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Valorization of neutral mine drainage using hydroponic floating wetland planted with Vetiver grass: A simplified approach to reclaim drinking water

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ABSTRACT

In this study, neutral mine drainage (NMD) was polished using hydroponic floating wetland (HFTW) planted with Vetiver grass. Specifically, the pilot HFTW was constructed using a plastic drum of 100 litres capacity and a rectangular plastic basin measuring 60 cm (length), 45 cm (width) and 20 cm (height). The water was flowing in continuous drops from the drum to the treatment cell where leachates were collected daily and analyzed for chemical species concentration for a period of 40 days. The results revealed a slight pH increase with an increment of 0.9, while the chemical species removal obeyed the following order: Cu (100%) = Ni (100%) = Zn (100%) > Fe (99.6%) > Al (99%) > Mn (94%) > SO_4^{2-} (75%) > EC (50%). The bioaccumulation factor and root/shoot ratio results revealed that various mechanisms including hydraulic control, phytoaccumulation, phytodegradation, phytostabilization, phytovolatilization, rhizodegradation and rhizofiltration were involved in inorganic pollutants removal. The high resolution-scanning electron microscopy-energy dispersive X-ray spectroscopy (HR-SEM-EDS), and powder X-ray diffraction (PXRD) analysis displayed the presence of chemical species in Vetiver grass roots while Brunauer-Emmett-Teller (BET) results revealed difference in roots surface area. The treated water met drinking water standard set by the World Health Organization (WHO) proving that potable fresh water can be reclaimed from NMD using HFTW.

Keywords: hydroponic floating wetland, neutral mine drainage, polishing, phytoremediation, Vetiver grass.

INTRODUCTION

Acid mine drainage (AMD) released following mining activities is an acidic mining influenced effluent with low pH (pH ≤ 4), rich in ions and mostly aluminum (Al^{3+}), iron ($Fe^{2/3+}$), manganese (Mn^{2+}), copper (Cu^{2+}), nickel (Ni^{2+}), zinc (Zn^{2+}), arsenic (As^{2+}), lead (Pb^{2+}) and sulphate ions (SO_4^{2-}) (Mahlohla et al., 2026; Nguegang and Ambushe, 2024). Consequently, the release of such harsh and unpleasant wastewater in the environment poses a significant threat to ecology and public health (Azizi et al., 2024; Wang et al., 2025). As such, various treatment methods including abiotic (use of alkaline chemical) and biotic (derived from living organisms) or the combination of both are commonly applied to treat AMD with the primary aim to satisfy stringent environmental regulations and requirement without considering a possible reclamation of drinking water (Muedi et al., 2025; Nepfumbada et al., 2023). Amongst the biotic methods, constructed wetlands (CWs) which are wastewater treatment technologies mimicking natural wetlands, however, in controlled environment, appear as the most viable alternative to abiotic or chemical treatment. Constructed wetlands attenuate inorganic pollutants via a combination of physical, chemical biological processes including sedimentation, filtration, microbial degradation, absorption, and plants uptake (Beauclair and Ambushe, 2024; Beauclair et al., 2022). Constructed wetlands are very promising in treating wastewater due

to their high efficiency, low capital and operational cost, and self-renewable system (Zhong et al., 2024). However, they are inefficient to treat very acidic mine water to required standard for environmental discharge (Yu et al., 2021; Yu et al., 2022). Typical components of CWs include sediments (rock, gravel, and soil amongst others), vegetation and water surface. Amongst the different type of CWs, hydroponic floating wetland (HFTW) are the cleanest one since they do not require substrate. Hydroponic floating wetland are buoyant, artificial structure that supports the growth of wetland plants on the water's surface thereby allowing their roots to hang down and accumulate pollutants and nutrients directly from the water column (Lucke et al., 2019). The HFTW improve water quality through biological filtration process, enhancing biodiversity and reducing algal blooms (Kiiskila et al., 2017; Calheiros et al., 2025). In HFTW, plants provide ideal conditions for various biological and physico-chemical process hence enhancing inorganic pollutants removal (Kiiskila et al., 2017). Furthermore, plants promote the growth of metal-oxidizing bacteria by transferring oxygen to the roots zone, provide carbon source (organic matter) for sulphate-reducing bacteria (SRB), thus, differentiating HFTW from sand filters (Carrillo et al., 2026). Furthermore, roots of plants serve as surface for iron plaque which is mostly hydroxides precipitate holding metal atoms together by the attraction between positively charged nuclei and their delocalized valance electrons (Kilian and Pawęska, 2025). Plants develop strategies such as the use of enzymes (enzymes degradation) to break down pollutants and resist their toxicological effects, while reducing their concentration in contaminated environment (Vymazal, 2022; Zhang et al., 2010). Overall, plants play a vital role in HFTW even though their contribution is slow and require an adaptation step to be fully effective. Amongst various species of plants used in CWs technology, Vetiver grass (VG) appears as the most prominent one due to its capacity to resist harsh environmental conditions with a stress tolerance index (STI) generally greater than 1 (STI > 1), and its large biomass of shoots, which survive grazing pressure and roots system which is finely structured, very strong and can grow 3-4 m deeps within the first year (Nuamah et al., 2020). Inorganic pollutants accumulated by Vetiver grass induce physiological regulations since plants use their micro-rhizo environment and more precisely plant growth-promoting rhizobacteria (PGPR) to develop strategies to survive stressful conditions and ensure continuous growth (Iqbal et al., 2024). Although HFTW is an environmentally friendly and cost-effective technique to clean up contaminated soil, water and sediment, different biochemical and biological mechanisms governing inorganic pollutants removal are still unclear. On the other hand, the reclamation of drinking water standard from neutral mine drainage (NMD) using HFTW is yet to be investigated. Considering that, this study was therefore motivated by the need to polish NMD with the ultimate goal to reclaim possible drinking water while gaining a deep understanding of different mechanisms governing inorganic pollutants attenuation in HFTW technology. In this study, the main venture will explicitly be to construct a HFTW planted with VG and assess its potential in polishing NMD to drinking water standard and have a clear understanding of mechanism governing inorganic pollutants attenuation in phytoremediation technology.

