Effect of nickel on nutrient removal ability of selected indigenous protozoan species in wastewater systems

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Abstract This study compared the effect of Ni²⁺ on nutrient-removal efficiency of four indigenous wastewater protozoan species. Isolates could simultaneously remove phosphate (66.499.36%), nitrate (56.19-99.88%) and Ni²⁺ (45.98-85.69%) with Peranema sp. having the highest removal of nutrients (Phosphate-99.36% and Nitrate-99.88%) and Paramecium sp. the highest Ni²⁺-removal: 85.69%. Increase in Ni²⁺ concentration had a significant effect on nutrient-removal efficiency of these indigenous isolates. Although Ni²⁺ appeared to be toxic to isolates, its effect at low concentration (10 mg-Ni²⁺/L) towards test isolates can be used to enhance the wastewater treatment process for nutrient-removals. Peranema sp. is candidate in bioremediation of wastewater systems.

Keywords wastewater, metal, nickel, bioremediation, protozoa, phosphate, nitrate, phosphate, pollution

Introduction
Human activities such as industrialisation, mining operations and urbanisation have been reported to negatively impact not only on the use of available water resources, but also on aquatic life (Kamika and Momba 2011). Metals are among these pollutants discharged in water resources. They have been reported as being capable of affecting the removal of other pollutants such as nitrate and phosphate during the biological treatment of wastewater (Ochoa-Herrera et al. 2011). However, little has been known regarding the protozoan ability to simultaneously remove/take up nitrate and phosphate in wastewater mixed liquor under metal stress. This study assessed the effect of Ni²⁺ on the simultaneous uptake of nitrate and phosphate by four protozoan isolates (Aspidisca sp., Peranema sp., Paramecium sp. and Trachelophyllum sp.).

Materials and Methods

Test organisms
The indigenous protozoan species used in this study included Aspidisca sp., Paramecium sp., Trachelophyllum sp. and Peranema sp. These protozoan species were isolated from wastewater mixed liquors collected from the aeration tanks of the Daspoort Wastewater Treatment Plant (Pretoria, South Africa). They have demonstrated the ability to successfully remove nitrate and phosphorus in modified mixed liquor media (Akpor et al. 2008) and also to tolerate V⁵⁺ and Ni²⁺ separately (Kamika and Momba 2011). The preparation of these protozoan species was carried out according to Akpor et al. (2008).

Sample collection and preparation of the culture medium
Wastewater samples (5 L) were collected on a monthly basis between November 2011 and May 2012 from the anaerobic and aerobic zone of the Northern Wastewater Treatment Works in Johannesburg. Modified mixed liquor (culture medium) from the collected wastewater samples was prepared as reported by Kamika and Momba (2011) and screened in terms of chemical oxygen demand (COD), dissolved oxygen (DO), pH, phosphate, nitrate and metal...
presence (Ni²⁺). Nickel nitrate [Ni(NO₃)₂] was used as a source of Ni²⁺ ions. The stock solution of Ni²⁺ at a concentration of 1000 mg/L was prepared using deionised water. A 200 mL wastewater mixed liquor medium was prepared with Ni²⁺ at a concentration of 10 to 50 mg/L Ni²⁺ (increased at a geometric scale of 10 mg/L). Nickel concentration was confirmed using ICP-OES. To maintain the nutrients in solution, the pH of the modified wastewater mixed liquor was maintained as acidic (6.5 ± 0.3) by adding 1.0 M HCl and 1.0 M NaOH (Merck, SA). The ICP-OES was used to confirm the Ni²⁺ concentrations in the wastewater mixed liquor media, while nitrate and phosphate concentration were confirmed using the standard methods (APHA 2001).

