Effect of vanadium toxicity at its different oxidation states on selected bacterial and protozoan isolates in wastewater systems

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This study assesses and compares vanadium toxicity in its different oxidation states towards bacterial isolates (*Pseudomonas putida* and *Bacillus licheniformis*) and protozoan isolates (*Peranema* sp. and *Trachelophyllum* sp.). The isolates were exposed to various concentrations of V in mixed liquors and their tolerance to V was assessed at 30°C at a pH of 4. The results revealed that the increase in V oxidation state increased its toxicity to bacterial isolates, whereas its toxicity decreased for protozoan isolates. Among the bacterial isolates, *P. putida* was found to be more tolerant to V^{3+}(24 h-median lethal concentration (LC_{50}): 390 mg/l), V^{4+}(24 h-LC_{50}: 230–250 mg/l) and V^{5+}(24 h-LC_{50}: 180–200 mg/l), whereas for the protozoan isolates, *Peranema* sp. appeared to be more tolerant to V^{3+}(24 h-LC_{50}: 110–120 mg/l), V^{4+}(24 h-LC_{50}: 160–170 mg/l) and V^{5+}(24 h-LC_{50}: 160–200 mg/l). A comparison of both groups of organisms revealed *Trachelophyllum* sp. as the most sensitive organism to V at its various oxidation states. The visual and spectrophotometric methods used to assess V reduction revealed that *P. putida* was the only isolate able to reduce V^{5+}, V^{4+} and V^{3+} to V^{2+} in mixed liquor media. Vanadium (+2) in concentrations of approximately 46.46 mg/l, 29.57 m mg/l and 38.01 mg/l found in the media was treated with V^{3+}, V^{4+} and V^{5+}, respectively, and inoculated with *P. putida*. This study revealed that the ability of V reduction, adopted with *P. putida*, can be an effective strategy to remove V from polluted environments. This study also showed that the toxicity of V, in terms of its oxidation states, differs from one species to another and in kingdoms.

Keywords: bacteria; heavy metals; protozoa; vanadium; wastewater; toxicity

Introduction

Heavy metals are an inherent part of nature and can be found deep in the Earth's crust.[1–3] Their presence is naturally limited to certain concentrations in water, soil and foods. In addition to natural sources, human activities (industrialization, population growth, etc.) have been reported to play an important role in the mobilization and transport of heavy metals in the environment.[4,5] Due to some biochemical activities, heavy metals can be classified as being essential and non-essential to both humans and animals.[6,7] However, essential or not, they are toxic to all forms of life at higher concentrations.[8,9] Several studies conducted to determine specific effects of heavy metals on humans were found to be difficult to conclude, since results of many studies are based on extrapolations from investigations conducted on animals or micro-organisms in laboratories.[10] Yet, it has been pointed out that the toxicity of a given compound can remarkably vary according to the oxidation state, the complexes formed and the biotransformation of the toxic ions in specific organisms.[11] In humans, the heavy metal toxicities can also be related to sex, age, physiological state, etc.[12] Among heavy metals, vanadium (V) is the 22nd naturally occurring element, widely dispersed at an average concentration of approximately 100 mg/kg.[13] It is also the most abundant transition metal in the aquasphere.[14] Regarded as one of the essential elements, V has been used in dietary supplements for human beings.[15] It has been found to express regulatory enzymatic activities in mammalian tissue.[16] Furthermore, V has been reported as an electron acceptor and growth factor and is involved in nitrogen fixation by certain micro-organisms.[17,18]

Studies have revealed that the possible toxic effects of V are related to its oxidation state since this metal can exhibit several oxidation states (−1 to +5).[10,19] Two of these oxidation states (V^{4+} and V^{5+}) are considered to be predominant in the environment, and inhalation and ingestion are the main routes of exposure.[20] Naturally, V (+4 and +5) can be found in air (0.05–0.18 mg/l), drinking water (0.2–100 µg/l) and food (19–50 µg/l).[21] According to Mannazzu,[19] the toxicity of V increases as the oxidation state increases, and V (+5) is the most soluble, toxic and stable in the aqueous environment. Chandy [22] investigated the toxicity of V on various micro-organisms and revealed that V toxicity was similar to nickel toxicity when tested on chromogenic and non-chromogenic marine bacteria. A study conducted on the effects of V also revealed that,

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at acute exposure, it can cause local irritation of eyes and in upper respiratory tracts, which is characterized by rhinitis, bronchitis, pneumonitis, bronchopneumonitis, wheezing, conjunctivitis, sore throat, chest pain and pharyngitis.[23] Vanadium pentoxide (V_2O_5), one of the V compounds in air, is a major concern. Acute exposure to V_2O_5 has been reported to cause bronchitis and pneumonitis.[21] Similar respiratory effects have been observed in a clinical study conducted by Zenz and Berg.[24] Vanadium compounds have been reported to be able to induce asthma bronchiole, provoke anaemia, leucopenia and punctatebasophilia of erythrocytes and also to reduce the cholesterol level in the blood.[25] Rehder [26] pointed out that vanadate (V^{5+}) was able to inhibit adenosine triphosphatase (ATPases) due to its similarity to phosphate.

Controversies on V toxicity and its beneficial effects on humans have been noted among researchers.[21,27,28] Due to the lack of evidence on V toxicity, especially in the aquatic environment and its potential to upset wastewater treatment during the biological process, the present study was conducted to assess the toxicity of V at its different oxidation states to selected bacterial and protozoan isolates in wastewater systems. Despite the fact that the selected bacterial and protozoan isolates are commonly found in wastewater,[29,30] they have been used in the present study due to their very diverse metabolism, including their ability to resist heavy metal toxicity as reported by several researchers.[31–33] As members of the wastewater system, bacteria and protozoa are generally the first category to be exposed to heavy metal toxicities and serve as very constructive models for investigating toxic effects of metals at the cellular level.[34] In addition, scientific interest has been raised over bacteria, especially the Gram negative bacteria, as they are considered to be the major group of micro-organisms in the wastewater treatment plants. Although protozoans also play an important role in the removal of biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, bacteria concentration as well as the turbidity of wastewater effluent,[35,36] their resistance ability to and the bioremoval of heavy metals have not been fully documented and, therefore, assessing their tolerance limit to V needs further investigation.[37,38] Therefore, the study was done to characterize the toxicity of V in terms of its oxidation states to two groups of microbial species (bacterial and protozoan isolates). The tolerance limit and removal ability of bacterial species as well as that of protozoan species to vanadium were ascertained. The effectiveness of the selected microbes in the detoxification process of the contaminants was conducted in laboratory-scale reactors, which operated in batches.

