ORIGINAL ARTICLE



Assessment of cobalt accumulation effect on growth and antioxidant responses in aquatic macrophyte *Hydrilla verticillata* (L.f.) Royle

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Received: 29 August 2019 / Accepted: 1 April 2020 / Published online: 14 April 2020 © Institute of Molecular Biology, Slovak Academy of Sciences 2020

Abstract

Cobalt is an essential trace metal and plays a pivotal role in the growth of all living organisms. The exposure of cobalt in the aquatic environment is ubiquitous via both natural and anthropogenic activities. The present study was carried out to investigate the accumulation response of macrophyte *Hydrilla verticillata* (L.f.) Royle towards the cobalt exposure at different concentrations (1, 25, 50, 75 and 100 μ M) for seven days. The results showed that the cobalt accumulation increased in treated *H. verticillata* (L.f.) Royle with increase in concentration. The Bioconcentration factor (BCF) values were high in plants exposed to higher concentration of cobalt. Increase in growth (shoot length) and pigments (chlorophyll a, b and total chlorophyll) were detected at 25 μ M cobalt concentration, whereas the growth and pigments were declined in 50, 75 and 100 μ M. There was no significant difference in the carotenoid content between control and treated plant. Antioxidant enzymes (superoxide dismutase, catalase, and peroxidase) were increased at higher concentrations of cobalt. The results indicated that the decline in growth and pigments, and an increase in antioxidant enzyme activities, were triggered by the accumulation of cobalt in the plant. These observations suggest that *H. verticillata* (L.f.) Royle is well equipped to accumulate low concentration of cobalt in tissues to increase its growth and detoxify ROS generation. The BCF results indicate the efficiency of using *H. verticillata* (L.f.) Royle as a phytostabilizer.

Keywords Cobalt · Hydrilla verticillata (L.f.) Royle · Antioxidant enzymes · Bioconcentration factor

Introduction

Trace metal contamination in the aquatic environment is a major environmental threat, which affects the aquatic ecosystems, agriculture and human health (Sasmaz et al. 2008). Even though the impacts of trace metals on health-related issues are known, the exposure of trace metals is continuing because of its use in many areas (Jarup 2003). Essential trace metals such as manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), zinc (Zn), and copper (Cu) are vital for growth at trace amount and act as cofactors for enzymes but toxic at elevated levels (Gadd

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and Griffiths 1977; Reed and Gadd 1989). Arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), plutonium (Pu), tungsten (W), and vanadium (V) are non-essential trace metals and potentially toxic even at low concentration (Johri et al. 2010). Trace metal contamination became a major risk to agriculture, aquatic ecosystem and human health (Ashraf et al. 2019).

The technologies such as ion-exchange, reverse-osmosis, adsorption, electrodialysis, etc. to remove trace metal pollution are expensive, metal-specific and need intensive energy. In contradictory to this, the promising technology is phytoremediation, which uses plants to remove trace metals from wastewater (Singh et al. 1996; Miretzky et al. 2004; Mishra and Tripathi 2008). Aquatic plants can utilize trace metals from wastewater by aggregating and assimilating in their tissues (Vymazal and Kropfelova 2008). Kuyucak and Volesky (1989) mentioned that aquatic plants are trace metal accumulators. Aquatic plants can be used in ecosystem to remove pollutants (Shehata 2019). Due to fast growth and biomass production, the aquatic macrophytes hold high potential for micro-nutrient remediation (Eid et al. 2020).

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Cobalt is an essential micronutrient, a unique example of an organometallic compound and the main constituent of Cobalamin (vitamin B12) which plays a vital role in humans and animals. Cobalt is essential in trace amount for the growth of animals, plants, algae and marine phytoplankton (Bruland et al. 1991), but at higher concentration, it becomes toxic to both terrestrial and aquatic life (Gal et al. 2008; Nagpal 2004). Toxicologists believe that cobalt becomes an environmental contaminant when released in higher concentration from industries (Lauwerys and Lison 1994). In the aquatic environment, cobalt naturally occurs at very low concentration, but due to natural and human activities the level is increased and causes menacing effects. Cobalt demand in industries such as steel, magnet, cement, paint, and fertilizers is extensive and limitless. Production of cobalt-containing chemicals, cobalt mining and processing, sewage effluents, and agricultural run-off are the main anthropogenic exposure of cobalt in the aquatic environment. Cobalt induces DNA damage via a series of oxidative stress (Zeeshan et al. 2017), and it is emphatically a genotoxicant (Reinardy et al. 2013). Ceyhun et al. (2011) indicated that the exposure of cobalt in aquatic bodies might cause significant changes in fish and affect their metal detoxification mechanisms. Suganthi et al. (2015) suggested that a possible remediation method should be adopted to prevent the exceeding concentration of cobalt in the aquatic environment.

