© 2025 IJNRD | Volume 10, Issue 4 April 2025 | ISSN: 2456-4184 | IJNRD.ORG

IJNRD.ORG ISSN : 2456-4184

INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Bio potential factors of the aquatic plant species Ceratophyllum submersum: BCF, BAF, MEF, and MTF

S.L.Jadhav[1] and M.G.Babare[2]
[1]Indian Institute of Food Science and Technology,
Chhatrapati Sambhajinagar -431005(India).
[2]Department of Environmental Science,
Dr.Babasaheb Ambedkar Marathwada University, Aurangabad-431004 (India).

Abstract:

Heavy metal contamination poses a significant environmental issue, arising from the release of toxic metals such as mercury, lead, cadmium, and arsenic into the environment due to human activities like mining, industrial operations, and agricultural methods. This type of pollution threatens both ecosystems and human health. It is crucial to comprehend the biological interactions of metals within plants to facilitate the phytoremediation process. Phytoremediation highlights the capacity of plants to absorb metals, their distribution within plant tissues, and their accumulation in various organs. This understanding is essential for selecting suitable macrophytes for treating metal-contaminated wastewater. Consequently, this study seeks to assess the biopotential factors, including Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF), Metal Enrichment Factor (MEF), and Metal Translocation Factor (MTF) of the submerged macrophyte species Ceratophyllum submersum through a hydroponic bioassay using a synthetic metal solution. The hydroponic bioassay was carried out from June 2018 to December 2022, exposing Ceratophyllum submersum to a mixed metal synthetic solution to evaluate its ability to absorb, transfer, accumulate, and enrich metals for phytoremediation purposes. The findings of this study highlighted the phytoremediation potential and accumulation patterns of ten heavy metals: As, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sr, and Zn across different parts of Ceratophyllum submersum. The BCF values for the analyzed metals are ranked as follows: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr. The MEF rankings mirror this order: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr. The BAF rankings for the metals studied are also consistent: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr, while the MTF is ranked as Fe > Zn > Cd > Mn > Co > Cu > Sr > Cr > Pb > As > Ni.

Key words:

Aquatic Macrophytes, *Ceratophyllum submersum*, Heavy Metals, Bioconcentration, Biomagnification, Metal Transfer, Metal Enrichment, Phytoremediation

1. INTRODUCTION:

Water pollution represents a significant health threat, particularly in developing countries, where around 80% of illnesses and deaths are attributed to this problem. The release of harmful pollutants in wastewater negatively impacts aquatic ecosystems and renders water sources unsafe for human use. A variety of dangerous substances, such as pharmaceuticals, pesticides, surfactants, acids, detergents, dyes, and toxic metals, have contaminated water supplies, posing ecological risks to humans, plants, and animals (Vardhan et al., 2019). Heavy metals (HMs) are identified as major contributors to global water pollution (Zaimee et al., 2021). Given the rising need for clean and safe drinking water, it is crucial to investigate solutions and technologies that enable effective wastewater treatment for reuse.

Heavy metals like cadmium (Cd), chromium (Cr), cobalt (Co), arsenic (As), zinc (Zn), strontium (Sr), and copper (Cu) are commonly linked to environmental pollution and toxicity, especially in their dissolved states. High levels of these heavy metals can significantly threaten natural ecosystems and human health. Their toxic properties can undermine various environmental benefits. Furthermore, heavy metals are resistant to biodegradation, making their removal more challenging. Consequently, it is essential to monitor, comprehend, manage, and remediate the pollution caused by these heavy metals.

Bioremediation is a modern technological method that employs microorganisms to either remove or stabilize waste materials (Shanahan, 2004). This technique plays a crucial role in the cleanup of groundwater, soils, lagoons, sludge, and various waste streams. Essentially, bioremediation utilizes biological treatments, primarily through microbial action, to tackle hazardous pollutants found in soil and both surface and subsurface waters. These microorganisms aid in transforming harmful substances into less toxic forms. As a pollution management strategy, bioremediation harnesses biological systems to facilitate the breakdown, transformation, or removal of a wide range of chemicals into safer alternatives (Atlas, 1995). In recent years, industrialized nations have increasingly emphasized environmental restoration, particularly focusing on preserving water quality and rehabilitating contaminated surface and groundwater (Brierly, 1991). As a result, bioremediation has become a cost-effective approach to improving environmental quality. Its economic benefits, especially when compared to chemical and physical treatment methods for dilute contaminants, significantly contribute to its growing adoption. While the primary focus has been on treating industrial effluents to remove organic pollutants, there has been a notable shift towards addressing metal contaminants in the past two decades (Brierly, 1991). The escalating levels of environmental pollution caused by heavy metals, due to their increased use in industrial processes, present serious challenges to human health and aquatic ecosystems. Bioremoval processes provide significant advantages, including their efficiency in substantially reducing heavy metal ion concentrations and their use of low-cost biosorbent materials (Wild and Bennemann, 1993).

Plants are essential components of ecosystems, as they enable the transfer of elements from the non-living environment to the living one (Martinez-Lopez et al., 2014). Aquatic macrophytes, in particular, have a remarkable ability to absorb metals from water, often achieving internal concentrations that can be several times greater than those present in their environment. These plants can accumulate heavy metals at concentrations up to 100,000 times higher than those in the surrounding water (Misshra and Tripathi, 2008). Although bioremediation holds significant promise for tackling complex environmental challenges, it is important to recognize that much of this potential has yet to be realized (Shingadgaon et al., 2018; Thete-Jadhav et al., 2018; Dashpute et al., 2018; Shingadgaon and Chavan, 2019). There remains a considerable gap in our understanding of the interactions between microorganisms and different hydrologic environments. As we enhance our knowledge of these interactions, the effectiveness and applicability of bioremediation are anticipated to improve substantially. This study seeks to assess the heavy metal absorption capabilities of three distinct hydrophytes, with a particular emphasis on evaluating the biopotential of Ceratophyllum submersum, a submerged macrophyte, in relation to MCF, MEF, MTF, and BAF.

2. MATERIALS AND METHODS:

A. Overview of Ceratophyllum submersum Macrophyte:

The genus *Ceratophyllum* is derived from the Greek words "keras," meaning 'horn,' and "phyllon," which translates to 'leaf,' highlighting the horn-like appearance of its leaf branches. *Ceratophyllum submersum*, commonly known as soft hornwort, is an aquatic plant that is submerged and free-floating, thriving in still or slow-moving waters. This species is notable for its lack of true roots and is well-suited to eutrophic conditions characterized by low light and high nutrient availability. Also referred to as coontail, *Ceratophyllum submersum* is recognized for its potential in phytoremediation, particularly in the extraction of heavy metals and other pollutants from aquatic environments. It is part of the *Ceratophyllaceae* family and is native to temperate regions, often found in ponds, lakes, and ditches. As a hydroannual or hydrosubshrub, it completes its life cycle entirely in aquatic settings, demonstrating continuous growth without a dormant phase when conditions are favorable. *Ceratophyllum submersum* (L.), also known as coontail and previously called 'honewort,' thrives just beneath the surface of shallow, calm, or slow-moving waters, as indicated in the BSBI List (2007). This species can flower while fully submerged and belongs to a unique group of two species that lack close relatives in the plant kingdom, making them potential candidates for classification as 'living fossils.' As a member of the *Ceratophyllum* genus and the *Ceratophyllaceae* family, coontail or honewort can grow rapidly, reaching heights of 1-4 inches (3-10 cm) per week when provided with adequate light and nutrients. The plant's stems typically branch three to four times, with the tips featuring 6 to 8 threadlike extensions (Goulder and Boatman, 1971).

Ceratophyllum submersum (L.) flourishes in shallow, submerged waters, often found in coastal or hard water habitats. It tends to be particularly abundant in local areas from May through September. This species is also popular as an aquarium plant, commonly known as tropical or spineless hornwort. It is a fully aquatic plant that exists entirely underwater and does not possess true roots. The plant showcases mid to dark green whorls of slender, repeatedly forked leaves that are arranged along the main stem, giving it an attractive feathery appearance, although the leaves are actually stiff and brittle, which contrasts with its common name. Small, subtle green flowers can be found in the leaf axils.

Under optimal light conditions, this plant can grow swiftly, forming dense clusters. It promotes its growth by releasing chemicals that suppress the growth of other aquatic plants, including algae, which can otherwise obscure the water and hinder sunlight penetration. The species has distinct male and female plants. During the summer, pollen-laden anthers from the male plants detach and float just below the water's surface, where they may encounter the stigmas of female plants, resulting in the production of fertile seeds that can be dispersed by waterfowl. However, vegetative reproduction is crucial, as flowers do not always yield mature seeds. The stems are fragile and can readily regenerate from broken pieces. In autumn, the vegetative structures die back, but not before specialized buds form, which detach and sink to the bottom, poised to initiate growth in the subsequent year (Smith and Wolfe-Murphy, 1991).