MATERIALS AND METHODS

Procurement of Vetiver grass shoots, reagents and sampling of neutral mine drainage

Shoots of VG plants were procured from Vetiver SA Ltd, White River, Mpumalanga Province, South Africa (25.3299° S and 31.0154° E). Reagents were procured from Sigma Aldrich South Africa, while NMD were sampled from an active AMD treatment plant, Gauteng Province, South Africa (26.2326° S and 27.2924° E). Thereafter the collection of NMD, in situ analysis was immediately performed to measure pH, electrical conductivity (EC) and total dissolved solids (TDS) using a multimeter parameter (HANNA instrument, Johannesburg, South Africa).

Growth of Vetiver grass and construction of hydroponic floating wetland

Once at the green house of the University's campus (University of Johannesburg, Auckland Park Kingsway campus), shoots of VG were grown as described in our previous studies (Nguegang et al., 2022) and used to construct the HFTW.

Hydroponic floating wetland design and description

Two identic pilots HFTW were constructed with the help of a plastic drum of 100 litres capacity and a rectangular plastic basin measuring 60 cm (length), 45 cm (width) and 20 cm (height). The first pilot plant was used for treatment while the second pilot plant with the plastic drum containing potable fresh water served as control. The plastic drum and the basin were interconnected using a polyvinyl chloride (PVC) pipe as shown in Figure 1.

Hydrology of the hydroponic floating wetland system

In CWs, hydrology plays a vital role since it significantly influences the performance of the treatment plant. Purposively for this study, the influent flow rate (IFR) within the treatment cell and holding residence time (HRT) were ascertained as described in the following sub-section.

Influent flow rate and holding residence time of the hydroponic floating wetland

The flow of water within the HFTW was calibrated with the help of medical plastic drip bottle. Specifically, the liquid (water) was allowed to flow from the plastic drum to treatment cell in continuous drops for 40 days. Thereafter, the IFR (1.2 litres/day) was determined from inflow rate and outflow rate while the HRT was calculated from IFR as shown in Equations 1 and 2 (Darcy's law).

$$HRT = \frac{V \times d}{\text{IFR}} \quad (1)$$

where: V (m^3) is the volume of the wetland cell, d (%) is the porosity of the substrate and IFR (m^3/d) is the influent flow rate within the wetland cell. Since hydroponic floating wetland without substrate was used in this study, the porosity is 1 and the Darcy's law becomes as shown in Equation 2.

$$HRT = \frac{L \times w \times h}{Q_{av}} \quad (2)$$

where: L (m) and w (m) represent the length and width of the treatment cell, respectively while h (m) is the depth of water in the treatment cell and Q_{av} (m^2/d) is the mean influent flow rate within the system.

$$HRT = \frac{0.6 \times 0.4 \times 0.2}{0.0012} = 40 \text{ days}$$

$$HRT = 40 \text{ days}$$

The low flow rate (1.2 litres/day) and long HRT (40 days) were applied in this study to ensure that the water spend enough time in the treatment cell since low flow rate and long HRT play a vital role in improving the efficiency of the CWs. In addition, this flow rate will require a landscape where fluctuation of water shapes the environment and allows plant and animal life (hydrophytes and specialized fauna) to evolve specific survival traits for water saturation, shifting currents, and changing nutrients levels.

Leachates collection and analysis

Neutral mine drainage samples and leachates (200 mL) collected every day at the outlet of the treatment and control HFTW were filtered using a 0.22 µm nylon syringe filter membrane and

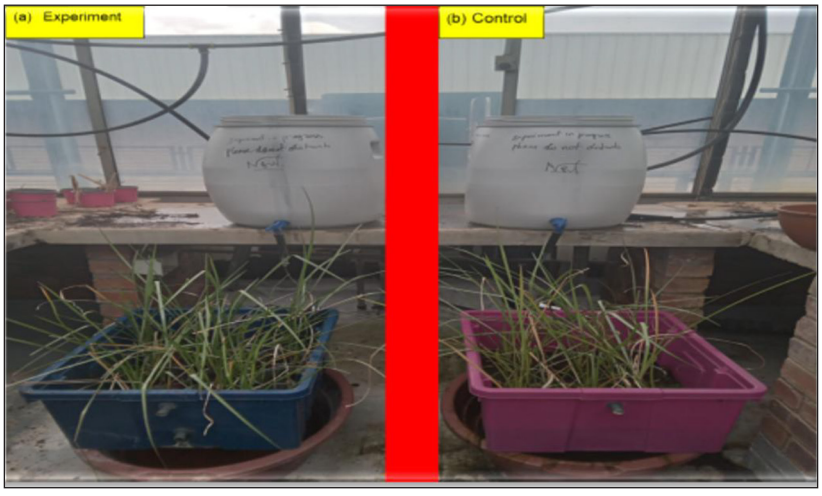

Figure 1. Illustration of the hydroponic floating wetland used to polish NMD in pilot scale

then divided in three sub-samples. Sub-sample one (50 mL) was left non-acidified and analyzed for SO_4^{2-}, sub-sample two (50 mL) was acidified by adding a few drops of 65% HNO_3 to prevent ageing and immediate precipitation of metals and analyzed for metals concentration while sub-sample three (100 mL) was also left non-acidified and used for the determination of pH and EC.

Removal efficiency of HFTW polishing neutral mine drainage

The efficacy or removal efficiency (RE) of the HFTW polishing the pre-treated or NMD was determined gradually following the analysis of leachates for inorganic pollutants concentration. The RE and pH rise were determined as shown in Equations 3 and 4, respectively.

$$RE = \frac{C_i - C_f}{C_i} \quad (3)$$

$$R = pH_f - pH_i \quad (4)$$

where: C_i and C_f are the initial and final concentration of chemical species in NMD and product water, respectively. The pHi is the initial pH of NMD, pHf is the pH of product water and R the rise of pH.

