METAL TOLERANCE AND PHYTOACCUMULATION POTENTIAL OF *DRYOPTERIS PENTHERI* (KRASSER) C. CHR

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Abstract. Globally, heavy metals from natural and anthropogenic activities cause serious health and environmental risks, especially in water systems. Phytoremediation is a promising approach for removing metal contaminants in the environment. The present study aimed to evaluate the ability of a fern, *Dryopteris pentheri* (Krasser) C. Chr. to take up, accumulate, and tolerate heavy metals. The study was conducted in a controlled environment where plants were cultivated in 20 L dishes filled with wetland water for 7 days under control, low and high metal concentrations. Overall, *D. pentheri* contained high concentrations of selected metals; however, composition and concentrations differed across plant parts, with roots containing exceptionally high concentrations of Na, Mg, and K. Both bioaccumulation and translocation factors were greater than 1 for some selected metals in the roots, indicating that *D. pentheri* is a hyperaccumulator and has shown the ability to tolerate metals by increasing chlorophyll content under high metal concentrations after prolonged exposure. Therefore, *D. pentheri* stands as a good candidate for removing metals from aquatic environments.
Keywords: *bioaccumulation factor, ferns, translocation factor, phytostabilisation, hyperaccumulator, phytoaccumulation*

Introduction

Heavy metals pose a significant threat to ecosystems and human health (Dalu and Tavengwa, 2022; López-Botella et al., 2021; Parui et al., 2024). Heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are among the most pervasive and hazardous environmental pollutants (Dalu and Tavengwa, 2022; Balali-Mood et al., 2021; Sharma et al., 2021). These metals are introduced into the environment through various anthropogenic activities, including mining, industrial processes, agricultural practices, and urban discharges (Rashid et al., 2023; Parui et al., 2024; Akhtar et al., 2021; Pandey and Kumari, 2023). In contrast to organic pollutants, heavy metals cannot be degraded and tend to accumulate in soils and water bodies, leading to long-term environmental and health risks (Yaashikaa et al., 2022). Prolonged exposure to heavy metals can have severe health implications for humans, including neurological disorders, kidney damage, and cancer (Shetty et al., 2023). Moreover, heavy metals can disrupt ecosystems by affecting soil fertility, plant growth, and both terrestrial (Gall et al., 2015) and aquatic life (Xu et al., 2022; Priyadarshanee et al., 2022). Given

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the widespread contamination of heavy metals and their potential detrimental effects, there is an urgent need for effective remediation strategies.

Phytoremediation is the use of plants to remove, stabilise, or degrade contaminants from soil, water, and air, which has emerged as a sustainable and cost-effective approach to environmental clean-up (Kafle et al., 2022; Shen et al., 2022). Various methods have been used to clean up metal contaminants from the environment. Among these include, traditional metal remediation methods, such as excavation and chemical treatment, which are often expensive, energy-intensive, and disruptive to the environment (Rajendran et al., 2022; Azhar et al., 2022). In contrast, phytoremediation offers an eco-friendly alternative that leverages the natural capabilities of plants to uptake and accumulate metals, thereby restoring contaminated sites (Shen et al., 2022).

Phytoremediation encompasses several mechanisms by which plants can mitigate metal contamination, including phytoextraction, phytostabilisation, phytovolatilisation, and rhizofiltration (Kafle et al., 2022; Kristanti et al., 2023). Phytoextraction involves the uptake of metals from the soil or water and their translocation to above-ground plant tissues, which can then be harvested and removed (Gul et al., 2021; Mudgal et al., 2023). Phytostabilisation focuses on immobilising metals in the soil or water, reducing their bioavailability and preventing their spread (Bhat et al., 2022; Moreira et al., 2021; Gavrilescu, 2022). Phytovolatilisation involves the uptake and transpiration of volatile metals, such as mercury and selenium, into the atmosphere (Somagattu et al., 2024). Rhizofiltration refers to the absorption or adsorption of metals by plant roots in aquatic environments (Kristanti et al., 2021; Woraharn et al., 2021; Green, 2024). Among these, phytoextraction is particularly promising for the remediation of heavy metal-contaminated soil, because it enables the plant to uptake metals from the soil to aboveground parts, which also detoxifies the metals inside the plant organs (Gavrilescu, 2022; Kafle et al., 2022). The success of phytoremediation depends on the selection of appropriate plant species that exhibit high metal uptake capacity, tolerance to metal toxicity, and the ability to thrive in contaminated environments (Yadav et al., 2023; Kafle et al., 2022; Liang et al., 2024). Hyperaccumulator plants, those that can accumulate exceptionally high concentrations of metals in their tissues, are particularly valuable for phytoextraction (Yaashikaa et al., 2022). However, they are often slow-growing and have limited biomass production, which can limit their practical application (van der Ent et al., 2024; Li and Liu, 2022). Therefore, there is a growing interest in identifying non-hyperaccumulator plants with moderate-high metal accumulation capacity but higher biomass production, as these may offer a more feasible solution for large-scale remediation efforts.

Fern plants have been recognised for their ability to tolerate and accumulate heavy metals, making them promising candidates for phytoremediation (Sharma and Sharma, 2022). Ferns are perennial species belonging to the family Dryopteridaceae (Maroyi, 2024). Specifically, plants of the genus *Dryopteris* are a promising group for phytoremediation due to their ability to uptake and accumulate heavy metals, as well as their tolerance to metal toxicity (Grosjean et al., 2019; Senila et al., 2013). *Dryopteris pentheri* (Krasser) C. Chr., commonly known as Penther's wood fern, has the potential as a phytoremediator of heavy metals, owing largely to its adaptability to harsh environmental conditions, including low nutrient availability and high metal concentrations (Hietz, 2010; Gao et al., 2018). The ability of *D. pentheri* to uptake and accumulate heavy metals can also be attributed to other factors such as root morphology, metal transporters, and chelation mechanisms (Sharma and Sharma, 2022). Despite this, research on *D. pentheri* and its metal uptake capabilities is relatively limited.

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Preliminary studies on *Dryopteris* have shown that it can accumulate moderate levels of metals such as cadmium, lead, and zinc in its fronds and roots (Senila et al., 2013; Grosjean et al., 2019; Sajeev et al., 2022). However, the extent of metal accumulation and the plant's tolerance thresholds vary depending on the metal species, soil properties, and environmental conditions (Sharma and Sharma, 2022). For example, *Dryopteris* may exhibit a higher accumulation of certain metals in acidic soils, where metal bioavailability is increased (Khan et al., 2024), while the presence of other nutrients and organic matter in the soil or water can influence metal uptake and translocation patterns (Yan et al., 2021). However, research on *D. pentheri* and its metal uptake capabilities is relatively limited.

One advantage of *Dryopteris* is its adaptability to harsh environmental conditions, including low nutrient availability and high metal concentrations (Hietz, 2010; Gao et al., 2018). Thus, understanding these mechanisms is crucial for assessing the fern plant's phytoremediation potential and optimising its use in contaminated environments. The ability of *D. pentheri* to uptake and accumulate heavy metals can be attributed to several factors, including root morphology, metal transporters, and chelation mechanisms (Sharma and Sharma, 2022). Ferns typically have extensive root systems that enhance their capacity to absorb metals from the soil or water (Sharma and Sharma, 2022). Once absorbed, metals are transported to various plant tissues, where they may be sequestered in vacuoles or bound to metal-chelating compounds such as phytochelatins (Cobbett, 2000a, b). The phytochelatins play a critical role in detoxifying metals and preventing their interference with essential cellular processes. Preliminary studies on *Dryopteris* have shown that it can accumulate moderate levels of metals such as cadmium, lead, and zinc in its fronds and roots (Senila et al., 2013; Grosjean et al., 2019; Sajeev et al., 2022). However, the extent of metal accumulation and the plant's tolerance thresholds vary depending on the metal species, soil properties, and environmental conditions (Sharma and Sharma, 2022). For example, *Dryopteris* may exhibit higher accumulation of certain metals in acidic soils, where metal bioavailability is increased (Khan et al., 2024). Additionally, other nutrients and organic matter in the soil or water can influence metal uptake and translocation patterns (Yan et al., 2021).

