# 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 $Ni^{2+}$ on nutrient-removal efficiency of four indigenous wastewater protozoan species. Isolates could simultaneously remove phosphate (66.4-99.36 %), nitrate (56.19-99.88 %) and $Ni^{2+}$ (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 $Ni^{2+}$-removal: 85.69 %. Increase in $Ni^{2+}$ concentration had a significant effect on nutrient-removal efficiency of these indigenous isolates. Although $Ni^{2+}$ appeared to be toxic to isolates, its effect at low concentration (10 mg-$Ni^{2+}$/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 $Ni^{2+}$ 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 $V^{5+}$ and $Ni^{2+}$ 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 ($Ni^{2+}$). Nickel nitrate [$Ni(NO_3)_2$] was used as a source of $Ni^{2+}$ ions. The stock solution of $Ni^{2+}$ at a concentration of 1000 mg/L was prepared using deionised water. A 200 mL wastewater mixed liquor medium was prepared with $Ni^{2+}$ at a concentration of 10 to 50 mg/L $Ni^{2+}$ (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 $Ni^{2+}$ concentrations in the wastewater mixed liquor media, while nitrate and phosphate concentration were confirmed using the standard methods (APHA 2001).

### *Determination of $Ni^{2+}$ 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 $Ni^{2+}$ and the negative control had the mixed liquor with 50 mg/L $Ni^{2+}$. The negative control was used to assess any external contamination in the samples during the experimental study. To check the effect of $Ni^{2+}$ on the ability of microbial isolates to remove nutrient, the protozoan isolates were separately inoculated in the culture media containing the mixture $Ni^{2+}$. 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 $Ni^{2+}$ median lethal concentration ($LC_{50}$) 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 $Ni^{2+}$ 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 $Ni^{2+}$. 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 $Ni^{2+}$ concentration resulted in a decrease in the rate of nitrate removal by protozoan isolates. The nitrate removal in the modified wastewater mixed liquor without $Ni^{2+}$ (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 $Ni^{2+}$, 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 $Ni^{2+}$ (10 to 50 mg/L). The removal efficiencies of the isolates were significantly higher in the controls than in the mixed liquor with $Ni^{2+}$. Although higher 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 $Ni^{2+}$. Even though the highest nitrate removal efficiency of all the isolates occurred in the mixed liquor containing 10 mg-$Ni^{2+}$/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-$Ni^{2+}$/L. While the nitrate uptake abilities of other protozoans decreased considerably in the nickel mixed liquor containing up to 50 mg-$Ni^{2+}$/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 $Ni^{2+}$ (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-$Ni^{2+}$/L. When comparing the uptake of nitrate and phosphate by test isolates, $Ni^{2+}$ toxicity appear to affect the nitrate uptake more than the phosphate uptake.

### *Determination of 24 h-$LC_{50}$ of $Ni^{2+}$ to and growth/die-off rate of test organisms*

Fig. 3 illustrates the effect of $Ni^{2+}$ 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 $Ni^{2+}$ concentration throughout the experimental study. Over the period of exposure, the growth response in the mixed liquor not treated with $Ni^{2+}$ ranged from 2 to 5$\log_{10}$ Cells/mL for *Peranema* sp., 2 to 6$\log_{10}$ Cells/mL for *Paramecium* sp., 2 to 5 $\log_{10}$ Cells/mL for *Trachelophyllum* sp. and 2 to 6 $\log_{10}$ Cells/mL for *Aspidisca* sp. However, in the mixed liquor treated with $Ni^{2+}$, the growth response of protozoan isolates ranged from 2 to 4 $\log_{10}$ Cells/mL for *Peranema* sp., 2 to 5 $\log_{10}$ Cells/mL for *Paramecium* sp., 2 to 4 $\log_{10}$ Cells/mL for *Trachelophyllum* sp. and 2 to 3 $\log_{10}$ 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

