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STUDY OF HEAVY METAL CONCENTRATION OF FOUR *CHEILANTHES* SPECIES AND SOIL SUBSTRATUM FROM NORTHERN WESTERN GHATS OF INDIA

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ABSTRACT: Northern Western Ghats of India is natural gift of biodiversity in the forests. The varieties of genes, species, and ecosystems, such as ferns, are rich and naturally plentiful. Therefore, specific fern species have a noble possibility for using in phytoremediation process. The aims of this research were to determine the accumulation of heavy metals of some fern species and to study the ecological effects on heavy metals absorption as plant substratum in soil. The study area was conducted at Northern Western Ghats of India. The soil substratum and fern samples were taken in July 2014 utilizing line transect method along the pathway as well as applied square plots size of 1×1 m 2 for the sampling method. Heavy metal accumulation in ferns was calculated as well as soil properties and element concentrations were analysed. It was detected that the ferns taken higher potentials of absorbing heavy metals than in the soil. From 4 *Cheilanthes* species, some fern species had high heavy metal accumulation due to the ecological effect. *C.farinosa.* showed significantly highest levels of Pb and Ni concentration in their leaves, while *C.anceps,* was the best Pb, Ni and Co absorption the outcome from this study can play an significant role in bioremediation process to improve concentration of heavy metals from the environment.

Keywords: Fern, Heavy metal, *Cheilanthes* species, Northern Western Ghats, phytoremediation.

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1.INTRODUCTION

Northern Western Ghats of India has a rich plant biodiversity for that reason it has good potential to provide appropriate species for phytoremediation which is a encouraging new method. Phytoremediation uses green plants to integrate or purify metals as well as organic compounds. The term was first invented in 1991 to define the practice of plants to accumulate metals from soil and groundwater [4]. The huge scale phytoremediation of metal pollutants from soils involve plant species that have high biomass, fast growing and accumulate metals. Plants that accumulate metals to high concentrations are sometimes called as "hyper accumulators" [14]. Hyperaccumulation of heavy metals or metalloids is a occasional phenomenon in terrestrial higher plants. About 400 taxa of hyper accumulator species have been identified [16]. The distribution of heavy metal in soils differs with the parent materials after which the soil is derived. Though, growing under exactly the same natural conditions all plant species demonstrated quite unique uptake characteristics [7]. Even though microorganisms have also been verified for remediation potential, plant has exposed the greater ability to bear up and accumulate high concentrations of toxic metals, chemical substances. An extensive range of plant species has been recognized as being arsenic resistant. Several researchers reported that ferns can extremely absorb toxic and carcinogenic substances, heavy metals, from contaminated soils, that released up the possibility for its practice for remediation of soils. Scientists in the USA discover the fern (*Pteris vittata*), identified as brake fern, can absorb arsenic from soils and translocate arsenic to its parts above the ground as well as leaves (fronds). This study revealed that other fern species which are hyperaccumulated and other heavy metals.

2. MATERIALS AND METHODS

Site characterization

The plant and soil substratum samples used in this study were collected from four *Cheilanthes* species were obtained from different localities of Northern Western Ghats of India (*Cheilanthes farinosa* (Forssk.) Kaulf. — Molem locality, GPS: 15^{0} $22^{l}09^{ll}$ N′,74^{0} 12^{l} 44^{ll} E; *Cheilanthes anceps Sw*, — Mahabaleshwar locality, GPS: 17^{0} 55^{l} 31^{ll} N, 73^{0} 39^{l} 45^{ll} E; *Cheilanthes tenuifolia (Burm.f.) Sw*, — Gaganbawada locality, GPS: 16^{0} 31^{1} 58^{ll} N′,73^{0} 49^{l} 5^{ll} E; *Cheilanthes albomarginata* Clarke — Amboli locality, GPS: 15^{0} 57^{l} 42^{ll} N, 73^{0} 59^{l} 48^{ll} E;). Specimens were authentically identified with help of Dr. Manisha Kale (Associate Professor Department of Botany, Jaysingpur College Jaysingpur, Maharashtra, India. The Whole Plant with rhizome of *Cheilanthes* sp. was collected from Northern Western Ghats, Maharashtra, India. The *Cheilanthes* species were cleaned and separated into dry powder form. The CSWPR was stored in a freezer (−20°C) until further analysis.

