# RISK ASSESSMENT OF POTENTIALLY TOXIC ELEMENTS IN THE HABITAT, AND DIET OF SMOOTH-COATED OTTER (*Lutrogale perspicillata*) IN TUNGABHADRA OTTER CONSERVATION RESERVE (TOCR), KARNATAKA


by

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**Dissertation Thesis**

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**For the partial fulfillment of the degree**

**Master of Science**
**In**
**Wildlife Science**

**Under the supervision of**

**Dr. J.A. Johnson, Scientist F**

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**Ms. Ruchika Sah, Principal Project associate (WII-NMCG Project)**

**JULY 2024**

# DECLARATION

I hereby declare that the work conducted under the thesis entitled **"Risk Assessment of Potentially Toxic Elements in the Habitat, and Diet of Smooth-coated Otters in Tungabhadra Otter Conservation Reserve, Karnataka",** is a record of original and independent research work done by me and subsequently submitted for the award of the degree of **Master's in Wildlife Science** at the **Academy of Scientific and Innovative Research**. This research work has been carried out under the guidance and supervision of **Dr. J.A. Johnson**, **Scientist - F**, co-supervision of **Dr. S.A. Hussain**, **Project manager (WII – NMCG Project),** and **Ms. Ruchika Sah, Principal Project Associate (WII – NMCG Project)** of Wildlife Institute of India, Dehradun. The work has not formed the basis for the award of any other degree, diploma, or any other qualification. I also declare that the thesis embodies my own work, analysis, observation, understanding and the particulars given in it are true to the best of my knowledge.

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# CERTIFICATE

This is to certify that the thesis by **K. NIYAZ AHAMED** entitled **"Risk Assessment of Potentially Toxic Elements in the Habitat, and Diet of Smooth-coated Otters in Tungabhadra Otter Conservation Reserve, Karnataka"** is an original and independent research work submitted to the **Academy of Scientific and Innovative Research**, for the award of the degree of **Master's in Wildlife Science**.

**K. NIYAZ AHAMED** has put one semester of research work embodied in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other University or Institute for the award of any degree, diploma or distinction.

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# CERTIFICATE OF PLAGIARISM CHECK

It is certified that the Master's dissertation thesis titled **"Risk assessment of Potentially Toxic Elements in the Habitat and Diet of Smooth-coated Otter (*Lutrogale perspicillata*) in Tungabhadra Otter Conservation Reserve (TOCR), Karnataka"** submitted by **K Niyaz Ahamed** has been examined by us for plagiarism check as per UGC (Promotion of Academic Integrity and Prevention of Plagiarism in Higher Educational Institutions) Regulations. The following inferences are drawn from this check:

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**K. Niyaz Ahamed**

# SUMMARY

Smooth-coated otter (*Lutrogale perspicillata),* is a vital indicator species for freshwater ecosystems, playing a crucial role in maintaining ecological balance. Despite their essential place in the food web, they are always neglected compared to other terrestrial carnivores. This study explores the distribution, diet, and, ecotoxicological exposures to smooth-coated otters in Tungabhadra Otter Conservation Reserve, Karnataka. The study area, encompassing a 36km stretch of the Tungabhadra River (TOCR), is a unique habitat facing significant anthropogenic pressures, including mining activities and pollution, which threaten the local biodiversity and otter populations. The distribution of smooth-coated otters in TOCR was assessed through intensive sign surveys, revealing a clumped distribution pattern. Otter signs, including spraints, pugmarks, and grooming sites were dominant signs found in the landscape. Dietary analysis was examined by analysing the spraints (scat), using two methods frequency of occurrence method, and the score bulk estimate method revealing a predominantly piscivorous diet. The diet consisted of about 14 species out of which 69.53% is constituted of non–native fish species (*Oreochromis mossambicus, Labeo rohita*, *Cyprinus carpio, and Cirrhinus cirrhosus*). Ecotoxicological assessments were performed on water, sediment, fish, and spraint samples to evaluate the presence of potentially toxic elements (PTEs). While PTE concentrations in water and sediment were generally below harmful thresholds, their presence poses a long-term risk through bioaccumulation. Risk assessment indicated significant potential health risks from metals like mercury and arsenic, which could impact otter health.

# 1.0 INTRODUCTION

## 1.1 A General Introduction to otter

Otters make up the sub-family Lutrinae of the family Mustelidae, which with some 67 species (Ewer, 1973), is one of the largest families of the order Carnivora (Mason and Macdonald 1986). Davis (1962) classified otters into nine species under three tribes (i) Lutrini (ii) Aonychini and (iii) Hydrictini based on differences in vocalization, baculum, and male external genitalia. However, in the present context, the Lutrinae sub-family encompasses a total of 13 species of otters distributed around the globe except for Australia, Antarctica, and some islands (Hussain and Choudhury 1995). Of the 13 species, 5 are endangered, 5 are near threatened, 2 are vulnerable and 1 is least concern (IUCN Red List 2015). The Indian subcontinent has three species of otter, the Smooth-coated otter (*Lutrogale perspicillata,* Geoffroy 1826), the Asian small-clawed otter (*Aonyx cinereus,* Illiger 1815), and the Eurasian otter (*Lutra lutra (*Linnaeus, 1758)) (Pocock 1941; Mason and Macdonald 1986; Hussain and Choudhury 1995).

The otter's body is elongated and the head is flattened with small ears. Long, stiff whiskers called vibrissae surround the large muzzle, aiding in finding prey in murky water (Green, 1977). They are piscivorous, semiaquatic mammals with short legs and have five webbed toes for better locomotion underwater. They possess dense and waterproof fur, consisting of two types of hairs: a dense underfur, approximately 10 to 15mm in length, which traps an insulating layer of air, keeping it dry while swimming, and overlying hairs, about 25mm long, providing additional waterproofing. This evolutionary strategy, eschewing blubber for air as insulation, proves advantageous over blubber, as air is a more efficient insulator compared to fat, with just 1cm of air being as effective as 4cm of fat (Kruuk 2006).

Additionally, this fur structure aids otters in agile movement on land, a task that would have been challenging with the presence of blubber. They show less sexual dimorphism in morphological characters but the males are about 28% larger than females (Law et al., 2016). They have a pair of anal scent glands that produce a liquid called mucous jelly which is added to spraint and conveys information about the sex and sexual state and receptivity of the individual (Green 1977; Mason and Macdonald 1986; Hussain and Choudhury 1995; Kruuk 2006).

### 1.1.1 The smooth-coated otter (*Lutrogale perscpicillata,* Geoffroy, 1826)

The smooth-coated otter inhabits a wide range across Asia, from the Indian subcontinent through Southeast Asia to parts of China (Pocock 1941)**.** There are three sub-species of smooth-coated otter (i*) L. p. perspicillata* from India, Nepal, Sumatran, and Javan region with a blackish brown coat, (ii) *L. p. sindica* from Pakistan (Sind province and Punjab) with a sandy coat, and (iii) *L. p. maxwelli* from Tigris River Iraq an altogether darker, with an iron-grey throat and a little paler (Mason and Macdonald 1986; Maxwell 1960).

Smooth-coated otters are the largest otters found in India, measuring between 1067 – 1300mm in total length, with a head-to-body length of 655 – 790mm and a tail length of 406 – 505mm (Duplaix and Davis 1981). They possess distinct physical characteristics, including flattened tails, and notably large and heavily webbed front paws and the common name of the species comes from its smooth and velvety coat. Their dorsal pelage coloration varies from dark, almost blackish brown to light brown with a rufous tinge, sometimes appearing pale sandy or tawny brown. The ventral side is lighter in comparison. Whitish coloring is present from the cheeks to the eyes and ears, along the upper lip to the edge of the rhinarium, as well as on the sides of the neck, chin, and throat (Pocock 1941; Prater 1971). The hairless rhinarium has an upper margin forming an inverted “V” which is distinct from other sympatric species found in India.

Sub-adults typically weigh around 6.5kg, while adults range from 7 – 11.4 kg (Harris 1968). They reach sexual maturity at around 22 months in captivity (Desai 1974). The gestation period ranges from 60 – 62 days, with litter size typically ranging from 1 – 5 offspring (Yadav 1967; Desai 1974). These are mainly nocturnal, although they can be diurnal when not disturbed. Smooth-coated otters form large, vocal family groups preying together on fish, shrimp, frogs, crabs, insects, and birds. Found mainly in the slow-flowing water typical of rice and floodplains, they may use large rivers in some regions. The smooth-coated otter needs thick riverside vegetation to hide. It has a large distribution that is slowly and steadily shrinking. A fact made obvious by the remnant population in Iraq – its range once spread from the Middle East to Southeast Asia (IUCN OSG).

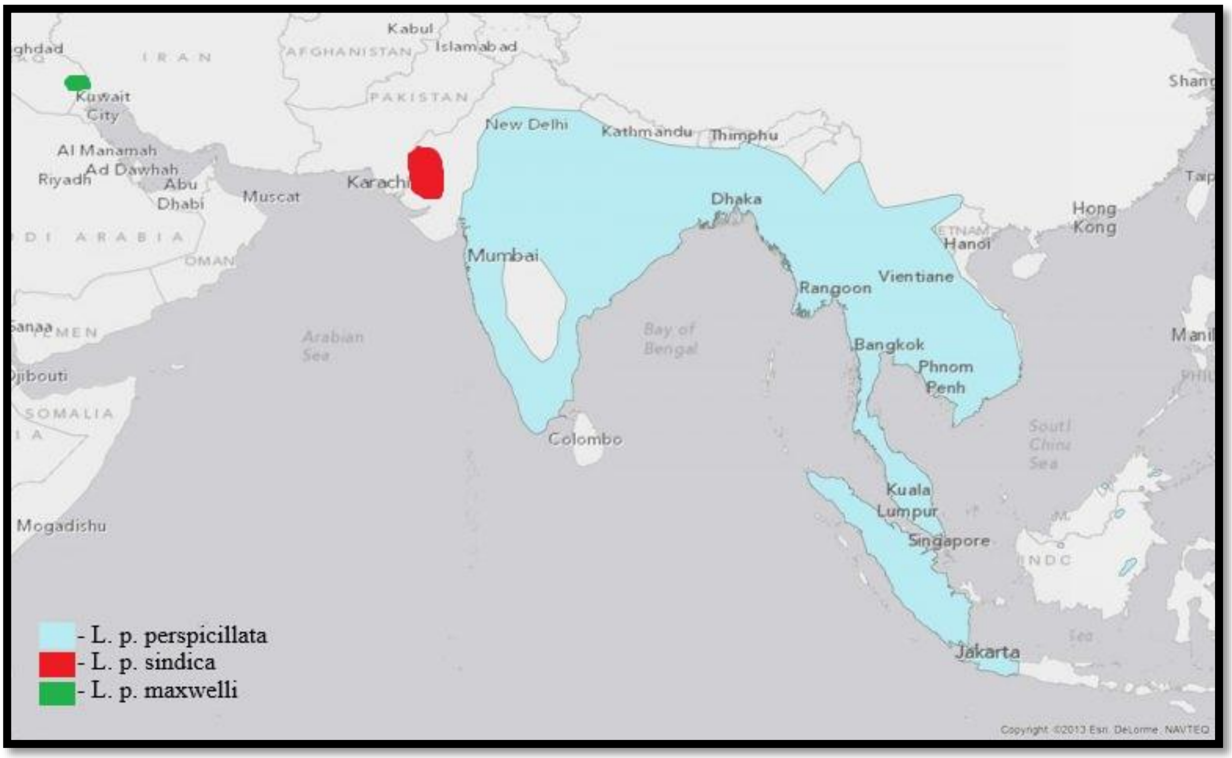



*Figure 1*: Range map of Smooth-coated otter and its sub-species

Source: IUCN Otter Specialist Group

## 1.2 Review of literature

### 1.2.1 Dietary studies on otters

The diet of otters is a crucial and easiest aspect of their ecological study, which has been extensively researched across various regions of the globe. Four primary methods are employed to determine the dietary habits of wild otters: (i) direct observation of feeding, (ii) examination of feeding sites, (iii) analysis of stomach content, and (iv) analysis of droppings, known as spraints (Webb, 1975). Direct observation can accurately identify prey items and estimate their size class (Kruuk and Moorhouse, 1990), though it is limited in diurnal regions and not possible in nocturnal regions. Feeding sites only provide information on large prey consumed out of water, as most fish are eaten in water, reducing the method's utility. Stomach content analysis is the most reliable but ethically challenging due to the necessity of killing animals, with only one notable study by Fairley (1972) analysing 33 otters drowned in fish traps. Analysis of spraint is the most effective and non-invasive method to understand the diet of the species. The portions of the diet that cannot be digested are contained in spraint (scales, hairs, feathers, bones, etc) (Mason and Macdonald, 1986).

In the Indian context, some studies have been carried out regarding the dietary aspect of smooth-coated otters and Asian small-clawed otters. A study on habitat selection and feeding habitats on smooth-coated otters in the Periyar Tiger Reserve revealed that a diet consists of 61% exotic fish, such as tilapia and European carp, which were introduced 10 – 15 years ago (Anoop and Hussain 2004). In the Hosur and Dharmapuri forest divisions, they found fish as the dominant prey element along with insects, molluscans, crabs, frogs, reptiles, and birds (Baskaran et al 2021). In the study on the Asian small-clawed otter from Karlapat Wildlife Sanctuary, Odisha found the major prey item to be crab followed by insects, frogs, fishes, reptiles, and mammals (Palei et al 2023).

