EVALUATION OF METAL CONTENT IN THE SCAT OF SMOOTH-COATED OTTERS (LUTROGALE PERSPICILLATA)

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, **"Evaluation of metal content in the scat of Smooth-coated Otters (*Lutrogale perspicillata)*"** is based on the results of investigations carried out by me in the Zoology at School of Biological Sciences and Biotechnology, Goa University under the Supervision of Dr. Avelyno H. D'Costa and the same has not been submitted elsewhere for any award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations/experimental or other findings given in the dissertation. I hereby authorize the University authorities to upload this dissertation repository or anywhere else as the UGC regulations, demand and make it available to any one needed.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report entitled **"Evaluation of metal content in the scat of Smooth-coated Otters (*Lutrogale perspicillata)*"** is a bonafide work carried out by Mr. Jonathan D'Costa under my supervision in partial fulfilment of the requirements for the award of the degree of Masters in Zoology in the Discipline Zoology at the School of Biological Sciences and Biotechnology, Goa University

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CHAPTER I

INTRODUCTION

Heavy metals are a group of toxic elements having high atomic weights and densities that are present in the environment and pose a significant threat to human health and the ecosystem. This study is aimed at evaluating the metal content in the scat of smooth-coated otters from two sites in Goa associated with two different river systems; rivers Mandovi and Zuari.

Heavy metals can be toxic to living organisms, including humans, when they are present in high concentrations in the environment. Some metals have the potential to bio-magnify up the food chain, resulting in higher contaminant loads in top-level consumers compared to their prey (Cardoso et al., 2014; Daley et al., 2014).

Heavy metals in otter scat can be an indicator of environmental pollution and can have negative impacts on the health of otters and other wildlife. Heavy metals such as lead, mercury, and cadmium can accumulate in the tissues of otters and other animals, causing a range of health problems, including neurological damage, reproductive failure, and immune system dysfunction.

Mid-sized and large carnivores, which are top predators and scavengers, have a crucial role in ecosystem functioning. However, they are susceptible to the accumulation of pollutants, including harmful heavy metals, due to their position in the food chain (Kalisinska et al., 2016).

Otters, being top predators in their ecosystem, can accumulate high levels of heavy metals in their bodies over time. Otters are good bio-indicators of the quality of the environment, especially aquatic habitats (Clarkson, 1995). According to Wainstein (2022), river otters are an exceptional bio-monitor of environmental contaminant exposure in river systems, as pollutant levels in their scat reflect the abiotic and biotic conditions of the region.

By studying the presence of heavy metals in otter scat, we can get an idea of the extent of heavy metal pollution in the environment and the potential risks to both wildlife and humans. Monitoring heavy metals in otter scat provides insight into the effectiveness of pollution control measures and inform future management strategies.

Study Animal

Smooth-coated otters (*Lutrogale perspicillata*) are semi-aquatic mammals that belong to the family Mustelidae, which also includes weasels, ferrets, badgers, and wolverines. They are found throughout much of South and Southeast Asia, including India, Bangladesh, Nepal, Bhutan, Myanmar, Thailand, Laos, Cambodia, Vietnam, and Indonesia. Smooth-coated otters are the largest of the otter species found in Asia, and can grow up to 1.3 meters (4.3 feet) in length, including their tail, and can weigh up to 11 kg (24 pounds). Smooth-coated otters are found in a wide variety of aquatic habitats, including rivers, lakes, swamps, and coastal areas (Smooth-coated otter, n.d ; Kruuk, 2006).

They are particularly common in freshwater and estuarine habitats, and are known for their ability to adapt to human-modified landscapes such as agricultural areas, urban parks, wetlands and Khazan lands in Goa. Smooth-coated otters are listed as “Vulnerable” on the IUCN Red List of Threatened Species, due to habitat loss and degradation, pollution, hunting, and the illegal pet trade. It has been classified as a Schedule 1 species under the Indian Wildlife Protection Act (IWPA).

On the Indian peninsula, smooth-coated otters are primarily found in plains, deserts, semi-arid areas, and the highlands of the Deccan, inhabiting canals, and irrigation tanks, using flooded fields, estuaries, coastal belts, and the open sea as hunting grounds (Prater 2005).

Local Scenario

In Goa, smooth-coated otters can most commonly be found in brackish water or estuarine habitats such as in the mangroves of rivers Zuari and Mandovi. These mangroves are ideal habitats for smooth-coated otters. These areas are highly productive and are hence used by the locals for culturing commercial species such as Indian white prawns, pearl spot, giant perch, etc (Sonak et al. 2013). Most areas around these mangrove forests are converted to Khazan lands. The Khazan system is a traditional method of agriculture practiced in the coastal regions of Goa. The system involves the cultivation of crops in low-lying marshy areas, which are flooded by seawater during high tide and drained during low tide. The Khazan system has been practiced in Goa for over 1,000 years and has played an important role in the social and economic development of the region. In Goa, Khazan lands are commonly found along the banks of the Mandovi and Zuari rivers and their tributaries, as well as along the other estuaries and brackish water ecosystems in the coastal regions. The Khazan system is based on the construction of bundhs or embankments along the creeks and estuaries, which trap the seawater during high tide and release it during low tide. Bundhs are embankments or levees built along the rivers, estuaries, and backwaters to prevent flooding and erosion, and to facilitate irrigation and agriculture. The bundhs are made of mud or stone and are usually planted with grass and shrubs to stabilize the soil and prevent erosion. Bundhs in Goa have multiple functions, including flood control, water storage, and irrigation (Rubinoff, 2001).

Bundhs in Goa also have cultural and social significance, as they are often associated with traditional fishing communities and agriculture. The bundhs provide a habitat for a variety of aquatic species, including fish, crabs, and molluscs, which are an important source of food for the local communities. These species are mainly cultured by the locals for commercial purposes and serve as a major source of income in such communities (Rubinoff, 2001).

The Khazan lands in Goa are particularly important habitats for otters, as they provide an abundance of prey in the form of fish and crabs. The bundhs in Khazan lands serve as defecation sites for smooth-coated otters. Smooth-coated otters feed on a variety of organisms such as fish, frogs, snakes, small mammals, molluscs, crustaceans such as crabs and prawn, etc. Being the top predator in the ecosystem, smooth-coated otters are prone to bioaccumulation and biomagnification of several pollutants such as heavy metals.

