season.

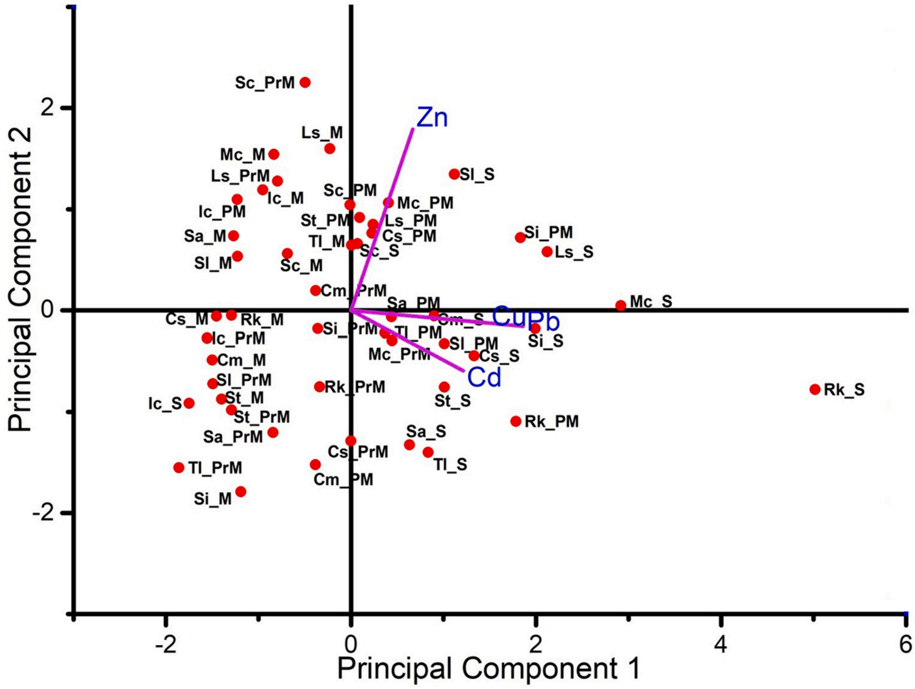

Fig. 3. Biplot showing the interrelationship between metal, season, and finfish species. Species — Ic - *Iniistius cyanifrons*; Cm - *Carangoides malabaricus*; Mc - *Mugil cephalus*; Sl - *Sardinella longicep*; Rk- *Rastrelliger kanagurta*; St - *Saurida thumbil*; Cs - *Cynoglossus* sp.; Ls - *Lagocephalus sapadiceus*; Sc - *Siganus canaliculatus;* Si - *Stolephorus indicus*; Sa - *Sphyraena acutipinnis*; and Tl - *Trichiurus lepturus*. Season — PrM - premonsoon; M - monsoon; PM – postmonsoon; and S – summer. Labels of scores in the biplot are expressed as 'Species_Season'.

Cu concentration was not detected in *Iniistius cyanifrons* during the postmonsoon and summer season, and the highest concentration of Cu was noted in *Mugil cephalus* (47.98 ± 1.74 μg/g) during the summer season. Many fish species were devoid of Pb during the premonsoon and monsoon seasons; however, the Pb content increased in the post-monsoon and summer seasons. Out of the detected values, the minimum was in *Rastrelliger kanagurta* (0.06 ± 0.05 μg/g) during the premonsoon season, and the maximum was also noted in *Rastrelliger kanagurta* (6.82 ± 0.40 μg/g) during the summer season. Zn was detected in all species across all seasons. The least concentration of Zn was noted in *Stolephorus indicus* (9.75 ± 1.51 μg/g), and the maximum level of concentration was recorded in *Siganus canaliculatus* (42.92 ± 1.65 μg/g). The overall order of mean concentration of metals in different season is as follows: pre-monsoon (Zn > Cu > Pb > Cd), monsoon (Zn > Cu > Pb > Cd), post-monsoon (Zn > Cu > Pb > Cd), and summer (Cu > Zn > Pb > Cd). Considering only the 9 pelagic fish, the mean concentrations of Cd, Cu, Pb, and Zn were 0.27, 9.87, 1.22, and 24.72 μg/g, respectively. The ranges of the four metals in the pelagic fish species were as follows: Cu (<LDL–0.95 μg/g), Cu (<LDL–47.98 μg/g), Pb (<LDL–5.12 μg/g), and Zn (9.27–38.46 μg/g). In the 3 benthic fish, the mean concentrations of Cd, Cu, Pb, and Zn were 0.24, 9.00, 1.08, and 31.84 μg/g, respectively. The ranges of the four metals in the benthic fish species were as follows: Cu (<LDL–0.75 μg/g), Cu (1.28–30.21 μg/g), Pb (<LDL–4.55 μg/g), and Zn (19.57–42.92 μg/g). In both benthic and pelagic fish, the mean metal concentrations were in the following descending order: Zn > Cu > Pb > Cd. A comparison of the metal concentrations in the present study with the permissible limits set by various international agencies for metals in the fish tissues are presented in Table 1. Average Cd concentration was higher in pelagic fish (0.27 μg/g) compared with benthic fish. Similarly, the Cu and Pb levels were also higher in the pelagic fish in comparison with benthic fish. The average level of Cu in pelagic fish was 9.87 μg/g, whereas it was only 9.00 μg/g in the benthic fish. Pb value was 1.22 μg/g and 1.08 μg/g in the pelagic and benthic fish, respectively. But there was a converse scenario in Zn, where the benthic fish had higher levels of Zn compared with pelagic fish. The mean value of Zn in the pelagic group was 24.72 μg/g and 31.84 μg/g in the benthic group.

Table 2

Percentage of variance, Eigenvalues, and principal component values of for metal concentration in finfish species across two significant principal components.

Elements	PC1	PC2
Cd	0.441	−0.313
Cu	0.538	−0.066
Pb	0.676	−0.083
Zn	0.243	0.944
Eigenvalue	1.862	0.956
Percentage of variance	46.56%	23.89%
Cumulative	46.56%	70.45%

The average levels of metals found in the tissue samples differ with the species of fish. A variety of factors influence metal accumulation; however, season may play a significant role (Kargin, 1996). As per the report of Vinothkannan et al. (2022), more metal contaminants may get flushed and diluted into the rivers and estuaries during the monsoon seasons due to increased runoff caused by the rainwater (Hossain et al., 2020). The higher levels of metals observed in the summer season might be due to the lack of any influx of freshwater causing the muddy sediments loaded with metals to get deposit in the estuarian ecosystem (Pande and Nayak, 2013). The variation in metal levels shows how each species' feeding habit and accumulating capacity changes over the seasons. The primary route of transition for many aquatic species is a direct transfer of metals from sediment to aquatic organisms. Metals tend to accumulate in the benthic species and transmit upwards through the food chain via biomagnification (Jovanovic et al., 2017). In a similar study, Pourang (1995) has already explored the effect of dietary habits on metal deposition in various fish organs and tissues, and the relative significance of water and food to the accumulation of metals by fish. Cadmium (Cd) and arsenic (As) are metals that are strongly linked with sediments which can act as long-term pollutants for benthic fish (Noel et al., 2013). The mean levels of Cd (0.26 μg/g) and Pb (1.18 μg/g) in the current study are higher than the permissible limit as per MAFF (2000), but they fall within the permissible limit set forth by FAO/WHO (1989) and USEPA (2000). The Cu (9.65 μg/g) and Zn (26.5 μg/g) levels are below the permissible limits set by the regulatory agencies worldwide (Table 1).

The PCA results are shown in Fig. 3. With eigenvalues of 1.86 and

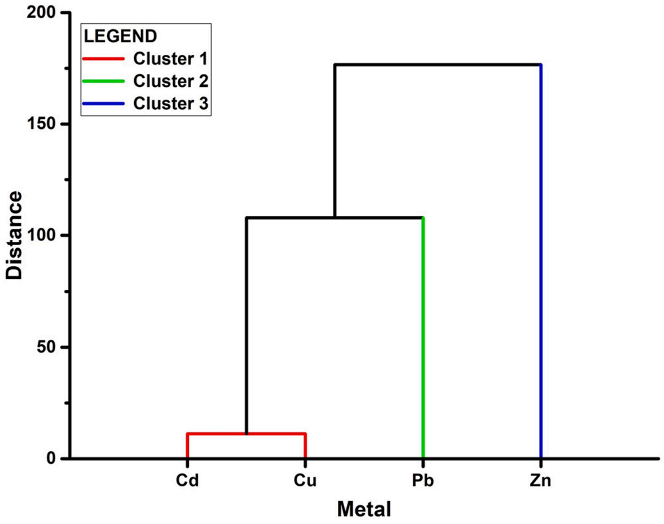

Fig. 4. Dendrogram showing the hierarchical cluster analysis of metal concentrations.

0.96, respectively, only two main components i.e., PC1 and PC2 were considered for discussion. Owing to the low eigenvalue (<1), the principal components PC3 and PC4 were not considered. PC1 contributed 46.56% of variance with significant positive loadings for Pb (0.67) and moderate loading for Cu (0.53). PC2 contributed 23.89% of variance with significant positive loading of Zn (0.94) (Table 2). PC1 exhibited a close collaboration with Pb followed by Cu, with the results indicating that greater levels of Pb in the environment are due to anthropogenic activities rather than geogenic sources. The possible sources of lead in the study areas are paint industries, automobile emissions, lead-acid batteries, and other industrial products. The scenario observed in this study has a strong correlation with an earlier study of Arumugam et al. (2018b). The loading of other metals in PC1 denoted the sources of the metals from a combination of both anthropogenic and natural sources. For PC2, only Zn had a strong positive loading and all other metals were negatively loaded. This indicates a natural source; nevertheless, an over abundance of Zn on the other hand, has the potential to harm organisms. Our PCA results revealed that first principal component is influenced by both manmade and natural sources, while the second principal component is influenced by natural source. This trend can be explained by the fact that monsoon dilution is a prominent factor in the Cuddalore coast. The interrelationship between the different metals is represented as a dendrogram in Fig. 4. The dendrogram was constructed using the dataset's interrelationships. There were three cluster groups. The elements Cu and Cd fell in Cluster 1. Pb and Zn individually were grouped under Cluster 2 and Cluster 3, respectively. The essential element Cu and the non-essential element Cd fall in a same cluster suggesting that their sources may be a combination of both anthropogenic and natural sources. They are largely linked by mixed source of both natural and anthropogenic factors at different seasons.

The BCF was determined to better understand how different elements accumulated in different species with respect to season (Fig. 5). The order of BCF value in pelagic fish was Cu > Zn > Pb > Cd, and the range of BCF for different metals was Cd (0–3.65), Cu (0–117.02), Pb (0–10.67), and Zn (5.62–23.31); while in benthic fish, the order was Cu > Zn > Pb > Cd, and the range was Cd (0–2.88), Cu (3.12–73.68), Pb (0–9.48), and Zn (11.86–26.01). The highest BCF value of Cd (3.65) in pelagic fish was observed for *Rastrelliger kanagurta* during the postmonsoon season. Maximum BCF value for Cu (117.02) was noted in *Mugil cephalus* during the summer season. Highest BCF value for Pb (10.67) was noted in *Rastrelliger kanagurta* during the summer season. BCF value for Zn (23.31) was highest in *Stolephorus indicus* during the postmonsoon season. Coming to the benthic species, the highest BCF for Cd (2.88) was noted in *Cynoglossus* sp., during the premonsoon season. BCF for Cu (73.68) was highest in *Lagocephalus sapadiceus* during the summer season. Maximum BCF value for Pb (9.48) was also observed in *Lagocephalus sapadiceus* in the summer season. Highest BCF value for Zn (26.01) was seen in *Siganus canaliculatus* during the premonsoon season. The higher BCF values for the four metals were recorded in the postmonsoon and summer seasons in the pelagic fish. And in the benthic group, the highest values were observed in the summer and premonsoon seasons. For most species, values of BCF fell within the threshold level (BCF < 1) in the premonsoon and monsoon seasons. Compared with premonsoon and monsoon seasons, the BCF values reported during the postmonsoon and summer seasons were higher than the threshold level (BCF > 1). The results indicate that the monsoon season clearly influences the Cuddalore coast and has a massive impact on the ecosystem with respect to the distribution of metal contaminants in the marine food web.

The concentrations of metals in fish could have a major impact on

Table 3
Estimated exposures and risk assessment due to consumption of finfish from the Cuddalore coast based on USEPA guidelines.

Fish Type	Metal	Mean Concentration (μg/g)	Exposure Dose (ED)	Target Hazard Quotient (THQ)
Pelagic Fish (*n* = 9)	Cd	0.27	0.28	0.28
	Cu	9.87	10.15	0.25
	Pb	1.22	1.25	0.35
	Zn	24.71	25.42	0.085
			HI =	0.97
Benthic Fish (*n* = 3)	Cd	0.24	0.25	0.25
	Cu	9.00	9.26	0.23
	Pb	1.08	1.11	0.31
	Zn	31.84	32.75	0.109
			HI =	0.90

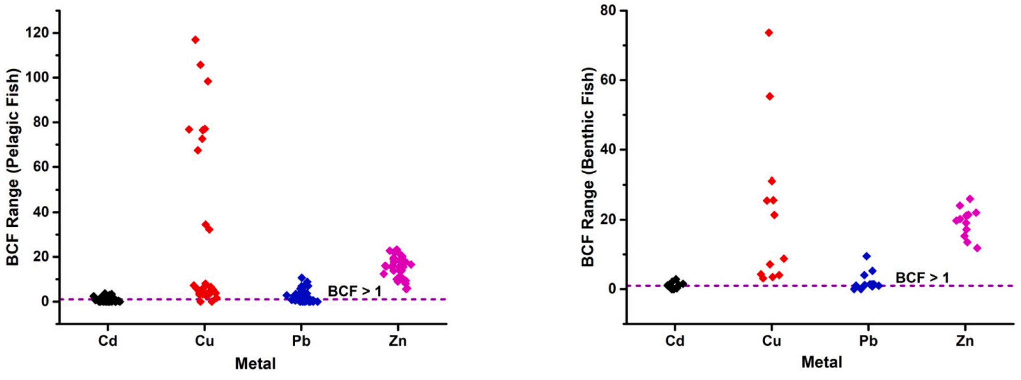

Fig. 5. Variations in the bioconcentration factors (BCF) in the pelagic and benthic finfish species.

