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Research article

Comparative evaluation of salicylic acid and EDTA chelant induced phytoremediation of lead and nickel using *Lemna minor* L.

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Abstract: The objective of the present study was to study the influence of natural organic agent SA (salicylic acid) and synthetic organic agent EDTA (ethylenediaminetetraacetic acid) on metal uptake by *Lemna minor* L. in Pb and Ni contaminated water. *L. minor* was treated with Pb and Ni, each at concentration of 10 $mg.l^{-1}$. EDTA and SA were added at 2.4 mM concentration. Samples were collected at an interval of 7 days for four weeks *i.e.* 7, 14, 21 and 28 day. The nickel accumulation capacity of *L. minor* for combined Pb+Ni treatment was lower than individual Ni treatment accumulation capacity. Salicylic acid significantly enhanced the uptake of Pb and Ni in single Pb and Ni treatments. However, addition of EDTA could not induce Pb and Ni accumulation. Based on results it could be said that removal of metals depends on the type and concentration of chelants and concentration of pollutants in water.
Keywords: Chelant - Duckweed - Heavy metals - Phytoremediation.

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INTRODUCTION

Heavy metals are persistent pollutants and they are of major concern in the environment due to their toxicity and harm to plant and animal life. They are released either by natural or anthropogenic sources. Lead and nickel heavy metals are detected in the waste streams emerging from mining operations, tanneries, electronic industries, electroplating, batteries and petrochemical industries as well as textile mill products (Johnson 1998).

Lead is an extremely toxic metal and is having many important industrial applications. The permissible limit of Pb in drinking water is 0.005 $mg.l^{-1}$ (Patterson *et al.* 1998). Pb effects kidney, nervous disorder and mental deficiency (Panchandaikar & Das 1994, Axtell *et al.* 2003). Pb is the most abundant, globally distributed, best-recognised dangerous among heavy metals that has been longest use since ancient times (Volesky & Holan 1995, Salt *et al.* 1998). Pb has been used as an anti-knocking gasoline additive since 1920 and remains an important component in the manufacturing of the several commercial items such as storage batteries, cable coverings, casting, sheet lead, pipes and ammunition. Pb has been tested and found to be carcinogenic, mutagenic, and teratogenic. It affects the reproductive, nervous, muscular and haemopoeitic systems (Volesky & Holan 1995). Ni causes dermatitis and bronchial problems (Gardea-Torresday *et al.* 1996).

Metal contamination in water can be treated using a variety of technologies that incorporate chemical, physical and biological processes. Conventional remediation technologies such as chemical precipitation, ionic exchange, filtration, electrochemical treatment, reverse osmosis, evaporative recovery and solvent extraction (Rahmani & Sternberg 1999) are generally too expensive and are not able to remove heavy metals completely and its byproducts (toxic sludge generation) again need disposal. Phytoremediation has emerged as a cost-effective and environmental friendly sustainable technique in the last decades (Debusk *et al.* 1996). It is the use of plants for pollutant removal from contaminated water or soil (Vujevic *et al.* 2000). Several studies indicate that aquatic plants have large potential for the removal of organic and inorganic pollutants from wastewater. Aquatic plants such as *Eichhornia crassipes*, *Elode Canadensis*, *Myriophyllum spicatum*, *Potamogeton pectinatus*, *Wolfia globosa*, *Lemna*, *Vellisnaria*, *Hydrilla verticilleta* and *Typha latifolia* have been extensively

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used in phytoremediation research. Floating macrophytes (macro algae, duckweed, and water hyacinth) provide advantages over emergent aquatic plants as they are much easier to harvest (Begonia *et al.* 2002).

Lemnoideae are limnic vascular plants that belong to the Araceae family and comprise the *Landoltia* spp., *Lemna spirodela*, *Wolfia* spp. and *Wolffiella* spp. They are commonly found in fresh water and brackish ecosystems in temperate climates and serve as an important food source for various water birds and fish. Additionally they provide habitats for invertebrates. Lamnaceae plants are easy to culture and handle, have a high growth rate and are highly sensitive to different pollutants. *Lemna minor* L. is frequently used in ecotoxicological research as a representative of higher aquatic plants. It can serve as a hyperaccumulator, keeping the metal from continuous reintroduction into the ecosystem (Turgut *et al.* 2004). *Lemna minor* can be used in phytotoxicity tests of contaminants, including heavy metals, phenolics and herbicides (Wu *et al.* 2004).