Ceratophyllum submersum (L.) is an aquatic species found in various regions, which has probably lost much of its significant potential over the years. It is capable of flourishing in deeper waters during the winter months and can reproduce both asexually via damaged or intact stems and sexually with other plants. This species displays strong biological resilience and exhibits relatively fast growth rates. Its concerning influence on aquatic ecosystems promotes its growth by changing nutrient distribution, while also enabling faster spread across water bodies, resulting in heightened competition with less competitive organisms.

B. Collection and Sampling of Ceratophyllum submersum:

Ceratophyllum submersum (L.) specimens were collected from their natural habitats, where they serve phytoremediation functions. Surveys were carried out in both natural and artificial aquatic settings, including stagnant and flowing waters, as well as reservoirs, to identify the presence of *Ceratophyllum submersum*. The macrophytes were meticulously observed, sampled, and gathered from the Marathwada study area, ensuring no physical harm was inflicted. Sampling adhered to established scientific protocols and involved rinsing the specimens with water. The collected samples were fresh and green, stored in appropriately sized transparent polyethylene bags or jars. Each sample consisted of at least ten healthy macrophytes. These were replanted within four to five hours of collection to acclimate to metal-mixed synthetic wastewater in rectangular test chambers measuring 1 meter by 1 meter and 10 cm in depth, with a maintained freeboard of 2 cm.

Furthermore, an additional set of samples was taken to the laboratory, where they were rinsed with gently flowing tap water while adhering to necessary safety measures, air-dried, and identified using various literature sources for qualitative floristic data. This identification process focused on species and community characteristics rather than solely on their physical structures and appearances. Photographs and samples were forwarded to botanical research experts in the relevant fields for verification and confirmation of the identified information, including critical details, prior to reaching final conclusions, thereby ensuring an authentic and supportive second opinion. The choice of the macrophyte *Ceratophyllum submersum* for this study was influenced by its local prevalence, allowing for collection from any area within the study region for further examination.

C. Investigation of Metal Accumulation Potential:

Healthy specimens of *Ceratophyllum submersum* macrophytes were selectively pruned by removing those that exhibited signs of infection or poor health. The remaining viable specimens were subsequently relocated to a laboratory-scale testing environment. These testing environments, constructed from small plastic tanks, were specifically designed to assess the adaptability of the macrophytes to altered environmental conditions. The selected healthy macrophytes were subjected to local climatic conditions for an adequate duration to ensure they acclimatized and achieved full growth in a synthetic wastewater test bath containing metals. The acclimatization period was set to one month. Following this phase, growth was monitored for an additional month in a synthetic wastewater test bath infused with metals, utilizing stock solutions at a concentration of 100 ppm. The bath solution was freshly prepared to contaminate tap water, allowing for the periodic creation of the synthetic wastewater test bath, alongside a control group that did not include synthetic wastewater. Various metal salts, such as $Na_3AsO_4 \cdot 12H_2O$, $CuSO_4 \cdot 8H_2O$, PbO, $FeSO_4 \cdot 7H_2O$, $CdSO_4 \cdot 8H_2O$, $NH_4SO_4 \cdot NiSO_4 \cdot 6H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $ZnSO_4 \cdot 7H_2O$, $Sr(OH)_2$, and $MnCl_2 \cdot 4H_2O$, were employed in accordance with established standard procedures (AOAC, 1975; APHA, 1980; Echem, 2014; Smith, 1983). After the designated growth period, the macrophyte plants were collected and analyzed to determine their capacity for metal accumulation. Adjustments were made to incorporate the findings from the control group, which utilized standard water. This evaluation aimed to determine their viability and potential for use in phytoremediation efforts. The detailed methodology used to assess the absorption capabilities of heavy metals is outlined below:

The plant materials of Ceratophyllum submersum were initially rinsed with tap water before being transported for laboratory analysis. Following this, they underwent a second wash with gently flowing tap water, and were subsequently rinsed with double distilled water to eliminate any remaining dirt and contaminants. The macrophyte samples were air-dried and then sectioned using stainless steel scissors to separate the shoots, roots, stems, and leaves. Each part was dried in an oven at 60 °C until a consistent weight was achieved, after which they were ground with a pestle and mortar to create a homogeneous mixture, which was then stored for future analysis. To determine metal content, 500 mg of each plant material was digested using H_2O_2 and H_2SO_4. The resulting digested aliquot was analyzed for various heavy metals employing suitable methodologies. The heavy metals from the prepared aliquot were assessed using Atomic Absorption Spectroscopy (AAS) and Gas Chromatography (GC) in different laboratories, adhering to sample and metal-specific protocols, or through appropriate methods referenced from credible literature or research publications, depending on the availability of necessary analytical resources. The analytical techniques utilized included a variety of methods, such as the cobalt analysis via the cobaltous pyridine method

International Research Journal
Research Through Innovation

as outlined by Nicolaysen (1941), iron (Fe) analysis using the Dichromate method, and zinc (Zn) evaluation through the EDTA Complexometry-Back Titration method (Tazul and Ahemad, 2013).

Manganese (Mn) was measured using Volhard's method, while Copper (Cu) was determined through Sodium Thiosulphate titration, with validation provided by the Spectrophotometric method (Ahmed and Zannat, 2012). Lead (Pb) was assessed using the EDTA Complexometric method, and Manganese (Mn) was further analyzed by the Periodate Oxidation Method. Chromium was evaluated through the Diphenylcarbazide Spectrophotometric method (IBM, 2012), and Cadmium (Cd) was investigated using a spectrophotometric technique (Ahamed and Chowdhury, 2004). Additionally, Chromium (Cr) was also analyzed via the Diphenylcarbazide Method (Yarbro, 1976), and Cobalt (Co) was examined using a colorimetric method (Hobart, 1920).

A series of samples were processed and digested using an automated program within the NuWav-Ultra Microwave Digestion Extraction System. The concentrations of metals, including As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, and Zn, were analyzed in a separate laboratory employing the Shimadzu Atomic Absorption Spectrometer 6300 model and the Agilent 725 ICP-OES instrument for necessary confirmation. Most results were validated through random cross-checks against established standard methods to ensure analytical accuracy and reliability, as referenced in various studies (Bendix and Grabenstetter, 1943; Kimura and Murakani, 1951; Sandall, 1965; Hackley et al., 1968; Loftberg, 1969; Rubeska, 1969; Baker et al., 1971; James and MacMohan, 1971; Song et al., 1976; Sarma et al., 2005; Soomro and Menon, 2009; Ahemad and Roy, 1969; Soomro and Shar, 2014; Wei, 2014; Shingadgaon and Chavaan, 2019).

D. Chemicals and Reagents:

All chemicals, reagents, and solvents utilized in this research were of analytical reagent grade or possessed the highest levels of purity, and they were prepared fresh prior to use. Doubly distilled water was consistently employed throughout the experimental procedures. The glassware underwent thorough cleaning by soaking in acidified solutions of potassium permanganate (KMnO4) or potassium dichromate (K2Cr2O7), followed by treatment with concentrated nitric acid (HNO3) and several rinses with doubly distilled water. Calibration curves, which served as standard references for solutions with known metal concentrations, were established. These curves were subsequently used to ascertain the concentrations of substances in unknown samples. The calibration curves for heavy metals were specifically applied in the analysis of concentrations within the test samples.

E. Assessment of Factors Affecting Heavy Metal Mobility Potential:

The study investigated the ability of *Ceratophyllum submersum* macrophytes to facilitate the transfer of heavy metals from contaminated substrates into their root systems, as well as their capability to accumulate these metals in different plant tissues. This evaluation included the transport of metals from the roots to the above-ground, harvestable parts of the plant and analyzed the potential for metal accumulation. A variety of metrics were employed in this assessment, including the bio-concentration factor (BCF), bioaccumulation factor (BAF), metal translocation factor (MTF), and metal enrichment factor (MEF). These indicators were utilized to evaluate the potential of *Ceratophyllum submersum* macrophytes for phytoremediation, offering significant insights into their effectiveness in restoring metal-contaminated environments.

I. Bioconcentration Factor (BCF):

The bioconcentration factor (BCF) is defined as the ratio of the total concentration of metals in plant roots to that in the surrounding environment, which may include contaminated soil or wastewater (Elkhatib et al. 2001; Gonzalez & Gonzalez-Chavez 2006; Yoon et al. 2006). The BCF quantifies the degree to which a chemical, particularly heavy metals, accumulates within an organism or biological system from its environment. This factor indicates the ability of plants to absorb metals from soil or water (Kamari et al., 2014). The BCF is calculated using the following formula:

BCF is defined as the ratio of the concentration of a heavy metal within an organism to its concentration in the surrounding water. The bioconcentration factor (BCF) for macrophytes was assessed using the formula established by Demina et al. (2009).