Heavy metals

Heavy metals are elements that have a high atomic weight and density. They are naturally occurring elements in the earth's crust, and can be released into the environment through human activities such as mining, manufacturing, and waste disposal. Heavy metals are a major environmental and health concern due to their toxicity and persistence in the environment. Some of the most common heavy metals include lead, mercury, cadmium, arsenic, and chromium. These metals are toxic to both humans and the environment, and can cause a range of health problems depending on the level of exposure (Jaishankar et al., 2014).

This study is aimed at studying the concentrations of heavy metals in the environment by analysing their concentrations in the faeces of smooth-coated otters. The heavy metals that are focused in this study include Lead (Pb), Cadmium (Cd) and Arsenic (As).

Lead (Pb)

Lead is a highly toxic metal that has been widely used in paint, gasoline, and plumbing materials. Exposure to lead can cause neurological and developmental problems, especially in young children. It can also cause anaemia, kidney damage, and reproductive problems.

Lead can be found in various sources, including:

1. Old paint: Lead-based paint was commonly used before the 1970s. Homes built before 1978 may still have lead-based paint on their walls, windows, and doors.
2. Soil: Soil can become contaminated with lead from lead-based paint or other sources, such as leaded gasoline, industrial activities, or nearby lead smelters.
3. Drinking water: Lead can leach into drinking water from lead pipes, lead solder, and lead-containing plumbing fixtures.
4. Consumer products: Some products, such as toys, jewellery, and ceramics, may contain lead, especially those made in countries with less stringent regulations.
5. Food: Lead can be found in certain types of food, such as some types of fish, fruits and vegetables grown in lead-contaminated soil, and certain types of candy or food imported from countries with less stringent regulations.
6. Air: Lead can be released into the air from sources such as industrial processes, vehicle exhaust, and lead smelters

   (Jaishankar et al., 2014).

Cadmium (Cd)

Cadmium is a heavy metal that is commonly used in batteries, pigments, and plastics. Exposure to cadmium can cause lung and prostate cancer, as well as kidney damage and osteoporosis.

Cadmium can be released into rivers from various sources, both natural and anthropogenic.

Here are some common sources of cadmium in rivers:

1. Natural sources: Cadmium can occur naturally in rocks, soils, and sediments, and can be released into rivers through erosion, weathering, and natural mineralization.
2. Industrial sources: Cadmium is used in various industrial processes such as electroplating, manufacturing of batteries, pigments, and plastics, and can be discharged into rivers through industrial effluents.
3. Agricultural sources: Cadmium is also used in fertilizers and can accumulate in agricultural soils, which can then be washed into rivers through runoff.
4. Mining activities: Cadmium is often found in association with zinc and other metals in ores, and can be released into rivers through mining activities and mine waste.
5. Sewage sludge: Cadmium can also be present in municipal sewage sludge, which can be applied to agricultural lands and can then leach into rivers through runoff or leaching.
6. Atmospheric deposition: Cadmium can also be deposited onto river surfaces through atmospheric deposition from sources such as incineration of municipal waste or fossil fuel combustion

   (Jaishankar et al., 2014).

Arsenic (As)

Arsenic is a toxic heavy metal that is found in the earth's crust and can be released into the environment through mining and agricultural activities. Exposure to arsenic can cause skin lesions, cardiovascular disease, and cancer.

Arsenic can be found naturally in the earth's crust and can be released into the environment through both natural and human activities. Here are some sources of arsenic in rivers:

1. Natural weathering of rocks and minerals: Arsenic is naturally present in rocks and minerals, and as these materials weather, arsenic can be released into water sources.

2. Agricultural practices: The use of arsenic-containing pesticides and fertilizers in agriculture can lead to contamination of water sources, particularly in areas where irrigation is used.

3. Mining and industrial activities: Arsenic is often present in metal ores, and mining and smelting activities can release arsenic into the environment. Similarly, industrial activities such as coal-fired power plants and metal processing can also contribute to arsenic contamination of water sources.

4. Contamination from waste disposal: Arsenic can be present in waste materials such as industrial waste, hazardous waste, and landfill leachate, which can contaminate nearby water sources.

5. Naturally occurring sources: Arsenic can occur naturally in some types of rock formations, and in some areas, groundwater can be naturally high in arsenic

   (Jaishankar et al., 2014).

Heavy metals can enter the environment through a variety of pathways, including air pollution, water pollution, and soil contamination. Once in the environment, they can persist for a long time and accumulate in the food chain. For example, fish that live in contaminated water can accumulate high levels of mercury, which can then be consumed by humans.

Human exposure to heavy metals can occur through a variety of pathways, including inhalation, ingestion, and skin contact. The study of heavy metals in the environment is crucial because they can pose a significant threat to the health and well-being of humans, animals, and ecosystems. Heavy metals naturally occur in the Earth's crust, but their concentrations can increase in the environment due to human activities such as mining, manufacturing, and waste disposal. Heavy metals are non-biodegradable, meaning that they cannot be broken down by biological processes, and can accumulate in the environment over time, making them persistent pollutants.

Heavy metals are toxic to living organisms. Even small amounts of heavy metals can cause severe health problems, such as damage to the nervous system, liver, kidneys, and reproductive system. Long-term exposure to heavy metals can lead to chronic health issues, including cancer. Heavy metals can disrupt the balance of ecosystems by affecting the growth and reproduction of plants and animals (Jaishankar et al., 2014).

CHAPTER II

LITERATURE REVIEW

A number of studies are available on the presence of metals in otters in different parts of the world. Several studies on the heavy metal content in the scat of other mammals are available and studies on the bioaccumulation and excretion of heavy metals in various organisms are also available.

Delibes et al. (2009) determined the amount of several heavy metals such as copper, cadmium, zinc (Zn) and lead (Pb) and metalloids (As) in otter scat. Concentrations of Zn, Pb and As were statistically higher in faces collected along the test river than in those collected along the control river.

Rodríguez-Estival et al. (2020) estimated daily intakes of Pb and Hg non-invasively from the proportion of crayfish remains and metal levels in otter faeces. A total of 44 spraints (16 from the Hg-AREA, 12 from the Pb-AREA, and 16 from the REF-AREA) were randomly collected along a 1–2 km stretch in each river course. Otter spraints from the Hg-AREA had about 13-fold and 8-fold higher Hg levels than those from the REF-AREA and the Pb-AREA, respectively ($F2,41 = 84.62$, $p < 0.001$). Otter spraints from the Pb-AREA had about 7-fold and 43-fold higher Pb levels than those from the REF-AREA and the Hg-AREA, respectively ($F2,41 = 14.77$, $p < 0.001$).

