Trace Metals in Seaweeds of Anjuna, Goa- West Coast of India

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This is to certify that the dissertation report **"Trace Metals in Seaweeds of Anjuna, Goa- West Coast of India"** is a bonafide work carried out by **Ms Sumedha S. Kamble** under my supervision in partial fulfilment of the requirements for the award of the degree of **Master of Sciences** in the Discipline of Marine Sciences at the School of Earth, Ocean and Atmospheric Sciences, Goa University.

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PREFACE

Seaweeds are increasingly utilized in various industries, including food, pharmaceuticals, and bioremediation. The presence of elevated trace metal concentrations in seaweeds can pose risks to human health through consumption or product use, underscoring the importance of assessing and managing metal pollution in marine environments.

Understanding the distribution and behavior of trace metals in seaweeds is essential as bioindicators of environmental quality due to their sensitivity to metal pollution. Monitoring trace metal levels in seaweeds can provide valuable insights into the health of marine ecosystems and help identify areas at risk of contamination.

Despite the significance of trace metals in seaweeds, studies investigating their distribution are still limited. Therefore, this study aims to fill this gap by examining the distribution of trace metals among seaweeds in Anjuna, Goa, a coastal area known for its biodiversity and environmental significance.

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Sumedha S. Kamble

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ABBREVIATIONS USED

Entity	Abbreviations
September	Sept
October	Oct
November	Nov
December	Dec
January	Jan
Bioaccumulation factor	BAF
Analysis of Variance	ANOVA
Metal Pollution Index	MPI
Pollution Load Index	PLI
Not Detected	ND

ABSTRACT

The study investigates the distribution of trace metals among seaweeds along the coast of Anjuna, Goa- the west coast of India. The trace metal concentrations in seawater and seaweeds were analysed using Atomic Absorption Spectrophotometry. Analysis of trace metal concentrations in seaweeds revealed higher Cu concentrations in Phaeophyta compared to other phyla, with Chlorophyta showing the highest concentrations of Zn, Ni, Fe, and Mn. Chlorophyta species exhibited the highest Bioaccumulation Factor (BAF), followed by Phaeophyta and Rhodophyta, with *Acrosiphonia orentalis*, *Padina tetrastromatica*, and *Gelidium pusiillum* showing the highest BAF, respectively. Pollution Load Index (PLI) values were highest in Chlorophyta, with *Acrosiphonia orentali*s and *Padina tetrastromatica* exhibiting the highest Metal Pollution Index (MPI). The high BAF, MPI, and PLI values of *Acrosiphonia orentalis* and *Padina tetrastromatica* suggest their suitability for biomonitoring the pollution status of Anjuna, Goa.

Keywords: Trace metals; Seaweeds; Bioaccumulation factor; Atomic Absorption Spectrophotometer; ANOVA

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Metal pollution is a global environmental issue of significant concern, with discharge from diverse industrial activities being a primary contributor to this problem **(Sharma and Dubey, 2005)**. The presence of trace metals in the marine environment poses a significant threat to both aquatic ecosystems and human health.

Trace metals are naturally occurring elements that can be introduced into marine ecosystems via geological weathering, industrial activities such as ore and metal processing, the utilization of metals and metal products, as well as the release of metals from waste disposal sites like garbage dumps and solid waste dumps. Additionally, heavy metals can enter the environment through animal and human excreta containing heavy metals **(Forster and Wittmann, 1979).** While trace metals are essential micronutrients at low concentrations, their accumulation in the environment can reach to toxic levels that have adverse effects on marine organisms and entire ecosystems. Metal ions dissolved in water can be taken up by aquatic organisms, including plants and animals, and may lead to toxicity if their concentration reaches sufficiently high levels **(Alloway and Ayres, 1993).**

The bioaccumulative nature of trace metals means that they can magnify through the food chain, leading to biomagnification and concentrating in higher trophic levels. Metals beyond the threshold have been found to deactivate proteins, denature enzymes, and disturb cell functions **(Hall, 2002)**. Analysing the total metal content in water or sediment does not predict the toxic effects of contaminants on biota **(Rainbow, 2006).** Therefore, aquatic organisms are used as a bio-monitors to access environmental pollution.

Bio-monitors are organisms that accumulate pollutants in proportion to their environmental concentrations, offering a method to evaluate and monitor the extent of contamination from toxic metals in marine environments **(Ganesan et al. 1991; Rajendran et al. 1993).** Bio-monitors need to have certain characteristics such as sedentary nature, widespread distribution with significant biomass, ease of identification and the ability to accumulate pollutants **(Rainbow 1995)**. Seaweeds possess a range of essential traits that make them ideal bio-monitor; thus, being recognized as effective bio-monitors for heavy metal contamination in seawater **(Akcali and Kucuksezgin 2011; Chaudhuri et al. 2007; Murphy et al. 2007).**

Seaweeds are macroalgae inhabiting both brackish and marine environments. They lack flowers and are characterized by their thallophytic nature. Unlike traditional plants, their thallus body comprises a holdfast, stipe, and blade instead of roots, stems, and leaves. Seaweeds typically adhere to hard surfaces such as boulders, rocks, and even the pneumatophores of mangrove species. They encompass a diverse array of multicellular, visible marine algae. Based on the presence of pigments, seaweeds are categorized into three groups, namely Chlorophyceae (green seaweed), Phaeophyceae (brown seaweed), and Rhodophyceae (red seaweed). Seaweeds serve critical ecological functions by influencing nutrient dynamics within coastal ecosystems and contributing to the modulation of water quality **(Wilson, 2002)**.

Seaweeds are well-known for their capacity to accumulate trace metals from the ambient environment. Their cell walls are composed of various polysaccharides and proteins, some of which contain anionic carboxyl, sulphate, or phosphate groups, providing excellent sites for metal retention. **Bryan (1969)** demonstrated the robust binding of metals by macroalgae, with minimal exchange between bound metals and the surrounding water. Macroalgae can accumulate trace metals to concentrations thousands of times higher than those found in seawater (**Conti and Cecchetti, 2003**). The ability of algae to accumulate metals is influenced by several factors, with the most significant being the availability of metals in the surrounding water and the algae's uptake capacity **(Sanchez-Rodriguez et al. 2001).**

The biosorption of metals takes place in two stages: (1) absorption onto the surface (2) accumulation into the cells **(Monteiroet al. 2011).** Firstly, there's a surface reaction where metals are absorbed onto algal surfaces via electrostatic attraction to negative sites. This process is unaffected by factors like temperature, light, pH, or the age of the plant but is influenced by the relative abundance of elements in the surrounding water. This mechanism appears to be the primary uptake pathway for zinc. The second mechanism involves a slower, active uptake, where metal ions are transported across the cell membrane into the cytoplasm. This method is more reliant on metabolic processes and appears to be relevant for copper, manganese, selenium, and nickel. It is subjected to variations due to changes in temperature, light, or the age of the plant **(Sanchez-Rodriguez et al. 2001)**.

Macrophytes typically serve as the basis of the aquatic food chain and consequently have the potential to impact the chemical composition of higher trophic levels **(Phillips, 1977; Netten et al. 2000**).

The interaction between seaweeds and trace metals is a critical aspect of marine ecology, influencing nutrient cycling, ecosystem dynamics, and human health. Understanding the dynamics of trace metals in seaweeds is essential for assessing the health of marine environments, monitoring pollution levels and exploring the potential use of seaweeds in environmental remediation strategies.

1.2 SCOPE

Seaweeds are increasingly being utilized for various purposes, including food, pharmaceuticals and cosmetics. However, if seaweeds accumulate high levels of toxic trace metals, there is a risk of human exposure through consumption or product usage. By monitoring the levels of trace metals accumulated in seaweeds, researchers can evaluate the extent of metal pollution in coastal waters. High levels of trace metals in seaweeds can have cascading effects on associated organisms, leading to alterations in community structure and dynamics. Changes in trace metal concentrations in seaweeds over time can indicate variations in pollution levels and help identify potential sources of contamination.

1.3 AIM AND OBJECTIVE

AIM

To study the trace metals in the seaweeds of Anjuna, Goa- West Coast of India.

OBJECTIVE

- The objective of this study is to investigate the distribution of trace metals in seawater and seaweeds of Anjuna, Goa.
- To derive Bioaccumulation Factor (BAF) of metals in seaweed and to check the pollution status.

1.4 STUDY AREA

The state of Goa has a coastline of 110 km in length and spreads into two districts i.e. North Goa and South Goa. This coastal expanse features a variety of natural features, including rivers, estuaries, beaches, rocky shores, cliffs, bays, and creeks, all bordered by lush coconut plantations. The major rivers are Mandovi, Zuari, Terekhol, Chapora, Kushavati river and the Sal. It has seven estuaries and three bays. The coastline is rockier in North Goa and supports more growth and diversity of seaweeds; among which Anjuna is one of the important places of algal growth at Goa coast. Anjuna is located about 18 km from Panjim and lies in Bardez taluka. The beach spans 30 km along the western coast of Goa, running alongside the Arabian Sea. It’s a purely marine area with no influence of fresh water. It has a broad and rocky intertidal region.

Anjuna beach is naturally endowed with beautiful landscape and attracts a large number of national and international tourists. It is known for its scenic beauty and is a hotspot for tourists.

CHAPTER 2

REVIEW OF LITERATURE

Agarwal et al. (2022) studied the heavy metals (Zn, Cu, and Pb) contamination in green seaweed *(Enteromorpha compressa*) for three seasons (pre-monsoon, monsoon, and post-monsoon) at the lower Gangetic delta complex. The concentration of heavy metals in both seawater and *Enteromorpha compressa* showed a trend with Zn > Cu > Pb. The seasonal variation was found to be highest in concentration during the monsoon followed by post-monsoon and pre-monsoon.

Ryan et al. (2012) studied the levels of total, intracellular, and surface-bound metals (Pb, Zn, As, Co, Cr, Cu, Mn, and Ni) associated with *Polysiphonia lanosa, Ascophyllum nodosum, Fucus vesiculosus,* and *Ulva sp*. at Fethard on Sea, Ireland. The total, intracellular, and surface-bound metal concentrations in seaweed showed that Zn, Mn, and As values were significantly higher than other metals studied. *Polysiphonia lanosa* exhibited exceptional bioaccumulation capabilities, boasting the highest Concentration Factor reported among seaweed species to date.

Khaled et al. (2014) studied the distribution of heavy metals (Cd, Ni, Cu, Fe, Pb, and Zn) in seagrass and seaweed of six different species at ten different locations along the Marsa-Matrouh coast, Egypt. *P. oceanica* recorded the highest concentration of Zn, Pb, Ni, Cu and Cd for the seasons winter, summer, spring, spring, and summer respectively. A positive correlation was observed between Fe concentrations in the seaweeds and those of Cu and Pb, suggesting a common source of these metals and/or synergistic interactions between them. Additionally, there were negative correlations between Cu and Ni, Pb and Zn.

Besada et al. (2009) studied the concentration of heavy metals in edible seaweeds collected from specialist shops throughout Spain, the Atlantic Ocean and the Pacific Ocean. Among the analysed seaweeds, *Hizikia fusiforme* showed the highest concentration for total and inorganic arsenic, and most of the seaweed samples collected showed a concentration of cadmium above the limit set by French Legislation.

Karthick et al. (2012) studied the concentration levels of manganese (Mn), lead (Pb), zinc (Zn), cadmium (Cd), copper (Cu) and chromium (Cr) in six seaweed samples collected from Wandoor, South Andaman Island. Mn exhibited the highest accumulation, while the lowest was Cd. Cd concentrations were relatively consistent across all seaweed species. Zn was only detected in *S. swartzi,* while Pb and Mn occurred at similar concentrations in *S. duplicatum* and *S. swartzi.* Cu and Cr were not detected in any of the samples. The heavy metal accumulation order in the seaweeds was Mn > Pb > Cd. Additionally, the MPI (Metal Pollution Index) of the six seaweed species was observed in the following descending order: *A. calyculus* > *Corallina sp.* > *D. bartayresiana* > *G. marginata* > *S. duplicatum* > *S. swartzi.*

In the study conducted by **Sudharsan et al. (2012),** the concentrations of Cd, Cu, Mn, Ni, Pb, Zn and Hg were analysed in eight seaweed species and two seagrasses collected from three stations (Kanyakumari, Ervadiand Thondi) along the Southeast coast of India. At the Kanyakumari station, Cd, Mn, Zn and Cu levels were highest in *S. hypnoides*. At the Ervadi station, Cu and Mn were highest in *G. acerosa*. At the

Thondi station, Cu, Zn and Mn content were highest in *S. isoetifolium*. Cd, Ni, Pb and Hg were recorded at levels below 1 ppm in both seagrasses.

Boutahar et al. (2021) studied the concentrations of seven trace elements in different tissues of *Cymodocea nodosa* (leaves, rhizomes and roots) as well as in sediment at Marchica Lagoon, Morocco. The average concentrations of metals in sediment followed the order of Al > Zn > Pb > Cu > Cr > Ni > Cd. In *Cymodocea nodosa,* Zn exhibited the highest concentration in leaf material, while Pb and Al concentrations were higher in roots, with comparable concentrations observed for Cu, Ni, Cr and Cd. This alternating accumulation pattern between leaves and roots suggests that C. nodosa in Marchica Lagoon might employ a mixed tolerance strategy, characterized by compartmentalization leading to element accumulation in roots and a removal strategy involving shedding temporary tissues (leaves). The results reported the lowest trace metals concentrations in *C. nodosa* rhizomes.

Ganesan et al. (1991) conducted a study in the Gulf of Mannar region of the Bay of Bengal on the distribution of manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) in 18 species of seaweeds gathered from two distinct stations. The concentration of Fe was observed to be lowest in *Dictyota dichotorna* at station 1 (Pudumadam), while it reached its highest levels in *Acanthophora spicifera* at station 2 (Tuticorin). Mn concentrations were found low in *Ulva reticulata* and highest in *Centroceras clavulatum* at station 2. Zn and Cu concentrations peaked in *Sarconema sp.* station 2, while they were lower in *Gracilaria corticata* and *Padina tetrastromatica* at station 1. Overall, the metal concentrations in the seaweeds followed the order Fe > Mn > Zn >

Cu, with a few exceptions such as *Sargassum wightii, Ulva reticulata and Sarconema sp*., which exhibited higher Zn levels compared to Mn.

Rajendran et al. (1993) investigated the levels of Mn, Fe, Cu and Zn in six species of marine macroalgae collected from five intertidal sites along the Tamil Nadu coast of India, namely Kasimedu, Covelong, Mahabalipuram, Tranquebar and Nagapattinam. Fe and Zn concentrations exhibited variations, with higher levels observed in green and red algae from Covelong and Kasimedu areas respectively, whereas Mn levels were lowest in green and red algae at Covelong but higher in green algae at Mahabalipuram and red algae at Nagapattinam. Cu concentrations showed discrepancies as well, being higher in red algae at Nagapattinam and lowest at Mahabalipuram. Among the six studied seaweed species, *Centroceras clavulatum*, a red alga, exhibited higher levels of Fe, Mn and Cu compared to others.

Agadi et al. (1978) found *Sargassum tenerrimum* had the highest concentration of Pb, *Padina tetrastromatica* of Fe and Mn a very high concentration of Cu recorded for *Acanthophora specifera* from Anjuna, Goa which could not be explained.

Filippini et al. (2020) examined 72 food products from Italy's markets containing seaweed intended for human consumption for the presence of 20 heavy metals. Results indicated that green seaweeds had the highest iron content, while red seaweeds exhibited the highest levels of zinc and manganese; except for iodine, which was most abundant in brown seaweed. It was also observed that the Rhodophyta class had a notably high concentration of trace elements and toxic metals. The obtained Hazard Index (HI) value for aluminium in children exceeds the threshold set by USEPA guidelines, indicating a moderate to high risk of adverse health effects in humans.

Pan et al. (2018) analyzed seaweeds at Dongtou Islands in East China Sea. Among the analysed seaweeds, Phaeophyta stands out with the highest concentrations of Cd and As. In comparison to Rhodophyta and Chlorophyta, Phaeophyta exhibits similar concentrations of Cr and Pb. Rhodophyta, on the other hand, demonstrates significantly elevated levels of Cu and Zn compared to Phaeophyta and Chlorophyta. Notably, Chlorophyta had the highest concentration of Ni among the examined seaweeds.

Tresnati et al. (2021) studied the bioaccumulative potential of *Kappaphycus alvarezii.* The concentrations of Cu, Cd and Pb found in the seaweed *K. alvarezii* were observed to be higher than those in the surrounding seawater. This suggests that *K. alvarezii* possesses the potential to serve as a bioaccumulator for these heavy metals.

