Original Research

Phytoremediation of Industrial Wastewater Containing Nitrates, Nitroglycerin, and Nitroglycol

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Abstract

Experiments focused on evaluating the ability of three aquatic plant species (sweet flag, common reed, and broadleaf cattail) to decontaminate industrial wastewater effluents from a nitroglycerin and nitroglycol production factory were carried out in the framework of this study. Toxicity of a nitrate-rich wastewater toward the studied plant species was determined by measurement of plant biomass gain, and subsequent mathematical modeling allowed for assessment of IC50 values. The studied plant species were cultivated in hydroponic cultures containing 1,500 mg/l of nitrates, 2.4 mg/l of nitroglycerin, and 1.0 mg/l of nitroglycol for 21 days, during which the decontamination efficiency of each nitrogen-based compound was analyzed. It was observed that the application of sweet flag and common reed contributed to a high nitrate removal efficiency (82 and 79%, accordingly). Sweet flag also was most efficient for decontamination of nitroglycerin (87%) and nitroglycol (42%), but the removal of the former compound was preferential.

Keywords: explosives, ethylene glycol dinitrate, nitroglycerine, phytoremediation

Introduction

In the 20th century the rapid development of industry and agriculture has resulted in frequent releases of xenobiotics into the environment. A perfect example includes nitrogen-based compounds, which are considered as common and arduous pollutants of anthropogenic origin. Some compounds, such as trinitrotoluene (TNT), have been a popular object of numerous studies [1], but more recently industrially-relevant nitrate esters such as nitroglycerin (1,2,3-trinitroxypropane, NG) or ethylene glycol dinitrate (EGDN) also have received much scientific attention [2, 3]. These substances are used as components for the production of explosives and are present as co-contaminants in nitrate-rich wastewater originating from manufacturing facilities. Due to repeated washing steps during the production process the wastewater from explosives production factories is generated in very large volumes (often above 240 m^3/day). Direct toxicity of nitroglycerin toward mammals ranges from 30 to 1,300 mg/l, while the LC50 value for aquatic organisms is between 1.5 and 3.0 mg/l [4]. This fact, in addition to considerable content of NG and EGDN, as well as a high concentration of nitrating mixture residues (nitric acid and sulfuric acid), makes them an exceptional environmental threat [5]. The reported concentration of NG in wastewaters originating from explosives production plants ranges from 180 mg/L at the Badger Army Ammunition Plant (Baraboo, WI, USA) to between 300

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and 600 mg/L at the Radford Army Ammunition Plant (Radford, VA, USA) [6]. Such wastewater requires pretreatment before discharge into wastewater treatment plants. Untreated post-production wastewater streams are frequently released into lagoons and may contaminate natural watercourses. Since nitrate esters are resistant to biodegradation and characterized by a slow decay rate, they tend to accumulate in the environment [7, 8]. Evaluation of the environmental pollution at military firing ranges in the United States and Canada has confirmed that NG is a major soil pollutant, with concentrations reaching up to 4,700 mg/kg [9]. Nitrate esters are capable of migrating over great distances (i.e. through groundwater), therefore they pose a threat not only in the primary contamination area but also to all ecological niches in the direct vicinity [10]. Due to this fact, the regulatory standards for nitroglycerin (2 μg/l for water supplies [11]) are frequently exceeded.

The treatment of wastewater containing nitrates and nitrate esters is a challenging task. The difficulty is mostly associated with the specific composition, which is determined by the type of nitrating mixture used during the production of explosives. This corresponds to a high concentration of nitrates (up to 7,500 mg N/l) and sulphates as well as a low pH value (usually between 1.0 and 1.5). Most of the conventional physicochemical methods of wastewater treatment are either severely limited in terms of efficiency or completely unsuitable for treating such industrial effluents. The high nitrate content excludes the use of ion exchange-based methods. The possible adsorption of nitroglycerin on membranes corresponds to a risk of microexplosions, which renders membrane-based methods inapplicable. Hence, only a limited number of physicochemical methods (such as microwave-assisted degradation [12] and reductive transformation by pyrite and magnetite [13]) may be applied. However these methods often involve high energy consumption or the need to employ specific reagents, which make the process economically inefficient.

An alternative and potentially more cost-effective way of waste treatment is the employment of biological methods. Most of the studies featuring biological treatment of nitrate-rich wastewater focused on aerobic biodegradation using bacteria [14-16], fungi [17], or under anaerobic conditions [18]. However, it should be pointed out that the efficiency of conventional biological methods (i.e. activated sludge-based treatment) may be significantly limited, since microbial inoculums are rarely capable of survival in raw industrial effluents. This is a common cause of failure when microorganisms with exceptional biodegradative potential under laboratory conditions are used for treatment of actual wastewater [19].