Metal tolerance in plants is a complex trait that involves multiple physiological and biochemical adaptations (Chen et al., 2022; Wang et al., 2023; Sajeev et al., 2022). In *D. pentheri,* similar to other fern species, tolerance mechanisms may include the production of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), which mitigate oxidative stress induced by metal toxicity (Alam et al., 2021; Zhang et al., 2019). The plant may also upregulate the synthesis of osmoprotectants, such as proline and glycine betaine, to maintain cellular homeostasis under metal stress (Drăghiceanu et al., 2016). Furthermore, *D. pentheri* may employ exclusion strategies, such as binding metals to cell walls or the secretion of organic acids, to limit metal uptake and translocation. Thus, understanding how *D. pentheri* moves metals is essential for enhancing the potential phytoremediation of the taxa.

Dryopteris genus is a promising group for phytoremediation due to its ability to uptake and accumulate heavy metals, as well as its tolerance to metal toxicity (Grosjean et al., 2019; Senila et al., 2013). Thus, further research is needed to fully characterise its metal accumulation capacity, tolerance mechanisms, and potential for large-scale application particularly in subtropical regions. By leveraging the natural capabilities of *D. pentheri*, it may be possible to develop sustainable and effective strategies for mitigating heavy metal contamination and restoring polluted ecosystems.

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This study aims to contribute to the growing body of knowledge by assessing the phytoremediation potential of *D. pentheri*, thereby paving the way for its practical application in environmental clean-up efforts. Specifically, the study aims to evaluate the ability of a fern *D. pentheri* to uptake, accumulate, and tolerate heavy metals (e.g., lead, cadmium, arsenic, iron, chromium, and magnesium) in a controlled environment. It was hypothesised that *D. pentheri* will uptake and accumulate heavy metals from contaminated water. Furthermore, they will exhibit differential tolerance and accumulation capacities for different metals among different tissues (i.e., roots, rhizomes, fronds).

Materials and methods

Reagents and chemicals

All chemicals (i.e., potassium nitrate AR (cas no. 7757-79-1), calcium nitrate tetrahydrate AR (cas no. C1843), potassium dihydrogen phosphate AR (cas no. 7778-77-0), magnesium sulphate AR (cas no. 10034-99-8), zinc sulphate heptahydrate AR (cas no. 7446-20-0), cupric sulphate pentahydrate crystals AR (cas no. 10099-74-8) used in the experiment were of analytical grade. Most of the chemicals were obtained from RadChem, South Africa, except for calcium nitrate tetrahydrate AR which was obtained from Minema Chemicals, South Africa.

Indigenous potted fern plants *Dryopteris pentheri* (n = 18) were sourced from a local warehouse store in Nelspruit, South Africa. The fern plants *D. pentheri* were taken to the University of Mpumalanga, where they were placed in dishes (diameter 80 cm, depth 45 cm) filled with 20 L of wetland water. The wetland water was collected from a nearby wetland system. The fern plants *D. pentheri* (n = 3 per dish) were randomly placed in the dishes, and the plants were left to acclimatise for 3 days before the experiment was initiated. After three days, the fern plants *D. pentheri* were briefly taken out of the water the water level was topped to the initial level and the chemicals were added before being thoroughly mixed while ensuring all chemicals dissolved in the water after which the fern plants *D. pentheri* were placed back in the dishes (*Table 1*). Metal concentrations in treatments simulate contaminated field conditions to help assess *D. pentheri* tolerance and phytoremediation potential, thus, the concentrations mimic real-world pollution levels. The experimental design consisted of two dishes for each of the treatments: control (n = 3 plants per dish), low (n = 3 plants per dish) and high (n = 3 plants per dish) metal concentrations (*Fig. 1*).

Physico-chemical water parameters (i.e., temperature, total dissolved solids, conductivity, pH, salinity, oxygen reduction potential, resistivity) were measured using a handheld HANNA multiparameter. The water measurements were recorded on days 1, 3, 5 and 7. The leaf chlorophyll-*a* concentration was measured from 20 randomly selected leaves per treatment dish (n = 40 per treatment) using a handheld chlorophyll SPAD 520 meter on days 1, 4 and 7. Furthermore, water and fern plant *D. pentheri* samples were collected at days 1 and 7 for metal analysis (i.e., sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), aluminium (Al), arsenic (As), boron (B), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), lithium (Li), manganese (Mn), nickel (Ni), phosphorous (P) and zinc (Zn). The fern plant *D. pentheri* sampled on day 1 was from the 3 extra pots to know the background values on metals in the plants and were placed in labelled Ziplock bags after dividing them into roots, stems and leaves. Water samples were collected in labelled 500 mL polyethylene water bottles.

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Table 1. *The concentrations of chemicals (g) added in water tanks (20 L) for the phytoremediation experiments*

Chemical	Control	High	Low
Pb	0	80	40
Cu	0	196.5	98.25
Zn	0	220	110
Mg ($MgSO_4 \cdot 7H_2O$)	0	51.5	25.75
K (KH_2PO_4)	0	60	30
Ca ($CaNO_3$)	0	45	22.5
NO_4^+ (KNO_3)	0	45	22.5

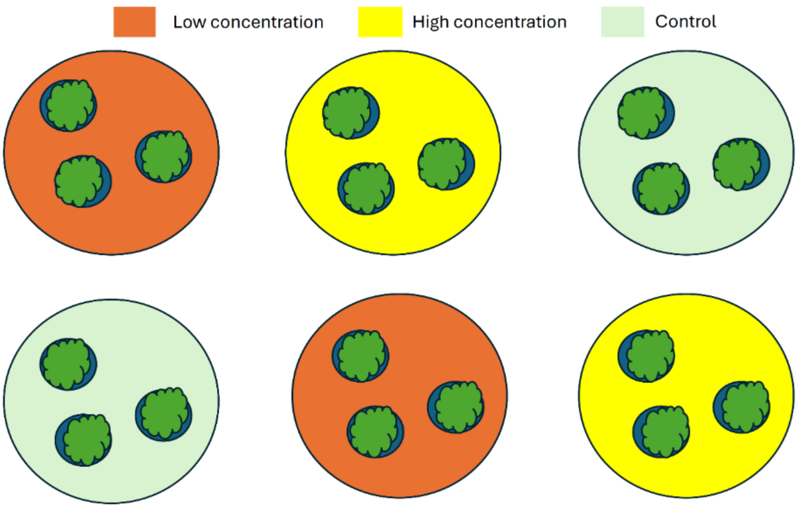

Figure 1. *Experimental design for the fern Dryopteris pentheri phytoremediation study*

Metal analysis

Water

At the conclusion of the experiment, collected water samples were sent to WaterLab, Pretoria, South Africa for metal analysis, where major anion samples were filtered through a 0.45 µm filter paper before analysis. The Na, K, Ca, Mg, Al, As, B, Cd, Cr, Co, Cu, Fe, Pb, Li, Mn, Ni, P and Zn were analysed using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Varian, Mulgrave). The reliability of the analysis results was determined using the ionic balance error of samples; the samples showed an ionic balance of ≤ ±10% error, with four samples showing an ionic balance error of ±10 to ±15%.