Smith et al. (2010) examined the heavy metal content of four seaweeds commonly found in commercial markets—*Macrocystis pyrifera, Undaria pinnatifida, Porphyra and Ecklonia radiata* alongside six species harvested from the wild—*Ulva stenophylla, Porphyra, Ecklonia radiata, Durvillaea antarctica, Hormosira banksii and Undaria pinnatifida.* Mercury was most concentrated in *E. radiata* both in commercially harvested and wild samples, while *U. stenophylla* contained the highest levels of lead. However, none of the seaweeds examined contained heavy metal levels that would pose harm when consumed in normal quantities.

Kumar et al. (1990) examined trace metal bioaccumulation by marine organisms near a caustic soda plant in Karwar. They assessed Cd, Pb, Cu, Zn and Mn levels in fishes, shellfishes and seaweeds, noting high concentrations in oysters, mussels and seaweeds. Oysters were superior accumulators of Zn, Cu and Cd, while mussels, and seaweeds accumulated Pb, and Mn effectively.

CHAPTER 3

MATERIALS AND METHODS

3.1 INTRODUCTION

Samples are obtained from the chosen study area, pretreated and subsequently subjected to analysis for multiple paraments. The analysis is conducted using standardized procedures to ensure the accuracy of results; therefore, internationally recognized protocols are used to achieve the goal of this dissertation.

3.2 SAMPLING

The sampling was conducted at Anjuna Goa, (15.5739° N and 73.7407° E) (Figure 3.1) for 6 months: May (2023), September (2023), October (2023), November (2023), December (2023) and January (2024).

Sea water sample was collected in plastic cans and then further processed when brought to laboratory.

The seaweeds which were distinctly exposed on rocky surfaces were collected from inter-tidal zone during low tides. Seaweeds attached to rocky substratum were plucked out. After washing off all associated sediments with the seawater, the collected samples were transferred into polyethylene bags. On returning to the laboratory, collected samples were rinsed under running tap water to wash off any epiphytes that may be present. After a thorough wash with deionized water, the seaweeds were kept in oven at 60°C until completely dried. The dried samples were powdered with an agate mortar and pestle; and stored in polyethylene bags which were then kept in the refrigerator.

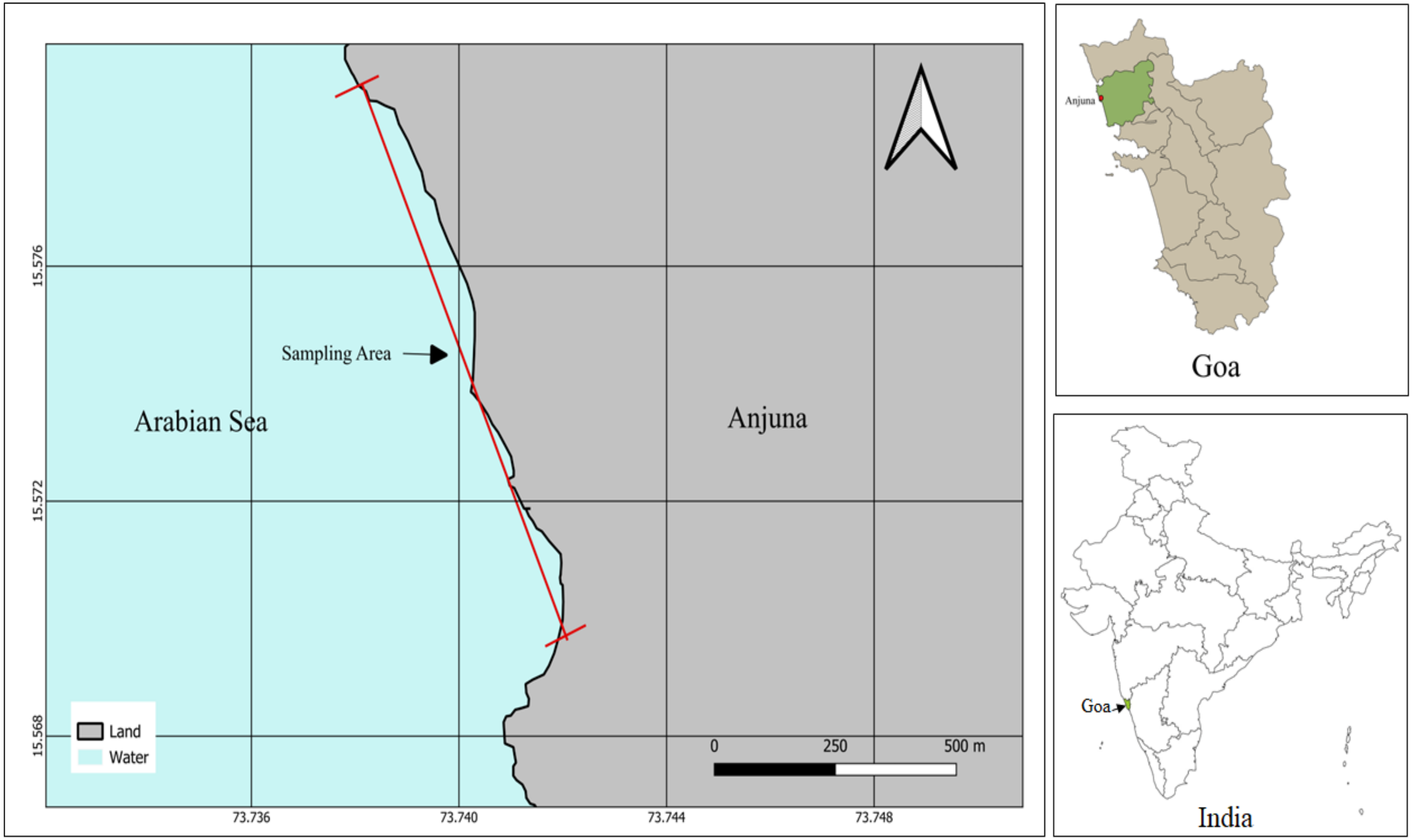

Figure 3.1 - Map showing location of sampling site at Anjuna, Goa- West Coast of India.

Table 3.1: Seaweeds collected from Anjuna, Goa.

Month	Species
May	▪ *Padina tetrastromatica* ▪ *Acrosiphonia orentalis* ▪ *Sargassum ilicifolium* ▪ *Sargassum linerifolium* ▪ *Gelidium pusillum* ▪ *Culerpa peltata*
September	▪ *Chaetomorpha antennina* ▪ *Chondracanthus acicularis* ▪ *Ulva lactuca*
October	▪ *Sargassum ilicifolium* ▪ *Padina tetrastromatica* ▪ *Sargassum cinctum* ▪ *Chaetomorpha antennina* ▪ *Acrosiphonia orentalis* ▪ *Dictyota bartayresiana* ▪ *Gelidium pusillum*
November	▪ *Sargassum cinctum* ▪ *Dictyota bartayresiana* ▪ *Sargassum linerifolium* ▪ *Padina tetrastromatica* ▪ *Sargassum ilicifolium*

	▪ *Sargassum tenerimmum*
December	▪ *Padina tetrastromatica* ▪ *Dictyota bartayresiana* ▪ *Gelidium pusillum* ▪ *Sargassum tenerimmum* ▪ *Sargassum polycystum* ▪ *Sargassum linerifolium*
January	▪ *Sargassum ilicifolium* ▪ *Sargassum tenerrimum* ▪ *Sargassum polycystum* ▪ *Sargassum cinctum* ▪ *Sargassum wightii* ▪ *Padina tetrastromatica* ▪ *Dictyota bartayresiana*

Figure 3.2 a. - *Chaetomorpha antennina*

Figure 3.2 b. - *Ulva lactuca*

Figure 3.2 c. - *Culerpa peltata*

Figure 3.2 d. - *Acrosiphonia orentalis*

Figure 3.2 e. - *Dictyota bartayresiana*

Figure 3.2 f. - *Padina tetrastromatica*

Figure 3.2 g. - *Sargassum tenerrimum*

Figure 3.2 h. - *Sargassum wightii*

Figure 3.2 i. - *Sargassum cinctum*

Figure 3.2 j. - *Sargassum polycystum*

Figure 3.2 k. - *Sargassum ilicifolium*

Figure 3.2 l. - *Sargassum linerifolium*

Figure 3.2 m. - *Gelidium pusiillum*

Figure 3.2 n. - *Chondracanthus acicularis*

3.3 ANALYTICAL METHODS

To fulfil the objectives detailed in Chapter 1, the analytical methods adopted are detailed below.

3.3.1 Temperature

Temperature was measured using a thermometer soon after the collection of the seawater sample.

3.3.2 pH

pH value was measured by using the pH meter. The instrument was calibrated with standard buffer of 4.0, 7.0 and 10.0 before measuring the samples.

3.3.3 Preconcentration of Trace Metals from the Seawater

500 ml of acidified filtered seawater sample was used for the preconcentration of the trace metals (Fe, Mn, Zn, Cu, Ni and Co) by ammonium pyrrolidine dithiocarbonic acid (APDC) and methyl isobutyl ketone (MIBK) extraction method **(Brooks et al. 1967)**. This was carried out after adjusting the pH in the range of 4-5 with ammonia. The sample was then transferred to a 1000 ml separating funnel; successively 10ml APDC and 15ml MIBK was added and the mixture was shaken vigorously for 2 minutes. The two layers were allowed to separate followed by the transfer of organic fraction to a 100ml separating funnel. This procedure was repeated again with the addition of 5ml APDC and 10ml MIBK and the organic fraction was collected.

To the combined organic layer (from the 2 extractions) 15ml of 1N HNO_3 was added followed by vigorous shaking for 2 minutes. The metals were back extracted

(Tsukaijan and Young, 1978) after being allowed to separate for 10 minutes. The aqueous (HNO_3) layer was separated and the back extraction procedure was repeated again with the addition of 10ml of 1 N HNO_3. The two aqueous layers were combined and collected in a polyethylene bottle, stored to analyse via AAS.

3.3.4 Digestion of The Seaweed Sample

A known weight (1.0g) of the seaweed powder was treated with a mixture of $HClO_4$- HNO_3- H_2SO_4 (1:5:1) in a Teflon beaker and heated to almost dryness so as to complete the digestion. The residue after cooling was dissolved, filtered and made upto100ml with 0.1 N HNO_3 and stored in a clean polyethylene bottle until further analysis via AAS **(Allen et al. 1976).**

3.3.5 Trace Metals

Atomic absorption spectroscopy, whether performed with or without a flame, is a versatile method for determining metals in water post preconcentration (3.3.3) and seaweed digestion (3.3.4). This technique is known for its speed, accuracy and minimal interference. It has been effectively utilized for analyzing Fe, Mn, Zn, Cu, Ni and Co.

The AAS was calibrated using standard stock solutions. These solutions were prepared using Merck solution of concentration 1ml=1000ppm. The analysis will be conducted by following the instrumental setting and precautionary measures recommended in the AAS operation manual. The instrument was calibrated for direct reading using the stock solutions and suitable hollow cathode lamps at the specified wavelengths

Aliquots of the sample, containing trace metals from the preconcentration step (3.3.3) and seaweed digestion (3.3.4), were aspirated into the AAS. The instrument readings were recorded based on calibration curves constructed using standards in deionized water.

The concentration of trace metals in water was computed using the below equation:

$$C = A \times V$$

Where,

C = Concentration of trace metal

V = Final volume after preconcentration

A= AAS response

The concentration of trace metals in seaweeds were calculated using the below formula

$$C = \frac{(A \times V \times d)}{W}$$

Where,

C= concentration of trace metal

A=AAS response

V= final volume

d= dilution factor (if any)

W= weight of the seaweed sample (g)

The dilution factor 'd' was calculated using the equation

$$d = \frac{V1 + V2}{V1}$$

Where,

V1- digested sample taken in ml.

V2- deionized water added for dilution in ml.

3.3.6 Calculations

3.3.6 a. Metal Pollution Index

To compare the total metal content in algal species metal pollution index (MPI) was used **(Usero et al. 2005).**

$$MPI = (M1 \times M2 \times M3 \times \ldots . Mn)^{1/n}$$

Where, M is the concentration of the metal and n is the metal number.

Bioconcentration factors were calculated as a ratio of the metal concentration in the macroalgae to the metal concentration in seawater, i.e. the soluble fraction **(Black and Mitchell, 1952).** Bioconcentration is the accumulation of the contaminant by aquatic organisms through non dietary uptake routes, example, from the soluble phase **(Zauke et al. 1998).**

$$\text{Bioaccumulation Factor} = \frac{\text{Concentration of X metal in seaweed}}{\text{Concentration of X metal in ambient medium}}$$

A low Concentration Factor (CF) is indicative of low accumulation of elements by the plants whereas high Concentration Factor (CF) indicates active uptake **(Poppy et al. 2003)**.

To access the health of the study area with respect to the metals studied, the Pollution Load Index (PLI) was used **(Tomlinson et al. 1980; Angula, 1996)**. PLI is used as an index of bioavailability of contaminants for organisms in coastal waters. It standardizes the data using the quotients obtained by dividing each concentration by a baseline concentration of the contaminant, i.e. the lowest concentration found during the study or reported in the literature. It is calculated using the following equation:

$$\text{PLI} = (\text{CF1} \times \text{CF2} \times \text{CF3} \ldots.\text{CFn})^{1/n}$$

Where ,

$$\text{CF} = \text{C}_{\text{metal}}/\text{C}_{\text{baseline}}$$

PLI values of ≥ 100 indicate a polluted site and those of ≥ 50 require intense monitoring of the site. A PLI value of < 50 indicates that no drastic rectification measures are needed **(Tomlinson et al. 1980).** The advantage of using PLI is that it does not show any dependence on the year of sampling or on species, but it does detect an annual variation in metal concentrations.

3.3.6 b. Statistical Analysis

ANOVA stands for Analysis of Variance. It's a statistical method used to analyse the differences among group means in a sample. ANOVA tests the null hypothesis that the means of several groups are equal. If the null hypothesis is rejected, it indicates that at least one group differs significantly from the others. ANOVA is commonly used in experimental research where there are three or more groups to compare.

There are different types of ANOVA, such as one-way ANOVA, two-way ANOVA and MANOVA (Multivariate Analysis of Variance), each suitable for different experimental designs and research questions. One-way ANOVA is used when there is one independent variable with three or more levels, while two-way ANOVA is used when there are two independent variables. MANOVA is used when there are two or more dependent variables. ANOVA calculates the F-statistic, which is the ratio of the variance between groups to the variance within groups.

The analysis of Variance (ANOVA) was performed to determine the variation of selected metals between months and species using Microsoft Excel. $P < 0.05$ was considered statistically significant.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 HYDROCHEMICAL PARAMETERS

Studying the inorganic constituents of seaweeds makes it important to consider the chemical composition of the surrounding seawater, especially since these organisms derive their nutrition from it. The accumulation of metals in these systems is generally influenced by factors like nutrient availability and water movement, which can be impacted by local phenomena such as upwelling, river discharge, urban runoff and human activities. To comprehend how these factors affect metal accumulation in seaweeds, an investigation into hydrochemical parameters in the study area has been conducted, with summarized results provided below.

4.1.1 Temperature

The surface seawater temperature (°C) during the study period varied from 29°C to 31°C. The monthly variation of temperature recorded the highest value in May (31.5°C) and the lowest value during September (29°C), and December (29°C). The maximum temperature observed in May can most likely be due to increased atmospheric heating, which is common in summers. On a bright sunny day, the solar intensity is higher and the water gets heated up, thereby increasing the temperature. The minimum temperature during September and December can be attributed to monsoons and winters, respectively. During monsoons, the water vapor is highly saturated, leading to the formation of clouds, thereby decreasing the incoming solar radiation and thus, decreasing the surface water temperature.

4.1.2 pH

The pH of the seawater varied from 7.67 to 8.05. Monthly variation of pH recorded the lowest values in September (monsoon) and the highest value in January (post-monsoon).

4.1.3 Salinity

Salinity of the surface seawater varies from 28.6 to 32.6 during the study period. Monthly variation of salinity showed the highest values in the month of May (Pre-monsoon) and the lowest values in the month of September (Monsoon). Due to monsoons, there is fresh water inflow and the rate of evaporation is less. This leads to a decrease in the salinity, whereas, an increase in salinity can be attributed to a high rate of evaporation.

