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A study on the application of phytoremediation ecological technology of heavy metals in water using *E. fluctuans*: A medicinal plant adapting to climate change

An The Huynh^{*}, Trung Minh Dao



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ABSTRACT

Phytoremediation can be used as a different approach to absorb heavy metals because of environmentally friendly and potential cost-effective. *E. fluctuans* is one species of herbaceous, semiaquatic, widely distributed in Vietnam, South and Southeast Asia, and tropical Africa. Its leaves are used as edible vegetables in daily meals, and it is also used to treat some diseases in traditional medicine. This paper aims to test the effectiveness of *E. fluctuans* in removing heavy metals from the aquatic environment. The experiments were performed using healthy, young, and acclimatized *E. fluctuans*. Water containing 0.5 mg/L cadmium, 0.5 mg/L arsenic, 2 mg/L lead, 5 mg/L zinc, and 5 mg/L copper concentrations were experimented with 100 g of *E. fluctuans* in 30-liter foam containers. The experiments were repeated three times. Throughout the course of the trial, water samples were tracked and their heavy metal concentrations were examined every 10, 20, and 30 days. Through the use of inductively coupled plasma-mass spectrometry, the heavy metals were identified. After 30 days, *E. fluctuans* could remove Cd at 83.4%, As at 60.8%, Pb at 60.4%, Zn at 40.5%, and Cu at 58.7%. Thus, the *E. fluctuans* show a high potential for effectively extracting heavy metals from industrial effluent.

Key words: phytoremediation, E. fluctuans, cadmium, arsenic, lead, zinc, copper, climate change, medicinal plant

INTRODUCTION

2 One of the most important resources for human sur-³ vival and daily activities is water¹. When the global 4 population continuously increases, it is also increas-5 ing the food demand and intensive activities of in-6 dustry and urbanization leading to the requirement of 7 water owing steadily increasing. However, the quanti-8 ties of water are gradually degraded in many areas due 9 to the expanded volume of huge amounts of danger-10 ous chemicals from intensive agriculture and industries as well as human activities that of contaminated 11 12 waste and wastewater released directly into the natural environment². Especially, ollution from heavy 13 14 metals is an emergency impact to the environment 15 and human health because of their toxic properties, ¹⁶ tendency, and persistent in nature³⁻⁵. Specifically, 17 excessive levels of heavy metals including Cd, A, Pb, 18 Zn, and Cu in wastewater will pollute water sources 19 and easily accumulated in the human body by drink-20 ing the water without or un-well treatment. Those ²¹ heavy metals can cause deleteriously affecting human $_{22}$ health even though at very low concentrations⁶.

²³ Among the most appealing research areas is the de-²⁴ velopment of environmentally friendly and effective

wastewater treatment systems. Phytoremediation is ²⁵ acknowledged as an ecological remediation technology that is climate change-adapted and is thought ²⁷ to be a viable technique for removing contaminants ²⁸ from wastewater⁷. ²⁹

Aquatic plants are essential for maintaining water re-30 sources in the phytoremediation system, as a part to 31 absorb energy, organic matter, and heavy metals in the water, which is greatly improving the water qual-33 ity⁸. Previous researches have found a number of 34 aquatic plants improving the water environment such 35 as Water Lily, Cyperus Alternifolius, Phragmites Aus-36 tralis, Water Hyacinths, Water Spinach, Typha Orien-37 talis... Besides, E. fluctuans is considered as a potential aquatic plant remove heavy metals in water. 39

E. fluctuans commonly known as helencha or harkuch 40 is a tropical herb. It is belonging to Asteraceae family 41 which is importance species for therapeutic process. 42 This herbaceous vegetable plant, which is edible and 43 semi-aquatic, has serrated leaves and is widely grown 44 in Vietnam. The plant is a prostrate herb with 1-3 inch 45 long, opposing sessile, linear oblong leaves. The herb 46 is often pubescent and glandular, with a glabrous tex-47 ture. The stems have a length of 0.3-0.6 m and can ei-48 ther simply extend or divide at the nodes. The leaves 49

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 $_{\rm 50}\,$ have a hint of bitterness, and they are good for treating

51 smallpox, bronchitis, leucoderma, nervous affliction,

52 laxative, and inflammation. The nutritional worth of

 $_{\rm 53}\,$ plants, such as β -carotene, saponins, cholesterol, glu-

54 coside, and enhydrin, among others. Moreover, E.