Baos et al. (2022) used the Eurasian otter (*Lutra lutra*) as sentinel species to assess the potential impact of the toxic spill on the river ecosystems and their recovery with time by studying the spatial and temporal variation (1999-2003, 2006) of selected trace element (Cu, Zn, Cd, Pb and As) concentrations in faeces. The levels of trace elements decreased with the time elapsed since the toxic spill, except for Cd ($F_{1,352} = 0.29$, $P = 0.59$). However, rebounds for some elements (Pb, As, and Cu) were also observed, especially in the Middle and Lower reaches of the river, which might be attributed to the residual contamination in abiotic compartments and/or new inputs from industrial and agricultural activities in the nearby areas. Concentrations were relatively high when compared to those reported for the reference areas and other metal-polluted zones. Authors found that the estimated amounts of Pb and As ingested during the first years after the spill would be high enough to cause reproductive issues. This could affect the local population recovery, although evidence on distribution range and numbers suggests otherwise, with thriving populations at regional scale.

A study was carried out by Gutleb et al. (1998) on otters in three different countries from 1989 to 1994. Their kidneys and livers were analysed for five heavy metals, including mercury, zinc, copper, lead, and cadmium. Results revealed that lead concentrations did not exceed 3.5 µg/g in both liver and kidney tissues, with the majority of concentrations below 1 µg/g. Similarly, cadmium levels were not higher than 4.6 µg/g of dry weight in kidneys, which is deemed safe since a concentration of 100 µg/g of fresh weight in kidneys is considered critical for rats according to Goyer et al. (1984). Nonetheless, a concentration of 96 µg/g of cadmium was identified in the liver of an otter. Mean concentrations of cadmium, lead, zinc, and copper in the livers and kidneys of the otters included in the study were lower than toxic levels for otters, and all values were consistent with previous studies. However, toxic amounts of mercury were detected in the otters.

Lemarchand et al. (2010) analysed the organochlorine pesticides, PCBs, heavy metals and anticoagulant rodenticides in tissues of Eurasian otters (*Lutra lutra*) from upper Loire River catchment (France). Tissues of the Eurasian otter *(Lutra lutra)* from a naturally expanding population along upper Loire River (France) catchment were used for contaminants analyses. nine organochlorine pesticides, 16 PCB congeners, five heavy metals (lead, cadmium, mercury, copper and arsenic) and three anticoagulant rodenticides were quantified in livers of road-traffic killed otters."

In a study conducted by Vaz et al. (2021) the impact of mining activity on water quality and fish growth was analysed by comparing water samples collected from upstream and downstream sites in four mining areas in South Goa: Kalay, Ponda, Codli, and Rivona. The upstream site was considered as uncontaminated water while the downstream site had mining effluents from surrounding mines. Physicochemical parameters and heavy metals in water samples were analysed and fish from these sites were examined for body length, width, weight, and heavy metals in gills, liver, and muscle tissues. Statistical analysis was performed using Fisher's exact test. Results showed a degradation of water quality in downstream sites due to mining effluents, with significant alterations in turbidity, total suspended solids, and sulphates. Metals detected included iron, copper, zinc, manganese, cadmium, and nickel. Fish in downstream sites exhibited decreased body weight, attributed to the combined effect of changes in water quality and increased heavy metal concentrations due to mining effluents.

In a study conducted by Brown et al. (2021) metal concentrations in various tissues of northern sea otters were evaluated along with the effect of the otter's length on the concentration levels. The study also investigated if biomagnification of metals from prey to other tissues occurred and if selenium and mercury concentrations indicated a health risk or benefit. Arsenic, cadmium, lead, and total mercury concentrations were significantly different in different tissues and related to the otter's length. Cadmium, copper, and selenium levels increased in the kidney and liver tissues due to biomagnification. Total mercury levels were found to increase in all tissues. The study also found that lead and arsenic were excreted easily. The stomach contents showed higher mean arsenic levels than other tissues, indicating that sea otters effectively process and eliminate arsenic from their diet.

In a study by Gupta et al. (2012), the heavy metal concentrations in the faeces and feed of various captive wild mammals at Jodhpur Zoo in Rajasthan were analysed. The study found that the faeces had higher concentrations of heavy metals such as lead, cadmium, chromium, zinc and copper than the feed. The concentration of lead analysed in faecal matter of captive zoo wild mammals was in the range of 72.8±1.36 (*Panthera leo*) to 22.4±1.97 (*Axis axis*) ppm d/w. Cadmium was in range between 3.80±0.69 (*Panthera leo*) to 0.60±0.32 (*Macaca mulatta*) ppm d/w. Chromium ranged from 1.14 ppm to 9.9 ppm, copper ranged from 0 to 22.23 ppm and zinc ranged from 0 to 25.53 Studies conducted on soil and vegetation of the areas show that heavy metals such as lead, cadmium, chromium, copper and zinc were present in background levels. The soil and water in the areas were also analysed. It was found that the heavy metal content was higher in the soil than the faeces, suggesting that the main source of heavy metals in the body of these captive mammals if through the soil and not the feed. An additional source could have been through aerial exposure. The results obtained also show that these captive animals had higher concentrations of heavy metals than wild animals of the same species.

In a study by Gupta et al. (2012), the heavy metal content of faecal samples from wild animals in selected Wildlife Sanctuaries and National Parks of Western Rajasthan was analysed. Cadmium was in range of 0.90 to 1.49 ppm d/w in DNP , whereas it was 0.96 to 2.6 ppm d/w in Gajner sanctuary. Lead was observed in the range of 1.02 to 1.88 ppm d/w whereas it was 0.40 to 2.17 ppm d/w in Gajner sanctuary.

A study conducted by Dias et al. (2022) aimed to evaluate the habitat selection of smooth-coated otters in the state of Goa, India. The results show that otter occurrence is influenced by high salinity and prevalence of fishing. Otter presence also highly correlated to mangrove cover. The study concluded that smooth-coated otter prevalence is attributed to estuarine environment that is characterized by fishing activities, mangrove cover and brackish water.