Table 4
Metal concentrations (µg/g) reported in the finfish in the present study compared with similar studies worldwide.

S. No.	Species	Location	Cd	Cu	Pb	Zn	References
1	*Iniistius cyanifrons*	Cuddalore coast, India	0	0.81	0.08	25.25	Present Study
2	*Carangoides malabaricus*	Cuddalore coast, India	0.09	12.57	1.20	20.80	Present Study
		Gulf of Mannar, India	–	0.52	ND	0.32	Nagarani et al., 2020
		Andaman Sea	–	0.36	–	4.74	Kureishy et al., 1981
		Qatari coast, Doha.	1.01	3.42	1.91	–	Kureishy, 1993
3	*Mugil cephalus*	Cuddalore coast, India	0.25	18.00	1.83	32.28	Present Study
		Iskenderun Bay, Turkey	–	0.24	3.59	21.62	Yilmaz, 2005
		Iskenderun Bay, Turkey	–	1.45	7.45	19.6	Yilmaz, 2005
		Black Sea coast of Bulgaria	0.024	–	0.07	–	Stancheva et al., 2013
		Black Sea coast of Bulgaria	0.012	–	0.05	–	Stancheva et al., 2013
		Machilipatnam Coast, Andhra Pradesh	–	5.5	8.4	25.2	Krishna, 2014
		Ghar El Melh lagoon, Tunisia	0.02	–	0.20	–	Chouba et al., 2007
		Bafa Lake, Eastern Aegean	0.02	1.18	0.73	16.01	Aydin-Onen et al., 2015
		Northeast Mediterranean Sea	0.96	4.48	6.25	26.13	Kalay et al., 1999
		North African coasts, Mediterranean Sea	0.46	7.54	1.31	111.5	Ouali et al., 2018
		Creek in Woji,southern, Nigeria	17.44	32.98	6.75	–	Ihunwo et al., 2020
		Amlik lagoon, Turkey	0.07	–	–	88.97	Dural et al., 2006
		Pulicat lake, southeast coast of India	0.77	2.72	10.25	11.02	Laxmi Priya et al., 2011
		Barmouth near Pulicat lake, southeast India	0.55	1.73	10.75	8.25	Laxmi Priya et al., 2011
		Koycegiz Lagoon System, Turkey	0.48	29.90	0.63	78.90	Genc and Yilmaz, 2018
		Manzala Lake, Egypt	–	2.55	7.04	–	El-Hak et al., 2021
		Coastal lagoons of NW, Mexico	0.17	1.06	1.63	21.77	Frias-Espericueta et al., 2011
		Lake Macquarie, Australia	0.05	3.6	–	14	Kirby et al., 2001
4	*Sardinella longiceps*	Cuddalore coast, India	0.13	9.18	1.37	27.03	Present Study
		Balochistan coast, Pakistan	0.17	3.18	0.25	3.1	Ahmed et al., 2016
		Northern United Arab Emirates	0.17	0.56	–	21.8	Malik et al., 2020
		Arabian Sea, Pakistan	0.48	1.47	2.65	8.34	Tariq et al., 1991
		Cochin, southwest coast of India.	–	1.54	–	20.52	Nair et al., 1997
		Gulf of Mannar, southeast coast of India	0.11	–	0.01	–	Rameshkumar et al., 2016
		Abeokuta, Ogun state, Nigeria	0.6	2.64	1.28	3.16	Akinhanmi et al., 2021
		Southeast coast of India	0.20	4.23	0.8	35.69	Sankar et al., 2018
		Coastal areas in Bangaladesh	0.21	3.61	ND	24.66	Bristy et al., 2021
5	*Rastrelliger kanagurta*	Cuddalore coast, India	0.62	22.98	2.62	25.10	Present Study
		Oman Sea	0.42	2.76	0.65	26.68	Sadeghi et al., 2021
		Dar es Salaam, Tanzania	0.04	2.73	0.02	31.04	Mziray and Kimirei, 2016
		Straits of Malacca	–	0.20	0.02	5.61	Khandaker et al., 2015
		Red Sea cost of Yemen	0.3	1.15	0.85	6.65	Heba et al., 2014
		Northeast coast of India	0.33	3.9	–	19.8	Kumar et al., 2012
		Cochin, India	5.18	2.75	0.56	37.4	Rejomon et al., 2010
		Cochin, India	–	2.01	–	14.99	Nair et al., 1997
		Port Dickson coastal water, Malaysia	0.09	2.15	12.44	–	Praveena and Lin, 2015
		Olavakkode fish market, Palakkad, India	6.88	6.2	11.6	13.9	Rini et al., 2020
6	*Saurida thumbil*	Cuddalore coast, India	0.27	8.49	0.72	22.54	Present Study
		Calicut region, Kerala, India	ND	1.5	ND	7.3	Sankar et al., 2006
		Southwest coast of India	0.36	3.5	1.2	16.1	Unnikrishnan et al., 2003
		Basra city, Iraq	2.86	8.52	3.19	8.75	Yesser et al., 2013
7	*Cynoglossus* sp.	Cuddalore coast, India	0.36	6.79	1.28	25.12	Present Study
		Azuabie creek, Bonny Esturary, Nigeria	0.01	–	0.18	–	Daka et al., 2008
		Western Part of Madura Strait	0.42	1.43	4.40	2.49	Charisma et al., 2013
		Lagos Metropolis, Nigeria	0.03	–	0.02	–	Lawal-Are et al., 2018
		Northeast coast of Bay of Bengal, India	1	47.97	19.96	44.74	De et al., 2010
		Lagos Lagoon, Nigeria	–	4.08	5.96	12.05	Taiwo et al., 2018
8	*Lagocephalus sapadiceus*	Cuddalore coast, India	0.22	9.6	1.52	35.60	Present Study
		Yeşjlovacık coasts of Turkey	–	1.54	–	42.66	Dogdu et al., 2021
	Male	Berdan River and Yeşilovacık Bay	0.81	1.33	2.68	47.32	Kosker et al., 2019
	Female	Berdan River and Yeşilovacık Bay	1.03	1.45	3.39	58.1	Kosker et al., 2019
9	*Siganus canaliculatus*	Cuddalore coast, India	0.15	10.61	0.44	39.80	Present Study
		Terengganu Coastal Area, Malaysia	0.1	0.68	0.15	11.6	Rosli et al., 2018
		Southeast coast of India	0.08	0.62	–	4.09	Shalini et al., 2021
		Arabian Gulf Coast at the Eastern Province,	0.54	1.67	0.66	8.83	Zyadah and Almoteiry, 2012
10	*Stolephorus indicus*	Cuddalore coast,India	0.40	13.66	1.81	25.16	Present Study
		UAE coast, Arabian Gulf	0.1	1.5	–	8.9	Alizada et al., 2020
		Palk Bay, Southeastern India	0.02	0.2	0.9	21.48	Arulkumar et al., 2017
11	*Sphyraena acutipinnis*	Cuddalore coast,India	0.36	8.12	0.67	23.12	Present Study
12	*Trichiurus lepturus*	Cuddalore coast,India	0.31	9.08	1.15	21.2	Present Study
		Oman Sea	0.50	0.62	0.84	11.04	Sadeghi et al., 2021
		Karachi Fish Harbour, Pakistan	0.42	2.23	0.2	20.34	Ahmed et al., 2018
		Mumbai Harbor, India	0.12	2.11	0.04	42.34	Velusamy et al., 2014
		Miri coast, Sarawak, Borneo	0.6	9	0.6	25.3	Anandkumar et al., 2018

Metal concentration values are represented as mean in µg/g (dry weight).

human health, especially to those who consume fish on a daily basis. As a result, health risk assessments are important for fish that come from polluted sites. The hazard index (HI) for the pelagic fish was 0.97 and for the benthic fish was 0.90. The health risk evaluations showed a threshold reaction, which assumed that the majority of substances had noncancerous effects. The ED, THQ, and HI of finfish from the Cuddalore coast are shown in Table 3. The fish mean metal concentrations of present study compared with the concentrations reported by various studies around the world represented in Table 4. Among the twelve fish species assessed in our study, there are no reports elsewhere on metal accumulations for two fish species — *Iniistius cyanifrons* and *Sphyraena acutipinnis*. Cd and Pb levels in *Carangoides malabaricus* in our study had considerably lower value compared with a study in the Qatari coast, Doha (Kureishy, 1993). Cu and Zn values in *Carangoides malabaricus* are higher in the current study in comparison with the values reported in the Andaman Sea and Gulf of Mannar, Chinnamuttom region (Kureishy et al., 1981; Nagarani et al., 2020).

The average Cd concentration in *Mugil cephalus* reported in our study was similar to several studies conducted worldwide (Frias-Espericueta et al., 2011; Genc and Yilmaz, 2018; Ouali et al., 2018); however, in a study from the creek in Woji, Southern Nigeria (Ihunwo et al., 2020), Cd values about hundred times higher than the present study were reported. In the case of Cu, the present study recorded several folds higher concentration when compared to the report of Yilmaz (2005). The Pb concentrations for *Mugil cephalus* in the current report were similar to values reported in the coastal lagoons of NW Mexico reported by Frias-Espericueta et al. (2011). The Zn values were similar to the reports of Krishna (2014) and Kalay et al. (1999), but three-fold higher than the values reported in the North African coasts of the Mediterranean Sea (Ouali et al., 2018).

In *Sardinella longiceps*, the values for Cd were comparable to the values reported all over world, except for the value from Abeokuta in Nigeria which was slightly higher (Akinhanmi et al., 2021). The Cu values were higher in present study compared with the data elsewhere. Pb level was lower in the present study compared with that reported in the Arabian Sea by Tariq et al. (1991) and in the Gulf of Mannar by Rameshkumar et al. (2016). Zn values were similar to that reported by Bristy et al. (2021), and lesser than that reported in the Balochistan coast in Pakistan by Ahmed et al. (2016). In *Rastrelliger kanagurta*, the Cd value in our study was ten-fold lesser than the values reported in the Olavakkode Fish Market in Kerala, India (Rini et al., 2020). Nevertheless, the values reported by us are similar to that of Kumar et al. (2012). The Cu and Pb values were higher when compared with the other works reported globally (Heba et al., 2014; Khandaker et al., 2015; Mziray and Kimirei, 2016; Praveena and Lin, 2015; Rejomon et al., 2010). In *Saurida thumbil*, the Cd, Cu, and Pb values in the current study were higher than the values reported by Yesser et al. (2013) in Basra city, Iraq, but were almost similar to the data of Yesser et al. (2013). Zn values were higher in the present study compared with globally reported data.

In *Cynoglossus* sp., the present values were on average comparable to the data reported from different regions of the world (Charisma et al., 2013; De et al., 2010; Taiwo et al., 2018). In *Lagocephalus sapadiceus*, the higher values of Cd, Pb, and Zn were noted in the female fish at Berdan River and Yeşilovacık Bay (Kosker et al., 2019) compared with the values reported in the present study; however, the Cu values were higher in the current study. In *Siganus canaliculatus*, the Cd and Pb values were higher in the Arabian Gulf Coast as reported by Zyadah and Almoteiry (2012). In *Stolephorus indicus*, all the four metal values were higher than those reported by Alizada et al. (2020) and Arulkumar et al. (2017). In *Trichiurus lepturus*, the Cd value was comparatively higher in the Miri coast of Borneo (Anandkumar et al., 2018) and lower in Mumbai Harbor, India (Velusamy et al., 2014). The Cu and Pb values were higher in the present study in comparison to an earlier report (Anandkumar et al., 2018). The Zn value was notably higher in Mumbai Harbor, India (Velusamy et al., 2014). In comparison to our data, lower level of Zn was reported in Karachi fish harbor in Pakistan (Ahmed et al., 2018).

In comparison with the global data, the results in our present study had higher concentrations of the essential metals (Cu and Zn) since many of the collected species may have accumulated these essential metals from the surrounding medium and also their food sources. Nevertheless, the non-essential metals Cd and Pb were found to reach maximum values in a few species of finfish compared with the global reports elsewhere. It is also evident that Pb may have originated from the industrial activities, which are prominent in the Cuddalore area, and distributed in the Uppanar Estuary before reaching the Bay of Bengal. The Cd levels reported in our study are comparably lower than global statistics; however, they are still higher than the international regulatory limits (FAO/WHO, 1989; USEPA, 2000). The bioconcentration of these toxic metals in the tissues of the fish pose a hazard to the humans who consume these metal-contaminated fish. The hazard indices for both the pelagic fishes and the benthic fishes are in the verge of exceeding the safe limits for consumption of these fish. In the near future, if the metal pollution is not controlled, the HI values make exceed the safe threshold limits. Hence, in the event of more metal pollution in the Cuddalore coast, consumption of fish from these contaminated waters can cause adverse health effects in humans. The continuous monitoring of the metal levels in the environment and the ichthyofaunal resources from the Cuddalore coast are necessary to ensure the safety of humans who consume these fish.

CRediT authorship contribution statement

A.V, P. EC, and R.R conceived and designed the experiments. A.V, R. R and A.G collected and identified the samples. Data were analyzed and wrote the paper by A.V, P.EC, A.G, and R.R. A.V, P.EC, and R.R involved in data analysis and language editing.

Declaration of competing interest

All the authors agree with the contents and to the submission and disclose that, there is no conflict of interest including financial, personal or other relationships with other people or organizations.

Acknowledgement

The authors are grateful to the Department of Marine Science, Bharathidasan University, Tiruchirappalli, India, for providing the facilities for the research work. The authors convey their gratefulness to the Ministry of Earth Science, Government of India (Ref. No.: MoES/36/OOIS/Extra./9/2013) for funding the AAS facilities.

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Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-021-13371-1

RESEARCH ARTICLE

Avian feathers as a biomonitoring tool to assess heavy metal pollution in a wildlife and bird sanctuary from a tropical coastal ecosystem

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Received: 26 November 2020 / Accepted: 4 March 2021

Abstract

In this study, we have assessed the concentrations of four heavy metals (Cu, Zn, Cd, and Pb) in the feathers of 11 species of birds from the Point Calimere Wildlife and Bird Sanctuary, a protected environment. Concentrations of copper and zinc were detected in all the bird species, cadmium was observed only in two bird species, and lead was below the detection limits for all birds. The order of concentration of metals in the feathers is Zn > Cu > Cd > Pb. Using the multivariate statistical analysis, principal component analysis (PCA), the metal origins were traced to natural, dietary, and manmade sources. In addition, sediment samples were also collected from the sanctuary, to assess the bioaccumulation factor (BAF). The BAF values follow the order Cd < Cu < Zn < Pb. In comparison with worldwide heavy metal reports in bird feathers, lower concentrations of metals are observed in our study area. The tropical marine ecosystem at Point Calimere Wildlife and Bird Sanctuary can be considered as pristine regarding heavy metal pollution. Continuous monitoring of the ecosystem is crucial to sustain the pristine nature of the sanctuary and to attract many more birds.