55 fluctuans's fuel extract contains antioxidant, hepato-

⁵⁶ protective, CNS depressant (Central Nervous System⁵⁷ (CNS) depressants), analgesic, and Antidiarrheal ac-

58 tivity⁹⁻¹¹.

59 Vietnam is a tropical country with year-round high

60 temperatures and humidity. The country has a long

₆₁ coastline of 3,260 km with more than 2,360 rivers and

⁶² streams and thousands of lakes and ponds ¹². This wa-

63 ter source is the habitat of animals, plants, and mil-

64 lions of people and is also the main source for produc-

65 tion. However, overuse and various levels of pollution

⁶⁶ are causing these water resources to be severely dam-⁶⁷ aged and destroyed. Even many rivers, river sections,

⁶⁸ ponds, and lakes are "dead" by the volume of waste,

⁶⁹ garbage and wastewater discharged into the environ-

70 ment without being treated.

71 However, there have been a few studies in Vietnam

⁷² about the ability of aquatic plants to treat wastewater
⁷³ pollution. Furthermore, it is still no research about
⁷⁴ using *E. fluctuans* to treat heavy metal pollution in
⁷⁵ wastewater, especially in industrial wastewater. It is

 $_{76}$ necessary to investigate the research on the use of *E*.

77 fluctuans - A medicinal plant with potential in phy-

78 toremediation of water contaminated by heavy met-

79 als. The study will send the significant theory and

⁸⁰ practical results of uptake heavy metals contaminants ⁸¹ from industrial wastewater by *E. fluctuans*.

PHARMACOLOGICAL ACTIVITY OF *E. FLUCTUANS*

⁸⁴ Medicinal plants are a class of plants that are used for
⁸⁵ medicinal purposes and have specific active medicinal
⁸⁶ components together with certain traits or attributes
⁸⁷ that allow them to be used as drugs or therapeutic

agents¹³.

89 A small genus of marsh herb known as E. fluctuans

90 (Family: Asteraceae) is found in tropical and subtrop-

91 ical regions. The plant is an annual herb that spreads

to the prostate. This is another edible, semi-aquatic

⁹³ herbaceous vegetable plant that grows throughout
 ⁹⁴ Vietnam and has serrated leaves¹⁴ Between novem-

⁹⁴ Vietnam and has serrated leaves¹⁴ Between novem-⁹⁵ ber and january, the wet roadside canals and marshy

⁹⁶ waste areas are the primary habitats for *E. fluctuans*¹³.

97 It is a plant with medicinal potential in Bangladesh,

98 Malaysia, Srilanka, Zambia, Zimbabwe, India, China,

99 Thailand, Indonesia, Southeast Asia, Tropical Africa,

100 and Vietnam. From ancient times, folk medicine has

been used in all of *E. fluctuans* to treat a wide range of illnesses, with different countries having different indications. The pharmacological activity of *E. fluctuans* are detailed in Figure 1.

E. Fluctuans may have some anti-cancer properties. ¹⁰⁵ Though it has only been shown against Swiss Albino ¹⁰⁶ Mice with Ehrlich's Ascites Carcinoma (EAC). Therefore, more investigation is required to ascertain the ¹⁰⁷ anti-cancer potential of the plant extracts ¹⁵. In addition to its antioxidant qualities, it possesses antimicrobial, cytoprotective, anti-inflammatory, analgesic, ¹¹¹ CNS depressant (Central Nervous System (CNS) depressants), and thrombolytic characteristics. The ¹¹³ abundance of biomolecules in the database suggests ¹¹⁴ that more study will advance the pharmaceutical industry. ¹¹⁶

