Asian Journal of Water, Environment and Pollution, Vol. 21, No. 6 (2024), pp. 31-37.
DOI 10.3233/AJW240070

Total Nitrogen, Nitrate and Ortho-Phosphate Elimination Effect in Common Reed and *Typha angustifolia* from Wastewater

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Received September 8, 2023; revised and accepted June 23, 2024

Abstract: The objective of this study is to evaluate the potential degree of *Common reed* (Pa) and *Typha angustifolia* (Ta) in the elimination of nitrate, total nitrogen and ortho-phosphate from wastewater using the sulfanilamide, Kjeldahl and ammonium molybdate methods, respectively. The results obtained show that aquatic plant species studied have a strong potential to reduce nitrate and total nitrogen charges from wastewater with rates of 62 and 61% respectively for the nitrate and 52% obtained with *Typha angustifolia* for the total nitrogen. For the orthophosphate, both plants always show a strong elimination effect with reduction rates of approximately 81 and 76%, respectively, compared to the positive control. In light of these results, treatment by plantation to eliminate nitrate, total nitrogen and orthophosphate represents a reliable and simple technology to operate. The research suggests that these particular plant species hold promise as a natural and cost-effective solution for reducing high nitrogen and phosphorus concentrations in polluted sites. Furthermore, their ability to remove these nutrients suggests potential for integration into wastewater treatment systems at wastewater treatment plants. This approach could improve treatment efficacy while reducing overall costs.

Key words: Common reed, *Typha angustifolia*, wastewater, pollution, phytoremediation.

Introduction

Water pollution is one of the most pressing environmental challenges facing many countries today. This contamination can stem from various sources, including households, industrial activities, agricultural practices, and improper waste disposal from humans and animals. A particular concern is the growing impact of animal waste on water quality. As human populations rise and the demand for meat increases, intensive livestock production intensifies. This trend leads to the generation of significant volumes of animal waste, often

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concentrated in confined spaces. This waste can be a significant source of organic pollutants and excessive nutrients, particularly nitrogen (N) and phosphorus (P), as highlighted in a study by Pincam and Jampeetong (2020).

Denitrification is the primary mechanism by which natural and constructed aquatic ecosystems permanently remove excess nitrogen. This process involves the anaerobic respiration of nitrate (NO_3^-) from the environment, ultimately converting it to di-nitrogen gas (N_2). Heterotrophic bacteria, both anaerobic and facultatively aerobic, residing in biofilms at the sediment-water interface and on submerged macrophytes, carry out this denitrification process (Soana et al., 2020).

Ortho-phosphates most often have an urban origin (components of detergents) or agricultural (leaching of fertilisers). They are present in water in several forms: phosphates, poly-phosphates, and organic phosphorus. The ortho-phosphate ion (PO_4^{3-}) is the most abundant form in water and comes mostly from animal waste and laundry products. It plays an important role in the respiration of living cells, and in the storage and transfer of energy. At moderate concentrations, phosphorus is not toxic to humans, animals or fish, but at high concentrations in water, it causes eutrophication, leading to excessive growth of algae and plants, which leads to the reduction of the oxygen content, sometimes to a lethal level for the aquatic environment.

The proliferation of certain algae can also lead to the production of toxins that are harmful to the health of aquatic organisms and, consequently, to human beings (Lawniczak-Malinska and Achtenberg, 2018; Moussa et al., 2005).

A large number of plants species and others have very interesting biological properties, which find their application in various fields, especially in ecosystem treatment (Belattar et al., 2012; Sellal et al., 2012, 2016).

Phytoremediation, a technology utilising constructed wetlands or natural water marshes, offers a natural solution for water pollution control and sustaining healthy wetlands (Sellal and Belattar, 2023; Sellal et al., 2024). These ecosystems, whether natural or man-made, can effectively purify wastewater. Their ability to degrade, absorb, or filter pollutants, along with their capacity to take up excess nutrients, makes them ideal for wastewater treatment. Consequently, constructed wetlands are gaining increasing popularity as a natural and sustainable wastewater treatment option (Belattar and Sellal, 2020; Sellal et al., 2019a, 2019b).

This study aimed to explore how de-nitrification and de-eutrophication increase NO_3^- and phosphorus (P) availabilities in aquatic vegetated (*P. australis* and *Typha angustifolia*) and unvegetated mediums.

