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Phytoremediation of heavy metals in battery industrial effluent using *Eichhornia crassipes*

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ABSTRACT

Phytoremediation of heavy metal ions present in battery industry effluent using *Eichhornia crassipes* through influence of ethylenediaminetetraacetic acid (EDTA) and cow dung manure (CDM) was investigated. Among considering 11 heavy metal ions (As^{2+}, Cd^{2+}, Ca^{2+}, Cr^{6+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, Mg^{2+}, Ni^{2+}, K^{+} and Zn^{2}), Cu^{2+} ions was higher in translocation factor (0.84), bioconcentration factor for root (1.01) and shoot (0.5) and removal % (54.78%). Highest degradation rate and transposition factor (TrF) was obtained for As^{3+} (19.46%) and K^{+} ions (2.24%), respectively. *E. crassipes* grown in direct effluent (DE) with CDM possess very good biochemical properties (chlorophyll a and b, total chlorophyll, carotenoid and proline) compared with plants under DE and DE with EDTA. *E. crassipes* with CDM provide an efficient and ecological alternative to accelerate the removal and degradation of heavy metal pollution from industrial effluent.

Keywords: Eichhornia crassipes; Phytoremediation; Translocation factor; Heavy metal; Aquatic plants

1. Introduction

Urbanization and rapid industrial growth are the main source for the contamination of aquatic bodies. Due to untreated discharge of domestic and industrial wastes to environment, quality of ground water is decreasing gradually to an alarming level [1,2]. Effluents discharged from electroplating, battery, textile, pigment and dyes, paint industries have significant quantities of heavy metal ions (Cd^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+}, etc.) [3,4].

Contaminants accumulated in the marine environment are biomagnified in aquatic plants [5]. Heavy metal ions are gradually incorporated in the food chain which affects plant growth and metabolism and causes physical illness and incurable diseases to living beings [6]. The effects of which include stunted growth, chlorosis, reduced crop yield, delayed germination, senescence, premature leaf fall, biochemical lesions, enzymatic changes and reduced biosynthesis [7,8]. Heavy metal toxicity often leads to chronic condition to human health. So, it is essential and important to eradicate such toxic metal ions from environments.

Conventional methods such as membrane filtration, precipitation, nanofiltration, ion-exchange, electrocoagulation, flotation and adsorption are found to be efficient [9–13]. But the operating cost and other form of effluent from conventional technologies made it difficult to implement in developing countries which produces a huge amount industrial wastes and releasing it to environment [14]. Scientists have turned their attention towards biological methods to overcome such drawback [15–17]. Researchers have identified phytoremediation, use of plants to remove heavy metal ions from environment, as an eco-friendly and economically alternative methodology [18,19]. Since it is cost-effective and requires less skill, phytoremediation is an effective approach to remove contaminants from environment [20,21].

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Phytoremediation is one of the recent technologies which guarantee an effective, economical and sustainable means to achieve pollution-free environment [18,21]. It has emerged as a feasible technology for the removal of elemental pollutants from soil and water using various hyperaccumulator plant species [22]. Phytoremediation has the ability to remove suspended particles, nutrients and contaminants from wastewater. Hence the contamination level can be reduced by the activity of plants during the process [23,24]. The success of phytoremediation mainly depends on the photosynthetic activity and the growth rate of plants [5].

Lemna sp., *Spirodela* sp., *Eichhornia* sp., *Azolla* sp. and *Pistia* sp. are some of the aquatic macrophytes that have been used in the removal of contaminants from wastewater [17,18,23,24,25]. *Eichhornia crassipes,* an aquatic plant, is one of the expandable plants from the environment which possess rapid growth and distribution rate. It is a perennial free-floating plant which has high tolerance to heavy metal contaminants and ability to accumulate contaminants from wastewater [26]. Studies have shown that *E. crassipes* was able to treat domestic sewage and a significant number of researches have been carried out to find the prospective of *E. crassipes* [27,28].

The main objective of this study was to understand the accumulation ability and biochemical properties of *E. crassipes* on treatment of heavy metal contaminants under influencing agents.

2. Materials and methods

2.1. Materials

Eichhornia crassipes was collected from local Ukkadam-Valankulam Lake, Coimbatore, India. Plants were acclimatized for 25 d in laboratory conditions (115 µmol m^{-2} s^{-1} light with 12 h photoperiod at 23°C ± 2°C) in 10% Hoagland's solution. Standard solutions were prepared according to the PerkinElmer Pure Atomic Spectroscopy Standards guidelines (NIST traceable CRM, PerkinElmer Corporation, USA and Merck, Germany). Working standard solutions were prepared by diluting the stock solution with 0.1 M HNO_3 for checking the linearity.

2.2. Phytoremediation experiment

5-L Perspex plastic container was filled with soil (sterilized in autoclave at 150°C) and industrial effluent (battery industry) in the ratio of 1:1. After 5 weeks of growth in Hoagland solution, evenly sized plants from the sides of *E. crassipes* were taken for experimentation. Plants were allowed to grow for 25 d under pH 6–7 using $C_2H_3NaO_2$ buffer. Experiment was carried out by direct effluent (DE), DE with ethylenediaminetetraacetic acid (EDTA) and DE with cow dung manure (DE + CDM). The heavy metal content in effluent, soil and plant (root and shoot) was estimated (triplicate manner) at an interval of 5 d.

2.3. Determination of heavy metal concentration

Root, shoot and soil samples were acid-digested to determine the heavy metal concentration. Perspex plastic containers and glassware were used for the preparation of solid samples. 0.5 g sample was cleaned (using distilled water) and placed in a muffle furnace at 450°C for 3 h. The residual ash was mixed completely with 5 mL of 6 M HCl and kept in a hot plate for digestion. The digested solution was diluted up to 50 mL using 0.1 M HNO_3 for analyzing the heavy metals. Atomic absorption spectrophotometer (Analyst 800, PerkinElmer Corporation, USA) equipped with flame and graphite furnace was used to determine the heavy metal concentration (Table 1). Air-acetylene flame was used for determination of metal content. Data obtained from AAS were rounded off suitably according to the value of standard deviation from measurements in triplicate.

Table 1
Heavy metal concentration in effluent

Heavy metal content	Concentration (mg/L)
Arsenic (As^{3+})	33.52
Cadmium (Cd^{2+})	1.84
Calcium (Ca^{2+})	26.56
Chromium (Cr^{6+})	1.35
Copper (Cu^{2+})	64.76
Iron (Fe^{2+})	177.33
Lead (Pb^{2+})	25.28
Magnesium (Mg^{2+})	25.32
Nickel (Ni^{2+})	42.18
Potassium (K^{+})	153.52
Zinc (Zn^{2+})	41.96

2.4. Determination of photosynthetic pigments

1 g of fresh leaves obtained from *E. crassipes* was washed with distilled water and homogenized using a pestle and mortar in 10 mL of 95% (v/v) ethanol. The solution was filtered through Whatman filter (2.5 µm) paper and the filtrate was made up to 25 mL with 95% ethanol for estimating chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll and carotenoid [8].

