INNSPUB

**Journal of Biodiversity and Environmental Sciences (JBES)**
**ISSN: 2220-6663 (Print) 2222-3045 (Online)**
**Vol. 13, No. 1, p. 84-91, 2018**
**http://www.innspub.net**

RESEARCH PAPER OPEN ACCESS

# Spore germination of silver fern (*Pityrogramma calomelanos* (L.) Link) using various lead (Pb) concentrations

**Cherie Cano-Mangaoang*[1], Florfe M. Acma[2]**

[1]*Department of Biological Sciences, University of Southern, Mindanao, Kabacan, Cotabato, Philippines*

[2]*Department of Biology, Central Mindanao, University Town, Musuan, Bukidnon, Philippines*

Article published on July 27, 2018

**Key words:** Fern, Phytoremediation, Prothallus, Spore, Rhizoid

**Abstract**

Utilization of spores to screen potential of certain species of ferns is not new in the field of phytoremediation. However, for *Pityrogramma calomelanos* this is not yet done thus, in this study spores were used to investigate capability of this species to tolerate lead. This species has been documented to hyperaccumulate arsenic in the environment hence its capacity to accumulate other heavy metals is of interest. This study aims to assess the spore germination of silver fern (*P. calomelanos*) exposed to different concentrations of lead nitrate. Spores were collected by scraping the sori and sowed in sterile soil with various lead nitrate concentrations- $T_0$-control, $T_1$-100ppm, $T_3$-150ppm and $T_3$-200ppm. Result of the study showed that *P. calomelanos* spores germinated in all treatments. Germination of spores occur 7 days after sowing both in control and in various concentration of lead nitrate used. However, at $T_3$ fewer rhizoids developed and a smaller prothallus was observed. Prothallial development is normal in all treatments exhibited by multiseriate and green in color similar to the control. Our result indicate that *P. calomelanos* may be a potential accumulator of lead. Utilization of spore in screening potential of plant species in phytoremediation could lessen the use of mature fern plant. This is a practical strategy in any conservation effort involving ferns species utilized in the field of phytoremediation.

***Corresponding Author:** Cherie Cano-Mangaoang ✉ cheriecano1201@gmail.com

**Introduction**

Environmental pollution affects the quality of our environment and it has increased quickly in recent years. Soil pollution due to increasing demands and resource utilization has unprecedently increased in recent years (He *at al.*, 2015). Rapid technological advancement and increasing industrial activity throughout the last century means heavy metal contamination of the environment has become a serious problem (Drăghiceanu *et al.*, 2014; Prabhu *et al.*, 2016). Removal of heavy metal contamination either in soil or water using plants is increasingly being adopted due to its being cost effective and user friendly compared with traditional methods (Irudayaraj *et al.*, 2011).

Plant groups such as pteridophytes have been found to hyperaccumulate metal such as Chromium (Cr), Cadmium (Cd), Arsenic (As), and Lead (Pb) (Woolhouse, 1983; Zhang *et al.*, 2002; Rathinasabapathi *et al.*, 2006; Baldwin and Butcher, 2007; Lone *et al.*, 2007; Tu and Ma, 2012; Baskaranand Jeyachandran, 2012; Borja*et al.*, 2016).Fern species *Adiantum philippinense* L. and most species of the genus *Pteris*have been recently found to accumulate elevated levels of heavy metal from soil and water(Srivastava *et al.*,2005; Kamachi *et al.*, 2005; Salido *et al.*, 2005; Oloyede *et al.*, 2011).

The fern species *Pityrogramma calomelanos* was also documented to hyperaccumulate arsenic with approximately 2% phytoremediation potential in arsenic contaminated per year (Cheruiyot, 2008). Study on the capacity of *P. calomelanos* in remediating copper contaminated soil had already been initiated to further exploit its potential in the field of phytoremediation Evaluation on the capacity of this species in remediating soil contaminated by other heavy metals such as Copper was already initiated to further explore the utilization of *P. calomelanos* in the field of phytoremediation (Bansa, 2016; Borja *et al.*, 2016; Dahilan and Dalagan, 2017). Unlike angiosperms hyperaccumulators, ferns have evolved with inherent biological characteristics that could be exploited in the phytoremediation strategies aimed at decontaminating polluted sites (Hansa *et al.*, 2013). Ferns could present new adaptations in the action of stress factors because they are of wide geographic distribution and have the ability to adapt to diverse habitats (Dahilan and Dalagan, 2017).

