INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 19, No. 6, p. 106-114, 2021
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

# Assessment of heavy metals concentrations in different parts of *Diplazium esculentum* in selected riverine system of Bunawan, Agusan del Sur, Philippines

**Vivian C Peligro***

*Agusan del Sur State College of Agriculture and Technology, Bunawan, Agusan del Sur, Mindanao, Philippines*

Article published on December 06, 2021

**Key words:** Assessment, Heavy metal, *D. esculentum*, Riverine system, Medicinal plant

**Abstract**

Heavy metals are essential for the proper functioning of the biological systems but their deficiency or excess could lead to several disorders. Thus, this study aimed to assess the levels of heavy metals (lead, chromium, cadmium) concentrations of *Diplazium esculentum* in the selected riverine system of Bunawan, Agusan del Sur. Three (3) sub-stations were established in each area. Selected physicochemical parameters were determined. Two (2) kilograms of plant samples were collected in each site separating the roots, stems, and fronds. Both water and plant samples were subjected to acid digestion and were analyzed using the MP-AES method. Results revealed that all the physicochemical properties were within the desirable limits except for the dissolved oxygen in station 1 (Agsao River). Heavy metals concentrations were compared to the standard value set by USEPA and WHO. Lead and chromium concentrations in water samples were within the standard limit. However, cadmium was below the detectible limit in both water and plant samples in all stations. Moreover, lead and chromium contents of *D. esculentum* in all parts across all stations were remarkably higher than tolerable limits. The highest accumulation was on its roots because it is responsible for the uptake of nutrients including pollutants. Hence, *D. esculentum* is a good Pb and Cr accumulator. Furthermore, constant monitoring and evaluation for the heavy metal's concentrations in the sampling stations both in water and plant samples are necessary. Likewise, the levels of heavy metal pollutants must be checked before consumption to ensure safe use of this plant.

***Corresponding Author:** Vivian C Peligro ✉ vpeligro@asscat.edu.ph

## Introduction

Water is considered essential for life on earth. It is the most important substance for human existence next to oxygen (Mahmoud *et al.,* 2001). Together with the land, they are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, because of anthropogenic activities, land and water were became severely polluted. Examples of these pollutions are the following namely: emission, effluents and solid discharge from industries, vehicle exhaustion, and metals from smelting and mining (point sources) and soluble salts, use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers (nonpoint sources) (McGrath *et al*., 2001; Nriagu and Pacyna, 1988; Schalscha and Ahumada, 1998; Lone *et al*., 2008). Each type of pollution causes different harmful effects not only to plants and animals but also to human (Garbisu and Alkorta, 2001; Gisbert *et al*., 2003; Lone *et al*., 2008). Many of these wastes are toxic and they find their way into the land, water, and air (Wold *et al*., 2016).

Although heavy metals are essential for the proper functioning of the biological systems their deficiency or excess could lead to several disorders (Prasannakumari *et al*., 2014). Furthermore, heavy metals in plants should be determined because prolonged human intake of toxic trace elements, even at low doses, results in organ malfunction and causes chronic toxicity. Hence, substantial information should be obtained from plants that grow on soils/waters containing high concentrations of heavy metals (Jasim *et al*., 2014). It is therefore is an issue of international distress at present (Prasannakumari *et al*., 2014).

According to World Health Organization (WHO), 80% of the world's population relies on herbal medicines, for their primary health care needs (WHO, 2007). The major merits of traditional or herbal medicine seem to be their perceived efficacy, low incidences of serious adverse effects, and comparatively low cost (Behera *et al*., 2016). However, environmental pollution especially with heavy metals poses a serious problem with the quality of medicinal plants and their products (Das, 2016).

In recent years, medicinal ferns play an important role in traditional medicines because of their increased use as a treatment for many human diseases and food (Rai *et al*., 2005; Jasim *et al*., 2014). The existence of heavy metals in some medicinal ferns such as cadmium, chromium, copper, nickel, and lead causes health risks. Plants function as important links that transfer metals from soil/water to humans (Rai *et al*., 2005). Moreover, medicinal plants may be contaminated during the growing and processing stages (Jasim *et al*., 2014). Further, ferns are a bioindicator of the presence of contaminated soils especially from mining activities (Kachenko *et al*., 2007).