Hydrophytes such as coontail (Ceratophyllum demersum), tape grass (Vallisneria spiralis), water hyacinth (Eichhornia crassipes), water fern (Azolla filiculoides), eurasian watermilfoil (Myriophyllum spicatum) and a number of other submerged and floating plants were exploited for the remediation of aquatic environment (Kumar et al. 2008). Kamal et al. (2004) indicated that plants could uptake metals by three patterns (a) true exclusion where metals are confined from entering the plant, (b) shoot exclusion where metals accumulate in root but translocation to shoot is restricted, and (c) accumulation in which metals are concentrated in plant parts. Harguintegy et al. (2014) reported the accumulation of cobalt in aquatic plant threadleaf-pondweed (Stuckenia filiformis). Wolverton and McDonald (1975) demonstrated that the water hyacinths (E. crassipes) and alligator weeds (Alternanthera philoxerides) could remove trace metals such as cobalt, strontium, and silver from static water systems. Singh et al. (2017) observed the elevated level of Cd, Co, Fe, Mn, Pb, and Zn in blue panicgrass (Panicum antidotale) and water hyacinths (E. crassipes) and suggested its hyperaccumulation property. Phytostabilization, another strategy of remediation, absorbs and accumulates trace metals, thus prevent the passage of trace metals to the food chain (Radziemska et al. 2017).

Hydrilla verticillata (L.f.) Royle is a submerged fastgrowing aquatic plant commonly known as Hydrilla or Water thyme, which develops dense mat on the water surface, has strong adaptability and multiplies in many ways (Wang et al. 2008; Haller and Sutton 1975; Langeland et al. 1992; Shearer et al. 2007). Dixit and Dhote (2010) observed that H. verticillata (L.f.) Royle has the capability of removing trace metals. Hydrilla verticillata (L.f.) Royle can potentially accumulate trace metals like Pb, Hg, Cu, Cd, Cr, As and Ni (Singh et al. 2013; Gupta and Chandra 1996; Srivastava et al. 2006; Rai et al. 1995; Sinha and Pandey 2003; Xue et al. 2010). Bunluesin et al. (2007) showed that biomass of H. verticillata (L.f.) Royle could be used as an efficient biosorbent material, for the removal of cadmium ion contaminants in aqueous solution. They clearly demonstrated that the accumulating potential of H. verticillata (L.f.) Royle can be useful in trace metals contaminated areas. So, the aim of this study was to determine the bioaccumulation potential of the plant H. verticillata (L.f.) Royle towards the acute exposure of different concentrations of cobalt to evaluate the growth, pigments and antioxidant responses of the plant.

Materials and methods

Plant material and metal treatment using hydroponic culture

Plants of Hydrilla verticillata (L.f.) Royle was purchased from an aquarium shop in Chennai (Tamil Nadu, India). It was grown in large cement tanks in the greenhouse environment. Prior to metal treatment, plants were acclimatized for 10 days in modified Hoagland solution (5 mM KNO3; 3 mM Ca (NO₃)₂·4H₂O; 1 mM NH₄H₂PO₄; 2 mM MgSO₄·7H₂O; 16 µM FeSO₄·7H₂O; 8 µM EDTA diNa-salt 2H₂O; 30 µM H₃BO₃; 9 µM MnCl₂·4H₂O; 0.50 µM CuSO₄·5H₂O; 0.50 μM H₂MoO₄; 1 μM CoSO₄·7H₂O; 0.80 μM ZnSO₄.7H₂O; pH 5.2) in laboratory condition at 24±2 °C (12 h light and 12 h dark). The acclimatized similar size plants were transferred to a plastic pot (one plant per pot) containing different increasing concentrations of cobalt (25, 50, 75, 100 µM) supplied as CoSO₄·7H₂O in Hoagland solution. The solution with 1 μ M CoSO₄·7H₂O acted as control. The plants were exposed to different concentrations of cobalt for 7 days in laboratory condition. The solution was replaced two days once to maintain the levels of nutrients and metals.