The use of spores to understand the capacity of some fern species in phytoremediation were previously explored (Hansa *et al.*, 2013; Biswas *et al.*, 2015; Apuan *et al.*, 2016). Although, the use of spores from *P. calomelanos*, which is a commonly occurring species remains under studied. Thus, the result of this study will provide important information on the potential of *P. calomelanos* spores to tolerate the effect of lead and evaluate its effect on spore germination. Thus, the study aimed to assess the ability of *P. calomelanos* spores to tolerate the effects of lead and morphologically describe the effect on spore germination as exposed to varying concentrations. Furthermore, it may provide the basis of utilizing this species in phytoremediation of lead-contaminated soils and lessen the utilization of mature plant in screening for phytoremediation thus promoting conservation of this important species of plant.

**Materials and methods**

*Pityrogramma calomelanos* is commonly called "silver fern". It is common in the tropics. It can grow as tall as 40 cm, at the abaxial side of the fertile frond are sori with silvery colour thus the common name was taken. *P. calomelanos* fertile leaves were collected from the Central Mindanao University fernery. The spores were removed from the fertile frond by scraping the brownish sori using a dissecting needle. Spores were separated from the sporangia by sieving with a fine mesh cloth.

*Preparation of soil*

Garden soil was used in the study. The soil was sterilized in an oven at $180^o$ centigrade for 2 hours. It is important to sterilize the germination mixture before sowing the fern spores so as to kill the spores of fungi and other plants that may germinate and crowd out the developing fern prothalli.

It was allowed to cool down and then transferred to microwavable cups. Each cup contained 50 grams of sterilized soil.

*Sowing Method*

Spores were sparsely sprinkled on the sterilized soil with varying concentration of lead nitrate approximately 20ml to each treatment ($T_0$ control- no lead nitrate, $T_1$-100ppm, $T_2$ - 150 ppm and $T_3$ - 200ppm). Each treatment has three replicates. After the spores were sown, the containers were covered and kept at around 20 °C and fluorescent tube is the only source of light until germination occurred.

*Incubation and data gathering*

All the cultures were kept in a closed cabinet and exposed to 20 watts fluorescent tubes (Phillips) for 24 hours until germination is visible. Samples for microscopy were taken twice, first on the $7^{th}$ day after sowing and the second was 15 days later. Spore samples were taken and mounted on a slide then examined under a microscope for the presence of rhizoid and the prothallial development was observed carefully.

Micro-morphological developments were observed and recorded using a simple compound microscope. Data on the number of rhizoids, occurrence of germination and rows of cells in the prothallus were recorded.

**Results and discussion**

Spores of *P. calomelanos* are light brown with dark brown ridges and are tetrahedral-globose (Fig. 1). The spores started to germinate 7 days after sowing.

The occurrence of germination was exhibited by the swelling of the spore and the cracking of the spore wall and the intine and the emergence of a germ tube (Fig. 2).

This result is the same when spores of *Pteris vittata* was exposed to 10ppm of chromium (Hansa *et al.*, 2013) even exposure to lead chloride, cadmium chloride and mercuric chloride with 0.1 ppm/L, 0.01ppm/L and 0.001ppm/L (Biswas *et al.*, 2015). However, it took a longer period for *Pteris confusa* and *Pteris argyraea* (10 days) to germinate when exposed to zinc (Irudayaraj*et al.*, 2011).