Studies have revealed that EDTA forms a metal-complex that enhances the mobility of the metal through the plant (Raskin 1992, Dat *et al.* 1998, Janda *et al.* 2000). Salicylic acid, which may act as a component of the signal transduction system important in defense mechanisms against pathogen attack (Lu *et al.* 2004), may also provide protection against certain abiotic stresses e.g. heat stress in mustard seedlings (Saygideger & Dogan 2004) or chilling damage in maize (Janda *et al.* 1999).

The objective of this research was to investigate the ability of chelants (EDTA and SA) to enhance the phytoremediation of Pb and Ni in contaminated water.

MATERIALS AND METHODS

Duckweed plants were picked up from a stream of water from the main Indian Institute of Technology Delhi campus. They were identified as *Lemna minor* L. and were cultured in a water tank in micro model IIT Delhi (the experimental site). Stock solutions were prepared by using lead nitrate and nickel nitrate salt. Plastic containers of ten litre capacity were filled with water. 6.0 g initial fresh weight of *L. minor* was used and treated with Pb and Ni, each at concentrations of 10 $mg.l^{-1}$. EDTA and SA were amended at 2.4 mM concentration as $C_{10}H_{14}K_2N_2O_8.2H_2O$ (MERCK) and $C_6H_4OH\text{-}COOH$ (Qualigens). A black line was drawn on the containers so a six litre water level could be kept constant. Every 1-2 days, the plants were checked and tap water was added to each container so the six litre water level line remained constant. Samples were collected in an interval of 7, 14, 21 and 28 days.

The biomass weight was taken by drying duckweed plants on filter paper for 10 minutes (fresh weight). Plants were dried and their dried weights were noted in the lab notebook. Relative growth of control and treated plants were calculated as follows (Lu *et al.* 2004):

$$\text{Relative growth} = \frac{\text{Final fresh weight(FFW)}}{\text{Initial fresh weight (IFW)}}$$

The dried plant samples were heated in a muffle furnace at 500°C for 6 hours. The ash of each sample was dissolved in 5 ml of 20% HCl to dissolve the residue. Samples were heated on a hot plate to boiling. Required amount of HCl (20%) was added to avoid sample drying. The resulting solutions were filtered and diluted to 50 ml with deionized water in volumetric flasks. The Pb and Ni content of these plant samples and water samples were determined using flame atomic absorption spectrophotometer (Electronics Corporation of India Limited AAS4129) with the following settings: for Pb - wavelength 217 nm, lamp current 5 mA, slit 1 nm, fuel – acetylene and oxidant air and for Ni - wavelength 232 nm, lamp current 5 mA, slit 0.2 nm, fuel – acetylene and oxidant air.

RESULTS AND DISCUSSION

Relative growth

Effect of chelants (EDTA and SA) on relative growth of *Lemna minor* L. treated to Pb and Ni is shown in figure 1. Relative growth of *L. minor* increased with duration of time. In Pb experiment sets, the highest relative growth was 2.54±0.13 in Pb+SA combination after 28 days. Furthermore, the lowest relative growth was 1.13±0.05 in individual Pb treatment after 7 days. However, in Ni experiment sets, the maximum value of relative growth 1.99±0.19 in Ni+SA combination after 28 days and the minimum relative growth was observed in Ni+EDTA combination after 7 days (1.07±0.025). Nevertheless, the highest and the lowest relative growth in Pb+Ni experiment sets were measured in Pb+Ni+SA combination after 28 days (2.48±0.18) and in Pb+Ni combination after 7 days (1.17±0.08) respectively.