Material and Methods

The Total Nitrogen, Nitrate and Ortho-Phosphoric Load Removing Effect of *Common reed* and *Typha angustifolia*

The young stems of the plants collected in January from the region of El Hammadia (Bordj Bou Arreridj, Algeria) are washed with tap water and then with distilled water to remove all debris and cultivate in a nutrient solution (KNOP) until growth. Among the growing plants, four similar in weight and biomass are selected and replanted separately in a tank containing the raw wastewater from the wastewater treatment plant (Soana et al., 2020). Then, temporal monitoring (at 0, 20 and 40 days) of the total nitrogen kjeldahl (TNK), nitrate and ortho-phosphtae for wastewater samples (three repetitions each time) taken separately from the planted tanks is carried out at National Sanitation Office laboratory (NSO). The results at day 0 are considered as controls.

Total Nitrogen

Total nitrogen includes all forms of nitrogen like nitrates, nitrites, ammoniacal nitrogen and nitrogen fixed to organic matter. The determination of the TNK is realised in two steps, in the first one; total nitrogenous organic matter is transformed to ammoniacal nitrogen by acid digestion. For the second step, the ammonium ions are measured by the method described by Mroczkowski and Stuczyñski (2006). About 20ml of sample or NH_4Cl as a standard (at different concentrations) and 2ml of complexing solution (sodium potassium tartrate 23%, sodium phenate 20% and nitroprusside of sodium 1%) are mixed. Then 0.5 ml of hypochlorite is immediately added to the reaction medium. Under the action of hypochlorite, two phenol rings fix one nitrogen atom to give indophenol blue having an absorption maximum at 625 nm.

Nitrate Ions (NO_3^-)

At 1 ml of the sample or KNO_3 solution (standard 0.5, 1, 2, 4, 6, 8, and 10 mg/L), 5ml of NaOH and 5ml of first reagent (Hydrazine 13g/L and $CuSO_4$ prepared previously at a ratio of 1:1 v/v) are added. After 1 hour of incubation in darkness, 40 ml of second reagent (sodium acetate, sulphanilic acid, ethylene di-amine tetra-acetic acid (EDTA), Naphthylamine and Acetone 1: 1: 1: 1: 0.5 ml) are added to the mixture which makes to obtain a light pink colour which becomes fuchsia pink after 15 minutes of incubation in the dark. Then the absorbance of the samples and the standards are read at 520 nm against a blank using a UV-visible spectrophotometer (UV/Vis spectrophotometer LAMDA 25, DR/2000 HACH) auto-analyser. The control is prepared in the same way, except that the sample is substituted by distilled water. The nitrate concentrations (mg/L) of the samples are obtained by projection on the calibration line (Mroczkowski and Stuczyñski, 2006).

Ortho-phosphate

Phosphate ions are measured using the ammonium molybdate method adapted from that reported by Hass et al. (2011) with modifications. The determination of total phosphorus is carried out in two steps. The first step is digestion in an acid medium which transforms all the phosphorus present into ortho-phosphate. In the second step, the ortho-phosphate ion reacts with the molybdate ion and the antimony ion to form a phospho-molybdate complex. The latter is reduced with ascorbic acid in an acid medium to cause the appearance of molybdenum blue, whose absorbance at 820 nm is proportional to the concentration of the ortho-phosphate ion.

To 5 ml of the water to be analysed, 3 ml of ammonium molybdate $(NH_4)_2 MoO_4$ 25% and 1 ml of ascorbic acid are added. The mixture is then incubated in a water bath at 80°C. For 10 min. After cooling, the reading is taken at a wavelength of 820 nm against distilled water.

Percentage Reduction

The percentage reduction of different removal effects is calculated according to the following formula:

$$\% \text{ Reduction activity} = [(Cc - Cs) / Cc] \times 100$$

Cc: Concentration of control.

Cs: Concentration of the sample.

Statistical Analysis

Results are presented as means ± SD in triplicate. Student's t test is used to compare the different samples (the three TNK, NO_3 or PO_4 repeats of different periods) to the control (concentration on day 0) for the photo-purifying test. $P \leq 0.01$ is considered significant, $P \leq 0.001$ is highly significant.

Results

Total Nitrogen

Determining the total Kjeldahl nitrogen is carried out in two stages, the first stage is devoted to digestion in an acid medium which transforms all the nitrogenous organic compounds into ammoniacal nitrogen.

In the second step, the ammonium ions react with salicylate, nitro-ferricyanide and sodium hypochlorite to form an ammoniacal salicylate complex in an alkaline medium, the absorbance of which at 625 nm is proportional to the nitrogen concentration ammoniacal.