Another possible solution involves the use of phytoremediation. This method relies on the application of plants that exhibit high survivability in contaminated sites and the ability to uptake pollutants, which leads to subsequent decontamination. Phytoremediation has been successfully used to remove heavy metal ions and organic contaminants from soil, as well as excessive amounts of biogenic elements (nitrogen and phosphorous) from municipal wastewater. This method is considered worldwide as an eco-friendly approach to environmental cleanup [20, 21]. Several reports have shown that plants may be used for efficient removal of nitroglycerin and nitroglycol from soil [22, 23]. Recent studies on phytoremediation in a constructed wetland confirmed that wetland species may be successfully used for treatment of wastewater with a high concentration of nitroesters [24].

The aim of the presented studies focuses on evaluating the ability of three aquatic plant species (*Acorus calamus*, *Typha latifolia*, and *Phragmites communis*) to decontaminate nitrate-rich industrial wastewater originating from a nitroglycerin and nitroglycol production plant. The experiments involved survivability assessment by determination of EC50 values as well as analysis of nitrates, nitroglycerin, and nitroglycol decontamination efficiency for each plant species.

Materials and Methods

Characteristics of Wastes

The nitrate processing wastewater was collected from a Polish explosives production plant (2012). Wastes of many production departments are combined into one stream and flow into a drain collector. No information regarding its detailed composition was obtained. Because of their low pH (pH=1.5), they are neutralized to pH=7.0 by the addition of lime. The determined composition of treated wastes was: N 3,000 mg/l, 21 mg/l chlorides, 1,880 mg/l sulfates. Moreover, the wastes contained organic compounds: 4.8 mg/l of nitroglycerin, 1.9 mg/l of nitroglycol, and a number of other unknown organic compounds (COD ranged from 1,200 to 4,000 mg/l). This means that 720 kg/day of nitrates, 2 kg/day of nitroglycerin, and 0.5 kg/day nitroglycol may be released to the environment.

Hydroponic Cultures

The experiments were carried out using rhizomes of sweet flag (*Acorus calamus* L.), broadleaf cattail (*Typha latifolia*), and common reed (*Phragmites australis*) obtained from the commercial market. Plants were taken out of pots and the soil was carefully removed by washing them under running water several times. Afterward, the plants were transferred to hydroponic containers (0.75 l) with 300 ml of Hoagland's medium ((g/l): NH_3PO_4 0.15; KNO_3 0.61; $Ca(NO_3)_2 \times 4H_2O$ 0.94; $MgSO_4$ 0.24. The microelements (mg/l): H_3BO_3 2.85; $CuSO_4 \times 5H_2O$ 0.08; $ZnSO_4$ 0.22; $MnCl_2 \times 4H_2O$ 1.81; $Na_2MoO_4 \times 2H_2O$ 0.01; $FeSO_4$ 2.78; Na_2EDTA 3.72) and left to grow for 14 days at 26±1ºC, light intensity of 20,000 luxes, and a 16:8 hour photoperiod (lighting/darkness) until its leaves were about 20 cm long. The plants in this phase were used in all experiments.

Toxicity Studies

The pre-cultured sweet flag, broadleaf cattail, and common reed plants (leaves were about 20 cm long) were carefully rinsed with distilled water and weighed and then were grown (one plant per sample) for 21 days at 26±1ºC, light intensity of 20,000 luxes, and 16:8 hour photoperiod (lighting/darkness) in pots containing 10-65% of wastewater (v/v), which corresponds to the concentration of nitrates 325-1,925 mg N_{NO_3}/l. Upon finishing the experiments, the plants were collected, carefully rinsed with distilled water, and weighed. The obtained wet biomass values were compared to control samples. All experiments were carried out in triplicate. Based on the results, a mathematical model describing wastewater toxicity toward the studied plant species was built in the form of an equation:

$$Y=A + (B-A)/(1+10^{((LogIC50-X)*C)})$$

...where:
A – the value of the lower theoretical asymptote
B – the value of the upper theoretical asymptote
C – the value determining the slope

The employed mathematical model allowed for evaluating the concentration of wastewater (expressed as mg N_{NO_3}/l), which caused a 50% inhibition of plant biomass gain (IC50). The estimations of coefficient values in the equation were carried out using the quasi-Newton method (Statistica, StatSoft, Inc. USA).

Plant Cultures in the Presence of Nitrates, NG and EDGN

In order to assess the ability of the studied plants to decontaminate the nitrogen-based compounds present in the wastewater effluent, sweet flag, broadleaf cattail, and common reed were transferred to hydroponic containers (0.75 l) with 200 ml of Hoagland's medium and 200 ml of waste water. Then the plants were left to grow for 21 days at 26±1ºC, light intensity of 20,000 luxes, and a 16:8 hour photoperiod (lighting/darkness). A parallel set of samples was prepared as negative controls (without plants). Every three days samples (2 ml) were taken in order to determine the decontamination efficiency of nitrates, EGDN, and NG. All experiments were carried out in triplicate.