### 1.2.2 Eco-toxicological studies on otters

Otters, being the top predator of aquatic ecosystems are particularly vulnerable to exposure of anthropogenic contaminants through bioaccumulation and bio-magnification (Fairbrother 2001; Mason and Wren 2001, Delibes et al., 2009; Chadwick et al., 2011; Roos et al., 2012, 2013). Because of this, otters are excellent sentinel species in aquatic ecosystems to monitor anthropogenic contaminants that pose a threat to human and environmental health (Rodríguez-Estival and Mateo, 2019). A total of approximately 53 research questions have been addressed in the ecotoxicological aspect by using otters as study species, 20 studies focused on heavy metals, about 33 studies focused on pesticides, organochlorines, organophosphates, rodenticides, polychlorinated biphenyls, endocrine disrupting chemicals, and many other chemical pollutants. Most of the studies have taken place in European countries, which gives insights into these pollutants' exposure, intake, and effects (Andrew et al., 2018; Charles et al., 2020; Mason & Macdonald., 1983, 1987, 1988).

Very few studies have estimated the content of pollutants in the otters using non-invasive methods like utilization of spraints and fur (Andrew et al., 2018; Rodríguez et al., 2019; Nadia et al., 2012; Nico et al., 2006;). In the context of selecting an appropriate method, there is often debate between choosing invasive versus non-invasive approaches. However, Mason and O'Sullivan (1993) demonstrated that PCB levels in spraint from Eurasian otters (*Lutra lutra)* show a significant correlation with PCB levels in liver tissue. This finding suggests that assessing spraints is a more advantageous and non-invasive method compared to collecting the tissue samples. Estival et al., (2019) used atomic absorption spectroscopy to assess the exposure and estimate the daily intake of two targeted metals through spraints of the Eurasian otter (*Lutra lutra*) from invasive red cramp fish. They found that the metal levels exceeded the minimum thresholds, leading to neurotoxicity and hispathological lesions.

However, there are very few studies on pollution exposure in aquatic ecosystems that use otters as the study species in the Indian context, highlighting a significant research gap in this area. This lack of research is concerning given the ecological importance of otters as apex predators and indicators of aquatic ecosystem health. Understanding the extent of pollution exposure and its effects on otter populations is crucial for conservation efforts and for developing effective environmental management policies. Further studies are needed to address this gap and provide a comprehensive assessment of the impact of pollution on otters and other aquatic species in Indian waterways. Numerous studies have examined the effects of ecotoxicological loads on fish and assessed the risk to humans (Rubalingeshwari et al., 2020; Anulipi et al., 2015; Velma et al., 2009).

Table 1: The effects on otters and their respective causative agents as identified in various studies

| Pollutants | Reference | Effects |
| --- | --- | --- |
| Heavy metals | Mazet et al., 2005; Sanders et al., 2020; Estival et al., 2019; Delibes et al., 2009; Eccles et al., 2017; Rosas et al., 2012; Lemarchand et al., 2011; Mason and Macdonald (1986); Lee et al., 2021; Sleeman et al., 2010; Harding et al., 1998 | 1) Impairment on reproduction 2) Lethal chronic health effects 3) Neurological disruptions 4) Renal necrosis |
| Persistent organic pollutants | Mazet et al., 2005; Kannan et al., 2004; Lemarchand et al., 2011; Brink et al., 2006; Leonards et al., 1998; Mason and Macdonald (1993); Wainstein et al., 2022; Huang et al., 2018; Kean et al., 2021; Ruhi et al., 2015 | 1) Weakened immune system 2) Affects reproduction 3) Reproductive abnormality 4) Endocrine disrupting capacity 5) Reduced fertility |
| Rodenticides | Lemarchand et al., 2011; Chambrillion et al., 2004 | 1) Anticoagulant |

### 1.3 Objectives of the study

The proposed study focuses on addressing Smooth-coated otters' feeding habits and exposure to Potentially Toxic Elements through habitat and dietary means.

1. Assessing the presence and distribution of smooth-coated otter in Tungabhadra Otter Conservation Reserve based on direct and indirect evidence.
2. Documenting the dietary spectrum of smooth-coated otter in human-modified riverscape.
3. Assessing the concentration of potentially toxic elements in otter diet, spraints, and habitat of smooth-coated otter.

### 1.4 Research questions and hypotheses

1. What is the distribution pattern of smooth-coated otters in the Tungabhadra otter conservation reserve?

   **Null hypothesis:** The distribution of smooth-coated otters in the Tungabhadra Otter Conservation Reserve is random, with no specific pattern.

2. What are the primary components of the diet of smooth-coated otters in Tungabhadra Otter Conservation Reserve?

   **Null hypothesis:** Prey species are uniformly distributed in their diet with no dominant species.

3. What is the correlation between otter sign density and concentration of heavy metals in sediment and water samples in the Tungabhadra otter conservation reserve?

   **Null hypothesis:** There is no significant relationship between otter distribution and environmental contaminant concentration in Tungabhadra Otter Conservation Reserve.

## 1.5 Research gaps

The aquatic ecosystem is increasingly under threat from various anthropogenic factors, including pollution, habitat destruction, and the introduction of invasive species. These disturbances can significantly alter the ecological balance, leading to the weakening of native species and the degradation of water quality. Despite numerous studies on water pollution and habitat loss, there remains a critical research gap in understanding the impact of these pollutants on human and environmental health (Rodriguex-Estival and Mateo, 2019). Existing studies often lack comprehensive data on the long-term effects of pollutants and their interactions within the ecosystem. Otters being the top predators are excellent sentinel species in aquatic ecosystems to monitor these pollutants. However, studies specifically focusing on otters as bioindicators are sparse, particularly in regions with significant anthropogenic pressures. This gap in knowledge hinders our ability to protect our ecosystems.

## 1.6 Rationale of the study

Freshwater habitats constitute only 0.01% of the world's water and roughly 0.8% of the planet's surface. Freshwater is home to at least 100,000 species out of the estimated 1.8 million total species or nearly 6% of all known species (Dudgeon et al., 2005). Over 21.5% of assessed freshwater species are classified as threatened by the IUCN Red List, with high spatial variation in the degree of threat (Linke et al., 2022). Freshwater ecosystems are inherently highly interconnected, and conserving them can be challenging. The consequences on river health and biodiversity are often transboundary and non-regional. Major threats to global freshwater biodiversity are grouped under six categories: (i) Overexploitation, (ii) Water pollution, (ii) Flow modification, (iv) Degradation of habitat, (v) Invasion by exotic species, and (vi) Climate change (Dungeon et al., 2006).

Over the past century, industrialization has rapidly expanded owing to rising demands for resources due to the increasing population. Thus, the rise in resource extraction using unsustainable techniques has made environmental contamination a global issue. Numerous inorganic and organic pollutants, radioactive isotopes, organometallic compounds, gaseous pollutants, and nanoparticles, have significantly contaminated the aquatic environment (Briffa et al., 2020). The emergence of Potentially Toxic Elements (PTEs) pollution in the aquatic environment is due to human activities such as mining, smelting, foundries, and other metal-related businesses operating along the water resources and usages in agricultural activities (Molo 2021). Metals are persistent in the environment (Ali et al., 2019). By enclosing the active component in a protein or storing the metal ions in intracellular granules in an insoluble state that may be eliminated in the organism's waste or stored for a long time, organisms can detoxify metal ions (Dungeon et al., 2006). In the case of heavy metals which bio-accumulate in animal body systems when they are ingested or breathed in. They are therefore categorized as hazardous. Physiological and metabolic issues are brought on by this bioaccumulation (Briffa et al., 2020). These metals through natural drainage streams from the mining areas mix with the river and persist in the freshwater ecosystems, continually accumulating and creating highly polluted habitats. The primary disadvantage of aquatic predators is that they spread into these seemingly ideal habitats in search of prey or dispersion, which reduces fitness and eventually acts as sink populations. These areas are called “ecological traps”, and the density of these traps is rising at the current date (Huang et al., 2018). The phrase "Otters are the indicators of pristine habitats or ambassadors of the clean environment" was frequently applied to them; however, with a further and better understanding of otter ecology, we can now refer to them as better “bio-monitors than bio-indicators” (Kruuk 2006).

Otters, being semi-aquatic, are entirely dependent on aquatic habitat for most of their activities in their life cycle, from feeding, locomotion to reproduction. As an apex carnivore in freshwater environments, the species is susceptible to heavy metals bio-magnification. The occurrence of heavy metals and other toxic elements in the freshwater environment will have long-term detrimental consequences on the otter population (Nadia et al 2012). The presence of these potentially toxic elements in mustelids is known to affect reproductive impairment, developmental abnormalities, neurotoxicity, etc. (Andrew et al., 2018; Charles et al., 2020).

# 2.0 STUDY AREA

## 2.1 Study site description

This study was carried out in a 36km stretch of Tungabhadra River which is notified for the conservation of smooth-coated otters as Tungabhadra Otter Conservation Reserve [TOCR]. Tungabhadra River derives its name from the Tunga, and the Bhadra Rivers, and the confluence of the two rivers at Koodli in Shimoga, Karnataka. It flows northeast and joins the Krishna River at Kurnool district in Andhra Pradesh. It is the largest tributary of the River Krishna with an annual discharge of 14,700 million $m^3$ and encompassing 27.6% of the Krishna basin. Tungabhadra Otter Conservation Reserve was notified in 2015 from Mudlapur village to Kampli in Ballari district and lies between 15°15'44.01" N and 15°26'27.90 "N latitudes; 76°20'17.67" E and 76°36'58.84 "E longitudes. The area comes under the administrative jurisdiction of Vijayanagara, Gangavathi, and Bellary districts in Karnataka state.

### 2.1.1 Climate

Tungabhadra Otter Conservation Reserve experiences a typical semi-arid climate with distinct wet and dry seasons as summer (March to May), monsoon (June to September), and winter (October to February). A major part of the rainfall is from the south-west monsoon and the annual rainfall is about 620mm. April and May are more likely to be the peak of the hot season with temperature rising up to 38.9$^o$C and December to be the coldest month with the temperature of 18.01$^o$C.

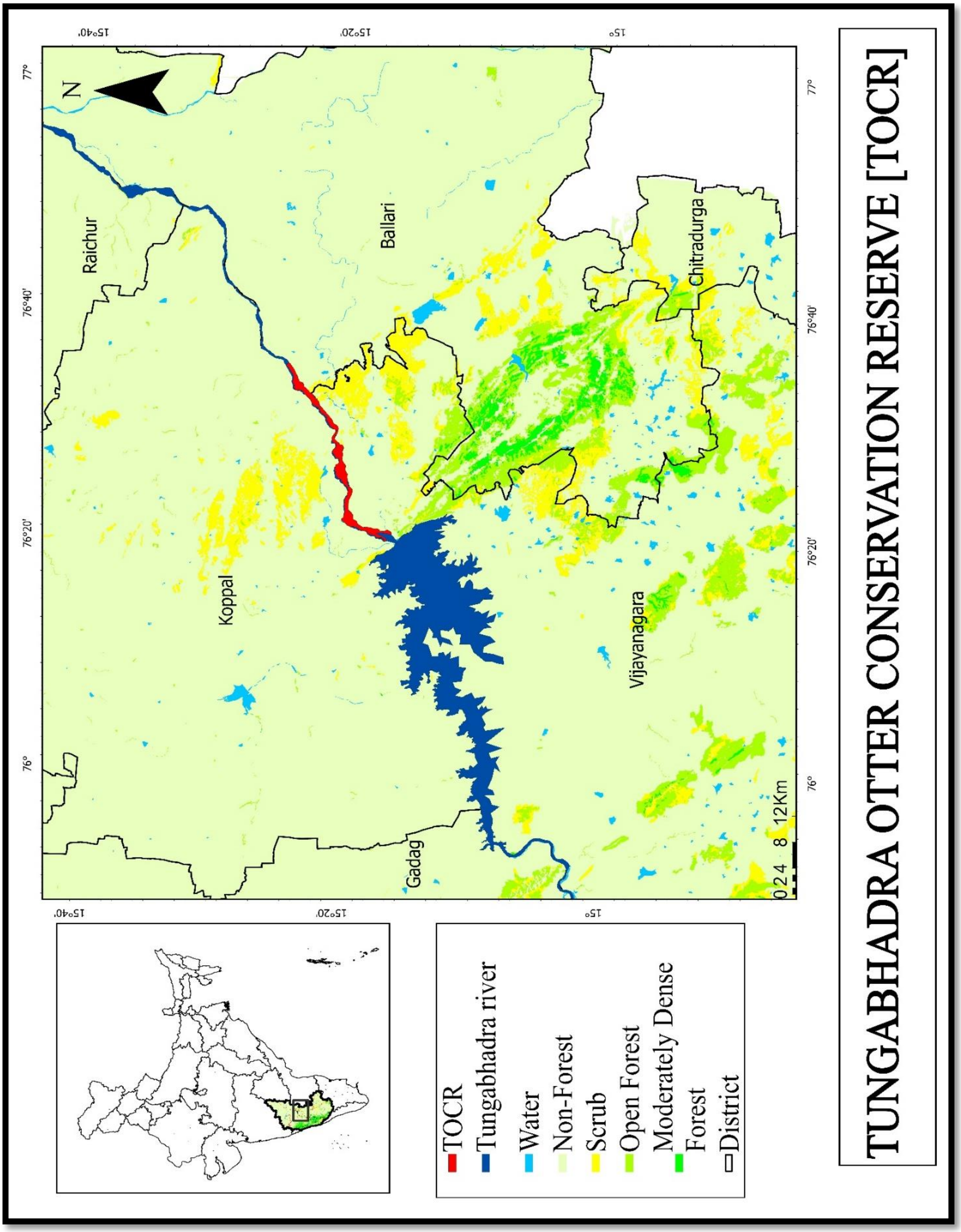



Figure 2: The map of Tungabhadra Otter Conservation Reserve, Karnataka

### 2.1.2 Geological setting

Tungabhadra Otter Conservation Reserve located in the southern part of the Deccan plateau lies 400 – 700m above MSL. It is dominated by two main geological formations (i) The Dharwar craton and (ii) The Cuddapah basin. This ancient Dharwar craton is estimated to be over 2.5 billion years old and forms the basement complex underlying Hospet. These iron-rich rocks have been instrumental in making Hospet and the surrounding township a major center for iron ore mining. The presence of such strong and stable rocks from the craton has also made the region suitable for the construction of the Tungabhadra reservoir.

