Abstract. This study aimed to discover and explore the impacts and interactions of applying Mn²⁺ and Co²⁺ metals in NH₄ removal performance under nitrification conditions through soil aquifer treatment. A laboratory scale column was used to simulate the soil aquifer treatment system using quartz sand as packed media to infiltrate the synthetic wastewater mixture from 35 mg/L of NH₄, 60 mg/L Mn²⁺ and 40 mg/L of Co²⁺ concentrations. The experimental and linear regression model results demonstrate a significant relationship between NH₃-N concentration with Mn²⁺ and Co²⁺ concentration with a p-value < 0.05, indicating a substantial influence of Mn²⁺ and Co²⁺ presence on ammonium bioremediation. Co²⁺ has a negative correlation interaction with NH₃-N, which means Co²⁺ has increased ammonium oxidation by stimulating the degradation bacteria and cell growth to supplement and improve the activity of ammonium degradation bacteria. However, applying a high concentration of Mn²⁺ works as an inhibitor for NH₃-N bioremediation. Using a high concentration of Mn²⁺ negatively impacts bacteria, causing a toxic effect and decreasing the bacteria's degradability for ammonium.

1 Introduction

Ammonium (NH₄) flowing freely from agricultural, industrial, and household pollution sources into water streams is currently a worldwide concern. The existence of NH₄ in unacceptable high concentration levels in water bodies causes growth in oxygen demand. It influences aquatic biodiversity by obstructing and decreasing the capability to remain alive, develop, and propagate [1]. Various ammonium (NH₄) removal methods are based on biological, physical, and chemical removal processes for nitrogen species from water and wastewater effluents. The most common ammonium (NH₄) removal method is carried out by biological degradation, which is known as nitrification and denitrification reactions to oxidize the nitrogen species that oxidize NH₄ to NO₂⁻ and NO₃⁻ at nitrifying response followed by denitrifying NO₃⁻ to N₂ gaseous [2].

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Ammonium biological degradation is considered the most environmentally friendly removal process, compared with physical and chemical removal methods. Nitrification and denitrification are naturally oxidation processes with low energy and operation requirements. Furthermore, the nitrogen species in wastewater effluent can be eliminated using aerobic denitrification bacteria [3]. Aerobic denitrification is a biological process that occurs in the presence of oxygen, where certain microorganisms convert nitrate (NO$_3^-$) into nitrogen gas (N$_2$) [4]. Unlike traditional denitrification, which occurs in oxygen-depleted environments, aerobic denitrification takes place under aerobic (oxygen-rich) conditions. It is a natural mechanism for reducing excess nitrate levels in environments such as oxygenated regions of aquatic systems or well-aerated soils. Aerobic denitrification is carried out by specific bacteria and fungi that possess the necessary enzymes to facilitate the conversion of nitrate into nitrogen gas, contributing to the removal of nitrogen compounds from ecosystems [5].

However, the biodegradation bacteria are sensitive to several parameters to achieve a high nitrogen species removal performance in the wastewater system, like as dissolved oxygen level, carbon resources components, temperature, pH and macro and micronutrients. These factors must be in sufficient amounts and suitable species to avoid any metabolic limitation that may harm the degradation of bacteria efficiency [6]. Nitrogen biodegradation bacteria need a sufficient amount of micronutrients like Manganese (Mn), Cobalt (Co), Zinc (Zn), Copper (Cu), Molybdate (Mo), Iodine (I), Boron (B) and Iron (Fe) to secure the bacterial cells metabolism [7].

