Evaluating Lettuce Growth in Controlled Hydroponic Systems Using AnMBR-Treated Municipal Wastewater Mixed with Compost Leachate

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Abstract

Global water scarcity and the heavy agricultural reliance on freshwater necessitate innovative approaches to reusing treated municipal wastewater. This study evaluates the integration of an Anaerobic Membrane Bioreactor (AnMBR) treating a mixture of municipal wastewater and compost leachate (90:10 ratio) with an indoor hydroponic lettuce cultivation system. We assessed four hydroponic conditions over 14 days: a tap water control (Case A), AnMBR effluent (Case B), aerated AnMBR effluent (Case C), and aerated AnMBR effluent with a 450 ppm nitrate addition (Case D). The raw AnMBR effluent (Case B) significantly outperformed the baseline, yielding a maximum wet plant weight of approximately 4.3 g—an over 4× increase compared to the ~1 g baseline—and the largest total green leaf area. In addition, element accumulation in lettuce tissues and irrigation water was analyzed using ICP-MS, and transfer factors were calculated to evaluate element uptake by the plants. However, tissue analysis revealed considerable differences in element accumulation among treatments, with several elements showing substantial transfer from the irrigation water into plant tissues. The results demonstrate that AnMBR effluent can effectively support lettuce growth under controlled hydroponic conditions, but the observed element accumulation highlights the importance of monitoring water-to-plant transfer and potential food-safety risks. Further long-term studies are needed to optimize nutrient conditions and assess the suitability of treated wastewater for practical hydroponic food production.

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1 Introduction

Global water scarcity currently affects 25 countries, with projections indicating that up to 3.1 billion people will face water supply shortages by 2050. Agriculture accounts for approximately 72% of all global freshwater withdrawals, making it the dominant driver of water demand and a primary target for conservation efforts (Mekonnen & Hoekstra, 2016). At the same time, the volume of water already used and discharged as wastewater constitutes a resource that remains largely untapped: an estimated 380 billion m^3 of wastewater is generated globally each year, most of it of municipal origin, with a large share released to the environment with little or no treatment (Qadir et al., 2020).

Saudi Arabia is one of the 25 countries currently facing extremely high water stress, a status driven by its hyper-arid climate. Average annual precipitation across the Kingdom is below 100 mm, and much of the interior receives markedly less; with no perennial rivers or lakes, the country depends instead on two energy-intensive sources for its freshwater (Odnoletkova & Patzek, 2023). Thermal and reverse-osmosis desalination supply roughly two-thirds of municipal demand, while non-renewable "fossil" groundwater is drawn down to meet approximately 80% of agricultural water needs (Odnoletkova & Patzek, 2023). Agriculture alone accounts for around 67% of the country's total freshwater consumption, while its municipalities treat more than 6.5 million m^3 of wastewater daily — roughly 2.4 billion m^3 per year — of which only about a quarter is currently reused (Odnoletkova & Patzek, 2023). In response, Saudi Vision 2030 and the National Water Strategy set a target of raising treated-wastewater reuse to 70% by 2030, while the Kingdom's Circular Carbon Economy framework recasts municipal wastewater treatment plants as resource-recovery facilities rather than disposal endpoints (Ali et al., 2022). Water conservation, food security, and wastewater reuse are consequently treated as linked national priorities rather than separate policy areas.

Anaerobic membrane bioreactors (AnMBRs) are one of the technologies being positioned to close the gap between the wastewater Saudi Arabia generates and the share it currently reuses. An AnMBR combines anaerobic digestion with membrane filtration in a single unit; because the membrane retains biomass independently of the flow of water through the reactor, the hydraulic retention time can be decoupled from the solids retention time, giving near-complete biomass retention, shorter hydraulic retention times, higher mixed-liquor solids concentrations, and higher volumetric organic loading rates than conventional anaerobic digesters achieve (Elmoutez et al., 2023). Because the process is anaerobic, it requires no aeration, and the methane recovered in the resulting biogas can offset most or all of the energy the reactor consumes, allowing AnMBR to function as a net energy producer under favorable conditions (Elmoutez et al., 2023). Reported methane content in AnMBR biogas treating municipal wastewater has reached 80–83%, with yields as high as 0.35 L CH_4 per gram of COD removed under stable mesophilic operation. The compact footprint that follows from this membrane-based design also makes AnMBR well suited to smaller, decentralized installations located closer to where the treated water and recovered energy are needed (Elmoutez et al., 2023).

Because AnMBRs remove very little nitrogen or phosphorus during treatment, ammonia and phosphate remain largely dissolved in the permeate rather than being incorporated into sludge or biomass, leaving a nutrient-bearing effluent that is better suited to fertigation than to potable reuse. A review of 50 pilot- and laboratory-scale AnMBR systems treating

municipal wastewater confirmed that this effluent can be used for agricultural irrigation under stable operation (Huang et al., 2024). Consistent with this, a study evaluating a Vegetated Biofiltration System (VBS) irrigated with AnMBR effluent found that plant grew taller (94.8 cm versus 52.6 cm), greener, and visibly more robust than an identical system fed with chlorinated aerobic membrane bioreactor effluent, with leaf chlorophyll concentrations roughly 2.9 times higher (Xiong et al., 2025). Together, these findings support the direct reuse of AnMBR effluent for landscaping and cultivation and where the application does not require potable-grade water quality.

While direct irrigation is viable, utilizing hydroponic (soilless) systems offers a superior alternative to traditional soil cultivation, particularly in Saudi Arabia. The region's native sandy soils are highly permeable and dry, leading to rapid water absorption and substantial losses through evaporation and transpiration. High soil-water tension in these arid environments also makes it difficult for plants to extract moisture, driving up overall agricultural water consumption. Hydroponic systems circumvent these issues by minimizing water loss and providing a highly controlled growth environment. Therefore, this research bridges these technologies by evaluating lettuce growth in controlled hydroponic systems using AnMBR-treated municipal wastewater mixed with compost leachate, providing a sustainable and water-secure agricultural solution

2 System Description

- **Objectives**.
    - To evaluate the effluent quality and treatment efficiency of an AnMBR when treating a mixture of municipal wastewater and compost leachate.
    - To assess the applicability of reusing the treated AnMBR effluent as a nutrient source for control hydroponic agriculture.
    - Evaluate the quality and nutrient composition of the AnMBR-treated wastewater and compost leachate mixture.
    - Assess lettuce growth under different hydroponic treatment conditions.
    - Monitor plant growth parameters, including plant height, root length, leaf number, leaf area, biomass, and leaf yellowing.
    - Evaluate changes in water quality and nutrient concentrations during plant cultivation.
    - Assess the accumulation of selected elements in lettuce tissues
- **Problem statement.**

    Saudi Arabia faces severe water scarcity due to its hyper-arid climate, limited rainfall, and heavy dependence on desalinated and groundwater resources. Concurrently, conventional soil-based agriculture consumes vast amounts of freshwater, a challenge exacerbated by dry and sandy soils that reduce water availability to plant roots and cause additional losses. While municipal wastewater presents a promising alternative water source, its direct agricultural reuse is currently hindered by concerns regarding water quality, nutrient balance, and overall plant performance. Consequently, there is a critical need to investigate sustainable treatment and agricultural systems that can safely recover and reuse municipal waste streams while significantly reducing freshwater consumption. To address this need, this project investigates the applicability of utilizing AnMBR-treated effluent for indoor hydroponic irrigation. The

AnMBR was selected because it is an energy-efficient treatment technology that produces high-quality effluent suitable for direct agricultural reuse. Furthermore, its compact design and reduced physical footprint make it highly viable for decentralized, urban installations.

- **Key assumptions or limitations of the problem.**

Ammonia Toxicity Limit: A significant limitation of the application is the sensitivity of the crops to specific nitrogen forms; utilizing ammonium proportions greater than 39% causes severe plant toxicity in lettuce, hindering ion absorption and reducing overall production yields (De Souza et al., 2021).

Element Accumulation: The treated wastewater may contain elevated concentrations of specific elements that can accumulate in the hydroponic solution and plant tissues, potentially affecting lettuce growth and food safety.