2.5. Estimation of proline

Fresh leaves of the plant were homogenized using 10 mL of 3% (w/v) $C_7H_6O_6S$ and centrifuged at 6,000 rpm for 10 min. 1 mL supernatant from centrifuge, CH_3COOH and $C_9H_6O_4$ were boiled at 100°C for an hour. Reaction mixture was cooled down after the formation of brick red colour in the tube. Extract was collected after vortexed with the addition of toluene [8]. Free proline content in plant is assessed from the standard curve made from the toluene as a blank solution using UV–visible spectrophotometer, Shimadzu, Japan, at 520 nm.

3. Results

3.1. Distribution of adsorption sites

Heavy metal concentration in effluent, soil, root and shoot of *E. crassipes* was determined from DE, DE + EDTA and DE + CDM treatment (Tables 2–4). SEM characterization

Table 2
Heavy metal concentration in *E. crassipes* (DE)

Period (d)	Root						Shoot						Soil						Effluent					
	0	5	10	15	20	25	0	5	10	15	20	25	0	5	10	15	20	25	0	5	10	15	20	25
As^{3+}	0.00	0.05	0.40	1.23	4.32	8.46	0.00	0.03	0.19	0.59	2.06	4.63	0.00	0.38	1.07	7.26	9.86	10.61	33.52	31.73	30.41	22.71	15.02	7.10
	±0.00	±0.00	±0.01	±0.02	±0.07	±0.10	±0.00	±0.00	±0.00	±0.02	±0.09	±0.07	±0.00	±0.01	±0.04	±0.11	±0.20	±0.22	±0.00	±0.01	±0.05	±0.11	±0.09	±0.22
Cd^{2+}	0.00	0.05	0.10	0.17	0.28	0.34	0.00	0.01	0.04	0.09	0.15	0.25	0.00	0.16	0.35	0.50	0.58	0.68	1.84	1.55	1.29	1.01	0.73	0.44
	±0.00	±0.00	±0.00	±0.01	±0.01	±0.01	±0.00	±0.00	±0.01	±0.00	±0.00	±0.00	±0.00	±0.01	±0.01	±0.01	±0.01	±0.01	±0.00	±0.01	±0.01	±0.00	±0.02	±0.02
Ca^{2+}	0.00	0.54	1.51	2.93	4.20	5.38	0.00	0.15	0.49	1.26	2.56	3.27	0.00	1.29	3.52	6.89	9.05	9.94	26.56	23.61	19.94	14.33	9.37	6.45
	±0.00	±0.01	±0.02	±0.08	±0.11	±0.17	±0.00	±0.00	±0.01	±0.04	±0.07	±0.14	±0.00	±0.04	±0.09	±0.10	±0.11	±0.03	±0.00	±0.04	±0.08	±0.14	±0.24	±0.15
Cr^{6+}	0.00	0.07	0.12	0.19	0.25	0.30	0.00	0.02	0.06	0.10	0.13	0.18	0.00	0.11	0.25	0.36	0.40	0.44	1.35	1.10	0.88	0.66	0.50	0.34
	±0.00	±0.00	±0.00	±0.01	±0.00	±0.01	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.01	±0.01	±0.01	±0.01	±0.00	±0.00	±0.01	±0.01	±0.01	±0.01
Cu^{2+}	0.00	0.96	4.18	10.68	17.90	24.49	0.00	0.14	0.78	2.89	7.13	10.98	0.00	1.48	6.13	8.96	14.73	15.93	64.76	59.70	51.12	39.61	21.65	9.53
	±0.00	±0.02	±0.09	±0.11	±0.18	±0.63	±0.00	±0.00	±0.03	±0.11	±0.18	±0.12	±0.00	±0.03	±0.12	±0.29	±0.15	±0.03	±0.00	±0.05	±0.09	±0.51	±0.44	±1.16
Fe^{2+}	0.00	4.72	7.84	12.64	20.46	26.37	0.00	0.29	0.90	2.42	8.08	13.06	0.00	12.76	24.19	39.44	66.82	73.32	177.33	153.18	136.12	113.82	71.76	53.05
	±0.00	±0.07	±0.16	±0.26	±0.20	±0.71	±0.00	±0.01	±0.03	±0.05	±0.25	±0.70	±0.00	±0.26	±1.02	±1.66	±2.43	±0.49	±0.00	±0.21	±1.27	±2.03	±3.02	±3.37
Pb^{2+}	0.00	0.05	0.23	0.60	1.51	2.60	0.00	0.03	0.09	0.44	1.27	1.92	0.00	0.40	1.12	6.39	9.27	10.83	25.28	23.81	22.77	16.47	11.74	8.41
	±0.00	±0.00	±0.01	±0.02	±0.02	±0.11	±0.00	±0.00	±0.00	±0.01	±0.01	±0.05	±0.00	±0.00	±0.01	±0.21	±0.09	±0.11	±0.00	±0.00	±0.01	±0.24	±0.13	±0.08
Mg^{2+}	0.00	0.93	2.97	5.02	6.43	7.42	0.00	0.22	0.73	1.93	3.33	4.07	0.00	1.81	4.69	6.74	7.04	7.91	25.32	21.46	15.77	10.09	6.65	3.92
	±0.00	±0.02	±0.08	±0.18	±0.13	±0.15	±0.00	±0.00	±0.01	±0.07	±0.03	±0.01	±0.00	±0.07	±0.14	±0.20	±0.18	±0.04	±0.00	±0.08	±0.11	±0.25	±0.30	±0.13
Ni^{2+}	0.00	0.46	1.52	4.13	11.19	15.49	0.00	0.24	0.58	1.77	3.53	5.95	0.00	0.49	2.75	5.28	9.63	10.85	42.18	39.34	35.55	28.93	15.01	6.75
	±0.00	±0.01	±0.03	±0.13	±0.36	±0.40	±0.00	±0.00	±0.01	±0.06	±0.11	±0.02	±0.00	±0.02	±0.09	±0.24	±0.35	±0.03	±0.00	±0.02	±0.10	±0.28	±0.54	±0.36
K^{+}	0.00	6.40	13.29	16.85	19.57	20.10	0.00	1.86	2.86	4.37	6.20	7.90	0.00	10.57	39.02	65.34	70.44	71.51	153.52	125.79	87.33	56.53	43.74	31.96
	±0.00	±0.24	±0.16	±0.24	±0.80	±0.20	±0.00	±0.04	±0.04	±0.12	±0.13	±0.16	±0.00	±0.15	±1.94	±1.59	±2.13	±1.23	±0.00	±1.05	±2.76	±1.77	±0.65	±0.56
Zn^{2+}	0.00	1.23	2.66	5.67	7.65	7.49	0.00	0.23	0.99	2.15	3.98	4.76	0.00	1.90	3.58	7.23	12.52	14.39	41.96	37.05	33.15	25.08	15.79	13.22
	±0.00	±0.04	±0.07	±0.03	±0.33	±0.34	±0.00	±0.00	±0.04	±0.05	±0.10	±0.12	±0.00	±0.05	±0.07	±0.15	±0.39	±0.22	±0.00	±0.08	±0.17	±0.19	±0.83	±0.61

Table 3
Heavy metal concentration in *E. crassipes* (DE + EDTA)