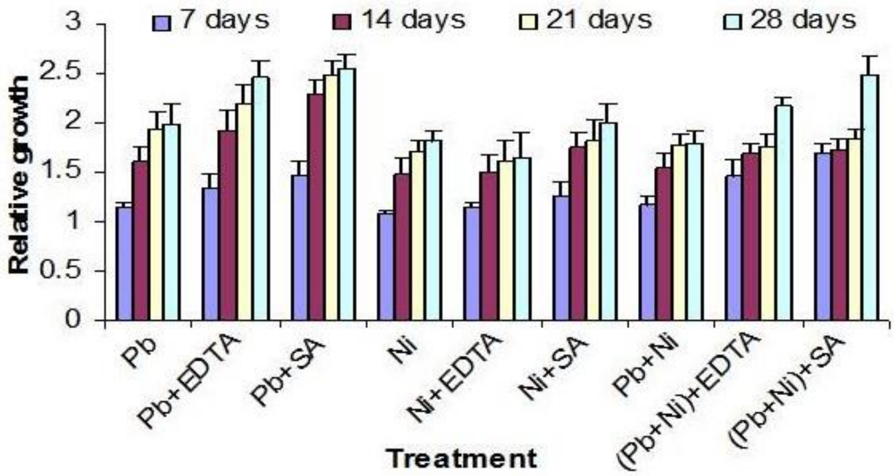

Figure 1. Effect of chelants on relative growth of *Lemna minor* L. treated with Pb and Ni concentrations (10+10 mg.l^{-1}).

Saygideger & Dogan (2004) observed that single and combined Cd and Pb with and without EDTA concentrations affected growth in *L. minor* and *Ceratophyllum demersum* L. after 7 days exposure. Both macrophytes were adversely affected in 50 mg.l^{-1} Pb plus 0.5 mg.l^{-1} Cd combination more than other tested concentrations. Liu *et al.* (2004) reported that when *Sedum alfredii* Hance plants were treated with 200 µM Pb+200 µM EDTA+1-100 µM IAA (Indole-3-acetic acid) for 12 days, EDTA and IAA had no effects on shoot biomass.

Metal accumulation

Effect of chelants on Pb and Ni accumulation by *Lemna minor* L. is shown in figure 2–3. Pb and Ni accumulation increased with time in all sets. The highest and the lowest Pb accumulation in Pb experiment sets were determined as 2.56±0.12 mg kg^{-1} for Pb+SA combination after 28 days and 1.10±0.10 mg kg^{-1} for individual Pb for 7 days respectively. Similarly, in Ni experiment sets, the highest Ni accumulation (910±101 mg kg^{-1}) was found in the same treatment and same time as in case of Pb. However, the lowest Ni accumulation (205±36 mg kg^{-1}) was obtained in Ni+EDTA after 7 days.

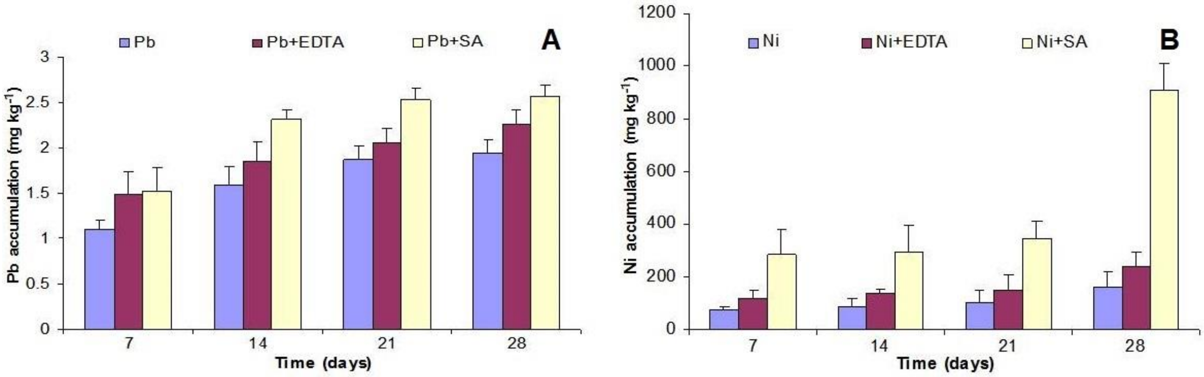

Figure 2. Effect of chelants on accumulation in *Lemna minor* L.: **A,** Pb; **B,** Ni.