Table 4.1- Monthly variation of pH, Salinity and Temperature

Months	pH	Salinity (‰)	Temperature (^oC)
May	7.98	32.6	31.5
September	7.67	28.6	29
October	7.7	29.1	31
November	7.93	30.5	31
December	8.01	31.01	29
January	8.05	31.6	31
Average	**7.89**	**30.51**	**30.33**

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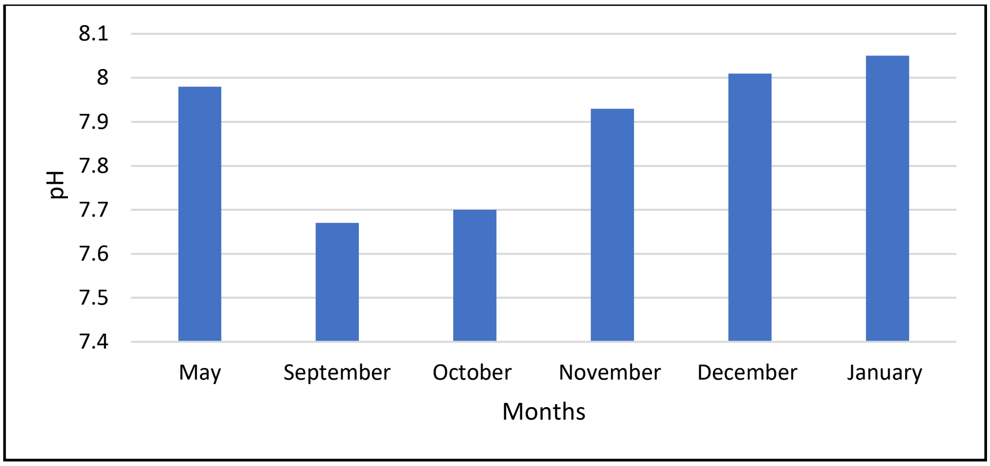

Figure 4.1 a. - Monthly variation of pH

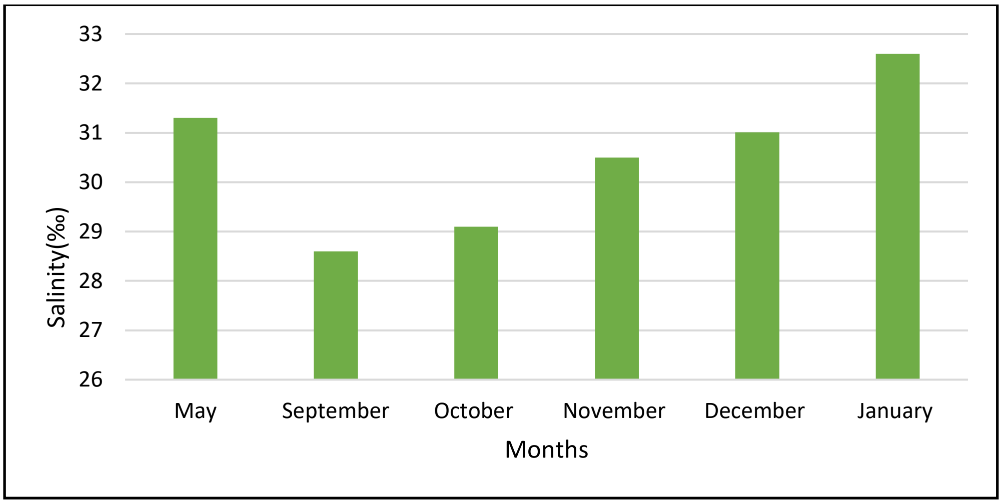

Figure 4.1 b. - Monthly variation of Salinity

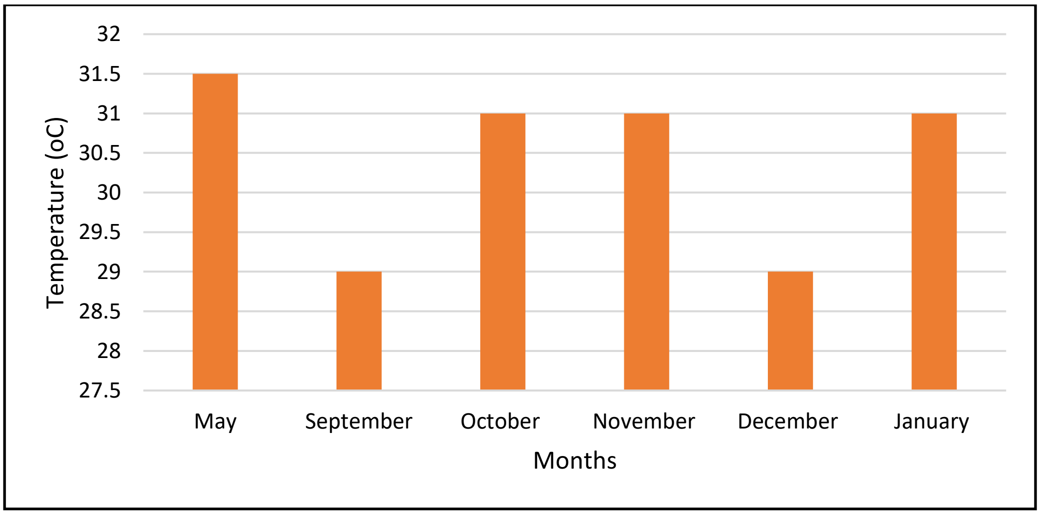

Figure 4.1 c. - Monthly variation of Temperature

4.2 TRACE METALS IN SEAWATER

Trace metal variation in seawater at Anjuna, Goa is given in Table 4.2

4.2.1 Copper

The Cu concentration ranged from 22.86 ppm to 54.74 ppm. The highest concentration was recorded in January (54.74 ppm) and the lowest was in October (22.86 ppm) (Figure 4.2 a.). The Cu concentration varied with the highest concentration in January> May> December> November> September> October. **Paul and Pillai, (1983)** suggested that elevated levels of copper observed during the non-monsoon period could be attributed to evaporation and increased sediment dissolution, which were influenced by higher temperatures and longer contact durations. The Cu concentrations obtained in this study are higher than the reported values by **Chakraborty et al. (2013), Robin et al. (2012)** and **Kumar et al. (2020)** at the south Australian coastline, south west coast of India and the south east coast of India respectively.

4.1.2 Zinc

The Zn concentration ranged from 36.05 ppm to 84.36 ppm. The highest concentration of Zn was observed in October (84.36 ppm) and the lowest concentration was observed in January (36.05 ppm) (Figure 4.2 b.). The Zn concentration varied in the order October> May> November> September> December> January. The high concentration of Zn in non-monsoon season can be reasoned as high temperature favours decomposition of organic matter, by setting the trace metals in the bottom sediments which can be resuspended by tides **(Martin and Whitfield, 1983)**. The Zn values obtained are higher than the ones recorded by **Robin et al. (2012)** south west coast of India.

Table 4.2- Trace metal variation in seawater at Anjuna, Goa

Months	Cu (ppm)	Zn (ppm)	Ni (ppm)	Co (ppm)	Fe (ppm)	Mn (ppm)
May	51.63	71.68	0.79	ND	72.25	2.72
September	29.34	51.97	3.45	0.75	3086.25	6.20
October	22.86	84.36	ND	ND	910.62	3.8
November	36.95	68.17	27.13	ND	2477.5	10.89
December	43.51	40.01	3.56	ND	1025	3.12
January	54.74	36.05	4.28	ND	77.45	6.63
Average	**39.84**	**58.70**	**7.84**	**0.75**	**1274.84**	**5.56**

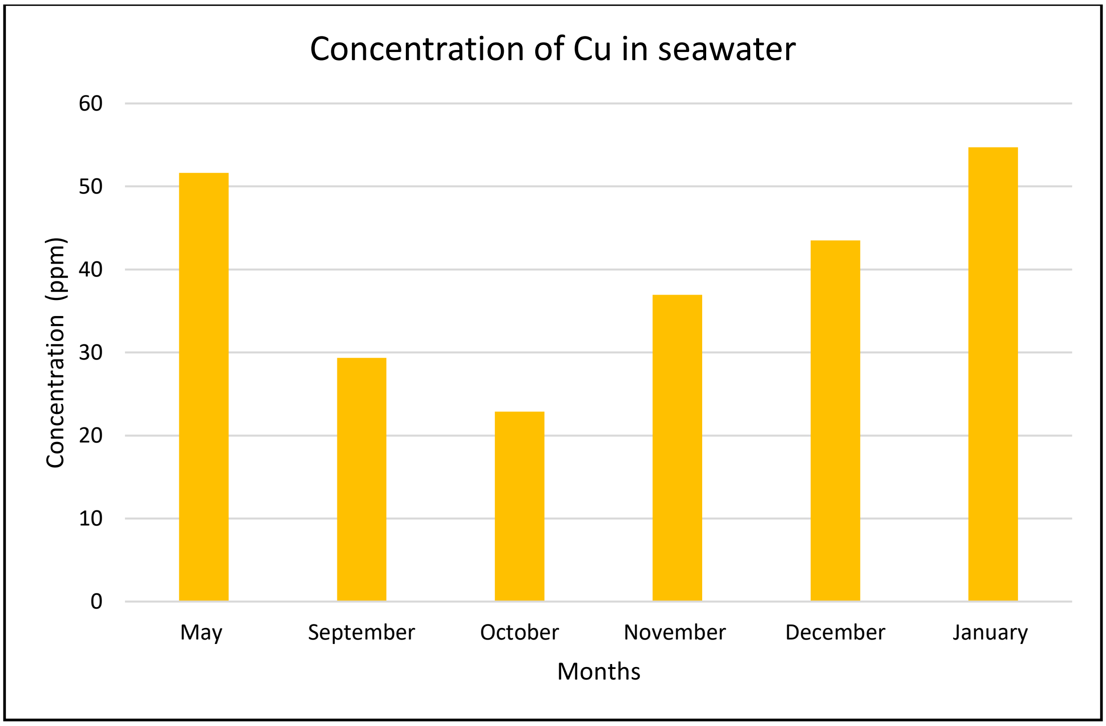

Figure 4.2 a. - Copper variation in seawater at Anjuna, Goa

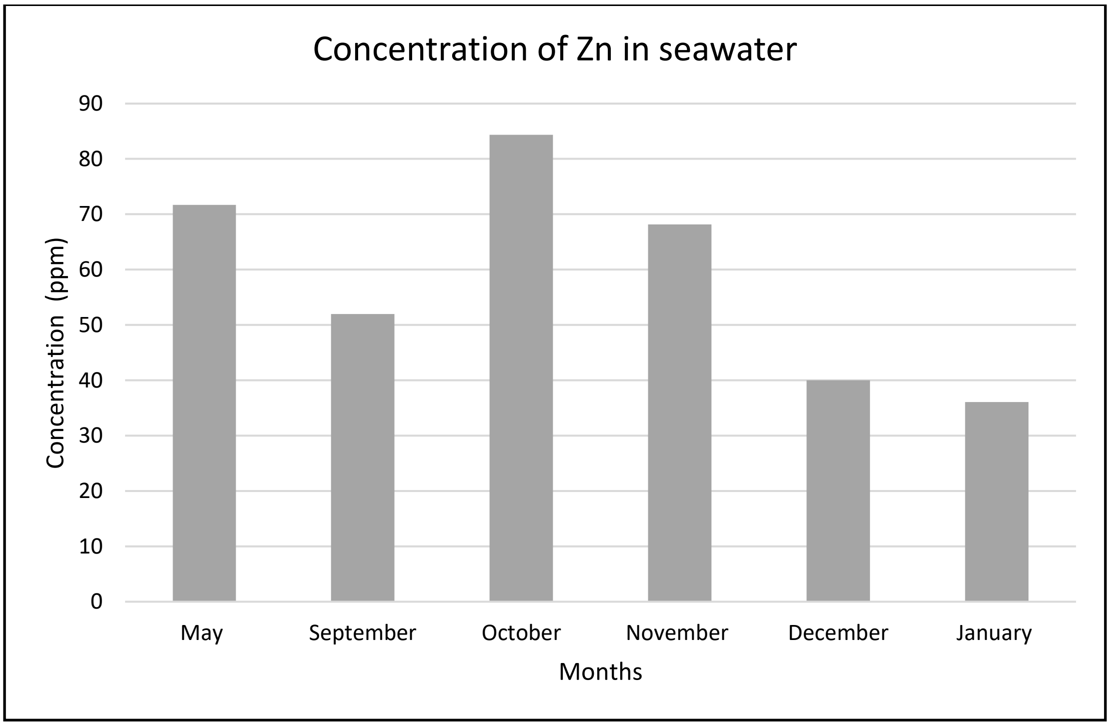

Figure 4.2 b. - Zinc variation in seawater at Anjuna, Goa

4.2.3 Nickel

The Ni concentration ranged from 0.79 ppm to 27.13 ppm. The highest concentration was recorded in November (27.13 ppm) and the lowest was recorded in May (0.79 ppm) (Figure 4.2 c.). Ni concentration was not detected in October. The Ni concentration varied in order November> January> December> September> May. The nickel concentration obtained are higher than the ones reported by **Lallu et al. (2022)** and **Kumar et al. (2020)** at the south west coast of India and South east coast of India respectively**.**

4.2.4 Cobalt

The Co concentration was only detectable in September with value as 0.75 ppm (Figure 4.2 d.). The lower values of Co in seawaters are due to removal of a substantial amount of Co from surface waters by phytoplankton **(Bruland and Franks, 1983).** The only detectable values observed in monsoon period can be due to the influx of land runoff.