Pathogen Effects on Plants: The potential effects of pathogens and microorganisms in the treated wastewater on lettuce plants, roots, and plant tissues were not investigated.

Short Experimental Duration: The system was operated for less than one month; therefore, the results provide preliminary evidence and cannot fully demonstrate the long-term stability and performance of the proposed system.

Laboratory-Scale Conditions: The study is conducted under controlled hydroponic conditions; therefore, the results may not fully represent the long-term performance or large-scale application of the proposed system.

- **Summary of the proposed system and how it solves the problem**

The hydroponic system consists of eight tanks where each two tanks represent one case, and we assess four different cases, and we do this to avoid bias and ensure random selection. Case A is our control sample with tap water used for irrigation (represent tank 1 and 7), Case B is the AnMBR effluent only used (tank 4 and 6), where Case C (tank 3 and 5) and D use AnMBR effluent we supply oxygen to both cases and add 450 ppm concentration of nitrate solution for case D to minimize the effect of high ammonia concentration.

The layout of the hydroponic system is shown in the figure bellow. The hydroponic system was housed inside a wooden cabinet equipped with two 30W Full-spectrum LED light. Environmental parameters were monitored via sensors installed on the left interior wall: one multi-sensor recorded CO_2, air temperature, and relative humidity, while a second sensor measured light intensity. Water temperature was recorded manually across each tank using an immersion probe. The setup contained eight 9.5 L tanks, each featuring 20 growth ports for lettuce plants.

A constant influent flow rate of 5 mL/min was maintained per tank, yielding a full liquid turnover approximately every 32 hours. For Case D, the additive stock (Nitrate solution) was introduced at 0.5 mL/min and mixed with 4.5 mL/min of AnMBR effluent through a Y-connector prior to entering the tanks.

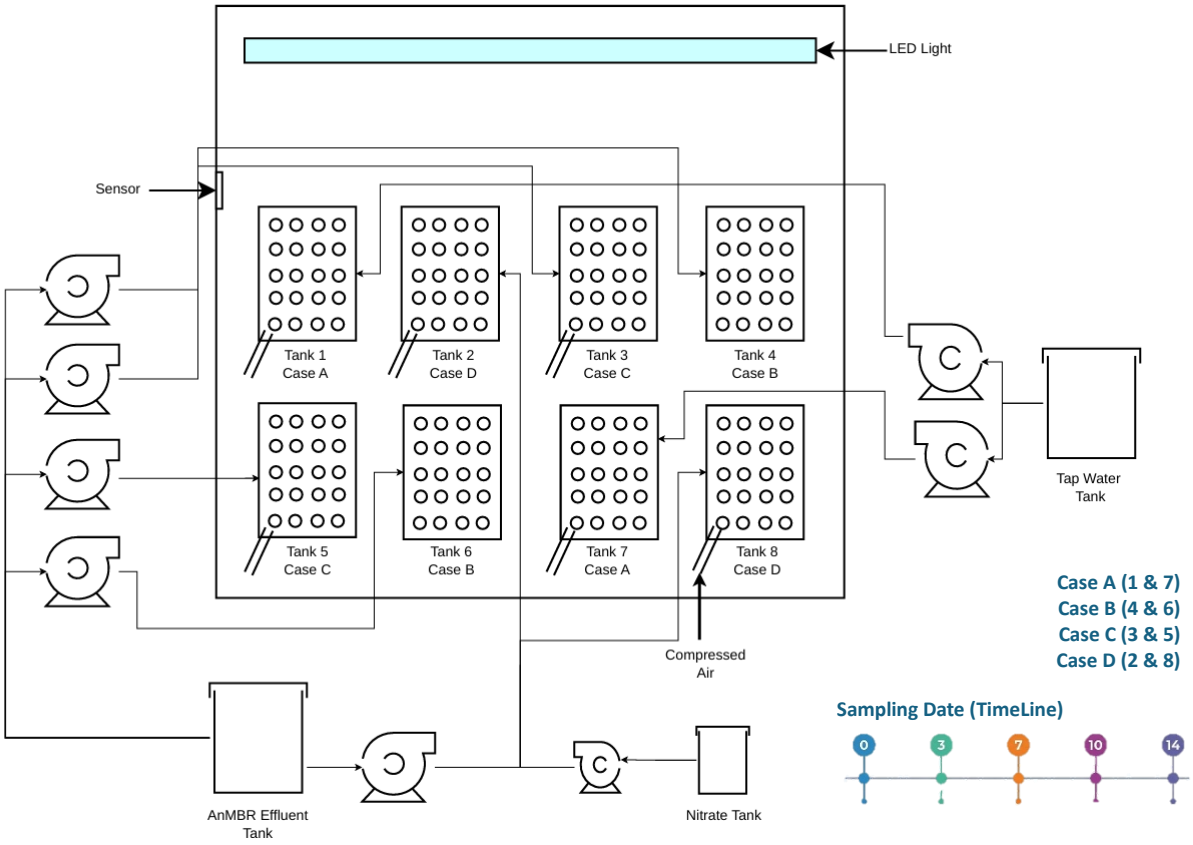


Figure 1: Hydroponic System Layout.

Additionally, the mixture of leachate and municipal wastewater is treated by AnMBR unit and this paragraph will introduce a brief description of the system layout and working flow. The municipal wastewater (MWW) is stored in tank 1, and the compost-extracted leachate is stored in tank 2. A mixture of 10% leachate and 90% MWW is prepared in tank 3 due to the lower organic strength, and then the mixture is pumped into the cylindrical bioreactor (R1) at a flow rate of 60 mL/min. In this stage, the complex organic compounds are digested, and anaerobic bacteria grow. The temperature of the digester is maintained between 40 °C and 45 °C using an external thermal heating jacket. Figure 1 illustrates the complete layout of the system.

The mixed liquor then moves into the adjacent column (R2), where it passes through the hollow-fiber membrane using peristaltic pump. The treated water is pulled through the microscopic pores, leaving the biomass behind, which is returned to the bioreactor. A continuous gas recirculation system is applied to scrub away the accumulating biofilm and mitigate fouling. In this system, biogas is collected from the headspace of R2, compressed, and bubbled up from the bottom of the membrane module. Concurrently, excess biogas is captured in a collection bag connected to the top of the R2 column. Additionally, the membrane is backwashed twice a week to prolong its lifetime. Finally, the treated effluent is used to irrigate the lettuce under indoor hydroponic system.

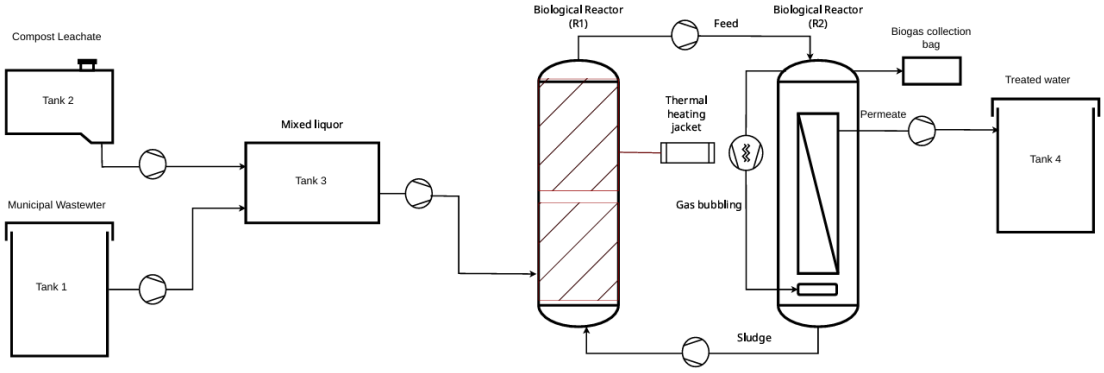


Figure 2: AnMBR system layout.

3 Methodology

3.1 Method details

Lettuce plants were germinated on rolled tissue paper saturated with tap water for 11 days, after which the water was replaced with a nutrient solution for an additional 6 days. The tissue was then carefully removed from the root systems, and the seedlings were transferred to the hydroponic system. Simultaneously, the pump, sensors, and supporting equipment for the hydroponic system were installed and calibrated to ensure proper operation.

