Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF), Metal Enrichment Factor (MEF), and Metal Translocation Factor (MTF) for the submerged macrophyte species *Ceratophyllum demersum*

S.L.Jadhav[1], 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 metals are prevalent environmental pollutants that pose significant concerns due to their toxic, persistent, and non-degradable characteristics. The biological factors associated with metals in plants are crucial for the phytoremediation process, as they provide insights into the plants' capacity to uptake metals, their translocation within plant tissues, and their storage in above-ground biomass. This information is essential for identifying suitable macrophytes capable of absorbing substantial amounts of these elements. Consequently, this study aims to assess and evaluate the Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF), Metal Enrichment Factor (MEF), and Metal Translocation Factor (MTF) of the submerged macrophyte species *Ceratophyllum demersum* through a hydroponic bioassay.**

The hydroponic bioassay was conducted from June 2018 to December 2022, exposing *Ceratophyllum demersum* to a mixed metal solution to evaluate its ability to absorb, transfer, accumulate, and enrich heavy metals, thereby determining its potential for phytoremediation applications. The findings of this study highlighted the phytoremediation capabilities and accumulation patterns of ten heavy metals: As, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sr, and Zn across various plant organs of *Ceratophyllum demersum*. The results indicated that the order of BCF for the metals analyzed is Fe > Zn > Mn > Cu > Pb > Cd > Cr > Co > As > Ni > Sr, while the order of MEF is Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > As > Sr > Ni. The BAF for the studied metals follows the order Zn > Fe > Mn > Cu > Cd > Pb > Cr > Co > Sr > As > Ni, and the MTF is ranked as Zn > Sr > Fe > Cu > Cd > Mn > Pb > As > Co > Cr > Ni. Notably, *Ceratophyllum demersum* acts as a hyperaccumulator for Zn, Fe, Mn, Cu, Cd, Pb, Ni, Co, Cr, and As, with the exception of Sr, which has a BCF of less than 1.

***Index Terms* Aquatic Macrophytes, *Ceratophyllum demersum*, Heavy Metals, Bioconcentration, Biomagnification, Metal Transfer, Metal Enrichment, Phytoremediation.**

I. INTRODUCTION

The contamination of aquatic ecosystems by heavy metals poses significant environmental and public health challenges. Unlike other pollutants, heavy metals are non-biodegradable and tend to accumulate within living organisms [1], [2]. This issue has become increasingly pressing in contemporary society due to its widespread occurrence, the complexities involved in remediation, and the substantial risks it poses to human health [3], [4]. In India, traditional physico-chemical methods for cleaning heavy metal contamination are often prohibitively expensive and technologically demanding, costing as much as Rs. 25,000 per cubic meter of soil or wastewater, making them largely impractical for developing nations. Consequently, there is a growing interest in alternative remediation strategies, such as phytoremediation. In recent decades, research has highlighted the potential of certain plant species to remediate metal-contaminated sites, particularly their remarkable ability to absorb heavy metals at concentrations up to 100 times greater than those typically found in their aerial parts [5],[6].

Numerous experts and authorities have acknowledged the potential of phytoremediation, referring to it as "green technology" [7], [8] & [9]. Despite this

recognition, there have been limited practical efforts to harness these potentials, particularly in light of the widespread heavy metal contamination across various regions of the country. Most phytoremediation research has primarily focused on mining areas [10] often overlooking or giving minimal attention to other locations that may also be affected by similar heavy metal pollution. Aquatic macrophytes found in rivers, marshes, and other water bodies present a promising solution to address heavy metal contamination. Plants are classified into three categories based on their growth strategies in metal-contaminated soils: metal excluders, indicators, and accumulators or hyperaccumulators [6], [9].

Metal excluders are plant species that effectively restrict the movement of heavy metals within their structure, maintaining relatively low concentrations in their aerial parts across a variety of soil conditions; however, they may still accumulate significant amounts of metals in their root systems. Metal indicators are plants that gather metals in their aboveground tissues, with the metal concentrations in these tissues typically mirroring the levels found in the surrounding soil. Nevertheless, persistent absorption of heavy metals can lead to the decline of these plants. Metal accumulators, also known as hyperaccumulators, are species that can concentrate metals in their aboveground tissues at levels significantly higher than those found in the soil, wastewater, or in nearby non-accumulating species. These plants have the ability to extract heavy metals from the soil and store them in their shoots. Given this context, it became evident that prior to developing phytoremediation strategies for wastewater, it was essential to first assess the response of aquatic macrophytes to heavy metals. The current study aims to evaluate the phytoremediation potential and accumulation characteristics of ten heavy metals—As, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Sr, and Zn—in various organs of the *Ceratophyllum demersum* macrophyte. The concentrations of metals measured were utilized to calculate the Bioconcentration Factor (BCF) and the Translocation or Transfer Factor (TF), which quantitatively indicate the plants' tolerance or avoidance of these metals.