Accumulation and bioconcentration factor of cobalt

After 7 days of cobalt exposure, plants were washed thoroughly with double distilled water and oven dried at 80 °C for 2 days. The plant shoots were digested with concentrated nitric chloride and allowed to evaporate at 100 C. The volume was diluted and filtered through Whatman filter paper. The accumulation of cobalt content was analyzed by atomic absorption spectrophotometer (PyeUnicam, Cambridge, UK). The bioconcentration factor (BCF) helps to compare the bioaccumulation capacity of *H. verticillata* (L.f.) Royle in relation to the cobalt concentration present in the solution. The bioconcentration factor was calculated by using the below formula:

 $\cdot = \frac{Concentration \ of \ metal \ in \ plant}{Concentration \ of \ metal \ in \ solution}$

Determination of plant growth and photosynthetic pigments

Plant growth was measured by observing the shoot length of the plants. Photosynthetic pigments such as chlorophyll (a, b and total) and carotenoid contents were measured by following the method of Arnon (1949) and Duxbury and Yentsch (1956) respectively. Plant material (100 mg) was homogenized in 80% chilled acetone in dark place. The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was read at 645, 663, 480, and 510 nm in UV-Spectrophotometer. The protein content was estimated by Lowry et al. (1951) using bovine serum albumin as standard.

Assay of antioxidant enzymes

Plant material (100 mg) was homogenized in 100 mM potassium phosphate buffer (pH 7.4) containing 1% PVP (polyvin y l p y r r o l i d o n e) a n d 0 . 1 m M E D T A (Ethylenediaminetetraacetic acid). The homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant was collected to measure the activity of antioxidant enzymes. The protein content of the supernatant was measured by the method Lowry et al. (1951).

Superoxide dismutase was assayed according to Marklund and Marklund (1974) by calculating the inhibition of pyrogallol auto-oxidation by SOD at 325 nm for 3 min. The reaction mixture (4.5 mL) contains 2 mL Tris-HCl buffer (0.1 M, pH 8.2), 0.5 mL of pyrogallol, 0.5 mL enzyme extract and 1.5 mL of double distilled water. The amount of enzyme required for 50% inhibition of pyrogallol auto-oxidation was considered as one unit of enzyme activity. The enzyme activity was expressed as Units/mg protein.

Catalase was determined by the method of Aebi (1984). The reaction mixture contains 1 mL phosphate buffer (0.5 M, pH 7), 0.4 mL of H_2O_2 (0.2 M) and 0.1 mL of enzyme extract. The decrease in the absorbance was noted at 240 nm. Enzyme activity was expressed as Units/mg protein. One unit of catalase activity was defined as the amount of enzyme required to decompose 1 μ Mol H_2O_2 /min/mg/protein.

Peroxidase activity was estimated by following the protocol of Maehly and Chance (1955). The reaction mixture of 3 mL contains 0.32 mL potassium phosphate buffer (100 mM, pH 6.0), 0.16 mL of H_2O_2 (0.5%), 0.32 mL Pyrogallol (0.5%), 0.1 mL of enzyme extract and 2.1 mL of double distilled water. The change in the absorbance was read at 420 nm for 2 min at the interval of 20 s in UV-spectrophotometer. Enzyme activity was expressed in Units/mg protein. One unit of peroxidase activity was defined as the amount of oxidation of pyrogallol into purpurogallin in 20 s.

Statistical analysis

The data were expressed as mean \pm SD and analyzed by oneway analysis of variance (ANOVA) using SPSS (version-20, SPSS Inc., Chicago, IL, USA), followed by Duncan's multiple range test (DMRT) to compare the significant differences among treatments at p < 0.05.

Results and discussion

The effect of cobalt in aquatic macrophytes have been reported in many literature (Duman et al. 2009; Prajapati et al. 2012; Saleh et al. 2017) but the ability of *H. verticillata* (L.f.) Royle to grow under increasing concentration of cobalt was rarely demonstrated. So, in this study, after exposing *H. verticillata* (L.f.) Royle to different concentrations of cobalt (25, 50, 75, 100 μ M) for 7 days, the plant was collected from each concentration and parameters such as accumulation, plant growth, and antioxidant enzymes were analyzed.