Materials and methods

Test organisms

Four different isolates (two bacterial species – *Bacillus licheniformis*-ATCC12759 and *Pseudomonas putida*-ATCC31483 – and two protozoan species – *Trachelophyllum* sp. and *Peranema* sp.) were used in this study. The selection of these bacterial species as well as protozoan species was based on their abilities to tolerate/remove heavy metals and their resistance to antibiotic effects as previously reported.[39–42] The two bacterial species were purchased from Quantum Biotechnologies (Strydompark Randburg, South Africa). To obtain a fresh culture of the bacterial isolates, an aliquot of each was separately inoculated in a 500 ml Erlenmeyer flask containing 100 ml of sterile nutrient broth in aseptic conditions and incubated at 30°C in a shaking incubator with an exception of *B. licheniformis* which was incubated at 50°C overnight. In order to determine the cellular concentration needed for the experiment, the growth of bacterial species was measured using the spread plate method every 30 min.[43]

The two protozoan species were obtained from the stock cultures of TUT-Water Research laboratory (South Africa). These protozoan species were previously isolated from wastewater mixed liquors collected from the aeration tanks of the Daspoort wastewater treatment plant (Pretoria, South Africa). The preparation of these protozoan species was done as reported elsewhere.[44] Briefly, each protozoan isolates was separately transferred from the stock culture to a 500 ml Erlenmeyer flask containing 100 ml of fresh media of proteose peptone glucose (PPG) medium at aseptic conditions. An antibiotic (streptomycin-50 μg/ml) to prevent bacterial contamination and a heat-killed *Escherichia coli*-WG4 were also added as sources of nutrients. To obtain the needed protozoan concentration, the inoculated flasks were incubated at room temperature (25°C) in the dark and the cell number was determined every hour using an inverted microscope (Axiovert S100, Carl Zeiss) at ×100 to ×400 magnifications.

Sample collection and preparation of the culture medium

Wastewater samples were collected between November 2011 and July 2012 from the secondary settling tanks of Northern Wastewater Treatment Works in Johannesburg. The wastewater samples were allowed to settle for 2 h before use and prepared as described by Akpor et al.[44] During the experimental study, sodium metavanadate anhydrous ($NaVO_3$), vanadium tetrachloride (VCl_4), vanadium trichloride (VCl_3) and vanadium(II) chloride (VCl_2) used as sources of V^{5+}, V^{4+}, V^{3+} and V^{2+} ions, respectively, were of analytical grade and purchased from Sigma-Aldrich (Cape Town, South Africa).

The stock solutions of vanadium at a concentration of 1000 mg/l were prepared in the deionized water. Aliquots were taken from the stock solutions to prepare modified wastewater mixed liquor containing various V concentrations (from 10 mg/l to 600 mg/l increased at a geometric scale of 10 mg/l) with a final volume of 150 ml. The pH of the final mixed liquor media was adjusted to 4.5 ± 0.3 by using 1.0 M HCl and 1.0 M NaOH, while inductively coupled plasma optical emission spectrometry (ICP-OES) was

used to confirm the metal concentrations of the mixed liquor medium after sterilization. The pH value 4 for the modified mixed liquor was chosen to avoid precipitation and to maintain V in ionic forms: $V(OH)_2^+$, VO^{2+} and $VO_2(OH)_2^-$ for V^{3+}, V^{4+} and V^{5+}, respectively. Prior to use, the modified wastewater mixed liquor was checked in terms of its sterility by inoculating 1 ml of aliquot into the sterile bacteriological agar and the plates were incubated at 37°C for 24 h. Only flasks containing the sterile media were considered for the study.

Vanadium toxicity versus oxidation states: experimental study

Tolerance limits of test organisms

In order to determine the tolerance limits of microbial isolates to V^{3+}, V^{4+} and V^{5+}, a series of experimental studies were conducted separately in 250 ml Erlenmeyer flasks containing 150 ml of the wastewater mixed liquor media. Separate flasks were aseptically inoculated with fresh culture of bacterial isolates (~100 cfu/ml) or protozoa cells (~100 ells/ml). Two supplementary controls (positive and negative) were used in this study. The positive control flask contained the mixed liquor without vanadium but inoculated with the specific micro-organism, while the negative controls contained only the mixed liquor with 600 mg/l of V^{5+}, V^{4+} and V^{3+} separately. All the inoculated flasks as well as the controls were incubated at 30°C ± 2°C and aliquots were taken every day for four days. The LC_{50} of the test metal for each of the test microbial isolates was determined as described previously.[37] The minimum inhibitory concentration (MIC) of the test metal was determined according to Shirdam et al.[42] During each sampling regime, aliquot samples were taken every 24 h for four days in order to estimate the microbial concentration. The growth of bacterial species was determined on nutrient agar using the spread plate method after dilution,[43] while the growth of the protozoan species was determined by visual count using an inverted microscope (Axiovert S100, Carl Zeiss) under ×100 to ×400 magnifications. The microbial species' growth rates were calculated according to Ferrier-Pagès and Rassoulzadegan.[45] After incubation, the microbial isolates were classified as sensitive or tolerant to V^{3+}, V^{4+} and V^{5+} according to the inhibition of growth cells. In addition, the variations of COD and dissolved oxygen (DO) concentrations in the mixed liquor were measured as described in the standard method.[43]

Determination of vanadium (+2) concentrations

Due to changing colour of the culture media inoculated with *P. putida* from green (for V^{3+}), blue (for V^{4+}) and yellow (for V^{5+}) to lilac colour corresponding to the presence of V^{2+}, the concentration of V^{2+} was determined by spectrophotometric measurements as described by Bredberg et al.[46] Prior to analysis, standard solutions of V^{2+} using VCl_2 (Sigma-Aldrich, Cape Town, South Africa) were prepared at concentrations varying from 20 to 400 mg/l with double-deionized water. The absorbance of the standard solutions (1 ml) was measured at 760 nm and the regression equation was also determined. Samples were filtered at first, using a 0.2 μm filter and the absorbance of the filtrate (1 ml) was measured by a spectrophotometer (Spectroquant Pharo300, Merck Chemicals, South Africa). The absorbance values of samples were then used in the regression equation to determine the concentration of V^{2+}.