Period (d)	Root						Shoot						Soil						Effluent					
	0	5	10	15	20	25	0	5	10	15	20	25	0	5	10	15	20	25	0	5	10	15	20	25
As^{3+}	0.00	0.46	1.14	2.48	3.87	5.22	0.00	0.11	0.38	0.74	1.53	2.08	0.00	1.19	3.47	7.92	10.22	11.72	33.52	31.37	27.33	20.59	14.36	10.91
	±0.00	±0.01	±0.01	±0.04	±0.08	±0.07	±0.00	±0.00	±0.01	±0.01	±0.05	±0.02	±0.00	±0.01	±0.09	±0.17	±0.32	±0.24	±0.00	±0.26	±0.44	±0.11	±0.12	±0.16
Cd^{2+}	0.00	0.04	0.09	0.14	0.25	0.33	0.00	0.00	0.02	0.05	0.12	0.18	0.00	0.21	0.31	0.41	0.49	0.57	1.84	1.57	1.37	1.13	0.78	0.49
	±0.00	±0.00	±0.00	±0.01	±0.01	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.01	±0.00	±0.05	±0.02	±0.01	±0.01	±0.01	±0.00	±0.06	±0.02	±0.02	±0.02	±0.02
Ca^{2+}	0.00	0.48	1.10	2.39	3.77	5.21	0.00	0.12	0.40	0.79	1.57	2.24	0.00	2.50	4.22	6.70	7.25	7.72	26.56	23.27	20.20	14.89	11.14	7.24
	±0.00	±0.01	±0.04	±0.06	±0.04	±0.11	±0.00	±0.00	±0.02	±0.04	±0.03	±0.03	±0.00	±0.77	±0.37	±0.48	±0.30	±0.19	±0.00	±0.82	±0.40	±0.54	±0.37	±0.15
Cr^{6+}	0.00	0.05	0.11	0.15	0.20	0.23	0.00	0.01	0.05	0.08	0.12	0.16	0.00	0.17	0.26	0.34	0.41	0.45	1.35	1.12	0.90	0.69	0.49	0.36
	±0.00	±0.00	±0.00	±0.00	±0.01	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.01	±0.00	±0.00	±0.02	±0.03	±0.01	±0.01	±0.00	±0.01	±0.02	±0.03	±0.01	±0.01
Cu^{2+}	0.00	0.81	3.90	5.89	7.75	9.34	0.00	0.12	0.72	2.49	5.54	7.85	0.00	3.12	8.28	13.32	16.67	20.71	64.76	59.61	50.32	40.66	29.98	18.25
	±0.00	±0.02	±0.06	±0.12	±0.12	±0.42	±0.00	±0.00	±0.01	±0.08	±0.06	±0.32	±0.00	±0.42	±0.85	±0.68	±0.56	±1.67	±0.00	±0.59	±1.07	±0.41	±0.35	±0.47
Fe^{2+}	0.00	4.18	7.04	11.11	16.24	19.15	0.00	0.24	0.77	1.97	5.99	9.09	0.00	19.57	28.32	38.80	51.63	66.36	177.33	151.86	136.97	115.13	89.57	54.39
	±0.00	±0.11	±0.11	±0.29	±0.16	±0.19	±0.00	±0.01	±0.01	±0.07	±0.06	±0.28	±0.00	±2.24	±1.72	±1.08	±1.80	±1.30	±0.00	±2.33	±2.09	±1.16	±2.45	±0.25
Pb^{2+}	0.00	0.05	0.22	0.56	1.31	2.43	0.00	0.02	0.07	0.33	0.93	1.52	0.00	2.18	2.03	6.18	7.36	8.89	25.28	22.84	22.67	17.05	13.92	8.95
	±0.00	±0.00	±0.01	±0.02	±0.04	±0.07	±0.00	±0.00	±0.00	±0.01	±0.03	±0.07	±0.00	±0.55	±0.11	±0.28	±0.36	±0.19	±0.00	±0.59	±0.13	±0.36	±0.49	±0.09
Mg^{2+}	0.00	0.80	2.32	4.33	5.90	6.19	0.00	0.13	0.61	1.33	2.47	3.69	0.00	2.66	5.35	6.30	6.39	6.60	25.32	21.53	16.24	11.67	8.08	4.70
	±0.00	±0.02	±0.06	±0.04	±0.06	±0.06	±0.00	±0.00	±0.02	±0.01	±0.07	±0.10	±0.00	±0.19	±0.09	±0.21	±0.16	±0.11	±0.00	±0.21	±0.16	±0.23	±0.22	±0.07
Ni^{2+}	0.00	0.34	0.94	2.34	4.97	6.35	0.00	0.18	0.44	1.22	2.49	4.04	0.00	2.95	4.43	6.78	8.99	11.82	42.18	38.48	35.71	30.04	21.94	14.88
	±0.00	±0.01	±0.01	±0.09	±0.10	±0.26	±0.00	±0.01	±0.01	±0.03	±0.03	±0.10	±0.00	±1.67	±0.84	±1.05	±0.08	±0.05	±0.00	±1.78	±0.95	±1.31	±0.59	±0.40
K^{+}	0.00	6.24	11.90	13.73	17.84	18.87	0.00	0.78	1.68	4.07	5.29	6.18	0.00	8.72	23.96	43.72	51.52	54.87	153.52	134.03	101.91	70.77	52.20	44.40
	±0.00	±0.06	±0.24	±0.52	±0.63	±0.77	±0.00	±0.02	±0.02	±0.15	±0.29	±0.22	±0.00	±0.18	±0.26	±2.27	±1.35	±0.56	±0.00	±0.74	±2.63	±0.78	±0.28	±0.02
Zn^{2+}	0.00	1.13	2.47	5.24	7.14	7.43	0.00	0.13	0.60	1.70	2.92	3.81	0.00	3.28	5.29	7.83	9.59	13.31	41.96	37.17	32.82	25.10	18.49	12.14
	±0.00	±0.05	±0.05	±0.16	±0.08	±0.08	±0.00	±0.00	±0.01	±0.03	±0.10	±0.04	±0.00	±0.38	±0.38	±0.61	±0.39	±0.15	±0.00	±0.42	±0.38	±0.68	±0.09	±0.22

Table 4
Heavy metal concentration in *E. crassipes* (DE + CDM)