B.C.F = Concentration of the element in the plant / Concentration of the element in the water

To calculate the bio-concentration of a particular metal, the following formula is applied:

BCFMetal = [Metal Concentration in root] / [Metal Concentration in Source]

= CR/CWW

In this formula, BCFMetal represents the bioconcentration factor for the specific metal, CR denotes the concentration of that metal in the root, measured in mg/kg, while CWW indicates the concentration of the metal in the growth medium, such as wastewater, also measured in mg/kg. In this context, "metal" encompasses any of the eleven metals that were analyzed.

II. Metal Enrichment Factors (MEF):

Metal Enrichment Factors (MEF) were employed to assess the degree of metal contamination in sediment, adhering to the methodology outlined by Buat-Menard and Chesselet (1979). A commonly accepted approach for evaluating anthropogenic impact involves the calculation of a normalized enrichment factor (EF), which is derived from metal concentrations measured against uncontaminated background levels, as referenced by Salomons and Forstner (1984), Dickinson et al. (1996), and Hornung et al. (1989). The purpose of calculating the EF is to reduce variability in metal concentrations that may result from different source ratios, thereby serving as an effective analytical instrument. This technique normalizes the observed concentrations of heavy metals relative to a reference metal, such as iron (Fe) or aluminum (Al), as detailed by Ravichandran et al. (1995).

MEFMetal = [Metal content in only shoot] / [Metal Concentration in Source]

= COS/CWW

In this formula, MEFMetal represents the specific metal enrichment factor, COS refers to the designated metal content in the shoot, measured in mg/kg, while Cww indicates the metal concentration in the environmental sources, such as wastewater, also measured in mg/kg.

III. Bioaccumulation Factor (BAF):

The bioaccumulation factor (BAF) quantifies the degree to which a substance, particularly a heavy metal, accumulates within an organism or biological system. In relation to heavy metals, the BAF is defined as the ratio of the concentration of the heavy metal present in the organism to its concentration in the surrounding environment.

The Bioaccumulation Factor (BAF) is determined using the following formula:

BAF = [Concentration of heavy metal in aerial parts] / [Concentration of heavy metal in the source]

For a specific metal, it can be expressed as:

BAFMetal = [Metal content in aerial parts] / [Metal Concentration in Source]

= CAP/CWW

In this formula, BAFMetal represents the bioaccumulation factor for a specific metal, where CAP signifies the concentration of that metal in the aerial parts, measured in mg/kg, and CWW refers to the metal concentration in the growth medium, such as wastewater, also measured in mg/kg.

IV. Metal Translocation Factor (MTF):

The metal translocation factor (MTF) is defined as the ratio of the total metal concentration found in the shoots to that in the roots (Mocko & Waclawek 2004; Yoon et al. 2006; Sanghamitra et al. 2012). This factor reflects the relative concentration of metals in the shoots in comparison to the roots. An MTF value greater than 1 indicates that the plant is effective in moving metals from the root system to the shoots (Rezvani and Zaefarian, 2011). The metal transfer factor is an important measure for evaluating the mobility of metals from their origin to macrophytes. The MTF for specific metals can vary considerably based on the species of macrophytes and the environmental conditions present. Key factors influencing MTF include the physical and chemical properties of the source, the behavior of trace metals in both the source and the macrophytes, and variations in environmental conditions. The transfer factor from soil to plants is determined by calculating the ratio of metal concentration in the plants to that in the source (Kumar et al., 2015; Akande and Ajayi, 2017; Ogoko, 2015).

MTFMetal = [Metal Content in shoots] / [Metal Concentration in roots]

= COS/CR

In this equation, MTFMetal represents the specific metal translocation factor, Cs signifies the metal content in the aerial parts measured in mg/kg, and CR denotes the metal concentration in the roots, also expressed in mg/kg. This is commonly referred to as the shoot-root quotient and is typically represented as MTF.

RESULTS AND DISCUSSIONS:

Wastewater contains a variety of pollutants from diverse sources, such as industrial discharges, trade effluents, and municipal sewage, which ultimately lead to the contamination of water bodies. Among these pollutants, both organic and inorganic compounds, particularly heavy metals, present significant threats to aquatic ecosystems and human health due to their potential to enter the food chain through bioaccumulation. High levels of trace metals in plant tissues can adversely affect animals, especially when ingested. For instance, while zinc and copper are trace metals that are vital for healthy growth in small quantities, excessive concentrations can lead to phytotoxicity in plants and zootoxicity in animals (Osundiya et al., 2014; Shingadgaon and Chavan, 2019).

The ability of macrophytes to absorb heavy metals from contaminated substrates into their roots, as well as their potential to accumulate these metals in different plant parts and transport them from roots to the harvestable aerial sections, was evaluated using the bioconcentration factor (BCF), bioaccumulation factor (BAF), metal translocation factor (MTF), and metal enrichment factor (MEF). This assessment seeks to explore the viability of employing native macrophyte species for phytoremediation, offering valuable insights for the cleanup of metal-contaminated environments.

Heavy metals have become widespread pollutants worldwide, leading to significant environmental challenges due to their persistence in non-degradable forms. These metals can negatively impact ecosystems by infiltrating the food chain, thereby presenting various health risks to humans (Chopra et al., 2009; Roberts, 1999; WHO, 2011). Their presence can be identified in contaminated aquatic environments, ranging from sediments at the bottom to surface waters. The behavior of heavy metals in both water and wastewater is influenced by several factors, such as sediment composition, water chemistry, salinity, redox potential, and pH levels (Connell et al., 1984). This situation highlights the necessity of utilizing submerged macrophytes for the remediation of water and wastewater at varying depths.

A. Metals Uptake Potentials of Ceratophyllum submersum:

i. Metal concentrations in plant parts:

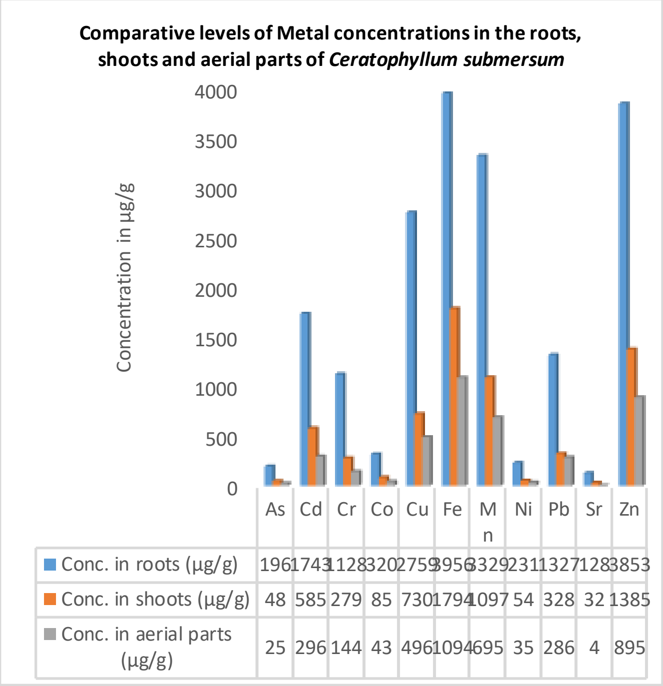
The aquatic macrophyte *Ceratophyllum submersum* exhibited variations in metal concentrations, reflecting its differing capacities to absorb various metals, a finding consistent with previous research (Freitas et al. 2004; Nouri et al. 2009; Nazareno & Buot 2015; Jones and Inocencio, 2017). Table 1 illustrates the metal concentrations detected in the roots, shoots, and aerial parts of *Ceratophyllum submersum*, providing a comparative analysis of these levels within the submerged macrophyte. The roots showed the highest concentration of iron (Fe) at 3956 µg/g, followed by zinc (Zn) at 3853 µg/g, manganese (Mn) at 3329 µg/g, and copper (Cu) also at 3329 µg/g. Lead (Pb) was measured at 1327 µg/g, cadmium (Cd) at 1743 µg/g, chromium (Cr) at 1128 µg/g, cobalt (Co) at 320 µg/g, nickel (Ni) at 231 µg/g, arsenic (As) at 196 µg/g, and the lowest concentration of strontium (Sr) at 128 µg/g. The hierarchy of metal accumulation in the roots of *Ceratophyllum submersum* was determined to be Fe > Zn > Mn > Cu > Pb > Cd > Cr > Co > Ni > As > Sr.