Plants

The collected fern plant *D. pentheri* parts (i.e., roots, stem and leaves) were oven-dried at 60°C for 3 days before being ground into powder. Approximately 10 g of the sample for each plant part were analysed at the Stellenbosch University Central Analytical Facility after microwave digestion (MARS®-5) at 280 PSI pressure and 180°C for 20 min using 6 mL (65%) nitric acid: 2 mL (30%) hydrochloric acid and 6.5 mL (65%) nitric acid: 0.5 mL (30%) hydrochloric acid, respectively (Environmental Protection Agency

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(EPA), 2007). Post-digestion, de-ionised water was added to make 50 mL final volume of each sample. Metal analyses were performed on an Agilent 7900 quadrupole Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) equipped with a High Matrix Introduction system and Agilent Mass Hunter software (version 4.4) for instrument control and data processing (McCurdy, 2009). For quality control, each set of matrix digestions included dual quality controls, with a blank acid mixture of 1 573a tomato leaf (~0.3 g) Certified Reference Material (National Institute for Science and Technology, Gaithersburg, USA) were used as controls for plants. The accuracy range was 81.8–113.4%), with a recovery range of 83.6–113.2% was recorded.

Data analysis

Bioconcentration factor (BCF) is indicative of the degree of enrichment of a heavy metal in a biota (i.e., fern plant *D. pentheri*) relative to that in its habitat (i.e., water). The BCF is calculated based on the equation:

$$BCF = \frac{C_{fern}}{C_{Water}} \quad \text{(Eq.1)}$$

where C_{fern} is the metal concentration in the fern tissue (i.e., root, rhizome or fronds) and C_{water} is the metal concentration in water where the ferns were kept. A BCF value > 1 indicates that the fern plant *D. pentheri* can accumulate more metals than are available in the water (i.e., hyperaccumulation). A BCF value ≤ 1 indicates that the fern plant *D. pentheri* can only absorb metals in proportion to what is available in the water.

The transportation index (T_i) gives the fronds/root or rhizome/root metal concentration, and it depicts the fern plant *D. pentheri* ability to translocate metal species from roots to the rhizome or fronds at different concentrations. It is calculated based on the equation:

$$T_i = \frac{Metals\ in\ leaves\ or\ stems}{metals\ in\ roots} \times 100 \quad \text{(Eq.2)}$$

A $T_i > 1$ means the fern plant *D. pentheri* can efficiently move the metal from its roots to its rhizome or fronds, and a $Ti < 1$ is ideal for phytostabilisation.

Statistical analysis

A repeated measures analysis of variance (ANOVA) was used to assess the differences in basic water variables (i.e., pH, conductivity, salinity, total dissolved solids, ORP, resistivity, temperature), metals (i.e., Na, K, Ca, Mg, Al, As, B, Cd, Cr, Co, Cu, Fe, Pb, Li, Mn, Ni, P, Zn) in water and plants across days, after testing for homogeneity of variances (Levene's test) and normality of distribution (Shapiro-Wilk test). For significant results, Tukey post-hoc tests were performed via estimated marginal means when the effects were significant. All statistical analyses were performed using IBM SPSS version 28.0.0.0 based on log (x + 1) transformed data.

Results

Water

Conductivity, TDS and salinity were generally high in the high-concentration treatment and low in the control treatment (*Table 2*). Resistivity was high in the control

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treatment. Based on repeated measures ANOVA analysis, temperature, salinity, resistivity and pH were found to be significantly ($p < 0.001$) different across days, conductivity, TDS, salinity and resistivity were significantly ($p < 0.001$) different among treatments (*Table 3*).

Table 2. *Mean (± standard deviation) of basic water quality variables measured over the experimental period*

Time	Treatment	Conductivity (µS cm^{-1})	Temperature (°C)	TDS (ppt)	Salinity (ppt)	Resistivity (Ω)	pH	ORP (mV)
Day 1	Control	0.2 ± 0.01	20.2 ± 1.0	0.1 ± 0.01	0.1 ± 0.004	5717 ± 492.1	7.5 ± 0.3	-45.6 ± 0.3
	Low	8.9 ± 0.1	19.6 ± 0.4	6.1 ± 3.7	5.0 ± 0.09	136.5 ± 6.0	7.4 ± 0.09	-46.0 ± 0.07
	High	15.3 ± 0.1	20.4 ± 1.2	6.2 ± 0.3	8.9 ± 0.01	79.9 ± 4.9	7.3 ± 0.01	-45.8 ± 0.2
Day 3	Control	0.2 ± 0.03	17.9 ± 0.1	0.1 ± 0.003	0.1 ± 0.0	5485.5 ± 613	7.8 ± 0.04	-46.1 ± 1.3
	Low	12.4 ± 4.5	17.4 ± 0.5	3.3 ± 0.08	5.1 ± 0.08	148.9 ± 2.4	7.8 ± 0.04	-46.9 ± 0.8
	High	15.7 ± 0.02	17.5 ± 0.2	5.8 ± 0.05	9.1 ± 0.002	85.8 ± 1.0	7.8 ± 0.001	-46.8 ± 0.5
Day 5	Control	0.3 ± 0.01	16.0 ± 0.2	0.1 ± 0.01	0.1 ± 0.01	4840 ± 257.3	7.7 ± 0.02	-39.2 ± 1.2
	Low	10.0 ± 0.07	14.9 ± 0.6	3.2 ± 0.1	5.6 ± 0.1	160.5 ± 0.1	7.7 ± 0.01	-38.6 ± 0.4
	High	17.2 ± 0.05	15.7 ± 1.1	5.6 ± 0.2	10.0 ± 0.1	88.7 ± 4.7	7.7 ± 0.01	-39.4 ± 0.1
Day 7	Control	0.3 ± 0.002	22.5 ± 0.4	0.1 ± 0.004	0.1 ± 0.003	3278 ± 59.3	7.6 ± 0.01	-36.2 ± 1.9
	Low	10.7 ± 0.01	22.8 ± 1.6	4.9 ± 0.3	6.1 ± 0.02	102 ± 8.5	7.6 ± 0.03	-35.8 ± 0.4
	High	18.3 ± 0.2	23.1 ± 0.5	8.4 ± 0.3	10.9 ± 0.1	59.1 ± 2.0	7.6 ± 0.02	-37.1 ± 0.3

Table 3. *Repeated measures analysis of variance of water quality variables and fern plant Dryopteris pentheri parts over the entire experimental period. Values in bold indicate significant differences at p < 0.05*