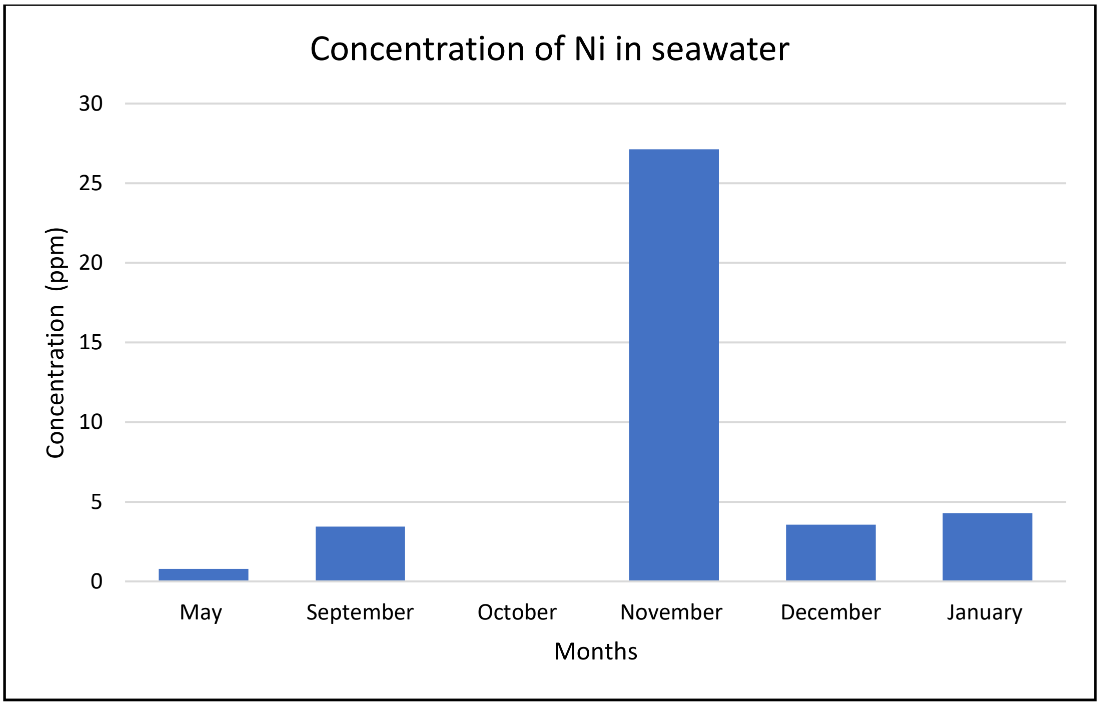

Figure 4.2 c. - Nickel variation in seawater at Anjuna, Goa

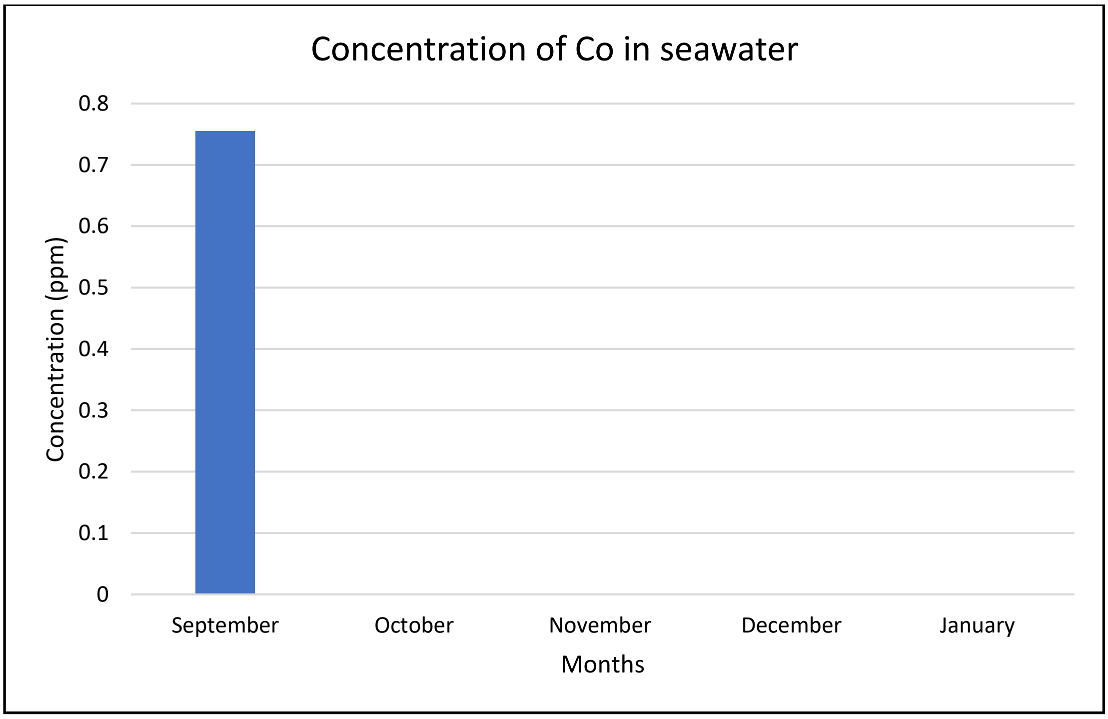

Figure 4.2 d. - Cobalt variation in seawater at Anjuna, Goa

4.2.5 Iron

The Fe concentration ranged from 72.45 ppm to 3086.25 ppm. The highest concentration was observed in September (3086.25 ppm) and the lowest in May (72.45ppm) (Figure 4.2 e.). The Fe concentration was highest in September> November> December> October >January> May. The high concentration of Fe can be attributed by the land runoff. The Fe concentration obtained in this study are higher than the values reported by **Kumar et al. (2020), Lallu et al. (2022)** and **Singh et al. (2008)** at the southeast coast of India, south west coast of India and Mandovi Estuary, Goa- west coast of India respectively.

4.2.6 Manganese

The Mn concentration ranged from 2.72 ppm to 10.89 ppm. The highest concentration was observed in November (10.89 ppm) and the lowest in May (2.725 ppm) (Figure 4.2 f.). The Mn concentration was highest in November>January> September> October > December> May. The Mn concentration obtained in this study are higher than the reported values by **Kumar et al. (2020)** and **Lallu et al. (2022)** at the southeast coast of India and south west coast of India respectively.

The mean trace metal concentration in seawater was in the order: Fe> Zn> Cu> Ni> Mn> Co**.** A similar trend was observed by **Kumar et al. (2020)** at the south east coast of India. The high trace metal concentration observed the non-monsoon period can be attributed due to high temperatures which favours decomposition of organic matter in releasing trace metals on the surface sediments, which can be resuspended by tides, currents and dredging. These combined effects might have resulted in higher concentrations of trace metals during non-monsoon period.

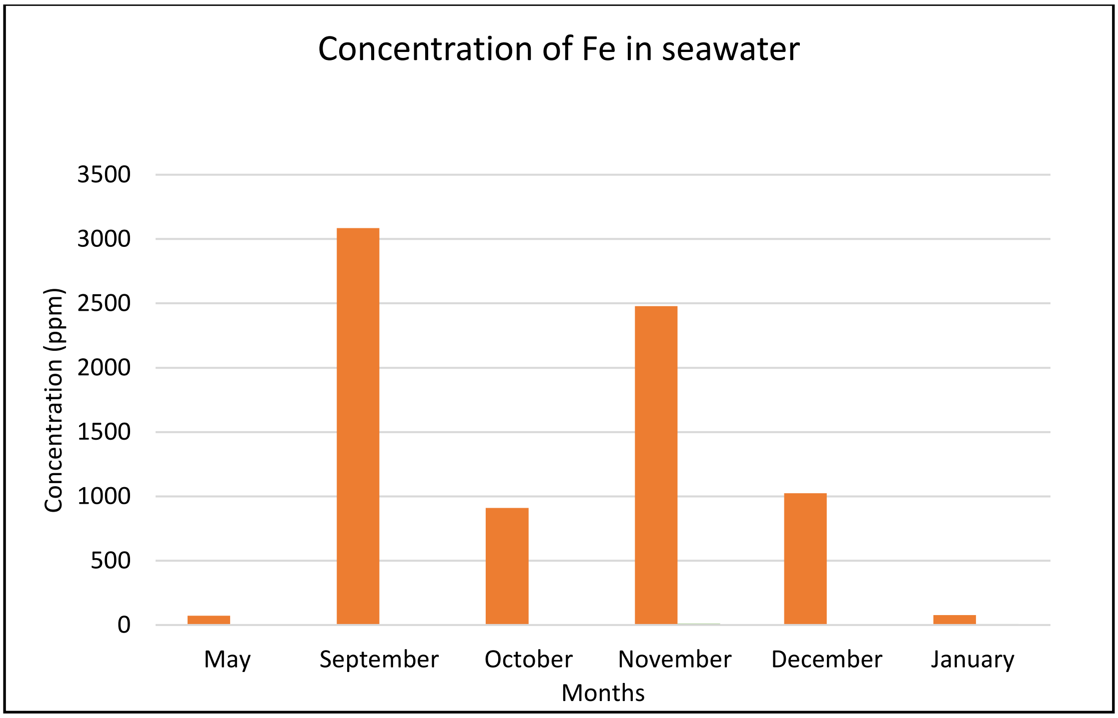

Figure 4.2 e. - Iron variation in seawater at Anjuna, Goa

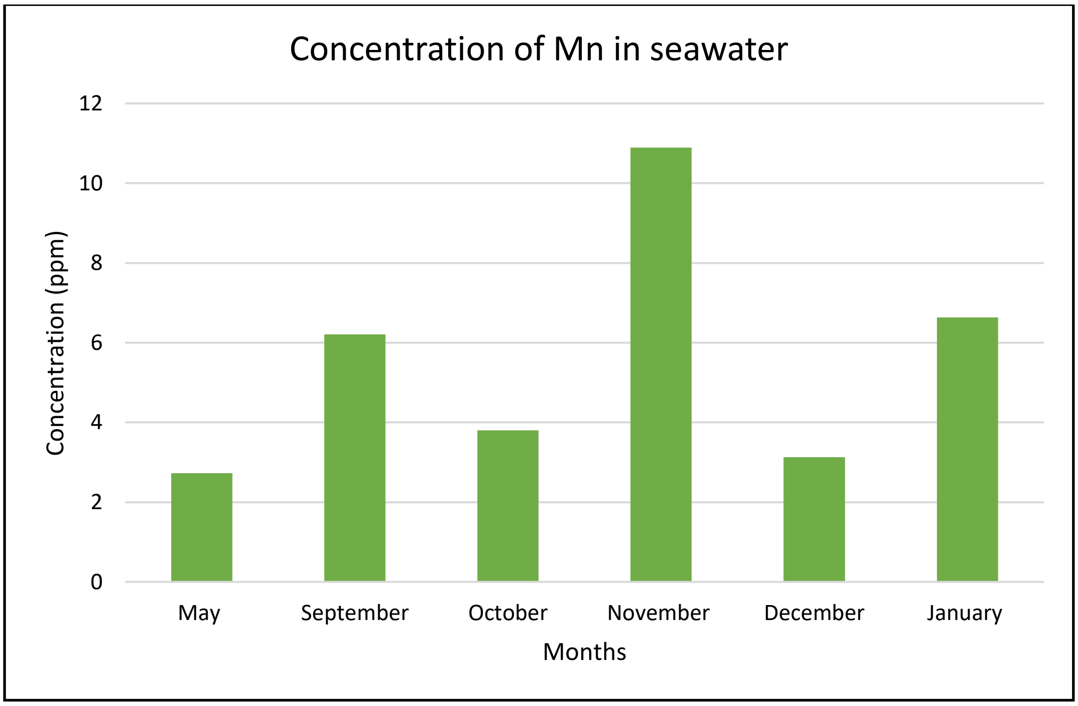

Figure 4.2 f. – Manganese variation in seawater at Anjuna, Goa

4.3 TRACE METALS IN SEAWEEDS

Monitoring the presence of metal pollutants in marine environments has gained increasing significance due to its detrimental effects on water quality, sediment composition and marine biodiversity. The Indian coastline, renowned for its rich seaweed growth, holds particular importance in this regard. Seaweeds have historically served as vital resources worldwide, utilized for both sustenance and industrial purposes **(Chapman, 1950).** Within marine ecosystems, algae play pivotal roles in primary production and the generation of detritus, which serves as a crucial food source for various marine organisms.

Despite the critical ecological functions of seaweeds, there remains a significant gap in understanding the distribution of trace metals within Indian seaweed populations. This knowledge deficit underscores the urgency for comprehensive studies to assess the extent of metal pollution and its potential impact on marine flora and fauna along the Indian coastline. By elucidating the distribution patterns of trace metals in seaweeds, researchers can better comprehend the ecological implications and formulate targeted mitigation strategies to safeguard marine ecosystems against pollution-induced threats.

Table 4.3 – Data on Copper (ppm) in seaweeds of Anjuna, Goa

Species	May	Sept	Oct	Nov	Dec	Jan	Average
Chlorophyta							
Ulva lactuca	-	16.57	-	-	-	-	**16.57**
Chaetomorpha antennina	-	31.64	37.52	-	-	-	**34.58**
Acrosiphonia orentalis	17.48	-	43.07	-	-	-	**30.27**
Culerpa peltata	8.96			-	-	-	**8.96**
Average	**13.22**	**24.10**	**40.29**	**-**	**-**	**-**	**22.59**
Phaeophyta							
Padina tetrastromatica	11.38	-	18.68	16.33	18.69	123.13	**37.64**
Dictyota bartayresiana	-	-	16.38	32.43	12.86	26.73	**22.1**
Sargassum linerifolium	5.73	-		32.82	100.11	11.36	**37.50**
Sargassum cinctum	-	-	36.78	27.54	-	89.63	**51.31**
Sargassum wightti	-	-	-	-	-	14.8	**14.8**
Sargassum tenerrimum	-	-	-	14.47	103.25	-	**58.86**
Sargassum polycustum	-	-	-	-	10.57	7.33	**8.95**
Sargassum ilicifolium	29.61		15.42	14.09	-	84.3	**35.85**
Average	**15.57**		**21.81**	**22.94**	**49.09**	**51.04**	**33.37**
Rhodophyta							
Gelidium pusiillum	5.79	-	14.79	-	12.27	-	**10.95**
Chondracanthus acicularis	-	16.22	-	-	-	-	**16.22**
Average	**5.79**	**16.22**	**14.79**	**-**	**12.27**	**-**	**13.58**

4.3.1 Copper

The concentration of Cu (ppm) in the seaweed species during the study period ranged from 5.73 ppm to 123.13 ppm (Table 4.3). Figure 4.3 shows the graphical representation of Cu concentration in analysed seaweed species.

For Chlorophyta the average Cu concentration ranged from 8.96 ppm to 34.58 ppm. The highest concentration was observed for *Acrosiphonia orentalis* (43.07 ppm) in October and lowest was observed for *Culerpa peltata* (8.96 ppm) in May. The highest average Cu concentration in species of Chlorophyta followed the order *Chaetomorpha antennina* > *Acrosiphonia orentalis* > *Ulva lactuca* > *Culerpa peltata.*

For Phaeophyta the average Cu concentration ranged from 8.95 ppm to 58.86 ppm. The highest concentration was observed for *Padina tetrastromatica* (123.13 ppm) in January and lowest was observed for *Sargassum linerifolium* (5.73 ppm) in May. The highest average Cu concentration in species of Phaeophyta followed the order: *Sargassum tenerrimum* > *Sargassum cinctum* > *Padina tetrastromatica*> *Sargassum linerifolium* > *Sargassum ilicifolium* > *Dictyota bartayresiana* > *Sargassum wightti* > *Sargassum polycustum.*

For Rhodophyta highest concentration was observed for *Chondracanthus acicularis* (16.22 ppm) in September and lowest was observed for *Gelidium pusiillum* (5.75 ppm) in May. The mean value of Cu in the three phyla were in the order Phaeophyta > Chlorophyta > Rhodophyta. The high concentration of Cu in Phaeophyta observed could be due to alginate which is a main component responsible for metal sorption and is present in a gel form in brown seaweed’s cell walls **(Fourest and Volesky, 1997).**

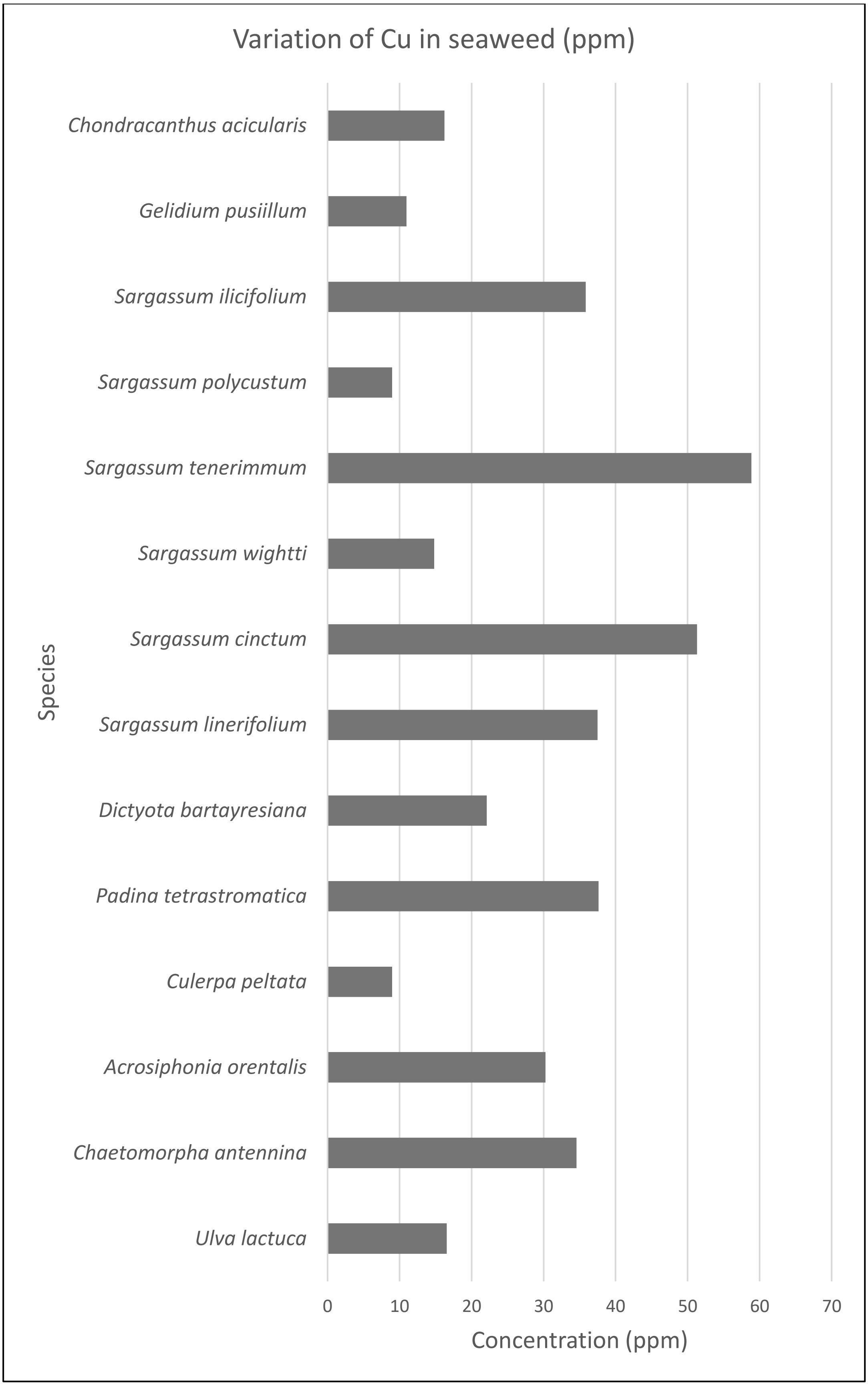

Figure 4.3 –Variation of Cu (ppm) in seaweeds of Anjuna, Goa

Table 4.4 – Data on Zinc (ppm) in seaweeds of Anjuna, Goa

Species	May	Sept	Oct	Nov	Dec	Jan	Average
Chlorophyta							
Ulva lactuca	-	93.79	-	-	-	-	**93.79**
Chaetomorpha antennina	-	57.08	49.83	-	-	-	**53.45**
Acrosiphonia orentalis	52.33	-	44.97	-	-	-	**48.65**
Culerpa peltata	38.37	-	-	-	-	-	**38.37**
Average	**45.35**	**75.43**	**47.4**	**-**	**-**	**-**	**58.56**
Phaeophyta							
Padina tetrastromatica	83.65	-	41.8	30.36	49.62	80.2	**57.12**
Dictyota bartayresiana	-	-	31.45	106.51	9.69	64.05	**52.92**
Sargassum linerifolium	21.79	-	-	33.95	35.96	16.31	**27.00**
Sargassum cinctum	-	-	24.92	32.73	-	26.26	**27.97**
Sargassum wightti	-	-	-	-	-	24.3	**24.3**
Sargassum tenerrimum	-	-	-	8.85	30.21	-	**19.53**
Sargassum polycustum	-	-	-	-	10.77	13.54	**12.15**
Sargassum ilicifolium	26.88	-	15.75	27.12	-	47.41	**29.29**
Average	**44.10**	**-**	**28.48**	**39.92**	**27.25**	**38.86**	**31.28**
Rhodophyta							
Gelidium pusiillum	72.1	-	23.08	-	9.17	-	**34.78**
Chondracanthus acicularis	-	58.66	-	-	-	-	**58.66**
Average	**72.1**	**58.66**	**23.08**	**-**	**9.17**	**-**	**46.72**

4.3.2 Zinc

The concentration of Zn (ppm) in the seaweed species during the study period ranged from 8.85 ppm to 93.79 ppm (Table 4.4). Figure 4.4 shows the graphical representation of Zn concentration in analysed seaweed species.