Period (d)	Root						Shoot						Soil						Effluent					
	0	5	10	15	20	25	0	5	10	15	20	25	0	5	10	15	20	25	0	5	10	15	20	25
As^{3+}	0.00	0.05	0.38	1.17	4.02	7.87	0.00	0.02	0.17	0.54	1.83	4.17	0.00	0.38	1.26	7.62	9.29	6.53	33.52	31.42	27.91	19.60	13.60	8.43
	±0.00	±0.00	±0.01	±0.04	±0.13	±0.29	±0.00	±0.00	±0.00	±0.01	±0.04	±0.08	±0.00	±0.02	±0.03	±0.15	±0.11	±0.10	0.00	±0.02	±0.02	±0.15	±0.18	±0.18
Cd^{2+}	0.00	0.05	0.10	0.15	0.24	0.31	0.00	0.01	0.03	0.08	0.15	0.25	0.00	0.15	0.31	0.51	0.62	0.59	1.84	1.55	1.24	0.90	0.61	0.46
	±0.00	±0.00	±0.00	±0.00	±0.01	±0.01	±0.00	±0.00	±0.00	±0.00	±0.00	±0.01	±0.00	±0.00	±0.01	±0.01	±0.00	±0.01	0.00	±0.01	±0.01	±0.01	±0.01	±0.01
Ca^{2+}	0.00	0.52	1.42	2.82	4.33	5.27	0.00	0.13	0.41	1.21	2.42	3.25	0.00	1.24	3.82	7.15	8.28	8.53	26.56	23.43	18.41	12.46	8.53	6.37
	±0.00	±0.01	±0.01	±0.04	±0.13	±0.16	±0.00	±0.00	±0.01	±0.01	±0.09	±0.05	±0.00	±0.03	±0.11	±0.23	±0.43	±0.13	0.00	±0.03	±0.10	±0.20	±0.24	±0.21
Cr^{6+}	0.00	0.06	0.12	0.16	0.22	0.27	0.00	0.02	0.06	0.09	0.12	0.16	0.00	0.12	0.25	0.39	0.46	0.46	1.35	1.08	0.82	0.58	0.41	0.31
	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±0.01	±0.00	±0.00	±0.00	±0.00	±0.00	±0.01	±0.01	0.00	±0.00	±0.01	±0.00	±0.01	±0.00
Cu^{2+}	0.00	0.90	3.98	10.31	17.31	22.22	0.00	0.12	0.72	2.70	6.80	11.08	0.00	1.36	5.80	10.00	9.27	6.89	64.76	59.26	47.75	33.82	23.22	15.16
	±0.00	±0.02	±0.13	±0.46	±0.45	±0.22	±0.00	±0.00	±0.02	±0.09	±0.29	±0.34	±0.00	±0.01	±0.03	±0.32	±0.29	±0.07	±0.00	±0.01	±0.13	±0.67	±0.35	±0.33
Fe^{2+}	0.00	5.12	8.28	11.50	19.14	24.46	0.00	0.27	0.86	2.30	6.85	10.32	0.00	12.65	25.27	43.56	60.67	69.57	177.33	151.32	125.77	97.17	67.10	48.90
	±0.00	±0.10	±0.08	±0.12	±0.47	±0.63	±0.00	±0.00	±0.02	±0.08	±0.25	±0.31	±0.00	±0.66	±0.54	±1.34	±0.35	±1.47	±0.00	±0.71	±0.55	±1.17	±0.19	±0.48
Pb^{2+}	0.00	0.05	0.23	0.59	1.44	2.51	0.00	0.02	0.08	0.38	1.11	1.71	0.00	0.37	1.22	7.46	9.68	10.17	25.28	23.59	20.91	13.64	9.66	7.29
	±0.00	±0.00	±0.01	±0.02	±0.01	±0.05	±0.00	±0.00	±0.00	±0.01	±0.02	±0.03	±0.00	±0.01	±0.04	±0.15	±0.11	±0.36	±0.00	±0.01	±0.04	±0.14	±0.11	±0.25
Mg^{2+}	0.00	0.89	2.72	5.62	6.40	7.15	0.00	0.23	0.74	1.70	3.24	4.14	0.00	1.78	5.19	6.92	7.20	7.10	25.32	21.30	14.66	8.97	6.27	4.64
	±0.00	±0.02	±0.07	±0.11	±0.39	±0.24	±0.00	±0.00	±0.02	±0.04	±0.05	±0.19	±0.00	±0.04	±0.19	±0.22	±0.47	±0.38	±0.00	±0.04	±0.18	±0.30	±0.08	±0.30
Ni^{2+}	0.00	0.40	1.12	3.19	7.97	13.86	0.00	0.22	0.53	1.45	3.11	5.15	0.00	0.47	2.81	5.52	7.29	5.36	42.18	39.04	33.20	25.94	17.63	10.16
	±0.00	±0.01	±0.03	±0.08	±0.08	±0.52	±0.00	±0.01	±0.01	±0.01	±0.11	±0.22	±0.00	±0.01	±0.06	±0.12	±0.18	±0.03	±0.00	±0.03	±0.09	±0.11	±0.15	±0.40
K^{+}	0.00	6.26	13.38	15.40	17.95	18.88	0.00	0.89	1.76	4.60	5.92	7.55	0.00	0.98	31.98	66.46	73.18	74.14	153.52	141.45	101.55	61.38	43.18	34.53
	±0.00	±0.21	±0.29	±0.23	±0.49	±0.40	±0.00	±0.01	±0.04	±0.18	±0.18	±0.04	±0.00	±0.03	±0.72	±2.15	±0.41	±2.81	±0.00	±0.35	±0.65	±2.51	±1.18	±1.38
Zn^{2+}	0.00	1.18	2.57	5.40	7.49	7.45	0.00	0.15	0.68	1.97	3.55	4.24	0.00	1.82	3.58	8.09	10.40	11.67	41.96	36.87	30.92	21.46	15.18	12.46
	±0.00	±0.03	±0.03	±0.21	±0.12	±0.23	±0.00	±0.01	±0.02	±0.02	±0.22	±0.20	±0.00	±0.05	±0.12	±0.36	±0.37	±0.12	±0.00	±0.06	±0.12	±0.35	±0.14	±0.23

has been done on *E. crassipes* before and after treating the effluent (Figs. 1(a) and (b)). Before treatment, the surface of *E. crassipes* (Fig. 1(a)) had definite and even texture. After treatment (Fig. 1(b)), the surface of *E. crassipes* became uneven and irregular textures [16]. Comparing surface morphology (Figs. 1(a) and (b)), it is understood that the presence of contaminants has affected the surface structure of *E. crassipes*. *E. crassipes* meticulously accumulates higher concentrations of Cu^{2+}, Mg^{2+} and Ni^{2+} ions. Uptake of essential elements by *E. crassipes* is higher compared with non-essential elements. Exudates from root have impact on heavy metal accumulation [6,22,29]. *E. crassipes* develops its own strategy and defensive mechanism to protect photosynthetic processes against the harmful effects of heavy metal contamination [23].

3.2. Analysis of translocation factor

Translocation factor (TF) was calculated from the ratio of heavy metal concentration in shoot to root. TF value of *E. crassipes* grown in DE, DE + EDTA and DE + CDM container was plotted (Figs. 2(a)–(c)). In DE, Pb^{2+} and Cd^{2+} ions had higher (0.74) TF value compared with other heavy metal ions, with K^+ ion as lowest (0.39). In EDTA, Cu^{2+} (0.84) and K^+ (0.33) ions obtain highest and lowest TF value. In DE + CDM, Cd^{2+} (0.82) and Ca^{2+} (0.40) ions obtain highest and lowest TF value. TF value of Ni^{2+} ion decreases from 5th day onwards for DE and DE + CDM but plant under DE + EDTA treatment, Pb^{2+} ion appears to be decreased after 20th day of experiment. TF mainly depends upon the rhizosphere environment, chelating compounds, pH and redox potential, in addition to that root exudates such as organic acids, amino acids, phytochelatins, siderophores, etc., enhanced the phytoremediation process. Accumulation ability, permeability