Keywords Bird feather · Trace metals · BAF · Bioaccumulation · Shore birds · Protected environment

Introduction

Unique to birds, feathers serve the birds in flight, camouflaging, insulation, waterproofing, and even to show off during mating rituals. Apart from these, they can also help humans understand the level of pollutants in the environment. Bird feathers can be used as effective biomonitoring tools to measure the heavy metal pollution. Numerous biomonitoring methods to assess the concentration of heavy metals in the environment have been developed in the recent decades; out of them, birds are considered as effective biomonitors due to the fact that most birds are long-living species and occupy high levels in their food pyramids (Burger 1993; Van Straalen and Ernst 1991). Even though the inner tissues of birds being proven as the best indicator for bioaccumulative compounds, the need for non-invasive biomonitoring choices has risen for a more ethical and eco-friendly methodology. Various non-invasive matrices such as eggs (Eens et al. 2013; Jaspers et al. 2005; Van den Steen et al. 2006) and feathers (Frantz et al. 2012; Kim and Koo 2007; Veerle et al. 2004) are used for biomonitoring instead of sacrificing the living birds. The increase in concentrations of heavy metals in the environment and their adverse effects on the ecosystem and biota are still a topic of imminent threats, specifically in the developing parts around the globe (Abbasi et al. 2015). Although there are various debates on the usage of feathers as biomonitoring tools, feathers offer many benefits as a useful non-destructive biomonitoring material and are every so often deemed to be an excellent means for monitoring pollutant concentrations (García-Fernández et al. 2013; Malik and Zeb 2009).

Responsible Editor: V. V.S.S. Sarma

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Metals naturally originate through natural ecosystems, but perhaps the human-induced causes of metals, namely, resource extraction, agricultural pollutants, residential pollutants, and environmental accumulation, significantly increase the concentration of metals especially in the aquatic ecosystem (Gu et al. 2015; Sapkota et al. 2008). Trace elements are generally classified into two subgroups viz., essential elements that are required for the vital functioning of the organism, and non-essential elements that are not needed for any physiological function but are highly toxic once accumulated inside the organisms (Burger 1993; Ceyca et al. 2016; Moura et al. 2018). According to Goede and De Bruin (1986), metals can get accumulated in the feathers through the following routes: (1) uptake through the food and assimilation by blood circulation during feather growth; (2) contamination through preening—heavy metals present in secretion products of salt and/or uropygial glands (preen glands) contaminate the feathers; (3) contamination through straight interaction with the environment (water, air, and soil). The coastal wetland habitats are of importance to birds as they provide vital resources for avian social activities such as foraging, reproduction, sanctuary, and protection. Many migratory birds utilize the sanctuaries for the above-said purposes. Recently, this habitat type is slightly contaminated due to natural disasters and anthropogenic activities like eco-tourism and industrialization (Pott and Wiedenfeld 2017). The contamination of the ecosystem is a tremendous threat to all biodiversity. Persistence of heavy metals in the ecosystem is a huge concern, despite its influence on ocean biodiversity. The heavy metal contamination in wetlands often disintegrates the water quality, either intentionally or unintentionally, negatively impacts the flora and fauna, and reduces the distribution of several bird species, due to the loss of biodiversity in wetlands (Gochfeld 1997). Therefore, to enhance community concern about heavy metal pollution, ecological damage needs to be assessed, tracked, reviewed, controlled, and remedied (Movalli 2000; Naccari et al. 2009). Organisms differ in their amount of accumulation of heavy metals in a living environment, based on its size, eating habits, age, gender, or various other internal or external criteria (Abbasi et al. 2015). However, awareness of the fate and impact of chemicals is important to determine the quality of environments as well as provide advanced warning of environmental changes which may represent harmful effects (Burger 2002).

Biomonitoring and environmental monitoring are the only appropriate remedies for preventing environmental degradation. Biological markers or monitoring systems would be relevant only if they could provide evidence of the transfer and accumulation of the metals in the environment. Certain advantages are achieved by using avian feathers as biomarkers. In fact, feathers could be obtained without sacrificing the bird, and this allows one to make repeated sampling possible even on a massive scale. In addition, consideration should be given to how such contaminants reach the feathers and represent the effect and strength of a pollutant in the bird’s atmosphere (Rose and Parker 1982). Numerous researches focused on metal accumulation by seabirds have been done worldwide and have reported the effectiveness of biomonitors. Throughout this aspect, seabirds are one of the great biomonitors for determining heavy metal pollutant levels in seashore or other wetlands (Borghesi et al. 2016; Durant et al. 2009). In our study area, Point Calimere Wildlife and Bird Sanctuary (Viji et al. 2018) have conducted research with respect to several environmental quality parameters, except heavy metals. They highlight that despite the sanctuary being a Ramsar Site and protected area, a few small-scale shrimp farms and chemical firms around the wetland have started to threaten the ecology and biodiversity of the sanctuary. The pH and salinity of the waters in the area have exceeded the permissible thresholds for the ecologically sensitive zones. So far, to the best of our knowledge, there is no comprehensive report on the bioaccumulation of trace metal concentration in the Point Calimere Wildlife and Bird Sanctuary. On highlight of this, our study was conducted to assess the heavy metal contamination in wetlands by using bird feathers as biomonitors. The main goal of this research was to document baseline data of two essential (Cu, Zn) and two non-essential (Cd, Pb) metals of different bird feathers collected from the Point Calimere Wildlife and Bird Sanctuary. In addition, we also have assessed the background concentrations of Cd, Cu, Pb, and Zn in the sediment samples collected from Point Calimere Wildlife and Bird Sanctuary.

Materials and methods

Study area

Point Calimere Wildlife and Bird Sanctuary (Kodiyakkarai) is situated on a low promontory in the Coromandel Coast above the Palk Strait in the Nagapattinam district of Tamil Nadu. Point Calimere Forest has an area of 1729 ha, comprising the Kodikkadu Reserve Forest and Kodikkadu Extension Reserve Forest. In 1988, the sanctuary was renamed as the Point Calimere Wildlife and Bird Sanctuary after enlarging the area to 37,733 ha by adding the Talaignayar Reserve Forest and the Great Vedaranyam Swamp. The sanctuary has been considered an Important Bird and Biodiversity Area (IBA), with code: IN275 and criteria: A1, A4i, A4iii. This IBA comprises mudflats scattered with numerous islets in the Siruthalaikkadu–Kodikkarai area, and a lagoon and mangrove forest in the Muthupet–Adirampattinam. It also covers tropical dry evergreen forest and low-lying coastal grazing lands. The swamp is supplied with freshwater by five channels only during the monsoon. The area has variable rainfall regimes and is not typical of tropical monsoon climate.

The northeast monsoon is the main contributor to this area, although some rainfall occurs during the southwest monsoon. There are prominent dry winds, but cause low-pressure depressions in the Bay of Bengal, thus causing cyclonic storms on the mainland. The sanctuary is dotted with several salt pans and wetlands that act as staging, foraging, and wintering grounds for the migratory birds that visit the sanctuary, mainly for flamingos, waders, ducks, terns, and gulls. It harbors many migratory flamingos and waders (Ali 1963). Out of 110 species of water birds documented from the swamp and saltpans, 34 species were winter migrants from the Palearctic region (Sugathan 1982). As on date, about 200 bird species have been reported in the sanctuary. The demarcations of the sampling sites are shown in Fig. 1. Sediment samples and feathers of seabirds were obtained from three separate sites around the swamp viz., Site-I: Pump house I (10°16′27.2″N; 79°48′28.6″E) to Pump house II (10°17′34.4″N; 79°48′57.5″E); Site-II: Manavaikal mouth (10°16′26.3″N; 79°48′10.0″E) to Sellakkani mouth (10°16′35.7″N; 79°50′24.9″E); and Site-III: Pump house II (10°17′34.4″N; 79°48′57.5″E) to Pump house III (10°18′53.6″N; 79°48′54.4″E). The sites near the swamps and mouths are mostly inhabited by many migratory birds, and so the bird feathers have been collected from the sites. The swamp area witnesses huge fluctuations in water level, depending on the rainfall, during the monsoon time. The swamps in Kodiyakkarai produce irrigation only during the monsoon, and the water gets dried in the summer. Most of the water from these swamps drains into the sea through the Manavaikal and Sellakkani mouths. The Kodiyakkarai swamps are mixed ecosystem influenced by freshwater, brackish water, and seawater.

Collection of bird feathers

The birds in the Point Calimere Wildlife and Bird Sanctuary were watched throughout the day using binoculars. The foraging and feeding grounds of each species of bird were identified and observed continuously. Once the birds had moved from the area, we visited the sites and collected the freshly molten feathers of the birds for each species. Molten feathers

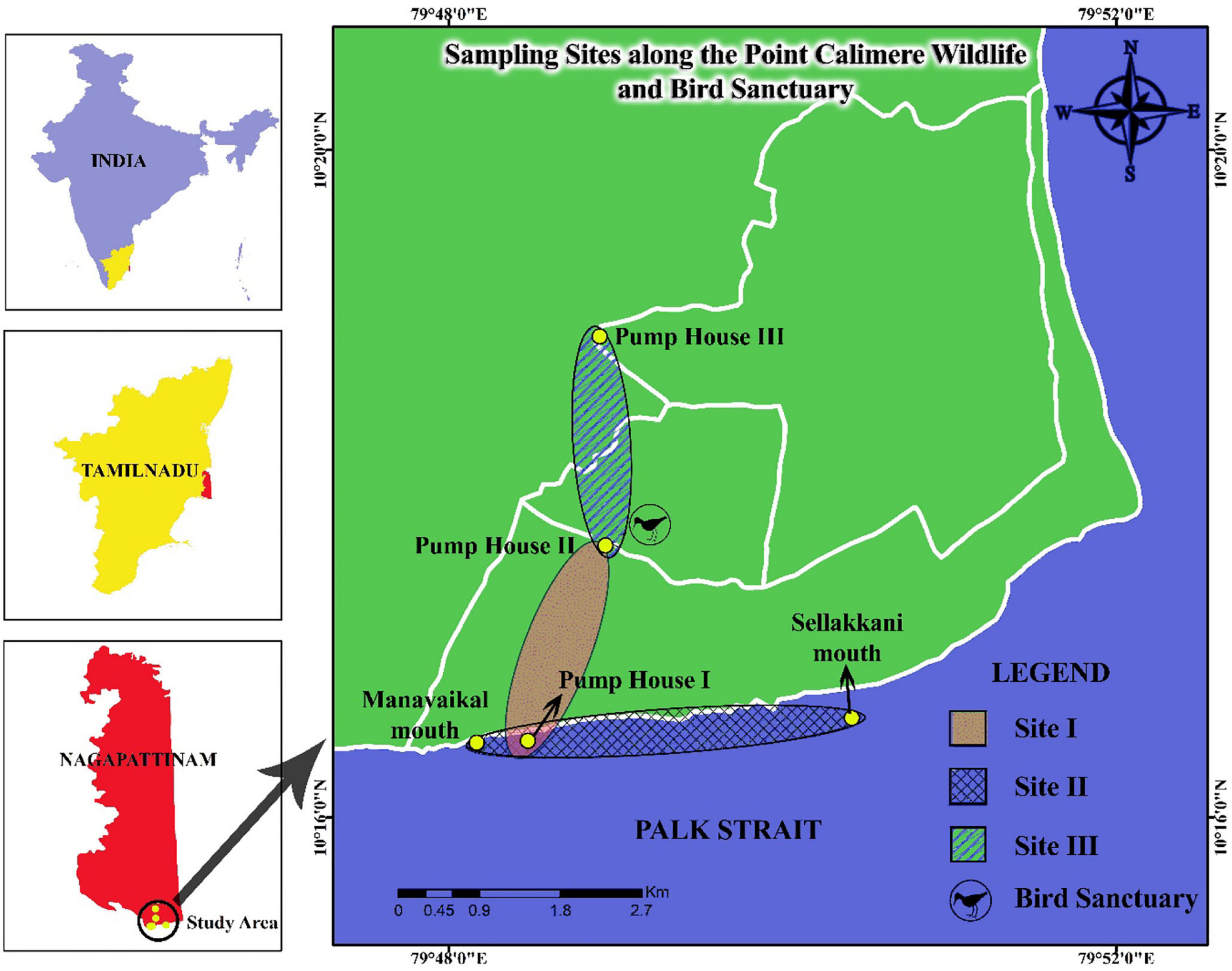

Fig. 1 Map of the sampling sites along the Point Calimere Wildlife and Bird Sanctuary

of birds were collected from January to March 2019 in the selected sites of Point Calimere Wildlife and Bird Sanctuary. Out of all the feathers collected from the study area, only the tail and wing feathers were used for the study. It is to be noted that only molten feathers of the birds were collected without interacting with the birds or causing any harm to them; therefore, no ethical clearance was needed for this study. The collected feathers were carefully sealed in sterile zip-lock bags and transported to the laboratory. The handbook on Indian wetland birds and their protection by Kumar et al. (2005) was used to identify the species of the birds that the feathers belonged to. The collected seabirds' feathers belonged to the following 11 species: Northern pintail (*Anas acuta*), Greater flamingo (*Phoenicopterus roseus*), Great egret (*Ardea alba*), Indian pond heron (*Ardeola grayii*), Painted stork (*Mycteria leucocephala*), Spot billed pelican (*Pelecanus philippensis*), Common tern (*Sterna hirundo*), Terek sandpiper (*Xenus cinereus*), Western reef egret (*Egretta gularis*), Caspian tern (*Hydroprogne caspia*), and Heuglin's gull (*Larus heuglini*). In addition to the feathers, sediments (500 g each) from across different locations in the bird sanctuary were collected in sterile plastic containers and transported to the laboratory.