For Chlorophyta the average Zn concentration ranged from 38.37 ppm to 93.79 ppm. The highest concentration was observed for *Ulva lactuca* (93.79 ppm) in September and lowest was observed for *Culerpa peltata* (38.37 ppm) in May. The highest average Zn concentration in species of Chlorophyta followed the order *Ulva lactuca* > *Chaetomorpha antennina* > *Acrosiphonia orentalis* > *Culerpa peltata*. Similarly, *Ulva lactuca* reported highest concentration of Zn in a study done by **Chakraborty et al. (2014)**

For Phaeophyta the average Zn concentration ranged from 12.15 ppm to 57.12 ppm. The highest concentration was observed for *Padina tetrastromatica* (83.65 ppm) in May and lowest was observed for *Sargassum tenerrimum* (8.85 ppm) in November. The highest average Zn concentration in species of Phaeophyta followed the order *Padina tetrastromatica* > *Dictyota bartayresiana* > *Sargassum ilicifolium*> *Sargassum cinctum* > *Sargassum linerifolium* > *Sargassum tenerrimum* > *Sargassum wightti* > *Sargassum polycustum.*

For Rhodophyta highest concentration of Zn was observed for *Chondracanthus acicularis* (58.66 ppm) in September and lowest was observed for *Gelidium pusiillum* (9.17 ppm) in December. The mean value of zinc in the three phyla were in the order Chlorophyta > Rhodophyta > Phaeophyta. **Chakraborty et al. (2014)** reported similar findings where Chlorophyta accumulated the highest concentration of Zn than Phaeophyta and Rhodophyta.

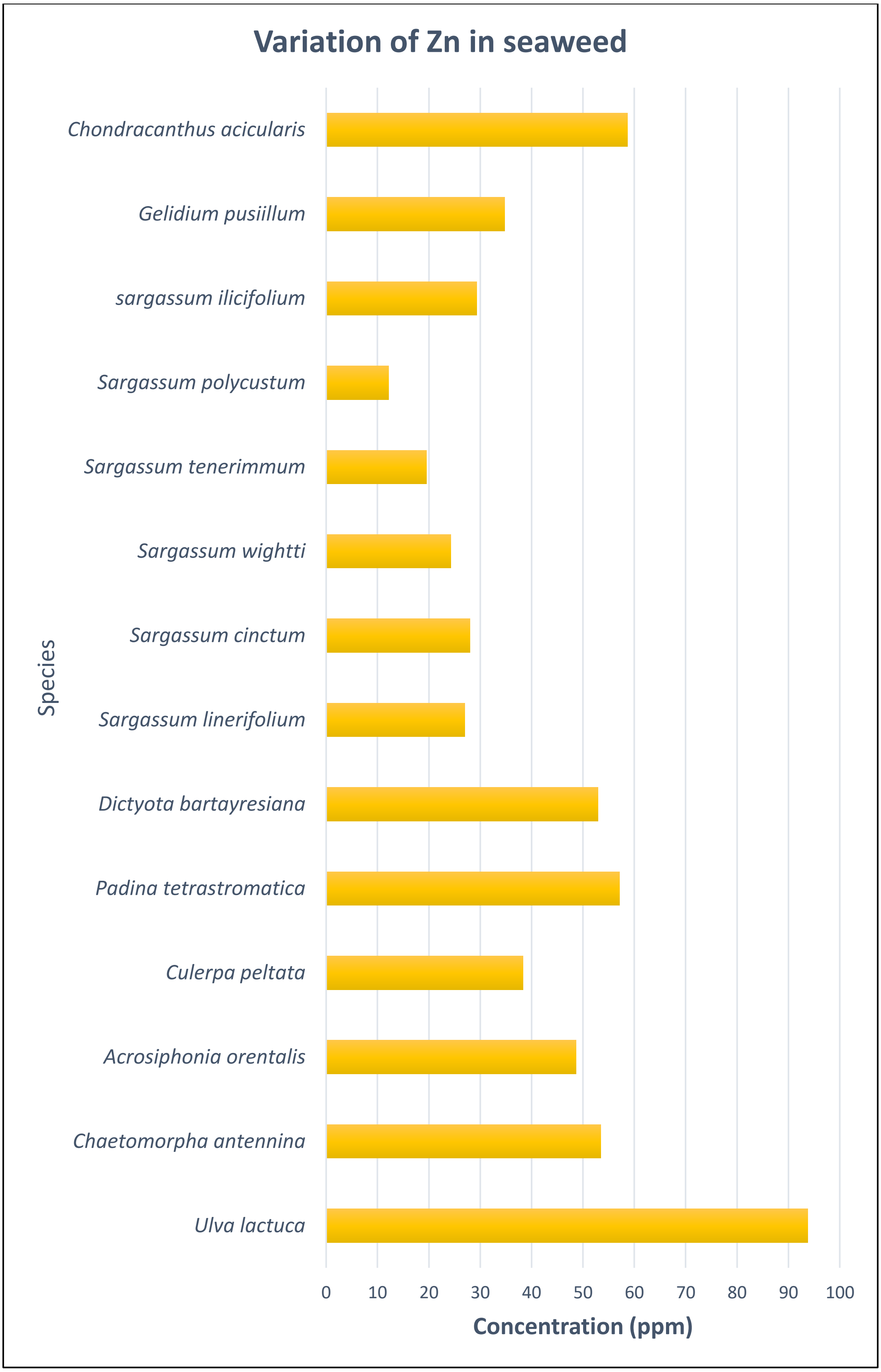

Figure 4.4 – Variation of Zinc (ppm) in seaweeds of Anjuna, Goa

Table 4.5 – Data on Nickel (ppm) in seaweeds of Anjuna, Goa

Species	May	Sept	Oct	Nov	Dec	Jan	Average
Chlorophyta							
Ulva lactuca	-	4.5	-	-	-	-	**4.5**
Chaetomorpha antennina		6.71	9.9	-	-	-	**8.30**
Acrosiphonia orentalis	16.95	-	32.16	-	-	-	**24.55**
Culerpa peltata	9.31	-	-	-	-	-	**9.13**
Average	**13.13**	**5.605**	**21.03**	**-**	-	-	**11.62**
Phaeophyta							
Padina tetrastromatica	11.08	-	7.91	7.34	14.84	8.26	**9.88**
Dictyota bartayresiana	-	-	6.57	7.16	0.38	12.59	**6.67**
Sargassum linerifolium	6.95	-	-	2.1	2.76	1.78	**3.39**
Sargassum cinctum	-	-	2	1.31	-	3.41	**2.24**
Sargassum wightti	-	-	-	-	-	4.61	**4.61**
Sargassum tenerrimum	-	-	-	ND	ND	-	**-**
Sargassum polycustum	-	-	-	-	ND	ND	**-**
Sargassum ilicifolium	5.87	-	1.89	3.4	-	2.71	**3.46**
Average	**7.96**		**4.59**	**4.26**	**5.99**	**5.56**	**5.04**
Rhodophyta							
Gelidium pusiillum	4.34	-	2.93	-	0.77	-	**2.68**
Chondracanthus acicularis	-	2.45		-		-	**2.45**
Average	**4.34**	**2.45**	**2.93**	**-**	**0.77**	**-**	**2.56**

4.3.3 Nickel

The concentration of Ni (ppm) in the seaweed species during the study period ranged from 0.77 ppm to 32.16 ppm (Table 4.5). Figure 4.5 shows the graphical representation of nickel concentration in analysed seaweed species.

For Chlorophyta the average Ni concentration ranged from 4.5 ppm to 24.55 ppm. The highest concentration was observed for *Acrosiphonia orentalis* (32.16 ppm) in October and lowest was observed for *Ulva lactuca* (4.5 ppm) in September. The highest average Ni concentration in species of Chlorophyta followed the order *Acrosiphonia orentalis* > *Culerpa peltata* > *Chaetomorpha antennina* > *Ulva lactuca.*

For Phaeophyta the average Ni concentration ranged from 1.31 ppm to 14.84 ppm. The highest concentration was observed for *Padina tetrastromatica* (14.84 ppm) in December and lowest was observed for *Sargassum cinctum* (1.31 ppm) in November. *Sargassum tenerimmum* and *Sargassum polycustum* did not show detectable concentrations for Ni. The highest average Ni concentration in species of Phaeophyta followed the order *Padina tetrastromatica* > *Dictyota bartayresiana* > *Sargassum wightt.*> *Sargassum ilicifolium* > *Sargassum linerifolium* > *Sargassum cinctum.*

For Rhodophyta highest (4.34 ppm) and lowest (0.77 ppm) concentration was observed for *Gelidium pusiillum* in May and December respectively. The mean value of Nickel in the three phyla were in the order Chlorophyta > Phaeophyta> Rhodophyta.

Pan et al. (2018) found Ni concentration to be highest in Chlorophyta and suggested its use as a bio-monitor for Ni pollution in South China Sea. Similarly, **Abdallah et al. (2005**) also observed high concentration of Ni in Chlorophyta.

Whereas, on the contrary **Chakraborty et al. (2014)** found Phaeophyta accumulated more Ni than either Chlorophyta or Rhodophyta.

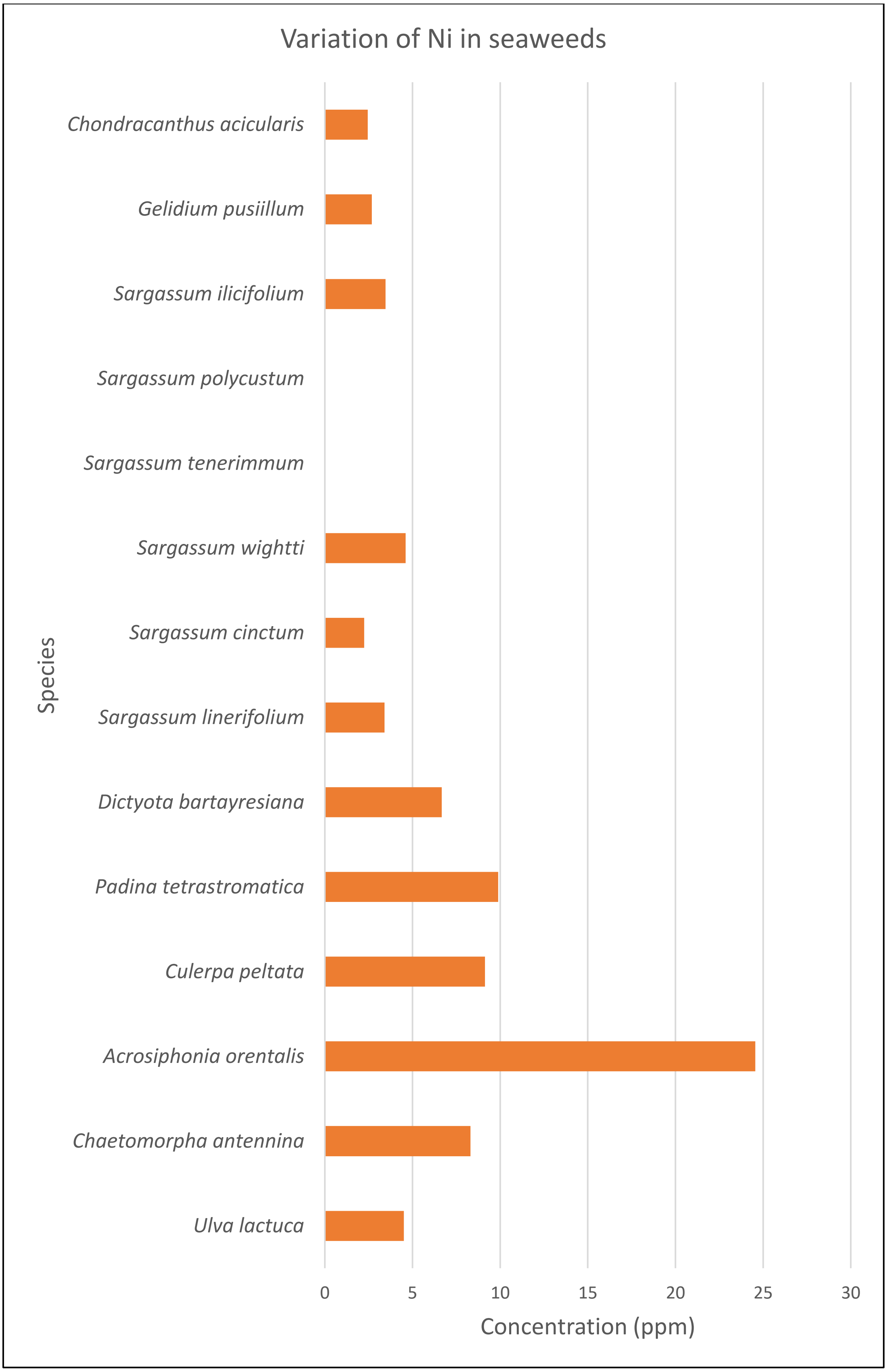

Figure 4.5- Variation of Nickel (ppm) in seaweeds of Anjuna, Goa

Table 4.6 – Data on Cobalt (ppm) in seaweeds of Anjuna, Goa

Species	May	Sept	Oct	Nov	Dec	Jan	Average
Chlorophyta							
Ulva lactuca	-	ND	-	-	-	-	**ND**
Chaetomorpha antennina	-	ND	ND		-	-	**ND**
Acrosiphonia orentalis	3.2	--	1.2	-	-	-	**2.2**
Culerpa peltata	ND	-	-	-	-	-	-
Average	**3.2**	**-**	**1.2**	**-**	-	-	**ND**
Phaeophyta							
Padina tetrastromatica	ND	-	ND	ND	ND	ND	**ND**
Dictyota bartayresiana	-	-	ND	ND	ND	ND	**ND**
Sargassum linerifolium	ND	-	-	ND	ND	ND	**ND**
Sargassum cinctum	-	-	ND	ND	-	ND	**ND**
Sargassum wightti	-	-	-	-	-	ND	**ND**
Sargassum tenerrimum	-	-	-	ND	ND	-	**ND**
Sargassum polycustum	-	-	-	-	ND	ND	**ND**
Sargassum ilicifolium	ND	-	ND	ND	-	ND	**ND**
Average	**-**	**-**	**-**	**-**	**-**	**-**	**ND**
Rhodophyta							
Gelidium pusiillum	ND	-	ND	-	ND	-	**ND**
Chondracanthus acicularis	-	ND	-	-	-	-	**ND**
Average	**-**	**-**	**-**	**-**	**-**		**ND**

4.3.4 Cobalt

Detectable levels of Co concentration were only present in *Acrosiphonia orentalis* (Chlorophyta), 3.2 ppm and 1.2 ppm in May and October respectively (Table 4.6) (Figure 4.6). Other species showed no detectable levels of Co present. This could be attributed by the low concentration of Co present in seawater at Anjuna, Goa (Table 4.2) (Figure 4.2 d.)