*Diplazium esculentum*, (fig. 1) the vegetable fern, is an edible fern found throughout Asia and Oceania (Copeland, 1942). It is known as pucuk paku in Malaysia (Jasim *et al*., 2014) and pakô in the Philippines (Copeland, 1942). *D. esculentum* is a huge perennial fern associated with about 20 cm rhizome and covered with about 1 cm long rufous scales. It is a bipinnate plant with long brownish petioles. The base of its petiole black and protected with short scales. The frond can reach 1.5 cm in length, and the pinnae are about 8 cm long and 2 cm wide (Tanaka *et al*., 2007). The leaves of *D. esculentum* are traditionally used as poultice to treat malaria, jaundice, constipation, earache, fever, measles, and dermatitis; the rhizomes of this plant are used to cure hemoptysis and cough (Anderson *et al*., 1993). Hence, this study aimed to assess the levels of concentrations of heavy metals in water and different parts of *D. esculentum* in the selected riverine system of Bunawan, Agusan del Sur.

**Fig. 1.** Image of *Diplazium esculentum*.

## Material and methods

*Study Area*

Three (3) waterbodies of Bunawan, Agusan del Sur were used as sampling sites namely: Agsao River (station 1), Bulong-bulongan Falls (station 2), and Bunawan Brook (station 3). In every sampling site, three (3) sub-stations, 100-m-apart was used to gather samples for the different study parameters (Fig. 2). Some tributaries that contribute to the waterbody are coming from small- and large-scale gold mining areas in Agusan del Sur.

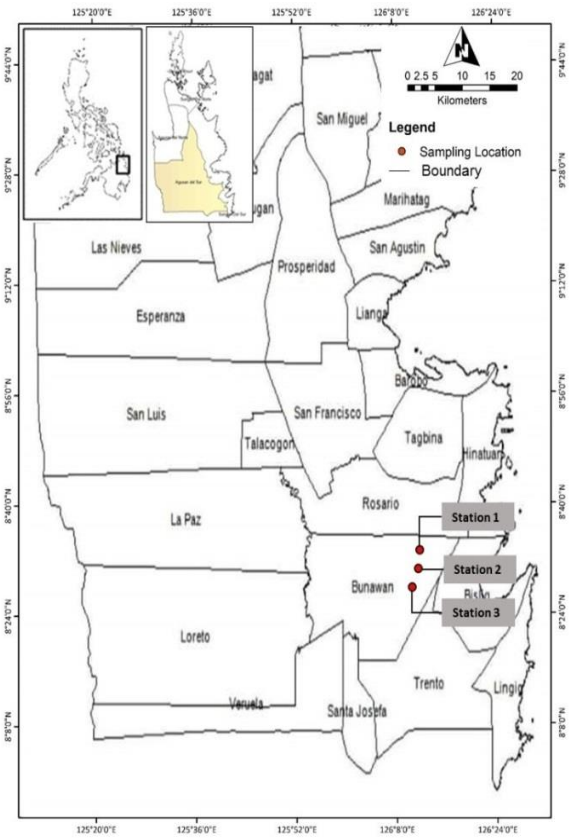


**Fig. 2.** Map showing the 3 sampling stations of the study.

*Water quality assessment*

There were triplicates at a 100-m distance within each of the stations in determining selected physicochemical parameters (Table 1). Dissolved oxygen, pH, Temperature, Total Dissolved Solids (TDS), and Conductivity were measured on-site using a Waterproof Cyberscan CD 650 multimeter kit (®Eutech Instruments) (Peligro and Jumawan 2015).

*Sample Collection*

*Water samples*

Three (3) water samples, one (1) L each were randomly collected over a distance of 100 m in each sampling site with glass bottle samplers, which were previously washed and rinsed with deionized water. Water samples were taken directly, with the bottle's mouth facing into the flow of the stream water. Bottles that already contained water samples were labeled according to the sampling station. The samples were then put into a cool box and taken to the laboratory for heavy metal analysis.