### 2.1.3 Flora

The vegetation of this conservation reserve belongs to Dry Deciduous Scrub (5DS1) and Southern thorn forests (6A/DS1) according to Champion and Seth classification. The riverine tracts consist mostly *of Prosopis juliflora, Samanea saman, Tamarindous indica, and Eucalyptus* plantations. The major grass species consist of *Typha anguistata, Polygonum barbatum*, *Cyperus iria*, *Ipomea carnea*, *Phragmites karka* and *Chrysopogaon zizinoides.* However, the aquatic habitat is highly infested by Water hyacinth (*Pontederia crassipes*) in this region.

### 2.1.4 Fauna

Tungabhadra Otter Conservation Reserve being a safe haven for smooth-coated otters (*Lutrogale perscipillata*) also supports many Rare, Endangered and Threatened (RET) species like Marsh Crocodile (*Crocodylus palustris*), freshwater turtles like Leithis soft shell Turtle (*Nilssonia leithi*), Narrow headed soft shell turtle (*Chitra indica*), Indian soft shell turtle (*Lissemys punctata*) and Indian star tortoise (*Geochelone elegans*). There are many fish species like Deccan mahseer (*Tor khudree*), Hump back mahseer (*Tor mussullah*), Ray finned fish (*Hypselobarbus pulchellus*), Sandkhol carp (*Thynnichthys sandkhol*), Rohu (*Labeo* *rohita*), Catla (*Carpio carp*), Tilapia (*Oreochromis mossambicus*), Indian Garr (*Xenentodon cancila*) and mammals include Sloth bear (*Melursus ursinus*), Striped hyena (*Hyaena hyaena*), Common Leopard (*Panthera pardus*), Indian pangolin (*Manis crassicaudata*), Indian wolf (*Canis lupus pallipes*), Indian fox (*Vulpes benghalensis*), Asian palm civet (*Pardoxurus hermaphroditus*). The region also supports diverse avifauna of around such as Yellow throated bulbul (*Pycnonotus xantholaemus*), Indian skimmer (*Rynchops albicollis*), lesser flamingos (*Phoeniconaias minor*), Painted sandgrouse (*Pterocles indicus*), Chestnut bellied sandgrouse (*Pterocles exustus*), Rock bush quail (*Perdicula argoondah*), Painted spurfowl (*Falloperdix lunulata*), and many more.

**2.2 Rationale for study area selection**

Tungabhadra Otter Conservation Reserve is encircled by one of the oldest formations of the earth's crust, and rich iron and manganese ore deposits are naturally present. The mining in this area started in 1907 and is still in place; 125 major mineral mining leases are sanctioned in the district, and about 107 mines and 42 steel-producing industries are around 60km radius of Tungabhadra Otter Conservation Reserve. All the heavy metals extracted from these mines get deposited, travel through natural runoffs (Qiao et al., 2022; Khalil et al., 2007; Elrashidi et al., 2007) and get incorporated into the Tungabhadra River as Fig 3. A reconnaissance survey was conducted from 26/10/2023 to 29/10/2023 to confirm the presence of heavy metals in water, sediment, fish, and spraints. The results showed the presence of heavy metals.

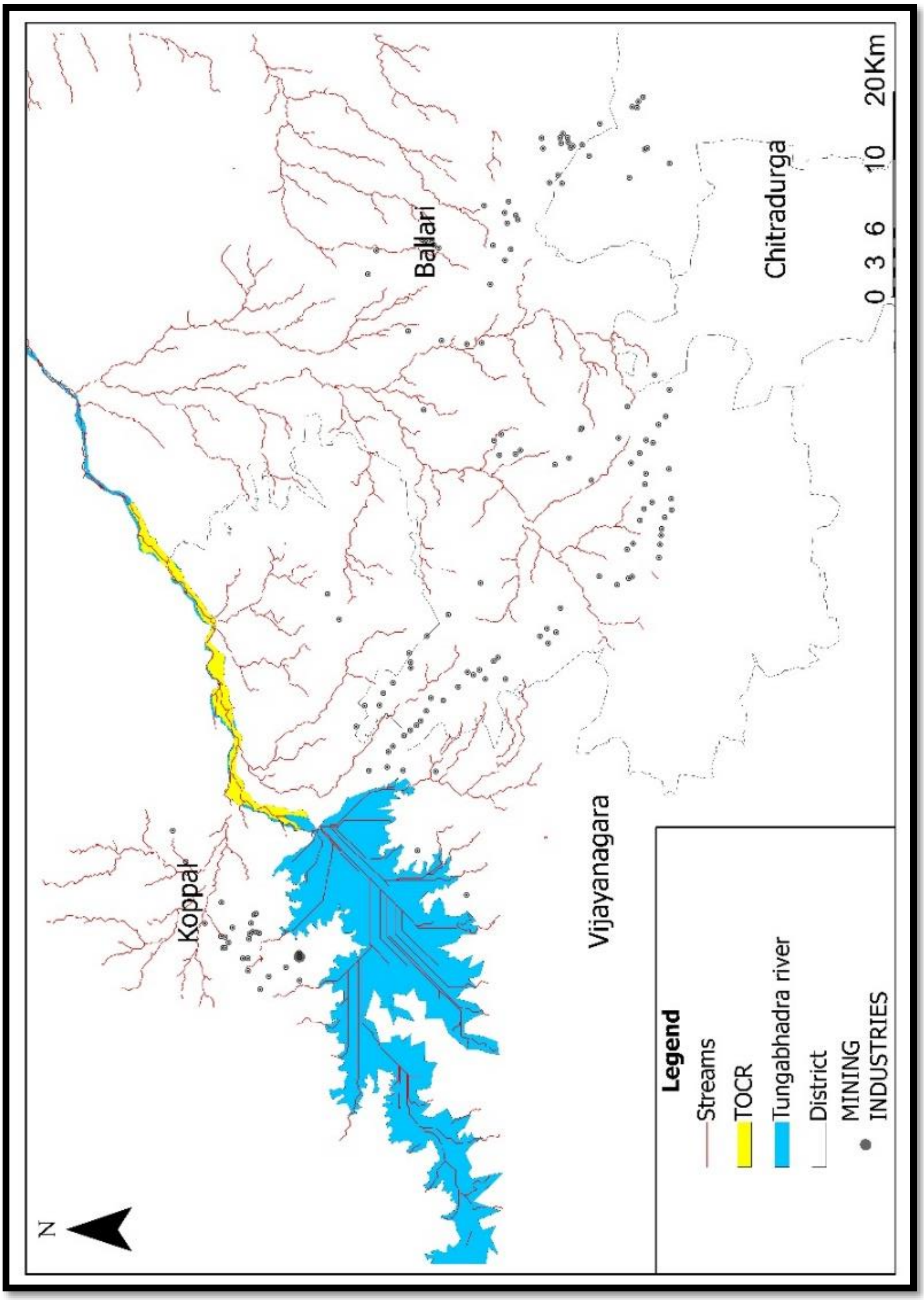



Figure 3: Mines and Steel industries around Tungabhadra otter conservation reserve, Karnataka

# 3.0 METHODS

## 3.1 Reconnaissance survey

The first 10 days were spent to gain an understanding of the riverscape and the industries present around the Tungabhadra Otter Conservation Reserve. Interacting with the local people regarding the species and the industries whether any possible cases of toxicity either in the habitat or in the species (Fish, Otter). Later 15 days were spent identifying the indirect signs made by smooth-coated otter such as tracks, active/inactive holts, grooming sites, feeding signs, and spraints (scat) were identified with the help of fish scales/crab exoskeleton present.

## 3.2 Distribution of Smooth-coated otter using sign survey

### 3.2.1 Measuring Sign Density

While performing the reconnaissance survey, all types of signs were examined and differentiated from those of porcupines and mongooses by thorough examination and calibration to avoid confusion.

The entire stretch of Tungabhadra Otter Conservation Reserve was divided into 500 x 500 m grids and surveyed intensively by foot and boat to record direct and indirect signs of smooth-coated otters during their activity period, i.e. at dawn and dusk. We used Locus mobile application to track the entire movement during the sign survey by foot and boat and mark the waypoints of different direct and indirect signs. After completing the sign survey, sign density per grid was calculated, as follows.

$$\text{Sign density (signs/m}^2\text{)} = \frac{\text{Number of signs encountered per Grid}}{\text{Total distance covered per Grid}}$$

The total distance covered in each grid was quantified using a GIS tool called as Intersect tool in ArcGIS, which gives the sum of all the tracks (in meters) present in the grid which represents the effort. Later dividing the entire grids into tertiles (three equal proportions of data) based on the sign density as high-density grids [HD] (>66%), moderate-density grids [MD] (33-66%), and low-intensity grids [LD] (<33%). Later based on the tertiles obtained, the distribution of smooth-coated otter was mapped in Tungabhadra Otter Conservation Reserve as Low-density, Moderate-density, and High-density areas. These data were tested for normality using the shapiro-wilk test and based on the results, we performed Kruskal- Wallis test between different otter density sites. Moran's – I test was used to determine the distribution of otter signs.

### 3.3 Food habits of smooth-coated otter

#### 3.3.1 Preparation of the standard reference collection of fish for spraint analysis

The preparation of the standard references and the collection of fish was done with the help of local fishermen. We daily visited the local fishermen and seven fish landing sites around the Tungabhadra reservoir for a month to collect 2 – 4 fishes of each species with varying lengths. The fish species were collected from the Ichthyofaunal list of Tungabhadra River which was made by studying the available literature (Nagabhusan 2022). Each fish species has its unique scale characteristics (Scale shape, Lines of the scales, Anterior and posterior design of the scale) which can be utilized later to identify them from the spraints (Webb 1976; Brager and Mortiz 2016).

The size and character of the scale varies in different regions of the fish but not the shape. Later scales were removed mainly from 3 different regions of the fish specimen (i) Base of the Head, (ii) Above the lateral line, and (iii) Near the tail. The scale was soaked in distilled water and it was scraped off using a small brush to remove the mucous layer. Staining was done using Alizarin red S, and DPX was used as a mounting agent. The slide was sealed using a cover slip and labelled for later use during spraint analysis.

For the fish species in which the scales are absent such as catfish and freshwater eel, we boiled the specimen for about 15 – 20 minutes and separated the muscle from the bones, later dorsal spine and razor ray fin bone were separated, dried and preserved for the further identification process.

**i. Collection of spraints**

During the sign survey about 45 communal spraint sites were identified and spraints were collected from these sites. Fresh spraints and also spraints about 1- 2 weeks old were collected and very old spraint were avoided from collecting. The spraints aging period was identified based on characteristics such as sliminess, colour, and smell. Fresh spraints were dark grey colour, fully intact, stiff, and had a pungent smell. In contrast, very old spraints turned completely white and scattered A total of 98 spraints samples were collected from the field, sundried, and the dry weight of each sample was taken using a weighing balance (d = 0.01g, e = 0.01g).

**ii. Sorting and identification of prey items**

Each spraint samples, was placed on a gridded petri dish. Initially, non – digested prey remains (such as scales, bones and exoskeletal fragments) were identified and categorized as fish and crab. Due to the time-consuming nature of analysing all scales and remains in the spraint, a sub-sampling method was employed. Ten scales were randomly selected from the different sections of the Petri dish. If all the scales were from the same species, the spraint was assumed to contain only that species. If a different scale was identified among the first ten, an additional ten scales were randomly selected, and this process continued until all species in the spraint were identified (Basak et al., 2021). The scales were stained and examined under 4X magnification of the compound microscope.

### 3.3.2 Estimation of proportions of prey items consumed

Two methods (frequency of occurrence and score bulk estimate method) were used to express the data obtained from spraint analysis. The most common is determining the Frequency of Occurrence. However, this can lead to overrepresentation of minor items and underestimation of major ones. To address this issue, a visual scoring method called Score Bulk Estimate can be used to assess the importance of a particular item in a spraint.

1) **Frequency of Occurrence:** The prey categories present in each spraint were identified and the relative frequency of each prey item was calculated i.e. the number of occurrences of a prey category was expressed as the percentage of samples having that category. This method of scat analysis is implemented in many of the studies (Erlinge, 1968 for the Eurasian otter; Melquist and Horncocker, 1983 for the Canadian river otter; Hussain and Choudhary, 1998 for the smooth-coated otter; Pardini, 1998 for Neotropical river otter; Perrin and Carugati 2000 for spot-necked otter and Cape clawless otter).
2) **Score-bulk estimate:** The proportion of each prey category was visually estimated and given a score from 1 to 10 on the coverage in the Petri dish. Then the score of each prey category was then multiplied with the dry weight of the spraint and the resulting figures were summed up for each prey category and expressed as a percentage (Wise et al., 1981; Hussain and Choudhary, 1997).

## 3.4 Heavy metal ecotoxicology in aquatic ecosystems

### 3.4.1 Collection of samples

1) **Water:** A total of 11 samples were collected from different density areas [Low density, Moderate density, and High-density area], Control sites [Tunga and Bhadra reservoir], and Mining stream. The samples were collected from 15 – 20 cm below the surface contaminants. At each site, water samples were taken from three locations (right bank, left bank, and centre of the river) and combined to create a composite sample. Approximately 200 ml of the composite sample was placed in a polypropylene (PP) bottle and immediately acidified with concentrated nitric acid (HNO3) to preserve the metals in a dissolved state. Subsequently, these samples were stored in cool ($4^{o}C$) and dark conditions.