Statistical analysis

Data were statistically analysed using the Stata computer software (version: STATA V10, STATA Corp. LP, 2009). The difference between physicochemical parameters and also growth performance, tolerance limit over the oxidation states was analysed using the one-way analysis of variance (ANOVA). The tests for relationships were carried out using the Pearson correlation index and the interpretation was performed at a two-sided 95% confidence limit.

Results

Profile of the activated sludge mixed liquor in Northern Wastewater Treatment Works

The physicochemical parameters of the wastewater samples collected from Northern Wastewater works in Johannesburg are summarized in Table 1. The DO profile throughout the sampling period varied significantly ($p < 0.05$) and ranged from 4.05 to 6.31 mg/l. However, samples collected in May 2012 had the highest DO value (6.31 mg/l), whereas July's samples had the lowest DO value (4.05 mg/l). The nutrient regimes (nitrate and phosphate) of the wastewater samples throughout the sampling period varied from 3.73 to 22.05 and from 0.17 to 1.13 mg/l, respectively. There was an indication of non-significant pH variations ($p > 0.05$) ranging between 6.17 and 6.42 in the wastewater samples throughout the experimental study. The temperature profile of the wastewater samples varied significantly ($p < 0.05$) between 15.20°C and 17.90°C with the sample collected on 2 April 2012 having the highest temperature of 17.90°C. Besides the above parameters, conductivity value, COD and total vanadium concentrations ranged from 77 to 187 μS/m, 2.67 to 70.25 mg/l and 0.03 to 0.08 mg/l, respectively.

Vanadium toxicity versus oxidation states experimental study

Vanadium toxicity in relation to its oxidation states (V^{3+}, V^{4+} and V^{5+}) on microbial isolates in modified mixed liquor is shown in Figure 1. In general, the difference of toxicity among V^{3+}, V^{4+} and V^{5+} on microbial isolates was noted throughout the experimental study. The effect of V toxicity based on its different oxidation states was revealed

Table 1. The profile of wastewater samples used during the present study.

Date of collection	DO (mg/l)	Nitrate (mg/l)	Phosphate (mg/l)	pH
27-11-2011	4.64 ± 0.12	14.41 ± 1.98	0.72 ± 0.01	6.34 ± 0.01
15-12-2011	5.97 ± 0.07	22.05 ± 4.25	0.62 ± 0.02	6.38 ± 0.20
01-03-2012	5.67 ± 0.04	4.48 ± 0.37	0.17 ± 0.21	6.42 ± 0.08
02-04-2012	4.38 ± 0.08	3.73 ± 0.97	1.05 ± 0.05	6.43 ± 0.35
09-05-2012	6.31 ± 0.07	10.95 ± 3.21	0.77 ± 0.06	6.34 ± 0.04
06-06-2012	4.96 ± 0.25	12.25 ± 2.36	0.32 ± 0.08	6.37 ± 0.07
03-07-2012	4.05 ± 0.11	4.53 ± 1.05	1.13 ± 0.09	6.17 ± 0.21
SA Std		15	10	5.5–9.5
Date of collection	Temperature (°C)	Conductivity (μ/Sm)	COD (mg/l)	Total Vanadium (mg/l)
27-11-2011	17.50 ± 0.03	164.00 ± 2.09	51.83 ± 7.98	0.05 ± 0.01
15-12-2011	17.70 ± 0.02	169.00 ± 3.18	23.96 ± 3.65	0.05 ± 0.01
01-03-2012	17.80 ± 0.09	187.00 ± 3.25	59.33 ± 8.12	0.04 ± 0.06
02-04-2012	17.90 ± 0.03	77.00 ± 16.31	26.83 ± 2.36	0.08 ± 0.02
09-05-2012	15.20 ± 0.08	126.00 ± 31.95	69.33 ± 4.25	0.03 ± 0.06
06-06-2012	15.70 ± 0.02	159.00 ± 10.35	70.25 ± 8.25	0.05 ± 0.07
03-07-2012	16.90 ± 0.01	119.00 ± 8.25	2.67 ± 0.32	0.07 ± 0.05
SA Std		250	75	0.1*

*FAO-recommended limit [19]; SA Std: South Africa Standard limit.