Soil substratum sampling and analysis

[A] Soil sampling and analysis

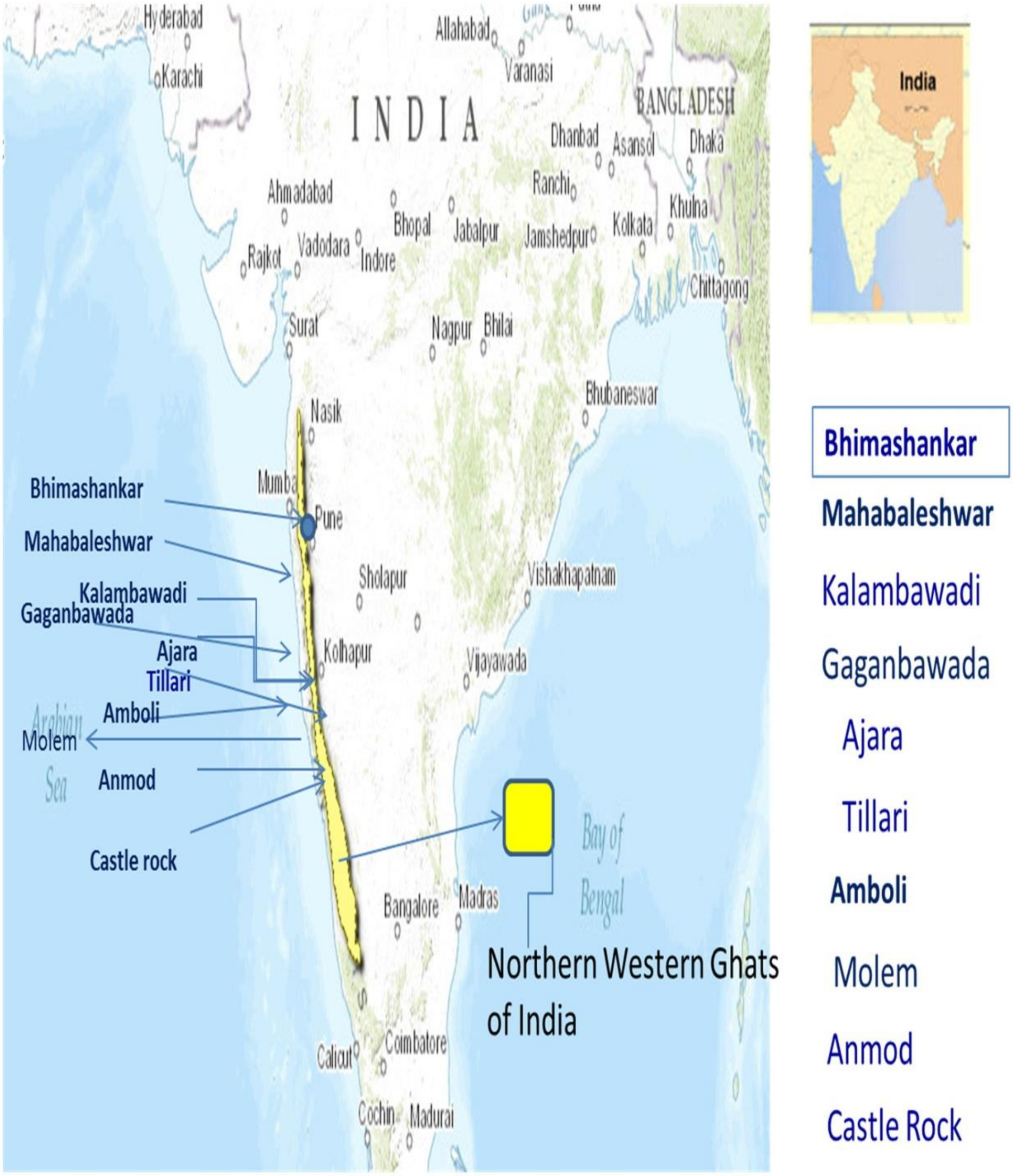
The study was carried out in the Four Cheilanthes plant species were collected in the surrounding area Northern Western Ghats of India. Samples of soil substratum were collected in July to Oct, 2012 as well as July to Oct 2014. The voucher specimens were deposited at Department of Botany, Shivaji University, Kolhapur Maharashtra, and Soil samples (0-15 cm, approx. 1000 g) was occupied by using line transects method along the pathway. The soil was air dried designed for a week and then it was sieved (2-mm mesh) to eliminate stones and plant materials. Subsequently that soil chemical properties such as soil pH, organic matter (%OM) and CEC: cation exchange capacity were measured. Determined soil pH was using glass electrode in a 1: 5 soil water ratio [9]. Organic matter was analysed conferring to the Walkley and Black method [5] as well as wet ashing with a K2Cr2O7-H2SO4 solution and titrated with FeSO4. CEC: CEC: Cation exchange capacity was analysed using ammonium saturation method [3]. Heavy metals such as Ni, Pb, and Co was determined using DTPA 0.005 M extracting solution with a soil: extractant ratio of 1:5 (Norvell, 1984). All metals were analysed using flame atomic absorption spectrophotometry (FAAS model GBC Avanta PM 05593).