*Plant samples*

Two (2) kilograms of *D. esculentum* that grow on the shore in every study site were randomly collected. The whole plant was then washed by water and then packed into polyethylene bags and was brought to the laboratory. It was then carefully washed with distilled water, separating the roots, stem, and fronds that were cut into small pieces and then oven-dried at 80°C for 3 days to attain constant weight (Tiwari *et al.* 2011). The dried plant samples were then pulverized using porcelain mortar and pestle, sieved through a 2-mm nylon sieve and stored in a polyethylene bottle in a refrigerator.

**Table 1.** List of Physico-chemical parameters with their corresponding unit of measurement, method of analysis, sample volume needed, reference and instrument.

| Parameters | Unit | Method of Analysis | Sample Volume | Reference/Instrument |
|---|---|---|---|---|
| 1.Conductivity | µS | Direct method (electrode) | Determined on site | Portable Conductivity probeCond600 |
| 2.pH | Range 0-14 | Direct method (electrode) | Determined on site | Portable pH Meter, probepH600 |
| 3.Temperature Portable | °C | Direct method (electrode) | Determined on site | Conductivity probeCond600 |
| 4.Total Dissolved Solids (TDS) Portable | ppm | Direct method (electrode) | Determined on site | Conductivity probeCond600 |
| 5. Dissolved Oxygen (DO) | mg/L | Direct method (electrode) | Determined on site | Portable Meter, probeDO600 |

*Digestion of Test Samples*

A 1.0 g sample was weighed and put into a 500mL Erlenmeyer flask. It was then added with 30mL hydrochloric acid and 10mL nitric acid and digested until brown fumes ceases completely without boiling to dryness. When decomposition of organic matter was not completed, it was washed inside of beaker with a small quantity of $H_2O$, added a few drops of $HNO_3$, and heating was continued until the completion of decomposition. A 100mL of hot distilled water was added and boiled several times. It was then filtered to a 250mL volumetric flask, cooled, and diluted to mark with distilled water. The samples were then analyzed using the MP-AES method in determining the Lead (Pb), Cadmium (Cd), and Chromium (Cr) concentrations of the water and plant samples.

*Statistical treatment*

One-way ANOVA was used to compare the differences of physicochemical data and heavy metals concentrations between elevations with $p<0.05$ set as a significant value using Paleontological Statistics Software (PAST®).

**Results and discussions**

Table 2 and fig. 3 revealed that all the selected physicochemical properties of the three (3) study sites were within the desirable limit (Tepe *et al.*, 2005; Peligro and Jumawan, 2015) such as total dissolved solids (TDS), pH, temperature, salinity, and conductivity except the Dissolved Oxygen (DO) of Agsao River.

The high value of DO in Bulong-bulongan Falls and Bunawan Brook which were 8.423 and 6.15 respectively indicated a good water quality in the sites. Furthermore, it also indicated that the three (3) sampling stations have almost the same physicochemical characteristics (fig. 3) that they are 98% similar based on the Bray-Curtis similarity.

**Table 2.** Physicochemical assessment.

| Physicochemical | Stations | | |
|---|---|---|---|
| Parameters | AR | BF | BB |
| TDS (mg/L) | 94.8±0.251 | 116.633±0.273 | 97.733±0.352 |
| pH | 7.953±0.013 | 8.006±0.009 | 8.316±0.201 |
| Temperature | 27±0.05 | 27.82±0.72 | 27.08±0.079 |
| Salinity (mg/kg) | 0.09±0.002 | 0.112±0.002 | 0.093±0.033 |
| Conductivity (mS/cm) | 0.212±0.001 | 0.243±0.002 | 0.213±0.002 |
| DO (mg/L) | 3.81±0.20 | 8.423±0.092 | 6.15±0.18 |
| Standard Value | | | |
| DO pH | TDS | Salinity | Conductivity |
| > 5 mg/L 6.5-8.5 | <1000 mg/L | <0.5 mg/L | <1,500 uS/cm |

AR-Agsao River

BF-Bulong-bulongan Falls

BB-Bunawan Brook

The results of this study indicated that the current status of Agsao River, Bulong-bulongan Falls, and Bunawan Brook in terms of heavy metals concentration specifically Pb, and Cr under permissible limits (US EPA, 2008) and (WHO, 2008) (Table 3). Cadmium (Cd) concentrations were below the detectible limit (BDL). However, the present heavy metal concentrations of the three (3) sampling areas should not be taken for granted though their values were lower than the acceptable limits.