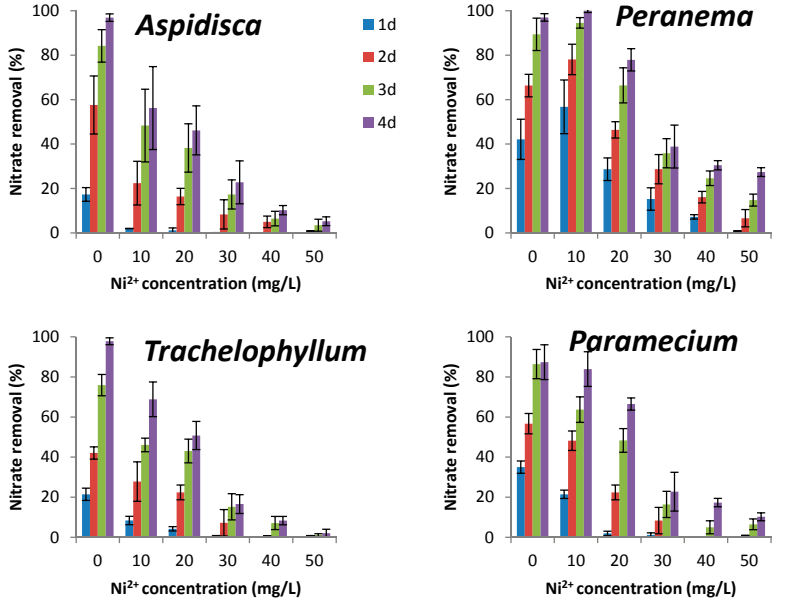


***Fig. 1*** *Percentage removal of nitrate by test organisms inoculated in the modified wastewater liquor and incubated at 30 °C, pH 6.5 for 4 days in the presence of various concentrations of $Ni^{2+}$. (Initial concentration of nitrate in the mixed liquor: 97.15 mg/L, Positive control: Flask with 0 mg-$Ni^{2+}$/L and inoculated with test isolates).*

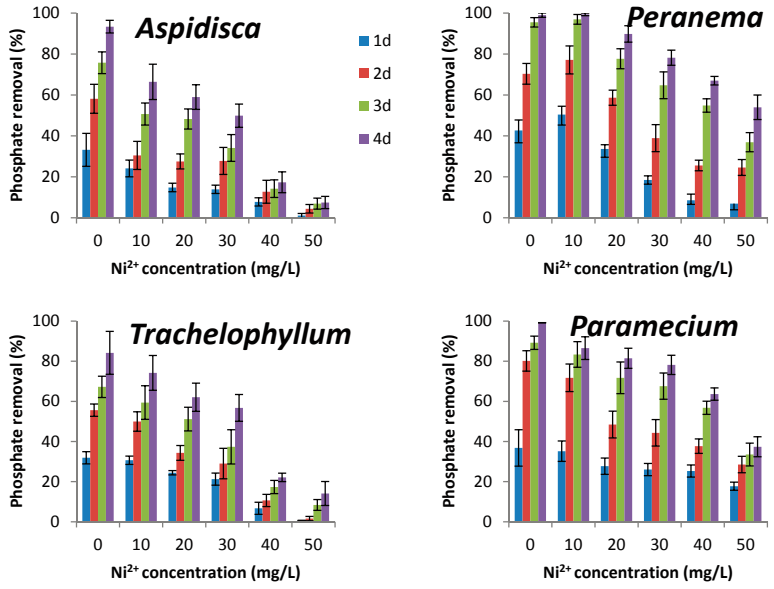


***Fig. 2*** *Percentage removal of phosphate in wastewater inoculated with test organisms and incubated at 30 °C, pH 6.5, for 4 days. Initial concentration of phosphate in the mixed liquor (120.32 mg/L). Positive control: Flask with 0 mg-$Ni^{2+}$/L and inoculated with test isolates.*

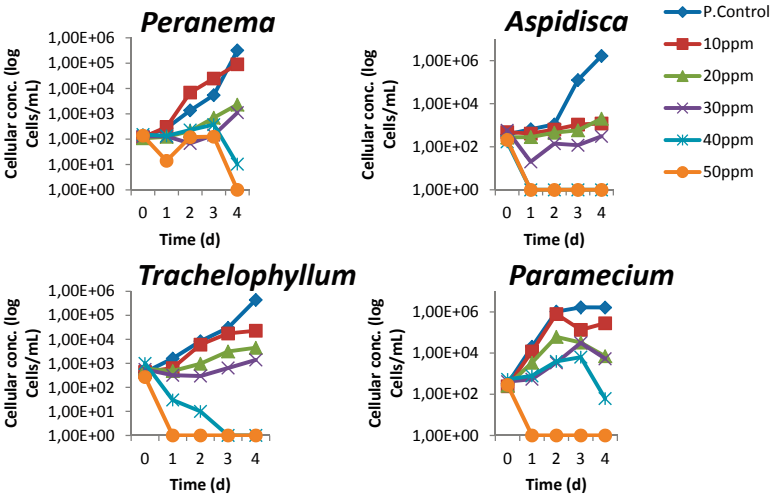


***Fig. 3*** *Nickel toxicity on isolates growth in the modified wastewater mixed liquor incubated at 30°C pH 6.5.*

higher than its respective positive control (1.98 $d^{-1}$) in culture media containing 10 $Ni^{2+}$.

Furthermore, protozoan isolates exposed to $Ni^{2+}$ 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-$LC_{50}$: 50 mg-$Ni^{2+}$/L), while *Paramecium* sp., *Trachelophyllum* sp. and *Aspidisca sp* showed die-off rate of approximately 38.12 % (24 h-$LC_{50}$ between 40–50 mg-$Ni^{2+}$/L), 62.62 % (24 h-$LC_{50}$ between 30–40 mg-$Ni^{2+}$/L) and 62.94 % (24 h-$LC_{50}$ between 20–30 mg-$Ni^{2+}$/L), respectively.