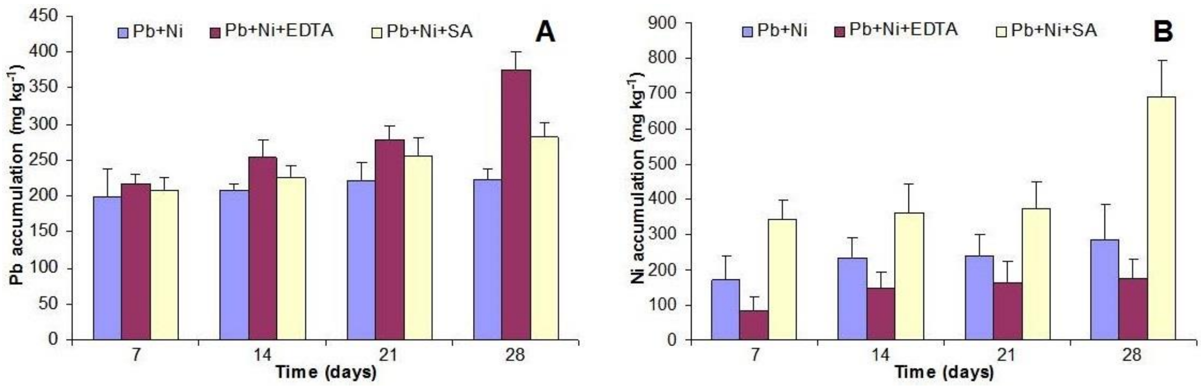

Figure 3. Effect of chelants on accumulation in *Lemna minor* L. by Pb+Ni treatments: **A,** Pb; **B,** Ni.

Effect of EDTA and SA on Pb and Ni accumulation in combined Pb+Ni treatment by *L. minor* is shown in figure 3 respectively. The highest and the lowest Pb accumulation were in Pb+Ni+EDTA treated plants and Pb+Ni treated plants respectively. The highest and the lowest Ni accumulation were in Pb+Ni+SA treated plants and Pb+Ni+EDTA treated plants respectively. Pb and Ni accumulation increased with time. It shows that in combined Pb+Ni treatment, Pb and Ni accumulation in *L. minor* was found to be the utmost in Pb+Ni+SA treatment. However, addition of EDTA could not induce Pb and Ni accumulation much. Salicylic acid enhanced the uptake of Pb and Ni in single Pb and Ni treatments. Nickel accumulation capacity of *L. minor* for combined Pb+Ni treatment was lower than individual Ni treatment accumulation capacity.

The Pb concentrations in the leaves and roots of *Typha orientalis* plant increased with increasing of Pb level in the nutrient solution of 0–500 $mg.l^{-1}$ (Li *et al.* 2008). Results from this study demonstrated that the plants had the highest accumulation and translocation under the condition of 500 $mg.l^{-1}$ Pb+0.5 m $moll^{-1}$ EDTA. Rahman *et al.* (2008) reported that the uptake of inorganic arsenic species into the aquatic plant *Spirodela polyrhiza* L. increased by EDTA when plants were exposed to different arsenic species at 6 µM and 50 µM EDTA for two weeks.

Metal remained in the residual solution

SA and EDTA absorbed/extracted greater Pb/Ni than control. The best results of Pb and Ni removal were obtained in SA treatments which may be due to the greater solubility of SA (0.2 g/100 ml at 20ºC) in water than EDTA (0.05 g/100 ml at 20ºC) and metal-SA complexes may have been more available to plants than metal-EDTA complexes.

In combined Pb+Ni treatments, Pb removal by EDTA was more than SA and Ni removal was best in SA. This may be due to the competition between Pb and Ni to bind with chelants EDTA and SA. EDTA has strong complexing capacity with Pb but in Pb treatment, uptake got reduced may be due to reduction in ion activity in water. SA acts as defender and the protective function of SA includes absorption and distribution of elements. SA is implicated in the high degree of cellular tolerance towards Ni in the genus *Thalspi* (Freeman *et al.* 2005). SA may help in metal uptake by chelating Pb/Ni in the solution and then release Pb/Ni metal in the plant system. The differences in uptake between the essential and non-essential metals and the effects of chelants on their uptake can be explained by two parallel pathways: a selective symplastic pathway and a nonselective apoplastic pathway (Tandy *et al.* 2006). Selective uptake of Ni is very efficient and uptake of Pb is very low under these conditions. Apoplastic (passive) uptake of metal complexes is a function of the complex concentration in the surrounding solution. This also suggests that higher chelate concentrations may lead to increased uptake of essential metals. There were no literature on EDTA and SA effect in removal of Pb and Ni from water. Based on presented results it could be said that removal of metals depends on the type and concentration of chelants in water.

Ni uptake was inhibited by EDTA. On the other hand, the addition of SA in the medium increased the uptake substantially. Our results are corroborated with Shi & Zhu (2008). They reported that addition of SA increased Mn concentration in cucumber plants under excess Mn condition. Similarly, content of Cd in barley treated with SA was higher than Cd alone treatment (Metwally *et al.* 2003).