**Table 1.**Characteristics of Spore germination of *Pityrogramma calomelanos* in varying concentrations of lead nitrate after 15 days of incubation.

| Treatments | Rhizoid (present/absent) | No. of Rhizoids | Germination | Prothallus | | Stage of Prothallus development |
|---|---|---|---|---|---|---|
| | | | | Rows of cells | Color of cells | |
| Control | Present | minimum of 12 | + | multiseriated | Green | Cordate |
| 100ppm | Present | minimum of 10 | + | multiseriated | Green | Spatulate |
| 150ppm | Present | minimum of 10 | + | multiseriated | Green | Cordate |
| 200ppm | Present but fewer | less than 10 | + | multiseriated | Green | Spatulate |

The responses of the spores in varying concentrations of lead nitrate ($T_0$- no lead nitrate, $T_1$-100ppm, $T_2$ - 150 ppm and $T_3$ - 200ppm) were observed twice with an interval of one week. An indication of spore germination was observed as the water in the medium change into green colour due to the formation of green prothallus. Spore germination is manifested by the appearance of a green film across the surface of the germination tray or the formation of pale green filaments on the medium (Irudayaraj *et al.*, 2011; Hansa *et al.*, 2013; Apuan *et al.*, 2016).

In the present study, 7 days after sowing, germination was observed in all treatments. In the process of germination, the spore wall splits, primary rhizoids start to emerge, followed by the development of the prothallus with rhizoids and consequently the appearance of the gametophyte.


**Fig. 1. a.** Habit, b. fertile frond and c. spore (400x magnification) of *Pityrogramma calomelanos*.

The development of the prothallus was normal (Oloyede *et al*., 2011). The appearance of rhizoids was observed to be happening in all treatments (Fig.3). Rhizoid of *P. vittata* was already noted after as early as 7 days which was also observed in 10ppm and 20ppm chromium treated spores (Hansa *et al*., 2013). At 25-100ppm copper concentrations, undamaged rhizomes and rhizoids of *P. calomelanos*were observed, however, deformations were observed at 200-400ppm (Borja *et al*., 2016).

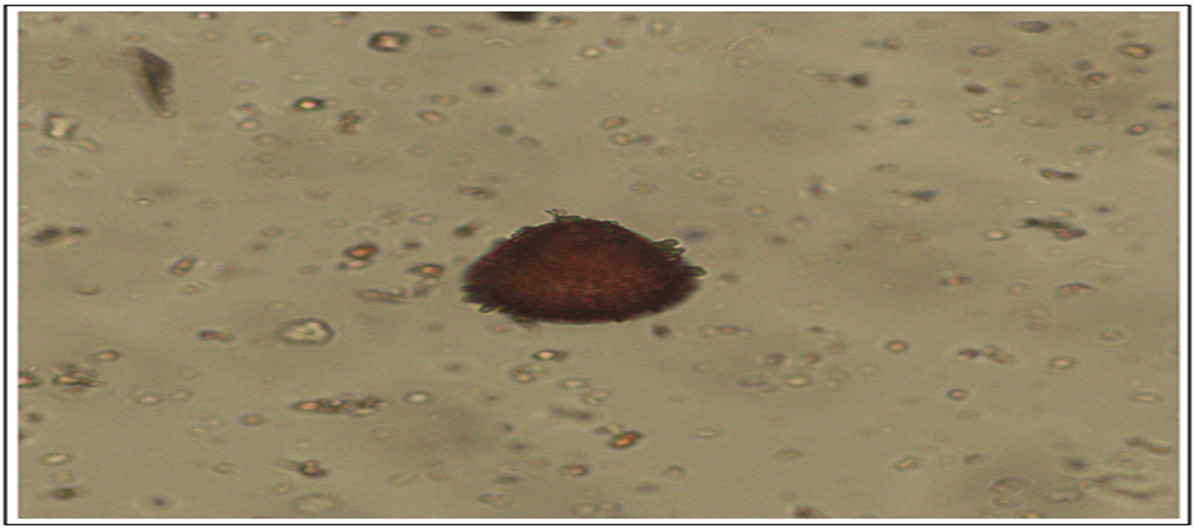

**Fig. 2.** Spore of *P. calomelanos* undergoing the initial stage of germination (400x).

Multiseriate prothalli were also observed to be normal even after 15 days of sowing (Fig. 4 A, B, C and D). However, morphological variations were observed in $T_3$ such as slow growth of the prothallus as manifested by its smaller size compared to the other treatments ($T_0$, $T_1$, and $T_2$), and the rhizoids present were noted to be few. The colour of the cells found in the prothalli was the same in all the treatments.