Harvest and characterization of vetiver grass – stress tolerance index (STI) determination

At the end of the experiment, one plant of VG was acquired from each HFTW (treatment and control), washed with ultrapure water (18.5 MΩ.cm) and air dried in a controlled environment for one month. Thereafter, plants were weighed, and the stress tolerance index (STI) was ascertained as shown in Equation 5.

$$STI = \frac{Y_p \times Y_s}{(Y_p)2} \quad (5)$$

Characterization of vetiver grass roots

Thereafter the determination of STI, plants were gently divided into underground and aboveground parts, grinded into powder using Bosch coffee grinder, Germany. One gram (1 g) of each portion was digested in the following conditions: ramping time = 15 min, holding time = 30 min, temperature = 180 °C, time = 1 h, in a blend of 5 mL of 65% HNO_3 and 2 mL of 35% H_2O_2 using microwave digestion system (CEM, Charlotte, USA). The digested samples were treated according to the method reported in literature (Nuamah et al., 2020) followed by the determination of metals and SO_4^{2-} concentrations. Morphology and other microstructural of VG roots from treatment and control wetland were determined with the help of high resolution-scanning electron microscopy-energy dispersive X-ray spectroscopy (HR-SEM-EDS), (TESCAN VEGA 3, LMH, Brno Czech Republic). The mineralogical composition of VG roots was ascertained using powder X-ray diffraction (PXRD) analysis, Philips PW 1710 diffractometer, Netherlands graphite secondary monochromatic source was used for analysis, while Brunauer-Emmet-Teller (BET) (Micromeritics Tritar II, Norcross, GA, USA) was used to determine the pore size, surface area and pore volume.

Bioaccumulation factor (BAF) and root/shoot (R/S) ratio

The R/S ratio allowed to understand the process of pollutants transfer from underground to aerial part of the plant while the BAF compared the content of inorganic pollutants in plant tissue biomass to their concentration in the wetland at the end of the experiment. The BAF and R/S ratio were ascertained as shown in Equations 6 and 7.

$$BAF = \frac{ion\ concentration\ in\ plant\ tissue}{Final\ ion\ concentration\ in\ wetland\ cell} \quad (6)$$

$$R/S = \frac{C_{shoot}}{C_{root}} \quad (7)$$

where: C_{shoot} and C_{root} represent the concentration of inorganic contaminants in shoot and root, respectively.

The *BAF* and *R/S* ratio are vital factors in phytoremediation since they indicate the ability of plants species to accumulate a particular ion from the contaminated environment.

RESULTS AND DISCUSSION

Effect of HFTW planted on pH

Effect of HFTW planted with vetiver grass on pH was determined gradually as the HRT increased and the results are shown in Figure 2.

As depicted in Figure 2, the pH of the NMD increased gradually as a function of HRT. Precisely, the pH increased from the initial pH (7.5) to pH 8.4 with 30 days being the optimum HRT after which further extension of HRT to 40 days has no effect. The increase in pH might be credited to various biochemical and biological mechanisms occurring within the HFTW and specifically around the rhizosphere of the plant. Since it is the HFTW, the main mechanism contributing to pH increase and inorganic pollutants attenuation may be rhizofiltration. In fact, in rhizofiltration process, the pH plays a crucial role in the bio-sorption of inorganic and organic pollutants by modifying their bioavailability and speciation for plants (Bakshe and Jugade, 2023). Furthermore, plants release organic acids, inorganic ions and carbon dioxide (CO_2) *via* respiration process to mineralize organic compound and stimulate nutrient biosorption leading to inorganic pollutants precipitation and attenuation of hydrogen ions (Calheiros et al., 2025). Microbial activity in the rhizosphere zone of the plant promotes the growth of SRB in the presence of SO_4^{2-} leading to an increase in alkalinity and rising of pH (Batool et al., 2022).

Effect of HFTW on inorganics contaminants attenuation

The change in inorganic pollutants concentration and RE of HFTW as function of HRT is reported in Table 1 and Figure 3, respectively.

The results in Table 1 underlines that the HFTW planted with VG removed inorganic pollutants (metals and SO_4^{2-}) and reduced EC gradually as function of HRT. Specifically, the RE of inorganic contaminants as a function of HRT was in the following order: Cu (100%) = Ni (100%) = Zn (100%) > Fe (99.6%) > Al (99.03%) > Mn (94.16%) > SO_4^{2-} (75%) > EC (50.32%) (Figure3). The results further highlighted that the removal of inorganic contaminants progressed slowly as the HRT increased and this may be credited to physiological and molecular process controlling metals accumulation by plants. They include: (i) Metals chelation where plants release enzymes into the wetland cell to bind metals; (ii) cell wall modification which is the change in the composition of plants cell walls in order to bind metals; (iii) metals transporters which are specific transporters present in plants plasma and move the metals into the plants; (iv) vacuole sequestration which is the transfer of metals from plants cytoplasm to their vacuole for storage; (v) metals detoxification which is the use of proteins by plants to detoxify metals; (vi) symbiosis with microorganisms which is the association between plants roots and microorganisms to absorb metals; and (vii) gene expression which is the change in the expression of gene controlling metals transporters (Choi et al., 2021). Metals play a vital role in plant metabolism; as such, the accumulation of metals greatly depends on their significance in plants metabolic function. In particular, Al assists in plant metabolism by promoting roots growth, nutrient extraction, development of plants immune system amongst others (Ofoe et al., 2023), while Fe prevents plants chlorosis, contribute to photosynthesis process by assisting to the synthesis of chlorophyll and ensure the maintenance of chloroplast structure and function (Rai et al., 2021). Likewise, other chemical species including Ni, Zn and SO_4^{2-} also play an important role in plants metabolism. Precisely, Zn participates in seed germination, ensures the coordination and interaction of different hormones in plants and boosts the plant immune system, whereas Ni contributes to the activation of urease (enzyme)

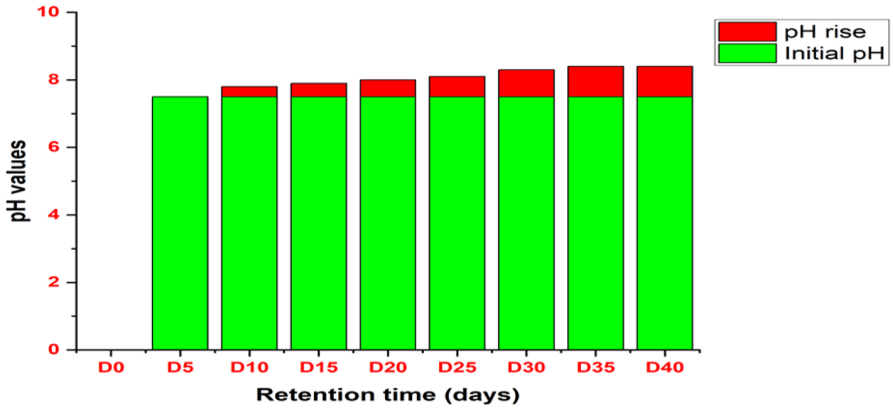

Figure 2. Variation in the pH of product water as a function of hydraulic retention time