2. MATERIALS AND METHODS

A. Overview of *Ceratophyllum demersum* Macrophyte:

Ceratophyllum derives its name from the Greek words "keras," meaning 'horn,' and "phyllon," meaning 'leaf,' which describes the horn-like appearance of its leaf branches. The term "demersum" comes from the Latin "demerge," which translates to 'sink' or 'plunge,' highlighting the plant's tendency to grow submerged. Commonly known as 'hornwort,' this plant is recognized for its distinctive leaf structure. In India, it is referred to by various names, including ambuchamar, ambutala, araka, hathaparni, haval, honaal, Jalaja, jalakesha, jalakuntala, jalanchana, jalamandapi, jalanili, jalaprishthaja, jalashuka, ka, karimpayal, kavara, Manjula, nasu, neeti sambraani, saivala, saivalah, salilakundala, sevar, shaiwal, shaiwar, sheoyala, sheshana, shevala, shivala, sivara, and souvala [11], [12].

Ceratophyllum demersum is a perennial macrophyte that typically grows with its stem base embedded in sandy or silty substrates. It is commonly found floating in stagnant or slow-moving waters. This delicate, free-floating aquatic plant lacks roots but is characterized by its dense foliage, which can resemble a bushy animal tail at the tips. It reproduces both vegetatively and through seeds. Hornwort thrives in warm, shaded waters with gentle flow (approximately 1 cm per second) and is sensitive to turbidity and salinity. This aquatic plant enhances water oxygenation, serves as a food source for herbivorous species, and is seldom problematic. *Ceratophyllum demersum*, commonly known as Coontail due to its compact whorls resembling a raccoon’s tail, or Hornwort, is a cosmopolitan, perennial, obligate aquatic plant. The name "hornwort" derives from the Greek words "keras," meaning horn, and "phyllon," meaning leaf. This species has been a part of the aquarium trade for many years and is readily available. It is also commonly found as a substrate in ponds [13]. Ceratophyllum demersum is a perennial submerged macrophyte that typically thrives with its stem base anchored in muddy or silty substrates.

B. Collection and Sampling of *Ceratophyllum demersum*:

Natural and artificial water bodies, including both stagnant and flowing waters as well as reservoirs, were

surveyed for the presence of *Ceratophyllum demersum*. The macrophytes were manually observed, sampled, and collected from the Marathwada study area without inflicting any physical harm. They were uprooted or sampled using appropriate scientific techniques, rinsed with water to eliminate excess dirt and mud, and carefully wrapped in paper. The collected specimens were in a fresh, green state and placed in adequately sized transparent polyethylene bags. Each sample contained at least ten healthy macrophytes. These were replanted within four to five hours of collection to acclimatize in metal-mixed synthetic wastewater within rectangular test chambers measuring 1 meter by 1 meter and 10 cm deep, maintaining a 2 cm freeboard.

A separate collection of samples was taken to the laboratory, where they were rinsed with gently flowing tap water while adhering to appropriate safety measures, air-dried, and identified using various literature sources for qualitative floristic data, focusing on species and community characteristics rather than solely on their physical structures and appearances. The photographs and samples were forwarded to botanical research specialists in the relevant fields for verification and confirmation of the identified information, including essential details, prior to drawing final conclusions, thereby providing an authentic and supportive second opinion. The macrophyte *Ceratophyllum demersum* was chosen for this investigation due to its local prevalence, allowing for collection from any area within the study region for further research.

C. Studies on Metal Accumulation Potential:

The macrophytes were selectively thinned by eliminating those that were infected or unhealthy. The remaining healthy specimens were then relocated to a laboratory-scale test bath. These test baths, constructed from small plastic tanks, were designed to assess the adaptability of the macrophytes to the new local conditions. The selected healthy macrophytes were subjected to the local climatic environment for a sufficient duration to ensure they acclimated and achieved full growth in a synthetic wastewater test bath containing metals. In this study, the acclimatization period was set at one month. Growth continued for an additional month in a metal-mixed synthetic wastewater test bath, utilizing stock solutions with concentrations 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 set without synthetic wastewater. Various metal salts, including $Na_3AsO_4 \cdot 12H_2O$, $CuSO_4 \cdot 8H_2O$, 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 utilized according to established standard procedures [14],[15],[16] & [17]. Following the designated growth period, the macrophyte plants were harvested and analyzed to determine their capacity for metal accumulation, with adjustments made to account for the control group results using normal water. This assessment aimed to evaluate their potential and suitability for application in phytoremediation processes. The complete methodology employed for assessing heavy metal absorption capabilities is outlined below:

The plant materials of *Ceratophyllum demersum* were initially rinsed with tap water before being transported for laboratory analysis. They underwent a second wash with gently flowing tap water, followed by a rinse with double distilled water to eliminate any dirt and impurities. The macrophyte samples were then air-dried and cut with stainless steel scissors to separate the shoots, roots, stems, and leaves. Each of these components was oven-dried at 60 °C until a constant weight was achieved, then ground in a pestle and mortar to create a homogeneous mixture, which was subsequently stored for analysis. For the determination of 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 using appropriate methods. The heavy metals from the prepared aliquot were examined using Atomic Absorption Spectroscopy (AAS) and Gas Chromatography (GC) in other laboratories, based on sample and metal-specific rates, or through suitable methods referenced from credible literature or research publications, depending on the availability of necessary facilities for the required analysis. The methods employed for analysis encompassed a variety of techniques, including the Cobalt by cobaltous pyridine method, the Iron (Fe) analysis via the Dichromate method, and the Zinc (Zn) assessment using the EDTA Complexometry-Back Titration method, Manganese (Mn) was analyzed using Volhard's method, while Copper (Cu) was determined

through Sodium Thiosulphate titration, with confirmation provided by the Spectrophotometric method [18]. Lead (Pb) was assessed using the EDTA Complexometric method, and Manganese (Mn) was also analyzed via the Periodate Oxidation Method. Chromium was evaluated using the Diphenylcarbazide Spectrophotometric method [19] (IBM, 2012), and Cadmium (Cd) was analyzed through a spectrophotometric approach [20]. Additionally, Chromium (Cr) was assessed using the Diphenylcarbazide Method [21]. and Cobalt (Co) was analyzed through a colorimetric method [22].