[B] Plant sampling and analysis

Four Cheilanthes plant species and its soil substratum were collected in the surrounding area Northern Western Ghats of India from July to Oct, 2012 to July to Oct 2014. Some plant samples were kept in a plant press as well preserved by drying at 50-55 oC up to 4 days by making them into a dried up herbarium in order to further classify the fern types. Pteridophytes specimens were identified with the keys as well as descriptions from taxonomic literature, such as flora, monograph, manual, as well as research articles. Botanical names of every specimen were confirmed by comparison to the voucher specimens were deposited at Department of Botany, Shivaji University, Kolhapur Maharashtra. After collection, each one plant sample was washed with running tap water, dipped with deionized water in whole plant. The samples were dried at 70oC for 48 hours. The dried samples were then ground to a powder by mortar for purpose of heavy metal determination.

[C] Determination of heavy metal in whole plant with rhizome

Whole Plant samples were prepared for analysis with the digestion method [17, ; Simmons et al., 2004). A portion (~ 0.2 – 0.5 g) of the dried powder was precisely weighed (± 0.001g) directly to a flask as well as mixed with 15 ml of mixed acid (HNO3 and HClO4 , 80:20, v : v). The samples were digested at 120 oC on a hot dish. While digestion was finished, samples were removed and diluted to 50 ml with deionized water and filtrated. This solution was analyzed and designed for Lead (Pb), Cobalt (Co) and Nickel (Ni) by frame atomic absorption spectrophotometry (FAAS, GBC Avanta PM 05593). Acid blanks were also analyzed in order to probable contamination. The FAAS analyses were done at Department of Environment science, Shivaji University, Kolhapur. Heavy metal concentrations in the samples were reported on a dried mass basis.



Study Area Showing Different Localities

Calculation of TF

The ability of plants to tolerate and accumulate heavy metals is useful for phytoremediation purpose measured using translocation factor (TF) which is defined as the ratio of metal concentration in the shoots to the roots ([Metal] Shoot/ [Metal] Root) to show metal translocation properties from roots to shoots (Kabata-Pendias and Pendias 1984).

RESULTS AND DISCUSSION

Soil properties

Geology is another significant factor that impacts element concentrations in the soil. The soil properties are pH, %OM and CEC, all played a role for the observed metal concentrations in whole plant along with rhizomes. The total means of pH, %OM and CEC were calculated. In order to inspect the deviation of any specific species from total means, the critical intervals of student-t distribution were applied to examine the importance of deviation. Analytical outcomes are summarized in Table 1 as well as distribution profiles were drawn as shown in Figures 2, 3 and 4.

Table 1: Elevation and soil properties (pH, %OM and CEC) of some fern species.