Feather sample processing

Further in the laboratory, feathers were cleaned with acetone and rinsed with deionized water thrice to get rid of all external contaminants, such as dust and other particles (Burger and Gochfeld 1993). Washing of feathers with water gets rid of the dust and external airborne particles effectively, while acetone being a surfactant wipes off the oil, lipid contents, and other sticky substances. Treating feathers using this method removes a considerable fraction of external contamination, but the contamination accumulated through preening probably cannot be removed entirely (Jaspers et al. 2008). The feathers were then washed twice with deionized water, followed by oven drying at 60 °C for 48 h. Thereafter, the feathers for each species were cut into small pieces. Then, a portion of 1 g of feathers was taken up for each individual species and transferred to labeled beakers for the acid digestion. A reagent comprising 5 ml of nitric acid (69%) and 5 ml of hydrogen peroxide (30%) was added to the samples in the same ratio, and the beaker was kept on a hot plate at 70 °C until the acid digestion process was complete. Final extract was cooled to room temperature, filtered using Whatman filter paper (grade 42; diameter 90 mm), and made up to 25-ml portions by adding deionized water. This analytical method was adopted by Gruz et al. (2018).

Sediment sample processing

Sediment samples were air-dried and ground into a fine powder using pestle and mortar, for easier acid digestion. In this method, 1 g of the powdered soil sample was added to 10 ml of acid reagent (HNO_3, H_2SO_4, and $HClO_4$ in the ratio of 5:2:1) and digested at about 60 to 100 °C on a hot plate until the total volume was reduced to 1 ml. Later, 5 ml of 2 N hydrochloric acid was added to perform digestion of the residues, and the solution was filtered using Whatman filter paper (grade 42; diameter 90 mm) and made up to 25 ml by adding distilled water. Following the above-mentioned procedure, the blank samples were also prepared without adding any samples (Arumugam et al. 2018).

Heavy metal analysis and quality control and assurance (QC/QA)

Using the atomic absorption spectrophotometer (AA7000-Shimadzu Company, Japan), the prepared samples were analyzed for the heavy metals at the following operating wavelengths of 213.85 nm for Zn, 324.75 nm for Cu, 228.80 nm for Cd, and 217.00 nm for Pb (Rajaram et al. 2017). To maintain the quality assurance, every sample processing was performed in a much more sterile and non-contaminated setting. In addition, all the chemical reagents including deionized water and acetone were freshly prepared. All glassware and storage containers were acid-washed and dried in a sterile condition to ensure that the entire process is contaminant-free. The quality assurance and quality control for the AAS analyses were maintained by analyzing the standards, and in addition the blank samples were run after each ten samples.

Statistical approach

The data obtained from the AAS analysis provided the essential information about the metal distribution among the feathers of different birds. All the data were processed using Microsoft Excel (Windows 2010) and presented in mean ± SD. One-way ANOVA was performed to analyze the species-specific variations of each elemental concentration using Origin 8.0 (Windows). Principal component analysis (PCA) revealed the interrelationship between the heavy metal concentrations based on the correlation matrix and was performed using PAST tool (version 3.0). The results of PCA were expressed in biplot. On the other hand, bioaccumulation factor (BAF) was calculated based on the ratio between the concentration of metal in environmental sources, including sediment samples, and the feathers of the birds (Rajaram et al. 2020).

Results and discussion

Heavy metal distribution across different species

Out of the four metals analyzed, the essential metals, copper (Cu) and zinc (Zn), were detected in all the bird species.

Cadmium (Cd) concentration was reported only in two bird species, and in the remaining nine bird species, the concentrations were below detection limit or were not detected. The concentration of lead (Pb) was below the detection limits or was not detected for all 11 species of birds studied. The metal concentrations observed in the bird feathers are presented as mean ± SD and tabulated in Table 1. The mean concentrations of metals across all species of birds ranged as follows: Zn (15.24–40.55 μg/g), Cu (0.34–7.65 μg/g), and Cd (0.04–0.82 μg/g). The concentrations of Cu and Zn among the different species of birds were statistically significant at 0.05 levels ($p < 0.0001$; $f = 77.07$) and ($p < 0.0001$; $f = 26.10$), respectively. The maximum mean concentration of Zn (40.55 ± 2.33 μg/g) was recorded in Terek sandpiper (*X. cinereus*), whereas Cu (7.64 ± 0.79 μg/g) was highest in Northern Pintail (*A. acuta*). The minimum mean concentration of Zn (15.24 ± 1.83 μg/g) was recorded in Heuglin's gull (*L. heuglini*), whereas Cu (0.34 ± 0.08 μg/g) was lowest in Western reef egret (*E. gularis*). Out of the two reported concentrations for Cd, the higher value of 0.82 ± 0.10 μg/g was observed in Heuglin's gull (*L. heuglini*), and the lower value of 0.04 ± 0.01 μg/g was in Northern pintail (*A. acuta*). The metal concentrations for the sediment and their comparison with the permissible limits are presented in Table 2. The metal concentration in sediments for Cu was 10.51 ± 5.66 μg/g, Zn was 70.89 ± 53.05 μg/g, Cd was 0.57 ± 0.16 μg/g, and Pb was 1.75 ± 0.46 μg/g, following the decreasing order of Zn > Cu > Cd > Pb. The mean concentrations of metals for all the bird species studied here, compared with the concentrations reported by various researchers worldwide, are shown in Table 3. Among the birds studied, the concentration of Zn was observed in the following descending order *X. cinereus* (40.55 ± 2.33 μg/g) > *A. acuta* (32.24 ± 3.06 μg/g) > *A. alba* (31.31 ± 2.9 μg/g) > *M. leucocephala* (29.98 ± 1.99 μg/g) > *P. philippensis* (26.28 ± 1.83 μg/g) > *A. grayii* (25.46 ± 2.9 μg/g) > *E. gularis* (23.98 ± 1.61 μg/g) > *H. caspia* (23.72 ± 1.41 μg/g) > *S. hirundo* (23.57 ± 1.49 μg/g) > *P. roseus* (21.44 ± 2.56 μg/g) > *L. heuglini* (15.24 ± 1.83 μg/g). The Cu concentrations were arranged as *A. acuta* (7.64 ± 0.79 μg/g) > *A. alba* (5.35 ± 0.79 μg/g) > *X. cinereus* (4.62 ± 0.55 μg/g) > *H. caspia* (2.24 ± 0.34 μg/g) > *P. philippensis* (1.88 ± 0.31 μg/g) > *M. leucocephala* (1.87 ± 0.34 μg/g) > *P. roseus* (1.8 ± 0.33 μg/g) > *L. heuglini* (1.05 ± 0.27 μg/g) > *A. grayii* (0.92 ± 0.42 μg/g) > *S. hirundo* (0.46 ± 0.16 μg/g) > *E. gularis* (0.34 ± 0.08 μg/g).

Principal component analysis (PCA)

The results of PCA are presented in Fig. 2. Three principal components were extracted, namely, PC1, PC2, and PC3, with the eigenvalues of 1.97, 0.82, and 0.20, respectively. Among them, PC3 is not considered due to the lesser eigenvalue. PC1 accounts for 49.34% variance with the positive loading of Zn (0.67) and Cu (0.55), while PC2 accounts for 20.56% of cumulative variance with the positive loading for Cu (0.63) and Cd (0.77). PC1 shows the combinations of both Cu and Zn, as they are essential elements. This denotes that their natural sources might be from the dietary habitat or other natural process. Our discussion is supported by Tsipoura et al. (2008), who claimed species-specific variations in the metal concentration due to the unusual diet nature (Tsipoura et al. 2008). In the case of PC2, both Cu and Cd showed positive loading, leading to the possibility that they might have originated from both natural and manmade activities. Cu is known to be the essential element of living system used to carry some of the important biological functions and occurs from either environmental exposure or from the dietary route. However, the excessive amount of Cu has the potential to negatively

Table 1 Heavy metal concentrations observed in 11 different species of bird feathers from Point Calimere Wildlife and Bird Sanctuary, Tamil Nadu, India (μg/g)

Sample number	Common name	Scientific name	Cu Mean ± SD	Zn Mean ± SD	Cd Mean ± SD	Pb Mean ±SD
1	Northern pintail	*Anas acuta*	7.64 ± 0.79	32.24 ± 3.06	0.04 ± 0.01	ND
2	Greater flamingo	*Phoenicopterus roseus*	1.80 ± 0.33	21.44 ± 2.56	ND	ND
3	Great egret	*Ardea alba*	5.35 ± 0.79	31.31 ± 2.90	ND	ND
4	Indian pond heron	*Ardeola grayii*	0.92 ± 0.42	25.46 ± 2.90	ND	ND
5	Painted stork	*Mycteria leucocephala*	1.87 ± 0.34	29.98 ± 1.99	ND	ND
6	Spot billed pelican	*Pelecanus philippensis*	1.88 ± 0.31	26.28 ± 1.83	ND	ND
7	Common tern	*Sterna hirundo*	0.46 ± 0.16	23.57 ± 1.49	ND	ND
8	Terek sandpiper	*Xenus cinereus*	4.62 ± 0.55	40.55 ± 2.33	ND	ND
9	Western reef egret	*Egretta gularis*	0.34 ± 0.08	23.98 ± 1.61	ND	ND
10	Caspian tern	*Hydroprogne caspia*	2.24 ± 0.34	23.72 ± 1.41	ND	ND
11	Heuglin's gull	*Larus heuglini*	1.05± 0.27	15.24 ± 1.83	0.82 ± 0.10	ND

Metal concentrations in mean ± SD (μg/g dry weight)

Table 2 Heavy metal concentrations in sediment samples from Point Calimere Wildlife and Bird Sanctuary, Tamil Nadu, India, compared with standard permissible limits (μg/g)

Comparison of metal concentration in sediment with permissible limits

Samples	Metals	Present report	Permissible limits (ppm)
Sediments	Cu	10.51	18.7 (CEQG 2001); 19 (EPA 1986)
	Zn	70.89	124 (CEQG 2001 and EPA 1986)
	Cd	0.56	0.7 (CEQG 2001); 0.68 (EPA 1986)
	Pb	1.75	30.2 (CEQG 2001)

Metal concentrations expressed in mean (μg/g dry weight)

impact the organisms. By considering the biplot (Fig. 2) in the present study, only Heuglin's gull (*L. heuglini*) has elevated level of Cd, and only a few birds like *Anas acuta*, *Ardea alba*, and *X. cinereus* were associated with elevated levels of Cu and Zn.

Bioaccumulation factor (BAF)

BAF values of bird feathers are presented in Fig. 3. The ranges of BAF values are as follows for Cu (0.03–0.73), Zn (0.22–0.57), Cd (0–1.43), and Pb (0). Irrespective of bird species, the BAF values were varied in the following order: Cd < Cu < Zn < Pb. As compared with all other metals, Cd concentration was found to be elevated only in *L. heuglini* showing the BAF value of 1.43. However, BAF values of other elements were found to be within the guided levels.

Comparison of metal concentrations with worldwide data

In *A. acuta*, the mean concentration of Cu (7.64 ± 0.79 μg/g) reported in our study is lower than the concentrations reported in China (24.5 μg/g) and Northern Iraq (11.4 μg/g) reported by Eun-Young et al. (1996) and Sadeghi et al. (2019), respectively. Also, lower concentrations of Zn (32.24 ± 3.06 μg/g) have been reported in our study area, compared with the higher concentrations reported in the studies conducted in China (74.6 μg/g) and Northern Iraq (53.650–105.54 μg/g) (Eun-Young et al. 1996; Karimi et al. 2016; Sadeghi et al. 2019). The concentration of Cd (0.04 ± 0.01 μg/g) reported for *A. Acuta* in our study is lower than the values (0.210–1.090 μg/g) reported in various studies conducted in the Freidounkenar International Wetland in Northern Iraq (Karimi et al. 2016; Sadeghi et al. 2019); however, no Cd value is observed by Eun-Young et al. (1996) in their study in Chuan, Russia. Pb concentrations in the range of 3.050–10.480 μg/g are reported by others in Northern Iraq and Mexico (Karimi et al. 2016; Rendón-von Osten et al. 2001; Sadeghi et al. 2019).

Considerably lower concentrations of Cu (1.8 ± 0.33 μg/g) and Zn (21.44 ± 2.56 μg/g) in *P. roseus* were reported in our study compared with concentrations ranging from 3.75 to 9.85 μg/g for Cu and 38.65–147.0 μg/g for Zn reported in Western Europe by Amiard-Triquet et al. (1991), Borghesi (2009), and Borghesi et al. (2016, 2017) in places such as Macchiareddu saltpans, Italy; Étang du Fangassier, Southern France; Comacchio saltpans, North Eastern Italy; Odiel marshlands, Southern Spain; Venice, Italy; Fuente de Piedra, Southern Spain; and French Mediterranean coast, France. Cd concentrations in the range of 0.052–1.933 μg/g and Pb in the range of 0.041–3.59 μg/g are reported by Amiard-Triquet et al. (1991), Borghesi (2009), and Borghesi et al. (2016, 2017). In *A. alba*, also referred to as *Egretta alba* in the past, Cd, ranging from 0.0305 to 0.12 μg/g, and Pb, ranging from 0.0543 to 4.80 μg/g, concentrations are assessed and reported by Burger (2013) in New Jersey and Burger and Gochfeld (1993) in Hong Kong. They did not assess concentrations of Cu and Zn in their studies; however, in the present study, we have observed mean concentrations of Cu (5.35 ± 0.79 μg/g) and Zn (31.31 ± 2.9 μg/g) only. In a similar study in Korea, the concentrations of Cu (10.2 ± 1.39 μg/g), Zn (114 ± 7.58 μg/g), Cd (0.024 ± 0.008 μg/g), and Pb (1.44 ± 0.42 μg/g) are reported in wet weight by Honda et al. (1986).

In *A. grayii*, mean concentrations of 0.92 ± 0.42 and 25.46 ± 2.9 μg/g were observed for Cu and Zn, respectively. In a similar study carried out during 1998–1999 in the Nilgiris, India, metal concentrations in the ranges of 11.12–235.43, 100.97–188.90, 0.70–11.01, and 3.22–9.19 μg/g were observed for Cu, Zn, Cd, and Pb, respectively (Muralidharan et al. 2004). In a study conducted in the outskirts of Lahore city, Pakistan, Tasneem et al. (2020) reported metal concentrations (Cu, 2.62 ± 0.53 μg/g; Zn, 100.97 ± 33.7 μg/g; Cd, 0.98 ± 0.23 μg/g; Pb, 31.62 ± 9.80 μg/g) in the tail feathers of *A. grayii.* The concentrations observed for *A. grayii* in our study area are lower than that reported by Muralidharan et al. (2004) and Tasneem et al. (2020).