Following seedling transfer, four plant samples were taken from each tank to measure physical parameters, including water content, plant height, root length, and leaf area (categorized by green, yellow, and brown regions). The plant samples were then preserved for ICP-MS analysis. Water samples were collected from each tank at the same intervals for heavy metal testing. Sampling commenced on Day 0 (system startup) and continued Days 3, 7, 10, and 14.

System conditions were monitored using environmental sensors for air and water temperature, CO2 levels, relative humidity, and light intensity. Water pH, electrical conductivity (EC), total dissolved solids (TDS), and salinity were measured using a multi-parameter meter, while dissolved oxygen (DO) inside each tank was also recorded. Additionally, concentrations of nitrite, nitrate, ammonia, phosphorus, total nitrogen (TN), and chemical oxygen demand (COD) were measured using Hach test kits at the first (Day 0) and third (Day 7) sampling points.

For heavy metal quantification, plant samples were dried, ground, and digested. Both digested plant tissue and water samples were analyzed via inductively coupled plasma mass spectrometry (ICP-MS) to quantify the concentrations of 12 elements:

- Vanadium (V)
- Chromium (Cr)
- Manganese (Mn)
- Iron (Fe)
- Cobalt (Co)

- Nickel (Ni)
- Copper (Cu)
- Zinc (Zn)
- Arsenic (As)
- Selenium (Se)
- Cadmium (Cd)
- Lead (Pb)

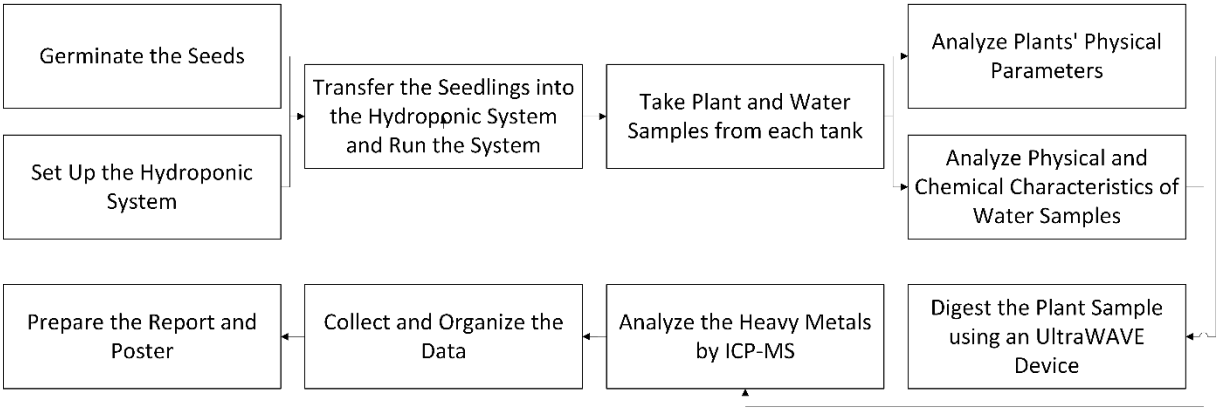


Figure 3: Flowchart of Project Methodology.

Finally, all experimental data were collected, organized, and analyzed to draw conclusions. These findings were then synthesized for the preparation of the final project report and poster. Figure 3 illustrates the complete project methodology flowchart.

4 Results

Water quality in each tank

Table 1: water quality in each tank

Tank	Nitrite (mg/L)	Nitrate (mg/L)	Ammonia (mg/L)	Phosphorous (mg/L)	TN (mg/L)	COD (mg/L)
1 (Case A)	0.003	0.001	0.1	0.039	2.45	2.25
2 (Case D)	0.323	15.6	18.1	4.12	42.5	35.8
3 (Case C)	0.102	0.429	18.6	4.48	70.1	39.2
4 (Case B)	0.005	0.394	20.1	4.23	23.4	49.4
5 (Case C)	0.061	0.412	18	4.26	22.6	39.5
6 (Case B)	0.004	0.393	18.3	4.18	30.7	48.5
7 (Case A)	-	-	0.4	0.047	-	6.7
8 (Case D)	0.659	16	18.1	3.79	36.5	36.8

4.1 Plant Height and Root Development

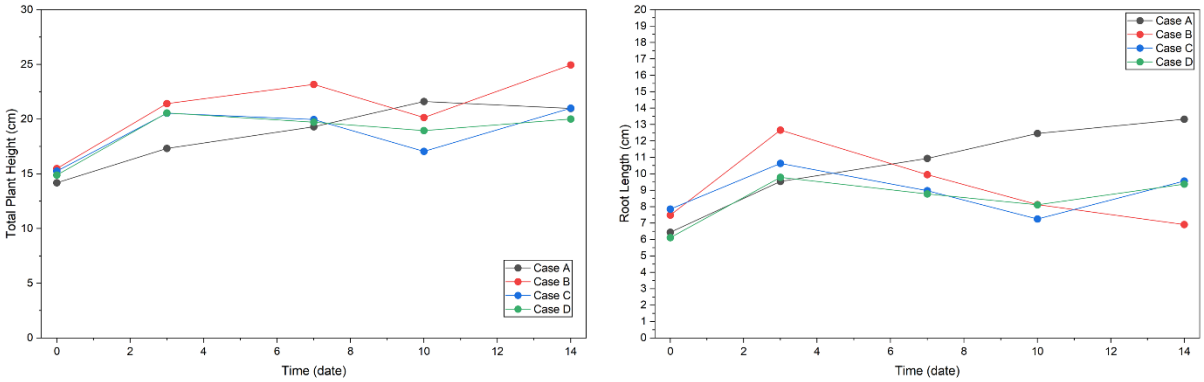


Figure 4: Total Plant Height and Root Length (cm) Over Different Days.

Plant height and root length were monitored over the 14-day cultivation period for the four experimental conditions. Case A, which received tap water, was used as the baseline for comparison because it represents the conventional water condition without AnMBR-derived nutrients. Cases B, C, and D represent increasing levels of modification to the AnMBR effluent, with Case C receiving aeration and Case D receiving both aeration and nitrate supplementation. The experimental configuration and treatment assignments are described in the system design.

Plant height increased in all four cases during the experiment, although the magnitude and temporal pattern differed among treatments. At Day 0, plant heights were approximately 14.2 cm for Case A, 15.5 cm for Case B, 15.0 cm for Case C, and 14.9 cm for Case D. By Day 3, Case B had increased to approximately 21.4 cm, while Cases C and D reached approximately 20.5 cm and 20.4 cm, respectively. Case A increased more gradually to approximately 17.4 cm. At Day 7, Case B reached approximately 23.1 cm, the highest value among the treatments at that sampling point. Case A, Case C, and Case D were approximately 19.3, 19.9, and 19.6 cm, respectively. At Day 10, Case A temporarily reached approximately 21.6 cm, while Case B decreased to approximately 20.2 cm, Case C decreased to approximately 17.1 cm, and Case D was approximately 19.0 cm. By Day 14, Case B again produced the greatest plant height at approximately 24.9 cm, followed by Case A at approximately 21.1 cm, Case C at approximately 21.0 cm, and Case D at approximately 20.0 cm.

Relative to the tap-water baseline, the final plant height in Case B was approximately 18% higher, while Cases C and D were approximately equal to or slightly below the control. These results indicate that AnMBR effluent alone supported strong shoot development during the experimental period.

Root development showed a different pattern from shoot development. All treatments showed an initial increase in root length, but the final root lengths did not follow the same ranking as shoot height. Case A increased from approximately 6.5 cm at Day 0 to approximately 13.3 cm at Day 14, representing the greatest final root length. Case B initially increased strongly, reaching approximately 12.7 cm at Day 3, but declined to approximately 7.0 cm by Day 14. Cases C and D reached approximately 9.6 and 9.5 cm at Day 14, respectively. Thus, although Case B produced the tallest plants, it produced the shortest roots at the end of the experiment. This difference between shoot and root responses is an important component-level result because it indicates that greater above-ground growth did not necessarily correspond to stronger overall plant development.