Variable	Days			Treatment			Days × treatment		
	Df	F	*P*	*df*	F	*P*	*df*	F	*p*
Basic water parameters									
Conductivity	3	2.248	0.135	**2**	**159.000**	**< 0.001**	6	0.944	0.500
Temperature	**3**	**105.611**	**< 0.001**	2	1.426	0.278	6	0.440	0.838
TDS	3	0.704	0.568	**2**	**45.986**	**< 0.001**	6	0.776	0.604
Salinity	**3**	**26.665**	**< 0.001**	**2**	**16.797**	**< 0.001**	**6**	**26.278**	**< 0.001**
Resistivity	**3**	**73.741**	**< 0.001**	**2**	**118.681**	**< 0.001**	**6**	**3.830**	**0.023**
pH	**3**	**17.741**	**< 0.001**	2	0.475	0.633	6	0.844	0.560
ORP (mV)	3	0.754	0.541	2	0.914	0.427	6	1.019	0.458
Water metals									
Na	1	1.667	0.244	2	2.317	0.180	2	0.817	0.486
K	1	3.522	0.110	**2**	**38.544**	**< 0.001**	2	0.452	0.656
Ca	**1**	**32.139**	**0.001**	**2**	**17.736**	**< 0.001**	**2**	**8.732**	**0.017**
Mg	1	0.791	0.408	**2**	**16.046**	**< 0.001**	2	0.056	0.946
Al	**1**	**17.415**	**0.006**	2	3.168	0.115	**2**	**13.048**	**0.007**
As	**1**	**3.769**	**0.100**	2	2.385	0.173	2	0.538	0.609
B	**1**	**7.361**	**0.035**	2	1.994	0.217	2	1.994	0.217
Cd	1	1.000	0.356	2	1.000	0.422	2	1.000	0.422
Cr	**1**	**2.601**	**< 0.001**	**2**	**19.000**	**0.003**	**2**	**8.103**	**< 0.001**
Co	**1**	**12.462**	**0.012**	**2**	**15.346**	**0.004**	**2**	**20.192**	**0.002**
Cu	**1**	**35.306**	**0.001**	**2**	**15.755**	**< 0.001**	**2**	**8.869**	**0.016**
Fe	**1**	**11.306**	**0.015**	**2**	**17.447**	**0.003**	**2**	**5.854**	**0.039**
Pb	1	1.775	0.231	2	1.503	0.296	2	1.234	0.356
Li	**1**	**9.000**	**0.024**	2	3.000	0.125	2	3.000	0.125
Mn	**1**	**57.629**	**< 0.001**	**2**	**10.467**	**0.011**	**2**	**22.983**	**0.002**
Ni	**1**	**16.427**	**0.007**	**2**	**8.515**	**0.018**	2	0.444	0.661
P	**1**	**21.921**	**0.003**	**2**	**134.268**	**< 0.001**	**2**	**9.508**	**0.014**
Zn	**1**	**45.496**	**0.001**	**2**	**255.096**	**< 0.001**	**2**	**11.858**	**0.008**

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Variable	Days			Treatment			Days × treatment		
	Df	**F**	***P***	***df***	**F**	***P***	***df***	**F**	***p***
Plant metals									
	Plant part			**Treatment**			**Plant part × treatment**		
Na	2	0.290	0.750	2	3.032	0.062	4	1.497	0.226
Mg	**2**	**183.390**	**< 0.001**	2	0.951	0.397	4	2.145	0.098
P	**2**	**30.481**	**< 0.001**	**2**	**13.104**	**< 0.001**	4	1.785	0.156
K	**2**	**27.678**	**< 0.001**	**2**	**4.750**	**0.016**	4	1.707	0.173
Ca	**2**	**70.443**	**< 0.001**	2	0.314	0.732	4	2.596	0.055
B	**2**	**78.823**	**< 0.001**	2	1.840	0.175	4	1.407	0.254
Al	**2**	**130.488**	**< 0.001**	2	1.930	0.162	4	2.580	0.056
Cr	**2**	**71.737**	**< 0.001**	2	0.760	0.476	4	0.699	0.599
Mn	**2**	**79.409**	**< 0.001**	2	0.124	0.884	**4**	**3.403**	**0.020**
Fe	**2**	**125.520**	**< 0.001**	2	0.140	0.870	4	0.172	0.951
Co	**2**	**42.100**	**< 0.001**	2	0.329	0.722	4	0.928	0.460
Ni	**2**	**29.418**	**< 0.001**	2	0.495	0.614	4	0.780	0.547
Cu	2	1.971	0.156	**2**	**9.218**	**0.001**	4	1.452	0.240
Zn	**2**	**4.524**	**0.019**	**2**	**12.356**	**< 0.001**	4	2.541	0.059
As	**2**	**103.024**	**< 0.001**	2	0.346	0.710	4	0.663	0.622
Cd	**2**	**15.243**	**< 0.001**	2	0.758	0.477	4	2.095	0.105
Pb	2	1.744	0.191	2	2.668	0.085	4	0.941	0.453

Metals (i.e., Al (range 0.100–0.375 mg L^{-1}), As (range 0.002–0.005 mg L^{-1}), B (range 0.025–0.025 mg L^{-1}), Cd (range 0.001–0.001 mg L^{-1}), Cr (range 0.025–0.025 mg L^{-1}), Co (range 0.025–0.025 mg L^{-1}), Fe (range 0.339–0.534 mg L^{-1}), Li (range 0.001–0.001 mg L^{-1}), Mn (range 0.025–0.139 mg L^{-1}), Ni (range 0.025–0.025 mg L^{-1}) were below 1 mg L^{-1} across all treatments, before experiment. The control treatment had low metal concentrations compared to the control and high metal concentration treatments (*Fig. 2*). For example, the Mg was 6.5 mg L^{-1}, 109 mg L^{-1} and 218.5 mg L^{-1} in control, low and high concentration treatments, respectively, with similar patterns being observed for Na, K, Ca, Cu, P and Zn before the experiment (*Fig. 2*). At the end of the experiment, an increase in Al (range 0.531–1.111 mg L^{-1}), Fe (range 0.231–1.350 mg L^{-1}) and Mn (range 0.736–3.920 mg L^{-1}) concentrations was observed across all treatments. For Na, K, Mg, Cu, Pb, P and Zn there was a decrease in concentrations, except for Ca which increased among treatments (*Fig. 2*). The repeated measures ANOVA test, indicated significant ($p < 0.05$) differences for Ca, Al, AS, B, Cr, Co, Cu, Fe, Li, Mn, Ni, P and Zn before and after the experiment, with K, Ca, Mg, Cr, Co, Cu, Fe, Mn, Ni, P and Zn being significantly ($p < 0.05$) different among treatments (*Table 3*). Based on the Tukey's post hoc analysis, most of the variables were significantly different among the dependent variables ($p < 0.05$), with Cr (high vs low, $p = 0.109$), Co (high vs low, $p = 0.495$), Fe (high vs low, $p = 0.820$), Mn (high vs low, $p = 0.903$) and Ni (high vs low, $p = 0.926$) being not significantly different (see *Table A1*).

Fern plant Dryopteris pentheri metal concentrations

In the plant roots, Ca, Mg and K were found in high concentrations exceeding 2000 mg kg^{-1}, with relatively high concentrations in the low (i.e., Ca, Mg) and high (i.e., K) concentration treatments (*Fig. 3a*). Potassium, Na, Ca, Mg, Cu, Pb, P, and Zn were high in plant stems with the high concentration treatment (*Fig. 3b*). In the plant leaves, Na, K, Mg, Cu, Pb, P and Zn were high in the high-concentration treatment (*Fig. 3c*). Repeated measures ANOVA indicated significant ($p < 0.05$) differences in Mg, P, K, Ca, B, Al, Cr, Mn, Fe, Co, Ni, Zn, As and Cd among the different plant parts, with P, K, Cu

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and Zn being significantly ($p < 0.05$) different among treatments (*Table 3*). Based on Tukey's post hoc analysis, most of the metals were found to be significantly ($p < 0.05$) different among the different plant parts except Mg (roots vs stems, $p = 0.816$), P (leaves vs stems, $p = 0.222$), Ca (leaves vs roots, $p = 0.990$), B (roots vs stems, $p = 0.055$), Cr (leaves vs stems, $p = 0.917$), Ni (leaves vs stems, $p = 0.875$), As (leaves vs stems, $p = 0.801$), Cd (leaves vs stems, $p = 0.222$), Fe (leaves vs stems, $p = 0.961$), Co (leaves vs stems, $p = 0.873$), and Zn (leaves vs roots, $p = 0.086$; leaves vs stems, $p = 0.262$; roots vs stems, $p = 0.785$) (see *Table A1*).