The results obtained (Figure 1) for total nitrogen show a statistically insignificant difference (P>0.05) for waters planted with *Common reed* or *Typha angustifolia*.

Total nitrogen concentrations show a temporal decrease (P≤0.01) in water treated with Ta with a value of 40.23 mg/L ± 1.4, respectively, obtained after 40 days of planting.

On the other hand, water planted with *Common reed* showed an increase in concentration with an average of 105 ± 2 mg/L compared to the positive control with an average of 85 ± 2.82 mg/L (P≤0.001). The Ta thus presents a maximum reduction of about 52.66%.

Nitrate Ions (NO_3^-)

In natural waters, nitrate (NO_3^-) reigns supreme as the primary form of combined nitrogen. This dominance stems from its position as the final product of nitrogen oxidation. Nitrite (NO_2^-), readily oxidised to nitrate, is rarely found in significant concentrations due to this conversion. For the determination of nitrate and nitrite ions, nitrate is first reduced to nitrite in an alkaline medium via hydrazine sulphate. In the presence of

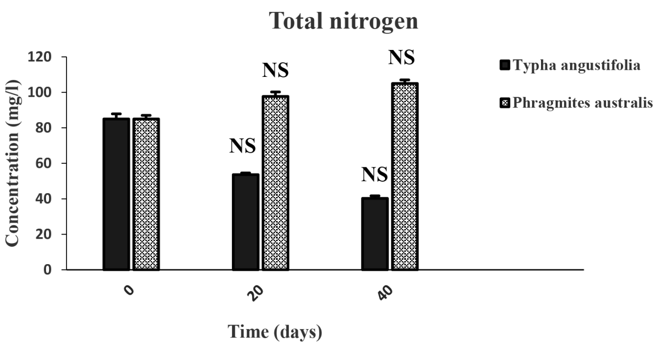

Figure 1: The ability of *Phragmites australis* and *Typha angustifolia* to reduce total nitrogen concentration compared to raw water on day zero. Results are presented as mean ± standard deviation (*n* = 3). Planted water vs positive control (raw water); NS represent a not significant difference (Student's *t* test).

copper sulphate which acts as a catalyst. Nitrite can be dosed without nitrate by eliminating the reduction step. Nitrite reacts with sulfanilamide to form a diazo compound which combines in an acid medium with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a pink to purple compound whose absorbance at 520 nm is proportional to the concentration nitrite and nitrate ions.

The results of the nitrate ions obtained (Figure 2) show a significant reduction with *Typha* and highly significant with Pa compared to the control ($P \leq 0.01$). The concentrations of nitrate ions obtained in the tanks containing the water treated by the two plants used and the untreated water are 0.507 ± 0.01 mg/L, 0.493 ± 0.02 mg/L and 1.31 ± 0.14 mg/L, respectively ($P \leq 0.001$). Thus, the concentrations of nitrate ions were reduced by about 61.27% and 62.36%, respectively.

Ortho-phosphate

Ammonium molybdate reacts in an acid medium in the presence of phosphate. It forms a phospho-molybdic complex reduced by ascorbic acid, which makes it possible to develop a blue color (molybdenum blue) proportional to the concentration of the ortho-phosphate ion capable of colorimetric assay at 820 nm.

The Pa and Ta plants present significant concentrations ($P \leq 0.01$) lower than that of the positive control (raw water) considered as 100% of the ortho-phosphate (maximum concentration).

Thus, at time 0, the concentration of the crude sample is 26 ± 3 mg/L, the latter decreases after 40 days to 4.8 ± 0.45 mg/L with Pa and to 6.16 ± 0, 37 mg/L with Ta (Figure 3) which corresponds to reduction percentages of approximately 81 and 76%, respectively, compared to the positive control.

Discussion

It is very important to understand the relationship between increased nitrate (NO_3^-) and total nitrogen loading and the corresponding de-nitrification effects of vegetation which is essential to design, restore and manage these wetlands to maximize their effectiveness against eutrophication. *Common reed* is frequently used to remediate nitrogen (N) pollution.

Our results are in agreement with those obtained by Soana et al. (2020). These are followed by the de-nitrification of sediments in two different environments (vegetated and non-vegetated). His results show that the de-nitrification induced by *P. australis* is considered optimal and is probably due to the assimilation of NO_3^- and the production of nitrogen and di-nitrogen gas (N_2).

