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RESEARCH ARTICLE

Growth and Phytoremediation Potential of Watercress *Nasturtium officinale* R. Br. in Ammonium-rich Wastewater

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Coordinates: 34°18'51" N; 47°03'54" E

Key words: Wastewater treatment, Ammonium Pollutant removal

Abstract

Phytoremediation is considered to be eco-friendly and efficient technology for the removal of pollutants present in wastewater. Nowadays the focus is to look for a sustainable approach in developing wastewater treatment capability in rapidly increasing. The main objective of this paper is to review the possibility of using watercress (*Nasturtium officinale*) for the removal of ammonium ion present in high-strength industrial wastewater. It was found that there was no significant reduction of shoot height, root length and total biomass of watercress due to an increase in NH4+ concentrations. Statistical analysis indicates that the length of roots were significantly ($P < 0.05$) increased at NH4+ rich waste water supply levels of 20-25% while compared to the control. The plants accumulated large amounts of ammonium ion or nitrogen in tissues under conditions of abundant supply. The removal efficiency of raw waste water was around 66 % for nitrogen, 23% for calcium,25 % for total hardness, 22% for biological oxygen demand and 38% of chemical oxygen demand. Nutrient removal efficiency was positively correlated with the initial nutrient supply. The results show that *N.officinale* grown in the fixed mat economic plant-based treatment system may be an effective, low-cost phytoremediation technology to treat water containing a higher concentration of ammonium.

Introduction:

Recently, promising technology using aquatic macrophytes for wastewater treatment has gained popularity because of it is an eco-friendly and environmentally sound approach (Vacca *et al.,* 2005). Therefore, researchers have recently paid attention towards the potential of aquatic macrophytes to remediate and recycle pollutants from urban and industrial wastewater (Tak *et al.,* 2010; 2012). Several processes exist for the removal of chemical and microbial pollutants from wastewater but they are expensive and produce high sludge content (Ruiz-Marin *et al.,* 2010). Wastewater phytoremediation approach using microalgae, macrophytes and different other water plants including, floating or submerged (Noemi *et al.,* 2004) is based on natural processes to remove different wastewater pollutants. Phytoremediation through aquatic macrophytes has the efficient capacity for removing various organic and inorganic pollutants through processes like absorption, degradation, or stabilization. Two main processes for nitrogen removal are: a) Oxidation of ammonium to nitrite, followed by oxidation of nitrite to nitrate in oxygen rich environment (nitrification process) b) Reduction of nitrate to nitrite, followed by the reduction of nitrite to nitrogen gas (denitrification under oxygen deficient conditions). In different municipal and industrial processes many nitrogen-rich streams are produced with a low content of biodegradable organic matter (Siegrist *et al.,* 1998; Zimmo *et al.,* 2004). In wastewater treatment plants with anaerobic sludge digestion, 15% of the inlet nitrogen load is recycled with the return of liquors from sludge dewatering. Separate treatment of this digester supernatant, containing 600 g.m-3 NH_4N would significantly reduce the nitrogen load of the mainstream and improve nitrogen elimination (Fux *et al.,* 2003). Chemical elimination, or with air stripping is feasible but is much more expensive (Siegrist, 1996).

In the recent past years de-ammonification processes,

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as a new technology for the treatment of nitrogen rich wastewaters, have been developed (Helmer *et al.,* 2000; VanDongen *et al.,* 2001). The introduction of de-ammonification process can reduce the cost of plant operation and make the management more sustainable. Due to low growth rates of microorganisms denitrification process favors systems with long sludge retention times (biofilm systems), a system which continuously receives waste water, rich in ammonium and nitrite (VanDongen *et al.*, 2001). Watercress, (*N.officinale*), is an edible aquatic plant. It commonly occurs in urban streams where it has been observed to achieve biomasses of up to 200 g m^{-2} and can dominate the aquatic plant assemblage (Ozturk *et al.,* 2010). Watercress as a medicinal plant has been traditionally considered a diuretic, purgative and tonic, and consumed as a salad green (Ozturk *et al.*, 2010). The properties of *N.officinale* that entail mental accumulation was extensively studied in the past. However, no studies to date have examined Nasturtium's growth, nutrient uptake responses and phytoremediation abilities to the large gradient of ammonium concentration typical of industrial wastewaters. Therefore, in this study, we investigated the in situ phytoremediation assays using the association of watercress plant during different proposed treatments in order to improve treated wastewater quality. The results of this study will provide a better understanding of the phytoremediation process.