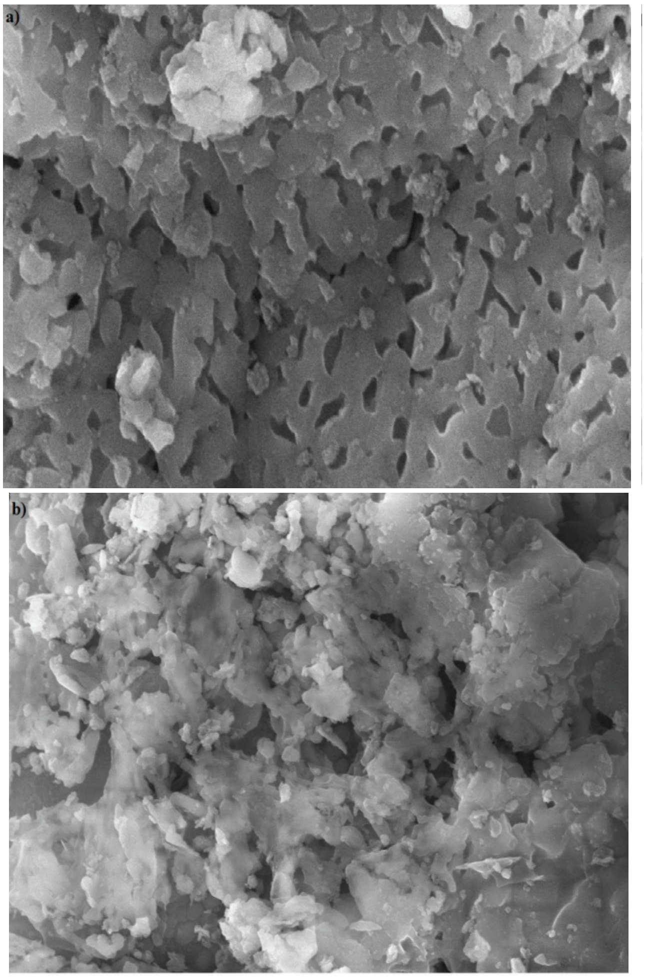

Fig. 1. SEM image of *E. crassipes* (a) before and (b) after treatment with the effluent.

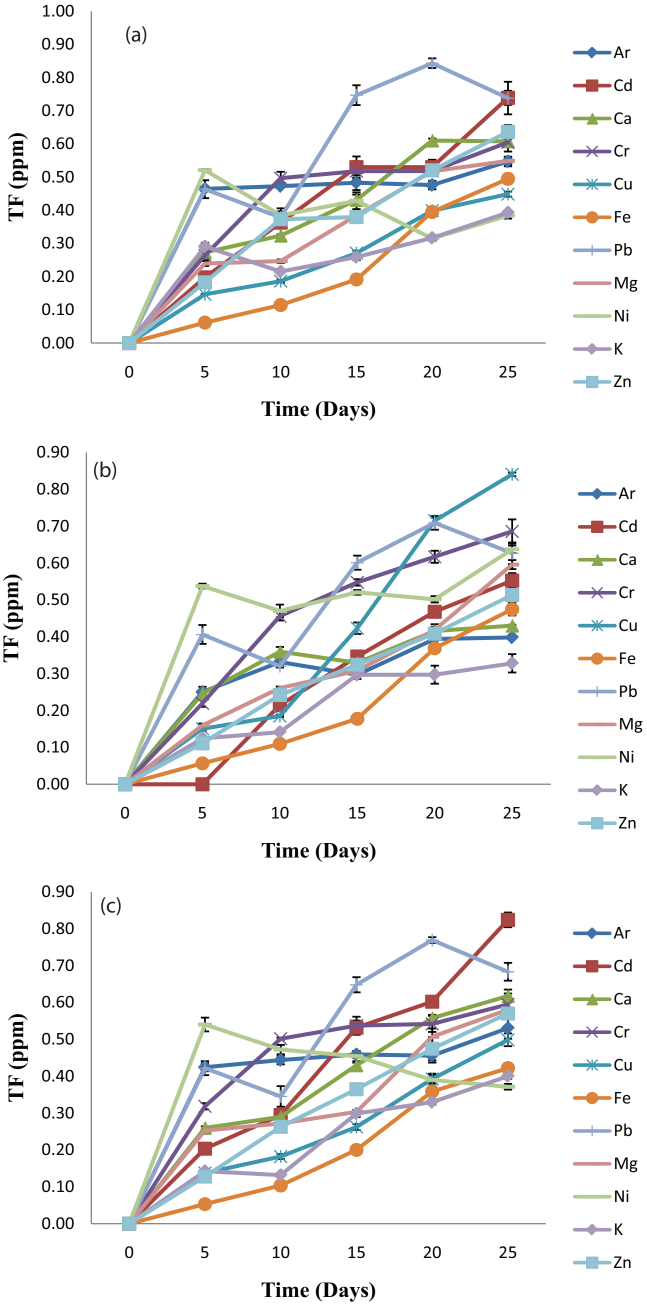

Fig. 2. TF value of *E. crassipes* under (a) DE, (b) DE + EDTA and (c) DE + CDM.

of cell walls, metabolic patterns, function of membranes and adaptation towards metal ions plays an important role in TF [30]. Accumulation of contaminant across the plant tissues depends on metal ions rather than plant. As the presence of heavy metal element is high, optimum result (TF should be >1) was not obtained.

3.3. Analysis of bioconcentration factor

Bioconcentration factor of root and shoot ($BCF_{Root\ and\ Shoot}$) values of *E. crassipes* was calculated from the ratio of heavy metal concentration in root and shoot to effluent (Figs. 3(a)–(c) and 4(a)–(c)). It is seen that Cu^{2+} (0.96, 1.01) and Ni^{2+} (0.88, 0.89) ions had higher values in BCF_{Root} for DE and DE + CDM, respectively. Meanwhile higher BCF_{Root} value of DE + EDTA is Mg^{2+} (0.55). Pb^{2+} ions (0.14) have been lowest at all the experimental containers. Similar to BCF_{Root}, BCF_{Shoot} has Cu^{2+} ion (0.43 and 0.50) as the highest value followed by Mg^{2+} ion (0.34, 0.35) for DE and DE + CDM, respectively. In DE + EDTA, Mg^{2+} (0.35) ions have the highest BCF_{Shoot} value. Compared with BCF_{Root}, K^+ ion has the lowest value in all the experimental containers with a range of 0.7–0.8. Accumulated contaminants from effluent were higher in BCF_{Root} than BCF_{Shoot}. Correlations between sediments and roots indicate that the sediments are the source of supply to the roots. Due to low bioavailability of metal ions in sediments, accumulation was decreased after certain period.

3.4. Analysis of transposition factor

Transposition factor (TrF) is the amount of contaminants transferred from one medium to another. TrF value of contaminants is calculated from the ratio of heavy metal concentration in soil to effluent, plotted (Figs. 5(a)–(c)). Obtained results indicated that the contaminants in effluent are easily transferred into the soil medium. Results obtained for DE and DE + CDM have higher TrF rate compared with the DE + EDTA. Overall average TrF value was higher in DE container (K^+ ions: 2.24) followed by DE + CDM (K^+ ions: 2.15) and DE + EDTA (Mg^{2+} ions: 1.40). Degradation rate of DE + CDM was higher compared with DE and DE + EDTA. Metal contaminants from soil sediments are important reason behind higher TrF rate in DE container.