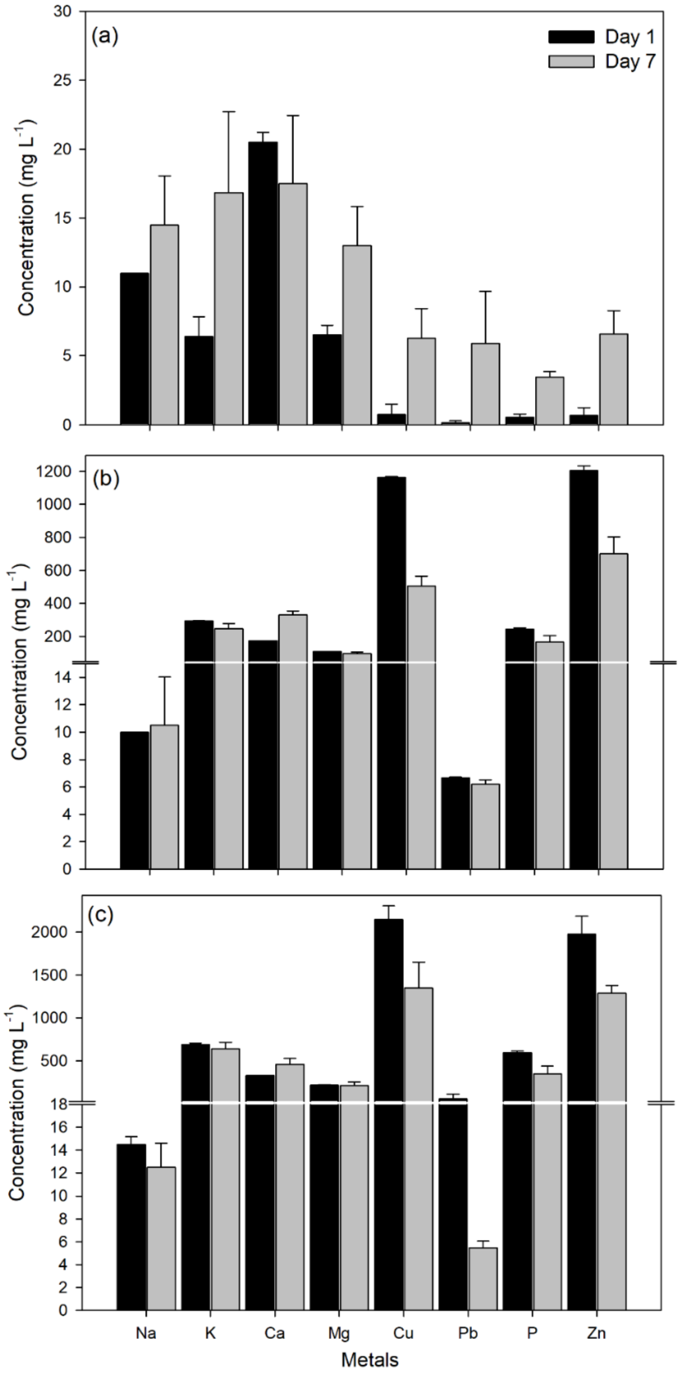

Figure 2. *Water metal variation (mean ± standard deviation) measured at the beginning and end of experiment: (a) control, (b) low and (c) high concentration treatment*

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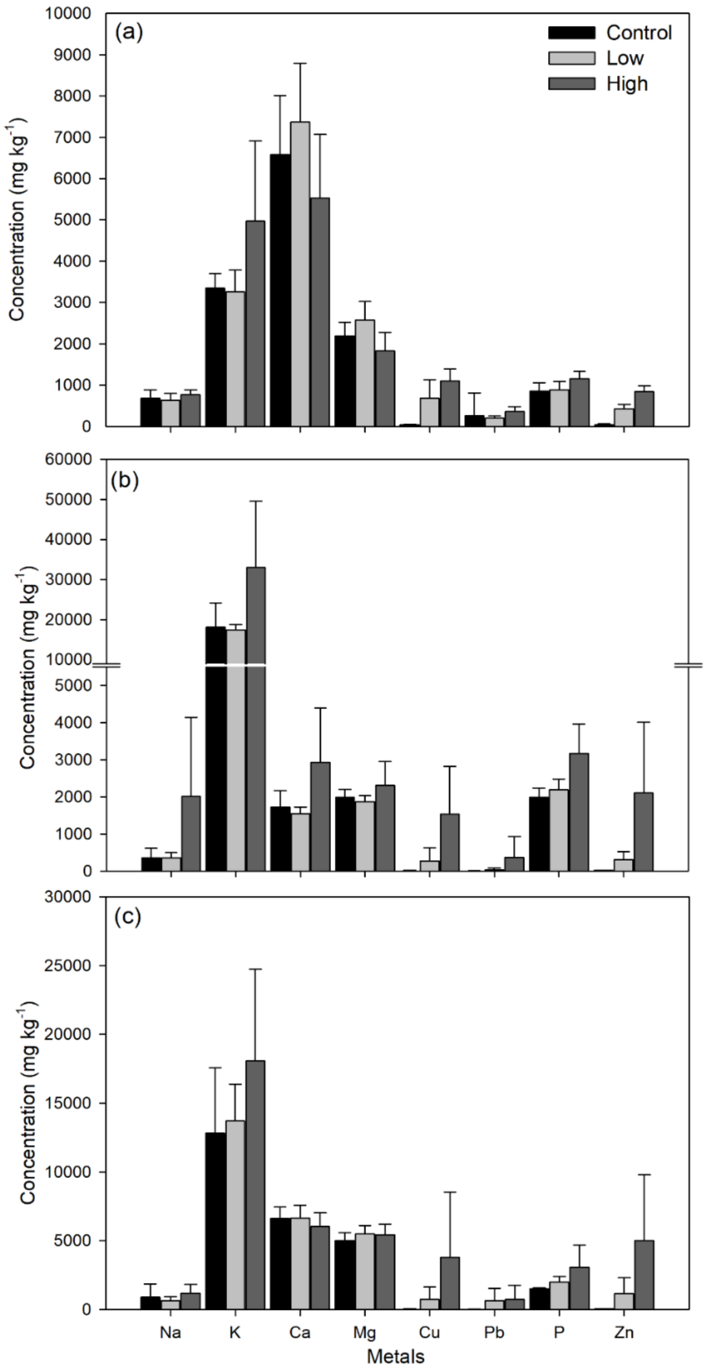

Figure 3. *Fern plant Dryopteris pentheri metal variation (mean ± standard deviation) measured at the end of experiment among different plant parts: (a) roots, (b) stems and (c) leaves*

Dryopteris pentheri leaf chlorophyll concentrations (F

The plant leaf chlorophyll concentrations in the control were 4.52 ± 3.39 SPAD units at day 1 before decreasing at day 3 (2.54 ± 1.49 SPAD units) and then increasing to day 7 (4.32 ± 2.83 SPAD units). In the low concentration treatment, chlorophyll concentrations decreased from day 1 (5.02 ± 3.85 SPAD units) to 7 (2.22 ± 0.95 SPAD units), with

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similar pattern being observed in the high concentration treatment, where concentration decreased from day 1 (2.88 ± 2.03 SPAD units) to day 3 (1.80 ± 0.76 SPAD units) and there was a slight increase at day 6 (1.96 ± 0.77 SPAD units) (*Fig. 4*). Repeated measures ANOVA indicated significant differences among days (F = 13.813, $df = 2$, $p < 0.001$) and treatments (F = 16.063, $df = 2$, $p < 0.001$). The Tukey's post hoc analysis indicated significant differences for day 1 vs 2 ($p < 0.001$) and day 1 vs 3 ($p < 0.001$), and furthermore for control vs high ($p < 0.001$) and low vs high ($p < 0.001$).