2) **Sediment:** Additionally, surface sediment samples were collected from the same sites. Sediments were taken from a 2-5cm layer, mixed, and stored in a 50 ml PP centrifuge vial. All samples were stored at $4^{o}C$ during transportation and after arrival.

3) **Fish sample:** A total of 39 freshly caught fish, representing approximately 13 species, were collected from the local fishermen as part of their routine fishing activities. The fishermen used non-metallic tools for capturing and handling the fish to avoid contamination, Fish of similar size were selected to reduce variability in heavy metal accumulation. The fish were kept in neatly labelled zip-lock bags and stored in the freezer at $-20^{o}C$ before transportation to the lab. During transportation, they were kept with ice in cold-storage boxes and then stored at $-20^{o}C$ in the lab until analysis.

4) **Spraint:** A total of 98 spraints were collected during the sign survey using gloves and stored in plastic petri dishes after dietary analysis. To avoid any metal contamination, only plastic spatulas and scalpels were used. The spraints were later transported in the same petri dishes.

### 3.4.2 Sample preparation and analysis

**1) Water:** The stored samples were transferred to 15ml polypropylene (PP) tubes after centrifuging for 15 minutes at 5,600 rpm.

**2) Sediment and Spraint:** The sediment and spraint samples were firstly dried for a week at room temperature, later homogenized using granite mortar and pestle, and sieved using a 1mm plastic sieve. Approximately 0.4 g of sample were digested using 4.0 mL of nitric acid (Merck, 69%) and 2.0 mL of hydrogen peroxide (Merck, 50%) and kept for overnight digestion. The following day the samples were run in Anton Paar Multiwave Go Microwave Digester at $120^{o}$ C for 15 min, ramped to $200^{o}$ C in 15 min, and digested at $120^{o}$ C for 30 min. After cooling, the digested sample was diluted to 25 mL with ultrapure water (Sah et.al. 2024).

**3) Fish:** Fish samples were homogenized using a REMI tissue homogenizer and approximately 0.4 g of sample were digested using 4.0 mL of nitric acid (Merck, 69%) and 2.0 mL of hydrogen peroxide (Merck, 50%) and kept for overnight digestion. The following day the samples were run in Anton Paar Multiwave Go Microwave Digester at $120^{o}$ C for 15 min, ramped to $200^{o}$ C in 15 min, and digested at $120^{o}$ C for 30 min. After cooling, the digested sample was diluted to 25 mL with ultrapure water (Sah et al. 2024).

All these samples, after being diluted up to 25mL with ultrapure water were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent ICP-MS-7850). ICP-MS was utilized to identify and quantify the PTEs Cr, Co, Ni, Cu, Zn, As, Cd, Hg, and Pb in the water, sediment, fish, and spraint samples. A nine-point calibration was performed using multi-element calibration standard-2A and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use/United States Pharmacopeia (ICH/USP) Oral Target Elements Standard 'A' (Agilent Technologies, USA). Scandium (Sc), Yttrium (Y), Indium (In), Terbium (Tb), and

Bismuth (Bi) were used as internal standards for Cr, Co, Ni, Cu, Zn, As, Cd, and Pb. Gold (Au) was used as an internal standard for Hg. Later, the concentrations of PTEs in water, sediment, fish, and scat were tested for normality using Shapiro-Wilk's test and we used pair plots to identify significant relationships between Otter sign density sites and water and sediment PTE concentrations.

### 3.4.3 Risk assessment

The ecological risk assessment screening level was employed to evaluate the risk of PTEs from dietary consumption. The Risk Quotients for the elements that were examined were estimated using the Reference Dose, or RfD. The goal of the dose-response assessment was to determine the maximum acceptable concentration (MAC) of a particular element in fish tissue, which is the maximum amount of any toxicant that can exist in fish without endangering smooth-coated otters. To do this, reference dose (RfD, mg/kg wet weight (ww) day $^{-1}$) was used to determine MACs based on the connections between concentrations of specific trace elements in the fish tissues and reaction in terms of unfavourable biological effects on the smooth-coated otters. The RfDs were developed using toxicity data, primarily the no observable adverse effect level (NOAEL) values obtained on testing mammals adjusted by uncertainty factors and reported by the USEPA in the Integrated Risk Information System (IRIS).

The maximum allowable concentration in food based on the RfD ($MAC_{RfD}$) is calculated using the equation for intake of a contaminant

**Intake (mgKg$^{-1}$day$^{-1}$)** = CF x IR x FI x EF x ED / BW x AT

**CF** = contaminant concentration in fish (mgKg$^{-1}$ ww); **IR** = ingestion rate (kg day$^{-1}$); **FI** = fraction digested from contaminated source; **EF** = exposure frequency (day year$^{-1}$);

**ED** = exposure duration (years); BW = body weight (Kg); **AT** = averaging time (period over exposure is averaged in days).

When the intake of a contaminant is at the highest rate at which health risk is at an acceptably low level, then

**Intake** = RfD and **CF** = $MAC_{RfD}$,

Thus,

**RfD** = $MAC_{RfD}$ x IR x FI x EF x ED / BW x AT;

Therefore **$MAC_{RfD}$** = RfD x BW x AT / IR x EF x ED (Hung et al., 2007)

# 4.0 RESULTS

### 4.1 Distribution of smooth-coated otter in Tungabhadra Otter Conservation Reserve

A total of 102km was intensively surveyed by foot and boat for direct sightings and indirect signs of smooth-coated otters in the 36km stretch of Tungabhadra Otter Conservation Reserve. A total of 132 signs of smooth-coated otter were observed during the study period from the 36 km stretch. A summary of the different signs encountered is given below Figure 4.

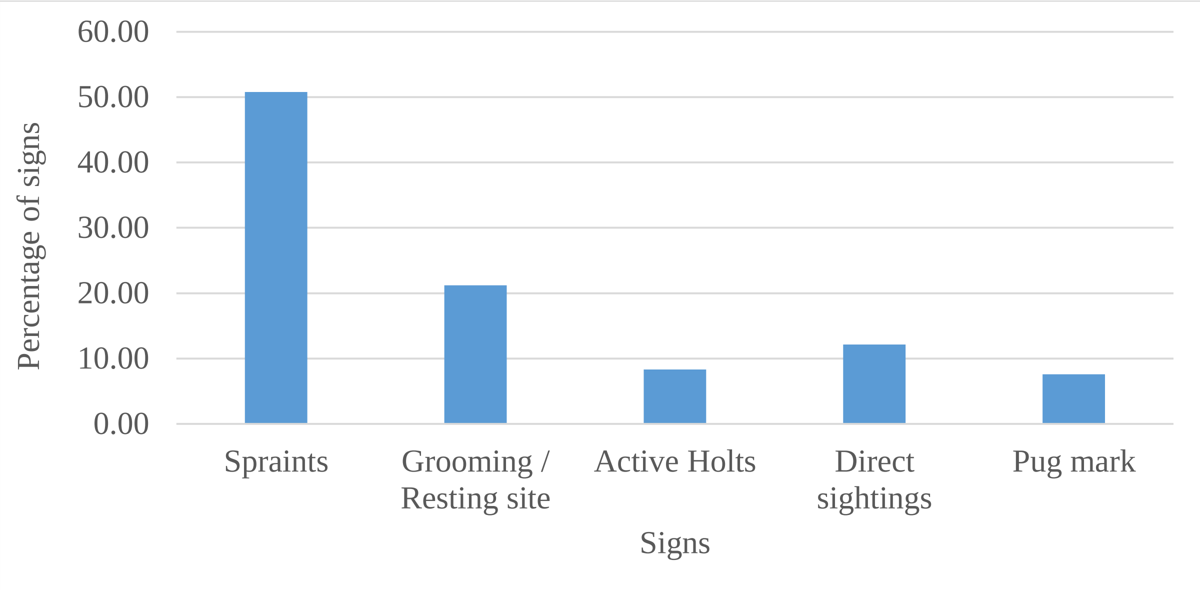



Figure 4: Summary of signs encountered during the sign survey in Tungabhadra Otter Conservation Reserve, Karnataka (N = 132)

The highest number of signs found were of spraints which included individual spraints, and sprainting sites followed by grooming/resting areas, the grooming sites are the areas where the otters rub their fur/skin to the soil/grass and these are always associated with the resting areas. The least signs we found were pugmarks because the rocky nature of the riverbed prevented them from being imprinted as they would be in soil.

The pattern of distribution appeared to be clumped in the map and also Moran's – I test indicates a highly significant positive spatial autocorrelation with a value of 0.99203 close to +1, indicating otter signs are highly clustered rather than randomly distributed across Tungabhadra Otter Conservation Reserve. Given these findings, we reject the null hypothesis that the distribution of smooth-coated otters in the Tungabhadra Otter Conservation Reserve is random. Instead, the results demonstrate a significant clustering of otter signs, suggesting that smooth-coated otters exhibit a non-random, clumped distribution pattern in this area.

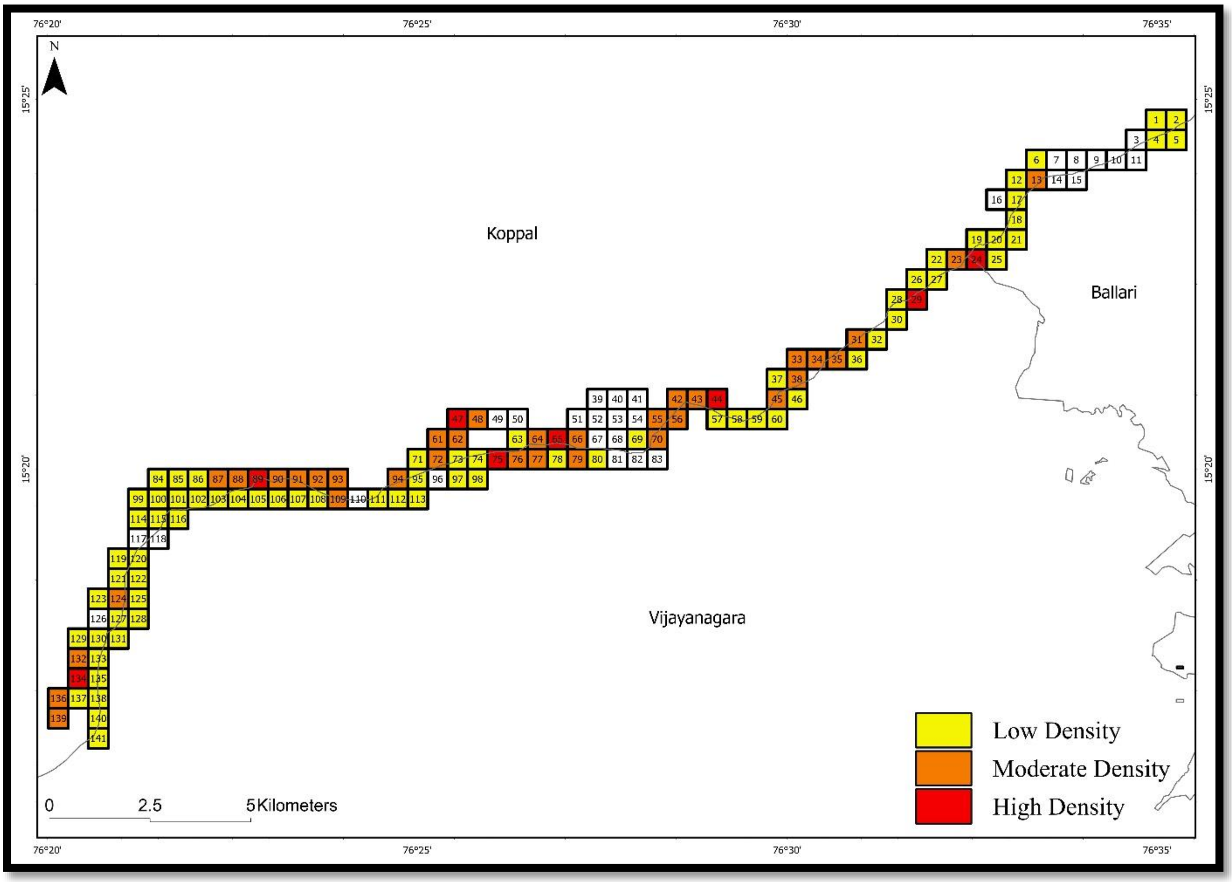



Figure 5: Distribution map of Smooth-coated otter in Tungabhadra Otter Conservation Reserve using sign density

**Plate 1: Smooth-coated otter from Tungabhadra Otter Conservation Reserve, Karnataka**

# Plate 2: Indirect signs of Smooth-coated otter



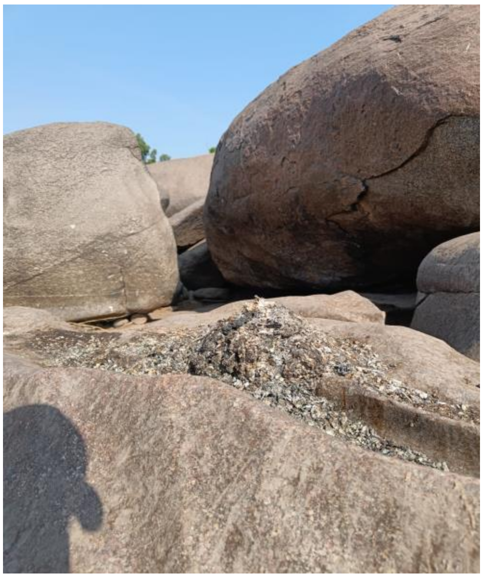
Grooming site

Sprainting site with fresh and old scat

Otter pugmark

Otter track (pugmark, and tail)

Half eaten fish (*Cyprinus carpio*) by Otter



Spraint mount (Unusual) near Holt (Near boulders)

## 4.2 Diet Composition of Smooth-coated Otter

### 4.2.1 Proportions of major prey categories encountered in otter spraints as estimated by the 'frequency of occurrence' method.