Materials and methods:

Plant collection cultivation and wastewater treatments: *Nasturtium officinale* samples (in seedling stage) were collected in May of 2015 from the Ghaisavand Stream in Kermanshah, Iran. Prior to the experiment, plant culture media (containers) were disinfected with 1% (v.v) NaClO for 3–5 min thereafter rinsed three times with distilled water (Hou et al., 2007). Collected samples were washed in tap water and acclimatized for 5 days in a climate chamber with a water temperature of 18°C, a relative humidity of 70% and light-dark photoperiod of16 h light.8 h dark. Containers were mildly aerated.

The experiments which arranged in this study were set-up in triplicate, wherein each replicate constituted approximately 6 g of the evaluated plants. The ammonium-rich wastewater solutions utilized in the present study were gained from Kermanshah ammonium refinery, Iran. Each watercress sample was exposed to five varied test concentrations (5, 10, 15, 20, and 25 percent) of ammonium-rich wastewater in separate 1000 mL conical flasks (Ozturk *et al.,* 2010). The plants that were not exposed to wastewater served as the control groups of this experiment. The flasks that comprise the plant and wastewater concentrates were placed in a climate chamber under the aforementioned conditions for 30 days. Flasks were not aerated during the experiment. The change that occurred in the volume of the solution within the flasks due to evapotranspiration was compensated for by the addition of double distilled water. During the experiment period, leaves were harvested (usually eight per treatment) on day 0 (before treatment, time t1), day 15 (time t2) and day 30 (time t3). The resultant plant samples in each harvesting time were collected and sieved with a plastic griddle. Each plant was rinsed with deionized water, drained, and then blotted on paper towels for 2 min. Determination of growth parameters During each harvesting time, shoot height was measured from the culm base to the tip of the longest leaf and root length was measured from the root–shoot junction to the tip of the longest root. Before treatment and on harvesting, the root and shoot biomass pot-1 were measured.

Analytical procedures: The Nessler method was used for analyses ammonium ion in wastewater, In the ammonia test, Nessler Reagent (K_2HgI_4) reacts with the ammonium ion present in the sample (under strongly alkaline conditions) to produce a yellow-colored species. The intensity of the color is in direct proportion to the ammonium ion concentration.

The total concentration of calcium ion in each wastewater treatment was determined using Shimadzu AA-6200 atomic absorption spectrometer (HG-AAS; Shimadzu, Tokyo, Japan). The reference standard for calibration of the AAS was made using 1000 mg L^{-1} (Beach leaves material FD8, Commission of the European Communities, Joint Research Centre ISPRA). The samples were analyzed in triplicate. All analytical reagent grade used in this study were of Merck, Darmstadt, Germany.

The chemical oxygen demand (COD) measurement was based on digestion with potassium dichromate in concentrated Sulphuric acid for 2h at 150°C. Biological oxygen demand (BOD) was determined titrimetrically, suspended solids were measured by using WHATMAN filter paper.

Physical parameters like pH and TDS were determined by a portable combine meter (Milwaukee, model $SM80_2$) and TH was measured by the following equation: TH = 2.497Ca + 4.115 Mg (Vasanthavigar *et al.,* 2010).

Statistical analysis Values of all data are expressed as mean ± SD. The one-tailed paired Student's t-test was used to determine statistical significance between the untreated and treated parameters at $P < 0.05$. All analyses were carried out in triplicate.

Results and Discussion :

Effects of ammonium rich wastewater on growth of watercress: the suppression of growth and yield in NH4+ -sensitive species can be severe, and for this reason, NH4+ toxicity is of major importance in agricultural and ecological settings. Certain plant species and even families are particularly sensitive to or tolerant of NH_4^+ as the sole

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nitrogen source. In general, the biomass of plants may be an important index in identifying them as a tolerant and an accumulator. Therefore, this parameter should not decrease significantly at the threshold concentration of plant growth inhibition. The total biomass was measured at the end of the experiment and is presented in figure 1. During the 30-day exposure to different content of ammonium rich wastewater (0-25%), the biomass of watercress did not show any visual symptoms.