4.2 Wet Weight, Dry Weight, and Water Content

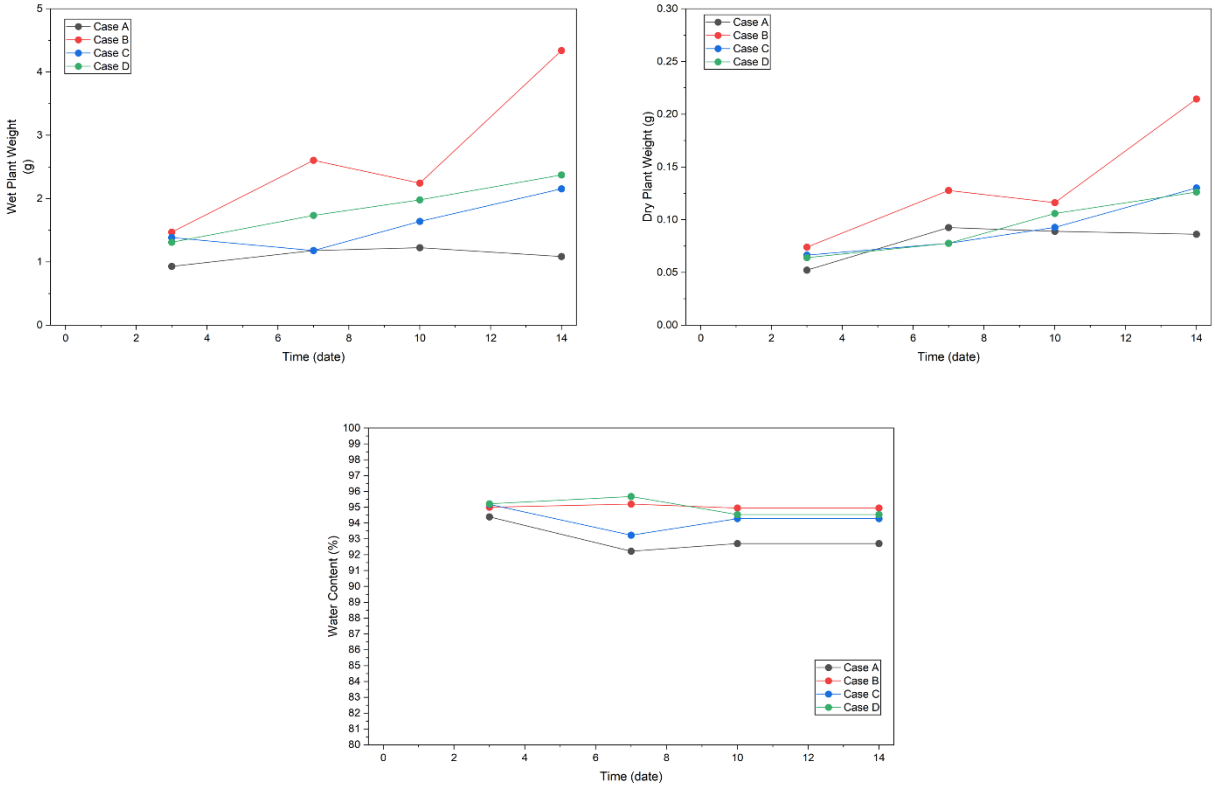


Figure 5: Wet Weight, Dry Weight, and Water Content Percentage of Plant Samples Over Different Days.

Biomass measurements showed clear differences among the treatments. At Day 3, wet plant weights were approximately 0.9 g for Case A, 1.5 g for Case B, 1.4 g for Case C, and 1.3 g for Case D. By Day 7, Case B reached approximately 2.6 g, while Cases C and D were approximately 1.2 and 1.7 g, respectively, and Case A remained approximately 1.2 g. At Day 10, Case B decreased slightly to approximately 2.25 g, whereas Case C increased to approximately 1.65 g and Case D to approximately 2.0 g. At Day 14, Case B reached approximately 4.35 g, the highest wet biomass recorded, followed by Case D at approximately 2.37 g and Case C at approximately 2.14 g. Case A remained close to 1.08 g.

Compared with the tap-water baseline at Day 14, the wet biomass of Case B was approximately four times greater. Cases C and D also exceeded the control, by approximately twofold and 2.2-fold, respectively. This indicates that the AnMBR-derived treatments generally provided more favorable conditions for biomass production than tap water alone during the 14-day period.

Dry weight showed a similar overall trend. At Day 14, Case A produced approximately 0.085 g dry biomass, while Cases B, C, and D produced approximately 0.215, 0.130, and 0.126 g, respectively. Therefore, Case B produced approximately 2.5 times the dry biomass of the control. The water-content measurements remained high for all treatments, generally between approximately 92% and 95%, indicating that most of the harvested lettuce mass consisted of water. Case B maintained approximately 95% water content at the final sampling point.

4.3 Leaf Area and Visual Plant Condition

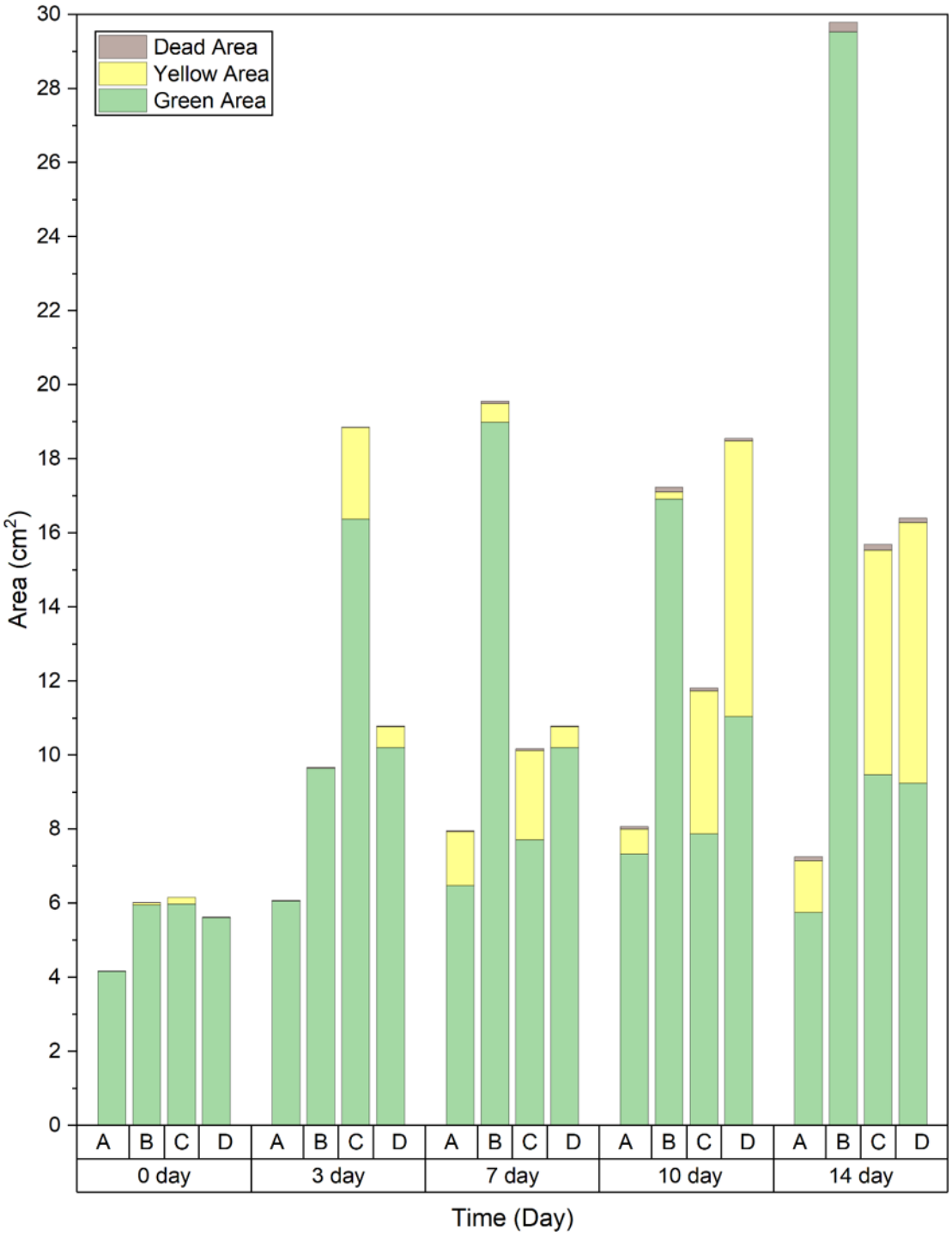


Figure 6: Changes in Lettuce Leaf Area (Green, Yellow, and Dead Regions) Across Four Experimental Conditions Over a 14-Day Period.