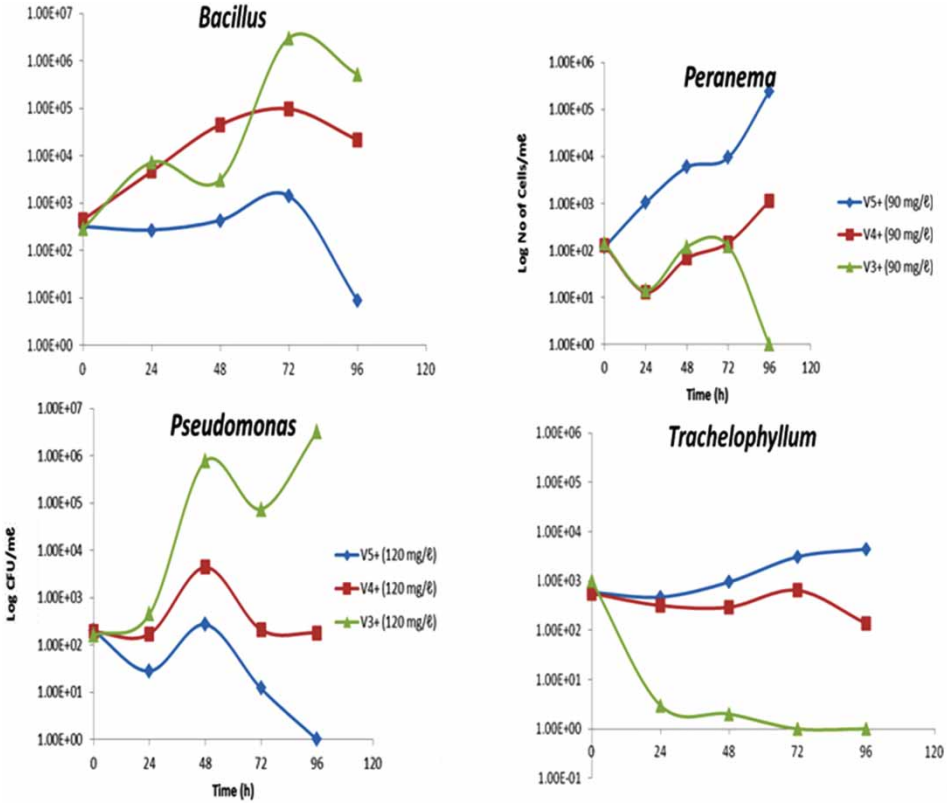

Figure 1. An illustration on vanadium toxicity versus oxidation states in modified mixed liquor (V^{3+}, V^{4+} and V^{5+} separately).

to be different from both bacteria and protozoan isolates. The increase in the oxidation states appeared to increase the toxicity of V to bacterial isolates with *P. putida* – V^{3+}(24 h-LC_{50}: 390 mg/l), V^{4+}(24 h-LC_{50}: 230–250 mg/l) and V^{5+}(24 h-LC_{50}: 180–200 mg/l) being the most tolerant, while decreasing the toxicity of V for protozoan isolates with *Peranema* sp. as the most tolerant protozoan – V^{3+} (24 h-LC_{50}: 110–120 mg/l), V^{4+}(24 h-LC_{50}: 160–170 mg/l) and V^{5+}(24 h-LC_{50}: 160–200 mg/l) (Figure 1, Table 2). Of all the microbial isolates used in this study, *Trachelophyllum* sp. was reported as the most sensitive organism to V at its different oxidation states with a 24-h

Table 2. The 24 h-LC_{50} and MIC of $V^{3+/4+/5+}$ to the test organisms in mixed liquor ($n = 7$).

	V^{3+} (mg/l)		V^{4+} (mg/l)		V^{5+} (mg/l)	
	24 h-LC_{50}	MIC	24 h-LC_{50}	MIC	24 h-LC_{50}	MIC
Pseudomonas putida	390	450	230–250	300	180–200	250
Bacillus licheniformis	260–280	360	190–210	270	140–150	200
Peranema sp.	110–120	140	160–170	180	160–200	230
Trachelophyllum sp.	60–70	100	100	110	120–130	150

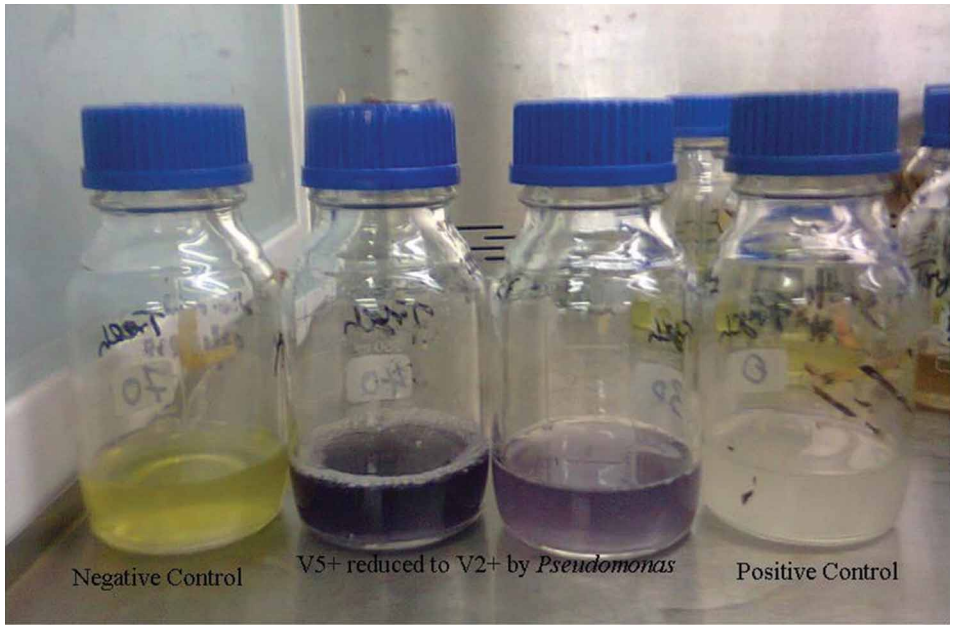

Figure 2. Reduction of V^{+5} to V^{+2} by *Pseudomonas putida* in the modified mixed liquor media at pH 4.5 and 30°C ($n = 7$). Positive control: mixed liquor not treated with V but inoculated with test isolates. Negative control: mixed liquor treated with V and inoculated with test isolates. (Source: Author).

LC_{50} of 60–70 mg/l, 100 mg/l and 120–130 mg/l for V^{3+}, V^{4+} and V^{5+}, respectively. However, *P. putida* was the most tolerant isolate of all test organisms (bacterial as well as protozoan). In addition, *Peranema* sp. (24 h-LC_{50}: 160–200 mg/l) also revealed more tolerance to V^{5+} than *B. licheniformis* (24 h-LC_{50}: 140–150 mg/l).

Statistical analysis carried out using an one-way ANOVA showed significant differences ($p < 0.05$) between MIC mean values of V^{5+}, V^{4+} and V^{3+} for bacterial isolates, whereas no significant differences ($p > 0.05$) were noted for protozoan isolates. Further statistical analysis showed no significant difference ($p > 0.05$) among the 24 h-LC_{50} mean values of bacterial species and those of protozoan species. In the presence of V^{3+}, a significant difference ($p < 0.05$) was exceptionally observed in terms of 24 h-LC_{50} mean values of bacterial and protozoan species with bacterial species having the highest 24 h-LC_{50} values.