Table 1. Variation in the concentration of chemical species as a function of hydraulic retention time

HRT	EC	Al	Cu	Fe	Mn	Ni	Zn	SO_4^{2-}
Day 0	1550 ± 0.7	2.08 ± 0.4	0.08± 0.00	20.1 ± 0.6	1.20 ± 0.2	0.18 ± 0.01	0.28 ± 0.01	2201 ± 0.6
Day 5	1439 ± 0.6	2.00 ± 0.3	0.06± 0.00	17.0 ± 0.3	0.98 ± 0.1	0.13 ± 0.01	0.19 ± 0.01	2113 ± 0.4
Day 10	1328 ± 0.4	1.07 ± 0.2	0.02± 0.00	12.5 ± 0.1	0.92 ± 0.1	0.09 ± 0.01	0.12 ± 0.01	1945 ± 0.3
Day 15	1112 ± 0.3	1.01 ± 0.2	< 0.002	7.05 ± 0.3	0.74 ± 0.3	0.06 ± 0.01	0.08 ± 0.01	1702 ± 0.3
Day 20	1020 ± 0.6	0.65 ± 0.1	< 0.002	2.28 ± 0.2	0.59 ± 0.2	0.04 ± 0.01	0.05 ± 0.01	1520 ± 0.2
Day 25	901 ± 0.4	0.46 ± 0.1	< 0.002	1.23 ± 0.1	0.43 ± 0.1	0.02 ± 0.01	0.02 ± 0.00	1325 ± 0.6
Day 30	882 ± 0.5	0.32 ± 0.1	< 0.002	0.95 ± 0.1	0.26 ± 0.0	0.01 ± 0.01	0.02 ± 0.00	1105 ± 0.4
Day 35	805 ± 0.6	0.05 ± 0.1	< 0.002	0.61 ± 0.02	0.16 ± 0.0	< 0.001	< 0.002	961 ± 0.6
Day 40	770 ± 0.4	0.02 ± 0.1	< 0.002	0.08 ± 0.01	0.07 ± 0.0	< 0.001	< 0.002	798 ± 0.3
RE of HFTW (%)	50.32	99.03	100	99.6	94.16	100	100	63.7

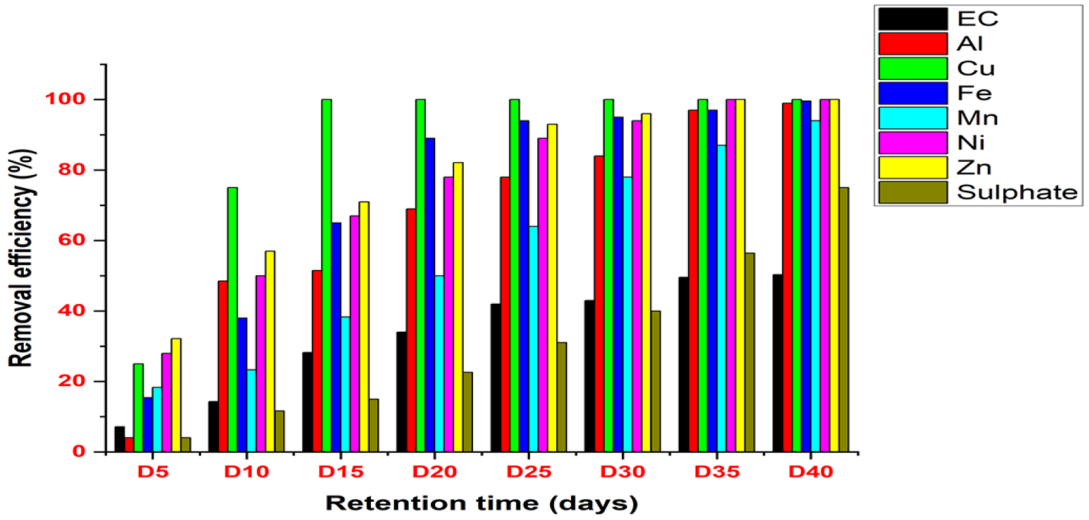

Figure 3. Variation in the percentage removal of chemical species of concern as a function of hydraulic retention time

in plants. Sulphate is a vital source of sulfur for plants since it is assimilated into organic products mostly cysteine which is used to synthesize glutathione and methionine. In addition, sulfur and sulfur-containing compounds in SO_4^{2-} act as signaling molecules in stress management and normal metabolic function (Fabiano et al., 2015; Sinche-Gonzalez and Fornasiero, 2021). Overall, the HFTW allowed to gradually reduce inorganic pollutants to MPLs as set for drinking water standard yielding a worthy of attention results in valorizing NMD.

Overall water quality

The chemical species of product water were compared with the quality of drinking water as set by regulatory bodies and the results are reported in Table 2.

As reported in Table 2, the pH of feed NMD water is 7.5 that is within acceptable level for drinking water standard which is 6.5–8.5 as set by regulatory bodies. However, the content of inorganic pollutants in NMD were above the maximum permissible levels (MPLs) for drinking water standard. After treatment using HFTW planted with VG, there was slight increase of pH from 7.5 to 8.5, moderate reduction of EC, complete removal of Cu, Ni and Zn with 100% RE each and partial attenuation of Al, Fe, Mn and SO_4^{2-} with a RE of 99%, 99.6% 94.16% and 63.6%, respectively. Comparing the physiochemical quality of polished NMD with drinking water standard as set by WHO, it follows that Al, Cu, Fe, Mn, Ni and Zn were within acceptable level while SO_4^{2-} and EC were slightly above the MPLs. From the findings of this study, standard drinking water can be reclaimed from NMD using this emerging passive approach if the HRT is

Table 2. Inorganic pollutants in NMD before and after treatment as compared to prescribed standards, specification, and guidelines for drinking water

Parameters of concern	Feed NMD	Unit	Polished NMD	RE (%)	DWS	WHO	References
pH	7.5		8.4	0.9 (Increment)	5.5–9.7	6.5–8.5	Emmanuel et al., 2024
EC	1550 ± 0.04	µS/cm	770 ± 0.01	50.32	0–150	0–150	de Sousa et al., 2014
Al	2.08 ± 0.03	mg/L	< 0.001	99.1	0–0.3	≤ 0.2	Wynn et al., 2008
Cu	0.08 ± 0.02	mg/L	< 0.002	100	0.100	2	Wynn et al., 2008
Fe	20.1 ± 0.06	mg/L	0.08± 0.00	99.6	0–0.01	0.1–0.3	Wynn et al., 2008
Mn	1.20 ± 0.01	mg/L	0.07± 0.00	94	0–0.05	0.4	Quattrini et al., 2016
Ni	0.18 ± 0.01	mg/L	< 0.001	100	0–0.07	0.07	Quattrini et al., 2016
Zn	0.28 ± 0.00	mg/L	< 0.003	100	0–0.07	5	Quattrini et al., 2016
SO_4^{2-}	1602 ± 1.22	mg/L	407 ± 1.01	75	0–500	0–250	Preethi et al., 2025

extended providing that microbiological analysis will be conducted and that the STI of plants will still be higher than 1 (STI > 1).