3.5. % Removal of heavy metal ions

% Removal of contaminants was calculated from the ratio of heavy metal concentration in plant to the initial concentration of effluent. It is shown in Figs. 6(a)–(c). Cu^{2+} ion had higher removal level from both medium with 54.78% and 51.41% for DE and DE + CDM. In case of DE + EDTA, Mg^{2+} (38.99) ion has higher removal rate. Application of chelating agents is the primary factor involved in the removal of contaminants. At all cases, non-essential element Pb^{2+} had lower removal rate with 17.88%, 15.66% and 16.70% at the end of analyzing period. Stable increase in removal rate has been observed at all cases. Effect of essential and non-essential might be the cause of variation in heavy metal removal [26].

3.6. Degradation % of heavy metal ions

Degradation of heavy metal contaminants was analyzed from initial concentration level to heavy metal contaminants present in soil, plant and effluent at each period. Degradation rate of heavy metal contaminants in DE, DE + EDTA and DE + CDM container was calculated (Figs. 7(a)–(c)). K^+ ion has higher degradation rate under DE and DE + EDTA. In the case of DE + EDTA degradation of K^+ ion was low compared with other heavy metal ions. It is due to the presence and degradation of other heavy metal ions in the treatment. Maximum degradation rates were attained for DE, DE + EDTA and DE + CDM were K^+ (14.37%), As^{2+} (19.46%) and K^+ (19.02%) ions, respectively. Chelating agents play a major role in degradation of heavy metal contaminants.

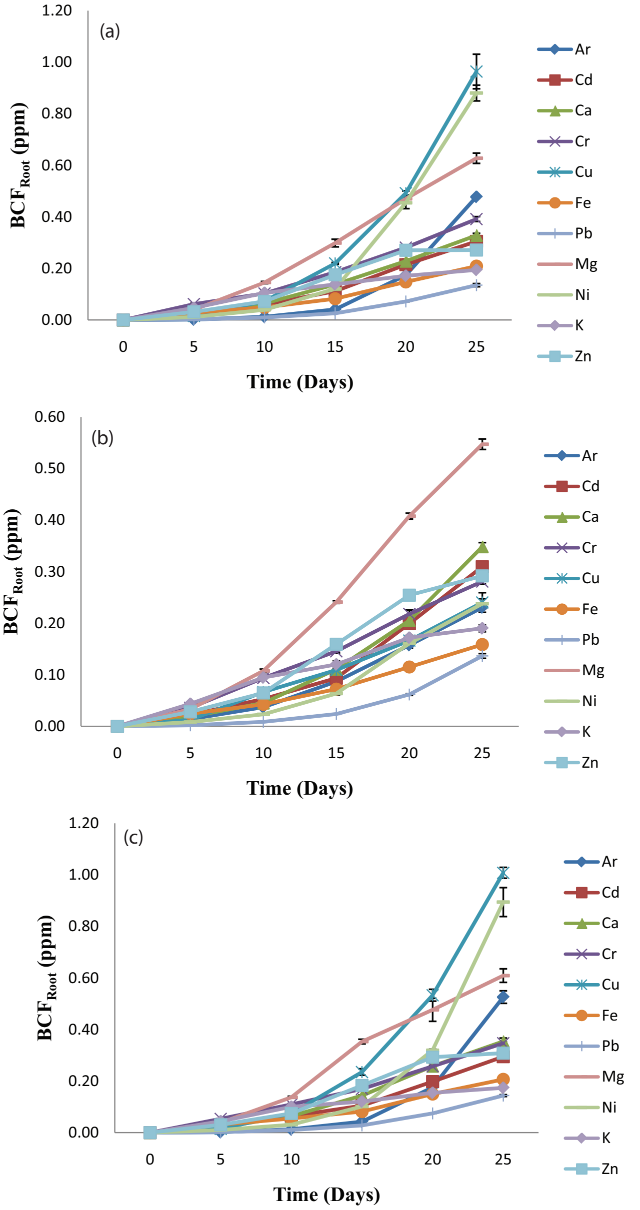

Fig. 3. BCF_{Root} value of *E. crassipes* under (a) DE, (b) DE + EDTA and (c) DE + CDM.

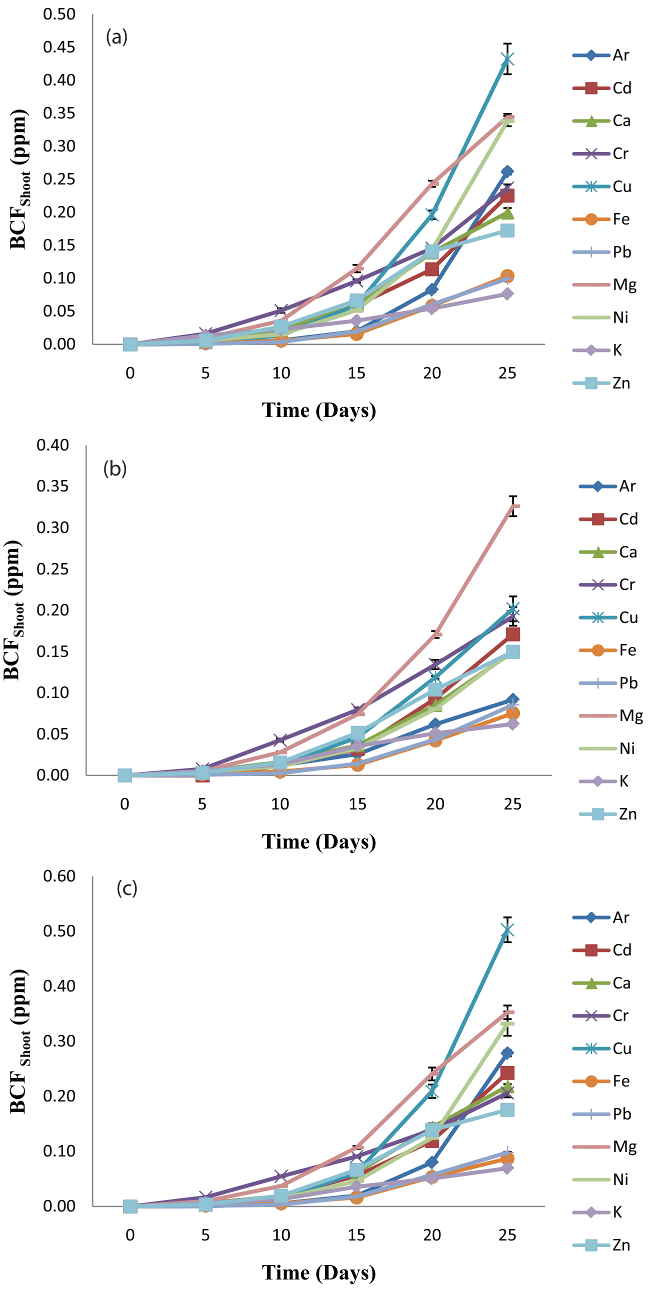

Fig. 4. BCF_{Shoot} value of *E. crassipes* under (a) DE, (b) DE + EDTA and (c) DE + CDM.

