Phytoremediation of Heavy Metals by *Salvinia natans* in a Kashmir Himalayan RAMSAR Site

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INTRODUCTION

Heavy metals are metals having a density greater or equal to 5 g/cc (Nies 1999). In water (both surface water and ground water) they pose a serious environmental problem threatening not only the aquatic ecosystem but also human health through contamination of drinking water. Being persistent, heavy metals accumulate in water, soil, sediment and living organisms (Gomes 2013; Miretzky et al. 2004). Exposure to heavy metals has been linked with developmental retardation, various cancers, kidney damage, autoimmunity, and even death in some instances when exposed to very high concentrations (Glover-Kerkvil et al. 1995). Essential metals such as copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) have normal physiological regulatory functions (Hodgstrand and Haux 2001) but many also bioaccumulate and reach toxic levels (Rietzler et al. 2001). Non-essential heavy metals are usually potent toxins and their bio-accumulation in tissues leads to intoxication, decreased fertility, cellular and tissue damage, cell death and disjunction of a variety of organs (Damek-Poprawa and Savicka Kapusta 2003; Oliveira et al. 2000, 2002). High levels of Cd (cadmium), Cu, Pb (lead), and Fe can act as ecological toxins in aquatic and terrestrial ecosystems (Alkorta et al. 2004; Guilizzoni 1991; Lenntech 2015).

Recent concerns regarding environmental contamination have initiated the development of appropriate technologies to assess the presence and mobility of metals in soil, water and wastewater (Shtangeeva and Ayrault 2004). The chemical methods commonly used for the treatment of surface waters may be successfully replaced by phytoremediation (Holtra 2010). Presently, phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil. Phytoremediation using vegetation to remove, detoxify or stabilize persistent pollutants is an accepted tool for cleaning polluted soil, water, and waste water. Phytoremediation has the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body (Hinchman et al. 1995). This technology is environment friendly and potentially cost-effective. The potential of aquatic macrophytes for heavy metal removal has been investigated and reviewed thoroughly (Ahmad et al. 2014, 2015; Devlin 1967; Ellis et al. 1994; Kara et al. 2003; Marques et al. 2009; Rahman et al. 2008; Rai 2005; Sharma and Gaur 1995; Sitarska et al. 2015; Vymazal 1995).

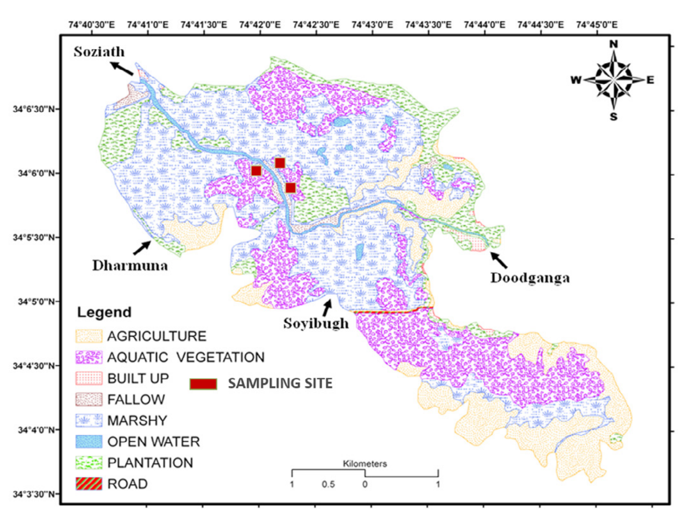
Ahmad et al. (2014, 2015) recently reported the heavy metal removal capability of *Ceratophyllum demersum, Phragmites australis* and *Potamogeton natans* in Hokersar wetland - an important Ramsar site in the Kashmir Himalaya, India. These species significantly reduced the supply of metals available for avifauna and prevented their bio-accumulation. The present study assesses the metal-removing capability of a most common macrophyte *Salvinia natans* in the Hokersar wetland.