Leaf area was separated into green, yellow, and dead regions to distinguish productive plant tissue from visible signs of deterioration. At Day 0, the total leaf areas were relatively similar among treatments, ranging from approximately 4.1 to 6.1 cm². Differences became more pronounced during cultivation.

At Day 3, Case C had the largest total leaf area at approximately 18.8 cm², while Case B reached approximately 9.6 cm², Case D approximately 10.8 cm², and Case A approximately 6.1 cm². At Day 7,

Case B showed approximately 19.5 cm^2, with approximately 19 cm^2 represented by green tissue, whereas Case A, Case C, and Case D were approximately 7.9, 10.2, and 10.8 cm^2, respectively.

At Day 10, Case D reached approximately 18.6 cm^2, followed by Case B at approximately 17.2 cm^2, Case C at approximately 11.8 cm^2, and Case A at approximately 8.1 cm^2. By Day 14, Case B showed the strongest leaf development, with a total leaf area of approximately 29.7 cm^2, of which approximately 29.5 cm^2 was green. Case C and Case D reached approximately 15.7 and 16.4 cm^2, respectively, while Case A reached approximately 7.2 cm^2.

The distribution of green, yellow, and dead tissue is particularly important. The large green area in Case B at Day 14 indicates that its high total leaf area was not primarily caused by yellow or dead tissue. In contrast, Cases C and D showed larger yellow regions relative to their total leaf areas, indicating more visible leaf stress than Case B. Case A had the smallest total leaf area and also showed a comparatively limited amount of green tissue.

4.4 Element Concentrations in Lettuce Tissue

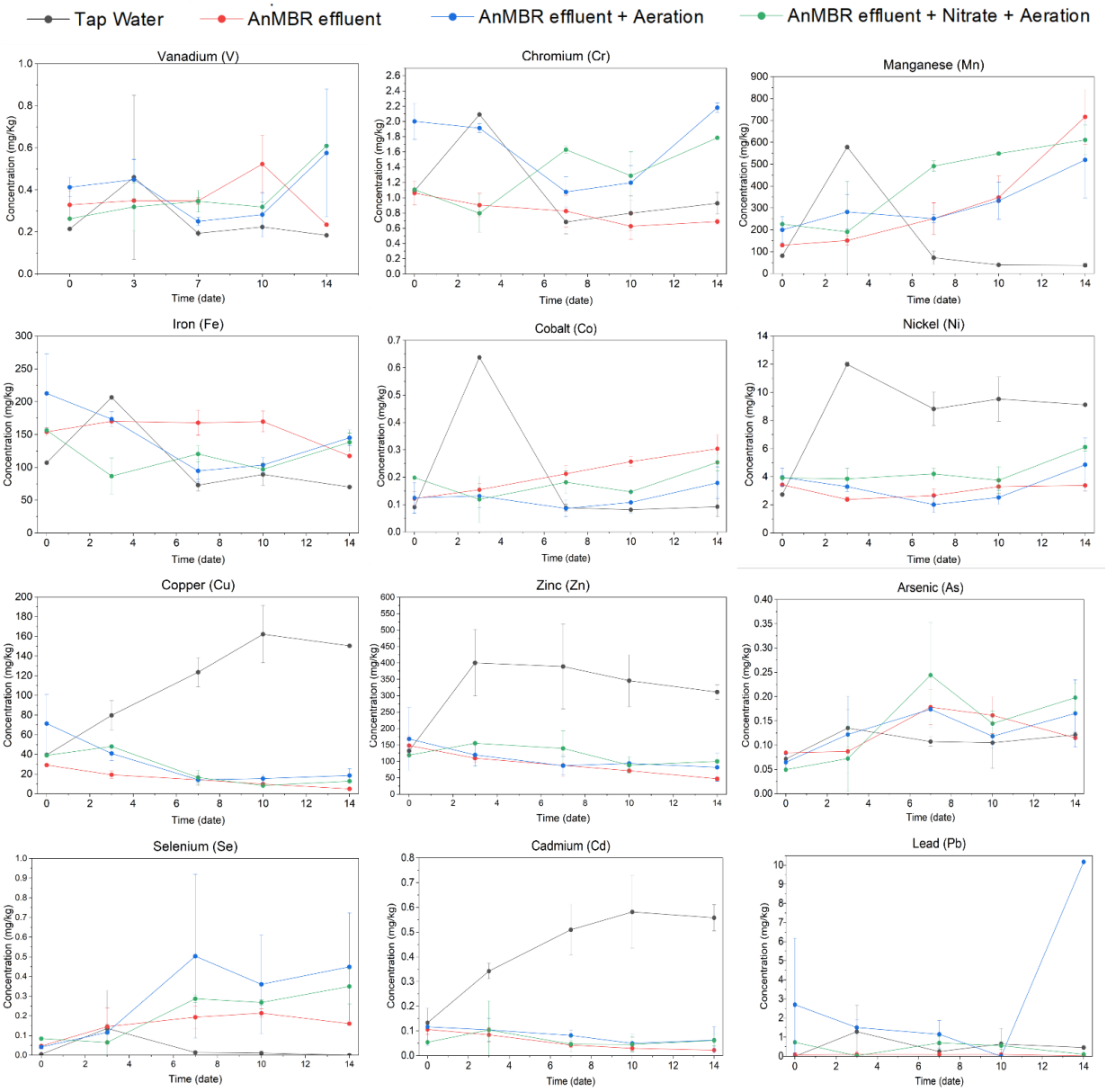


Figure 7: Concentrations (mg/kg) of 12 heavy metals in lettuce plant tissue across four cases and over a 14-day period: Data points represent sampling at Days 0, 3, 7, 10, and 14.

ICP-MS analysis of the plant tissues across the 14-day period (Figure 7) revealed distinct element accumulation patterns depending on the water source.

Tap Water Baseline (Case A): The control plants absorbed exceptionally high concentrations of copper and zinc, with copper reaching approximately 160 mg/kg by day 10 and zinc plateauing near 400 mg/kg.

AnMBR Effluent (Case B): Plants grown in untreated AnMBR effluent showed a steady, aggressive accumulation of manganese, climbing from ~150 mg/kg at day 0 to over 700 mg/kg by day 14.

Aerated and Nitrate-Added Effluent (Cases C & D): Case C experienced a severe, late-stage spike in lead (Pb), jumping from near-zero to approximately 10 mg/kg on day 14. Chromium (Cr) levels also peaked in Case C at around 2.2 mg/kg.