Accumulation and bioconcentration factor

The accumulation factor can be used to evaluate the phytoremediation potential of a plant (Eid et al. 2019). In this study, the accumulated cobalt content in the treated *H. verticillata* (L.f.) Royle plant tissue was 0.031 ± 0.004 , $1.285 \pm 0.055, \ 2.798 \pm 0.023, \ 5.387 \pm 0.024, \ 8.95 \pm 0.104$ mg/kg dry weight after treating the plant with cobalt at 1, 25, 50, 75, 100 μ M of cobalt respectively (Fig. 1). Accumulation of cobalt increased significantly (p < 0.05) in H. verticillata (L.f.) Royle plant with increasing concentration of cobalt. The accumulation of cobalt at 25 μ M enhanced the growth of H. verticillata (L.f.) Royle as compared to control $(1 \mu M)$. Jaleel et al. (2009) observed increase in growth of maize plants (Zea mays) at low concentration of cobalt. The increase in biomass growth was noted in algae Monoraphidium minutum and Nitzchia perminuta at low concentration of cobalt (El-Sheekh et al. 2003). Essential trace metal such as cobalt enhances the growth of H. verticillata (L.f.) Royle at low concentration (Srivastava et al. 2006). On increase of cobalt concentration in Hoagland solution, the BCF values were significantly increased (Fig. 2). The high BCF value of 0.317 was observed in 100 µM cobalt concentration. The BCF values in the range of 0.1-0.5 are recognized

as potential phytostabilization (Balabanova et al. 2015). In this study, the values of BCF were from 0.071 to 0.317. These results indicate that the plant *H. verticillata* (L.f.) Royle is suitable for phytostabilization, which is an important strategy of remediation to control trace metal polluted site.

Effect of cobalt on growth

In this study, H. verticillata (L.f.) Royle showed various responses to growth in different concentrations of cobalt. The shoot length of plant treated with different concentrations of cobalt was shown in Table 1. A significant increase (p < 0.05) in shoot length (5.57 \pm 0.155 cm) was observed at 25 μ M whereas, in 50, 75 and 100 µM concentrations of cobalt exposure, significant decline in shoot length $(4.48 \pm 0.327, 4.03)$ ± 0.208 and 3.34 ± 0.150 cm respectively) was noted. Duman et al. (2009) observed increased growth rate of watercress (Nasturtium officinale) at lower concentration of cobalt. However, the growth rate was negative, when N. officinale was exposed to higher concentration. Patel et al. (1976) observed 45% of growth reduction in chysanthemum (Chrysanthemum morifolium) at higher level of cobalt concentration. The excess level of cobalt affect cell metabolites, alter nutrient transport and effect on cellular functioning in crop plants (Singh et al. 2010).

Photosynthetic pigments and protein content

Photosynthetic pigments (chl a, chl b, total chlorophyll, and carotenoid) were examined after 7 days of cobalt exposure. There was a significant increase (p < 0.05) in chlorophyll pigments (chl a = 0.36 ± 0.03 , chl b = 0.30 ± 0.03 and total chlorophyll = 0.66 ± 0.05) at 25 µM and gradually decreased





Fig. 1 Accumulation of cobalt by *Hydrilla verticillata* (L.f.) Royle at different concentrations of cobalt exposure. C = control (1 μ M of cobalt concentration). Values are interpreted by triplicate results (mean ± SD). Different uppercase letters indicate statistical differences (p < 0.05)



Cobalt concentration in μM

Fig. 2 Bioconcentration factor of cobalt at different concentrations in *Hydrilla verticillata* (*L.f.*) *Royle*. C = control (1 μ M of cobalt concentration). Values are interpreted by triplicate results (mean ± SD). Different uppercase letters indicate statistical differences (p < 0.05)

in 50, 75, and 100 μ M (Table 2). However, the reduction in chlorophyll pigments in other concentrations were not significant. Kumar et al. (2018) also observed that at lower concentration of metals Cu and Cd increased chlorophyll content and increasing concentration of these metals reduced chlorophyll a and b in vetiver grass (Vetiveria zizanioides). Trace metal stress induces the chlorophyllase enzyme which is responsible for the degradation of chlorophyll (Abdul-Basset et al. 1995). Particularly, cobalt inhibits 5-aminolevulenic acid (ALA) synthase, ALA dehydratase, prophobilinogenase and unporphyrinogen III decarboxylase which are needed for chlorophyll biosynthesis (Shalygo et al. 1999). No significant changes were observed in carotenoid content when compared to control (Table 2). Increased protein content was observed at 25 and 50 µM cobalt concentration, but in higher concentrations (75 and 100 µM) protein contents were decreased (Table 3). The declined protein and chlorophyll content of the leaves affect the photosynthetic activity of the plant (Swaminathan et al. 1990).