MATERIALS- METHODS

Chemicals and instruments

Standard solutions containing 100 μg/mL of copper,119zinc, lead, arsenic, and cadmium were made. All of120the compounds were pure substances produced by121Merck Chemical in Darmstadt, Germany. The water122used to make the solutions was double-distilled.123The research employed various instruments such as124an analytical balance with a precision of 0.0001 mg, a125flask, a test tube, an electric stove, and a micropipette.126ICP-MS (Inductively Coupled Plasma - Mass Spec-127trometry) equipment made by Perkin Elmer ELAN1289000 was used to analyze the samples.129

Experimental Setup

100 g of *E. fluctuans* were utilized in the pot for the course of the 30-day trials. Thirty-liter foam contain- 132 ers were used for planting, after they had been cleaned 133 with distilled water to remove any dust and soil (Plant 134 with a 35-cm body length that is selected based on a 135 set of characteristics, such as the absence of insects 136 and the roots being three months old). According to 137 the ational technical regulation on industrial wastewater in Vietnam (QCVN 40:2011/BTNMT), concen- 139 tration selection tests were conducted based on the allowed threshold levels for Cd, As, Pb, Zn, and Cu in 141 aquatic environments. Three to five times the permit- 142 ted threshold was exceeded by the experimental concentration. E. fluctuans was cultivated in irrigation 144 water that had chosen concentrations of the heavy 145 metals Cd (II), As (III), Pb (II), Cu (II) and Zn (II). Ta-146 ble 1 shows the concentrations selected for perform-147 ing the experiment. 148

Plants in distilled water were planted as a matched 149 plant control sample. 150

Heavy metals (Cd, As, Pb, Zn, and Cu) in water were 151 the analyzed parameters. 152

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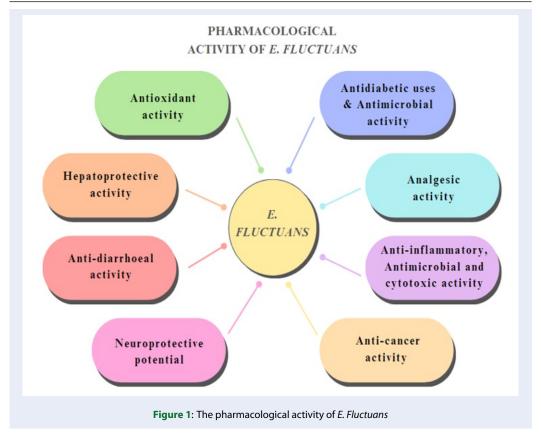


Table 1: Concentration selected for experiment

No.	Heavy metal	Concentration
1	Cadmium (Cd)	0.5 mg/L
2	Arsenic (As)	0.5 mg/L
3	Lead (Pb)	2 mg/L
4	Zinc (Zn)	5 mg/L
5	Copper (Cu)	5 mg/L

153 Analysis Sample

Following planting, three analyses of the heavy metal
concentration in the water were conducted on the
sample at 10, 20, and 30 day intervals in order to track
the evolution of the amount of metal in the treated water with *E. fluctuans*. Through the use of inductively
coupled plasma-mass spectrometry, the heavy metal
content of the plants was determined to be Cd, As, Pb,
Zn and Cu.

162 Analyzing Data

¹⁶³ The researched data were evaluated and contrasted
¹⁶⁴ with the most recent tandards for Vietnam (QCVN
¹⁶⁵ 40:2011/BTNMT). Software from Statgraphics and

Excel were used to process the data.

RESULTS AND DISCUSSIONS

Growth of *E. fluctuans* in heavy metals polluted water 169

Normally, when the concentration of a metal in water is not higher than the critical tolerance value of the plant, it does not affect the growth and height of the plant, so the biomass of the plant will not affect the plant growth. Because lant growth necessitates specific heavy metals and upkeep¹⁶. However, when the metal concentration in the water exceeds the limit value, the plant growth will be inhibited and manifest external states such as yellow leaves, which 178