Reduction of Vanadium (5+, 4+ and 3+) by microbial isolates

Throughout the study, in the modified mixed liquor containing V^{3+}, V^{4+} and V^{5+} separately and inoculated with *P. putida*, the colour of the media was observed to change from green, blue and yellow, respectively, to approximately lilac (Figure 2). There was no change in colour during the process in the media inoculated with the rest of microbial isolates (*B. licheniformis*, *Peranema* sp. and *Trachelophyllum* sp.). The colour change in the culture media was observed only in the flasks showing growth and controls revealed no colour change phenomena.

The presence of V^{2+} was then confirmed with the use of spectrophotometric analysis of the samples (Figure 3). A general increase in V^{2+} concentrations in the culture media was noted over time. Average concentrations of V^{2+} of approximately 46.46 mg/l, 29.57 mg/l and 38.01 mg/l were noted in the media treated with V^{3+}, V^{4+} and V^{5+}, respectively, when inoculated with *P. putida*. Besides the media inoculated with *P. putida*, V^{2+} was found at a concentration of lower than 1 mg/l in the culture media inoculated with *B. licheniformis*, *Peranema* sp. and *Trachelophyllum* sp.

Figure 4 summarizes the profile of COD in the wastewater modified mixed liquors inoculated with test isolates and various concentrations of V^{5+}, V^{4+} and V^{3+}. A general observation shows a decrease in COD concentrations with

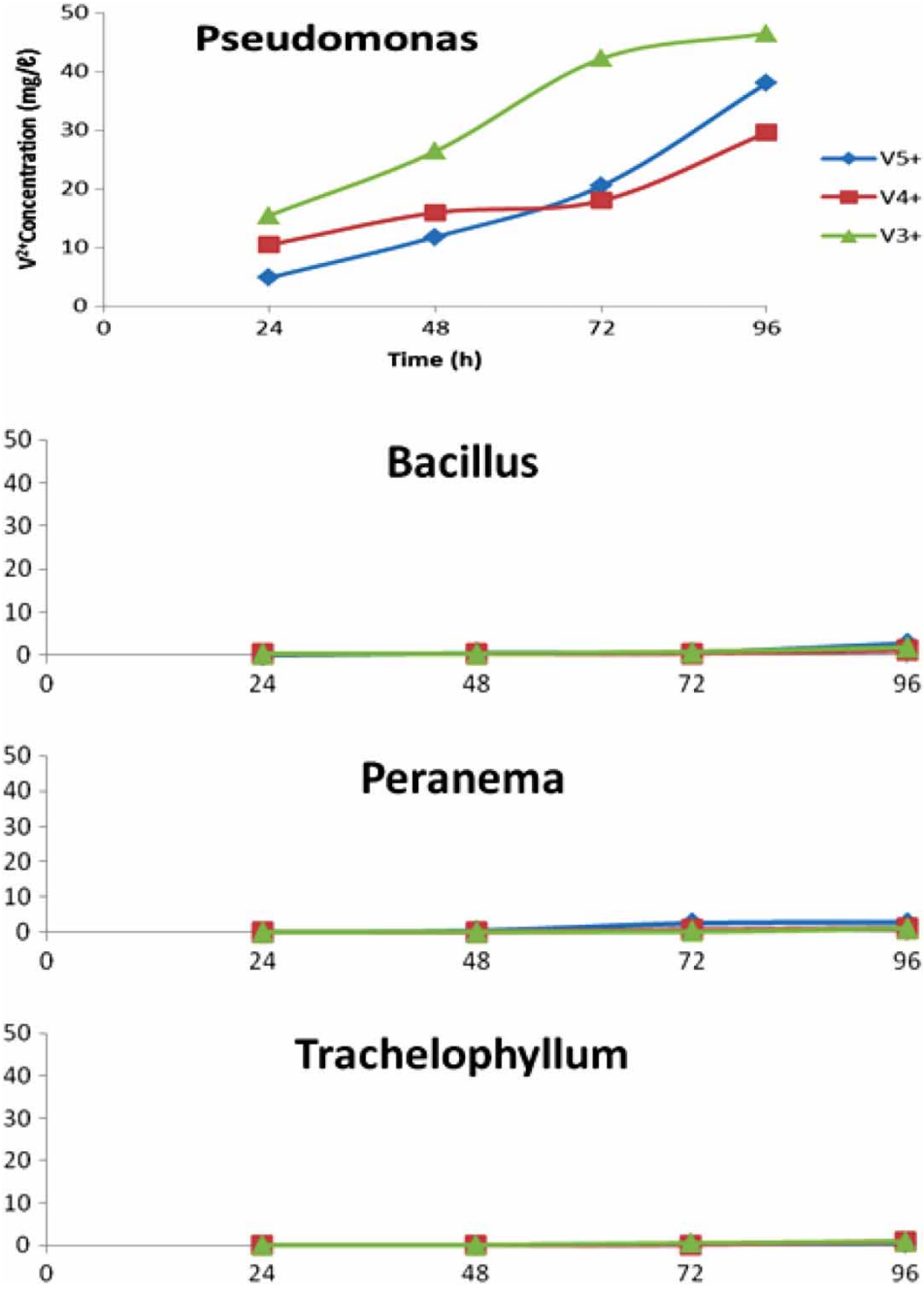

Figure 3. Vanadium (+2) concentration in culture media highlighting the reduction of V^{5+}, V^{4+} and V^{3+} to V^{2+} over time by microbial isolates ($n = 7$). The media analysed was prepared with 100 mg/l of each V, separately.***

an increase in V concentrations in the modified mixed liquors inoculated with test isolates; this was also influenced by V oxidation states. For bacterial isolates, the media containing V^{3+} was revealed to have high COD concentrations at the end of the experiment, followed by V^{4+} and V^{5+}. In the media inoculated with *B. licheniformis*, average COD concentrations increased from 1459.34 to 2393.61 mg/l (64.02%), from 1309.82 to 2013.85 mg/l (53.75%) and from 1224.34 to 1792.8 mg/l (46.43%) in the presence of V^{3+}, V^{4+} and V^{5+}, respectively. *Pseudomonas putida* also showed an increase in average COD concentrations from 1488.5 to 2252.84 mg/l (51.86%), from 1322.67 mg/l to 1997.10 mg/l (50.99%) and from 1219.33 mg/l to 1784.86 mg/l (46.38%) in the culture media treated with V^{3+}, V^{4+} and V^{5+}, respectively. Significant inhibition ($p < 0.05$) of COD release was observed in the media treated with vanadium at concentrations above 100 mg-V^{5+}/l, 300 mg-V^{4+}/l and 300 mg-V^{3+}/l for *P. putida*, and in the media treated with 80 mg-V^{5+}/l, 100 mg-V^{4+}/l and 100 mg-V^{3+}/l for *B. licheniformis*.