Fish contributed the maximum to the otter diet with 97.87 % followed by the carb with 2.13 % (Table 2).

### 4.2.2 Proportions of fish species encountered in otter spraints as estimated by the 'frequency of occurrence' and 'score-bulk' method.

A total of 14 fish species were identified in the otter spraints, with one species remaining unidentified. *Oreochromis mossambicus* (Tilapia) formed the maximum contribution (FOC = 37.70%, SBE = 39.77%), followed by *Cyprinus carpio* (Common carp) (FOC = 11.48%, SBE = 12.32%) (Table 3).

The results obtained from Frequency of occurrence and Score-bulk estimate method suggests that the smooth-coated otter in Tungabhadra Otter Conservation Reserve have a diet that is not uniformly distributed among prey species. Instead, certain species, particularly *Oreochromis mossambicus* and *Cyprinus carpio*, dominate their diet. Therefore, the null hypothesis that prey species are uniformly distributed in their diet with no dominant species is rejected.

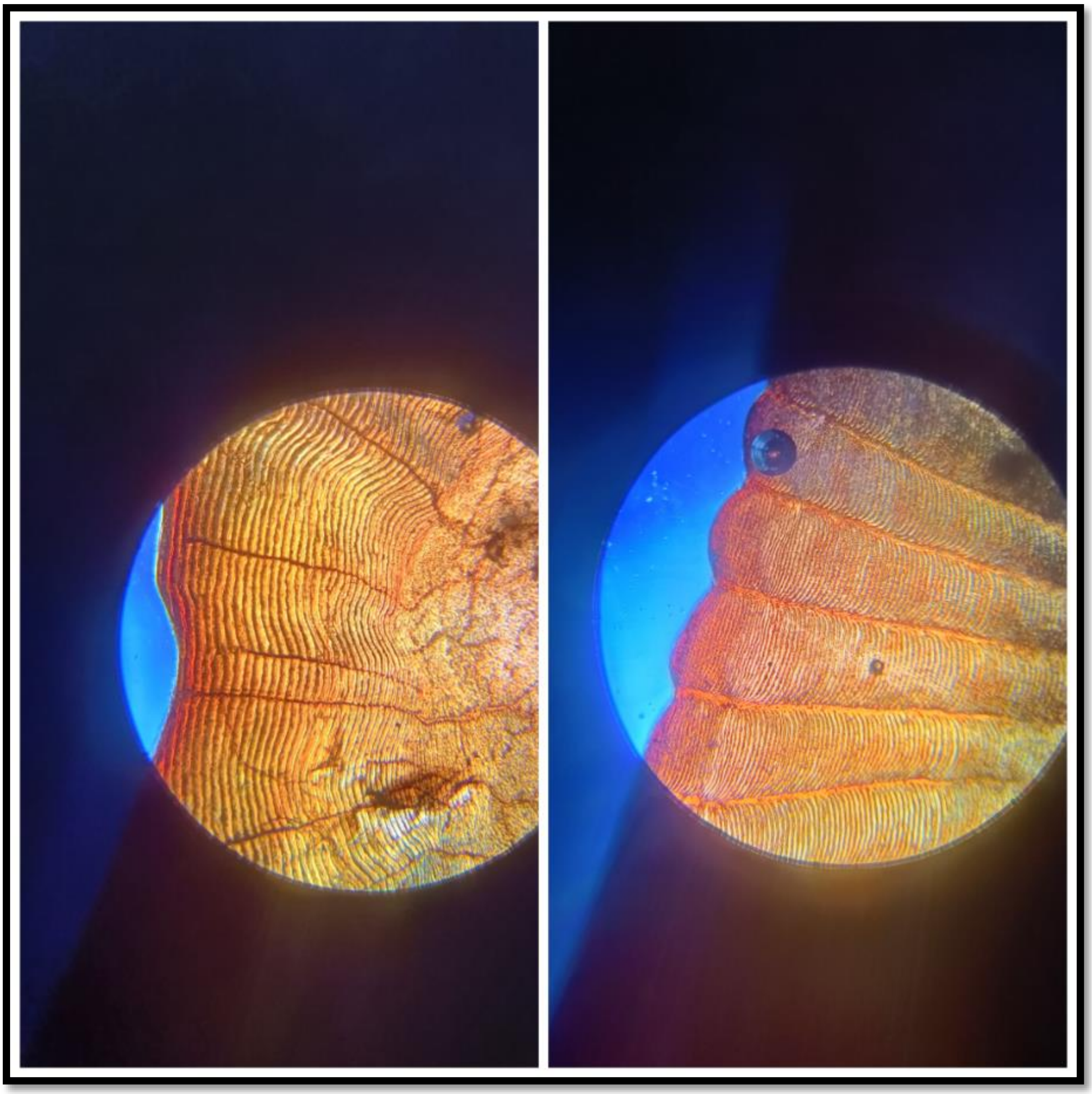


**Plate 3: The scales of the anterior part of *Cyprinus carpio* (Left) and *Oreochromis mossambicus (*Right)**

**Table 2: Major prey categories represented in the diet of Smooth-coated otter**

| Prey Items | Occurrences (N) | Frequency of Occurrence (%) |
|---|---|---|
| Fish | 92 | 97.87 |
| Crab | 2 | 2.13 |
| **Total** | **94** | **100** |

**Table 3: Relative percentage of major prey species represented in the diet of Smooth-coated otter**

| Species | Occurrences (N) | Frequency of Occurrence (%) | SBE (%) |
|---|---|---|---|
| *Oreochromis mossambicus* | 46 | 37.70 | 39.77 |
| *Cyprinus carpio* | 14 | 11.48 | 12.32 |
| Unknown - 1 | 12 | 9.84 | 7.98 |
| *Channa striatus* | 10 | 8.20 | 8.29 |
| *Labeo rohita* | 10 | 8.20 | 11.11 |
| *Labeo gonius* | 9 | 7.38 | 7.05 |
| *Mystus sengtee* | 4 | 3.28 | 2.72 |
| *Cirrhinus reba* | 3 | 2.46 | 2.10 |
| *Mastacembelus pancalus* | 3 | 2.46 | 1.11 |
| *Mystus spp.* | 3 | 2.46 | 1.84 |
| *Mystus vittatus* | 3 | 2.46 | 0.65 |
| *Labeo calbasu* | 2 | 1.64 | 1.99 |
| *Cirrhinus cirrhosus* | 1 | 0.82 | 0.77 |
| *Rita kuturnee* | 1 | 0.82 | 0.37 |
| *Tor khudree* | 1 | 0.82 | 1.92 |
| **Total** | **122** | **100** | **100** |

### 4.3 Heavy metal ecotoxicology in aquatic ecosystems

#### 4.3.1 Concentration of Potentially Toxic Elements (PTEs) in aquatic ecosystems

**1) Environmental concentrations:** In Tungabhadra Otter Conservation Reserve out of 13 PTEs we found the presence of 10 PTEs in water and 12 PTEs in sediment. Their concentration (µg/L) is given in Figure 6.

Aluminium (Al) was observed to have the highest concentration in water, followed by Tin (Sn) (111.36 ± 265.74) > Manganese (Mn) (40.46 ± 58.55) > Zinc (Zn) (12.90 ± 20.73) > Nickel (Ni) (1.86 ± 4.55) > Copper (Cu) (1.62 ± 2.48) > Chromium (Cr) (0.47 ± 0.75) > Cobalt (Co) (0.67 ± 1.64) > Lead (Pb) (0.14 ± 0.33). In sediment, the PTE concentrations followed the order Al (1703.33 ± 675.90) > Mn (102.37 ± 125.73) > Cr (7.69 ± 1.71) > Zn (3.49 ± 1.81) > Ni (3.06 ± 1.11) > Pb (2.27 ± 1.07) > Cu (1.96 ± 0.73) > Co (1.42 ± 0.53) > Arsenic (As) (0.42 ± 0.19) > Antimony (Sb) (0.006 ± 0.008) > Cadmium (Cd) (0.005 ± 0.004) > Mercury (Hg) (0.004 ± 0.003) as given in Figure 7.

A high collinearity was observed for environmental metal concentrations and otter densities. Interestingly, the sediment concentrations for Al, Cr, Co, and Ni showed a significant ($p < 0.05$) positive relationship with otter density sites while Hg showed a significant ($p < 0.01$) inverse relationship. However, there was no significant relationship between water concentration and PTEs Figure 8 & 9.

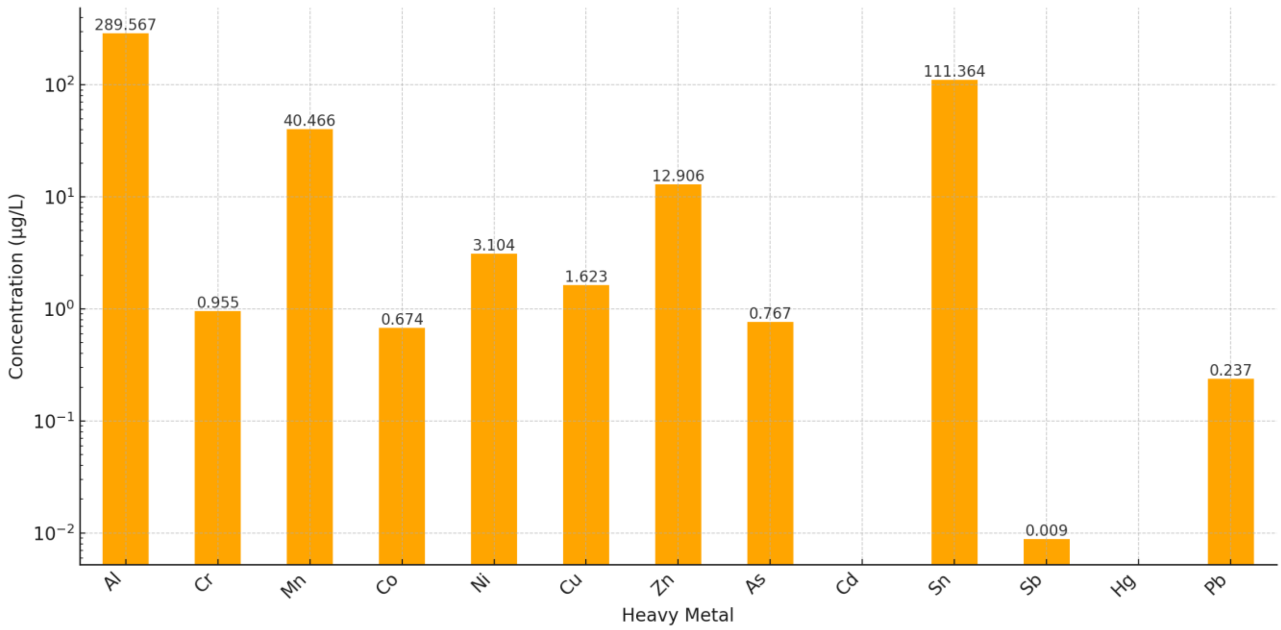



Figure 6: Concentration of PTEs in Water sample of Tungabhadra Otter Conservation Reserve (N = 10)

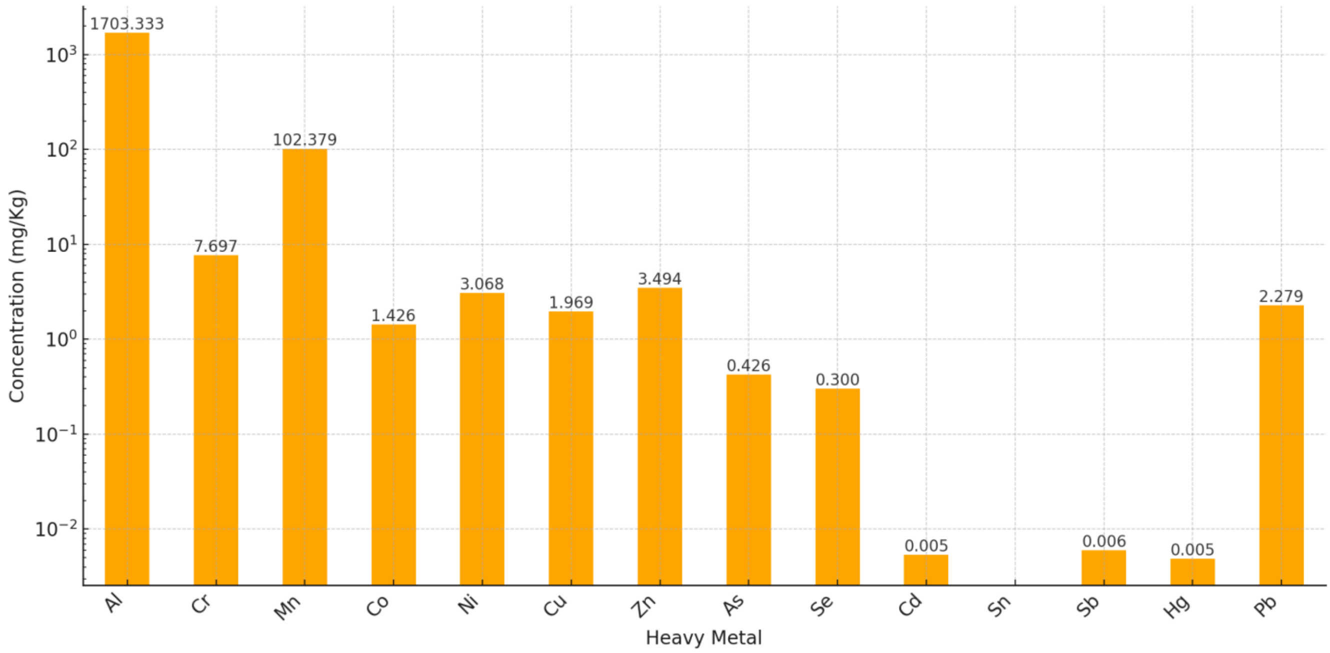



Figure 7: Concentration of PTEs in Sediment sample of Tungabhadra Otter Conservation Reserve (N = 10)