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¹⁷⁹ reduces its height and biomass ¹⁷. Depending on the ¹⁸⁰ specific heavy metal involved in the process, different ¹⁸¹ heavy metals have different effects on plant growth. ¹⁸² Table 2 displays the impact of water contaminated ¹⁸³ with heavy metals on *E. fluctuans* plant growth. The ¹⁸⁴ changes in plant growth in these samples could plau-¹⁸⁵ sibly be explained by significant differences in height

growth rates. (p < 0.05). 186 Table 2 presents the results, which indicate that one of 187 the key indicators for evaluating the growth of plants 188 containing various heavy metals in the environment 189 (Cd, As, Pb, Zn, and Cu) is height. The plants' abil-100 ity to absorb nutrients determined the E. Fluctuans ' 191 height growth rate. The heavy metals in the water, in 192 the following order: Cd > As, Cu > Pb > Zn, were cor-193 related with the decreasing height. The following is a description of the growth condition: 195

¹⁹⁶ The growth of plants in the environment with 0.5
¹⁹⁷ mg/L Cd and As content. *E. fluctuans* showed good
¹⁹⁸ Cd and As absorption capacity, the plants were less
¹⁹⁹ likely to die, had good growth ability. There was an
²⁰⁰ increase in height.

201 Growth of plants in a water environment with 2 mg/L
202 of lead. *E. fluctuans* grew and developed well. How203 ever, after 15 days, it was observed that the plants had
204 yellowing of leaves.

The plants in the water containing 5 mg/L of zinc 205 showed symptoms of stunted growth; the stems and 206 leaves had gone yellow. Ninety percent of the plants 207 had softened leaves and stems by day 20 due to yellow-208 209 ing. The plants began to wilt, turn yellow, and show no signs of survival at the end of the experiment. The 210 plants in 5 mg/L of Cu in water demonstrated that the 211 212 growth of the plants was still maintained and there was growth in height. However, at the end of experi-213 mental period, many foliage had turned to yellow. 214

When plants are grown in water contaminated with 215 heavy metals, their growth is reduced as a result of al-216 tered physiological and biochemical activities. This is 217 particularly true when the heavy metal in question has 218 no favorable effect on the growth and development of 219 plants¹⁸. It is therefore clear from the height of the E. fluctuans in each pot that height growth was taken 221 into consider. However, the various metals had vary-222 223 ing effects on the E. fluctuans' height growth; some 224 even wilted, perished, and failed to develop young plants. After that, the pot's biomass was drastically 225 226 decreased.

227 Potential of *E. fluctuans* to accumulate228 heavy metals

Plant species with the potential to process heavy met-als must meet at least two of the following conditions:

(i) have the ability to accumulate large amounts of pol lutants (100 times greater than normal plants); (ii) ca pable of generating large biomass under the simplest
 cultivation conditions^{19,20}.

Removing potentially hazardous metals from the environment through phytoremediation is thought to be an efficient, aesthetically pleasing, economical, and environmentally benign approach. Through their roots, plants in phytoremediation gather pollutants, which they then transfer to the portion of their bodies above ground^{21,22}. Many terms, including agro-remediation, green remediation, vegetative remediation, green technology, and botano remediation^{23,24} are used to refer to phytoremediation. 244

E. fluctuans was used in a phytoremediation procedure with the aim of evaluating its efficacy in treating heavy metals. In order to track the amount of metal in 247 water that *E. fluctuans* has treated over time, the sample was planted and the heavy metal concentration in 249 the water was tested three times, after 10, 20, and 30 250 days. Table 3 presents the findings. 251

According to the experimental procedure, the find- 252 ings indicate that as the duration of treatment with $E_{1,253}$ fluctuans increased, the amounts of Cd, As, Pb, Zn, 254 and Cu in the water steadily reduced. In particular, 255 the starting levels of Cu, Zn, Pb, As, and Cd in the 256 water were 0.5 mg/L, 0.5 mg/L, 2 mg/L, 5 mg/L, and 257 5 mg/L, correspondingly. The amounts of Cd, As, Pb, 258 Zn, and Cu in the water were 0.304 mg/L, 0.392 mg/L, 259 1.617 mg/L, 4.087 mg/L, and 3.261 mg/L, respectively, 260 ten days after the E. fluctuans was planted. The con- 261 centration of heavy metals (Cd, As, Pb, Zn, and Cu) 262 dramatically dropped by day 30 of the experiment. 263 Growing E. fluctuans in water that contains heavy 264 metal contamination (Cd, As, Pb, Zn, and Cu) has 265 been shown to allow the plant to grow and flourish 266 to a certain extent. The effects of various heavy met- 267 als on plant growth and development vary depending 268 on the particular heavy metal involved in that process. 269 Metals like Pb, Cd, and As have been demonstrated to 270 negatively affect plant growth even at extremely low 271 concentrations and to play no beneficial role in plant 272 growth¹⁸. The examination of the water's content of 273 heavy metals revealed a trend of gradually declining 274 concentration. As a result, E. fluctuans is highly effec- 275 tive at cleaning water tainted with heavy metals. 276