METHODS

Study area

Hokersar wetland (34° 06′ N, 74° 05′ E) is a protected and world-famous game reserve situated at an altitude of 1,584 m (amsl). The wetland is about 12 kms Northwest of Srinagar city in Kashmir Himalaya, India (Figure 1). This wetland with a fluvial origin is a permanent but relatively shallow water body subject to a sub-Mediterranean climate (Figure 2). The area of the wetland comprises mostly Karewa table lands with lacustrine deposits (clay, silt, sand particles, conglomerates, boulders, and pebbles) of Pleistocene age. Once spread over an area of 19.5 km^2, the wetland has presently shrunk to just 13.26 km^2. The wetland is fed by a perennial channel at Dharmuna and Soyibugh. The water leaves the wetland through needle weir gate at Soziath village on the western side. Hokersar wetland is an ideal habitat for many migratory and residential birds and during winter harbors about two million waterfowl that migrate from Central Asia, China, northern Europe, and

FIGURE 1. Map showing the Hokersar wetland.



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Siberia. The wetland attains a maximum depth of 2.5 m in spring due to increased runoff from the snow meltwater in the upper reaches of Doodhganga catchment, whereas in autumn the water is just 1 m deep.

Geological weathering and anthropogenic activities such as municipal wastes and use of pesticides in the adjoining rice fields and heavy vehicular traffic running on highway adjacent to the wetland have introduced heavy metals into the wetland. Being an important game reserve, the past practice of hunting/poaching of birds with lead shot has been another source of heavy metals for the wetland.

Study species

Salvinia natans L. (floating fern, floating water moss or water butterfly wings) is a pantropical aquatic fern belonging to the family Salviniaceae (Figure 3). It is an annual free-floating species that appears similar to moss and reproduces by sexual and asexual (vegetative) means. The macrospore develops into the female gametophyte and the microspore has pouches of air sacs that help in floating. Fertilization takes place on the water surface, producing a zygote which becomes the sporophyte (Szmeja and Gałka 2013). Available fossil data indicate that *Salvinia* was distributed in tropical latitudes as well as in temperate latitudes of the Northern Hemisphere throughout most of the Cenozoic. Its modern pantropical distribution could be the result of Pleistocene extinction of *Salvinia* in temperate regions due to global cooling climate trend (Consuegra et al. 2017).

Sampling

Leaf, water, and sediment samples were collected from the Hokersar wetland for analysis of heavy metals. Leaf samples were randomly collected from different sites in the wetland and were sealed in airtight polythene bags. The samples were then transported to the laboratory and stored at 4°C. One liter of water was collected in high density polyethylene (HDPE) bottles from each site during the spring season to determine the metals in solution. The samples were preserved with 2 ml of concentrated HNO_3 (Ultrex) per liter and were kept at 4°C until analyzed. The sampling quality control in water was ensured by introducing bottle blanks (distilled water) and field samples in duplicate which were analyzed to measure the integrity of the samples and reproducibility, respectively. Sediment samples were collected with the help of a dredger and taken to the laboratory in plastic bags. The samples were stored at 4°C for about 1 week. Prior to sampling all the sampling equipment was pre-treated as specified by American Public Health Association (APHA 1998).

Analysis

The plant samples were thoroughly washed with distilled water in the laboratory. Plant samples were oven-dried at 60°C for 24 hours. The dried samples were weighed and ground using a Wiley mill to pass through a 40 mesh screen. The analysis of plants was carried out by acid digestion of dry samples with an acid mixture (9 parts nitric acid: 4 parts perchloric acid) at about 80°C. All the reagents were of analytical grade. The reaction vessels were washed well to avoid external contributions of the metals. Sample blanks consisting of only chemical reagents used for sample analysis were analyzed to correct for possible external contributions of the metals while replicate samples were also evaluated and all the analyses were done in triplicate to ensure reproducibility of the results. The digested samples were analyzed for nine metals (Al

FIGURE 2. View of the Hokersar wetland showing different macrophytic species.

FIGURE 3. Closeup of *Salvinia natans*. (Photo courtesy of Le.Coupe.Gris).

[aluminum], Mn [manganese], Zn [zinc], Cu [copper], Pb [lead], Co [cobalt], Cr [chromium], Ni [nickel] and Cd [cadmium]) using an atomic absorption spectrometer (AAS; Perkin Elmer, model Analyst 800 with a detection limit of ppb).

Water samples (50 mL) were digested with 2M HNO_3 at 95°C for 2h and were increased to 100 mL in volumetric flask with demineralized water. The digestion was done in glassware previously soaked in nitric acid and washed with demineralized water. Sample blanks were also analyzed to correct for any contamination in the course of analysis. The digested samples were analyzed for metals on an AAS.