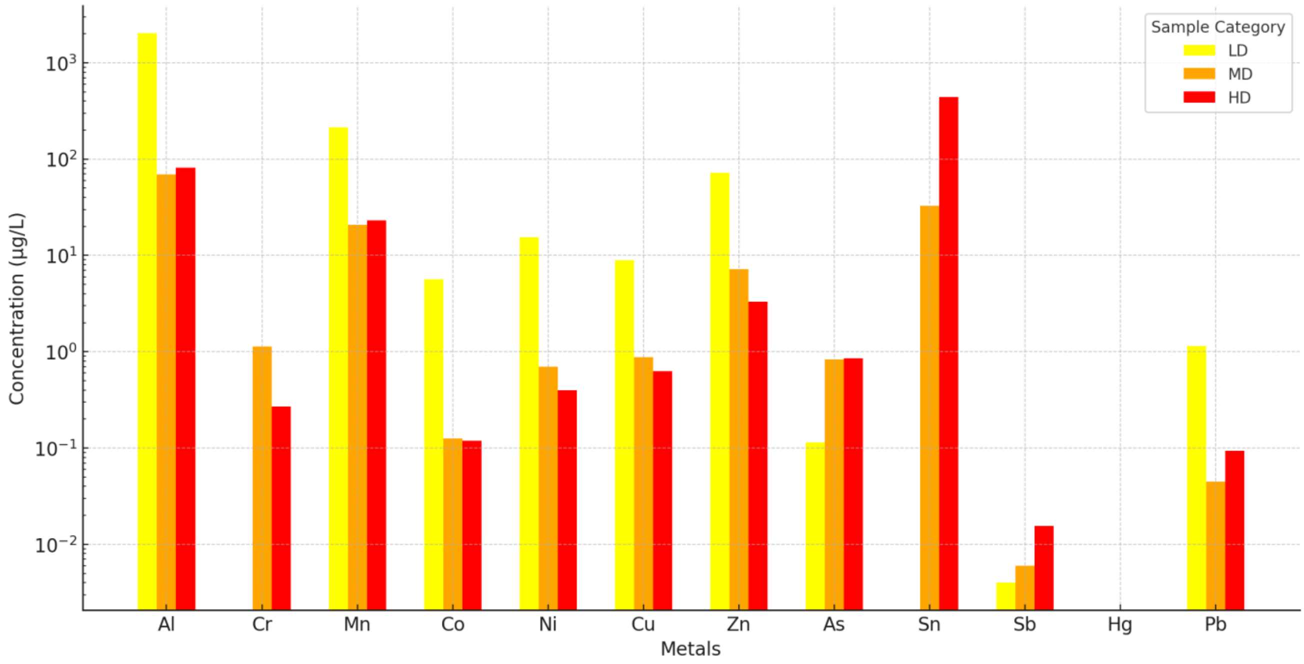



Figure 8: Comparison of PTEs in water samples across different otter density sites

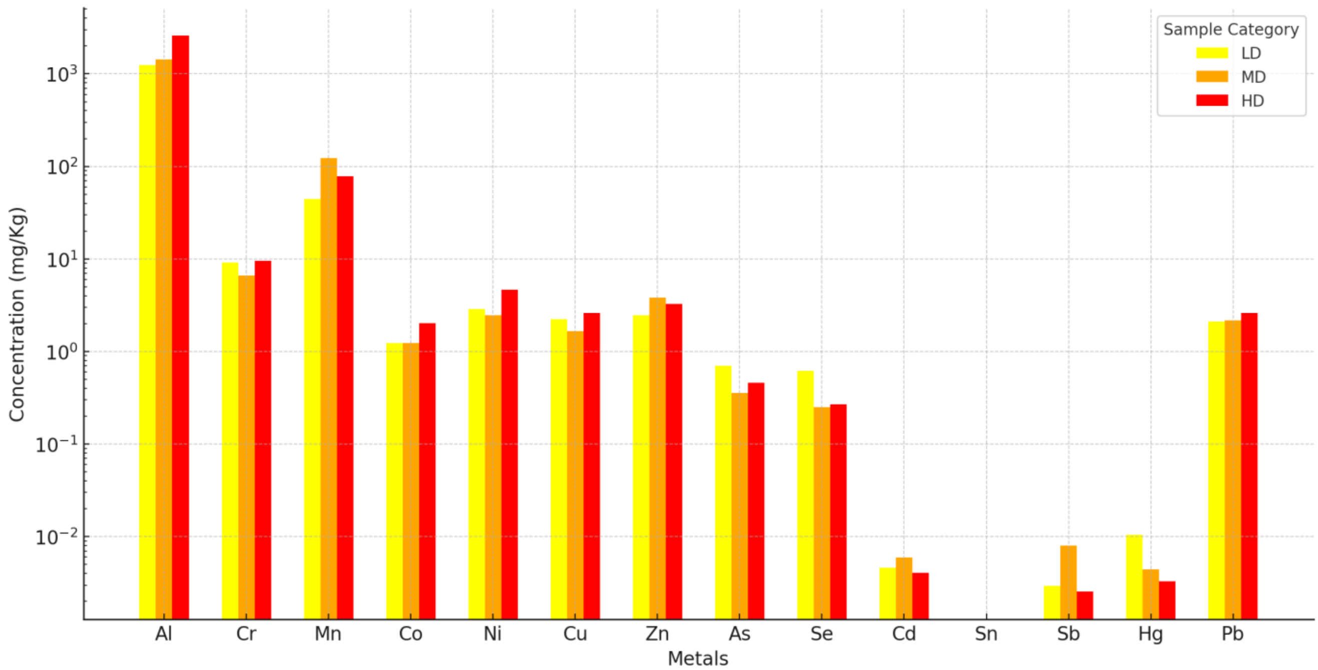



Figure 9: Comparison of PTEs in sediment samples across different otter density sites

**2) Diet:** Approximately 13 fish species were analysed for the presence and concentration of PTE. The analysis detected all the PTEs, with their concentrations shown in Figure 10. On an average, the PTE concentrations in fish followed the order Sn (1607 ± 3040.9) > Al (37.94 ± 62.04) > Zn (22.52 ± 10.61) > Mn (13.52 ± 16.29) > Cu (6.18 ± 6.52) > Cr (0.44 ± 0.97) > Pb (0.12 ± 0.32) > As (0.08 ± 0.05) > Ni (0.06 ± 0.23) > Hg (0.049 ± 0.035) > Co (0.033 ± 0.042) > Cd (0.011 ± 0.006) > Sb (0.003 ± 0.005). In terms of Σ PTE concentrations, the fish species followed the order *Labeo calbasu* > *Rita kuturnee* > *Oreochromis mossambicus* > *Wallago attu* > *Barbonymus gonionotus* > *Cirrhinus reba* > *Garra bicornuta* > *Labeo rohita* > *Garra lamta* > *Tor khudree* > *Osteochilus nashi* > *Chagunious chagunio* > *Labeo dyocheilus.*

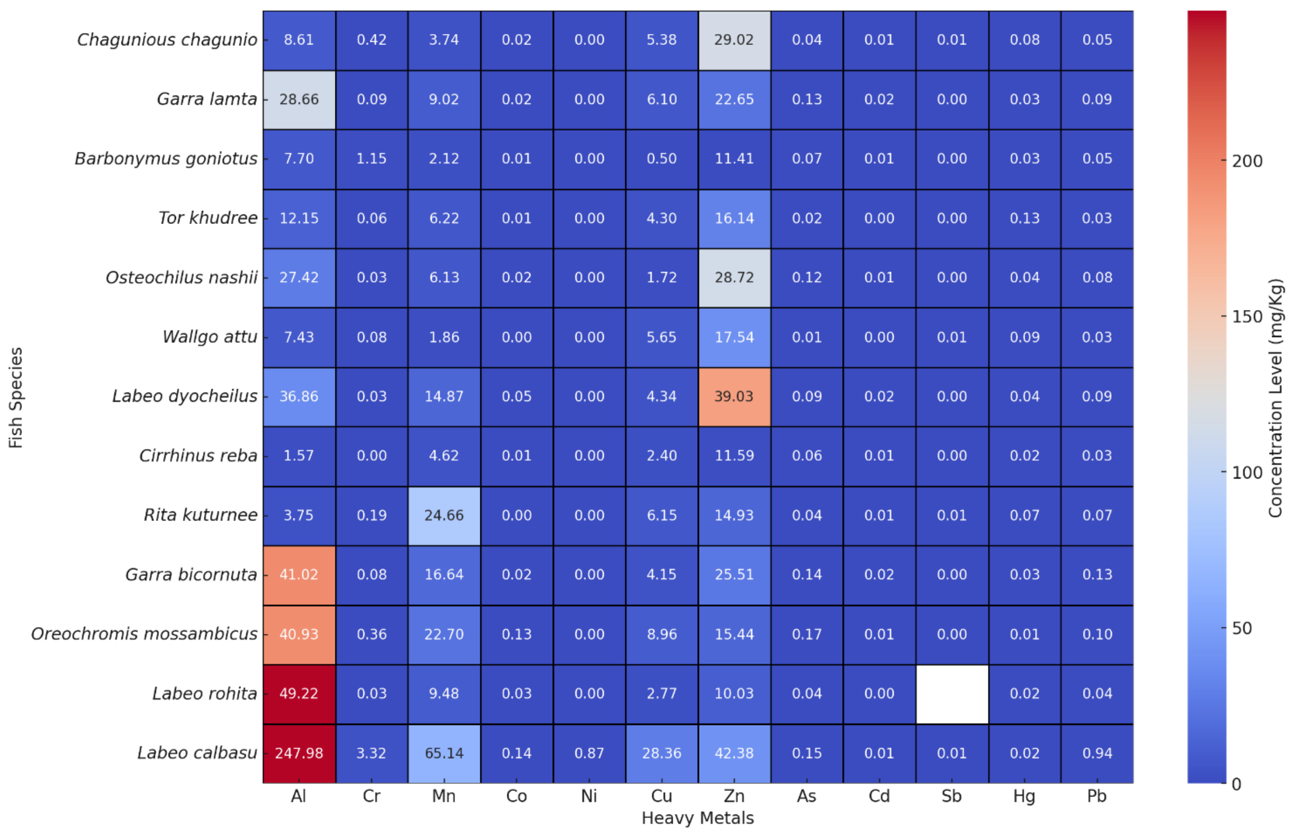



Figure 10: Concentration of PTEs in Fishes of Tungabhadra Otter Conservation Reserve, Karnataka (N = 39)

3) **Scat:** A total of 92 scat samples with 13 replicates that is a total of 117 scat samples were analysed for the PTEs. On average, the PTE concentrations in scat followed the order Sn (5808.9 ± 9161.42) > Al (4438.1 ± 3285.8) > Se (2277.8 ± 4792) > Mn (474.81 ± 275.37) > Zn (331.65 ± 180.39) > Cu (29.96 ± 32.76) > Cr (10.36 ± 10.99) > Ni (8.83 ± 11.07) > Pb (2.688 ± 1.67) > Co (2.56 ± 2.62) > As (2.27 ± 1.88) > Cd (0.15 ± 0.19) > Hg (0.091 ± 0.073).

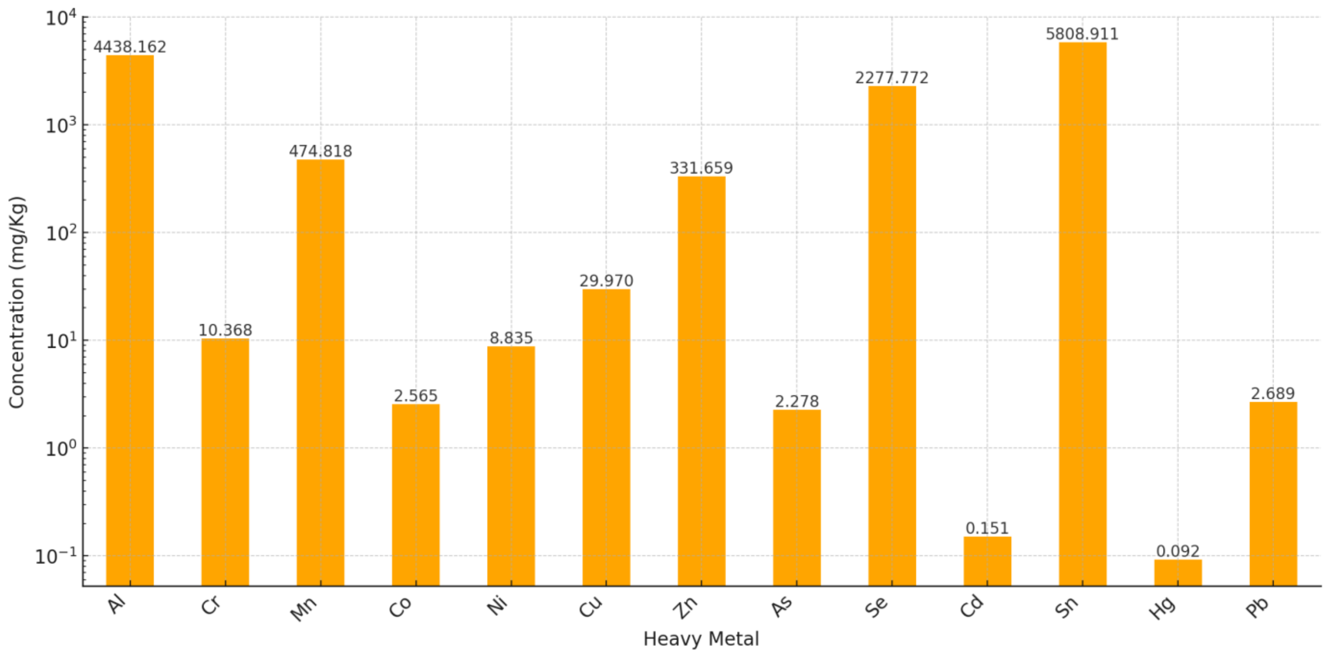



Figure 11: Concentration of PTEs in Scat of Smooth-coated otter from Tungabhadra Otter Conservation Reserve, Karnataka (N = 117)

### 4.3.2 Risk Assessment

**1) Environmental exposure:** Aquatic Life Criteria values specified by the United States Environmental Protection Agency (USEPA) were used to calculate the Risk Quotients for each metal in the water. Figure 12 shows the RQ values calculated for each element in the water. Individual elements posed an ecological risk in the order Al > Mn > Zn > Ni > Cr > Cu > As > Pb > Sb.