In the latest study by Pandiyan et al. (2020), metal concentrations have been reported in *M. leucocephala* from Pichavaram Mangrove Forest, which lies approximately 130 km north of Point Calimere Bird Sanctuary—our study area. In their study, Cu (0.6 ± 0.18 μg/g) and Zn (1.6 ± 0.17 μg/g) concentrations were found to be lower than concentrations of Cu (1.87 ± 0.34 μg/g) and Zn (29.98 ± 1.99 μg/g) observed in our present study. However, concentrations of Pb (8.1 ± 0.85 μg/g) and Cd (0.4 ± 0.11 μg/g) are reported in their study which is not detected by us.

For *P. philippensis*, there are no reports of metal concentrations in the feathers. Our study is the first to report the metal concentrations in the feathers of *P. philippensis* in the Point Calimere Wildlife and Bird Sanctuary, India. We report the mean metal concentrations of 1.88 ± 0.31 and 26.28 ± 1.83 μg/g for Cu and Zn, respectively.

Table 3 Heavy metal concentrations in avian feathers in the present study compared with similar studies worldwide (µg/g)

Sample number	Common name (*Scientific name*)	Location	Copper	Zinc	Cadmium	Lead	Studied by
1	Northern pintail(*Anas acuta*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	7.64 ± 0.79$_a$	32.24 ± 3.06$_a$	ND	ND	Present study
		Chaun, Russia	24.500$_b$	74.600$_b$	ND	NA	Eun-Young et al. (1996)
		Fereydoon Kenar International Wetland site	11.400$_b$	105.540$_b$	0.210$_b$	10.480$_b$	Sadeghi et al. (2019)
		Freidounkenar International Wetland, southern Caspian Sea, Northern Iraq	NA	53.650$_c$	1.090$_c$	3.050$_c$	Karimi et al. (2016)
		Freidounkenar International Wetland, southern Caspian Sea, Northern Iraq	NA	67.630$_c$	1.010$_c$	3.510$_c$	Karimi et al. (2016)
		Pabellon Inlet, Sinaloa, Mexico	NA	NA	NA	10.320$_b$	Rendón-von Osten et al. (2001)
2	Greater flamingo (*Phoenicopterus roseus*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	1.8 ± 0.33$_a$	21.44 ± 2.56$_a$	ND	ND	Present study
		Macchiareddu saltpans, Cagliari, Sardinia, Italy	3.76 ± 0.40$_a$	111 ± 7$_a$	EWR	0.46 ± 0.08$_a$	Borghesi et al. (2016)
		Étang du Fangassier, Camargue, Southern France	4.66 ± 0.48$_a$	90 ± 6$_a$	EWR	0.57 ± 0.15$_a$	Borghesi et al. (2016)
		Comacchio saltpans, North Eastern Italy	3.95 ± 0.36$_a$	118 ± 8$_a$	EWR	1.93 ± 0.51$_a$	Borghesi et al. (2016)
		Odiel marshlands, Huelva, Southern Spain	4.20 ± 0.39$_a$	133 ± 6$_a$	EWR	0.68 ± 0.13$_a$	Borghesi et al. (2016)
		Cagliari, Italy	3.95 ± 0.6$_a$	114 ± 15.1$_a$	NA	0.49 ± 0.20$_a$	Borghesi (2009)
		Comacchio, Italy	4.12 ± 0.53$_a$	127.2 ± 13.5$_a$	NA	1.97 ± 0.76$_a$	Borghesi (2009)
		Odiel, Spain	4.35 ± 0.59$_a$	130.9 ± 16.1$_a$	NA	0.64 ± 0.16$_a$	Borghesi (2009)
		Venice, Italy	4.65 ± 0.66$_a$	147.0 ± 7.7$_a$	NA	2.12 ± 0.59$_a$	Borghesi (2009)
		Camargue Biosphere Reserve, France	4.31 ± 1.64$_a$	85.0 ± 16.1$_a$	0.062 ± 0.031$_a$	3.59 ± 1.21$_a$	Amiard-Triquet et al. (1991)
		Camargue Biosphere Reserve, France	3.75 ± 1.03$_a$	90.5 ± 17.7$_a$	0.052 ± 0.024$_a$	3.25 ± 1.11$_a$	Amiard-Triquet et al. (1991)
		Aigues-Mortes, southern France	9.848$_c$	43.312$_c$	1.656$_c$	0.061$_c$	Borghesi et al. (2017)
		Fuente de Piedra, southern Spain	7.225$_c$	40.482$_c$	1.289$_c$	0.041$_c$	Borghesi et al. (2017)
		Odiel Marshes, southern Spain	8.509$_c$	38.652$_c$	1.933$_c$	0.148$_c$	Borghesi et al. (2017)
3	Great egret (*Ardea alba*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	5.35 ± 0.79$_a$	31.31 ± 2.9$_a$	ND	ND	Present study
		Barnegat Bay, New Jersey	NA	NA	0.0305 ± 0.0107$_d$	0.0543 ± 0.0173$_d$	Burger (2013)
		Mai Po Nature Reserve heronry, Hong Kong	NA	NA	0.072$_d$	1.500$_d$	Burger and Gochfeld (1993)
		Mai Po Nature Reserve heronry, Hong Kong	NA	NA	0.12± 12$_d$	4.8± 0.67$_d$	Burger and Gochfeld (1993)
4	Indian pond heron (*Ardeola grayii*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	0.92 ± 0.42$_a$	25.46 ± 2.9$_a$	ND	ND	Present study
		Manga mandi and Muridke in the outskirts of Lahore, Pakistan	2.62 ± 0.53$_a$	122.1 ± 47.5$_a$	0.98 ± 0.23$_a$	31.62 ± 9.80$_a$	Tasneem et al. (2020)
		Nilgiris, Tamil Nadu, India	11.12–235.43$_e$	100.97–188.9$_e$	0.70–11.01$_e$	3.22–9.19$_e$	

Table 3 (continued)

Sample number	Common name (*Scientific name*)	Location	Copper	Zinc	Cadmium	Lead	Studied by
							Muralidharan et al. (2004)
5	Painted stork (*Mycteria leucocephala*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	$1.87 \pm 0.34_{a}$	$29.98 \pm 1.99_{a}$	ND	ND	Present study
		Pichavaram Mangrove Forest, Tamil Nadu, India	$0.6 \pm 0.18_{d}$	$1.6 \pm 0.17_{d}$	$0.4 \pm 0.11_{d}$	$8.1 \pm 0.85_{d}$	Pandiyan et al. (2020)
6	Spot billed pelican (*Pelecanus philippensis*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	$1.88 \pm 0.31_{a}$	$26.28 \pm 1.83_{a}$	ND	ND	Present study
7	Common tern (*Sterna hirundo*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	$0.46 \pm 0.16_{a}$	$23.57 \pm 1.49_{a}$	ND	ND	Present study
		Bird Island, Massachusetts, USA	NA	NA	$0.104 \pm 0.01_{d}$	$1.15 \pm 0.102_{d}$	Burger et al. (1994)
		New York Bight, USA	NA	NA	$0.0471–0.051_{e}$	$1.3653\text{-}1.6159_{e}$	Burger and Gochfeld (1991)
8	Terek sandpiper (*Xenus cinereus*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	$4.62 \pm 0.55_{a}$	$40.55 \pm 2.33_{a}$	ND	ND	Present study
		Okgu Mudflat, Korea	$11.7 \pm 3.21_{a}$	$96.5 \pm 22.4_{a}$	$0.46 \pm 0.41_{a}$	$5.95 \pm 2.08_{a}$	Kim and Oh (2012)
9	Western reef egret (*Egretta gularis*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	$0.34 \pm 0.08_{a}$	$23.98 \pm 1.61_{a}$	ND	ND	Present study
		Hara biosphere reserve, southern Iran	$10.1 \pm 2.1_{d}$	$60.88 \pm 10_{d}$	$1.13 \pm 0.4_{d}$	$6.40 \pm 4.3_{d}$	Mansouri et al. (2012)
		Hara biosphere reserve, southern Iran	$10.3 \pm 1.7_{d}$	$52.69 \pm 9_{d}$	$1.87 \pm 0.7_{d}$	$4.30 \pm 1.8_{d}$	Mansouri et al. (2012)
10	Caspian tern (*Hydroprogne caspia*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	$2.24 \pm 0.34_{a}$	$23.72 \pm 1.41_{a}$	ND	ND	Present study
		Penguin Island, Western Australia	NA	NA	0.001_{b}	0.074_{b}	Dunlop and McNeil (2017)
11	Heuglin's gull (*Larus heuglini*)	Point Calimere Bird Sanctuary, Tamil Nadu, India	$1.05 \pm 0.27_{a}$	$15.24 \pm 1.83_{a}$	ND	ND	Present study
		Hara biosphere reserve, southern Iran	$4.10 \pm 0.1_{d}$	$43.79 \pm 6_{d}$	$1.04 \pm 0.4_{d}$	$8.75 \pm 1.8_{d}$	Mansouri et al. (2012)
		Hara biosphere reserve, southern Iran	$5.06 \pm 0.9_{d}$	$47.53 \pm 5_{d}$	$1.28 \pm 0.6_{d}$	$6.52 \pm 3.7_{d}$	Mansouri et al. (2012)

Mean concentrations of heavy metal concentrations expressed in μg/g dry weight in the feathers of birds

EWR excluded while reporting, *NA* not assessed, *ND* not detected

a—mean ± SD; b—mean; c—median; d—mean ± SE; e—range of mean

In the 1990s, studies conducted in the USA have reported concentration ranges of Cd (0.0471–0.104 μg/g) and Pb (1.15–1.616 μg/g) in the feathers of *S. hirundo*; however, those studies have not assessed the concentrations of Cu and Zn (Burger and Gochfeld 1991; Burger et al. 1994). For *S. hirundo*, we report here the metal concentrations of Cu and Zn to be 0.46 ± 0.16 and 23.57 ± 1.49 μg/g, respectively. In the feather of *X. cinereus*, the concentrations of Cu and Zn are reported to be 4.62 ± 0.55 and 40.55 ± 2.33 μg/g, respectively. In a study in the Okgu Mudflat, Korea, more than twofold concentrations of Cu (11.7 ± 3.21 μg/g) and Zn (96.5 ± 22.4 μg/g) are reported. The study also reports Cd (0.46 ± 0.41 μg/g) and Pb (5.95 ± 2.08 μg/g) concentrations (Kim and Oh 2012).

In the present study, for *E. gularis* only metal concentrations of Cu (0.34 ± 0.08 μg/g) and Zn (23.98 ± 1.61 μg/g) are observed. In comparison, Mansouri et al. (2012) has analyzed the feathers of both male and female birds from Hara

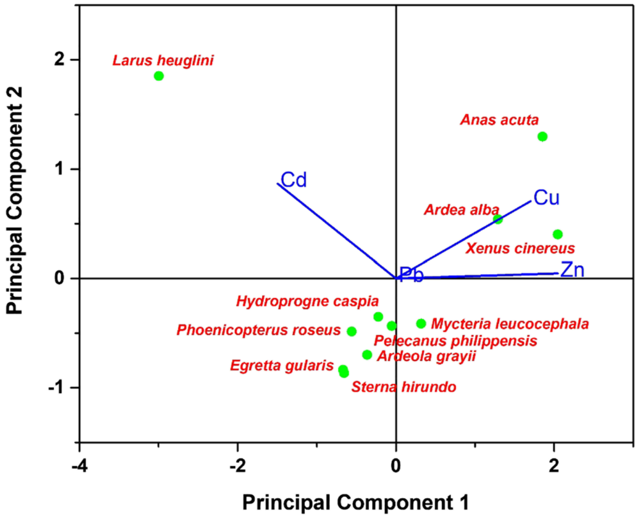

Fig. 2 Representation of interrelationship of metal concentration among the feather samples by biplot analysis

Biosphere Reserve, Southern Iran, and has reported the range of metal concentrations as follows: Cu (10.1–10.3 μg/g), Zn (52.69–60.88 μg/g), Cd (1.13–1.87 μg/g), and Pb (4.30–6.40 μg/g). The concentrations of metals in *E. gularis* feathers from Point Calimere Wildlife and Bird Sanctuary are way lower that those reported in Hara Biosphere Reserve, Iran. In our study, we report the concentrations of Cu and Zn to be 2.24 ± 0.34 and 23.72 ± 1.41 μg/g, respectively, in the feathers of *H. caspia*. In a similar study from Penguin Island, Western Australia, trace concentrations of Cd (0.001 μg/g) and Pb (0.074 μg/g) in the feathers of *H. caspia* are reported (Dunlop and McNeill 2017).

With regards to the feathers of *L. heuglini*, metal concentrations of Cu (1.05 ± 0.27 μg/g), Zn (15.24 ± 1.83 μg/g), and Cd (0.82 ± 0.10 μg/g) are observed. Similarly, in a study by Mansouri et al. (2012) in the Hara Biosphere Reserve, Southern Iran, metal concentrations in feathers of *L. heuglini* are studied in both male and female birds. The metal concentrations reported by them fall in the ranges as follows: Cu (10.1–10.3 μg/g), Zn (52.69–60.88 μg/g), Cd (1.13–1.87 μg/g), and Pb (4.30–6.40 μg/g). The concentrations of metals in *L. heuglini* from Point Calimere Wildlife and Bird Sanctuary are considerably lesser that those reported by Mansouri et al. (2012).