A study by Harika et al., (2023) analysed the scats of the fishing cat (*Prionailurus viverrinus*) collected from five locations in the Godavari estuary mangrove habitats, Coringa Wildlife Sanctuary, Andhra Pradesh, India, to determine the level of various metals. The study applied an opportunistic method to collect scats in the mangrove forest and found a variety of prey species in the scats, including crabs, fishes, birds, rodents, plants, plastics, and unidentifiable prey matters. The study analysed the concentration of select metals, such as chromium (Cr), copper (Cu), and lead (Pb), since they intensively influence the physiology and behaviour of top predators. The concentration of Cu in fishing cat scats was found to be higher than the other two metals assessed, and the metals showed statistically substantial variation across locations. The study concluded that heavy metals may significantly threaten the fishing cat in the Coringa Wildlife Sanctuary, which is a vulnerable species according to the ICUN categories.

OBJECTIVES

- To assess the metal content in the scat of Smooth-coated Otters from Divar in North Goa, and Ambora in South Goa.
- To understand the risks of heavy metals in the food chain of otters.

CHAPTER III

MATERIALS AND METHODS

Study Organism

Smooth-coated otters are one of the largest species of otters, weighing up to 11 kilograms and measuring up to 1.4 meters in length. They have sleek, dark-brown fur that appears black when wet, and a white chin and throat. They have webbed feet and long, muscular tails that help them swim and manoeuvre in water (Smooth-coated otter, n.d ; Kruuk, 2006).

Smooth-coated otters are opportunistic feeders and consume a wide range of prey, including fish, crustaceans, molluscs, and amphibians. They are known to be particularly fond of crustaceans like crabs and prawns.

Classification:

Kingdom: Animalia

Phylum: Chordata

Class: Mammalia

Order: Carnivora

Family: Mustelidae

Genus: *Lutrogale*

Species: *Lutrogale perspicillata*

FIG. 1. IMAGE OF A SMOOTH-COATED OTTER. *https://roundglasssustain.com/conservations/smooth-coated-otter-unexpected-visitor-*

Study sites

The scat samples were collected from two different locations in Goa. The first site is located on Divar island, an island formed within the river Mandovi in North Goa, and the second site is located in a village called Ambora, alongside the river Zuari in South Goa. Both areas are influenced by two different river systems and have dense mangrove forests associated with Khazan lands. Dense mangroves associated with brackish water having high salinity along with presence of Khazan lands favour the presence of otters in a region (Dias et al., 2022).

The study areas were identified using GPS/Google earth. These two areas were chosen because they both lie downstream of their respective rivers, and pollutants such as heavy metals can accumulate in higher concentrations in the sediments and waters of rivers in the downstream regions. Also, the data obtained from the maps showed that there was high density of mangrove forests associated with Khazan lands in both the areas.

A preliminary survey was conducted over the course of a month, to detect the presence of smooth-coated otters in both areas. The bundhs within each of the study areas were surveyed, and the bundhs having the highest number of defecating areas were selected as transects for collection. After the transects were finalized, collection of the scat begun. The transects were surveyed for 3 months; October, November and January. Since the availability of fresh scat is indefinite, the transects were surveyed multiple times during the particular month until fresh scat was obtained.

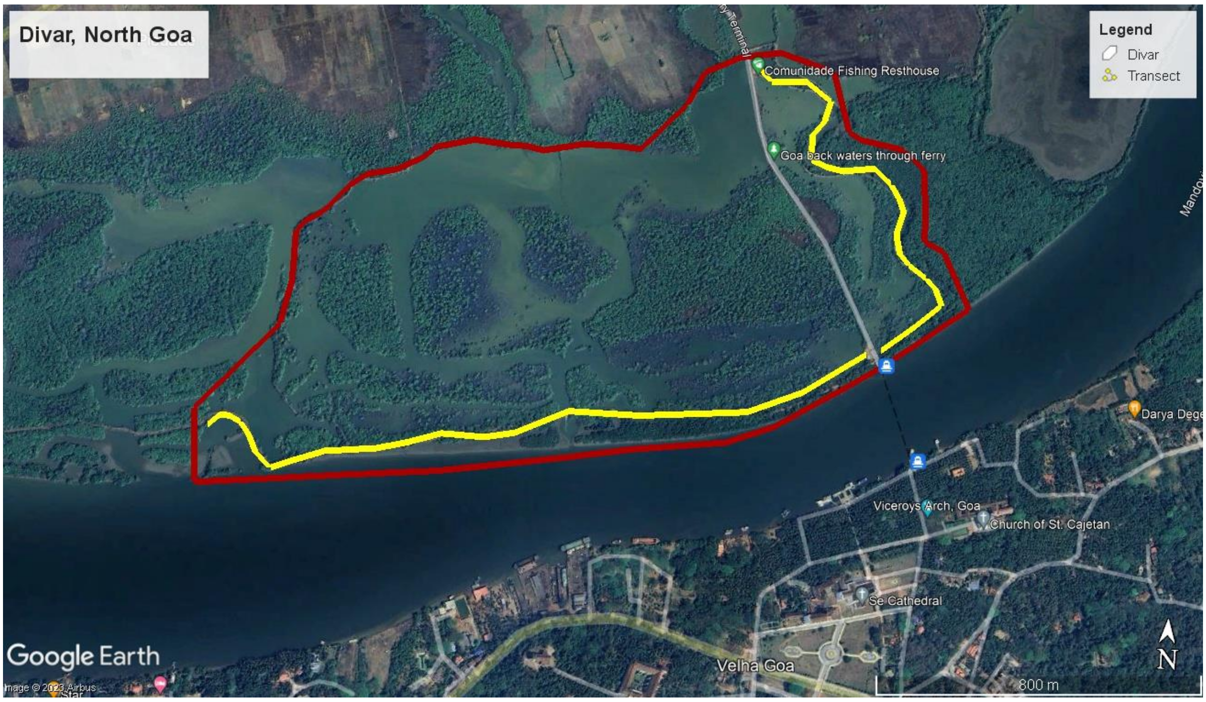


FIG. 2. MAP SHOWING LOCATION OF DIVAR, NORTH GOA. GOOGLE EARTH, EARTH.GOOGLE.COM/WEB/

FIG. 3. MAP SHOWING LOCATION OF AMBORA, SOUTH GOA. GOOGLE EARTH, EARTH.GOOGLE.COM/WEB/

Methodology for collection and storage of otter scat:

Upon finding fresh otter scat, the scat was picked by using a latex glove. The glove was then turned inside out to store the scat within the glove. The glove was then placed in a container or a bag which was then stored in a deep freezer until processing of the scat was commenced with. The scat that was collected had to be such that it should not have touched the mud on the ground. Only fresh scat that lies at the top, which does not touch the ground was collected (Rodríguez-Estival, et al., 2020). This precaution was carried out to prevent cross contamination of heavy metals from the mud to the scat sample. Older scat was not collected because the metal content in it might have been affected due to exposure to natural elements such as rain, wind, contamination by soil and other organisms.