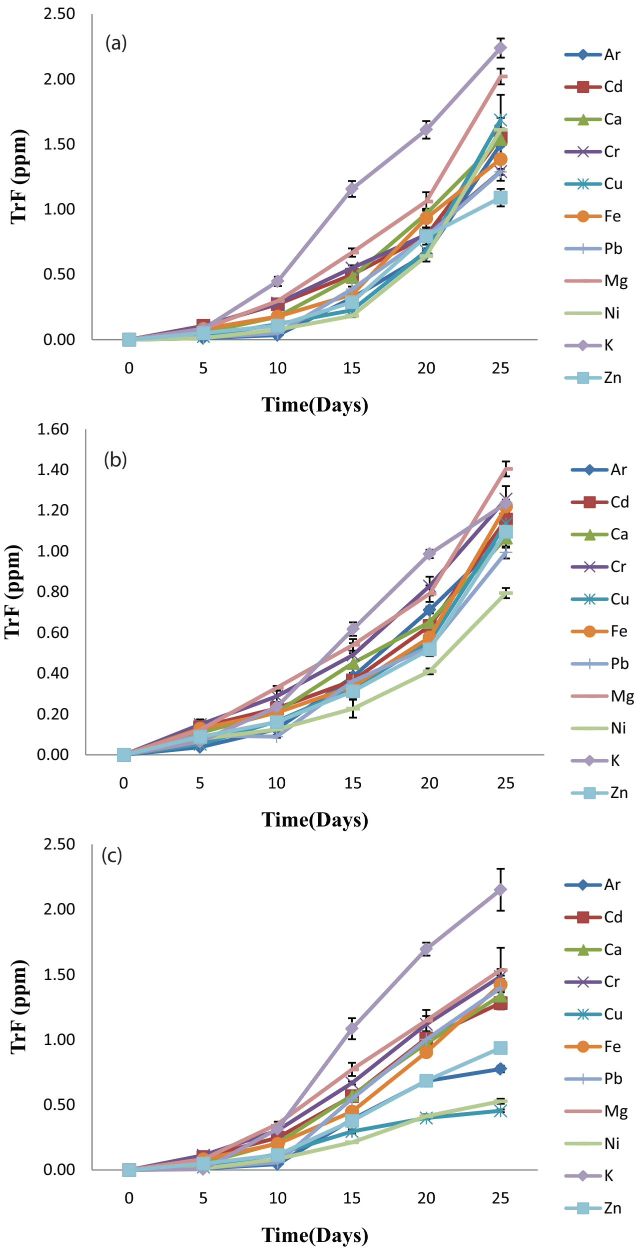

Fig. 5. TrF value of *E. crassipes* under (a) DE, (b) DE + EDTA and (c) DE + CDM.

3.7. Biochemical characteristics of E. crassipes

Biochemical characteristics of *E. crassipes* were tabulated and compared with the controlled plant (Tables 5(a)–(e)). Biochemical characteristics of *E. crassipes* are susceptible to contaminants present in effluent. Biochemical properties were in the order: DE + CDM > DE + EDTA > DE. Biochemical properties such as chl a, b, total chl, carotenoid and proline were 112.24, 128.04, 249.76, 52.45 and 8.82 ppm, respectively, in controlled plant at the end of 25th day. In case of plants in DE, DE + EDTA and DE + CDM have lower biochemical properties with 70.33, 73.61, 151.09, 23.39, 4.47; 76.03, 78.29, 168.26, 34.96, 4.73; 78.18, 87.39, 172.49, 36.70, 5.56 for chl a, b, total chl, carotenoid and proline, respectively. *E. crassipes* exhibits the ability to remove contaminants and recover from the toxicity of effluent, indicated by the rate of recovery from 20th to 25th day of experiment. Decrease in carotenoid and proline content, photosynthetic process of *E. crassipes* has been affected. Plants grown under DE + CDM have shown the ability to control the chl a, chl b and total chl content compared with DE and DE + EDTA. Chlorophyll content of the plant directly resembles the strength of the plant [8,19], it is been affected relentlessly by the toxicity of contaminants. Synthesis of chlorophyll contents by *E. crassipes* was inhibited and annihilated by the toxicity of metal ions.

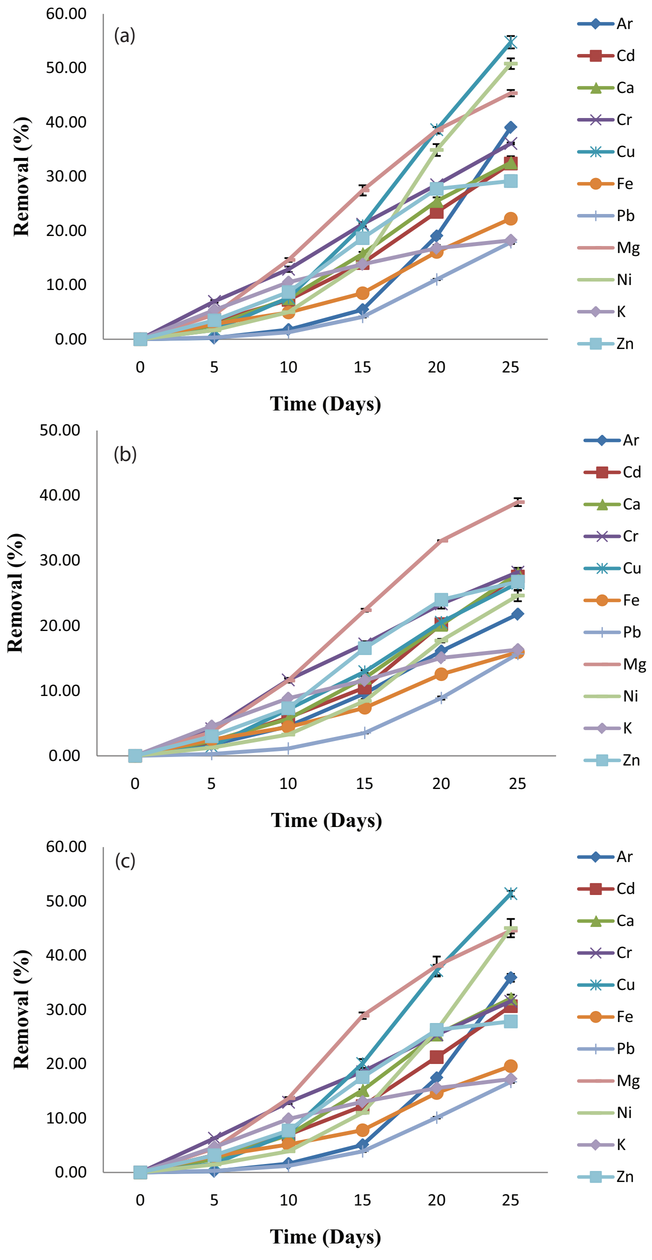

Fig. 6. Removal % of *E. crassipes* under (a) DE, (b) DE + EDTA and (c) DE + CDM.