The rise in nitrate (NO_3-N) concentrations likely stemmed from nitrification, an aerobic process where nitrifying bacteria convert ammonium (NH_4^+) into nitrite (NO_2^-) and ultimately nitrate. This process relies on oxygen, potentially supplied by plant roots. Plants also contribute to nitrogen removal by directly absorbing both ammonium and nitrate. Mass balance calculations suggest that plant tissues accounted for roughly 57% of the removed inorganic nitrogen (combined NH_4-N and NO_3-N), representing approximately 36% of the initial inorganic nitrogen load. Interestingly, plants grown on wastewater with higher nitrogen concentrations exhibited a tendency to accumulate more nitrogen in all tissues, resulting in a lower average carbon-to-nitrogen

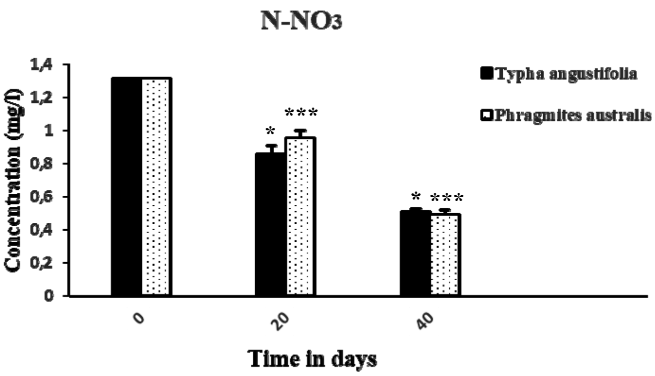

Figure 2: Concentrations of nitrate ions obtained with raw and treated water by *Typha angustifolia* and *Common reed*, respectively. Results are presented as mean ± standard deviation (*n* = 3). Planted water vs positive control (raw water); **P* ≤ 0.01 significant difference and ****P*≤0.001 highly significant difference (Student's *t* test).

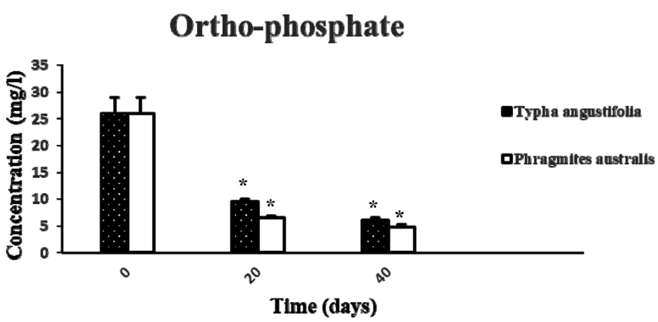

Figure 3: The capacity of *Typha angustifolia* and *Common reed* on the reduction of water ortho-phosphate concentration in comparison with raw waters (concentration in day zero). Results are presented as mean ± standard deviation (*n* = 3). Planted water vs positive control (raw water); **P* ≤ 0.01 significant difference (Student's *t*-test).

(C/N) ratio across all tissues (Daniel et al., 2009).

Constructed wetlands offer a multi-pronged approach to nitrogen removal from wastewater. Beyond plant uptake, microbial assimilation, ammonia volatilisation, denitrification, and adsorption all contribute to this process (Pincam and Jampeetong, 2020).

The increase in the concentration of nitrogen in the tanks planted by *P. australis* is explained by the effect of assimilation of N by aquatic plants which may contribute to the retention of N.

This retention of N by the plants is only temporary because during their senescence, the plants are decomposed and release the assimilated N into the environment.

As mentioned before, the results of Soana et al. (2020) show that *P australis* is able to consume NO_3^- and denitrify it into total nitrogen. The significant decrease in nitrate and nitrogen concentration with *Typha* is still explained by Soana et al. (2020), who showed that aquatic plants influence denitrification in several ways: either by modifying the conditions hydrological of the environment (dense vegetation reduces the speed of the current and increases the retention time of water in the ecosystem) or by influencing bio-geo-chemical factors such as the contributions between nitrogen and organic carbon of their immediate environment (water and sediment).

The production of organic matter during plant growth and senescence increases denitrification by providing a source of carbon needed by denitrifying bacteria. Additionally, by altering oxygen concentrations during photosynthesis, aquatic plants can stimulate or inhibit denitrification (Pincam and Jampeetong, 2020).

For a long time, aquatic plants such as *Common reed* species have been known for their purifying potential. The tests of Finlayson and Chick (1983) on the treatment of slaughterhouse effluents prove the strong capacity possessed by *Typha* and *Phragmites* in reducing the concentration of phosphorus with a rate of 68 and 79% respectively, which works with our results obtained.