FIG. 4. IMAGE SHOWING A DEFECATING AREA ON A BUNDH IN ONE OF THE STUDY SITES

FIG. 5. IMAGE SHOWING A FRESH OTTER SPRAINT/SCAT

Methodology for processing the scat for heavy metal analysis

The methods used for processing and analysing the scat for heavy metals were adopted from studies conducted by Gutleb et al., (1998), Rodríguez-Estival et al. (2020) and Gupta et al. (2012).

1. **Drying of scat**: The collected scat samples were dried in an oven at 60-80°C until they became brittle. The scat had to placed in an apparatus having a large surface area such as a petri plate to maximize evaporation. Oven heating was carried out for 5 to 6 hours.

2. **Weighing of scat**: One gram of the dried scat was then weighed on a weighing scale.

3. **Sample digestion**: The 1g scat samples were then digested with 10 ml nitric acid (HNO3) on a hot plate at 80° C till the organic matter got dissolved completely (Gutleb et al., 1998). The digestion process helps to break down the organic matter and release the heavy metals from the sample.

4. **Filtration**: The digested sample was then filtered using a filter paper.

5. **Dilution**: The filtrate was then diluted with distilled water and the volume was brought up to 50 ml.

6. **Storage:** The samples were transferred to sterilized borosil glass vials and stored in the dark at room temperature prior to analysis.

7. **Heavy metal analysis**: The digested sample was then analysed for heavy metal content using atomic absorption spectrometry. This method can detect a range of heavy metals

including lead, cadmium, arsenic, copper, zinc, and mercury among many others (Rodríguez-Estival, et al., 2020).

Quality control: It is important to perform quality control checks during the analysis to ensure the accuracy and precision of the results. This involves using non-metal items for storage or for processing the samples.

CHAPTER IV

RESULTS

The heavy metal concentrations of cadmium, lead, and arsenic were measured in otter scat collected from the two study sites: Divar in North Goa and Ambora in South Goa.

Three samples were collected from each site in the months of October, November, and January.

Table 1: Results for heavy metal analysis from AAS (Atomic Absorption Spectroscopy)

SAMPLE ID	LOCATION	MONTH	CADMIUM (PPM)	LEAD (PPM)	ARSENIC (PPM)
1	Divar	October	0.02	0.10	0.002
2	Ambora	October	0.02	0.09	0.003
3	Divar	November	0.02	0.05	0.0005
4	Ambora	November	0.02	0.06	0.001
5	Divar	January	0.03	0.09	0.001
6	Ambora	January	0.02	0.08	0.003

The above table shows the sample results obtained through Atomic Absorption Spectroscopy (AAS). The actual concentrations of the heavy metals in the samples analysed can be calculated by using the following formula.

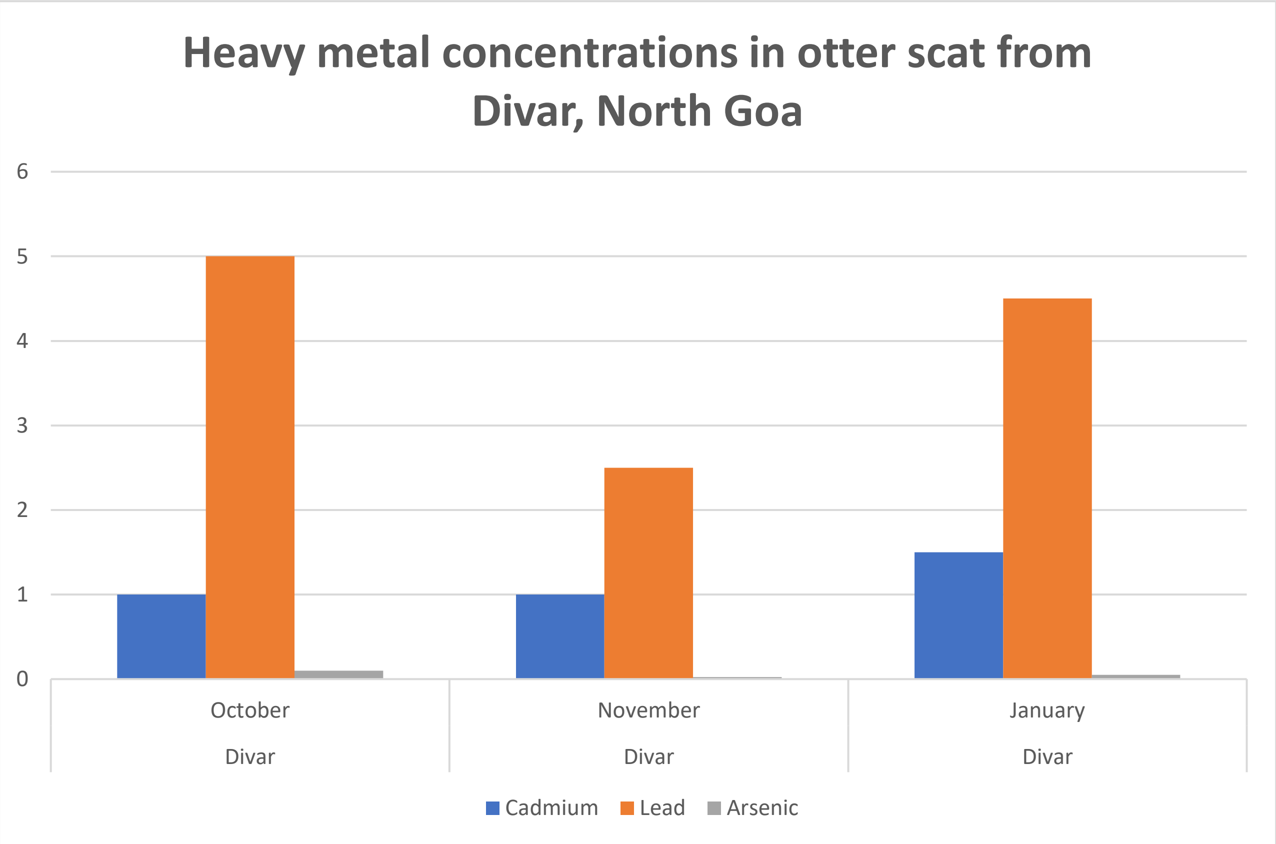
Concentration of Element (ppm, mg/kg) = [AAS results (ppm) x Dilution Vol. (ml)]/ Mass of sample (g)