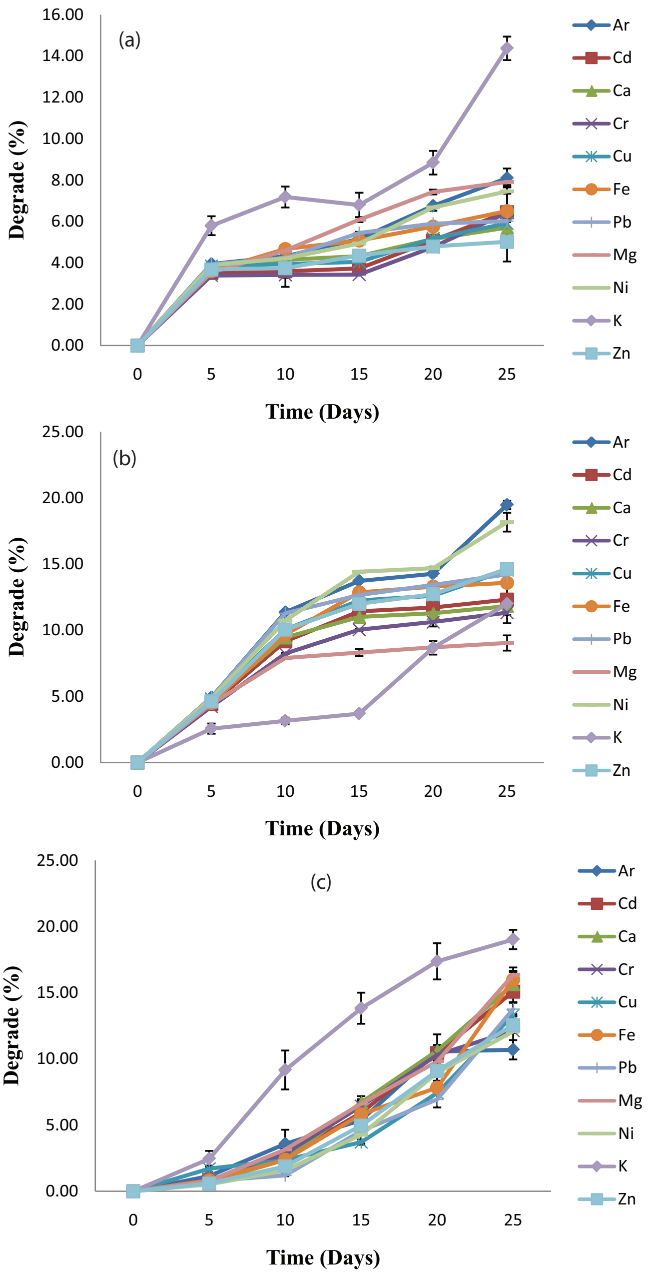

Fig. 7. Degradation % of *E. crassipes* under (a) DE, (b) DE + EDTA and (c) DE + CDM.

Carotenoid content in the sample containers was decreased dramatically at initial stage and regains its potency later. At 5th day of sample analysis, carotenoid content in DE, DE + EDTA and DE + CDM (32.24, 35.19 and 39.10 ppm, respectively) was decreased from initial day observation (45.20). Synthesis of carotenoid was increased on 25th day (20.87) from 20th day (23.39). In case of DE + EDTA and DE + CDM, carotenoid syntheses were increased from 29.89 to 34.96 and 33.12 to 36.70, respectively (15th day to 25th day). A significant decrease in carotenoid level has been observed in the samples under DE compared with DE + EDTA and DE + CDM [31]. Presence of various contaminants in the effluent affects the carotenoid level in plant for a period until the effects of toxicity diminishes. Deleterious nature of contaminants has caused damage to carotenoid and successively to *E. crassipes* [21].

Proline content in *E. crassipes* was observed to be decreased from 5th day onwards. DE and DE + EDTA have recovered from its decreasing rate by 15th day onwards with 3.47 to 4.47 (25th day) and 4.25 to 4.73 (25th day), respectively. Plants under DE + CDM treatment were invariably decreasing to the whole experimented period but the level of decreasing rate is very low compared with other treatments. Proline accumulation is based upon the toxicity level in the environment [8]. The increased level of proline after a certain period in *E. crassipes* is due to continuous contact and reduced effect of toxicity.

Table 5
Biochemical characteristics of *E. crassipes*

Period (D)	0	5	10	15	20	25
(a)	Chlorophyll a					
Control	102.76±2.65	104.77±3.29	110.13±2.28	111.85±2.36	112.25±0.66	112.24±1.12
DE	102.76±2.65	100.51±2.63	95.56±0.96	78.77±2.83	75.76±0.88	70.33±3.24
DE + EDTA	102.76±2.65	96.84±2.08	91.45±3.01	84.23±3.24	77.43±2.32	76.03±1.94
DE + CDM	102.76±2.65	99.54±3.49	92.86±3.05	88.89±1.35	85.18±2.56	78.18±2.01
(b)	Chlorophyll b					
Control	110.38±2.88	115.39±2.00	119.91±3.60	125.37±1.25	127.22±4.46	128.04±2.27
DE	110.38±2.88	101.81±3.05	101.25±2.65	90.73±1.86	81.87±2.07	73.61±3.08
DE + EDTA	110.38±2.88	103.49±1.83	91.40±2.44	85.16±0.50	83.24±0.96	78.29±3.21
DE + CDM	110.38±2.88	104.55±4.24	96.33±3.14	94.18±0.95	89.02±1.91	87.39±4.03
(c)	Total chlorophyll					
Control	220.39±6.87	225.11±8.14	242.02±6.66	246.99±4.88	246.29±5.20	249.76±4.46
DE	220.39±6.87	210.81±5.20	207.65±5.70	183.57±7.76	161.10±1.99	151.09±5.39
DE + EDTA	220.39±6.87	210.47±4.58	187.52±6.81	174.59±1.88	172.49±7.29	168.26±10.43
DE + CDM	220.39±6.87	217.70±12.14	201.28±6.14	198.82±10.60	180.76±5.37	172.49±8.65
(d)	Carotenoid					
Control	45.20±0.27	49.11±0.97	49.43±1.33	52.36±1.08	50.65±1.89	52.45±2.36
DE	45.20±0.27	32.24±0.56	24.64±0.89	21.29±0.80	20.87±0.49	23.39±0.49
DE + EDTA	45.20±0.27	35.19±0.61	25.03±1.03	29.89±0.18	31.52±1.21	34.96±2.21
DE + CDM	45.20±0.27	39.10±1.77	35.05±1.58	33.12±0.51	34.28±1.03	36.70±1.47
(e)	Proline					
Control	7.69±0.16	7.99±0.08	7.87±0.20	8.16±0.38	8.46±0.08	8.82±0.22
DE	7.69±0.16	6.34±0.17	4.87±0.09	3.47±0.06	3.93±0.12	4.47±0.18
DE + EDTA	7.69±0.16	6.42±0.14	4.97±0.05	4.25±0.13	4.55±0.14	4.73±0.08
DE + CDM	7.69±0.16	6.97±0.31	6.24±0.11	5.72±0.18	5.57±0.10	5.56±0.15

4. Conclusion

Current work on *E. crassipes* on treatment of heavy metal contamination was found to have positive approach on phytoremediation of battery industrial effluent. Heavy metal concentration was increased in root and shoot parts of *E. crassipes*. Due to several elements present in the effluent and the contact period was less compared with the plant grown in polluted environment, *E. crassipes* acts as a very good accumulator in eradicating the contaminants from aquatic environment for short period of time with the addition of cow dung manure as influencing agent. Frequent cultivation of aquatic plants led to complete removal of contaminants in soil and effluent.

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