HPLC Analysis of Nitroglycerine and Nitroglycol

Determination of nitroglycerine and nitroglycol were carried out using a MERCK-HITACHI system consisting of an autosampler (model L-7250), pump (model L-7100), and DAD (model L-7455) set at 220 nm. Analyses were performed isocratically at a flow rate of 0.60 ml/min, at 30ºC on a Lichrospher® RP-18 column (5 µm, 205 × 4.6 mm, MERCK, Germany). Methanol, acetonitrile, and 0.01 M NaH_2PO_4 (pH = 4.5) were used as a mobile phase with isocratic flow rate at 1 ml·min^{-1}. The assay was performed at 36ºC. The sample volume was 50 µl. Samples were filtered prior to injection (0.22 µm, Millex-GS, Millipore). A standard was used as reference. This was accomplished via computer integration (Chromatography Data Station Software, MERCK-HITACHI) operated in the mode of external standard. All experiments were carried out in triplicate.

Determination of Nitrates

Determination of nitrates in the wastewater was conducted with the aid of spectrophotometry (λ=410 nm) via the reaction with sodium salicylate (Specord 40, Jena Analytic, Germany) as previously described by Cyplik et al. [25]. All experiments were carried out in triplicate.

Results

Toxicity Assessment

The first experimental step focused on assessing the toxicity of industrial wastewater toward the selected plant species. The application of a mathematical model for analysis of the obtained results allowed a wastewater dose that caused 50% inhibition of plant biomass gain (IC50) to be determined. The plants exhibited various resistances to the toxic substances present in the industrial effluent. The tolerance toward nitrate-based compounds, measured by shifts in plant biomass gain, was dependant on the plant species and decreased with increasing concentration of the wastewater. All the presented results are expressed as values relative to the control sample (plants cultivated without wastewater).

The highest tolerance was observed for sweet flag (Fig. 1) and common reed (Fig. 2). Both plant species exhibited biomass gain when cultured in pots containing up to 40% wastewater (corresponding nitrate concentration of 1,285 mg N_{NO_3}/l). For sweet flag, the maximum biomass gain value after 21 days of experiments reached 21% for samples containing wastewater at 965 mg N_{NO_3}/l.

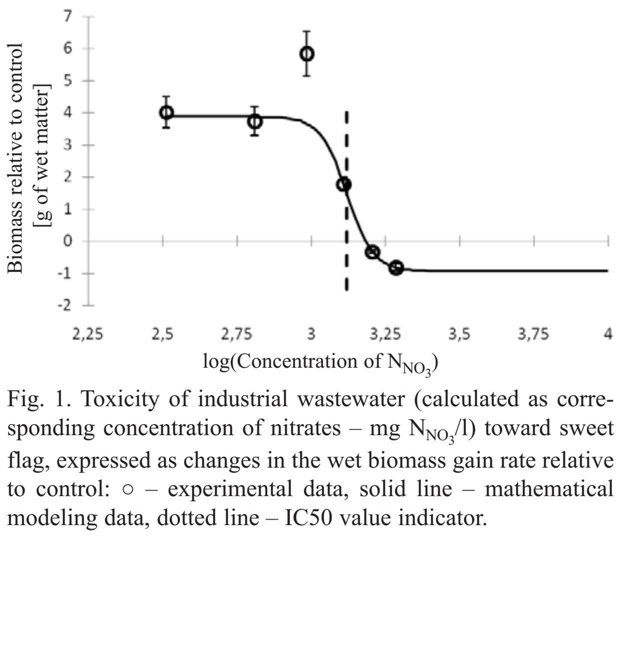

Fig. 1. Toxicity of industrial wastewater (calculated as corresponding concentration of nitrates – mg N_{NO_3}/l) toward sweet flag, expressed as changes in the wet biomass gain rate relative to control: ○ – experimental data, solid line – mathematical modeling data, dotted line – IC50 value indicator.

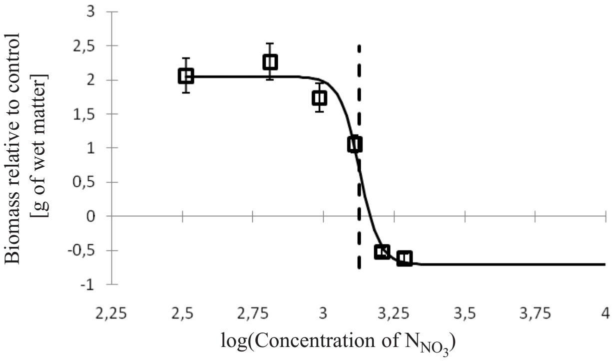

Fig. 2. Toxicity of industrial wastewater (calculated as corresponding concentration of nitrates – mg N_{NO_3}/l) toward common reed expressed as changes in the wet biomass gain rate relative to control: □ – experimental data, solid line – mathematical modeling data, dotted line – IC50 value indicator.