Family	**Species**	**pH**	**OM (%)**	**CEC me/100g**
Pteridaceae	*Cheilanthes farinosa* (Forssk.) Kaulf	4.91 ± 0.10	4..93 ± 2.35	6.79 ± 1.93
Pteridaceae	*Cheilanthes anceps Sw*	5.91 ± 0.13	1.17 ± 0.23	5.26 ± 0.55
Pteridaceae	*Cheilanthes tenuifolia*	5.68 ± 0.38	2.61 ± 1.21	7.67 ± 3.11
Pteridaceae	*Cheilanthes albomarginata* Clarke	5.46 ± 0.43	2.45 ± 1.08	8.32 ± 0.83
Values of pH OM and CEC are reported in means ± SD				

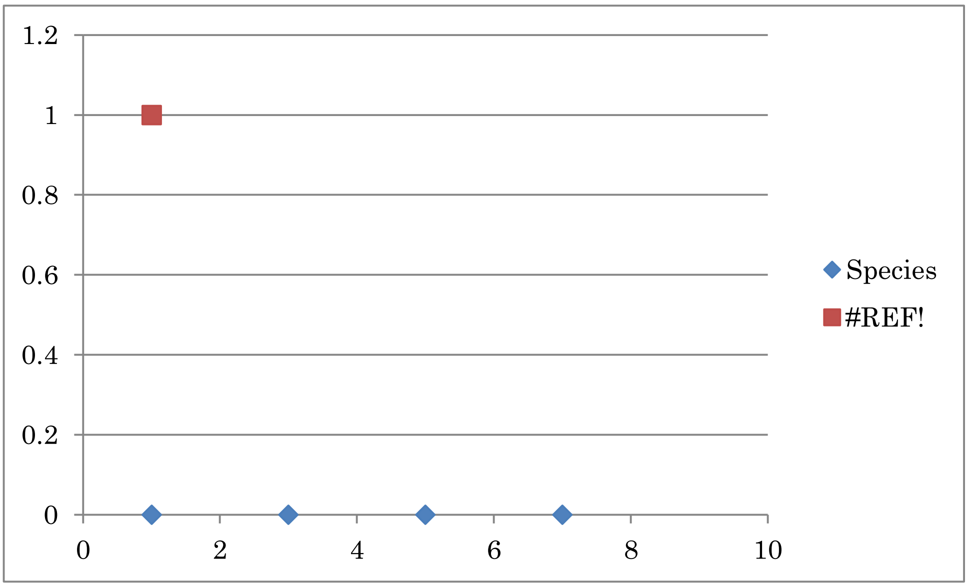


Figure 2: Specific distribution of pH in soil. Solid dots indicates the mean value of four *Cheilanthes* Species and dot line shows the significant range with a critical value of α = 0.01%

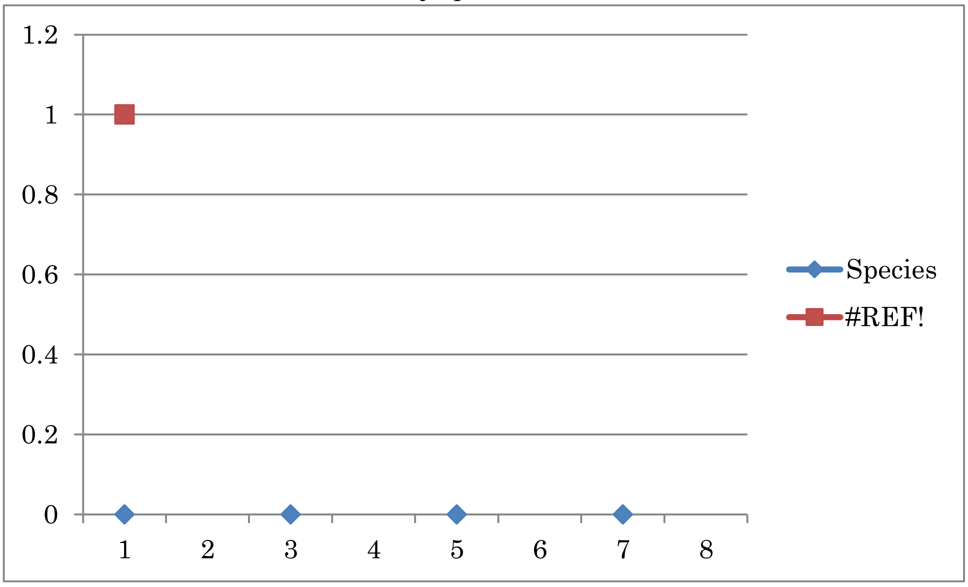


Figure 3: Specific distribution of OM in soil. Solid line indicates the mean value of four Cheilanthes Species and dot line shows the significant range with a critical value of α = 0.01%