Table 1: Levels of metal concentrations found in the roots, shoots, and aerial parts of the submerged macrophyte species *Ceratophyllum submersum*, as recorded during the phytoremediation bioassay.

Metal	**Conc. in roots (µg/g)**	**Conc. in shoots (µg/g)**	**Conc. in aerial parts (µg/g)**
As	196	48	25
Cd	1743	585	296
Cr	1128	279	144
Co	320	85	43
Cu	2759	730	496
Fe	3956	1794	1094
Mn	3329	1097	695
Ni	231	54	35
Pb	1327	328	286
Sr	128	32	4
Zn	3853	1385	895

In contrast, the concentration of metals in the shoots was consistently lower than that observed in the roots across all metals evaluated. The highest concentration of Iron (Fe) in the shoots was 1794 µg/g, followed by Zinc (Zn) at 1385 µg/g. Manganese (Mn) was measured at 1097 µg/g, while Copper (Cu) reached 730 µg/g. Other metals detected in the shoots included Cadmium (Cd) at 585 µg/g, Lead (Pb) at 328 µg/g, Chromium (Cr) at 279 µg/g, Cobalt (Co) at 85 µg/g, Nickel (Ni) at 54 µg/g, and Arsenic (As) at 48 µg/g. The metal with the lowest concentration was Strontium (Sr) at 32 µg/g. The pattern of metal accumulation in the shoots differed from that in the roots, following this order: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr.

The accumulation of metals in parts other than the roots and shoots is categorized as aerial parts. The concentrations of metals in the aerial parts of *Ceratophyllum submersum* were found to be lower than those in the roots and shoots, as depicted in Fig. 1. Among the metals, Iron (Fe) exhibited the highest concentration in the aerial parts, recorded at 1094 µg/g, followed by Zinc (Zn) at 895 µg/g. Both Manganese (Mn) and Copper (Cu) were measured at 695 µg/g, while Cadmium (Cd) was at 296 µg/g, Lead (Pb) at 286 µg/g, Chromium (Cr) at 144 µg/g, Cobalt (Co) at 43 µg/g, Nickel (Ni) at 35 µg/g, Arsenic (As) at 25 µg/g, and Strontium (Sr) at a minimal 4 µg/g. The order of metal accumulation in the aerial parts of *Ceratophyllum submersum* is as follows: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr.



	As	Cd	Cr	Co	Cu	Fe	Mn	Ni	Pb	Sr	Zn
🟦 Conc. in roots (µg/g)	196	1743	1128	320	2759	3956	3329	231	1327	128	3853
🟧 Conc. in shoots (µg/g)	48	585	279	85	730	1794	1097	54	328	32	1385
⬜ Conc. in aerial parts (µg/g)	25	296	144	43	496	1094	695	35	286	4	895

Figure 1: Comparison of metal concentration levels in the roots, stems, and above-ground parts of the submerged macrophyte species Ceratophyllum submersum.

ii. Biopotential Factors:

The Metal Transport Factor (MTF) refers to a plant's intrinsic ability to move metals, as described by Nouri et al. (2009). Yoon et al. (2006) emphasize that both the Bioaccumulation Factor (BCF) and MTF are critical for assessing a plant's efficiency in metal phytore mediation. The BCF measures the extent to which a plant can accumulate metals in its roots, while the MTF evaluates the plant's ability to transfer these metals from the roots to its aerial parts. Plants with BCF values below one are deemed ineffective for phytoextraction (Yoon et al. 2006). Conversely, plants that exhibit both BCF and MTF values exceeding one (BCF>1, MTF>1) are considered appropriate for phytoextraction. Additionally, plants with a BCF greater than one and an MTF less than one (BCF>1 and MTF<1) are recognized for their potential in phytostabilization. A hyperaccumulator plant is characterized by having either a BCF or MTF greater than one, along with total metal accumulation exceeding 1000 mg kg−1 for Cu, Co, Cr, or Pb, or surpassing 10000 mg kg−1 for Fe, Mn, or Zn (Kabata-Pendias 2011).

The bioconcentration factor (BCF) is a widely used indicator for assessing the bioaccumulation of heavy metals (Parkerton et al. 2008). This study aims to investigate the bio-factors, including bioconcentration, bioaccumulation enrichment, and metal transfer of heavy metals in the macrophyte *Ceratophyllum submersum*. In this research, we calculated the bioconcentration factor, metal enrichment factor, bioabsorption factor, and metal transfer factor for each metal analyzed in the submerged aquatic plant *Ceratophyllum submersum*, as presented in Table 2. A comparative overview of BCF, BAF, MEF, and MTF in *Ceratophyllum submersum* is illustrated in Fig. 2.

Table 2: The Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF), Metal Enrichment Factor (MEF), and Metal Translocation Factor (MTF) recorded in the submerged macrophyte species *Ceratophyllum submersum* throughout the phytoremediation bioassay.

Metal	BCF	MEF	BAF	MTF
As	1.96	0.48	0.25	0.2449
Cd	17.43	5.85	2.96	0.3356
Cr	11.28	2.79	1.44	0.2473
Co	3.20	0.85	0.43	0.2656
Cu	27.59	7.30	4.96	0.2646
Fe	39.56	17.94	10.94	0.4531
Mn	33.29	10.97	6.95	0.3295
Ni	2.31	0.54	0.35	0.2338

International Research Journal
IJNRD
Research Through Innovation

Pb	13.27	3.28	2.86	0.2472
Sr	1.28	0.32	0.04	0.2500
Zn	38.53	13.85	8.95	0.3595

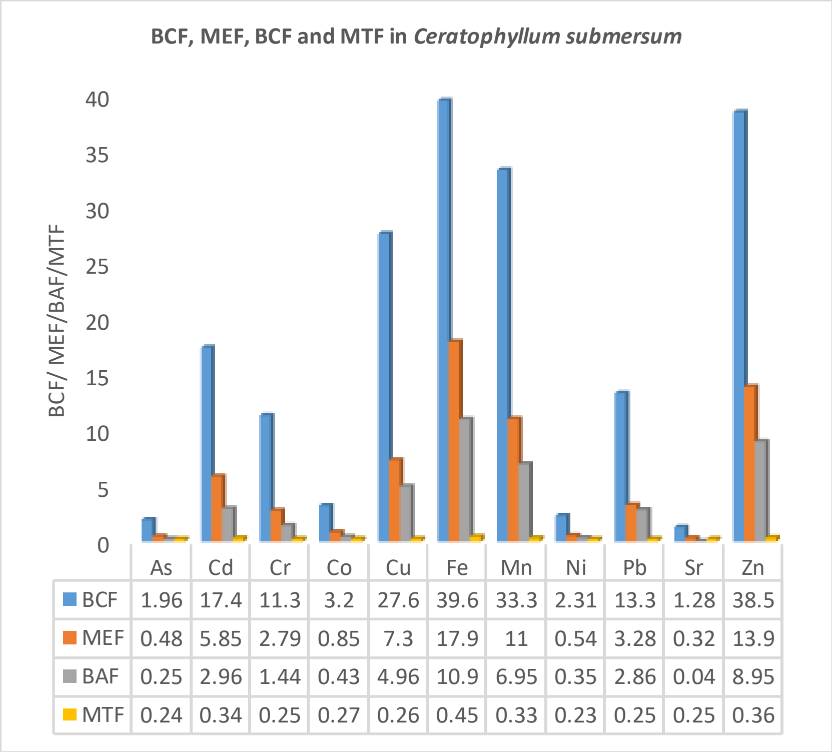


Figure 2: Comparison of BCF, BAF, MEF, and MTF in Ceratophyllum submersum.

The bioconcentration factor (BCF) for iron (Fe) was measured at 39.56, making it the highest among the metals examined. Following iron, zinc (Zn) recorded a BCF of 38.53, manganese (Mn) was at 33.29, copper (Cu) at 27.59, cadmium (Cd) at 17.43, lead (Pb) at 13.27, chromium (Cr) at 11.28, cobalt (Co) at 3.20, nickel (Ni) at 2.31, arsenic (As) at 1.96, and strontium (Sr) at 1.28. A graphical representation of this comparison can be found in Fig. 3.

International Research Journal
IJNRD
Research Through Innovation

Iron is a crucial micronutrient vital for the metabolic processes in plants (Kumari and Tripathi 2015). The typical manganese concentration in plant tissues ranges from 10 to 25 mg/kg (Parzych et al. 2016), which satisfies the physiological needs of most plant species. Levels above this range are often deemed toxic to various plants (Bonanno and Lo Giudice 2010, Bonanno 2013). Nevertheless, no phototoxic effects were detected in this study. The order of bioconcentration factors for the metals analyzed is as follows: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr.