Accumulation effect of cobalt on antioxidant enzymes

Plants are very well equipped with antioxidant enzymes to detoxify and protect the damage of oxidative stress. Among the antioxidant enzyme activities, there were no significant differences in Superoxide dismutase (SOD) activity at lower concentration of Co. The SOD values in 1, 25, and 50 μ M were 21.66 ± 3.73, 22.47 ± 2.98, and 26.79 ± 3.74 respectively, while significant increase (p < 0.05) was noted in 75 μ M (35.00 ± 3.09) and 100 μ M (46.40 ± 2.55) concentration of cobalt (Table 3). Similarly, Tewari et al. (2002) reported that increasing level of cobalt in the growth medium induced the activity of SOD in mung bean (*Phaseolus aureus*). The

Cobalt concentration (µM)	Shoot length (cm)	Percentage activity with respect to control		
С	4.96 ± 0.152^{b}	100		
25	$5.57 \pm 0.155^{\rm a}$	112		
50	$4.48 \pm 0.327^{\circ}$	91.42		
75	4.03 ± 0.208^{d}	81.25		
100	3.34 ± 0.150^{e}	67.33		

Table 1 Shoot length of *Hydrilla verticillata* (L.f.) Royle treated with different concentrations of cobalt

 $C = control (1 \ \mu M \text{ of cobalt concentration})$

Values are given in mean \pm SD. Mean values within the column sharing different letters are statistically significant at p < 0.05

increase in SOD activity in aquatic plants was due to higher concentrations of trace metals (Garcia et al. 1999; Singh et al. 2013). The level of O_2^- is increased under stress condition and that can lead to increase the level of SOD to maintain and protect the free radical damage in plants (Yan et al. 2008).

Catalase plays a crucial role to nullify the toxic effect of oxidative damage by crumbling the H_2O_2 into water and oxygen. The increase in catalase activity due to metal stress was observed in higher plants and also in aquatic plants (Duraipandian et al. 2016). In this experiment, in 100 μ M of cobalt concentration, the activity was significantly increased (28.16 ± 2.78) when compared to control (22.23 ± 3.19) (Table 3). As the stress increases, the plant tissues accumulate active oxygen beyond its adaptive capabilities (Lin et al. 2015).

Stroinski (1994) reported that the induction of peroxidase activity is a common response of higher plants to metal toxicity. In this study, peroxidase (POD) activity increases when the concentration of cobalt is increased (Table 3). At 25, 50, and 75 μ M of cobalt concentration, POD activities were 2.01 \pm 0.47, 2.50 \pm 0.38, and 3.14 \pm 0.29 respectively. Highest activity of POD was observed significantly (p < 0.05) at 100 μ M (3.84 \pm 0.42) when compared to control (1.70 \pm 0.25). Nayek et al. (2010) also observed enhanced activity of POD in aquatic macrophytes after exposing to a high concentration of various metals. Pandey et al. (2009) analyzed that Co and Cd enhanced POD activity as compared to Ni, Cu, and Zn in

spinach (*Spinacia oleracea* L.). Increased activity of POD in macrophytes species was due to the accumulation of metals in leafy vegetative parts (Rai et al. 2004).

Ali et al. (2010) suggested that cobalt at lower concentration favoured nitrogen fixation, assimilation, and nodulation in chickpea (Cicer arietinum L.) but generated oxidative stress at higher concentration. Tewari et al. (2002) reported that the excess amount of cobalt supply increased the activities of oxidative stress-responsive enzymes in mung bean (Phaseolus aureus). In cauliflower (Brassica oleracea L.), the translocation of Zn, Mn and Cu from roots to apical parts were affected by high level of cobalt exposure, and also decreased the transpiration rate and water potential significantly (Chatterjee and Chatterjee 2000). Zhang et al. (2020) reported that the efficiency of tolerance, accumulation and combat was high in leaves than the stems of H. verticillata (L.f.) Royle and even suggested that H. verticillata (L.f.) Royle is best in treating Pb polluted water after comparing its physiological response and defense mechanisms against Ni, Cd and Pb metals stress. Rai et al. (2004) mentioned that the biochemical response of aquatic macrophytes is species-specific and depended on plant's inherent characteristics. On the other hand, the data of this study indicate that H. verticillata (L.f.) Royle has characteristics to be useful in phytostabilization strategies. In addition, the phytovolatilization property of H. verticillata (L.f.) Royle had already been reported (Carvalho et al. 2001). So, further the investigation of