For protozoan isolates, the highest COD release was contrary to that observed in the media containing V^{5+},

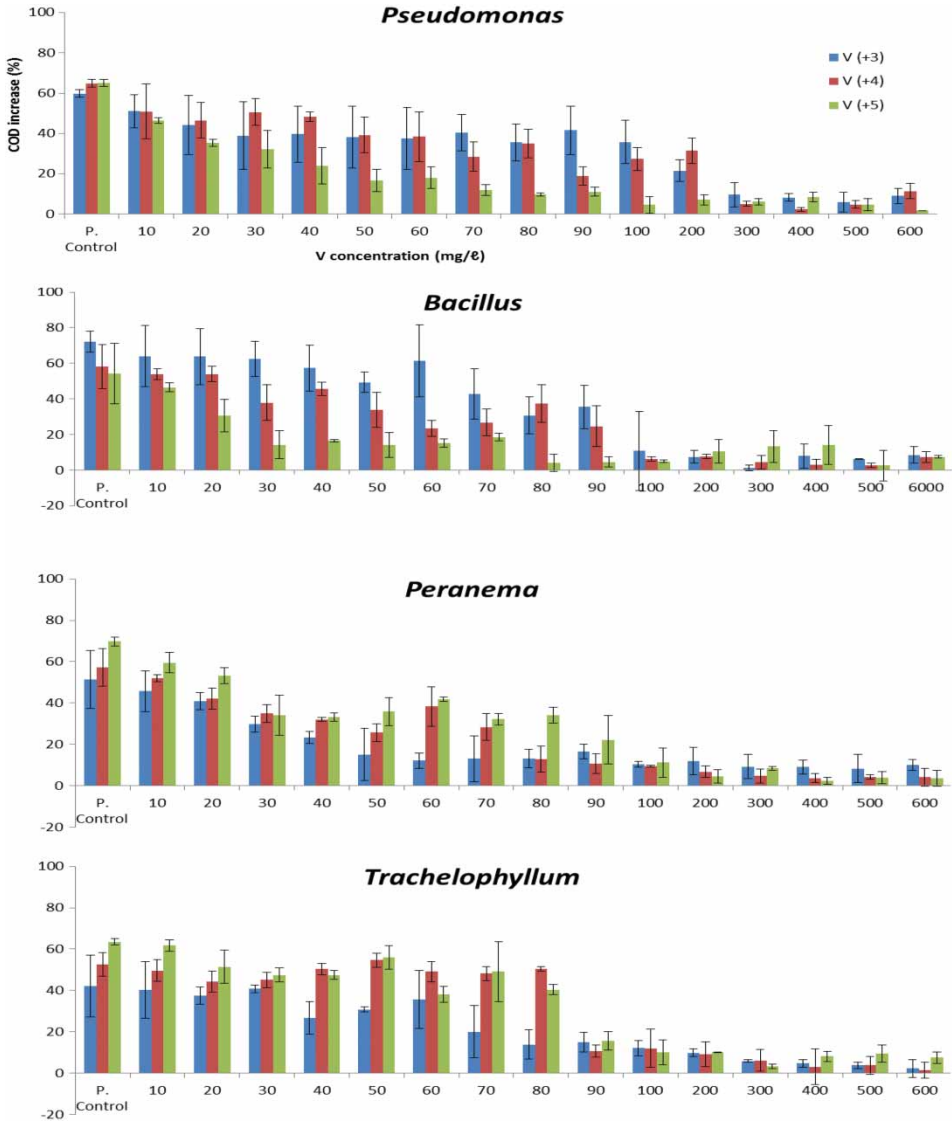

Figure 4. Percentage of COD increasing in the modified mixed liquor containing V (+3, +4 and +5, separately) after four days of incubation and inoculated with test isolates at pH 4.5 and 30°C. P. control: positive control (mixed liquor not treated with V but inoculated with test isolates.)

followed by V^{4+} and V^{3+}. The presence of *Peranema* sp. in mixed liquor media containing V^{3+} or V^{4+} generated the highest COD concentrations increasing from 1464.65 to 2134.73 mg-V^{3+}/l (45.74%) and from 1298.65 to 1973.95 mg-V^{4+}/l (52%), respectively, whereas the media inoculated with *Trachelophyllum* sp. revealed the highest COD release at 61.69% (ranging from 1347.67 to 2179.05 mg-V^{5+}/l) in the presence of V^{5+}. Similar to bacterial isolates, for protozoan isolates a significant inhibition ($p < 0.05$) of the increase in COD concentrations was also revealed in the media treated with V at concentrations above 100 mg-V^{5+}/l, 90 mg-V^{4+}/l and 90 mg-V^{3+}/l for *Peranema* sp. and 90 mg-V^{5+}/l, 90 mg-V^{4+}/l and 80 mg-V^{3+}/l for *Trachelophyllum* sp.

In Table 3, a gradual decrease in DO uptake occurred with a gradual increase in V concentrations in the modified mixed liquors. The uptake rate of DO was high in the untreated modified mixed liquor (positive control) [for *P. putida* (96.37%-V^{3+}, 95.77%-V^{4+} and 97.71%-V^{5+}), for *B. licheniformis* (92.69%-V^{3+}, 82.13%-V^{4+} and 99.35%-V^{5+}), for *Peranema* sp. (91.68%-V^{3+}, 87.02%-V^{4+} and 96.92%-V^{5+}) and for *Trachelophyllum* sp. (81.86%-V^{3+}, 93.09%-V^{4+} and 90.43%-V^{5+})] when compared with the mixed liquors treated with V [for *P. putida* (91.26%-V^{3+}, 74.58%-V^{4+} and 70.57%-V^{5+}), for *B. licheniformis* (82.66%-V^{3+}, 81.7%-V^{4+} and 67.19%-V^{5+}), for *Peranema* sp. (66.72%-V^{3+}, 85.37%-V^{4+} and 96.22%-V^{5+}) and for *Trachelophyllum* sp. (45.59%-V^{3+}, 69.13%-V^{4+} and 80.84%-V^{5+})].