A collection of samples was processed and digested using an automated program in the NuWav-Ultra Microwave Digestion Extraction System. The metal contents, including As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sr, and Zn, were analyzed in another laboratory using the Shimadzu Atomic Absorption Spectrometer 6300 model and the Agilent 725 ICP-OES instrument for confirmation, as required. Most results were validated through random cross-checks against established standard methods to ensure analytical simplicity and accuracy, as referenced in various studies [23, [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35] , [36].

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 application. Doubly distilled water was consistently employed throughout the experimental procedures. The glassware was meticulously cleaned by soaking in acidified solutions of potassium permanganate ($KMnO_4$) or potassium dichromate ($K_2Cr_2O_7$), followed by treatment with concentrated nitric acid (HNO_3) and several rinses with doubly distilled water. Calibration curves, which acted as standard references for solutions with known concentrations of the respective metals, 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 capacity of *Ceratophyllum demersum* macrophytes to facilitate the movement of heavy metals from contaminated substrates into their root systems, as well as their ability to store these metals in various plant tissues, was analyzed. This evaluation encompassed the transfer of metals from the roots to the above-ground harvestable parts and the potential for metal accumulation. Several 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 determine the viability of using *Ceratophyllum demersum* macrophytes for phytoremediation, offering significant insights into their effectiveness in restoring metal-polluted environments.

I. 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. For 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) was determined using the following formula:

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

For specific metal, it can be expressed as;

$$BAF_{Metal} = \frac{\text{[Metal content in aerial parts]}}{\text{[Metal Concentration in Source]}}$$

$$= C_{AP}/C_{WW}$$

Where BAF_{Metal} stands for bioaccumulation factor for a specified metal, C_{AP} represents specific Metal Concentration in aerial parts expressed in mg/kg and C_{WW} represents Metal Concentration in growth environment-source like wastewater expressed in mg/kg.

II. Bioconcentration Factor (BCF):

The bioconcentration factor (BCF) is defined as the ratio of the total concentration of metals in the roots to that in the surrounding environment, which may

include contaminated soil or wastewater [37], [38], [39]. 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 the soil [40]. The BCF is determined using the following formula:

BCF = [Concentration of heavy metal in the organism] / [Concentration of heavy metal in water]

The BCF for macrophytes was assessed using the equation established [41].

For a specific metal, the bio-concentration of a specified metal is calculated using the following equation;

$$BCF_{Metal} = \frac{[\text{Metal Concentration in root}]}{[\text{Metal Concentration in Source}]} = C_R/C_{WW}$$

Where BCF_{Metal} stands for bio-concentration factor for a specified metal, C_R represents specified Metal Concentration in root expressed in mg/kg and C_{WW} represents Metal Concentration in growth environment-source like wastewater expressed in mg/kg, wherein for this particular study the metal refers to any of the all eleven metals studied.

III. Metal Enrichment Factors (MEF)

Metal Enrichment Factors (MEF) were employed to assess the degree of metal contamination in sediment, adhering to the methodology proposed [42]. A commonly accepted approach for evaluating anthropogenic impact involves the calculation of a normalized enrichment factor (EF), which is based on metal concentrations derived from uncontaminated background levels, as highlighted by [43], [44], [45]. The purpose of calculating the EF is to reduce the 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 against a reference metal, such as (Fe) iron or (Al) aluminum [46].

$$MEF_{Metal} = \frac{[\text{Metal content in only shoot}]}{[\text{Metal Concentration in Source}]} = C_{OS}/C_{WW}$$

Where MEF_{Metal} stands for specific metal enrichment factor, C_{OS} represents specified Metal content in only shoot expressed in mg/kg and C_{ww} represents Metal Concentration in growth environment-sources like wastewater expressed in mg/kg.

IV. Metal Translocation Factor (MTF):

The metal translocation factor (MTF) is defined as the ratio of the total concentration of metals in the shoots compared to that in the roots [47], [48]. This factor reflects the relative concentration of metals in the shoots in relation to the roots. An MTF value greater than 1 indicates that the plant is capable of effectively transporting metals from the root system to the shoots [49]. The metal transfer factor is a useful measure for evaluating the mobility of metals from their source to macrophytes. The MTF for specific metals can vary significantly 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, as well as 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 [50], [51], [52].

$$MTF_{Metal} = \frac{[\text{Metal Content in only shoot}]}{[\text{Metal Concentration in root}]} = C_{OS}/C_R$$

Where MTF_{Metal} stands for specific metal translocation factor, C_s represents_Metal content in aerial parts expressed in mg/kg and C_r represents Metal Concentration in root expressed in mg/kg. It is also called as shoot-root quotient and may be denoted as MTF in general.