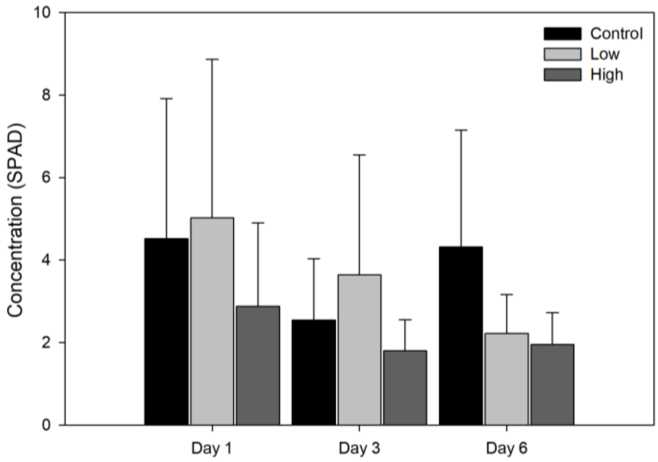

Figure 4. *Fern plant Dryopteris pentheri chlorophyll content measured over the course of the experiment*

Transportation index (translocation factors) of metals

The stem/root metal concentration index (T_i) indicated less ideal for phytostabilisation as most metal concentration values were less than 1, with the exception of K and B which were greater than 1, indicating that the fern plant *D. pentheri* could efficiently move these two metals from its roots to its shoots among all treatments (*Table 4*). Based on one-way ANOVA analysis, no significant ($p > 0.05$) differences were observed for the stem/root metal concentration index. The leaf/root metal concentration index (T_i) indicated that Na, Mg, B, Cu, Zn and Pb metals could be efficiently moved from the roots to the leaves as the value was greater than 1 across all treatments, with Ca being > 1 in the control treatment only. The high-concentration treatment generally had high values (*Table 4*). Based on one-way ANOVA, only Co (F = 11.111, $df = 2$, $p = 0.004$) was the only significantly different for the leaf/root metal concentration index among treatments, with the Tukey's post hoc indicating significant differences for control vs high ($p = 0.009$) and control vs low ($p = 0.008$).

Bioaccumulation factors

Al, Cr, As, Cd, Mn, Fe, Co, and Ni were hyperaccumulating in the roots > fronds > rhizomes in all treatments (*Table 5*). Lead in the control treatment was hyperaccumulating the roots, whereas in the low and high concentrations, it was hyperaccumulating in the fronds. Copper and Zn did not show accumulation in roots and rhizomes for low and high-concentration treatments. Trace metals (i.e., Na, Mg, K, Ca) were below 1, meaning that the fern plant *D. pentheri* was only able to absorb what was available in the water except for K in rhizomes and fronds of the control treatment that

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showed hyperaccumulation (*Table 5*). The low-concentration treatment had high BCF values compared to other treatments. Based on the two-way ANOVA, all metals except Na ($p = 0.057$), Cu ($p = 0.407$) and Pb ($p = 0.379$) were significantly different among fern plant *D. pentheri* parts. Among treatments Mg, K, Ca, Al, Mn, Cu, As and Zn were significantly ($p < 0.01$) different among the treatments (*Table 6*).

Table 4. *The stem/root and leaf/root concentration ratios (transportation index) of metals in fern plant Dryopteris pentheri grown for 7 days. Red – values < 1 = less ideal for phytoremediation, and green – values > 1 = plant could efficiently move these two metals from its roots to its shoots*

Metals	Stem/root			Leaf/root		
	Control	Low	High	Control	Low	High
Na	0.51 ± 0.18	0.55 ± 0.14	0.64 ± 0.18	1.17 ± 0.23	1.06 ± 0.33	1.51 ± 0.06
Mg	0.9 ± 0.14	0.73 ± 0.11	0.89 ± 0.16	2.31 ± 0.2	2.16 ± 0.2	2.56 ± 0.09
K	5.76 ± 1.71	5.44 ± 0.68	5.89 ± 0.89	3.96 ± 0.57	4.2 ± 0.26	5.05 ± 0.23
Ca	0.27 ± 0.07	0.21 ± 0.04	0.28 ± 0.03	1.02 ± 0.06	0.91 ± 0.08	0.92 ± 0.03
B	1.13 ± 0.2	1.33 ± 0.25	1.39 ± 0.14	3.66 ± 0.1	3.51 ± 0.3	3.72 ± 0.13
Al	0.07 ± 0.06	0.02 ± 0.01	0.01 ± 0	0.09 ± 0.02	0.07 ± 0.03	0.04 ± 0.03
Cr	0.06 ± 0.04	0.03 ± 0.02	0.02 ± 0	0.1 ± 0.02	0.1 ± 0.06	0.06 ± 0.02
Mn	0.15 ± 0.04	0.17 ± 0.05	0.21 ± 0.04	0.54 ± 0.14	0.75 ± 0.24	0.82 ± 0.02
Fe	0.06 ± 0.06	0.01 ± 0	0.01 ± 0	0.07 ± 0.01	0.06 ± 0.03	0.03 ± 0.02
Co	0.05 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.07	0.18 ± 0.05	0.19 ± 0
Ni	0.07 ± 0.04	0.05 ± 0.02	0.09 ± 0.05	0.12 ± 0.02	0.15 ± 0.05	0.17 ± 0.01
Cu	0.29 ± 0.37	0.46 ± 0.66	0.53 ± 0.05	1.07 ± 0.82	1.28 ± 1.62	2.6 ± 0.12
Zn	0.55 ± 0.45	0.81 ± 0.79	0.87 ± 0.15	1.54 ± 1.94	3.23 ± 4	5.42 ± 0.17
As	0.06 ± 0.04	0.03 ± 0.01	0.02 ± 0	0.13 ± 0.02	0.10 ± 0.04	0.08 ± 0.02
Cd	0.22 ± 0.06	0.19 ± 0.1	0.21 ± 0.04	0.12 ± 0.06	0.18 ± 0.1	0.24 ± 0.01
Pb	0.17 ± 0.14	0.2 ± 0.22	0.13 ± 0.08	1.04 ± 1.3	3.61 ± 4.28	2.77 ± 0.03

Discussion

The present study indicate that *Dryopteris pentheri* accumulated different metal concentrations in various plant parts. Moreover, bioaccumulation and translocation factors were greater than 1 for some selected metals in the roots, showing that *D. pentheri* is a hyperaccumulator and also showed metal tolerance ability due to the increased chlorophyll content under the high metal concentration over time.

Plants can accumulate metal levels differently in their different parts. The present study indicates that more metals were accumulated in the roots than in the fronds and rhizomes. The increased chlorophyll content as shows that *D. pentheri* is tolerant to the metal concentrations in the water. The current study demonstrated that most metals accumulated were remarkably increased in the roots, then in the aboveground plant parts. Numerous studies observed similar results (Eid et al., 2021; Kumar et al., 2019; Galal et al., 2018; Kumar et al., 2020). The bioaccumulation factors (BCF) are used to determine plant's ability to transport metals from soil or water to the roots (Eid et al., 2021). If BCF is greater than 1, then it means the plant can accumulate metals in the roots (Ang et al., 2023). The fronds/roots translocation factor (TF) showed that the plants can translocate the metals from the roots to the leaves in high concentrations. The TF value of

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rhizome/roots was less of phytostabilisation, with TF values being less than 1. As it was reported by Laptiev et al. (2024) that if the TF value is less than one then the plant is less for phytostabilisation.