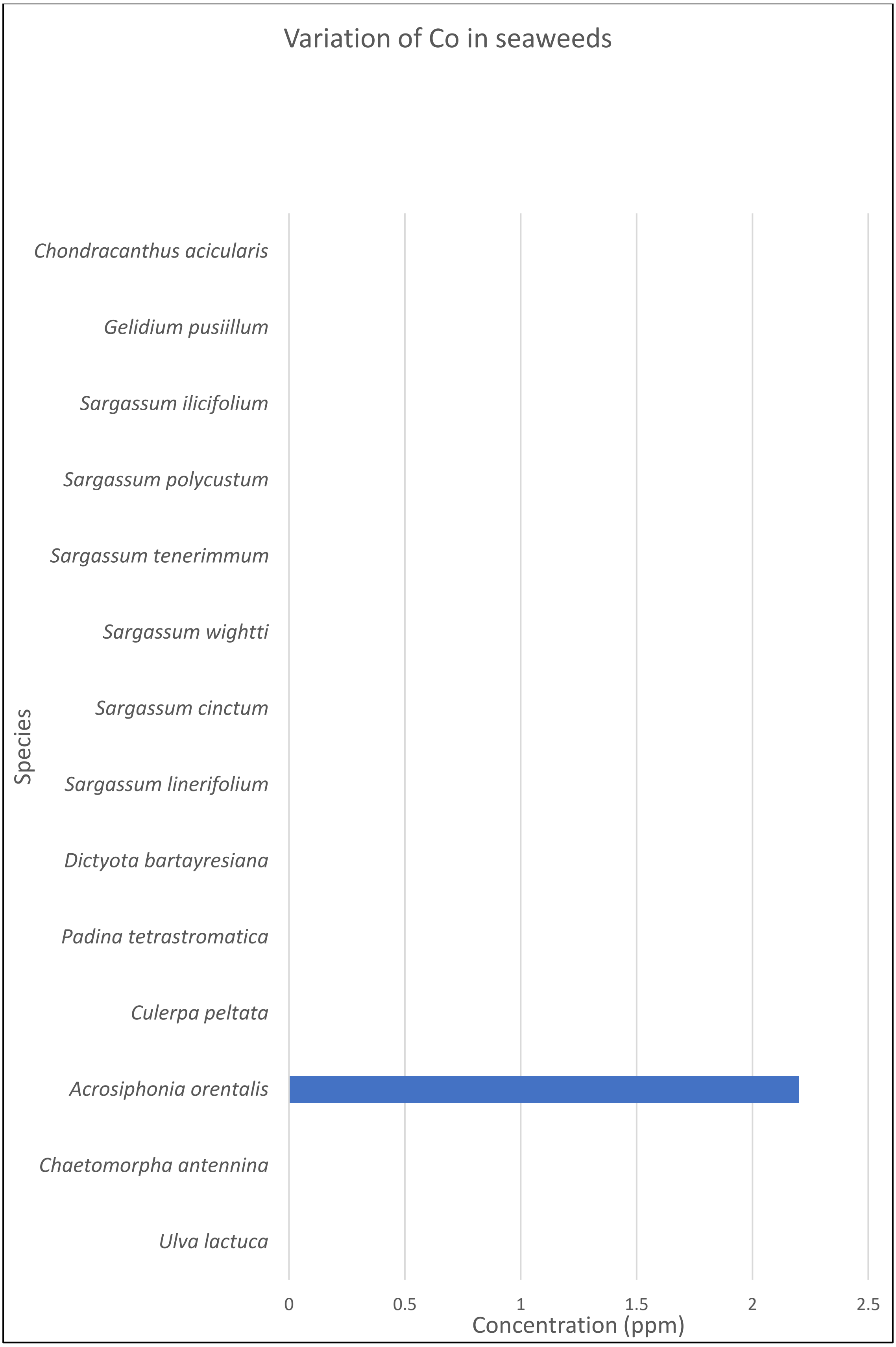

Figure 4.6 – Variation of Cobalt (ppm) in seaweeds of Anjuna, Goa

Table 4.7 – Data on Iron (ppm) in seaweeds of Anjuna, Goa

Species	May	Sept	Oct	Nov	Dec	Jan	Average
Chlorophyta							
Ulva lactuca	-	448.46	-	-	-	-	**448.46**
Chaetomorpha antennina	-	4262.5	8482.5	-	-	-	**6372.5**
Acrosiphonia orentalis	7417.5	-	16008.75	-	-	-	**11713.13**
Culerpa peltata	5125	-	-	-	-	-	**5125**
Average	**6271.25**	**2355.48**	**12245.63**	**-**	**-**	**-**	**5914.77**
Phaeophyta							
Padina tetrastromatica	6643.75	-	5035	4912.5	9845	6653.75	**6618**
Dictyota bartayresiana		-	2913.75	2790	537.36	5730	**2992.77**
Sargassum linerifolium	442.06	-	-	251.3	332.38	276.48	**325.55**
Sargassum cinctum	-	-	491.31	418.86	-	294.54	**401.57**
Sargassum wightti	-	-	-	-	-	765.9	**765.9**
Sargassum tenerrimum	-	-	-	110.63	155.3	-	**132.96**
Sargassum polycustum	-	-	-	-	201.97	181.83	**191.9**
Sargassum ilicifolium	478.57	-	250.12	431.32	-	317.9	**369.47**
Average	**22521.46**	**-**	**2172.54**	**1485.76**	**2214.40**	**2031.48**	**2974.76**
Rhodophyta							
Gelidium pusiillum	5559.2	-	546.55	-	602.3	-	**2236.01**
Chondracanthus acicularis	-	344.23	-	-	-	-	**344.23**
Average	**5559.2**	**344.23**	**546.55**	**-**	**602.3**	**-**	**1290.12**

4.3.5 Iron

The concentration of iron (ppm) in the seaweed species during the study period ranged from 110.65 ppm to 16008.75 ppm (Table 4.7). Figure 4.7 shows the graphical representation of iron concentration in analysed seaweed species.

For Chlorophyta the average Fe concentration ranged from 448.46 ppm to 11713.13 ppm. The highest concentration was observed for *Acrosiphonia orentalis* (16008.75 ppm) in October and lowest was observed for *Ulva lactuca* (448.46pmm) in September. The highest average Fe concentration in species of Chlorophyta followed the order *Acrosiphonia orentalis* > *Chaetomorpha antennina* > *Culerpa peltate* > *Ulva lactuca.*

For Phaeophyta the average Fe concentration ranged from 132.96 ppm to 6618 ppm. The highest concentration was observed for *Padina tetrastromatica* (6653.75 ppm) in January and lowest was observed for *Sargassum tenerrimum* (110.63 ppm) in November. The highest average Fe concentration in species of Phaeophyta followed the order *Padina tetrastromatica* > *Dictyota bartayresiana* > *Sargassum wightti* > *Sargassum cinctum* > *Sargassum ilicifolium* > *Sargassum linerifolium* > *Sargassum polycustum* > *Sargassum tenerrimum.*

For Rhodophyta highest Fe concentration was observed for *Gelidium pusiillum* (5559.2 ppm) in May and lowest was observed for *Chondracanthus acicularis* (344.23 ppm) in May as well. Concentration of Fe obtained in this study falls in a higher range then the concentrations reported by **Ganesan et al. (1991)** at Gulf of Mannar and **Chakraborty et al. (2014)** at Gulf of Kutch.

The mean value of Fe in the three phyla were the in order Chlorophyta > Phaeophyta > Rhodophyta.

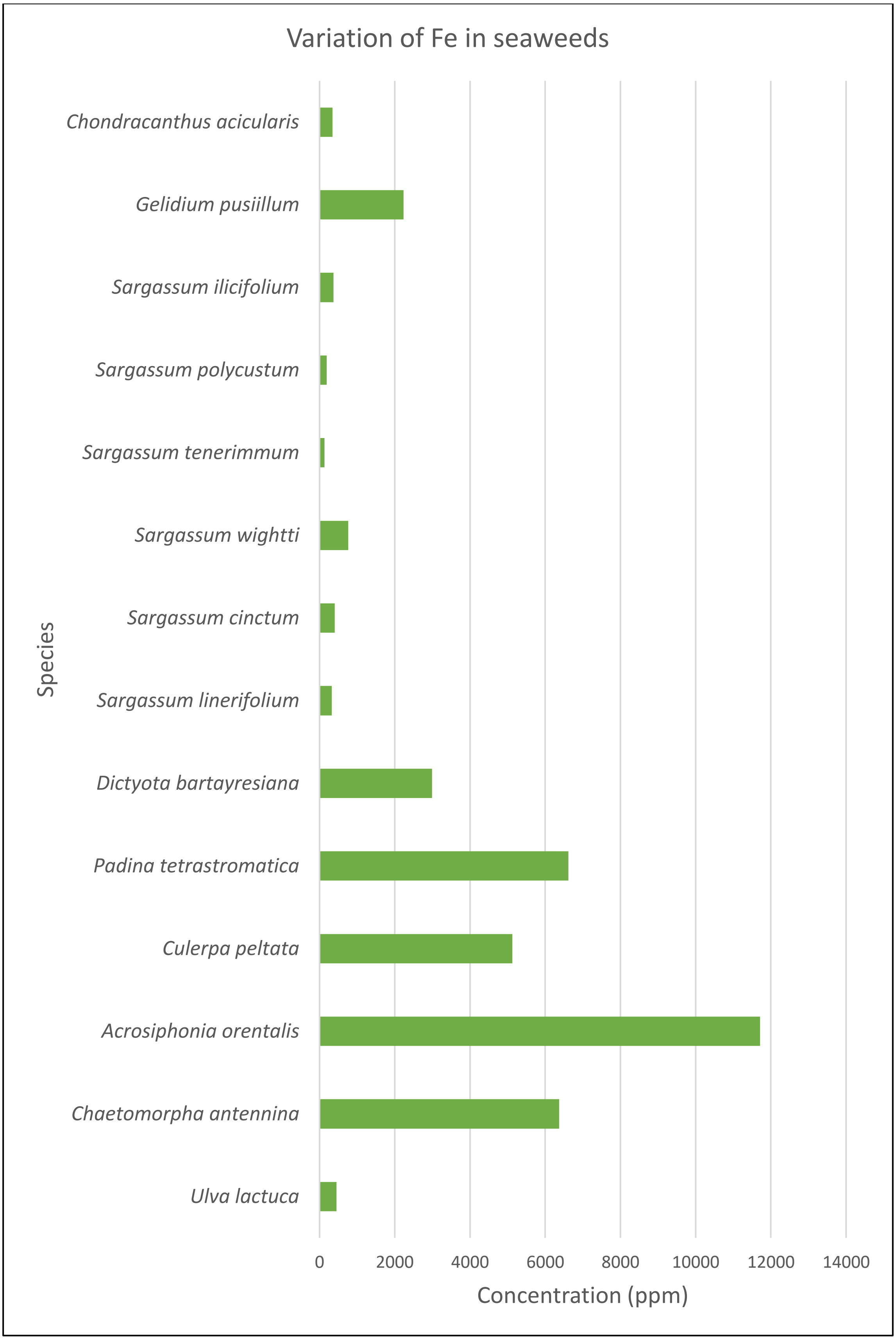

Figure 4.7 – Variation of Iron (ppm) in seaweeds of Anjuna, Goa

Table 4.8 – Data on Manganese (ppm) in seaweeds of Anjuna, Goa

Species	May	Sept	Oct	Nov	Dec	Jan	Average
Chlorophyta							
Ulva lactuca	-	87.49	-	-	-	-	**87.49**
Chaetomorpha antennina	-	112.52	150.56-	-	-	-	**131.54**
Acrosiphonia orentalis	2140	-	387.5	-	-	-	**1263.75**
Culerpa peltata	123.551	-	-	-	-	-	**123.55**
Average	**1131.77**	**100.00**	**269.03**	**-**	-	-	**401.58**
Phaeophyta							
Padina tetrastromatica	264.39	-	119.69	124.46	267.89	84.16	**172.11**
Dictyota bartayresiana	-	-	71.26	57.95	19.42	41.88	**47.62**
Sargassum linerifolium	93.09	-	-	29.85	21.44	8.32	**38.17**
Sargassum cinctum	-	-	38.18	40.26	-	4.48	**27.64**
Sargassum wightti	-	-	-	-	-	32.39	**32.39**
Sargassum tenerrimum	-	-	-	14.52	21.09	-	**17.80**
Sargassum polycustum	-	-	-	-	9.13	8.87	**9**
Sargassum ilicifolium	57.45	-	24.38	47.29		17.32	**36.61**
Average	**138.31**	**-**	**63.37**	**52.38**	**67.79**	**28.20**	**47.67**
Rhodophyta							
Gelidium pusiillum	90.85	-	61.23	-	40	-	**64.02**
Chondracanthus acicularis	-	59.54	-	-	-	-	**59.54**
Average	**90.85**	**59.54**	**61.23**	**-**	**40**	**-**	**61.78**

4.3.6 Manganese

The concentration of Mn (ppm) in the seaweed species during the study period ranged from 8.87 ppm to 2140 ppm (Table 4.8). Figure 4.8 shows the graphical representation of manganese concentration in analysed seaweed species.

For Chlorophyta the average Mn concentration ranged from 87.49 ppm to 1263.75 ppm. The highest concentration was observed for *Acrosiphonia orentalis* (2140 ppm) in May and lowest was observed for *Ulva lactuca* in September. The highest average Mn concentration in species of Chlorophyta followed the order *Acrosiphonia orentalis* > *Chaetomorpha antennina* > *Culerpa peltate* > *Ulva lactuca.*

For Phaeophyta the average Mn concentration ranged from 172.11 ppm to 9 ppm. The highest concentration was observed for *Padina tetrastromatica* (267.89 ppm) in December and lowest was observed for *Sargassum polycustum* (9.13 ppm) in November. The highest average Mn concentration in species of Phaeophyta followed the order *Padina tetrastromatica* > *Dictyota bartayresiana* > *Sargassum linerifolium* > *Sargassum ilicifolium* > *Sargassum wightti* > *Sargassum cinctum* > *Sargassum tenerrimum* > *Sargassum polycustum.* Padina species was found to accumulate high concentration of Mn by **Chakraborty et al. (2014)**

For Rhodophyta highest Mn concentration was observed for *Gelidium pusiillum* (90.85 ppm) in May and lowest was observed for *Gelidium pusiillum* (40 ppm) in December. The mean value of Mn in the three phyla were in the order Chlorophyta > Rhodophyta > Phaeophyta.

Chlorophyta showed high concentration to Mn; however, **Sawidis et al. (2001)** demonstrated that Mn levels in Rhodophyta were significantly greater than those of the Phaeophyta and Chlorophyta species.