Cobalt (Co$_2^+$) is usually detected in polishing and painting wastewater from the porcelain and steel industries [8]. Co$_2^+$ has been demonstrated as the minimal poisonous metal between Ni$_2^+$, Zn$_2^+$, and Cu$_2^+$ in nitrifying conditions [9]. Gikas [10] abbreviated the impacts of Co$_2^+$ on activated sludge and discovered that Co$_2^+$ is performed as a cell development incentive instead of a controller or inhibitor. Also, he found that low concentrations of Co$_2^+$, less than 2 mg/L, have never turned toxic to microorganisms. Likewise, manganese (Mn$_2^+$) is regularly initiated in enriched wastewater involving wastewater with high nitrogen species concentrations. However, the nitrogen species purification in nitrifying anaerobic ammonia-oxidizing capacity is developed by adding Mn$_2^+$ in long time reactions [11]. Huang, Gao, PengTao [12] investigated nitrogen purification capacity in activated sludge, which will be multiplied when adding an Mn$_2^+$ concentration of 2.8 mg/L. Many studies have discussed and discovered the effects of heavy metals in ammonium oxidation on aerobic denitrification and activated sludge. However, rare studies deal with heavy metals' impact on ammonium removal under aerobic nitrification conditions. Therefore, this study will present the implications and interrelations of infiltrating two types of heavy metals (Mn$_2^+$ and Co$_2^+$) with ammonium (NH$_4^+$) through soil aquifer treatment columns under aerobic nitrification.

### 2 Materials and methods

A plexiglass laboratory scale column using to simulate the soil aquifer treatment system with a 3 cm internal diameter packed with quartz sand at a 30 cm depth applied to explore the impact and relations of adding Mn$_2^+$ and Co$_2^+$ metals with the NH$_4^+$ in the same synthetic wastewater solution and infiltrating through sand media at room temperature of 22 ℃. Quartz sand is used as filtrate-packed media with particle sizes range 0.50 – 0.125 mm, 0.42 porosity and 1.48 dry bulk density. A synthetic wastewater solution was designed by mixing NH$_4$CL, MnSO$_4$.4H$_2$O and CoCL$_2$.6H$_2$O in sufficient amounts to prepare concentrations of 35 mg/L NH$_4^+$, 60 mg/L Mn$_2^+$ and 40 mg/L Co$_2^+$ and pH 6.5. NH$_4^+$ was measured as NH$_3$-N using the direct reading spectrophotometer device according to the Nessler method, while Mn$_2^+$ and Co$_2^+$ were measured using a microwave plasma–atomic emission spectrometry device (MP-AES). A linear regression model applies to explore the interaction relationship and the impact
behavior of Mn$^{2+}$ and Co$^{2+}$ presence on NH$_4$ performance through filtrating in the quartz sand column under the nitrification conditions.

### 3 Results and discussions

Dissolved oxygen (DO), pH and oxidation-reduction potential (ORP) are the critical functions for nitrification oxidizing efficiency. An average DO > 2 mg/L, pH 6.5 and ORP range of 180 – 220 mV were performed to save a nitrifying behaviour through the sand column. Fig. 1 presents the breakthrough curves for NH$_3$-N, Mn$^{2+}$ and Co$^{2+}$ through quartz sand column during six days of synthetic wastewater infiltration.

![Fig. 1. NH$_3$-N, Mn$^{2+}$ and Co$^{2+}$ breakthrough curves with time profile through quartz sand column.](image)

Fig. 1. NH$_3$-N, Mn$^{2+}$ and Co$^{2+}$ breakthrough curves with time profile through quartz sand column.

Fig. 2 presents the interaction curves between NH$_3$-N with Mn$^{2+}$ and Co$^{2+}$ and the concentration fluctuation of Mn$^{2+}$ and Co$^{2+}$ over the NH$_3$-N mean. Figure 2 shows a substantial change in NH$_3$ concentration to the Mn$^{2+}$ and Co$^{2+}$ concentrations, indicating a strong interrelationship and impaction between NH$_3$ with Mn$^{2+}$ and Co$^{2+}$.