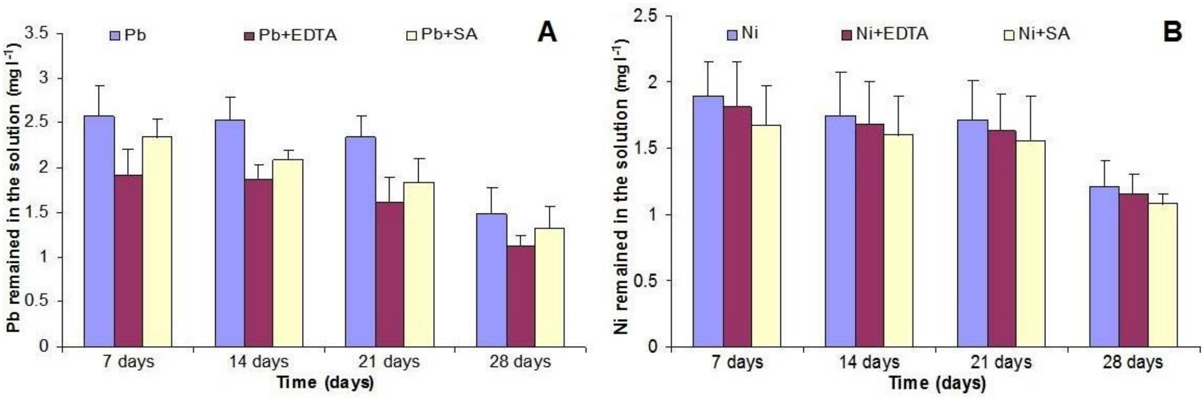

Figure 4. Concentration of remained in the residual solution after treatments: **A,** Pb; **B,** Ni.

Concentrations of Pb and Ni ($mg.l^{-1}$) remained in the water samples after treatments are represented in figures 4–5. Pb and Ni content in water were decreased with the passage of time. The lowest value of Pb

(1.12 ± 0.12 mg.l^{-1}) remained in the solution after 28 days was in treatment Pb+Ni+EDTA (97% removal) while the highest value (2.58 ± 0.34) in the same treatment was after 7 days. Removal potential of Ni was highest in Pb+Ni+SA treatment for 28 days (99.5%).

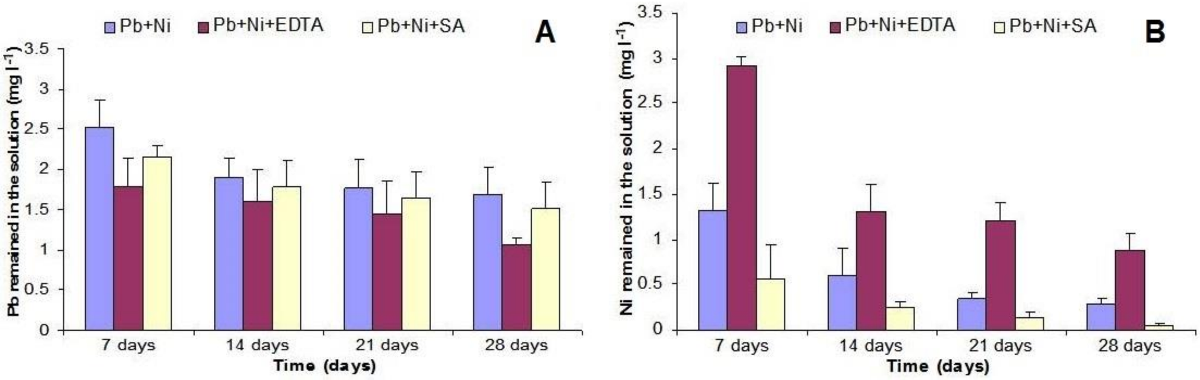

Figure 5. Concentration of remained in the residual solution in Pb+Ni treatments: **A,** Pb; **B,** Ni.