The gametophyte of *Cyathea medullaris* as exposed to less than 500 µMPb showed various morphological changes including stunted growth, browning or brown patches, shrinkage and stress (Wanninayake, 2007) which was not manifested in the gametophyte of *P. calomelanos* in this study.

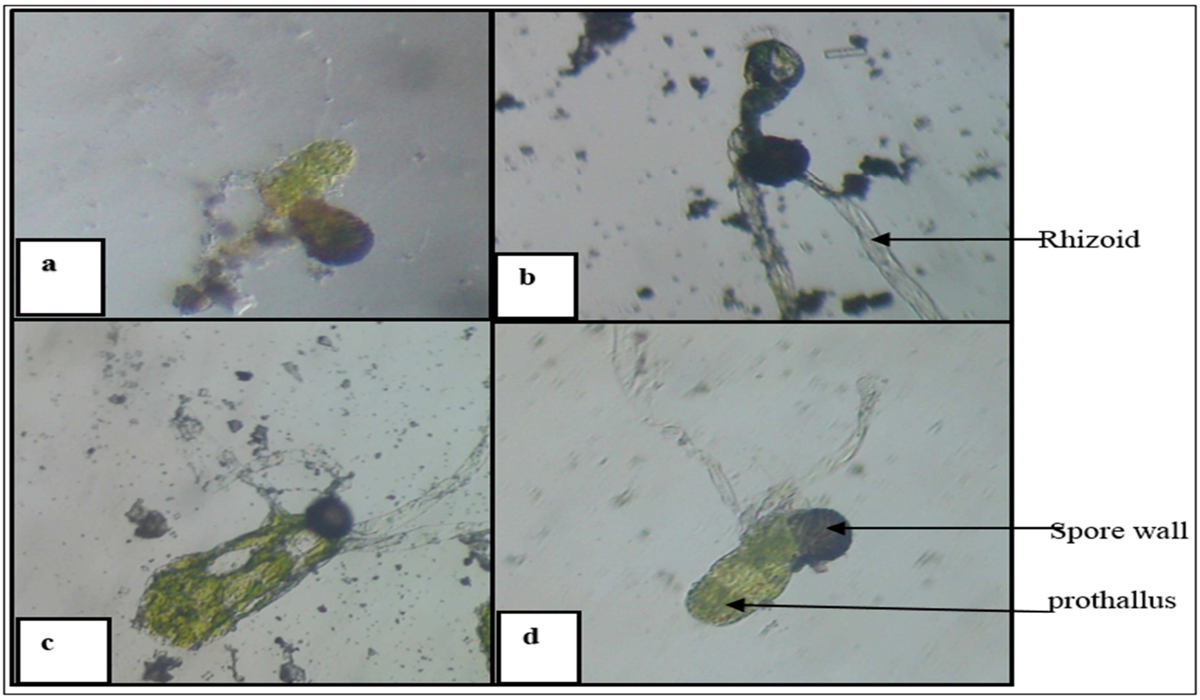


**Fig. 3.** Initial stage spore germination of *P. calomelanos* (7 days after sowing): a. $T_0$ – control, b. $T_1$ – 100ppm, c.$T_2$ – 150ppm and d. $T_3$ – 200 ppm.