4.5 Element Concentrations in Water

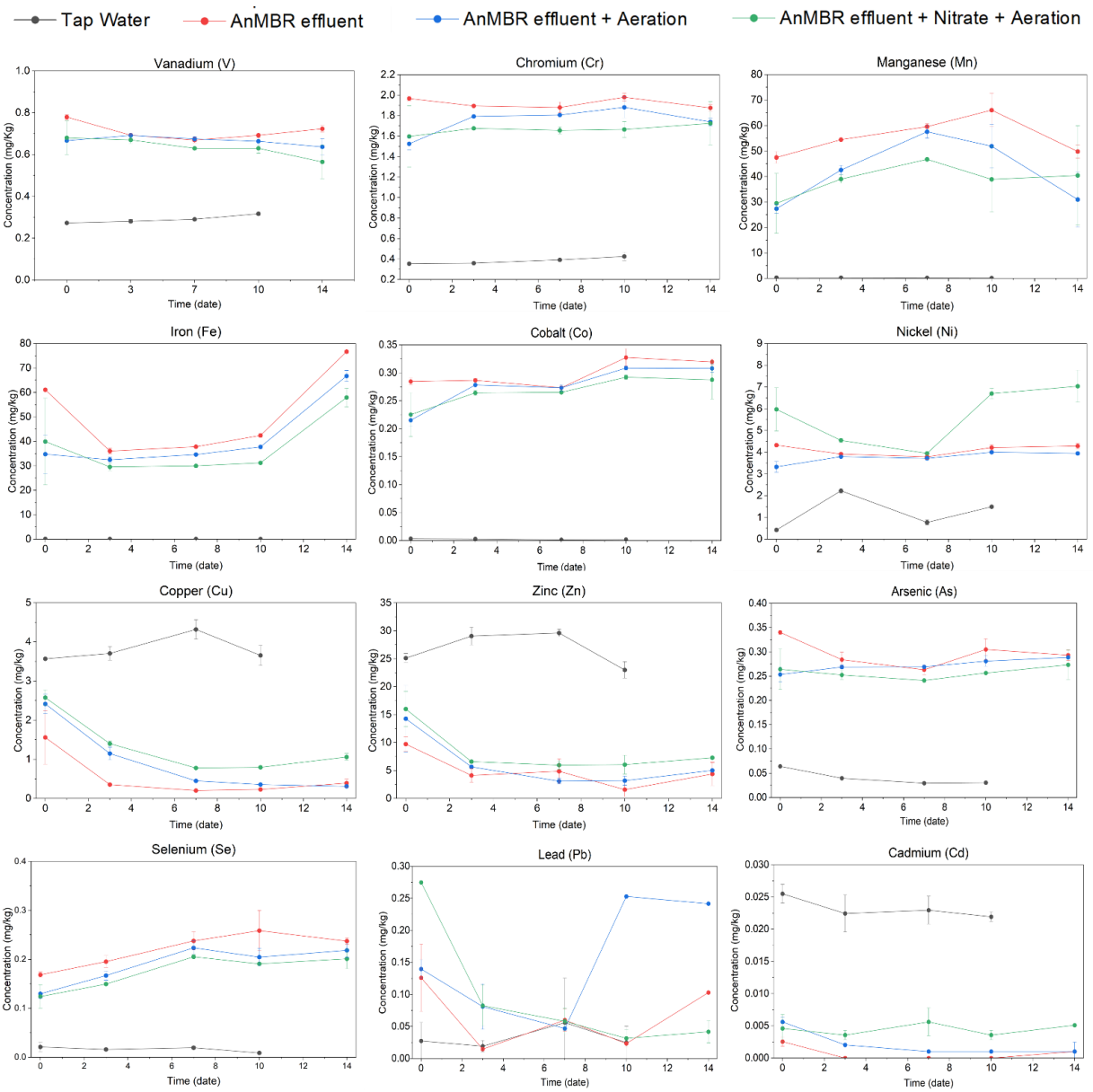


Figure 8: Concentrations (mg/kg) of 12 heavy metals in water sample across four cases and over a 14-day period: Data points represent sampling at Days 0, 3, 7, 10, and 14.

The water samples showed treatment-specific patterns for the same 12 elements. Compared with the tap-water baseline, AnMBR-derived water generally contained higher concentrations of several elements. Chromium, manganese, iron, cobalt, nickel, arsenic, and selenium were among the elements for which the AnMBR treatments commonly showed higher water concentrations than the tap-water condition.

For example, water chromium in Case B remained around 1.9–2.0 mg/kg during the experiment, compared with approximately 0.35–0.42 mg/kg in the tap-water treatment. Water manganese in Case B increased from approximately 47 mg/kg to approximately 66 mg/kg before declining to approximately 50 mg/kg at Day 14, while the tap-water concentration remained near zero. Iron in Case B increased markedly to approximately 76 mg/kg at Day 14, while the tap-water concentration remained near zero. These differences demonstrate that the AnMBR-derived irrigation water introduced a substantially different elemental environment from the tap-water control.

Conversely, some elements were present at higher concentrations in the tap-water treatment. Copper and zinc are notable examples. Tap-water copper remained approximately 3.5–4.3 mg/kg, whereas the AnMBR treatments generally remained below approximately 1.5 mg/kg. Tap-water zinc remained approximately 23–30 mg/kg, while the AnMBR treatments were generally below approximately 16 mg/kg.

Table 2: Comparative Element Accumulation Under AnMBR and Tap-Water Treatments.

Element	Biological role	Effect of AnMBR vs Tap Water	Interpretation
Mn	Essential	**Strongly increased**	**Main concern**
Fe	Essential	Generally higher	Potentially beneficial, but monitor
Cu	Essential	**Much lower**	Favorable control of accumulation
Zn	Essential	**Much lower**	Favorable control of accumulation
Se	Essential trace element	Higher	Potential benefit, but concentration matters
Co	Trace/essential role	Increased	Monitor
Ni	Trace/essential role	**Lower**	Favorable relative to control
Cr	Form-dependent	Generally lower	No obvious AnMBR enrichment
As	Toxic/non-essential	Similar	Continue monitoring
Cd	Toxic/non-essential	**Much lower**	**Very favorable**
Pb	Toxic/non-essential	Low in Case B	**Not an AnMBR-only problem**
V	Possible biological role	Variable	No clear major AnMBR effect

Overall Element Accumulation

ICP-MS analysis revealed that the effect of AnMBR effluent on elemental accumulation was element-specific rather than a general increase in heavy-metal uptake. Compared with the tap-water control, lettuce grown with AnMBR effluent showed lower accumulation of several elements, including Cu, Zn, Ni, Cr, and Cd. However, a pronounced increase in Mn accumulation was observed, particularly toward the end of the cultivation period. These results indicate that AnMBR treatment altered the elemental uptake pattern of lettuce rather than uniformly increasing the accumulation of potentially harmful elements.

Essential Elements

Among the essential elements, manganese (Mn) showed the most pronounced accumulation under AnMBR treatment. Mn concentrations increased substantially during the later stages of cultivation and reached approximately 710 mg/kg at Day 14, making it the main elemental concern associated with the

AnMBR treatment. Although Mn is an essential micronutrient required for plant growth, its excessive accumulation warrants further investigation and monitoring.

Iron (Fe) was generally higher in the AnMBR treatment during the later sampling stages. As an essential plant nutrient, this increase may contribute to plant nutritional status; however, the observed concentrations should be evaluated further before concluding that the increase is beneficial.

The AnMBR treatment also resulted in changes in cobalt (Co) and selenium (Se) accumulation. Both elements showed higher concentrations than the tap-water control at several sampling points. Because these elements have biological roles at low concentrations, their accumulation should be interpreted according to their concentration ranges rather than being classified simply as beneficial.

Elements Showing Lower Accumulation Under AnMBR

A particularly important finding was the substantially lower accumulation of copper (Cu) and zinc (Zn) in the AnMBR treatment compared with the tap-water control. The tap-water treatment showed pronounced accumulation of both elements over time, whereas their concentrations remained considerably lower in lettuce exposed to AnMBR effluent. Since Cu and Zn are essential micronutrients but can become harmful at excessive concentrations, the lower accumulation observed under AnMBR may indicate better control of excessive elemental uptake.

Nickel (Ni) also remained considerably lower under AnMBR treatment than under tap water, particularly during the later stages of cultivation. Similarly, chromium (Cr) was generally lower under AnMBR than in the tap-water treatment, with no clear evidence from the dataset of substantial Cr enrichment caused by AnMBR effluent.

Toxic and Potentially Toxic Elements

The most favorable result from a food-safety perspective was observed for cadmium (Cd). Cd accumulation was substantially lower in lettuce grown with AnMBR effluent than in the tap-water control, where Cd increased progressively during cultivation. This suggests that, under the conditions tested, AnMBR effluent did not promote Cd accumulation and may have reduced its uptake relative to tap water.

Arsenic (As) showed relatively similar and variable concentrations among treatments, with no clear evidence of substantial As enrichment specifically attributable to AnMBR effluent. Nevertheless, because As is a toxic, non-essential element, its accumulation should continue to be monitored in future experiments.

For lead (Pb), the major late-stage increase occurred in the aerated AnMBR treatment (Case C) rather than the non-aerated AnMBR treatment (Case B). Therefore, the observed Pb increase should not be attributed solely to AnMBR effluent. This distinction suggests that aeration or the combined treatment conditions may have influenced Pb accumulation.