However, the uptake rate of DO by *P. putida* appeared to be the highest in the media treated with V^{3+} (91.26%), whereas with *Peranema* sp., it appeared to be the highest in the media treated with V^{4+} (85.37%) and V^{5+} (96.22%). A significant difference ($p < 0.05$) was noted between all the

Table 3. Variations of dissolved oxygen (%) in the modified mixed liquor inoculated with V (+3, +4 and +5, separately).

	Pseudomonas			*Bacillus*			*Peranema*			*Trachelophyllum*		
V conc.	V^{+3}	V^{+4}	V^{+5}	V^{+3}	V^{+4}	V^{+5}	V^{+3}	V^{+4}	V^{+5}	V^{+3}	V^{+4}	V^{+5}
0	96.37	95.77	97.71	92.69	82.13	99.35	91.68	87.02	96.92	81.86	93.09	90.43
10	91.26	74.58	70.57	82.66	81.70	67.19	66.72	85.37	96.22	45.59	69.13	80.84
20	84.30	58.05	56.65	67.03	64.40	61.73	54.32	66.72	80.89	41.87	70.43	75.33
30	75.45	60.63	30.96	65.08	58.65	47.31	47.01	62.18	64.48	26.50	65.18	78.81
40	65.64	45.10	37.71	65.45	56.77	47.56	40.92	61.71	61.77	12.79	56.52	69.84
50	53.83	43.50	13.27	61.89	51.90	31.52	33.49	40.57	52.89	6.82	29.28	32.07
60	46.75	29.32	14.46	47.95	33.33	30.50	20.71	35.76	40.38	5.36	32.17	31.12
70	40.70	28.55	9.63	40.39	25.63	22.10	9.32	10.17	15.06	2.59	20.98	33.74
80	39.95	15.90	10.63	33.02	22.16	19.68	5.95	1.82	0.63	0.96	1.66	10.62
90	27.49	13.89	5.23	30.74	34.95	15.30	1.26	2.24	1.95	2.55	1.73	9.46
100	26.44	13.47	3.11	30.04	26.41	1.61	2.65	1.15	0.16	1.76	1.73	7.37
200	14.47	8.43	0.45	22.92	21.35	1.37	2.59	7.73	2.34	1.04	1.25	5.47
300	14.48	1.20	1.58	9.06	11.34	4.09	1.68	1.61	2.58	1.15	1.88	2.09
400	2.20	1.20	0.18	1.45	0.94	0.32	1.41	4.39	3.64	1.95	1.98	1.47
500	5.87	1.60	1.15	7.69	1.26	0.47	0.30	3.27	1.55	1.81	2.88	0.49
600	2.77	0.27	1.13	1.37	1.10	0.49	1.66	2.71	0.76	1.67	1.24	2.13

Note: Conc.: concentration.

isolates when comparing their DO-uptake rates. The tests for relationships carried out using the Pearson correlation index revealed a strong negative correlation between DO uptake and COD release in the media inoculated with bacterial isolates ($r = -0.818, p < 0.05$) as well as protozoan isolates ($r = -0.89, p < 0.05$). Further statistical analysis (Pearson's correlation) showed a strong positive correlation between the DO uptake and the growth of the microbial isolates ($r = 0.923$ for bacterial isolates, $r = 0.901$ for protozoan isolates).

Discussion

Pollution caused by chemical compounds, including heavy metals, is a global issue and a direct consequence of all human activities.[47,48] In recent years, disposal of heavy metals has increased the environmental concern, especially into water bodies.[49] It is indeed believed that heavy metal toxicity exceeds that of all other radioactive and organic wastes released into the environment.[50] Vanadium is widely distributed in nature and possesses a noteworthy chemistry with its toxicity, depending on its oxidation states.[51] Due to the lack of solid evidence on its toxicity and dispersion in South Africa, a country that is considered as the main producer of this metal, the present study assessed the toxicity of V at its different oxidation states (+3, +4 and +5) to selected bacterial and protozoan isolates. The selected bacterial and protozoan species used in the present study are commonly found in the wastewater system.[29,30] It has been reported that the success of a toxicological study on specific ecosystem is dependent, to a great extent, on the species that constitute the community.[37,52]

During the study period, wastewater effluent samples collected from the Northern Wastewater Treatment Works in Johannesburg were screened for COD, DO and pH and also for the presence of vanadium. The results revealed that the pH values of the wastewater were within the permissible range 5.5–9.5 pH units, set by the South African Water Act No. 36 of 1998. In addition, the electrical conductivities of the collected wastewater, the nitrate, the phosphate and the COD concentrations were found at levels below the permissible maximum limits of 250 µS/cm, 15 mg/l, 10 mg/l and 75 mg/l, respectively, as set by the Water Act No. 36 of 1998.[53] As there is no limit set for V in the South African Water Act., the limit set by the United Nations Food and Agriculture Organization was considered in this study. The V concentrations in the wastewater samples were found below the most permissible maximum limit of 0.1 mg/l, as set by the United Nations Food and Agriculture Organization Standard.[54]

The effect of heavy metals such as iron, nickel, chromium, etc. on micro-organisms has been extensively studied compared with that of vanadium. However, it has been reported that V is widely used and seen as one of the most important metals in modern technology.[14] Therefore, its high distribution in water has become a major environmental concern. The outcomes of this study revealed that V is toxic to microbial isolates and its toxicity is dependent on oxidation states.[19–22] However, *P. putida* was the most tolerant to V at its various oxidation states than any other microbial isolates (Table 2). The increase in the oxidation states appeared to increase the toxicity of V to bacterial isolates, while decreasing the V toxicity for protozoan isolates. Results also showed that V^{5+} was more toxic than V^{3+} for bacterial isolates but, less toxic than V^{3+} for protozoan isolates (Figure 1, Table 2). Several studies have indicated that an increase in oxidation states is directly proportional to the increase in the toxicity of metal, which places V^{5+} as being the most toxic form of V.[19,51,55]