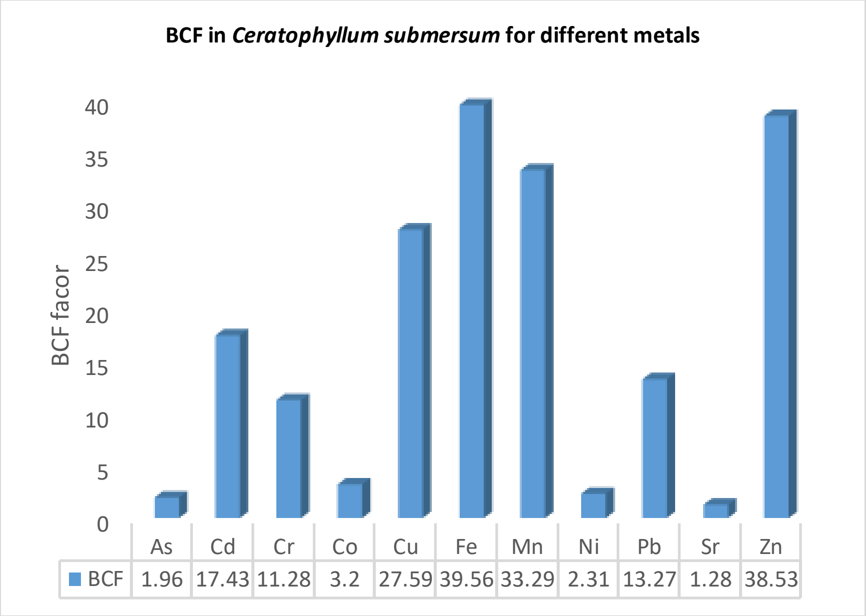


Figure 3:

Comparison of the Bioaccumulation Factor (BCF) for different metals in *Ceratophyllum submersum*.

In the examination of aquatic macrophytes, the Metal Enrichment Factor (MEF) and Metal Translocation Factor (MTF) are utilized as metrics to assess the degree of metal accumulation and movement within the plants and their surrounding ecosystems. The MEF reflects the capacity of macrophytes to absorb metals, with iron (Fe) demonstrating the highest MEF value of 17.94. Following iron, zinc (Zn) recorded an MEF of 13.85, manganese (Mn) had a value of 10.97, copper (Cu) showed 7.30, cadmium (Cd) was measured at 5.85, and lead (Pb) had an MEF of 3.28. Furthermore, chromium (Cr) exhibited an MEF of 2.79, cobalt (Co) was at 0.85, nickel (Ni) at 0.54, arsenic (As) at 0.48, and strontium (Sr) at 0.32. These MEF values are visually represented in Fig. 4 for easy comparison. The hierarchy of MEF values for the metals analyzed is as follows: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr.

The Metal Enrichment Factor (MEF) is an essential indicator that assesses the phytoremediation potential of specific plant species (Zhao et al., 2003). It functions as a means to determine both the presence and concentration of pollutants. Some researchers propose that in the realm of phytoremediation for contaminated soils, MEF values between 0.5 and 1.5 suggest that the levels of trace metals can be attributed exclusively to natural weathering processes (Zhang and Liu, 2002; Barbieri, 2018). In contrast, an MEF exceeding 1.5 indicates that a significant portion of trace metals is derived from non-crustal sources, suggesting that these metals are introduced through various pathways, including both point and non-point sources of pollution, as well as biological activities (Zhang and Liu, 2002; Klerks and Levinton, 1998; Sutherland et al., 2000). This research employed a synthetic metal bath to create a known source of metals. An enrichment factor above 1 signifies a plant's enhanced ability to absorb and translocate metals, which are then stored in different plant tissues (Sasmaz et al., 2008). In this study, MEF values varied from 17.94 for iron (Fe) to 0.32 for strontium (Sr). The MEF was utilized to assess the contamination levels of each metal examined.

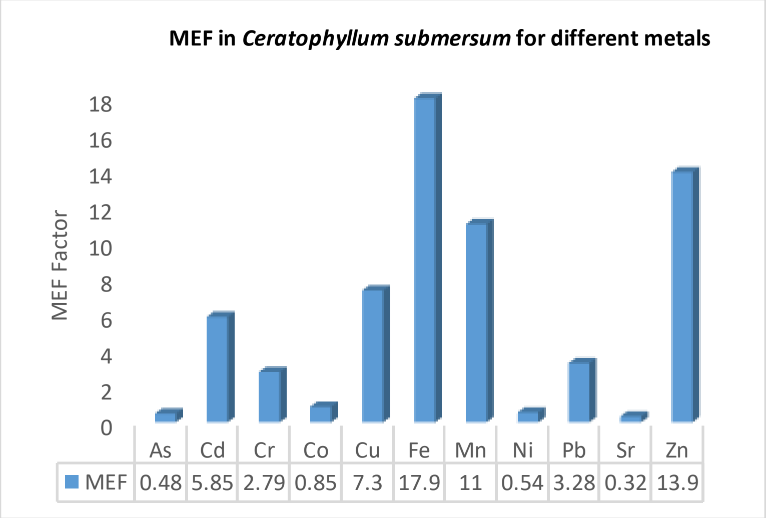


Figure 4: Comparison of the MEF factor across various metals in *Ceratophyllum submersum*.

In the examination of aquatic macrophytes, the Bioaccumulation Factor (BAF) quantifies the degree to which a pollutant accumulates within an organism relative to its surrounding environment, considering all possible exposure routes, including water, diet, and sediment. The bioaccumulation factors (BAFs) for macrophytes concerning sediment vary according to the type of metal and the ecological classifications of the macrophytes. Studies suggest that the capacity of hydrophytes to accumulate substantial amounts of metals is affected by the distinct characteristics of the plant species and the concentration of ions in the water (Kadukin, 1982; Sood, 2012). The macrophyte Ceratophyllum submersum displayed diverse accumulation patterns for the metals analyzed in the current research. The highest recorded bioaccumulation factor (BAF) was for iron (Fe), with a value of 10.94. This was followed by zinc (Zn) at 8.95, manganese (Mn) at 6.95, copper (Cu) at 4.96, cadmium (Cd) at 2.96, lead (Pb) at 2.86, chromium (Cr) at 1.44, and cobalt (Co) at 0.43, as illustrated in Fig. 5. The BAF for nickel (Ni) was 0.35, while arsenic (As) and strontium (Sr) had BAF values of 0.25 and 0.04, respectively. The ranking of BAF values for the analyzed metals is as follows: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr.

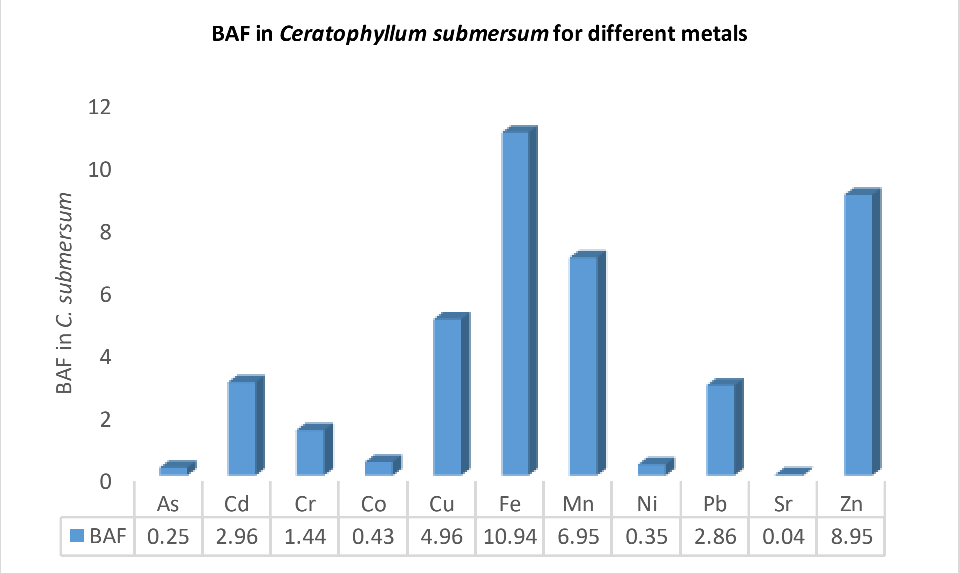


Figure 5: Comparison of the BAF factor across various metals in *Ceratophyllum submersum*.

Similar BAF values have been documented in previous studies by Ogunkunle et al. (2016), El-Amier (2017), Zurayk et al. (2001), and Bonanno and Giudice (2010) for various macrophyte species, corroborating the results of the current research. The study revealed that the accumulation of heavy metals varied among different plant organs, a trend also observed by several researchers (McLaughlin et al., 1999; Jadhav and Babare, 2025). Furthermore, factors such as the sampling locations, timing, and meteorological conditions can greatly influence metal accumulation in plants (Hofman et al., 2013), which aligns with the findings of this study.