Toxicity and detoxification response of Vetiver grass

A STI value allows to assess how a plant or animal can withstand stressful environmental conditions. The calculated STI of this experiment was 1.4 (STI > 1) reflecting a net increase in plant biomass indicating that VG has developed strategies to tolerate harsh or unpleasant environmental conditions confirming the physical appearance of plants that seemed healthy and green at the end of the experiment. This indicates the ability of VG to survive in alkaline pH (8.5). In fact, VG is tolerant to both acidic and alkaline pH as revealed in literature (Beauclair et al., 2022; Kiiskila et al., 2017; Kiiskila et al., 2019) and the findings of this study proved that this plant species adapts itself to different environmental conditions and this may be attributed to its cytogenetic composition, which allows the plant to modify its metabolic functions to withstand stressful conditions.

Bioaccumulation factor (BAF) and root/shoot (R/S) ratio

The BAF is vital to demonstrate the capability of plants to uptake inorganic contaminants while R/S ratio indicates the mechanisms of phytoremediation. Both BAF and R/S were determined, and the results are reported in Table 3 and Figure 4, respectively. It should be noted that the BAF of Cu, Ni and Zn couldn't be calculated since their final concentration NMD were below limit of detection.

The BAF indicates the potential of the plants species to be used in phytoremediation technique. A BAF greater than 1 means a particular ion can be easily accumulated by the plant species. The findings from this study showed that the BAF obeyed the following order: SO_4^{2-} (3.4) > Fe (2.3) > Mn (1.1) > Al (0.8). The BAF values of Cu, Ni and Zn could not be calculated since their final content in treatment wetland were below the limit of detection. Following the BAF values, it can be concluded that there is dissimilitude in ions accumulation by VG and this may be credited to the importance of each ion in plant metabolic function. The BAF of Fe, Mn and SO_4^{2-} were greater than 1 while that of Al was less than 1 meaning VG has accumulated and translocated Fe, Mn and SO_4^{2-} in its shoots. The moderate accumulation of Al by the plants may be credited to plants stress management. In fact, Al has antagonist effect in plants; firstly, it helps plant absorbs phosphorus (P), nitrogen (N) and potassium (K) as well as tolerating abiotic stress, at elevated concentration. Secondly, it slows reactive oxygen species, which can damage cell organelles (Nguegang et al., 2022). Its moderate accumulation by VG means the plant just accumulated the quantity required for its metabolic function. From results in Figure 4, it follows that R/S ratio greater than 1 were recorded for Fe, Mn and SO_4^{2-} while R/S ratio less than 1 were recorded for Al, Cu, Ni and Zn. The findings indicated that VG was able to transfer enough Fe, Mn and SO_4^{2-} from roots (underground parts) to aboveground (shoots) of the plants meaning the main mechanisms used to accumulate these inorganic contaminants is

Table 3. Bioaccumulation factor of the chemical species of concern

Ions	Al	Cu	Fe	Mn	Ni	Zn	SO_4^{2-}
BCF	0.8 ± 0.01	–	2.3 ± 0.04	1.1 ± 0.02	–	–	3.4 ± 0.08

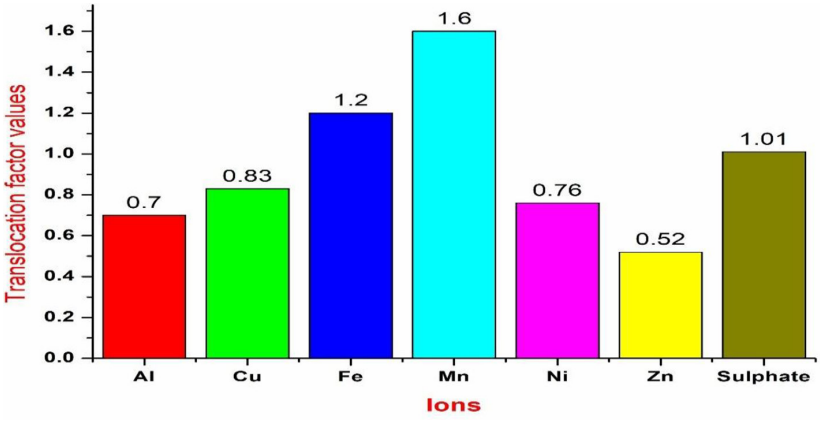

Figure 4. Root/shoot ratio of ions

phytoextraction. The low translocation of Cu, Ni and Zn which are essential ions for plants metabolic function means they were stabilized by plants in the underground parts (phytostabilization). In addition, other essential ions such as Cu, Ni and Zn cause leaves chlorosis, thus, reducing photosynthesis process and this might be the reason why the plants kept them in the roots.

Characterization of the solid samples

PXRD results of vetiver grass roots

Powder X-ray diffraction were performed for roots of VG and the results are shown in Figure 5.

As shown in Figure 5 (a,b), the PXRD patterns of VG roots from both control and treatment cell showed various peaks varying from 2θ = 18° to 2θ = 40°. The roots from control plant showed peak at 2θ = 18 and 21° and this could be indicating cellulose type I and cellulose type II structure following the recrystallization of amylopectin and amylase, respectively; thus, corroborating with the results reported in literature (Ju et al., 2015). Contrary to the roots from control wetland, that from experimental cell showed a sharp diffraction peaks likely indicating the presence of microcrystalline following the contact of VG roots with H_2SO_4 from NMD, both roots showed a peak at 2θ = 40°, which could be indicating the presence of cellulose, thus, corroborating with findings reported in literature (Ju et al., 2015).