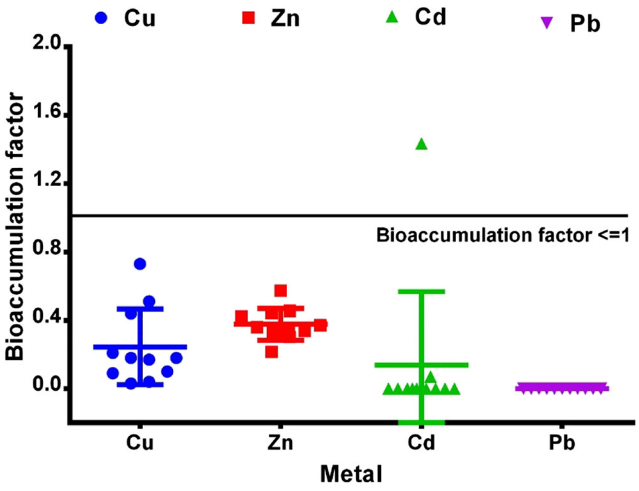

Fig. 3 Variations in the level of bioaccumulation factors (BAF) with reference to metal concentration. BAF greater than one is an indicative of potent accumulation.

Conclusion

Overall, our results highlight that the essential metals (Cu and Zn) are present in the feathers of birds of Point Calimere Wildlife and Bird Sanctuary. Cd, which is a non-essential metal, was present only in two species of birds, namely, Heuglin’s gull (*Larus heuglini*) and Northern pintail (*Anas acuta*) in trace levels. Pb, another non-essential metal, was totally not detected in the bird feathers collected during the study. In worldwide comparison, our result shows that the ecosystem of Point Calimere Wildlife and Bird Sanctuary is still not polluted with respect to heavy metals. Metal concentrations in the sediments of the sanctuary fall below the permissible limits. The bioaccumulation factors for the metals studied in bird feather followed the order of Cd < Cu < Zn < Pb. With the inference to our results and comparison of our data with worldwide reports in bird feathers, we conclude that the sanctuary is pristine with respect to the heavy metal pollution. Regular monitoring of the sites regarding water quality parameters and heavy metal pollutions is to be mandated. In the future, monitoring and rehabilitation programs are to be excessively implemented by the government to preserve the wetlands and mangrove ecosystems of Tamil Nadu, which are crucial for the avian population. In short, as per the proverb “a stitch in time saves nine,” an effort today to preserve the Point Calimere Wildlife and Bird Sanctuary could prevent losing the habitat forever.

Acknowledgments All the authors thank the Department of Marine Science, Bharathidasan University, Tiruchirappalli, India for facilitating the study, and convey their gratitude to the Ministry of Earth Science, Government of India (Ref. No.: MoES/36/OOIS/Extra./9/2013) for providing AAS facilities. The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this Research Group Project No. RGP-289.

Authors’ contributions V.A.: conceived and designed the experiments, analyzed data, wrote the paper. E.C.P.: conceived and designed the experiments, analyzed data, wrote the paper. G.A.: analyzed data, wrote the paper. V.S.: collected and identified the samples. R.R.: conceived and designed the experiments, collected and identified the samples, analyzed data, language editing. B.A.P.: data analysis, language editing. M.K.A.-S.: data analysis, language editing. A.R.A.-M.: data analysis, language editing.

Funding This work was supported by the Ministry of Earth Science, Government of India (Ref. No.: MoES/36/OOIS/Extra./9/2013) by providing the AAS facilities. This work also supported by the Deanship of Scientific Research at King Saud University through Research Group Project No. RGP-289.

Availability of data and materials The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare that they have no competing interests.

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Identification of heavy metal pollution source due to idol immersion activity across the Cauvery river basin, Tamil Nadu, South India

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Idol immersion activities alter the hydrological parameters of an aquatic body. However, relevant research in the Cauvery river basin in terms of idol immersion activity has been limited. In the present study, a total of 29 water and topsoil samples were collected from the Cauvery basin before and after idol immersion, and evaluated for the presence of metals. The experimental results showed elevated Cd and Pb levels in water and sediment samples of both Cauvery and Kollidam rivers. Strong statistical significance was observed for all the elements studied in the soil samples collected before and after idol immersion ($P < 0.01$). Industrial effluents, textile waste, untreated sewage, municipal waste and agricultural activities are the most common causes of elevated levels of heavy metals in the study area. Further, geo-accumulation index and pollution load index studies showed lesser impact of idol immersion on metal distribution compared to other sites reported from India. However, strict regulatory policies of the concerned authorities help maintain the quality of the Cauvery basin.

Keywords: Geo-accumulation index, heavy metals, idol immersion, pollution load index, river basin.

WATER plays a vital role in the survival of life on Earth. Several sources fulfil the requirement of water like rainwater, rivers, lakes, ponds, etc. Among them, the river ecosystem is considered as essential as it serves many purposes like irrigation, increasing the groundwater table, etc.[1]. In India, about 37,000 km^3 of water and 13.5×10^9 tonnes of sediments are transported into the sea by rivers[2–4]. The Cauvery, Vaigai and Thamirabarani are the three major rivers of southern Tamil Nadu, India. The Vaigai flows through Theni, Madurai, Sivagangai and Ramanathapuram districts of the state. The inhabitants of Tirunelveli and Tuticorin depend on the Thamirabarani for water[5,6]. The Cauvery provides irrigation for five major districts of Tamil Nadu, viz. Karur, Namakkal, Tiruchirappalli, Thanjavur and Nagapattinam[7,8].

The source of the heavy metals could be traced to the weathering of rocks, and these metals get accumulated in the aquatic ecosystems. Due to urbanization and anthropogenic activities, the concentration of heavy metals has increased[9–11]. These changes create unfavourable conditions for the survival of living organisms by altering the physicochemical parameters, nutrient levels, and water quality index. About 99% of heavy metals deposited in river sediments are from various routes[12]. Bioaccumulation of heavy metals and bio-magnification occur through the food-chain. Finally, when the river water reaches the sea, the marine ecosystem gets polluted with these toxic metals, ultimately causing health issues in human beings who rely upon the marine organisms for their diet[13–15].

Not only industrial and agricultural pollutants, activities like idol immersion play an important role in river pollution. In India, every year during September–October, at the end of the festival season idols of Gods/Goddesses are immersed into the rivers, lakes, ponds, etc.[16]. Most of the idols are made of clay, plaster of Paris, cement and decorated by paint, clothes, bamboo and varnishes[17,18] which contain metals like lead, cadmium, copper, iron, zinc, chromium as well as different organic and inorganic substances. After idol immersion, these chemicals are dissolved in water and settle down as sediments eventually transferred to the food-chain[19–22]. Idol immersion contaminates the water bodies which are the source of irrigation. Thus crops are affected by heavy metals and there is a major impact of ecotoxicology[23].

Tamil Nadu is one of the diverse biospheres in India, consisting of both biologically and economically important zones. Currently, several anthropogenic activities are disturbing the life of aquatic organisms[24,25]. The present study was conducted in River Cauvery from Mettur dam to Poombuhar, and a branch of the river called Kollidam from Trichy, Mookumbu to Pazhayar. We examined the impact of immersion of idols in South India, especially in the Cauvery river basin. The objectives of the study were as follows: (i) Assessment of concentration of both essential (Cu, Zn) and non-essential (Cd, Pb) elements in river samples before and after idol immersion. (ii) Comparison of metal concentrations before and after the immersion of

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Table 1. Study area description and geographical coordinates

Station no.	Possible activities causing pollution	Latitude	Longitude
Description of Cauvery river sampling sites			
C1	Textiles industries and spinning mills	77°75′98″	11°65′04″
C2	Mettur Thermal Power Station dump	77°74′37″	11°57′60″
C3	Textiles industries and spinning mills	77°69′06″	11°45′16″
C4	Textiles industries and spinning mills	77°43′37″	11°45′16″
C5	Textiles industries and spinning mills	77°88′98″	11°07′76″
C6	Dye and bleaching industries	78°23′51″	10°96′02″
C7	Dye and bleaching industries	78°41′83″	10°94′76″
C8	Medical waste and sewage	78°57′87″	10°88′25″
C9	Medical waste and sewage	78°69′85″	10°83′84″
C10	Agricultural run-off and domestic sewage	78°81′90″	10°83′03″
C11	Agricultural run-off and domestic sewage	78°94′99″	10°85′47″
C12	Agricultural run-off and domestic sewage	79°08′58″	10°87′60″
C13	Agricultural run-off and domestic sewage	79°25′54″	10°94′14″
C14	Agricultural run-off and domestic sewage	79°38′30″	10°96′99″
C15	Agricultural run-off and domestic sewage	79°49′23″	11°03′39″
C16	Agricultural run-off and domestic sewage	79°56′14″	11°08′04″
C17	Agricultural run-off and domestic sewage	79°65′40″	11°10′48″
C18	Agricultural run-off and domestic sewage	79°78′16″	11°14′95″
C19	Agricultural run-off and domestic sewage	79°81′94″	11°14′51″
C20	Agricultural run-off, domestic sewage and fishing activities	79°85′71″	11°13′62″
Description of Kollidam river sampling sites			
K1	Agricultural run-off and sewage	78°70′36″	10°87′25″
K2	Agricultural run-off and sewage	78°83′33″	10°84′21″
K3	Agricultural run-off and sewage	78°97′82″	10°88′67″
K4	Agricultural run-off and sewage	79°03′70″	10°88′24″
K5	Sand mining	79°36′07″	11°05′36″
K6	Sand mining	79°45′48″	11°13′49″
K7	Sand mining	79°61′48″	11°24′10″
K8	Sewage, plastics household waste	79°70′71″	11°33′47″
K9	Fishing is major activity	79°83′02″	11°35′60″

idols. (iii) Identification of the potential source of heavy metals. (iv) Assessment of pollution load index and geo-accumulation index of analysed elements.

Materials and methods

Description of the study area

The Cauvery is a predominant river system of South India that provides water to most areas of Karnataka and Tamil Nadu. In the subcontinent, the Cauvery is the eighth longest river[26]. It influences the traditional life of the people in peninsular India, and also plays a major role in the flourishment of the region[27]. The Cauvery has 29 crucial creeks and branches. Water flow occurs during the southwest monsoon and northeast monsoons, except in Kodagu district, Karnataka. The temperature of the river basin is approximately 25°C and decreases at higher elevation[26]. The study area is surrounded by several industrial estates like textile, steel and automobiles. Agricultural activity is also present, which contributes to the economy of the people. Table 1 describes the exact geo-coordinates of the sampling points and possible source of pollution.

Sampling

To understand the impact of idol immersion, two different samples were collected, i.e. before and after the immersion. Triplicate water and sediment samples in one-month intervals were collected before (August 2018) and after (October 2018) idol immersions (Figure 1). One litre of water sample from each station was collected in acid-washed polystyrene containers and 1 ml of concentrated nitric acid was added[28]. A PVC pipe was used to extract the top 2-cm layer of the soil, and the samples were packed in sterilized zip-lock polythene covers. All the collected samples were stored at 4°C until further analysis[29].

Sample preparation

Water samples were filtered using the Millipore filtration unit to avoid debris-mediated contamination. In order to acidify the sample and to chelate the available elements, 1% freshly prepared APDC solution was added to the water sample. In addition, MIBK solvent was added to extract the metal–APDC complex. Finally, the samples containing organic layer were re-extracted using 50% HNO_3.

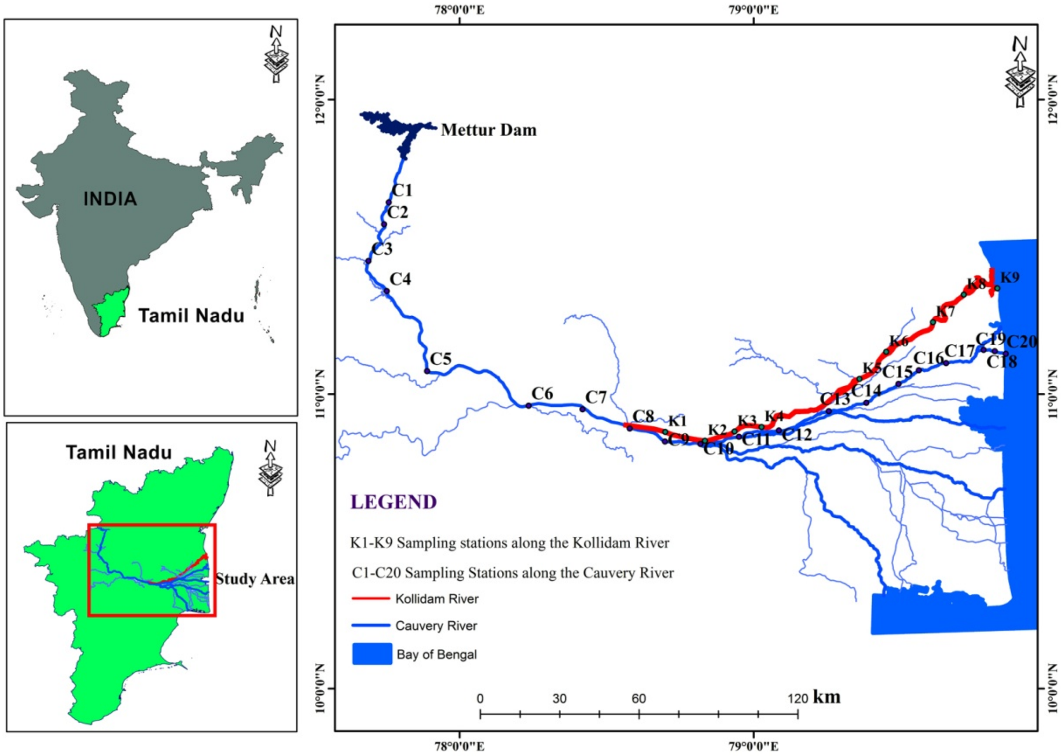

Figure 1. Location of sampling sites along the Cauvery and Kollidam rivers (Cauvery basin).

The extract was made up to 25 ml by adding distilled water[30]. The soil samples were air-dried and ground into a fine powder to perform acid digestion[31]. For this, 1 g of powdered soil sample was digested using 10 ml of acid reagent (HNO_3, H_2SO_4, and $HCLO_4$ in the ratio 5 : 2 : 1) at 60°–100°C on a hot plate until the volume was reduced to 1 ml. Then 5 ml of 2N hydrochloric acid was added to perform complete digestion. The solution was filtered and made up to 25 ml by adding distilled water. Following the above procedure blank samples were prepared[30].

Statistical analysis

One-way ANOVA was performed using SPSS software to determine the concentration of heavy metals in the water and soil samples at 0.05% level. The source of heavy metals was identified by multivariate statistical analysis such as principal component analysis (PCA) using PAST software.