The Metal Translocation Factor (MTF) serves as a vital indicator of a plant's ability to move specific elements, including metals, from the roots to the shoots or other aerial parts. This factor plays a crucial role in comprehending phytoremediation and the uptake of contaminants. The MTF values obtained from this study are presented in Fig. 6. Fluctuations in MTF indicate the selective uptake and movement of metals. Species recognized as metal accumulators demonstrated translocation factors exceeding 1, whereas metal excluders generally recorded MTF values below 1. An MTF greater than 1 implies an efficient mechanism for metal transport from roots to shoots, likely attributed to an effective metal transport system and the storage of metals in leaf vacuoles and apoplasts (Lasat et al., 2000). Elevated MTF values (≥1) suggest improved metal absorption and translocation from the environment by the plants. In contrast, an MTF value under 1 indicates a restricted ability of the plant to absorb metals (Shingadgaon and Chavan, 2018). The MCF values noted among the species examined, particularly Ceratophyllum submersum, exhibited a wide range of variability.

International Research Journal
Research Through Innovation

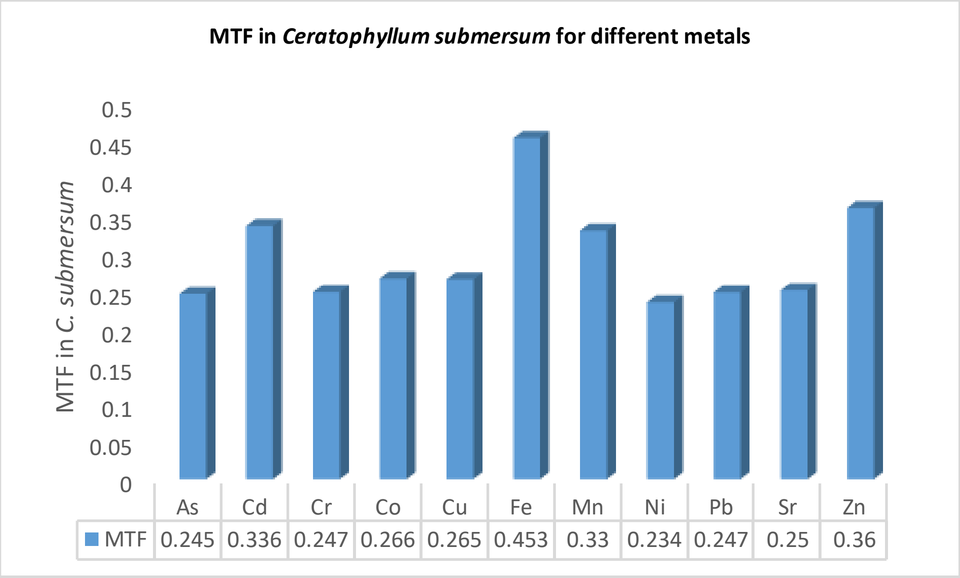


Figure 6: Comparison of the MTF factor across various metals in *Ceratophyllum submersum*.

The metal transfer factor (MTF) in plant species is crucial for determining how metals are distributed among different plant tissues (Xiong, 1998). A notable difference in pattern was observed when comparing the MTF to the metal enrichment factor (MEF). Iron (Fe) exhibited the highest MTF at 0.4531, followed by zinc (Zn) at 0.3595, cadmium (Cd) at 0.3356, manganese (Mn) at 0.3295, cobalt (Co) at 0.2656, copper (Cu) at 0.2646, strontium (Sr) at 0.2500, chromium (Cr) at 0.2473, lead (Pb) at 0.2472, and arsenic (As) at 0.2449. Nickel (Ni) had the lowest MTF, recorded at 0.2338. The hierarchy of MTF values for the analyzed metals is as follows: Fe > Zn > Cd > Mn > Co > Cu > Sr > Cr > Pb > As > Ni.

CONCLUSION

The results obtained from phytoremediation bioassays suggest that the submerged macrophyte species Ceratophyllum submersum has considerable potential for the remediation of metal-contaminated wastewater. This is supported by its bioconcentration factor (BCF), bioaccumulation factor (BAF), metal enrichment factor (MEF), and metal translocation factor (MTF). The BCF values for the analyzed metals are ranked in the following order: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr. The MEF exhibits the same ranking: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr. The BAF rankings for the metals studied also align consistently: Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > Ni > As > Sr, while the MTF is ranked as Fe > Zn > Cd > Mn > Co > Cu > Sr > Cr > Pb > As > Ni. Ceratophyllum submersum shows hyperaccumulation abilities for the metals analyzed, with the exception of strontium, which is indicated by a BCF of less than 1. In summary, the findings of this study affirm that Ceratophyllum submersum can effectively absorb and accumulate high levels of these metals, excluding strontium. Consequently, it is advisable to employ Ceratophyllum submersum for the phytoremediation of aquatic environments affected by these metals.

ACKNOWLEDGEMENTS:

The authors would like to express their sincere gratitude to Dr. B. L. Chavan, Professor and Head of the Department of Environmental Science at Dr. Babasaheb Ambedkar Marathwada University in Chhatrapati Sambhajinagar, for his invaluable assistance in the experimental work and for his expert guidance.

REFERENCES:

1) Jamaluddin, A. M., & Zannat, T. (2012). A straightforward spectrophotometric technique for quantifying copper in various real-world samples, including environmental, biological, food, and soil matrices, Pak. J. Anal. Environ. Chem., 13(1), 22-35.
2) Jamaluddin, A. M., & Chowdhury, M. T. I. (2004). An uncomplicated spectrophotometric approach for measuring cadmium in industrial, environmental, biological, and soil samples utilizing 5,7-Dibromo-8-hydroxyquinoline, Analytical Science, June 2004, The Japan Society for Analytical Chemistry, 987-990.
3) Jamaluddin, A. M., & Roy, U. K. (2009). A simple spectrophotometric method for the determination of iron(II) in aqueous solutions, Turk J Chem, 33, 709-726.
4) Akande, F. O., & Ajayi, S. A. (2017). Evaluation of heavy metal concentrations in soil and vegetables cultivated in peri-urban farms in Osun State and the related human health risks, International Journal of Environment, Agriculture and Biotechnology (IJEAB), 2(6), 3250-3261.
5) AOAC (Association of Official Analytical Chemists). Official Methods of Analysis, 1975, 12th edition, William Hormitz, pages 9-12.