Scanning electron microscope results

The HR-SEM spectra of Vetiver grass roots from treatment and control wetland is shown in Figure 6. The morphological structure of VG roots from both HFTW (treatment and control) showed a clear difference. That from treatment cell depicted a leafy like structure across the surface with isolated bright structure, which could represent silica as revealed by EDS spectra. In addition, the SEM image revealed a decaying feature denoting the disruption of macro-fibers resulting from the attenuation of cross-linking structure between cellulose, hemicellulose and lignin leading to the increase of surface area as revealed by BET results (Figure 6b). Contrary to roots from treatment cell, the roots from control wetland (Figure 6a) showed a sort of small palettes clustered together to form a large mass of palettes occupying the whole surface and displaying stomata and rigid structure of cellulose, hemicellulose and lignin arranged into macro-fibers corroborating results reported in previous study (Masilela et al., 2025).

Energy-dispersive X-ray spectroscopy results

The EDS results of VG roots from both HFTW (experiment and control) are shown in Figure 7.

The EDS spectra of roots from control wetland showed the presence of C and O as major elements with percentage composition of 56.6% and 38.9%, respectively while K was present as minor element with percentage composition of

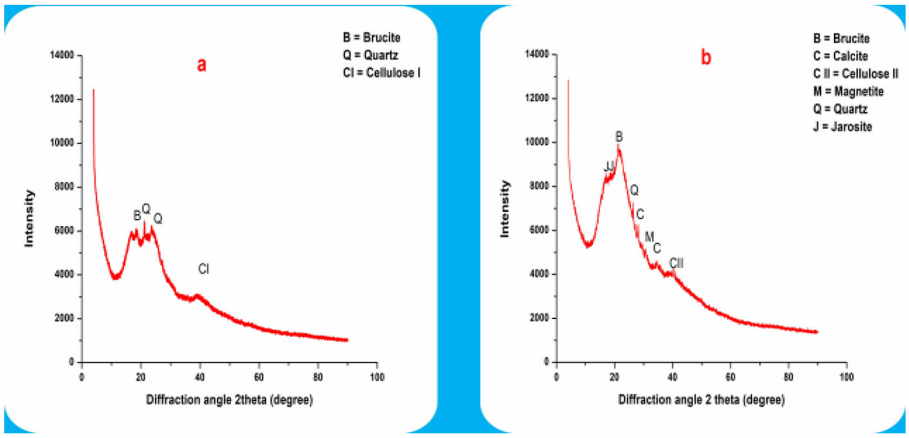

Figure 5. p-XRD patterns of Vetiver grass roots: (a) control and (b) treatment

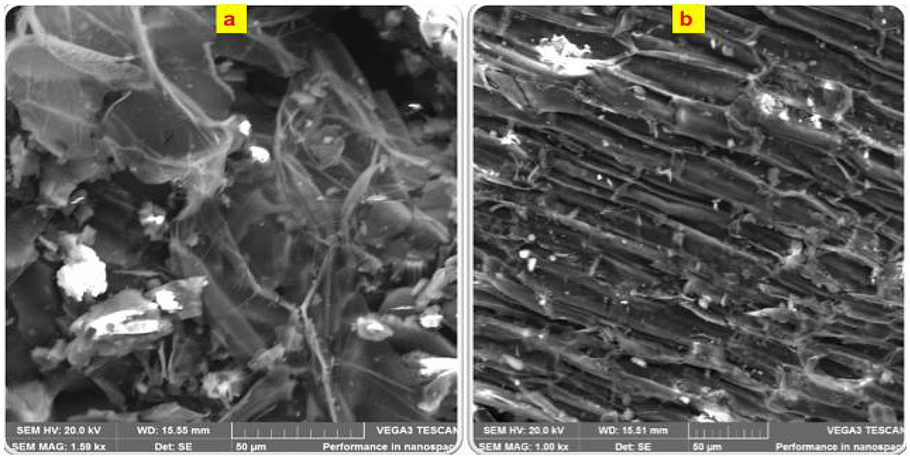

Figure 6. Scanning electron microscopy of vetiver grass roots (a) from control wetland and (b) from treatment wetland

2.1% and traces or impurities of S, Cl, Ca, Mg, Zn and Si with percentage composition of less than 0.7% each. The presence of these chemical species could be assigned to the chemical composition of compost soil used for the germination of plants. The EDS spectra of VG roots from treatment wetland disclosed an increase in the percentage of major elements (O and C) with a percentage composition of 57.0% and 39.2%, respectively and impurity of K, Si, Fe Cl, Al, Cu Mn, Ni and P. Comparing the two EDS spectra, it follows that Al, Fe and Mn were present only in roots from treatment wetland confirming their presence in NMD, thereby, corroborating previous findings (Suelee et al., 2017).

Brunauer- Emmett-Teller results

The surface area, pore diameter and pore volume of VG roots from both treatment and control wetlands were determined using BET method of N_2 adsorption-desorption at –196 °C and the results are reported in Table 4 while the nitrogen adsorption-desorption isotherm curves are shown in Figure 8.

The BET surface area and pore volume of VG roots from treatment wetland slightly increased compared to that from control wetland. Specifically, the surface area of roots from treatment wetland and control wetland were 7.2 and 5.1 m^2/g, respectively, whereas the total pore volume was 0.013 and 0.012 cm^3/g and a pore diameter of 2.65 and 2.78 nm for roots from treatment and control wetland, respectively. The surface area of VG indicates that the macrophytes is hyper accumulator plant species with pore structure, which enhances the uptake and sequestration of pollutants, thereby, confirming the STI as revealed in this study and previous study (Madadgar et al., 2023). These findings corroborated with the nitrogen adsorption-desorption isotherm curves results shown in Figure 8 (a and b), which displayed