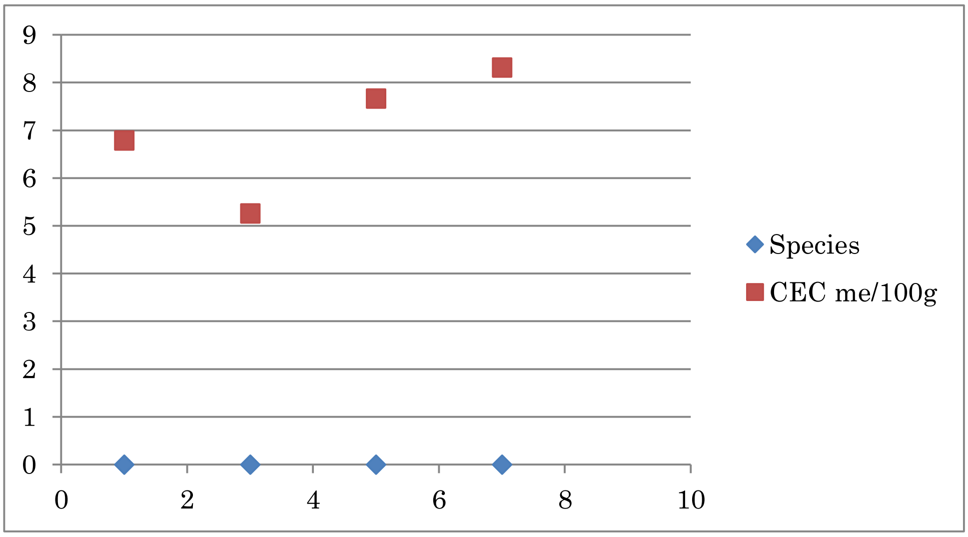


Figure 4: Specific distribution of CEC in soil. Solid line indicates the mean value of all *Cheilanthes* species and dot line shows the significant range with a critical value of α = 0.01%

pH

All soil substratum contain acidic values in the ranged of 4.91 – 5.91. *Cheilanthes farinosa* (Forssk.) Kaulf were found lowest pH value in (Table 1). *Cheilanthes anceps Sw*. was found the highest value of pH as shown in Table 1. *Cheilanthes tenuifolia* and *Cheilanthes albomarginata* Clarke showed expressively highest levels of pH value in their soil showed significantly highest levels of pH value in their soil at $p \leq 0.05$ (Figure 1).

OM

The organic matter are in the ranged of 1.17 – 4.93. *Cheilanthes farinosa* (Forssk.) Kaulf was found the highest organic matter content in Table 1 and it showed pointedly highest organic matter content in their soil at $p \leq 0.05$ (Figure 2). Cheilanthes anceps Sw. was found the lowest of organic matter content in Table 1. Though, the Cheilanthes genus such as *Cheilanthes tenuifolia*, and *Cheilanthes* *albomarginata* Clarke showed significantly maximum levels of organic content in their soil at $p \leq 0.05$.in figure 2.

CEC

The cation exchange capacity: CEC is in the ranged of 5.26 – 8.32 me/100g as shown in Table 1. The lowest of cation exchange capacity was found in *Cheilanthes anceps Sw* and it revealed significantly lowermost cation exchange capacity in their soil at $p \leq 0.05$ *Cheilanthes albomarginata* Clarke was found the greatest value of cation exchange capacity and it exposed significantly highest cation exchange capacity in their soil at $p \leq 0.05$ (Figure 3).

Heavy metal concentrations in soil substratum and whole plant with rhizome

Leaves are the physiologically active organs of a plant converting the inorganic matters into coordination compounds. Total samples of leaves were collected from terrestrial fern over 5 cm in height. Sampling ferns were identified as 19 species, 11 genera and 11 families. The overall means of the concentrations of metal (Pb, Ni and Co) were calculated. The means of particular species for the leaves were compared with the overall means in order to examine the deviation of any particular species. When the value of any particular species was found greatly deviated from the mean, the critical intervals of T-test distribution and DMRT were applied to examine the significance of deviation. When the concentration of certain elements was significantly high in certain species, the species was regarded as accumulator of the element [8]. The mean of the concentration for heavy metals as Pb, Ni and Co in soil and leaves of some fern species are also given in Table 2. Some value showed the concentrations in leaves and soil along the transect reveals the high standard deviation, this reason displays by the location of the plot sampling was collected in different lithologies, typical of the greater area and different soil horizons along transect. Figure 6 is drawn by plotting the heavy metal concentration in leaves of different fern species.