3. RESULTS AND DISCUSSIONS

There are various types of pollutants in the wastewaters from various sources such as industrial discharges, trade effluents, municipal sewage which enter the water sources. Among these organic and inorganic pollutants, including heavy metals, most of these can cause danger directly on the lives of most aquatic organisms and human life by entering the food chain due to their accumulation potentials. The presences of elevated levels of the trace metals in plant tissues have detrimental toxic effects on the animals, especially on the consumption. For instance, the zinc and copper are trace metals those are needed in minute quantities for the healthy growth. Though are essential

for plant growth as well as animal and human nutrition, but at elevated concentrations they can lead to phytotoxicity in plants and zootoxicity in animals [53], [54].

The mobility potentials of macrophytes for the heavy metals from the polluted growth media into the roots of the plants and the ability to accumulate in different parts, translocate the metals from roots to the harvestable aerial part and potential to enrich by metal in *Ceratophyllum demersum* were evaluated respectively by means of the bioconcentration factor (BCF), bioaccumulation factor (BAF), the metal translocation factor (MTF) and the metal enrichment factor (MEF) to assess the feasibility of the macrophytes for phytoremediation to provide insight for the use of native macrophyte plants to remediate metal contaminated sites.

Heavy metals have become widespread pollutants globally, raising significant environmental concerns due to their persistence in non-degradable forms. They can have toxic effects on ecosystems by infiltrating the food chain, ultimately posing various threats to human health [55], [56], [57]. Their presence is evident throughout contaminated water bodies, from the bottom sediments to the surface. The behavior of heavy metals in water and wastewater is influenced by factors such as sediment composition, water chemistry, salinity, redox potential, and pH [58]. This situation necessitates the implementation of submerged macrophytes for the remediation of water and wastewater across varying depths.

Uptake Potentials of Metals in *Ceratophyllum demersum*:

The aquatic macrophyte *Ceratophyllum demersum* exhibited variations in metal content, suggesting differing capacities for the uptake of each metal, a finding consistent with previous research [59], [60], [61], [62]. The concentrations of metals in the roots, shoots, and aerial parts of *Ceratophyllum demersum* are detailed in Table 1. Figure 1 provides a comparative analysis of metal concentrations in the roots, shoots, and aerial components of this submerged macrophyte species. The highest concentration of iron (Fe) was found in the roots, measuring 3739 µg/g, followed by zinc (Zn) at 2643 µg/g, manganese (Mn) at 2189 µg/g, and copper (Cu) at 1549 µg/g. The lowest concentration of strontium (Sr) recorded in the roots was 86 µg/g. The sequence of metal accumulation in the roots of *Ceratophyllum demersum* was determined to be Fe > Zn > Mn > Cu > Pb > Cd > Cr > Co > As > Ni > Sr.

In contrast, the accumulation of metals in the shoots was generally lower than that in the roots across all measured metals. The highest concentration of iron in the shoots was 1285 µg/g, followed by zinc at 960 µg/g, manganese at 649 µg/g, and copper at 528 µg/g. Other metals accumulated in the shoots included cadmium (Cd) at 317 µg/g, lead (Pb) at 298 µg/g, cobalt (Co) at 63 µg/g, arsenic (As) at 35 µg/g, strontium (Sr) at 31 µg/g, and nickel (Ni) at 25 µg/g, which was the lowest among the metals measured. The order of metal accumulation in the shoots differed from that in the roots, with the sequence being Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > As > Sr > Ni.

The accumulation of metals in the aerial portions of *Ceratophyllum demersum* was found to be lower than that in the roots and shoots, as indicated in Table 1. Among the metals, zinc exhibited the highest concentration in the aerial parts, measuring 526 µg/g, followed by iron at 504 µg/g, manganese at 354 µg/g, copper at 274 µg/g, cadmium at 138 µg/g, lead at 123 µg/g, chromium at 37 µg/g, cobalt at 25 µg/g, strontium at 16 µg/g, and both arsenic and nickel at 14 µg/g each. The hierarchy of metal accumulation in the aerial parts of *Ceratophyllum demersum* is as follows: Zn > Fe > Mn > Cu > Cd > Pb > Cr > Co > Sr > Ni & As.

Table 1: Metal concentrations in the roots, shoots and aerial parts of submerged macrophyte species *Ceratophyllum demersum* recorded during phytoremediation bioassay

Metal	Conc. in roots (µg/g)	Conc. in shoots (µg/g)	Conc. in aerial parts (µg/g)
As	149	35	14
Cd	965	317	138
Cr	685	142	37
Co	286	63	25
Cu	1549	528	274
Fe	3739	1285	504
Mn	2189	649	354
Ni	139	25	14
Pb	1078	298	123

Sr	86	31	16
Zn	2643	960	526

The Metal Transport Factor (MTF) refers to the internal metal transportation capability [60], [39]. It states that both the Bioaccumulation Factor (BCF) and MTF are useful for assessing a plant's potential for metal phytoremediation. The BCF measures a plant's capacity to accumulate metals in its roots, while the MTF evaluates its ability to move these metals from the roots to the aerial parts of the plant. Plants with BCF values below one are deemed ineffective for phytoextraction [39]. Conversely, plants that exhibit both BCF and MTF values exceeding one (BCF > 1, MTF > 1) are considered suitable 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 TF greater than one, along with a total metal accumulation exceeding 1000 mg kg−1 for Cu, Co, Cr, or Pb, or over 10000 mg kg−1 for Fe, Mn, or Zn [63].