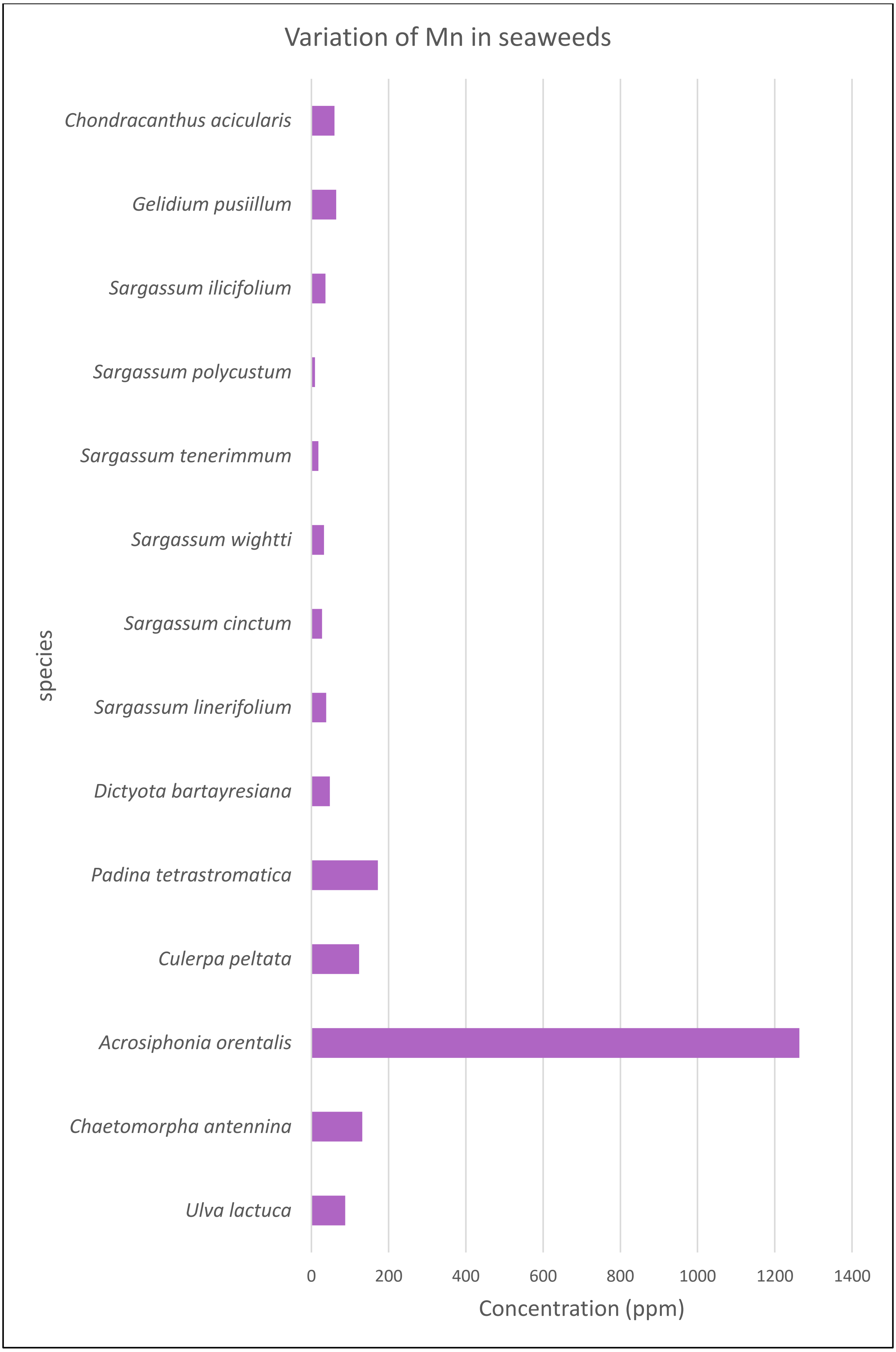

Figure 4.8 – Variation of Manganese (ppm) in seaweeds of Anjuna, Goa

Table 4.9-Data on mean concentration of trace metals in seaweeds of Anjuna, Goa.

Mean concentration (ppm)						
Species	Cu	Zn	Co	Ni	Fe	Mn
Chlorophyta						
Ulva lactuca	16.57	93.79	ND	4.5	448.46	87.49
Chaetomorpha antennina	34.58	53.45	ND	8.305	6372.5	131.54
Acrosiphonia orentalis	30.27	48.65	2.2	24.55	11713.12	1263.75
Culerpa peltata	8.96	38.37	ND	9.13	5125	123.551
Average	**22.59**	**58.56**	**2.2**	**11.62**	**5914.77**	**401.58**
Phaeophyta						
Padina tetrastromatica	37.642	57.12	ND	9.88	18618	172.11
Dictyota bartayresiana	22.1	52.92	ND	6.67	2992.77	47.62
Sargassum linerifolium	37.505	27.00	ND	3.39	325.55	38.17
Sargassum cinctum	51.31	27.97	ND	2.24	401.57	27.64
Sargassum wightti	14.8	24.3	ND	4.61	765.9	32.39
Sargassum tenerrimum	58.86	19.53	ND	ND	132.96	17.80
Sargassum polycustum	8.95	12.15	ND	ND	191.9	9
Sargassum ilicifolium	35.85	29.29	ND	3.46	369.47	36.61
Average	**33.37**	**31.28**	**ND**	**5.04**	**2974.76**	**47.67**
Rhodophyta						
Gelidium pusiillum	10.95	34.78	ND	2.68	2236.01	64.02
Chondracanthus acicularis	16.22	58.66	ND	2.45	344.23	59.54
Average	**13.58**	**46.72**	**ND**	**2.56**	**1290.12**	**61.78**

Based on the mean concentration of trace metals (Table-4.9) the distribution of trace metals in the three phyla of macroalgae are mentioned below:

Cu: Phaeophyta > Chlorophyta > Rhodophyta

Zn: Chlorophyta > Rhodophyta > Phaeophyta

Ni: Chlorophyta > Phaeophyta > Rhodophyta

Fe: Chlorophyta > Phaeophyta > Rhodophyta

Mn: Chlorophyta > Rhodophyta > Phaeophyta

The above data suggests that the Chlorophyta and Phaeophyta species are found to accumulate higher concentration of trace metals than Rhodophyta. Similar results were obtained by **Nabil et al. (2007)** the uptake of heavy metals by different marine algal divisions was in the order of Chlorophyta> Phaeophyta> Rhodophyta.

The order of trace metal concentration in the phyla of seaweeds are mentioned below.

Chlorophyta: Fe> Mn> Zn> Cu> Ni> Co

Phaeophyta: Fe> Mn> Cu> Zn> Ni> Co

Rhodophyta: Fe> Mn> Zn> Cu> Ni> Co

Chlorophyta and Phaeophyta show similar trend for trace metal concentration whereas, Phaeophyta differs slightly. The difference observed in the trace metal concentrations of marine macroalgae during this study can be attributed to a combination of factors, including environmental and metabolic influences. Seasonal changes in temperature, salinity, pH, wave activity and other environmental factors can affect the accumulation of metals by algae. Variations in metal concentrations in seawater throughout the seasons, as well as interactions between metals and other elements present in seawater, can also lead to changes in metal levels in algae over time. Metabolic factors such as seasonal variations in growth rates and differences in energy requirements for metal uptake also contribute to variations.

Bioaccumulation Factor (BAF), Pollution Load Index (PLI) and Metal Pollution load (MPL) values of trace metals in seaweeds of Anjuna, Goa are given in the Table 4.10.

The highest BAF from Chlorophyta, Phaeophyta and Rhodophyta was shown by *Acrosiphonia orentalis, Padina tetrastromatica* and *Gelidium pusiillum* respectively. The highest BAF values for Cu, Zn, Co, Ni, Fe and Mn are found for *Sargassum tenerrimum* (4.47), *Ulva lactuca* (2.09), *Acrosiphonia orentalis* (1), *Acrosiphonia orenta* (9.50), *Padina tetrastromatica* (923.78) and *Padina tetrastromatica* (232.24) respectively (Figure 4.10 a). The BAF in the three phyla was observed in the order:

Cu: Phaeophyta > Chlorophyta > Rhodophyta

Zn: Chlorophyta > Rhodophyta > Phaeophyta

Ni: Chlorophyta > Phaeophyta > Rhodophyta

Fe: Chlorophyta > Phaeophyta > Rhodophyta

Mn: Chlorophyta > Phaeophyta > Rhodophyta

The BAF of the trace metals in three phyla followed the order:

Chlorophyta: Fe> Mn> Ni> Cu> Zn> Co

Phaeophyta: Fe> Mn> Cu> Ni> Zn> Co

Rhodophyta: Fe> Mn> Ni> Cu> Zn> Co

The PLI for Chlorophyta ranged from 17.45 to 3.72. The PLI for Chlorophyta was highest for *Acrosiphonia orentalis* (17.45) and the lowest was for *Ulva lactuca* (3.72).

The PLI for Phaeophyta ranged from 1.44 to 19.74. The PLI for Phaeophyta was highest for *Padina tetrastromatica* and the lowest was for *Sargassum polycustum*.

The PLI for Phaeophyta ranged from 2.67 to 4.62. The PLI for Chlorophyta was highest for *Gelidium pusiillum* and the lowest was for *Chondracanthus acicularis*.

The PLI value in the three phyla were in the order: Chlorophyta > Phaeophyta > Rhodophyta

The PLI observed are all a below 50 indicating there is no drastic rectification measures required.

The MPI for Chlorophyta, Phaeophyta and Rhodophyta ranged from 48.71 to 259.54, 11.34 to 146.77 and 34.34 to 42.94 respectively. The highest MPI in Chlorophyta was found for *Acrosiphonia orentalis* (259.54) and for Phaeophyta the highest MPI was found for *Padina tetrastromatica* (146.77).

Table 4.10 – Bioaccumulation Factor, Pollution Load Index and Metal Pollution load values of trace metals in seaweeds of Anjuna, Goa

Species	Bioaccumulation Factor							PLI	MPI
	Cu	Zn	Co	Ni	Fe	Mn	Avg		
Chlorophyta									
Ulva lactuca	1.26	2.09	-	1.74	22.25	6.98	6.86	3.72	48.71
Chaetomorpha antennina	2.63	1.19	-	3.21	316.19	80.24	80.69	12.07	105.17
Acrosiphonia orentalis	2.30	1.08	1	9.50	581.18	117.28	118.72	17.45	259.54
Culerpa peltata	0.68	0.85	-	3.53	254.29	6.13	53.09	5.03	72.38
Average	**1.96**	**1.25**	**1**	**5.12**	**345.21**	**70.00**	**84.71**	**12.50**	**171.26**
Phaeophyta									
Padina tetrastromatica	2.86	1.27	-	3.82	923.78	232.24	232.79	19.74	146.77
Dictyota bartayresiana	1.68	1.18	-	2.58	148.49	38.13	38.41	7.81	64.45
Sargassum linerifolium	2.85	0.60	-	1.31	16.15	4.88	5.16	2.82	33.59
Sargassum cinctum	3.90	0.62	-	0.86	19.92	5.99	6.26	3.02	32.39
Sargassum wightti	1.12	0.54	-	1.78	38.00	10.04	10.29	3.34	33.32
Sargassum tenerrimum	4.47	0.43	-	-	6.59	3.49	3.75	2.14	19.36
Sargassum polycustum	0.68	0.27	ND	-	9.52	3.52	3.49	1.44	11.34
Sargassum ilicifolium	2.72	0.65	ND	1.34	18.33	5.43	5.69	2.98	34.55
Average	**2.53**	**0.69**	**ND**	**1.95**	**301.93**	**37.96**	**69.01**	**8.31**	**77.36**
Rhodophyta									
Gelidium pusiillum	0.83	0.77	ND	1.03	110.94	28.33	28.38	4.62	42.94
Chondracanthus acicularis	1.23	1.31	ND	0.94	17.08	5.22	5.16	2.67	34.34
Average	**0.93**	**0.91**	**ND**	**1.014**	**87.47**	**22.56**	**22.57**	**4.42**	**42.905**
Average	2.04	0.92	1	2.65	189.25	39.91	39.29	8.23	

All species studied showed capacity to accumulate trace metals. Given the significant role of algal species as primary producers in coastal waters, their ability to concentrate metals should not be underestimated. As primary producers are consumed by fish and invertebrates, they transfer their metal content up the food chain. *Acrosiphonia orentalis* and *Padina tetrastromatica* have high BAF, PLI and MPI showing great potential for being used for biomonitoring the pollution status of Anjuna Goa. Although the trace metal accumulation in seaweeds along the Anjuna, Goa is not currently alarming, it warrants further investigation.

Table 4.11- ANOVA results showing variation of metals (Cu, Zn, Ni, Co, Fe and Mn) in seaweeds, ambient seawater, months and BAF

	Parameter	Source of Variation	F	P-value	F crit
Ambient seawater	Metals	Between months	0.7432	0.600777	2.740058
		Between metals	4.352525	0.008276	2.740058
Seaweeds	Cu	Between species	0.463745	0.915824	2.397254
		Between months	1.108429	0.394433	2.852409
Seaweeds	Zn	Between species	2.137686	0.075756	2.397254
		Between months	2.736015	0.056902	2.852409
Seaweeds	Ni	Between species	9.053694	0.000134	2.565497
		Between months	3.029758	0.046482	2.958249
Seaweeds	Fe	Between species	0.793001	0.659511	2.397254
		Between months	1.048748	0.423671	2.852409
Seaweeds	Mn	Between species	4.424128	0.003099	2.397254
		Between months	3.298231	0.030914	2.852409
BAF	Metals	Between species	1.444248	0.172962	1.921429
		Between metals	4.393072	0.002156	2.400409
Phylum	Metals	Between phyla	0.951628	0.425841	4.45897
		Between metals	6.940363	0.008717	3.687499
BAF (Phylum)	Metals	Between phyla	0.979773	0.416295	4.45897
		Between metals	6.943652	0.008705	3.687499
Chlorophyta	Metals	Between species	1.059203	0.402508	3.490295
		Between metals	4.744083	0.01268	3.105875
Phaeophyta	Metals	Between species	1.533361	0.200087	2.388314
		Between metals	1.53415	0.221581	2.742594
Rhodophyta	Metals	Between species	1.622809	0.271686	7.708647
		Between metals	1.790436	0.293233	6.388233

4.3 ANOVA

Two-way ANOVA without replication was performed to understand the variability among the trace metals, months, species, phylum and BAF. Table 4.10 shows the results obtained through the statistical test.

For ambient seawater: The p-value of 0.601 (Between months) suggests that the variation of metal concentration among months is not statistically significant since the p-value is greater than the typical significance level of 0.05. The p-value of 0.008 indicates that the variation between the analysed metal concentration is statistically significant since the p-value is less than 0.05. While there isn't a significant difference among the months, there is a significant difference among the concentrations of different trace metals.

For Cu in seaweed species: The p-value of 0.916 (Between species) suggests that the variation of Cu concentration among species is not statistically significant since the p-value is greater than the typical significance level of 0.05. The p-value of 0.394 (Between months) indicates that the variation of Cu concentration among months is also not statistically significant since the p-value is greater than 0.05. **Agarwal et al. (2022)** also observed no significant variation of Cu between seasons at lower Gangetic Delta Complex

For Zinc in seaweed species: The p-value of 0.076 (Between species) suggests that the variation of Zn concentration among species is not statistically significant since the p-value is greater than the typical significance level of 0.05. The p-value of 0.057 (Between months) indicates that the Zn concentration variation among months is not statistically significant since the p-value is greater than 0.05. The variation of accumulated Zn concentration among species and the variation among months does not appear to be statistically significant at the conventional significance level of 0.05. However, the p-values are close to this threshold, suggesting that there may be some trends worth further investigation. **Agarwal et al. (2022)** found significant spatial-seasonal variation of bioaccumulated Zn in *E. compressa.*

For Nickel in seaweed species: The p-value of 0.000134 (Between species) suggests that the Ni concentration variation among species is statistically significant since the p-value is less than the typical significance level of 0.05. Indicating some species accumulate significantly higher concentration of Ni than others. The p-value of 0.0465 (Between months) indicates that the variation among months is statistically significant since the p-value is less than 0.05. There is also a significant difference in the nickel concentration among different months.

For Iron in seaweed species: The p-value 0.6595 (Between species) is greater than the significance level 0.05 indicating that there is no significant difference among the mean Fe concentration of different species. The p-value 0.4237 (Between months) is also greater than the significance level, suggesting no significant difference among the mean Fe concentration of different months.

For Manganese in seaweed species: The p-value 0.0031 (Between species) is less than the significance level 0.05, indicating that there is a significant difference among the means of Mn concentration of different species. The p-value 0.0309 (Between months) is also less than the significance level, suggesting a significant difference among the means of Mn concentration of different months. The obtained p-values suggest there is a significant difference in means among different species and months.

For BAF values in seaweed species: The p-value 0.172 (Between species) being greater than 0.05 indicates there's no significant difference in the mean BAF values among different species of seaweeds. The p-value 0.002 (Between metals) is less than 0.05. Thus, there's a significant difference in the mean BAF values among different trace metals. Indicating some species are better accumulators for certain trace metals than others.

For metal concentration in phyla: The p-value 0.425 (Between phyla) being greater than 0.05 indicates there's no significant difference in the mean accumulated metal concentrations values among different phyla of seaweeds. The p-value 0.008 (Between metals) is less than 0.05. Thus, there's a significant difference in the mean values of different trace metals accumulated.