It could be elevated beyond the tolerable limits because of several pollutant sources such as animal faces, precipitation, vegetation, dust and dirt, agricultural activities, fertilizers, pesticides, detergent, and mining activities (Wold *et al.*, 2016). The decrease in heavy metal concentrations in water was due to heavy metal accumulation in sediments, aquatic plants and animals (Sriuttha *et al.*, 2016). Heavy metal contaminations such as Pb, Cr, and Cd were found from groundwater (Tantemsapya, 2011).

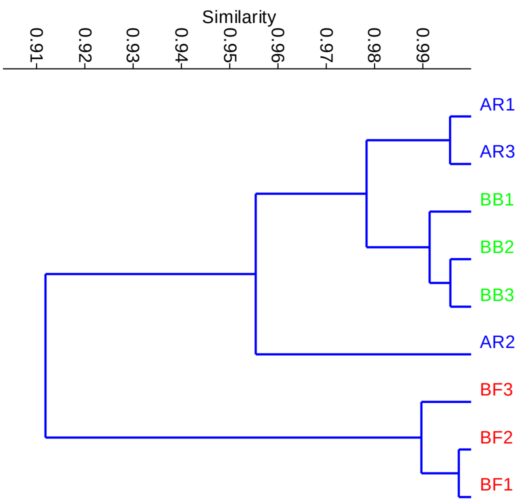


**Fig. 3.** Multivariate relationships between study stations based on their physicochemical features. A dendrogram of Bray-Curtis similarity of the study stations based on physicochemical features for Agsao River (blue), Bulong-bulongan Falls (red), and Bunawan Brook (green).

**Table 3.** Heavy metal concentrations (ppm) of water samples in three (3) sampling stations.

| Station | Pb | Cr | Cd |
|---|---|---|---|
| AR | 0.015±0.005 | BDL | BDL |
| BF | BDL | BDL | BDL |
| BB | 0.015±0.005 | BDL | BDL |
| Standard | 0.015*/0.01** | 0.1*/0.05** | 0.005*/0.003** |

* Environmental Protection Agency (US EPA 2008)

** World Health Organization (WHO 2008)

BDL- Below Detectible Limit, AR-Agsao River

BF-Bulong-bulongan Falls, BB-Bunawan Brook

**Table 4.** The mean of heavy metals concentrations (ppm) in various parts of *D. esculentum* in three (3) study sites.

| Stations | Plant Parts | Pb | Cr | Cd |
|---|---|---|---|---|
| AR | Roots | 30.5±0.5 | 35.5±0.5 | BDL |
| | Stem | 24±1 | 57±0 | BDL |
| | Fronds | 13.5±0.5 | 9.5±1.5 | BDL |
| BF | Roots | 21±1 | 22±0 | BDL |
| | Stem | 11±1 | 5.5±0.5 | BDL |
| | Fronds | 8.5±0.5 | BDL | BDL |
| BB | Roots | 18.5±0.5 | 25.5±0.5 | BDL |
| | Stem | 10±1 | 5±0 | BDL |
| | Fronds | 9.5±0.5 | BDL | BDL |
| Standard | | 10* | 2* | 0.3* |

* Based on the standard value set by the World Health Organization (WHO 2007) for the raw herbal plants.

BDL= Below Detectible Limit, AR-Agsao River

BF-Bulong-bulongan Falls, BB-Bunawan Brook

The present study assessed the three (3) heavy metals such as Lead (Pb), Chromium (Cr) and Cadmium (Cd) in the roots, stems, and fronds of *D. esculentum* in Agsao River, Bulong-bulongan Falls, and Bunawan Brook, Bunawan, Agusan del Sur (Table 4 and fig. 4).