In the case of common reed, the maximum biomass gain observed after the same time period was similar (20%), but at a lower concentration of wastewater (325 mg N_{NO_3}/l). The IC50 value for sweet flag and common reed was at 1,318 and 1,309 mg N_{NO_3}/l, accordingly. The broadleaf cattail was characterized by a notably lower tolerance toward the presence of nitrogen-based compounds in the cultivation broth (Fig. 3). Although this plant species displayed the highest maximum biomass gain (29%) at the lowest concentration of wastewater (325 mg N_{NO_3}/l), an increase in the wastewater content led to a significant reduction of biomass gain rate (2% at 965 mg N_{NO_3}/l), and the determined IC50 value was lower (739 mg N_{NO_3}/l) compared to the other plant species.

After exceeding the IC50 value, rapidly progressing morphological changes could be observed for all the studied plant species. In the primary stage several necrotic changes appeared on the leaves and afterward the plants started to dry out, which resulted in a drastic decrease of their biomass.

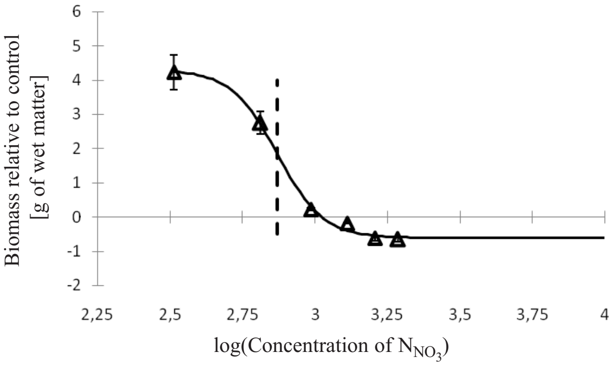

Fig. 3. Toxicity of industrial wastewater (calculated as corresponding concentration of nitrates – mg N_{NO_3}/l) toward broadleaf cattail expressed as changes in the wet biomass gain rate relative to control: Δ – experimental data, solid line – mathematical modeling data, dotted line – IC50 value indicator.

Removal of Nitrates

The second experimental step was carried out in order to evaluate the nitrate removal efficiency for each plant species used in the study. The uptake and removal rate was influenced by the resistance of the plants as well as the incubation time.

The most rapid removal of nitrates in the cultivation broth was observed for sweet flag and common reed, since the N_{NO_3} content in the cultivation broth was decreased over threefold (from 1.5 to 0.4 g/l) after nine days (Fig. 4). In the following days the nitrate removal rate was notably lower and at the end of the experiment (day 21) the final concentration of nitrates was at 0.27 g/l for sweet flag and 0.32 g/l for common reed, which corresponded to a decontamination efficiency of 82 and 79%, accordingly. In the case of broadleaf cattail the nitrate uptake and removal occurred at a notably slower rate throughout the whole incubation period. Ultimately, for samples cultivated in the presence of cattail species, approximately 65% of the initial nitrate load remained at the 21st day of the experiment. The parallel negative control showed that an insignificant decrease of the nitrate concentration (below 5%) occurred for samples without any plant species.

Removal of NG and EGDN

During the final experimental step, the ability of the selected plant species to remove nitroglycerin and nitroglycol from the industrial effluent was analyzed. The experiments revealed that the susceptibility of specific wastewater components to phytoremediation with the use of sweet flag, common reed, or broadleaf cattail may vary. In an effluent where both nitroglycerin and nitroglycol were present, the removal of NG was preferential (Fig. 5). In the case of sweet flag and common reed the concentration of nitroglycerin was reduced by 50% after the 3rd day. At the end of the experiment (day 21), decontamination efficiency reached 87 and 84% for sweet flag and common reed, accordingly. During the experiments with broadleaf cattail the NG removal rate was slower, reaching 52% decontamination efficiency after 21 days of incubation.

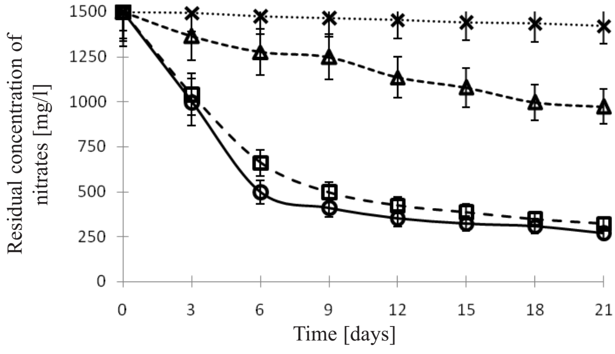

Fig. 4. Shifts in the concentration of nitrates during wastewater decontamination studies with the use of studied plant species: ○ – sweet flag, □ – common reed, Δ – broadleaf cattail, x – control.