For BAF values in phyla: The p-value 0.416295 (Between phyla) is greater than the significance level. Therefore, there is no significant difference between the means of BAF for the phyla.

The p-value 0.008705 (Between metals) is less than the significance level. Hence, there is a significant difference between the means of BAF for the trace metals accumulated by the different phyla.

For metal concentration in Chlorophyta: The p-value 0.402 (Between species) is greater than 0.05. Therefore, there's no significant difference in the mean metal values among different species of Chlorophyta. The p-value 0.013 (Between metals) is less than 0.05. Thus, there's a significant difference in the average concentrations of different trace metals accumulated in the Chlorophyta.

For metal concentration in Phaeophyta: The associated p-value (Between species) 0.200 is greater than 0.05. Therefore, there's no significant difference in the mean metal concentration values among different species of Phaeophyta. The p-value 0.222 (Between metals) is greater than 0.05. Thus, there's no significant difference in the mean average concentrations of different accumulated trace metals.

For metal concentration in Rhodophyta: The p-value 0.27 (Between species) is greater than the typical significance level of 0.05 indicates there's no significant difference in the mean metal concentration values among different species of Rhodophyta. Similarly, the p-value (Between metals) of 0.2932, indicates that there's no statistically significant difference among the mean average concentrations of different accumulated trace metals.

CHAPTER 5

SUMMARY AND CONCLUSION

The study was conducted to understand the trace metal distribution among seaweeds at Anjuna, Goa- west coast of India. Hydrochemical parameters, trace metal concentration in seawater and seaweeds were analysed with Atomic Absorption Spectrophotometer. The obtained results were further processed to derive BAF, MPI, PLI and to perform ANOVA.

The mean trace metal concentration in seawater was in the order: Fe> Zn> Cu> Ni> Mn> Co.

The trace metal analysis in seaweeds revealed high Cu concentrations in Phaeophyta compared to other two phyla. Chlorophyta showed the highest concentration of Zn, Ni, Fe and Mn. The Co concentration was only detected in *Acrosiphonia orentalis* (Chlorophyta).

Based on the mean concentration of trace metals the distribution of trace metals in the three phyla of macroalgae are mentioned below:

Cu: Phaeophyta> Chlorophyta> Rhodophyta;

Zn: Chlorophyta> Rhodophyta> Phaeophyta;

Ni: Chlorophyta> Phaeophyta> Rhodophyta;

Fe: Chlorophyta> Phaeophyta> Rhodophyta

Mn: Chlorophyta> Rhodophyta> Phaeophyta.

Chlorophyta (Fe> Mn> Zn> Cu> Ni> Co) and Phaeophyta (Fe> Mn> Zn> Cu> Ni> Co) show similar trend for trace metal concentration whereas, Phaeophyta (Fe> Mn> Zn> Cu> Ni> Co) differs slightly.

Chlorophyta species showed the highest BAF followed by Phaeophyta and Rhodophyta. The highest BAF from Chlorophyta, Phaeophyta and Rhodophyta was shown by *Acrosiphonia orentalis, Padina tetrastromatica* and *Gelidium pusiillum* respectively.

The PLI value in the three phyla were in the order: Chlorophyta> Phaeophyta> Rhodophyta. The highest MPI in Chlorophyta was found for *Acrosiphonia orentalis* and for Phaeophyta the highest MPI was found for *Padina tetrastromatica.* The high BAF, MPI and PLI shown by *Acrosiphonia orentalis* and *Padina tetrastromatica*, makes them ideal for bio-monitoring the pollution status of Anjuna, Goa- west coast of India.

ANOVA results demonstrated that the variation between the analysed metal concentration in seawater is statistically significant. Cu, Zn, Fe concentrations showed no statistically significant variability among seaweed species; whereas, Ni and Mn concentrations showed variability. BAF variability among metals were significant indicating different species are better accumulator of one metal than other metals. The mean metal accumulation between the three phyla showed variability. There was variability seen among metal concentration accumulated by the different species of Chlorophyta but the same was not observed for Phaeophyta and Rhodophyta.

REFERENCES

1. Abdallah, A. M. A., Abdallah, M. A., & Beltagy, A. I. (2005). Contents of heavy metals in marine seaweeds from the Egyptian coast of the Red Sea. *Chemistry and Ecology*, *21*(5), 399-411.
2. Agarwal, S., Albeshr, M. F., Mahboobb, S., Atique, U., Pramanick, P., & Mitra, A. (2022). Bioaccumulation Factor (BAF) of heavy metals in green seaweed to assess the phytoremediation potential. *Journal of King Saud University-Science*, *34*(5), 102078.
3. Akcali, I., & Kucuksezgin, F. (2011). A biomonitoring study: heavy metals in macroalgae from eastern Aegean coastal areas. *Marine pollution bulletin*, *62*(3), 637-645.
4. Allen, S.E., Grimshaw, H.M., Parkinson, J. A., Quarmby, C. and Roberts, J.D. 1976.Chemical Analysis. In: Methods in plant Ecology, edited by S.B. Champman, Blackwell Scientific Publications, Oxford, Chapter 8, 411-466.
5. Alloway, B. J. and Ayres, D. C. (1993). In: "Chemical Principles of Environmental Pollution", Eds. Blackie Academic and Professional, an imprint of Chapman and Hall, Glasgow. 109-164.
6. Al-Shwafi, N. A., & Rushdi, A. I. (2008). Heavy metal concentrations in marine green, brown and red seaweeds from coastal waters of Yemen, the Gulf of Aden. *Environmental Geology*, *55*, 653-660.
7. Anbazhagan, V., Partheeban, E. C., Arumugam, G., Arumugam, A., Rajendran, R., Paray, B. A., ... & Al-Mfarij, A. R. (2021). Health risk assessment and bioaccumulation of metals in brown and red seaweeds collected from a tropical marine biosphere reserve. *Marine Pollution Bulletin*, *164*, 112029.

8. APHA, 1989. Standard Methods for the Examination of Water and Wastewater, Part 3, Determination of Metals. *American Public Health Association*, Washington DC, p. 164.
9. Balasubramanian, T. (2012). Heavy metal contamination and risk assessment in the marine environment of Arabian Sea, along the southwest coast of India. *American Journal of Chemistry*, *2*(4), 191-208.
10. Besada, V., Andrade, J. M., Schultze, F., & González, J. J. (2009). Heavy metals in edible seaweeds commercialised for human consumption. *Journal of Marine Systems*, *75*(1-2), 305-313.
11. Black, W. A. P. and Mitchell, R. L. 1952. Trace elements
12. Boutahar, L., Espinosa, F., Sempere-Valverde, J., Selfati, M., & Bazairi, H. (2021). Trace element bioaccumulation in the seagrass Cymodocea nodosa from a polluted coastal lagoon: Biomonitoring implications. *Marine Pollution Bulletin*, *166*, 112209.
13. Brooks, R. R., Presley, B. J., & Kaplan, I. R. (1967). APDC-MIBK extraction system for the determination of trace elements in saline waters by atomic-absorption spectrophotometry. *Talanta*, *14*(7), 809-816.
14. Bruland, K. W. and Franks, R. P. 1983. InT race Metals in Seawater" Edited by S.Wong (Plenum Press, New York) 414.
15. Bryan, G. W. (1969). The absorption of zinc and other metals by the brown seaweed Laminaria digitata. *Journal of the Marine Biological Association of the United Kingdom*, *49*(1), 225-243.
16. Caliceti, M., Argese, E., Sfriso, A., & Pavoni, B. (2002). Heavy metal contamination in the seaweeds of the Venice lagoon. *Chemosphere*, *47*(4), 443-454.

17. Chakraborty, S., & Owens, G. (2014). Metal distributions in seawater, sediment and marine benthic macroalgae from the South Australian coastline. *International Journal of Environmental Science and Technology*, *11*, 1259-1270.

18. Chakraborty, S., Bhattacharya, T., Singh, G., & Maity, J. P. (2014). Benthic macroalgae as biological indicators of heavy metal pollution in the marine environments: A biomonitoring approach for pollution assessment. *Ecotoxicology and environmental safety*, *100*, 61-68.

19. Chapman, V, J. 1950. Seaweeds and their uses. Methuen, London. Edwards, Peter. 1972. Benthic algae in the polluted estuaries. Mär. Poll. Bull 3: 55-60.

20. Chaudhuri A, Mitra M, Havrilla C, Waguespack Y, Schwarz J (2007) Heavy metal biomonitoring by seaweeds on the Delmarva Peninsula, east coast of the USA. Bot Mar 50:151–158

21. Conti, M. E., & Cecchetti, G. (2003). A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. *Environmental research*, *93*(1), 99-112.

22. Davis, T. A., Volesky, B., & Vieira, R. H. S. F. (2000). Sargassum seaweed as biosorbent for heavy metals. *Water research*, *34*(17), 4270-4278.

23. Filippini, M., Baldisserotto, A., Menotta, S., Fedrizzi, G., Rubini, S., Gigliotti, D., ... & Vertuani, S. (2021). Heavy metals and potential risks in edible seaweed on the market in Italy. *Chemosphere*, *263*, 127983.

24. Förstner, U., Wittmann, G. T., & Prosi, F. (1981). Heavy metals in aquatic organisms. *Metal pollution in the aquatic environment*, 271-323.

25. Ganesan, M., Kannan, R., Rajendran, K., Govindasamy, C., Sampathkumar, P., & Kannan, L. (1991). Trace metals distribution in seaweeds of the Gulf of Mannar, Bay of Bengal. *Marine pollution bulletin*, *22*(4), 205-207.

26. Hall, J. Á. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of experimental botany*, *53*(366), 1-11.

27. Kalesh, N. S., & Nair, S. M. (2005). The accumulation levels of heavy metals (Ni, Cr, Sr, & Ag) in marine algae from southwest coast of India. *Toxicological & Environmental Chemistry*, *87*(2), 135-146.

28. Karthick, P., Sankar, R. S., Kaviarasan, T., & Mohanraju, R. (2012). Ecological implications of trace metals in seaweeds: Bio-indication potential for metal contamination in Wandoor, South Andaman Island. *The Egyptian Journal of aquatic research*, *38*(4), 227-231.

29. Khaled, A., Hessein, A., Abdel-Halim, A. M., & Morsy, F. M. (2014). Distribution of heavy metals in seaweeds collected along Marsa-Matrouh beaches, Egyptian Mediterranean Sea. *The Egyptian Journal of Aquatic Research*, *40*(4), 363-371.

30. Khan, N., Ryu, K. Y., Choi, J. Y., Nho, E. Y., Habte, G., Choi, H., ... & Kim, K. S. (2015). Determination of toxic heavy metals and speciation of arsenic in seaweeds from South Korea. *Food chemistry*, *169*, 464-470.

31. Kumar, S. B., Padhi, R. K., Mohanty, A. K., & Satpathy, K. K. (2020). Distribution and ecological-and health-risk assessment of heavy metals in the seawater of the southeast coast of India. *Marine Pollution Bulletin*, *161*, 111712.

32. Lallu, K. R., John, S., Muraleedharan, K. R., Gireeshkumar, T. R., Udayakrishnan, P. B., Mathew, D., ... & Balachandran, K. K. (2023). Input-

export fluxes of heavy metals in the Cochin estuary, southwest coast of India. *Environmental Science and Pollution Research*, *30*(2), 2771-2786.

33. Martin, J. M., & Whitfield, M. (1983). The significance of the river input of chemical elements to the ocean. *Trace metals in sea water*, 265-296.
34. Monteiro, C. M., Fonseca, S. C., Castro, P. M., & Malcata, F. X. (2011). Toxicity of cadmium and zinc on two microalgae, Scenedesmus obliquus and Desmodesmus pleiomorphus, from Northern Portugal. *Journal of Applied Phycology*, *23*, 97-103
35. Murphy, V., Hughes, H., & McLoughlin, P. (2007). Cu (II) binding by dried biomass of red, green and brown macroalgae. *Water resarch*, *41*(4), 731-740.
36. Pan, Y., Wernberg, T., de Bettignies, T., Holmer, M., Li, K., Wu, J., ... & Xiao, X. (2018). Screening of seaweeds in the East China Sea as potential bio-monitors of heavy metals. *Environmental Science and Pollution Research*, *25*, 16640-16651.
37. Paul, A. C., & Pillai, K. C. (1983). Trace metals in a tropical river environment-distribution. *Water, Air, and Soil Pollution*, *19*, 63-73.
38. Phillips, D. J. (1977). The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments—a review. *Environmental Pollution (1970)*, *13*(4), 281-317.
39. Poppy, M. V. C. and Phoebe, M. M. 2003. Distribution of trace metals in red algae, seawater and sediments of Tuticorim coast. Seaweeds Res. Util, 24 (1&2): 63-68.
40. Rainbow, P. S. (1995). Biomonitoring of heavy metal availability in the marine environment. *Marine pollution bulletin*, *31*(4-12), 183-192.

41. Rajendran, K., Sampathkumar, P., Govindasamy, C., Ganesan, M., Kannan, R., & Kannan, L. (1993). Levels of trace metals (Mn, Fe, Cu and Zn) in some Indian seaweeds. *Marine pollution bulletin*, *26*(5), 283-285
42. Rubio, C., Napoleone, G., Luis-González, G., Gutiérrez, A. J., González-Weller, D., Hardisson, A., & Revert, C. (2017). Metals in edible seaweed. *Chemosphere*, *173*, 572-579.
43. Ryan, S., McLoughlin, P., & O'Donovan, O. (2012). A comprehensive study of metal distribution in three main classes of seaweed. *Environmental pollution*, *167*, 171-177.
44. Sánchez-Rodrıguez, I., Huerta-Diaz, M. A., Choumiline, E., Holguın-Quinones, O., & Zertuche-González, J. A. (2001). Elemental concentrations in different species of seaweeds from Loreto Bay, Baja California Sur, Mexico: implications for the geochemical control of metals in algal tissue. *Environmental Pollution*, *114*(2), 145-160.
45. Sawidis, T., Brown, M. T., Zachariadis, G., & Sratis, I. (2001). Trace metal concentrations in marine macroalgae from different biotopes in the Aegean Sea. *Environment International*, *27*(1), 43-47.
46. Sharma, P., & Dubey, R. S. (2005). Lead toxicity in plants. *Brazilian journal of plant physiology*, *17*, 35-52.
47. Singh, K. K., Matta, V. M., Sharma, B. M., & Usha, K. (2008). Distribution of some trace metals in the mandovi estuary of Goa, west coast of India.
48. Smith, J. L., Summers, G., & Wong, R. (2010). Nutrient and heavy metal content of edible seaweeds in New Zealand. *New Zealand Journal of Crop and Horticultural Science*, *38*(1), 19-28.

49. Sudharsan, S., Seedevi, P., Ramasamy, P., Subhapradha, N., Vairamani, S., & Shanmugam, A. (2012). Heavy metal accumulation in seaweeds and sea grasses along southeast coast of India. *Journal of Chemical and Pharmaceutical Research*, *4*(9), 4240-4244.

50. Tomlinson, D. L., Wilson, J. G., Harris, C. R., & Jeffrey, D. W. (1980). Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. *Helgoländer meeresuntersuchungen*, *33*, 566-575.

51. Tresnati, J., Yasir, I., Aprianto, R., & Tuwo, A. (2021, May). Metal bioaccumulation potential of the seaweed Kappaphycus alvarezii. In *IOP Conference Series: Earth and Environmental Science* (Vol. 763, No. 1, p. 012059). IOP Publishing.

52. Tsukaijan, K. and Young, D. R. 1978. Determination of microgram amounts of some transition metals in seawater by methyl isobutyl ketone-nitric acid absorption spectrophotometry. Anal. Chem. 50 : 1250 – 1253.

53. Usero, J., Morillo,J.,&Gracia,I.(2005). Heavy metal contamination in molluscs from the Atlantic coastof southern Spain. Cheamosphere, 59, 1175-1181

54. Van Netten, C., Cann, S. H., Morley, D. R., & Van Netten, J. P. (2000). Elemental and radioactive analysis of commercially available seaweed. *Science of the Total Environment*, *255*(1-3), 169-175.

55. Wilson, S. (2002). Nutritional value of detritus and algae in blenny territories on the Great Barrier Reef. *Journal of Experimental Marine Biology and Ecology*, *271*(2), 155-169

56. Zauke, G. P., Ritterhoff, J., & Rinderhagen, M. (1998). Concepts and applications in aquatic biomonitoring–internal review paper. *Aquatic Ecology Group, ICBM, CvO Universitat, Oldenburg, Germany*, 38.