In addition, lead (Pb) is the prominent heavy metal being detected in all parts of *D. esculentum* in all sampling sites (Table 4 and fig. 4A, 4B, and 4C). All its values fall beyond the permissible limit set by the World Health Organization (WHO 2007) which is 10ppm except for the fronds of Bulong-bulongan Falls which is 8.5ppm found to be below the limit.

It indicates further lead accumulation efficiency of the species (Prassannakumari *et al.*, 2014). Moreover, its high concentrations were found on the roots of *D. esculentum* which confirms the results of Jasim *et al.*, 2014 and Kabata-Pendias *et al.*, 2001. The order of lead concentrations on the plant were arranged as follows: roots>stems>fronds in all sampling areas. Lead is a stable and the most frequently occurring heavy metal in nature.

It is considered extremely dangerous for plants, animals, and micro-organisms (Behera and Bhattacharya, 2016) and a major chemical pollutant in the environment (Jasim *et al.*, 2014). Lead can cause anemia, headache, chronic nephritis, brain damage, convulsion, and central nervous system disorders when it is used for long-term above the tolerable limit (Klaassen, 2001; Tong, 2000). In plants, the high concentrations of Pb can induce oxidative stress by increasing the production of reactive oxygen species (Reddy *et al.*, 2005).

Moreover, analysis of all parts of *D. esculentum* exhibited high concentrations of Chromium (Cr) (Table 4 and fig. 4D,4E and 4F) in all study areas except on the fronds of Bulong-bulongan Falls, and Bunawan Brook which were below the detectible limit (BDL). Its values range from 5 ppm to 35.5 ppm which is far above the tolerable limit which is 2 ppm (WHO 2007). Their orders of concentration in all sampling sites were as follows: roots>stems>fronds.