Spore germination in *Athyrium yokoscense* was more tolerant to lead (Pb), compared to that in other fern species, such as *Pteridium aquilinum*, *Lygodium japonicaum* and *P.* vittata (Lombi*et al.*, 2002). In 1ppm concentration of $PbCl_2$, $CdCl_2$ and $HgCl_2$ become lethal for the spores of *Pteris vittata* and no germination took place (Biswas *et al.*, 2015). No germination of spores of *Pteris vittata* at higher concentrations ($\geq 20ppm$) of chromium treatment (Hansa *et al.*, 2013). In addition, the early gametophyte development of *Athyrium yokoscense* was not much affected by 10 M Pb2+, as evaluated from the prothallial growth and rhizoid development (Salido *et al.*, 2003). Gametophytes are able to grow normally in a medium containing 20mM arsenate and accumulate 2.5% of their dry weight as arsenic (Gumalius *et al.*, 2004). Spatulate stage and cordate gametophyte – the two important stages in the development of *P. vittata*, developed in 10ppm and 20ppm chromium concentration but alterations in the anatomical organizations were noticed (Hansa *et al.*, 2013). There is considerable genetic variation in the abilities of various species to tolerate otherwise toxic amounts of non-essential lead, cadmium, silver, aluminum, mercury, tin and other metals (Woolhouse, 1983).Plants have both constitutive and adaptive mechanisms for coping with the elevated metal concentrations. The adaptive mechanism differs from species to species (Mehrah, 1994).

The result of the present study showed that *P. calomelanos* can be a good phytoremediator for lead as shown in Table 1. At concentrations of $T_1$ - 100ppm to $T_3$ - 200ppm, spores of *P. calomelanos* germinated same as $T_0$. However, at $T_3$ rhizoids developed but were fewer. Though $T_3$ is multiseriate but with fewer rows compared with other treatments. Furthermore, morphological characteristics of the cells in the prothallus in $T_3$ manifested some variations compared with other treatments such as lighter in colour, which might be due to fewer chloroplast present. These morphological appearance of *P. calomelanos* treated with various concentrations of lead nitrate were observed 15 days after sowing.

In the study conducted by Hansa *et al.*, they observed that in more than 20ppm concentration of chromium, spatulate gametophyte of *P. vittata* developed late with some changes in the anatomical structures and cordate gametophyte is totally absent.

Thus, result of this study showed that spores of *P. calomelanos* can germinate in soil with up to 200ppm of lead nitrate which might be a good indicator of its tolerance towards said heavy metal. Furthermore, it might be an indication of its potential of surviving in a lead contaminated soil thus a possible candidate species in the field of phytoremediation.