Assessment of health of the study area

To assess the pollution status of the study area, we used the geo-accumulation index (I_{geo}), pollution load index (PLI) and contamination factor (CF).

$$I_{\text{geo}} = \log 2\left(\frac{C_{\text{n}}}{1.5\times B_{\text{n}}}\right),$$

where C_{n} is the heavy metal concentration in the sediment, and B_{n} is the background value of element in the earth crust. A factor of 1.5 was introduced to compensate the background content due to lithogenic effects[24].

$$\text{CF}_i = C_i/B_i,$$

where C_i is the concentration of individual elements studied and B_i is the background concentration of a particular element.

$$\text{PLI} = (\text{CF}_1 \times \text{CF}_2 \times \text{CF}_3 \times \ldots\ \text{CF}_n)1/n.$$

The PLI values were interpreted in two levels as polluted (PLI > 1) and unpolluted (PLI < 1)[25].

Quality assessment and assurance of the instrument

All the analyses of Cu, Cd, Pb and Zn were performed using atomic absorption spectrophotometer (AA7000-Shimadzu, Japan) based on the standardized laboratory method[29,30]. The air–acetylene combination was maintained in all samples. According to the manufacture's protocol, all the basic spectrometric corrections were

Table 2. Variation of metal concentration in Kollidam water (mg/l) and soil (mg/kg)

		Cu		Cd		Pb		Zn	
Sample	Station	Before	After	Before	After	Before	After	Before	After
Water	K1	0.3 ± 0.21	0.13 ± 0.04	0.27 ± 0.15	0.24 ± 0.08	ND	ND	ND	ND
	K2	ND	ND	0.45 ± 0.12	0.25 ± 0.05	ND	ND	ND	ND
	K3	ND	ND	0.62 ± 0.11	0.04 ± 0.06	ND	ND	ND	ND
	K4	ND	ND	0.44 ± 0.1	0.24 ± 0.11	ND	ND	ND	ND
	K5	ND	ND	0.36 ± 0.08	0.31 ± 0.10	ND	2.48 ± 0.50	ND	ND
	K6	ND	ND	0.18 ± 0.04	0.06 ± 0.07	ND	2.88 ± 1.63	ND	ND
	K7	ND	ND	0.32 ± 0.1	0.12 ± 0.11	ND	0.65 ± 0.10	ND	ND
	K8	ND	ND	0.37 ± 0.06	0.11 ± 0.04	ND	0.52 ± 0.09	ND	ND
	K9	ND	ND	0.27 ± 0.06	0.14 ± 0.07	ND	0.52 ± 0.09	ND	ND
Soil	K1	5.06 ± 1.29	2.89 ± 0.8	5.69 ± 1	1.33 ± 0.58	ND	4.79 ± 0.5	0.32 ± 0.1	6.89 ± 0.71
	K2	6.38 ± 1.16	2.73 ± 1.2	1.87 ± 0.65	2.12 ± 0.2	ND	0.85 ± 0.1	5.01 ± 1.1	0.16 ± 0.1
	K3	4.68 ± 1.09	2.53 ± 0.71	2.52 ± 1.01	1.42 ± 0.42	ND	0.74 ± 0.09	3.13 ± 0.96	0.11 ± 0.05
	K4	5.88 ± 0.95	2.77 ± 0.67	0.85 ± 0.13	ND	ND	1.12 ± 0.23	6.42 ± 0.8	ND
	K5	4.57 ± 0.87	2.03 ± 0.44	1.57 ± 0.53	ND	ND	1.92 ± 0.2	2.65 ± 0.67	5.64 ± 0.78
	K6	3.39 ± 0.22	3.11 ± 1.03	1.74 ± 0.86	ND	ND	1.69 ± 0.45	4.89 ± 1.99	3.58 ± 1.26
	K7	4.54 ± 0.63	3.85 ± 0.8	5.14 ± 1.14	ND	ND	0.68 ± 0.11	0.33 ± 0.1	2.15 ± 0.2
	K8	5.39 ± 0.91	5.36 ± 1.04	1.86 ± 0.55	ND	ND	1.65 ± 0.37	7.21 ± 1.1	3.7 ± 0.56
	K9	5.11 ± 0.48	3.03 ± 0.87	5.76 ± 1.32	0.06 ± 0.08	ND	7.62 ± 0.76	3.26 ± 0.95	4.2 ± 0.36

ND, Not detectable; values represent mean ± standard deviation.

performed. Calibrations of each element were settled by standard solutions made by stepwise dilution of the stock solution. The absorption wavelength was 228.8 nm for Cd, 324.7 nm for Cu, 217.0 nm for Pb and 213.9 nm for Zn.

Results and discussion

Heavy metal concentration in Kollidam river samples

Table 2 gives the concentration level of metals before and after idol immersion. Concentration of metals in the Kollidam sediment samples ranged from 3.39 ± 0.22 to 6.38 ± 0.22 mg/g for Cu, 0.85 ± 0.13 to 5.69 ± 0.99 mg/g for Cd, no traces of Pb and 0.32 ± 0.10 to 9.21 ± 1.10 mg/g for Zn. It varied significantly after idol immersion ranging from 2.03 ± 0.44 to 5.36 ± 1.04 mg/g for Cu, 0.06 ± 0.08 to 2.12 ± 0.20 mg/g for Cd, 0.68 ± 0.11 to 7.62 ± 0.76 mg/l for Pb and 0.11 ± 0.05 to 6.89 ± 0.71 mg/g for Zn. Before idol immersion in the Kollidam river water, the concentration range of Cu and Cd was 0 to 0.30 ± 0.21 mg/l and 0.18 ± 0.04 to 0.62 ± 0.11 mg/l) respectively. However, no traces of Pb and Zn were found in the samples. The concentration of heavy metals varied with respect to different stations, which could be attributed to a direct relation with idol immersion activity or by any other source of pollution.

In the Kollidam samples the trend of metal distribution was entirely different. Before idol immersion traces of Cd alone were detected in the water sample; Pb > Cd > Cu > no Zn (after idol immersion). In the soil samples, Cu > Zn > Cd > no Pb (before idol immersion); Cu > Zn > Pb > Cd (after idol immersion). In order to understand the significant impact of idol immersion in the both samples, metal concentration was evaluated using statistical analysis (non-parametric). The water samples showed significant variation in Cd and Pb concentration before and after idol immersion ($P < 0.01$). Similarly, strong statistical significance was observed for Cu, Cd, and Pb in the collected soil samples before and after idol immersion ($P < 0.01$).

In water samples before and after idol immersion, the mean concentration of Cu and Cd was higher than the BIS, IS-10500 (Bureau of Indian Standards); 2012, WHO (World Health Organization) and CPCB (Central Pollution Control Board) standards. Although no traces of Pb were recorded before idol immersion in both samples, after immersion Pb levels were elevated to several fold than the BIS, IS-10500 in water and less than the Canadian Environmental Quality Guidelines (CEQG, 2001) in the sediments. During the entire study period, Zn concentration was recorded below the permissible limits given by BIS, IS-10500 and CEQG 2001 (Table 3).

Heavy metal concentration in Cauvery River samples

Tables 4 and 5 list the mean and range of metal concentration among different sites of River Cauvery before and

Table 3. Permissible level of different metals in water and soil samples recommended by national and international agencies

Sample	Metal	Present study	BIS, IS-10500; 2012	WHO/CPCB
Water (mg/l)	Cu	0.20–1.73	0.05	2
	Cd	0.13–2.02	0.003	0.003
	Pb	0.52–2.88	0.01	0.01
	Zn	ND	5	No health-based guideline value has been proposed
Soil (mg/kg)	Metal	Present study	CEQG	
	Cu	1.53–16.39	18.7	–
	Cd	0.85–7.21	0.7	–
	Pb	0.17–7.62	30.2	–
	Zn	0.32–34.70	124	–

BIS, Bureau of Indian Standards; WHO, World Health Organization; CPCB, Central Pollution Control Board; CEQG, Canadian Environmental Quality Guidelines.

Table 4. Distribution of different elements in water samples (mg/l) of the Cauvery basin

	Cu		Cd		Pb		Zn	
Station	Before	After	Before	After	Before	After	Before	After
C1	0.89 ± 0.08	0.21 ± 0.03	0.6 ± 0.13	0.19 ± 0.06	ND	0.18 ± 0.05	ND	ND
C2	1.57 ± 0.27	0.25 ± 0.11	0.5 ± 0.36	0.31 ± 0.1	ND	0.85 ± 0.10	ND	ND
C3	1.73 ± 0.40	0.37 ± 0.15	2.02 ± 0.50	0.25 ± 0.1	ND	1.57 ± 0.63	ND	ND
C4	0.56 ± 0.12	0.31 ± 0.12	0.63 ± 0.15	0.28 ± 0.04	ND	0.86 ± 0.19	ND	ND
C5	0.50 ± 0.09	0.23 ± 0.10	0.38 ± 0.10	0.27 ± 0.05	ND	0.65 ± 0.10	ND	ND
C6	0.34 ± 0.06	0.18 ± 0.06	0.53 ± 0.15	0.31 ± 0.11	ND	0.16 ± 0.12	ND	ND
C7	0.01 ± 0.01	0.2 ± 0.11	0.31 ± 0.17	0.23 ± 0.04	ND	ND	ND	ND
C8	0.33 ± 0.20	0.24 ± 0.08	0.68 ± 0.38	0.34 ± 0.12	ND	ND	ND	ND
C9	0.05 ± 0.06	0.25 ± 0.08	0.56 ± 0.21	0.28 ± 0.04	ND	ND	ND	ND
C10	0.24 ± 0.06	0.23 ± 0.12	0.36 ± 0.2	0.2 ± 0.07	ND	ND	ND	ND
C11	0.29 ± 0.13	0.17 ± 0.05	0.55 ± 0.26	0.24 ± 0.05	ND	ND	ND	ND
C12	0.24 ± 0.09	0.25 ± 0.10	0.77 ± 0.14	0.31 ± 0.1	ND	ND	ND	ND
C13	0.04 ± 0.05	0.14 ± 0.11	0.61 ± 0.12	0.16 ± 0.09	ND	ND	ND	ND
C14	0.07 ± 0.10	0.15 ± 0.06	0.56 ± 0.1	1.47 ± 1.5	ND	ND	ND	ND
C15	0.12 ± 0.05	0.09 ± 0.07	0.67 ± 0.24	0.45 ± 0.51	ND	ND	ND	ND
C16	0.04 ± 0.06	0.31 ± 0.10	0.77 ± 0.21	0.32 ± 0.11	ND	ND	ND	ND
C17	0.12 ± 0.17	0.17 ± 0.10	0.65 ± 0.15	0.19 ± 0.08	ND	ND	ND	ND
C18	0.05 ± 0.08	0.09 ± 0.07	0.67 ± 0.09	0.14 ± 0.12	ND	ND	ND	ND
C19	0.13 ± 0.18	0.12 ± 0.13	0.55 ± 0.16	0.09 ± 0.15	ND	ND	ND	ND
C20	0.11 ± 0.08	0.14 ± 0.11	0.61 ± 0.15	0.31 ± 0.1	ND	ND	ND	ND

ND, Not detectable; values represent mean ± standard deviation.

after the idol immersion. The abundance of metals in the Cauvery samples varied in the following order: Cu > Cd > no traces of Pb and Zn (before idol immersion); Pb > Cd > Cu > no traces of Zn (after idol immersion) in water, and Zn > Cu > Cd > Pb (before idol immersion); Cu > Zn > Pb > Cd (after idol immersion) in the soil. Heavy metal concentration in water and sediment samples of different sites in the Cauvery before idol immersion ranged from 0.01 ± 0.01 to 1.73 ± 0.40 mg/l and 4.76 ± 0.85 to 16.39 ± 2.17 mg/kg for copper, 0.31 ± 0.17 to 2.02 ± 0.50 mg/l and 2.36 ± 1.09 to 7.21 ± 1.17 mg/kg for cadmium, no traces and 0.72 ± 0.79 to 2.92 ± 2.57 mg/kg for lead, and no traces and 2.62 ± 1.25 to 34.70 ± 1.48 mg/kg for zinc. The concentration of metals in water and sediment samples after idol immersion varied as follows: 0.20 ± 0.11 to 0.37 ± 0.15 mg/l and 1.53 ± 0.31 to 9.58 ± 0.86 mg/kg for copper, 0.09 ± 0.15 to 1.47 ± 1.50 mg/l and 0.09 ± 0.13 to 2.20 ± 0.40 mg/kg for cadmium, 0.16 ± 0.12 to 1.57 ± 0.63 mg/l and 0.17 ± 0.10 to 3.80 ± 0.67 mg/kg for lead, and no traces and 0.09 ± 0.07 to 14.57 ± 1.10 mg/kg for zinc.