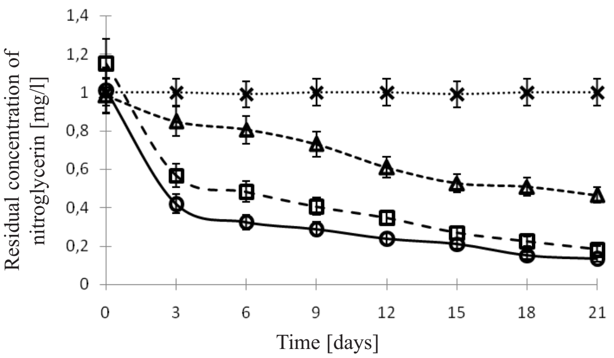

Fig. 5. Shifts in the concentration of nitroglycerin during wastewater decontamination studies with the use of studied plant species: ○ – sweet flag, □ – common reed, Δ – broadleaf cattail, x – control.

In contrast to nitroglycerin, the removal of nitroglycol from the hydroponic cultures occurred at a notably lower rate for all the studied plant species. Similarly to the experiments with NG, the highest decontamination efficiency was observed for cultures with sweet flag or common reed, while a lower efficiency was noted for broadleaf cattail. However, the differences were not as notable as before. Ultimately, on the 21st day of the experiment the EGDN decontamination efficiency was at approximately 42% for sweet flag and common reed and at 29% for broadleaf cattail (Fig. 6). No changes in the concentration of either NG or EGDN were observed for negative controls after 21 days.

Discussion

Aquatic plant species, macrophytes and hydrophytes are commonly used to remove pollutants in wastewater or for remediation of the environment. For such purposes, the application of free-floating plants such as water hyacinth (*Eichhornia crapssipes*), duckweed (*Lemna* sp.), and other species from the *Araliaceae* family, or emergent plants such as cattails (*Typha* sp.), common reed (*Phragmites australis*), and others, is a frequent practice [26, 27].

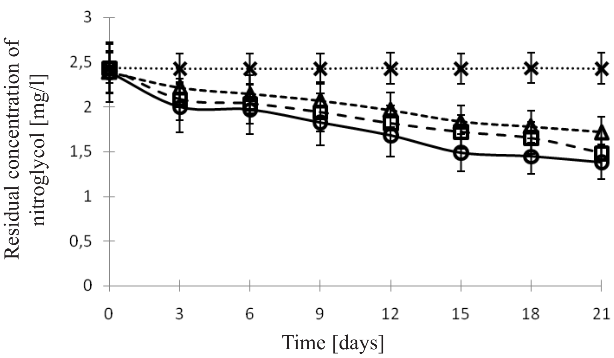

Fig. 6. Shifts in the concentration of nitroglycol during wastewater decontamination studies with the use of studied plant species: ○ – sweet flag, □ – common reed, Δ – broadleaf cattail, x – control.

These plants are among species that exhibit rapid biomass increase rate and the ability to utilize nutrients present in wastewater. These traits may be helpful during removal of nitrogen-based compounds [28-30].

Phytoremediation of an environment contaminated with compounds exhibiting explosive properties is a challenging task due to considerable toxicity of such pollutants and a limited number of plants that are capable of their removal. Out of the three main groups of explosive materials – nitroaromatics, nitroamines, and nitrate esters – most studies including the use of plants as a treatment method were focused on the former two. For example, numerous studies involving phytoremediation were conducted for areas contaminated with hexahydro-1,3,5-trinitro-1,3,5-triazine or trinitrotoluene [31-37].

The number of studies dedicated to removal of NG or EGDN by employing plants is much more limited. The ability of *Beta vulgaris* plant species to uptake and metabolize nitroglycerin was reported by Goel et al. [38]. This plant species was capable of growth in a broth containing 2 mM of NG as well as the transformation of this compound into glycerol dinitrate (GDN) and further into glycerol mononitrate (GMN). The ability to transform NG at an initial concentration of 10 mg/l by hydroponic cultures of *Cyperus escalantus*, *Juncus effuses,* and *Setaria glacula* also was studied by Rifler and Medina [10]. Experiments involving the toxicity of NG and EGDN as well as their removal by flax (*Linum usitatissimum*), rush (*Juncus inflexus*), reed (*Phragmites australis*), and mustard (*Sinapis alba*) were carried out by Podlipna et al. [7]. The Authors established that flax was able to uptake and transform NG into 1,2-dinitroglycerine and 1,3-dinitroglycerine (1,2-DNG and 1,3-DNG). After 24 days of experiments with initial concentration of nitroglycerin at 300 mg/l, only 40% of its degradation products remained. Toxicity assays for NG using 3-day seedling of *Sinapis alba* revealed that the inhibition of root growth occurred at 50 mg/l, while at 200 mg/l the root length was reduced by 80% compared to control samples. Tests carried out with *Juncus inflexus* and *Phragmites australis* also confirmed the toxicity of EGDN. The plants turned brown and withered after just 3 days of exposure to EGDN at a concentration of 500 mg/l. The same authors used *in vitro* regenerants of *Juncus inflexus* and *Phragmites australis* for removal of NG and EGDN [24]. During 20 days *Juncus inflexus* was capable of completely removing NG and EGDN at initial concentrations of 600 and 100 mg/l, accordingly. The use of *Phragmites australis* allowed for the decontamination of an industrial effluent from an explosives production factory. Upon dilution the wastewater contained approx. 190 mg/l of EGDN, 60 mg/l of NG, and 10 mg/l of both DNG and MNG. Over the course of 40 days the removal of NG was very rapid, followed by subsequent formation of DNG and its further transformation, which ultimately resulted in the accumulation of MNG. The concentration of EGDN was reduced to approximately 5% of the initial concentration.