Heavy metal remaining ercentages in water 277 and removal efficiency ercentage 278

Figure 2 displays the percentage of removal efficiency 279 for the plants' capacity to take up heavy metals (Cd, 280 As, Pb, Zn and Cu) in the water over time. 281

Factor	Initial Height (cm)	After thirty days, Height(cm)
Cd	35	$38.0a\pm0.2$
As	35	$37.2b\pm0.8$
Pb	35	$37.0b\pm0.2$
Zn	35	$36.1b\pm0.5$
Cu	35	$37.2c \pm 0.4$
= 0.0069*		

Table 2: Effect of heavy metal polluted water on plant growth of E. fluctuans

1. The values represented by a, b, and c are statistically distinct.

2. * Significant means with p<0.05.

Table 3: Results of analysis of heavy metal concentrations in water over time.

Days	Cd (mg/L)	As (mg/L)	Pb (mg/L)	Zn (mg/L)	Cu (mg/L)
0	0.5	0.5	2	5	5
10	0.304 ± 0.001^a	0.392 ± 0.001^a	1.617 ± 0.0006^a	4.087 ± 0.002^a	3.261 ± 0.002^a
20	0.215 ± 0.0006^b	0.297 ± 0.001^b	1.250 ± 0.001^b	3.428 ± 0.002^b	2.782 ± 0.001^b
30	0.083 ± 0.002^{c}	0.196 ± 0.002^{c}	0.793 ± 0.0006^c	2.977 ± 0.001^{c}	2.067 ± 0.002^c
QCVN 40:2011/BTNMT (Column B)	0.1	0.1	0.5	3	2

* The data are presented as mean \pm SD; values indicating significant differences at P < 0.05 are indicated by various superscripts in the same column.

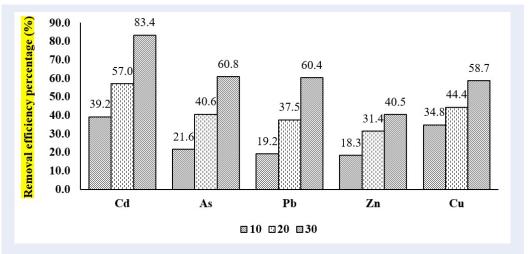


Figure 2: Heavy metal removal efficiency percentage in water

Cadmium has the best absorption performance 282 among metals. Zinc is the metal with the worst ab-283 sorption performance. Over the course of the 30-day 284 trial, E. fluctuans was able to achieve treatment effi-285 ciencies for Cd, As, Pb, Zn, and Cu of 83.4%, 60.8%, 286 60.4%, 40.5%, and 58.7%, respectively. These results 287 corresponded to the beginning concentrations of 0.5 288 mg/L, 0.5 mg/L, 2 mg/L, 5 mg/L, and 5 mg/L. All 289 heavy metals (Cd, As, Pb, Zn, and Cu) had cleaning rates of 40.5-83.4% for E. fluctuans by the end of the 291 30-day survey period. The analysis results show that 292 after 30 days compared with the values in column B QCVN 40:2011/BTNMT, the Cd content (reaching 294 the treatment rate of 83.40%) is within the allowable 295 296 limit.