Determination of Ni²⁺ effects on nutrient removal
The experiments were conducted in 250 mL Erlenmeyer flasks containing 200 mL of the modified mixed liquor. The flasks were aseptically inoculated with a fresh culture of protozoan (= 100 Cells/mL) isolates separately. For each microbial isolate, positive and negative controls were used in this experimental study. The positive control flask contained the mixed liquor without Ni²⁺ and the negative control had the mixed liquor with 50 mg/L Ni²⁺. The negative control was used to assess any external contamination in the samples during the experimental study. To check the effect of Ni²⁺ on the ability of microbial isolates to remove nutrient, the protozoan isolates were separately inoculated in the culture media containing the mixture Ni²⁺. All the inoculated samples as well as the controls were incubated at 30 °C ± 2 °C for four days. After each 24 h period, samples were homogeneously shaken, an aliquot of 30 mL was taken, and analyses for growth/die-off of the microbial isolates, pH, COD and DO, phosphate, nitrate concentrations were performed. The Ni²⁺ median lethal concentration (LC₅₀) was estimated according to the inhibition concentration approach. The first-order die-off rate of microbial species was calculated using the formula reported by Peng et al. (2008), whereas the growth rates were calculated according to by Kamika and Momba (2011). The data were statistically analysed using the Stata computer software.

Effect of Ni²⁺ on nitrate and phosphate removal in wastewater
Fig. 1 illustrates the percentage removal of nitrate by specific test organisms in the modified wastewater mixed liquor containing various concentrations of Ni²⁺. In general, the nitrate uptake was observed throughout the experimental study in both the positive control and the inoculated mixed liquor media. While a gradual increase in the nitrate uptake was observed over the incubation period in each of the culture media, an increase in the Ni²⁺ concentration resulted in a decrease in the rate of nitrate removal by protozoan isolates. The nitrate removal in the modified wastewater mixed liquor without Ni²⁺ (Positive controls) ranged from 42.12 to 96.98 % for Peranema sp., 35.03 to 87.36 % for Paramecium sp., 21.44 to 97.81 % for Trachelophyllum sp. and from 17.32 to 96.91 % for Aspidisca sp. However, in the mixed liquor media containing Ni²⁺, the ranges were as follows: 0.85 to 99.88 % for Peranema sp., 0.02 to 83.92 % for Paramecium sp., 0.03 to 68.84 % for Trachelophyllum sp. and 0.02 to 56.19 % for Aspidisca sp. Overall, significant differences were noted between the removal efficiency of protozoan isolates inoculated in the controls and those inoculated in the mixed liquor with Ni²⁺ (10 to 50 mg/L). The removal efficiencies of the isolates were significantly higher in the controls than in the mixed liquor with Ni²⁺. Even though the highest nitrate removal efficiency of all the isolates was significantly noted during day 4 of exposure (p < 0.05), Peranema sp. was found to have the highest nitrate uptake and Aspidisca sp. the lowest nitrate uptake in the mixed liquor with Ni²⁺. Even though the highest nitrate removal efficiency of all the isolates occurred in the mixed liquor containing 10 mg-Ni²⁺/L, this nickel concentration appeared to significantly
enhance the ability of *Peranema* sp. to uptake more nitrate compared to other protozoan isolates. Furthermore, *Peranema* sp. was the only protozoan isolate able to remove more than 30% of nitrate concentration in the modified wastewater mixed liquor containing 30 mg-Ni²⁺/L. While the nitrate uptake abilities of other protozoans decreased considerably in the nickel mixed liquor containing up to 50 mg-Ni²⁺/L, the removal efficiency of *Peranema* was still palpable (fig. 1).

Similar to the nitrate uptake, the phosphate uptake was observed throughout the experimental study in both the positive control and the mixed liquor media treated with Ni²⁺ (fig. 2) with *Peranema* sp. having the highest phosphate-removal efficiency (6.91 to 99.36%) and *Aspidisca* sp. the lowest (0.27 to 66.40%). However, statistical evidence revealed no significant difference (*p* > 0.05) between the removal efficiency of the isolates. In addition, the phosphate-removal efficiency of all test isolates appeared to be higher in the mixed liquor media treated with 10 mg-Ni²⁺/L. When comparing the uptake of nitrate and phosphate by test isolates, Ni²⁺ toxicity appear to affect the nitrate uptake more than the phosphate uptake.