Although in our studies the concentrations of NG and EGDN were notably lower compared to those used by Podlipna et al. [24], the apparent differences in removal rates for both compounds may be explained by the high content of the nitrating mixture residues present in the wastewater that was employed. Based on the obtained results, it may be concluded that removal of nitrates and NG is preferential compared to EGDN, as confirmed by the relatively low decontamination efficiency of this compound in the industrial wastewater effluent sample. Our studies suggest that sweet flag may be as effective for removal of nitrate esters as common reed.

Studies focused on the removal of nitrogen from a synthetic medium with the use of water hyacinth, water lettuce, and salvinia were carried out by Ayyasamy et al. [27]. The Authors observed that the application of water hyacinth enabled the reduction of the initial nitrogen concentration by 64-83% after a 10-day period. Increasing the initial concentration from 100 to 300 mg/l resulted in increased removal rate, although after exceeding 500 mg/l the decontamination efficiency of the studied plants was notably limited. On the other hand, such a high concentration of nitrogen resulted in a considerable biomass gain value (37%) after 10 days of the experiment. During the same time period the biomass gain for water lettuce and salvinia was at 24 and 15%, accordingly. The end-treatment of wastewater from a municipal treatment plant by means of phytoremediation was reported by Kim [39]. By employing a constructed wetland system (area of 5,500 m^2) based on the activity of *Phragmites australis,* a high removal efficiency (approximately 70%) was achieved for both nitrogen and phosphorous, with a retention time equal to only 2.5 days. In comparison, the results obtained in the framework of this study showed that the use of sweet flag may contribute to an enhanced nitrate decontamination rate, as a considerably higher initial nitrate concentration (1,500 mg N_{NO_3}/l) was reduced by 73% after 10 days.

Although the possible detoxification mechanisms were not determined in the scope of this study, it is plausible that the processes were carried out in a similar manner to that described by Podlipna et al. [24]. According to the established pathway, the phytoremediation of nitrate esters is mainly focused on progressive denitrification. Nitroglycerin is transformed into two possible dinitroglycerin homologues (either 1,2- or 1,3-), with subsequent degradation to mononitroglycerin and finally to glycerol. Upon denitrification nitroglycol is transformed into mononitroglycol and then degraded into ethylene glycol. Both denitrification products can then be incorporated into biological cycles of plants and further altered to obtain plant hormones or other biologically relevant compounds.

Several advantages of phytoremediation may be elucidated by a comparison of results with those obtained in our previous study, where activated sludge microorganisms and a microbial consortium DNC2 were used for biological denitrification of industrial nitrate-rich wastewater [5]. For example, the addition of an external carbon source is not necessary for phytoremediation, whereas an addition of glycerol was necessary to achieve complete removal of nitrates with the use of microorganisms. Additionally, the nitrate removal rate for microorganisms was strongly influenced by aeration conditions. The best performance occurred under strictly anaerobic conditions (complete removal of 3 g/l after 6 days); however, under aerobic conditions no removal was observed. The use of plants allows for satisfactory nitrate removal under aerobic conditions, without the necessity to provide anaerobic conditions. It is also worth noting that the phytoremediation-based methods are usually more economically efficient, since biodegradation carried out in bioreactors under strictly specified conditions corresponds to energy usage and maintenance costs. On the other hand, the use of microorganisms enables better removal of both nitroglycerin and nitroglycol, which suggests that a combined phytoremediation/biodegradation approach may potentially be most appropriate for efficient treatment of industrial effluents. Taking this into consideration, future studies will be focused on the combined application of aquatic plant species and microbial consortia that exhibit a high biodegradation potential in order to enhance the decontamination efficiency *via* potential rhizosphere interactions.