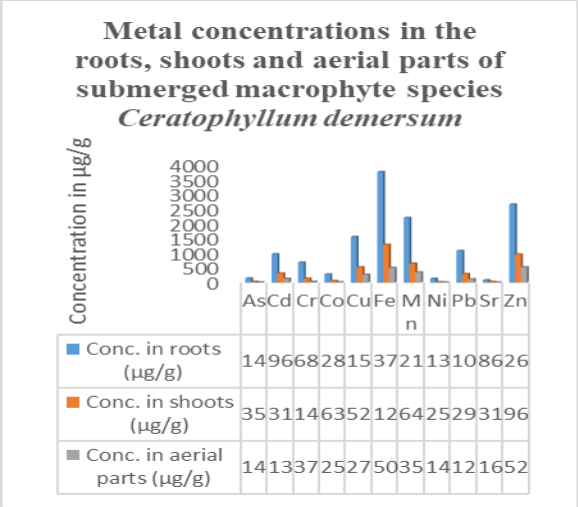

Fig. 1: Comparative levels of Metal concentrations in the roots, shoots and aerial parts of *Ceratophyllum demersum* submerged macrophyte species

Table 2: Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF), Metal Enrichment Factor (MEF) and Metal Translocation Factor (MTF) in submerged macrophyte species *Ceratophyllum demersum* recorded during phytoremediation bioassay

Metal	BCF	MEF	BAF	MTF
As	1.49	0.35	0.14	0.2349
Cd	9.65	3.17	1.38	0.3285
Cr	6.85	1.42	0.37	0.2073
Co	2.86	0.63	0.25	0.2203
Cu	15.49	5.28	2.74	0.3409
Fe	37.39	12.85	5.04	0.3437
Mn	21.89	6.49	3.54	0.2965
Ni	1.39	0.25	0.14	0.1799
Pb	10.78	2.98	1.23	0.2764
Sr	0.86	0.31	0.16	0.3605
Zn	26.43	9.60	5.26	0.3632

In this study, the bioconcentration factor, metal enrichment factor, bioabsorption factor, and metal transfer factor for each tested metal were determined for the submerged aquatic plant *Ceratophyllum demersum*, as shown in Table 2. The bioconcentration factor for iron (Fe) was the highest at 37.39, followed by zinc (Zn) at 26.43, manganese (Mn) at 21.89, copper (Cu) at 15.49, lead (Pb) at 10.78, cadmium (Cd) at 9.65, chromium (Cr) at 6.85, cobalt (Co) at 2.86, arsenic (As) at 1.49, nickel (Ni) at 1.39, and strontium (Sr) at 0.86. The ranking of bioconcentration factors for the metals analyzed is Fe > Zn > Mn > Cu > Pb > Cd > Cr > Co > As > Ni > Sr.

The metal enrichment factor (MEF), which reflects the capacity of macrophytes to accumulate metals, was highest for iron (Fe) at 12.85, followed by zinc (Zn) at 9.60, manganese (Mn) at 6.40, copper (Cu) at 5.28, cadmium (Cd) at 3.17, lead (Pb) at 2.98, chromium (Cr) at 1.42, cobalt (Co) at 0.63, arsenic (As) at 0.35, strontium (Sr) at 0.31, and nickel (Ni) at 0.25. The order of MEF for the metals examined is Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > As > Sr > Ni.

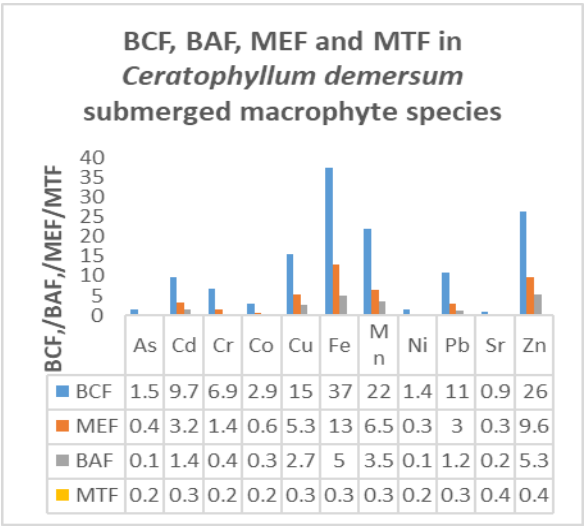

Fig. 2: Comparision BCF, BAF, MEF and MTF in *Ceratophyllum demersum*.