*ppm, ug/g, mg/kg are interchangeable units

Results for Divar, North-Goa

Table 2: Heavy metal concentrations in otter scat from Divar, North Goa after calculations

Sample ID	Month	Cadmium (ppm)	Lead (ppm)	Arsenic (ppm)
1	October	1	5	0.1
2	November	1	2.5	0.025
3	January	1.5	4.5	0.05
Standard deviation		0.288675135	1.322875656	0.038188131
Mean		1.1666	4	0.05833

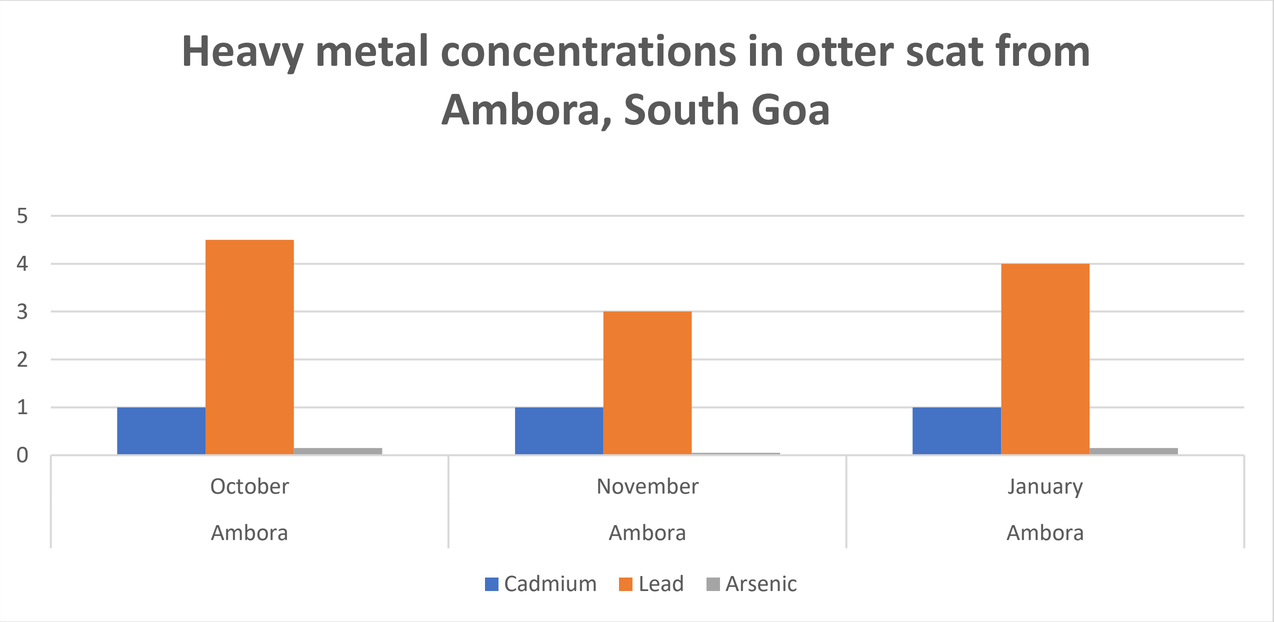


Graph representing the heavy metal concentrations in smooth-coated otter scat from Divar, North Goa

Results for Ambora, South-Goa

Table 3: Heavy metal concentrations in otter scat from Ambora, South Goa after calculations

Sample ID	Month	Cadmium (ppm)	Lead (ppm)	Arsenic (ppm)
1	October	1	4.5	0.15
2	November	1	3	0.05
3	January	1	4	0.15
Standard Deviation		0	0.763762616	0.057735027
Mean		1	3.8333	0.1166



Graph representing the heavy metal concentrations in smooth-coated otter scat from Ambora, South Goa

The results of the analysis showed that all six scat samples contained measurable concentrations of cadmium, lead, and arsenic. The mean concentrations of each metal in the scat samples from Divar were 1.16666 ppm (cadmium), 4 ppm (lead), and 0.05833 ppm (arsenic). The mean concentrations of each metal in the scat samples from Ambora were 1 ppm (cadmium), 3.833333333 ppm (lead), and 0.116666667 ppm (arsenic).

The highest concentration of cadmium was found in the January sample of Divar, with concentrations of 1.5 ppm and the highest concentration of lead was found in the October sample of Divar 5 ppm. The highest concentration of arsenic was found in the October and December samples of Ambora, with a concentration of 0.15 ppm.

Cadmium

The statistical analysis of the results showed that the mean concentration of cadmium in Divar was 1.16666 ppm, while the mean concentration in Ambora was 1 ppm. The standard deviation of cadmium concentrations in Divar was 0.288675135, which was higher than that of Ambora (0.00000), indicating greater variation in cadmium concentrations in Divar. For cadmium, the concentrations ranged from 1 to 1.5 ppm in all six samples, with no significant difference between the two sites or between the different sampling months.

Lead

For lead, the concentrations ranged from 2.5 to 5 ppm. The highest concentration of lead was found in Divar in the month of October, while the lowest was found in Divar in the month of November. The mean concentration of lead in the scat samples from Divar was 4 ppm, while the mean concentration in the scat samples from Ambora was 3.8333 ppm. The standard deviation of lead concentrations in the scat samples from Divar was 1.322875656, which was higher than that in the scat samples from Ambora (0.763762616), indicating greater variation in lead concentrations in the scat samples from Divar. These results suggest that there is no significant difference in the concentration of lead in otter scat between the two sites, but there is greater temporal variation in lead concentrations in the scat samples from Divar.

Arsenic

For arsenic, the concentrations ranged from 0.025 to 0.15 ppm. The highest concentration of arsenic was found in Ambora in the month of October and December, while the lowest was found in Divar in the month of November. The mean concentration of arsenic in Divar was 0.058333333 ppm, while the mean concentration in the scat samples from Ambora was 0.116666667 ppm. The standard deviation of arsenic concentrations in the scat samples from Divar was 0.038188131, which was lower than that of the Ambora scat samples (0.057735027), indicating less variation in arsenic concentrations in the scat samples from Divar. These results suggest that there is a significant difference in the concentration of arsenic in otter scat between the two sites, with higher concentrations found in Ambora.

CHAPTER V

DISCUSSION

The results of this study indicate that smooth-coated otters in both the sites are exposed to heavy metals, specifically cadmium, lead, and arsenic.