Conclusions

The obtained results suggest that the studied plant species may be successfully applied for treatment of industrial wastewater with a high concentration of N_{NO_3}, nitroglycerin, and nitroglycol. Each of the studied plant species displayed an ability to decontaminate nitrogen-based compounds, but the highest removal efficiency was observed for sweet flag (*Acorus calamus*). Common reed (*Phragmites communis*) exhibited both a similar removal rate as well as a high tolerance toward the presence of nitrogen-based compounds. The performance of broadleaf cattail (*Typha latifolia*) was notably lower, as this species was more susceptible to the toxic effect. Upon adequate dilution the industrial effluents generated in explosives production plants may be efficiently treated with the use of phytoremediation, either as a single, independent treatment process or as an end-point clean-up stage in a hybrid treatment method. This method makes for an economically justified step that allows for enhanced treatment efficiency. Additionally, due to its simplicity, phytoremediation may be easily integrated into the overall treatment process in wastewater treatment plants. Depending on the available space, the employment of constructed wetlands with the use of the studied plants may be an excellent strategy, enabling optimization of the flux ratio in order to achieve maximum detoxification efficiency.

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References

1. HANNINK N.K., ROSSER S.J. BRUCE NC. Phytoremediation of Explosives. Crit. Rev. Plant Sci. **21**, 511, **2002**.
2. HALASZ A., THIBOUTOT S., AMPLEMAN G., HAWARI J. Microwave-assisted hydrolysis of nitroglycerin (NG) under mild alkaline conditions: New insight into the degradation pathway. Chemosphere, **79**, 228, **2010**.
3. ROCHELEAU S., KUPERMAN R.G., DODARD S.G., SARRAZIN M., SAVARD K., PAQUET L., HAWARI J., CHECKAI R.T., THIBOUTOT S., AMPLEMAN G., SUNAHARA G.I. PHYTOTOXICITY and uptake of nitroglycerin in a natural sandy loam soil. Sci. Total Environ. **409**, 5284, **2011**.
4. WENDT T.M., CORNELL J.H., KAPLAN A.M. Microbial degradation of glycerol nitrates. Appl. Environ. Microbiol. **36**, 693, **1978**.
5. CYPLIK P., MARECIK R., PIOTROWSKA CYPLIK A., OLEJNIK A., DROŻDŻYŃSKA A., CHRZANOWSKI Ł. Biological denitrification of high nitrate processing wastewater from explosives production plant. Water Air Soil Poll. **223**, 1791, **2012**.
6. OH S.-Y., CHA D.K., KIM B.J., CHU P.C. Reduction of Nitroglycerin with Elemental Iron: Pathway, Kinetics, and Mechanisms. Environ. Sci. Technol. **38**, 3723, **2004**.
7. PODLIPNA R., FIALOVA Z., VANEK T. Toxic effect of nitroesters on plant tissue cultures. Plant. Cell. Tiss. Org. **94**, 305, **2008**.
8. CLAUSEN J., ROBB J., CURRY D., KORTE N. A case study of contaminants on military ranges: Camp Edwards, Massachusetts, USA. Environ. Pollut. **129**, 13, **2004**.
9. JENKINS T.F., HEWITT A.D., GRANT C.L., THIBOUTOT S., AMPLEMAN G., WALSH M.E., RANNEY T.A., RAMSEY C.A., PALAZZO A.J., PENNINGTON J.C. Identity and distribution of residues of energetic compounds at army live-fire training ranges. Chemosphere **63**, 1280, **2006**.
10. RIFLER R.G., MEDINA V.F. Phytotreatment of propellant contamination. Chemosphere. **63**, 1054, **2006**.
11. US EPA. Standards and Priority Pollutants Designated Uses Criteria or Toxic Concentrations. http://water.epa.gov/scitech/swguidance/standards/wqslibrary/upload/2009_5_19_standards_wqslibrary_nc_nc_numeric.pdf
12. HALASZ A., THIBOUTOT S., AMPLEMAN G., HAWARI J. Microwave-assisted hydrolysis of nitroglycerin (NG) under mild alkaline conditions: New insight into the degradation pathway. Chemosphere **79**, 228, **2010**.
13. OH S.Y., CHIU P.C., CHA D.K. Reductive transformation of 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and nitroglycerin by pyrite and magnetite. J Hazard. Mater. **158**, 652, **2008**.
14. BHAUMIK S., CHRISTODOULATOS C., KORFIATIS G.P., BRODMAN B.W. Aerobic and anaerobic biodegradation of nitroglycerin in batch and packed bed bioreactors. Water Sci. Technol. **36**, 139, **1997**.
15. YE J., SINGH A., WARD O.P. Biodegradation of nitroaromatics and other nitrogen containing xenobiotics. World J Microbiol. Biotechnol. **20**, 117, **2004**.