There was no significant reduction ($P < 0.001$) of shoot height observed due to an increase in NH_4^+ concentrations. It is revealed that the length of roots was significantly ($P<0.05$) increased at $NH4^+$ wastewater supply levels of 20-25% compared to the control. The maximum root length at 25 % NH_4^+ was about 1.23 fold of the control (Fig.- 2).

Based on these growth traits, it is suggested that watercress was considerably tolerant to NH_4^+ riches wastewater. Because tolerance to metal toxicity is a crucial characteristic for hyperaccumulators or accumulators, therefore watercress had the potential for use in phytoremediation of NH_4^+-polluted wastewaters. The results of the physicochemical parameters of wastewater study are presented in Table 1, after multiplying the values by the dilution factor. The hydrogen-ion concentration (pH) is an important quality parameter of wastewater. Table 1 shows that the pH of the influent (raw wastewater) was measured to be 8.5 compared to 8.8 with treated effluent. On 5%, pH of the effluent increased from 8.5 to 8.8 and maintained thereafter, furthermore total dissolved solids (TDS) was enhanced from 0 to 25% wastewater treatment. In each $NH4^+$ wastewater treatment, TDS was increased from 0- 30 days of treatment periods (Table-1). The values show TDS are in the permissible limit as compared with Indian standards. The watercress treatment resulted in a significant reduction in total hardness in each treatment by about 25%. Free ammonia levels were reduced by 66% at 30th days in 25% wastewater treatment (Fig.- 3). Calcium also followed a similar trend with 23% reductions (Figure 4). Reductions in biochemical oxygen demand (BOD) and chemical oxygen demand (COD) levels were 22% and 38%, respectively, during the Phytoremediation process (Table 1). The BOD and COD levels of influent wastewater varied from 4.73-4.8 mg.l and 9.4-22.9 mg.l respectively. Whereas the BOD and COD levels of effluent varied from 3.33-2.86 mg.l and 11.033 –20.33 mg.l respectively. Fig.-5&6 shows the levels of BOD to a certain extent due to the biological treatment process for which the effluent is treated which consists of equalization, primary clariflocculator, aeration tank and the secondary clariflocculator. Biological treatment process results in oxidation of organic matter, which provides energy for the microbial metabolic process.

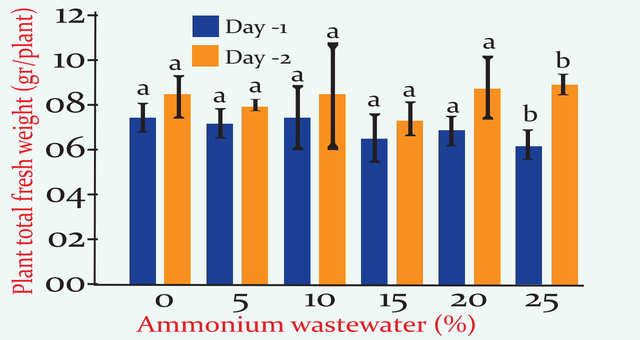

Figure-1: Effect of ammonium-rich wastewater on plant fresh mass of watercress (*N. officinale*). The bars having similar letters do not significantly different at P < 0.05 (T-test). N=5.

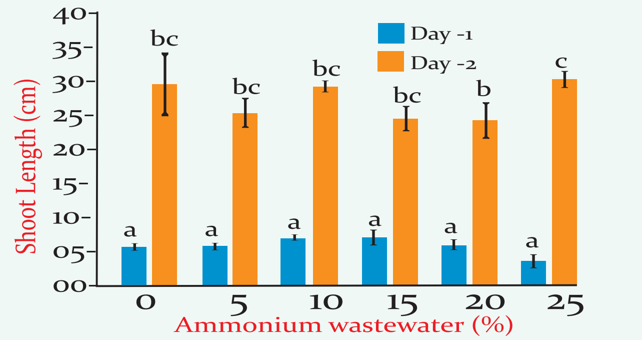

Figure-2: Effect of ammonium-rich wastewater on shoot length (cm) of watercress (*N. officinale*). The bars having similar letters do not significantly different at P < 0.05 (T-test). N=5