Overall Interpretation

Overall, the elemental analysis indicates that AnMBR effluent did not cause a generalized increase in heavy-metal accumulation in lettuce. Instead, it was associated with lower accumulation of several elements, including Cu, Zn, Ni, Cr, and particularly Cd, while the principal concern was the strong accumulation of Mn at the later growth stage. The results therefore suggest that AnMBR-treated wastewater has potential for hydroponic reuse, but element-specific monitoring is necessary, especially for Mn and other elements that may accumulate under particular treatment conditions.

4.6 Transfer Factor (TF)

The transfer factor was calculated as the concentration of an element in plant tissue divided by its corresponding concentration in the water tank at the same sampling time. The report also presents the tap-water experiment separately as the baseline for comparison.

$$TF = \frac{C_{pt}}{C_{wt}}$$

C_{pt} : the concentration of certain element in plant tissue at t day

C_{wt} : the corresponding concentration of certain element in water tank at same time of C_{pt}

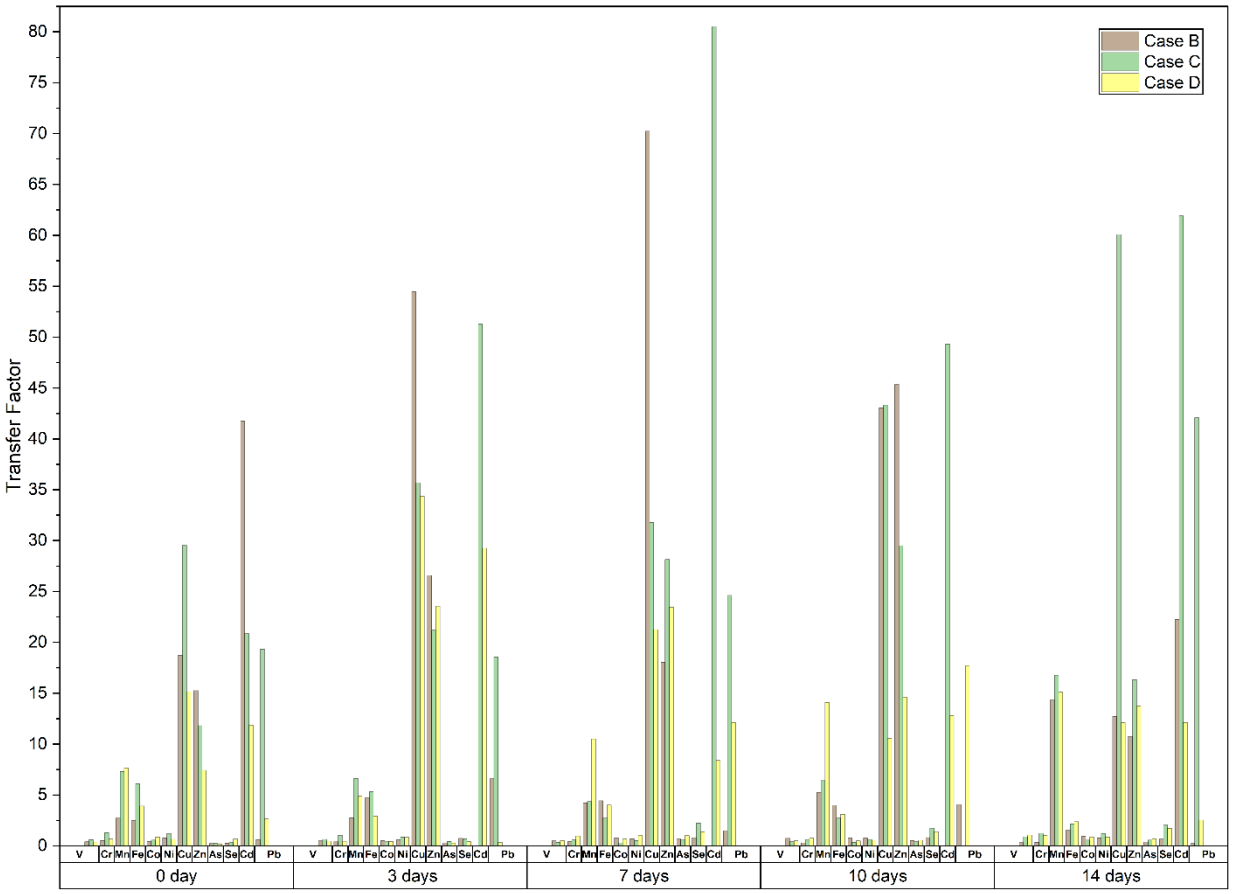


Figure 9: Transfer Factor of 12 elements under three cases (B, C, and D) over different time.

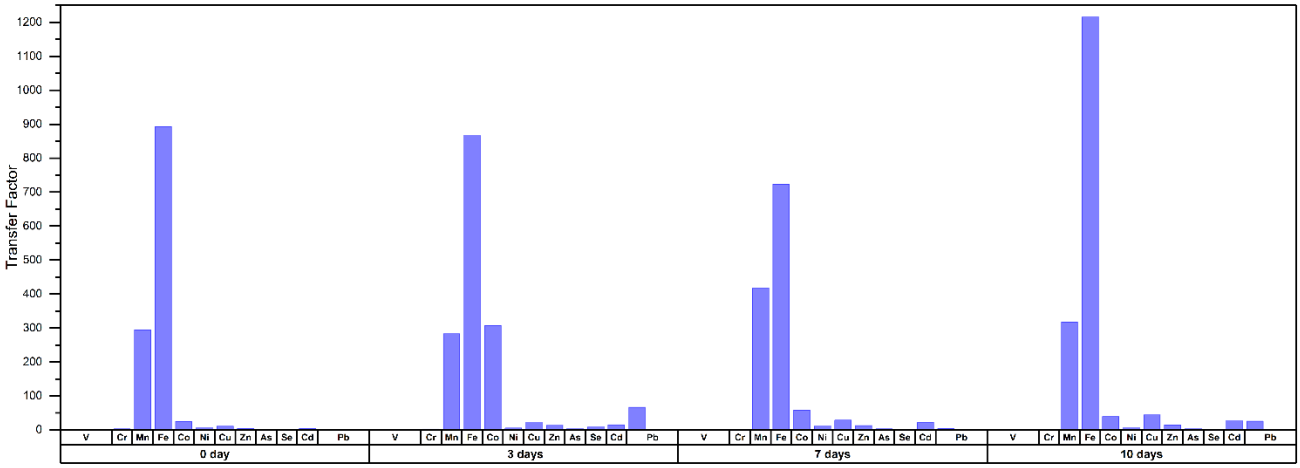


Figure 10: Transfer Factor of 12 elements for tap water experiment (case A), which is the baseline, over different time.

The transfer-factor results showed strong element-specific variation. Several elements produced transfer factors substantially greater than 1, demonstrating that plant concentrations could exceed the corresponding measured water concentrations. In particular, high transfer factors were observed for elements such as manganese, iron, copper, zinc, arsenic, selenium, and cadmium at selected time points. Case C showed some of the largest transfer factors, including values exceeding 80 for selected elements, while Case B also showed values above 70 for selected measurements.

The tap-water baseline also produced very high transfer factors for several elements, including manganese and iron. Therefore, high transfer factors cannot be attributed exclusively to AnMBR exposure. Instead, the results indicate that plant uptake and accumulation depend on both the treatment water composition and the plant's ability to concentrate individual elements relative to their water concentrations.

Overall, the component-level comparison indicates that the addition of aeration and nitrate did not uniformly improve plant growth or reduce element accumulation. Case D produced more biomass than Case C at Day 14, but it did not consistently outperform Case B, which received AnMBR effluent without the additional nitrate or aeration.