16. DARIO A., SCHROEDER M., NYANHONGO G. S., ENGLMAYER G., GUEBITZ G.M. Development of a biodegradable ethylene glycol dinitrate-based explosive. J Hazard. Mater. **176**, 125, **2010**.
17. SUNDARAM S. T., ZHANG Y. Z., SHARMA A., BRODMAN B.W. Screening for the involvement of the hydroxyl radical in the biodegradation of glyceryl trinitrate by *Penicillium corylophilium* Dierckz. Waste Manag. **17**, 437, **1997**.
18. CHRISTODOULATOS C., BHAUMIK S., BRODMAN B.W. Anaerobic biodegradation of nitroglycerin. Water Res. **31**, 1462, **1997**.
19. THOMPSON I.P., VAN DER GAST C.J., CIRIC L., SINGER A.C. Bioaugmentation for bioremediation: the challenge of strain selection. Environ. Microbiol. **7**, 909, **2005**.
20. RYLOTT E.L., BRUCE N.C. Plant disarm soil: engineering plants for the phytoremediation of explosives. Trends Biotechnol. **27**, 73, **2008**.
21. PENG J., SONG Y., YUAN P., CUI P., QUI G. The remediation of heavy metals contaminated sediment, J. Hazard. Mater. **161**, 633, **2009**.
22. MEAGHER R.B. Phytoremediation of toxic elemental and organic pollutants. Curr. Opin. Plant Biol. **3**, 153, **2000**.
23. EAPEN S., SINGH S., D'SOUZA S.F. Advances in development of transgenic plants for remediation of xenobiotic pollutants. Biotechnol. Adv. **25**, 442, **2007**.
24. PODLIPNA R., FIALOVA Z., VANEK T. Degradation of nitroesters by plant tissue cultures. J Hazard. Mater. **184**, 591, **2010**.
25. CYPLIK P., SCHMIDT M., SZULC A., MARECIK R., LISIECKI P., HEIPIEPER H.J., OWSIANIAK M., VAINSHTEIN M., CHRZANOWSKI Ł. Relative-quantitative PCR to assess bacterial community dynamics during degradation of diesel and biodiesel fuels under various aeration conditions. Biores. Technol. **102**, 4347, **2011**.
26. KARPISCAK M.M., FOSTER K.E., HOPF S.B., BANCROFT J.M., WARSHALL P.J. Using water hyacinth to treat municipal wastewater in the desert southwest. J Am. Water Works Ass. **30**, 219, **1994**.
27. AYYASAMY P.M., RAJAKUMAR S., SATHISHKUMAR M., SWAMINATHAN K., SHANTHI K., LEE S., LAKSHMANAPERUMALSAMY P. Nitrate removal from synthetic medium and groundwater with aquatic macrophytes. Desalination. **242**, 286, **2009**.
28. GERSBERG R.M., ELKINS B.V., LYON S.R., GOLDMAN C.R. Role of aquatic plants in wastewater treatment by artificial wetlands. Water Res. **20**, 363, **2986**.
29. SOOKNAH R.D., WILKIE A.C. Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. Ecol. Eng. **22**, 27, **2004**.
30. HUETT D.O., MORRIS S.G., SMITH G., HUN N. Nitrogen and phosphorus removal from plant nursery runoff in vegetated and unvegetated subsurface flow wetlands. Water Res. **39**, 3259, **2005**.
31. LEWIS T.A., NEWCOMBE D.A., CRAWFORD R.L. Bioremediation of soils contaminated with explosives. J. Environ. Manage. **70**, 291, **2004**.
32. MAKRIS K.C., SHAKYA K.M., DATTA R., SARKAR D., PACHANOOR D. High uptake of 2,4,6 trinitrotoluene by vetiver grass – potential for phytoremediation? Environ. Pollut. **146**, 1, **2007**.
33. LEE I., BAEK K., KIM H., KIM S., KIM J., KWON Y., CHANG Y., BAE B. Phytoremediation of soil co-contaminated with heavy metals and TNT using four plant species. J. Environ. Sci. Health Part A., **42**, 2039, **2007**.
34. RYLOTT E.L., LORENZ A., BRUCE N.C. Biodegradation and biotransformation of explosives. Curr. Opin. Biotech. **22**, 434, **2011**.

35. BEST E.P., KVESITADZE G.K., KHATISAHVILI G., SADUNISHVILI T. Plant processes important for the transformation and degradation of explosives contaminants. Z. Naturforsch. C. **60**, 340, **2005**.
36. ROSSER S.J., FRENCH C. E., BRUCE N.C. Engineering plants for the phytodetoxification of explosives. In Vitro Cell Dev.-Pl. **37**, 330, **2011**.
37. HANNIK N., ROSSER S.J., FRENCH C.E., BASRAN A., MURRAY J.A.H., NICKLIN S., BRUCE M.C. Phytodetoxification of TNT by transgenic plants expressing a bacterial nitroreductase.. Nat. Biotechnol. **19**, 1168, **2001**.
38. GOEL A., KUMAR G., PAYNE G.F., DUBE S.K. Plant cell biodegradation of a xenobiotic nitrate ester, nitroglycerin. Nat. Biotechnol. **15**, 174, **1997**.
39. KIM D.G., PARK J., LEE D. KANG H. Removal of nitrogen and phosphorus from effluent of a secondary wastewater treatment plant using a pond-marsh wetland system. Water Air Soil Poll. **214**, 37, **2012**.