The current study results indicated an increase of water parameters (conductivity, total dissolved solids and salinity) in high concentration and resistivity had increased in control. Similar results by Helard et al. (2021) reported that high metal concentrations had increased water parameters. Our study reported that metal accumulation by *D. pentheri* differed among the treatments. This suggests that it might be due to the solubility and mobility of the metals in the water (Munyai and Dalu, 2023). Trace metals such as Ca, Mg, Na and K were accumulated in roots and transferred to the shoots, irrespective of the treatments. Ferns typically have extensive root systems that enhance their capacity to absorb metals from the soil or water (Sharma and Sharma, 2022). Once absorbed, metals are transported to various plant tissues, where they may be sequestered in vacuoles or bound to metal-chelating compounds such as phytochelatins (Cobbett, 2000a, b). These compounds play a critical role in detoxifying metals and preventing their interference with essential cellular processes.

Table 5. *Comparison of metal accumulation in Dryopteris pentheri leaves, stems and roots across different treatments after 7 days. Red shade – values ≤ 1 = plant can only absorb metals in proportion to what is available in the water, and green shade – values > 1 = plant can accumulate more metals than are available in the water (i.e., hyperaccumulation)*

Variable	Control			Low			High		
	Roots	Stems	Leaves	Roots	Stems	Leaves	Roots	Stems	Leaves
Na	0.06 ± 0.01	0.03 ± 0.02	0.08 ± 0.08	0.06 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.03 ± 0.01	0.08 ± 0.04
Mg	0.33 ± 0.05	0.30 ± 0.03	0.77 ± 0.08	0.02 ± 0.004	0.02 ± 0.001	0.05 ± 0.01	0.01 ± 0	0.01 ± 0.001	0.02 ± 0.003
K	0.52 ± 0.05	2.91 ± 1.02	2.00 ± 0.74	0.01 ± 0.001	0.06 ± 0.004	0.05 ± 0.01	0.01 ± 0	0.03 ± 0.01	0.03 ± 0.01
Ca	0.37 ± 0.08	0.09 ± 0.02	0.37 ± 0.04	0.04 ± 0.01	0.01 ± 0.001	0.04 ± 0.01	0.02 ± 0.001	0.01 ± 0	0.02 ± 0.003
B	260.4 ± 46.0	291.5 ± 46.3	944.6 ± 269.3	260.8 ± 42	340.1 ± 26.2	906.8 ± 72.5	264.7 ± 32.9	369.2 ± 66.1	975.5 ± 186.6
Al	18947 ± 6720	1323 ± 1611	1760.3 ± 826.4	87836 ± 34636	1643 ± 720.5	6097 ± 1333	84573 ± 7332	1256 ± 286.9	3475 ± 1845
Cr	1200 ± 368.0	85.4 ± 88.9	111.4 ± 28.8	1496.5 ± 782.2	39.6 ± 22.1	111.3 ± 28.4	1172.3 ± 100.9	25.9 ± 6.1	75.1 ± 27.6
Mn	4328 ± 752.5	678.0 ± 147.1	2281 ± 468.3	22431 ± 7412	3661 ± 256.2	15646 ± 1931	16365 ± 1762	3478 ± 417.4	13487 ± 936.8
Fe	16488 ± 4518	977.6 ± 1422	1073 ± 413.5	28094 ± 12107	437.7 ± 180.3	1532 ± 355.5	23833 ± 945.2	333.3 ± 64.8	950 ± 445.4
Co	241.8 ± 157.7	10.9 ± 8.2	11.6 ± 1.4	187.9 ± 57.4	9.1 ± 2.5	31.9 ± 4.5	179 ± 11.2	10.4 ± 2.4	34 ± 9.8
Ni	506.1 ± 379.8	29.8 ± 16.5	53.6 ± 24.9	445.6 ± 172.6	22.8 ± 4.6	59.9 ± 4.4	382.1 ± 26.5	34.9 ± 19.7	65.5 ± 9.1
Cu	43.34 ± 18.2	12.7 ± 9.6	50.8 ± 60.6	0.5 ± 0.3	0.2 ± 0.3	0.6 ± 0.7	0.5 ± 0.1	0.3 ± 0.1	1.7 ± 2.2
Zn	60.6 ± 21.6	31.5 ± 6.6	89.7 ± 27.4	0.3 ± 0	0.2 ± 0.1	0.9 ± 0.9	0.4 ± 0	0.3 ± 0.1	2.5 ± 2.4
As	1298 ± 366.2	75.7 ± 65.7	162.5 ± 41.7	1594 ± 610.1	44.2 ± 12.3	147.4 ± 21.1	525.7 ± 17.8	11.7 ± 1.2	44.7 ± 11
Cd	81.2 ± 36.0	17.2 ± 6.1	9.5 ± 1.9	87.6 ± 32.3	15.0 ± 3.6	14.1 ± 4.1	76.4 ± 22	16 ± 2.3	17.8 ± 5.2
Pb	1755 ± 3717	20.6 ± 10.8	109.2 ± 116.2	1331 ± 382.4	256.3 ± 313.4	4433 ± 5943	5.8 ± 2.3	0.6 ± 0.3	14 ± 18.5

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Table 6. *Two-way analysis of variance of bioaccumulation factors for the different fern plant Dryopteris pentheri parts. Bold values indicate significance at p < 0.05*

Variable	Plant parts			Treatment			Plant parts × treatment		
	df	F	*p*	*Df*	F	*P*	*Df*	F	*P*
Na	2	3.197	0.057	2	0.049	0.953	4	0.128	0.971
Mg	**2**	**61.347**	**< 0.001**	**2**	**503.125**	**< 0.001**	**4**	**51.775**	**< 0.001**
K	**2**	**8.299**	**0.002**	**2**	**58.198**	**< 0.001**	**4**	**8.614**	**< 0.001**
Ca	**2**	**30.454**	**< 0.001**	**2**	**201.677**	**< 0.001**	**4**	**21.602**	**< 0.001**
B	**2**	**105.493**	**< 0.001**	2	0.291	0.750	4	0.194	0.939
Al	**2**	**99.039**	**< 0.001**	**2**	**16.914**	**< 0.001**	**4**	**14.559**	**< 0.001**
Cr	**2**	**62.656**	**< 0.001**	2	0.486	0.620	4	0.487	0.745
Mn	**2**	**59.375**	**< 0.001**	**2**	**69.423**	**< 0.001**	**4**	**10.158**	**< 0.001**
Fe	**2**	**93.310**	**< 0.001**	2	2.533	0.098	4	2.684	0.053
Co	**2**	**32.236**	**< 0.001**	2	0.172	0.843	4	0.599	0.667
Ni	**2**	**24.727**	**< 0.001**	2	0.151	0.860	4	0.224	0.923
Cu	2	0.930	0.407	**2**	**8.797**	**0.001**	4	0.945	0.453
Zn	**2**	**6.360**	**0.005**	**2**	**83.386**	**< 0.001**	**4**	**6.299**	**0.001**
As	**2**	**69.216**	**< 0.001**	**2**	**7.210**	**0.003**	**4**	**4.714**	**0.005**
Cd	**2**	**47.871**	**< 0.001**	2	0.085	0.918	4	0.217	0.926
Pb	2	1.006	0.379	2	1.912	0.167	4	1.407	0.258

Our current findings show that the chlorophyll content increased in the control group after a period of 7 days, confirmed by Drăghiceanu et al. (2021) who reported an increase in the chlorophyll content of *Dyropteris* sp. in control. The results of our study indicate that chlorophyll content decreased at low concentrations over time, while it increased slightly in both the control and high concentration groups after 7 days. In contrast, a study by Drăghiceanu et al. (2021) reported that the chlorophyll content of *Dryopteris affinis* and *Dryopteris filix-mas* has been reduced by high metal concentrations after long exposure to metals. In addition, various other studies including (Doganlar et al., 2012; Singh and Pandey, 2011; Shah et al., 2017) reported contradicting findings to the current study where they reported low metal concentrations stimulated the chlorophyll over time.