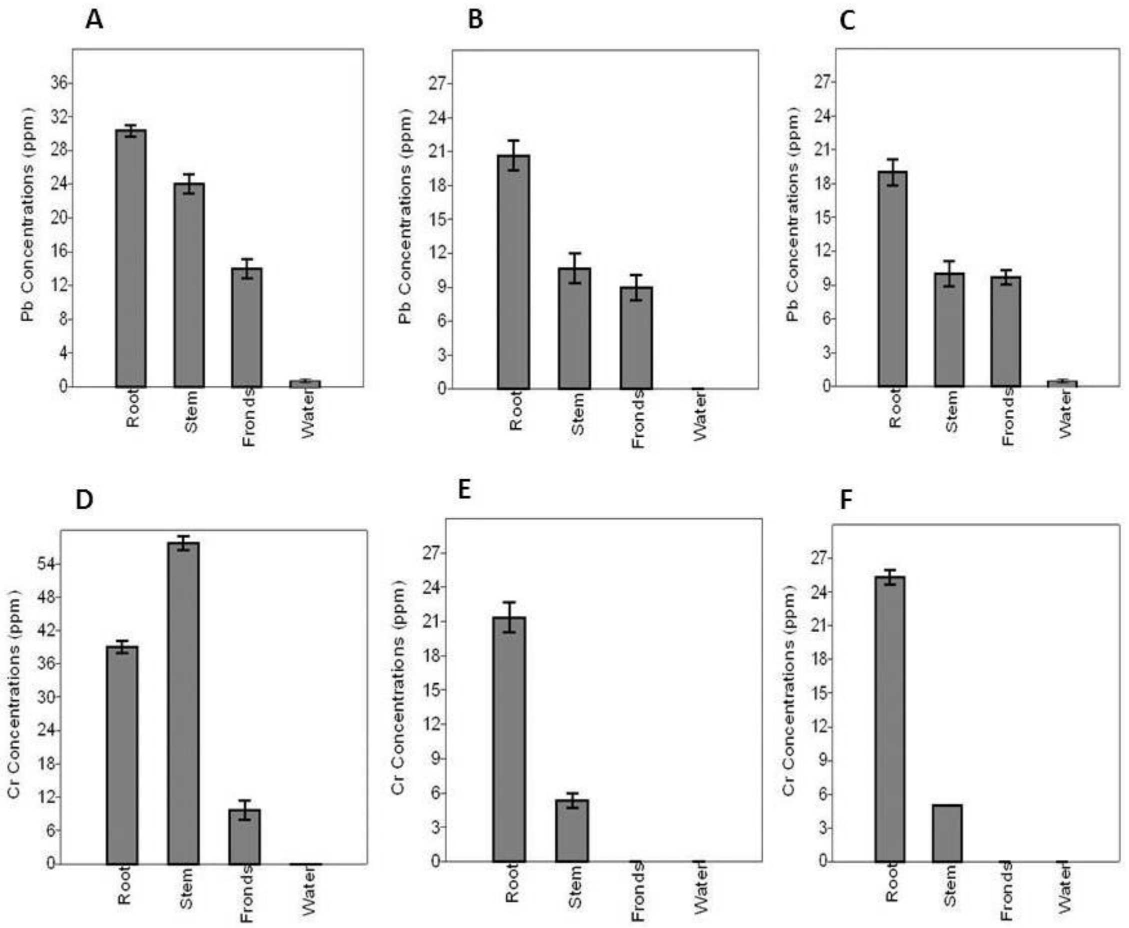


**Fig. 4.** Heavy metal concentrations in different parts of *D. esculentum* and water samples in three (3) sampling stations. A. Pb concentrations in Agsao River; B. Pb concentrations in Bulong-bulongan Falls; C. Pb concentrations in Bunawan Brook; D. Cr concentrations in Agsao River; E. Cr concentrations in Bulong-bulongan Falls; F. Cr concentrations in Bunawan Brook.

On the other hand, human bodies require chromium (Cr) in small amounts but when it is in large dose it is considered toxic already (Sriuttha *et al.*, 2016). Skin rash, nose irritations, bleeds, stomach upset, kidney and liver damage, and lung cancer are considered toxic effects of chromium. Disturbance in glucose lipids and protein metabolism are some of its deficiencies (Rai *et al.*, 2005; Shanker *et al.*, 2005). Carbohydrate, nucleic acid, and lipoprotein metabolisms were regulated by chromium as well as the insulin action was being potentiated by this metal (Yap *et al.*, 2010).

Another heavy metal that was tested in the present study was cadmium. It is a non-essential trace element with uncertain direct functions in both plants and humans and responsible for several cases of poisoning through food. Recently, its wide occurrence in water, soil, milk, dietary, and herbal medicinal products gaining more attention (Singh *et al.*, 2014). It can cause adverse changes in the arteries of the human kidney leading to kidney failure in small quantities. It replaces zinc biochemically and causes hypertension, liver and kidney damage when it accumulates in the human body. Itai-itai is a disease characterized by softening of bones, anemia, renal failure, and ultimately death caused by cadmium poisoning (Nordberg, 1999).

However, cadmium (Cd) concentrations in this study were found below the detectible limit (BDL) in all plant parts across all study sites. The permissible value of cadmium is 0.3 ppm (WHO 2007). Hence, the level of this metal is within the safe range for household consumption irrigational use, and protection for aquatic life (Wold *et al.*, 2016).

Generally, the highest levels of heavy metals were found in the roots of *D. esculentum* due to the following reasons: First, the higher surface area for adsorption and absorption of heavy metals is in the roots and root hairs compared to leaves. That's why it can be noted in this plant organ the highest concentrations of heavy metals. Second, the first organ to take up all the heavy metals from the soil/water before distributing them to other parts of the plant is the root. Third, the only organ covered with soil is the root. Therefore, its major function in the uptake of nutrients and pollutant is all manifest due to physiological feature of this organ (Yap *et al.*, 2010).

The results of this study indicate that the level of heavy metal concentration is associated with habitats (Sriuttha *et al.*, 2016). The increased heavy metal accumulation in plants and animals was due to heavy metal contamination in soil/water where local people consumed these as regular foods (Promsid, 2014).