As evident in Table 1, all test isolates were able to remove $Ni^{2+}$ from the modified wastewater mixed liquor. Whereas, a general decrease of $Ni^{2+}$ removal was observed throughout the experimental study in the mixed liquor. Similarly to the removal of nutrients, the $Ni^{2+}$ removal ability of isolates appeared to decrease with an increase in the $Ni^{2+}$ concen-

| $Ni^{2+}$, mg/L | Peranema | Paramecium | Tachelophyllum | Aspidisca |
|---|---|---|---|---|
| 10 | 84.02 | 85.69 | 79.36 | 45.98 |
| 20 | 79 | 82.31 | 45.65 | 35.32 |
| 30 | 75.3 | 79.65 | 26.25 | 30.28 |
| 40 | 67 | 70.69 | 11.29 | 5.32 |
| 50 | 65.32 | 69.32 | 3.68 | 1.78 |

***Table** 1 Average percentage removal of nickel in the modified wastewater mixed liquor incubated at 30 °C, pH 6.5 (n=3)*

tration in the culture media. *Paramecium* sp. (85.69 %) was the protozoan isolates with the highest percentage removal of $Ni^{2+}$ in the culture media, followed by *Peranema* sp. (84.02 %). However, *Aspidisca* sp. could not remove more than 50 % of the $Ni^{2+}$, with the highest (45.98 %) removal rate being from the wastewater mixed liquor containing 10 mg-$Ni^{2+}$/L.

For COD variation (Table 2), a general increase in COD concentrations was observed in the mixed liquor without $Ni^{2+}$ (positive control) and the mixed liquor treated with $Ni^{2+}$. However, COD concentration gradually decreased with the gradual increase of $Ni^{2+}$ in the treated mixed liquor media. In the mixed liquor media without $Ni^{2+}$, 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 $Ni^{2+}$, 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-$Ni^{2+}$/L. Moreover, the antimicrobial action of $Ni^{2+}$ was evident by the decrease of DO uptake with the gradual increase of $Ni^{2+}$ 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 $Ni^{2+}$, 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 $Ni^{2+}$ 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-$Ni^{2+}$/L. No explanation could be given for *Tachelophyllum* sp. displaying a higher DO-percentage uptake in the mixed liquor con-

| $Ni^{2+}$, mg/L | Peranema | | Paramecium | | Tachelophyllum | | Aspidisca | |
|---|---|---|---|---|---|---|---|---|
| | Initial | final | Initial | Final | Intial | Final | Initial | Final |
| 0 | 267.8 | 916.64 | 281.28 | 903.52 | 274.04 | 560.88 | 257.98 | 604.32 |
| 10 | 231.76 | 968.32 | 303.04 | 937.6 | 236.87 | 464.96 | 295.53 | 541.04 |
| 20 | 276.56 | 715.92 | 226.88 | 701.04 | 254.98 | 528.56 | 199.93 | 416.56 |
| 30 | 219.04 | 724.32 | 278 | 647.83 | 314.74 | 394.88 | 237.01 | 267.6 |
| 40 | 291.21 | 368.32 | 276.64 | 297.63 | 231.98 | 316.64 | 199.17 | 245.12 |
| 50 | 300.69 | 282.56 | 300.64 | 280.75 | 234.98 | 280.32 | 298.01 | 297.52 |

***Table 2** Average concentration of COD release in the modified wastewater mixed liquor incubated at 30 °C, pH 6.5 (n=3).*

| $Ni^{2+}$, mg/L | Peranema | Paramecium | Tachelophyllum | Aspidisca |
|---|---|---|---|---|
| 0 | 91.14 | 95.85 | 65.97 | 43.94 |
| 10 | 97.58 | 86.18 | 52.34 | 41.27 |
| 20 | 54.38 | 47.05 | 68.84 | 40.76 |
| 30 | 55 | 46.26 | 36.79 | 37.07 |
| 40 | 29.67 | 27.05 | 28.7 | 30.8 |
| 50 | 14.16 | 33.18 | 2.25 | 15.11 |

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

taining 20 mg-$Ni^{2+}$/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-$Ni^{2+}$/L. Findings of the present study corroborate those of Awasthi and Rai (2005), who investigated the toxicity of $Ni^{2+}$ and $Cd^{2+}$ to nitrate uptake in free and immobilised cells of *Scenedesmus quadricauda.* Tsai *et al.* (2006) pointed out that $Cd^{2+}$ at 2 mg/L could affect the biological reaction of phosphate removal, while at 5 mg/L, the $Cd^{2+}$ removal efficiency of total nitrogen and nitrification dropped substantially.

## Conclusion

The overall aim of the study was to assess the effect of $Ni^{2+}$ 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 $Ni^{2+}$ 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, $Ni^{2+}$ 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 $Ni^{2+}$. Further studies are needed to identify more protozoan species able to simultaneously remove nutrients and metals during the treatment of wastewater.

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