**Determination of 24 h-LC₅₀ of Ni²⁺ to and growth/die-off rate of test organisms**

Fig. 3 illustrates the effect of Ni²⁺ on the growth responses of each test isolate in the modified wastewater mixed liquor. A general decrease in growth response was observed with an increase of Ni²⁺ concentration throughout the experimental study. Over the period of exposure, the growth response in the mixed liquor not treated with Ni²⁺ ranged from 2 to 5 log₁₀ Cells/mL for *Peranema* sp., 2 to 6 log₁₀ Cells/mL for *Paramecium* sp., 2 to 5 log₁₀ Cells/mL for *Trachelophyllum* sp. and 2 to 6 log₁₀ Cells/mL for *Aspidisca* sp. However, in the mixed liquor treated with Ni²⁺, the growth response of protozoan isolates ranged from 2 to 4 log₁₀ Cells/mL for *Peranema* sp., 2 to 5 log₁₀ Cells/mL for *Paramecium* sp., 2 to 4 log₁₀ Cells/mL for *Trachelophyllum* sp. and 2 to 3 log₁₀ Cells/mL for *Aspidisca* sp. with an increase in growth rate ranging from 0.02 to 2.03 d⁻¹, 0.27 to 4.04 d⁻¹, 0.05 to 1.31 d⁻¹ and 0.04 to 0.50 d⁻¹, respectively. All protozoan isolates appeared to exhibit a low growth rate with *Paramecium* sp. (4.04 d⁻¹) being the isolate with the highest growth of all the protozoan isolates. *Peranema* sp. was the only isolate that showed the growth rate as being...
higher than its respective positive control (1.98 d⁻¹) in culture media containing 10 Ni²⁺.

Furthermore, protozoan isolates exposed to Ni²⁺ appeared to be sensitive when comparing their growth to that of respective positive controls (fig. 3). After one day of incubation, a significant percentage die-off rate of 46.41 % was revealed in the modified wastewater mixed liquor inoculated with Peranema sp. (24 h-LC₅₀: 50 mg-Ni²⁺/L), while Paramecium sp., Trachelophyllum sp. and Aspidisca sp showed die-off rate of approximately 38.12 % (24 h-LC₅₀ between 40–50 mg-Ni²⁺/L), 62.62 % (24 h-LC₅₀ between 30–40 mg-Ni²⁺/L) and 62.94 % (24 h-LC₅₀ between 20–30 mg-Ni²⁺/L), respectively.

As evident in Table 1, all test isolates were able to remove Ni²⁺ from the modified wastewater mixed liquor. Whereas, a general decrease of Ni²⁺ removal was observed throughout the experimental study in the mixed liquor. Similarly to the removal of nutrients, the Ni²⁺ removal ability of isolates appeared to decrease with an increase in the Ni²⁺ concen-
Paramecium sp. (85.69%) was the protozoan isolates with the highest percentage removal of Ni²⁺ in the culture media, followed by Peranema sp. (84.02%). However, Aspidisca sp. could not remove more than 50% of the Ni²⁺, with the highest (45.98%) removal rate being from the wastewater mixed liquor containing 10 mg-Ni²⁺/L.

For COD variation (Table 2), a general increase in COD concentrations was observed in the mixed liquor without Ni²⁺ (positive control) and the mixed liquor treated with Ni²⁺. However, COD concentration gradually decreased with the gradual increase of Ni²⁺ in the treated mixed liquor media. In the mixed liquor media without Ni²⁺, COD concentration varied from 267.8 to 916.64 mg/L for Peranema sp., 281.28 to 903.52 mg/L for Paramecium sp., 274.04 to 560.88 mg/L for Trachelophyllum sp. and 257.98 to 604.32 mg/L for Aspidisca sp.