## Conclusion

All the physicochemical parameters of the study sites were found within the desirable limits except for the dissolved oxygen in station 1 (Agsao River) which found to be less than the accepted value. Moreover, the current status of the three (3) sampling areas based on the heavy metal content was within the allowable limits set by the different organizations such as US-EPA and WHO. Hence, it can be used by the local community for household consumptions and irrigation purposes as far as the concentrations of these heavy metals are concerned.

However, it was opposite to the heavy metal contents in various parts of *D. esculentum* such as roots, stems, and fronds found remarkably higher from the acceptable limits in Pb and Cr while Cd is below the detectible limit. The highest heavy metal accumulation was detected in the roots followed by the stems and lastly the fronds. Likewise, the noticeably high values of lead and chromium in the areas, which are extremely higher from the standard limits, could be an indication of inadequate protection of the environment against the penetration of the toxic substances. Hence, *D. esculentum* can be considered a good Pb and Cr accumulator.

Thus, constant monitoring and evaluation for the heavy metals' concentrations in the sampling stations both in water and plant samples are necessary. Furthermore, the levels of heavy metal pollutants must be checked before consumption to ensure the safe use of medicinal plants. Consuming medicinal plants contaminated with heavy metals can cause serious effects on human health. People in the locality are further advised to collect medicinal plants used for human and animal consumptions from unpolluted areas. Lastly, a test for other heavy metals, plants, and sediments is necessary to compare its concentrations on the samples.

## References

**Copeland EB.** 1942. "Edible Ferns". American Fern Journal **32(4),** 121-126. doi:10.2307/1545216.

**Das DK.** 2015. In: Introductory Soil Science, 4 edn; Kalyani Publishers, Ludhiana/New Delhi, India.

**Das DK.** 2016. Assessment of heavy metal accumulation in medicinal plants and possible remedial measures. Journal *of* Pharmaceutical Research **15(3),** 63-66.

**Garbisu C, Alkorta I.** 2001. Phytoextraction: A cost effective plant-based technology for the removal of metals from the environment. Biores. Technol **77(3),** 229-236. [doi:10.1016/S0960-8524(00)00108-5].

**Gisbert C, Ros R, de Haro A, Walker DJ, Pilar Bernal M, Serrano R, Avino JN.** 2003. A plant genetically modified that accumulates Pb is especially promising for phytoremediation. Biochem. Biophys. Res. Commun **303(2),** 440-445.
DOI: 10.1016/S0006 -291X(03)00349-8.

**Jasim HS, Idris M, Abdullah A, Kadhum AAH.** 2014. Effects of Physicochemical Soil Properties on the Heavy Metal Concentrations of Diplaziumesculentum (medicinal plant) from the UKM and TasikChini, Malaysia. International Journal of ChemTech Research **6(14),** 5519-5527; ISSN : 0974-4290.

**Jasim HS, Idris M, Abdullah A, Kadhum AAH.** 2014. Determination of Heavy Metals in Soil and Different Parts of *Diplazium esculentum* (Medicinal Fern). AIP Conference Proceedings **1614,** 713.

**Kachenko AG, Singh B, Bhatia NP.** 2007. Heavy metal tolerance in common fern species. Australia Journal of Botany **300,** 207-219.

**Klaassen CD.** 2001. Casarett and Doull's toxicology: the basic science of poisons. (New York, USA: McGraw-Hill).

**Lone MI, He Z, Stoffella PJ, Yang X.** 2008. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. Journal of Zhejiang University SCIENCE B ISSN 1673-1581 (Print); ISSN 1862-1783.

**Mahmoud AS, Emmanuel E, Joseph J, Bobby LW.** 2001. Chemical Evaluation of Commercial Bottled Drinking Water from Egypt, J. Food Composition Analysis **14,** 127-152.

**McGrath SP, Zhao FJ, Lombi E.** 2001. Plant and rhizosphere process involved in phytoremediation of metal-contaminated soils. Plant Soil **232(1/2),** 207-214. [doi:10.1023/A:1010358708525.

**Neeratanaphan L.** 2016. Cadmium, chromium, and lead accumulation in aquatic plants and animals near a municipal landfill. Human and Ecological Risk Assessment: An International Journal.
DOI: doi.org/10.1080/10807039.2016.1248893.