6) APHA (American Public Health Association), Standard Methods for the Examination of Water and Wastewater, 1980, 15th edition, American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, D.C.
7) Atlas, R.M. (1995). "Bioremediation." Chemical Engineering News, 3:32-42.
8) Baker, J.M., Teggins, J.E., and Hoffman, J.W. A Colorimetric Method for Determining Trace Copper Concentrations in Water. Journal of the American Water Resources Association, 1971; 7(6): 1246-1249.
9) Barbieri, M. (2016). The Significance of Enrichment Factor (EF) and Geoaccumulation Index (Igeo) in Assessing Soil Contamination. Journal of Geology and Geophysics, 5: 237.
10) Bonanno, G. and Giudice, R. L. (2010). Bioaccumulation of heavy metals in the organs of Phragmites australis (common reed) and their potential as indicators of contamination. Ecological Indicators, 10, 639-645.
11) Bonanno, G. (2013). A comparative study of trace element bioaccumulation and biomonitoring in the plant species Typha domingensis, Phragmites australis, and Arundo donax. Ecotoxicology and Environmental Safety, 97, 124-130.
12) Bendix, G. H. and Grabenstetter, D. (1943). A rapid method for the determination of copper using dithizone. Industrial & Engineering Chemistry, Analytical Edition, 15(10), 649-652.
13) Brierly, C. (1991). Bioremediation of metal-contaminated surface and groundwater. Geomicrobiology Journal, 8, 201-223.
14) BSBI List. (2007). Botanical Society of Britain and Ireland. Retrieved from the original (xls) on January 25, 2015.
15) Buat-Menard, P. and Chesselet, R. (1979). The variable influence of atmospheric flux on the trace metal chemistry of oceanic suspended matter. Earth and Planetary Science Letters, 42, 398-411.
16) Chopra, A. K., Pathak, C., & Prasad, G. (2009). Addressing heavy metal contamination in agricultural soils and exploring management strategies. Journal of Applied Natural Science, 1, 99-108.
17) Claveria, R. R., De Los Santos, C. Y., Teodoro, K. B., Rellosa, M. A., & Valera, N. S. (2010). Identification of metallophytes in iron and copper-rich regions of Brookes Point, Palawan, and Mankayan, Benguet, and their significance for phytoremediation. Science Diliman, 21(2), 1–12.
18) Connell, B. S., Cox, M., & Singer, I. (1984). Nickel and chromium. In F. Brunner & J. W. Coburn (Eds.), Disorders of Mineral Metabolism (pp. 472–532). Academic Press: New York.
19) Daspute-Taur, A. B., Thete-Jadhav, R. G., Jadhav, S. L., & Shankar, S. (2018). Utilizing a floating constructed wetland reactor for the phytoremediation of sewage. International Research Journal of Natural and Applied Sciences, 5(2), 136-144.
20) Demina, L. L., Galkin, S. V., & Shumilin, E. N. (2009). Bioaccumulation of trace elements in the biota of hydrothermal fields in the Guaymas Basin (Gulf of California). Boletin De La Sociedad Geologica Mexicana, 61(1), 31-45.
21) Dickinson, W. W., Dunbar, G. B., & McLeod, H. (1996). A historical analysis of heavy metals from sediment cores in Wellington Harbour, New Zealand. Environmental Geology, 27, 59–69.
22) Echem Ogbonda G. (2014). Assessment of heavy metal uptake (Cu, Fe, Ni, Pb, and Cd) in pumpkin (Telfairia occidentalis) leaves grown in contaminated soil. Journal of Applied Sciences and Environmental Management, 18(1), 71-77. The full text is accessible online at www.ajol.info and www.bioline.org.br/ja.
23) El-Ameir, Y. A. (2017). Analysis of heavy metal contamination in the Damietta Branch of the Nile River, Egypt, utilizing metal indices and phyto-accumulators. Journal of Environmental Sciences, 46, 89-102.
24) Freitas, H., Prasad, M. N. V., & Pratas, J. (2004). Plant communities that exhibit tolerance to trace elements in the degraded soils of the São Domingos mine in southeastern Portugal: Environmental implications. Environment International, 30, 65–72.
25) Goulder, R., & Boatman, D. J. (1971). Evidence indicating that nitrogen availability affects the distribution of the freshwater macrophyte, Ceratophyllum demersum. Journal of Ecology, 59, 783-791.
26) Hackley, B. M., Smith, J. C., & Halsted, J. A. (1968). A simplified technique for determining plasma zinc levels using atomic absorption spectrophotometry. Clinical Chemistry, 14, 1-5.
27) Hobart, F. B. (1920). A colorimetric technique for estimating cobalt levels. Thesis submitted for the Bachelor of Science degree in Chemistry, College of Liberal Arts and Science, University of Illinois.
28) Hofman, J., Stokkaer, I., Snauwaert, L., & Samson, R. (2013). Assessment of the spatial distribution of particulate matter in an urban street canyon through biomagnetic leaf monitoring of particles deposited on tree crowns. Environmental Pollution, 183, 123-132.
29) Hornung, H., Karm, M. D., & Cohen, Y. (1989). The distribution of trace metals in the sediments and benthic fauna of Haifa Bay, Israel. Estuarine, Coastal and Shelf Science, 29, 43–56.
30) Indian Bureau of Mines. (2012). Manual of Procedure for Chemical and Instrumental Analysis of Ores, Minerals, Ore Dressing Products, and Environmental Samples. Edited by C.S. Gundewar, Revised Edition. Nagpur: Controller General.
31) Jadhav, S. L., & Babare, M. G. (2025). Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF), Metal Enrichment Factor (MEF), and Metal Translocation Factor (MTF) for the submerged macrophyte species Ceratophyllum demersum. International Journal of Innovative Research in Technology, 11(11), 1903-1913.
32) James, B. E., & Macmahon, R. A. (1971). The effect of trichloroacetic acid on the determination of zinc using atomic absorption spectroscopy. Clinical Chemistry Acta, 32, 307-309.
33) Kabata-Pendias, A. (2011). Trace Elements in Soils and Plants (3rd ed.). Boca Raton: CRC Press.
34) Kadukin, A. I., Krasintseva, V. V., & Romanova, G. I. (1998). Accumulation of Fe, Mn, Zn, Cu, and Cr in various aquatic plants. Hydrobiological Journal, 18, 79-82.

35) Kamari, A., Yusoff, M., Najiah, S., Putra, W. P., Ishak, C. F., Hashim, N., Mohamed, A., & Phillip, E. (2014). Metal uptake in water spinach cultivated on contaminated soil amended with chicken manure and coconut tree sawdust. Environmental Engineering Management Journal, 13(9), 2219–2228.
36) Kimura, K., & Murakami, Y. (1951). A novel modified colorimetric technique for quantifying copper in biological samples using sodium diethyldithiocarbamate. Microchimica Acta, 36(2), 958-966.
37) Klerks, P. L., & Levinton, J. S. (1989). Rapid development of metal resistance in a benthic oligochaete species from a metal-contaminated environment. Biological Bulletin, 176, 135-141.
38) Kumari, M., & Tripathi, B. D. (2015). The effectiveness of Phragmites australis and Typha latifolia in removing heavy metals from wastewater. Ecological Engineering and Environmental Safety, 112, 80-86.
39) Kumar, A., Ahirwal, J., Maiti, S. K., & Das, R. (2015). Evaluation of metal content in fly ash and its translocation and bioaccumulation in perennial grasses at reclaimed opencast mining sites. International Journal of Environment, 9(3), 1089-1096.
40) Lasat, M. M., Pence, N. S., Garvin, D. F., Ebbs, S. D., & Kochian, L. V. (2000). Molecular physiology of zinc transport in the hyperaccumulator Thlaspi caerulescens. Journal of Experimental Botany, 51, 71-79.
41) Lofberg, R. T. (1969). A highly sensitive visible spectrophotometric method for copper detection using bis-substituted thiocarbamoyl trisulfides. Analytical Letters, 2(8), 439-448.
42) Maha Ahmed Mohamed Abdallah (2012). Phytoremediation of heavy metals from aqueous solutions using two aquatic macrophytes, Ceratophyllum demersum and Lemna gibba L. Environmental Technology, 33(14), 1609-1614.
43) Martinez-Lopez, J., Carreno, M. F., Palazon-Ferrando, J., Martinez-Fernandez, A. J., & Esteve, M. A. (2014). Utilizing remote sensing of plant communities to evaluate the condition of semiarid Mediterranean saline wetlands in agricultural catchments. International Journal of Applied Earth Observation and Geoinformation, 26, 193-204.
44) McLaughlin, M. J., Parker, D. R., & Clark, J. M. (1999). Food safety concerns regarding metals and micronutrients. Field Crops Research, 60, 143–163.
45) Mishra, V. K., & Tripathi, B. D. (2008). Simultaneous removal and accumulation of heavy metals by three aquatic macrophytes. Bioresource Technology, 99, 7091–7097.
46) Mohan, J., Pandey, A., & Singh, V. (2023). Utilizing the aquatic plant Ceratophyllum demersum (L.) for the phytoremediation of wastewater. Agricultural Science Digest, 43(5), 655-660. doi: 10.18805/ag.D-5443.
47) Mocko, A., & Waclawek, W. (2004). A three-step extraction method for assessing the availability of heavy metals to vegetables. Analytical and Bioanalytical Chemistry, 380, 813-817.
48) Nazareno, P. G., & Buot, I. E. Jr. (2015). The impact of cadmium and chromium on plants in a Philippine landfill and its implications for future remediation of metal-contaminated soils. Journal of Ecology and Environment, 38(2), 123–131.
49) Nicolaysen, H. F. (1941). Quantitative volumetric titration of heavy metals. Retrospective Theses and Dissertations, 13747.
50) Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A. H., & Yousefi, N. (2009). The accumulation of heavy metals in soil and their uptake by plant species with potential for phytoremediation. Environment and Earth Science, 59, 315–323.
51) Ogoko, E. C. (2015). The accumulation of heavy metals in soil and their transfer to leafy vegetables with potential for phytoremediation. American Journal of Chemistry, 5(5), 125-131.
52) Ogunkunle, C. O., Mustapha, K., Oyedeji, S., & Fatoba, P. O. (2016). Evaluation of metallic contamination levels in surface water and aquatic macrophytes of earthen dams in Ilorin, North-Central Nigeria, as indicators of environmental health. Journal of King Saud University-Science, 28, 324-331.
53) Osundiya, M. O., Ayejuyo, O. O., Olowu, R. A., Bamgboye, O. A., & Ogunlola, A. O. (2014). Accumulation of heavy metals in commonly consumed leafy vegetables grown along the Nigeria-Benin Seme Border, West Africa. Advances in Applied Science Research, 5(1), 1-7.
54) Parkerton, T. F., Arnot, J. A., Weisbrod, A. V., Russom, C., Hoke, R. A., Woodburn, K., Traas, T., Bonnell, M., Burkhard, L. P., & Lampi, M. A. (2008). Recommendations for assessing in vivo fish bioaccumulation data. Integrated Environmental Assessment and Management, 4(2), 139-155.
55) Parzych, A., Cymer, A., & Macheta, K. (2016). The leaves and roots of Typha latifolia L. and Iris pseudacorus L. as indicators of heavy metal contamination in bottom sediments. Limnological Review, 16(2), 77-83.
56) Ravichandran, M., Baskaran, M., Santschi, P. H., & Bianchi, T. (1995). A historical perspective on trace metal pollution in the Sabine-Neches Estuary, Beaumont, Texas. Environmental Science and Technology, 29, 1495–1503.
57) Roberts, J. R. (1999). The effects of metal toxicity on children. In Training Manual on Pediatric Environmental Health. Children's Environmental Health: Emeryville, CA.
58) Rezvani, M., & Zaefarian, F. (2011). Bioaccumulation and translocation of cadmium and lead in Aeluropus littoralis. Australian Journal of Agricultural Engineering, 2, 114-119.
59) Rubeska, I. (1969). Atomic Absorption Spectrophotometry. Chemical Rubber Co., Cleveland, Ohio, 97-100.
60) Salomons, W., & Förstner, U. (1984). Metals in the Hydrocycle. Springer, Berlin Heidelberg Tokyo.
61) Sasmaz, A., Obek, E., & Hasar, H. (2008). Heavy metal accumulation in Typha latifolia L. cultivated in a stream with secondary effluent. Ecological Engineering, 33, 278-284.
62) Sandell, E.B. (1965). Colorimetric Determination of Traces of Metals. Interscience, New York, 269.
63) Sanghamitra, K., Prasada Rao, P.V.V., & Naidu, G.R.K. (2010). Uptake of Zn (II) by the invasive weed species Parthenium hysterophorus L. Applied Ecology and Environmental Research, 10(3), 267-290.