It was found that the levels of cadmium, lead, and arsenic varied between the two sites. It was observed that the mean levels of cadmium and lead were higher in Divar than in Ambora, while the mean level of arsenic was higher in Ambora than in Divar. Cadmium and lead showed minimal variations between the sites where as the concentrations of arsenic were wide ranging.

Cadmium

The mean cadmium level in the samples collected from Divar was 1.1666 ppm, while in the samples collected from Ambora site, it was estimated to be 1 ppm. The concentrations of cadmium did not vary profoundly, with the only difference in concentrations between the two sites being in January. The concentrations of cadmium analyzed from the rest of the months in both the sites were the same. Thus, it can be inferred that the concentration of cadmium in the otter scat did not differ significantly between the two sites and remained consistent, suggesting that there may be a stable source of cadmium pollution in both the areas that is persisting over time, such as industrial or agricultural practices that are ongoing and releasing cadmium into the environment and also perhaps due to the absence of any new sources of cadmium contamination. Upon reviewing similar studies conducted on heavy metal concentrations in otter scat, no such articles were found that included the estimation of cadmium in their study. However, studies on bioaccumulation of cadmium in various other organisms are available. Studies on cadmium concentration in the scat of other mammals show that cadmium was found to be between the range of 3.80 ppm (*Panthera leo*) to 0.60 ppm (*Macaca mulatta*) (Gupta et al., 2012). In another study conducted, it was found that cadmium was in between the range of 0.90 ppm to 1.49 ppm in one site whereas it was found to be between 0.96 ppm to 2.6 ppm in the other (Gupta, 2012). According to research conducted by Shenai-Tirodkar et al. (2016), oysters from Goa had cadmium levels in their tissues ranging from 7.1 to 88.5 mg/kg. Similarly, Kumari et al. (2006) found high levels of cadmium in *Paphia malabarica* clams, ranging from 1.4 to 8.4 mg/kg, along the coast of Goa. This may imply that cadmium gets bioaccumulated to a high extent in organisms inhabiting Goa's estuarine and marine ecosystems. According to Asagba (2013), in most animal species, the absorption of cadmium after exposure through diet falls between 0.5% and 3.0%. This goes in accordance with the current study wherein the cadmium levels obtained from the scat samples was found to be between the range of 1 to 1.5 ppm mg/kg. Genchi et al. (2020) state that crustaceans, bivalve molluscs, oysters, and crabs have been found to contain high concentrations of cadmium. This suggests that smooth-coated otters are more prone to cadmium bioaccumulation if the above species are consumed in higher amounts. The Bureau of Indian Standards (BIS) value for cadmium in water in India is 0.01 ppm which is lower than the values of cadmium estimated from the samples. Since about 97 %– 99.5% of ingested cadmium is excreted by most animals (Asagba, 2013), it can be assumed that the environmental concentration of cadmium may be higher than the permissible limit.

Lead

The mean lead level was 4 ppm in the samples collected from Divar, while it was 3.8333 ppm in the samples collected from Ambora. Lead showed a greater temporal variation as compared to cadmium, with an increase in concentrations in the months of October and January from both sites, and a decrease in concentrations during the month of November. This may be due to differences in the sources of contamination or the timing of exposure. For example, if the otters are exposed to lead during a particular period when agricultural or other anthropogenic activities are more prevalent, this could result in higher temporal variation in lead concentrations.

A study conducted on the heavy metal content in crayfish and otter scat by (Rodrigues-Estival et al., 2019) found that three of the otter feces from one site showed lead levels relatively high (3.54, 4.35 and 13.87 mg/kg) and two of the otter scats from the second site showed extremely high lead levels (202.9 and 291.9 mg/kg, respectively). The estimated daily intake (EDI) of the otters in this study was found to be above the oral exposure level of 300 µg/kg per day. Metal concentrations in otter spraints ranged from 0.07 to 3.82 mg/kg in a study conducted by (Gutleb, 1994). In other similar studies conducted, Mason et al., (1986) found lead values ranging between 23.3 and 32.4 mg/kg in otter spraints from contaminated areas, and Delibes et al. (2009) found mean lead levels of 41.79 mg/kg, ranging between 2.28 and 159.8 mg/kg, in otter spraints collected from areas where a toxic spill had occurred. Results obtained from these studies show that the mean concentrations of lead in the current study that was estimated from the samples from both the sites are lower than the concentration of lead estimated in otter scat from most of these studies.

Upon ingestion, approximately 5 to 10% of lead is absorbed, and typically less than 5% of what is absorbed is retained, according to Goyer (1986). As a result, roughly 99.5% of the total ingested lead is excreted through feces. Of this amount, 90% is excreted without being absorbed, while 9.5% is excreted after being absorbed and metabolized, leaving only 0.5% to be deposited in various body tissues. Lead that is absorbed through the gastrointestinal tract and lungs exits the body in urine, with the majority of ingested Pb being excreted in faeces without being absorbed, according to Kalisinska et al. (2016). The absorption of lead via the gastrointestinal tract is dependent on the stomach's acidic environment, among other factors. As a result, carnivorous animals such as canids, with lower gastric pH levels (around 1-3), may absorb more lead from their diets than piscivorous carnivores such as otters with higher gastric pH levels (Kalisinska et al., 2016). Thus, it can be stated that otters are less susceptible to lead biomagnification than other carnivores such as canids. The amount of lead that was detected in the samples from the current study was comparatively lower than the amount of lead detected in otter scats from similar studies even though it had the highest concentrations amongst the three metals within the current study.