The findings demonstrate that, in comparison to the 297 initial concentrations, after 30 days, E. fluctuans' ca-298 299 pacity to accumulate heavy metals steadily declined with the residual heavy metal contents in the water 300 in the order Cd < As < Pb < Cu < Zn. In particular, 301 302 the residual percentage of cadmium was just 16.6%, meaning that the initial concentration was 0.1 mg/L. 303 According to the study, E. fluctuans has a strong ca-304 pacity to absorb heavy metals in wastewater, includ-305 ing lead, zinc, copper, cadmium, and arsenic. The 306 analysis's findings supported the buildup of various 307 metals within E. fluctuans and the related decline in 308 metal levels in the water. As has been noted in a 309 number of other macrophyte species, E. fluctuans ex-310 hibits a high capacity for heavy metal removal from 311 the aqueous medium. This capacity may be facili-312 tated by their greater biomass, quick development, 313 and capacity for metal absorption from the aqueous 314 315 medium.

316 CONCLUSIONS

As opposed to other environmental pollutants, heavy
metals are now the main cause for concern. because
heavy metals can't be destroyed by degradation. So,
choosing a low-cost, environmentally friendly technology is a top priority. An additional option as a
green method to treat areas affected by heavy metals
is phytoremediation. Choice of the appropriate plant
is the most significant feature in phytoremediation.

E. fluctuans was evaluated as a possible plant for the 325 removal of Cd, As, Pb, Zn, and Cu based on the 326 amounts of heavy metals in water using phytoreme-327 328 diation technology. E. fluctuans was able to lower the concentrations of Cd by 83.4%, As by 60.8%, Pb by 329 60.4%, Zn by 40.5%, and Cu by 58.7%, per the ex-330 periment results. Sixty-eight percent less heavy metal 331 332 was present overall. Furthermore, E. fluctuans is a ³³³ medicinal plant that has been useful in the treatment

of a wide range of illnesses. These characteristics, ³³⁴ along with its capacity for fragmentation-based asexual propagation, make it a plant that is worthwhile investigating for its potential use in both safe and environmentally friendly wastewater treatment methods that integrate natural ecosystems and phytoremediation of heavy metal-contaminated water. 340

CONFLICT OF INTEREST

There is no conflict of interest declared by the authors. 342

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AUTHOR CONTRIBUTIONS

(A.T.H) Original draft writing, writing reviews, editing, conceptualization, methodology, research, and experimentation. The conceptualization, review, and supervision were completed by (TMD).

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TÓM TẮT

Phương pháp xử lý ô nhiễm môi trường bằng thực vật có thể được sử dụng như một giải pháp thay thế để hấp thụ kim loại nặng vì thân thiện với môi trường và có khả năng tiết kiệm chi phí. Cây Ngổ trâu là loài cây thân thảo, bán thủy sinh, phân bố rộng rãi ở vùng nhiệt đới Châu Phi, Nam Á, Đông Nam Á và Việt Nam. Lá của nó được dùng làm thức ăn và trong y học cổ truyền ó cũng được sử dụng để điều trị một số bệnh. Bài báo này nhằm mục đích kiểm tra hiệu quả của cây Ngổ trâu trong việc loại bỏ kim loại nặng khỏi môi trường nước. Các thí nghiệm được thực hiện bằng cách sử dụng cây Ngổ trâu khỏe, trưởng thành và đã thích nghi với khí hậu. Nước chứa ồng độ 0,5 mg/L cadimi, 0,5 mg/L asen, 2 mg/L chì, 5 mg/L kẽm và 5 mg/L đồng đã được thử nghiệm với 100 g Ngổ trâu trong thùng xốp 30 lít. Các thí nghiệm được lặp lại ba lần. Các mẫu nước được theo dõi và phân tích hàm lượng kim loại nặng tại các thời điểm 10, 20 và 30 ngày của quá trình thí nghiệm. Các kim loại nặng được xác định bằng phương pháp hổ khối-plasma kết hợp cảm ứng. Sau 30 ngày thí nghiệm, cây Ngổ trâu có khả năng loại bỏ Cd là 83,4%, As là 60,8%, Pb là 60,4%, Zn là 40,5% và Cu là 58,7%. Do đó, cây Ngổ trâu tiểm năng cao trong việc loại bỏ kim loại nặng khỏi nước thải công nghiệp một cách hiệu quả.

Từ khoá: xử lý môi trường bằng thực vật, cây Ngổ trâu, cadimi, asen, chì, kẽm, đồng, biến đổi khí hậu, cây thuốc

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