Similarly, RQs were calculated for the sediments using the Threshold Effect Levels specified under International Sediment Quality Guidelines. Figure 13 shows the RQ values calculated for each element in the sediment. Individual elements posed an ecological risk in the order Cr > Zn, Ni, Cu > Pb > As > Cd > Hg. All the elements were observed to be within the recommended threshold limits.

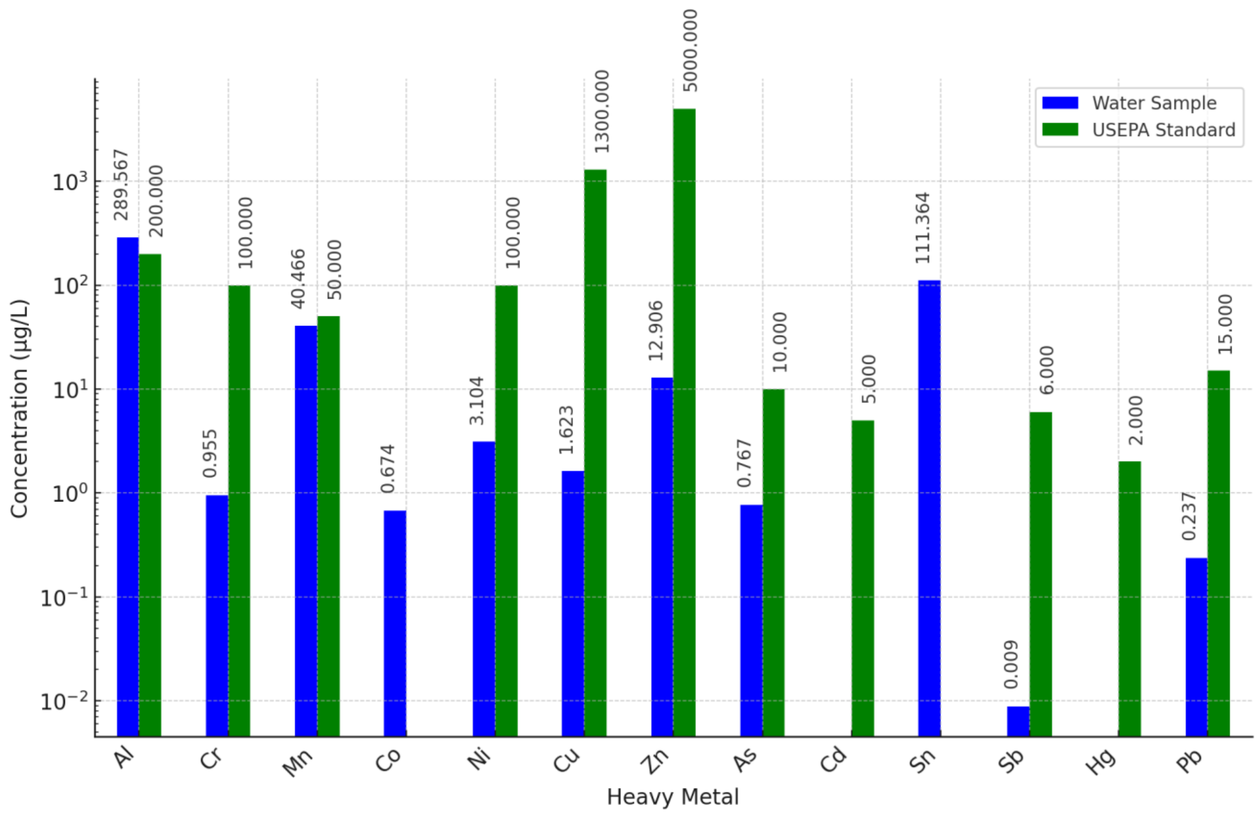



Figure 12: Comparison between the concentration of PTEs in water samples from Tungabhadra Otter Conservation Reserve and Standard Threshold values of United States Environmental Protection Agency

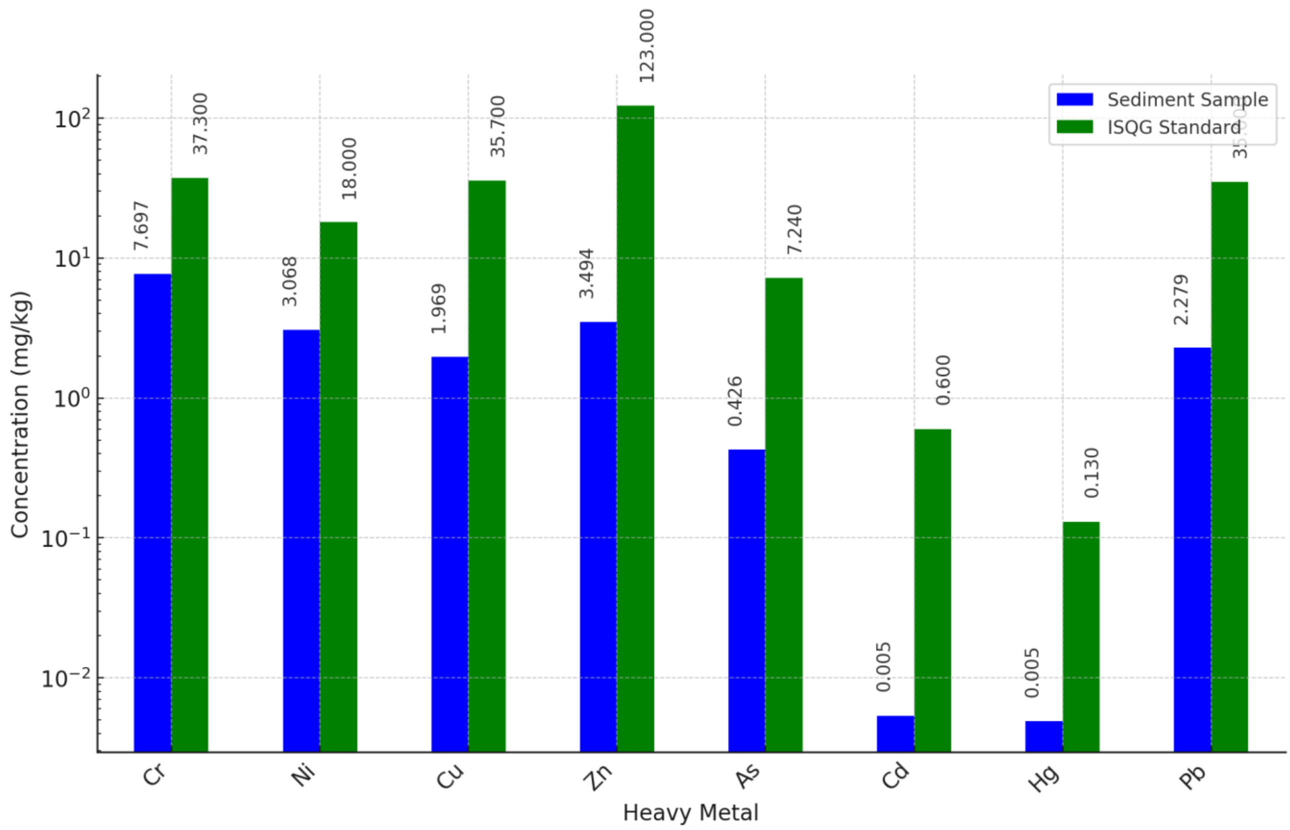



Figure 13: Comparison between the concentration of PTEs in sediment samples from Tungabhadra Otter Conservation Reserve and Standard Threshold values of International Sediment Quality Guidelines

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**2) Dietary Exposure:**

For each element, the Risk Quotient (RQ) was estimated using the Maximum Allowable Concentrations based on Reference Dose (MACRfD). The RQ was estimated based on the 5th, 50th, and 95th percentile, with the 95th percentile RQs representing the worst-case scenario. The elements posed a risk in the order As > Cu > Cr > Hg > Cd based on MAC RfD, Owing to the nature of its values, MAC RfD is used as the reference for the worst-case scenario, prompting proactive measures for the respective elements. As shown in Figure 14, the RfD-based RQs show high risk for As, Cu, Hg, and Zn at all three percentiles.

| PTE | RFD (mg/kgwwday) | MACRfd. (mg/kgww) | RQ @ 5% RfD | RQ @ 50% RfD | RQ @ 95% RfD | RQ AVERAGE |
|---|---|---|---|---|---|---|
| **Arsenic** | 0.0003 | 0.003 | 5.445 | 22.424 | 52.741 | 27.542 |
| **Cobalt** | 0.06 | 0.505 | 0.002 | 0.038 | 0.257 | 0.068 |
| **Cadmium** | 0.001 | 0.008 | 0.323 | 0.993 | 2.892 | 1.355 |
| **Chromium** | 0.003 | 0.025 | 0.667 | 3.123 | 80.607 | 17.959 |
| **Copper** | 0.04 | 0.337 | 3.651 | 12.877 | 49.619 | 18.443 |
| **Lead** | - | - | - | - | - | - |
| **Nickel** | 0.02 | 0.168 | 0 | 0 | 2.08 | 0.4 |
| **Mercury** | 0.0007 | 0.006 | 2.340 | 5.006 | 18.293 | 7.925 |
| **Zinc** | 0.3 | 2.524 | 4.301 | 6.949 | 15.996 | 8.667 |

Table 4: Reference dose, Maximum allowable reference dose, and Risk Quotient values at 5%, 50%, and 95%

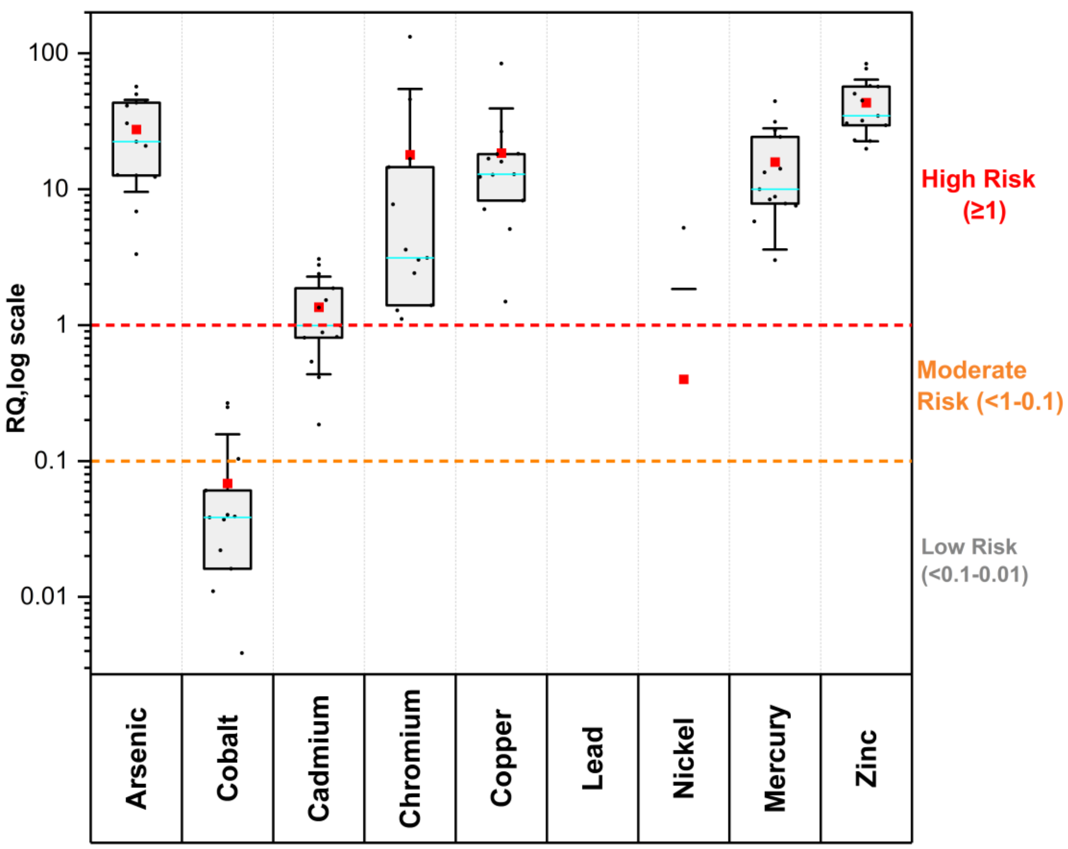


Figure 14: PTEs with Risk quotient categories

### 4.3.3 Heavy metal excretion in otters: Egestion to Ingestion ratios

The daily ingestion rate of a smooth-coated otter is 15 – 20% of its body weight which is about 2.2 - 2.8 kg per day (Mason and Macdonald 1986). Even with an average intake of 2.5kg/day. So we calculated the Egestion/Ingestion ratio based on the concentration of PTEs in prey (Ingestion) and scat (Egestion). We found that with high egestion/ingestion ratios for metals, like Copper and Nickel, the otters are effectively egesting them, potentially reducing the risk of bioaccumulation. Conversely, metals with low ratios, like Mercury, Lead, and Chromium suggest a higher risk of accumulation and toxicity.