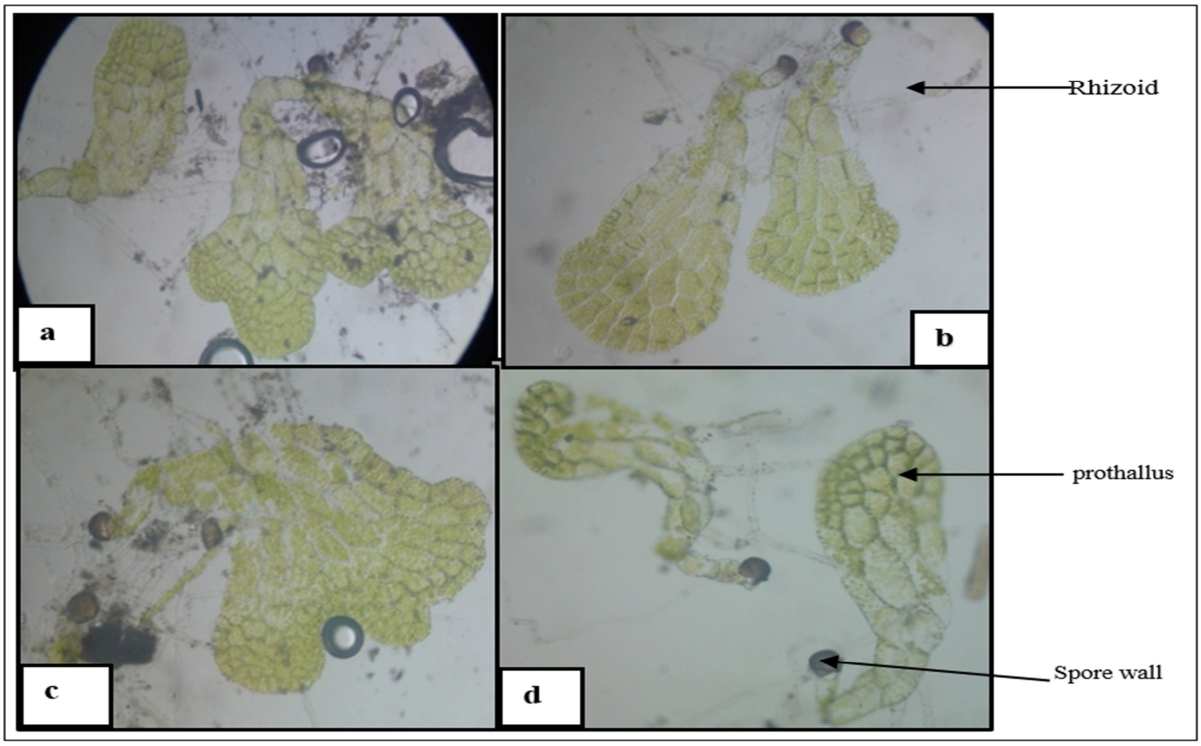


**Fig. 4.** Prothallial development after 15 days of incubation: a. $T_0$-no lead nitrate, b. $T_1$-100ppm, c. $T_2$-150ppm and d. $T_3$- 200ppm.

**Conclusion and Recommendation**

The spores of *Pityrogramma calomelanos* exposed to various lead nitrate concentration showed that it could tolerate said heavy metal up to 200ppm which might be an indicator that this fern species could be utilized in accumulating lead-contaminated soil. However, it is recommended that higher amount of lead will be used in order to establish the maximum amount of lead that can be tolerated or can affect the development of the spores.

**Acknowledgment**

The authors would like to thank Charissa Joy Gumban and Vince Abarquez for the help extended during field collection. We also would like to thank Dr James Gregory C. Salem and Krizler Tanalgo for helping in the improvement of the paper.

**References**

**Apuan DA, Apuan MJB, Perez TR, Perez RE, Claveria RJR, Doronilla A, Tan M.** 2016. Propagation protocol of *Pteris vittata* L. using spores for phytoremediation. International Journal of Biosciences **8(6),** 14-21.

**Baldwin PR, Butcher DJ.**2007. Phytoremediation of arsenic by two hyper-accumulators in a hydroponic environment. Microchemical Journal **85,** 297–300.

**Bansa KJ.**2016. Phytoremediation Potential of Plants Grown in Reclaimed Spoil Lands, Ghana Mining Journal **16(1),**68-75.

**Baskaran X, Jeyachandran R.** 2012. In vitro spore germination and gametophyte growth assessment of a critically endangered fern: *Pteris tripartita* Sw. Gayathri Teknological Publication **1(1),** 4-9.

**Biswas M, Khare P, Kumari N.** 2015. Effect of heavy Metals like Mercury ($Hg^{++}$), Cadmium ($Cd^{++}$) and Lead ($Pb^{++}$) on the spore germination of Pteris vittata; the common road side fern. International Journal of Science, Technology & Management **4(1),** 194-198.

**Borja KM, Luzano CR, Chang A, Sia GL, Sia S, Banez G, Agoo EMG.** 2016. Growth and Morphology of the Rhizome and Rhizoid of *Pityrogramma calomelanos* (L.) Link (Pteridaceae) at Varying Copper sulfate concentrations **11(4),** 121-126.

**Cheruiyot DJ.** 2008. Tolerance and Accumulation of Copper, Lead and Zinc by *Ceratopteris richardii*. A Thesis in Environmental Science. Columbus State University.

**Dahilan JKA, Dalagan JQ.** 2017. Bioavailability and Accumulation Assessement of Copper in *Pityrogramma calomelanos*, Philippine Journal of Science**146(3)**,331-338.

**Drăghiceanu OA, Dobrescu CM, Soare LC.**2014. Applications of Pteridophytes in Phytoremediation. Current Trends in Natural Sciences **3(6),** 68-73.

**Gumaelius BL, Salt DE, Banks JO.** 2004. Arsenic Hyperaccumulation in Gametophytes of Pterisvittata. A New Model System for Analysis of Arsenic hyperaccumulation. Luke Plant Physiology **136,** 3198-3208.