The results of the current study indicated that translocation factor (TF) values of rhizome/roots were less than 1, which suggests that D*ryopteris pentheri* is the metal excluder (Ang et al., 2023). A TF value of less than 1 might be due to the exclusion strategy and restriction of metal movement from the roots to the shoots (Pang et al., 2023). Additionally, plant physiology also plays an important role in excluding certain metals that are not required by the plant itself, thereby protecting the shoots (Netshiongolwe et al., 2020; Ang et al., 2023). *Dryopteri*s *pentheri*, being a metal excluder, has showed to have translocated only potassium (K) and boron (B) from roots to the rhizomes, which were explained by their TF values being greater than 1. Thus, indicates that *D. pentheri* the has a potential to remove K and B from the environment. The fronds/roots TF values of Na, Mg, B, Cu, Zn, Pb and Ca were more significant than 1, and our current study confirms that *D. pentheri* is a hyperaccumulator of these metals. *Dryopteris pentheri* showed the phytoaccumulation ability. The findings in the current study however confirms observation by Munyai and Dalu (2023) who reported that trace metals (Na, B, Cu and Zn) have increased from the roots to the fronds.

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The present study had high bioaccumulation factors (BCF), indicating the ability of the plant to hyperaccumulate metals such as Al, Cr, As, Cd, Mn, Fe, Co and Ni in the roots followed by fronds and then rhizomes among all concentrations. Our results reflect those reported by Cornara et al. (2007); Senila et al. (2013) and Singh et al. (2010) who reported that most ferns, such as *D. fillix-mas, Pteris cretica* and *P. vittata* are hyperaccumulators of As, Mn, Fe, Al and Cr than any other non-fern plants, and further mentioned that control stimulated the hyperaccumulation of lead (Pb) in the roots. Our present study indicates that K had BF > 1; this might suggest that K hyperaccumulation in the control concentration might have been influenced by As in the roots. Arsenic is known to induce growth stimulators such as K at low concentrations (Singh et al., 2010).

Conclusions

The study concludes that *Dryopteris pentheri* accumulated different metals in different parts (root, rhizome and fronds) at different concentration levels. Furthermore, the study indicates potential of removing and up taking metals from the aquatic environment. There is a high translocation of metals of some metals from the roots into the fronds. In addition, the results revealed that both BF and TF values were > 1, revealing that *D. pentheri* to hyperaccumulate metals, especially, As, Cr, As, Cd, Mn, Fe, Co and Ni among all treatments. Generally, *D. pentheri* indicates to be tolerant to the metals, as it was shown by the increase in chlorophyll content among the treatments. After assessing *D. pentheri* for phytoremediation, the results indicate that this species is a good candidate for hyperaccumulating the metals from an environment.

Author contributions. Conceptualization, E.K., C.M., T.D.; methodology, E.K., P.M., T.D.; validation, All authors; formal analysis, E.K., P.M., T.D.; investigation, E.K., P.M., L.F.M., T.D.; resources, E.K., C.M., F.D., T.D.; data curation, E.K., T.D.; writing—original draft preparation, E.K., T.D, L.F.M.; writing—review and editing, All authors; visualization, All authors; supervision, C.M., T.D.; project administration, T.D.; funding acquisition, E.K., F.D., T.D. All authors have read and agreed to the published version of the manuscript.

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Conflict of interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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APPENDIX

Table A1. *Tukey's post hoc analysis of water and plant material among different water treatments and plant parts*

Dependent variable			***P***	**Dependent variable**			***p***
Water treatment							
K	Control	High	**<0.001**	Fe	Control	High	**0.007**
	Control	Low	**<0.001**		Control	Low	**0.004**
	High	Low	**<0.001**		High	Low	0.820
Ca	Control	High	**<0.001**	Mn	Control	High	**0.014**
	Control	Low	**<0.001**		Control	Low	**0.023**
	High	Low	**0.001**		High	Low	0.903

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Mg	Control	High	**<0.001**	Ni	Control	High	**0.022**
	Control	Low	**0.001**		Control	Low	**0.035**
	High	Low	**<0.001**		High	Low	0.926
Cr	Control	High	**0.024**	P	Control	High	**<0.001**
	Control	Low	**0.002**		Control	Low	**0.001**
	High	Low	0.109		High	Low	**<0.001**
Co	Control	High	**0.004**	Zn	Control	High	**<0.001**
	Control	Low	0.015		Control	Low	**<0.001**
	High	Low	0.495		High	Low	**<0.001**
Cu	Control	High	**<0.001**				
	Control	Low	**<0.001**				
	High	Low	**<0.001**				
Plant parts							
Mg	Leaves	Roots	**<0.001**	Ni	Leaves	Roots	**<0.001**
	Leaves	Stems	**<0.001**		Leaves	Stems	0.875
	Roots	Stems	0.816		Roots	Stems	**<0.001**
P	Leaves	Roots	**<0.001**	Zn	Leaves	Roots	0.086
	Leaves	Stems	0.222		Leaves	Stems	0.262
	Roots	Stems	**<0.001**		Roots	Stems	0.785
K	Leaves	Roots	**0.001**	As	Leaves	Roots	**<0.001**
	Leaves	Stems	**0.008**		Leaves	Stems	0.801
	Roots	Stems	**<0.001**		Roots	Stems	**<0.001**
Ca	Leaves	Roots	0.990	Cd	Leaves	Roots	**<0.001**
	Leaves	Stems	**<0.001**		Leaves	Stems	0.222
	Roots	Stems	**<0.001**		Roots	Stems	**0.001**
B	Leaves	Roots	**<0.001**	Fe	Leaves	Roots	**<0.001**
	Leaves	Stems	**<0.001**		Leaves	Stems	0.961
	Roots	Stems	0.055		Roots	Stems	**<0.001**
Al	Leaves	Roots	**<0.001**	Co	Leaves	Roots	**<0.001**
	Leaves	Stems	0.939		Leaves	Stems	0.873
	Roots	Stems	**<0.001**		Roots	Stems	**<0.001**
Cr	Leaves	Roots	**<0.001**	Mn	Leaves	Roots	**<0.001**
	Leaves	Stems	0.917		Leaves	Stems	**<0.001**
	Roots	Stems	**<0.001**		Roots	Stems	**<0.001**
Plant treatments							
P	Control	High	**<0.001**	Zn	Control	High	**<0.001**
	Control	Low	0.574		Control	Low	0.524
	High	Low	**0.009**		High	Low	**0.017**
K	Control	High	**0.028**	Sb	Control	High	**<0.001**
	Control	Low	0.989		Control	Low	0.877
	High	Low	**0.032**		High	Low	**0.001**
Cu	Control	High	**0.002**				
	Control	Low	0.545				
	High	Low	**0.045**				

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