| | Egestion (conc. In spraint, mg/kg) | Average PTE content of prey | Ingestion (Daily intake of PTE , mg/Kg) | Egestion/Ingestion Ratio |
|---|---|---|---|---|
| **Arsenic** | 2.28 | 0.08 | 0.13 | 17.77 |
| **Cobalt** | 2.56 | 0.06 | 0.09 | 27.33 |
| **Cadmium** | 0.15 | 0.01 | 0.01 | 13.93 |
| **Chromium** | 10.37 | 0.79 | 1.22 | 8.48 |
| **Copper** | 29.97 | 0.06 | 0.09 | 319.34 |
| **Lead** | 2.69 | 0.24 | 0.36 | 7.41 |
| **Nickel** | 8.83 | 0.17 | 0.27 | 32.84 |
| **Mercury** | 0.09 | 0.05 | 0.08 | 1.17 |
| **Zinc** | 331.66 | 19.78 | 30.47 | 10.89 |

Table 5: Egestion/Ingestion ratios of PTEs

# 5.0 DISCUSSION

Smooth-coated otters are the top aquatic predators of Indian rivers. Several factors directly or indirectly influence the decline of otter populations. However, the significant causes of decline in this landscape could be the degradation of their habitat quality due to increased land use practices, pollution, and changes in the composition and health of its prey. Habitat degradation due to direct chemical addition occurs when pollutants enter the ecosystem through runoff from agricultural areas, industry outflows, mining areas, and rainfall from contaminated skies (Qiao et al., 2022; Khalil et al., 2007; Elrashidi et al., 2007). Numerous organochlorines, including PCBs and heavy metals, particularly mercury, are implicated. All of these substances have the potential to be fatal when they build up in the bodies of mammals (Kruuk 2006). The presence of these potentially toxic elements in mustelids is known to affect reproductive impairment, developmental abnormalities, neurotoxicity, etc. (Andrew et al., 2018; Charles et al., 2020). This study aimed to assess the presence and distribution patterns of smooth-coated otters in Tungabhadra Otter Conservation Reserve, and also document their dietary spectrum in human-modified river-scape also evaluate the concentration of Potentially Toxic Elements (PTEs) in their diet, spraint, and habitat.

## 5.1 Distribution of Smooth-coated otter using sign survey

Sign surveys measure spatial patterns of animals based on detection or non-detection of animal signs (Heinemeyer et al., 2008). Signs surveys are best non-invasive, inexpensive method to study animal distribution (Humphrey and Zinn 1982). The study revealed that the smooth-coated in Tungabhadra Otter Conservation Reserve exhibits clumped distribution, with high-density areas serving as core regions of otter activity. The clumped pattern distribution suggests that the otters prefer specific areas within the Tungabhadra Otter Conservation

Reserve. The high-density areas could be indicative of optimal habitat features such as abundant food resources, adequate escape cover, and availability of water with suitable quality, minimal human disturbance, and favourable shelter sites which are crucial for otter survival.

In relation with previous studies the distribution of Eurasian otter spraints densities found to be non-random and in clumped distribution (Jenkins and Burrows, 1980). This similarity could be attributed to the comparable methodology followed. Other studies have also documented clumped distribution in otter populations. For instance, Mason and Macdonald (1986) observed that otter spraint distribution in British river systems showed significant clustering around specific habitats such as large rocks and tree roots. Further, Kruuk (2006) noted that otter populations tend to aggregate in areas with high prey availability and low human disturbance, leading to clumped distribution patterns. Similarly, a study by Romanowski et al., (2013) on the habitat correlates of Eurasian otter reported that otters preferentially selected river stretches with higher fish densities and lower levels of pollution, resulting in non-random, clumped distribution.

Through effective conservation and management strategies, the high-density areas of otter distribution identified in this study should be prioritized for protection by the forest department. These high-density regions could be crucial for the survival and reproductive success of the smooth-coated otter population in Tungabhadra Otter Conservation Reserve. Ensuring the protection of these areas can help maintain the necessary habitat conditions.

### 5.2 Food Habits of Smooth-coated otter

This study reveals that smooth coated-otters have a heavy reliance on fish, indicating that they are predominantly piscivorous constituting about 98% of fish diet followed by 2% of crab. Previous studies on smooth-coated otters in other regions have similarly reported a preference for fish-dominated diets (Hussain 2013; Anoop and Hussain 2005; Nawab and

Hussain 2012; Basak et al., 2021). During the study, there were no signs of birds, amphibians, or mammals in the otter diet indicating otters were able to meet their entire energy requirements through fish and crabs. Consumption of such secondary prey categories has been reported in previous studies (Anoop and Hussain 2005; Basak et al., 2021; Baskaran et al., 2021).

The diet composition of otters in Tungabhadra Otter Conservation Reserve consists of approximately 69.53% non-native fish species (*Oreochromis mossambicus, Cyprinus carpio, Labeo rohita, and Cirrhinus cirrhosus*) reflecting high local availability of these non-native fishes over native fishes. However, the mandates of the National Fisheries Development Board (NFDB) have significantly altered the natural ichthyofaunal assemblages by introducing exotic fish species into these ecosystems to achieve their objectives, without considering the long-term impacts on the native fish communities (Milardi et al., 2019; Anna et al., 2019). The dietary diversity of carnivores fluctuates inversely with prey availability (Tinker et al., 2008) which is evident in the smooth-coated otter, where over 60% of its diet is comprised of just three non-native fish species.

Estimating the diet through two different methods "frequency of occurrence" and the "score-bulk estimate" method, has its advantages and disadvantages. The frequency of occurrence method can overestimate prey items that are present in small amounts and underestimate those that are present in large amounts, leading to a skewed perception of their dietary significance; this method has been widely criticized for this issue (Jacobsen and Hansen 1996). On the other hand, the score-bulk estimate method can be subjective, as it relies on visual estimation and scoring, which might vary between researchers. In the study, there is a fairly similar percentage (+/- 5) of prey categories in both methods. It is recommended that the 'score-bulk estimate' give a better estimate of prey than any other method (Jacobson and Hansen, 1995).

## 5.3 Heavy metal ecotoxicology in aquatic ecosystems

Heavy metals are a major group of persistent toxic pollutants and these can be acquired by otters from the fish they eat, with bioconcentrations reaching 90-95% (Ruiz-Olmo et al., 2000a, Ruiz-Olmo et al., 2000b). Prey and direct contamination are known to have contributed to the local extinction of the Eurasian otter (*Lutra lutra)* in several European regions (Gutleb, 2000). Apart from the build-up of these impurities in otter tissues, there are proof that mothers pass these components on to their young (NOM-027-SSA1-1993; Chen et al., 2009; White et al., 2009; Basu et al., 2005; Croteau et al., 2005). When compared to healthy individuals, ill and malnourished sea otters had higher amounts of Co, Zn, and Cd. This suggests that these PTEs may have immunotoxin effects on otters (Kannan et al., 2006).

The study reveals the presence of all the PTEs in the habitat, diet, and scat of smooth-coated otters. In water and sediment, the concentration of the PTEs is below the standard threshold values which indicate no harmful effects to the aquatic species except aluminium in water which could cause possible effects such as neurological effects, respiratory disorders, renal damage, and also decline in reproductive health. Though these PTEs are below the standard threshold values they pose a potential health risk to the otters through bioaccumulation.

The analysis of PTE concentrations in water across different otter-density sites. Specifically, LD sites, which represent areas with fewer otter signs, exhibit higher concentrations of metals compared to MD and HD. This trend suggests that regions with fewer otters are more heavily contaminated with various PTEs. This pattern may imply that smooth-coated otters, being top predators and bio-indicators, either avoid heavily contaminated areas or that their presence influences metal concentration. Interestingly these results contrast with the findings of Huang and Nelson (2018) on river otters (*Lontra canadensis*), where they explored the concept of ecological traps and concluded that otters are incapable of recognizing ecological traps in their habitat. Furthermore, when examining the concentrations of PTEs in sediment, the results indicate that HD sites have higher concentrations compared to MD and LD sites. The contrast between water and sediment contamination patterns highlights the complex interactions between otter and their habitat, suggesting that while otters might avoid contaminated water bodies, they may not exhibit the same avoidance behaviour for sediment contamination.

Assessing the risk assessment of otters has given that Rfd based RQ values indicating high risk from all the elements except for Cobalt. Arsenic has relatively high RfD values at all percentiles compared to MAC, indicating significant potential risk. The most concerning metal affecting the health of otters is Mercury and the high Rfd values could be affecting the otters significantly but the ability of otters to eliminate huge amounts of Hg and other PTEs through their hair during molting could be the reason for otters being present in Tungabhadra Otter Conservation Reserve (Mason and Macdonald, 1986; Hyvarinen et al., 2003).

The results reveal that Arsenic has the highest RQ, with values reaching up to 52.47 mg/kg at the 95th percentile suggesting severe contamination. Elevated arsenic levels can lead to severe health issues, including carcinogenic effects and metabolic disruptions similar results have been observed in other studies. Copper also shows a high RQ, with an average value of 26.979mg/kg indicating substantial contamination. Similar findings were reported by Mason and Macdonald (2004), who highlighted the adverse effects of copper on aquatic ecosystems and top predators. Elevated levels of copper can lead to kidney damage. Mercury with an average RQ of 15.85 mg/kg, poses a significant risk, particularly at the 95$^{th}$ percentile. Mercury contamination has been extensively studied in various otter species indicating bioaccumulation and adverse health effects. Experiments on Canadian otters (*Lontra canadensis*) by O’Connor and Nielsen (1981) showed clinical signs of mercury poisoning at 2,4, and 8 p.p.m and they concluded that regular intake of food containing 2 p.p.m would be lethal to otters in the wild

(Mason and Macdonald, 1986). Zinc also shows a high risk with an average RQ of 43.318, which is considerably higher compared to other elements. Previous research by Roos et al., 2012, has shown that elevated zinc levels can lead to toxic effects in otters, including impaired growth and weakening immune function.

The study of Egestion/Ingestion ratios for various PTEs in smooth-coated otters offers significant insights into their ability to manage heavy metal exposure through diet. Metals such as mercury, lead, and chromium exhibit low egestion/ingestion ratios. This indicates a higher risk of these metals accumulating within otters. Mercury, in particular is known for its high toxicity and ability to bioaccumulate in the food chain, leading to health impacts. But the metals such as copper, nickel, cobalt, and arsenic have high egestion/ingestion ratios indicating effective excreting of these elements. The reasons for the effective elimination of heavy metals could be due to the role of gut microbiota which would detoxify heavy metals by binding with them and preventing their absorption and facilitating their excretion which is called Bioremediation (Monachese et al., 2012; Daisley et al., 2018; Zhai et al., 2016). It could also be through the composition of the diet which can influence the egestion of heavy metals and also to the fast metabolism which aids in the rapid processing and elimination of waste, including heavy metals. The efficient metabolic processes of otters help them prevent the accumulation of toxic substances by ensuring they are quickly processed and excreted from the otter gut (Bist and Choudhary 2022).

### 5.4 Limitations of the study

Despite all the efforts made using all possible methods we didn’t get permission to catch the fish in the TUNGABH to quantify the fish availability which forced us to collect fish only from the fishermen and also prepare the reference slides for diet analysis and collection of fish for the ecotoxicological objective. There were difficulties in collecting water as well as sediment samples from the middle stretch of the river. Retreating monsoon rains just before the start of the study also posed significant challenges, as they affected the collection of otter signs for the sign survey by washing away the old spraints and pugmarks. Furthermore, logistical constraints and limited access to certain remote areas of the reserve hindered comprehensive data collection. Finally, the reliance on non-invasive methods may have limited the depth of information that could have been obtained through direct sampling techniques for the ecotoxicological part.

## 5.5 Conservation implications

Identifying the high-density areas of otter activity can inform conservation efforts to prioritize and protect these critical habitats. Also, it will be easy for the forest department just to monitor and protect these habitats rather than monitoring the entire stretch. The study highlights the presence of high concentrations of PTEs in water and sediment and there is a need for stricter pollution control measures to reduce the contamination levels in these habitats. The diet of otters in Tungabhadra Otter Conservation Reserve is about 63.27% which consists of non-native fishes which means otters are indirectly controlling the population of non-native fishes and preventing the complete suppression of the native fish species. Without otters in Tungabhadra Otter Conservation Reserve, there is a risk that many native fish species could be quickly displaced by these non-native fish.

# 6.0 CONCLUSION

The study reveals that smooth-coated otters in Tungabhadra Otter Conservation Reserve exhibit a clumped distribution pattern, with a preference for specific areas. Their diet is predominantly composed of non-native fish species, which reflects the altered ichthyofaunal assemblages in the region. The otters are exposed to PTEs in their environment, with water and sediment PTE concentrations generally below harmful thresholds. Whereas the concentration of PTEs was very high in the diet of otter posing a high risk. The egestion/ingestion ratio revealed the efficient removal of most heavy metals mainly copper and also the low egestion rate of mercury which indicates the bioaccumulation of mercury and its high risk.

## 6.1 Way forward

Furthermore, the research will be extended to other regions to explore the relationship between otter distribution and environmental pollutants in different habitats. By comparing data across various landscapes, we aim to develop a comprehensive understanding of how anthropogenic pressures influence aquatic ecosystems. This study in Tungabhadra Otter Conservation Reserve will continue to investigate seasonal variations in diet and heavy metal loads in this landscape. Baseline data on the population of smooth-coated otters will be estimated using spatially explicit capture-recapture (SECR) methods. Major emphasis will be placed on studying the basic ecology of smooth-coated otters, including behaviour and hunting ecology using unmanned aerial vehicles (UAVs), and Holt ecology using camera trap.

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