However, the findings of this study conflict with those of previous studies in reporting a decrease in V toxicity to protozoan isolates with an increase in oxidation states. Based on the biochemical properties of V, the situation of V^{5+} being less toxic than V^{3+} on protozoan isolates was unexpected and not understood.[15,17,26,56,57] This could have been due to the pH of the culture media adjusted at 4.5 ± 0.3 and other metabolites produced by protozoan isolates, which favoured the polymerization of V^{5+} products (decavanadates); this might be less toxic than compounds formed by other forms of vanadium (V^{4+} and V^{3+}).[15,58] It should be mentioned that pH could not be the only parameter involved in the reduction of V^{5+} toxicity since culture media inoculated with bacterial isolates at the same conditions did not reveal similar observation. According to Wei et al.[59] the toxicity of V^{5+} may be associated with the type of polymers formed. It has been previously reported that the toxicity of V is related to its capacity to induce the formation of intracellular reactive oxygen, which impairs the mitochondrial function.[60,61] The toxicity of V^{5+} (vanadate) has also been linked with its ability to inhibit several enzymes, which could be due to its similarity and phosphate chemistry.[62]

Despite numerous studies on V biochemistry and physical properties, the toxicity to micro-organisms is still poorly understood. Shirdam et al.[42] reported that bacterial species such as *P. putida*, *Bacillus cereus* and *Pseudomonas peudoalkaligenes* were able to tolerate Cd, Ni and V by removing approximately 40–50%, 5–6% and 10–12%, respectively, in the media. Lyalkova and Yurkova [63] found that various microbial species belonging to the taxon of *Pseudomonas* were highly resistant to V. This corroborates to the finding of the present study that also revealed that *P. putida* was able to reduce V^{5+}, V^{4+} and V^{3+} to V^{2+} as indicated by the characteristic lilac colour developed in the culture media (Figure 2).

The change of colour from yellow to lilac (violet) revealed the presence of V^{2+} at high concentrations in the media and implied that this strategy was adopted by this microbial isolate to support their growth and also to reduce the toxicity of this metal in the culture media. This was confirmed with the methodical analysis of the culture media revealing high concentrations of V^{2+} in the media inoculated with *P. putida* (Figure 3). According to Lovley,[64] micro-organisms enzymatically reduce a variety of metals (Fe, Mn, U, Se, Cr, Hg, etc.) in metabolic processes. Ortiz-Bernad et al.[14] reported the reduction of V^{5+} to V^{4+} by *Geobacter metallireducens*. Lyalkova and Yurkova [63] also pointed out that some *Pseudomonas* species were able to reduce V^{5+} to lower oxidation states under anaerobic conditions. Furthermore, Briand et al.[65] indicated that *Acidithiobacillus* spp. was able to reduce V^{5+} to V^{4+} as well. The reduction of V^{5+} to V^{4+} by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* was also reported by Bredberg et al.[46] Carpentier et al.[66] pointed out that *Shewanella oneidensis* was able to reduce V^{5+} to V^{4+} in an anaerobic atmosphere. Although it has been reported that the pH of the media plays a major role in the reduction/oxidation of vanadium,[67] the absence of V^{2+} in the controls as well as in the culture media inoculated with other microbial isolates in the present study showed that the pH did not play a significant role in the reduction of V in the media inoculated with *P. putida*.

During this study, DO and COD variations in the inoculated media appeared to be inversely proportional in the media inoculated with bacterial isolates ($r = -0.818$, $p < 0.05$) and also with those inoculated with protozoan isolates ($r = -0.89$, $p < 0.05$). A decrease in the DO uptake rate and the COD release in the culture media were noted with an increase in V concentrations. Nonetheless, the DO uptake in the mixed liquor was found to be inversely proportional to the gradual increase in $V^{5+/4+/3+}$ concentrations, but strongly correlated to the growth of the microbial isolates ($r = 0.923$ for bacterial isolates, $r = 0.901$ for protozoan isolates). The DO depletion during the experimental study was clearly linked to the growth of the isolates, while the absence of DO removal at high $V^{5+/4+/3+}$ concentrations could be due to the toxicity of the test metals which prevented the microbial growth and activity in the mixed liquors.[40] The inhibition of the DO uptake rate was found to be higher in the presence of V^{5+} than in the presence of V^{3+} for bacterial isolates, while for protozoan isolates, this phenomenon was noted in the presence of V^{3+} more than in the presence of V^{5+}. A similar observation was noted in the case of COD concentrations where the inhibition of COD release was mostly marked in the media treated with V^{5+} than in the one treated with V^{3+} for bacterial isolates and with V^{3+} than V^{5+} for protozoan species. These findings are in agreement with those reported by Kamika and Momba [40] who noted a strong positive correlation between COD release and DO uptake for test bacterial ($r = 0.9166$) and protozoan isolates ($r = 0.8810$). A study by Akpor et al. [44] when working on nutrient removal from an activated sludge mixed liquor by wastewater protozoa also indicated a strong positive correlation ($r = 0.806$, $p < 0.01$) between COD concentrations and growth rates of the protozoan isolates (including *Aspidisca* sp., *Peranema* sp. and *Trachelophyllum* sp.). A negative significant correlation ($r = -0.602$, $p < 0.01$) was also found between COD and DO, which expressed a positive relationship between COD release and DO uptake.

In conclusion, the toxicity of V in its different oxidation states to selected bacterial species was assessed and compared with those of protozoan isolates. The outcome of this study revealed that an increase in vanadium oxidation states stimulates a further increase in toxicity of the test metal on bacterial isolates, whereas its toxicity on protozoan isolates decreases. The study has also demonstrated the ability of *P. putida* to reduce V^{5+}, V^{4+} and V^{3+} to V^{2+} in the modified mixed liquor. Based on scientific evidence that V^{5+} is more toxic than other V (+2, +3 and +4) to humans and other animals, the ability of *P. putida* to reduce V^{5+}, V^{4+} and V^{3+} to V^{2+} showed that this isolate is a potential

candidate for the reduction of the toxicity of V in water and can be used in the remediation of V-polluted wastewater system. Further investigations are needed to delineate components required in the electron transport chain that are needed for V (+5, +4, +3) reduction.

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