The bioaccumulation factor (BAF) was found to be highest for zinc (Zn) at 5.26, followed closely by iron (Fe) at 5.04, manganese (Mn) at 3.54, copper (Cu) at 2.74, cadmium (Cd) at 1.38, lead (Pb) at 1.23, cobalt (Co) at 0.25, strontium (Sr) at 0.16, and both arsenic (As) and nickel (Ni) at 0.14 each. The ranking of BAF for the analyzed metals is as follows: Zn > Fe > Mn > Cu > Cd > Pb > Co > Sr > As & Ni.

In contrast, the metal transfer factor (MTF) exhibited a different trend compared to the metal enrichment factor (MEF). The highest MTF was observed for Zn at 0.3632, followed by Fe at 0.3437, Cu at 0.3409, Cd at 0.3285, Mn at 0.2965, Pb at 0.2764, As at 0.2349, Co at 0.2203, Cr at 0.2073, and Ni at 0.1799. The sequence of MTF for the studied metals is as follows: Zn > Sr > Fe > Cu > Cd > Mn > Pb > As > Co > Cr > Ni.

4. CONCLUSION

The bioconcentration factor (BCF), bioaccumulation factor (BAF), metal enrichment factor (MEF), and metal translocation factor (MTF) in the submerged macrophyte species *Ceratophyllum demersum*, as revealed by phytoremediation bioassays, indicate its potential for the remediation of metal-contaminated wastewater. The order of BCF for the metals examined is Fe > Zn > Mn > Cu > Pb > Cd > Cr > Co > As > Ni > Sr, while the order of MEF is Fe > Zn > Mn > Cu > Cd > Pb > Cr > Co > As > Sr > Ni. The ranking of BAF for the metals studied is Zn > Fe > Mn > Cu > Cd > Pb > Cr > Co > Sr > As & Ni, and the order of MTF is Zn > Sr > Fe > Cu > Cd > Mn > Pb > As > Co > Cr > Ni. The aquatic species *Ceratophyllum demersum* acts as hyperaccumulator for the metals studied except Sr indicated by BCF<1.

ACKNOWLEDGEMENT

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

REFERENCE

[1] Rai, P.K. and B.D. Tripathi, 2009. A comparative evaluation of Azolla pinnata. International Journal of Phytoremediation, 10: 430-439.

[2] Malik, R.N., S.Z. Husain, and I. Nazir, 2010. Assessment of heavy metal contamination and accumulation in soil and wild plant species from an industrial area in Islamabad, Pakistan. Pakistan Journal of Botany, 42: 291-301.

[3] Adriano, D.C., Chlopecka, A., Kaplan, D.I., Clijsters, H., and Vangrosvelt, J. (1995). The philosophy, science, and technology of soil contamination and remediation. In: R. Prost (Ed.). Contaminated Soils. Paris: INRA, 466–504.

[4] Bini, C. (2010). Transitioning from soil contamination to land restoration. In: R.V. Steinberg (Ed.). Contaminated soils: Environmental impact, disposal, and treatment. New York: Nova Science Publisher. https://doi.org/10.1201/b10197-9

[5] Baker, A.J.M. (1981). Strategies of accumulators and excluders in plant responses to heavy metals. Journal of Plant Nutrition, 3: 643–654.

[6] Baker, A.J.M. and Walker, P.L. (1990). The ecophysiology of metal uptake in tolerant plants: Heavy metal tolerance in plants. In: A.J. Shaw (Ed.). Evolutionary aspects. Boca Raton: CRC Press.

[7] Paz-Alberto, A. M., & Sigua, G. C. (2013). Phytoremediation: An eco-friendly technology for the removal of environmental pollutants. American Journal of Climate Change, 2, 71–86.

[8] Sadeghi, S. A. T., Sadeghi, M. H., & Dashtizadeh, M. (2014). Analysis of heavy metals, including lead, nickel, and cadmium, in coastal forage from Tangistan, Bushehr Province, Iran. Bulletin of Environment, Pharmacology and Life Sciences, 3(3), 266–270.

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

[10] 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 environments of Brookes Point, Palawan, and