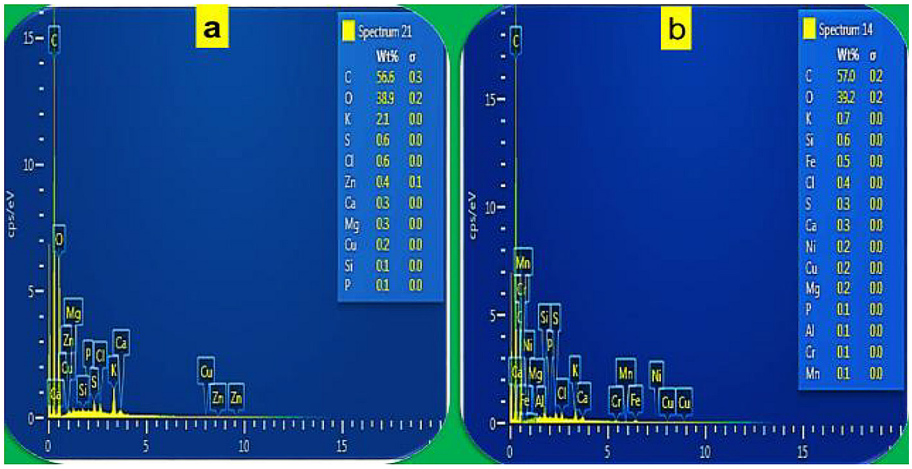

Figure 7. The EDS spectra of vetiver roots: (a) form control wetland, (b) from treatment wetland

Table 4. Surface areas pore volume and pore diameters of roots from control and treatment wetland

Materials	Surface area (m^2/g)	Pore volume (cm^3/g)	Pore diameter (nm)
Control root	5.1 ± 0.01	0.002 ± 0.00	2.78 ± 0.01
Treatment root	7.2 ± 0.03	0.003 ± 0.00	2.65 ± 0.01

with type H_2 hysteresis loop. Considering the IUPAC nomenclature, the P/Po axis with elevated relative pressure (P/Po > 0.4) denotes the super saturation and stability of nitrogen (Bakshe and Jugade, 2023). Furthermore, the classic type H_2 hysteresis loop may be credited to the formation of pores in the clustering of particles while the adsorption isotherm plateau denotes the presence of macro-pores range resulting from the pores sizes distribution.

Mechanism of phytoremediation involved in this study

Various mechanisms involved in phytoremediation technique include hydraulic control, phytoaccumulation, phytostabilization, phytoextration, phytovolatilization and phytofiltration while BAF and R/S ratio are vital parameters indicating the mechanism of phytoremediation. The R/S ratio values of greater than 1 indicate plants capability to transfer inorganic contaminants from roots to shoots. The R/S ratio less than 1 indicates plants inability to effectively transfer inorganic contaminants from roots to shoots. Following the findings of this study, Fe, Mn and SO_4^{2-} had a R/S ratio of 1.2, 1.6, and 1.01, respectively meaning VG was able to effectively transfer these ions from roots to shoots and the mechanism involved is phytoextraction. Contrary to Fe, Mn and SO_4^{2-}, the R/S ratio values of other ions of concern (Al, Cu, Ni and Zn) were less than 1, meaning they were extracted and stored more in the roots and the mechanism involved is phytostabilization. Storing Al, Cu, Ni and Zn in the roots is self-protective strategy for VG to limit their effects on plants growth. Therefore, different phytoremediation mechanisms and their implication in inorganic contaminants removal are described below.

Hydraulic control

The hydraulic control in phytoremediation is a process by which plants act as natural pumps to regulate the water flow around the rhizome of the plants. In fact, plants take up water and use it for their metabolic function and return the excess to the environment through respiration as illustrated by Equation 8.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + \\ + 6H_2O + E\,(ATP) \quad (8)$$

In CWs, the hydraulic control limits the migration of water around the plants rhizome, thus, facilitating the accumulation of inorganic contaminants by plant species (Muthusaravanan et al., 2018). Vetiver grass has a large rhizome that produce dense and aromatic roots, stout and fibrous, which can grow deep into the bottom or

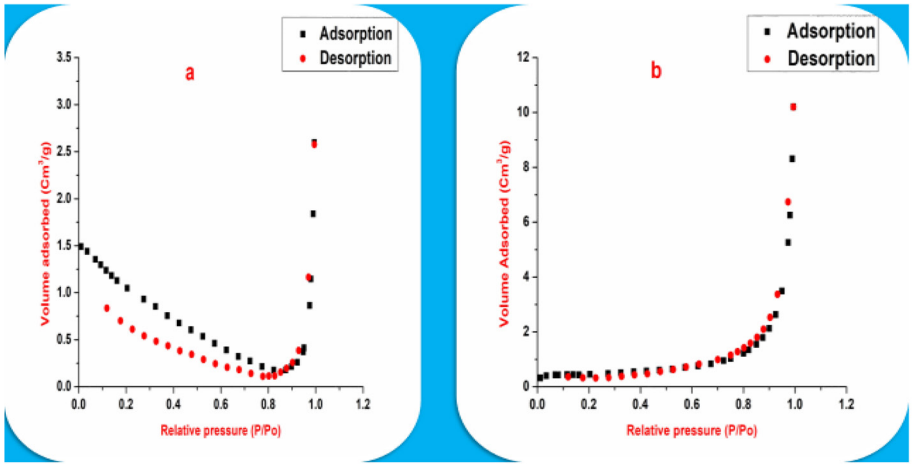

Figure 8. Nitrogen adsorption-desorption of Vetiver grass roots: (a) from treatment wetland and (b) from control wetland

ground helping to accumulate inorganic contaminants and thereby purifying water in the CWs (HFTW in the case of this study). By limiting the flow of water around the rhizome of the plants in HFTW, the hydraulic control then prevents the leaching out of pollutants in the roots zone.

Phytoaccumulation

Phytoaccumulation is a phytoremediation mechanism where plants absorb pollutants from soil or water through their roots and transfer them to the harvestable biomass (shoots and leaves). The specificity of phytoaccumulation is its ability to accumulate inorganic contaminants and store them in different parts (roots, stems and leaves) of the plants without destroying them (Singh and Jain, 2003). The phytoaccumulation is conditioned by the capacity of plants to store elevated concentration of inorganics contaminants without experiencing cytotoxicity. Following the findings of this study, phytoaccumulation is one of the main mechanisms that actively contributed to pollutants removal since the calculated STI was greater than 1 (STI = 1.4) while inorganic contaminants removed from HFTW were stored in different portal of the plant. This results corroborated with previous studies where VG was used as macrophyte plants in CWs treating very acidic mine water (Suelee et al., 2017). The results of this study also revealed that VG is a hyperaccumulator plant species since high level of inorganic contaminants accumulated were stored in the tissues of plants as revealed by greater BAF (> 1) for Al, Fe, Mn and SO_4^{2-}.