Submerged macrophytes like common reed and *Typha angustifolia* act as water quality guardians in aquatic ecosystems. Their presence limits sediment resuspension, keeping the water clear. They compete with phytoplankton for nutrients, preventing excessive algal blooms that can harm water quality. Additionally, they improve the overall health of the ecosystem by promoting oxygen production, and organic matter decomposition, and providing a habitat for zooplankton and young fish. In lakes with a high abundance of these plants, phytoplankton blooms are less frequent, further slowing down the process of eutrophication, where nutrient enrichment leads to excessive algal growth and oxygen depletion (Lawniczak-Malińskaand Achtenberg, 2018).

The work of Pincam and Jampeetong (2020) shows that the concentration of dissolved oxygen in the effluent of the anaerobic digester is increased over time due to the oxygen released by the roots of *T. angustifolia*. Their research suggests that this particular plant can develop aerenchyma (air pockets) in its root cortex even under stressful conditions, such as those encountered in high-strength sewage. This unique adaptation allows the plant to tolerate harsh environments. Additionally, the plant's roots release oxygen, which benefits the growth of microorganisms and enhances biodegradation processes, contributing to a highly efficient wastewater treatment system. The study proposes two possible mechanisms for the decrease in ortho-phosphate concentration: precipitation with metal cations (like Fe, Al, and Mg) present in the wastewater, or absorption by microorganisms and the plant itself.

His results show a decrease in P of about 80% over 49 days, of which they referred this capacity to the accumulation potential possessed by aquatic plants such as *Typha angustifolia* and *Common reed*.

A study by Jakubaszek (2021) investigated reeds in constructed wetlands for their ability to remove pollutants from wastewater. They found that reed leaves were most effective at accumulating nitrogen (32.21 gN/kg dry weight) and phosphorus (1.54 gP/kg dry weight), with accumulation decreasing with depth and towards the treated water outflow. This suggests efficient nutrient removal throughout the system, further supported by the higher reed biomass and nitrogen content in the wetland's initial sections. These findings highlight the potential of reeds as natural filters in constructed wetlands for wastewater treatment.

Studies by Sellal et al. (2019a) and Sellal and Belattar (2024) show they enhance soil oxygenation, leading to a 74% reduction in Chemical Oxygen Demand (COD). This oxygen boost promotes the growth of beneficial aerobic bacteria, which effectively break down organic matter in the wastewater. Consequently, compared to unplanted systems, reeds facilitate better mineralization and oxidation of pollutants.

Research confirms that reeds (like common reed and *Typha angustifolia*) can store large quantities of various elements they absorb from the water. These elements are then transported and safely locked away within special compartments (vacuoles) inside the plant's above-ground parts. They are stored as complexes with various molecules, including fatty acids and organic acids (like propionate, butyrate, and citrate). Studies by Sellal et al. (2019a, 2019b, 2024) and Belattar and Sellal (2020) support this idea of complexation. They found that extracts from the reeds' fatty substances (hexane extracts) had a high capacity (56%) to bind (chelate) these elements.

Conclusion

In the present study, the aquatic plants common reed and *Typha angustifolia* are tested in the treatment and purification of wastewater particularly for the elimination or reduction of nitrates, total nitrogen and orthophosphate. These two species show a tolerance to polluted aquatic ecosystems as well as a very high efficiency in the elimination of the elements mentioned, after 40 days of contact only. The reduction rates obtained for nitrate with *Common reed* and *Typha angustifolia* are 62 and 61%, respectively, while the total nitrogen is reduced only with *Typha angustifolia* by 52%, finally, both plants also present a very acceptable potential towards orthophosphate with rates of 81 and 76%, respectively. According to these results obtained, the treatment of wastewater by planting *Common reed* and *Typha angustifolia* is significant, very effective and therefore represents a reliable technology, simple to operate. This research suggests that these particular plant species hold promise as a natural and cost-effective solution for reducing pollution levels in contaminated sites. Furthermore, their ability to remove pollutants from water makes them suitable candidates for integration into wastewater treatment systems at treatment plants, potentially leading to reduced treatment costs.

Acknowledgements

This work was carried out at the National Sanitation Office Laboratory (NSO), Relizane, Algeria.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication and dissemination of the information provided herein.

Author's contribution

All authors participated in conceived and designed research, Material preparation, data collection and analysis and in the redaction of the manuscript.

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