Mankayan, Benguet, and their implications for phytoremediation. Science Diliman, 21(2), 1–12.
[11] William, W.T., and Parsons, Thomas, E.G. 2004. Noxious Weeds of Australia. Cuthbertson, CSIRO PUBLISHING, Australia, 2nd Edition, pp. 376-377.
[12] Syed, Ibrahim, Fatima, Humaira, Mohammed, Abrar, and Siddiqui, Mohsin Ali. 2018. "Ceratophyllum demersum: A Free-floating Aquatic Plant - A Review." Indian Journal of Pharmaceutical and Biological Research (IJPBR), 6(2): 10-17.
[13] Quattrocchi, Umberto, F.L.S. 2012. World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology. CRC Press, Taylor & Francis Group, Florida, USA, 892 pages.
[14] AOAC (Association of Official Analytical Chemists), Official Methods Of Analysis, 1975, 12th ed., William Hormitz, pp 9 -12.
[15] APHA, 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.
[16] Echem Ogbonda G., Determination of the levels of heavy metal (Cu, Fe, Ni, Pb and Cd) up take of pumpkin (Telfairia occidentalis) leaves cultivated on contaminated soil, J. Appl. Sci. Environ. Manage. March 2014 Vol. 18 (1) 71-77, also its full-text Available Online at www.ajol.info and www.bioline.org.br/ja
[17] Smith R., A laboratory manual for the determination of metals in water and wastewater by 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. 1983.
[18] Jamaluddin Ahmed M., and Tasnima Zannat, A Simple Spectrophotometric Method for the Determination of Copper in Some Real, Environmental, Biological, Food and Soil Samples Using Salicylaldehyde Benzoyl Hydrazone, Pak. J. Anal. Environ. Chem. 2012, Vol. 13, No. 1, 22-35.
[19] IBM, Manual of Procedure for Chemical and Instrumental Analysis of Ores, Minerals, Ore Dressing Products and Environmental Samples Issued by Controller General Indian Bureau of Mines NAGPUR, C.S.Gundewar (Ed), Revised Edition February, 2012.
[20] Jamaluddin Ahmed M. and M Tauhidual Islam Chowdhury, 2004, A simple spectrophotometric method for the determination of Cadmium in industrial, Environmental, Biological and soil samples using 5,7-Dibromo-8-hydroxyquinoline, Analytical Science, June-2004, The Japan Society for Analytical Chemistry, pp. 987-990.
[21] Stanley Keith Yarbro, 1976, Two photometric methods for the determination of chromium in biological materials, A thesis Presented to the faculty of the division of graduate studies in partial fulfillment of the requirements for the degree Doctor of philosophy in the school of Chemistry, Georgia institute of technology. June, 1976.
[22] Hobart F. B. (1920), A Colorimetric method for the estimation of Cobalt, Thesis submitted for the Degree of Bachelor of Science in Chemistry, College of Liberal Arts and Science, University of Illinois.
[23] Bendix GH, Grabenstetter D. A Dithizone Method for the Rapid Determination of Copper. Ind.Eng. Chem. Anal.Ed. 1943; 15910): 649-652.
[24] Kimura K, Murakami Y. New Modified Colorimetric Method of Determination of Copper in Biological Materials with Sodium Diethyldithiocarbamate. Microchim. Acta1951; 36(2): 958-966.
[25] Sandell E.B. “Colorimetric Determination of Traces of Metals”, *Interscience*, New York. 1965, p. 269.
[26] Hackley BM, SmithJC, HalstedjA, A simplified method for plasma zinc determination by atomic absorption spectrophotometry. Clin Chem, 14:1-5, 1968.
[27] Lofberg RT. A Sensitive Visible Spectrophotometric Method for Copper Using Bis-Substituted Thiocarbamoyl Trisulfides. Anal. Lett. 1969; 2(8): 439-448.
[28] Rubeska I, Atomic Absorption Spectrophotometry. Chemical Rubber Co., Cleveland, Ohio, 1969, pp 97-100.
[29] Baker JM, Teggins JE, Hoffman JW. A Colorimetric Method for the Determination of Trace Copper Concentrations in Water. J. Am. Water Resour. As. 1971; 7(6): 1246-1249.
[30] James BE, Macmahon RA, An effect of trichloroacetic acid on the determination of zinc by atomic absorption spectroscopy. Clin Chim, Acta 32:307-309, 1971.

[31] Song M. K., N. F. Adham and H. Rinderknecht, A Simple, Highly Sensitive Colorimetric Method for the Determination of Zinc in Serum, *AJ.C.P.*, Feb.1976, *Vol. 65, 229-233.*Availabe and Downloaded from https://academic.oup.com/ajcp/article-abstract/65/2/229/1770841 as guest on 01 March 2018.

[32] Sarma LS, Kumar JR, Reddy KJ, Reddy AV. Development of an Extractive Spectrophotometric Method for the Determination of Copper (II) in Leafy Vegetable and Pharmaceutical Samples Using Pyridoxal-4-phenyl-3-thiosemicarbazone (PPT)J. Agric. Food Chem. 2005; 53 (14): 5492-5498.

[33] Rubina Soomro, M. Jamaluddin Ahmed, and Najma Menon, 2009, Simple and rapid spectrophotometric determination of trace level chromium using bis (salicylaldehyde) orthophenylenediamine in nonionic micellar media, Turk J Chem., 35 (2011), 155 – 170.

[34] Jamaluddin Ahmed M., Uttam Kumer Roy, A simple spectrophotometric method for the determination of iron (II) aqueous solutions, Turk J Chem., 33 (2009), 709-726.

[35] Gul Afshan Soomro, and Ghulam Abbas Shar, A Simple Spectrophotometric Method for the Determination of Cadmium (II) using 1-(2-pyridylazo)-2-naphthol in Micellar Aqueous Solution of CTAB, Ultra Chemistry Vol. 10(2), 95-104 (2014). Available at www.journalofchemistry.org

[36] Chong Kian Wei, Determination of heavy metals in water samples using sodium Sulfide precipitation and inorganic coagulation followed by FAAS detection, The Experiment, 2014., Vol. 28(1), 1888-1895. Available at www.experimentjournal.com

[37] Elkhatib, E.A., Thabet, A.G., Mahdy, A.M., 2001. Phytoremediation of cadmium contaminated soils: role of organic complexing agents in cadmium extraction. Land Contam. Reclamation 9 (4), 359e366.

[38] R. Carrillo Gonzalez a and M.C.A. Gonzalez-Chavez, 2006. Metal accumulation in wild plants surrounding mining wastes, Environmental Pollution 144 (2006) 84e92.