Removal potential of *L. minor* with chelant in combined Pb+Ni treatment was better as compared to individual Pb and Ni treatments (Fig. 6–7). Akin *et al.* (1994) observed that removal of lead was decreased by increasing the concentration of EDTA in *Eichhornia crassipes* (water hyacinth). Kruatrachue *et al.* (2002) studied the combined effects of Pb and humic acid on Pb uptake by *L. minor*. Humic acid did not significantly decrease Pb uptake at 50 and 100 mg.l^{-1} Pb treatment. In the 200 mg.l^{-1} treatment, the lowest Pb contents were observed in the presence of 160 mg.l^{-1} humic acid. Therefore, a high concentration of humic acid could significantly decrease the Pb uptake. Miretzky *et al.* (2006) investigated the mechanism of simultaneous metal removal of Cd, Ni, Cu, Zn and Pb by *Spirodela intermedia*, *L. minor* and *Pistia stratiotes*. *L. minor* biomass presented the highest mean removal percentage and *P. stratiotes* the lowest for all metals tested. Pb and Cd were more efficiently removed by the three of them. No significant differences were observed in the metal exchange amounts while using multi-metal or individual metal solutions.

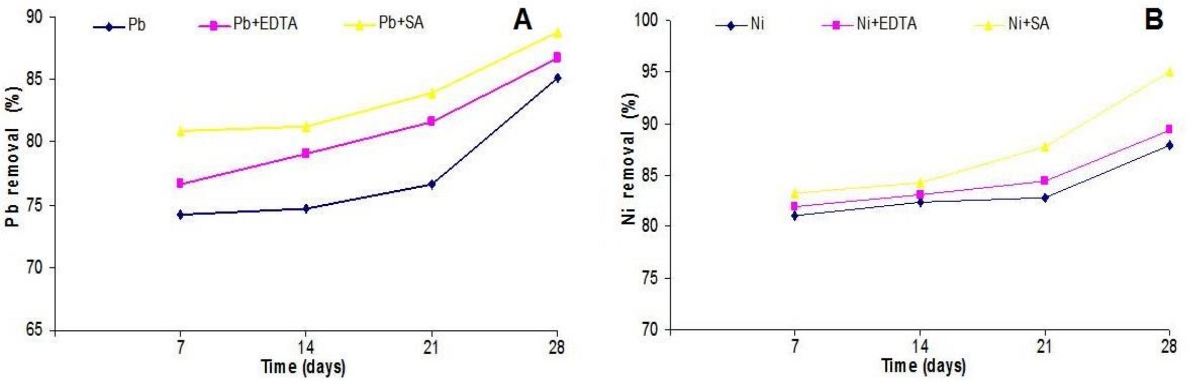

Figure 6. Removal potential of *Lemna minor* L. after different days: **A,** Pb; **B,** Ni.

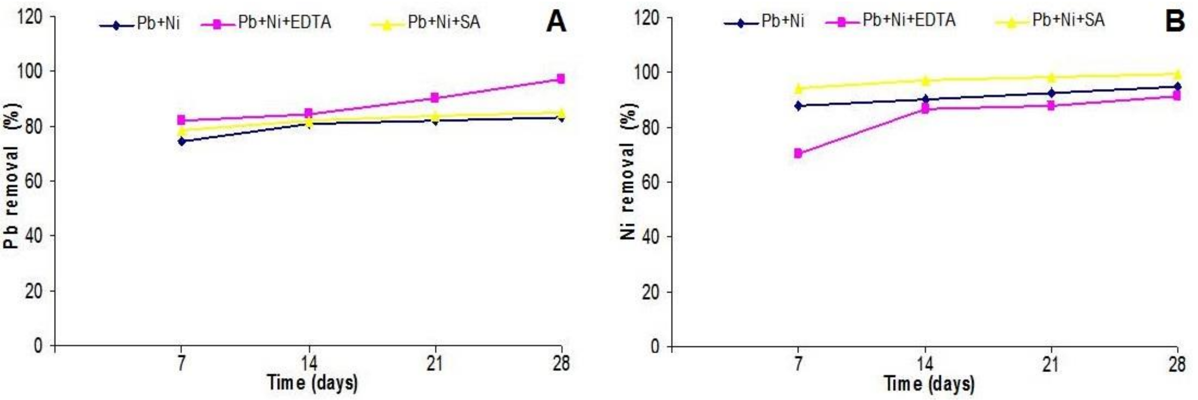

Figure 7. Removal potential of *Lemna minor* L. in Pb+Ni treatments with or without chelants: **A,** Pb; **B,** Ni.

CONCLUSION

The results showed that growth of *Lemna minor* helped in the accumulation of Pb and Ni. SA significantly increased more Pb and Ni accumulation in *L. minor* as compared to EDTA when added as chelants in water. This suggests that *L. minor* can be used for cleaning water polluted by heavy metals with the help of chelants.

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