The water samples showed significant alteration variation in Cd concentration before and after idol immersion ($P < 0.01$). Strong statistical significance was observed for all the elements studied in the soil samples collected before and after idol immersion ($P < 0.01$). In most of the stations, Cu and Cd levels in the water samples were high while Cu, Cd and Zn levels were high in sediment samples before idol immersion. In both water and sediment samples Pb concentration increased after idol immersion. Before and after immersion, the mean concentration of metals such as Cu, Cd and Pb in the water

Table 5. Distribution of different elements in soil samples (mg/kg) of the Cauvery basin

	Cu		Cd		Pb		Zn	
Station	Before	After	Before	After	Before	After	Before	After
C1	12.75 ± 2.62	9.58 ± 0.86	3.97 ± 1.54	0.12 ± 0.1	ND	0.17 ± 0.1	34.7 ± 1.48	14.57 ± 1.1
C2	11.07 ± 2.21	5.78 ± 1.05	2.51 ± 0.89	0.45 ± 0.15	ND	0.23 ± 0.06	11.34 ± 1.1	7.14 ± 0.98
C3	16.39 ± 2.17	5.06 ± 0.71	3.82 ± 1.31	0.12 ± 0.04	ND	0.33 ± 0.11	13.51 ± 0.66	2.24 ± 0.31
C4	12.27 ± 2.19	6.78 ± 1.31	3.36 ± 1.12	1.79 ± 0.77	ND	0.65 ± 0.08	10.13 ± 0.98	9.53 ± 0.80
C5	8.35 ± 1.34	4.61 ± 0.66	2.62 ± 1.09	0.09 ± 0.13	ND	0.52 ± 0.11	9.66 ± 0.87	1.63 ± 0.60
C6	12.36 ± 2.79	7.22 ± 0.99	2.97 ± 1.11	0.27 ± 0.1	ND	1.01 ± 0.2	17.12 ± 1.65	3.96 ± 0.74
C7	12.31 ± 2.6	5.03 ± 0.66	2.97 ± 1.05	0.93 ± 0.1	ND	0.62 ± 0.1	15.29 ± 1.21	10.6 ± 0.83
C8	5.54 ± 0.82	4.31 ± 1.02	2.28 ± 0.97	ND	ND	3.24 ± 1.08	3.35 ± 1.02	1.67 ± 0.76
C9	5.90 ± 1.35	5.37 ± 1.09	3.58 ± 1.05	ND	ND	2.42 ± 0.45	9.93 ± 1.64	7.52 ± 0.75
C10	4.76 ± 0.85	3.66 ± 0.45	3.06 ± 0.82	0.24 ± 0.1	ND	0.68 ± 0.1	9.56 ± 0.86	1.45 ± 0.52
C11	5.95 ± 2.03	1.53 ± 0.31	3.28 ± 1.10	1.7 ± 0.72	2.92 ± 2.57	0.44 ± 0.1	3.27 ± 1.03	ND
C12	5.25 ± 1.14	4.2 ± 0.63	7.18 ± 1.01	0.52 ± 0.2	0.72 ± 0.79	0.44 ± 0.1	3.17 ± 0.68	1.77 ± 0.70
C13	5.17 ± 1.59	3.09 ± 0.63	3.67 ± 0.8	0.23 ± 0.1	1.86 ± 0.46	1.25 ± 0.27	2.62 ± 1.25	0.36 ± 0.16
C14	13.17 ± 2.09	3.68 ± 0.52	4.22 ± 1.37	0.73 ± 0.1	ND	3.8 ± 0.67	22.03 ± 1.36	0.64 ± 0.30
C15	8.63 ± 1.20	2.44 ± 0.53	5.8 ± 1.57	0.26 ± 0.1	ND	0.62 ± 0.1	16.73 ± 0.85	0.09 ± 0.07
C16	10.84 ± 2.07	3.93 ± 0.63	3.72 ± 1.09	0.31 ± 0.1	ND	0.66 ± 0.12	15.77 ± 1.38	3.58 ± 0.83
C17	8.13 ± 1.48	3.53 ± 1.0	4.87 ± 1.36	0.25 ± 0.09	ND	1.59 ± 0.6	6.33 ± 1.01	ND
C18	5.51 ± 0.57	2.31 ± 0.33	7.21 ± 1.17	0.15 ± 0.05	ND	1.9 ± 0.44	4.63 ± 0.84	2.74 ± 0.66
C19	11.23 ± 1.31	8.36 ± 0.95	4.63 ± 1.11	0.54 ± 0.15	ND	0.26 ± 0.08	13.44 ± 1.21	9.40 ± 1.00
C20	6.32 ± 1.15	2.63 ± 0.56	2.36 ± 1.09	2.20 ± 0.40	ND	0.63 ± 0.11	6.84 ± 1.05	ND

ND, Not detectable; Values represent mean ± standard deviation.

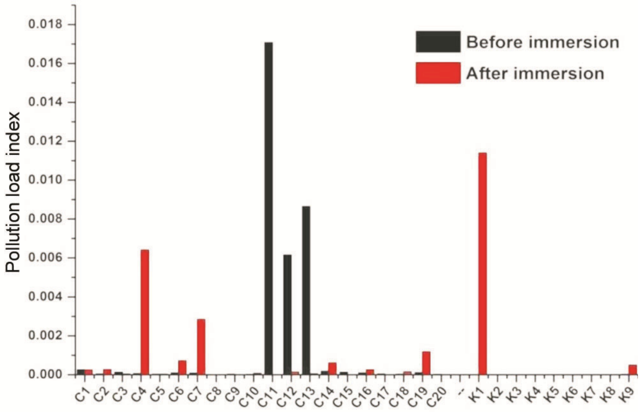

Figure 2. Pollution load index of different elements studied before and after idol immersion across the Cauvery river basin.

samples was relatively higher than the permissible limits of drinking water quality of BIS standards and WHO (Table 3).

The metals like Cu and Cd showed elevated levels in water samples of the Cauvery river point before idol immersion than after immersion. The same pattern was observed for metals such as Cu, Cd and Zn in the sediments during the entire study period. These might be influenced by industrial activities, municipal waste, untreated effluents from the nearby SIPCOT industries and agricultural activities. In Tamil Nadu, the Cauvery delta is the major contributor to paddy irrigation. In the due course of time, tonnes of contaminated forms of Cu, Cd, Pb and Zn as insecticides and fertilizers were directly applied to land[32,33]. This has increased two to three-fold during the past 20 years[34].

A unique pattern of Pb distribution was observed during the study. Among the 20 stations of the Cauvery, only a few stations (C1–C5) were reported with elevated Pb concentration in water. These stations are rich in industrial sites, where lead and its components are widely used for several purposes, e.g. lead acetate (dyeing of textiles, insecticides, chrome pigments), lead chromate (pigment in plastics), lead tetrafluoroborate (salt for electroplating), lead molybdenum chromate (pigments) and lead phosphate (stabilizing agent in plastics)[35–37]. According to the recent report by Shalini[38], around 30,000 idols are immersed into the Hussain Sagar lake every year that contain 10 µg lead/kg of paint[38]. The drastic increase in the concentration of Pb in water and sediment samples is evidence that is due to idol immersion activities. This scenario is directly correlated with several studies in different parts of India[38–40]. Several studies have confirmed the elevated metal levels in specific aquatic bodies during the idol immersion activity throughout India.

Principal component analysis

In order to identify the potential sources of heavy metals in the present study, PCA method was employed to determine the concentration of heavy metals in the entire Cauvery river using eigen values and varimax rotation. Based on the concentration of all the elements analysed in the water samples, one principal component (PC) was extracted with 39.24% and 42.10% of total variance before and after immersion respectively. The coefficient of PC1 showed positive loading for Cu (0.707) and Cd (0.707) before immersion of the idols, also positive

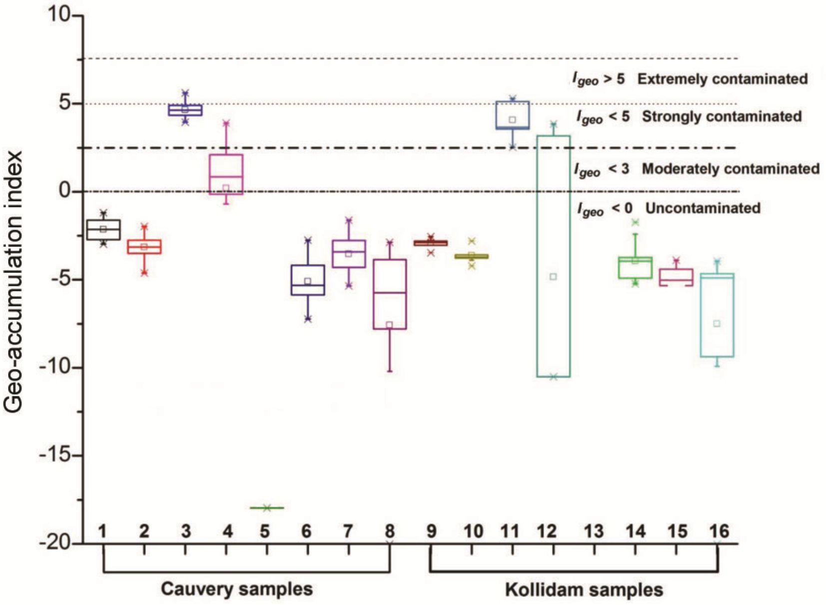

Figure 3. Geo-accumulation index of different elements studied before and after idol immersion across the Cauvery river basin.

loading was observed in Cu (0.695) and Pb (0.692) after immersion of the idols, thus indicating that the metals were from an anthropogenic origin. Followed by the pre- and post-idol immersions, sediment samples were extracted with two PCs showing 75.98% and 82.36% of the total variance. In PC1 for the period before and after immersion, Cu (0.613; 0.625) and Zn (0.614) showed strong positive correlation, while Cd and Pb were in negative loading; this shows the impact of lithogenic origin of metals. However, in PC2 both before and after immersion Cd had strong loading (0.953; 0.740), which confirms the source of anthropogenic origin.

In the Kollidam river water samples, only one PC was extracted with an eigen value greater than one, which accounted for 31.89% and 35.05% of the total variance before and after immersion respectively. Before immersion of the idols, there was a strong positive loading for Cd (0.707), while after the immersion of idols there was a positive loading observed for Cu (0.690). Thus we can confirm that elevated metal concentration before immersion is linked to anthropogenic origin[41], while after immersion it is directly linked with lithogenic sources. In case of sediment samples, pre-immersion activity was extracted by one PC with a total variance of 46.82%, especially positive loading for Zn (0.684). Post-immersion, two PCs were extracted; however, PC1 alone could be contributed by an anthropogenic component having strong loading for both Pb (0.593) and Zn (0.660).

Moreover, the loading is evidence of alteration in the water bodies during idol immersion, especially the elevated Pb levels. The concentration of lead in unpolluted waters is less than 0.01 mg/l, but an excessive amount of 1.57 mg/l was reported with an average of 0.71 mg/l. This might be due to contaminants of untreated industrial effluents, improper handling of solid waste, battery manufacturing units, pigment industries, textile industries, and untreated effluents of sewage treatment plants. Elevated levels of some heavy metals and nutrients in the groundwater were reported from Mettur Dam; the possible sources are SIDCO Industrial Estate, thermal power plant, disposal of industrial effluents, municipal sewage and agrochemical leaching[32]. In the Cauvery river basin, monsoonal variation occurs that could be the possible source of pollutants, which might bring all the dumps and solid waste[42]. A significant change in metal concentration both before and after idol immersion was observed. However, the result lies within the BIS, IS-10500 limits.

Assessment of metal pollution in river soil samples

Figure 2 shows the calculated pollution load index (PLI) values of different metals during this study. The PLI values were relatively lower both before and after idol immersion in the Cauvery and Kollidam rivers. In all the sites PLI was less than 1, indicating least contamination of all the elements studied. The I_{geo} was calculated using the metal concentration present in the soil samples of the study area (Figure 3). The I_{geo} values for Cu, Pb, and Zn before and after idol immersion showed uncontaminated status of both river samples with reference to the background concentration of metals. Cd alone showed positive loading for all the sampling sites of both rivers (Cauvery – C1 to C20; Kollidam – K1 to K9) before idol immersion. However, the trend gradually altered after immersion and I_{geo} values were between strongly contaminated and moderately contaminated zones. CF of Cu,

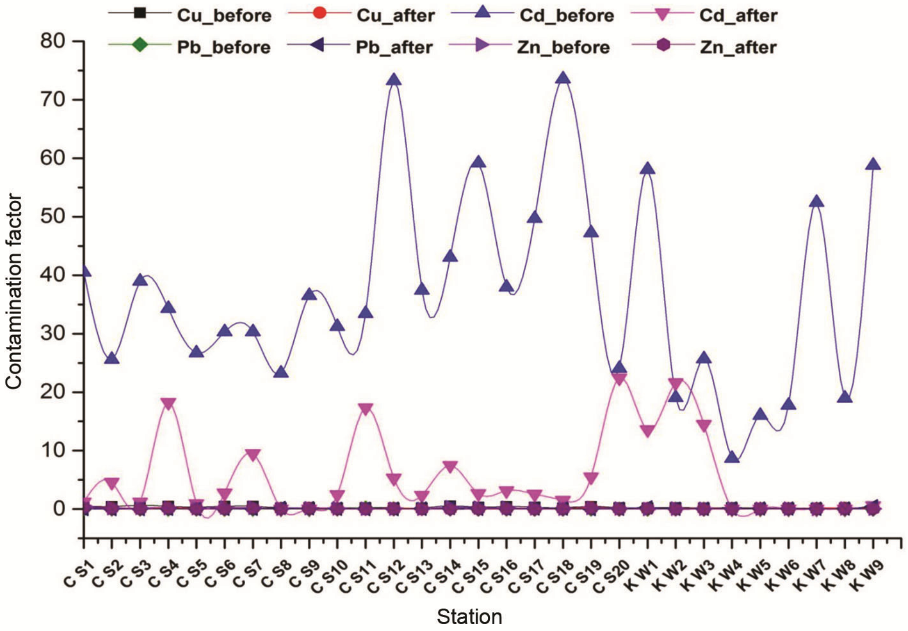

Figure 4. Contamination factor of different elements studied before and after idol immersion across the Cauvery river basin.

Cd, Pb, and Zn. Considerable variation exists for Cd before immersion ranging from 23.27 to 73.57 in case of Cauvery river and 8.67 to 58.78 for Kollidam river samples (Figure 4). Such discrepancy might be influenced by water run-off during monsoon. Moreover, the obtained I_{geo} values indicate that the rivers of Tamil Nadu are strongly influenced by Cd-medicated toxicity that may impact human and aquatic life.

Conclusion

In recent decades due to several anthropogenic activities, the Cauvery river basin is being polluted by metals and other pollutants. Among them, seasonal idol immersion activity was reported as one of the main sources of pollution. In the present study traces of elements were detected in water and soil samples of the Cauvery river basin, which might be influenced by several anthropogenic activities. The concentration factor and geo-accumulation index revealed that soil samples of the Cauvery river basin were highly contaminated by Cd and less contaminated by other elements respectively. The immersion of the seasonal idol may not create any strong impacts on the distribution of metal in the Cauvery river because of its water flow. However, when the river water ends up into the sea, these metal pollutants arising from the immersions of idols would have a negative impact in the waters and sediments of the Bay of Bengal.

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ACKNOWLEDGEMENTS. We thank the authorities of Bharathidasan University, Tiruchirappalli, for providing the necessary facilities to carry out this study, and the Ministry of Earth Science, Government of India (Ref. No.: MoES/36/OOIS/Extra./9/2013) for providing AAS facilities. We also thank Mr P. Vignesh and Mr K. Sridhar (Bharathidasan University) for help in preparing the map of the study area.

Received 21 September 2019; revised accepted 29 September 2020

doi: 10.18520/cs/v120/i1/200-208