[39] Yoon, J., Cao, X., Zhou, Q., & Ma, L. Q. (2006). The accumulation of lead, copper, and zinc in native plants at a contaminated site in Florida. Science of the Total Environment, 368, 456–464.

[40] Kamari A, Yusoff M, Najiah S, Putra WP, Ishak CF, Hashim N, Mohamed A, Phillip E 2014. Metal uptake in water spinach grown on contaminated soil amended with chicken manure and coconut tree sawdust. Environmental Engineering Management Journal, volume 13, issue 9, pages 2219–2228.

[41] Demina. L.L; Galkin. S.V. & Shumilin. E.N. Bioaccumulation of some trace elements in the biota of hydrothermal fields of the Guaymas Basin (Gulf California). Boletin De LA Sociaeded Geologica Mexicana, 2009; 61(1): 31-45

[42] Buat-Menard, P. and Chesselet, R. (1979) Variable Influence of Atmospheric Flux on the Trace Metal Chemistry of Oceanic Suspended Matter. Earth and Planetary Science Letters, 42, 398-411.

[43] Salomons, W., & Förstner, U. (1984). Metals in the hydrocycle. Springer, Berlin Heidelberg Tokyo.

[44] Dickinson, W. W., Dunbar, G. B., & McLeod, H. 1996. The historical record of heavy metals from sediment cores in Wellington Harbour, New Zealand. Environmental Geology, 27, 59–69.

[45] Hornung, H., Karm, M. D., & Cohen, Y. 1989. The distribution of trace metals in sediments and benthic fauna of Haifa Bay, Israel. Estuarine, Coastal and Shelf Science, 29, 43–56.

[46] Ravichandran, M., Baskaran, M., Santschi, P. H., & Bianchi, T. 1995. A historical overview of trace metal pollution in the Sabine-Neches Estuary, Beaumont, Texas. Environmental Science and Technology, 29, 1495–1503.

[47] Mocko A, Waclawek W (2004) Three-step extraction procedure for determination of heavy metals availability to vegetables. Analytical and Bioanalytical Chemistry 380, 813-7.

[48] Sanghamitra, K., Prasada Rao, P.V.V. Naidu, G.R.K Uptake of Zn (II) by an invasive weed species Parthenium hysterophorus L. Applied Ecology And Environmental Research 10(3): 267-290.

[49] Rezvani, M. and Zaefarian, F. (2011). Bioaccumulation and translocation factors of cadmium and lead in *Aeluropus littoralis*. *Australian Journal of Agricultural Engineering*, 2: 114-119.

[50] Kumar, A., Ahirwal, J., Maiti, S.K., and Das, R. 2015. An assessment of metals in fly ash and their translocation and bioaccumulation in perennial grasses growing at the reclaimed opencast mines, International Journal of Environment, volume 9, issue 3, pages 1089-1096.

[51] Akande, F.O. and Ajayi, S.A. 2017. Assessment of Heavy Metals Level in Soil and Vegetables Grown in Peri-Urban Farms around Osun State and the Associated Human Health Risk, International Journal

of Environment, Agriculture and Biotechnology (IJEAB), volume 2, issue 6, pages 3250-3261.
[52] 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.
[53] Osundiya, M. O., Ayejuyo, O. O., Olowu, R. A., Bamgboye, O. A., & Ogunlola, A. O. (2014). Bioaccumulation of heavy metals in commonly consumed leafy vegetables cultivated along the Nigeria-Benin Seme Border, West Africa. Advances in Applied Science Research, 5(1), 1-7.
[54] Shingadgaon S.S., Jadhav S.L., Thete-Jadhav R.B., Zambare N.S., Daspute-Taur A.B., Babare M.G. and Chavan B.L. "Potentials of aquatic macrophyte species for bioremediation of metal contaminated wastewater," *International Journal of Current Research*, vol.10, issue 12, pp.76384-76390, December, 2018.
[55] Chopra, A. K., Pathak, C., & Prasad, G. (2009). The issue of heavy metal contamination in agricultural soils and its management strategies. Journal of Applied Natural Science, 1, 99-108.
[56] Roberts, J. R. (1999). The impact of metal toxicity in children. In Training Manual on Pediatric Environmental Health. Children's Environmental Health: Emeryville, CA.
[57] World Health Organization (WHO). (2011). Guidelines for Drinking-water Quality (4th ed.). Malta: Gutenberg. ISBN 978 92 4 154815 1.
[58] 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.
[59] Freitas, H., Prasad, M. N. V., & Pratas, J. (2004). Plant communities tolerant to trace elements in the degraded soils of the São Domingos mine in southeastern Portugal: Environmental implications. Environment International, 30, 65–72.
[60] 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.
[61] Nazareno, P. G., & Buot, I. E. Jr. (2015). The effects 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.
[62] Jones, T. N., & Buot, I. E. Jr. (2017). Assessment of floral diversity in Balili River as potential phytoremediators. Journal of Wetlands Biodiversity, 7, 17-28.
[63] Kabata-Pendias, A. (2011). Trace elements in soils and plants (3rd ed.). Boca Raton: CRC Press.