Pigments	Different concentrations of cobalt in μM					
	С	25	50	75	100	
Total Chlorophyll	0.63 ± 0.04^{ab}	0.66 ± 0.05^a	0.58 ± 0.03^{bc}	0.55 ± 0.02 ^{cd}	0.51 ± 0.03^d	8.915
Chlorophyll a	0.35 ± 0.02^{ab}	0.36 ± 0.03^a	0.32 ± 0.02^{abc}	0.31 ± 0.02^{bc}	0.29 ± 0.03^c	3.675
Chlorophyll b	0.27 ± 0.03^{ab}	0.30 ± 0.03^a	0.25 ± 0.03^{abc}	0.23 ± 0.04^{bc}	$0.21\pm0.02^{\rm c}$	3.736
Carotenoid	0.25 ± 0.03^{a}	0.25 ± 0.02^{a}	0.25 ± 0.01^{a}	0.26 ± 0.03^{a}	0.26 ± 0.04^{a}	0.054

Table 2 Pigment contents in Hydrilla verticillata (L.f.) Royle treated with different concentrations of cobalt

 $C = control (1 \ \mu M \text{ of cobalt concentration})$

Values are interpreted in mean \pm SD. Mean values within the same row sharing different letters are statistically significant at p < 0.05. Mean values within the same row sharing the same letters are not statistically significant

Parameters	Different concentrations of cobalt in µM					
	С	25	50	75	100	
Protein (mg/ml)	12.67 ± 1.45^{ab}	13.55 ± 2.01^{a}	13.84 ± 2.45^{a}	12.12 ± 1.78^{ab}	9.76 ± 2.04^{b}	2.113
SOD (U/mg protein)	$21.66 \pm 3.73^{\circ}$	$22.47 \pm 2.98^{\circ}$	$26.79\pm3.74^{\rm c}$	${\bf 35.00 \pm 3.09^{b}}$	46.40 ± 2.55^{a}	30.498
CAT (U/mg protein)	22.23 ± 3.19^{b}	23.57 ± 2.61^{ab}	23.67 ± 3.14^{ab}	24.54 ± 2.92^{ab}	28.16 ± 2.78^{a}	1.747
POD (U/mg protein)	1.70 ± 0.25^d	$2.01 \pm 0.47 ^{cd}$	2.50 ± 0.38^{bc}	3.14 ± 0.29^b	3.84 ± 0.42^a	16.310

Table 3 Effect of cobalt on protein, SOD, CAT, POD of Hydrilla verticillata (L.f.) Royle

 $C = control (1 \ \mu M \text{ of cobalt concentration})$

Values are interpreted in mean \pm SD. Mean values within the same row sharing different letters are statistically significant at p < 0.05. Mean values within the same row sharing the same letters are not statistically significant

H. verticillata (L.f.) Royle in the aspect of phytovolatilization could be of interest that ensures the enrichment cobalt contaminated aquatic environment.

Conclusion

The results of the present study show that the aquatic macrophyte *H. verticillata* (L.f.) Royle could accumulate the cobalt in its tissue, and low level of cobalt concentration induces the plant growth. If the accumulation of cobalt is high in an aquatic plant, it affects the physiological properties of the plant. Through this analysis, we conclude that the aquatic plant *H. verticillata* (L.f.) Royle could be useful in cobalt affected aquatic environment as a phytostabilizer.

Acknowledgements The authors thank University Grants Commission - Special Assistance Programme - Departmental Research Support (UGC-SAP-DRS-II) (F.3-9/2013[SAP-II]), Department of Science and Technology Promotion of University Research and Scientific Excellence (DST PURSE Phase - II) (Ref. No. SR/PURSE PHASE 2/16(G) /& 16(C) Date. 21.02.2017) and Department of Science and Technology Fund for Improvement of S & T Infrastructure in Higher Educational Institutions (DST FIST) Level - I (stage-II) (Ref. No. SR/FST/LSI-647/2015(C) Date.11.08.2016) for the instrumentation support to Department of Animal Science, Bharathidasan University, Tiruchirappalli.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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