Phytodegradation

This mechanism also known as phytotransformation is the mechanism by which plants accumulate inorganic contaminants and break them down into harmless substances. This is done through plant metabolic function, which produce specific enzymes to perform the task (Zheng et al., 2022). The characteristics of contaminants (hydrophobicity and hydrophilicity) play a vital role in phytodegradation process. Inorganic pollutants (metals and SO_4^{2-}) in NMD water are hydrophilic (with surface attracting water) and since the water passes through the plant rhizome where the hydraulic control prevents its leaching out, inorganic pollutants are then accumulated and breakdown into harmless substances (phytotransformation).

Phytostabilization

Phytostabilization is the mechanism of phytoremediation, which limits the movement of pollutants by immobilizing them around the rhizosphere of the plants thereby reducing their movement and toxicological effects (Bakshe and Jugade, 2023). Phytostabilization prevents the transfer of inorganic pollutants from roots to leaves by keeping them immobilized in the rhizosphere. In fact, inorganic pollutants present in HFTW were immobilized by VG *via* various chemical process including adsorption, absorption and precipitation in the rhizosphere. Stabilized inorganic contaminants were sequestered within the roots tissues or adsorbed onto roots cell walls and this can justify the high content of Al, Cu, Ni and Zn in the roots of plants as revealed by a R/S less than 1. Plants play a vital

role in phytostabilization process by reducing the water flow *via* their roots, thereby, stabilizing or immobilizing inorganic contaminants in the rhizosphere leading to the changes in water biochemical properties. The major change in water biochemical properties include acidification, release of organic acids and increase the rate of microbial metabolic functions (Bakshe and Jugade, 2023).

Phytovolatilization

Phytovolatilization is the biochemical mechanism of phytoremediation, which consists to accumulate inorganic contaminants from the polluted sites and convert to harmless substance and then release them to the atmosphere *via* photosynthesis process through the leaves of the plants (Limmer and Burken, 2016). In the case of this study, the phytovolatilization mechanism was used by the plant to purify NMD in HFTW by removing inorganic contaminants and release them onto the atmosphere and this can be justified by the high content of Fe, Mn and SO_4^{2-} in the shoots of VG as revealed by R/S ratio greater than 1. However, phytovolatilization is limited by the toxicity of the inorganic contaminants. In addition, phytovolatilization is effective if contaminants are easily degraded once released onto the atmosphere. Or since the STI obtained from this study is greater than 1, it can be concluded that inorganic contaminants accumulated were easily converted into harmless form and released onto the atmosphere.

Rhizodegradation

Rhizodegradation is a phytoremediation process consisting of the breaking down of contaminants by microbial activity in the plant rhizosphere (Singh and Jain, 2003). Like many plant species, Vetiver grass contains various compounds including carbohydrates and acids which are released in the rhizosphere to enhance the growth of microorganisms. Those microorganisms such as sulphate reducing bacteria (SRB) use sulphate as an electron acceptor to convert SO_4^{2-} into hydrogen sulphide (H_2S) which is then accumulated by plant. Rhizodegradation was vital to degrade SO_4^{2-}, which could not be taken up directly by the plants without the conversion by bacteria. The findings from this study proved that SO_4^{2-} where attenuated by VG likely with the assistance of SRB *via* endophytic activities in the roots zone (Li et al., 2019). In fact, microorganisms present in the rhizosphere were spread and colonized the whole treatment cell, thereby, degrading high concentration of SO_4^{2-} and converted them into H_2S.

Rhizofiltration

Rhizofiltration is a hydroponic based phytoremediation that use aquatic or terrestrial plant to absorb heavy metals from aquatic medium. In Rhizofiltration process, plants grow hydroponically and filter contaminated water through a mass of roots to remove inorganic contaminants. For the present study, rhizofiltration played a vital role in pollutants removal since plant roots were used to absorb pollutants from the NMD and concentrated them in the root system or converted into insoluble form.

Cost-effectiveness of the proposed treatment technology compared to conventional method

Hydroponic floating wetland are highly cost-effective, and low maintenance emerging passive alternatives to active chemical neutralization for treating AMD. Capital costs typically range from 15 to 780 US dollar per square meter, and annual operational costs run from 0.5 to 38 US dollar per square meter, thus, drastically undercutting mechanical plant expenditures (Kiiskila et al., 2017; Calheiros et al., 2025). Cost was mostly driving by the purchase of materials, plants stock and other items used for the construction of floating wetland. During operational phase, expenses are restricted to occasional structural checks, periodic harvesting of metal-laden plant biomass, and managing accumulated metal hydroxide sludge. The use of HFTW eliminates continuous alkaline reagent consumption, which drives the high recurring price tags of conventional AMD treatment plants.

CONCLUSIONS

Hydroponic floating wetland planted with VG was successfully explored for the polishing of NMD and possible reclamation of drinking water. Findings from this study further disclosed that VG is tolerant to NMD as revealed by greater STI (STI > 1) which is confirmed by zero to trivial sign of toxicity after 40 days' HRT. Interestingly, the HFTW removed Al, Cu, Fe, Mn, Ni and Zn to the MPLs as required for drinking water standards whereas, SO_4^{2-} content and EC value were

slightly higher than the MPLs. The findings further revealed that various mechanisms including hydraulic control, phytoaccumulation, phytodegradation, phytostabilization, phytovolatization, and rhizofiltration altogether contributed to inorganic contaminants attenuation as confirmed by BAF and R/S ratio results. This study divulged the efficacy of HFTW planted with VG in polishing NMD and remove inorganic contaminants to prescribed standards, specification, and guidelines for drinking water except for SO_4^{2-} and EC within 40 days retention time. Findings from this study showed that HFTW planted with VG could likely yield the expected results in reclaiming drinking water from NMD providing that the HRT is extended beyond 40 days. This HFTW could be integrated downstream to an active acid mine drainage treatment plant to reclaim drinking water. Furthermore, the accumulated biomass could be managed by scheduled harvesting of dead parts of plants combined with proper disposal or resource recovery to prevent system clogging, nutrient recycling back into the water, and mosquito breeding. Specifically, the harvest of accumulated biomass will be done at the end of growing season before senescence to physically remove trapped nutrients (nitrogen and phosphorus) from the system. The harvest biomass will then be mixed with drier agricultural or yard waste to create safe soil amendments and compost or by processing high-yield non-woody biomass through anaerobic digestion for biogas production.

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