64) Sarma, L.S., Kumar, J.R., Reddy, K.J., & Reddy, A.V. (2005). Development of an extractive spectrophotometric method for determining copper (II) in leafy vegetables and pharmaceutical samples using pyridoxal-4-phenyl-3-thiosemicarbazone (PPT). Journal of Agricultural and Food Chemistry, 53(14), 5492-5498.

65) Shanahan, Peter. (2004). Bioremediation. In Waste Containment and Remediation Technology, Spring 2004, Massachusetts Institute of Technology, MIT OpenCourseWare. Retrieved from http://ocw.mit.edu/NR/rdonlyres/Civil-and-Environmental-Engineering/1-34Spring2004/335613D5-6D6F-413F-9098-453E8AC20BC2/0/lecture12.pdf.

66) Shingadgaon, S. S., & Chavan, B. L. (2019). Evaluation of Bioaccumulation Factor (BAF), Bioconcentration Factor (BCF), Translocation Factor (TF), and Metal Enrichment Factor (MEF) in aquatic macrophyte species subjected to metal-contaminated wastewater. International Journal of Innovative Research in Science, Engineering and Technology, 8(1), 329-347.

67) Shingadgaon, S. S., Jadhav, S. L., Thete-Jadhav, R. B., Zambare, N. S., Daspute-Taur, A. B., Babare, M. G., & Chavan, B. L. (2018). The potential of aquatic macrophyte species for the bioremediation of metal-contaminated wastewater. International Journal of Current Research, 10(12), 76384-76390.

68) Smith, R. (1983). A laboratory manual for the determination of metals in water and wastewater using atomic absorption spectrophotometry. National Institute for Water Research, Council for Scientific and Industrial Research (CSIR) Technical Guide K63, pp. 1-29. ISBN 0 7988 2482 4, Pretoria, South Africa.

69) Smith, S. J., & Wolfe-Murphy, S. A. (1991). Ceratophyllum submersum L. (Soft Hornwort), a new species record for Ireland. Irish Naturalists' Journal, 23, 374-376.

70) Song, M. K., Adham, N. F., & Rinderknecht, H. (1976). A straightforward and highly sensitive colorimetric technique for measuring zinc levels in serum. AJ.C.P., 65(2), 229-233. Retrieved from https://academic.oup.com/ajcp/article-abstract/65/2/229/1770841 as a guest on March 1, 2018.

71) Sood, A., Uniyal, P. L., Prasanna, R., & Ahluwalia, A. S. (2012). The phytoremediation capabilities of the aquatic macrophyte, Azolla. AMBIO, 41, 122-137.

72) Soomro, G. A., & Shar, G. A. (2014). A straightforward spectrophotometric approach for the detection of Cadmium (II) using 1-(2-pyridylazo)-2-naphthol in a micellar aqueous solution of CTAB. Ultra Chemistry, 10(2), 95-104. Available at www.journalofchemistry.org.

73) Soomro, R. M., Ahmed, J., & Menon, N. (2009). A rapid and simple spectrophotometric method for the determination of trace levels of chromium using bis(salicylaldehyde) orthophenylenediamine in nonionic micellar media. Turkish Journal of Chemistry, 35, 155-170.

74) Sutherland, R. A., Tolosa, C. A., Tack, F. M. G., & Verloo, M. G. (2000). Analysis of selected element concentrations and enrichment ratios in both background and anthropogenically affected roadside areas. Archives of Environmental Contamination and Toxicology, 38, 428-438.

75) Syed, I., Fatima, H., Mohammed, A., & Siddiqui, M. A. (2018). "Ceratophyllum demersum: A review of the free-floating aquatic plant." Indian Journal of Pharmaceutical and Biological Research, 6(2), 10-17.

76) Tazul Islam M. and M. Jamaluddin Ahmed, 2013. A straightforward spectrophotometric technique for the trace analysis of zinc in various real samples, including environmental, biological, pharmaceutical, milk, and soil matrices, utilizing 5,7-Dibromo-8-hydroxyquinoline. Pak. J. Anal. Environ. Chem. Vol. 14, No. 1 (2013) 01-15. Accessible at http://lib.dr.iastate.edu/rtd/13747.

77) Thete-Jadhav Renuka G.*, Asmita B. Daspute, S. L. Jadhav, Shankar S. Shingadgaon**, and B. L. Chavan**, 2018. Treatment of sewage using a floating constructed wetland reactor system. JETIR December 2018, Volume 5, Issue 12, 406-413.

78) Vardhan, K. H.; Kumar, P. S.; Panda, R. C. 2019. A comprehensive review on heavy metal pollution, its toxicity, and remedial measures: Current trends and future perspectives. J. Mol. Liq. 290, 111197; DOI: 10.1016/j.molliq.2019.111197.

79) Wei Chong Kian. 2014. Analysis of heavy metals in water samples through sodium sulfide precipitation and inorganic coagulation, followed by FAAS detection. The Experiment, Vol. 28(1), 1888-1895. Available at www.experimentjournal.com.

80) Wilde, E. and J. Benemann Biore. 1993. The removal of heavy metals using macro-algae. Biotechnol Adv. 11, 781-812.

81) World Health Organization (WHO). 2011. Guidelines for Drinking-water Quality (4th ed.). Malta: Gutenberg. ISBN 978 92 4 154815 1.

82) Xiong Z. T. 1998. Lead uptake and its effects on seed germination and plant growth in the Pb hyperaccumulator Brassica pakinensis Rupr. Bull Environ Contam. Toxicol. 60, 285-291.

83) Yarbro Stanley Keith (1976). Two photometric techniques for measuring chromium levels in biological materials. A thesis submitted to the faculty of the Division of Graduate Studies in partial fulfillment of the requirements for the Doctor of Philosophy degree in the School of Chemistry at the Georgia Institute of Technology. June 1976.

84) Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). The uptake of lead, copper, and zinc by native plants in a contaminated area in Florida. Science of the Total Environment, 368, 456–464.

85) Zaimee, M. Z. A., Sarjadi, M. S., & Rahman, M. L. (2021). Removal of heavy metals from water using effective adsorbents. Water, 13(19), 2659; DOI: 10.3390/w13192659.

86) Zhang, J., & Liu, C. L. (2002). The composition of riverine and estuarine particulate metals in China: weathering characteristics, human impact, and chemical fluxes. Estuarine, Coastal and Shelf Science, 54, 1051-1070.

87) Zhao, F. J., Lombi, E., & McGrath, S. P. (2003). Evaluating the potential of the hyperaccumulator Thlaspi caerulescens for the phytoremediation of zinc and cadmium. Plant and Soil, 249, 37-43.

88) Zurayk, R., Sukkariah, B., & Baalbaki, R. (2001). Common hydrophytes as bioindicators of nickel, chromium, and cadmium pollution. Water, Air, and Soil Pollution, 127, 373-388.

International Research Journal
IJNRD
Research Through Innovation