Arsenic

The mean arsenic level was 0.05833 ppm in the samples collected from Divar, while it was 0.1166 ppm in the samples collected from Ambora. Thus, the mean concentration of arsenic in the scat samples obtained from Ambora was almost twice the amount as that of the average arsenic content found in the scat collected from Divar, suggesting higher arsenic contamination in the area. Also, arsenic showed high temporal variation in concentrations in both the sites. Estimation of arsenic from all the 6 scat samples revealed that Arsenic was found to be lowest during the month of November. Studies indicate that arsenic does not undergo biomagnification up the food chain, although it has the potential to accumulate in various aquatic mammals, including otters, as observed in studies by Eisler (1988) and Kubota et al. (2001). A study conducted by Clarke, (2018) on the concentrations of heavy metals in crab samples and otter scat samples showed that the arsenic content was lower in the scat samples (2.5 mg/kg) and higher in the crab samples (35.636 mg/kg) that the otter feeds on. This suggests that arsenic gets bioaccumulated to a high extent within the tissues of the otters since the intake of arsenic far exceeds the concentration levels of arsenic found in the scat. This may provide a possible explanation as to why arsenic was found to be the lowest amongst the three metals in the current study. Arsenic bioaccumulation varies from organism to organism. Several factors such as time of retention of food in the gut, pH, presence of iron oxides, etc influences the absorption of arsenic from the food into the body. According to Kubota et al. (2001), otters that consume crabs have been found to have higher concentrations of arsenic than those that mainly feed on fish. In higher trophic level organisms, the ingestion of food is a critical pathway for total arsenic intake, accounting for over 90% of arsenic bioaccumulation, as indicated in studies by Casado Martinez et al. (2010), Falconer et al. (1983), McIntosh (1991), and Williams et al. (2010). In benthic grazers, the accumulation of arsenic from food is approximately ten times higher than from water, as observed in the study conducted by Maeda et al. (1990).

As per Dovick et al. (2016), the bioaccumulation of arsenic in the study conducted by them follows a pattern of decreasing concentration with increasing trophic levels, with primary producers exhibiting the highest concentrations followed by tadpoles, macroinvertebrates, and finally fish. Thus, it can be assumed that the variation in the arsenic levels in the scat samples from both the sites throughout the study period could be linked to the type of organism that the otters fed on. Higher levels of arsenic in some of the scat samples could be because the otter probably fed on organisms susceptible to arsenic bioaccumulation such as crabs, prawns or shellfish as opposed to less susceptible organisms such as fish.

On comparing the results with findings from other studies conducted on the concentrations of heavy metals in otter scat, as well as the scat of other mammals, it appears that the results obtained from this study suggest that the concentrations of cadmium analysed in the smooth-coated otter scat were within almost comparable ranges as related to similar studies conducted on the scat of other mammals. Arsenic and lead content in the scat on the other hand was found to be lower in concentration in the current study than in similar studies conducted. Although heavy metals may cause toxic effects in different organisms at different concentrations, it should be noted that the concentrations of heavy metals found in the scat samples in this study were higher than the threshold limits for the three metals set by the Bureau of Indian Standards (BIS) in drinking water and the tolerable weekly intake values according to the World Health Organization (WHO). It may be noted that these values do not apply to the concentration of metals found in the scat and are hence, not the reference limit for these metals in the scat. Nonetheless, it does indicate that these elements were found to be in higher amounts in both the environments.

According to Mayer et al. (1989), Gossiaux et al. (1992), de Lisle & Roberts (1994), Wang et al. (1996) the uptake and toxicity of cadmium generally increases with an increase in salinity. Both the study sites are in close proximity to the sea and thus exhibit high levels of salinity. This may explain why the metal was found in high concentrations in the scat of otters from both sites.

Lead was found to be in the highest concentrations amongst the three metals, and the concentrations were found to be way above the permissible limits of BIS in water and weekly tolerable intake values of WHO. Even though the above limits are calculated specifically for humans, the concentrations of lead in the scat samples are present in amounts that could potentially harm the organism.

Also, the presence of these three heavy metals in the scat samples indicates that these elements are circulating within the food-chain irrespective of their concentrations. Smooth-coated otters feed on a variety of organisms ranging from arthropods such as prawns and crabs, to animals such as fishes, amphibians, reptiles and smaller mammals. Otters are opportunistic animals and will feed on prey species that are readily available within their territories. Smooth-coated otters in Goa are known to mainly feed on Indian white prawns, mud crabs, fiddler crabs and fishes such as red snappers, lady fish, Asian sea bass, pearlspot, several species of silver biddys, etc. Most of these species are cultured within the Khazan lands by the locals (Sonak et al. 2013). These commercial species are cultured on a large scale, and hence most of the otters' diet consists of these organisms. Since cadmium, lead and arsenic were detected in moderate to high amounts from the scat samples collected from both sites, an assumption can be made that most of the heavy metals analysed from the scat samples were the result of consumption of these commercial species. Food sources are the main route of exposure of harmful contaminants such as iron, zinc, copper, selenium, manganese, lead, cadmium, arsenic, mercury, uranium, and nickel in mammals (Clarkson, 1995). Other inputs such as absorption of heavy metals from the river water may have also contributed to the presence of the heavy metals in the scat. Since these commercial species are also consumed by humans, the findings of this study suggests that people consuming these species may be prone to metal bioaccumulation of these three heavy metals. Heavy metals such as cadmium are efficiently retained within the body and accumulate within tissues throughout life since it has a half-life of 25-30 years (Genchi et.al., 2020). Chronic exposure to cadmium may be associated with certain cancers such as lung cancer, breast cancer, cancers of the nasopharynx, prostate urinary bladder and pancreas (Genchi et.al., 2020). Exposure to even small amounts of Cd can result in harm to the liver, kidneys, skeletal system, and cardiovascular system, as well as a decline in vision and hearing. (Genchi et.al., 2020).

CONCLUSION

From the study conducted on heavy metal content in smooth-coated otter scats from both the sites, it is evident that the three metals tested for were in moderate to high amounts. This study found that the concentrations of cadmium in smooth-coated otter scat were within comparable ranges to other studies conducted on otter scat and the scat of other mammals. However, the concentrations of arsenic and lead were lower in this study than in similar studies conducted. The presence of these heavy metals in the scat samples indicates that they are circulating within the food chain, likely due to the consumption of species such as prawns, crabs, and fishes, which are also consumed by humans. Chronic exposure to these metals in the form of such food sources may have long term effects on the health and proper functioning of the body.

It is worth noting that the sample size in this study was relatively small, with only three samples from each site. This limited sample size may affect the statistical significance of the results. Additionally, there may be other factors at play that influence heavy metal concentrations in otter scat other than food sources, such as absorption through air and water. It is important to note that the concentrations of heavy metals in otter scat may not directly reflect the concentrations in their environment, as they may metabolize or excrete heavy metals differently. Further research with larger sample sizes and more frequent sampling would be necessary to confirm these findings and to determine if there are any patterns or trends in the heavy metal concentrations over time. Additionally, it would be important to investigate the potential sources of these heavy metals in the otter's environment, such as pollution from human activities or naturally occurring geological features. Further research is needed to identify the specific sources of contamination in each site. It is important to continue monitoring heavy metal pollution in these regions, as it can have negative impacts on wildlife and human health.

CHAPTER VI

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