Table No. 2 Heavy metal concentrations in soil substratum and whole plant with rhizome of *Cheilanthes* species

Name of Cheilanthes Genus	**Pb (mg/kg)**		**Ni (mg/kg)**		**Co (mg/kg)**	
	Soil Substratum	Whole Plant	Soil Substratum	Whole Plant	Soil Substratum	Whole Plant
C. farinosa	19.635 ± 2.42	28.370 ± 9.90	3.464 ± 1.65	9.550 ± 1.86	2.135 ± 0.44	ND
C.anceps	11.903 ± 2.91	18.966± 13.89	2.309 ± 1.13	11.440 ± 1.90	1.886 ± 0.05	1.682 ± 0.55
C. tenuifolia	8.818 ± 6.11	6.729 ± 3.68	2.583 ± 0.48	14.652 ± 11.46	2.233 ± 0.70	1.219 ± 0.13
C.albomarginata	11.628 ± 1.75	9.245 ± 1.46	1.989 ± 0.09	12.955 ± 3.42	1.545 ± 0.50	2.835 ± 0.41
ND : Not detected, lower limit of detection was 0.0044 ppm , Values are reported in means ± SD						

Pb

Lead concentrations in soil of all species showed a weak positive correlation with OM and CEC. *C. farinosa* revealed highest levels of Pb concentration in soil. The heavy metal concentration of four *cheilanthes* species discover that Pb has the concentration in leaves ranged from 6.729 – 28.370 mg/kg. The specific distribution profile of Pb concentration in leaves is given in Figure 3. *C.anceps, C. tenuifolia C.albomarginata revealed* significantly higher concentration than the mean value. The cheilanthes species revealed significantly lower Pb concentration in such species as *C. tenuifolias*. *C.anceps, C.albomarginata* showed significantly highest levels of Pb concentration in their whole plant (28.37 mg/kg) at $p \leq 0.01$.

Ni

Nickel concentrations in soil substratum of all species presented a weak negative correlation with OM. *C. farinosa* Sw. exposed highest levels of Ni concentration in soil substratum. Ni concentration in whole plant of cheilanthes species ranged from 9.550 – 14.652 mg/kg, *C.anceps, C. tenuifolia C. albomarginata*. Showed significantly maximum accumulated Ni. On the opposing *C. farinosa* revealed significantly lower concentration than the mean value in their whole plant at $p \leq 0.01$ as shown in Figure 3.

Co

Cobalt concentrations in soil substratum of all species displayed a weak positive correlation with OM and CEC. All cheilanthes species showed non significantly of Co concentration in soil. The concentration of Co whole plant of cheilanthes ranged from 1.68 – 2.83 mg/kg. The specific distribution summary of Co whole plant is demonstrated in Table 2. The cheilanthes species *C. farinosa* Sw., *C.anceps, C. tenuifolia* and *C. albomarginata* revealed significantly higher concentration than the mean value. *C. farinosa* Sw showed significantly highest accumulated Co at $p \leq 0.01$.

4. CONCLUSION

Our study has shown that Cheilanthes species species of medicinal plants, growing in different geographical locations, cumulates different levels of heavy metals. Cheilanthes species such as *C. farinosa* Sw., *C.anceps, C. tenuifolia* and *C. albomarginata* are terrestrial fern species, revealed that significantly highest levels of Pb and Ni concentration in whole plant and soil substratum , while *C. farinosa* Sw., was the best Pb, Ni and Co absorption. It was observed that the *C. farinosa* Sw., *C.anceps, C. tenuifolia* and *C. albomarginata* ferns had higher potential for heavy metals accumulation than in the soil. The uptake rate of metal is associated with organic matter content**.** Thus, medicinal plants for the invention of herbal medicines should be harvested from pollution-free natural habitat. Our findings further point out that the medicinal plants, used for pharmaceutical purposes, should be collected from areas not contaminated with heavy metals. For that reason, advised that the metal content in medicinal plants be checked for levels of heavy metals before their use for Traditional as well as pharmaceutical purposes.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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None

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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