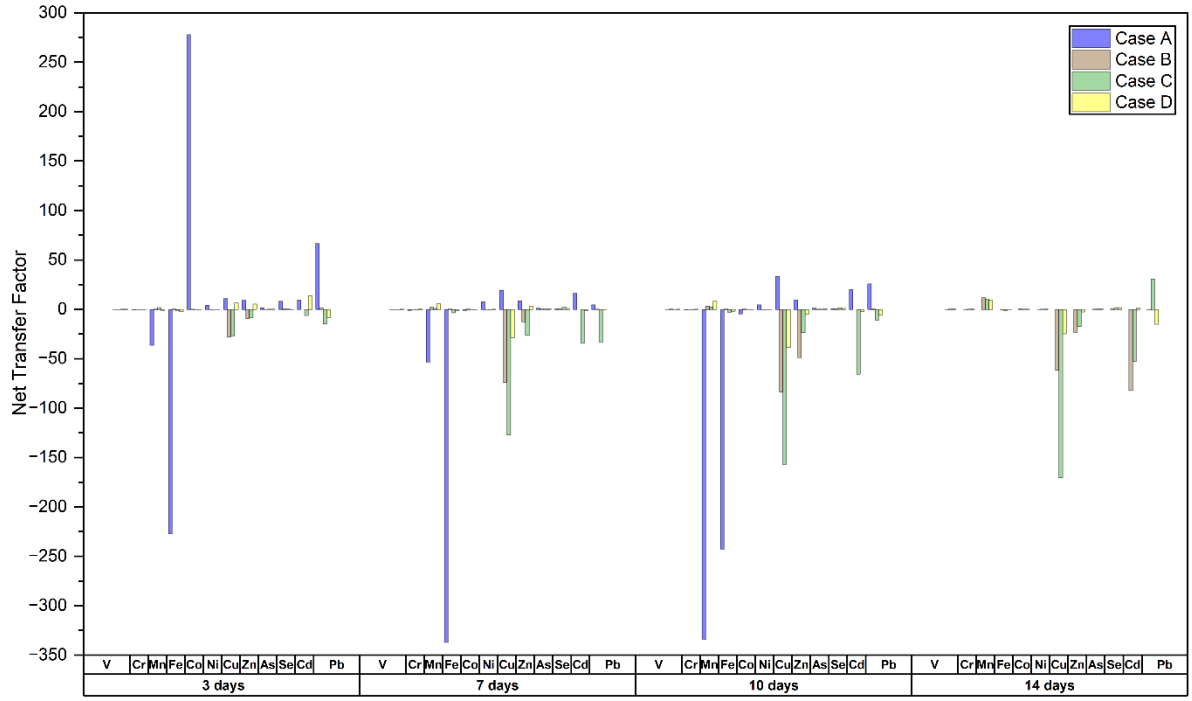
Net Transfer Factor (NTF):

$$NTF = \frac{C_{pt} - C_{p0}}{C_{wt}}$$

C_{p0} : the concentration of certain element in plant tissue at day zero (initial concentration)

C_{pt} : the concentration of certain element in plant tissue at t day

C_{wt} : the corresponding concentration of certain element in water tank at same time of C_{pt}


Net Transfer Factor
300
250
200
150
100
50
0
−50
−100
−150
−200
−250
−300
−350
Case A
Case B
Case C
Case D
V Cr Mn Fe Co Ni Cu Zn As Se Cd Pb
3 days
7 days
10 days
14 days

5 Discussion

The results show that lettuce growth was strongly influenced by the chemical characteristics of the irrigation water, particularly ammonia concentration, dissolved oxygen, and nutrient availability. Case B, using AnMBR effluent without aeration, produced the strongest overall growth, reaching approximately 24.9 cm plant height, 4.35 g wet weight, and 29.7 cm^2 leaf area at Day 14. However, Tanks 4 and 6 in Case B contained relatively high ammonia concentrations of 18.7 and 19 mg/L, respectively. Therefore, although the AnMBR effluent supported rapid growth, its high ammonia concentration may create an unfavorable nitrogen balance for longer-term cultivation. The nitrate supplementation applied in Case D did not produce better growth than Case B, indicating that increasing nitrate was not sufficient to optimize the nitrogen balance.

Yellow and white leaves were observed particularly in the aerated treatments and the tap-water treatment, which also showed relatively high dissolved oxygen. These symptoms may be related to reduced iron availability rather than high oxygen itself. Langenfeld and Bugbee reported that increasing aeration and agitation in hydroponic systems can disrupt the rhizosphere and reduce iron uptake, resulting in chlorosis. They also found that aeration increased iron accumulation around roots while reducing iron concentration in leaves. This provides a possible explanation for the leaf discoloration observed in the present study. Therefore, high DO should not necessarily be interpreted as a beneficial condition when it is achieved through strong aeration and root-zone agitation.

The ICP-MS analysis showed that element accumulation in lettuce tissues varied considerably among treatments and did not always correspond directly to the concentrations measured in the irrigation water. This demonstrates that lettuce can selectively accumulate elements from the water. The Transfer Factor and Net Transfer Factor provided additional information about this process by evaluating element movement from the water into plant tissues and changes relative to the initial tissue concentrations. These results are important because high element accumulation may represent a potential food-safety concern even when the corresponding water concentration is relatively low.

Overall, the results indicate that AnMBR effluent has potential as both an irrigation water and nutrient source for hydroponic lettuce. However, the high ammonia concentration in Case B, leaf discoloration under aerated conditions, and element accumulation in plant tissues demonstrate that maximizing plant growth alone is not sufficient to identify the best treatment. Future optimization should focus on controlling the ammonia-to-nitrate balance, improving iron availability, and monitoring element accumulation while maintaining suitable dissolved oxygen conditions.

6 Limitations and Future Work

1. High ammonia concentration in the AnMBR effluent.

The most important water-quality limitation was the high ammonia concentration measured in Tanks 4 and 6 (Case B), approximately 18.7 and 19 mg/L, respectively. Although Case B produced strong plant growth, the elevated ammonia concentration may create a risk of nitrogen imbalance or toxicity during longer cultivation periods. The current experiment was too short to determine whether this condition would eventually reduce plant productivity.

Future work: Measure ammonium and nitrate more frequently throughout the experiment and evaluate several nitrate-to-ammonium ratios. Rather than using only 450 ppm nitrate, future experiments should test multiple nitrate additions to determine the optimum nitrogen composition for lettuce.

2. Limited experimental duration.

The experiment lasted only 14 days after the plants were transferred to the hydroponic system. This period is sufficient to identify early growth responses but is not sufficient to evaluate full crop development, harvest-stage quality, or long-term accumulation of ammonia and elements.

The aerated treatments produced substantial changes in DO compared with the non-aerated conditions, while yellow and white leaf symptoms were observed in the aerated treatments and in the tap-water treatment. Because aeration also causes continuous movement and agitation of the root-zone solution, the effects of high DO and mechanical agitation cannot be clearly separated. Previous research has shown that aeration can affect rhizosphere conditions and iron availability, which may contribute to chlorosis.

Future work should therefore include an additional experiment without aeration, while maintaining comparable water-quality and nutrient conditions, to determine the plant response under lower and naturally changing DO conditions.

7 Conclusion

This study demonstrated that AnMBR-treated municipal wastewater mixed with compost leachate can support lettuce growth in a controlled hydroponic system, but the results also revealed important water-quality and nutrient-management challenges. The AnMBR effluent treatment produced strong biomass development, particularly in Case B; however, Tanks 4 and 6 showed high ammonia concentrations of approximately 18.7 and 19 mg/L, indicating that high growth alone does not represent an optimal treatment condition. Yellow and white leaf symptoms observed in the aerated treatments and tap-water tanks were also associated with high dissolved oxygen conditions, but the literature suggests that the accompanying aeration and root-zone agitation may disrupt the rhizosphere and reduce iron availability rather than high oxygen concentration itself being directly harmful. The ICP-MS analysis further demonstrated that element accumulation in lettuce cannot be predicted from water concentrations alone, making the Transfer Factor and Net Transfer Factor important for evaluating water-to-plant element movement. Overall, the main finding is that AnMBR effluent has promising potential as both an irrigation water and nutrient source, but successful reuse for lettuce requires careful control of ammonia, aeration, iron availability, and element accumulation. Longer experiments with optimized nitrogen ratios, controlled low-agitation oxygenation, direct iron-availability measurements, increased replication, and food-safety and pathogen assessments are necessary before the system can be recommended for practical edible-crop production.

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