The bioconcentration factor (BCF) is defined as the ratio between heavy metal concentration in the plant tissues by dry weight and heavy metal concentration in water (Zayed et al. 1998). Zayed et al. (1998) reported that the appropriateness of a plant for phytoremediation potential is often judged by its BCF. BCF values over 1000 are generally considered evidence of a useful plant for phytoremediation (Ahmad et al. 2015; Matache 2013).

For statistical analysis metal concentrations were expressed in µg/L in water and mg/kg in plant tissues. Standard error was determined from three replicates for every set of data.

RESULTS

The results of the concentration of heavy metals in *S. natans* and water collected from the Hokersar wetland are given in Table 1. Al and Mn are bioaccumulated in much higher concentrations than the rest of the heavy metals.

TABLE 1. Concentration of heavy metals in *S. natans*, water and bioconcentration factor (BCF) of *S. natans* depicting transfer of heavy metals from water to plant tissues. (Note: Plant species having BCF greater than 1000 are generally considered positive plants for phytoremediation.)

Heavy metal	Concentration (mg/kg)	Water (mg/L)	BCF
Pb	39.61±2.31	0.062±0.00	638
Mn	3858±179.66	0.67±0.06	5758
Al	3667±217.4	7.85±0.78	467
Cr	9.32±0.63	0.025±0.00	372
Co	9.65±0.25	0.01±0.00	965
Zn	30.62±2.05	0.06±0.00	510
Cu	16.75±0.44	0.30±0.01	55
Cd	3.63±0.24	0.004±0.00	907
Ni	9.59±0.21	0.03±0.08	319
Mo	18.87±0.41	0.36±0.02	52
Ba	158.82±2.22	0.26±0.00	610

The accumulation of the different elements in *S. natans* was in order of Mn > Al > Ba > Pb > Zn > Mo > Cu > Co > Ni > Cr > Cd.

The phytoremediation potential of *S. natans* was determined by calculating the BCF, which is defined as the ratio of metal concentration in the roots to that in water (Table 1). In *S. natans* the highest BCF corresponded to Mn metal (5758) followed by Co (965) and Cd (907) in decreasing order. Consequently, *S. natans* has high capacity to hyperaccumulate these heavy metals.

DISCUSSION

Kamel (2013) reported that plants show critical toxicity (affecting plant and inhibiting its growth) between the range of Cd 10-30, Co 1-8, Cu 25-90, Ni 10-50 Pb 30-100, and Zn 100-400. Dhir and Srivastava (2013) suggested that *S. natans* has fairly high levels of tolerance to Zn, Cd, Co, Cr, Fe, Cu, Pb and Ni with the levels of tolerance varying from metal to metal. Thilakar et al. (2012) reported that *S. natans* is a hyperaccumulator of heavy metals and has great potential to accumulate heavy metals and can be effectively used to clean up aquatic ecosystems. Dhir et al 2011 reported that accumulation of osmolytes under heavy metal stress might help in imparting tolerance to the heavy metals. *S. natans* possesses effective metabolic machinery that is capable of overcoming the osmotic stress induced by heavy metals including mercury (Hg) (Dhir and Sitarska 2011; Sitarska 2014). Sen and Bhattachariya (1994) reported that in the absence of other pollutants *Salvinia* plants may be used for the removal of Ni (II) in effluents and as an indicator of Ni pollution.

CONCLUSION

S. natans is among the more dominant species in Himalayan wetlands. Demonstration of its heavy metal accumulation potential and growing incidence of heavy metal contamination around the world make this species among the choicest for heavy metal remediation in regions where *S. natans* naturally occurs. This plant can be easily transferred to heavy metal-contaminated sites and encouraged to grow for bioaccumulation particularly of Mn, Co and Cd. The species can also be easily planted in constructed wetlands designed to treat wastewater containing different heavy metals. The present study and other studies conducted across the world confirm that *S. natans* serves as a potent phyoremediation plant for the removal of heavy metals particularly Mn, Co and Cd. It is important to note, however, that this species is viewed as an invasive species (e.g., a noxious weed in the United States; https://plants.usda.gov/core/profile?symbol=SANA5), so caution must be exercised in using this plant beyond its natural range. ■

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