**Nriagu JO, Pacyna JM.** 1988. Quantitative assessment of worldwide contamination of air water and soils by trace metals. Nature **333(6169),** 134-139.
DOI: doi:10.1038/333134 a0.

**Peligro VC, Jumawan JC.** 2015. Aquatic macroinvertebrates diversity and Riparian Channel and Environmental Inventory in Gibong River, Philippines. Journal of Entomology and Zoology Studies **3(5),** 398-405.

**Prasannakumari AA, Gangadevi T, Jayaraman PR.** 2014. Absorption potential for heavy metals by selected ferns associated with Neyyar River (Kerala), South India. International Journal of Environmental Sciences **5(2),** ISSN 0976 - 4402.

**Promsid P.** 2014. Chromosomal Aberration Assessment of Fish in Reservoir Affected by Leachate in Municipal Landfill. Master' Thesis, Department of Environmental Science, Faculty of Science, Khon Kaen University, Nong Khai, Thailand.

**Rai V, Agnihotri AK, Khatoon S, Rawat AK, Mehrotra S.** 2005. Chromium in some herbal drugs. BullEnviron Contam Toxicol **74,** 464-469.

**Reddy AM, Kumar SG, Jyonthsnakumari G.** 2005. Lead induced changes in antioxidant metabolism of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and bengalgram (*Cicer arietinum* L.). Chemosphere **60,** 97-104.

**Schalscha E, Ahumada I.** 1998. Heavy metals in rivers and soils of central chile. Water Sci. Technol **37(8),** 251-255. [doi:10.1016/S0273-1223(98)00255-8].

**Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S.** 2005. Chromium toxicity in plants. Environ Int **31,** 739-753.

**Sriuttha M, Tengjaroenkul B, Intamat S, Phoonaploy U, Thanomsangad P, Singh KP, Bhattacharya S, Sharma P.** 2014;Assessment of heavy metal contents of some Indian medicinal plants. American-Eurasian J Agric Environ Sci **14,** 1125-1129.

**Tantemsapya N, Naksakul Y, Wirojanagud W.** 2011. Mathematical modeling of heavy metals contamination from MSW landfill site in Khonkaen, Thailand. Water Sci Technol **64(9),** 1835-42.

**Tepe Y, Mutlu E.** 2005. Physico-chemical characteristics of Hatay Harbiye Spring water, Turkey, J of the Inst. Of Sci and Tech. of Dumlupinar University **6,** 77-88.

**Tiwari KK, Singh NK, Patel MP** 2011. Metal contamination of soil and translocation in vegetables growing under industrial wastewater irrigated agricultural field of Vadodara, Gujarat, India. Ecotoxicol Environ Saf **74,** 1670-7.

**Tiwari KK, Singh NK, Patel MP.** 2011. Metal contamination of soil and translocation in vegetables growing under industrial wastewater irrigated agricultural field of Vadodara, Gujarat, India. Ecotoxicol Environ Saf **74,** 1670-7.

**Tong S, von Schirnding YE, Prapamontol T.** 2000; Environmental lead exposure: a public problem of global dimension. Bull World Health Organ **78,** 1068-1077.

**Vanek A, Boruvka L, Drabek O, Mihaljevic M, Komarek M.** 2005. Mobility of Lead, Zinc and Cadmium in Alluvial Soils Heavily Polluted by Smelting Industry, Plant, Soil and Environment **51(7),** 316-321.

**WHO.** 2007. WHO Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues. (Geneva, Switzerland; World Health Organization).

**Wold F, Ayenew B, Ahmad T.** 2016. Assessment of heavy metals concentration in Togona river of goba town, oromia region, Ethiopia. Int. J. Chem. Sci **14(4),** 3207-3214; ISSN 0972-768X.

**Yap CK, Fitri MRM, Mazyhar Y, Tan SG.** 2010; Effects of metal-contaminated soils on the accumulation of heavy metals in different parts of *Centella asiatica*: A laboratorystudy. Sains Malaysiana **39,** 347-352.