**Hansa J, Chakraborty B, Laskar BA, Behera SK, Patel AK.** 2013. *Pteris vittata* Propagation through Different Exposure of Chromium Concentration: and Experiment to Comprehend Phytoremediation Properties. Advances in Bioresearch **4(1),** 43-49.

**He Z, Shentu J, Yang X, Baligar VC, Zhang T and Stoffella PJ.** 2015. Heavy metal contamination of soils: Sources, indicators and assessment. Journal of Environmental Indicators **9,**17-18.

**Irudayaraj V, Johnson M, Priyakumari AS, Janani, Prabha A.**2011.Effect of Heavy metal stress on spore germination of *Pteris confusa* T. G. Walker and *Pteris argyraea* T. Moore. Journal of Stress Physiology & Biochemistry **7(4),** 207- 216.

**Kamachi H, Ippei K, Hideo T, Sawa Y, Ichirou K, Honma KY, Wada N, Kawabata T, Matsuda K, Ikeno S, Noguh M, Inoue H.** 2005. Lead tolerance and accumulation in the gametophytes of the fern *Athyrium yokoscense*. Journal of Plant Research **118(2),**37-145.

**Lone MI, Zhen-li HE, Stoffella PJ,Yang X.** 2007. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. J Zhejiang UnivSci B, **9(3),**210-220.

**Lombi A, Zhao FJ, Fuhrmann M, Ma LQ, McGrath SP.** 2002. Arsenic distribution and speciation in the fronds of the hyperaccumulator *Pteris vittata*. New Phytologist**156,**195-203.

**Mehrah AA.** 1994. Integrated tolerance mechanisms: Constitutive and adaptive plant responses to elevated metal concentrations in the environment. Plant Cell Env, **17,**989-993.

**Oloyede FA, Abu B, Akomolafe FG.** 2011. Spore germination, Gametophyte morphology and Gametangia Development in a Tropical Fern, *Cyclosorus striatus* (Schum.) Ching (Thelypteridaceae). Nigeria Journal of Botany **24(2),** 239-244.

**Prabhu SG, Srinikethan G, Hegde S.** 2016. Potential of Pteridophytes in Heavy Metal Phytoremediation. International Journal of Research in Engineering and Technology **5(17),** 1-9.

**Rathinasabapathi B, Lena QM, Srivastava M.** 2006.Arsenic Hyperaccumulating Ferns and their Application to Phytoremediation of Arsenic Contaminated Sites Floriculture, Ornamental and Plant Biotechnology Volume III. Global Science Books.

**Srivastava M, Ma LQ, Cotruvo JA.** 2005. Uptake and distribution of selenium indifferent fern species. Int J. Phytoremediation **7(1),**33-42.

**Salido AL, Hastly KL, Lim JM, Buthcher DJ.** 2003. Phytoremediation of arsenic and lead in contaminated soils using Chinese brake ferns (Pteris vittata) and Indian mustard (*Brassica juncea*). IntJ. Phytoremediation, 2003, **5(2),** 89-103. http://dx.doi.org/10.1080/713610173

**Tu C, Ma LQ.** 2002. Effects of arsenic concentration and forms on arsenic uptake by the hyperaccumulator ladder brake. Journal of Environmental Quality **31,**641-647.

**Wanninayake SP.** 2002. Response of a New Zealand tree fern (*Cyathea medullaris*) to Lead (Pb) treatments. A thesis in Plant Biotechnology, University of Canterbury.

**Woolhouse HW.** 1983. Toxicity and tolerance in the responses of plant to metals. In: Lange, O.L., Nobel, P.S., Osmond, C.B. and Ziegler, H. (eds.), Encyclopedia of Plant physiology, New Ser. Vol. 12c, Physiological plant ecology III, Response to the chemical and biological environment. Springer-Verlag, Berlin245-300 p.

**Zhang WH, Cai Y, Tu C, Ma LQ.** 2002. Arsenic speciation and distribution in an arsenic hyper accumulating plat. Sci Total Environ **300(1-3),** 167-177. http://dx.doi.org/10.1016/S0048-9697(02)00165-1