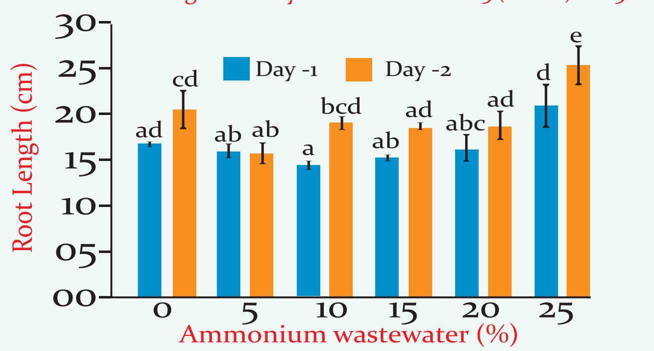

Figure-2: Effect of ammonium-rich wastewater on root length (cm) of watercress (*N. officinale*). The bars having similar letters do not significantly different at P < 0.05 (T-test). N=5

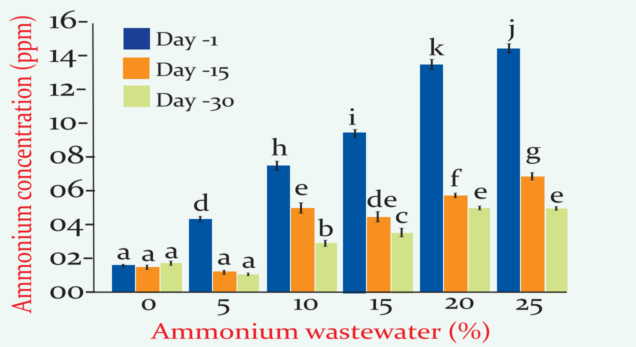

Figure-4: Effect of ammonium-rich wastewater on shoot ammonium concentration of watercress (*N. officinale*). The bars having similar letters do not significantly different at P < 0.05 (T-test). N=5.

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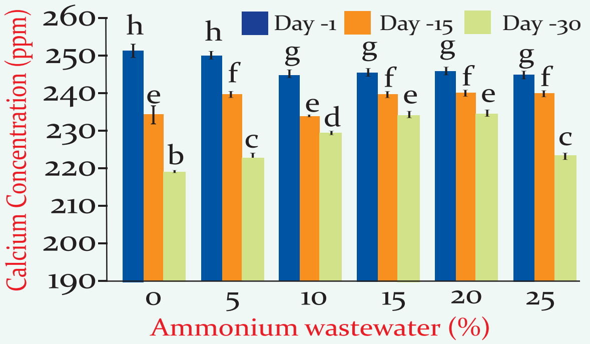

Figure-5: Effect of ammonium-rich wastewater on shoot calcium concentration of watercress (*N.officinale*). The bars having similar letters do not significantly different at P < 0.05 . N=5.

Table 1.: Physico-chemical parameters of ammonium-rich wastewater after purification by watercress (*N. officinale*).

EP(day)*	pH	TDS	TH	BOD	COD
Treatment (wastewater percentage) Control					
0	8.5	15.00	576.66	4.80	9.40
15	8.5	18.00	379.33	3.93	9.00
30	8.5	21.00	365.66	3.33	11.03
Treatment (wastewater percentage) 5					
0	8.8	15.50	580	4.50	11.00
15	8.8	17.50	393.66	4.26	8.99
30	8.8	19.50	374.00	3.80	12.99
Treatment (wastewater percentage) 10					
0	8.8	16.166	583.33	4.03	19.99
15	8.8	19.166	419.00	3.46	17.00
30	8.8	21.166	389.00	2.90	18.00
Treatment (wastewater percentage) 15					
0	8.8	16.33	580.66	4.76	17.99
15	8.8	18.50	435	4.033	14.00
30	8.8	22.16	400	3.53	18.99
Treatment (wastewater percentage) 20					
0	8.8	16.16	575	4.60	22.99
15	8.8	19.16	444.33	3.80	21.00
30	8.8	21.50	404.00	2.90	20.33

*Exposure period (day)

Conclusion:

Our study concluded that *Nasturtium officinale* is a very efficient in remediating Nitrogen (ammonium) contaminants.The treatment also improved the Physico-chemical characteristics of the wastewater such as colour, pH, TDS, BOD and COD values. The treated effluent from the system has met the wastewater effluent Standards, making it acceptable for recycling in flushing the urinals and water closets, then for gardening and other related purposes. Thus *N.officinale* could be recommended for Nitrogen bioremediation in domestic and industrial wastewater.

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