![Fig. 2. Interaction behavior between NH$_3$-N with Mn$^{2+}$ and Co$^{2+}$.](image)

Fig. 2. Interaction behavior between NH$_3$-N with Mn$^{2+}$ and Co$^{2+}$. 
Perform a linear regression model to show and model the relationship behaviour between NH$_3$-N and Mn$^{2+}$, and Co$^{2+}$ based on P-value and variance inflation factor (VIF) as statistically significant parameters. The interrelation model between NH$_3$-N and Mn$^{2+}$ as linear regression gives a 0.020 p-value and 66.30 VIF value. P-value < 0.05 indicates a substantial relationship between NH$_3$-N and Mn$^{2+}$ with a high correlation behaviour between NH$_3$-N and Mn$^{2+}$ according to a high VIF value. Moreover, the interrelation model between NH$_3$-N and Co$^{2+}$ as linear regression gives a 0.037 p-value and 69.40 VIF value. P-value < 0.05 indicates a substantial relationship between NH$_3$-N and Co$^{2+}$ with a high correlation behaviour between NH$_3$-N and Co$^{2+}$ according to a high VIF value. Fig. 3 presents the linear factorial regression model relation between NH$_3$-N with Mn$^{2+}$ and Co$^{2+}$.

As shown in fig. 3, Co$^{2+}$ is negatively correlated to the NH$_3$-N concentration. The biodegradation of NH$_3$ through quartz sand is increased when the Co$^{2+}$ concentration is increased. This means the appearance of Co$^{2+}$ metals are developed the nitrification oxidation as a result of Co$^{2+}$ stimulating the degradation of bacteria and motivating the bacteria growth cells. The positive effect of cobalt (Co$^{2+}$) on ammonium (NH$_4$) degradation in soil column could be due to its role in stimulating the activity of nitrifying bacteria. Co$^{2+}$ is an essential component of vitamin B12, which is an important cofactor in many enzymatic reactions in the microbial community, including nitrification. Therefore, Co$^{2+}$ can enhance the growth and activity of nitrifying bacteria, which in turn can increase the rate of NH$_4$ oxidation to nitrate (NO$_3^-$) in soil. Many researchers have demonstrated this, like Kim, Park, Cho, Nam, ParkBajpai [13] and He, Xie, Ni, LiLi [3]. Despite that, Mn$^{2+}$ is positively correlated to the NH$_3$-N concentration. This means the Mn$^{2+}$ appearance did not stimulate the NH$_3$ oxidation and works as an inhibitor for NH$_3$ degradation that is related to the high applied Mn$^{2+}$ concentration, which may cause toxic bacteria that reduce the NH$_3$-N removal capacity. Mn$^{2+}$ can act as an inhibitor of the microorganisms that perform nitrification, One possible mechanism for this inhibition is the adsorption of Mn$^{2+}$ onto the nitrifying bacteria cells, which can interfere with their metabolism and reduce their activity. Additionally, Mn$^{2+}$ can compete with the nitrifying bacteria for the oxygen and other nutrients that they require for their growth and activity. Overall, the presence of Mn$^{2+}$ in high concentrations can slow down the nitrification process, leading to the accumulation of NH$_4$ in the system. This concluded relation between the NH$_3$-N and Mn$^{2+}$ was demonstrated by He, Xie, Ni, LiLi [3]. However, many studies indicate that Mn$^{2+}$ metals significantly improve anaerobic ammonium oxidation bacteria by applying a low Mn$^{2+}$ concentration. Huang, Gao, PengTao [12] found a double ammonium removal capacity when adding Mn$^{2+}$. Li et al. (2018) also discovered that nitrogen species removal developed when adding Mn$^{2+}$ to the nitrifying anaerobic
ammonium oxidizing process. These results indicate that Mn$^{2+}$ is essential for nitrogen species bioremediation.

4 Conclusion

These results demonstrated the strong relationship between NH$_4$, Mn$^{2+}$ and Co$^{2+}$. There is a negative correlation between NH$_3$-N concentration with Co$^{2+}$ concentration, and the appearance of Co$^{2+}$ stimulates the bacteria degradation activities and the growth of cells. These factors increase the NH$_4$ oxidation and removal capacity. While Mn$^{2+}$ has positively correlated with the NH$_3$-N concentration, which means Mn$^{2+}$ metals have worked as an inhibitor for NH$_4$ degradation capacity according to the high Mn$^{2+}$ concentration under aerobic nitrification conditions. In general, the interaction between ammonium NH$_4$ and metals can stimulate or inhibitor for NH$_4$ degradation process according to the concentration level and the degradation environment conditions.

References