While in the mixed liquor media with Ni²⁺, the COD concentration varied from 219.04 to 968.32 mg/L (increase: 366.08% for Peranema sp., 226.88 to 937.6 mg/L (increase: 335.56%) for Paramecium sp., 231.98 to 464.96 mg/L (increase: 204.32%) for Trachelophyllum sp. and 199.17 to 541.04 mg/L (increase: 218.22%) for Aspidisca sp. A significant difference was noted between the COD variation in the treated and untreated mixed liquor media with Peranema sp. and Paramecium sp. exhibiting a higher COD increase in the treated sample than in the untreated ones (positive controls). An overall observation of COD variation in the treated mixed liquor revealed the highest COD increase in mixed liquor with 10 mg-Ni²⁺/L. Moreover, the antimicrobial action of Ni²⁺ was evident by the decrease of DO uptake with the gradual increase of Ni²⁺ concentrations (Table 3). In the untreated mixed liquor (positive controls), the DO-percentage uptake varied from 0 to 91.14% for Peranema sp., 0 to 95.85% for Paramecium sp., 0 to 65.97% for Trachelophyllum sp. and 0 to 43.93% for Aspidisca sp.

However, in the mixed liquor media with Ni²⁺, the DO-percentage uptake varied from 0 to 97.58% for Peranema sp., 0 to 86.18% for Paramecium sp., 0 to 52.34% for 68.84% for Trachelophyllum sp. and 0 to 41.27% for Aspidisca sp. In general, the test isolates were able to remove above 50% of DO in the culture media containing low Ni²⁺ concentrations with exception of Aspidisca sp (41.27%).

A slight difference (p>0.05) however, was observed in the DO uptake by the positive controls and the mixed liquor treated with 10 mg-Ni²⁺/L. No explanation could be given for Trachelophyllum sp. displaying a higher DO-percentage uptake in the mixed liquor con-

### Table 1

Average percentage removal of nickel in the modified wastewater mixed liquor incubated at 30 °C, pH 6.5 (n=3).

<table>
<thead>
<tr>
<th>Ni²⁺, mg/L</th>
<th>Peranema</th>
<th>Paramecium</th>
<th>Tachelophyllum</th>
<th>Aspidisca</th>
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### Table 2

Average concentration of COD release in the modified wastewater mixed liquor incubated at 30 °C, pH 6.5 (n=3).
taining 20 mg-Ni²⁺/L when compared to other concentrations and their specific positive control. *Peranema* sp. was the only isolate able to reach a 50 % of DO removal in the samples containing 30 mg-Ni²⁺/L. Findings of the present study corroborate those of Awasthi and Rai (2005), who investigated the toxicity of Ni²⁺ and Cd²⁺ to nitrate uptake in free and immobilised cells of *Scenedesmus quadricauda*. Tsai *et al.* (2006) pointed out that Cd²⁺ at 2 mg/L could affect the biological reaction of phosphate removal, while at 5 mg/L, the Cd²⁺ removal efficiency of total nitrogen and nitrification dropped substantially.

**Conclusion**

The overall aim of the study was to assess the effect of Ni²⁺ on the simultaneous uptake of nitrate and phosphate by indigenous protozoan isolates. Nickel antimicrobial action negatively affects the nutrient-uptake ability of all the protozoan isolates. Nitrate and phosphate removal appears to gradually decrease with a gradual increase of Ni²⁺ concentrations in the modified wastewater mixed liquor over the period of exposure. *Peranema* sp. was the isolate with the highest removal efficiency of nutrients. At 10 mg/L, Ni²⁺ could enhance the nutrient-removal ability of *Peranema* sp. Nickel uptake is also observed in the modified wastewater mixed liquor with *Paramecium* sp. (85.69 %) which demonstrates the highest uptake compared to other protozoan isolates. This study suggests that protozoan isolates, especially *Peranema* sp., is a potential candidate for the bioremediation of pollutants such as nitrate and phosphate in mine water containing Ni²⁺. Further studies are needed to identify more protozoan species able to simultaneously remove nutrients and metals during the treatment of wastewater.

**References**


**Table 3** Average percentage of DO uptake in the modified wastewater mixed liquor incubated at 30 °C, pH 6.5 (n=3).

<table>
<thead>
<tr>
<th>Ni²⁺, mg/L</th>
<th>Peranema</th>
<th>Paramecium</th>
<th>Tachelophysum</th>
<th>Aspidisca</th>
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